

Methods for Percent Oil Analysis of Avocado Fruit

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The 8% oil criterion for avocado was established in 1925 to insure minimum maturity and quality. However, determination of the oil content of avocado fruit is expensive, time consuming, and tedious, especially for growers who lack the necessary laboratory and equipment. A simpler method would be more useful for growers and also for packers.

The standard method for analyzing the oil content is done by the petroleum ether extraction of dried material in a Soxhlet extractor. However, this method is too expensive and requires too much time to be generally useful in the avocado industry. Lesley and Christie (1929) described a relatively short method which uses Halowax oil (monochloronaphthalene) as a solvent and a refractometer for determination of oil content in avocado tissue.

Two other methods, the NMR method and the simplified method (oil- plus-water constant method), have been tested in connection with oil content determination. The detailed procedures of the Soxhlet, Halowax, NMR, and oil-plus-water constant methods are described and compared.

[A] Soxhlet Method

Soxhlet extraction of oil is the American Official Agricultural Chemists' method for determination of oil content in plant materials. Oil is extracted with a continuous reflux of petroleum ether over dried tissue material in a Soxhlet extractor.

Apparatus and Solutions

1. Soxhlet extractor
2. microwave oven
3. balance (sensitive to 10 mg)
4. petri dish or weighing dish
5. potato peeler
6. mortar and pestle
7. porcelain thimble

8. drying oven
9. desiccator
10. solvent (petroleum ether)

Procedures

1. Weigh petri dish and record the weight (P).
2. Cut the fruit lengthwise into quarters, removing the seed, seed coat, and skin.
3. Take slices off one cut surface of each quarter with a potato peeler.
4. Transfer approximately 10 g of slices to the petri dish.
5. Weigh the dish containing fresh tissue and record the weight (F).
6. Place the dish in a microwave oven and cook the sample until dried to constant weight (about 15 minutes for 10 samples).
7. Weigh the dish containing dried tissue and record the weight (D).
8. Grind the dried tissue with a mortar and pestle.
9. Weigh a porcelain thimble and record the weight (T).
10. Transfer ground tissue to the thimble.
11. Weigh the thimble containing ground tissue and record the weight (G).
12. Place the thimble in a Soxhlet extractor apparatus. The apparatus consists of Soxhlet condensers, flat bottom flasks, and hot plates.
13. Add petroleum ether and boiling chips into the flat bottom flasks.
14. Turn on the burner and reflux the thimble for about 15 hours until the petroleum ether washing appears colorless.
15. Remove the thimble from the extractor and allow to drain.
16. Dry the thimble in a drying oven at about 90°C for 30 minutes and cool in a desiccator.
17. Weigh the thimble containing extracted tissue and record the weight (E). Calculate the percent oil.

$$\% \text{ Oil} = [(D-P)/(F-P)] \times [(G-E)/(G-T)] \times 100$$

P: Petri dish tare weight

F: Fresh tissue gross weight

D: Dried tissue gross weight

T: Thimble tare weight

G: Ground tissue gross weight

E: Extracted tissue gross weight

Difficulties

This method is cumbersome, too expensive, and requires too much time

[B] Halowax Method

Lesley and Christie (1929) described the Halowax method, using a refractometer for determining the oil content of avocados. Avocado tissue is ground with Halowax oil and the refractive index of the mixture is measured. The refractive index is calibrated from results obtained by Soxhlet extraction. This is the official method for determination of oil in avocado fruit (Sweet, 1955). Shannon (1949) revised this method and corrected the oil content and refractive index chart.

Apparatus and Solutions

1. Abbe-type refractometer and attachments including water circulation for temperature control
2. balance (sensitive to 10 mg)
3. food chopper or grater
4. steel ball grinder
5. hot water bath
6. 5 ml pipette
7. filter paper (Whatman #4)
8. Halowax oil #1000
9. ethyl ether

Procedures

The following procedures are summarized from the California Fruit and Vegetable Standardization Code (Article 11 Avocado).

1. Cut the avocado lengthwise into homologous halves, discarding the seed.
2. Cut each half into equal quarters.
3. Remove the skin and seed coat from alternate quarters.
4. Grind the four peeled pieces through a food chopper.
5. Collect the pulp and juice on a suitable dish.
6. Thoroughly mix the pulp and juice and, as rapidly as possible, weigh 5 g of the mixture on a piece of filter paper in a weighing dish.
7. Transfer the weighed sample together with the filter paper into the grinding shell

- containing one ball bearing.
8. Wipe the weighing dish clean with a piece of filter paper and place this into the shell, followed by the second ball bearing.
 9. Pipette exactly 5 ml of Halowax oil into the shell.
 10. Keep the shell in a vertical position until the cap is fastened tightly with a wrench.
 11. Clamp the shell containing the sample and Halowax oil into the grinding machine.
 12. The grinder should be operated for approximately 15 minutes. The resulting paste in the shell must be smooth and uniform.
 13. Transfer the bulk of the mixture to a large watch glass and press out liquid with the spatula.
 14. If separation of the liquid does not readily occur, the mixture should be heated for not more than 1 minute on a hot water bath.
 15. Cool the mixture, if heated, and transfer a few drops of the separated liquid to the refractometer prism.
 16. Refractometer temperature should be stabilized to 25°C by means of water circulating through the instrument.
 17. Read the index of refraction of the Halowax oil mixture to the fourth decimal figure.
 18. If the index of refraction of Halowax oil used is less than 1.6353 the difference is added to the reading on the sample; if greater, the difference is subtracted from the refractometer reading.
 19. The prism surfaces of the refractometer should be cleaned before each determination by wiping with lens paper.

Difficulties

1. The refractive index of different lots of Halowax oil may vary as much as 0.002 and may also change with age or exposure to light.
2. Refractive index readings are temperature dependent. To use the oil and refractive index chart, a correction must be made by adding 0.004 for every degree below 25°C.
3. It is necessary to read the refractive index to the fourth decimal figure in order to get 0.1% oil accuracy. However, it is not easy to read to that extent on a refractometer scale.
4. Many transfers of material are made in the Halowax method. Each transfer invites the possibility of error.
5. Because of the equipment needed, a grower cannot make determination himself.

[C] NMR Method

Oil content of avocado fruit can be tested with a Newport Analyser Mark III (Newport Oxford Instruments). This analyser is a unique instrument which provides a direct reading of the quantity of liquid oil. It is a low resolution NMR spectrometer which can measure the nuclear magnetic resonance of hydrogen contained in liquid (e.g. oil, water). Therefore, water must be removed by drying before the oil is measured. The main features of this machine are its rapidity, accuracy, and simplicity of operation.

Apparatus

1. Newport Analyser Mark III (Newport Oxford Instruments)
2. NMR vial
3. microwave oven
4. balance (sensitive to 1 mg)
5. petri dish or weighing dish
6. potato peeler
7. mortar and pestle
8. desiccator

Procedures

1. Weigh petri dish and record the weight (P).
2. Cut the fruit lengthwise into quarters, removing the seed, seed coat, and skin.
3. Take slices off one cut surface of each quarter with a potato peeler.
4. Transfer approximately 10 g of slices to the petri dish.
5. Weigh the dish containing fresh tissue and record the weight (F).
6. Place a dish in the microwave oven and cook the sample until dried to constant weight (about 15 minutes for 10 samples).
7. Weigh the dish containing dried tissue and record the weight (D).
8. Grind the dried tissue with a mortar and pestle.
9. For a reference, add pure avocado oil into the NMR vial and record the reference oil weight (R).
10. Insert the reference vial into the NMR apparatus.
11. Press reset with the integration time set at 32 and records the reference reading on the display (O).
12. Weigh an NMR vial and record the weight (V).
13. Add ground avocado sample into the vial.

14. Weigh the vial containing ground tissue and record the weight (G).
15. Insert the vial into the NMR apparatus.
16. Press reset and records the sample reading on the display (S).

$$\% \text{ Oil} = [(D-P)/(F-P)] \times [S/(G-V)] \times (R/O) \times 100$$

P: Petri dish tare weight

F: Fresh tissue gross weight

D: Dried tissue gross weight

R: Reference oil weight

O: Reference oil reading

V: Vial tare weight

G: Ground tissue gross weight

S: Sample reading

Difficulties

1. The NMR analyser is expensive.
2. Complete dryness of sample is necessary.

[D] Oil-Plus-Water Constant Method

For many varieties, the sum of the amount of oil and water in percent by weight is nearly constant during fruit development. Therefore, the percent oil can be calculated by subtracting percent water from the constant. The water content is determined by drying samples in a microwave oven. This method is so simple that growers would not need a laboratory for this operation. This would be a practical method for both growers and packing houses.

Apparatus

1. microwave oven
2. balance (sensitive to 10 mg)
3. petri dish or weighing dish
4. potato peeler

Procedures

1. Weigh a petri dish and record the weight (P).
2. Cut the fruit lengthwise into quarters, removing the seed, seed coat, and skin.

3. Take slices off one cut surface of each quarter with a potato peeler.
4. Transfer approximately 10 g of slices to the petri dish.
5. Weigh the dish containing fresh tissue and record the weight (F).
6. Place the dish in a microwave oven and cook the sample until dried to constant weight (about 15 minutes for 10 samples).
7. Weigh the dish containing dried tissue and record the weight (D).
8. Calculate the percent water, then subtract the percent water from the constant.

$$\% \text{ Water} = [(F-D)/(F-P)] \times 100$$

$$\% \text{ Oil} = \text{Constant} - [(F-D)/(F-P)] \times 100$$

P: Petri dish tare weight

F: Fresh tissue gross weight

D: Dried tissue gross weight

Difficulties

The constant may not be the same for all varieties and locations. Constants for more varieties and more locations should be checked in the future.

Idea behind the simplified method

As the avocado fruit matures the oil content increases and the water content decreases. Figure 1 shows the increase in the percent oil and decrease in percent water during development of Fuerte fruit at Fallbrook. There is a close relationship between the increase in the percentage of oil and the decrease in the percentage of water during maturation. At any time during maturation, percent oil plus percent water is nearly constant. This implies that the rate of increase in the percentage of oil is the same as the rate of decrease in the percentage of water during fruit development. Therefore, the percentage of oil can be calculated simply by subtracting the percentage of water from the constant. $\% \text{ Oil} + \% \text{ Water} = \text{Constant}$. $\% \text{ Oil} = \text{Constant} - \% \text{ Water}$.

This also suggests that the non-oil dry matter is constant at any time during maturation because avocado fruits are composed of water, oil, and non-oil dry matter. Schroeder (1958) has reported that during early fruit life the process of cell division is very intense, and a moderate but constant increase in the size of individual cells occurs. When the fruit has attained approximately half its ultimate size most of the cells will have reached their maximum dimensions, and subsequent increases in fruit size result primarily from cell divisions. This means that the composition of the fruit in later stages of development will be determined by cells that have uniformly attained their maximum dimensions.

It is reasonable to find that the non-oil dry matter will have a constant composition. Since the maximal dimensions of the cells are fixed, the continued metabolism leading to the formation of oil displaces or replaces an equivalent volume of water.

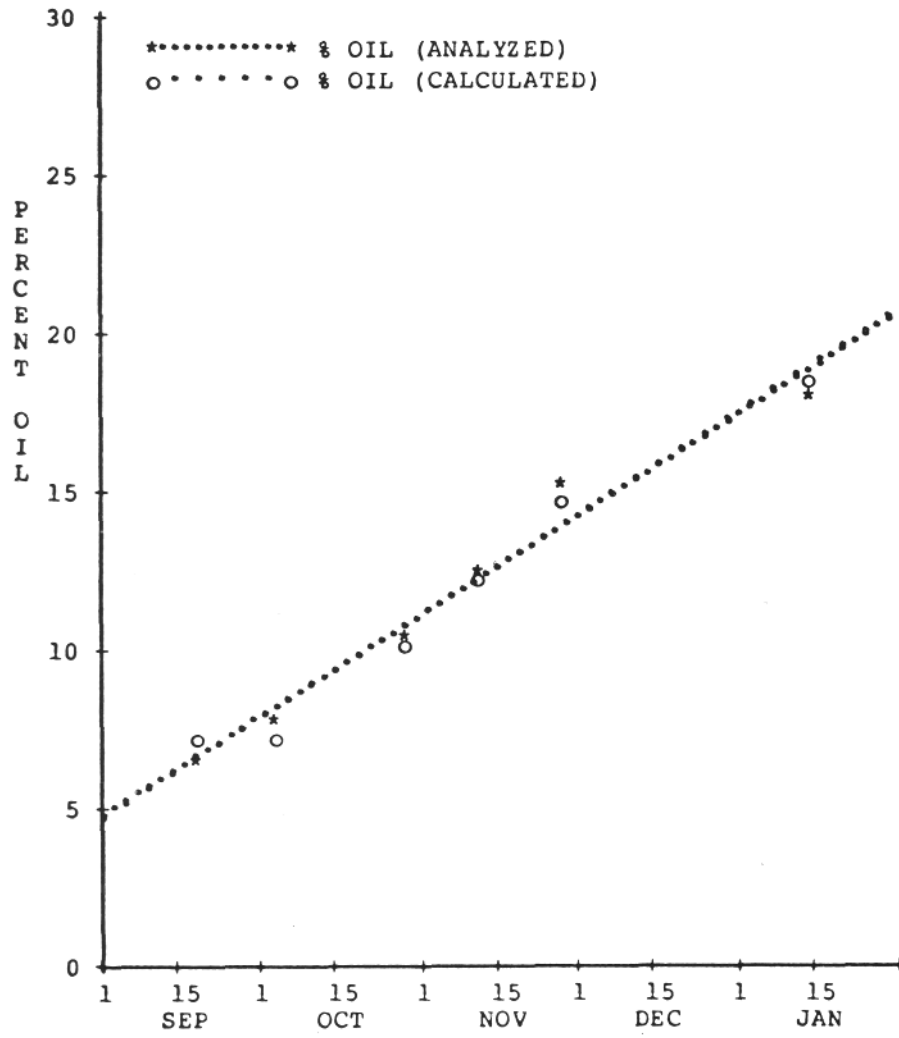


Figure 2: Difference between Percent Oil calculated from the Constant and Percent Oil obtained by Chemical Analysis.

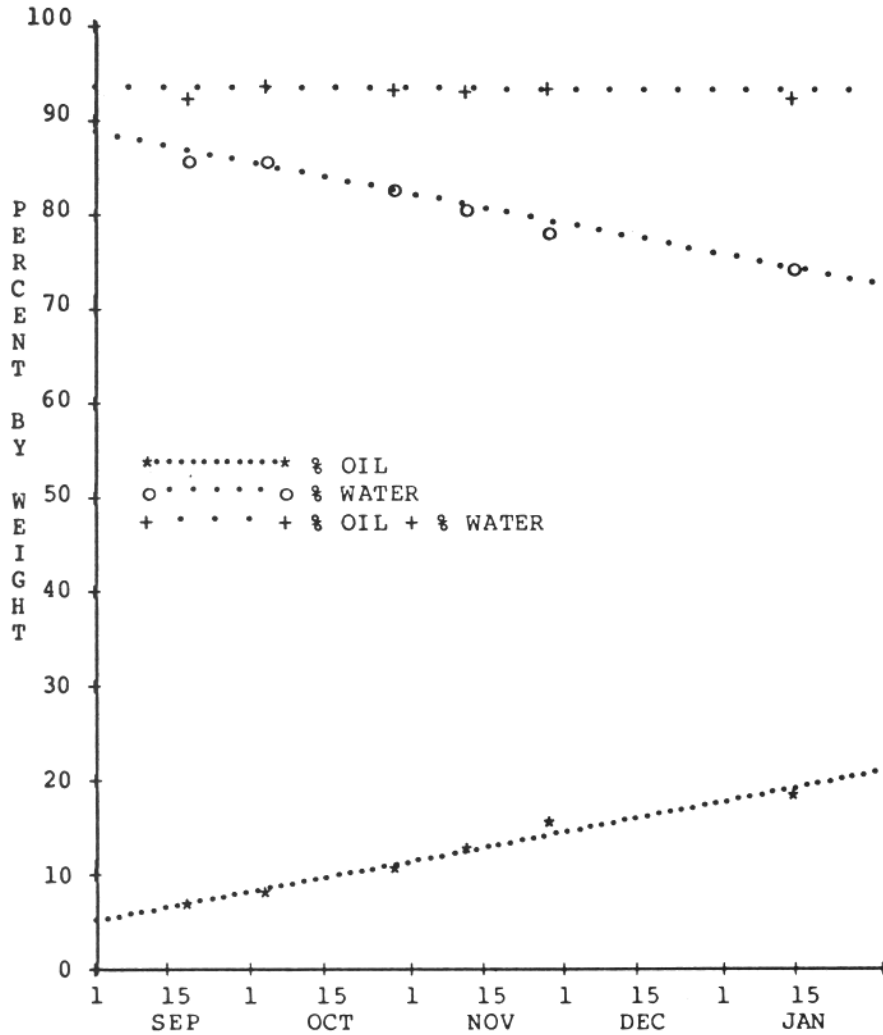


Figure 1: Increase in Percent Oil and Decrease in Percent Water during development of Fuerte Fruit at Fallbrook.

Figure 2 shows the difference between percent oil calculated from the constant and percent oil obtained by chemical analysis for Fuerte fruit at Fallbrook. Starred points indicate the percentage of oil obtained by chemical analysis. Open circles are the percentage of oil calculated by subtracting the percentage of water from the constant. The linear regression plots are indicated by the dotted lines. Apparently, there is no significant difference between the calculated percentage of oil and the percentage of oil obtained by chemical analysis.

References

- Lesley, B. E., and A. W. Christie (1929) Use of the refractometric method in determination of oil in avocados. *Industrial and Engineering Chemistry (Analytical Edition)* 1: 24
- Schroeder, C. A. (1958) Growth and development of the avocado fruit. *Calif. Avocado*

Soc. Yearbook 42: 114

Shannon, A. F. (1949) Refractive index and other extraction methods for oil in avocados. Bull. Calif. Dept. of Agric. 38: 127 - 132

Sweet, R. H. (1955) Official method for the determination of oil in avocados. Calif. Dept. of Agri. Bull. 44: 37