agree closely with the theoretical values. For example, it is possible to prepare a mixture consisting entirely of linoleic acid and stearic acid which would have the theoretical iodine, acid, and saponification numbers for pure oleic acid, whereas actually it would contain no oleic acid. A thiocyanogen number determination would immediately reveal the true composition. It is suggested that specifications for oleic acid and related materials be amended to include a thiocyanogen number analysis under controlled conditions.

Acknowledgment

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Summary

1. Oleic acid, methyl oleate, and oleyl alcohol of high purity (93 to 96%) were prepared from readily available and inexpensive commercial materials in 65 to 70% yields by fractional distillation and a single low-temperature fractional crystallization.

2. In the fractional distillation of red oil and its methyl esters, lower fractions amounting to about 20% of the starting material were obtained. These are suggested for use in soap manufacture.

3. A fraction containing more than 50% linoleic acid was obtained from the C-18 fraction of red oil by fractional crystallization. This fraction amounted to about 20% of the total starting material and had approximately the same percentage composition of fatty acids as several important semi-drying oils.

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The Isolation and Determination of Chlorophylls A and B in the California Avocado*

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In conjunction with the investigation of the oil soluble components of the California avocado, the identification and determination of its green pigments was undertaken. Methods for the isolation, purification, and identification of the chlorophylls have been recently developed (1-6), based largely on chromatography. However, due to its high oil content (up to 30%), the avocado presents a more difficult problem than does ordinary plant tissue. This oil rapidly inactivates the adsorbents used and thus prevents the isolation of the chlorophylls in any appreciable quantities. This difficulty was overcome by various modifications of the chromatographic method as described below. The quantitative determination of the chlorophylls was carried out spectrophotometrically on the crude avocado extract before decomposition of the very sensitive chlorophylls became appreciable (7, 8).

Experimental

Two varieties of California avocados were investigated in these experiments, the Fuerte and the Nabal. In all cases, the edible portion (whole fruit minus seed and skin) was tested. In the methods to be described the reagents used were as follows:

Methyl Alcohol, Absolute, C. P. Baker's
Calcium Carbonate, C. P. Baker's
Ether, Absolute, C. P. Baker's (diethyl)
Anhydrous Sodium Sulfate, Purified Grade
Distilled water saturated with C.P. Calcium Carbonate used in all cases
Skellysolve F, B.P. 30-60°C.
Skellysolve H, B.P. 70-100°C.
Cellite 545, obtained from John's Manville Sales Corp.

* Supported by a grant from Avocado Growers of California.

The Isolation of Chlorophyll from Avocado. All experiments were carried out in semi-darkness, the only illumination being that from a 25-watt tungsten filament lamp which was used in such a manner that the apparatus could be manipulated, yet would not receive any direct light.

Approximately 50 grams of ripe avocado (edible portion) was weighed into the micro cup of the Waring Blendor; 0.5 gram of $CaCO_3$, and 100 ml. of CH_3OH were added. The mixture was blended for approximately 30 seconds. If the avocado is not properly ripe this short blending time may not bring about a complete breakdown of the tissue. In this case the blending time should be increased to as long as five minutes. Sufficient ice was added directly to the mixture from time to time to keep the temperature at all times below 20°C.

To the completely broken down avocado mixture was added 15 grams of Celite 545, and 50 ml. of CH_3OH. This was mixed by blending for from 10 to 20 seconds and then filtered through rapid flowing qualitative filter paper on a buchner funnel with vacuum. The filter cake was removed from the funnel, separated from the filter paper and placed again in the blendor cup. The filter paper was placed in the buchner funnel for the next extraction.

One hundred ml. of CH_3OH and 50 ml. of skellysolve F were added to the cup, the mixture blended for 30 seconds, filtered as before, and the filter cake placed again in the blendor cup. These extractions were continued until the last extract came through the filter colorless. Usually a total of five extractions was sufficient.

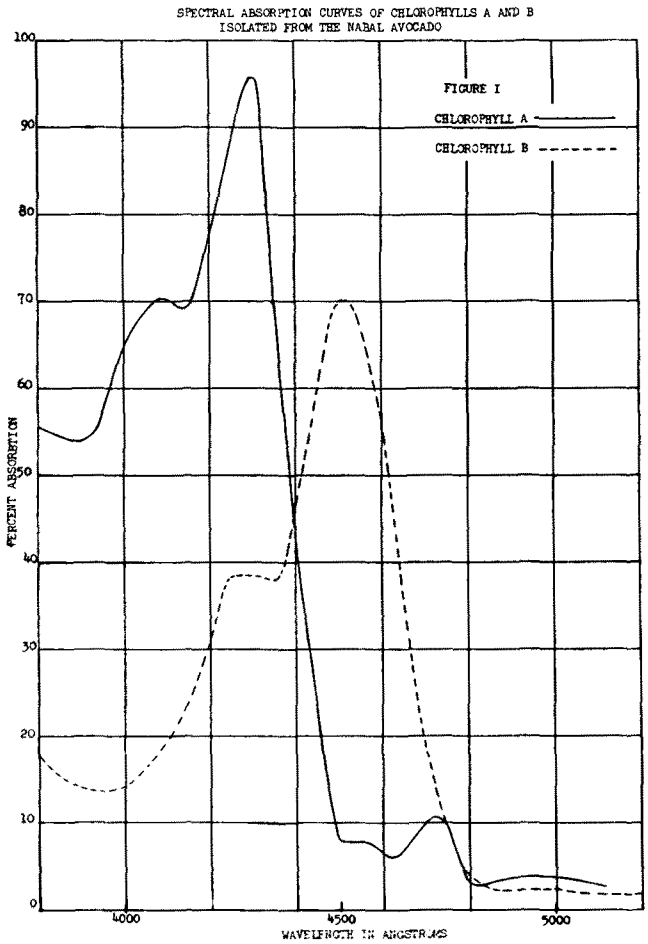

FIG. 1.

The combined filtrates were placed in a two-liter separatory funnel and an amount of water equal to the amount of CH_3OH used in the extraction was added. Two hundred ml. of a saturated aqueous sodium sulfate solution was added and the lower alcohol-water layer was removed. The blue green skellysolve layer was placed in a 1-liter separatory funnel containing 100 ml. of water, and the alcohol-water layer re-extracted with 100-ml. portions of skellysolve F until the last extract showed no color. Usually five extractions were sufficient.

The combined skellysolve F extracts in the 1-liter separatory funnel were washed with water (200 ml.) six times to remove the last traces of CH_3OH. This extract was dried over anhydrous sodium sulfate (the minimum amount, 1 to 5 grams) in a 500-ml. glass stoppered erlenmeyer flask, and then evaporated to approximately 50 ml. in vacuo at 30°C. under nitrogen in a 1-liter Claissen flask. The residue was quantitatively transferred to a 100-ml. volumetric flask with skellysolve H and made up to volume with this solvent.

A 10-ml. aliquot from the 100 ml. of extract was chromatographed on a powdered sugar column 35 mm. by 180 mm. The blue green material was all adsorbed on the upper two-thirds of the column, and it separated into a lower blue green band and an upper green band when developed with 10 to 30% ethyl ether in skellysolve H. Before starting the development, the column was washed with pure skellysolve H until all of the yellow color was washed out the bottom and the washings were coming out clear. Then the development was started first with 10% ether, and continued with greater percentages of ether as the bands formed. The development was stopped just before the lower blue band reached the bottom of the column and the column was extruded. The two chlorophyll bands were excised and the chlorophylls eluted with ethyl ether in glass-stoppered erlenmeyer flasks. The ether was evaporated in vacuo under nitrogen and the residues taken up with a small volume of skellysolve H (5 to 10 ml.). These solutions were rechromatographed on powdered sugar columns 20 mm. by 200 mm. as before. The final two chlorophyll fractions were dissolved in pure ethyl ether, made to a volume of 10 ml. and their spectral absorption curves determined in the Beckman Spectrophotometer.

The Determination of Chlorophyll in Avocado. This work was carried out in semi-darkness, as described in the isolation experiments. It was found essential that the entire determination be carried out in one day in order to insure that no decomposition would take place.

Approximately 50 grams of ripe avocado (edible portion) were weighed into the micro cup of the Waring Blendor; 0.5 gram of $CaCO_3$, and 100 ml. of CH_3OH were added, and the mixture was blended until the tissue was completely broken down. This may require from 30 seconds to five minutes, depending upon the degree of ripeness of the fruit. Sufficient ice was added directly to the mixture from time to time to keep the temperature at all times below 20°C.

To the completely broken down avocado mixture was added 15 grams of Celite 545, and 50 ml. of diethyl ether. This was mixed by blending for from 10 to 20 seconds and then filtered through rapid flowing qualitative filter paper on a buchner funnel with vacuum. The filter cake was removed from the funnel, separated from the filter paper, and placed again in the blendor cup. The filter paper was placed again in the buchner funnel for the next extraction.

Seventy-five ml. of alcohol and 50 ml. of ether were added to the cup, the mixture blended for 30 seconds, filtered as before and the filter cake placed again in the blendor cup. These extractions were continued until the last extract came through the filter colorless. Usually a total of six extractions was sufficient.

The combined filtrates were placed in a 2-liter separatory funnel and an amount of water equal to twice the total volume of CH_3OH used in the extraction was added. Four hundred ml. of saturated sodium sulfate solution and 200 ml. of ether were added, the mixture was gently shaken and the lower alcohol-water layer removed. The blue green ether layer was placed in a 1-liter separatory funnel containing 100 ml. of water, and the alcohol-water mixture was re-extracted with 100-ml. portions of ether until the last extract showed no color. Six extractions should be sufficient.

The combined ether extracts in the 1-liter separatory funnel were washed with 200-ml. portions of water six times to remove the last traces of methyl alcohol. The ether extract was then dried over anhydrous sodium sulfate (the minimum amount, 1 to 5 grams) in a 500-ml. glass-stoppered erlenmeyer flask, and these evaporated to approximately 50 ml. in vacuo at 30°C. under nitrogen in a 1-liter Claissen flask. The residue was quantitatively transferred to

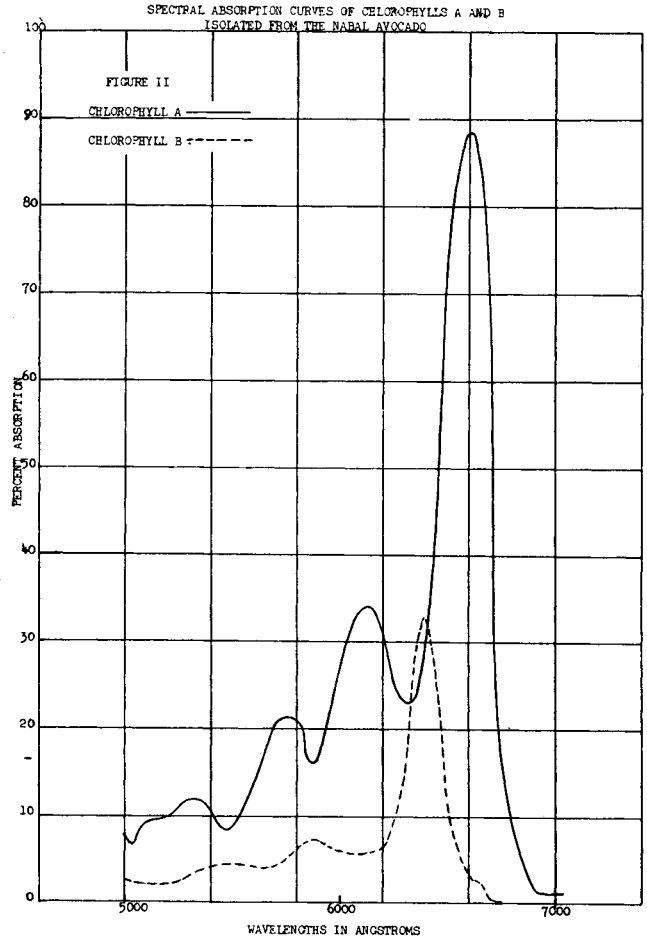

FIG. 2.

a 100-ml. volumetric flask with ether, and made to volume with this solvent.

The optical density of this solution was determined directly in the Beckman Spectrophotometer at wavelengths of 652.5 and 660.0 millimicrons.

Results

Chlorophylls A and B were isolated from the Fuerte and the Nabal varieties of the California avocado. Figures 1 and 2 show the spectral absorption curves of ether solutions of the chlorophylls isolated from the Nabal variety.* The absorption maxima of both the chlorophylls isolated from both varieties are in fair agreement with those obtained by Zscheile and Comar (2). The difficulty of preparing highly purified chlorophyll solutions from the avocado is indicated by the presence of several small peaks in the wavelength range of 4500 to 5500 Å in the curve for chlorophyll A, Figures 1 and 2. These are probably due to the presence of decomposition products such as Pheophytin A. The results obtained indicate that chlorophylls A and B are the principal green pigments of the edible portion of the Fuerte and Nabal varieties of the California avocado.

The quantitative determination of the chlorophylls by calculation from the optical densities at wavelengths of 652.5 and 660.0 millimicrons was conducted on both the Fuerte and the Nabal varieties according to the method of Comar and Zscheile (7) and Comar (8). By this method the Fuerte avocado was found to contain 21.57 micrograms of chlorophyll A and 15.57 micrograms of chlorophyll B per gram of edible portion. The Nabal avocado contained 19.93 micrograms of chlorophyll A and 11.90 micrograms of chlorophyll B per gram of edible portion. No attempt was made in this work to relate chlorophyll content to degree of maturity, degree of ripeness, or to season of the year. Further investigations along this line are contemplated.

Summary

It has been shown that chlorophylls A and B are the principal green pigments of two varieties of the California avocado. Chlorophylls A and B have been determined quantitatively in those two varieties.

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* Figure 1 covering the range of 3800 Å to 5000 Å, inclusice. Figure 2 covering the range of 5000 Å to 7000 Å, inclusive.

A Rapid Method for Estimating Glycerol in Kettle Soap

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Soapboiling has not yet completely emerged from the state of art to one of science. This is probably because the initial processing of kettle soap during the saponifying and salt washing procedures is easily controlled by eye and taste. It is not necessary to control them chemically at these stages of manufacture in order to make a suitable soap. The "fitting" stage, which is the final adjustment of caustic soda and salt content must be done with more care if a good product is desired. At this stage, the "art" of the soapboiler calls into play his sense of taste and his sense of feel as well as his eyesight. By touching the soap to his tongue he determines whether or not the proper adjustment of caustic has been made and by sight he judges whether the kettle soap is "boiling properly." He may even use a trowel to see how the soap slides off the flat blade. Then using his "art" he adjusts the kettle with salt, caustic soda, or water until a proper "fit" is obtained.

After the fitted soap has stood for a few days and the kettle has settled out its excess salt and caustic, the soap is ready for further processing. If the "fit-