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Analysis of Changes in Monoterpene Content of Ripening 'Hass' Avocado, and Comparison with Other Varieties

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Benefit to the Industry

It has been previously shown that a cytochrome P450 protein induced during ripening of the Hass variety metabolizes monoterpenes very efficiently *in vitro.* This project will reveal the chemical nature of monoterpenes in avocado fruit, if there are any. Monoterpenes often play a role in aroma and therefore flavor, and high levels of specific ones like 1,8 cineole have been shown to play a role in anti-herbivory. This project will begin to identify if there are any monoterpenes in six varieties including Hass, which may correlate with unique flavor, or herbivore preference of each variety. This understanding of the chemical changes that occur with ripening, and the unique chemical properties of each variety, could form the basis of tartetted genetic enginering of superior varieties.

Objectives

Presently it is known which terpenes are present in avocado leaves, but not the fruit. Toward this end, this research will begin to *characterize which terpenes are present in avocado fruit tissues, with the tissues and associated goals as follows:*

- various avocado varieties in order to determine variety-specific differences This could be used to understand properties contributing to flavor through aroma.
- unripe and ripe Hass To determine if levels of compounds such as 1,8-cineole (which presumably will decrease with ripening due to action of CYP71 Al) correlate with decreasing resistance to herbivory as ripening progresses.
- to determine if varieties showing higher levels of CYP71A1 during ripening have lower levels of 1,8-cineole.

To determine if varieties less susceptible to herbivory contain higher levels of 1,8-cineole

Summary of Proposed Research

Today there are various commercially available varieties of avocado, each with a unique size, flavor, and oil content which has not yet been precisely chemically defined. Much of a fruit's aroma and oil content come from the volatile terpenes which includes the 10-

carbon monoterpenes. The analysis of terpenes in avocado leaf has been performed, but as yet there has been no published analysis of terpenes from avocado fruit mesocarp tissue. This grant proposal proposes firstly to **characterize the terpenes in both unripe and ripe fruit mesocarp from various avocado varieties** grown on the Cal Poly Pomona campus, and the associated Pine Tree Research Station: *Bacon, Fuerte, Hass, Macarthur, Pinkerton, Zutano,* in an effort to begin understanding what components are involved in creating the unique characteristics of each variety.

The ultimate commercial value of understanding how and which of the various terpenes contribute to the best tasting varieties, is that through genetic engineering of the larger varieties we may someday be able to engineer improved flavor in the larger sized varieties. Another reason to characterize the ripe fruit monoterpenes is to compare them with the unripe profile of monoterpenes, particularly in the Hass variety. In an effort to understand what occurs at the genetic level during ripening of avocado, various genes have been characterized that respond to ripening. One of these is a cytochrome P450 which typically acts as a mixed-function oxidase. This avocado gene was heterologously expressed in yeast cells and its ability to metabolize various compounds was tested. The rate of activity toward various compounds is shown in Table 1. Apparently it is most catalytically active towards monterpenes, *particularly 1,8-cineloe.* Therefore, it may be degrading 1,8-cineole during ripening of avocado. This is especially interesting in light of studies which show 1,8-cineole plays an anti-herbivory role particularly in eucalyptus, where it is the major component of the essential oils. Therefore, it may be that a high level of 1,8-cineole in unripe avocado fruit must be broken down during ripening to make the fruit palatable to potseed dispersers (herbivores). Toward this end, we will be determining whether the levels of 1,8-cineole are reduced during ripening, and whether those varieties with lower level of 1,8-cineole are more susceptible to attack. Therefore, the levels of P450 protein in ripe fruit of each variety have been measured and the results shown in Figure 1. It appears that each variety has a different abundance of P450 in ripe tissue, with Pinkerton being the lowest (lane 2) and Hass being the highest (lane 12). Therefore a second goal of the proposal is to establish if changes in 1,8-cineole occurs during ripening of the Hass variety (and then the others) according to the extraction and analysis methods in the avocado leaf monoterpene analysis.

Table 1. *Activity of CYP71A1 toward various compounds*

Compound tested	Turnover number (min-1)	V/K (mM-1 min-1)
geraniol	1	38
S-(-)-limonene	3	not calculated (NC)
nerol	4	150
terpinolene	5	NC
p-menth-1-ene	6	NC
1,8-cineole	8	40,000
pCMA	8	40

Figure 1. Western Blot analysis of ripening-induced CYP71A1 in six avocado varieties. Lanes 1,3,5,7,9,11 contain unripe tissue, whereas lanes 2,4,6,8,10,12 contain ripe tissue of the varieties: Pinkerton (lans 1 and 2); Fuerte (lanes 3 and 4); Bacon (lanes 5 and 6); Zutano (lanes 7 and 8); Macarthur (lanes 9 and 10); Hass (lanes 11 and 12). 60 micrograms of microsomal protein was loaded in each lane.