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Pectin fractions in guava (*Psidium guajava* L.) cultivars 'Shweta' and 'Lalit'

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Abstract
Pectin fractions were studied in developing fruits of guava cvs 'Shweta' with white mesocarp and 'Lalit' with pink mesocarp. Flowers were tagged, fruits were harvested at 10 days intervals and estimated alcohol insoluble solids (AIS) and pectin fractions, (viz. water (WSP), ammonium oxalate (AOP) and alkali soluble (ASP)). Fruits of cultivar 'Shweta' contained 3.73 percent, while cultivar 'Lalit' had 3.97 percent AIS throughout development period of 132 days. Proportion of AIS in fruits decreased continuously from 62 to 112 days of fruit development and thereafter it remained almost constant in both the varieties. Total pectin (TP) content was significantly higher in cultivar 'Lalit' than cultivar 'Shweta' throughout the developmental period and attained maximum at 112 days after fruit set. Water soluble pectin was significantly higher in cultivar 'Shweta' towards maturity and ripening of fruits. AOP (pectinic acid) content was higher in cultivar 'Lalit' with respect to cultivar 'Shweta'. Pectinic acid increased continuously in cultivar 'Shweta' till 122 days (26.02%), thereafter; it decreased up to 132 days coinciding with ripe fruits. As fruit development advancement, ASP (protopectin) of cultivar 'Shweta' was found higher than that of cultivar 'Lalit'.

Keywords: *Psidium guajava*, maturity, ripening, pectin, protopectin, pectinic acid

Introduction
Guava (*Psidium guajava* L.), belongs to the family Myrtaceae, is known as apple of tropics. Guava fruits are exquisite and nutritionally valuable. From the date of flowering to fruit maturity is completed in 110-150 days. It has been observed that pectin content is higher in winter guava than the monsoon fruits (Salunke and Kadam, 1995) [1]. Guava fruits are important source of natural pectin and dietary fibre. The therapeutic significance attributed to pectic substances is the decrease in cholesterol, especially those linked to low density lipoprotein (LDL) (Hillman *et al*., 1985) [2]. It has also beneficial to diabetic patients reducing blood-sugar (Bolton *et al*., 1981) [3]. Pectin is used in food products as an inexpensive source of natural food thickener and gelling agent. Pectic polysaccharides solubilization occurs in fruit involving polygalacturonate hydrolysis, change in side chains and interruption of ionic bonds (Gross, 1990; Liyama *et al*., 1994) [4, 5]. Major component of cell wall are pectin that changes in the course of fruits development and ripening thereby, fruits changes in firmness and softens (Proctor and Peng 1989) [6]. Though there are reports on pectic enzymes during ripening of guava fruits but the studies on pectin fractions of guava fruits during development stages are meager. Reports in guava on total pectin are few (El Buluk 1995; Tiwari *et al*., 2007; Das and Majumdar, 2010) [7, 9] and limited studies on pectin in cultivar 'Shweta' (white pulp) and cultivar 'Lalit' (pink pulp). Cultivar 'Shweta' and cultivar 'Lalit' are the two recent selections developed at the Institute, which are gaining popularity. The fruits are large (150-250g), round in shape with smooth yellow coloured pericarp, the mesocarp of CV 'Shweta' is white, while that of cultivar 'Lalit' is pink in colour. Both the varieties are rich in vitamin-C content and total soluble solids. Therefore, keeping in view the importance of pectic substances, the present investigation was undertaken to study the pectin fractionation in cultivar 'Shweta' (white pulp) and cultivar 'Lalit' (pink pulp) during growth and development of the fruits.

Materials and Methods
Eight year old guava trees of cultivar 'Shweta' and cultivar 'Lalit', grown at the Institute farm, were selected for the study. Two hundred flowers in each were tagged on 28th August and fruit set was observed. Twenty fruits from each cultivar were harvested at 10 days intervals from

30th October to 4th January, i.e. for a period of 62 to 132 days after fruit set consisting of eight stages of fruit growth and development. Fruits were washed in running tap water, air dried and weight was recorded. Alcohol insoluble solids (AIS) extracted in the pulp were further separated into three pectin fractions (Roe and Bruemmer, 1981) [10]. For AIS extraction fruit pulp of 20 g were weighed and added with 100 ml ethanol. It was boiled for 15 minutes, cooled and filtered through Buchner funnel. The precipitates were washed thoroughly with ethanol followed by acetone until it turned colourless. It was dried at 45°C till weight was constant. Three pectin fractions were obtained by successive extractions of AIS with distilled water, ammonium oxalate and sodium hydroxide.

For pectin soluble in water (WSP), AIS (0.5g) and added 40 ml distilled water followed by incubation for 2 hours at 30°C. Then it was centrifuged at 10,000 rpm for 10 minutes and the precipitate was extracted by repeating the above process. The supernatants were collected in a 100 ml volumetric flask. The left over precipitate was extracted with 0.75 percent for ammonium oxalate pectin (OSP) following the above process and the volume was made up to 100 ml. The precipitate was further extracted to estimate alkali soluble pectin (ASP) in similar manner with 0.05 M NaOH and made up to 100 ml volume.

To the 2 ml of pectin solution, were added 1.0 ml of carbazole reagent and 12.0 ml of conc. H_2SO_4 with constant shaking. The tubes were allowed to stand for 10 min and colour was observed at 525 nm in a spectrophotometer (ECIL, India) (Rouse and Atkins, 1955) [11]. Galacturonic acid (100µg/ml) was used as standard and blank was taken as 1 ml of alcohol instead of 1.0 ml of carbazole.

All the estimations in the experiment were carried out in triplicate in CRD and subjected to statistical analysis by SAS 9.3 and the CD at 0.05 level was worked out.

Results and Discussion

The fruits were small, green in colour and immature on the 62nd to 82nd day which later developed to larger green mature fruits by 92 to 112 days and mature light green fruit by 122 day in CV 'Shweta' and CV 'Lalit' as indicated by maximum weight of fruits (Fig.1). Average fruit weight of CV 'Shweta' was higher than that of CV 'Lalit' throughout the period of fruit growth and development. There was a significant difference ($p \leq 0.05$) in average weight of fruits in both cvs 'Shweta' and 'Lalit' during growth and development. Fruit weight increased continously from 62 to 82 days after fruit set in CV 'Shweta' which decreased on 92 day and started increasing from 102 to 112 days of fruit set. In CV 'Lalit' the fruit size had increased continously till 132 days after fruit set. The typical double sigmoid curve of rapid increase in weight, followed by slow change in weight and lastly attainment of final size was observed in CV 'Shweta', while CV 'Lalit' differed in the growth curve. The sigmoid growth curve differs for different cultivars (Yusof and Suhaila, 1987; Jagatiani *et al*., 1988) [12, 13]. In winter growth period is more due to existing low temperatures during fruit development and ripening (Edmundo Mercado-Silva *et al*., 1998) [14]. The changes in contribution percentage of cell wall materials and fresh fruit weight are indicated by AIS. With the increase in fruit weight there was a decrease in proportion of AIS continuously upto 132 days maximum when fruits reached maturity. It can be noticed from the Fig.2 that the proportion of alcohol insoluble solids (AIS) of CV 'Shweta' was higher than that of CV 'Lalit' from the initial stage (62nd day) of fruit development to the last stage of fruit maturity and ripening. The AIS of 'Lalit' attained maximum on the 62nd days of fruit growth, which decreased continuously up to 102 days of fruit growth and development after which the AIS almost became constant during 122 to 132 days coinciding with maturity stages of fruit. The proportion of AIS of cultivar 'Shweta' increased in the initial stages i.e. up to 72 days; thereafter it decreased during 82 days to 102 days after fruit set and it became constant in 112 to 122nd days of fruit development. Decrease in AIS coincided with fruit softening and ripening in the field. Reduction in AIS may be due to increase in fruit size in different fruits (Proctor and Peng, 1989; Tiwari *et al*., 2007; Tandon and Kalra, 1984; Fils-Lycaon and Buret, 1990) [6, 8, 15, 16].

The water soluble pectin (WSP) of cvs 'Shweta' and 'Lalit' varied significantly (Fig.3.). Pectinic acid content of CV 'Shweta' was lower in the initial period (62 and 72 days) 26.48 and 26.98 percent of AIS, respectively. Later on the magnitude of increase in (WSP) pectinic acid was higher in 82, 92, and 102 days coinciding with green mature stage of fruit set. As the fruit growth advanced towards maturity and ripening i.e. 112th to 132nd days, it increased constantly in fruits. In CV 'Lalit' the WSP was significantly higher at 62 days of fruit growth and development. The water soluble pectin further decreased in 92 days and thereafter increased up to 112 days towards maturity and ripening of fruits. Significantly there was higher WSP in CV 'Lalit' compared to CV 'Shweta' towards the maturity and ripening of fruits. The decrease in water soluble pectins may be due to de-polymerization of pectin which are in-soluble in water and are soluble in oxalate solvent (El Kashif and Huber,1988) [17]. As fruits ripe, they became soft as cell bound pectin are de-esterified and de-polymerized by enzymes resulting in water soluble pectin in guava and other fruits (Proctor and Peng,1989; El-Buluk,1995; Das and Majumdar,2010; Silacci and Morrison,1990; Majumdar and Mazumdar, 2002) [6, 7, 9, 18, 19]. Increase in water soluble pectin caused by the solubilization of esterified pectin by enzymes (Sawamura, 1978) [20].

The ammonium oxalate soluble pectin (AOP) varied significantly during the fruit development in guava cvs 'Shweta' and 'Lalit' (Fig. 4). The AOP content was higher in CV 'Lalit' than CV 'Shweta' throughout the development. There was continuous increase of AOP in CV 'Shweta' from 62 till 122 days (16.29 to 26.02%) then decreased on 132nd day or the last stage of development where fruits are ripe. The AOP content in CV 'Lalit' increased (25.70 to27.42%) in the initial stage of growth, i.e., 62 to 92 days after fruit set. With the increase in growth and development of fruits there was an increase in AOP content of cv 'Lalit' from 82 to 92 days and decreased after 102 days of fruit growth and development coinciding with maturity of fruits. The increasing trends of AOP due to enzymatic de-esterification and de-polymerization of cell bound pectin which eventually resulted in water soluble pectins (high methoxyl) and ammonium oxalate (low methoxyl) pectin fractions during growth and development of guava and other fruits (Proctor and Peng, 1989; EL-Buluk, 1995; Das and Majumdar, 2010; Silacci and Morrison, 1990; Majumdar and Mazumdar, 2002) [6, 7, 9, 18, 19].

The alkali soluble pectin (ASP) varied significantly in cvs 'Shweta' and 'Lalit' (Fig.5). With the advancement in fruit development, the protopectin content of CV 'Shweta' was higher than that of CV 'Lalit'. ASP content in CV 'Shweta' decreased continuously up to 102 days (45.82%) and remained constant till 122 days and decreased marginally

thereafter. The magnitude of decrease in ASP content was lower in CV 'Lalit' up to the last stage of fruit development. There was a significant decrease in ASP or proto-pectin with the advancement of fruit development indicating the ripeness of fruits. ASP increases prior to onset of physiological maturity and then decreases with onset of fruit ripening. Alkali soluble pectin or protopectin along with other binding agents maintained fruit firmness. Contradicting to WSP and AOP, alkali soluble pectin was of declining quantitative pattern in guava (Proctor and Peng, 1989; EL-Buluk, 1995; Das and Majumdar, 2010; Silacci and Morrison, 1990; Majumdar and Mazumdar, 2002) [6, 7, 9, 18, 19].

The total pectin (TP) content, as a component of AIS, of CV 'Lalit' was significantly higher than CV 'Shweta' throughout the period of growth and development (62 to 132 days) (Fig.6.). The total pectin content of CV 'Lalit' has steadily increased from 11.27 percent on the 62nd day to 20.73 percent on the 132nd day of fruit development. Similarly, there was an increase in TP content of CV 'Shweta' from 7.37 to 13.28 percent of AIS on the 62nd and 132nd day, respectively. Total pectin recorded highest fruits of 112 days, thereafter it declined. Increase in TP during fruit development revealed the synthesis of new cell-wall (Tsantili, 1990) [21]. As the fruit weight increased, there was a decrease in pectin content until 122 days. Thus, there is increase in water content of fruits during growth and development. The enzymatic conversions of pectin substances are the cause of increase or decrease in different fractions of pectin. The increase in TP in fruit development is an implication of galacturonic acid synthesis (Silacci and Morrison, 1990) [18]. Similar results of higher pectin content in mature than in the ripe fruit of guava as reported by (Chyan *et al*., 1992) [22].

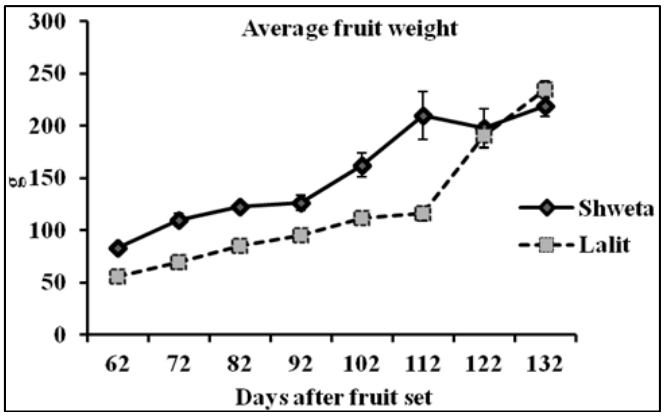

Fig 1: Average fruit weight (g) in cvs 'Shweta' and 'Lalit'during fruit growth and development

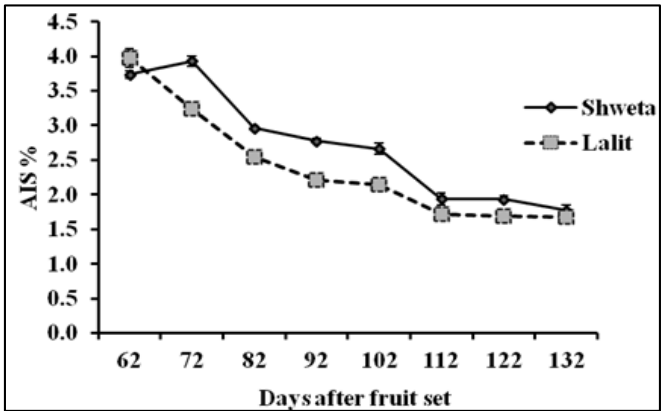

Fig 2: Alcohol insoluble solids (AIS) during fruit growth and development in cvs 'Shweta' and 'Lalit'.

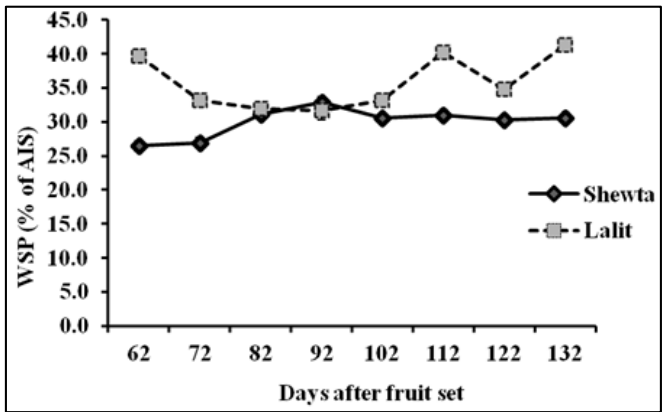

Fig 3: Water soluble pectin (percent of AIS) during fruit growth and development in cvs 'Shweta' and 'Lalit'.

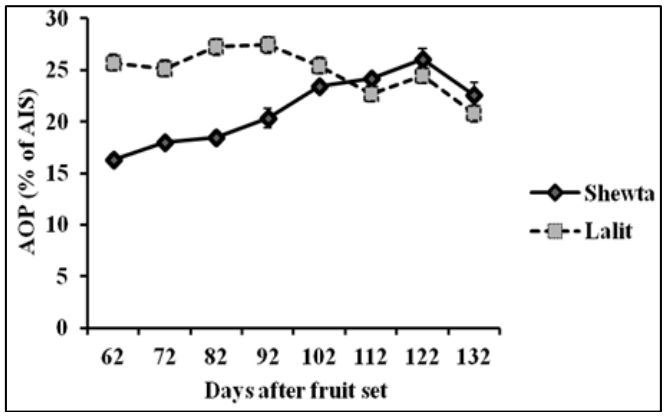

Fig 4: Ammonium oxalate soluble pectin (percent of AIS) during fruit growth and development in cvs 'Shweta' and 'Lalit'.

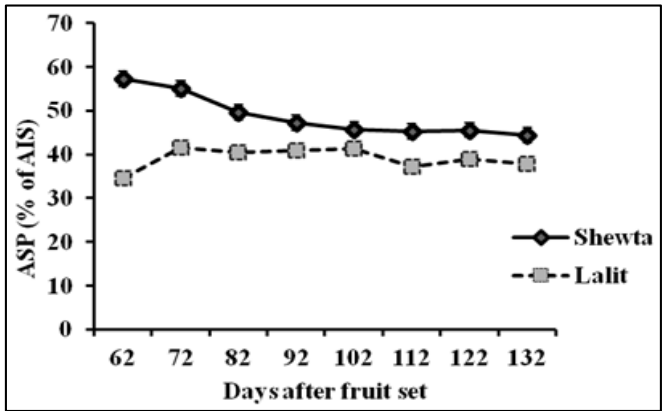

Fig 5: Alkali Soluble pectin (percent of AIS) during fruit growth and development in cvs 'Shweta' and 'Lalit'.

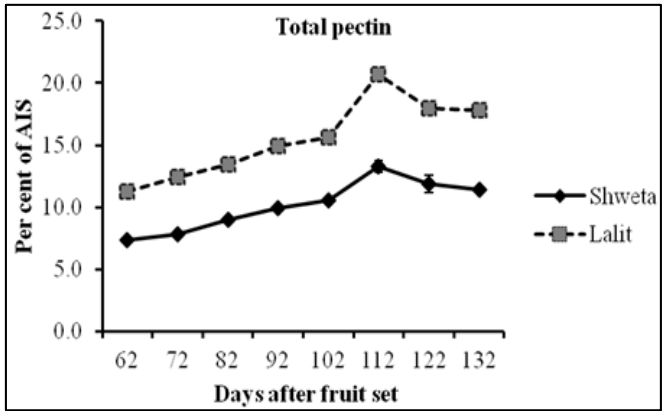

Fig 6: Total pectin (% of AIS) during fruit growth and development in cvs 'Shweta' and 'Lalit'.

Conclusion

It can be inferred from the results that the increasing trends of WSP are due to enzymatic reactions of bound pectin which consequently resulted in water soluble pectins (high methoxyl) and ammonium oxalate (low methoxyl) pectin fractions. ASP increases prior to physiological maturity and then decreases with fruit ripening. TP in CV 'Lalit' (pink mesocarp) is higher as compared to CV 'Shweta' (white mesocarp), consequently the fruits of cultivar 'Lalit' have better firmness.

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