

## ACTIVITY OF PECTIC ENZYMES (PECTINESTERASE AND POLY-GALACTURONASE) DURING THE RIPENING OF GUAVA FRUIT.

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A study of the changes in various physio-chemical characteristics of guava fruit, during the development and ripening, on the tree revealed that rapid growth in size and weight of the fruit occurred during the pre-ripening period, while most of the chemical changes leading to the attainment of proper eating quality occurred after the initiation of ripening. An increase in three pectin fractions was noted before ripening, followed by a decrease in the insoluble fractions, while water soluble pectin increased throughout, except a slight decrease in over-ripe fruits. PE activity increased with the development of the fruit while PG activity appeared only after the initiation of ripening. A slight decrease in enzymic activity occurred in the over-ripe fruits. Total solids, acidity and proteins decreased while soluble solids increased, gradually, with ripening.

### INTRODUCTION

Guava (*Psidium guajava* L.), native to tropical America, is now widely distributed throughout the world and is one of the major fruit crops grown in Pakistan. The fruit is a rich source of Vitamins, minerals and carbohydrates. It is quite suitable for the extraction of commercial pectin and is widely used for the preparation of jams and jellies. Changes in soluble solids, acid, Brix/acid ratio and colour, during maturation, have long been used as standard procedures for quality selection of the fruit for various purposes, but additional information regarding the enzymic activity, especially the pectic enzymes (PE, PG) at successive stages of growth, is also necessary due to their specific role in the ripening of the fruit. These enzymes cause a lot of physio-chemical changes resulting in over-ripening, loss of texture and ultimately the deterioration of the fruit quality.

The study was, therefore, aimed at determining the changes in pectic enzymes activity, pectic substances and various other physio-chemical characteristics during the ripening of guava fruit on the tree.

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## REVIEW OF LITERATURE

Carre (1922, 1925) suggested that the process of ripening and physiological breakdown were intimately associated with progressive solubilization of insoluble pectic material present in middle lamella, with maturation of apples. Archbald (1928) reported a gradual decrease in total solids and acidity and an increase in soluble solids during ripening of apples. Hobbs (1963, 1964) observed a fall in total and insoluble pectic contents of ripening tomatoes. PE activity increased with ripening. PG activity developed after green stage and increased sharply followed by a slight decrease in over ripe fruits. Deminque and Phaff (1965) attributed the increase in PG activity, with ripening to the formation of active PG from inactive proteins which were broken down to soluble form or due to the elimination of substances inhibitory to the enzyme activity. PG was not detected before the initiation of ripening. Seshadri and Vasista (1965) reported gradual decrease in polyphenolic inhibitory substances with ripening of guava fruit. Decrease in total pectin contents, during ripening of guava was observed by Verma and Srivastava (1966). Hasegawa *et al.* (1969) while working on dates, reported that PG activity, which was virtually absent at green, began to develop as the maturity progressed. Total proteins decreased with ripening. Paynter and Jom (1971) observed a gradual decrease in molecular weight of pectinic acids and increase in preparation of pectic acid in ripening peaches. Pressey *et al.* (1971) reported that insoluble pectin contents increased during early stages of maturity in peaches when no PG was present, followed by a rapid increase in water soluble pectin with development of PG in advanced stages of ripening. Decrease in total pectin occurred, after ripening. Al-Jasim and Al-Delaimy (1972) attributed the increase in PE activity, in different varieties of ripening dates, to the inactivation of inhibitors with advancing maturity, followed by a general decrease in advanced stages of ripeness.

## MATERIALS AND METHODS

The fruit was picked at weekly intervals from the tree. Extraction and estimation of pectic enzymes (PE, PG) was carried out according to the procedure described by Nagel and Patterson (1967). Water soluble ( $(\text{NH}_4)_2\text{C}_2\text{O}_4$  soluble and NaOH soluble - pectin fractions were determined after Rouse and Atkins (1955). Total pectin was calculated by adding the three fractions. Methods described in A.O.A.C. (1960) were used for the estimation of total solids, soluble solids, acidity and proteins.

## RESULTS AND DISCUSSION

The study revealed that rapid growth in size and weight of the fruit occurred during the pre-ripening period and it acquired a weight of 109 gm, in the seventh week of growth. Firmness of the fruit decreased rapidly after the initiation of ripening and only a slight increase in weight and size was noted. Slight decrease in weight of the fruit occurred in over-ripe fruits which may be attributed to transpirational losses. Total solids and acidity of the fruit gradually decreased as the maturation advanced, followed by a slight increase in the acid contents in the over-ripe fruits, due to the breakdown of reducing sugars to acidic substances. The decrease in acidity during ripening may be due the oxidative removal of volatile acids through respiration. Gradual conversion of insoluble substances like starch and protopectin to soluble sugars and pectin resulted in an increase in soluble solids with ripening of the fruit. The results are in accordance with these of Archbold (1928).

TABLE 1. *Physio-chemical changes in guava fruit during ripening.*

Determinations	DAYS AFTER FRUIT SET									
	7	14	21	28	35	42	49	56	63	70
PE activity	1.66	1.66	2.22	2.27	3.88	4.44	5.00	5.00	4.44	3.88
PG activity	1.00	1.00	1.00	1.00	1.05	1.07	1.10	1.17	1.22	1.20
Water soluble pectin	0.04	0.08	0.11	0.15	0.25	0.40	0.56	0.64	0.73	0.69
(NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> Soluble pectin	0.19	0.21	0.23	0.26	0.33	0.40	0.34	0.25	0.05	0.03
NaOH soluble pectin	0.24	0.25	0.28	0.31	0.40	0.46	0.40	0.21	0.05	0.02
Total pectin	0.47	0.54	0.62	0.72	0.98	1.26	1.30	1.10	0.83	0.74
Total solids	30.00	29.88	28.97	28.00	26.96	25.71	23.50	21.24	19.76	19.17
Total soluble solids	8.15	8.31	8.47	8.87	9.48	10.51	11.23	11.35	11.42	11.42
Acidity	1.20	1.22	1.20	1.15	1.06	0.94	0.80	0.73	0.72	0.74
Fresh weight	4.5	13.00	29.00	48.00	69.00	94.00	109.00	114.00	113.00	113.00
Per cent proteins	2.27	2.32	2.18	1.96	1.75	1.48	1.35	1.22	1.22	1.22

1. Pectin fraction and total pectin are expressed as per cent AGA.
2. Pectinesterase is expressed as (PEU)/g x 10<sup>-3</sup>.
3. Total solids, total soluble solids and acidity (Citrus acid) are expressed in per cent and each value is an average of two

Prominent changes occurred in pectic substances during the development and ripening of the fruit. NaOH and (NH<sub>4</sub>)<sub>2</sub> C<sub>2</sub>O<sub>4</sub> soluble - pectin increased until the initiation of ripening followed by a rapid decrease during successive stages due to their conversion to H<sub>2</sub>O soluble pectin through enzymatic degradation, resulting in a constant increase in water soluble pectin of the fruit. Total pectin increased until the beginning of eight week, followed by an abrupt decrease which may be attributed to the further degradation of water soluble pectin to substances no longer recognised as pectin. The result are in agreement with these of Carre (1922, 1925), Verma and Srivastava (1966) and Paynter and Jan. (1971).

An increase in the pectinesterase activity was observed with the ripening of the fruit, followed by a gradual decrease in over ripe fruits due to lack of the substrate. This increase in PE activity may be attributed to the gradual inactivation of effective quantities of inhibitory substances, observed by Sehadri and Vasista (1966) during ripening of Guava fruit. Similar results were obtained by Hobson (1963) in tomatoes and Al-Jasim and Al-Delaimy (1972) in dates.

Polygalacturonase activity was not detected in unripe fruits. It appeared with the initiation of ripening and increased rapidly followed by a slight decrease in the over-ripe fruits. Fruit firmness decrease more rapidly after the appearance of the enzyme activity. A decrease in the protein content was noted until the ripening of the fruit after which it remained constant. The rapid increase in PG activity may be due to the formation of active PG from inactive proteins, which are broken down to soluble form or elimination of inhibitory substances as suggested by Dominique and Phaff (1965). The results are also in agreement with those of Hobson (1964), Hasegawa *et al.* (1969) and Pressey *et al.* (1971).

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