THE DISTRIBUTION OF MOISTURE, ASCORBIC ACID, AND CARBOHYDRATES IN THE BANANA DURING RIPENING

By

Miin-Shyong Chen

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ABSTRACT

THE DISTRIBUTION OF MOISTURE, ASCORBIC ACID, AND CARBOHYDRATES IN THE BANANA FRUIT DURING RIPENING

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The moisture content in the banana core decreased with time while that of the periphery increased indicating some moisture transfer from core to periphery during ripening.

The distal portion had more total sugar than the stem portion during ripening, but for starch content the order was reversed. The starch content in the periphery was slightly greater than in the core. In terms of carbohydrate metabolism, the ripening of the core preceded that of the periphery; the distal portion ripened faster than the stem portion.

The ascorbic acid content was low in green bananas, increased during the turning stage, reached a maximum just before the green-tip stage and was low again at full ripeness. The ascorbic acid content of the Cavendish banana at the green-tip stage was almost twice as much as in the fully ripe bananas.

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INTRODUCTION

The banana is an important world food crop, the total annual production of the world being about 18 million metric tons, second only to grapes. Africa produces about 50% of the total, Asia (including the Pacific Islands and Australia) 20% and the Americas 30%. Bananas represent 40% by weight of all world trade in fruits, fresh or dried (Palmer, 1971). Some 7 million metric tons of bananas entered international trade in 1978 which is equal to 40 billion bananas.

Until only 17 years ago the dominant banana in the world trade was the Gros Michel variety which had reigned supreme for some 70 years. Due to a fusarial wilt disease it had to be replaced by the Cavendish group of bananas, which are resistant to the disease (Stove, 1972). Most publications prior to 1960, therefore, refer to a variety which has been almost completely replaced. The differences in nutrient composition between both varieties are relatively minor (Forsyth, 1982).

Biochemical and compositional changes associated with ripening bananas have been reviewed extensively (van Loesecke, 1950; Simmonds, 1966; Palmer, 1971). The most noticeable change in the banana pulp during ripening is the conversion of starch to sugars. Starch decreases from 20 to 25% of the pulp in the green fruit to only 1 to 2% of the pulp in the fully ripe fruit. Sugars, at the same time, increase from 1 to 2% in the pulp of the green fruits to 15 to 20% in the ripe pulp. Total carbohydrates decrease by 2 to 5% during ripening, presumably as sugars are utilized in respiration. The sugars at each stage of ripeness are roughly in the ratio glucose 20: fructose 15: sucrose 65 (Poland, 1938). By using modern liquid chromatgraphic techniques, Palmer and Brandes (1974) confirmed the early work on sugar concentrations and ratios with Cavendish bananas.

However, many physical and biochemical changes take place during ripening, many occuring at varying rates at different regions within the banana. Von Loesecke (1950), for example, reported that the first indications of the ripening are in the placental region where initial softening occurs and then progresses toward the peel. Moreover, the distal or stylar end of the banana ripens before the stem end. Rouhani (1979) also reported that the color change in banana peel during ripening start from the distal end of the fruits; the rate of degradation of chlorophyll and other changes,such as fruit softening, also begin at this end.

Little has been done with vitamins, except to tabulate data on vitamin content (McCance and Widdowson, 1960). Harris and Poland (1939) have made a comprehensive study of the variations in the ascorbic acid content of the banana and found that it increased during ripening, but decreased in the overripe stage. Thornton (1938) reported that ascorbic acid is quickly destroyed when banana pulp is macerated in air. He also showed (Thornton, 1943) that during ripening at 66.2°F (19°C) ascorbic acid increased slightly in the pulp at the time of early development of yellow color, then fell gradually with completely yellowing to 10 to 12 mg/100 g fresh weight.

The present investigation was undertaken as an attempt to map the changes in carbohydrates, ascorbic acid and moisture within the edible part of the banana fruit as the latter ripens.

LITERATURE REVIEW

The edible bananas handled in international commerce belong to the genus Musa of Musaceae family. The genus Musa contains four groups; Eumusa, Rhodochlamys, Australimusa and Callimusa. Australimusa species are utilized in large areas of the Pacific as a cooked vegetable. The Callimusa and Rhodochlamys species are of ornamental interest only. The Eumusa group is the most widespread geographically and contains all the major edible species (Palmer, 1971). The Eumusa bananas were distributed before the modern age of exploration from South India to Japan and Somoa in the mid-Pacific. The cultivars in this group derive from *Musa accuminata colla* or from hybridization between 2 Southeast Asian species, *M. accuminata* and *M. balbisiana colla* (Simmonds, 1966).

In commercial practice, bananas are never allowed to ripen on the tree. If so ripened, the peel splits, rendering the fruit easy prey to insects and disease (von Loesecke, 1950). All bananas for export are harvested in the green stage. The maturity at harvest is governed largely by the time required to transport the bananas to market, although there are clonal and seasonal considerations (Simmonds, 1966).

Growers try to harvest the fruit at the most advanced stage consistent with the fruit arriving green at the ripening rooms. This stage is determined by experience and judged largely by the visual appearance of the hanging "stem" or "bunch" and particularly by the angularity of the individual banana "finger". The terminology and criteria vary, but the common practice is to designate a banana as three-quarters (fruits at about one-half their possible maximum size, with clearly visible regions), full three quarters (fruit with less prominent angles) and full (fruit from which the angles have virtually disappeared). Intermediate designations are often adopted (Palmer, 1971). Von Loesecke (1950) provided with color plates and designated eight stages of ripeness for the Gros Michel variety.

Export bananas are generally transported on refrigerated ships at 14-15^{o}C in corrugated boxes. In some areas, transport is via ventilated rail car (Palmer, 1971). Boxing eliminates many sources of handling damage previously encountered in shipping. Rough handling of ripe or turning fruit may cause darkening and bruising of the interior of bananas, but show no external evidence of damage (United Fruit Sales Corporation, 1964).

In ripening bananas the endogenous starch of the pulp (20-25%) is almost quantitatively converted to sucrose, fructose, and glucose (von Loesecke, 1950). The ripening can be controlled within limits by temperature regulation, ethylene application and modification of the oxygen and carbon dioxide concentrations in the storage environment. Ethylene is used to "trigger" ripening, while the rate of ripening is controlled largely by temperature regulation (Charles, 1973). Loesecke (1950) defined fast, medium-speed, and slow ripening as holding the fruit at 68-70^{o}F, 64-66^{o}F, 60-62^{o}F, respectively. The relative humidity is 90-95%.

Both green and ripe bananas are susceptible to chilling injury, but green fruit is slightly more susceptible. Chilling is mainly a peel injury where certain cells are killed. The dead cells darken and give the peel a characteristic smoky or dull-yellow appearance after ripening rather than a bright-yellow color. Ripe fruit, if chilled develops a dull-brown color when later exposed to higher temperature, and is very susceptible to handling marks; the slightest pressure causes discoloration. Due to possible chilling injury, the refrigeration temperature for holding Cavendish bananas must not be below 13^{o}C on extended voyages. After bananas have ripened, the best holding temperature is 14^{o}C. However, even at this temperature, the ripened fruit cannot ordinarily be held for more than 2 to 4 days. Ripening should be timed so that holding ripened fruit is kept to a minimum. On the contrary, exposing ripened bananas to higher temperatures will hasten the ripening and resulting in softening and decay, weakening the neck and peel, and causing poor color. This kind of injury is usually termed "cooked" or "boiled" (USDA, 1977). Peacock (1980) examined the banana ripening at temperature ranges from 57^{O}F to 90^{O}F for their effect on eating quality, rate of color development, shelf-life, and pulp firmness.

Burg and Burg (1965) have studied the relation between ethylene production and ripening in bananas. Fruit at harvest contained 0.2 ppm of ethylene. About 4 hours before initiation of ripening, the ethylene content abruptly increased, averaging about 0.5 ppm at initiation. Application of ethylene concentrations of 0.1 ppm or higher to green bananas accelerates the onset of the climacteric. Concentrations of about 1 ppm or higher generally induce the climacteric within 12 hours; lower concentrations must be applied for longer induction periods. Temperature also markedly alters the effect of ethylene. At 56-60^{O}F ripening was not initiated by 24 hours of exposure to 100 ppm ethylene. Ripening was initiated by 16-20 hours exposure at 65-73^{O}F. Lustre (1976) conducted experimentation with acetylene-induced ripening of "SABA" bananas (a Philippine variety). He found that treatment of the fruit with acetylene brings about similar ripening changes as that of ethylene.

Mapson (1966) found that the initiation of ripening can be delayed for weeks or months by holding the green fruit in an atmosphere of 1-10% oxygen, 5-10% carbon dioxide or a combination of low O_2 and high CO_2. Smith (1963) noted that while laboratory experiments on the storage of bananas in modified atmospheres were successful, sea-transport trials failed under commercial conditions. The reason being that it was impossible to exclude fruit that had already started to ripen. Such fruits produce a considerable amount of ethylene which promotes ripening in less mature fruit.

Scott (1966) utilized polyethylene bags to delay ripening of bananas during transport and storage. In 1969, he further studied the effect of potassium permanganate and calcium hydroxide on the ethylene, carbon oxide, and oxygen concentrations in the atmosphere of polyethylene bags containing bananas. His conclusion was that the use of potassium permanganate as ethylene absorbent extended the storage life by about two weeks and reduced deterioration of the fruit due to softening during storage. Liu (1976) indicated that applying 10 to 100 ppm ethylene to the storage air of bananas can inhibit the development of superficial senescent spots on ripe bananas. Liu (1978) also ripened bananas with Ethephon ((2-chloroethyl)-phosphoric acid) in three polymeric film packages and concluded the resulting bananas had good eating quality and a longer shelf-life than the bananas ripened in air. Rouhani (1978) treated the pre-climacteric banana with 0 to 800 ppm $AgNO_3$ and stored for 28 days at 15^oC. He noted that the color change in banana peel started from the tip of the fruit; the rate of chlorophyll degradation and firmness change also begin at this end. The application of $AgNO_3$ decreased the weight loss, retarded the degradation of chlorophyll and reduced loss of firmness.

The banana has been classified as "climacteric" fruit. The climacteric stage is an upsurge in respiratory activity which may be observed at the end of the maturation phase. It is considered as a transition stage between the development phase of maturation and the final protoplasmic disorganization during senescence. During climacteric, the tissues pass from a low level of metabolic activity to a higher one (Biale, 1968). This phenomenon is well illustrated by the rapid rise of the respiration rate at this stage. Loesecke (1950) reported that when bananas were ripened at 20^oC, the respiration rate of banana rises in 2-4 days from a steady value of about 20 mg CO_2/kg/hour in the hard, green fruit to about 125 mg CO_2/kg/hour at climacteric peak and then falls to about 100 mg CO_2/kg/hour as ripening proceeds.

Barker et al. (1962) had reported an increase in the level of fructose-1, 6-diphosphate paralleling the climacteric rise. Salminen (1972) confirmed Barker's result by quantitative analysis of the relative change (twenty-fold) in the level of fructose-1,6-diphosphate between the preclimacteric to the climacteric stage. He suggested that phosphofructokinase (PFK) can account for this observed increase. Later, Salminen (1975) investigating the role of PFK in the climacteric bananas showed that the initial level of glucose-6-phosphate as well as fructose-6-phosphate was high (440 nmole/g dry weight in both cases) but increased less than 100% during the climacteric peak. Triose-phosphates were initially 60 nmole/g dry weight, and increased 3.7-fold. Fructose 1,6-diphosphate on the other hand, was initially present at only 7 nmole/g dry weight but increased 21-fold at the climacteric peak. He also observed that glucose-6-phosphate dehydrogenase activity increased 50% between the preclimacteric and climacteric stages, whereas aldolase activity increased only 9% during the same interval. On the other hand, PFK activity increased 155%. He concluded that the activity of PFK is consistent with the increase in respiratory activity during the climacteric stage of bananas.

Moisture

Stomata occur on the surface of mature banana peel at average densities of 400/cm^2. These stomata are functional in excised green fruits and can be induced to open by exposure to low intensity light (10 f.c.) when the fruits are held at 90-100% relative humidity (Palmer, 1970). Transpiration is relatively constant in the mature green fruit. Once ripening is initiated, a plot of transpiration rate versus time for individual bananas is remarkedly similar to the climacteric curve for respiration. The maximum transpiration rate is about twice that of green fruit. Despite transpirational losses, the moisture content of banana pulp normally increases during ripening, from about 69% (±4%) to about 74% (±3%) (Simmonds, 1966).

The water could be derived either from breakdown of carbohydrates, presumably during respiration, or from the osmotic withdrawal of moisture from the peel. The osmotic pressure of pre-climacteric peel and pulp is about 6 atmospheres. The peel pressure increases only slightly during the climacteric, then increases to about 11.5 atmospheres at the fully ripe stage. The pulp osmotic pressure increases to about 6.5 atmospheres during the climacteric and then rises sharply, reaching about 25-27 atmospheres when fully ripe. The difference in pressure between pulp and peel results in a migration of water from the peel to the pulp (Palmer, 1970). Loesecke (1950) explained that the pressure difference is due to starch, the major constituent of the pulp in the green fruit. Starch does not affect the pressure to any appreciable extent, while hydrolysis of this starch to sugars during ripening causes an increase in osmotic pressure, and this increase is in proportion to the sugar formed. However, the sugar content of the peel is much lower than that of the pulp, so the pressure is lower than that of the pulp.

The osmotic transfer of moisture can be reflected in change in the weight ratio of pulp-to-peel which has been termed "coefficient of ripeness" and was used as an index of fruit maturity. This ratio is about 1.2-1.6 in the green fruit and rises to 2.0-2.7 when the fruit is fully ripe (Loesecke, 1950).

Carbohydrate

About 20-25% of the pulp in the fresh green fruit is starch. In the week or so from initiation to completion of ripening, the starch is almost completely hydrolyzed, only 1-2% remaining in the fully ripe fruit. Sugars, normally 1-2% in the pulp of firm green fruits, increase to 15-20% in the ripe pulp. Total carbohydrate decreases 2-5% during ripening presumably as sugars are utilized in respiration (Simmonds, 1966).

Wolfson (1928) has carried out studies on the morphology of the pulp of the Gros Michel banana. He found that the cells from the center of the pulp of the green fruit are long, boxlike in shape, thin-walled and contained numerous large starch grains, in addition to the cytoplasm and nuclei. There are few or no intercellular spaces and the cells adhere firmly to one another. In the tissue of the corresponding ripe banana, the starch grains have almost entirely disappeared; those remaining are small and irregular in shape. Tissues of the outer portion of the pulp show cells of different shape but in other respects they are similar to those of the center.

Carson (1972) pointed out that banana starch has two major grain sizes. The irregular shapes of grain of banana starch make it difficult to specify their size. She gave an approximation of $22 \pm 7\mu$ for the smaller grains and $39 \pm 10\mu$ for the large grains. Fuwa (1979) using SEM (scanning electron microscopy) observed that the surface of the green banana starch granules appeared smooth, while that of the ripened fruits (green bananas treated with ethylene gas for 24 hours and kept for 8 days at 20-22^{o}C) showed parallel striations. These striated structures are due to the action of amylose during ripening. Kayisu (1980) confirmed Fuwa's observations of both green and ripe banana starch, and concluded that banana starch granules were irregularly shaped with spheroid and elongated forms being predominant. The size of the granules were 7-25 μm in width and 20-25 μm in length. Both the Brabender amylograms and the two-stage swelling patterns of the banana starches were similar to those reported for mung bean starch.

Loesecke (1950) noted that the first indications of banana ripening are in the placental region where the tissues become soft, acquire a honey-like color, and are sweet in taste. The distal or stylar end of the fruit ripens slightly in advance of the proximal, stem end. Poland et al. (1937, 1938) identified sucrose, glucose and fructose as the major sugars in banana pulp. All three sugars increased during ripening, maintaining nearly constant proportion of 66% sucrose, 14% fructose and 20% glucose. Maltose has also been detected in trace amounts. Marriott (1981) found that the ratio of glucose to fructose was approximately unity for Cavendish bananas at all stages of ripeness, and sucrose comprised more than 70% of the total sugars in fully ripe bananas. Henderson (1958) reported the presence of a trisaccharide (6^F-β-fructosucrose) during the ripening process of Cavendish banana.

Finney et al. (1967) reported that the firmness in banana was closely related to reducing sugars and starch content during ripening at 60°F (15.6°C). He defined firmness as modulus of elasticity (ratio of stress to strain within a material) and found that this modulus was directly correlated with starch content, but inversely correlated with luminous reflectance and the logarithm of percent reducing sugars. Charles (1973) compared physical, rheological, and chemical properties of ripening bananas at two different temperatures (16 and 25°C). He concluded that the rate of peel color change during ripening at 16°C was roughly half that at 25°C. The fruits ripened at 16°C were firmer than those ripened at 25°C. The pattern of total sugar increase in pulp tissues was the same at both ripening temperatures. However, sugar content was slightly higher in fruit ripened at 25°C. Lizana (1976) investigated the hydrolysis of starch in ripening banana under elevated temperature. He noted that bananas ripened at 30°C had apparently the same degree of sweetness as that at 20°C. No sweetness was detected in fruit ripened at 40°C in ethylene-free air, but some developed when the fruit was continuously gassed with 10 ppm ethylene. No sweetness developed when ripened at 50°C, even with ethylene treatment. Of the monosaccharides, fructose was in greater concentration than glucose in preclimacteric fruit but in the post-climacteric stage this relation was reversed. In both the pulp and peel of banana, ripened at 20°C and 30°C, sucrose was the predominant sugar in the pre-climacteric climacteric stages; in the post-climacteric period, glucose and fructose were the predominant sugars. There is a noticeable suppression of sucrose concentration in banana ripening at 40°C, but fructose and glucose remain unchanged. Total sugar increase was less than 1.5% when ripening occurred at 50°C. There was a very rapid increase in sucrose concentration at 20°C at the time of the respiratory climacteric.

Despite the massive synthesis and hydrolysis of starch in the developing and ripening bananas, virtually little is known about the enzyme or the mechanisms involved. Yang et al. (1958) suggested phosphorylase might be more important than amylase based on the observation of an increase in activity during the climacteric. A similar observation was made by Surendranatha et al. (1973). Baijal et al. (1972) and Shukla (1973) demonstrated low β-amylase activity in the banana fruit pulp, but did not analyze for α-amylase activity. Young et al. (1974) noticed a rise of α-amylase activity of banana during ripening, but their results did not provide a conclusion about its involvement in starch hydrolysis. They also detected the presence of β-amylase and phosphorylase in all stages of ripening, but were not able to assay these enzymes due to the enzyme inhibitors in the preclimacteric phase. Sornsrivichai (1976) showed that seven starch hydrolyzing enzymes could be detected in banana pulp: 2 α-amylases, 2 β-amylases, and 3 phosphorylases. Isozyme activity increased toward ripening and α-amylase activity predominated. Singh and Sanwall (1973, 1975, 1976) partially purified three phosphorylase forms from ripe bananas and observed different biochemical characteristics but did not investigate the behavior of these enzymes during ripening. Adimilson (1981) found that for

"Marmelo" (a Brázilian variety) bananas phosphorylase activity was constant during the preclimacteric phase but dropped after the climacteric peak and paralleled the decrease in starch content. However, α-amylase activity increased during the climacteric. He concluded that there is no strict dependence of starch breakdown upon increased activity of α-amylase, and although phosphorylase activity drops during climacteric and ripening, it could still be involved in the process of starch transformation. Richard (1982) proposed a cyclic pathway for the synthesis and hydrolysis of sucrose in the pulp of ripening banana fruit.

Ascorbic Acid

L-ascorbic acid (vitamin C) has the distinction of being the first nutritional adjunct whose deficiency was recognized as a cause of disease. Probably as early as 1700 it was observed that a lack of fresh fruits and vegetables resulted in scurvy and that this disease could be prevented and cured by the proper diet. It is recognized as having antisorbutic property.

Ascorbic acid may be easily oxidized in the presence of oxygen by both enzymatic and non-enzymatic catalysis to its oxidation product dehydroascorbic acid. Both copper and iron salts catalyze its oxidation. In fact, the oxidation in many processed productsmay be due to these salts. Enzymes containing copper and iron in their prosthetic groups are even more efficient catalysts. There are at least four enzymes that occur in fruits which may be responsible for the oxidative destruction of ascorbic acid; those are ascorbic oxidase, phenolase, cytochrome oxidase, and peroxidase. Only with ascorbic acid oxidase there is a direct reaction between enzyme, substrate and molecular oxygen; the other three enzymes oxidize the ascorbic acid indirectly. In the intact fruit these enzyme systems are controlled. Only where cellular disorganization occurs, as a result of mechanical damage, rot or senescence do these oxidative activities become effective (Mapson, 1970).

The ease of oxidation of ascorbic acid, and its ready reversal, have led to suggestions that it may function in the plants as a means of electron transfer in the respiratory chain. Mapson (1970) has suggested that it might also act as a hydrolxylating reagent.

The proportions of the oxidized and reduced forms in plant material are variable, but the balance usually falls much on the side of the reduced form. The reversible oxidation of ascorbic to dehydroascorbic acid leads to no loss of vitamin C activity. Loss can occur, however, by the irreversible conversion of dehydroascorbic acid to 2,3-dioxo-L-glulonic acid, which is then further metabolized:

$$\text{ascorbic acid} \rightleftharpoons \text{dehydroascorbic acid} \longrightarrow \text{2,3-dioxo-L-glulonic acid}$$

This reaction is very pH-dependent, being slow in acidic pH, rapid at neutral pH and extremely rapid at alkaline pH. We would expect the cellular environment in the ripening banana to become less inductive to loss of vitamin C activity as the pH falls from about 5.4 in preclimacteric to about 4.5 in post-climacteric stage (Burton, 1982).

The importance of the proper handling of samples for ascorbic acid determination cannot be overemphasized. The sampling and extraction of the material under examination must be carried out with minimum delay so that no significant change in ascorbic acid takes place prior to analysis. After raw materials are cut, bruised, or chopped, they must be handled very rapidly and blended with a stabilizing acid as quickly as possible to prevent undue oxidation of ascorbic acid. This is particularly true of raw products high in ascorbic acid oxidase activity like fruits (Freed, 1966).

Considerable effort has been expended in an attempt to define the most suitable extraction medium for ascorbic acid. Metaphosphoric acid, first suggested by Fujita (1935), possesses several advantages. It retards the oxidation of ascorbic acid by inactivating the catalytic effect of ascorbic acid oxidase and copper. In addition, it is a protein precipitant and thereby aids in the removal of enzymatic oxidase and facilitates clarification of the extract. Ponting (1943) compared a large number of potential extractants and found that metaphosphoric and oxalic acids were the most suitable. Oxalic acid is attractive because of its relatively lower cost and greater stability; however, it is not a protein precipitant and, therefore, is inapplicable for use in the extraction of animal tissues.

There are a variety of analytical procedures for detecting ascorbic acid, but none is entirely satisfactory because lack of specifity and because most foodstuffs contain numerous interfering substances. Analysis usually involves oxidation by a redox dye, such as 2,6-dichlorophenolindophenol. This procedure does not take into account dehydroascorbic acid (Fennema, 1980).

Little has been done with ascorbic acid in the banana, except to tabulate data on its content. Thornton (1938, 1943) reported that ascorbic acid is quickly destroyed when banana pulp is macerated in air. The green bananas contained approximately 15 mg of ascorbic acid per 100 g of fresh pulp and increased slightly, then decreased with continued ripening so that they contained not less than 10 mg at desirable eating stage of ripeness (ripened at 19^oC). Other reported ascorbic acid contents of ripe banana (expressed as mg/100 g fresh pulp) are: 9.1 (Loesecke, 1950), 10-30 (Mapson, 1970), 10 (Burton, 1981), 10 (USDA, 1981) and 12 (Forsyth, 1982).

MATERIAL AND METHODS

Plant Material

The non-gassed "Banacol" brand banana fruit (*Musa cavendishii* var. Valery) from Columbia was purchased from a Detroit wholesaler. The fruit reached a maturity stage at light full three-quarters according to the prepared chart by von Loesecke (1950, Figure 13).

All fruit in the 40 lb commercial carton was used in the ripening trials which were carried out in a closed cubicle at 16±0.5^{o}C and relative humidity 85-90%. The banana was treated with thiabendazole by "Banacol" company before distribution to avoid microbial growth. The bananas were covered by perforated polyethylene film.

Sample Preparation

Two fingers of fruit were picked randomly out of storage every 2 or 3 days for determination of moisture, ascorbic acid, sugars, and starch. The ratio of pulp weight to peel weight (coefficient of ripeness) and the peel color, as judged by von Loesecke (1950) color plates, were used to assure the uniformity of maturity in the samples.

Each banana was divided into three equal-length portions, designated as stem, middle, and distal portions, respectively. The No. 8 corer (diameter 1.5 cm) was used to extract core sections from each portion. After coring and peeling, each finger yielded six specimens for measurement (Figure 1). The green banana was estimated to be stage 2 of maturity. The weight of individual fingers (unpeeled) varied between 15.6 and 18.0 grams, while length varied between 16.3 and 18.5 cm.

Moisture Content

Moisture were determined by procedure 7.003 of the "Official Method of Analysis - A.O.A.C." (1980).

Ascorbic Acid

Ascorbic acid was determined by using Kyaw (1978) with some modifications.

Color Reagent (12-tungstophosphoric acid)

20 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$; Merck & Co., Inc.) and 10 g of disodium hydrogen phosphate were dissolved ($Na_2HPO_4 \cdot 2H_2O$; Fisher Scientific Company) in 30 ml warm water. 20 ml of 25% (v/v) sulfuric acid was added and the mixture was boiled gently for 2 hours under reflux. The resulting solution was cooled to room temperature.

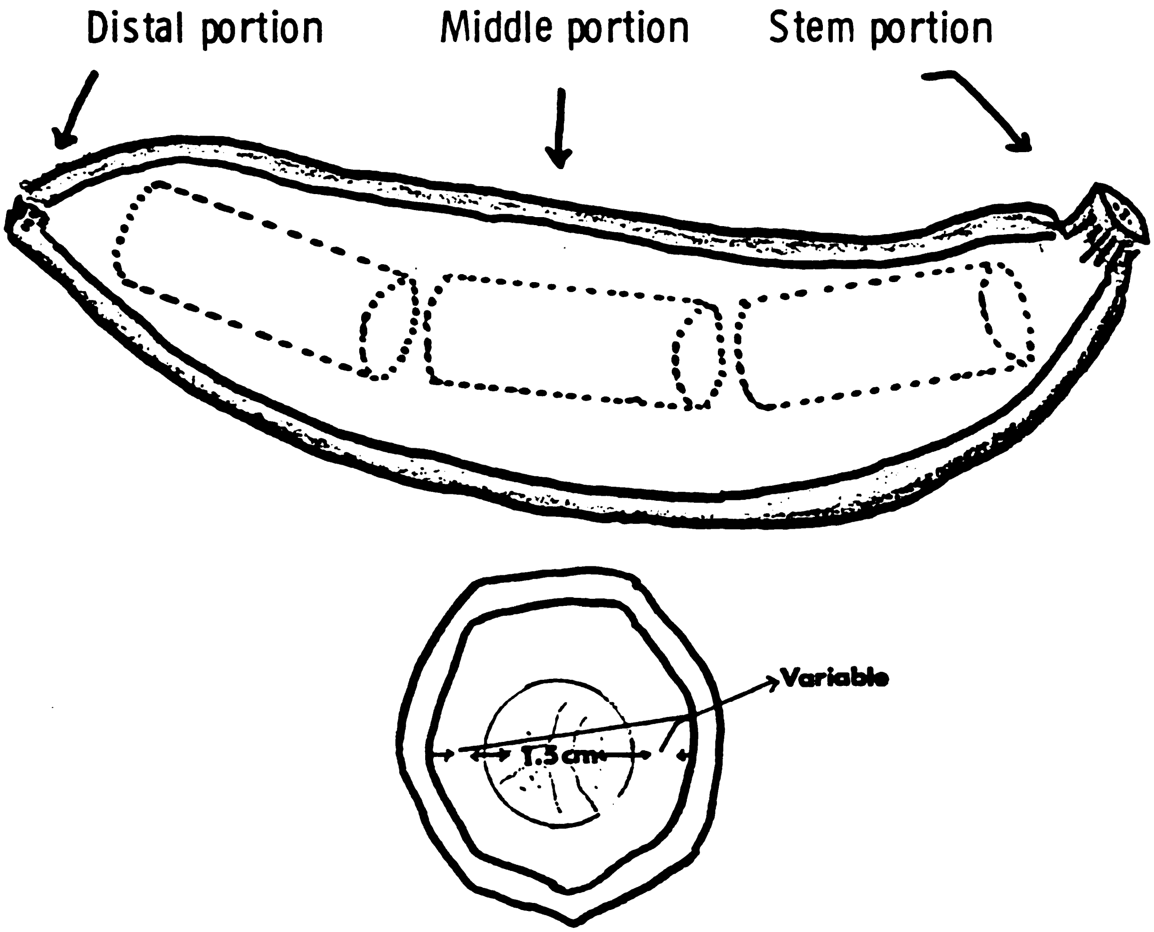

Fig 1. --- Longitudinal and cross sections of a banana showing the portions used for mapping several banana constituents.

(Core diameter was constant, peripheral thickness varied.)

Standard Curve

A stock solution was prepared by dissolving 64 mg of ascorbic acid (General Biochem. Co.) in 100 ml of 0.5% oxalic acid solution (w/v; Merck & Co., Inc.). By diluting the stock solution with 0.5% oxalic acid the following standard solutions were prepared: 6.4, 4.2, 3.2, 1.6, 0.8, and 0.4 mg/100 ml. Two ml of each ascorbic acid standard solution was mixed thoroughly with 2.0 ml of color reagent and allowed to stand for 30 min at room temperature. The solution was then centrifuged at 3000 rpm for 15 min. The blue-colored supernatant was transferred to a Beckman quartz cell (light path 1 cm) and its absorbance was measured at 700 nm against a blank (0.5% oxalic acid solution) in a Beckman DU Spectrophotometer (Beckman Instrument Co.). The reference curve is shown in Figure 2. The best-fit line was determined by linear regression.

Ascorbic Acid in Banana

A 1.5 cm thick slice of pulp was dropped into a pre-weighed beaker (W_3) containing 50 ml of 0.5% oxalic acid solution. The beaker with pulp was weighed again (W_4). The mixture was blended in an Omni-Mixer (Sorvall Inc.) to yield a homogeneous slurry and 2 ml of slurry was pipetted into a centrifuge tube. Color reagent was added and the measurement of absorbance was made as for the standard solutions, after carefully transferring

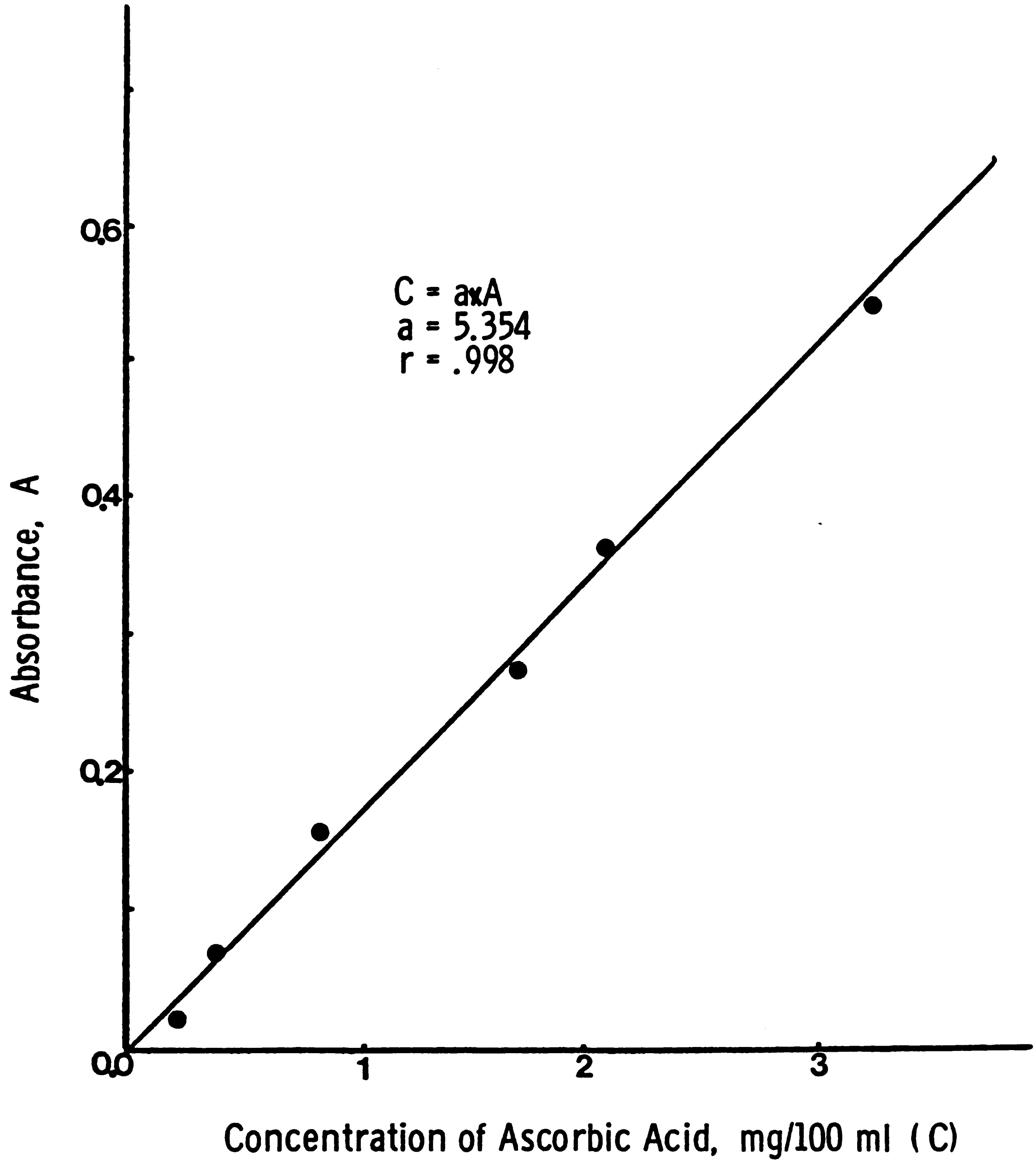

Fig 2. Reference curve for ascorbic acid determination.

the supernatant to the cell without distributing the precipitate.

The ascorbic acid content of banana expressed as mg/100 g fresh pulp was calculated according to the following equation:

$$\text{ascorbic acid content } \left(\frac{\text{mg}}{\text{100 g fr. wt.}}\right) = \left(\frac{5.354A}{W_4 - W_3}\right) \times 100$$

Sugars

The simple sugars (fructose, glucose, sucrose) were determined by HPLC.

HPLC System

A Waters liquid chromatographic model 201 (ALC CPS 201), equipped with a Model-600 solvent delivery system and a model R401 differential refractometer detector (1×10^8 refractive index units) was used. A μ Bondapak-Carbohydrate column (Waters Assoc.), 30 cmx3.9 mm I.D. stainless steel was used. A recorder (Kontes Inc.) model 100 was attached. The usual speed was 1 cm/min.

The mobil phase was water-acetonitrile (HPLC grade). The sample, 20 μl, was injected with a syringe (Bio-Rad, Inc.) via a model 7010 injection valve (Rheodyne Inc.). Millipore all-glass filter apparatus and Millipore pre-filters (0.3 μm pore size) and aqueous filters (0.45 μm pore size) were used to filter the eluent and sample solutions. An ultrasonic cleaner (Beckman Co.) was used to degas the mobile phase before use.

Standard Curve

The sugar standards were purchased from Mallinckrodt Inc., weighed, mixed in a 50 ml volumetric flask and brought to volume with distilled water. The concentrations (mg/ml) of fructose, glucose and sucrose in the standard solutions were 14.7, 15.0, 16.7; 11.1, 11.3, 10.6; 8.8, 8.5, 0.0; 2.9, 2.7, 2.5; 0.4, 0.5, 0.4, respectively. The chromatograph was carried out at ambient temperature. Before injection, the column was equilibrated for at least 30 min with mobile phase at a flow rate of 2.0 ml/min. Each standard was injected twice. The attenuation of refractometer was 4 X, and the chart speed of recorder was 1 cm/min. Fructose, glucose and sucrose eluted in that order and exhibited retention times of 3,3, 4.6, and 8 min, respectively. The reference lines are shown as Figure 3, 4 and 5.

Sugars in the Banana

A weighed portion of each specimen (A_0) was chopped into fine pieces and added to the beaker containing about 150 ml hot 80% (v/v) ethanol solution. A small amount of $CaCO_3$ (Mallinckrodt Inc.) was added to neutralize the acidity. The beakers were placed in a steam bath for 1 1/2 hours. Periodically, a glass rod was used to macerate the softened tissue during boiling. After the

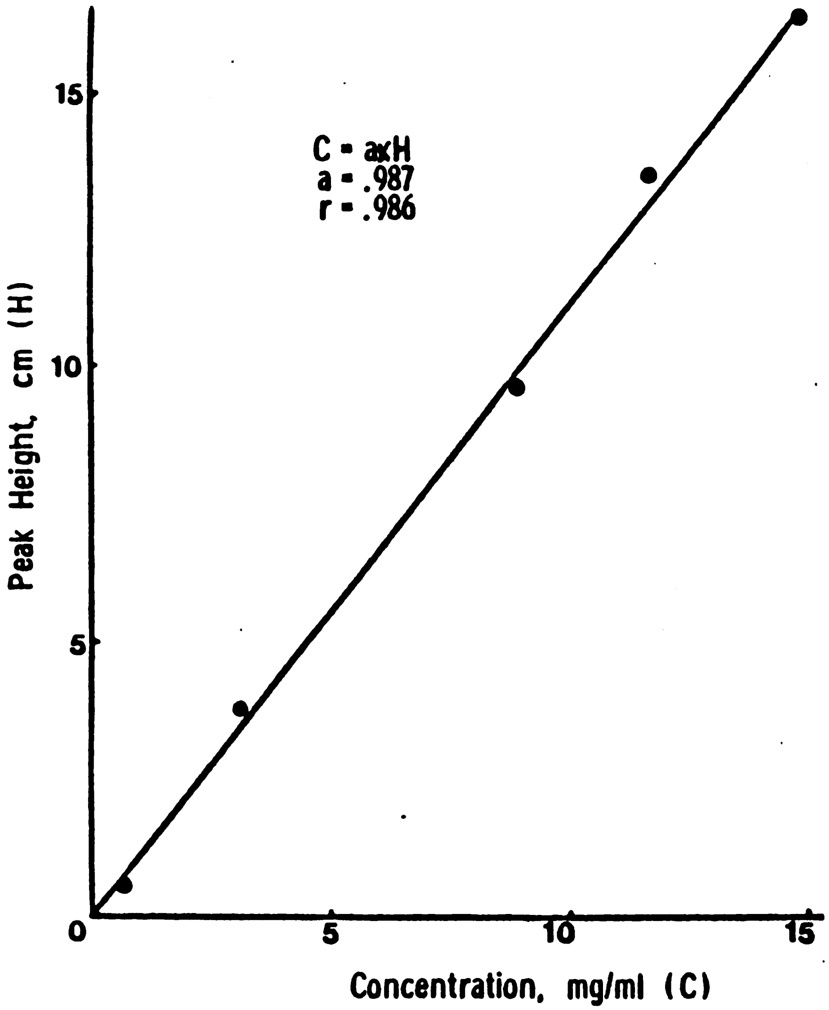

Fig 3. Reference curve for fructose determination.

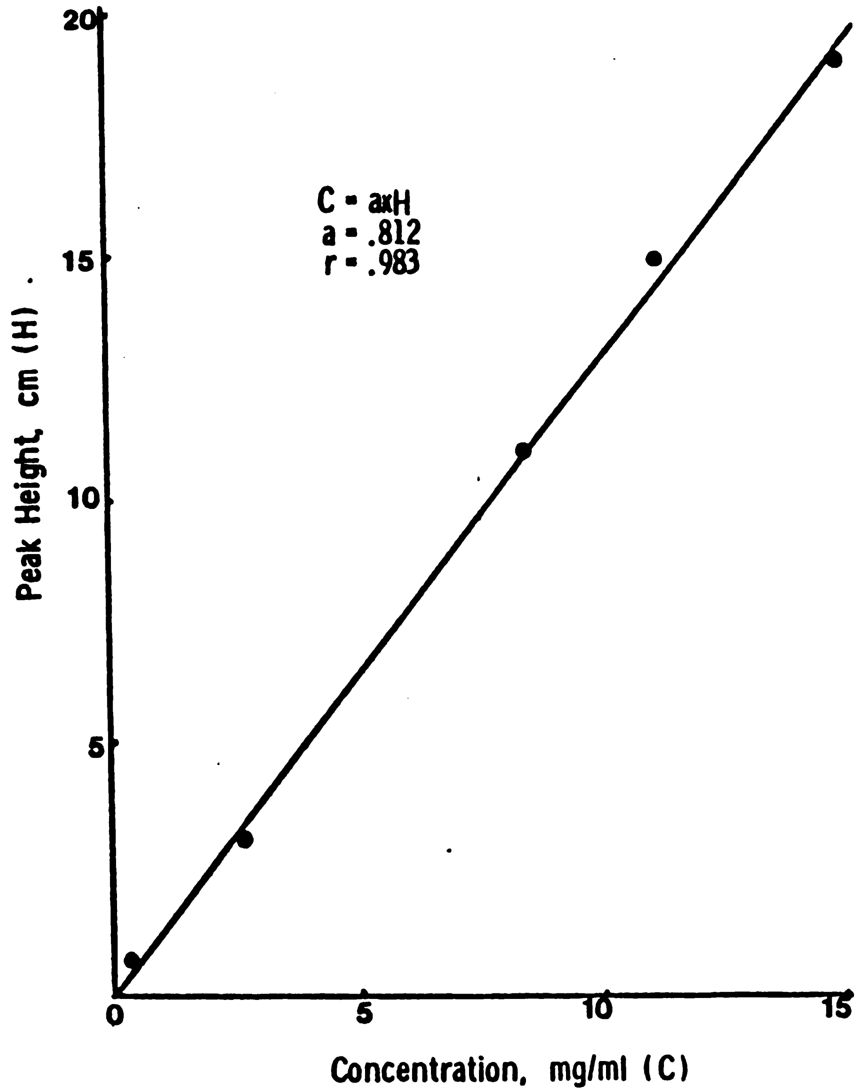

Fig 4. Reference curve for glucose determination.

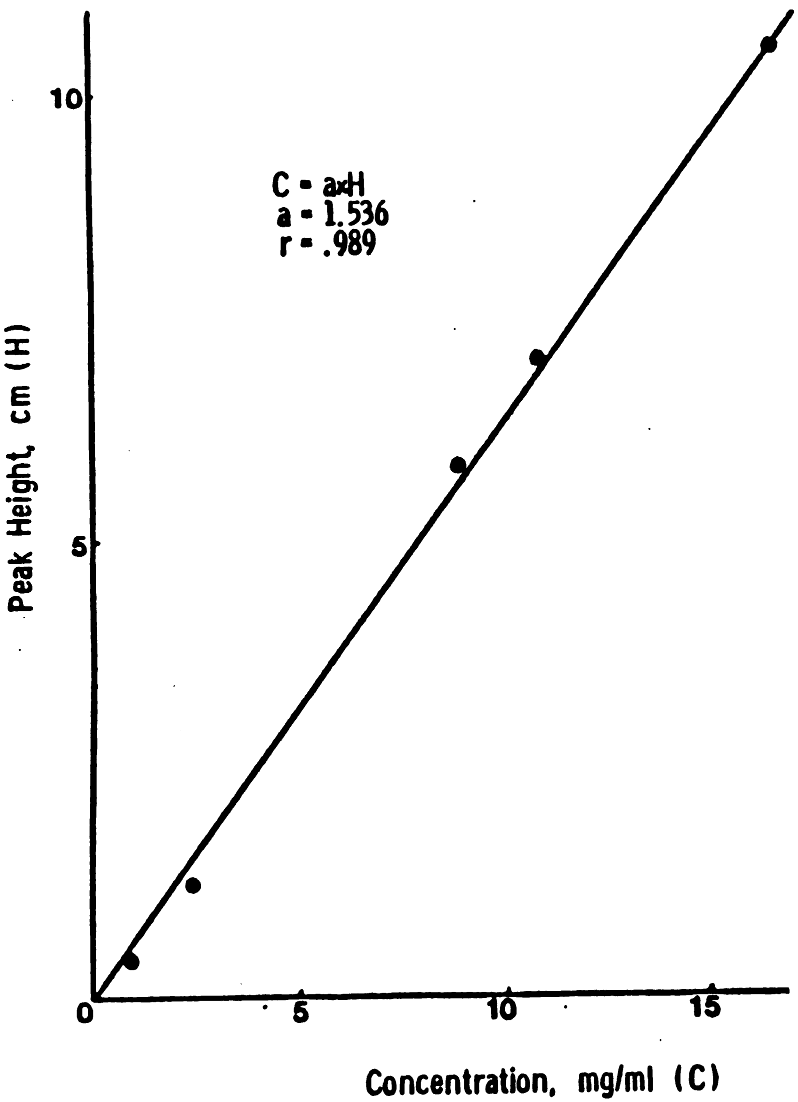

Fig 5. Reference curve for sucrose determination

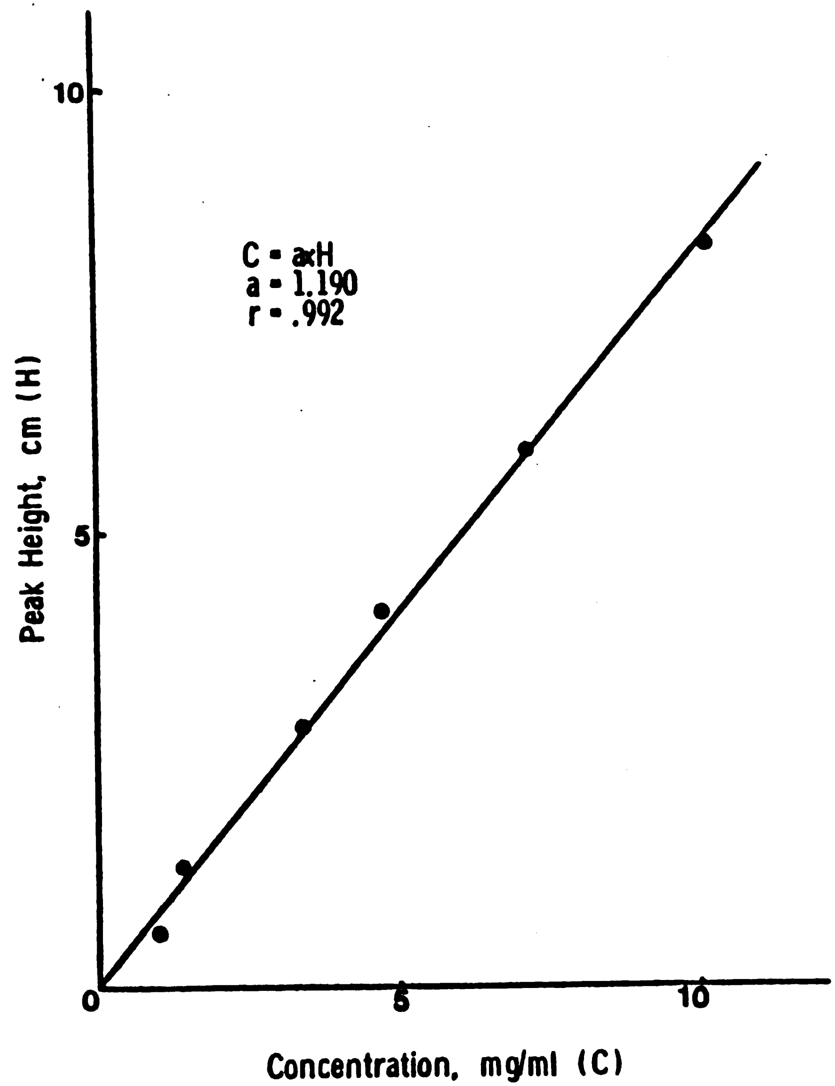

Fig 6. Reference curve for determination of glucose from the starch hydrolysate

steam bath, the macerates were cooled to room temperature and centrifuged at 1,700 G (Serval Superspeed centrifuger) for 30 min. The supernatant is transferred to 25 ml volumetive flask and brought to volume with distilled water. The sample was filtered through Millipore filter before injecting into the column.

From the regression equations, the concentration of each sugar in the sample were estimated. The percentage of the individual sugar extracted from the pulp were calculated according to the equation below:

$$\text{Sugar (\%, w/fr. wt.)} = \frac{C(\text{mg/ml}) \times V(\text{original sample volume,ml})}{A_0 \text{ (banana used, mg)}} \times 100\%$$

Starch

Enzyme Solution

A 0.2 M acetate buffer, pH=4.5, was prepared. One gram of amyloglucosidase was dissolved in 200 ml 0.2 M acetate buffer solution. The amyloglucosidase (glucoamylase 1,4-α-D-glucanglucohydrolase; EC 3.2.1.3 from Rhizopus mold,10,000 units/g solids) was purchased from Sigma Chemical Co.

Standard Curve

Six standard solutions of glucose were made by weighing certain amount of glucose anhydrous (Mallinckrodt Inc.) and dissolved in 50 ml distilled water. The concentrations were 10.12, 7.1, 4.79, 2.98, 1.62, and 1.09 mg/ml. The same procedure as in the sugar determination was followed to get the HPLC peak. Figure 6 showd the reference curve for determining glucose from starch.

Starch in the Banana

The residue obtained after the extraction of the sugars was dried in a vacuum oven at 60^oC for 12 hours and weighed (A_1). The dry residue was crushed into a powder by using a porcelain mortar and pestle and a 10 mg aliquots was placed in a centrifuge tube.

Two ml of 0.2 N NaOH was pipetted into each tube. The tubes were capped and shaken. After dispersion, 2 ml of 0.2 N CH_3COOH were added to neutralize the NaOH. The solution was allowed to stand for a few minutes and 6 ml of enzyme were added to each tube. The incubation was completed by placing the tubes in a water bath at 55±2^oC for approximately 40 hours.

After incubation, the tubes were cooled to room temperature and centrifuged at 3000 rpm for 10 min. One ml of supernatant was transferred to a 10 ml volumetric flask, and deproteinized (Somogyi, 1945) by adding distilled water (5 ml), 0.3 N NaOH (2 ml), and 5% $ZnSO_4$ (2 ml). After thorough mixing, the liquid was filtered through a Millipore filter and injected into the HPLC column. The deproteinization procedure was essential. Because even protein comes off the column before water, it could form films and clog syringes and tubings; it also increased the pressure required to force the solvent through.

Hydrolysis of 100 mg pure soluble starch (Ditco Lab., Detroit) by the enzyme solution after incubation yielded 122.3 mg glucose, thus giving the glucose to starch conversion factor of 0.90.

The glucose concentration in the hydrolyzate was calculated from the regression equation.

The starch content of the banana, expressed as the percentage of fresh-pulp was calculated as follows:

$$\text{starch (\%, w/fr. wt.)} = \frac{\text{N(concentration, mg/ml)} \times 10\ \text{ml} \times 10 \times 0.9 \times \frac{100\ \text{mg}}{\text{A(mg)}}}{\text{Ao (mg)}} \times 100\%$$

0.9 = conversion factor

10 = dilution factor

10 ml: original volume (before deproteinization)

RESULTS AND DISCUSSION

The data on the moisture content of six different banana portions during various stage of ripening are shown in Figures 7 and 8. The pulp-to-peel weight ratios at the same ripening stages are shown in Figure 9.

The percent moisture content increased steadily in the periphery with a concomitant decrease in the core of stem, middle, and distal portions. The distal portion had the highest percent moisture content followed by the stem and middle portions in both periphery and core.

The percent moisture in the core decreased with time, indicating transfer of moisture toward the periphery, which always seemed to have less moisture than the core during the ripening process. The highest percent moisture in the distal portion also indicated that the ripening process began from this portion which softened faster than other portions.

The pulp-to-peel weight ratio values ranged from 1.46 in green fruit to 2.19 in full-ripe fruit. This is in accordance with Loesecke (1950) and Simmonds (1966). The ratio of pulp-to-peel weight ratio increased to 1.77 on day 15, with a slight decrease till day 22 to 1.69, which later increased to 2.19 at full-ripe stage. A similar

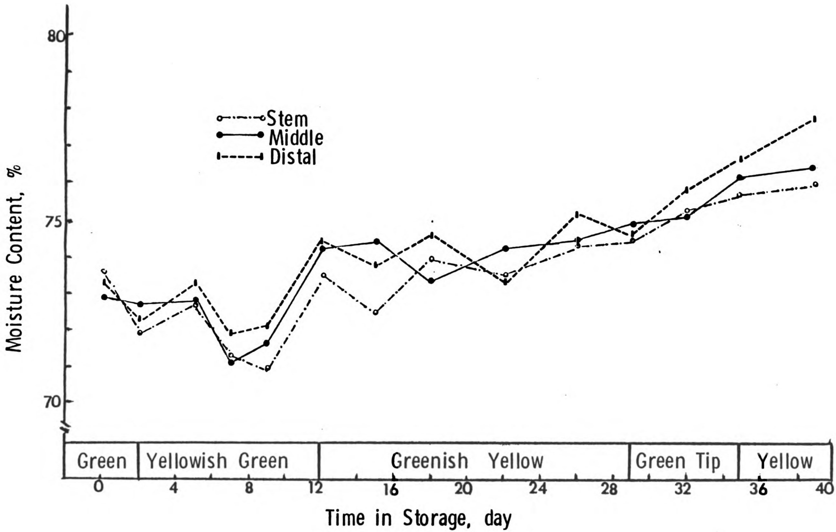

Fig 7. Moisture content of three peripheral portions of banana during ripening.

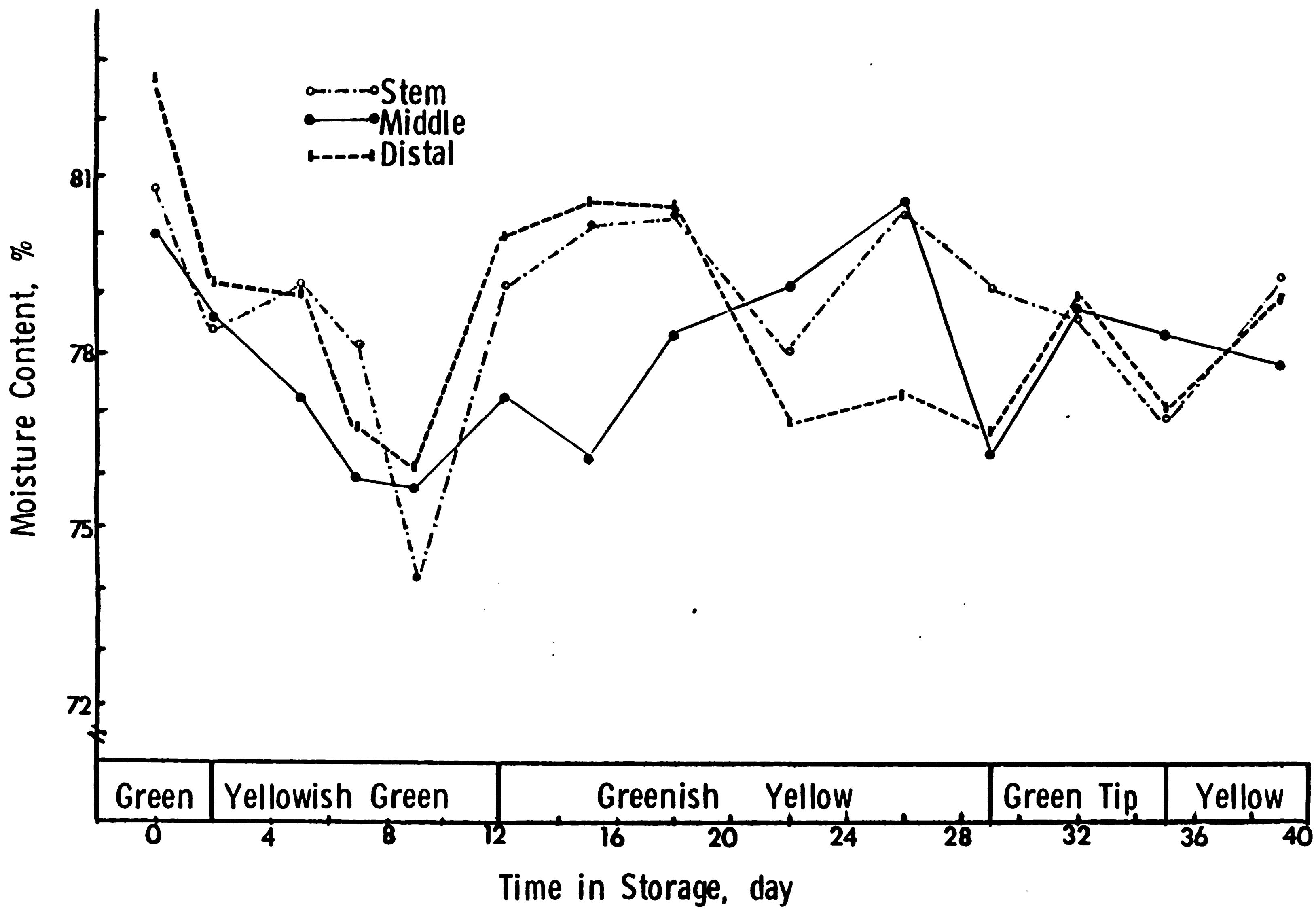

Fig 8. Moisture content of three core portions of banana during ripening

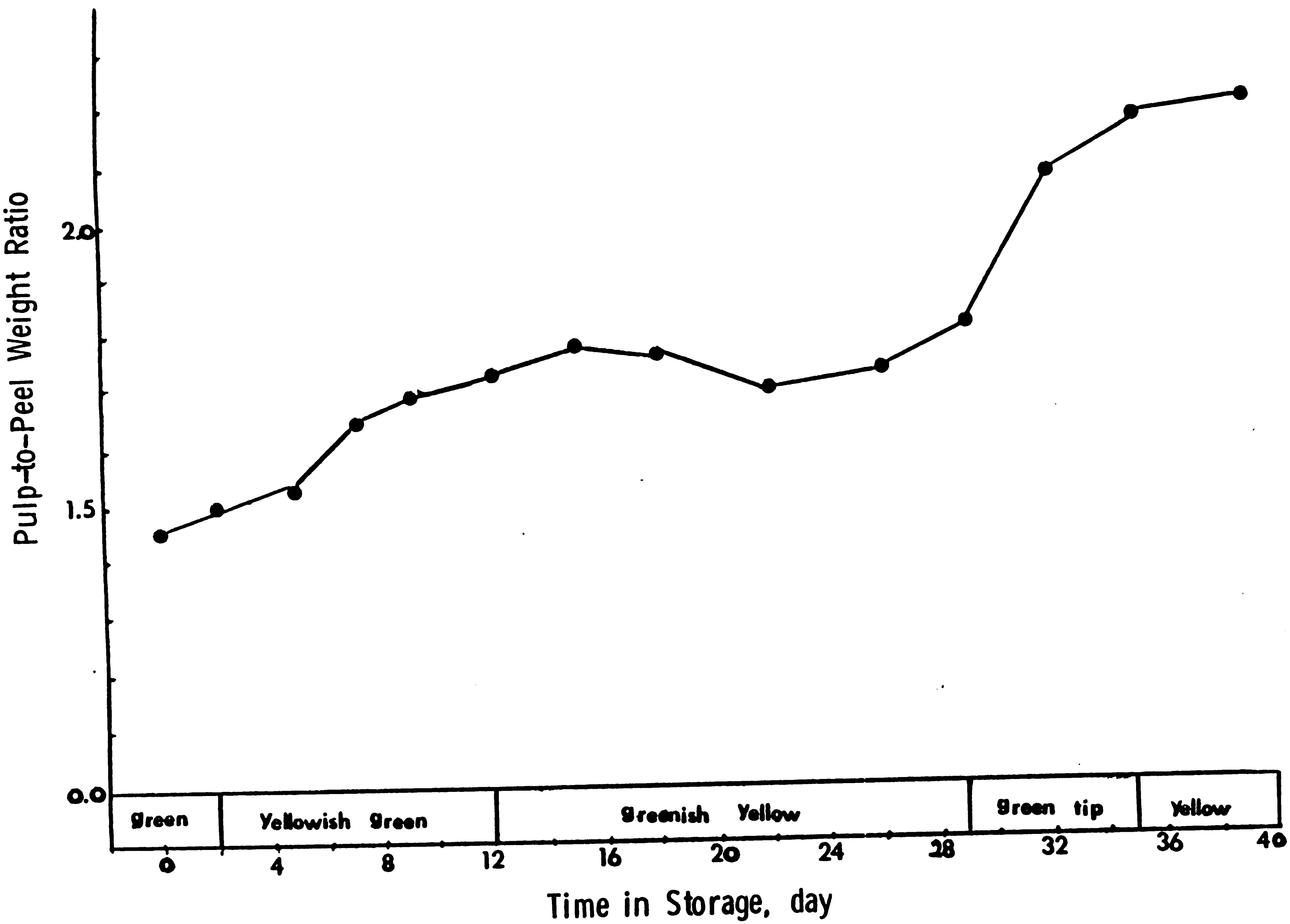

Fig 9. Pulp to peel weight ratio of banana during ripening

pattern was observed by Charles (1973), who used color index as x-axis instead of time. Although usual estimation of banana ripeness by color index was of greater speed and simplicity, the assignment of index value is very subjective. For example, the color index from day 15 to day 26 roughly fell between 3 and 4, making it difficult to assign any objective value. Using a time scale is preferable to color index.

The increase of pulp-to-peel weight ratio was due to the osmotic transfer of moisture from peel to pulp and the breakdown of carbohydrates during the ripening process.

The data for ascorbic acid content in the periphery and core are shown in Figures 10 and 11. Both graphs show two peaks. The first peak appears in the yellowish green stage and is smaller than the second peak appearing in the greenish yellow stage.

In the periphery, the ascorbic acid content in the distal portion was highest, followed by that in the stem and middle portions during the first week. No significant changes were observed between day 10 and day 22 in all locations. After day 22, there was a dramatic increase in ascorbic acid content in all three portions of the banana peaking at day 26, after which it fell off till day 35 and then levelled off. During the green-tip and yellow stages the stem portion showed the highest ascorbic acid content, followed by the distal and middle portions.

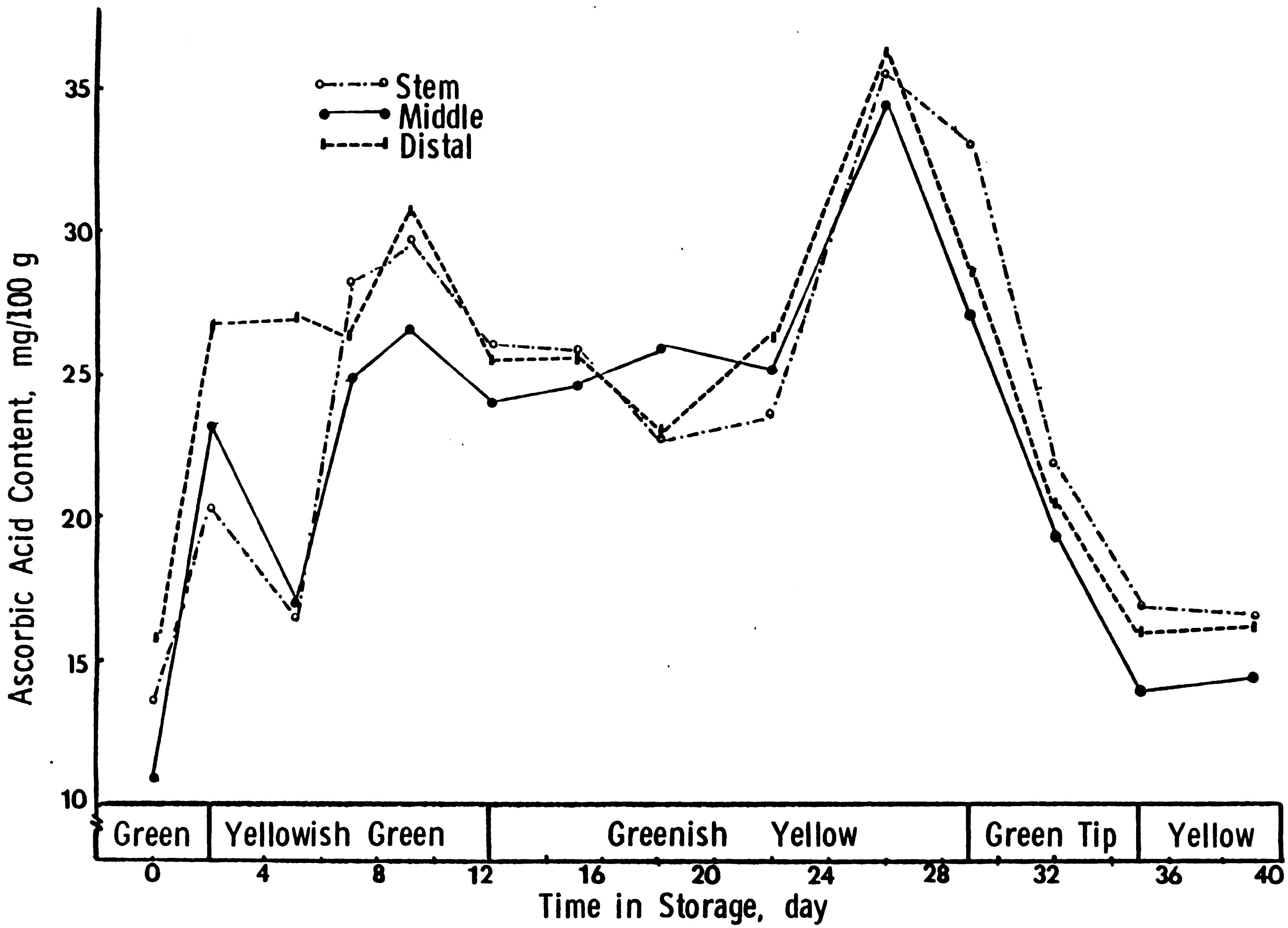

Fig 10. Ascorbic acid content of three peripheral portions of banana during ripening

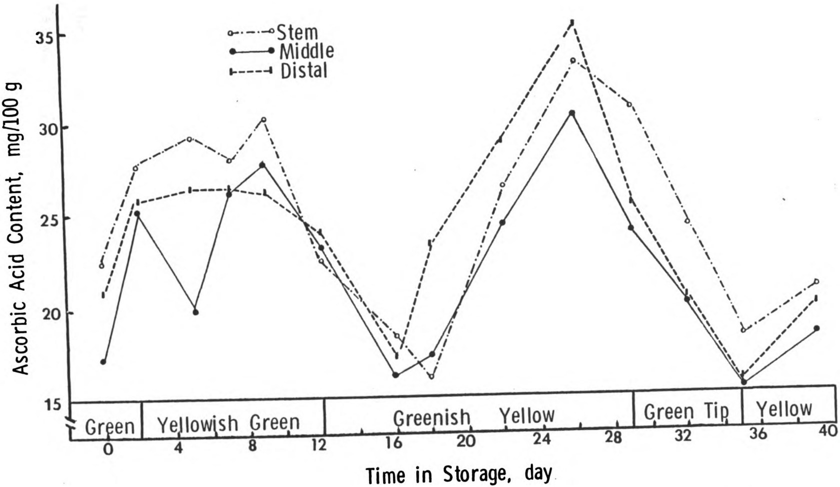

Fig 11. Ascorbic acid content of three core portions of banana during ripening

In the core, the ascorbic acid content in the stem portion was highest followed by the distal and stem portions during the first week. No significant changes were observed between day 10 to day 15 in all the locations. After day 18, there was a dramatic increase in all the three portions of the banana, and the distal portion had the highest amount of ascorbic acid followed by the stem and middle portions. There was a decrease of ascorbic acid content in all three locations of the banana after day 26, but the stem portion had the highest concentration of ascorbic acid, followed by the distal and middle portions. There was a small rise in ascorbic acid content for all locations at the full-ripe (yellow) stage.

The ascorbic content of the core was greater than that of the periphery during the first week. Later the order was reversed until day 29; subsequently, the core content in ascorbic acid again surpassed that of the periphery.

The ascorbic acid content increased during ripening in all six locations until day 29 and then started declining till the full-ripe stage. The maximum increase was almost double its value at green stage which is in contrast to the finding of Thornton (1943), who used Gros Michel banana in his studies.

The average ascorbic acid content in the full-ripe bananas was 17.7 mg/100 g fresh weight. Several investigations reported ascorbic acid content (mg/100 g fresh weight) in the range of 9.1 (Loesecke, 1950), 10-30 (Mapson, 1970), 10 (Burton, 1981). The differences may be due to variety, plant nutrition, and the conditions of plant growth.

The results suggest that there is a blanace between ascorbic acid synthesis and its destruction within the banana, and there may be some relationship between the rate of respiration and the ascorbic acid content of the fruit, since the respiratory rate increases along with the ripening process. Earlier, Mapson (1970) suggested that the synthesis of the ascorbic acid proceeded independently of its function in respiration, and its level in the cell might simply reflect the excess formed over that used in metabolism.

Fruits synthesize ascorbic acid from hexose sugar precursors (Mapson, 1970). In general, two sugar precursors have been identified in the synthesis of ascorbic acid in the plant: glucose and galactose. There was only glucose present in the banana, thus glucose is probably the major precursor for ascorbic acid formation.

The separations of sugars in full-ripe banana extract and the glucose from hydrolyzate of banana starch are shown in Figure 12. Fructose, glucose, and sucrose eluted in that order and exhibited retention time of 3.3, 4.6 and 8 min, respectively.

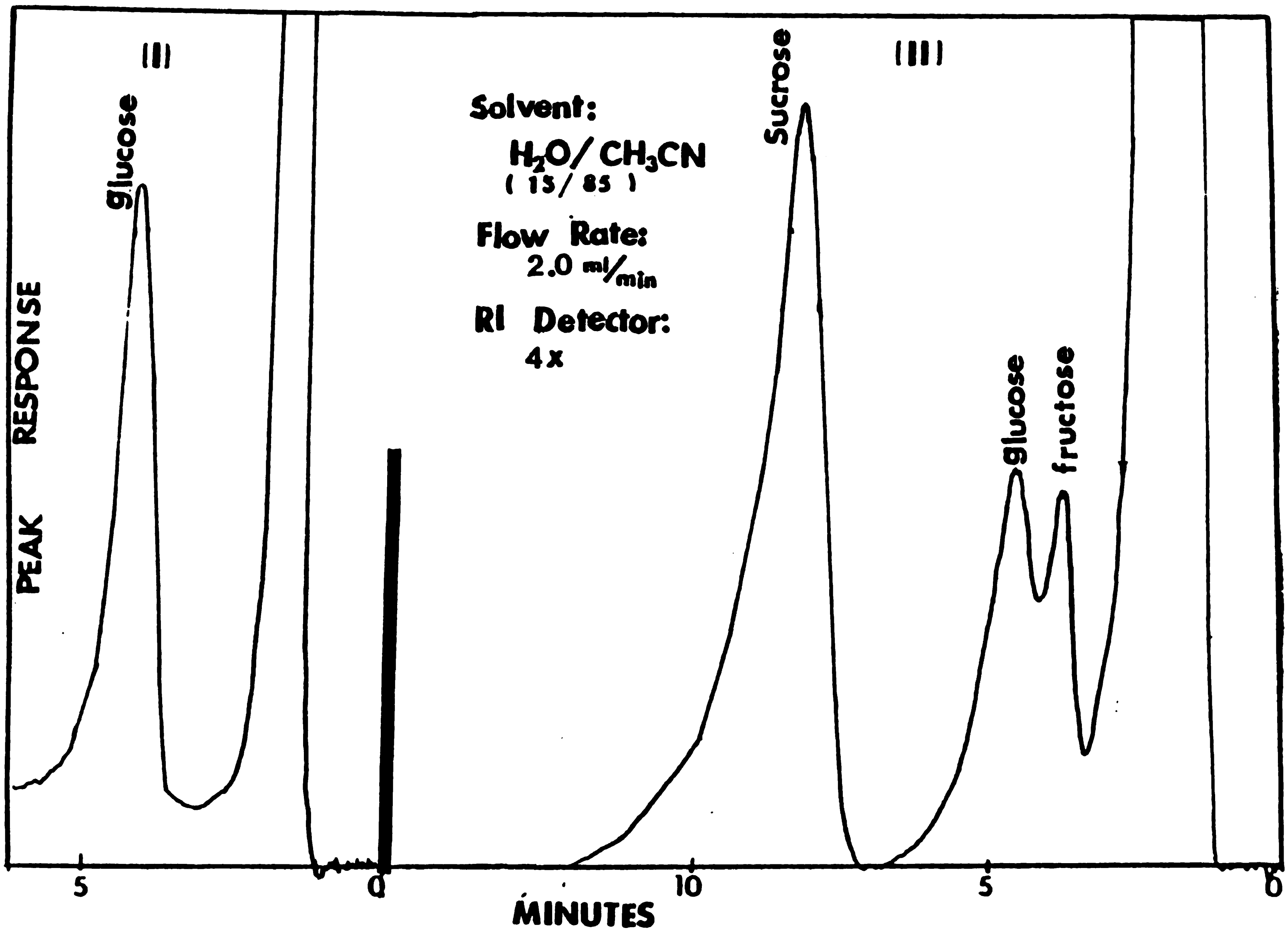

Fig 12. HPLC chromatograms : (I) of banana starch after hydrolysis by amyloglucosidase, (II) of a sugar extract from bananas

The data on the distribution of sugars in the six portions of the ripening are shown in Figures 13-20. The ratio of glucose to fructose was approximately unity in each location of the banana at all stages of ripening. Sucrose is the dominant sugar in both periphery and core, regardless of the stages of ripeness. This is in accordance with the earlier report by Marriott (1981) who ripened banana at $20^{o}C$.

All figures (13 to 20) show a small rise in all sugars at day 12, which may be due to the climacteric rise in respiration. Lizana (1976) reported a similar observation on bananas ripened at $20^{o}C$. He correlated this to respiratory rates.

In the periphery, all sugars were present at the highest concentrations in the distal portion, while the stem portion had the lowest concentrations (Figure 13, 15, 17 and 19). This is probably due to greater breakdown of starch in the distal portion as the concentration of starch decreased faster in this portion (see discussion on starch).

In the core, the glucose and fructose contents were highest in the distal portion until day 15. After that day, the middle portion became the richest in glucose and fructose, followed by distal and stem portions (Figures 14 and 16). Regarding sucrose (Figure 18), there was no particular distributional pattern in the core locations

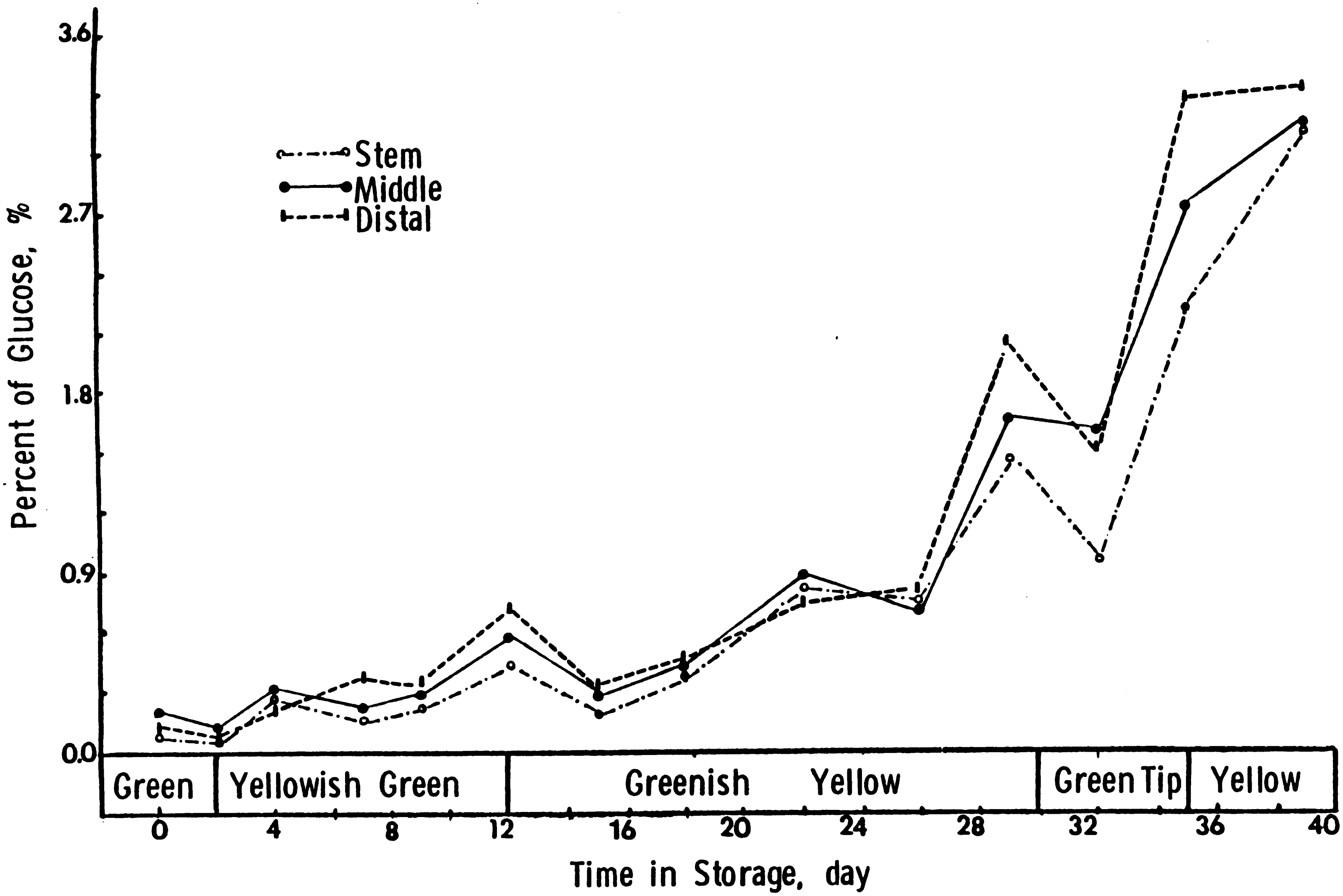

Fig13. Glucose content of three peripheral portions of banana during ripening.

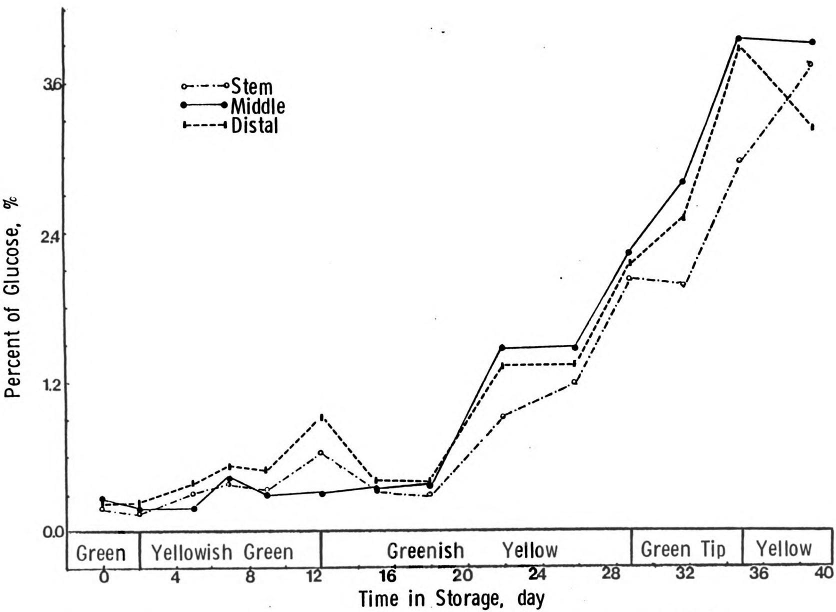

Fig 1′. Glucose content of three core portions of banana during ripening.

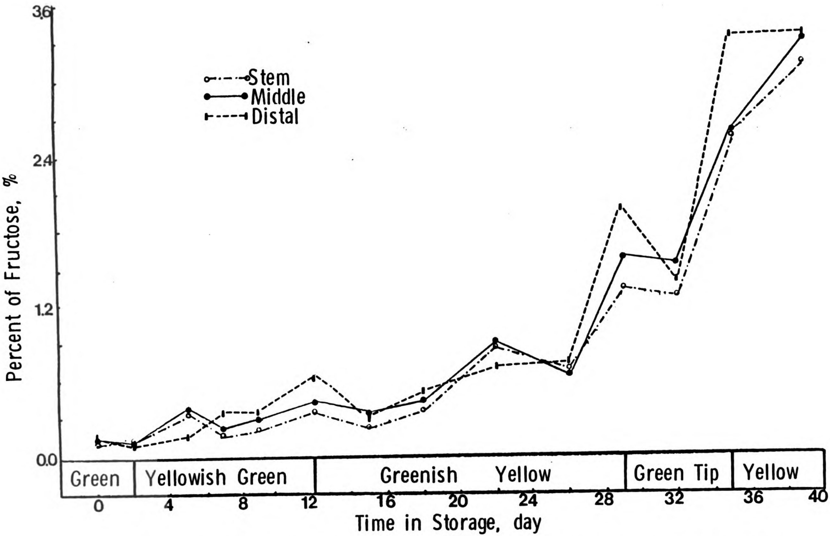

Fig 15. Fructose content of three peripheral portions of banana during ripening.

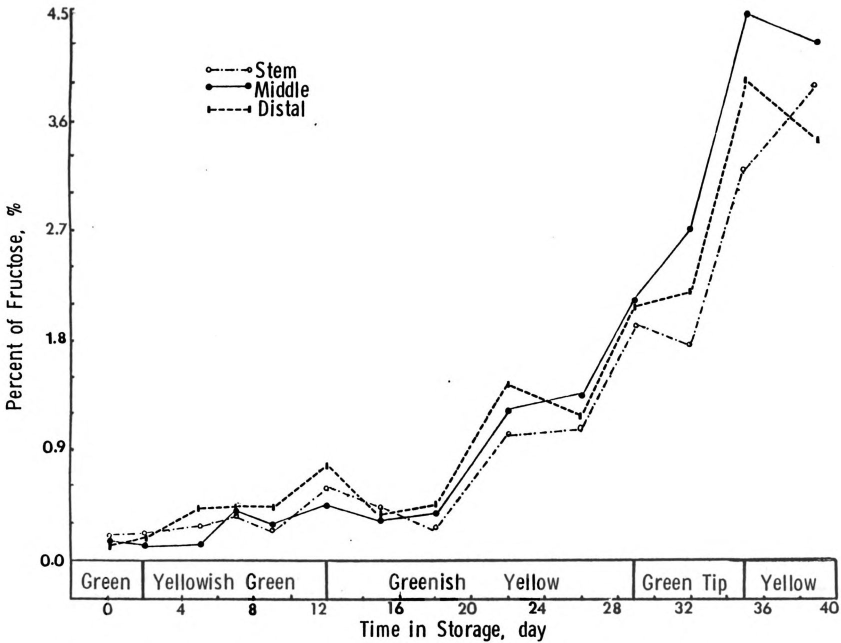

Fig 16. Fructose content of three core portions of banana during ripening.

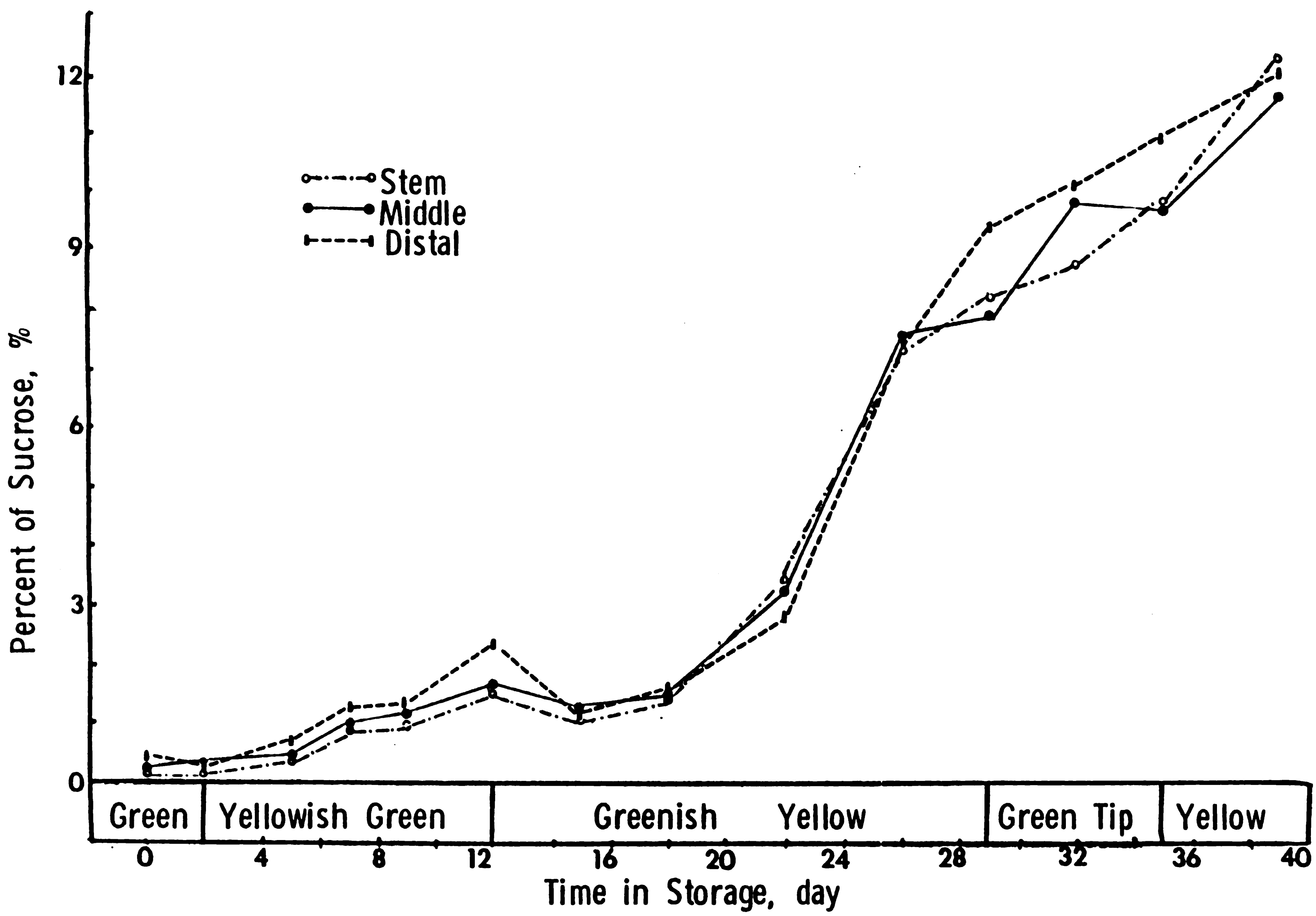

Fig 17. Sucrose content of three peripheral portions of banana during ripening.

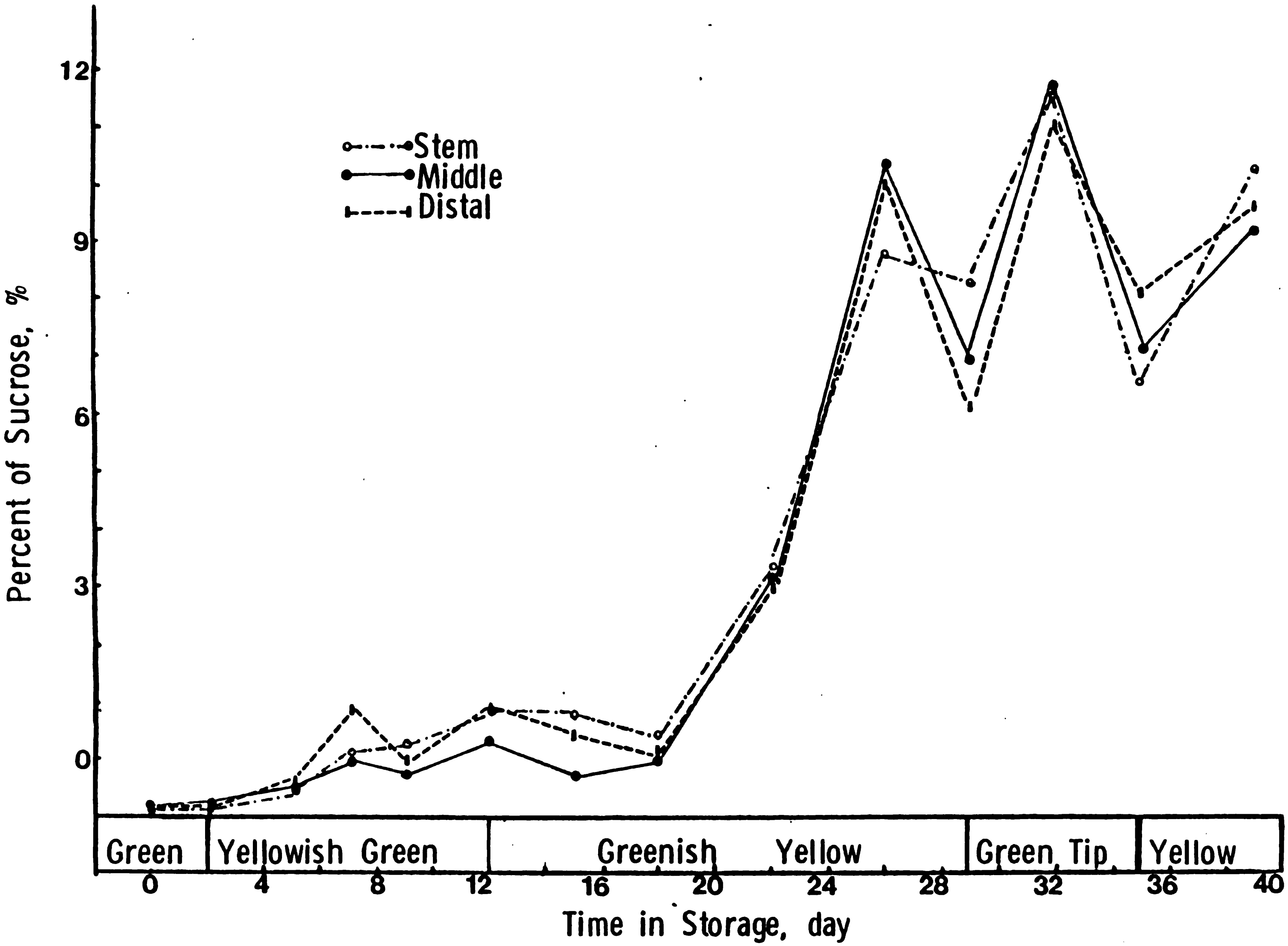

Fig 18. Sucrose content of three core portions of banana during ripening.

during ripening.

The amount of total sugars (Figures 19 and 20), in both the core and periphery, was the highest in the distal portion and the lowest in the stem portion. The differences, however, were not very large.

The data on starch content, periphery and core, stem, middle and distal portions, are shown in Figures 21 and 22. The starch content in both periphery and core fluctuated during the first 18 days, after which there was a significant decrease in all locations until the full-ripe stage. The fluctuation in the first 18 days may indicate starch resynthesis proceeded along with starch degradation at the early stages of ripening.

The starch content in the periphery seemed to be greater than that in the core during ripening. The distal portion had less starch than stem portion. Finney (1967) reported that the firmness of the banana during ripening was directly correlated with the starch content in the pulp, and the softening began from the distal portion as ripening progressed.

As starch hydrolysis proceeded during ripening, the total sugar content increased correspondingly (Figure 23). There was a loss of 1.4% in total carbohydrates during ripening and this may be due to the utilization of sugars in the respiratory process.

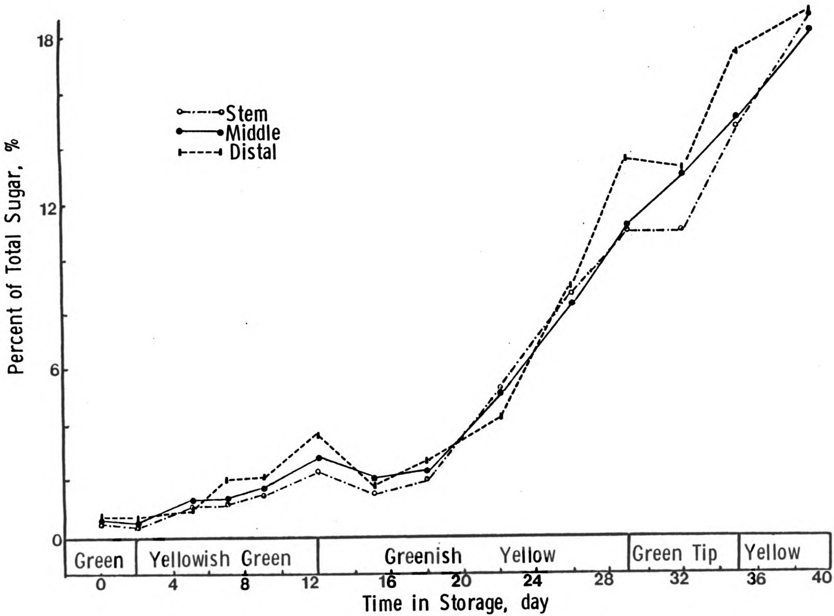

Fig 19. Total sugar of three peripheral portions of banana during ripening.

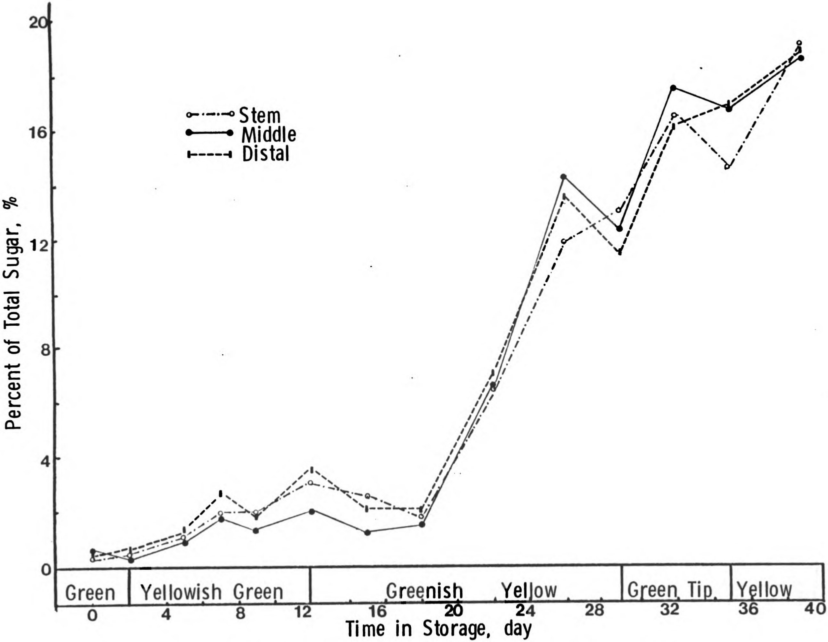

Fig 20. Total sugar of three core portions of banana during ripening.

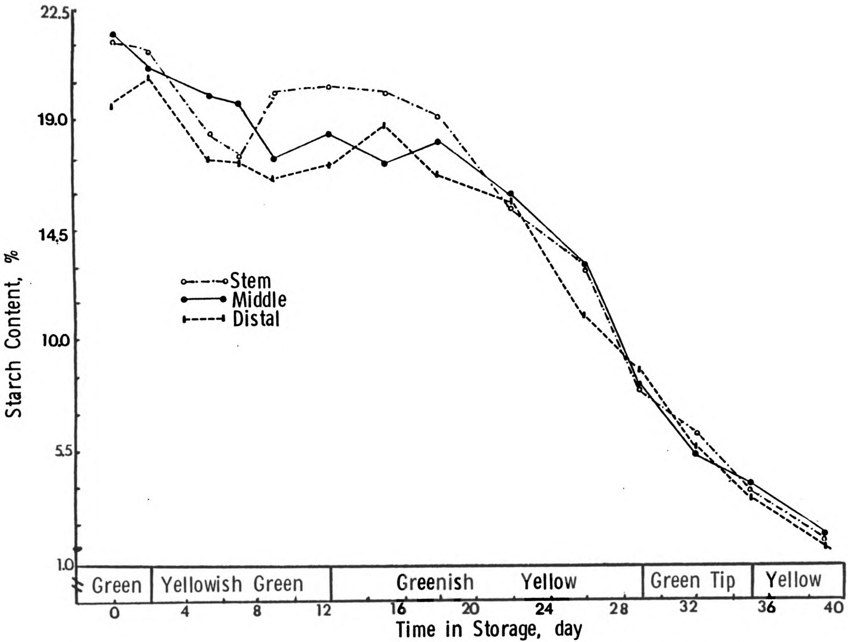

Fig 21. Starch content of three peripheral portions of banana during ripening.

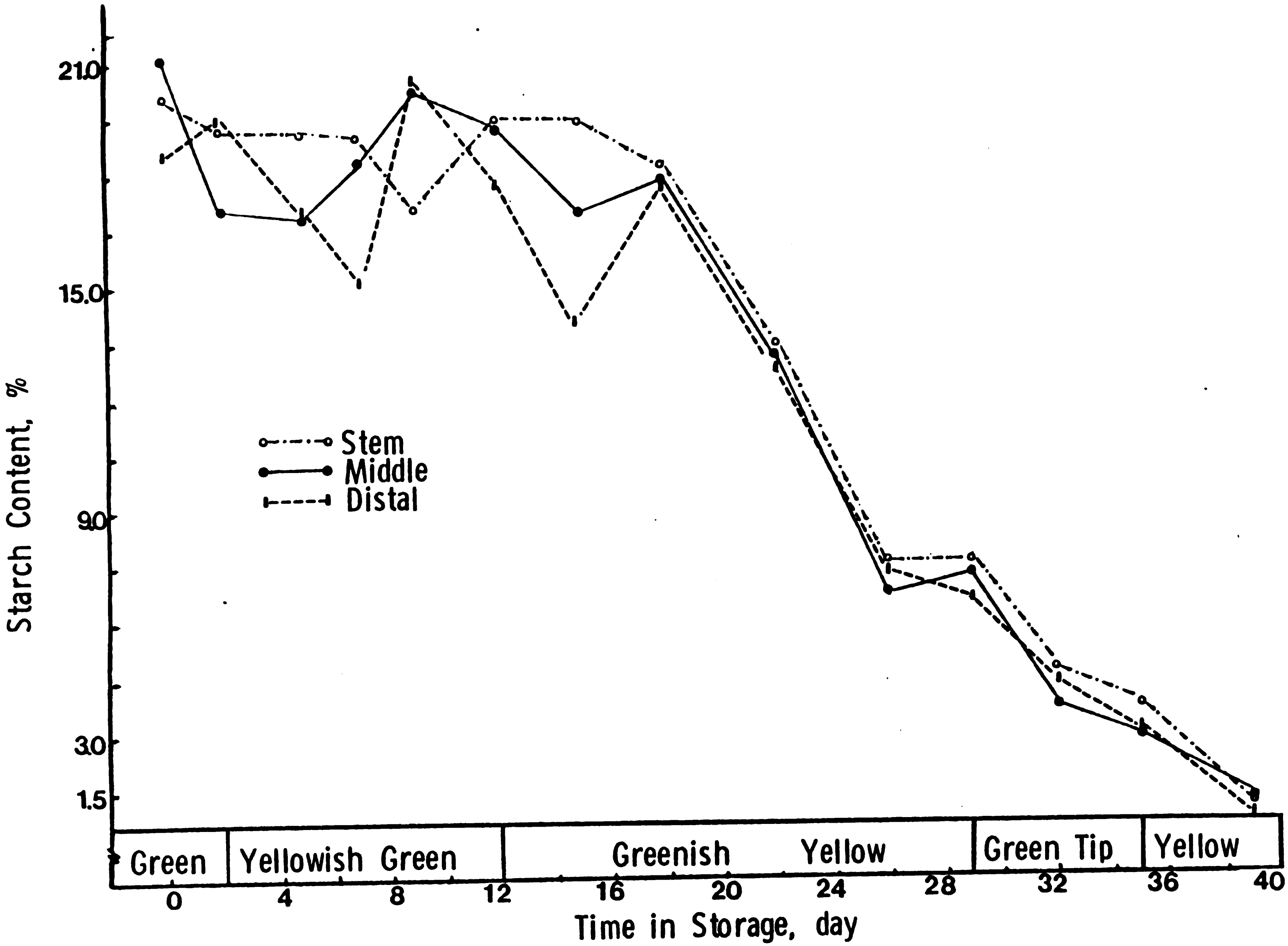

Fig 22. Starch content of three core portions of banana during ripening.

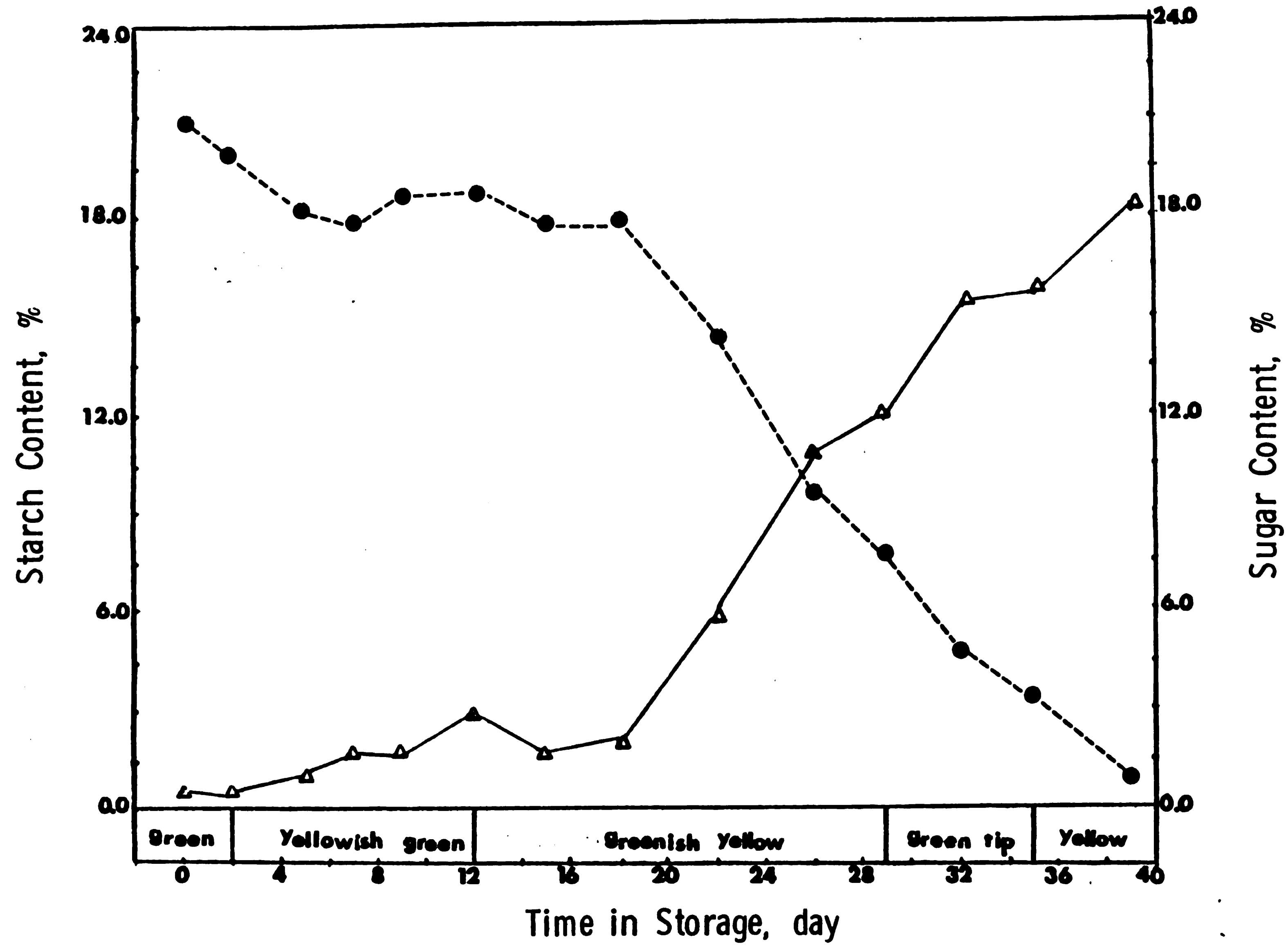

Fig 23. Total sugar (▲) and starch (●) contents in banana during ripening.

CONCLUSION

The moisture content in the core decreased with time while that of periphery increased. This indicated some moisture transfer from core to periphery during ripening. The increase in pulp-to-peel weight ratio must be due to osmotic transfer of moisture from peel to pulp.

The ascorbic acid content was low in green bananas, increased during the turning stage, reached a maximum just before the green-tip stage and was low again at full ripeness. The Cavendish banana at the green-tip stage contained almost twice as much ascorbic acid (about 31 mg/100 g) as fully ripe bananas (about 16 mg/100 g). There was some variation in ascorbic acid content among portions in the pulp.

The glucose to fructose ratio was approximately unity in each studied location of banana during ripening. Sucrose was the dominant sugar in both the periphery and the core, regardless of the stage of ripeness. There was more total sugar in the distal portion than that in the stem portion. The starch content in the periphery was greater than in the core. The distal portion had less starch than stem portion. There was a close correspondence between starch degradation and sugar accumulation, yet about 1.4% of total carbohydrate disappeared during ripening. In terms of carbohydrate metabolism the ripening of the core preceded that of the periphery; the distal portion ripened faster than the stem portion.

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LITERATURE CITED

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