A STUDY OF THE CARBOHYDRATE COMPOSITION OF PAPAYA FRUIT

I. IDENTIFICATION OF PRINCIPAL SUGARS.
II. REFRACTIVE INDEX AS A MEASURE OF SUGAR LEVEL.
III. SEASONAL AND MATURITY RELATIONSHIPS.

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INTRODUCTION

Horticultural investigations with papaya (Carica papaya L.) have been in progress for a number of years, particularly in the tropics and sub-tropics, but thus far little information on the biochemistry or physiology of this crop has been made available. It is hoped that this investigation will provide a renewed foundation and serve as an impetus to further research in these aspects. Working with the fruit, specific objectives pursued in this study are (1) identification of constituent sugars, (2) establishing reliability of refractive index as a measure of sugar level, and (3) determining the relationship of sugar content to season of harvest and stage of maturity. It is evident from the review below that the sugars in papaya fruit were not accurately identified in previous investigations. Moreover, no systematic attempt was made to demonstrate the applicability to papaya of the common practice of estimating sugar contents by simply determining sap refractive index. Finally, it is known that in Hawaii fruit flavor varies with season of harvest and other factors but chemical evidence has not been presented to show any probable cause.

REVIEW OF LITERATURE

All previous reports revealed that in the papaya fruit reducing sugars comprised the bulk of the total sugar with sucrose being a minor constituent. Pratt and del Rosario (11) reported in 1913 that total sugar averaged 6.0, reducing sugars 5.9, and sucrose 0.1%. These figures and all those shown below are on a fresh weight basis. In 1915 Thompson (13) surveyed several genetic lines and found that total sugar content ranged from 6.4 to 15.3; reducing sugar, 6.1 to 13.2; and sucrose, 0 to 2.0%. A few other genetic lines subsequently examined by Pope (10) showed a range in total sugar concentration of 8.6 to 13.8; reducing sugar, 8.4 to 13.4; and sucrose 0.4 to 0.8%. In each of these investigations, the compounds comprising the reducing sugar fraction were not specifically identified and all data were expressed as "glucose". Even the identification of sucrose was based only upon the difference in reducing sugar contents before and after hydrolysis of sugar extracts.

Thompson (13) also reported that no iodine reactive starch could be observed in any papaya line studied. Furthermore, sugar content increased steadily during fruit maturation and even up to a day before full ripeness. This relationship between fruit maturity and sugar content was subsequently substantiated by Jones *et al*. (4) in 1941. Thompson's observation revealed that the increased sugar content was due solely to increase in reducing sugars; sucrose showed no trend with maturation and ripening. On the other hand, Jones *et al*. found the increased sugar content to be associated with decrease in sucrose as well as an increase in reducing sugar.

Awada and Ikeda (1) observed that the sugar level in papaya fruit was inversely related to soil moisture content. Fruits obtained during September and October from orchards under low rates of irrigation contained 9.6 to 11.8% sugar, whereas those obtained during October through February from fields subjected to high irrigation contained 8.4 to 9.7%.

Refractive index determined with a hand refractometer has been used widely among sugar and horticultural crops to estimate sugar concentrations (3), and in these instances, the sap content in dissolved substances is adequately correlated to sugar concentration and/or flavor.

EXPERIMENTAL SECTION

I. IDENTIFICATION OF PRINCIPAL SUGARS

A. MATERIALS AND METHODS

1. Sources and Kinds of Samples:

The fruit samples used for sugar identification were obtained from the Waimanalo, Poamoho and Mid-Pacific branch farms of the Hawaii Agricultural Experiment Station. Identifications were performed on fruits representing nine genetic lines, five stages of fruit maturity, and two seasons of harvest. The genetic lines considered were Solo, Peterson 170, Hybrid #5, Guinea Gold, IAC-VA-32, IAC-MA-16, IAC-23, IAC-39, and Bettina 100-A. In examination of both genetic lines and seasons of harvest, fruits half yellow at picking and subsequently allowed to develop full color in the laboratory were used. In the study with genetic lines, only one fruit of each line was used, whereas in the seasonal study, three fruits at each harvest were examined. Of the latter, only average observations are reported.

To consider identity of sugars associated with fruit maturity, the stages full yellow, half yellow, a trace of yellow, mature green, and immature green were considered. These stages refer to maturity at time of picking; the colors describe external appearance of fruit. Mature green fruits were characterized by brown, full-size seeds, whereas the immature green fruits contained small, white seeds. A complete set of fruits depicting each stage of maturity was obtained from each experimental tree. Two sets of fruits were picked at each of two separate dates -- November 29, 1961, and January 3, 1962. At each harvest the sugars of one set were immediately extracted. The other set was allowed to attain full yellowness in the laboratory and then extracted. Only Solo fruits were used in this and the seasonal study. In the seasonal study two to three harvests were made during each of summer and winter.

2. Preparation of Extracts:

The procedure developed and employed in this study to prepare sugar extracts from papaya fruit consisted of the following steps:

a. Fifty grams of pulp were removed from one-half of each fruit.

b. One gram $CaCO_3$ was added to maintain near neutral pH and retard inversion of sucrose.

c. One-hundred milliliters of distilled water was then added and the mixture was homogenized for 5 minutes in a Waring blender.

d. The homogenate was boiled for 5 minutes to inactivate enzymes.

e. Five grams of washed Filter-Cel was added and the hot mixture was filtered.

f. The residue was rinsed with two 50-ml. portions of boiling distilled water.

g. The combined filtrates were cooled and diluted to 250 ml.

Storage of the extracts over extended periods was achieved by freezing in polyethylene plastic bottles. The degree of thoroughness of sugar extraction by this procedure was evident in the virtual absence of anthrone-reactive substances further extractable from the residue. The residue remaining after sugar extraction was re-extracted by the same procedure, and in a typical test the remaining contents in anthrone-reactive substances ranged from 0.033 to 0.144% of the sugars already removed.

3. Chromatography:

In preliminary explorations diverse methods described by Block et al. (2) and Lederer and Lederer (5) were tested. The following

a. Paper:

Whatman #1, sheets 7 1/2 x 18 inches.

b. Solvent System:

Upper phase of n-butanol: 95% ethanol: H_2O, 4:1:5, V:V:V.

c. Spotting:

Twenty-five microliters of the fruit extract were spotted 2 inches from the base of the sheet. Reference glucose, fructose, sucrose and other sugars were spotted in amounts of 500 micrograms each.

d. Development:

The descending technique was used, and development was allowed to proceed over a 3-day period.

e. Detection:

A 3% solution of p-anisidine·HCl in n-butanol was sprayed upon the chromatagrams and yellow or brown spots formed by sugars were detected after heating 10 minutes at 100°C.

Since it is often possible that sugar extracts prepared by the above outlined steps may contain artifacts, the data based upon these extracts were eventually verified by direct examinations of unheated and otherwise unmodified fruit saps. The chromatographic data are quantitative only to the extent that area and color intensity of spots were considered in estimating relative amounts of sugars.

4. Relative Content of Sucrose

Progress in the chromatographic identification work disclosed the sucrose level in extracts to be insignificant in comparison to that of either glucose or fructose. Thus the relative amounts of sucrose in extracts from Solo fruits showing undetectable, moderate, and high chromatographic levels were further determined by the method of reducing sugars described elsewhere in the section entitled, Refractive Index as a Measure of Sugar Level.

5. Starch Detection:

Also apparent during the course of investigation was the conspicuous absence of reserve carbohydrate in papaya fruit. Thus an attempt was made to detect starch in fruit tissue of varying stages of maturity and ripening. A drop of aqueous iodine reagent (0.3 grams I_2 and 1.5 grams KI in 100 ml distilled water) was placed upon the tissues; immediate development of blue color indicated presence of amylose. More critical observations were obtained by microscopic examinations of the treated tissue sections.

B. DATA AND DISCUSSION

1. Identity of Sugars in Diverse Genetic Lines:

The anisidine-reactive substances separated from sugar extracts of ripe papaya fruits of several genetic lines are shown, with relative amounts, in Table 1. The most striking characteristic of all lines examined was the prominence of both fructose and glucose and virtual absence of sucrose. Sucrose was detected in only the Solo line and even then in only a small amount. The data obtained here, together with previously reported observations of other genetic lines (10, 13), do not preclude widespread occurrence of sucrose in Carica papaya, but emphasize the relative insignificance of sucrose as an accumulated sugar in the species.

An unidentifiable substance, showing positive reaction with p-anisidine·HCl and a low Rf identical to lactose in butanol: ethanol:water, was also observed in Solo but not other lines. Neither maltose nor raffinose could be identified with this substance. This unknown did not reduce silver nitrate, and thereby

TABLE I

COMPONENT SUGARS IN RIPE FRUITS OF NINE GENETIC LINES OF PAPAYA

Genetic Line	Relative Level In Extract*			
	Fructose	Glucose	Sucrose	Unidentified**
Peterson 170	3	3	0	0
Hybrid #5	3	3	0	0
Guinea Gold	3	3	0	0
IAC-MA 32	3	3	0	0
IAC-MA-46	3	3	0	0
IAC-23	3	3	0	0
IAC-39	3	3	0	0
Bettina 100-A	3	3	0	0
Solo	3	3	1	1

*0 = non-detectable, 1 = trace, 2 = moderate amount, 3 = large amount.

**Aniline-reactive substance with Rf in butanol:ethanol:H_2O solvent identical to lactose, but not raffinose or maltose.

suggested a non-reducing compound.

2. Component Carbohydrates in Solo Papaya as Related to Stage of Fruit Maturity:

Regardless of the stage of fruit maturity, fructose and glucose were again the most prominent soluble sugars. This is evident in the data of Table II. The detectability of sucrose, at least in the present tests with Solo materials, seemed to depend upon fruit maturity. Sucrose was present in low amounts only in fruits which were harvested at a stage when some degree of yellowing, a characteristic associated with ripening, was evident. The green fruits contained no detectable sucrose. This contradicts the finding by Jones et al. (4) who reported an inverse relationship between fruit maturity and sucrose content. The basis for the discrepancy remains to be investigated. Permitting immature fruits to ripen in the laboratory caused no change in the kinds of extractable sugars. Detectability of the unidentified anisidine-reactive substance paralleled that of sucrose, i.e., it was only detectable when sucrose was present and undetectable in the latter's absence.

Reserve carbohydrate is conspicuously absent in papaya

TABLE II

COMPONENT CARBOHYDRATES IN SOLO PAPAYA FRUITS AS RELATED TO STAGE OF MATURITY

Date Harvested	Stage of Fruit Maturity at Harvest*	Stage of Maturity when Extracted*	Relative Level of Sugar in Extract** Fructose	Glucose	Sucrose	Unidentified***	Starch in Tissue
11/29/61	5	5	3	3	1	1	0
	4	4	3	3	1	1	0
	3	3	3	3	1	1	0
	2	2	3	3	0	0	0
	1	1	3	3	0	0	0
	5	5	3	3	1	1	0
	4	5	3	3	1	1	0
	3	5	3	3	1	1	0
	2	5	3	3	0	0	0
	1	5	3	3	0	0	0
1/3/62	5	5	3	3	1	1	-
	4	4	3	3	1	1	-
	3	3	3	3	1	1	-
	2	2	3	3	0	0	-
	1	1	3	3	0	0	-
	5	5	3	3	1	1	-
	4	5	3	3	1	1	-
	3	5	3	3	1	1	-
	2	5	3	3	0	0	-
	1	5	3	3	0	0	-

*1 = immature green, 2 = mature green, 3 = a trace of yellow, 4 = half yellow, 5 = full yellow.
**0 = non-detectable, 1 = trace, 2 = moderate amount, 3 = large amount.
***Anisidine-reactive substance with Rf comparable to that of lactose in butanol:ethanol:H_2O.

fruits. This is shown in part by the lack of starch in fruits of varying stages of maturity (Table II). Absence of starch in papaya fruit had been observed earlier by Thompson (13). Further evidence showing lack of a sugar reserve in papaya is described in a section to follow in this report.

3. Sugar Composition as Related to Season of Harvest:

Table III related the soluble sugar constituents to the season of harvest. Analysis of Solo fruits obtained from the three branch farms -- Waimanalo, Poamoho and Mid Pacific -- showed no consistent seasonal difference in kinds of sugars. Fructose and glucose were again very prominent and sucrose relatively insignificant. According to these data, the unidentified substance was absent in winter fruits; but analysis of further samples did not bear this out.

4. Range of Sucrose Content in Solo Papaya Fruit:

As is evident in Table IV, the fruit sample with a chromatographically undetectable level of sucrose contained 0.12%; that with a moderate level, 1.75%; and that with a large amount, 2.65% by fresh weight of this sugar. These percentages represent a range of only one to 20 percent of the total sugar in the extracts, and agree with reports of previous investigators.

These data, which attach special prominence to the reducing sugars fructose and glucose, may cast some doubt on the reported

TABLE III

COMPONENT SUGARS IN RIPE SOLO PAPAYA FRUIT AS RELATED TO SEASON OF HARVEST

Summer					Winter				
Date Harvested	Relative Level of Sugar in Extract* Fructose	Glucose	Sucrose	Unidentified**	Date Harvested	Relative Level of Sugar in Extract* Fructose	Glucose	Sucrose	Unidentified**
WAIMANALO									
6/17/61	3	3	1	0	12/6/61	3	3	1	0
7/14/61	3	3	1	1	1/17/62	3	3	1	0
8/25/61	3	3	1	1	2/14/62	3	3	1	0
POAMOHO									
7/13/61	3	3	1	1	1/17/62	3	3	1	0
8/10/61	3	3	1	1	2/14/62	3	3	1	0
MID-PACIFIC									
7/19/61	3	3	1	1	1/17/62	3	3	0	0
8/16/61	3	3	1	1	2/14/62	3	3	2	0

*0 = non-detectable, 1 = trace, 2 = moderate amount, 3 = large amount.

**Anisidine-reactive substance with Rf in butanol:ethanol:water comparable to that of lactose.

TABLE IV

RANGE OF SUCROSE CONTENT IN RIPE SOLO PAPAYA FRUIT

Fruit Sample Number	Chromatographic detectability[a]	Percent of Fresh Weight of Fruit: Reducing Sugars (as Glucose) pre-hydrolysis	Percent of Fresh Weight of Fruit: Reducing Sugars (as Glucose) post-hydrolysis	Percent of Fresh Weight of Fruit: Sucrose	Sucrose as percent of total Sugar
96	3	9.88	12.50	2.62	20.96
61	2	10.75	12.50	1.75	14.00
133	0	12.125	12.25	0.125	1.02

[a]0 = sucrose non-detectable, 2 = moderate sucrose, 3 = high sucrose.

vitamin C content (50 mg/100 g) of papaya (8). This vitamin is usually estimated by measuring the reducing capacity of extracts, and inadequate removal of the reducing sugars contained or improper choice or reagent can possibly result in erroneous data.

The exact proportion of glucose and fructose in papaya fruit has not been established in this study, but chromatography suggested that these sugars occur in equal amounts.

The identification of the anisidine-reactive substance with Rf in butanol:ethanol: water identical to lactose may prove to be significant. Whether or not this substance is a sugar remains to be established. It is interesting that this compound is evident in fruit extracts only when sucrose is present, and the exact relationship between the two may provide challenging study.

II. REFRACTIVE INDEX AS A MEASURE OF SUGAR LEVEL

A. MATERIALS AND METHODS

1. Source and Kinds of Fruit Samples:

One fruit each of 6 genetic lines of papaya were used to determine the correlation between refractive index and actual sugar content. Samples were picked at half yellow stage, allowed to attain complete yellowness in the laboratory, and then analyzed for refractive index and extracted for sugar determination.

2. <u>Methods of Analyses:</u>

a. <u>Refractive Index:</u>

The refractive index, or percent substances in solution, was measured with a Zeiss F-3 hand refractometer on the freshly expressed sap of the fruit pulp. The measurements were made on undiluted, unfiltered sap, this being the usual practice by horticulturists.

b. <u>Determination of Actual Sugar Content:</u>

1. <u>Preparation of Extracts:</u>

Extracts for chemical determination of sugar content of fruits were prepared in the manner described under Section I, <u>Identification of Principal Sugars</u>.

2. <u>Reducing Sugar Method:</u>

The reducing sugar approach is almost universally employed to determine sugar content of biological materials. This approach is often elaborate and tedious, as well as relatively non-specific and less sensitive than the anthrone method described below. The method adopted in this study, except for clarification and hydrolysis, was Method I of Ward and Johnston (14). The method consisted of the following steps:

a. Clarification and Hydrolysis of Extracts:

The method of Loomis (7) with slight modification was used. To 25 milliliters of extracts, a solution of saturated neutral lead acetate ($Pb(C_2H_3O_2)_2.3H_2O$) was added dropwise with constant stirring until a yellow precipitate was obtained in an external indicator of 5% potassium chromate (K_2CrO_4). The suspension was either filtered through acid-washed asbestos or centrifuged five minutes at circa 1000 x G and the clear solution was subsequently deleaded with crystalline potassium oxalate ($K_2C_2O_4.H_2O$). A weight of potassium oxalate equivalent to that of the lead acetate required for clarification was used. The deleaded suspension was further filtered or centrifuged. The clarified solution was then hydrolyzed by adding 2.5 ml. concentrated HCl and standing overnight (at least 12 hours) at room temperature. It was subsequently neutralized to pH 7.0 with 2 N NaOH, and diluted to 250 ml. One milliliter of this solution diluted with 4 ml. distilled water was carried through the analytical procedure described below. Note that the final dilution of extract used in sugar determination by the reducing sugar method was 50-fold.

b. Reagents:

1. Potassium ferricyanide ($K_3Fe(CN)_6$), 8.25 g., and sodium carbonate (Na_2CO_3.), 10.60 g. Both salts were dissolved in one liter distilled water.

2. Potassium iodide (KI), 12.5 g.; zinc sulfate ($ZnSO_4 . 7H_2O$), 25.0 g.; sodium chloride (NaCl), 125.0 g. The three salts were dissolved in 500 ml. distilled water.

3. Acetic acid ($C_2H_4O_2$), 5% (V/V).

4. Starch indicator. One gram starch was added to 20 ml. water, mixed and poured into 60 ml. boiling water, and boiled for 2 minutes; 20 g. sodium chloride was then added, and the solution was cooled and made to a volume of 100 ml.

5. Potassium Iodate (KIO_3), 0.080% solution.

6. Potassium iodide (KI), 2% solution.

7. Sodium Thiosulfate ($Na_2S_2O_3 . 5H_2O$), 0.333% solution.

8. Glucose ($C_6H_{12}O_6$.).

c. <u>Standardization of $Na_2S_2O_3$ solution:</u>

A mixture of 5 ml. reagent 5, 5 ml. reagent 6 and 3 ml. reagent 3 was titrated with reagent 7 using starch indicator. Normality of $Na_2S_2O_3$ =

$$\frac{\text{g. } KIO_3\text{/liter} \times \text{ml. } KIO_3 \text{ solution used}}{35.67 \times \text{ml. thiosulfate solution required}}$$

d. <u>Preparation of Standard Curve:</u>

Five milliliter solutions containing 0-4 mg. glucose were carried through the procedure in step (e) below, and the 0.01N thiosulfate equivalent determined:

$$0.01N \text{ thiosulfate equivalent} = (WB-R) \times \frac{N}{0.01}$$

where WB = water blank titer, R = sugar solution titer, and N = normality of thiosulfate solution.

A typically obtained standard curve relating glucose content and 0.01N thiosulfate equivalent is shown in Figure 1.

e. <u>Analytical procedure:</u>

Five milliliters of sugar solution or extract were mixed with 5 ml. reagent <u>1</u> in a boiling tube, covered loosely with a glass bulb, and placed in boiling water for 15 minutes. The reaction mixture was then cooled for 3 minutes in a cold water bath, 5 ml. reagent <u>2</u> and 3 ml. reagent <u>3</u> were added, and titration with standard sodium thiosulfate (reagent <u>7</u>) using starch as indicator (reagent <u>4</u>), was performed. A blank determination was made simultaneously in place of sugar solution.

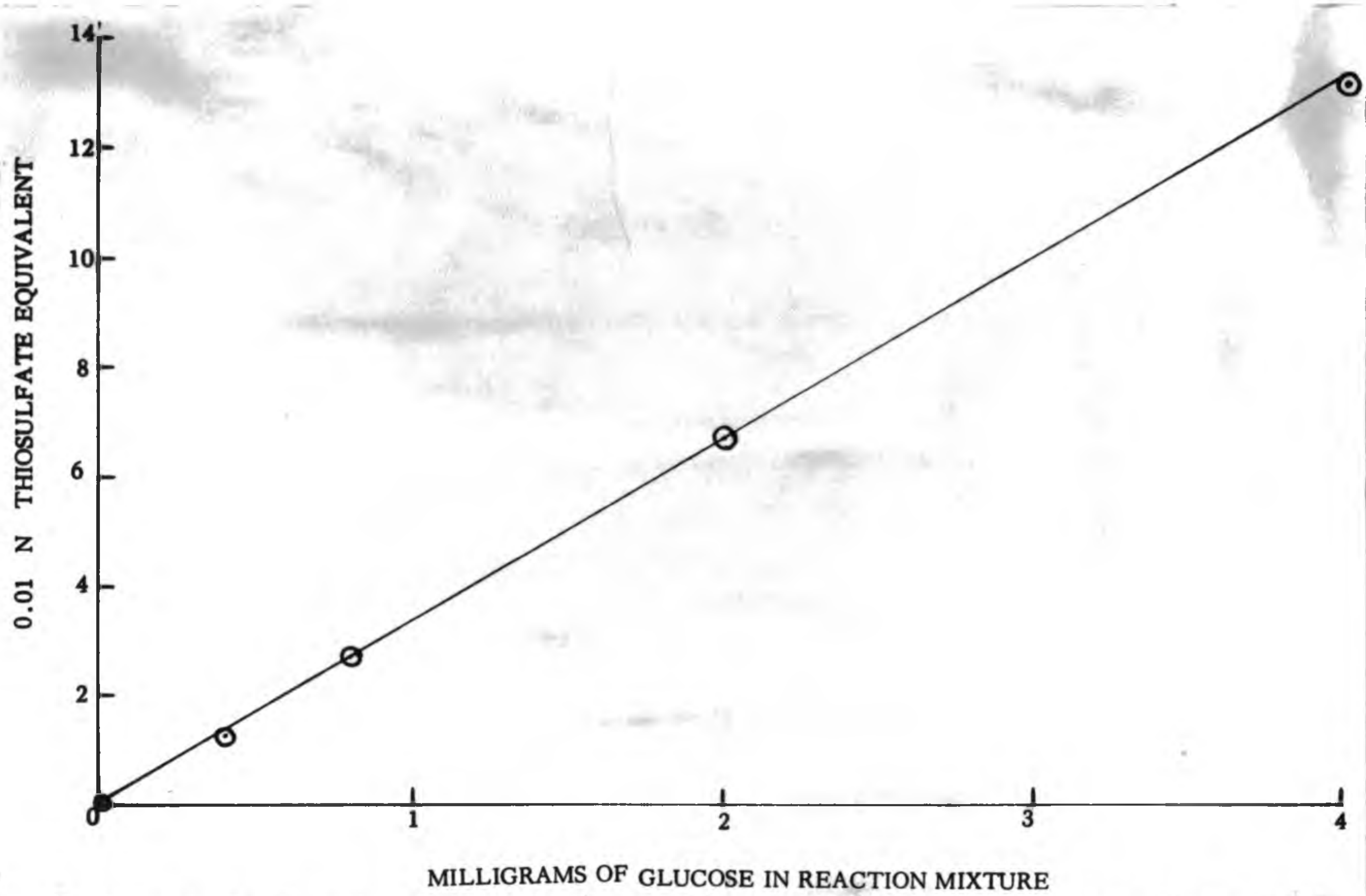

Figure 1. Standard thiosulfate equivalent as related to the amount of reducing sugar in reaction mixture.

3. Anthrone Method:

The anthrone method is reportedly more specific for carbohydrates than reducing sugar methods. Furthermore, it is much more sensitive, simple, and rapid, and it measures all forms of carbohydrates. The method used in this study was adapted from those of Morris (9) and Loewus (6), and consisted of the following:

a. Reagent:

Two grams (2.0 g.) anthrone (9-oxyanthracene) were dissolved in 1 liter of 95% of H_2SO_4, prepared by cautious addition of 1 liter concentrated H_2SO_4 to 50 ml. distilled H_2O.

b. Procedure:

Three milliliters of the solution to be determined were measured into an 18 x 150 mm. test tube, and 6 ml. of the reagent was added. The solutions were mixed thoroughly at once by swirling. The tube was placed in a boiling water bath for 3 minutes and then cooled. The color developed was measured in a Bausch and Lomb Spectronic

20 colorimeter at a wavelength of 620 millimicrons compared against a blank containing only water and reagent. Linearity of the reaction in the range 0 - 120 micrograms glucose is evident in Figure 2. A 60-microgram glucose standard was carried throughout.

All chemical analyses were performed in duplicates. The coefficients of correlation between refractive index and total sugars as determined by reducing sugar and anthrone methods were calculated. The relationships were further examined by constructing regression lines. All statistical analyses performed here and elsewhere in the work were based upon procedures described by Snedecor (12).

B. DATA AND DISCUSSION

The data showing ranges in refractive index and corresponding sugar content of papaya fruits are presented in Table V. The correlation between refractive index of fruit sap and sugar content in the fruit was indeed significant; the coefficients between refractive index and total sugar were 0.997 by the anthrone and 0.998 by the reducing sugar method. These relationships are further illustrated by the regression lines in Figure 3. Note that sugar determinations by the anthrone method were consistently lower than those by the reducing sugar method, and the

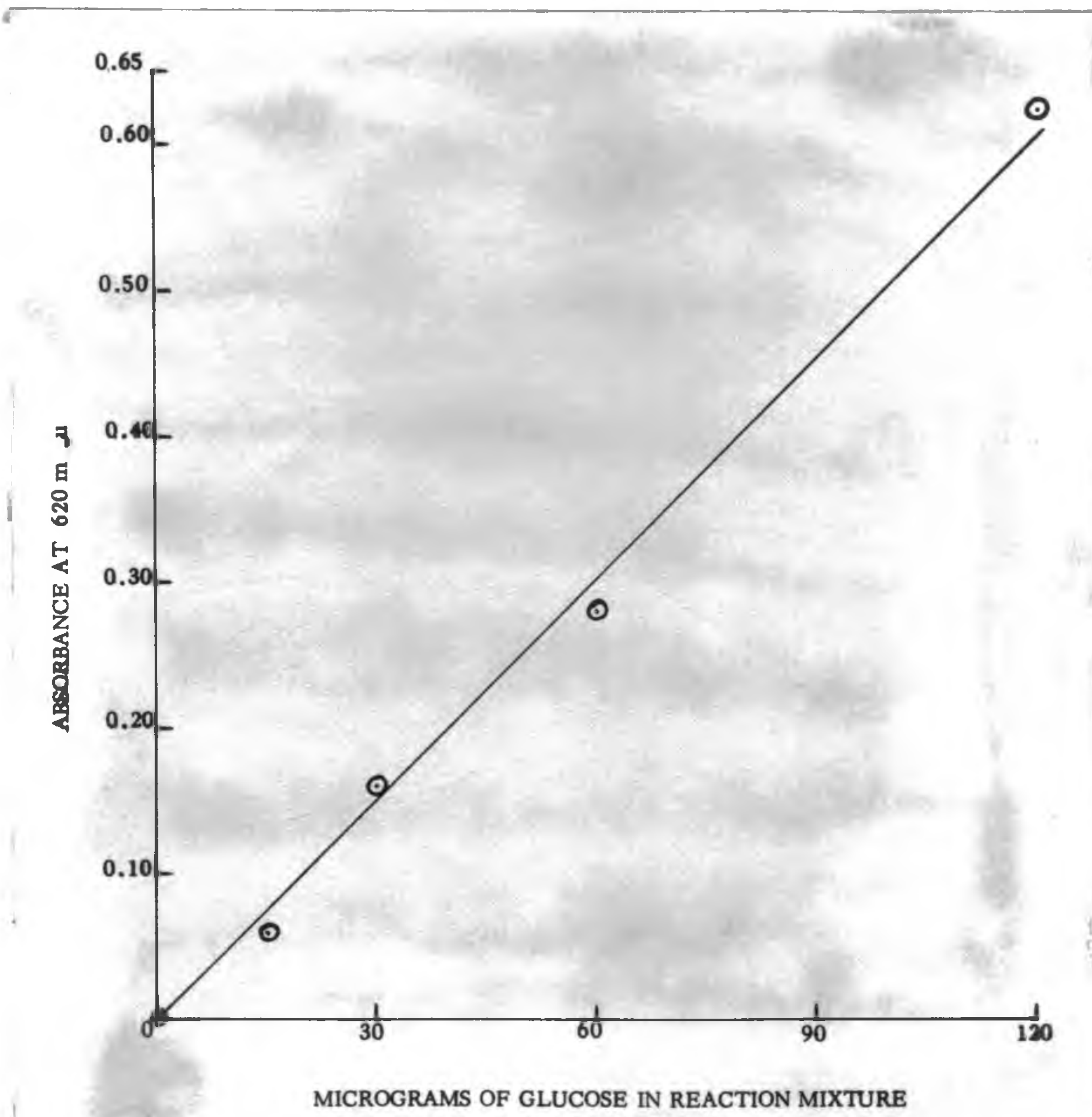

Figure 2. Relationship between sugar content and anthrone reaction.

TABLE V

RELATIONSHIP BETWEEN REFRACTIVE INDEX OF FRUIT SAP AND SUGAR CONTENT OF FRUIT
AS DETERMINED BY ANTHRONE AND REDUCING SUGAR METHODS

Genetic Line	Refractive Index of Sap, %	Total Sugars in Fruit (as Glucose, % Fresh Weight)	
		Anthrone Method	Reducing Sugar Method
IAC - 39	8.4	5.99	7.15
Bettina 100-A	8.8	5.11	7.00
60-6	10.8	7.16	8.95
Hybrid #5	12.8	8.86	11.20
60-9	12.8	10.26	11.70
Solo	15.2	11.44	13.90

Correlation coefficient r = 0.997 (anthrone/refractive index) and 0.998 (reducing sugar method/refractive index).

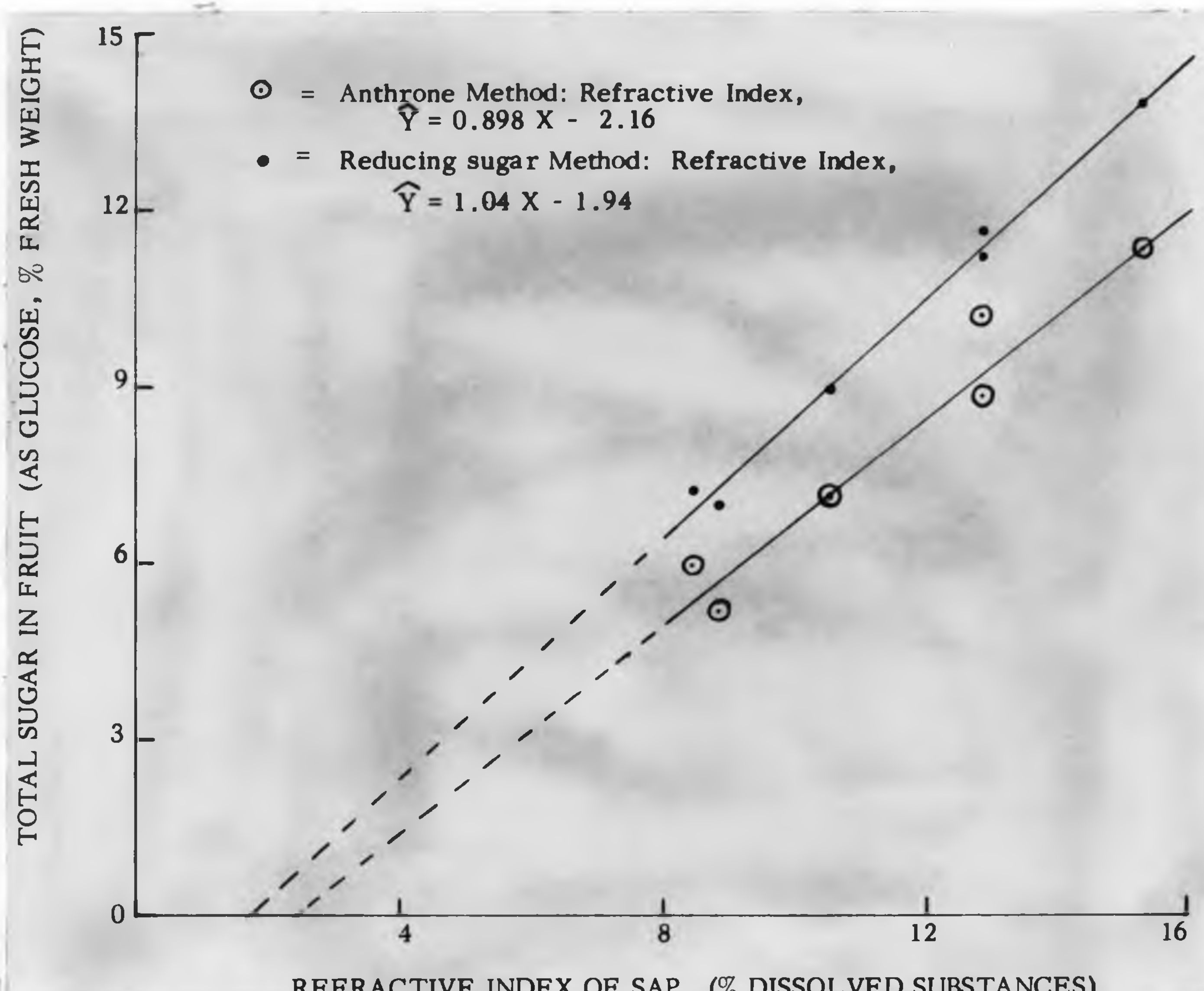

Figure 3. Relationship between refractive index of sap and sugar content of papaya fruit as determined by anthrone and reducing sugar methods.

more accurate of the two needs to be further determined. By extrapolating the regression lines to bisect the refractive index axis, it is apparent that the sap contained approximately 2% of dissolved substances other than sugars. While refractive index does not furnish an absolute measure of sugar content of papaya, its judicious use provides relative estimates suitable in many comparative situations, for example, in genetic selection studies.

III. SEASONAL AND FRUIT MATURITY RELATIONSHIPS

A. MATERIALS AND METHODS

1. Seasonal Relationships:

The same Solo fruits employed in the sugar identification study (Section I) were used to explore the quantitative relationship between season of harvest and sugar content. After fruits had attained full ripeness in the laboratory, refractive index of the sap and moisture contents were determined, and extracts were prepared for chemical sugar determination by the reducing sugar and anthrone methods described under Section II, Refractive Index as a Measure of Sugar Level.

To determine moisture content, a cylinder of pulp was obtained with a number 5 cork-borer from the unextracted half of each fruit, weighed, and reweighed after 120 hours in an 80°C oven. According to preliminary trials constant weight was obtained by this method. The loss in tissue weight after dessication was used as index of tissue moisture content.

T-test of group comparisons between summer and winter fruits was performed on data of each of the three locations from which fruit samples were obtained. Standard error of means was also calculated.

2. Fruit Maturity Relationship:

The same fruits and extracts employed in part of the identification study (Section I) were also used to determine the quantitative relationship between maturity and sugar content. Thus two sets of fruits representing five states of maturity, each picked on each of two separate dates -- November, 1961, and January, 1962 -- were examined. One set was analyzed immediately after harvest and the other when the fruits had ripened fully in the laboratory. The data obtained were refractive index and total sugar by the anthrone method. Steps to obtain each of these data were identical to those previously described.

B. DATA AND DISCUSSION

1. Seasonal Relationship:

Refractive indices, moisture contents and total sugars by anthrone and reducing sugar methods of ripe Solo fruit obtained during summer and winter of 1961-62 from the three branch farms are shown in Tables VIa and VIb. T-tests showed highly significant differences between summer and winter refractive indices, moisture contents, and total sugars by the reducing sugar method. Summer fruits consistently showed higher refractive indices and total sugars and lower moisture contents. It thus may be expected that fruits obtained during summer will be sweeter than those of winter. This relationship may be expected in Hawaii since higher intensity and longer duration of light is characteristic of the summer, whereas less light and greater rainfall is more typical of winter. These factors directly influence the rates of synthesis and accumulation of carbohydrate in all plants. Awada and Ikeda (1) observed earlier that sugar content of papaya was inversely related to the supply of soil moisture.

No difference was evident between seasons in total sugar as determined by the anthrone method. This is perhaps because the method is so sensitive that a drastic dilution (1000 fold) of

TABLE VIa

COMPOSITION OF SOLO PAPAYA FRUIT AS RELATED TO SEASON OF HARVEST

S u m m e r			W i n t e r		
Harvest Date	Refractive Index, %	Moisture, %	Harvest Date	Refractive Index, %	Moisture, %
		W A I M A N A L O			
6/17/61	15.4 ± 0.6	84.6 ± 0.2	12/6/61	13.6 ± 0.6	86.3 ± 0.4
7/14/61	15.8 ± 0.2	83.7 ± 0.1	1/17/62	12.3 ± 0.3	88.6 ± 0.6
8/11/61	15.6 ± 0.2	84.6 ± 0.4	2/14/62	12.5 ± 0.5	88.3 ± 0.4
		P O A M O H O			
7/13/61	14.4 ± 0.3	85.1 ± 0.1	1/17/62	12.5 ± 0.2	89.1 ± 0.5
8/10/61	14.6 ± 0.1	85.1 ± 0.2	2/14/62	12.7 ± 0.3	89.5 ± 0.2
		MID - PACIFIC			
7/19/61	14.5 ± 0.1	84.9 ± 0.3	1/17/62	10.7 ± 0.8	91.0 ± 0.8
8/16/61	15.1 ± 0.3	84.6 ± 0.1	2/14/62	12.8 ± 0.1	89.3 ± 0.1

TABLE VII

COMPOSITION OF SOLO PAPAYA FRUIT AS RELATED TO SEASON OF HARVEST

Summer			Winter		
Harvest Date	Total Sugar by Anthrone (as Glucose, % Fresh Weight)	Total Sugar by Reducing Sugar (as Glucose, % Fresh Weight)	Harvest Date	Total Sugar by Anthrone (as Glucose, % Fresh Weight)	Total Sugar by Reducing Sugar (as Glucose, % Fresh Weight)
			W A I M A N A L O		
6/17/61	9.2 ± 0.1	13.0 ± 0.3	12/6/61	9.6 ± 0.0	11.7 ± 0.5
7/14/61	10.5 ± 0.3	13.4 ± 0.0	1/17/62	10.5 ± 0.9	11.0 ± 0.4
8/11/61	9.8 ± 0.3	12.3 ± 0.1	2/14/62	10.4 ± 0.5	10.7 ± 0.3
			P O A M O H O		
7/13/61	9.5 ± 0.4	13.6 ± 0.4	1/17/62	10.2 ± 0.1	12.2 ± 0.4
8/10/61	10.2 ± 0.8	13.5 ± 0.3	2/14/62	10.1 ± 0.3	12.5 ± 0.6
			MID - PACIFIC		
7/19/61	8.5 ± 0.2	13.7 ± 0.3	1/17/62	8.7 ± 0.6	10.7 ± 1.3
8/16/61	9.6 ± 0.0	14.3 ± 0.3	2/14/62	10.1 ± 0.3	11.9 ± 0.5

extract was required and any error in preparing the dilution was magnified proportionately. In effect, these data suggest that the anthrone method cannot be applied to detect small differences in sugar content of papaya fruit. However, when large differences are expected, such as among certain genetic lines or as evidenced below among fruits differing widely in maturity, the anthrone method may be advantageous.

2. Fruit Maturity Relationships:

Sugar content with respect to stage of maturity of papaya fruit is shown in Table VII. Admittedly the data may not be based upon adequate replication; nevertheless, definite trends are evident. Total sugars were substantially lower in fruits picked before any externally evident yellow color. Allowing green fruits to ripen in the laboratory resulted in little if any change in sugar level. These observations, together with that in a preceding section where tissue amylose was shown to be absent, establish that papaya fruits lack reserve carbohydrate. Thus, the report by Thompson (13) is further substantiated, and the implication is made that bulk of the sugars in a papaya fruit must result from the continual mobilization of soluble sugars from other parts of the plant and accumulation in the fruit during maturation and possibly during the ripening process. In

TABLE VII

SUGAR CONCENTRATION OF SOLO PAPAYA FRUIT AS RELATED TO MATURITY AT HARVEST

Harvest Date	Stage of Fruit Maturity at Harvest*	Stage of Maturity at Extraction*	Refractive Index, %	Total Sugars by Anthrone (as Glucose, % Fresh Weight)
11/29/61	5	5	—	10.3
	4	4	—	10.4
	3	3	—	11.2
	2	2	—	6.4
	1	1	—	3.9
	5	5	—	10.3
	4	5	—	12.1
	3	5	—	11.2
	2	5	—	3.7
	1	5	—	2.8
1/3/62	5	5	13.8	11.7
	4	4	15.0	12.1
	3	3	13.6	12.1
	2	2	5.0	3.4
	1	1	4.0	2.6
	5	5	14.4	12.5
	4	5	14.2	11.3
	3	5	13.6	10.7
	2	5	9.0	6.0
	1	5	6.8	1.9

*1 = immature green, 2 = mature green, 3 = trace of yellow, 4 = half yellow, 5 = full yellow.

commercial practice it would be critical that fruits be harvested none too soon during maturation if maximum sugar content is desired. Too early removal from trees is perhaps the reason that fruits sold in markets substantially distant from orchards are not as sweet as those sold in nearby markets. Any evidence of external yellow color should indicate adequate maturity.

SUMMARY AND CONCLUSIONS

This study with papaya fruits attempted to (1) identify the principal sugars, (2) establish the relationship between sap refractive index and actual sugar content, and (3) determine sugar content as related to season of harvest and stage of maturity.

Regardless of genetic line, stage of fruit maturity, or season, the reducing sugars fructose and glucose were the most prominent. The exact proportions of each were not determined, although chromatography suggested nearly equal levels. Sucrose, observed only in Solo, was detectable in relatively small amount, ranging from one to 20 percent of the total sugar. Even in Solo, green fruits contained no sucrose. On the basis of these findings, together with similar observations made by earlier investigators, it is concluded that sucrose is not a significantly accumulated sugar in the papaya fruit.

Starch as amylose was completely absent. An unidentified substance giving positive reaction with p-anisidine · HCl but not silver nitrate, and possessing an Rf in n-butanol:ethanol:water identical to lactose but not maltose or raffinose, was detected in small amounts in Solo. Detectability of this substance coincided with the presence of sucrose, but its significance and relationship to sucrose remain unestablished.

The prominence of reducing sugars in papaya fruit extracts may cast some doubt on the reported vitamin C content. This vitamin is usually estimated by the reducing ability of fruit extracts, and inadequate removal of sugars or improper choice of reagent could result in erroneous data.

A near perfect correlation between refractive index of fruit sap and sugar content of fruit as measured by either the anthrone or reducing sugar method was revealed. Nevertheless, refractive index consistently disclosed more than dissolved sugars, hence should not be inferred as absolute measure of sugar. In comparative situations, for example, in genetic selection studies, its judicious use furnishes adequate relative information. Total sugar as determined by the anthrone method was invariably lower than by the reducing sugar method; furthermore, small differences could not be detected. The anthrone method, while simpler and more rapid, has questionable reliability in some work with papaya fruits. Its extreme sensitivity requires extracts to be drastically diluted prior to analysis and errors in preparing dilutions are magnified proportionately.

Papayas obtained in summer may be expected to be sweeter than those of winter since in the former sugar content is higher and moisture level is lower.

Fruits removed from trees prior to any externally visible yellow color contained markedly less sugar than those which showed some degree of yellowing. Permitting green fruits to ripen after harvest did not result in any change in sugar level. This finding, together with the observed absence of amylose starch in fruit tissue at any stage of maturity, established that papaya fruits contain no reserve carbohydrate. It implies that the bulk of the sugars in the fruit must result from continued mobilization from other parts of the plant and accumulation in the fruit during maturation and possibly ripening process. Hence, it is critical not to pick fruits prematurely if maximum sugar content is desired.

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