

## CORRELATIONS BETWEEN CUTICLE WAX AND OIL IN AVOCADOS

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Cuticle wax, or bloom, is the waxy material which may be observed on leaves, stems, and fruits of many plants. The wax is usually extruded as platelets or rodlets through submicroscopic pores in the cuticle, the lacquer-like covering on the epidermal cells. In the avocado fruit, the cuticle wax may be observed readily at maturity. This fact led Coit (2) to comment that the appearance of the bloom on avocados was an indication of coming maturity. Although he considered bloom a useful indicator, he preferred to rely on oil content for a more positive indication of the state of maturity.

Nothing further was done with cuticle wax as a possible diagnostic characteristic for indicating avocado maturity until Braithwaite and Robinson (1) prepared a report for the California Avocado Advisory Board using the infrared spectrophotometer to study absorption and reflectance spectra. The possibility of using a reflectance spectrum was clearly an advantage in developing a non-destructive test for maturity. However, the weak signal obtained from reflectance measurements resulted in emphasis being placed on the absorption spectra which were obtained from samples of wax removed from the fruits with solvents.

The results of their study indicated that the absorption spectrum of the cuticle wax changed as the fruit developed. The ratio of absorbance at  $13.95/3.4\mu$  was greater in mature than in immature fruit.

The interest in the possibility of using cuticle wax to develop a nondestructive test for avocado maturity led to the detailed study which is now in progress. The present report summarizes the findings on the amount of wax present on Hass avocados and on infrared absorption peak ratios of both Hass and Fuerte avocados.

### Materials and Methods

Ten fruit samples were collected at 2- or 4-week intervals during most of the period of fruit development. The fruits were weighed and measured to obtain the desired data on size and surface area. In preliminary trials, chloroform was found to be the most effective solvent for removing the cuticle wax and was therefore used in this investigation. The wax was removed from the fruits by rinsing them with solvent while gently rubbing with cotton or glass wool swabs. It was necessary to de-wax the cotton prior to use because it contained wax with an absorption spectrum similar to that of avocado wax. The chloroform solution of wax was strained through a wad of glass wool and then gently heated to drive off the solvent. The weight of wax residue was used as the value for total wax per fruit.

Infrared absorption spectra were made of wax samples deposited on sodium chloride plates. A known amount of wax dissolved in chloroform was applied to the salt plate and

the solvent was evaporated with the aid of an air stream and a heat lamp. The wax was confined to the central part of the salt plate with a Teflon ring pressed against the plate. The amount of wax used varied between 0.15 and 0.40 mg, being adjusted to give an absorbance of between 0.7 and 1.0 at  $3.42/\mu$ , the wavelength of greatest absorbance.

After removal of the cuticle wax, oil determinations of the fruit flesh were made by the Halowax oil method (3,4).

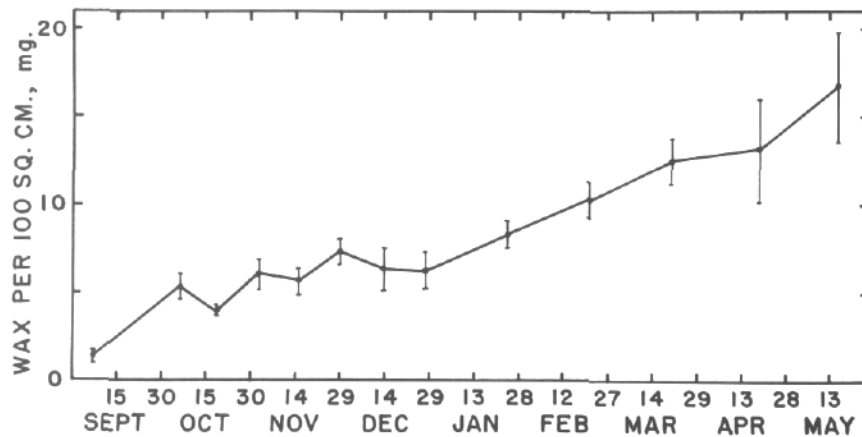


Figure 1. Amount of cuticle wax in mg per 100 sq. cm of fruit surface recovered from developing Hass avocados using chloroform as a solvent. Vertical bars indicate standard deviations.

## Results

*Cuticle wax on Hass avocados.* The cuticle wax on Hass avocados increased in amount per unit surface area during the entire period of fruit development (Fig. 1). In young fruit, the wax amounted to about 2 mg for 100 sq. cm of fruit surface. During a 3-month period of rapid fruit enlargement, the wax remained in a range of from 4 to 7 mg per 100 sq. cm. A long steady increase in wax began in January when the oil content was about 15 percent. In the final sampling, in May, the wax averaged nearly 17 mg per 100 sq. cm. This large increase in cuticle wax during the latter part of fruit development verifies that a conspicuous bloom on the fruit is a useful indication of fruit maturity.

Although the removal of the wax from the fruit was readily accomplished, the solvent damaged the fruit to the extent that the procedure could not be considered non-destructive. It is conceivable that with practice a person might develop proficiency in evaluating fruit maturity by merely estimating the amount of cuticle wax on the fruit. Such a procedure would permit selective harvesting simply by careful inspection of the fruit.

*Infrared absorption spectra.* Infrared absorption spectra of avocado cuticle wax exhibited absorption peaks in four regions: at 3.42, 5.85, 6.85, and 13.95 microns. Figure 2 (upper) shows the absorption spectrum for an immature Fuerte avocado (2.4 percent oil) while Figure 2 (lower) shows it for a mature Fuerte (19.9 percent oil). Peaks at 3.42 and  $6.85\mu$  remained relatively constant with respect to one another during the entire

sampling period and their ratio showed a low correlation with change in oil (Table 1).

TABLE 1. Correlation between Infrared Absorption Peak Ratios of Cuticle Wax and Oil Content in Fuerte Avocado.

RATIO (Wavelengths in microns)	REGRESSION OF RATIO ON OIL	CORRELATION COEFFICIENT (n = 100)
13.95/6.85	$Y = .181 + .00337 + .361 \log X$	.948
13.95/5.85	$Y = .220 + .313 X$	.936
6.85/5.85	$Y = 1.11 + .365 X$	.901
3.42/5.85	$Y = 8.00 + 1.83 X$	.868
13.95/3.42	$Y = .052 + .00461 X$	.824
6.85/3.42	$Y = .171 + .00111 X$	.241

The peak at 5.85 $\mu$  decreased with respect to that at 3.42 $\mu$  as the fruit matured, while the peak at 13.95 $\mu$  increased. Although the ratio between 13.95 and 5.85  $\mu$  might have been expected to show the greatest correlation with oil content, another ratio, 13.95/6.85, gave the highest correlation ( $r = .948$  in Fuerte). The degree of scattering of the points for the Fuerte variety is shown in Figure 3.

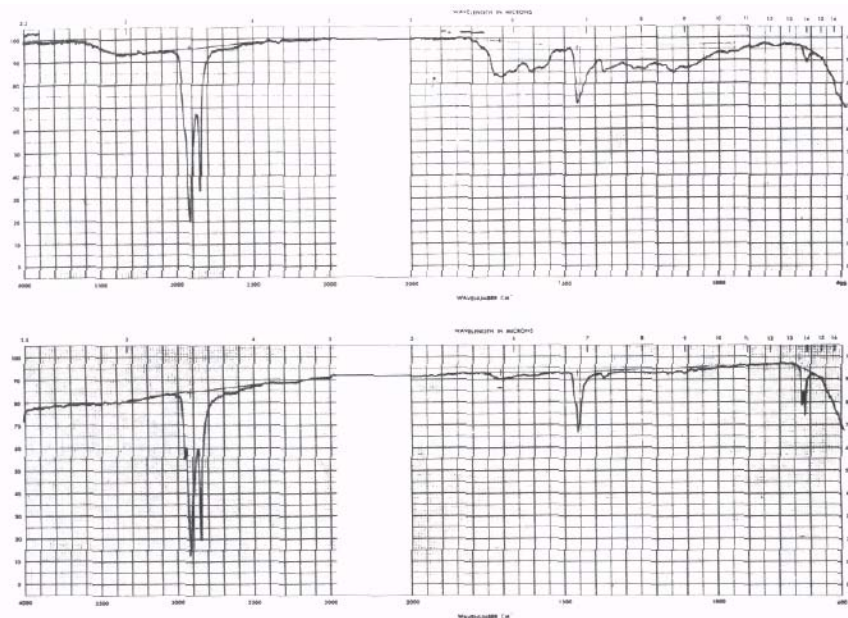


Figure 2. Infrared absorption spectra of cuticle wax from immature (upper) and mature (lower) Fuerte avocados. The immature fruit had 2.4 percent oil and the mature one had 19.9 percent.

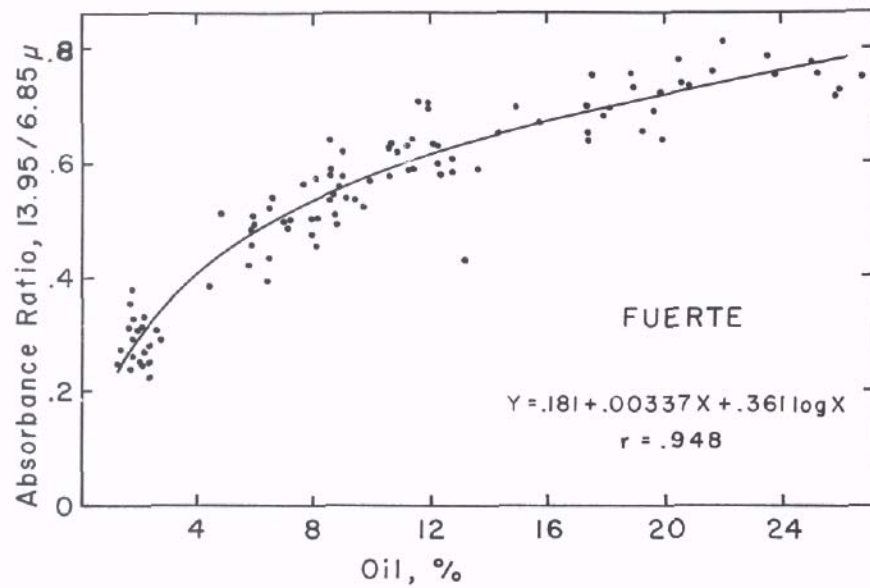


Figure 3. Regression of infrared absorbance ratio 13.95/6.85μ on oil content of Fuerte avocados.

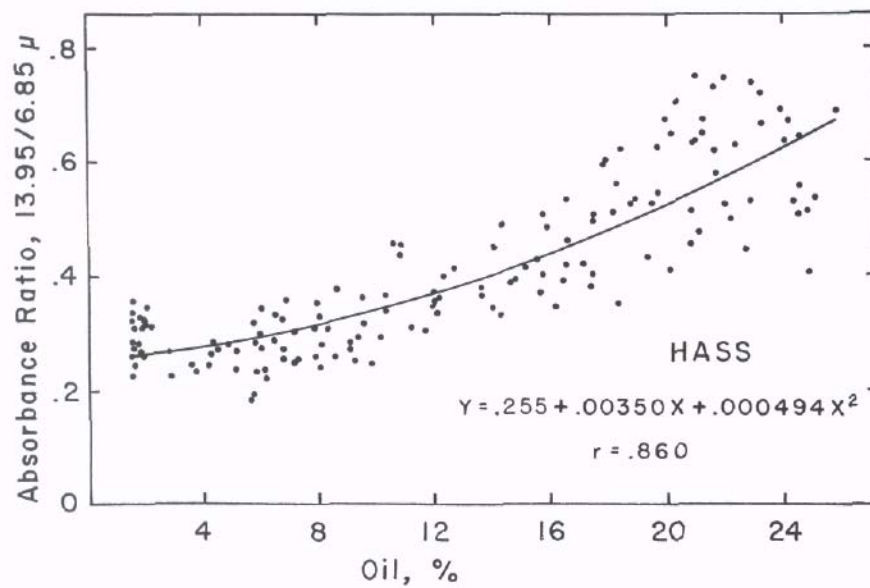
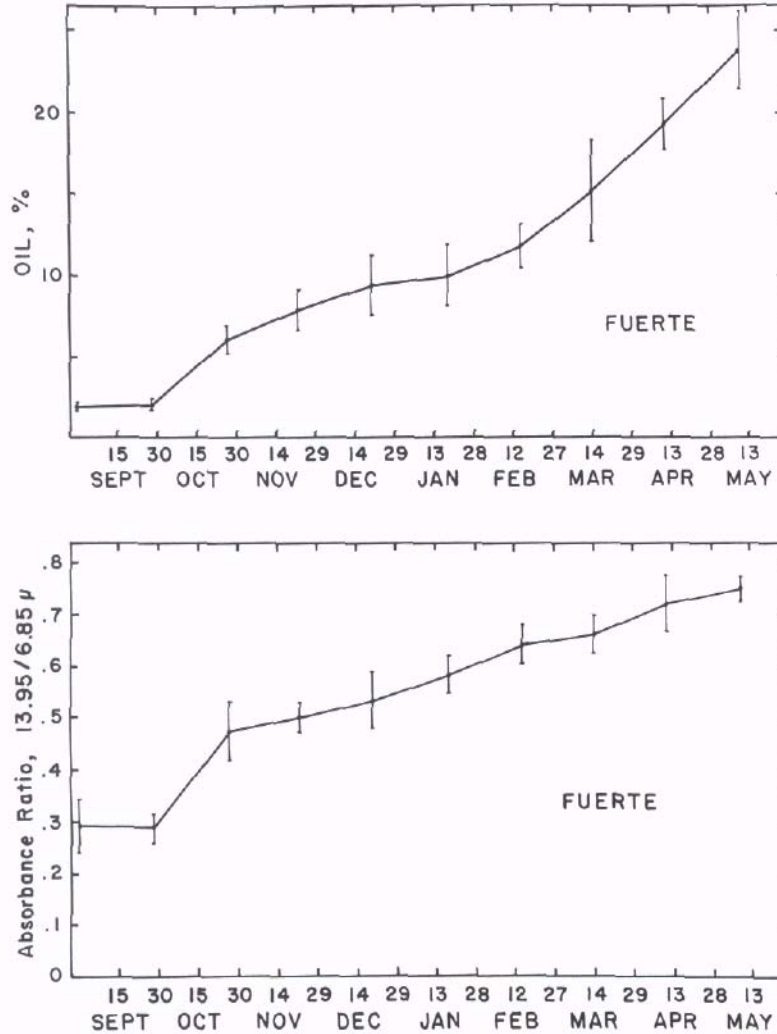


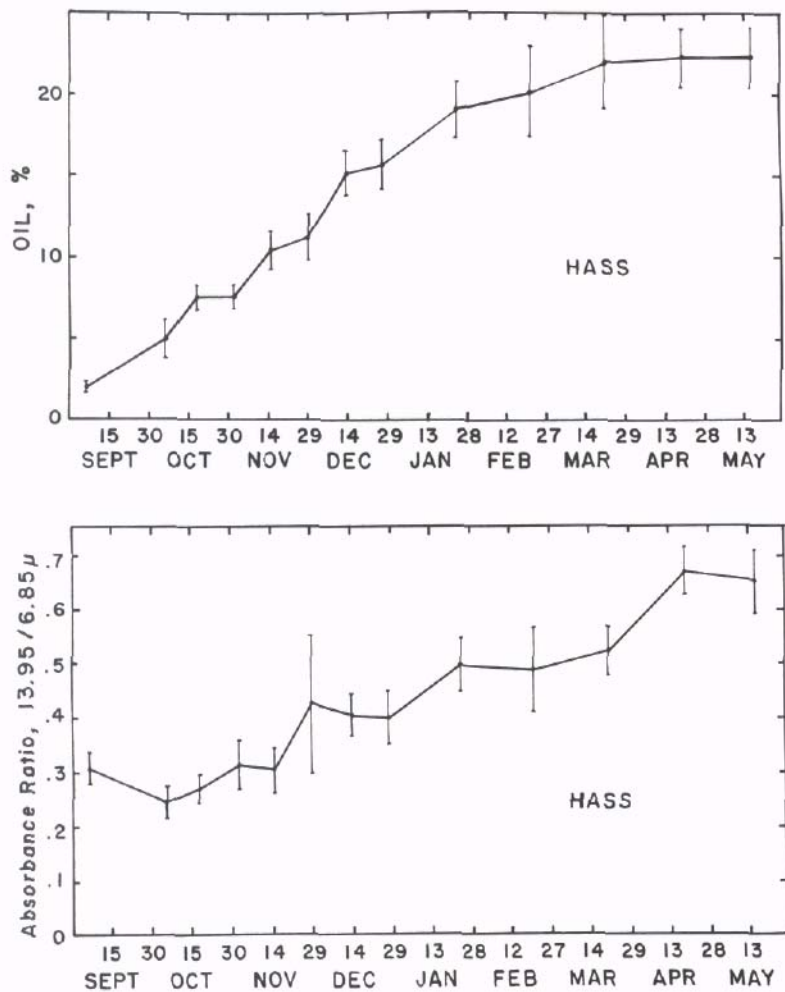
Figure 4. Regression of infrared absorbance ratio 13.95/6.85μ on oil content of Hass avocados.



**Figure 5. Changes in oil content (upper) and in infrared absorbance ratio at 13.95/6.85 $\mu$  (lower) in Fuerte avocados during fruit development. Vertical bars indicate standard deviations.**

Similar results were obtained with the Hass avocado (Fig. 4) although the coefficient of correlation between 13.95/6.85 $\mu$  and oil was somewhat lower ( $r = .860$ ).

While the correlation between ratio and oil content indicates how well these measures may be substituted for each other in evaluating a third factor, such as maturity, it does not reveal their individual variability. Figure 5 (upper) plots the mean oil content for the Fuerte avocados during their development. At each sampling the standard deviation is shown as a vertical bar extending above and below the mean.



**Figure 6. Changes in oil content (upper) and in infrared absorbance ratio at 13.95/6.85 $\mu$  (lower) in Hass avocados during fruit development. Vertical bars indicate standard deviations.**

About 2/3 of all determinations fall within the limits indicated by the standard deviation. Fig. 5 (lower) shows a similar set of values for the absorbance ratio 13.95/6.85 $\mu$ . When the standard deviation is expressed as a percentage of the mean, the variability in the oil content and the variability in the absorbance ratio may be compared. Thus, for the Fuerte, the standard deviation as a percentage of the oil content had a mean of 15.7 percent, while the standard deviation as a percentage of the absorbance ratio 13.95/6.85 $\mu$  had a mean of 8.5 percent. This comparison of variability in the oil content and in the infrared absorbance ratio showed that the oil was nearly twice as variable as the ratio. On the other hand, the Hass variety (Fig. 6) had essentially equal variability in the oil and ratio (12.0 and 12.4 percent, respectively). From the data available it is concluded that the oil content and the ratio are both variable and that neither is consistently less variable than the other.

*Wax samples from attached fruit.* It has been proposed (1) that wax samples could be obtained from fruit still attached to the trees by rubbing the fruit with a tissue (Kleenex) moistened with solvent. Several solvents were tried in this manner (chloroform, diethyl ether, petroleum ether, freon 11, and freon 113). In every instance blemishes developed on the fruits from their contact with the solvents. Perhaps less solvent or less rubbing might eliminate damage to the fruit. However, Kleenex and Kimwipes were found to contain waxes similar to avocado wax and were therefore unsuitable for use in their original state. In part, the success in developing an infrared absorbance method for estimating fruit maturity depends on obtaining a representative sample of wax without damage to the fruit.

### **Summary**

Hass avocados produced cuticle wax which accumulated on the fruit surface during fruit development to the extent of about 17 mg per 100 sq cm by the middle of May.

Of six infrared absorption peak ratios of cuticle wax, 13.95/6.85/ $\mu$  gave the highest correlation with oil in both Hass and Fuerte fruits. The variability in the infrared absorption peak ratios was not consistently higher or lower than the variability in the oil content.

### **REFERENCES**

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