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BIOCHEMICAL REACTIONS OF AVOCADOS IN RELATION TO STANDARDS OF MATURITY

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The avocado is hardly unique in the fruit world in being subject to a certain amount of variation in characteristics of flavor and quality, depending on variety and growing conditions. Still, in some respects, it seems to have some particularly exasperating traits for the grower and packer. The fact that it is not possible to judge the real quality of a given avocado until several days after it has been picked is one of these bothersome items. The grower, interested in picking his crop to meet market demand and commitments, may have difficulty in judging whether his fruit is really ready for picking. If he leaves it long enough to be certain that the fruit is in perfect condition, he may miss *tree* best prices. If he picks the fruit and then finds that it doesn't come up to standards, he loses by having the fruit put in an inferior grade classification.

This question of maturity is only one of the many problems which the Avocado Society has been trying to overcome in the interest of improving the quality and uniformity of the fruit.

A great deal of work has been done previously on obtaining workable standards of maturity and, at the present time, a standard has been set up on the basis of the oil content of the avocado. If the fruit has attained a minimum oil content of eight percent, then it is considered ready for marketing. This standard is the best found to date, but it still has some troubles associated with it. For certain varieties, the standard is set too low. With these varieties, when an eight percent oil content is reached, it is still no guarantee that the fruit will ripen to give the maximum in quality. On the other hand, some varieties grown under certain conditions may have difficulty in attaining the minimum requirement, even though they will make fruit of high quality for consumption. It was with such difficulties in mind that the University of California recently instituted a long term study of the biochemistry of maturation in avocados to obtain more basic information which might lead to establishment of more reliable and significant standards.

The difficulties with the oil content standard arise from the fact that the oil is a storage product rather than an active metabolite in the fruit. Thus, the quantity of oil will reflect the growing conditions of the fruit more than it will the physiological age. Because the methods available were limited, previous studies of maturity and ripening in avocado have been largely limited to the examination of the storage products, oils, fats, sugars and starches, in attempts to correlate content with age of the fruit. In recent years rapid advances in the methodology of chemistry, biochemistry and physiology have made it feasible to study concentrations of materials present in plants in quantities on the order

of hundredths or even thousandths of one percent of the total in the tissues. This means that concentrations of physiologically significant material, individual enzymes, complex compounds such as the coenzymes, rapidly metabolized intermediates, such as the sugar phosphates or fat intermediates, may be individually determined and followed through the life of the tissue. Use of such newly available techniques, as paper partition chromatography in conjunction with radioactive tracers, have made it possible, in recent years, to expose completely the entire mechanism of the formation of sugars from carbon dioxide through photosynthesis in plants. The availability of these new tools, which can measure tiny quantities of transitory material, makes it feasible to re-examine the maturation problem in a new light, with hope that better results might be obtained.

Let us examine the growth of the avocado fruit as it matures and ripens, to see what direction it might be best to take in the attack on the maturity problem. In the very early stages, for the first one or two months, the cells in the fruit are rapidly multiplying and expanding. This is a process which uses large amounts of energy, so the respiration rate of the fruit is correspondingly high. There is not much chance for the storage of large amounts of carbohydrates and fats in this stage, because as fast as these materials are received or synthesized they must be used to produce the energy for expansion. The fat and oil content is relatively low and the seed does not have even a trace of starch in it. If picked now, the fruit rapidly loses water and shrivels. In the next phase of growth, some of the cells have time to catch their breath and begin storing the excess material that is transported to them from the leaves of the tree. This is deposited in the form of oil in the pulp and starch in the seed. The over-all respiration rate goes down somewhat. During this stage, the multiplication continues but at a reduced rate. Toward the end of this period, the avocado has just about gained full size and might look ready to pick and ripen. However, if picked too soon, the fruit will take a very long time to soften and then will possibly merely become limp and tough instead of soft and creamy. This premature stage frequently merges quite imperceptibly into the final mature state. In this last stage, the fruit has attained essentially its maximum size. In some avocados, onset of maturity is heralded by changes in color which set this stage apart quite definitely, but in many varieties, fruit. Thus, the quantity of oil will reflect the growing conditions of the fruit more than it will the physiological age. Because the methods available were limited, previous studies of maturity and ripening in avocado have been largely limited to the examination of the storage products, oils, fats, sugars and starches, in attempts to correlate content with age of the fruit. In recent years, rapid advances in the methodology of chemistry, biochemistry and physiology have made it feasible to study concentrations of materials present in plants in quantities on the order of hundredths or even thousandths of one percent of the total in the tissues. This means that concentrations of physiologically significant material, individual enzymes, complex compounds such as the coenzymes, rapidly metabolized intermediates, such as the sugar phosphates or fat intermediates, may be individually determined and followed through the life of the tissue. Use of such newly available techniques, as paper partition chromatography in conjunction with radioactive tracers, have made it possible, in recent years, to expose completely the entire mechanism of the formation of sugars from carbon dioxide through photosynthesis in plants. The availability of these new tools, which can measure tiny quantities of transitory material, makes it feasible to re-examine the maturation problem in a new light, with hope that better results might be obtained.

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Therefore, it would seem some attempt should be made to determine what factor permits ripening in the mature fruit and prevents it in the premature fruit. The real differences will probably be found in the highly active enzymes, coenzymes or metabolic intermediates. These substances vary greatly with changes in metabolic patterns. Oils, sugars and similar storage products are merely the end-products of a long line of reactions. All the reactions involved in the growth of the plant, as well as formation and ripening of the fruit are controlled by some biologically active proteins called enzymes. They are the substances that take the carbon dioxide from the air and convert it in the leaves to sugars which can be transported to the other parts of the plant to be converted into energy, structural material and storage products. Reactions carried on by these enzymes build the fruit, ripen it and cause its deterioration after ripening. Since the enzymes are very complex compounds, they are subject to many manners of control within the plant. The interaction of these controls in the plant determines the order of growth of a given system. If some of the controls are interrupted or changed in some way, a different type of reaction may occur.

In the avocado, the continuing growth of the fruit is dependent on a continued interchange of materials between the fruit and the leaves of the plant. When the flow is interrupted by picking, the controls are changed and different sets of reactions may take over. In the mature fruit, the enzymes which attack the cell walls go to work and cause a softening of the whole pulp. In the immature fruit, these enzymes, for some reason, are not as active yet and do not accomplish the softening completely before other

processes cause the deterioration of the fruit. It should be possible to determine the reason for this difference. Such a difference could occur because an insufficient amount of the enzyme has been synthesized in the immature avocado; or it could be due to the presence of a large amount of enzyme inhibitor in the immature fruit; or the enzyme may be present but lack of an activating agent in the immature fruit prevents its action. A number of possibilities exist. Actually, the problem is not one of a single enzyme, but of a large number of systems. To determine what the real picture is a better understanding of the basic processes of maturing and ripening must be obtained.

The work being carried out at the University of California at Los Angeles is contributing greatly to knowledge on the nature of the ripen-process. The task at Riverside will be to determine the differences that exist in the micro-compounds and enzyme patterns in the mature and immature avocado, and to find what effect these differences have on the metabolism of the fruit. It may be possible to obtain a rapid insight into the difference in the general metabolism through the use of key compounds tagged with radioactive tracers. Using some of the knowledge gained from the use of the tracers, the activities of certain enzymes in the fruit will be checked at all stages of growth to determine whether a direct correlation with readiness for ripening can be found with activities for any of them. A similar study should be made on some of the highly active energy metabolism intermediates. Already, some preliminary experiments, using radioactive sugars, have indicated that there is a significant difference in the metabolic pattern for sugars in the immature, mature and ripe fruit. By following such leads, it should be possible, in due time, to come to a better understanding of the nature of maturation of the avocado fruit and perhaps arrive at a consistent standard of maturity. Other benefits may accrue along the path. By knowing what controls the maturation of the fruit, it is conceivable that artificial controls of the maturation rate could be obtained. In finding what enzymes are responsible for the development of the ripened flavor and texture and for development of off-flavors, methods of controlling the flavor and texture may be found so that it will be certain that each and every avocado on the market will be of the highest possible quality. Such controls may be dreams at present but, at the start of a project such as this, one should do a certain amount of star-gazing to insure that the work does not stop before the highest goal is reached.