A Study on the Activity of Selected Enzymes in the Ripening of Banana and Guava

BY

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Introduction

I. INTRODUCTION

A fruit is the edible, more or less succulent product of a tree or plant and consists of ripened seeds and adjacent tissues. These are appealing and attractive food which add texture to the diet while also providing valuable source of nutrients.

The ripening of fruits may be defined as the sequence of changes in colour, flavour and texture which lead to the state at which the fruit is acceptable to eat. Good quality is obtained when harvesting is done at the proper stages of maturity. Immature fruits when harvested will give poor quality and erratic ripening. In some cases if the produce is to be shipped to distant markets or stored to wait for a better price it should be picked in the mature but unripe stage.

Fruit ripening clearly involves the induction of a variety of enzymatically catalysed metabolic reactions. Enzymes associated with ripening of fruits increase in quantity either in consequence of changes in cell permeability or the synthesis of enzymes (Desai and Deshponde, 1978).

The results of many investigations have shown that the characteristic feature of fruit ripening is a change in texture, colour and sweetness. The most striking modification related to the softening process is the degradative changes in the cell wall components. Protopectin is converted to more soluble forms of pectic substances and the fruit firmness decreases. Softening during the ripening of many fruits is probably caused by changes in the activity of enzymes already present in cells or by de novo synthesis of these enzymes. Two enzymes pectin methyl esterase and polygalacturonase seem to be involved in this process (Houreneo, 1984).

During ripening acid phosphatase regulates respiration in fruits partly by holding sugars as sugar phosphates. The marked increase in the activity of acid phosphatase during ripening might be the result of the readily available substrate provided by the increased activities of both and ∝ - amylase and starch phosphorylase (Desai and Deshponde, 1978).

Marketability is largely determined by flavour and colour. Phenolic ~~subs~~ substances contribute in many fruits to both of these properties and hence the factors which determine the content of these substances whether as a group or individually are, from the economic stand point of prime importance. The production of phenolics is a consequence of the induction of enzymes by hormonal and environmental triggers. The level of phenolic compounds and the types which are present, are a function of these enzymes and also of those enzymes which destroy phenolics (Butt, 1980).

Banana (Musa paradisica) is an important and oldest fruit cultivated in India, ranking next to mango. It is one of the few fruits available through the year. Being rich in carbohydrates and minerals and also easily digestible, it is consumed by a majority of the population (Subrahmanyan, 1985). It is harvested in a nearly ripe state for home use, but for export they must be shipped unripe. During ripening the skin colour changes from dark to light green and greenish yellow to bright yellow. Meanwhile the pulp softens outwards from the core and from tip to stalk. If left too long the pulp becomes watery, the skin turns brown and finally the whole fruit rots away. Even in the tropics the quality of banana is improved if they are ripened properly at the right temperature and humidity (Samson, 1980).

Guava (Psidium guajava L.) occupies fourth position after Mango, Banana and Citrus in the country (Tandon et al., 1983). The round oval fruit is green yellow and shows a light yellow or pink pulp. It has a characteristic odour and is eaten fresh or cooked but is principally used for the preparation of jams.

Therefore, Banana and Guava were selected for the present study on the activity of selected enzymes in the ripening of fruits. The assay of enzymes was carried out at half ripe, full ripe and over ripe stages. The activity of pectic enzymes that help in softening of tissues, amylase and invertase in starch sugar transformation and acid phosphatase and polyphenol oxidase that are responsible for the phenolic content and therefore the colour and flavour of the fruits was assayed. It is hoped that the findings of the study may throw some light on the time of harvest and marketability of the selected fruits.

Review of Literature

II. REVIEW OF LITERATURE

The literature pertinent to the present Study on the Activity of Selected Enzymes in the Ripening of Banana and Guava are discussed under the following heads :-

I. Physico Chemical Changes associated with ripening :

1. Physical changes.
2. Chemical changes.
   - i Sugar content
   - ii Acidity
   - iii Volatile aroma constituents
   - iv Total soluble solids
   - v Pectin and
   - vi Ascorbic acid

II. Enzymatic Changes during ripening :

1. Pectic enzymes
2. Polyphenol oxidase
3. Enzymes involved in starch sugar transformation
4. Acid phosphatase
5. Enzymes related to gluconeogenesis in ripening
6. Alcohol dehydrogenase
7. Glycolytic enzymes associated with ripening.

III. Hormonal regulation of some hydrolytic and oxidative enzymes during ripening :

1. Physico chemical changes associated with ripening :

1. Physical changes :-

The readily apparent phenomena associated with the ripening of the majority of fruits include changes in weight, pulp : skin ratio, colour, texture, flavour, etc.

Dwarf cavendish variety of banana exhibited a concava growth curve and reached full maturity 130 days after inflorescence emergence. Fruit weight increase was maximum during the last four weeks of growth. (Thomas et al., 1983).

The pulp : skin ratio of banana increased from an initial value of 0.25 : 1.0 to 1.9 : 1.0 at full maturity (Thomas et al ., 1983).

Banana fruits kept at room temperature passed through four stages of maturity green, green-yellow, yellow-green and yellow. During this time the chlorophyll a and b contents decreased and the chlorophylls were not present in the ripe fruits. Total carotenoids decreased to half the original level by the green-yellow stage and then increased to their original level in the ripe fruit (Gross and Flugel, 1982).

Less mature guava fruits lost their green colour more slowly than more mature fruits. Post harvest changes in guava fruit of different maturity were studied. The fruits were soft at harvest and softened still more than less mature fruit during storage (Brown and Wills, 1983).

Changes of flavour during post harvest ripening typically result from an increase in sugar at the expense of reserve carbohydrate, a decrease in acids, which may be respired and a considerable increase in the production of volatile aroma compounds. (Burton, 1982).

2. Chemical Changes : -

i Sugar Content : -

The conversion of starch to sucrose, glucose and fructose was studied by Morroitt et al. (1981) and Morroitt et al.(1983) during ripening at 20°C of two Horn type and one French type plantains. Changes in these three varieties were similar and differed from those in cavendish banana. Starch degradation of plantain was slower than in banana and was not complete in over ripe fruits. Banana contained 1.0 per cent starch when fully ripe and none when over ripe whereas plantains contained about 9 per cent starch when fully ripe and 3 per cent when over ripe. Total sugar content continued to increase after full maturity and was still increasing in over ripe fruits. Sucrose comprised about 70 per cent of total sugars when plantains became fully yellow but this proportion fell to about 50 per cent when the fruits became over ripe. The ratio of glucose to fructose was approximately unity for banana and plantain at all stages of ripeness.

In guava reducing sugars mainly fructose and glucose increased slowly during the immature and mature stages and increased sharply at ripening upto the fully ripe stage. When fully ripe, fructose comprised 55.93 per cent and 58.28 per cent of the sugar in the white and pink guava fruits respectively. Sucrose and inositol present in small quantities also increased during ripening (Mowlah and Itoo, 1982).

ii Acidity :-

The loss of acidity which may accompany the ripening of fruit appears to result, atleast in part from the use of the acids as respiratory substrates via the Krebs cycle. Mowlah and Itoo, (1983) reported a fall in acidity in both white and pink guava varieties. The PH changed from 4.2 to 5.0 in the white variety and 4.0 to 5.2 in the pink variety over the three phases.

The metabolism of organic acids during ripening of banana was followed by incorporation of labelled organic acids in various fraction of fruits. It retained radio activity in organic acid fraction to the maximum extent which increased during ripening, thus indicating lesser utilization of organic acid in ripening (Satyan and Patwardhan, 1983). Burton (1982) found an increase in the acid content of banana during ripening accompanied by a fall in PH from about 5.4 ± 0.4 before the climacteric to about 4.5 ± 0.3 after the climacteric.

iii Volatile aroma compounds :-

In banana increased volatile production started on the day of the climacteric peak in respiration. Separation of the volatiles by gas chromatography showed mainly a few low boiling point substances in yellow-green fruit. Ripening to the full yellow stage was accompanied by an approximately 10 fold increase in the production of these and by increases in the production of high boiling point substances which in yellow green fruit was slight (Burton, 1982).

Banana ripened at 20°C or 30°C produced a large quantity of volatile compounds including isoamylacetate (Yoshioka, et al. 1982).

iv Total Soluble Solids :-

Tripathi et al. (1981) noted that there was an increase in total soluble solids during ripening of the banana varieties Bhos, Basari, Dwarf, Dudhia, MalBhog and Barsain.

v Pectin :-

Changes in pectic components during maturation, ripening and storage of guava at 20°C were examined. Total pectin content was 346-396 mg per 100g of fresh weight during maturation and 750-804mg per 100g of fresh weight during ripening. Protopectin predominated in mature and soluble pectin in ripening fruits (Mowlah and Itoo, 1983).

The relative levels of pectin in mature fruits of different varieties of banana varied from 0.41 per cent to 0.65 per cent, which further rose to the extent of 0.75 per cent to 0.85 per cent at the stage of ripening (Tripathi et al., 1981).

vi Ascorbicacid :-

A decrease in the ascorbic acid content during ripening of banana was noticed by Tripathi et al., (1983). Brown (1983) has reported that ascorbic acid content was retained during storage at 20-30°C for one week.

II Enzymatic Changes during ripening :-

1. Pectic enzymes :-

The edible part of fruit consists very largely of parenchyma with cell walls made up of arabinogalactan, rhamnogalactan, xyloglucan, glycoprotein and cellulose. The contigous cells being joined together by a layer (middle lamella) of arabinogalactan and rhamnogalactan derived from both adjacent cell walls.

The structure as described can be too hard to be edible even without the participation of strengthening tissue. Ripening of the fruit involves some degree of senescent change in the structure in which certain amount of degradation of cell wall occurs, rendering it sufficiently soft to be eaten (Burton, 1982).

Basic structure of pectin consists of long chains of polygalacturonic acid in which the carboxylic acid groups are partially esterified. The carboxylic acid groups also react with calcium forming calcium pectate an insoluble pectin, located in the middle lamella between adjacent cell walls and in the outer region of the cell walls. These protopectin present in the unripe fruit which is transformed enzymically to soluble pectin during the course of ripening.

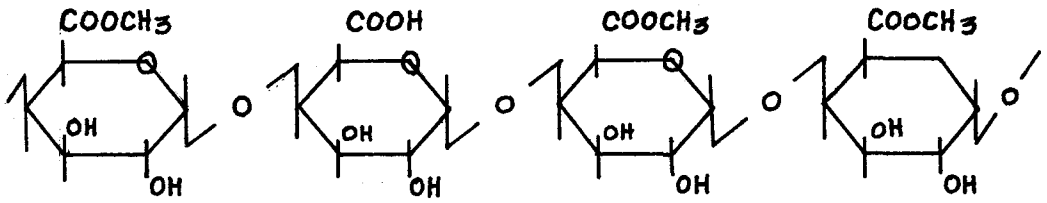
Cell wall degradation during ripening follows an increase in polygalacturonase and pectin methyl esterase activity. This probably results mainly from synthesis of the enzymes. Certainly there may be considerable protein synthesis during ripening (Burton, 1983).

The enzymes pectin methyl esterase and polygalacturonase and presumably protopectinase are thought to be active during fruit ripening and contribute to textural changes which take place during the postharvest storage of fruits. The overall reaction of these enzymes results in a hydrolytic cleavage of the methoxyl group from the chain followed by a hydrolytic splitting of the chain itself (Eskin, 1971) which is shown below.

Protopectin

Insoluble

↓ [Protopectinase]



Pectin

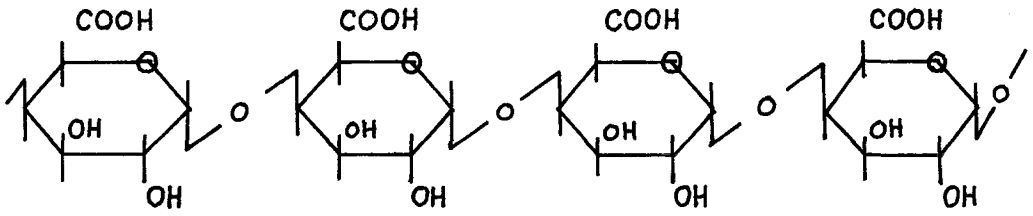
CH_3OH ← H_2O [Pectin methyl esterase]

↓

Pectinic acid

CH_3OH ← H_2O [Pectin methyl esterase]

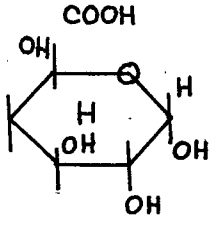
↓



Pectic acid

H_2O [Polygalacturonase]

↓



α-D-galacturonic acid

In guava polygalacturonase activity was not marked until full ripeness and increased thereafter. Pectin methyl esterase and cellulase activity also increased between maturation and ripening (Mowlah and Itoo, 1983).

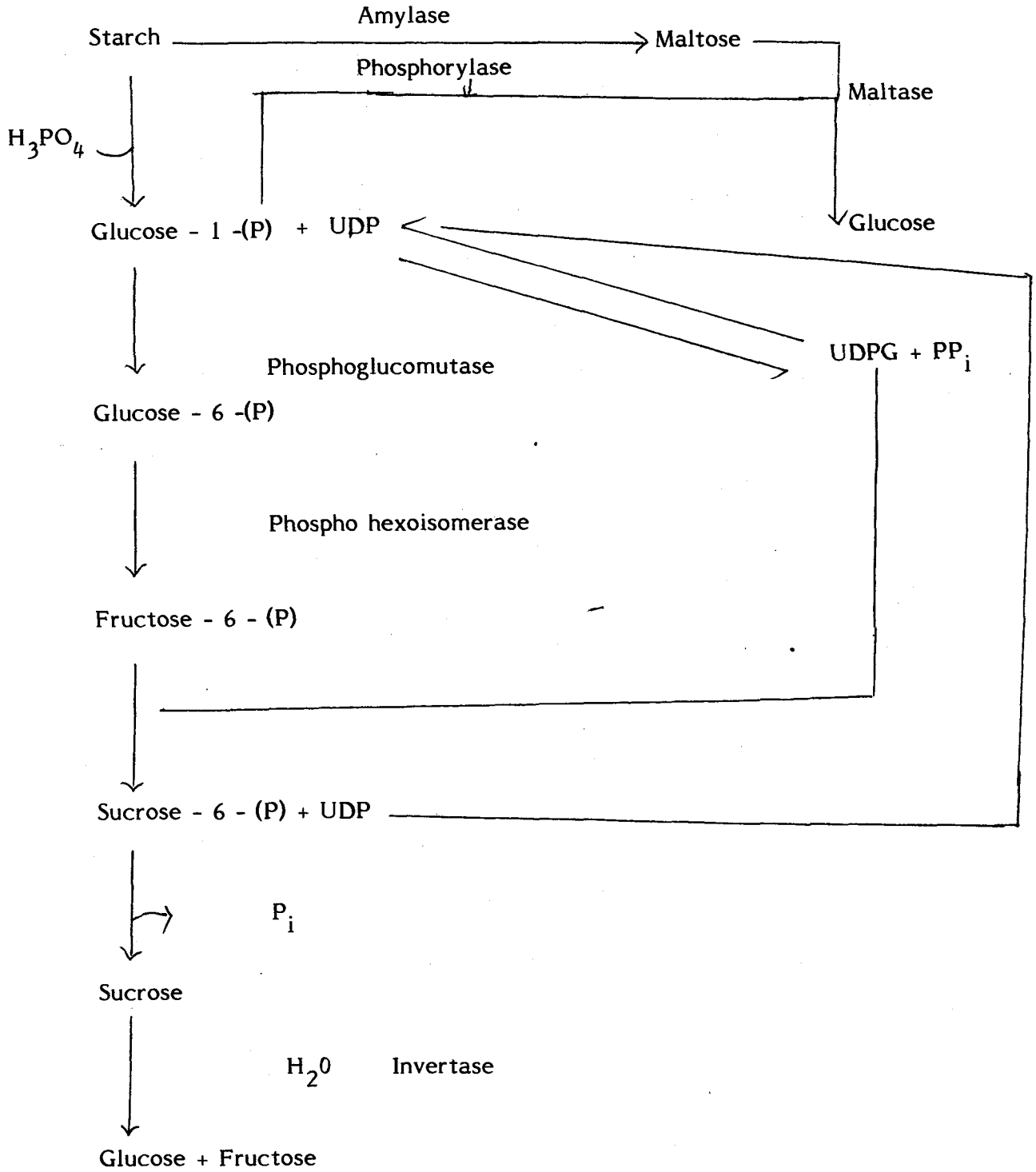
2. Polyphenol Oxidase :-

Polyphenol oxidase may play a vital role in the biochemical processes leading to fruit ripening and plant senescence in general as a means of controlling ehtylene biosynthesis. (Hulme, 1970).

During ripening the activity of polyphenol oxidase increases which may be associated with the decreased level of polyphenolics and thereby with the diappearance of astringency. Fruit of the white guava and the pink were examined at four stages viz. 26-29 per cent of full size, very hard and astrigent; 62-65 per cent of full size, hard, astringent and green, 81-88 per cent of full size, astringent and yellow; and full sized white to yellow. The phenolic compounds present in the guava were indentified as nontanninphenolics, tannin-phenolics, hydrolysable tannin, simple phenolics, non tannin flavans, condensed tannin, vanillin reactive phenolics, soluble leucoanthocyanin, and insoluble leucoanthocyanin. Changes in polyphenol oxidase activity were also recorded. The marked increase in this activity during ripening may be associated with disappearance of astringency (Mowlah and Itoo, 1982).

3. Enzymes involved in Starch Sugar transformation :-

Starch having been formed in the storage cells and tissues may become transformed into sugars particularly sucrose, glucose and fructose during the postharvest period. This change is largely dependent upon the conditions of storage such as temperature and time and upon the physiological state of the fruit. The conversion of starch to sugars may involve the possible metabolic scheme (Eskin et al.,1971).


Amylase
Starch
Maltose
Phosphorylase
Maltase
H_3PO_4
Glucose - 1 -(P) + UDP
Glucose
UDPG + PP_i
Phosphoglucomutase
Glucose - 6 -(P)
Phospho hexoisomerase
Fructose - 6 - (P)
Sucrose - 6 - (P) + UDP
P_i
Sucrose
H_2O
Invertase
Glucose + Fructose

Phosphatase and α-amylase activity in whole fruit of the banana Cv marmelo increased during the climacteric while phosphorylase decreased as starch degradation proceeded. Starch degradation had a different temperature coefficient than that of α - amylase activity when the storage temperature was raised from 20°C to 25°C and stopped when 25 per cent of the starch was still present. Activity of α-amylase in slices infiltrated with water increased before the onset of the climacteric as a result of injury and at the same time starch was being hydrolysed. Cycloheximide inhibited this induced activity increase but not starch break down or the increase of activity which occured during the climacteric (Chitarra and Lajolo 1981).

In ripening bananas starch degradation was accompanied by an increase in sucrose content, followed by glucose and fructose formation. Sucrose synthetase and invertase activities increased while UDP glucose pyrophosphorylase activity remained constant. These changes indicate that the conversion of starch to sucrose via. glucose-1-phosphate, UDP-glucose may be an important pathway for starch disappearance during ripening. When this fruit slices were infiltrated with labelled glucose-1-phosphate the label was incorporated into sucrose 3 times faster in climacteric compared with preclimacteric fruits. (Terra et al., 1983).

Guava sugar components and related enzymes at stages of fruit development and ripening were studied. Invertase activity increased during ripening and was greatest in fully ripe fruits. Amylase activity increased after maturation, again reaching a maximum in fully ripe fruits. Invertase activity was greater in the white variety than in the pink. Amylase in the pink variety was 2.1 times greater than in the white variety (Mowlah and Itoo 1982).

4. Acid phosphatase :-

Phosphatases as a class of enzymes are categorized according to substrate specificity and pH optima. It hydrolyses phosphoric acid esters making phosphate available for other metabolic processes.

Mature-green bananas stored at a constant 40°C in contrast with controls stored at 20°C showed a reduction in acid phosphatase activity and failed to ripen. If fruit was held for several hours at 40°C before being transferred to 20°C the acid phosphatase activity increased earlier than in ripening controls. Ethylene treatment also advanced the rise in activity at 20°C. However ethylene treatment at 40°C did not advance the start of ripening in fruit transferred to 20°. Seven acid phosphatase isozymes were shown to be present in mature green fruit and three more in fruit ripened at 20°C(but not in fruit held at 20°) (Yoshioka et al 1980). The activity of acid phosphatase increased progressively almost throughout the whole storage period of 35 days, the most rapid increase took place between the 21st day and 28 days of storage. An enhancement of about 3.9 to 4.9 fold of the initial level of acid phosphatase was observed during the ripening of the 3 cultivars (Desai and Deshponde, 1978).

5. Enzymes related to gluconeogenesis in ripening :-

Traces of phosphoenol pyruvate carboxykinase were noticed in Dwarf cavendish bananas throughout ripening and was confined to the soluble cytoplasm of the pulp. Succinic, malic, aconitic and α -keto glutaric acids inhibited, the enzyme's activity competitively and pyruvic acid inhibited it non-competitively. D-Glucose-6 phosphate D-Glucose-1 phosphate and DL-3-glycerophosphate activated the enzyme by decreasing the Km for phosphoenol pyruvate. The role of the enzyme was the accumulation of malic acid during ripening (Satyan and Patwardhan, 1984).

6. Alcohol dehydrogenase :-

Alcohol dehydrogenase activity markedly increased during ripening of banana fruit in association with the rise in ethylene, production and the respiration climacteric. The marked rise in alcohol dehydrogenase activity was followed by a sharp rise in ethanol formation in the tissue (Hyodo et al., 1983).

7. Glycolytic enzyme associated with ripening :-

The levels of the 6-glycolytic intermediates and the activity of phosphofructokinase were determined in Dwarf Cavendish banana at different stages of ripening (without ethylene stimulation) between harvest and senescence. There was a 2.3 fold increase in the level of fructose - 1, 6 - diphosphate between the preclimacteric and climacteric peak stages. Electrophoretic studies with the enzymes preparations of fruit at these two stages of ripening indicated the presence of two forms of phosphofructokinase at both stages. (Nair Darak, 1981).

III Hormonal regulation of some hydrolytic and oxidative enzyme :-

Abscisin 1 ppm or Indoleacetic acid 10 ppm markedly enhanced the activity of α - amylase, starch phosphorylase, acid phosphatase catalase and peroxidase in the fruit of the cultivars Pachabale, Rasabale and Rajbale held in polyethylene bags at 20° for upto 35 days. However gibberlic acid 50 ppm or kinetin 2ppm retarded the activities of all five enzymes. The fruit was sampled at weekly intervals. The activities of all enzymes were much lower in Rasabale than in the other two cultivars. (Desai et al., 1984).

Experimental Procedure

III EXPERIMENTAL PROCEDURE

The experimental procedure of the Study on the Activity of Selected Enzymes in the Ripening of Banana and Guava is detailed under the following headings :-

I. Collection of fruits :

II. Assay of enzymes :

a. Amylase

b. Invertase

c. Pectin methyl esterase

d. Polygalacturonase

e. Acid phosphatase

f. Polyphenol oxidase.

III. Pectin :

IV. Starch :

Collection of fruits :

Fruits necessary for the experiments were obtained from Tamil Nadu Agricultural University. The fruits selected for the experiment were Guava (Bapatla) and Banana Co 1. These two fruits were analysed at three different stages of ripening.

1. Half ripe
2. Full ripe
3. Over ripe

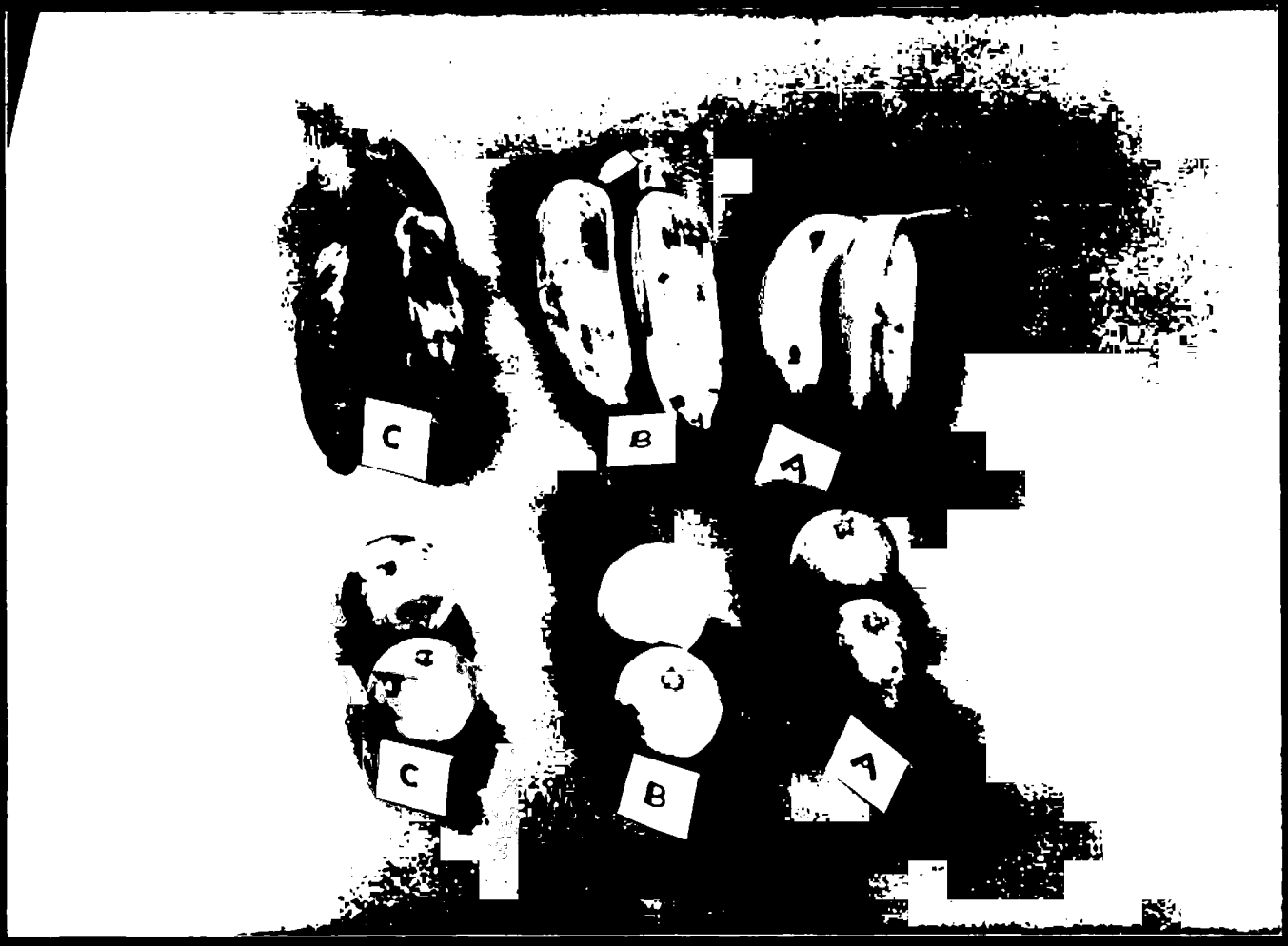


PLATE - I

(Banana) and 45 days (Guava)

The fruits harvested at 120 days of maturity were considered as half ripe stage. These fruits were kept at room temperature for ripening. Half ripe bananas were slight yellow in colour and guavas were light green in colour. Eight days after harvesting, the fruits yellow in colour were considered as ripe fruits. After thirteen days of harvesting the fruits which became delicate and very soft to touch were considered as 'over ripe' fruits. Plate-1 illustrates Banana and Guava in the three different stages (A = half ripe ; B = Full ripe ; C = Over ripe) of ripening.

II. Assay of enzymes :

The analysis was carried out in duplicates for each enzyme.

a. α -Amylase : (EC 3.2.1.1) Catalyses the endohydrolysis of 1,4 α - glycosidic linkages in polysaccharides containing three or more 1,4 α - linked D-glucose unit. During ripening it acts on starch and converts it into monisaccharides. It's activity was determined by the method of Mahadevan (1982). The details of the procedure are given in Annexure I. The amylase activity may be expressed as the amount of reducing groups released per gram of fresh weight of the tissue.

b. Invertase : (EC 3.2.1.26) Hydrolyses the terminal non-reducing β -D fructofuranoside residues in β -D fructofuranosides. Enzyme activity was determined by the method of Mahadevan (1982) as shown in Annexure II. Activity may be expressed as the amount of reducing groups released per gram of fresh weight of the tissue.

c. Pectin esterase : (EC 3.1.1.11) converts pectin into pectic acid. During this reaction carboxyl groups are released which decreases the pH. To maintain the pH, alkali is added. From the amount of alkali added the enzyme activity can be calculated as per the details of the procedure described in Annexure III.

Pectin esterase activity = PE U x 10^2/g of tissue.

$$= \frac{\text{ml of sodium hydroxide required x 1N NaOH}}{\text{gram of sample x Titre period in min.}} \times 10^2$$

d. Polygalacturonase :

(EC 3.2. 1.15) Catalyses the random hydrolysis of 1,4 α -D galactosiduronic linkages in pectate and other galacturonans. The activity of polygalacturonase was determined by the method of Mahadevan (1982). The details of the method are presented in Annexure IV. Protein content of the enzyme was estimated by the method of Lowry et al.,(1951).

e. Acid Phosphatase : (EC 3.1.3.2) Catalyses the hydrolysis of phosphate esters to phosphoric acid and alcohol. The amount of phosphoric acid produced during hydrolysis is a measure of enzyme activity. The liberated phosphoric acid containing the inorganic phosphorus was estimated by Fiske and Subbarow method (1925) as instructed in Annexure V. The specific activity of the enzyme may be expressed as the units of enzyme per mg of protein per minute.

f. Polyphenol Oxidase : (EC 1.10.3.1) The oxidative polymerization of phenol is primarily catalysed by polyphenol oxidase. The activity of this enzyme was estimated by the method of Mahadevan (1982). The details of the procedure for the same is presented in Annexure VI.

III. Pectin :

Pectin was estimated by the method of Pearson (1970).

IV. Starch :

Starch was determined by the method of Clegg (1956).

Results and Discussion

IV RESULTS AND DISCUSSION

The results obtained in the present investigation, on the Activity of Selected Enzymes in the Ripening of Banana and Guava are discussed under the following headings :

a. Activity of amylase and starch content of Banana and Guava during ripening,

b. Activity of invertase during ripening of Banana and Guava,

c. Activity of polygalacturonase during ripening of Banana and Guava,

d. Pectin esterase activity and the pectin content of Banana and Guava during ripening,

e. Ripening of Banana and Guava and the activity of acid phosphatase,

f. Activity of polyphenol oxidase in Banana and Guava during ripening.

a. Activity of amylase and starch content of Banana and Guava during ripening :

The activity of amylase and the starch content in the banana and guava at three different stages of ripening namely, half ripe, full ripe and over ripe are presented in Table I.

TABLE I

ACTIVITY OF AMYLASE AND STARCH CONTENT OF BANANA AND GUAVA

Stages	BANANA		GUAVA	
	* Activity of amylase (mg of reducing groups relased per gram)	* Starch (Percentage dryweight)	* Activity of amylase (mg of reducing groups relased per gram)	* Starch (Percentage dryweight)
Half ripe	3.40	56.70	3.88	40.05
Full ripe	5.92	34.20	5.32	27.90
Over ripe	4.56	18.90	5.16	19.35

* Mean of the duplicates

It is evident from Table I, that the activity of amylase was 3.40, 5.92, and 4.56mg of reducing groups relased per gram of tissue in the half full and over ripe banana respectively and it was found to be 3.88, 5.32 and 5.16 for the above mentioned stages respectively in guava.

The activity of amylase was found to be increased in the full ripe guava by 2.52mg of reducing groups relased per gram of tissue over and above that found in the half ripe banana. But it had decreased in the over ripe banana. These results are in agreement with those of Desai and Deshponde, (1978).

A similar trend in the activity of amylase was found in guava too. The activity of the enzyme was found to be increased from 3.88mg of reducing groups per gram

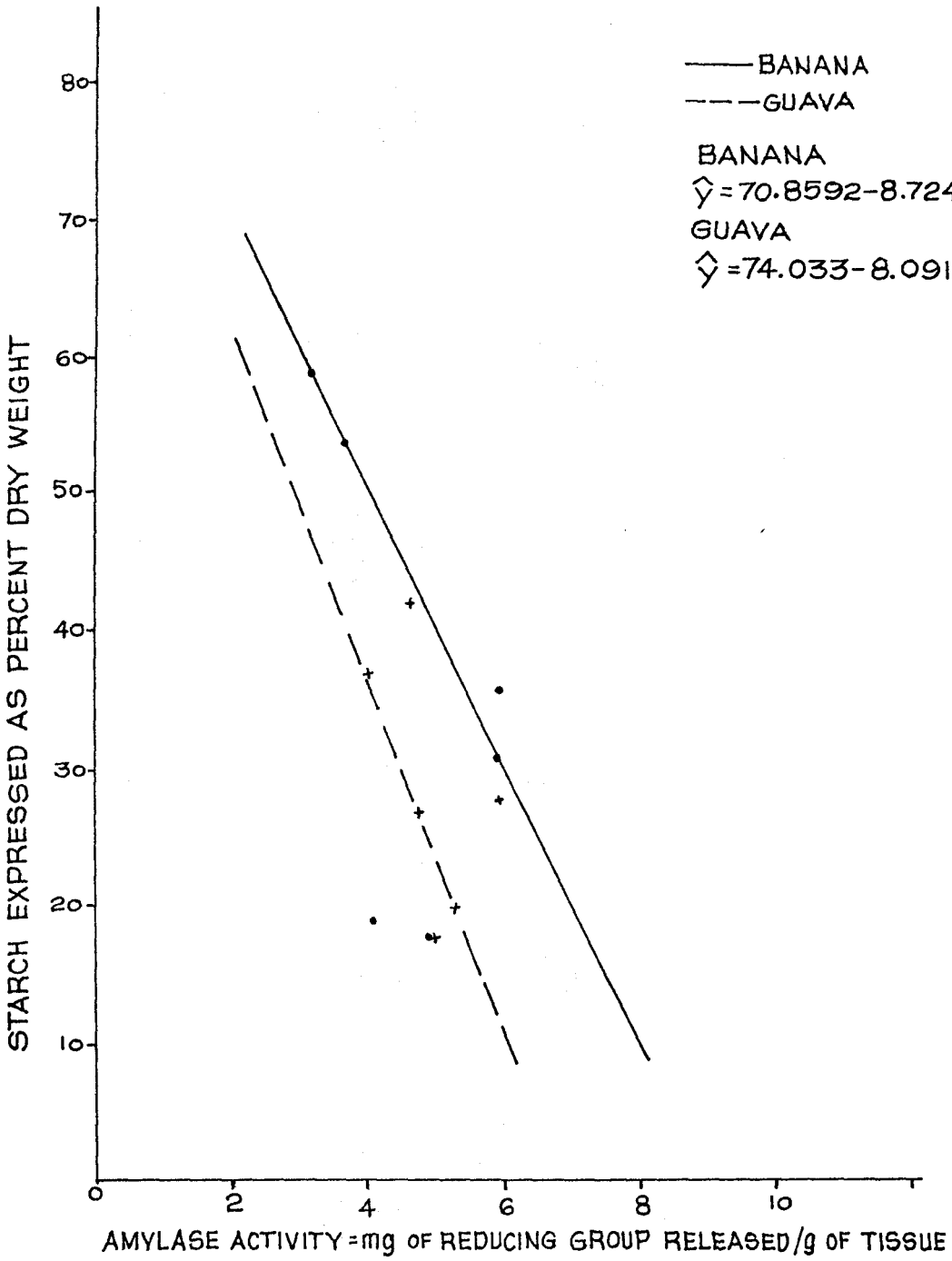


Figure.1 :- CORRELATION BETWEEN ACTIVITY OF AMYLASE AND STARCH IN BANANA AND GUAVA DURING RIPENING

of tissue in the half ripe stage to 5.32 in the full ripe stage and then decreased to 5.16 in the over ripe stage. Mowlah and Itoo, (1980) have also reported a similar trend in the activity of amylase in guava during ripening.

The substantial increase in the relative activity of amylase in both the fruits during ripening might be due to increased synthesis of this enzyme in response to the increased demand for the oxidisable substrates in the intensely respiring tissue, like a ripeining tissue as suggested by Desai and Desh ponde (1978).

The starch content of the banana was 56.70, 34.20 and 18.90 per cent dry weight in the half ripe, full ripe and over ripe stage respectively. While it was found to be 40.05, 27.90 and 19.35 for guava in the above mentioned stages respectively. In both the fruits a significant fall in the starch content was noticed with the progress of ripening. This was more evident in banana in which the starch content got reduced to 18.9 per cent of dry weight in the over ripe stage from 56.7 per cent of dry weight in the half ripe stage, in a span of just thirteen days.

This decrease in the starch content of the two fruits selected for the study might be due to the hydrolysis of starch which is one of the most important changes that characterises ripening of some climacteric fruits.

A negative correlation ($r = -0.55$ for banana and $r = -0.76$ for guava) between the activity of amylase and the starch content was observed in guava and banana as illustrated in Figure 1.

b. Activity of invertase during ripening of Banana and Guava :-

The activity of invertase during ripening of banana and guava is presented in Table II and in Figure 2.

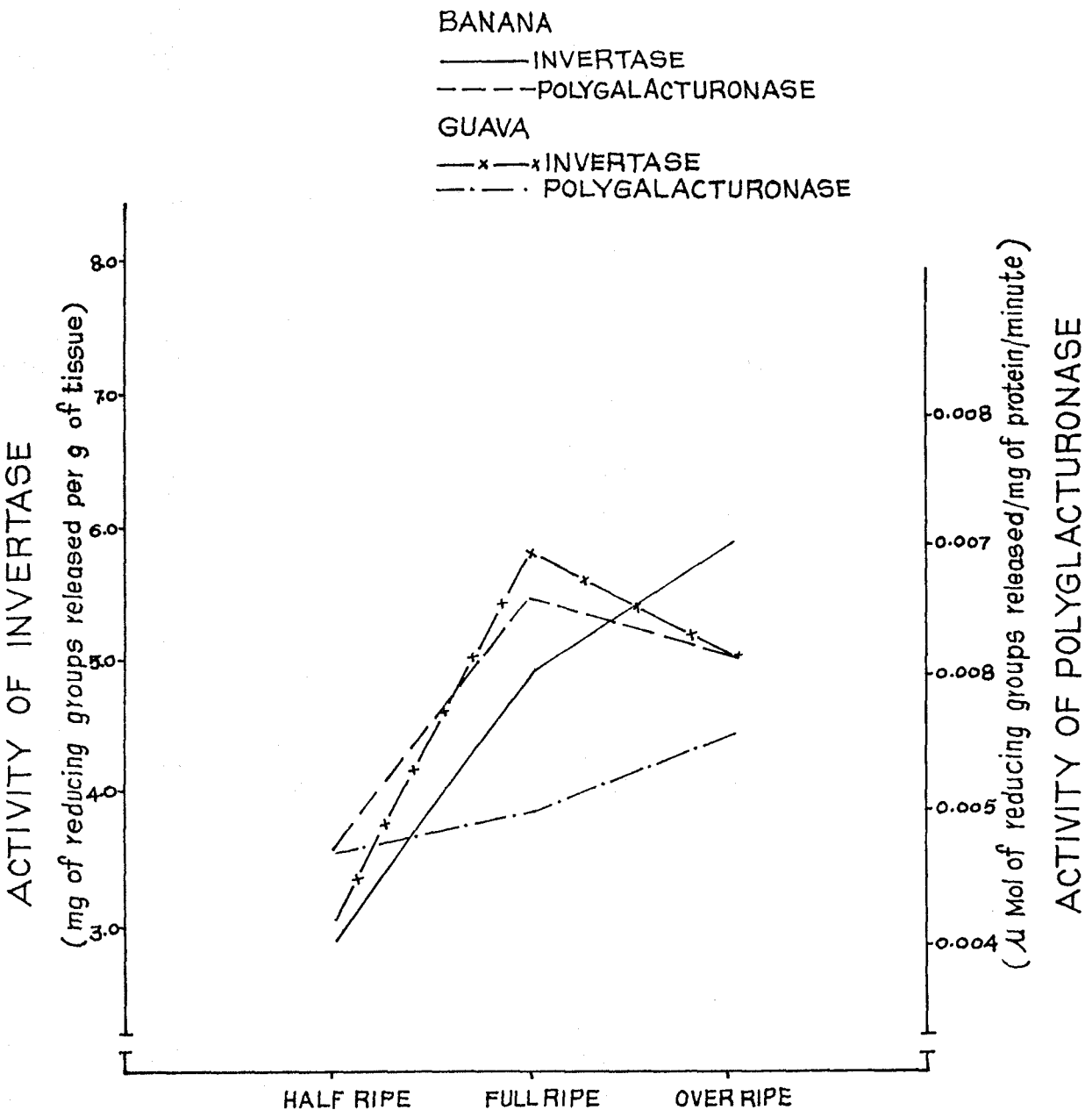


Figure.2:- ACTIVITY OF INVERTASE AND POLYGALCTURONASE DURING RIPENING OF BANANA AND GUAVA

TABLE II

ACTIVITY OF INVERTASE DURING RIPENING OF BANANA AND GUAVA

Stages	BANANA	GUAVA
	* Activity of invertase (mg of reducing groups relased per gram)	* Activity of invertase (mg of reducing groups relased per gram)
Half ripe	3.60	3.12
Full ripe	5.52	5.92
Over ripe	5.16	5.16

* Mean of the duplicates.

The relative activity of invertase in banana was found to be 3.60, 5.52 and 5.16mg of reducing groups released per gram of tissue in the half, full and over ripe. As in guava, in banana too the activity of invertase was found high in the full ripe fruit than in the half ripe and over ripe fruit.

Terra et al. (1983) have also noticed a similar change in the activity of invertase during ripening of banana.

Table II shows that the activity of invertase was 3.12, 5.92 and 5.16mg of reducing groups released per gram of tissue in half ripe, full ripe and over ripe guava respectively. A significant increase in the activity of invertase from 3.12 in

the half ripe to 5.92mg of reducing groups released per gram of tissue in the full ripe guava was noticed which was found to be 5.16mg of reducing groups released per gram of over ripe tissue indicating a fall in this stage.

The results are in agreement with the report of Mowlah and Itoo, (1982) who have found a parallel trend in the activity during ripening of guava.

c. Activity of Polygalacturonase during ripening of Banana and Guava :-

The activity of polygalacturonase of banana and guava was determined at half ripe, full ripe and over ripe stages and the values are given in Table III. Figure 2 illustrates the activity of Polygalacturonase in banana and guava during ripening.

TABLE III

ACTIVITY OF POLYGALACTURONASE DURING RIPENING OF BANANA AND GUAVA

Stages	BANANA	GUAVA
	* Activity of Polygalacturonase (Micromole of reducing groups released per mg of protein per minute)	*Activity of polygalacturonase (Micro mole of reducing groups released per mg of protein per minute)
Half ripe	0.0042	0.0046
Full ripe	0.0059	0.0049
Over ripe	0.0068	0.0055

* Mean of the duplicates.

As depicted in Table III the activity of Polygalacturonase was found to be 0.0042, 0.0059 and 0.0068 micromoles of reducing groups released per mg of protein per minute in banana and 0.0046, 0.0049 and 0.0055 micromoles of reducing groups released per mg of protein per minute in guava of the half, full and over ripe fruits respectively.

An increase in the activity of polygalacturonase from 0.0042 micromole of reducing groups released per mg of protein per minute in half ripe to 0.0059 in the full ripe banana was noticed and it further increased to 0.0068 micromole of reducing groups released per mg of protein per minute in the over ripe fruit.

In guava too, the Polygalacturonase activity was maximum in the over ripe stage (0.0055 micromole of reducing groups released per mg of protein per minute) when compared to the half ripe (0.0046 micromole of reducing groups released per mg of protein per minute) and full ripe (0.0049 micromole of reducing groups released per mg of protein per minute) stages.

With the increase in Polygalacturonase activity the fruits too became more and more soft indicating a positive relationship between the activity of the enzyme and the softening of the fruit during ripening.

d. Pectin ~~methyl~~ esterase activity and pectin content of Banana and Guava during ripening :-

Pectin content and pectin ~~methyl~~ esterase activity of banana and guava during ripening are given in Table IV.

TABLE IV

PECTIN ESTERASE ACTIVITY AND THE PECTIN CONTENT OF BANANA AND GUAVA DURING RIPENING :

Stages	BANANA		GUAVA	
	* Activity of pectinesterase (PEU x 10^2/g)	*Pectin (Percentage dryweight)	* Activity of pectinesterase (PEU x 10^2/g)	*Pectin (percentage dryweight)
Half ripe	1.133	2.3	0.866	1.70
Full ripe	1.733	1.7	1.665	1.20
Over ripe	1.933	1.3	1.466	0.85

* Mean of the duplicates.

The Pectin esterase activity of half ripe, full ripe and over ripe fruits as shown in Table IV was found to be 1.133, 1.733 and 1.933 PEU x 10^2 per gram of tissue respectively. While the pectin content of banana was found to be 2.3, 1.7 and 1.3 per cent of dry weight for the above indicated stages.

A similar trend in the activity of this enzyme and the pectin content was found in guava also during ripening. As shown in Table IV, the pectin content was found to decrease with the steady increase in the pectin esterase activity as the ripening process progressed. Figure 3. shows the relationship between pectin esterase

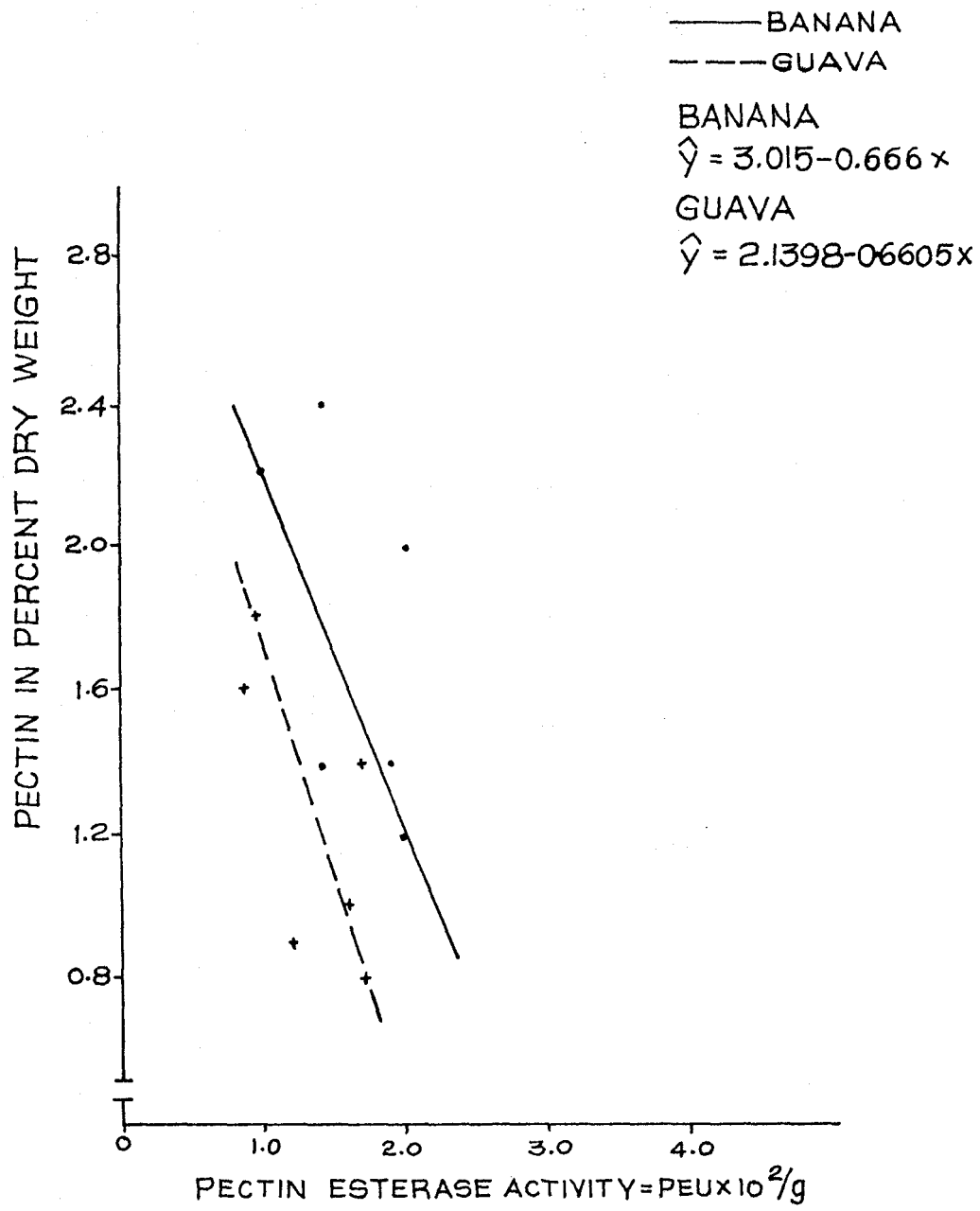


Figure.3 :- CORRELATION BETWEEN ACTIVITY OF PECTIN ESTERASE AND PECTIN IN BANANA AND GUAVA DURING RIPENING

activity and the pectin content. A negative correlation (r = -0.65 for banana, r= -0.68 for guava) was found between the pectin esterase activity and the pectin content of banana and guava during ripening.

These findings are in agreement with those of Pal and Selvaraj (1979). Along with the changes in the activity of pectin esterase and the pectin content, progressive softening of the fruits was also noticed indicating a close association between textural changes and pectin esterase activity while ripening as has been reported (Pal and Selvaraj, 1979) in a number of fruits.

e. Ripening of Banana and guava and the activity of acid phasphatase :

The activity of acid phosphatase in banana and guava during ripening is presented in Table V.

TABLE V

ACTIVITY OF ACID PHOSPHATASE IN BANANA AND GUAVA DURING RIPENING

Stages	BANANA	GUAVA
	* Specific activity (unit of enzyme per mg of protein per minute at 37°C)	* Specific activity (Unit of enzyme per mg of protein per minute at 37°C)
Half ripe	9.3×10^{-4}	1.0×10^{-3}
Full ripe	1.0×10^{-3}	1.1×10^{-3}
Over ripe	1.2×10^{-3}	1.3×10^{-3}

* Mean of the duplicates.

The specific activity of acid phosphatase in banana during ripening was 9.3×10^{-4}, 1.0×10^{-3} and 1.2×10^{-3} in the half, full and over ripe fruit respectively and it was 1.0×10^{-3}, 1.1×10^{-3} and 1.3×10^{-3} respectively in the half, full and over ripe guava.

A marked increase in the activity of this enzyme was noticed during ripening of both the fruits. This might be, as reported by Desai and Deshponde (1978) due to readily available substrates provided by the increased activities of both α- amylase and starch phosphorylase.

f. Activity of Polyphenol oxidase in Banana and Guava during ripening :-

Table VI gives the activity of Polyphenol oxidase in banana and guava during ripening. This is illustrated in Figure 4.

TABLE VI

ACTIVITY OF POLYPHENOL OXIDASE IN BANANA AND GUAVA DURING RIPENING

Stages	BANANA	GUAVA
	* Activity of enzyme (unit per mg of protein per minute)	* Activity of enzyme (Unit per mg of protein per minute)
Half ripe	6.808	13.400
Full ripe	15.035	20.665
Over ripe	19.265	23.945

* Mean of the duplicates.

one unit of pholyphenol oxidase activity = 0.001 change in absorbance per minute.

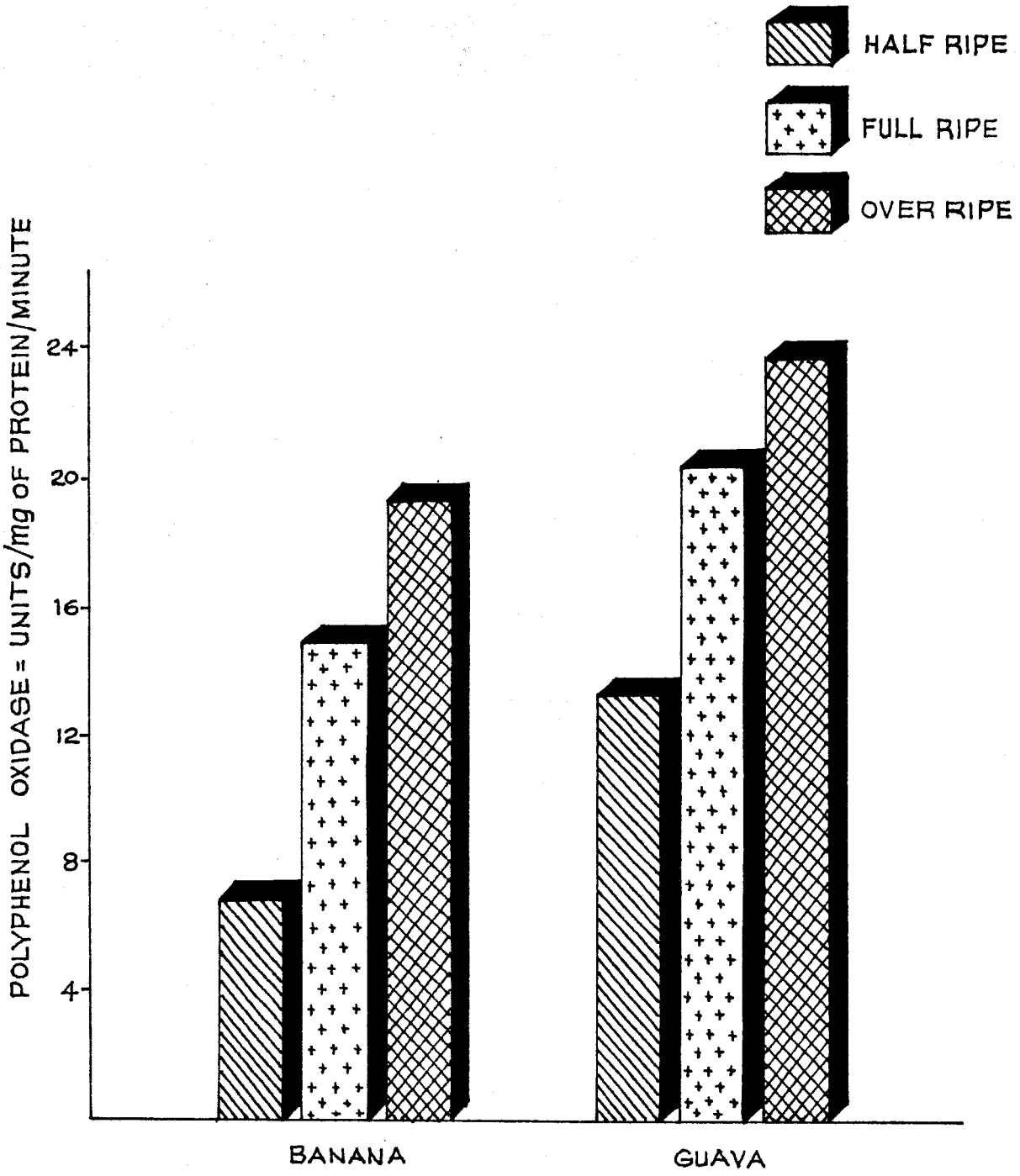


Figure.4 :- ACTIVITY OF POLY PHENOL OXIDASE IN BANANA AND GUAVA DURING RIPENING

As seen from Table VI, the polyphenol oxidase of banana in half ripe, full ripe and over ripe fruit was 6.808, 15.035 and 19.265 units per mg of protein per minute and that in guava was 13.400, 20.665 and 23.945 units per mg of protein per minute during the above mentioned stages.

A marked increase in the activity of polyphenol oxidase in both the fruits during ripening was noticed.

These findings in guava are in agreement with those of Mowlah and Itoo (1982) who found a marked increase in the activity of polyphenol oxidase during ripening. This may be associated with the decreased level of polyphenolics.

Summary and Conclusion

V. SUMMARY AND CONCLUSION

The present investigation was carried out on the activity of selected enzymes in the ripening of Banana and Guava. Banana Co 1 and Guava(Bapatla) were obtained from Tamil Nadu Agricultural University and were analysed to study the activity of amylase, invertase, pectin esterase, polygalacturonase, acid phosphatase and polyphenol oxidase and the levels of pectin and starch at half ripe, full ripe and over ripe stages. The fruits harvested at 120 days of maturity were considered as half ripe and 8 days after harvesting the fruits yellow in colour were considered as ripe fruits, whereas the fruits after 13 days of harvesting were considered as over ripe.

The activity of amylase was found to be 3.40, 5.92 and 4.56 mgof of reducing groups released per gram of tissue in the half, full and over ripe banana respectively and it was found to be 3.88, 5.32 and 5.16 for the above mentioned stages respectively in guava. The activity of amylase was found to be maximum in the full ripe fruit and then it decreased in the over ripe stage in both the fruits.

The starch content of the banana was 56.7, 34.2 and 18.9 per cent dry weight in the half, full and over ripe stages respectively, while it was found to be 40.05, 27.90 and 19.35 for guava in the above mentioned stages respectively. In both the fruits a significant fall in the starch content was noticed with the progress of ripening. This might be due to the hydrolysis of starch which is one of the most important changes that characterises ripening of some climacteric fruits. A negative correlation ($r = -0.55$ for banana and $r = -0.76$ for guava) between the activity of amylase and the starch content was observed in guava and banana.

The activity of invertase in banana was found to be 3.60, 5.52 and 5.16 mg of reducing groups released per gram of tissue in the half, full and over ripe stages. In guava it was found to be 3.12, 5.92 and 5.16 mg of reducing groups released per gram of tissue in half, full and over ripe stages respectively. In both the fruits the activity of invertase was found high in the full ripe stage when compared to the other two stages studied.

The activity of polygalacturonase increased from 0.0042 micromole of reducing groups released per mg of protein per minute in half ripe to 0.0059 in the full ripe banana and it further increased to 0.0068 micro mole of reducing groups released per mg of protein per minute in the over ripe fruit. In guava too, the polygalacturonase activity was maximum in the over ripe stage (0.0055 micro mole of reducing groups released per mg of protein per minute) when compared to the half ripe (0.0046 micro mole of reducing groups released per mg of protein per minute) and full ripe (0.0049 micro mole of reducing groups released per mg of protein per minute) stages. A positive relationship between the activity of the enzyme and the softening of the fruit during ripening was noticed.

The pectin esterase activity of half ripe, full ripe and over ripe banana was found to be 1.133, 1.733 and 1.933 PEU x 10^{2}/g respectively, while the pectin content of banana was found to be 2.3, 1.7 and 1.3 per cent dry weight for the above indicated stages. The ripe guava (1.665 PEU x 10^{2}/g) showed maximum activity of pectin esterase when compared to the half ripe (0.8661 PEU x 10^{2}/g) and over ripe (1.4661 PEU x 10^{2}/g) fruits. The pectin content of both the fruits was found to decrease with the ~~study~~ steady increase in the pectin esterase activity as the ripening process progressed.

The specific activity of acid phosphatase in banana during ripening was 9.3×10^{-4}, 1.0×10^{-3} and 1.2×10^{-3} in the half, full and over ripe fruit respectively and it was 1.0×10^{-3}, 1.1×10^{-3} and 1.3×10^{-3} respectively in the half, full and over ripe guava. The marked increase in the activity of this enzyme might be the result of the readily available substrates provided by the increased activities of both α -amylase and starch phosphorylase.

The activity of polyphenol oxidase of banana in half, full and over ripe fruit was 6.808, 15.035 and 19.265 units per mg of protein per minute and that in guava was 13.400, 20.665 and 23.945 units per mg of protein per minute during the above mentioned stages. There was a marked increase in the activity of polyphenol oxidase in both the fruits during ripening.

The present investigation revealed that the activity of the selected enzymes increased with the ripening process. Therefore it is recommended that the fruits may be harvested at half ripe stage for marketing.

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Appendices

ANNEXURE I

ESTIMATION OF AMYLASE (MAHADEVAN, 1982)

Preparation of the enzyme extract :

Weighed the tissues, cut into pieces, transferred to a blender and added chilled 20% aqueous glycerol. Ground at low speed for 2 - 3 minutes. Filtered the extract through three layers of cheese cloth to remove the pulp. The clear extract was used as enzyme source,

Reagents :

1. 20 per cent Aqueous glycerol.
2. 1.0 per cent Starch solution.
3. 1M acetic acid - sodium acetate buffer pH 6.
4. Copper Reagent A.

Dissolved 25g of sodium carbonate (anhydrous) 25g Rochelle Salt (sodium potassium tartarate) 20g of sodium carbonate and 200g of sodium sulphate (anhydrous) in 800ml of water and diluted to one litre with water. Stored at room temperature (A sediment may form after a few days which may be filtered off before use).

5. Copper reagent B.

15 per cent copper sulphate. $7H_20$ containing one or two drops of concentrated sulphuric acid per 100ml. Distilled water was used to dissolved the chemical.

6. Arsenomolybdate colour reagent :

Dissolved 25g of ammonium molybdate in 450ml of distilled water, added 21ml of concentrated sulphuric acid and mixed. Added 3g of sodium arsenate. $7H_2O$ dissolved in 25ml of water, mixed and placed in an incubator at 37°C for 24 - 48 hours. This reagent should be stored in a glass stoppered brown bottle and is stable for a few months.

Methods :

Placed 5.0ml of the crude enzyme extract in a 100ml flask. Added 10ml of 1M acetic acid - sodium acetate buffer pH6 and 5.0ml of 1.0 per cent starch. Incubated the reaction mixture at 37°C for 24 hours. Pipetted out aliquots of 1.0ml of the reaction mixture and stopped the reaction by the addition of 1.0ml of a mixture (prepared on the day of use) of 25 parts of reagent A to one part of reagent B and mixed the solution. Heated the tubes in a boiling water bath for 20 minutes. Cooled the tubes under a running tap and added one ml of arsenonmolybdate reagent to each tube. The colour develops very rapidly. Diluted the mixture to the mark (25ml) after 15 minutes and measured the absorbance of the solution in a colorimeter at 500nm. Reagent blank was used to adjust the absorbance 0. Standard graph was prepared from glucose. Enzyme activity was expressed as mg of reducing groups released per gram of tissue.

ANNEXURE II

ESTIMATION OF INVERTASE (MAHADEVAN, 1982)

Preparation of enzyme extract :-

Method of preparation was similar to that of amylase.

Reagents :-

1. Aqueous sucrose solution 2.5 percent.
2. 1 M acetic acid - sodium acetate buffer pH5.
3. Copper Reagent A.

Dissolved 25 g of sodium carbonate (anhydrous) 25g of Rochelle Salt (sodium potassium tartarate), 20g of sodium carbonate and 200g of sodium sulphate (anhydrous) in 800ml of water and diluted to one litre with water. Stored at room temperature (A sediment may form after a few days which may be filtered off before use).

4. Copper reagent B.

15 per cent copper sulphate. $7H_20$ containing one or two drops of concentrated sulphuric acid per 100ml. Distilled water was used to dissolved the chemical.

5. Aresenomolybdate colour reagent.

Dissolved 25g of ammonium molybdate in 450ml of distilled water, added 21ml of concentrated sulphuric acid and mixed. Added 3g of sodium arsenate. $7H_20$ dissolved in 25ml of water, mixed and placed in an incubator

at 37°C for 24-48 hours. This reagent should be stored in a glass stoppered brown bottle and is stable for a few months.

Method :

Placed 5.0ml of the crude enzyme extract in a 100ml flask. Added 10ml of 1 M acetic acid - sodium acetate buffer pH5 and 5.0ml of 2.5 per cent sucrose. Incubated the reaction mixture at 37°C for 24 hours. Pipetted out aliquots of 1.0ml of the reaction mixture and stopped the reaction by the addition of 1.0ml of a mixture (prepared on the day of use) of 25 parts of reagent A to one part of reagent B and mixed the solution. Heated the tubes in a boiling water bath for 20 minutes. Cooled the tubes under a running tap and added one ml of arsenomolybdate reagent to each tube. The colour develops very rapidly. Diluted the mixture to the mark (25ml) after 15 minutes and measured the absorbance of the solution in a colorimeter at 500nm. Reagent blank was used to adjust the absorbance 0. Standard graph was prepared from glucose. Enzyme activity was expressed as mg of reducing groups released per gram of tissue.

ANNEXURE III

ESTIMATION OF PECTIN ESTERASE (MAHADEVAN, 1982)

Preparation of pectic enzymes :-

Weighed 20g of tissue, cut into pieces of 1-2cm, transferred to blender or mortar and added 0.15m sodium chloride at the rate of 3-5ml for every gram of tissue. Blended for about 3-5 minutes, filtered through two layers of cheese cloth, centrifuged at 2,000g for about 30 minutes and dialysed the supernatant in cellophane tubing against several volumes of distilled water for about 24 hours at 2-4°C. Changed the water twice during dialysis. Dialysed solution was used as the crude pectic enzyme. Added a few drops of toluene and stored at 4°C. (Do not use the enzyme extract if it is more than one week old).

Reagents :-

1. One per cent pectin dissolved in 0.15M sodium chloride.
2. 0.02N sodium hydroxide prepared from 1N sodium hydroxide on the day of use.

Method :

Pipetted out 20ml of pectin solution in a 50ml beaker, adjusted the pH to 7.0 with a pH meter. Added 10ml of the enzyme solution and adjusted the pH immediately to 7.0 in the pH meter by adding 1N sodium hydroxide. This was the zero time. Placed the beaker in a water bath at 30°C. Maintained control as heated enzyme. The enzyme substrate mixture can be (was) incubated for 24 hours and the pH was readjusted with 0.02N sodium hydroxide.

ANNEXURE IV

ESTIMATION OF POLYGALACTURONASE (MAHADEVAN, 1982)

Reagents :

1. 0.15M sodium chloride.
2. Toluene
3. Pectic acid 0.714 per cent dissolved in sodium acetate acetic acid buffer pH 5.2.
4. 1M Sodium carbonate (anhydrous).
5. 0.1N iodine solution.
6. 2M Sulphuric acid.
7. 0.1N Sodium thiosulphate.

Method :

Added 15ml of the enzyme adjusted to pH 5.2 to 35ml of pectic acid dissolved in buffer pH 5.2 placed in Ehrlenmeyer flask to give a final concentration of the substrate to 0.5 per cent. Incubated the mixture in the water bath. Removed the aliquots of 5.0ml at one hour interval and added to 0.9ml of 1M sodium carbonate in a glass stoppered Ehrlenmeyer flask, followed by 5.0ml of 0.1N iodine solution. After exactly 20 minutes acidified the reaction mixture with 2.0ml of 2M sulphuric acid. Titrated the residual iodine with 0.1N sodium thiosulphate. Calculated the micromole of reducing groups liberated from pectic acid from a standard curve prepared from glucose which has the same value as that of galacturonic acid monohydrate. Enzyme activity was expressed as micromole of reducing groups liberated from pectic acid per minute per mg of protein.

ANNEXURE V

ESTIMATION OF ACID PHOSPHATASE (FISKE AND SUBBAROW 1925).

Principle :-

This enzyme catalyses the hydrolysis of phosphate ester to phosphoric acid and alcohol. The amount of phosphoric acid produced during hydrolysis is a meausre of enzyme activity. The liberated phosphoric acid containing the inorganic phosphorus is estimated by Fiske and Subbarow method.

Reagents :-

1. Citrate buffer of pH 5.6
2. Substrate :- 0.1M solution of β-glycerophosphate (3.154g per 100ml of water).
3. 15 per cent Trichloro acetic acid.
4. Ammonium Molybdate I.
5. Ammonium molybdate II.
6. Aminonaphthol sulphonic acid reagent.
7. 0.2M solution of Magnesium acetate.
8. Standard phosphate solution :

35.1mg of potassium dihydrogen posphate was dissolved in water. Added 1.0ml of 10N sulphuric acid and made upto 100ml with water.

10.0ml of the above solution was diluted to 100ml with water. 1.0ml of this solution contains 8 microgram of phosphorus.

9. Preparation of enzyme extract :-

10 grams of fruit sample was taken and homogenized with water. The homogenate was filtered through a cheese cloth. The filtrate was taken as the crude enzyme.

Procedure :-

3.0ml of buffer of optimum pH 5.6 was added to the 0.5ml of magnesium acetate. This was followed by the addition of 2.0ml of the substrate and 1.0ml of enzyme. The total volume was made upto 10ml with the buffer. The mixture was incubated at 37°C for one hour and at the end of the incubation period the reaction was stopped by the addition of 2.0ml of 15 per cent trichloroacetic acid. Along with this controls were also prepared by adding enzyme at the end of the incubation period. The mixture was centrifuged and transferred 1.0ml of the supernatant into clean dry tubes. To this added 1.0ml of molybdate II and 0.4ml of aminonaphthol sulphonic acid. The above mixture was made upto 10ml with water. Read the colour developed at 660nm in a colorimeter after 20 minutes. Potassium dihydrogen phosphate as a standard was used to establish the calibration curve.

ANNEXURE VI

ESTIMATION OF POLYPHENOL OXIDASE (MAHADEVAN, 1982)

Preparation of the enzyme extract :-

Weighed the tissues, cut into pieces, transferred to a blender and added chilled 0.1M phosphate buffer of pH6. 5.0ml of buffer was used for each gram of tissue. Ground at low speed for 2-3 minutes. Filtered the extract through three layers of cheese cloth to remove the pulp and centrifuged the extract for 30 minutes at 2,000g at 4°C. Decanted the supernatant and used the clear extract as enzyme source.

Regents :-

1. 0.1M phosphate buffer pH 6.0
2. 0.01M catechol dissolved in 0.1M phosphate buffer pH 6.0.

Method :-

Pipetted out 2.0ml of the extract and 3.0ml of 0.1M phosphate buffer into the cuvetts. Mixed the contents by inverting; placed in a colorimeter set at 495nm and adjusted the absorbance to zero. Removed the cuvette, added 1.0ml of 0.01M catechol in 0.1M phosphate buffer and mixed. Placed the tube immediately in the colorimeter and recorded the changes in absorbance at 30 seconds upto 3 minutes. Plotted the changes in absorbance between the first 30 seconds and 150 seconds of incubation and calculated the enzyme activity. Maintained control reactions with heated enzyme.

ANNEXURE VII

OF

ESTIMATION PROTEIN (LOWRY et al., 1951)

Principle :-

The method is based on the principle that different proteins contain different amounts of aromatic residues which react with Folin-Ciocalteu reagent giving a blue colour, which is read in a colorimeter.

Reagent :-

A. Alkaline sodium carbonate solution. (two per cent sodium carbonate in 0.1N sodium hydroxide).

B. Copper sulphate - sodium potassium tartorate solution. (0.5 per cent copper sulphate, $5H_20$ in one per cent sodium potassium tartarate (Prepared fresh).

C. Alkaline copper reagent.
Mixed 50ml of reagent A and 1.0ml of reagent B.
(Prepared freshly)

D. Folin-Ciocalteu reagent :

Dissolved 100g of sodium tungstate and 25g sodium molybdate in 700ml of water in one litre flask. Added 50ml of 85 per cent orthophosphoric acid and 100ml of concentrated hydrochloric acid and boiled under reflux gently for about 10 hours. Cooled and added 150g of lithium sulphate dissolved in 50ml of water and 4-5 drops of liquid bromine. Boiled the mixture without condenser for about 15 minutes to remove the excess bromine. Cooled and

diluted to volume with water and filtered. The reagent should be golden yellow in colour; stored the reagent in brown bottle. It is stable for many months. Just before use diluted one volume of this stock solution with two volumes of water.

Method :

0.2ml of the enzyme solution was made upto 1.0ml with water. Added 5.0ml of the reagent C to it and allowed the mixture to stand at room temperature for 10 minutes. ~~or longer~~. Added 0.5ml of Folin-Cio-calteu reagent rapidly and mixed the contents in the tube immediately. After 30 minutes measured the absorbance of the solution at 750nm. Calculated the amount of protein in the sample with a standard curve prepared using serum albumin.

ANNEXURE VIII

ESTIMATION OF STARCH (CLEGG, 1956)

Reagents :-

1. 80 per cent ethanol.
2. 52 per cent perchloric acid (Mixed 270ml of 72 per cent perchloric acid with 100ml of water).
3. Anthrone reagent :- 2 gram of anthrone per litre of sulphuric acid.

Method :-

Placed 100-200mg of dried sample into a 50ml centrifuge tube. Added 25ml of 80 per cent ethanol and heated the tubes in a water bath at 80-85°C for 10 minutes. Centrifuged at 2,000g for 15 minutes and decanted the supernatant liquid into a 50ml beaker. Repeated the extraction thrice. The residue contained starch.

Added 5ml of water to the residue left behind in the centrifuge tubes followed by 6.5ml of 52 per cent perchloric acid while stirring. Stirred the contents constantly with a glass rod for 5 minutes and then occasionally for the next 15 minutes. Added 20ml of water and centrifuged. Decanted the supernatant liquid into a 100ml volumetric flask. Added 5.0ml of water to the residue and repeated the extraction with perchloric acid, stirred occasionally for the next 30 minutes. Transferred the contents of the tubes into a volumetric flask made up the volume to 100ml with water and filtered through Whatman No. 42 filter paper. Discarded the first few ml of the filtrate. Diluted an aliquot of the filtrate to a known volume and analysed the sugar with anthrone reagent.

Added 4.0ml of the anthrone reagent to 1.0ml of a protein free carbohydrate solution and rapidly mixed. Placed the tubes in a boiling water bath for 10 minutes with a marble on top to prevent loss of water by evaporation cooled and read the extinction at 620nm against a reagent blank.

Prepared standard curves for the glucose and compared them.

ANNEXURE IX

ESTIMATION OF PECTIN (PEARSON, 1970)

Extracted repeatedly 5g of finely powdered sample with cold water, boiled the pooled extract and filtered. Diluted the filtrate to 300ml added 100ml of 0.1N sodium hydroxide and allowed to stand over night. Then added 50ml of N-acetic acid followed five minutes later by 50ml of 2N Calciumchloride solution. Allowed to stand for one hour, boiled for few minutes and filtered, washed the residue with boiling water and filtered, washed and dried at 100°C and weighed as calcium pectate.