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Biochemical changes in guava (*Psidium guajava*) fruits during different stages of ripening

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ABSTRACT

The present study was undertaken at Experimental Orchard of the Division of Fruit Science, Faculty of Agriculture, Udheywalla, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, J & K, India. The study was undertaken in seven guava (*Psidium guajava* L.) cultivars at three different developmental stages, viz. green mature stage (GMS), half ripe stage (HRS) and full ripe stage (FRS). The results of the present study revealed that with the advancement of fruit maturity at different stages, the total soluble solids (TSS), sugar (total sugars, reducing and non-reducing sugar), ascorbic acid contents increased significantly while, during fruit ripening acidity and pectin decreased. Of all the cultivars studied, L-49 showed highest TSS (12.25^{0}B) followed by Allahabad Safeda (10.75^{0}B). However, total sugar (8.50%) and ascorbic acid (265.09%) were also maximum in L-49 followed by Allahabad Safeda at FRS, L-49 showed minimum acidity (0.26%) and maximum pectin content (0.77%) followed by (0.23 and 0.73%, respectively) in Allahabad Safeda. Pectin methyl esterase (PME) activity increased progressively in all the cultivars up to HRS and subsequently decreased at FRS. Maximum PME activity was found in L-49 (56.25 units/g.f.wt) at HRS whereas, it showed a decrease at FRS (52.25 units/g fresh weight (FW) followed by Allahabad Safeda and Lalit. It is thus concluded that L-49 was superior among all the commercial cultivars of guava grown under sub-tropic condition followed by Allahabad Safeda and Lalit.

Key words: Ascorbic Acid, Pectin, Pectin methyl esterase, Ripening

Being a climacteric fruit, guava (*Psidium guajava* L.) fruits exhibit a typical increase in respiration and ethylene production during ripening. A large number of physiological, biochemical and structural changes occurs during ripening of fruits which include the degradation of starch or other storage polysaccharides, the production of sugars, the synthesis of pigments and volatile compounds, and the partial solubilization of cell wall. In guava, these changes take place over a relatively short period of time and therefore, have a very short shelf-life, which in turn makes transportation and storage difficult. Therefore, to improve the quality, storage, and processing characteristics of the fruits, it is necessary to understand the biochemistry of ripening of guava. Information on biochemical changes during ripening is available for many fruits such as mango (Preethi 2014) and olive (Azadeh *et al.* 2015). However, little is known regarding biochemical changes occurring during ripening of guava fruits. Such information is prerequisite in developing technologies for enhancing shelf-life of fruits and providing proper handling, transportation, and storage conditions. Hence, in the present investigation, changes in chemical composition and the activities of pectin methyl esterase enzymes during ripening of different commercial cultivars of guava under sub-tropical conditions of Jammu region have been studied.

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MATERIALS AND METHODS

Ten years old, uniformly growing and bearing habit trees of seven different guava cultivars, viz. Allahabad Safeda, L-49 (Sardar), Hisar Surkha, Hisar Safeda, Lalit, Pant Prabhat and Arka Amulya, grown in sandy loam soil under irrigated conditions in the Experimental Orchard of the Division of Fruit Science, Faculty of Agriculture, Udheywalla, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, J & K, India were chosen for the present investigation. The experimental orchard was situated at an elevation of 300 m above mean sea level and lies at 32.43^0 North latitude and 74.54^0 East

longitude. All the trees were given uniform cultural operation as per the package and practices of SKUAST-Jammu. The experiment was laid out in RBD, planted at a distance of 5 × 5m with seven cultivars as seven treatments with four replications i.e. single plant makes one replication. Observations were recorded for the winter season crop at the time of harvesting from all the cultivars because the fruit of the rainy season crops are watery, insipid and prone to fruit fly attack. For studying the biochemical parameters, 20 fruits were taken from each cultivars, harvested with secateurs keeping small intact pedicel with each fruit, on the basis of visual observation and firmness at three maturity stages, viz. green mature stages, GMS (100% green fruit); half ripe stages, HRS (50% yellow and 50% green) and full ripe stages, FRS (80% yellow and 20% green fruit). These stages were decided by visually observing the fruit skin colour and firmness was checked by putting pressure by hand and fruits which were not compressed by applying pressure with hand were categorized into GMS, while those fruits which were slightly compressed were categorized as HRS and those which were punctured were categorized as FRS.

T.S.S (%) was obtained from the fruit juice using Erma hand refracto meter. Titrable acidity was estimated by titrating against 0.1 N sodium hydroxide (Ranganna 1986) however reducing sugar, non-reducing sugar, total sugar, Ascorbic acid and pectin content was analyzed by adopting the standard procedure suggested by AOAC (1977). Activity of PME was assayed by the method of Hagerman and Austin (1986). The statistical analysis was done using SAS software.

RESULTS AND DISCUSSION

The perusal of data on biochemical characteristics of fruits revealed that most of the characters, i.e. TSS, acidity, ascorbic acid, reducing sugar and total sugar showed significant differences among the cultivars. Result related to change in the TSS, total sugar, reducing sugar, non-reducing sugar and ascorbic acid of all cultivars increased significantly at early stages of development and gradually at the later stages of development where as acidity and pectin content decrease with the advancement of fruit ripening.

Total soluble solids

It is evident that cultivar L-49 showed highest TSS at all the three stages of fruit maturity, i.e GMS, HRS and FRS with TSS of 6.63, 10.15 and 12.25° B, respectively. Allahabad Safeda followed it with TSS of 6.45, 9.04 and 10.75° B at GMS, HRS and FRS, respectively (Table 1). These results are in line with the finding of Hedge and Chharia (2004) and Soares *et al.* (2007) who also observed an increasing trend with fruit ripening. Increased TSS was attributed due to the conversion of starch into soluble solid and with the advancement of ripening, starch contents decreased progressively, while TSS increased. More increased at full ripe stage may be attributed to the increased in activity of enzymes responsible for starch hydrolysis (Bashir *et al*. 2003).

Titratable acidity

Among all the commercial cultivars at different maturity stage (Table 1), L-49 showed the lowest acidity followed by Allahabad Safeda whereas, highest acidity was recorded in Pant Prabhat followed by Hisar Safeda. The drop in acidity from GMS to HRS was 0.36 to 0.30 % and HRS to FRS was 0.30 to 0.26% in L-49. Whereas, in Pant Prabhat which recorded highest acidity content the drop in acidity was from 0.54 to 0.49 % from GMS to HRS was and 0.49 to 0.43 % from HRS to FRS. The acidity contents of all cultivars decreased slightly upto half ripe stage and then sharply decline in full ripe stage. The results are in conformity with the findings of Selvaraj *et al.* (1999) who observed similar trend of decrease in acidity with the advancement of fruit ripening. These results support the view that acids can be used as substrates for respiration when sugars have been consumed or participated in the synthesis of phenolic compounds, lipids and volatile aromas and provided in addition, a series of metabolites which are used in many processes that reflect dominance of sweet flavor in fruits (Ulrich 1970).

Ascorbic acid

L-49 fruit contained maximum amount of ascorbic acid in all the three stage of maturity (Table 1) which varied

Table 1 Total soluble solids (TSS), acidity (%) and ascorbic acid (mg/100g) of different guava cultivars at green mature stage (GMS), half ripe stage (HRS) and full ripe stage (FRS) under Jammu sub-tropics

Cultivars	TSS (^{0}B)			Acidity (%)			Ascorbic acid (mg/100g)		
	GMS	HRS	FRS	GMS	HRS	FRS	GMS	HRS	FRS
Arka Amulya	4.98	0.49	0.49	0.49	4.26	7.07	145.18	174.99	218.16
Hisar Surkha	5.75	0.44	0.44	0.44	5.87	7.96	76.84	99.20	112.50
Hisar Safeda	4.84	0.52	0.52	0.52	3.84	6.69	99.93	171.33	199.99
L-49	6.63	0.36	0.36	0.36	6.43	8.50	180.50	238.10	265.09
Allahabad Safeda	6.45	0.39	0.39	0.39	6.18	8.20	169.07	227.15	245.24
Lalit	5.26	0.46	0.46	0.46	5.69	7.59	86.98	139.99	179.16
Pant Prabhat	4.72	0.54	0.54	0.54	3.12	6.00	67.00	87.05	104.00
CD (P=0.05)	0.69	N.S	N.S	N.S	1.44	1.40	1.78	0.79	0.59

from 180.50 mg/100g in GMS to 265.09 mg/100g in FRS followed by Allahabad Safeda with 169.07mg/100g, 227.15 mg/100g and 245.24 mg/100g at GMS, HRS and FRS, respectively however, the cv. Pant Prabhat registered lowest ascorbic acid content of 67.00 mg/100g, 87.05 mg/100g and 104.00 mg/100g at GMS, HRS and FRS, respectively. It was significantly increased with the advancement of fruit ripening. These results are in the agreement with those of Singh and Jain (2007). The higher and lower values for all these characters showed inheritance, which is quite helpful in finding the suitable elite type as per requirements. The overall superiority of Lucknow-49, Allahabad Safeda and Lalit possess good quality characters and superior over all the germplasm/cultivars under this study.

Sugar

Wide variation was observed among different cultivars with respect to various types of sugar content (Table 2). The sugar content increased significantly with the advancement of ripening in all cultivars. Similar findings were reported by Bashir *et al*. (2003) who also observed increased sugar content with ripening. Among all the cultivars, highest total sugar content at FRS was recorded in L-49 (8.50%) followed by Allahabad Safeda (8.20%) whereas, Pant Prabhat showed lowest total sugars content of 6.00 per cent at FRS. The increase in total sugar during fruit ripening could be due to hydrolysis of starch (Prabha and Bhagyalakshmi 1998) by starch hydrolyzing enzymes. Starch which is the main storage polysaccharide in many unripe fruits and is degraded with ripening resulting in sweetness and textural change. Degradation of certain cell wall components such as pectin and hemicelluloses may also contribute to increase in reducing sugar content (Stahl and Camp 1971).

Pectin

Pectin content is correlated with fruit firmness and softening (White 2002). The pectin content decreased significantly throughout the ripening process in all cultivars of guava (Fig. 1). These lines are in the agreement with Jain *et al*. (2003) who also observed decreased pectin content with the progressive ripening. The maximum pectin content of 1.14% was observed at GMS which decreased progressively during ripening and attained a minimum value 0.77% at FRS in cv. L-49, while minimum pectin content was observed in Arka Amulya with 0.88% at GMS which with the advancement of fruit ripening decreased to 0.53% at FRS. The presence of appreciable amounts of cell wall components in guava pulp is because it contains stone cells which have highly lignified secondary cell walls (Marcelin *et al.*1993). Decrease in pectin, a cell wall degrading component is due to increase in the activity of cell wall hydrolyzing enzymes during ripening (Prabha and Bhagyalakshmi 1998).

Pectin methyl esterase (PME)

An initial increase in the specific activity of pectinmethyl esterase up to half ripe stage and then gradual decrease at full ripe stage was observed during ripening in all cultivars. Activity of PME was found highest in L-49 and reached its maximum of 56.25 unit/g FW at half ripe stage then (Fig 2), declined to a value of 52.25 unit/g FW at FRS. The lowest activity of PME was found in Pant Prabhat (35.50 unit/g FW) at full ripe stage which decreased to 29.75 unit/g FW at full ripe stage. It has been postulated that PME assists PG enzyme for methylation or esterification of galacturonide chains of pectin (Huber 1983). Thus, decreased in pectin methyl esterase activity after half ripe stage indicate that PME play a vital role up to half ripe stage and the role was over after making pectin substrate like polygalactonide for polygalacturonase (Ali and Lazen 1997) during cell well synthesis for further softening of fruit or dehydrolysis of cell wall component. Similar observations were made earlier in guava (Jain *et al.* 2003 and Sharma *et al.* 2012).

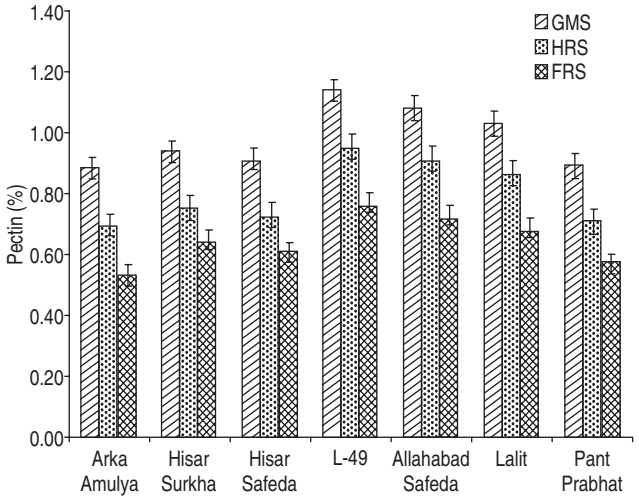

Fig 1 Pectin (%) of different guava cultivars at green mature stage (GMS), half ripe stage (HRS) and full ripe stage (FRS) under Jammu sub-tropics

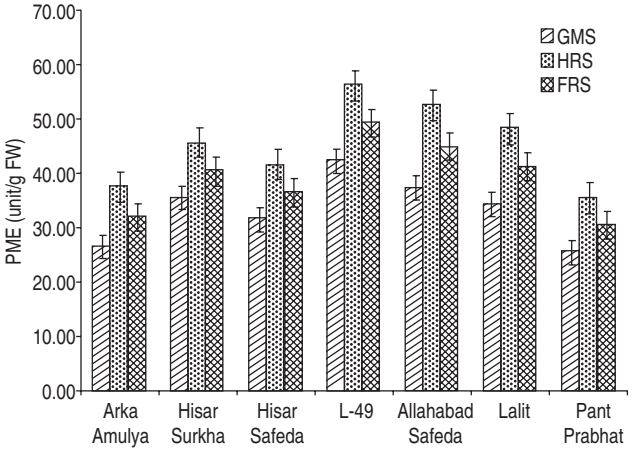

Fig 2 Pectin methyl esterase (PME) (unit/g FW) of different guava cultivars at green mature stage (GMS), half ripe stage (HRS) and full ripe stage (FRS) under Jammu sub-tropics

Table 2 Total sugar (%), reducing sugar (%) and non-reducing sugar (%) of different guava cultivars at green mature stage (GMS), half ripe stage (HRS) and full ripe stage (FRS) under Jammu sub-tropics

Cultivars	Total sugars (%)			Reducing sugar (%)			Non-reducing sugar (%)		
	GMS	HRS	FRS	GMS	HRS	FRS	GMS	HRS	FRS
Arka Amulya	3.26	4.26	7.07	2.49	2.93	4.37	0.77	1.33	2.70
Hisar Surkha	3.91	5.87	7.96	2.70	3.84	4.83	1.21	2.03	3.13
Hisar Safeda	2.81	3.84	6.69	2.20	2.86	4.21	0.61	0.92	2.48
L-49	4.26	6.43	8.50	2.93	4.03	5.13	1.32	2.38	3.37
Allahabad Safeda	4.12	6.18	8.20	2.84	3.96	4.91	1.28	1.97	3.41
Lalit	3.79	5.69	7.59	2.61	3.76	4.67	1.18	1.93	2.92
Pant Prabhat	2.57	3.12	6.00	2.09	2.61	3.98	0.47	0.51	2.01
CD (P=0.05)	0.11	1.44	1.40	N.S	0.76	0.66	0.34	0.33	0.66

From the results of the present investigation, it can be concluded that since transition of guava fruits from GMS (100% green fruit) to HRS (50% yellow and 50% green) is accompanied by major metabolic changes, it will be appropriate to harvest the fruits at HRS stage. This will facilitate safer handling and transportation of these fruits and will avoid postharvest losses. Further, as ripening is associated with changes in colour and activities of hydrolytic enzymes in fruits, these could be taken as good indicators of ripening process and exploited at the molecular level to increase the shelf life of fruits.

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