

*Bioactive and Biochemical Changes
During Growth & Development of
Spondias mangifera Fruits*

All great truths begin as blasphemies

– George Bernard Shaw

INTRODUCTION

Consumption of fruit at its peak accumulation of nutritional and bioactive compounds is governed by vast set of traits, which, in turn a function of fruit species. Thus, identifying the peak accumulation of nutritional and nutraceutical compounds, which associates with fruit growth and development is of vital importance. In post harvest physiology "mature" is defined as "that stage at which a commodity has reached a sufficient stage of fruit growth and development. Biochemical and bioactive changes during this period is critical and dictate harvesting and post harvest quality ultimately, the minimum acceptable characteristic quality to the consumer and/or food industry. Maturity stage differs with individual preferences: appearance and few visual defects are the criteria for growers and shippers, receivers. While, distributors and traders concerned about firmness and shelf life (Parker et al. 1990). Quality of the fruit is perceived by consumers in terms of fruit appearance, firmness, flavor, nutritional and nutraceutical value. Hence, correct maturity stage is essential for harvesting for maximum benefit in terms of catering every individual's need. The problem is multifold, because *Spondias mangifera* cv. Hal fruits tend to harden with the maturity. Consequently, the hog plum fruits have to be harvested after the attainment of maximum size but, prior to the hardening of the endocarp. Such index does not exist for Indian hog plum.

Coordinated biochemical alterations during fruit growth and development of *Spondias mangifera* cv. Hal determine the quality of fruits in terms of maturity, peak accumulation of bioactive compounds. Extensive review of literature showed lack of any maturity index for harvest. This is critical in horticulture to know, when to harvest the fruits. In the present study, successful identification of two bioactive compounds in *Spondias mangifera* cv. Hal gave an impetus to test teleological role of these bioactive compounds to define the maturity of fruits for harvest with preferred nutritional or nutraceutical or pharmaceutical quality. Participation of bioactive compounds in an array of teleological functions as precursors in imparting characteristic flavour, color, defense intermediaries and health benefiting factors in fruits, vegetables, and rhizomes were well documented [Tholl, 2006]. Temporal variation in the concentration of bioactive molecules

is regulated by a complex interactions between intrinsic plant factors and external storage factors [Herms and Mattson, 1992; Beckman, 2000; Booij-James *et al.*, 2000]. Lack of such studies in *Spondias mangifera* cv. Hal is apparent despite its pharmaceutical importance and exotic taste.

In the present study, among the isolated compounds, Spondiol exhibited the highest antioxidant, platelet-aggregation inhibitory activity and antimicrobial activity. Since, it is highly stable and easy to estimate, accumulation pattern of Spondiol and other changes in soluble and storage components as a function of physiological maturity in *Spondias mangifera* cv. Hal fruits were studied. A time course study of these changes from the time of fruit set to harvest, which ranged from 1 to 13 week at a time interval of 2 week were carried out. The details were presented in this chapter.

Sample collection

The fruits of Indian hog plum (*Spondias mangifera* Willd.) were procured from the local orchard of Mysore, Karnataka, India. The first sampling time (one week after fruit set) was conducted after fruit formation. Subsequently, the samples were collected at 1, 3, 5, 7, 9, 11 and 13 weeks after fruit set. Each sample was prepared from fruits obtained from *Spondias mangifera* trees that were harvested randomly from five different trees. All the biochemical analysis and other experiments were carried out in triplicates.

Physical changes

Physical measurement

Three replicates of 10 fruits for each stage of maturity were individually analyzed for physical characteristics. The length and diameter of the fruits were measured with a vernier caliper. The length was measured at the polar axis of fruit, i.e., between the apex and the stem. The maximum width of the fruit, measured in the direction perpendicular to the polar axis, defines the diameter of the fruit.

Firmness measurement

Firmness was evaluated according to the method described by Soliva-Fortuny et al. (2002), with some modification. Force of penetration was measured by using Lloyd texture instrument. Analysis was used to measure the force required for a 5 mm diameter probe to penetrate the *Spondias mangifera* fruits to a depth of 4-5mm at a rate of 50 mm/min using 100 Kg load cell. Samples were placed so that the rod penetrated their geometric centers.

Colour measurement

Skin color and flesh color were determined with a colorimeter (Minolta Spectrophotometer CM-3500d, Osaka, Japan). Color measurements were recorded using the CIE L*a*b* color space. Color values for each fruit were computed as means of 2 measurements taken from opposite sides at the equatorial region of the fruit (Abbott, 1999).

Physiological changes

Respiration

Weighed fruits of each stage were enclosed in 1300 ml hermetic containers for one hour and one ml of the atmosphere of the container was withdrawn and injected into GC model HP 6890 series having poropak Q column with TCD detector using nitrogen as the carrier gas at a flow rate of 30 ml/min. The percentage of CO₂ was calculated by simultaneous running of the standard CO₂ gas. The CO₂ evolution was calculated in mg/Kg-h by using the formula:

$$\text{CO}_2 = \frac{\text{Density of CO}_2 \times \text{CO}_2 \text{ released (\%)} \times \text{Container Volume} \times 60}{\text{Weight of the Sample (Kg)} \times \text{Enclosure time (min)} \times 100}$$

Biochemical analysis

Sample preparation

About 500 g of *Spondias mangifera* fruits were sliced, homogenized, and squeezed in two-layered muslin cloth, to extract the complete juice. The juice was centrifuged at 8000 rpm for 20 min at 4°C and used to determine pH, titrable acidity, total soluble solids (TSS), sugar content, protein content, and phenolic content.

Chlorophyll determination

Chlorophyll content was determined by using a UV-VIS spectrophotometer. Sample preparation and spectrophotometric measurements were conducted as described in AOAC method (1990). Chlorophyll content was calculated as follows:

$$\text{Total chlorophyll} = 7.12A_{660.0} + 16.8A_{642.5},$$

$$\text{Chlorophyll a} = 9.93A_{660.0} + 0.777A_{642.5},$$

$$\text{Chlorophyll b} = 17.6A_{660.0} + 2.81A_{642.5}.$$

Carotenoids determination

Total carotenoid content was determined by spectrophotometric method described by Ranganna (2001). In brief, about 10 g of *Spondias mangifera* fruit pulp was blended with 100 ml cold acetone in a pestle and mortar and filtered over cotton pad. Extraction was repeated until residue was colorless. Acetone extract was placed in the separating

funnel and agitated with 25 ml petroleum ether and 5 ml water. The mixture was left to stand for 30 min. The yellow colored petroleum ether extract was collected and filtered over anhydrous sodium sulphate on a Whatman filter paper No. 1. The extract was made up to 25 ml and the color intensity of carotenoid extract was measured at 450 nm in a UV-Visible spectrophotometer (UV-160A, Shimadzu Co. Japan). The total carotenoid content was calculated on the basis of the calibration curve of β -carotene and expressed as β -carotene equivalents mg/ 100 gm *Spondias mangifera*.

Ascorbic acid, total sugar reducing sugar and total protein content

Ascorbic acid was determined according to the AOAC method (1990). Total Sugar estimations was carried out by the method described by Dubois (1956) and reducing sugar by Miller (1959). The total protein content was determined by the Bradford method (1976), using bovine serum albumin (BSA) (Sigma Chemical, St. Louis, USA) as a standard protein.

pH, titrable acidity and total soluble solids

pH of the fresh juice was measured using pH meter calibrated with standard buffer at pH 4. Titrable acidity was determined by AOAC (1990) method. The total soluble solids (TSS) were determined by an RX-5000 digital refractometer (ATAGO, Japan) calibrated with distilled water. *Spondias mangifera* juice was passed through a filter paper (Whatman No.1) using vacuum before analysis.

Determination of phenolics

The total phenolic content in *Spondias mangifera* fruit was determined with the modified method of Taga et al. (1984). In brief, 100 μ L of sample was mixed with 2 mL of 2% aqueous sodium carbonate solution. After 3 min, 100 μ L of 50% Folin-Ciocalteu phenol reagent was added to the mixture. After 30 min of incubation at room temperature, absorbance was measured at 750 nm against a blank. Total phenolic content was calculated on the basis of the standard curve of Gallic acid.

Statistical analysis

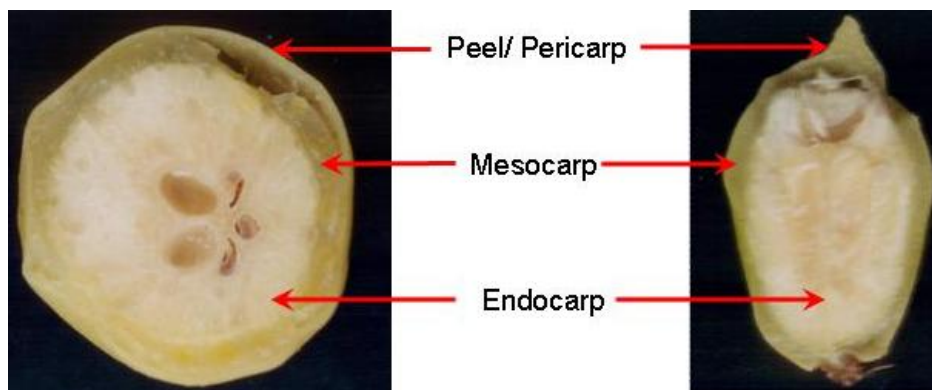
The data was subjected to Duncan's Multiple Range Test (DMRT) to determine significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Fig. 3.1: Growth and development of *Spondias mangifera* fruit.



Fig. 3.2: Transverse and vertical sections of *Spondias mangifera* fruit.



Depending upon number of weeks from the fruit initiation set, three distinct stages were categorized including the following stages for the convenience of explanation of the results carried out:

Table 3.1: Maturity indices of *Spondias mangifera*

| Stage | Weeks after fruit set |
|---------|-----------------------|
| Stage 1 | 1-5 |
| Stage 2 | 7-9 |
| Stage 3 | 11-13 |

PHYSICAL

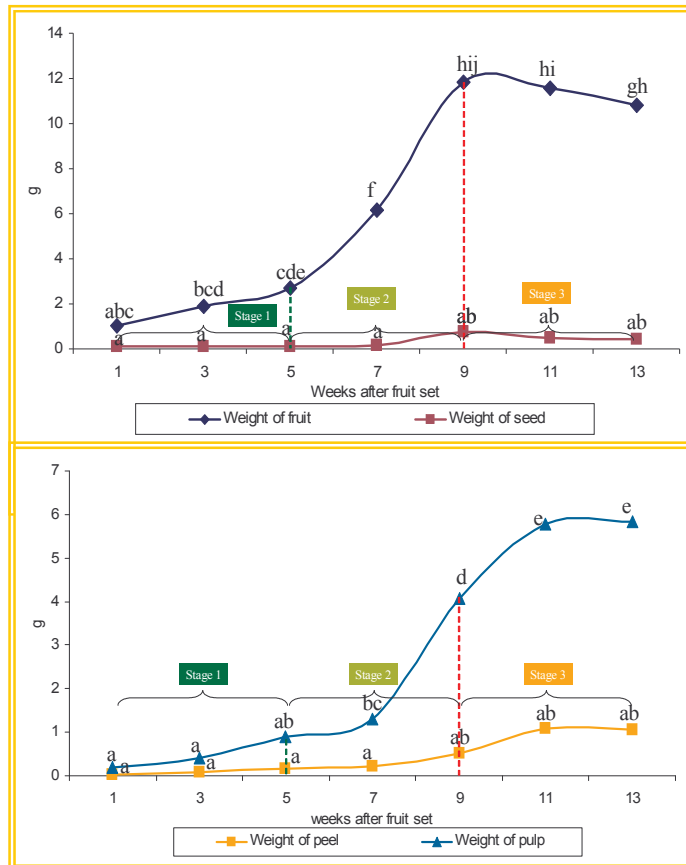
Length, Diameter, weight, texture, colour

Spondias mangifera

fruits showed a simple sigmoid curve with three distinct stages, when all the contingent of fruit growth was plotted against time from fruit set. There is a moderate increase in weight up to 5 weeks after fruit set, followed by two fold increase in weight for every fortnight from fifth to ninth week of development of fruit and finally a static or slight increase in growth for a period of two weeks from 9th week, which coincides with hardening of endocarp. This pattern of growth has been similar to the simple sigmoid growth curve reported in almond, apple and mango (Murkhejee and Dutta, 1967;

Rodriguez et al., 1971; Yusof and Suhaila, 1987; Jagtiani et al., 1988). However, this in contrast with development of fleshy drupe fruits of temperate zone like apricot, cherries and plums (Kennard, 1955). The longer growth period of fruits during initial stage of 5 weeks period is probably a response to cooler temperatures. Fruit mass changes showed a trend similar to that observed for diameter [Fig. 3.3]. Sigmoid growth curve was exhibited by all the contingencies of other fruit growth parameters such as length, diameter, fresh weight, of exocarp, mesocarp, endocarp and seeds were plotted against time. The ratio between the seed and the fruit increase with increase in time from fruit set. Increase in weight of the fruit from 0.85 to 5.28 g with advance of time was observed till 9th week of fruit set. The exponential increase in weight of the fruit till 9th week was observed.

Fig. 3.3: Changes in weight of *Spondias mangifera* during fruit development.

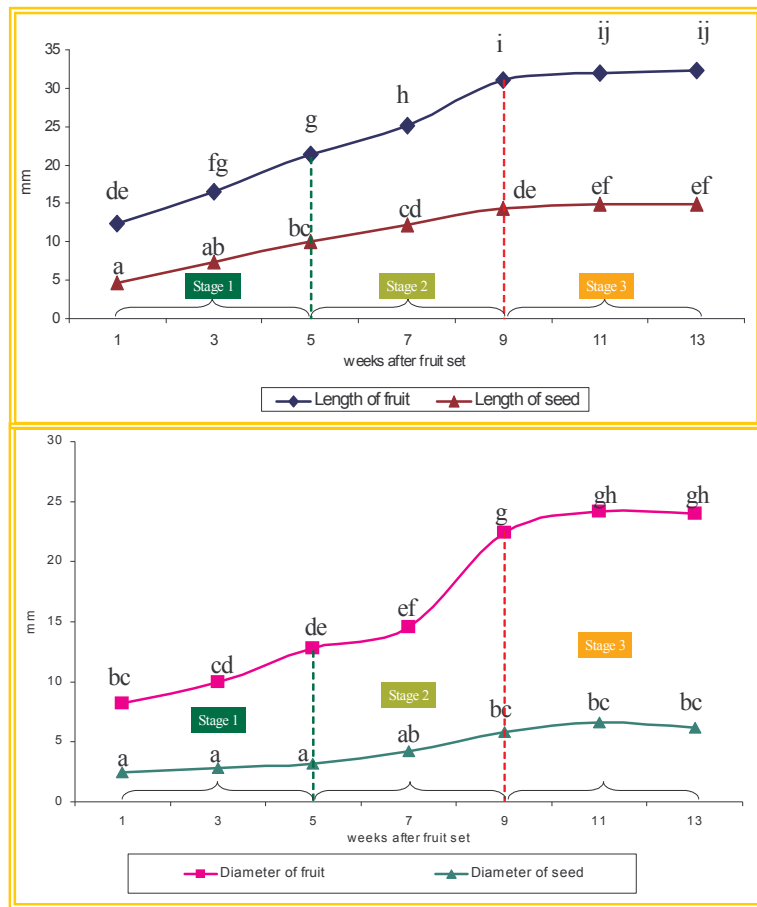


Each value is a mean of three different observations.

Values showed by different letters for each line are significantly different at $p < 0.05$.

Some of structural features of nectarines and peaches resemble to those of *Spondias mangifera* fruit. These are the some of the striking similarities have been observed in *Spondias mangifera* fruit: The fruit is a drupe developed from superior ovary and has no floral residue around the peduncle (Brady, 1993). These fruits have a thin outer skin (epicarp/exocarp), soft flesh of varying thickness under the skin (mesocarp) and an endocarp that is highly lignified (referred to as the stone or pit) that develops from the inner ovary wall. The skin is composed of cuticle, epidermis and some hypodermal cell layers. Peaches have trichomes or hair (“fuzz”) whilst, nectarines and *Spondias mangiferas* have smooth surfaces with no hair (Kader and Mitchell, 1989).

Fig. 3.4: Changes in diameter of *Spondias mangifera* during fruit development.



Each value is a mean of three different observations.
Values showed by different letters for each line are significantly different at $p < 0.05$.

The growth patterns of *Spondias mangifera* fruit unlike other fruits appear to be form of a simple, rather than double sigmoid curve. Coloring and softening of the flesh is from seed outwards; at this stage, the latter has become surrounded by a cartilaginous and finally, strong endocarp. These readily observable changes have been used as a means of assessing the optimal picking date for immediate consumption or for storage. Indian hog plum normally reach maturity in 2 to 3 months from flowering or 9 to 11 week from fruit set. They are harvested at a mature green stage and used as vegetable when unripe and as fruit

The distribution of the various weight components of the fruit is presented in **Table 3.2**. Taking into consideration of all the maturities, it was observed that, on an average, the seed, skin and pulp constituted 4.56 %, 43.00 %, 52.40 % of the weight of the whole fruit respectively. The flesh was found to be white in colour and the skin was green in colour at all the maturity stages. But, the skin turned into yellow colour. When the fruit is still in its immature green, sour and with its seed is not hardened. The total weight of the fruit increased from stage 1 to stage 7.

Table 3.2 Weight of various components of *Spondias mangifera* fruit

| Parameter (mm) | No. of weeks after fruit set | | | | | | |
|-------------------------------|------------------------------|-------|-------|---------|-------|---------|-------|
| | Stage 1 | | | Stage 2 | | Stage 3 | |
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 |
| Weight of fruit | 0.85 | 1.05 | 2.12 | 2.56 | 5.28 | 11.46 | 12.68 |
| Weight of peel | 0.15 | 0.16 | 0.20 | 0.22 | 0.36 | 0.42 | 0.53 |
| Weight of mesocarp | 0.25 | 0.36 | 0.56 | 0.68 | 0.90 | 3.96 | 4.77 |
| Weight of endocarp | 0.36 | 0.44 | 1.26 | 1.52 | 3.84 | 6.86 | 7.12 |
| Weight of seed | 0.09 | 0.094 | 0.096 | 0.14 | 0.18 | 0.22 | 0.26 |
| Ratio of fruit to Seed weight | 9.44 | 11.17 | 22.08 | 18.29 | 29.33 | 52.09 | 48.77 |
| Ratio of pulp to Peel weight | 1.67 | 2.25 | 2.80 | 3.09 | 2.50 | 9.43 | 9.00 |

The mature fruits are ovoid or ellipsoidal in shape, measuring 2.5-3.5 cm in length. The fruits are penta-carpellary with single seed in each carpel [**Table 3.3**, **Fig. 3.1**]. The endocarp is edible when it is soft. With advance of maturity the endocarp toughened with longitudinal interwoven fibers thus become inedible. The results indicate that hardening of endocarp begins after 9 weeks of fruit set]. Thus it reduces 80% of the fruit unavailable for consumption. It

Table 3.3 Physical parameters of the *Spondias mangifera* fruit and its components

| Parameter (mm) | No. of weeks after fruit set | | | | | | |
|------------------------------------|------------------------------|------|------|---------|------|---------|------|
| | Stage 1 | | | Stage 2 | | Stage 3 | |
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 |
| Length of fruit | 12.4 | 16.6 | 21.4 | 25.2 | 31 | 38.6 | 46.6 |
| Diameter of fruit | 8.2 | 10 | 12.8 | 14.6 | 17.4 | 24.2 | 31.2 |
| Length of seed | 4.6 | 7.4 | 10 | 12.2 | 13 | 15.8 | 20 |
| Diameter of seed | 2.5 | 2.8 | 3.2 | 4.2 | 5 | 5 | 5.2 |
| Thickness of endocarp | 2.8 | 2.8 | 3 | 3 | 4 | 4.8 | 5.4 |
| Thickness of mesocarp | 0.4 | 0.6 | 0.8 | 1.2 | 1.4 | 1.6 | 1.8 |
| Thickness of peel | 0 | 0 | 0 | 0.2 | 0.4 | 0.6 | 0.8 |
| Length/ diameter of fruit | 1.5 | 1.7 | 1.7 | 1.7 | 1.8 | 1.6 | 1.5 |
| Ratio of fruit to seed diameter | 3.3 | 3.6 | 4.0 | 3.5 | 3.5 | 4.8 | 6.0 |
| Ratio of fruit to seed length | 2.7 | 2.2 | 2.1 | 2.1 | 2.4 | 2.4 | 2.3 |
| Thickness (endocarp+mesocarp+peel) | 3.2 | 3.4 | 3.8 | 4.4 | 5.8 | 7 | 8 |

is interesting to note that hardening of endocarp associated with various physiological and biochemical changes in the fruit. Hardening of endocarp is the cause or consequence of these changes remains to be solved.

For the first time different stages in fruit growth in *Spondias mangifera* is defined based on physical parameters. Maturity indices depend upon the nature of the fruits. The various maturity indices that are followed for different stone fruits were Size and shape (Lill et al., 1989). Flesh firmness (Kader and Mitchell, 1989a; Kader and Mitchell, 1989b), Color (Rood, 1957; Romani and Jennings, 1971; Ryall and Pentzer 1982), Soluble Solids Concentration (SSC) (Mitchell et al., 1990; Mitchell et al., 1991), Titratable acidity (Reid, 1992), TSS : TA ratio Batten (1989) sugar, acid, sugar : acid ratio, Underhill and Wong (1990). However there is no report on use of endocarp development as maturity index for any stone fruits including mango. The present study, development and differentiation of endocarp in the Indian Hog plum, was found to be a function of maturity. It can be used as maturity index in India hog plum.

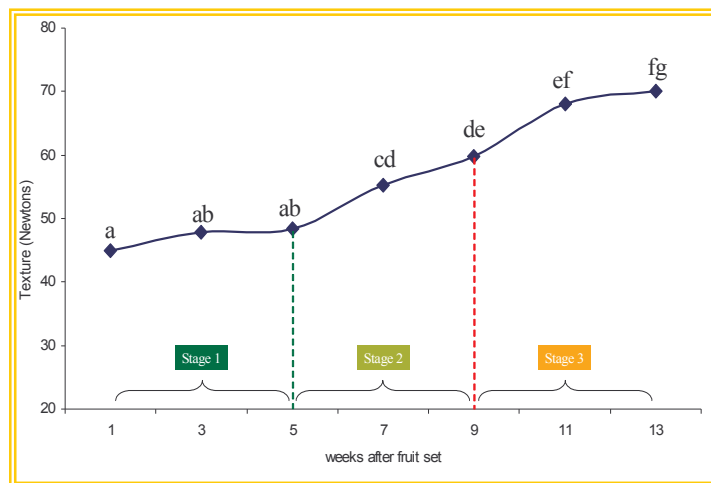
Texture

Fruit firmness usually follows a declining trend during maturation and ripening. Unlike the maturation of the fruit, firmness of the *Spondias mangifera* increases during development as the endocarp hardens. The force required for penetration into the fruit

gradually increased from fruits harvested after one week to thirteen weeks. During first stage of fruit development increase in percentage of firmness was 7 %. While at the end of stage 2 and stage 3 development, force required for penetration increased to 19 % and 15 % respectively [Fig. 3.5].

Significant increase in pressure during stage 2 & 3 compared to the stage 1 may be due to the hardening of the endocarp during the maturation of the fruit. During Stage 2 fruit developed fully preparing for the hardening of the endocarp during stage 3 of the development of the fruit, wherein, recorded highest firmness of 70N. The rise may also be

Fig. 3.5: Changes in texture in *Spondias mangifera* during fruits development



Each value is a mean of three different observations.
Values showed by different letters for each line are significantly different at $p < 0.05$.

attributed to the lignification of secondary cell wall with the advancement of the fruit maturity.

Fruit firmness is one of the practical and an excellent indicator for judging maturity. Though this is a destructive method, development of advanced techniques such as near infrared (NIR) spectroscopy has been used to evaluate internal quality of fresh fruits such as peach (Kawano et al., 1992, 1995), satsuma mandarin (Kawano et al., 1993) and mango (Guthrie and Walsh, 1997; Peiris et al., 1999; Schmilovitch et al., 2000). This can be quantified by hand or mechanical devices (Thompson, 1996). Similar trend in firmness at commercial maturity of early, mid and late season nectarines increased gradually unlike other fruits, where there was decline due to ripening and other associated changes (Kader and Mitchell, 1989a).

ENDOCARP

Endocarp formation and hardening

The fruit of the *Spondias mangifera* attains maturity on 9th week from fruit set. With no or little change in weight of the fruit accompanied with hardening of endocarp was observed till 11th week after fruit set. These visible observable changes can be considered to determine the harvest maturity in Indian hog plum fruits [Fig. 3.1]. There is an increase in thickness of the fruit with increase in duration after fruit set. Endocarp contributes more to the fruit thickness than the mesocarp. Thin, papery pericarp contributes to the least either to the thickness or to the weight of the fruit. Dimensions of the *Spondias mangifera* fruit and its components are shown in Table 3.1. The fruit is ovoid to ellipsoidal in shape, being the ratio of length to the diameter of the fruit is 1.7 for all maturities. The thickness of the pulp increased from 0.4 cm in stage 1 to 1.8 cm in stage 7 fruits.

The endocarp is white and cartilaginous and restricted itself to the respective carpels. With advance of development and maturity of the fruits they finally fuse together. This process of carpellary fusion and demarcation of endocarp from surrounding mesocarp completes on 9th week after fruit set [Fig. 3.1]. Later the physiological activities may concentrate more on hardening of endocarp, which was achieved by longitudinal

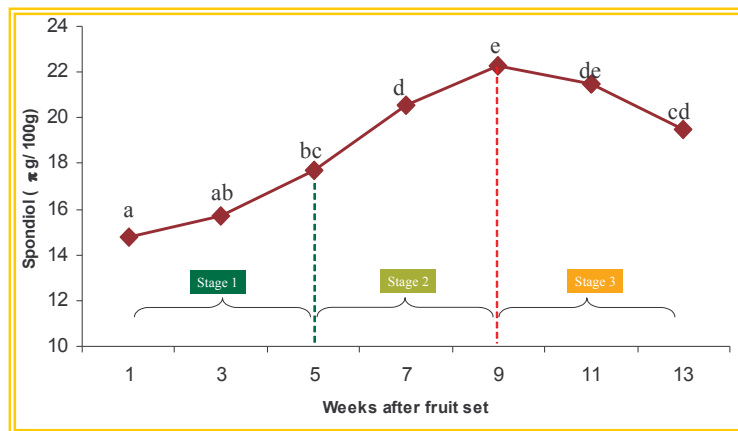
interwoven of fibers for a period of two to three weeks as in mango and drupes such as nectarines and peaches (Kader and Mitchell, 1989b). Thus extending these readily observable changes have been used as a means of assessing the optimal picking date for immediate consumption or for storage.

BIOACTIVE

Spondiol

Concentration of the bioactive compound 'Spondiol' increased with the development of the *Spondias mangifera* fruit. There was significant increase in the accumulation of Spondiol after five weeks after fruit set, which reached highest (22.25 mg/ 100g) after nine weeks [Fig. 3.6]. This followed by a decrease during third stage of development.

Fig. 3.6: Changes in Spondiol during fruit development of *Spondias mangifera* fruit.



Each value is a mean of three different observations.

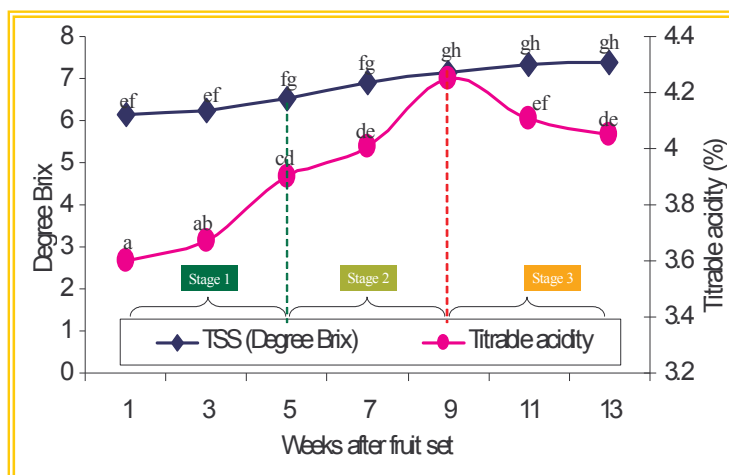
Values showed by different letters for each line are significantly different at $p < 0.05$.

BIOCHEMICAL

pH, titrable acidity and total soluble solids

pH, acidity and ascorbic acid content were highest in the early maturity stages compared to the late maturity stages. Similarly in mango (Rao et al., 1998). *Spondias mangifera* fruit, like stone fruits, lose acidity during maturation and ripening. This maturity feature is also affected by cultivar and seasonal variability (Boggess et al., 1974; Rood, 1957; Salunkhe et al., 1968).

Fig. 3.7: Changes in total soluble solids and titrable acidity in *Spondias mangifera* during fruit development



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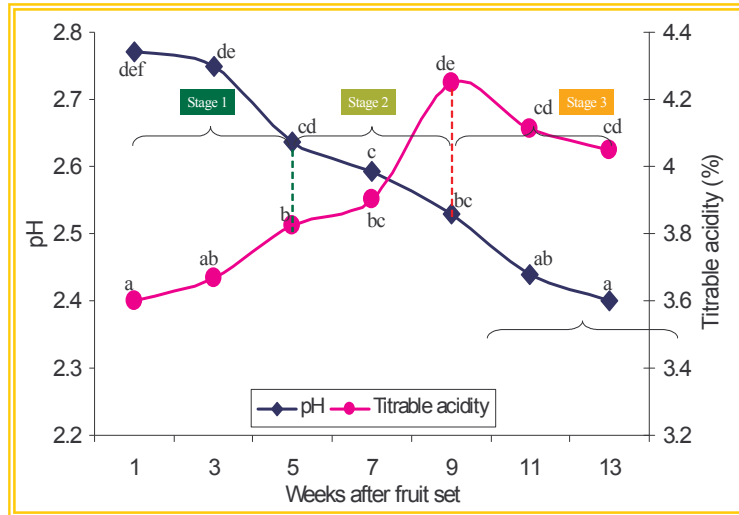
Maximum amount of brix (7.35), total sugars (0.88 mg/100g), reducing sugars (0.07 mg/ 100g) and non-reducing sugars (0.82 mg/ 100g) were observed in stage 7 [Fig. 3.7]. Similar trend in increase of total soluble solids observed in stone fruit, peach (Kakiuchi et al., 1981). Generally, sugars constitute major quantity in total soluble solids of the fruit juice. Measuring soluble solids in fruit juice can give a reliable measurement of sugar content and serve as a guide to maturation making it one of the indicators of eating quality. But, this may vary widely with varieties, production area and season (Dann and Jerie, 1988; Kader and Mitchell, 1989a) even fruit the orientation in the canopy (Mitchell et al., 1990; Mitchell et al., 1991) rendering its limitations as maturity index.

Conversion of polysaccharides into sugars may be the reason for the increase in sugar content. Gradual decrease in pH, acidity and ascorbic acid may be correlated to the

ripening changes associated with the maturity of the fruit. As the fruit developing from early maturity stage to the fully matured state, there are number of ripening changes associated, involving conversion of polysaccharides into reducing and non-reducing sugars, which may be the reason for the increase in the amount of total sugar content.

Fig. 3.8: Changes in pH and titrable acidity in *Spondias mangifera* during fruit development

The fruit being highly acidic in nature, it tends to show the gradual rise in the acidity and concomitant decline in pH indicating the nature of the fruit. In the developing Indian hog plum fruits, acidity increased at the early growth phase, reached a peak on the 9th week after fruit set and then declined gradually until harvest [Fig. 3.8]. In fruit the acidity reached maximum in about 9 weeks and declined slowly at the time of harvest.

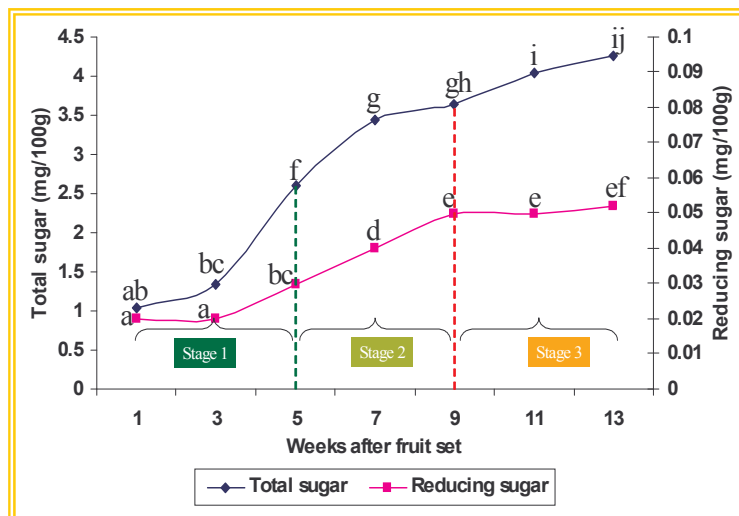


Each value is a mean of three different observations. Values showed by different letters for each line are significantly different at $p < 0.05$.

Total sugars and reducing sugars

Fig. 3.9: Changes in total sugar and reducing sugar content in *Spondias mangifera* during fruit development

At initial stages of fruit development, no systematic trend was observed in the sugar content, but after 3rd week both reducing and non-reducing sugars were found to be increasing. The total and reducing sugar content in fresh fruit varied between



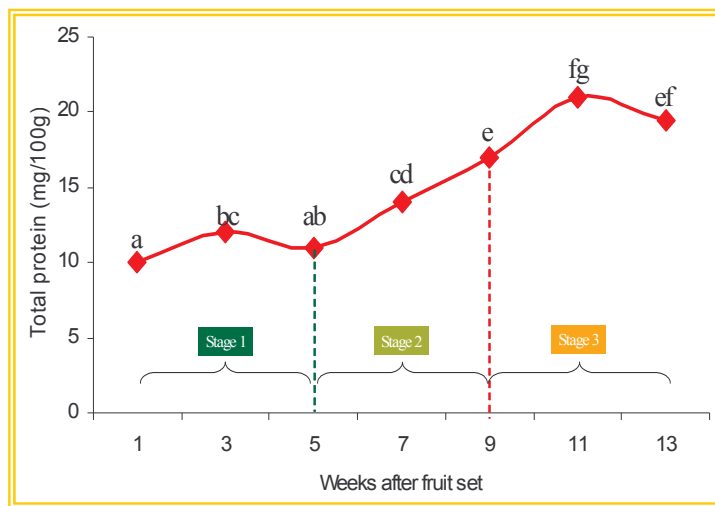
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1.0 and 4.5 mg/100g and 0.02 to 0.05 mg/100g respectively. There was a steep increase in the concentration of total sugars till 7th week, but slow down towards the end of maturity [Fig. 3.9]. While reducing sugar after 9th week, showed no change or slight decrease towards the end of maturity. Interestingly accumulation pattern of reducing sugar showed similarity with sigmoid growth curve. The soluble sugars of the fruit pulp consisted mainly of glucose, fructose, and sucrose.

Total protein

Protein content of the fruit was increasing as the fruit was developing from immature juvenile phase to matured fruit. Maximum protein content of 21mg/100g was observed in the fruits harvested after eleven weeks [Fig. 3.10]. Chaturvedi (1974) reported that alcohol-insoluble solids (AIS) and protein contents of guava fruit, at different stages of maturity, decreased with fruit development.

Fig. 3.10: Changes in total protein content in *Spondias mangifera* during fruit development

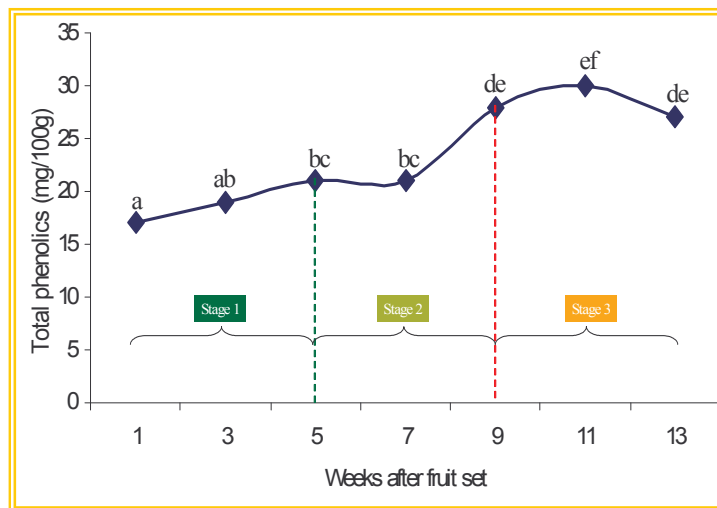


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Values showed by different letters for each line are significantly different at $p < 0.05$.

Total phenolics

Total phenolic content was observed to be an index of fruit development and maturation. The rate of phenols accumulation is slow at the beginning of fruit growth but during the exponential growth phase

Fig. 3.11: Changes in total phenolic content in *Spondias mangifera* during fruit development



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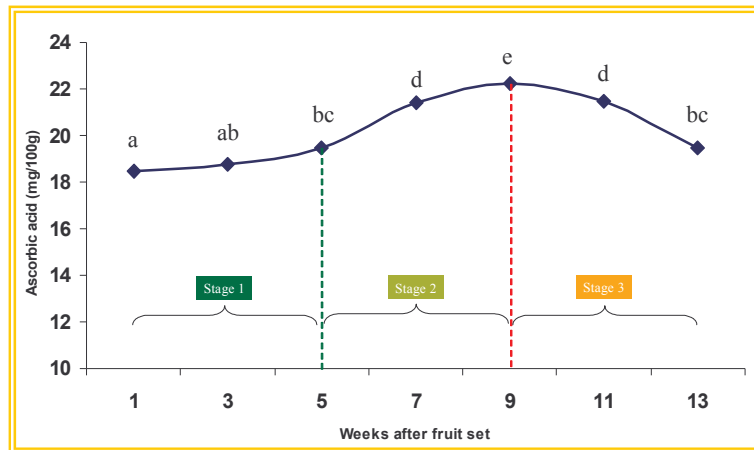
of the fruit, total phenolic content increased significantly (28 mg/ 100g) till 9th week after fruit set [Fig. 3.11]. During development accumulation of total phenols appear to follow the similar pattern of simple single sigmoid growth the fruit. Decline in total phenolics after eleven weeks after fruit set may be due to the oxidation of phenolic content by polyphenol oxidase (Amiot et al., 1995). Phenolic content is influenced by fruit maturity and similar trends were observed in grapes and pears (Fernandez de Simon et al., 1993; Amiot et al., 1995; Mayr et al., 1995).

Ascorbic acid

Ascorbic acid content was observed to be one of the indexes for the optimum stage of harvest of the *Spondias mangifera* fruits. Maximum ascorbic acid content of 22.63 mg/ 100g is accumulated till the fruit reached to the full maturation phase and then it started declining as the

maturation advanced [Fig. 3.12]. A similar trend was reported in mango and guava fruits (Bashir & Abu-Goukh, 2003). Similar studies on ascorbic acid in guava (El-Bulk et al. 1995, Rodriguez et al., 1971) showed that the ascorbic acid content for developing guava increased slowly during the initial growing period, followed by rapid increase during maturation and ripening. These cumulative changes leading to the increase in total soluble solids, sugars, carotenoids and ascorbic acid, while decrease in titrable acidity, total chlorophyll and total phenolics with fruit maturity rendering optimum eating quality attainment at the climacteric peak is in agreement with the Al-Niami et al. 1992.

Fig. 3.12: Changes in ascorbic acid content in *Spondias mangifera* during fruit development.

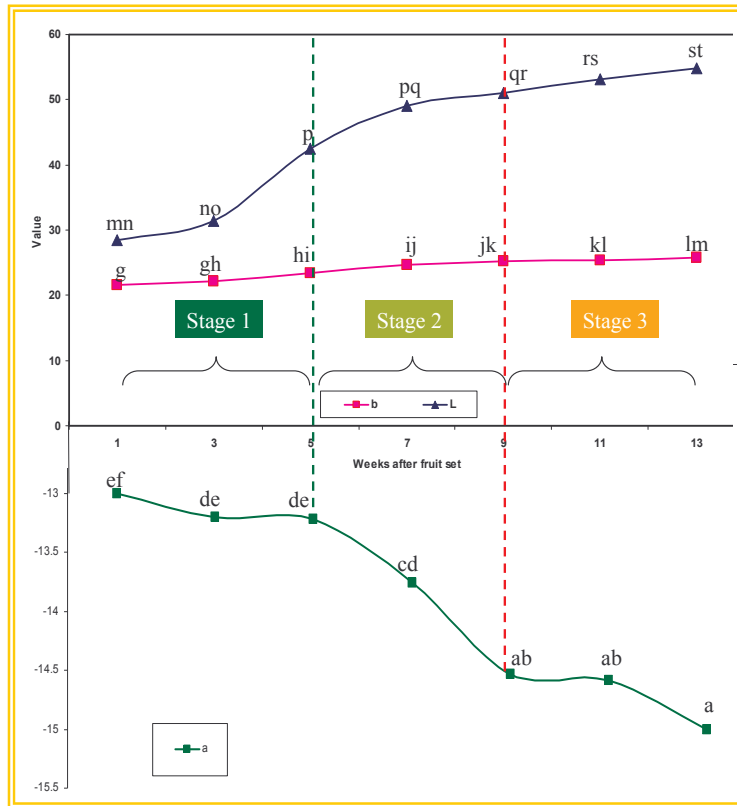


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Pigments and Colour

The L^* and Hue values at harvest were higher with increasing maturity stage; Lightness ‘L’ values were associated with the accumulation of carotenoids and/or deterioration of the chlorophyll pigments. It was highest in late harvested fruits (which appeared more yellowish), consistent with the colour alteration, total chlorophyll content was highest in early harvested fruits (1704.25 $\mu\text{g}/100\text{ g}$) and decreased gradually and reached

Fig. 3.13: Changes in colour readings of *Spondias mangifera* during fruit development.



Each value is a mean of three different observations.
Values showed by different letters for each line are significantly different at $p < 0.05$.

lowest chlorophyll content in fruits harvested after thirteen weeks (789.36 $\mu\text{g}/100\text{ g}$) [Fig. 3.14]. The dark nature of the fruit may be due to the high chlorophyll. Arias et al. (2000) and Shewfelt et al. (1988) reported the decrease of L^* with maturity reflecting the darkening of the tomatoes while in case of *Spondias mangiferas* it is the converse, showing increase of L^* may be due to the absence of dark pigmentation unlike tomatoes.

Individual colour values such as ‘a’, which is associated with green colour, decreased with fruit maturation. Colour differences during various maturity stages showed a decrease from -13 value to ‘a’ in stage 1 to -15 in stage 7 (Fig. 3.5). The a^* values were different for each stage of maturity and increased after fruit set. These values correspond to yellowish green fruit. The ‘b’ values also increased with the gradual change from green to yellow in the skin colour. An increase in yellow colour from 21.56 value of ‘b’ in stage 1 to 25.75 in stage 5, may be due to the accumulation of carotenoids. These changes are caused by the degradation of chlorophyll and the accumulation of carotenoid

pigments (Song et al., 1997). Increased lightness accompanied with accumulation of carotenoids with concomitant decrease in total chlorophyll converges on ninth week period after fruit set [Fig. 3.13] may indicate maturation of the fruit. This is the first report of nondestructive objective determination of external color as a reliable harvest index in Indian hog plum.

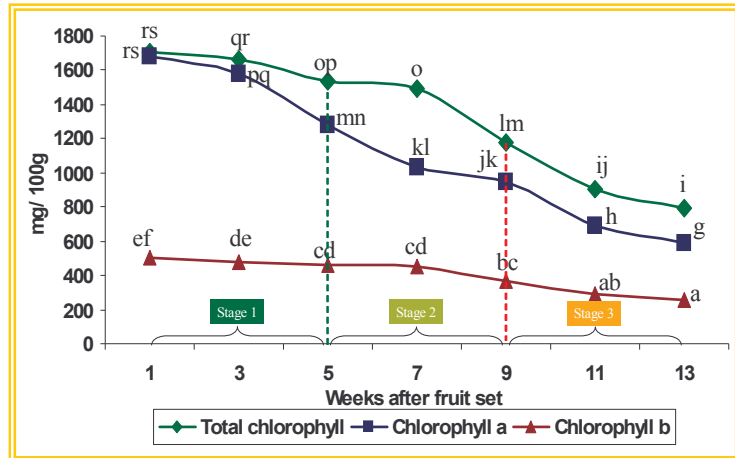
Pigments such as chlorophyll a, chlorophyll b and total chlorophyll also followed a decreasing trend with the advancement of maturity of the fruit [Fig. 3.14]. Maximum and less content of chlorophyll pigments in early and late developmental stages respectively may be due to

the degradation of chlorophyll as the fruit develops into fully matured fruit as in other drupaceous fruits such as mango (Va'squez-Caicedo et al., 2004) and *Spondias cytherea* Sonn (Ishak et al., 2005). Contrarily peak accumulation of carotene featured in the fully matured fruits. Total phenolics were found to be highest in case of fully matured fruits.

Visual observation of the skin pigmentation is the most evident change during ripening (Kader and Mitchell, 1989a; Lill et al., 1989; Robertson et al., 1990; Romani and Jennings, 1971; Ryall and Pentzer, 1982). Quick, reliable, convenient and ease of using color measurements correlated the long, tedious, and costly chemical methods of quantifying pigment, several studies have correlated the color with the pigment in different food stuffs. Apart from wide variety of leafy vegetables and other food stuffs, tomato (Arias et al. 2000), blueberries (Francis, 1985), grapes (Watada and Abbott, 1975), peaches (Morrison, 1990), are some of the food materials in which the color has been correlated with the pigment content.

Colour of the skin is quantified by determining various pigments in the skin and underlying fruit tissue. As the maturation of the fruit progresses, apparent changes are

Fig. 3.14: Changes in chlorophyll pigments in *Spondias mangifera* during fruit development

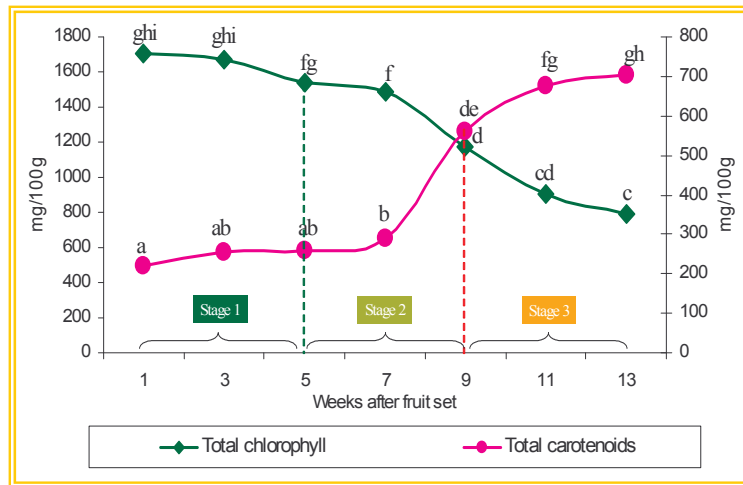


Each value is a mean of three different observations.
Values showed by different letters for each line are significantly different at $p < 0.05$.

brought about by the degradation of chlorophyll and concomitant biosynthesis of either anthocyanins or carotenoids (Tucker, 1993).

Therefore, as *Spondias mangifera* fruit mature and ripen, like nectarines and peaches, show a change in ground colour from green to white or yellow (Kader and Mitchell, 1989b) and this change is a major criterion for determining fruit maturity.

Fig. 3.15: Changes in total chlorophyll and total carotenoids in *Spondias mangifera* during fruits development



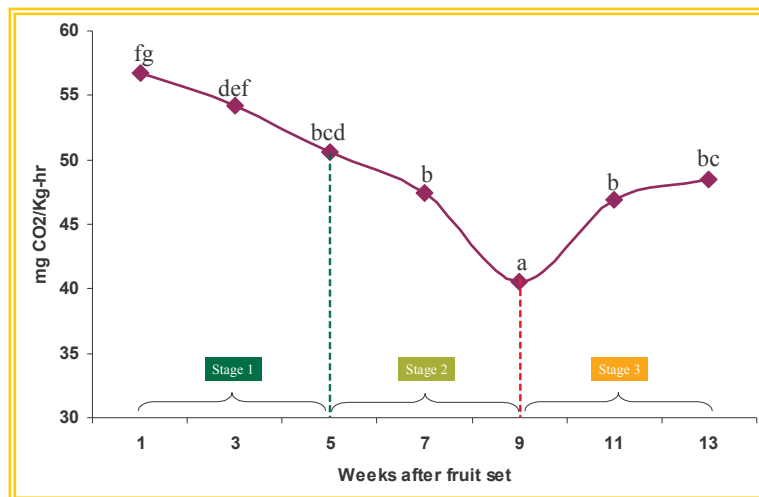
Each value is a mean of three different observations. Values showed by different letters for each line are significantly different at $p < 0.05$.

PHYSIOLOGICAL

Rate of Respiration

Rate of respiration in *Spondias mangifera* fruits showed a decreasing trend as a function of development till nine weeks during development leading to a pre-climacteric minimum of 40.57 mg CO₂/ kg-hr during 9 weeks after fruit set at the end of stage 2 followed by a rise in the rate of respiration [Fig. 3.16]. The high respiration rate (56.75 mg CO₂/ kg-hr) during initial harvest stage may be matched to the

Fig. 3.16: Changes in rate of respiration in *Spondias mangifera* during fruit development



Each value is a mean of three different observations. Values showed by different letters for each line are significantly different at $p < 0.05$.

higher surface area and the phase of maximum cell division, rapid growth phase marked by cell expansion phase, diminished surface area may result in decrease in rate of respiration, a phenomenon commonly recorded in climacteric fruits (Biale and Young, 1981, Kader, 2000). Wills et al. (1989) also reported that respiration rate per unit weight was observed to be highest for immature tissues and then steadily decreased with age. The upsurge of respiration prior to harvest may indicate the completion of maturity or herald the onset of ripening of fruit.

In the present study, the following multiple maturity indices has been evolved in *Spondias mangifera*, depending upon changes in various physical, bioactive and biochemical attributes during fruit growth and development.

Maturity Indices

At present, best time for harvest of fruits has been based on experience. This may vary with the individual's preferences and tastes. For the first time a set of development related physical, physiological and biochemical quality attributes have been identified for determining harvest maturity in Indian hog plum fruit. All the contingencies of physical growth like length, diameter, fresh weight of the fruit, when plotted over the development period of fruit showed simple sigmoid growth curve. Accordingly the bioactive and biochemical changes associated with fruit growth and maturation are discussed. Notable, chemical indices evolved during the present study being; the equilibrium concentration of chlorophyll and carotenoid on the 9th week may mark the attainment of optimum maturity and onset of ripening. The pre-climacteric dip followed by an upsurge in the rate of respiration heralds the optimum maturity and/or onset of ripening.

Field Chart

Maturity indices of *Spondias mangifera*



| MATURITY ATTRIBUTES | Stage 1 | Stage 2 | Stage 3 |
|--|-----------------------------|---|------------------------------|
| Weeks after fruit set | 1-5 <i>Immature</i> | 7-9 <i>Optimally matured</i> | 11-13 <i>Over matured</i> |
| Endocarp differentiation | <i>Indistinct endocarp</i> | <i>Distinct demarcation of endocarp</i> | <i>Dominant endocarp</i> |
| Physical and chemical attributes (texture, carotene, TSS, sugars) | Increasing/ accumulative | Peak increase and Stabilization | Declining |

Thus, in the present study, multiple maturity indices have been evolved and presented in tabular form, which can be used as a field chart along with colour illustrations presented in this chapter. Use of endocarp differentiation as a maturity index was carried out for the first time. Based on this facet, the fruit development was classified in to three phase viz. Indistinct, Distinct and dominant phases. This can be adopted as one of the method for determining the maturity index in other stone fruits. Popularization of nutraceutical food supplements posed new challenges and provided new dimension in identifying the maturity indices in fruits and vegetables. In the present study, use of bioactive molecule isolated from *Spondias mangifera* fruit has been effectively used as a biomarker for maturity. This may facilitate harvesting of fruits with high nutraceutical compounds, which is preferred for pharmaceutical industry. Thus the present study enabled to evolve multiple indexes to cater the needs of producers, distribution market vendors, consumers and also to pharmaceutical and/or nutraceutical industry.

CONCLUSION

For the first time a set of development related physical, physiological and biochemical quality attributes have been identified for determining harvest maturity in Indian hog plum fruit. Endocarp hardening was contingent and function of time during development and post maturity process in Indian hog plum fruit. The inevitable process of endocarp hardening turned out as a hallmark of maturity indices in *Spondias mangifera* fruit. Interestingly, contingents of fruit growth, pre-climacteric plunge in rate of respiration, coordinated changes in pigments with peak accumulation pattern of ascorbic acid, carotene and phenols were defined and featured as maturity indices for *Spondias mangifera* fruit. Based on bioactive and biochemical changes, the physiological/horticultural harvest maturity in *Spondias mangifera* plum fruits was fixed as 9th week after fruit set. Since hardening of endocarp is a post maturity process, commercial harvest time of fruit can be stretched till 11th week or little later.

Table 3.4. Maturity Standards for Harvest of *Spondias mangifera* fruit maturity markers index

| S. No. | Maturity Standard | Index |
|--------|------------------------------------|--|
| | <i>Physiological</i> | |
| 1 | Pre-climacteric dip in respiration | 7 th - 9 th week |
| | <i>Biochemical</i> | |
| 2 | Ascorbic acid (mg/100 g) | 20-23 |
| 3 | Titration acidity (%) | 4-4.5 |
| 4 | Total soluble solids (%) | 6-7 |
| 5 | Phenolic content(mg/ 100 g) | 21-28 |
| 6 | Total chlorophyll (µg/ 100 g) | 1400-1200 |
| 7 | Carotenoid (µg/ 100 g) | 300-600 |
| | <i>Morphological</i> | |
| 8 | Fusion of the endocarp (weeks) | 7-9 |
| | <i>Fruit Characteristics</i> | |
| 9 | size of fruit : (a) length (mm) | 25-35 |
| 10 | : (b) diameter (mm) | 15-20 |
| 11 | weight of fruit (g) | 3-6 |

Thus, measuring optimum stage of maturity is of paramount importance in dictating the terms of nutraceutical value, market value as well as consumer acceptance. Immature fruits characterized by low quality parameters viz., shriveling, internal breakdown, mechanical damage and inferior quality, when ripe, including low accumulation of bioactive compounds. While, the over-mature ones either may become soft and mealy and attain insipid flavour, may become hard stony and significant decrease in edible portion or total loss of palatability. Optimum maturation is the end point of final fruit growth with preferred texture, taste, characteristic aroma, nutritional and nutraceutical components. It also heralds the beginning of ripening subsequently senescence in *Spondias mangifera* fruits. Thus the present study enabled to evolve multiple indexes to cater the needs of producers, distribution market vendors, consumers and also to pharmaceutical and/or nutraceutical industry.