International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 3359-3363

Received: 05-01-2018
Accepted: 06-02-2018

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Ripening associated biochemical changes with relation to jelly seed formation in mango cv. Dashehari, Langra and Chausa

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Abstract
Mango fruit during ripening shows considerable changes in physiological and biochemical processes. Ripening associated biochemical changes viz., physiological weight loss (PLW), chlorophyll content, respiration rate, ethylene level, pectin methyl esterase (PME) and polygalacturonase (PG), catalase and peroxidase activities and their relation with softening of tissue in Chausa, Langra and Dashehari mangoes were studied at different period of their storage. Wide variations with superiority in Chausa in these attributes were recorded. The maximum PLW (24.36%) was recorded in Dashehari and minimum (13.19%) in Chausa on 9 day after storage. Maximum decrease in all type of chlorophyll was found in Dashehari at 9 day after storage. The rate of respiration and ethylene began to increase from 0 day to 7 day in all cultivars and rate of respiration was considerably low on 9 day of storage in case of Chausa, however Dashehari and Langra on 9 day after storage showed the decrease level in both the attributes.
The pectin methyl esterase (PME) activity increased initially up to 4 days (895 unit / min / g FW) and decreased slowly on 7 day (600 unit / min / g FW). On the other hand the activity of PME was decreased sharply (120 unit / min / g FW) on 9 day of fruit storage in Dashehari, Chausa and Langra. Contrary to the PME activity, the polygalacturonase (PG) activity was increased consistently with increasing the storage period up to 7 day in all the cultivars, thereafter, its activity gradually declined in Chausa and Dashehari. However, cultivar Langra maintained its activity up to 9 day of storage (400 mg glucose released / g FW). Thus, maximum activity of PME and PG in Dashehari cultivar at early stage of ripening may be associated with the incidence of softening in Dashehari.

Keywords: mango, *Mangifera indica* L., jelly seed, Dashehari, seed softening

Introduction
Mango (*Mangifera indica* L.) fruit during ripening shows considerable changes in physiological and biochemical processes. They are associated with increase in the activity of several hydrolytic and gluconogenic enzymes along with elevation in ethylene level and changes in pigment content. It is well known that the cultivars with high rate of ethylene production soften and ripen faster than cultivars that are low ethylene producers. Based on these ideas the experiments was performed to establish the change in physiological and biochemical parameter such as fruit weight loss, chlorophyll (a, b and total) content, TSS, pH, respiration rate, ethylene production, pectin methyl esterase (PME) and polygalactouronase activity (PG) in fruit of Dashehari (highly susceptible) Langra (less susceptible) and Chausa (resistant) cultivars at different period of storage.

Materials and Methods
The physiologically mature fruits of different cultivars were taken for these studies. Forty fruits of each cultivars set in CRD were taken for this purpose. Ten fruits each on 0, 6 and 9 days of storage was examined for physical and biochemical changes. Jelly seed (softening around stone) was observed after cutting the fruits. The relationship of these parameters with its incidence of this disorder was evaluated in the test cultivars. Total soluble solids content of fruits was recorded by using Hand refractometer (Erma Japan). The respiration was measured with Portable Infrared Gas Analyser as mol CO_2 h^{-1} kg^{-1} FW. Ethylene (C_2H_4) was measured with the help of Ethylene gas detector (Nucon). The polygalacturonase (PG) was estimated in fruit pulp of different cultivars at different stage of ripening with the following methods (Jansen *et al*., 1945) [11]. Pectin methyl esterase in most plant tissue is rarely in solution but is absorbed on the insoluble cellular solids. Express the results in pectin methyl esterase units PE

Unit (U) / g. The expression for milliequivalents of ester hydrolysed per minute per gram of enzyme as given below:

$$PE\ (U/g) = \frac{\text{ml of 0.02N NaOH consumed x 3.1 x 1 min}}{\text{ml of enzyme preparation x total time of determination in min}}$$

Results and Discussion

Ripening associated biochemical changes in terms of weight loss, chlorophyll (a, b and total) content, TSS, pH, CO_2, ethylene level, pectin methyl esterase (PME) and polygalacturonase activities (PG) were studied in Dashehari (susceptible), Langra (less susceptible) and Chausa (resistant) cultivars at different period of fruit storage. These cultivars differed in their maturity period, therefore, they have harvested at their fixed maturity for analysis of different parameters.

Changes in physiological weight and pigment content in mango cultivars

The data presented in Table-1 clearly revealed that the physiological weight loss was maximum in Langra (11.83%) and minimum in Chausa (7.05) on 7 day after storage. It is interesting to note that there were significant differences in weight loss on 7 and 9 days after storage in Dashehari and Langra cultivar. Physiological loss weight (PLW) percent also followed the same trend but there were significant differences in PLW (%) amongst all the test cultivars on 9 day after storage. The maximum PLW (24.36%) was recorded in Dashehari and minimum (13.19%) in Chausa on 9 day after storage.

Table 1: Physiological weight loss of mango cultivars at different period of storage

Cultivar	Weight (g)		PLW (%)		
	0 day	7 day	9 day	7 day	9 day
Chausa	346.0	321.6	300	7.05	13.19
Dashehari	264.4	235.5	200	10.93	24.36
Langra	263.6	232.4	216	11.83	18.06
CD (p = 0.05)	64.37	64.66	73.04	2.20	3.41

There were gradually decrease in chlorophyll a, b and total in Chausa and Dashehari at different period of storage and maximum decrease in all type of chlorophyll was found in Dashehari at 9 day after storage (Table 2). In case of Langra the chlorophyll b content was not changed significantly even after 9 days after storage as indicated by 23.33% decrease only as compared to 52.63% in Dashehari and 44.44 in Chausa. Similar trend with different magnitude was found in total chlorophyll content. The results indicated that upon ripening possibly a derivative of chl. b accumulate giving the fruit a 'stay green' characters in Langra mango.

Table 2: Chlorophyll content in the skin of mango cultivars at different days of storage during 2004 – 05 and 2005 – 06

Cultivar	Chlorophyll a (mg g^{-1} FW)			Chlorophyll b (mg g^{-1} FW)			Total Chlorophyll (mg g^{-1} FW)		
	Days			Days			Days		
	0	7	9	0	7	9	0	7	9
Chausa	0.40	0.19	0.12	0.18	0.13	0.10	0.58	0.32	0.22
Dashehari	0.35	0.16	0.10	0.19	0.11	0. 09	0.54	0.27	0.19
Langra	0.45	0.40	0.25	0.30	0.24	0.23	0.75	0.64	0.45
CD (p = 0.05)	0.04	0.12	0.07	0.06	0.06	0.41	0.10	0.19	0.12

In order to interpret the softening behaviour in different commercial cultivars (Chausa, Dashehari and Langra), ripening associated biochemical characteristics such as pigments (chlorophylls) degradation, PLW, TSS, pH respiration rate, enzyme production, chromacity value, pectin methyl esterase and polygalactouronase activities at different days of storage were worked out. The results pertaining to degradation of chlorophyll content in peels clearly showed the gradual decline in chlorophyll a, b total content in Chausa and Dashehari at different period of storage.

The maximum decrease in chlorophyll content was found in Dashehari at 9 day after storage. However, in case of Langra the chlorophyll content specially chl. b did not degrade significantly even after 9 days of storage as a result giving the Langra fruit a 'stay green' character and remain green at table – ripe stage. Disappearance of green colour is the first visible result of degradation of chlorophyll as a consequence of maturation and ripening of fruits (Hortensteiner, 2006) [8]. Chlorophyll breakdown is a regulatory process and various enzymes catalyzing the different reactions have been identified (Jacob-Wilk *et al*., 1999; Yamauchi and Watada, 1994; Takamiya *et al*., 2000) [9, 24, 20]. During ripening of fruit disappearance of chl is normally associated with unmasking of carotenoids and the fruit acquiring bright yellow – red colour. The Dashehari and Chausa cultivar acquired these characters after ripening, whereas the Langra fruits, despit their pleasant pulp colour, flavour and general acceptance failed to develop yellow colour may be due to incomplete degradation of chlorophyll even after 7 – 9 day of storage as per our data revealed.

These 'green – ripe' fruits affect consumer preference and consequently fetch a lower price. It is known that Langra mangoes in unripe stage are very sour due to high acidity thereby resulting in conversion of chl to pheophytins. As pheophytins are not on route in enzymic chl degradation (Janave, 1997; Vicentini *et al*., 1995) [10, 21], its accumulation may be resulting in 'stay – green' characters. It is also clear from the data that degradation of chl 'a' was faster than the degradation of chl 'b'.

Yah *et al*. (1998) [23] also observed that Kent variety of mango remained green upon ripening. Similar problem was noticed in Cavendish bananas (Janave, 1997) [10]. Blackbourn *et al*. (1990) [3] have suggested the inhibition may be due to the retention of thylakoid membranes and loss of electron transport capacity due to non-functional chlorophyll. Apart from this, no reports are available to understand the mechanism of inhibition of chl degreening on enzymatic basis. Therefore, more works are needed in this line, which may also helps to understand their role in different degree of softening in different mango cultivars. However, with this study the role of chlorophyll in the development of soft tissue is not clear and required more works in the line of energy generating system through electron transport of chlorophyll, which may involve protecting mango mesocarp from internal breakdown.

Variation in respiration rate and ethylene evolution in mango cultivars at different days of storage

Significant variation in rate of respiration and ethylene evolution was noticed among the cultivars at different period of storage being highest respiration (0.54 µ mol CO_2 m^{-2} s^{-1})

and ethylene (0.54 ppm) in Dashehari at 0 day and 7 day after harvest (18.35 µ mol CO_2 m^{-2} s^{-1}) respiration rate and 4.6 ppm ethylene) and lowest in Chausa in this corresponding period of storage (Table 3). It is clear from the data that the rate of respiration and ethylene began to increase from 0 day to 7 day in all cultivars. Its rate of respiration was considerably low on 9 day of storage in case of Chausa, however Dashehari and Langra on 9 day after storage showed the decrease level in both the attributes.

Table 3: Rate of respiration and ethylene evolution after different days of storage in mango cultivars during 2004–05 and 2005–06

Cultivar	0 day		7 day		9 day	
	Res	Eth	Res	Eth	Res	Eth
Chausa	0.31	0.24	10.25	3.0	13.15	2.4
Dashehari	0.54	0.50	18.35	4.6	11.35	1.5
Langra	0.32	0.40	12.62	4.1	11.89	2.6
CD (p = 0.05)	0.17	0.17	0.66	1.11	2.22	0.79

Res = Respiration (µ mol CO_2 m^{-2} s^{-1}), Eth = Ethylene (ppm)

Variations in TSS and pH level in mango cultivars

Variation in TSS and pH were also observed in these cultivars at various days of storage and maximum TSS was obtained in Chausa (19.2 °Brix) followed by Dashehari (19.0 °Brix) at 7 day after storage and minimum TSS was in Langra (18.6 °Brix) (Table 4). Statistically the Dashehari and Chausa did not show significant difference in TSS level. The same was with Langra and Dashehari when compared to each other for TSS. Its level was gradually decreased with increasing the storage period as indicated by its low level at 9 day after storage.

The pH level did not vary significantly at 0 day in Langra and Dashehari, however Chausa had significantly more pH as compared to these two cultivars. Significant difference in pH of pulp was obtained in different cultivars at 7 day after storage. The minimum pH (4.56) on 7 day of storage was recorded in Langra, which indicates the higher acidity as compared to Chausa (5.59) and Dashehari (5.78) indicating low acidity. On 9 day after storage there was no significant variation in Chausa and Dashehari in respect to their pH value, however, Langra showed significantly lower level of pH as compared to other two cultivars.

Table 4: Variation in TSS and pH of different cultivars of mango at various days of storage during 2004 – 05 and 2005 – 06

Cultivar	Storage period					
	0 day		7 day		9 day	
	TSS (°Brix)	pH	TSS (°Brix)	pH	TSS (°Brix)	pH
Chausa	6.98	4.33	19.2	5.59	17.9	5.26
Dashehari	10.2	3.96	19.0	5.78	16.8	5.75
Langra	8.10	3.30	18.6	4.56	16.6	4.15
CD (p = 0.05)	2.22	0.70	0.41	0.89	0.95	1.11

Changes in pectin methylesterase and polygalactouronase activities in mango cultivars

It is clear from the data (Tabale 5) that the Dashehari had maximum PME activity on 0 day of storage (800 unit / min / g FW) and minimum in Chausa (325 unit / min / g FW). Its activity increased initially up to 4 days (895 unit / min / g FW) and decreased slowly on 7 day (600 unit / min / g FW). On the other hand the activity of PME was decreased sharply (120 unit / min / g FW) on 9 day of fruit storage. Similar pattern of PME activity at different magnitude was obtained in Chausa and Langra. Contrary to the PME activity, the PG activity was increased consistently with increasing the storage period up to 7 day in all the cultivars, thereafter, its activity gradually declined in Chausa and Dashehari. However, cultivar Langra maintained its activity up to 9 day of storage (400 mg glucose released / g FW). Data also revealed that there were cultivar variations in PG activity at different days of storage as Dashehari registered maximum activity (570 mg glucose released / g FW) on 7 day after storage as compared to Langra (230 mg glucose released / g FW) and Chausa (285 mg glucose released / g FW). On the other hand the percent increase in its activity in Chausa and Dashehari was at par (47.36%) as compared to minimum percent increase in activity (13.0 mg glucose released / g FW) in case of Langra. The PG activity was found sluggish on 9 day of storage in Dashehari and Chausa cultivars and showing decreasing trend. PME is responsible for the de-esterfication of pectin required before polygalacturonase starts the depolymerization of pectin associated with fruit softening. The maximum activity of PME and PG in Dashehari cultivar at early stage of ripening may be associated with the incidence of softening in Dashehari.

Table 5: Changes in pectin methyl esterase (PME) and polygalacturonase (PG) activities in mango cultivars during 2004 – 05 and 2005 – 06

Cultivar	PME activity (Units equivalent / min / g FW)				PG activity (mg glucose equivalent released / g FW)			
	Days storage				Days storage			
	0	4	7	9	0	4	7	9
Chausa	325	369	300	145	150	188	285	215
Dashehari	800	895	600	120	300	476	570	380
Langra	500	580	300	250	200	215	230	400
CD (p = 0.05)	42.20	101.54	35.52	93.79	103.85	52.35	48.16	38.05

A considerable cultivar variation in ripening associated attributes like ethylene production, respiration rate, cell wall hydrolases (PME and PG) was noted in present study. It has been reported that cell separation and cell wall degeneration occur in disordered mesocarp of fruit affected with jelly seed, whereas cell cohesion is maintained in the healthy mesocarp (Burdon *et al*., 1991) [4]. It was also found that the tissues with jelly seed symptoms contained fewer fibre strands than healthy mesocarp (Singh, 2005) [5]. This may have been caused by a distruction or dissolution of fibres early in the onset of the disorder. This breakdown may have isolated the rest of the mesocarp from the seed, interrupting the supply of nutrients to the mesocarp. However, based on temporal and spatial differences in symptom development in fruits different cultivars, it appeared that variation in jelly seed development in three test cultivars may be due to variation in the activities of the parameters which are involve in this process.

Present observations show that the ethylene and respiration rate was highest in Dashehari on 7 day of storage as compared to Langra and Chausa. Similarly, the PME and PG activities varied significantly in these cultivars. In the pulp, their activity was much higher in Dashehari than in Chausa and Langra. After reaching a peak, the enzyme activities in the pulp of Dashehari decreased more rapidly than that of Chausa and Langra. Burst in ethylene production, increased in respiration rate followed by series of biochemical changes is a characteristics of climacteric category of fruit like mango.

In early date Burg and Burg (1962) [5] reported that mangoes are producing small amount of ethylene at the time of the commencement of the respiration climacteric. They suggested that the rapid rate of ripening which commences as soon as the mature fruit is picked from the tree is due to a ripening inhibitor which becomes inactivated on picking, rendering the fruit more susceptible to the low concentration of ethylene present in the fruit at this stage. When once the respiration climacteric commences autocatalytic production of ethylene proceeds as found in other climacteric class fruits. In respect to this concept the high rate of respiration and ethylene production in Dashehari as compared to Chausa particularly showed that this cultivar might be more susceptible to the ethylene production for loosing of shelf life and appearance of early softening.

Similar to this study, White *et al*. (2005) [22] evaluated 14 species of *Actinidia* for softening characteristics of fruit. They found that softening behaviours are differentially affected by genotype x harvest maturity. The mango fruit ripens only after harvest and shows a rapid transition from a hard to a soft flesh consistency. This change is intimately related to activity of cell wall degrading enzymes (PME and PG). PME is responsible for the de-esterification of pectin required before polygalactouronase starts the depolymerization of pectin associated with fruit softening (Mc Cready *et al*., 1995) [13].

Here, in mango, PME activity initially increases for a couple of days then it started to decline in all these cultivars. Its activity was much higher in Dashehari than Chausa cultivar. Contrary to the PME, the PG activity was increased consistently up to 7 day of storage being higher in Dashehari as compared to Chausa and Langra. The middle lamella of the cell wall of mango fruit is rich in pectic polysaccharides and is believed to be the region of the wall most affected during fruit softening. Different fruit contain varied levels of structural and non-structural carbohydrated thus requiring differential action by cell wall hydrolases (Muda *et al*., 1995; Ali *et al*., 2004) [15, 1]. These enzymes in turn are responsible for the differences in softening rates observed between fruits. Cell wall hydrolase, are thus considered to be the major factors responsible for softening in fruit (Rose and Bennett, 1999) [16]. Considerably high PME and PG activity in Dashehari cultivar seems to be responsible for quick softening and loosening of fruits as compared to Chausa and Langra.

On the other hand, PG activity observed higher in the inner mesocarp than the outer mesocarp (Mitcham and Mc Donald, 1992) [14]. The data also suggest that the softening is initiated on the inside of the fruit (Out side the endocarp / seed) and proceeds outward. Thus this portion soften earlier and becomes / jelly like at the time when the outer mesocarp started to soften, which may ultimately leads to jelly seed formation. PG activity is dependent on the substrate, therefore, being higher activity in Dashehari might be due to the variation in the molecular size and concentration of their substrate. Similar observation was made in tomato and avocado (Mc Cready *et al*., 1995) [13]. Further PG activity in mango seemed to reach a peak later than PME. Partial desterification of pectin by PME procedes the commencement of PG activity (Hobson, 1965) [7] appears to be necessary to provide attachment sites for polygalacturonase (Awad and Young, 1980) [2], thus the need for PME activity may decline early in the ripening process of mango this finding virtually corroborate the present report. Similarly the relationshiop of PME and PG activities were reported in tomato (Dahodwala *et al*., 1974; Lee and Macmillan, 1968) [6, 12] and apple (Ross *et al*., 1994) [17]. Recently Sane *et al*. (2005) [18] reported that softening in Dashehari mango is correlated with the expression of an early ethylene responsive, ripening related expansion gene, Mi Exp A1.

Cultivars variation in ripening attributes in respect to trend and magnitude clearly indicate that for regulation of the shelf life of fruit and control internal breakdown, each variety of mango has to be considered separately before any physical, physiological or molecular approach is made. Physiological weight loss, TSS, pH, chromacity values was also varied in these cultivars. The present results clearly showed that the difference in softening behaviour of fruit from different cultivars were largely associated with these parameters. Therefore, approach to regulate respiration, ethylene biosynthesis, enzymes hydrolases and other associated parameters at the initiation of post climacteric stage in mango may lead to slow down softening ripening process and enhance its shelf life. However, the present study opens up possibilities for the manipulation of softening / breakdown of tissue in mango.

Ripening associated biochemical changes viz., physiological weight loss (PLW), chlorophyll content, respiration rate, ethylene level, pectin methyl esterase (PME) and polygalacturonase (PG), catalase and peroxidase activities and their relation with softening of tissue in Chausa, Langra and Dashehari mangoes were studied at different period of their storage. Wide variations with superiority in Chausa in these attributes were recorded.

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