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Changes in physiological and biochemical parameters during growth and development of mango (*Mangifera indica* L.) fruit in Vietnam

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Abstract

This paper presents research results on the changes in physiological and biochemical parameters during growth and development of Elephant mango in Vietnam, thereby determining the physiological maturity time of the fruit. The results showed that significant changes occurred in pigments, reducing sugar, starch, total organic acid, vitamin C, protein, lipid, pectin and tannin content and changes in the activity of α-amylase, catalase, peroxidase enzymes in mango from fruit formation to ripening. Based on the results, we concluded that fruit should be harvested at physiological maturity (16 weeks old) before completely ripening on the tree to ensure the high nutritional value and quality of the fruit during storage.

Key words: Mango fruit, biochemical indicators, physiological maturity, ripening.

Introduction

Mango (*Mangifera indica* L.), sometimes called "The king of fruits", is by volume the second largest tropical fruit crop in the world after bananas. It is ranked fourth in total fruit production after bananas, citrus and apples. The mango has been known to Indians since very early times and scientific fossil evidence indicates that the mango made its first appearance 25-30 million years ago in northeast India, Myanmar and Bangladesh, from were it travelled down to southern India. Mangoes belong to genus *Mangifera* which consists of about 69 species of tropical fruiting trees in the flowering plant family Anacardiaceae (Ram and Rajan, 2003).

The mango fruits are available from April to August in most of the tropical countries. Mango fruit is one of the most popular, nutritionally rich fruits with unique flavor, fragrance, taste, and heath promoting qualities, making it numero-uno among new functional foods, often labeled as "super fruits." Mango is one of the delicious seasonal fruits grown in the tropics and has a high nutritional value and health benefits due to important constituents.

Mango components can be grouped into macronutrients (carbohydrates, proteins, amino acids, lipids, fatty, and organic acids), micronutrients (vitamins and minerals), and phytochemicals (phenolic, polyphenol, pigments, and volatile constituents). The mango is a good source of sugars, vitamins A and vitamin C and minerals. Mango pulp from Colombian varieties contains 0-0.6% of protein (Corrales-Bernal *et al.*, 2014), while in Peru, mango contains 1.5 to 5.5 % total protein; in other cultivars from Java, it has 1-2 %, and in India, cultivars exhibit low content of total protein (0.5-1 %) (Saleem-Dar *et al.*, 2016).

Vietnam is growing mango for many years and it is being grown in entire country, but mainly produced in the Southern provinces. In 2019, Vietnam had 92,746 hectares and production of 788,233 tons of mango, of which the Mekong Delta was the largest mango producing area, accounting for 46.1 % of the area and 64.4 % of the mango production of the country; followed by the South East (accounting for 19.2 % of the total area and 64.4 % of the total mango production). Mangoes are grown in 59 among 63 localities in Vietnam. About 96 per cent of Vietnamese mangoes are produced and sold locally. There are many varieties of mangoes, including Cat mango (Ben Cat mango), Hon mango, Thanh Ca mango, Tuong mango (Elephant mango), Xiem mango (Siamese mango), Coc mango (Toad mango), in which Elephant mango is relatively more popular.

Elephant mango has been exported to many countries such as South Korea, Japan, Singapore, Australia, New Zealand, China and Thailand. It is a important fruit of Vietnam and one can find Elephant mango at almost every corner of the street. Elephant mango is the most famous variety because of its delicious, sweet and big fruits (600-700 g) and has high economic value. In Vietnam, Elephant mango is also used as fresh vegetables, it can be used with some wild leafy vegetables in rice meals, green mangoes can be used in mango salad with fish sauce and dried shrimp. However, the harvesting and preservation of Elephant mango in Vietnam has no scientific basis rather it is based on the experience of gardeners. Due to this reason, qulaity of majority of mangoes in the market is not ensured. The mango fruit ripening process involves a series of physiological, biochemical and organoleptic changes that lead to the development of a soft, edible, ripe fruit with desirable qualities (Tharanathan *et al.*, 2006). Therefore, we conducted fruit sampling and analyzed the physiological and biochemical indicators of Elephant mango from fruit formation to ripening to find out the physiological maturity time for better shelf life and quality for the benifit of consumers and traders.

Materials and methods

Research material: Elephant mango was harvested at Bac Giang, Vietnam (21°16′29″N and 106°12′06″E). Analysis on physiological and biochemical parameters were undertaken at the Department of Plant Physiology and Application, Hanoi National University of Education. Physiological and biochemical indicators were analyzed at the Plant Laboratory, Hong Duc University and the Department of Plant Physiology and Application, Hanoi National University of Education.

Sampling: Samples were collected according to the mixed sampling method. Across the experimental area, we collected samples at many points, on many plants, these plants were growing normally, pest-free, and getting uniform cultural operations. When the fruit has just been formed, we conducted the fruit marking on the experimental trees, recording data by day and month. At each stage of the study, we collected samples from all plants: 5-10 fruits per tree. The collected samples were mixed well, then put into plastic bags and labeled. Samples were collected in the morning, then refrigerated and transferred to the laboratory. Part of the sample is used to immediately analyze indicators of pigments content, enzymes, vitamin C. The rest of the sample was stored at -80 °C to analyze other indicators.

Pigment content in the peel: Determination of pigment content in the peel by spectral methods were made as per Ma *et al.* (2013). Chlorophyll content was calculated by the formula: C_a (mg L^{-1}) = 9.784 x E_{662} - 0.990 x E_{644}. C_b (mg L^{-1}) = 21.426 x E_{644} - 4.650 x E_{662}. $C_{(a+b)}$(mg L^{-1}) = 5.134 x E_{662} + 20.436 x E_{644}. Carotenoids content was calculated by the formula: $C_{carotenoids}$(mg L^{-1}) = 4.695 x $E_{440.5}$ - 0.268 x $C_{(a+b)}$. Then the pigment content per 1 g of fresh fruit peel was calculated by the formula:

$$A = \frac{C \times V}{P \times 1000}$$

Where, E_{662}, E_{644} and $E_{440.5}$ are the results of absorbance at wavelengths of 662 nm, 644 nm and 440.5 nm; C_a, C_b, C_{a+b}, respectively chlorophyll content a, b and total; *A* is the content of chlorophyll in 1 g of fresh fruit peel; *C* is the chlorophyll content of the pigment extract (mg L^{-1}); *V* is the volume of pigment extract (10 mL); *P* is the sample mass (g).

Reducing sugar content, starch: Bertrand method (Mui, 2001) was followed. Reducing sugar content was calculated using following formula:

$$X = \frac{a \times V_1 \times 100}{V \times b \times 1000}$$

Where, *X* is the reducing sugar content (%); *a* is the weight (mg) of glucose obtained when examining the table for volume $KMnO_4$ 1/30 N (mL) used for titration of the laboratory sample minus the volume $KMnO_4$ 1/30 N (mL) titration in the control sample; *V* is the volume of the diluted sample solution (mL); V_1 is the volume of the analyzed sample solution (mL); *b* is the weight of the test sample (g); 100 is the conversion factor to %; the coefficient converts g to mg.

The starch content was calculated by the formula:

$$Y = \frac{a \times V_1 \times 100 \times 0.9}{V_2 \times b}$$

Where, *Y* is the content of starch (%); *a* is the amount of reducing sugar; V_1 is the volume of analyzed sample solution (mL); V_2 is the volume of diluted sample solution (mL); *b* is the weight of analyzed sample (g); 100 is the conversion factor to %; 0.9 is the coefficient of converting glucose into starch.

α-amylase: The enzyme activity was determined using spectrophotometer at 656 nm wavelength (Mui, 2001). α-amylase enzyme activity was calculated by the formula:

$$HdA = \frac{6.889 \times C - 0.089388}{W}$$

and C was calculated by the formula:

$$C = \frac{OD_1 \times OD_2}{OD_1} \times 0.1$$

Where, With C is the amount of starch hydrolyzed; OD_1 is the optical density at the control vessel; OD_2 is the optical density at the experimental flask; 0.1 is the amount of starch analyzed; *W* is the amount of analytical enzyme composition (g).

Catalase: The activity was estimated by Bac and Oparin method (Mui, 2001). Catalase enzyme activity was calculated by the formula:

$$X = \frac{(V_1 - V_2) \times 1.7 \times V_x}{V_c \times 30 \times 0.034 \times a} \times 0.1$$

Where, *X* is the catalase activity calculated by the number of micromol H_2O_2 resolved in 1 minute under the action of catalase enzyme in 1 g sample at 30 °C; V_1 is the volume of $KMnO_4$ 0.1N used to titrate H_2O_2 in the control vessel (mL); V_2 is the volume of $KMnO_4$ 0.1N used to titrate H_2O_2 in the experimental flask (mL); *Vx* is the total volume of enzyme extract (mL); *Vc* is the volume of analytical extract (mL); *a* is the weight of the crushed sample (g); 1.7 is the conversion coefficient from the titrant $KMnO_4$ 0.1N to mg H_2O_2 resolved; 30 is the duration of enzyme action (min); 0.034 is the conversion factor of mg to micromol.

Peroxidase: Peroxidase activity was determined using Boiarkin method on spectrophotometer (Mui, 2001): Peroxidase enzyme activity was calculated by the formula:

$$A = \frac{E \times a \times b}{p \times d \times t}$$

Where, *A* is the peroxidase activity in 1 g of sample; *E* is the selected optical density; *a* is the total volume of extract (ml); *b* is the degree of extract dilution; *p* is the weight of the plant sample (g); *d* is the cup thickness (cm); *t* is the time (s).

Protein: Protein content was estimated by Microkjeldahl method (Mui, 2001): The amount of NH_3 was calculated by the formula:

$$A = \frac{V \times V_a \times 0.142 \times 5.595 \times 100}{V_c \times g}$$

Where, X is the lipid content (%); V_a: is the volume of H_2SO_4 0.01 N used to titrate BO_3^-. *V*: is the total volume of enzyme extract (mL); V_c: is the volume of NH_3 in analytical extract (mL); g is the weight of the crushed sample (g); 0.142: mg N is equivalent to 1 mL H_2SO_4 0.01N; 5.595: is the conversion factor to indicate the result of protein; 100 is the conversion factor to indicate the result in %.

Lipid: Determination of lipid content was performed using Soxhlet method (Mui, 2001): The lipid content was calculated by the formula:

$$X = \frac{(G_m - G_c) \times 100}{G}$$

Where, *X* is the lipid content (%); G_m is the volume of of dry sample pack (g); G_c is the volume of lipid-extracted dry sample pack (g); *G* is the total volume of dry sample for analysis (g).

Total organic acid content: Total organic acid content was measured as per method outlined by Mui (2001). Total organic acid content was calculated by the formula:

$$X = \frac{a \times V_1 \times 100 \times 0.9}{V_2 \times P}$$

Where, *X* is the amount of total organic acid present in the extract; *P* is the amount of analytical sample (g); V_1 is the total volume of extract (mL); V_2 is the volume to be titrated (mL); *a* is the amount of 0.1N NaOH titration (mL).

Vitamin C: Determination of vitamin C content was performed

by titration method (Arya *et al.*, 2000). Vitamin C content was calculated by the formula:

$$X = \frac{V \times V_1 \times 0.00088 \times 100}{V_2 \times P}$$

Where, X is vitamin C content in the sample (%); V is the volume of diluted sample solution (mL); V_1 is the volume of 0.01 N I_2 solution (mL); V_2 is the volume of analyzed solution (mL); P is the weight of sample (g); 0.00088 is the weight (g) of vitamin C which was equivalent to 1 mL of 0.01 N I_2.

Pectin: Determination of pectin content by calcium pectate precipitation method (Mui, 2001): The content of pectin (P) was calculated by the formula:

$$X = \frac{A \times 0.92 \times 100}{B}$$

Where, A is the amount of the calcium pectate precipitate (g); B is the amount of pectin taken for saponification (g); 0.92 is the transfer coefficient except for the calcium content of the precipitate; 100 is the conversion factor to indicate the result in %.

Tanin: Determination of tannin content was done by Leventhal method (Chau *et al.,* 1998): The tannin content was calculated by the formula:

$$X(\%) = \frac{(a-b) \times V \times k \times 100}{V_f \times g}$$

Where, X is the tannin content (%); a is the volume of $KMnO_4$ used for titration in the flask (mL); b is the volume of $KMnO_4$ used for titration in the control vessel (mL); V is the total volume of extract (mL); V_f is the volume of the analyzed extract (mL); g is the weight of the analyzed sample (g); k is the tannin coefficient = 0.00582.

Statistical analysis: All experiments were conducted three times independently. The results are expressed as mean values and standard deviation (SD). The results were subjected to analysis of variance. Data were compared according to Tukey's test using IRRISTAT software (version 5.0) for Windows.

Results and discussion

Pigment content of mango during maturation: The color of the fruit peel is an important factor of mango maturation indices and quality, which changes from green to orange, yellow or red blush, depending on the type of cultivar. In mango fruit, the green pigmentation is attributed to the presence of chlorophyll (Sudhakar *et al.*, 2016).

The data from Table 1 shows that, in the first week, the content of chlorophyll in mango peel was low. The content of chlorophyll *a* was 0.027 mg g^{-1} fresh peel, chlorophyll *b* was 0.056 mg g^{-1} fresh peel and total chlorophyll was 0.083 mg g^{-1} fresh peel at 1 week. From 1 to 7 weeks, the content of chlorophyll *a* and chlorophyll *b* increased rapidly and reached the highest value at 7 weeks (Chlorophyll *a* was 0.097 mg g^{-1} fresh peel, chlorophyll *b* was 0.205 mg g^{-1} fresh peel, chlorophyll *a+b* was 0.302 mg g^{-1} fresh peel). After 7 weeks, the content of chlorophyll *a,* chlorophyll *b* and chlorophyll *a+b* gradually decreased and more rapidly at 16 and 17 weeks, this was because fruits begin to reach the stage of ripening, decomposed chlorophyll pigment and carotenoid pigment were synthesized.

Carotenoids content in mango peel increased with age of fruit during development. In the first week, low carotenoids content (0.011 mg g^{-1} fresh peel) was observed. From 1 to 9 weeks, the content of carotenoids increased slowly, then rapidly as per the maturity stage of the fruit. At 17 weeks, mango fruit was rich in carotenoids, the content of carotenoids reached 0.087 mg g^{-1} fresh peel. These molecules are lipid-soluble pigments contributing to yellow-orange colors of mango fruit and red color when mango is ripe, although the reddish color of peel in several varieties is due to anthocyanins (Masibo and Qian, 2008; Sivankalyani *et al.*, 2016). Chlorophyll and carotenoids are responsible for the color in some fruits. Several studies describe that chlorophyll breakdown is associated with the maturity of some fruits (Du *et al.*, 2014; Wei *et al.*, 2019). When fruit appears green, an abundance of chlorophyll masks the carotenoids. The yellow color of carotenoids is unmasked by chlorophyll degradation during ripening (Charoenchongsuk *et al.*, 2015). Taking this into account, the content of chlorophyll could be used as an indicator for the harvest in some fruits but not in others.

Reducing sugar content and starch content: Ripened mango fruit is a major source of sugars (glucose, fructose and sucrose) and other carbohydrates such as starch (Bello-Pérez *et al.*, 2007). All these are significant compounds from a nutritional and flavor aspects.

The results in Table 2 show that the content of reducing sugar in the early period of mango fruit (3 weeks) was relatively low, reaching 1.935 % weight of fresh fruit flesh. From 3 to 9 week old, the content of reducing sugar increased slowly and reached 3.568 % when fruit was 9 weeks. In the fruit development period from 9 to 16 weeks, the content of reducing sugar increased rapidly and reached 6.523 % when fruit was 16 weeks. At 17 weeks, the content of reducing sugar decreased to 6.487 %.

Table 1. Pigment content in mangoes peel at different maturation stages[1]

Age of fruit development	Chlorophyll a (mg g^{-1} fresh peel)	Chlorophyll b (mg g^{-1} fresh peel)	Chlorophyll a+b (mg g^{-1} fresh peel)	Carotenoids content (mg g^{-1} fresh peel)
1 week	$0.027^{c} \pm 0.004$	$0.056^{c} \pm 0.002$	$0.083^{d} \pm 0.004$	$0.011^{e} \pm 0.001$
3 weeks	$0.076^{c} \pm 0.006$	$0.094^{bc} \pm 0.001$	$0.170^{bc} \pm 0.011$	$0.013^{e} \pm 0.002$
5 weeks	$0.085^{b} \pm 0.008$	$0.116^{b} \pm 0.003$	$0.201^{b} \pm 0.005$	$0.018^{de} \pm 0.002$
7 weeks	$0.097^{a} \pm 0.009$	$0.205^{a} \pm 0.005$	$0.302^{a} \pm 0.009$	$0.021^{d} \pm 0.003$
9 weeks	$0.094^{a} \pm 0.002$	$0.126^{b} \pm 0.001$	$0.220^{b} \pm 0.008$	$0.025^{d} \pm 0.001$
11 weeks	$0.087^{b} \pm 0.001$	$0.109^{b} \pm 0.009$	$0.196^{b} \pm 0.014$	$0.039^{c} \pm 0.001$
13 weeks	$0.077^{c} \pm 0.004$	$0.097^{bc} \pm 0.008$	$0.174^{bc} \pm 0.021$	$0.047^{c} \pm 0.007$
15 weeks	$0.053^{d} \pm 0.005$	$0.091^{bc} \pm 0.008$	$0.144^{c} \pm 0.013$	$0.065^{b} \pm 0.005$
16 weeks	$0.035^{e} \pm 0.002$	$0.073^{c} \pm 0.003$	$0.108^{d} \pm 0.002$	$0.083^{a} \pm 0.009$
17 weeks	$0.032^{e} \pm 0.001$	$0.066^{c} \pm 0.002$	$0.098^{d} \pm 0.001$	$0.087^{a} \pm 0.008$

[1]Numbers represent mean values of three independent replicates ± SD. In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant ($P \leq 0.05$) difference by Tukey's test

Table 2. Content of reducing sugar and starch in mangoes at different maturation stages[1]

Age of fruit development	Reducing sugar content (% weight of fresh fruit flesh)	Starch content (% weight of fresh fruit flesh)
3 weeks	$1.935^{d} \pm 0.027$	$0.462^{e} \pm 0.041$
5 weeks	$2.451^{d} \pm 0.021$	$1.383^{d} \pm 0.009$
7 weeks	$3.073^{c} \pm 0.009$	$2.655^{c} \pm 0.021$
9 weeks	$3.568^{c} \pm 0.032$	$3.442^{b} \pm 0.015$
11 weeks	$4.372^{c} \pm 0.028$	$5.018^{a} \pm 0.074$
13 weeks	$4.980^{c} \pm 0.018$	$4.579^{ab} \pm 0.043$
15 weeks	$5.395^{b} \pm 0.007$	$3.907^{b} \pm 0.032$
16 weeks	$6.523^{a} \pm 0.092$	$3.142^{bc} \pm 0.081$
17 weeks	$6.487^{a} \pm 0.081$	$3.008^{c} \pm 0.026$

[1]Numbers represent mean values of three independent replicates ± SD. In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant ($P \leq 0.05$) difference by Tukey's test

When the fruit has just formed, low starch content reached 0.462 % weight of fresh fruit flesh (3 weeks). The highest starch content was 5.018 % at 11 weeks. After 11 weeks, the content of starch in the fruit decreased due to the strong metabolism in the fruit. During this period, the activity of α-amylase enzyme also increased. Due to increased α- amylase enzyme activity, starch converts into sugar required for respiration. From unripe to ripe stage, the starch content declined (Yashoda *et al.*, 2005). During ripening, starch is hydrolyzed to glucose, thus, glucose, fructose, and sucrose generally increases (Bernardes *et al.*, 2008). When fruit enters the ripening period, starch decomposes into sugar to increase the amount of reducing sugar to create sweetness in the fruit.

Total organic acid and Vitamin C content: Organic acids are characterized by weak acidic properties. These are necessary for aerobic metabolism and as flavor constituents that contribute to fruit quality, organoleptic properties and fruit acidity (Vallarino and Osorio, 2019).

The data in Table 3 shows that, at the stage when fruit formation starts, the organic acid content was 93.412 lđl 100 g^{-1} fresh fruit flesh. During the growth period from 3 to 11 weeks, the total organic acid content increased gradually and reached the highest value of 186.002 lđl 100 g^{-1} fresh fruit flesh at 11 weeks. Degradation of protein and lipids lead to production of intermediate products such as amino acids increasing the organic acid content.

The fruit growth period from 11 to 17 weeks, organic acid content decreased because organic acids are used in respiration. On the other hand, energy continues to be needed for the biosynthesis of fruit-specific ripening substances such as enzymes for hydrolysis, esters to create fruit aroma during ripening period and synthesis of sugar to create fruits sweetness resulting into decrease in total acid content (Prasanna *et al.*, 2007).

The content of vitamin C from 3 to 11 weeks increased rapidly. This is the period of major fruit development and the accumulation of vitamin C along with other nutrients takes place. After 11 weeks, vitamin C content continued to increase, but at a slower rate, the highest value (31.729 mg 100 g^{-1} fresh fruit flesh) reached at the 16th week, thereafter vitamin C content decreased. The vitamin C decrease may be due to the involvement of different metabolic pathways such as ethylene, oxalate, and tartrate biosynthesis because vitamin C is a coenzyme of their respective enzymes (Singh *et al.*, 2011).

Table 3. Content of total organic acid and vitamin C in mangoes at different maturation stages[1]

Age of fruit development	Total organic acid (lđl 100g^{-1} fresh fruit flesh)	Vitamin C (g 100 g^{-1} fresh fruit flesh)
3 weeks	$93.412^{e} \pm 0.961$	$10.802^{d} \pm 0.074$
5 weeks	$100.235^{e} \pm 0.823$	$14.250^{c} \pm 0.093$
7 weeks	$125.769^{d} \pm 0.637$	$16.927^{c} \pm 0.195$
9 weeks	$165.215^{b} \pm 0.445$	$21.423^{c} \pm 0.137$
11 weeks	$186.002^{a} \pm 0.719$	$25.046^{b} \pm 0.245$
13 weeks	$144.012^{c} \pm 0.545$	$26.185^{b} \pm 0.089$
15 weeks	$141.050^{b} \pm 0.602$	$29.621^{a} \pm 0.438$
16 weeks	$98.920^{e} \pm 0.340$	$31.729^{a} \pm 0.218$
17 weeks	$39.016^{f} \pm 0.401$	$30.750^{a} \pm 0.125$

[1]Numbers represent mean values of three independent replicates ± SD. In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant ($P \leq 0.05$) difference by Tukey's test

Protein and lipid content: The data in Table 4 show that the content of protein in mango fruit was relatively high at 3 weeks and declined thereafter from 3 to 11 weeks (from 1.017 g 100g^{-1} dried fruit weight to 0.452 g 100 g^{-1} dried fruit weight). After 11 weeks, protein content continued to decrease, but at a slower rate. This is a period of fruit maturity, a strong decrease in protein content during this period due to the increase in the activity of protease enzyme that dissolved protein. This result is consistent with previous studies about protein (Corrales-Bernal *et al.,* 2014; Saleem-Dar *et al.*, 2016).

Lipid content in mango fruit are relatively high from 3 weeks (reached 0.501 g 100 g^{-1} dried fruit weight), then increased rapidly according to the ripening of the fruit. The highest lipid content was 0.971 g 100 g^{-1} dried fruit weight at 11 weeks. After 11 weeks, the content of lipids in the fruit decreased due to the strong metabolism in the fruit. Under the action of lipase enzyme, the lipid hydrolyzes rapidly when fruit enters the ripening period. At 17 weeks, the content of lipids decreased to 0.641 g 100 g^{-1} dried fruit weight.

Pectin and tannin content: Pectins are responsible for fruit texture. The content of pectin in mangoes increased rapidly from 3 weeks to 9 weeks (from 4.285 to 7.362 % of fresh fruit weight). Pectin content decreased sharply in the period from 9 to 17 weeks (from 7.362 to 2.847 %), leading to the fruit softening because of enzymatic degradation and solubilization of protopectin.

Mango fruit pulp is composed of parenchymatous tissues that consist of calcium salts of pectin located in the cell wall during the early stages of cell growth. The deesterification of pectins and losses of calcium ions are characteristic of ripening fruit because of cell wall breakdown and dissolution of middle lamella (Tharanathan *et al.,* 2006). Pectin is a structural carbohydrate abundant in mango pulp and is considered an important component as a gelling sugar. When fruit is unripe, pectin is accumulated, but during ripening, its molecular weight decreases, this is attributed to the activity of hydrolysis of pectin enzymes in this stage (Prasanna *et al.*, 2004).

Tannin content in fruit was relatively high at 3 week (reached 0.410%). The high tannin content in the early growth period

Table 4. Protein and lipid content in mangoes at different maturation stages[1]

Age of fruit development	Protein (g 100 g^{-1} dried fruit weight)	Lipid (g 100 g^{-1}dried fruit weight)
3 weeks	$1.017^{a} \pm 0.018$	$0.501^{d} \pm 0.024$
5 weeks	$0.994^{a} \pm 0.016$	$0.652^{c} \pm 0.008$
7 weeks	$0.851^{a} \pm 0.059$	$0.762^{b} \pm 0.071$
9 weeks	$0.653^{b} \pm 0.014$	$0.932^{a} \pm 0.049$
11 weeks	$0.452^{c} \pm 0.020$	$0.971^{a} \pm 0.078$
13 weeks	$0.445^{c} \pm 0.013$	$0.898^{a} \pm 0.092$
15 weeks	$0.431^{c} \pm 0.078$	$0.767^{b} \pm 0.027$
16 weeks	$0.405^{c} \pm 0.041$	$0.698^{bc} \pm 0.060$
17 weeks	$0.368^{c} \pm 0.012$	$0.641^{c} \pm 0.067$

[1]Numbers represent mean values of three independent replicates ± SD. In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant ($P \leq 0.05$) difference by Tukey's test

Table 5. Pectin and tannin content in mangoes at different maturation stages[1]

Age of fruit development	Pectin (% dried fruit weight)	Tanin (% dried fruit weight)
3 weeks	$4.285^{d} \pm 0.081$	$0.410^{a} \pm 0.005$
5 weeks	$6.012^{b} \pm 0.105$	$0.375^{a} \pm 0.008$
7 weeks	$6.923^{a} \pm 0.110$	$0.201^{b} \pm 0.007$
9 weeks	$7.362^{a} \pm 0.098$	$0.160^{b} \pm 0.002$
11 weeks	$6.238^{b} \pm 0.082$	$0.152^{b} \pm 0.011$
13 weeks	$5.932^{b} \pm 0.077$	$0.069^{c} \pm 0.003$
15 weeks	$5.425^{c} \pm 0.012$	$0.032^{c} \pm 0.001$
16 weeks	$3.557^{d} \pm 0.024$	$0.028^{c} \pm 0.005$
17 weeks	$2.847^{e} \pm 0.021$	$0.026^{c} \pm 0.002$

[1]Numbers represent mean values of three independent replicates ± SD. In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant ($P \leq 0.05$) difference by Tukey's test

of fruit made it acrid. The tannin content in mango gradually decreased with age and rapidly declined in the period of 3 to 13 weeks. This decline is due to hydrolytic tannin being decomposed into pyrogallol and CO_2, making the mango turn to ripening (Del Bubba *et al.*, 2009). In the period of fruit maturity, tannin content decreased to only 0.026 % at 17 weeks, makes mangoes ripen soft, not acrid.

Activity of α-amylase, catalase, peroxidase: Table 6 shows that, when mangoes were just formed at 3 weeks, α-amylase activity was low (0.125 UI g^{-1} h^{-1}) and increased slowly between 3 and 9 weeks. In this period, the fruit had accumulation of starch reserves.

From 9 weeks onwards, α-amylase activity in fruits increased rapidly and reached a peak at 15 weeks (0.713 UI g^{-1} h^{-1}). At this time the fruit enters the ripening stage, so there is a strong resolution of starch under the action of amylase enzyme to create sugar as a material to provide respiratory breakdown and to create sweetness for the fruit. Therefore, at this stage, the content of reducing sugar will increase and the amount of starch in the fruit gradually decrease (Jain *et al.*, 2001). After 15 weeks, the enzyme amylase activity decreased. Amylase activity has been reported in mangoes to increase fruits growth period and decrease towards maturity (Luiz Carlos *et al.,* 2001).

Since, the fruit has just formed, The catalase activity was high (4.425 μM H_2O_2 g^{-1} min^{-1}) at 3 weeks at the start of fruit growth

Table 6. Activity of enzymes α -amylase, catalase, peroxidase in mangoes at different maturation stages[1]

Age of fruit development	α -amylase activity (UI g^{-1} h^{-1})	Catalase activity (μM H_2O_2 g^{-1} min^{-1})	Peroxidase activity (UI g^{-1} sec^{-1})
3 weeks	$0.125^{d} \pm 0.005$	$4.425^{d} \pm 0.012$	$0.035^{d} \pm 0.001$
5 weeks	$0.230^{cd} \pm 0.003$	$6.649^{c} \pm 0.046$	$0.060^{c} \pm 0.004$
7 weeks	$0.274^{bc} \pm 0.002$	$8.835^{b} \pm 0.071$	$0.068^{c} \pm 0.002$
9 weeks	$0.387^{b} \pm 0.008$	$9.128^{ab} \pm 0.023$	$0.075^{c} \pm 0.003$
11 weeks	$0.502^{ab} \pm 0.009$	$9.598^{a} \pm 0.017$	$0.086^{b} \pm 0.008$
13 weeks	$0.629^{a} \pm 0.009$	$8.726^{b} \pm 0.020$	$0.089^{b} \pm 0.006$
15 weeks	$0.713^{a} \pm 0.004$	$6.515^{c} \pm 0.080$	$0.106^{b} \pm 0.006$
16 weeks	$0.654^{c} \pm 0.007$	$5.063^{d} \pm 0.057$	$0.175^{b} \pm 0.012$
17 weeks	$0.537^{cd} \pm 0.006$	$3.550^{e} \pm 0.089$	$0.347^{a} \pm 0.024$

[1]Numbers represent mean values of three independent replicates ± SD. In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant ($P \leq 0.05$) difference by Tukey's test

stage. Catalase activity increased gradually from 3 to 11 weeks, reached the highest value (9.598 μM H_2O_2 g^{-1} min^{-1}) at 11 weeks. During this period, the high rate of metabolism took place resulting in a rapid increase in mass, strong oxidation reactions, and production of H_2O_2. In the period from 11 to 17 weeks, catalase activity decreased and as a result accumulation of sugar, starch, water, oxidation reactions slowed down.

From 3 to 15 weeks stage, peroxidase enzyme activity was low and increased slowly (from 0.035 UI g^{-1} sec^{-1} to 0.106 UI g^{-1} sec^{-1}). Because at this time, the oxidation process of substances is strong, has created a large amount of H_2O_2, the evolution of H_2O_2 is associated with catalase activity. From 15 to 17 weeks, peroxidase enzyme activity increased rapidly (from 0.106 UI g^{-1} sec^{-1} to 0.347 UI g^{-1} sec^{-1}). This is due to the oxidation of reduced substances, lower H_2O_2 concentration in the fruit, H_2O_2 evolution process is undertaken by peroxidase. At this time the peroxidase enzyme catalyzes the decomposition reaction of tannin so that the fruit enters the ripening stage, creating polyphenolic compounds. The increase in activity of several of the carbohydrate-degrading enzymes, which resulted in solubilization of the various polysaccharide fractions, correlated with fruit-softening phenomenon **(**Hosakote *et al.*, 2005).

The content of chlorophyll *a* and chlorophyll *b* in mango peel reached the highest value at 7th week and decreased rapidly at 17th week. In contrast, low carotenoids content from fruit formation was observed upto 9th week, then increased rapidly during fruit ripening.

The starch content increased gradually from the beginning and reached the maximum when the fruit was 11 weeks, then gradually decreased. Reducing sugar content increased continuously and reached maximum at 16th week, thereafter decreased slightly. Total organic acid content increased slightly from 3 weeks to 11 weeks. From 11 to 17 weeks, total organic acid content decreased sharply. The vitamin C content increased continuously and reached maximum at 16th week and then decreased slightly.

The content of protein in mango fruit was relatively high at 3rd week and decreased at 17th week. The lipid content in mango increased rapidly from 3 weeks to 11 weeks, after 11 weeks, the content of lipids in the fruit decreased.

The α-amylase activity fluctuated as per the changes in starch

and reducing sugar content at different development stages of the fruit. The catalase activity gradually increased and reached its maximum at 11th week, then gradually decreased. Peroxidase activity increased continuously until the fruit was ripe.

The study revealed that the 16 weeks old Elephant mango fruit attains best quality at ripe stage. Therefore, it is most appropriate time to harvest for excellant quality. If harvested earlier or later, the fruits quality is adversely affected.

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