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SEASONAL CHANGES OF AVOCADO LIPIDS DURING FRUIT DEVELOPMENT AND STORAGE

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In a study of the maturity of avocado fruits it is important to elucidate the lipid metabolism in the fruit during growth and storage, since the avocado stores a large amount of lipids in the edible pulp of the fruit. From morphological and physiological viewpoints of fruit development, Schroeder (10) observed that the avocado fruit deviated from, most investigated fruits in its method of development in that cell division remained an important factor in fruit growth as long as the fruit remained on the tree. Furthermore, a large droplet of reserve lipids was deposited in each indioblast, a large specialized type of cell found in the mesocarp tissue.

Davenport and Ellis (4) showed that the accumulation of reserve lipids in the mesocarp during fruit development was accompanied by a decrease in alcohol soluble as well as alcohol insoluble sugars. Such changes are consequently of interest in a study of lipid metabolism in the fruit of avocado.

Recent development of analytical methods for the separation and identification of lipids has materially facilitated studies of lipids and their metabolism, *i.e.* silicic acid column chromatography, thin layer chromotography and gas liquid chromatography. Thus, in 1965 Mazliak (6) could identify six saturated and five unsaturated fatty acids in the mesocarp of avocado fruits. Among these fatty acids he showed that only four acids (palmitic and palmitoleic acids with sixteen carbons and oleic and linoleic acids with eighteen carbons) represented more than 95% of the fatty acids in the fruits. As far as deposited lipids were concerned, more than 60% of the total fatty acids was in the form of oleic acid. Prior to the research of Mazliak, Davenport and Ellis (4) indicated that the major fatty acid constituent was a monoenoic acid which was synthesized during a long period of fruit development, while the saturated and polyunsaturated fatty acids were synthesized only in the primary stage of growth.

In 1957, Stumpf and Barber (11) demonstrated that mitochondria isolated from avocado mesocarp incorporated labelled acetate into long-chained fatty acids in the presence of ATP, CoA, NADPH, Mn++, and HCO^{3}— under aerobic conditions. Palmitic and oleic acids were the most labelled fatty acids found to accumulate, with the greater radioactivity appearing in the later.

The biosynthesis of triglyceride in avocado fruit was demonstrated by Barron and

Stumpf (2) in 1962. Microsomes isolated from avocado mesocarp appear to synthesize glycerides via a pathway essentially similar to the system in animal tissue. The route, called the Kennedy and Kornberg pathway (8) proceeds from glycerophosphate to phosphatidic acid to diglycerides and finally to triglycerides.

Studies on the avocado fruit were undertaken primarily to elucidate chemical composition of the lipid materials and their changes during growth and development of the fruit.

MATERIALS AND METHODS

Plant materials

The Fuerte variety of avocado. *Persea americana* Mill. (*P. grafissima* Gaerth) primarily was used in the studies on the seasonal changes of the lipids in the fruits, although some data were obtained for the Hass variety. The fruits were grown on the Riverside campus of the University of California, and after harvest were stored at 5°C for at least 24 hours before being used for experiments.

Crude lipid extraction

Ten grams of freshly grated tissue of avocado mesocarp were homogenized with 10 *ml* of chloroform-methanol mixture (2:1, by volume) in a stainless steel ball hammer mill (9). The resulting mixture was filtered through a Buchner funnel using two layers of Whatman No. 42 filter paper. The residue was again extracted with cholorform-methanol mixture and filtered. An additional 10 *ml* of the mixture was used for washing the mill and funnel. The combined extract was evaporated to dryness in a vacuum rotary evaporator. The dry lipids was dissolved in n-hexane to a volume of 10 *ml.* One half of the sample was dried overnight at 100°C and weighed. The remainder was used for the silicic acid column chromatographic separation of the classes of lipids described by Barran and Hanahan (1).

Separation and identification of lipids

The lipids extracted from avocado mesocarp were separated by silicic acid column chromatography as well as thin layer chromatography. The isolated lipids were characterized by infrared absorption spectroscopy and the fatty acid composition was determined by gas liquid column chromatography.

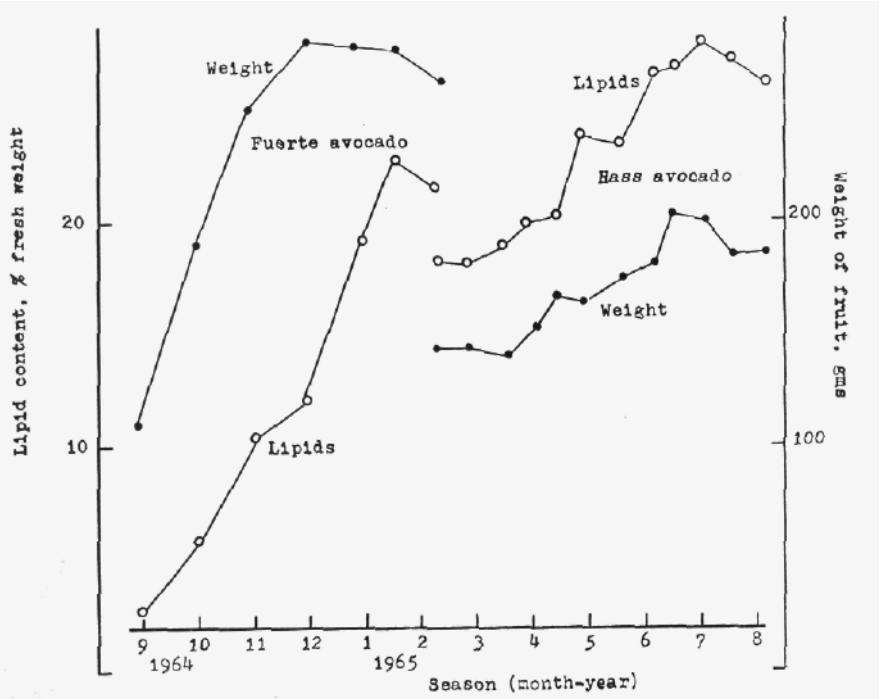

Figure 1. Seasonal changes in lipids and weight of Fuerte and Hass avocado fruits.

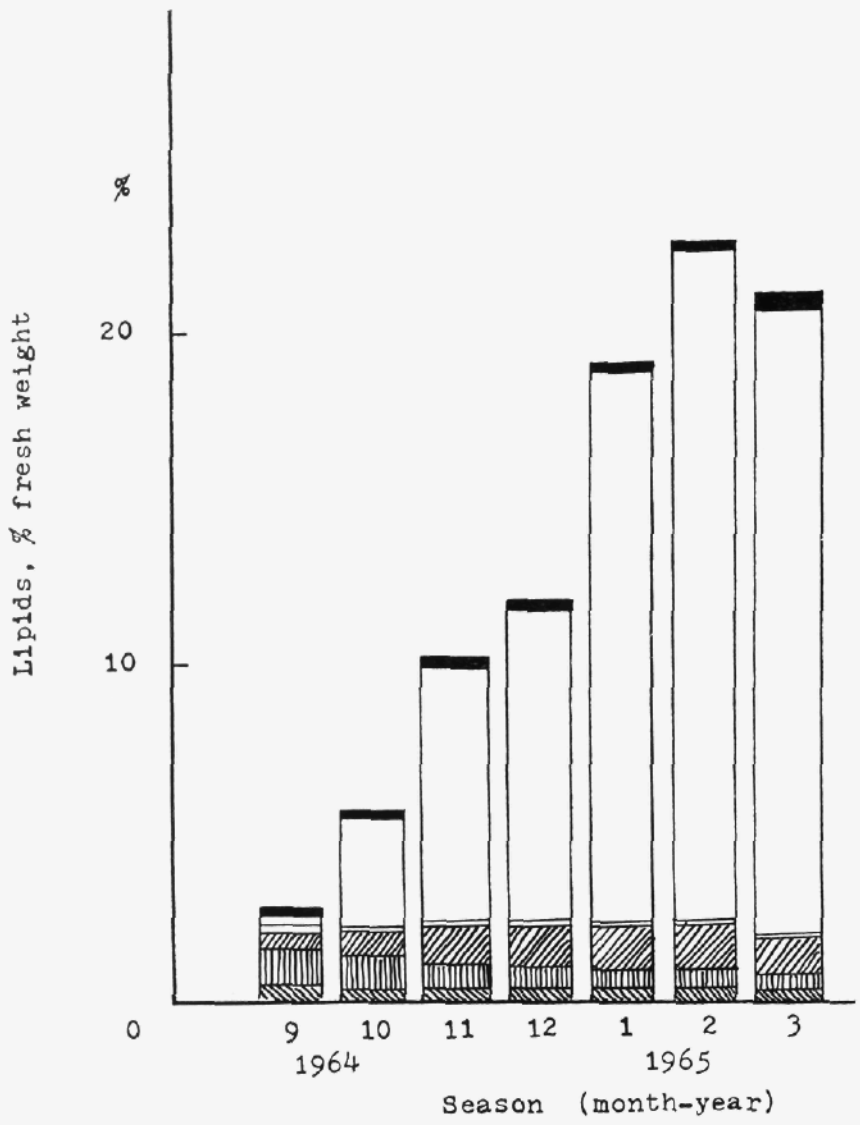

Figure 2. Seasonal changes of classes of lipids in the mesocarp of Fuerte avocado fruit. Fractions from top to bottom in each histogramare: hydrocarbons, triglycerides, free fatty acids, diglycerides, monoglycerides, and phospholipids.

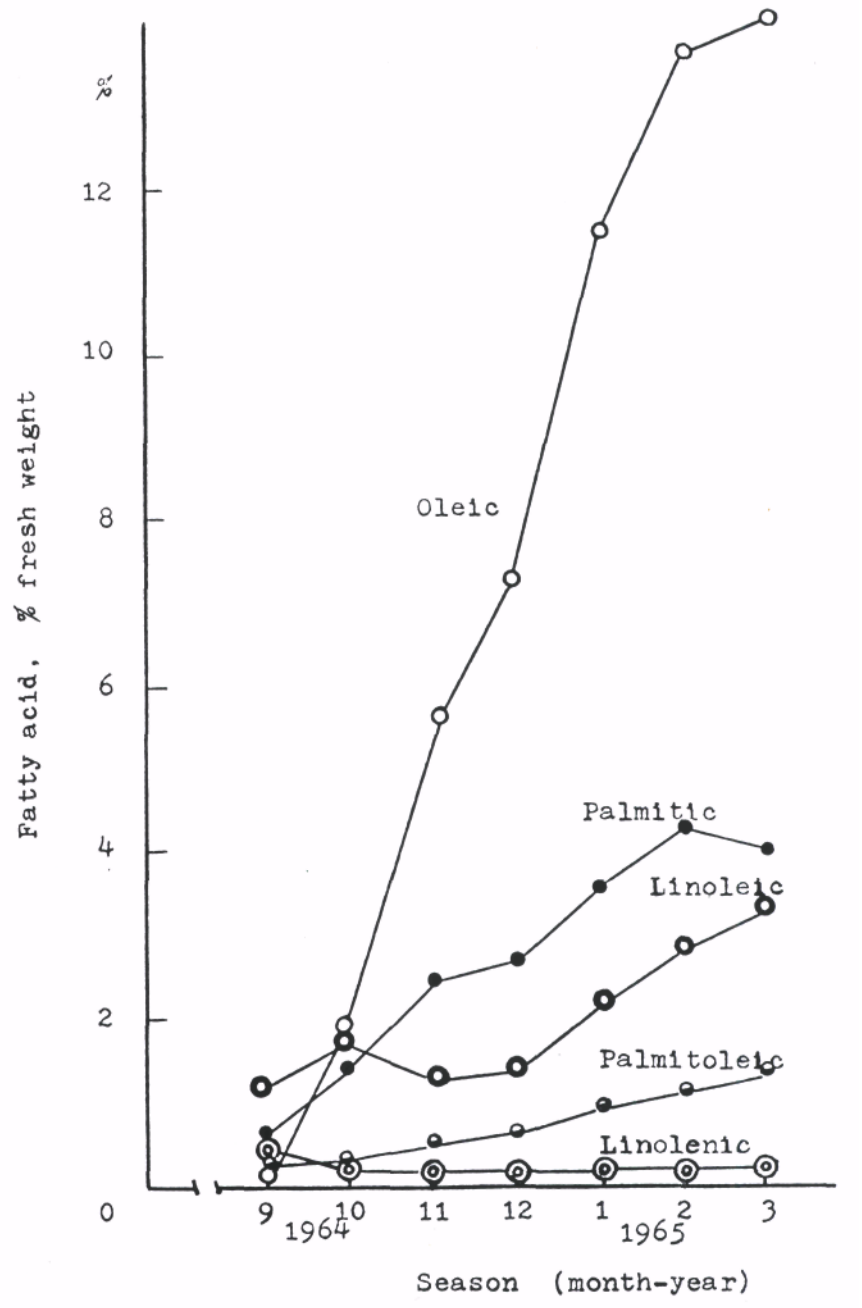

Figure 3. Seasonal changes of fatty acid composition in the fruit of Fuerte avocados.

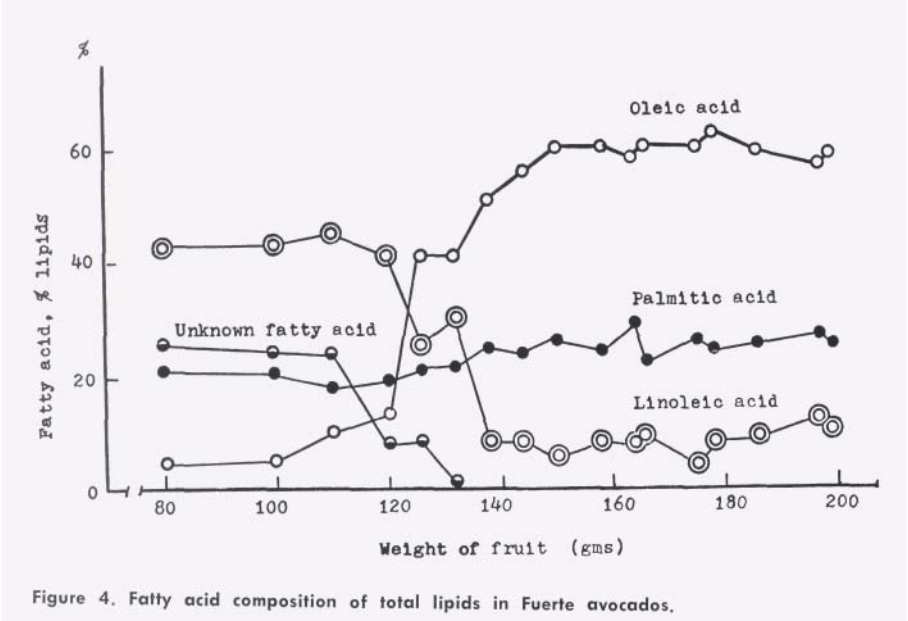

Figure 4. Fatty acid composition of total lipids in Fuerte avocados.

RESULTS

The lipid content of avocado mesocarp during the fruit growth has been plotted in figure 1. Fruit weights are also shown. Although the Fuerte and Hass avocado differed in size and lipid content at a given time, it was apparent that the growth of both varieties almost ceased before the fruit showed its maximum, accumulation of reserve lipid. As the lipid content of the mesocarp increased during fruit development, the content of water was reduced.

The marked increase in lipid content in Fuerte avocado fruit took place mainly over a four months' period (October to January). In the Hass avocado, the lipid accumulation activity seemed to be prolonged for about nine months. The large increase in lipids during fruit development was found to be mainly a large increase in the triglyceride fraction (figure 2). The phospholipid fraction remained constant over the examined period of fruit growth. The monoglyceride fraction appeared to be slightly decreased with a corresponding increase in the diglyceride fraction. The data of figure 3 indicate that it was principally oleic acid which was being synthesized during this period. Palmitic, palmitoleic, linoleic acids increased slightly while linolenic acid remained constant. The fatty acid composition of the total lipid from Fuerte avocado mesocarp has been plotted with respect to fruit weight in figure 4. Twenty fruits were sampled from September to December. Drastic changes between linoleic and oleic acids were observed while palmitic acid together with palmitoleic acid remained constant. It seems probable that these changes were due to the initiation of reserve lipid formation in the mesocarp of the fruit, while in younger fruit the bulk of fatty acids had arisen from phospholipids and glycolipids which were uniquely associated with these lipids.

The changes in lipid content during the ripening and storage period were determined and are shown in Table I for two lots of fruit harvested on January 19, 1965, or on February 17, 1965. The fruits were stored for 9, or 14 days, respectively. There were

slight increases in the total lipids during storage in both lots of fruit. However, there were large increases in the monoglyceride and free fatty acid fractions, with some relative decreases in the diglyceride and phospholipid fractions. This sequence of change suggests that the rate of triglyceride formation was exceeded by the increased rate of degradation of triglyceride to form monoglyceride and free fatty acid in the fruit during storage.

TABLE I

Percentages of Various Classes of Lipids in Fuerte Avocado Mesocarp Tissue after Storage

Date harvested	January 19, 1965			February 17, 1965		
Days after harvest	1	9		1	14	
Class	%	%	diff.	%	%	diff.
Triglyceride	16.40	17.10	0.70	19.96	19.51	-0.45
Diglyceride	1.63	1.28	-0.35	1.29	1.24	-0.05
Monoglyceride	0.50	1.33	0.38	0.78	2.26	1.48
Free fattty acid	0.12	0.29	0.17	0.10	0.50	0.40
Phospholipid	0.32	0.30	-0.02	0.39	0.20	-0.19
Others*	0.23	0.25	0.02	0.28	0.31	0.03
Total lipids	19.20	20.55	1.35	22.80	24.02	1.22
Weight loss		5.0			7.5	

*Other substances extracted with chloroform-methanol (2:1) included hydrocarbons and sterols.

DISCUSSION

Since the growth of the fruit almost ceased before the fruit showed its maximum accumulation of reserve lipids, a decrease in water content as a percent of fresh weight of the fruit during development results from a displacement of water by lipids.

During the phase of rapid lipid synthesis, oleic acid was predominantly synthesized, and eventually deposited as triglyceride in the mesocarp tissue of the fruit. The early rise in linoleic acid appeared to be associated with glycolipid which was a unique component in chloroplasts of young fruit (3) and with the phospholipid fraction which did not change during development of the fruit. This evidence supports the observation of an early establishment of the presence of polyunsaturated fatty acids in fruit, which then remain fairly constant throughout subsequent development (4).

Since the fruits are harvested and stored until they are consumed, they continuously undergo a loss in weight, which is largely a loss of water from the fruits. However, Erickson and Kikuta (5) observed that there appeared to be a slight loss of reserve lipids during ripening and an increase during storage following ripening of Hass avocado fruits.

Changes in classes of avocado lipids were observed during storage. There were reductions in the rate of triglyceride synthesis and diglyceride content as well as marked increases in monoglyceride and free fatty acid fractions, suggesting that lipids were involved to some extent in metabolic changes during the ripening process. Davenport and Ellis (4) studied fatty acid composition during storage and found that, the saturated fatty acids and the polyunsaturated fatty acids tended to increase. Mazliak (7) also examined the changes of fatty acid composition of the fruit under artificially modified

atmosphere and drew the general conclusion that in all the classes of lipids and in all the regions of the fruit, the percentage of unsaturated fatty acid was greater as the concentration of oxygen was increased in the atmosphere in which the fruit ripened, while saturated fatty acids increased under oxygen poor or carbon dioxide rich atmosphere. Interestingly, short-chained fatty acids in the phospholipid fraction increased in the presence of high oxygen pressure. These results suggest that oxygen is definitely required for oleic acid synthesis in avocado fruit during storage.

LITERATURE CITED

1. Barron, E. J. and D. J. Hanahan. 1958. Observation on the silicic acid chromatography of the neutral lipids of rat liver, beef liver and yeast. Jour. Biol. Chem. 23: 493-503.
2. Barron, E. J. and P. K. Stumpf. 1962. Fat metabolism in higher plants, XIX. The biosynthesis of triglycerides by avocado mesocarp enzymes. Biochim. Biophys. Acta 60: 329-337.
3. Benson, A. A. 1964. Plant membrane lipids. Ann. Rev. Plant. Physiol.15: 1-16.
4. Davenport, J, B. and S. C. Ellis. 1959. Chemical changes during growth and storage of the avocado fruit. Aust. Jour. Biol. Sci. 12:445-454.
5. Erickson, L.C. and Y. Kikuta. 1964. Ripening Hass avocado in high and low humidities. Calif. Avocado Soc. Yearbook 48: 90-91.
6. Mazliak, P. 1965. Les lipides de l'avocat. I. Composition en acides gras des diverses parties du fruit. Fruit 20: 49-57.
7. Mazliak, P. 1965. Les lipides de l'avocat. II. Variation de la composition en acides gras des lipides du pericarpe selon la compositionde l'atmosphere autor des fruits en maturation. Fruits 20: 117-120.
8. Kennedy, E. P. 1957. Biosynthesis of phospholipids. Federation Proc.16: 847-852.
9. Porter, R. S. 1947. Official method for the determination of oil in avocado. State of Calif., Dept. Agr. Bull. 36: 20-26.
10. Schroeder, C. A. 1953. Growth and development of the Fuerte avocado fruit. Amer. Soc. Hort. Sci. 61: 103-109.
11. Stumpf, P. K. and G. A. Barber. 1957. Fat metabolism in higher plants. IX. Enzyme synthesis of long chain fatty acids by avocado particles. Jour. Biol. Chem. 227: 407-417.