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SUGAR ESTIMATION

Refractometric Dry Solids as an Indicator of the Sugar Content of Papaya Fruit

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Where the range in sugar contents among examined fruits was substantial, near perfect correlations, $r = 0.987$ and 0.991 , between the refractometric dry solids (RDS) of the fruit juice and the sugar content of papaya (*Carica papaya* L.) fruit were obtained. The correlation was less impressive, $r = 0.610$, where sugar concentrations differed only slightly from one lot of fruits to another, but was adequate enough not to preclude the use of RDS as an indicator of sugar content.

OFTEN an estimate of the sugar content in plant tissue can be quickly obtained from the refractometric dry solids (RDS) of the cell sap. It is not precise, but where sugar is the predominant water-soluble constituent, the method has been used routinely and advantageously—e.g., in grape, melons, etc. (1, 2, 4, 7).

In breeding trials and in some cultural experiments with papaya, a fruit crop commercially important in Hawaii and in other tropical-subtropical areas, the juice RDS has been accepted as a measure of sugar content. Unfortunately, the relationship between the two characteristics, or the reliability of the former as indicator of the latter, has escaped any previous establishment. The refractometric dry solids denote content of any solute, and do not selectively disclose the level of sugar as sometimes inferred. In this study the degree of correlation has been determined.

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Materials and Methods

Samples of papaya (*Carica papaya* L.) fruit were provided by R. A. Hamilton of the station. The plants were grown at the Manoa, Poamoho, and Waimanalo farms. Three evaluations were performed. In the first, fruits of six strains with a substantial range in sugar contents were employed; another test considered Solo papaya fruits harvested during the two extremes in season, summer and winter. In both trials, samples were harvested at a stage of maturity when at least 50% of the fruits' external surface was yellow, and were analyzed when they attained complete yellowness after storage in the laboratory. The third evaluation explored Solo fruits which were in varying stages of maturity or ripeness when harvested. The stages were fully-yellow, half-yellow, a trace-of-yellow, mature-green, and immature-green. The first four were recognized by their external coloration; the mature-green fruit was distinguished from the immature-green by its larger size and content of fully-developed, dark seeds.

Samples for analyses consisted of three replicate fruits. The RDS was obtained with a Zeiss F₃ hand refractometer. The freshly expressed, unfiltered sap of the pulp was examined. Expectedly, the RDS's vary slightly with the morphologic position within a given fruit wherein the sample is obtained. Thus, the pulp from an entire fruit was first blended mildly and then examined.

Extracts for sugar determination were prepared from 50 grams of pulp by these steps: mixed with 1 gram of CaCO₃ and 100 ml. of distilled water, homogenized 5 minutes in a Waring Blendor, boiled 5 minutes and filtered hot with 5 grams of washed Filter Cel, rinsed with two 50-ml. portions of boiling distilled water, combined filtrates, cooled, and diluted to 250 ml. The absence of anthrone-reactive substances in further extracts of the residue was considered as evidence of complete extraction. Aliquots of the extracts were clarified by Loomis's method (3) and hydrolyzed by adding 1/10 their volumes in concentrated HCl and allowing to stand at room temperature overnight. After neutralization, the sugar content was

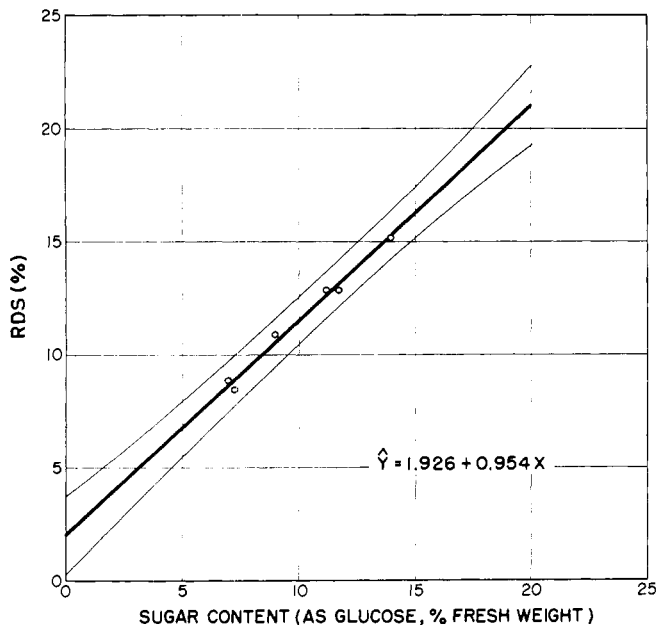


Figure 1. Regression of refractometric dry solids of fruit sap on sugar content of papaya fruit

Six strains employed; thin lines denote 95% confidence limits

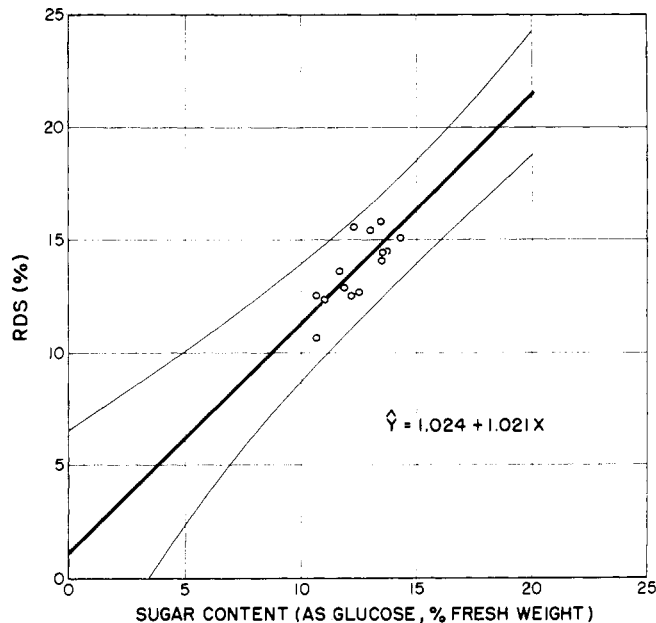


Figure 2. Regression of refractometric dry solids of fruit sap on sugar content of papaya fruit

Summer and winter fruits compared; thin lines denote 95% confidence limits

determined by the ferricyanide reduction method (6).

Statistical analyses were performed according to Snedecor (5).

Results and Discussion

Table I shows mean RDS's and corresponding sugar contents of fruits of the six strains of papaya. A near perfect correlation was obtained, with $r = 0.987$. Strains with higher sugar contents showed correspondingly higher soluble solids, and vice versa. The regression line, with its 95% confidence bands, is shown in Figure 1. Its intercept would suggest that the RDS to an extent 2% in value denoted solutes other than sugar. From this and from the regression equation, $\hat{Y} = 1.926 + 0.954X$, a close approximation of the sugar concentration is obtainable for any value of RDS by simply subtracting 2%.

Table II contains the average RDS's and the corresponding sugar contents of Solo papaya fruits harvested at five stages of maturity. The two attributes differed on the average by nearly 2%. Again, a high degree of correlation, $r = 0.991$, was obtained. Accordingly, the younger the fruit when harvested, the lower the refractometric dry solids and the sugar concentration.

In the above two instances, the range in sugar contents among the considered fruits was substantial. Where the contents differed only slightly from one lot to another, the degree of correlation was noticeably lower. The RDS's and corresponding sugar concentrations of Solo fruits harvested during the summer

Table I. Refractometric Dry Solids of Fruit Sap and Corresponding Sugar Contents of Fruits of Six Strains of Papaya

Strain	RDS (%)	Sugar Content (as Glucose, % Fr. Wt.)
IAC-39	8.4	7.2
Bettina 100-A	8.8	7.0
60-6	10.8	9.0
Hybrid No. 5	12.8	11.2
60-9	12.8	11.7
Solo	15.2	13.9

Coefficient of correlation, $r = 0.987$.

Table II. Refractometric Dry Solids and Corresponding Sugar Contents of Solo Papaya Fruits of Varying Stages of Maturity or Ripeness at Harvest

Harvest Stage	RDS (%)	Sugar Content (as Glucose, % Fr. Wt.)
Fully-yellow	13.8	11.7
Half-yellow	15.0	12.1
Trace-of-yellow	13.6	12.1
Mature-green	5.0	3.4
Immature-green	4.0	2.6

Coefficient of correlation, $r = 0.991$.

Table III. Refractometric Dry Solids and Corresponding Sugar Contents of Solo Papaya Fruits from Summer and Winter Harvests

Summer			Winter		
Harvest date	RDS (%)	Sugar content (as glucose, % fr. wt.)	Harvest date	RDS (%)	Sugar content (as glucose, % fr. wt.)
WAIMANALO					
6/17/61	15.4	13.0	12/6/61	13.6	11.7
7/14/61	15.8	13.4	1/17/62	12.3	11.0
8/11/61	15.6	12.3	2/14/62	12.5	10.7
POAMOHO					
7/13/61	14.4	13.6	1/17/62	12.5	12.2
8/10/61	14.6	13.5	2/14/62	12.7	12.5
MANOA					
7/19/61	14.5	13.7	1/17/62	10.7	10.7
8/16/61	15.1	14.3	2/14/62	12.8	11.9

Coefficient of correlation, $r = 0.610$.

and winter months can be seen in Table III. According to the *t*-test, the differences from one season of harvest to the other were significant; fruits from summer contained 0.8 to 3.0% more sugar and yielded correspondingly higher RDS's, from 1.4 to 3.8% more, than winter fruits. A variation among orchards in the degree of difference between summer and winter fruits is apparent, but the trend in each was the same. The coefficient of correlation was in this evaluation only 0.610; nevertheless it was significant at the 95% level of probability. The regression line, with its relatively broad confidence bands, is seen in Figure 2. The difference between the RDS and the sugar content was about 1%.

Sugar is indeed the predominant water-soluble substance in papaya fruit; it comprises about 90% of the sap

solutes. Thus, the correlation between juice soluble solids and sugar content is expectedly excellent, and the former is applicable as an indicator of the latter in certain situations. In variety trials, those yielding fruits with low juice RDS's—e.g., the first three in Table I—may be quickly eliminated from further screening. According to the Table II data, the sugar level in green fruits is markedly lower than in those which have attained a degree of yellowness. Growers ordinarily harvest fruits, particularly those which are intended for the more distant markets, when still green. A minimum content of sugar, or a standard of quality, in the marketed fruit may be assured by including, perhaps in the packing step, a systematic examination of RDS's. The critical solid content will have to be agreed upon first.

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BELL PEPPER CAROTENOIDS

The Carotenoids of Green Bell Peppers

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The most abundant carotenoid in green bell peppers was lutein, with beta-carotene, violaxanthin, and neoxanthin also as major pigments; minor pigments included phytoene, phytofluene, alpha-carotene, and zeta-carotene. No keto carotenoids such as capsanthin or capsorubin were found, nor was capsolutein, a lutein-like pigment occurring in place of lutein in the ripe red fruit.

THE CAROTENOID MIXTURE in red bell peppers (*Capsicum annuum*) (5) was quite complex and contained a number of pigments not found in any of the numerous other fruits examined in this laboratory. Seven of these carotenoids contained keto groups, including capsanthin and capsorubin, the structures of which were shown recently (7, 9) to contain one and two cyclopentane rings, respectively. At least six other pigments apparently also contained cyclopentane rings, including capsolutein, a lutein-like pigment for which the structure 6'-deoxocapsanthin was proposed (5). No lutein was found. Capsolutein was indistinguishable from lutein in visible and ultraviolet absorption spectra, and differed only slightly in behavior on countercurrent distribution, but unlike lutein did not contain an allylic hydroxyl group.

The carotenoids of green bell peppers have now been investigated, to see if any of the keto carotenoids found in red bell peppers were present, and in

particular to ascertain if lutein and/or capsolutein occurred. Another object was to obtain information on the carotenoids in green fruit, of which green bell peppers are a readily available example.

Experimental

Three lots of green bell peppers were obtained at a local market in April, June, and July. One kilogram (of each lot) of destemmed fruit was blended with 1 liter each of water and methanol, and 10 grams of magnesium carbonate; 100 grams of Celite 503 was then added and the mixture filtered on a Büchner funnel precoated with filter aid. The filter cake was worked up as previously described (8), including saponification.

Four solvent systems were used in countercurrent distribution runs in a Craig apparatus; systems I, II, and III were previously described (3, 4). System IVB (hexane and 70% methanol, 1 to 1 by volume) is similar to system IV (4). Aliquots of certain fractions

obtained on countercurrent distribution were chromatographed on magnesia (Sea Sorb 43) 14 by about 90 mm., without a diluent. The magnesia was added to a column partially filled with hexane; air pressure was applied to the top of the column to ensure even packing, and also that the top be level. A topping of about 10 mm. of anhydrous sodium sulfate was then added in a similar manner (6). Spectral data were obtained with a Beckman DK-2 recording spectrophotometer. The test for allylic hydroxyl groups was previously described (5).

Results and Discussion

The total carotenoids obtained from the three lots of green peppers were 10.6 (April), 11.2 (June), and 9.0 (July) mg. per kg. (measured in an Evelyn photoelectric colorimeter at 440 m μ and calculated as beta-carotene); these values are much lower than those obtained with red bell peppers (248 and 127 mg. per kg.) (5).