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# Pectin and Related Constituents in Avocado Fruit during Ontogeny[1]

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ABSTRACT. **Pectic substances in 4 avocado cultivars were determined as anhydrouronic acid (AUA) during ontogeny and related to fruit maturity, alcohol-insoluble solids (AIS), alcohol-soluble solids (ASS), ASS minus oil, total oil, fresh weight and dry weight. The concentration of pectic substances in avocado pulp varied among different cultivars and increases during growth and maturation. AUA varied between 0.7 to 1.5% on a fresh weight basis. However, values on a dry weight basis are relatively constant at about 5.0% and independent of the state of maturity or cultivar. AIS, ASS, alcohol-soluble acid and oil increase as the fruit mature, ASS minus oil and water content decreased during the growth and maturation periods. Change in oil content during ontogeny was the only constituent of those examined which was related to maturity.**

Pectic substances are usually associated with the tissue through which they are dispersed as bonding material. Non-uronide constituents are closely associated with the polygalacturonides. Highly purified pectic and pectinic acids frequently are found to contain non-uronide constituents such as L-arabinose, D-galactose, L-rhamnose and other neutral sugars. It is less likely to be dynamically related to oil or to other individual systems of substrate which occur in macro-concentrations throughout the organism. On the other hand, equilibrium between pectic substances and plant tissue constituting the whole of the organism is necessary during the growth process and may serve as an index to maturity.

Cellulose, protein, water, fatty ester (oil), simple carbohydrate and micro-concentration of organic acids make up the greater portion of avocado fruit (9). Changes in sugars (1), oil, water, fiber, protein, and carbohydrate (7) and pectin methylesterase and polygalacturonase (10) during growth and development have been reported. Changes in pectic substances during ripening have been determined (2, 5) but those in pectic substances during growth and maturation have not been investigated. However, it is expected that pectic metabolism in avocado will display a correlation positive to fibrous tissue and to total dry matter and negative to individual systems of substrate during

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development. Knowledge of its position in the scheme of avocado growth and maturation appears both necessary and useful in view of the ulterior role played by the pectic substances in pathological metabolism in fruits (6).

Levels of pectic substances in 4 avocado cultivars were determined as the anhydrouronic acid (AUA) and related to fruit maturity, alcohol-insoluble solids (AIS), alcohol-soluble solids (ASS), ASS minus the oil, total oil, fresh weight and dry weight.

## Materials and Methods

Representative 'Fuerte', 'Hass', 'Clifton' and 'McArthur' avocado fruit were picked periodically and pulp from each cultivar was shredded to facilitate efficient isolation of the fractional components. Ten g samples of pulp were treated for 4 or more hours with 95% ethyl alcohol in Soxhlet extraction assemblies. Alcohol-soluble and sparingly alcohol-soluble solids, oils and water were separated from AIS in this manner.

All samples of AIS were washed with 75% ethyl alcohol. Several samples of each cultivar were set aside, dried over $CaCl_2$ and weighed. Each of the remaining samples were dispersed in 200 ml 0.5% disodium versenate. Each solution was brought to pH 11.50 with dilute NaOH and saponification of all ester groups existing within the AIS was accomplished in 48 hr at 24°C. This supposition is justified by the results of preliminary experiments where the pH of a specific solution was measured at regular intervals for 9 days. Cumulative increases in molar acidity indicated complete saponification in 48 hr. Measurements were made following saponification, to determine the change in pH in each solution and a molar value for saponified esters in the AIS was calculated. The AUA monomer of a pectic chain as the theoretical molecular weight was employed to obtain the percentage methylated anhydrouronic acid in the samples. Finally, each solution was acidified with acetic acid to a pH of about 5.2. Pectic chains were broken into monomers of galacturonic acid with pectinase and the visible spectrum of the carbazole reaction described by McComb and McCready (3) and by McCready andMcComb (4) was employed to measure AUA.

Standard procedures were employed to separate ASS and sparingly ASS from oils, water and alcohol within each of the alcoholic extracts. Water, alcohol and trace amounts of volatile oils were evaporated away in each instance under reduced pressures at 80°C. Oils, traces of chlorophyll and other minor ether-soluble solids were taken up in petroleum ether, following determination of the combined gravimetric weight of oils and ASS and sparingly ASS in each sample. Ether-soluble oils were determined gravimetrically after insoluble solids were filtered off and the solvent was evaporated away with steam.

Water contents were estimated by the difference between sample weight and the combined weight of the corresponding AIS and ASS and sparingly ASS which were considered to be dry weight fraction. Alcoholic extracts of each cultivar were diluted with $H_20$ and total available alcohol-soluble and sparingly alcohol-soluble acid was determined by titration with standard NaOH to a phenolphthalein end point. Oil contents of the pulp of representative samples of the avocados were also determined by the method described by Sweet (8).

## Results and Discussion

**ALCOHOL-INSOLUBLE SOLIDS.** The pulp of avocado which remains insoluble in alcohol after extraction consists principally of polysaccharides; i.e., cellulose, hemicellulose, starch, and pectin. The last will be presented under a separate heading later. AIS expressed on a fresh weight basis (Table 1) fluctuates throughout the season, except for 'Hass' which increased substantially during maturation. However, AIS expressed as a percentage of dry weight vs. dry weight (Fig. 1) were quite consistent with a slight decrease in 'Hass' which had relatively high dry weight. AIS does not appear useful as a maturity index.

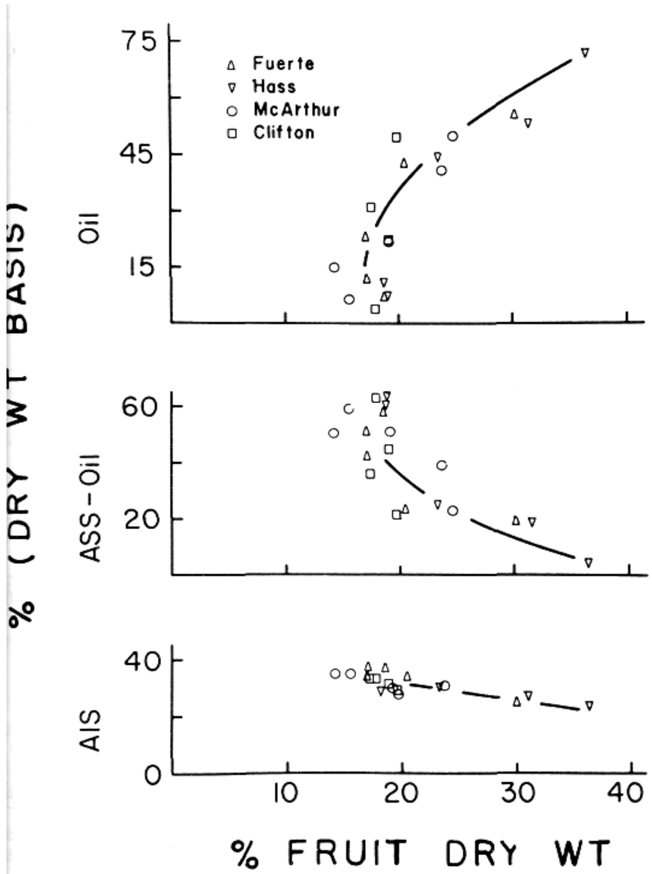

Fig. 1. Changes in oil (top), ASS minus oil (middle) and AIS (bottom) as % of the dry weight vs. % fruit dry weight.

**ALCOHOL-SOLUBLE SOLIDS.** The soluble and sparingly soluble portion of avocado pulp after alcohol extraction can be divided into 3 fractions; (a) water and negligible amounts of volatile oil, (b) ether-insoluble solids such as simple carbohydrates, acids and molecular constituents defined by electrovalent bonds and (c) ether-soluble solids such as oils and negligible amounts of organic molecules defined by covalent bonds. ASS increased in avocado pulp during growth and development (Table 1) primarily because of the increase in the oil during maturation (Table 1). Therefore, the ASS minus oil on a fresh weight basis (Table 1) decreased as the season advances and the decrease is more striking when expressed as % dry weight vs. dry weight (Fig. 1). The water content of all cultivars was relatively constant during growth. Water comprised about 80% or more of the weight of immature avocado pulp of all cultivars although some dependency is expected relatively to regional relative humidity and irrigation practices. This value did not change significantly in 'McArthur' or 'Clifton' at maturity, but decreased in 'Fuerte' and 'Hass' at maturity (Table 1). The alcohol-soluble acid as determined excludes a portion of the amino acids and includes functional groups that behave in an acidic manner when treated with a strong base, however the results provide estimates of total acid. The data show that 300 to 400 moles of hydrogen ion are present in 10 g of 'Fuerte', 'Hass', or 'Clifton' pulp before and at maturity and that the acidity of 'McArthur' pulp increased with maturity from 520 to 680 moles. Therefore, acid in avocado is minimal and does not play an important role nor is it useful as an index to maturity.

Table 1. Concentration of different fractions of avocado pulp during growth and maturation.

| Harvest date | Avg fruit wt (g) | Fraction concn (% fresh wt) | | | | | | | Alc. Sol. Acid (moles/10 g) |
|---|---|---|---|---|---|---|---|---|---|
| | | AUA | Methylated AUA | AIS | ASS | ASS minus Oil | Oil | $H_2O$ | |
| | | | | | *Fuerte* | | | | |
| July | 44 | 1.05 | 1.40 | 6.8 | 11.9 | 10.4 | 1.5 | 81.3 | 344 |
| August | 115 | 1.02 | 1.23 | 6.3 | 10.9 | 8.8 | 2.1 | 82.8 | 345 |
| Sept. | 170 | 1.17 | 1.18 | 5.8 | 11.6 | 7.4 | 4.2 | 82.6 | 435 |
| Nov. | 285 | 1.09 | 1.09 | 6.9 | 13.7 | 4.8 | 8.9 | 79.4 | -- |
| Feb. | 266 | 1.47 | 1.47 | 7.4 | 22.8 | 5.9 | 17.1 | 69.8 | 513 |
| | | | | | *Hass* | | | | |
| July | 26 | 0.82 | 0.96 | 5.3 | 13.6 | 12.2 | 1.5 | 81.3 | 300 |
| August | 61 | 0.96 | 0.88 | 5.1 | 13.2 | 11.1 | 2.1 | 81.7 | 293 |
| Nov. | 166 | 1.17 | 0.70 | 7.0 | 16.4 | 5.9 | 10.5 | 79.6 | 364 |
| Feb. | 180 | -- | -- | 8.44 | 23.0 | 6.0 | 17.1 | 68.6 | 372 |
| June | 218 | 1.47 | 0.88 | 8.6 | 27.8 | 4.3 | 23.5 | 63.5 | 385 |
| | | | | | *McArthur* | | | | |
| July | 17 | 0.67 | 1.66 | 5.4 | 10.4 | 9.3 | 0.9 | 84.4 | 720 |
| Sept. | 98 | 0.83 | 1.22 | 4.9 | 9.4 | 7.2 | 2.2 | 85.7 | 525 |
| Oct. | 163 | 0.97 | 0.88 | 5.7 | 13.5 | 8.8 | 4.2 | -- | -- |
| Feb. | 266 | 1.03 | -- | 4.8 | 14.0 | 5.3 | 8.7 | 76.2 | 631 |
| June | 220 | 0.84 | 0.79 | 5.5 | 14.3 | 4.4 | 9.9 | 80.3 | 680 |
| | | | | | *Clifton* | | | | |
| July | 47 | 0.92 | 1.32 | 6.0 | 12.0 | 11.4 | 0.6 | 82.1 | 394 |
| August | 105 | 0.96 | 1.25 | 5.9 | 3.2 | 8.7 | 4.5 | 80.9 | 330 |
| Sept. | 108 | 1.01 | 0.97 | 5.8 | 13.8 | 6.4 | 7.4 | 82.47 | 383 |
| Oct. | 192 | 1.04 | 1.07 | 5.7 | 14.1 | 4.3 | 9.8 | 80.2 | 415 |

**PECTIN.** Pectic substances expressed as % AUA on a fresh weight basis in the 4 avocado cultivars during ontogeny are listed in Table 1. The concentration of AUA in the fresh pulp varied somewhat among cultivars and increased as maturity approaches. However, variation in concentration of a dry weight basis from month to month or among cultivars was negligible during growth and maturation (Fig. 2). AUA expressed as % AIS varied only slightly during the season or between cultivars with a tendency to be slightly higher at mid-season compared with young or mature fruit (Fig. 3). There was an increase during the season when AUA is expressed as % ASS minus oil, which decreased during the season and differed among cultivars (Fig. 4). 'Fuerte' and 'Hass' showed similar patterns, with 'Clifton' being higher when mature and 'McArthur' being lower throughout the later part of the season. Pectic substances expressed as % oil decreased rapidly during growth and development and changed only slightly during maturation for 'Fuerte', 'Hass', and 'McArthur' with only slight variations among these cultivars (Fig. 5). 'Clifton' showed only a decline and the values were less than for the other cultivars. Esterified AUA decreased as the fruit matured (Table 1). The concentration of AUA increased during the season but the variation between cultivars limits its use as a maturity index. However, the increase in AUA and the decrease in esterification during the season is in agreement with reported lack of detectable polygalacturonase and decrease in pectin methylesterase during development (10). Changes in the concentration of other constitutents, in themselves or in relation to pectic substances, appear to have no tangible relationship other than the role they play as a part of the entire fruit.

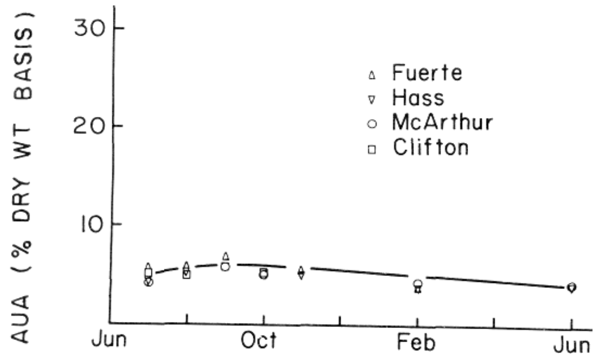


Fig. 2. Seasonal changes in anhydrouronic acid (AUA) expressed as % dry weight.

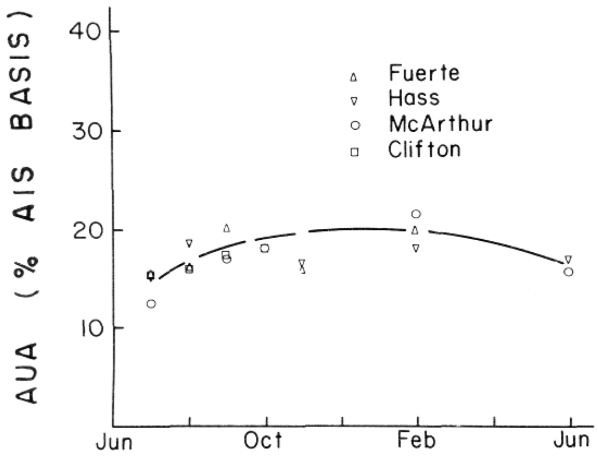


Fig. 3. Seasonal changes in anhydrouronic acid (AUA) expressed as % of alcohol-insoluble solids (AIS).

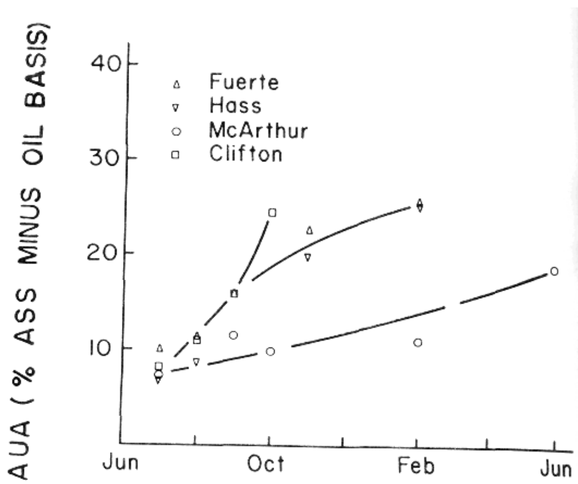


Fig. 4. Seasonal changes in anhydrouronic acid (AUA) expressed as % of alcohol-soluble solids other than oil (ASS minus oil).

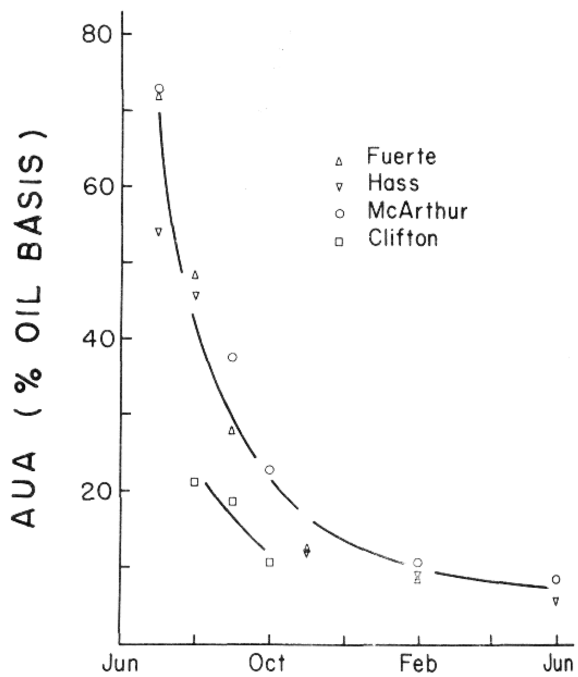


Fig. 5. Seasonal changes in anhydrouronic acid (AUA) expressed as % total oil content.

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