SOME CHEMICAL CHANGES DURING THE POST-HARVEST RIPENING OF PAPAYA FRUIT[1]

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Since 1823, the papaya was introduced in Hawaii, comparatively little work on biochemical changes during the ripening of papaya fruits has been done. With regard to sugar changes occurring in the fruit during ripening, a low percentage of sucrose was found. (Pratt and del Rosari, 1913, Thompson, 1941, Pope, 1930). Thompson (1941) noted that the sucrose content showed no appreciable change during ripening on the tree. Pope (1930) found an increase in reducing sugars during ripening. In 1940, Jones and Kubota reported that the ripe fruits contained 79.04% sugar on a dry weight basis, 14.4% of which was due to sucrose; whereas in the mature green fruits, they found only 71.68% total sugars but 30.98% sucrose. Presumably, a large amount of sucrose was hydrolyzed during ripening. A climacteric rise in carbon dioxide production with the ripening of the fruits was reported by Jones *et al.* in 1941, Wardlaw and Leonard in 1953. Orr *et al.* (1953) in their study of the sugar and ascorbic acid content of papaya, found that the amount of total sugars in papaya was correlated with the production of ascorbic acid. The purpose of the present study has been to investigate further possible chemical changes in the constituents of harvested papaya fruits.

Material and Methods

Papaya fruits, Solo strain, used in this study were obtained from the Waimanalo Branch Station of the Hawaii Agricultural Experiment Station. Mature green papayas were delivered and were allowed to ripen in the laboratory. The changes during the ripening process were studied.

Color Change

The external color as well as the internal color of the fruits were observed

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daily until the fruits reached full ripening. The microscopic observation for pigment distribution in the flesh was also performed.

Change in Water Content

Two-gram samples were taken daily from intact fruits by means of a cork borer. The tissue freed from seeds and skin was sliced and extracted with hot 80% ethanol in a tared beaker. Then the constant weights of alcohol soluble and insoluble materials were obtained, their percentages were calculated on a fresh weight basis, and the loss in weight was taken as the water content. The weights of the intact fruits were also recorded daily during the ripening period.

Determination of Changes in Pigmentation

The pigment in the two grams of the sliced tissue was completely extracted by 40 ml. of anhydrous methyl alcohol within a short period of time. The extract was filtered and made up to 50 ml., the absorbance was measured at the absorption maximum, 443 mμ, using a model DB Beckman spectrophotometer.

Identification of Sugars, Amino Acids, and Organic Acids in the Alcohol Extract of Papaya Fruit

Two dimensional paper partition chromatographic techniques were adapted from those described by Block *et al.* (1958). and were used in the study of these fractions. In these determinations the alcohol extracts from the 2 gram fresh tissue samples were used. Aliquots of these extracts were applied on 47×56 cm. Whatman No. 1 filter paper sheets. In all cases water-saturated phenol was used as a first solvent. The second solvent for both the sugar and the amino acid chromatograms was a mixture of n-butanol, acetic acid, and water, 62:10:28; for the organic acids was n-butanol which had been equilibrated against 4 M formic acid. The positions of the sugars on the developed chromatograms were revealed by spraying with p-anisidine; the positions of, the amino acids by treatment with ninhydrin; and the positions of the organic acids with bromthymol blue. In order to get the better chromatograms, the columns of Dowex-50 and Doulite-A[4] were used for isolation of amino acids and organic acids respectively.

Sugar Determinations

Analyses were performed in duplicate every day, from the first day to the fifth day after harvesting. The alcohol soluble fraction of each 2-gram tissue sample was dissolved in water and made up to 50 ml. Part of this solution was incubated with a few drops of 1% invertase and acetate buffer, pH 4.5, at 37°C for 2 hours. By the method of Somogyi-Nelson (1944), the total sugars were determined from this inverted solution. Part of the original sample solution was appropriately diluted and used to determine total fructose by Roe's colorimetric method (1934) and free reducing sugars by the method of Somogyi-Nelson. The amount of free glucose, fructose and sucrose were obtained by calculation. An Evelyn colorimeter was used in this experiment.

Determination of Pectic Substances

Pectic substances were determined on the alcohol extracted residues of the tissue samples used for sugar determinations. The "water soluble" pectin was extracted with hot water and the "acid soluble" pectin was extracted with 0.05N HCl from the residue after hot water extraction. The method used for the determination of galacturonic acid residues was the carbazole method of Dische (1947). Glucuronolactone was used to standardize the hexuronic acid in the samples.

Results and Discussion

Mature green papayas allowed to ripen at room temperature, 28°C., reached the full ripe stage after 5 days. Five ripening stages are defined according to the external color development as follows: Stage 0 or the mature green stage is the stage when the fruits are picked from the tree; Stage 1 or quarter ripe stage is the stage when one quarter of the external area has turned yellow. Stage 2 and Stage 3 are the stages at which the fruits have turned one-half and three-fourths yellow, respectively; and Stage 4 is the stage at which the fruits reach the full ripening and have full orange yellow color developed over the entire surface.

At the mature green stage, the fruits may be turning from dark to light green at the blossom end. Light orange color appears in the seed-coats, placenta and the flesh within about 0.5 cm. the central cavity, while the rest of the flesh appears to be white or greenish white. Under the microscope, it can be seen that there are seven to twelve layers of epidermal cell containing chloroplasts which give rise to the green color of the skin. The cells near the central cavity contain orange—colored chromoplasts. They do not contain chloroplasts. The cells in the middle part of the pericarp contain neither chloroplasts nor chromoplasts. Seeds appear to be full-sized and brown-colored with a transparent, orange-colored seed coat. The funiculi and placenta are full—sized with a silvery spongy structure, and are difficult to separate from the flesh. As the ripening progresses, the orange-yellow coloration increases from the inside of the fruit toward the outside. Rapid development of internal color occurs between Stage 0 and Stage 1, while rapid development of external yellow color appears between Stage 1 and Stage 2. When Stage 4 is reached the central cavity increases in volume. The placenta loses its silvery spongy structure. It becomes shrunken and along with the seeds is now easily separable from the flesh. Few, if any, chloroplasts are present in the epidermis, but it is full of carotenoids. Carotenoid granules in the flesh of the fruit are scattered throughout the cells to a greater degree than at any other stage. From these observations it is understood that color change in papaya fruit is brought about by the loss of chlorophyll and the unmasking and development of the carotenoids in the cells.

On a fresh weight basis the moisture content of a Stage 0 papaya is approximately 86.6%, the alcohol soluble fraction is 11.8%, and the alcohol insoluble fraction is 2.6%. When the fruit reaches Stage 4, the moisture content drops to about 84.0%. The alcohol soluble fraction and insoluble fraction are then 13.0% and 2.9%, respectively. The values obtained by Jones and Kubota (1940) for the moisture content of papaya fruit at mature green stage and full ripe stage are 86.1% and 85.3%, respectively. As the fruit reaches Stage 4, about 4.5% of the initial weight of the fruit is lost as shown in Table 1. That this loss in weight is due primarily to water loss is attested to by the fact that the weight loss after four days storage at 28°C but with 100% relative humidity is less tnan 1%. Accompanying the loss of water, there is also a loss of latex and luster. Shrinking and softening of the fruit are also noted.

Table 1. *Percentage weight change of papayas stored at 28°C.*

Days in Storage	Percent Initial weight*	Percent Weight Loss
0	100	0
1	98.9	1.1
2	97.8	2.2
3	96.6	3.4
4	95.6	4.4

*Average of ten fruits

Befor the mature green stage, the fruit shows no external trace of yellow but is entirely dark green in appearance. Inside the fruit, there is no pigment present. Both the pericarp and the seeds are white and the seeds have opaque seed coats. Such an immature green fruit when picked from the tree does not follow the normal ripening process at room temperature. It remains green and firm for a considerable length of time, and never ripens.

Change in Carotenoid Content

The increase in amount of carotenoid as expressed by the absorbancy at 443 mμ is shown in Table 2. The results indicate that there is a sharp increase in carotenoid content after harvest, as can also be judged by the rapid change in appearance of the fruits The pigment content continues to increase gradually, reaching a peak on the third day and remaining stationary thereafter. The carotenoid development was observed to start from the tissue near the central cavity. The development extended toward the skin as the ripening proceeded, so the color change of the fruit as judged externally was somewhat slower than the actual change in the carotenoid content as measured by the absorbancy.

Jones and Kubota (1940) in their study of papaya respiration noticed that

Table 2. *Increase in 443 mμ absorbancy following harvest of Solo papayas*

Days in Storage	Stage of Maturity	Absorbancy
0	0	0.25±.03
½	1	0.31±.02
1	2	0.34±.02
2	3	0.37±.02
3	4	0.38±.02
4	4	0.38±.02

as the color developed, the rate of respiration increased and reached a peak simultaneously with the full color development. They also claimed that respiration inside the fruit began long before the development of external color development. In this connection, it is also interesting to find that the carotenoid development started before the external color change, as shown in this experiment.

In order to obtain more information concerning the pigments, 20 grams of full ripe tissue free from skin and seeds were sliced and the pigments were extracted with anhydrous methanol. The extract was then partitioned between hexane and water. The hexane layer was separated and washed repeatedly with water in order to free it of methanol. The hexane solution was passed over a small magnesium oxide column, 1×9 cm., When a 60:40 hexane-benzene mixture was used as an elutriant the pigments absorbed at the top of column separated into five distinct bands. When viewed under ultra violet light it became apparent that a sixth band, fluorescent band, was located at the solvent front. The four lower pigments, were successively eluted from the column and, after appropriate dilution, absorption spectra were prepared by means of a Beckman DB recording spectrophotometer. On the basis of their adsorption characteristics on magnesium oxide the easily elutable components were identified as non-oxygenated hydrocarbons. Because of its green fluorescence and absorption maxima at 332, 350, and 370 mμ the most mobile pigment was identified as phytofluene. The second pigment eluted, with absorption maxima at 380, 405, 428, was assumed to be δ-carotene, octahydrolycopene. The third pigment, present in greatest amount (about 4 times that of the others), with 450, 480 absorption maxima was an ester of cryptoxanthin. The fourth elutable pigment with a 5mμ absorptive shift to 445, 475 mμ, was identified as α-carotene.

Because of their low mobilities it was assumed that the pigments remaining on the column were oxidized forms of the hydrocarbons. This is not unlikely in view of the 1933 reports by Yamamoto and Tin as cited by Karrer and Jucker (1948) that cryptoxanthin, 3-OH β-carotene, and violaxanthin, the dihydroxydiepoxide of β-carotene, both occur as esters in papaya fruits.

Analysis by Paper Chromatography

In the paper partition chromatograms, three sugar spots were detected. By referring to a standard chromatograms, these were identified as sucrose, fructose, and glucose. Sucrose appeared to be present in greatest amount, as much as the sum of fructose and glucose, and glucose seemed to be present in greater amount than fructose.

Five major amino acids were identified in the alcohol extract of the fruit; namely, aspartic acid, glycine, γ-amino butyric acid, glutamic acid and alanine. Aspartic acid appeared to be the most prominent amino acid, as judged from the chromatogram. Glutamic acid and alanine were present in lesser amounts. The distribution of the amino acids in the mature green fruit was only slightly different from that in the full ripe fruit. Whereas the green fruit appeared to contain more aspartic acid, the ripe fruit contained increased amounts of glutamic and γ-aminobutyric acids. Glycine and alanine seemed to remain about the same in the green and ripe fruits.

γ-amino butyric acid is found to be present in widely different fruits. Townsley et al. (1953) and Underwood and Rockland (1953) found that γ-amino butyric acid was present in all commercial citrus fruits. In many other non-citrus fruits, such as apples, apricots, avocadoes, and prunes, γ-amino butyric acid was found to be present in the juice, as shown in the study of Joslyn and Stepka (1949). Clements and Leland (1962) found that there was a seasonal change in amino acids. Proline and γ-amino butyric acid increased in amount during maturation. Recently, by means of paper chromatography, Silber and his students (1960) studied the amino acids in 32 fruits. They found alanine in all fruits they studied, γ-amino butyric acid in all but blueberry, and glutamic acid in all but date. They also found the smallest number of amino acids, 5 to 8, in apple, blueberry, gooseberry and rhubarb, and as many as 21 amino acids in red grapes. In present study, only the 5 amino acids which were present in sufficient concentration to be detected easily in the chromatograms of the papaya extracts have beeen considered. The actual number of amino acids in the tissue is undoubtedly more than 5, as can be demonstrated by cation exchange resin concentration of the amino acids in the juice.

When papaya extract anion concentrates were subjected to one dimensional chromatography with a butanol-formic acid solvent, malic and citric acids were obvious constituents. The presence of succinic and fumaric acid were indicated, but despite further concentration these two acids could not be positively identified on two-dimensional chromatograms. During the transition from mature green to full ripe the citric acid content of the papaya appears to remain constant whereas the concentration of malic acid decreases markedly.

Quantitative Analysis of sugars

The results of the analyses are presented in Table 3. They show that in papaya fruits 10 to 11% of the fresh weight is sugar. This is about 6% higher than the value reported by Pratt and del Rosari (1913), but in close agreement with the result of Orr *et al.* (1953) who reported 11.0 to 9.7% total sugar in the papaya fruit. There was no apparent change in the total sugar content throughout the ripening period. Fruit at color-turning or mature green contained 10.7% total sugar, and it was 10.9% at full ripening stage.

Table 3 *Sugar content as percentage of the fresh weight of papaya fruits during ripening*

Ripening Stage	Total Sugars	Sucrose	Fructose	Glucose	Reducing Sugars
0	10.7	6.87	1.4	1.9	3.38
1	11.1	6.70	1.7	2.8	4.42
2	10.9	6.45	1.9	2.6	4.56
3	10.2	6.24	1.8	2.2	4.03
4	11.1	6.45	1.8	2.6	4.30

Allen (1932) found an increase in the amounts of both sucrose and reducing sugars in peach, pear, and apple after harvest, but constant levels of sugars throughout the ripening in the case of plum. Papaya resembles plum in this respect.

Sucrose constituted the main part of the total sugars, amounting to 58.1 to 64.4%. The glucose content was slightly higher than that of fructose, the amounts being 21 to 25% for glucose and 14 to 18% for fructose, respectively.

That the sucrose content showed no appreciable change during ripening on the tree was reported by Thompson (1914). Pope (1930), however, reported that reducing sugar increased in amount during the ripening of papaya. Jones and Kubota (1940) reported that during the ripening of papaya a large portion of sucrose was hydrolyzed. This is not the case in the present study.

The source of this disagreement may be traced back to a fact of enzymic activity in the fruit tissue. It was found that an extremely active invertase is present in papaya fruits and reaches its maximum activity when the fruits full ripe. (personal communication) Therefore, it is suspected that the reports of sucrose being hydrolyzed during ripening of papaya fruits might be a misleadingness of not inactivating the enzyme before preparing the tissue for the analysis.

Pectic Substance

The experimental results shown in Table 4. indicate that the amount of water soluble pectic substances in papaya decreased in amount slowly during the ripening process till the fruit reached the stage when three-fourths of the external surface was colored yellow, then dropped sharply from 0.66% to 0.46%. The amount of water soluble pectic substances present at the mature green stage was significantly higher than that at the full ripe stage. The amount of acid soluble pectic substances was only one-third of the amount of water soluble pectic substances, and there was no significant change in the amount throughout the ripening process. The decrease in the amount of total pectic substances was significant at 5% level. Total pectic substances decreased rapidly from the three-fourths ripe to the full ripe stage. This suggests that, during the ripening process, water soluble pectic substances were converted to non-uronide forms, since the amount of acid soluble pectic substances remained unchanged.

Table 4. *Amount of pectic substances as percentage of the fresh weight of papaya fruits during ripening*

Ripening Stage	Water Soluble Pectin	Acid Soluble Pectin	Total
0	0.68%	0.25%	0.93%
1	0.74	0.23	0.97
2	0.63	0.21	0.84
3	0.66	0.20	0.86
4	0.46	0.18	0.64

Carre (1927) studied the pectic materials in the apple and found that during storage the amount of pectin increased while that of protopectin decreased, and the total pectic materials remained unchanged. Haller (1929) suggested that the softening of the apple in storage was due to the conversion of insoluble pectic substances to soluble forms. Similar information was obtained by Smock and Allen (1937) in apple and pear. Gerhardt and Ezell (1938) also found the same relationship between softening and ripening in Bartlett pears. They even proposed the use of soluble pectin as an index of maturity. Barnell (1943) noticed that accompanying the increase of water soluble pectin at the time the banana turned yellow, there was simultaneously a rapid decrease in total pectic substances from an initial content of 0.5% to 0.7% on fresh weight. Sterling and Kalb (1959) found that during the ripening of Elberta peaches, the amount of acid soluble pectic substances decreased and that of water soluble pectic substances increased while the total amount of pectic substances decreased.

Papaya fruits show a change in texture from firm to soft during ripening in the laboratory. However, the change of pectic substances observed in this study does not fully support thesis that softening is the result of the transformation of insoluble pectic substances to soluble forms as is the case with other fruits.

Summary

Chemical changes during the post-harvest ripening of papaya fruits were investigated. Most prominent, a change in color from green to orange yellow, is due to the destruction of chlorophyll in the epidermal tissue and the unmasking and development of carotenoids in the flesh. The color development starts in the placenta and spreads outward to the epidermis. Ripening of the fruit from mature green to full ripe requires five days at 28°C; however, the carotenoid content of the pericarp reaches its maximum at the three-fourth ripe stage and remains at that level. In addition to the principal constituent, an ester of cryptoxanthin, at least five other carotenoid pigments are present in the papaya fruit.

During ripening, the texture of the fruit changes from firm to soft, and there is an apparent increase in the sweetness of the fruit. The subepidermal latex disappears and the weight of the fruit decreases. Loss of water by transpiration is responsible for the decrement in weight.

Sugars comprise about 10% of the fresh weight of the fruits. The sugars present are sucrose, glucose, and fructose. Sucrose is the most abundant constituting about 60% of the total sugars. Glucose is present to the extent of about 24% and fructose about 16%. The amounts of these sugars remain at the same level throughout ripening.

The most abundant amino acids are, in order of amount present, aspartic acid, glycine. γ-amino butyric acid, alanine, and glutamic acid.

Citric and malic acids are the principal organic acids in green fruits. The amount of malic acid decreases as the fruits ripen.

There appears to be a decrease in pectic substances as the fruits ripen. This decrease involves both the water soluble and the acid soluble fractions which are present in a ratio of about 3:1.

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木瓜果實收成後的一些化學變化

陳 金 鑾

商業上，在木瓜果實表面剛呈現一點黃色斑痕時便施採果，此時採下的木瓜在 28°C的溫度下經過五天便達到完全成熟的階段。在此過程中最顯著的變化，是顏色由綠色轉變成金黃色，這是由於果皮的葉綠素消失，果肉內胡蘿蔔素的產生及增加的結果，至少有六種的胡蘿蔔素存在木瓜果實中。其他顯著的變化尚有果肉由硬而變軟。甜味增加，乳汁的消減及因蒸散作用而致果實重量的減少等。

約有新鮮果重的10%爲糖類，其中蔗糖佔全部糖類的 60%，其次爲葡萄糖及果糖，各佔全糖的 24%及 16%。這些糖類的含量在成熟過程中並沒有顯著的變化。

在果實中最主要的氨基酸有天門冬酸等五種，主要的有機酸則有檸檬酸及蘋果酸兩種。此外木瓜果實內，水溶性果膠質和酸溶性果膠質以 3 比 1 的比率存在，於成熟的過程中此兩種果膠質都有減少的趨勢。

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