



Physiological and biochemical changes in longan fruit (*Dimocarpus longan* Lour.) cultivated in Vietnam during growth and ripening

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Received 17 January 2020; Revised 15 September 2020

This paper presents research results on some physiological (pigments content) and biochemical indicators (reducing sugar content, starch, total organic acid, vitamin C, α -amylase enzyme, catalase enzyme, peroxidase enzyme) of longan fruit grown in Vietnam during growth and ripening, thereby determining the physiological ripening time of the fruit (the most appropriate time for fruit harvest). The results showed that significant changes occurred in pigments content, reducing sugar content, starch, total organic acid, vitamin C, protein, lipid, α -amylase enzyme, catalase enzyme, and peroxidase enzyme of longan from formation to fruit ripening. Through the research process, it was observed that the longan achieved the best quality to harvest when fruits were 19 weeks old. The results of this study provide important data for the harvesting and storage of longan fruit.

Keywords: Biochemical indicators, *Dimocarpus longan* Lour., Longan fruit, Physiological indicators, Ripening.

IPC code; Int. cl. (2015.01)-A01G

Introduction

Longan (*Dimocarpus longan* Lour.) is a subtropical fruit tree crop native from Southern Asia, extending from Myanmar to Southern China, Southwestern India, and Sri Lanka¹. Longan is commonly grown in China, Thailand, and Vietnam^{2,3}. The main longan producing countries are China with about 1,300 million tons, Vietnam with more than 600 million tons, and Thailand with over 500 million tons in 2010. In China, Vietnam and Thailand, fresh longan fruit has been appreciated for centuries due to their excellent nutritive and pharmaceutical properties⁴. Longan with about 150 genera and 2000 species includes species of interest for their valuable wood, such as maple (*Acer* spp) and buckeye (*Aesculus* spp), or as ornamental plants as the Taiwanese rain tree [*Koelreuteria elegans* (Seem) A.C.Sm.]. Most importantly, the Sapindaceae is a rich family in edible fruit species such as lychee (*Litchi chinensis* Sonn.), rambutan (*Nephelium lappaceum* L.), guarana (*Paullinia cupana* Kunth), korlan (*Nephelium hypoleucum* Kurz), pitomba (*Talisia esculenta* Radlk.), Spanish

lime (*Melicoccus bijugatus* Jacq.), pulasan (*Nephelium mutabile* Blume) or ackee (*Blighia sapida* K.D.Koenig)⁵. Longan fruits can also be marketed in various processed ways such as frozen, canned, or dried. The mature longan fruit is small (1.5-2 cm diameter), conical, heart-shaped or spherical in shape and light brown in colour². The seed is round, dark black and shiny with a circular white spot at the base, which looks like dragon eyes⁶. Longan and its product are popular among the Chinese people since they believe that eating longan with dragon-eye seeds is good for health.

There have been many studies on the physiological and biochemical changes of fruits at different stages of development⁷⁻⁹. In longan, information is available on flowering, and fruit development appears to be similar to lychee¹⁰. The reproductive process including pollination biology, the fertilization process, early embryology, aril initiation, fruit growth¹¹⁻¹⁵, and fruit contents have been studied in this species. Longan fruit contains significant amounts of bioactive compounds such as corilagin, ellagic acid and its conjugates, 4-*O*-methylgallic acid, flavone glycosides, glycosides of quercetin and kaempferol, ethyl gallate 1- β -*O*-galloyl-*D*-glucopyranose, grevifolin and 4-*O*- α -

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L-rhamnopyranosyl-ellagic acid¹⁶. The retention of cis-ocimene and beta-ocimene was found to be the highest in longan flesh dried at 70 °C¹⁷.

In Vietnam, longans are grown relatively popular with many new varieties for high and stable yield. However, the harvesting and preserving of longans based on the experience of gardeners, this makes the majority of longans in the market not yet ensure quality, affecting the health of consumers. Therefore, we conducted fruit sampling, analyzing the physiological and biochemical indicators of longans from formation to fruit ripening. Thereby finding out the physiological ripening time of longans to help consumers use and preserve longans better.

Materials and Methods

Research materials

Longan was harvested in Hung Yen, Vietnam (20°51'16"N and 106°00'58"E). Physiological and biochemical indicators were analyzed at the Plant Laboratory, Hongduc University and the Department of Plant Physiology and Application, Hanoi National University of Education.

Sample collection method

The longan fruit, according to the mixed sampling method was collected in the morning throughout the experimental area. The samples were collected at many points from 10 trees: 10 fruits per tree. The longan fruit was mixed well after harvesting, then put into plastic bags and labeled brought to the Laboratory of Plant Physiology, Hanoi National University of Education. Samples were numbered by weeks: week 1, week 3, week 5, week 8, week 10, week 12, week 15, week 16, week 18, week 19, and week 20. Part of the sample was used to immediately analyze indicators of pigment content, enzyme activity, vitamin C content, and the rest was stored at -80 °C to analyze other indicators.

Determination of pigment content in the peel by spectral method¹⁸

The chlorophyll content was calculated by the formula:

$$C_a(\text{mg/l}) = 9.784 \times E_{662} - 0.990 \times E_{644}$$

$$C_b(\text{mg/l}) = 21.426 \times E_{644} - 4.650 \times E_{662} \\ C_{(a+b)}(\text{mg/l}) = 5.134 \times E_{662} + 20.436 \times E_{644}$$

The carotenoids content was calculated by the formula:

$$C_{\text{carotenoids}}(\text{mg/l}) = 4.695 \times E_{440.5} - 0.268 \times C_{(a+b)}.$$

Then the pigment content per 1g 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 measuring chlorophyll colour at wavelengths of 662, 644, and 440.5 nm; Ca, Cb, Ca+b are respectively chlorophyll content a, b and total; A is the content of chlorophyll in 1g of fresh fruit peel; C is the chlorophyll content of the pigment extract (mg/L); V is the volume of pigment extract (10 mL); P is the sample mass (g).

Determination of reducing sugar content, starch by Bertrand method¹⁹

The reducing sugar content is calculated to the 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; 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 %; 1000 is the coefficient converts g to mg.

The starch content is 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 the analyzed sample (g); 100 is the conversion factor to %; 0.9 is the coefficient of converting glucose into starch.

Determination of α -amylase enzyme activity on a spectrophotometer at 656 nm wavelength¹⁹

The α -amylase enzyme activity is calculated by the formula:

$$HdA = \frac{6.889 \times C - 0.029388}{W}; C = \frac{OD_1 - OD_2}{OD_1} \times 0.1$$

where 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).

Determination of catalase enzyme activity by A. N. Bac and A. I. Oparin method¹⁹

The catalase enzyme activity is 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 H₂O₂ resolved in 1 minute under the action of catalase enzyme in 1 g sample at 30 °C; V₁ is the volume of KMnO₄ 0.1N used to titrate H₂O₂ in the control vessel (mL); V₂ is the volume of KMnO₄ 0.1N used to titrate H₂O₂ in the experimental flask (mL); V_x is the total volume of enzyme extract (mL); V_c 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 KMnO₄ 0.1N to mg H₂O₂ resolved; 30 is the duration of enzyme action (min); 0.034 is the conversion factor of mg to micromol.

Determination of peroxidase enzyme activity by A.N.Boiarkin method on spectrophotometer¹⁹

The peroxidase enzyme activity is 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 the 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).

Determination of protein content by Micro kjeldahl method¹⁹

The amount of NH₃ is calculated by the formula:

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

where X is the lipid content (%); V_a is the volume of H₂SO₄ 0.01N used to titrate BO₂; V is the total volume of enzyme extract (mL); V_c is the volume of NH₃ in analytical extract (mL); g is the weight of the crushed sample (g); 0.142 mg N is equivalent to 1 mL H₂SO₄ 0.01N; 5.595 is the conversion factor to

indicate the result of protein; 100 is the conversion factor to indicate the result in %.

Determination of total organic acid content¹⁹

The total organic acid content is calculated by the formula:

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

where X is the amount of total organic acid present in the extract; P is the amount of analytical sample (g); V₁ is the total volume of extract (mL); V₂ is the volume to be titrated (mL); a is the amount of 0.1N NaOH titration (mL).

Determination of lipid content by Soxhlet method²⁰

The lipid content is 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 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).

Determination of vitamin C content by titration method²¹

The vitamin C content is calculated by the formula:

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

where X is the content of vitamin C in the materials (%); V is the volume of diluted sample solution (mL); V₁ is the volume of 0.01N I₂ solution (mL); V₂ is the volume of analyzed solution (mL); b is the weight of the sample (g); 0.00088 is the weight (g) of vitamin C which was equivalent to 1 mL of 0.01N I₂.

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 an analysis of variance. Data were compared according to Tukey's test using IRRISTAT software (version 5.0).

Results and Discussion

Changes in the pigment content of longan peel

The data from Table 1 showed that, in the first week, the content of chlorophyll in longan peel was

Table 1 — Content of pigment systems in longan fruit

Age of fruit (week)	Chlorophyll a (mg/g fresh peel)	Chlorophyll b (mg/g fresh peel)	Chlorophylla+b (mg/g fresh peel)	Carotenoids content (mg/g fresh peel)
1	0.017 ^d ±0.003	0.479 ^a ±0.041	0.496 ^a ±0.051	0.001 ^f ±0.001
3	0.021 ^d ±0.001	0.310 ^b ±0.020	0.330 ^b ±0.002	0.003 ^f ±0.005
5	0.030 ^c ±0.005	0.300 ^b ±0.071	0.327 ^b ±0.028	0.008 ^f ±0.002
8	0.042 ^b ±0.009	0.205 ^c ±0.032	0.247 ^c ±0.012	0.025 ^e ±0.001
10	0.070 ^a ±0.013	0.120 ^d ±0.011	0.127 ^d ±0.015	0.034 ^{de} ±0.009
12	0.024 ^b ±0.001	0.126 ^d ±0.060	0.149 ^d ±0.017	0.041 ^d ±0.005
15	0.032 ^c ±0.007	0.061 ^e ±0.005	0.092 ^{de} ±0.003	0.121 ^c ±0.006
16	0.033 ^c ±0.003	0.041 ^{ef} ±0.002	0.073 ^{ef} ±0.005	0.228 ^b ±0.001
18	0.041 ^b ±0.002	0.033 ^f ±0.005	0.071 ^{ef} ±0.006	0.332 ^{ab} ±0.003
19	0.042 ^b ±0.008	0.026 ^g ±0.002	0.067 ^f ±0.002	0.391 ^a ±0.008
20	0.043 ^b ±0.006	0.024 ^g ±0.002	0.066 ^f ±0.004	0.336 ^a ±0.009

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant differences ($\alpha = 0.05$) by Tukey's test.

Table 2 — Content of reducing sugar, starch and total organic acid in longan fruit

Age of fruit (Week)	Reducing sugar content (% weight of fresh fruit flesh)	Starch content (% weight of fresh fruit flesh)	Total organic acid content (mg/100g fresh fruit flesh)
8	2.798 ^e ±0.120	0.328 ^e ±0.031	38.750 ^d ±0.428
10	2.667 ^e ±0.095	0.381 ^{de} ±0.018	45.002 ^b ±0.056
12	2.939 ^e ±0.193	0.617 ^c ±0.009	53.125 ^a ±0.029
15	3.907 ^d ±0.217	1.126 ^{ab} ±0.023	42.500 ^c ±0.160
16	5.506 ^c ±0.251	1.320 ^a ±0.037	41.875 ^c ±0.281
18	8.728 ^a ±0.018	0.951 ^b ±0.014	40.313 ^c ±0.084
19	9.290 ^a ±0.097	0.480 ^d ±0.051	36.875 ^e ±0.310
20	8.070 ^b ±0.084	0.321 ^e ±0.013	35.625 ^e ±0.079

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant differences ($\alpha = 0.05$) by Tukey's test.

low. The content of chlorophyll *a* was 0.017 mg/g fresh peel, chlorophyll *b* was 0.479 mg/g fresh peel and total chlorophyll was 0.496 mg/g fresh peel at 1 week old. The content of chlorophyll *a* in longan peel reached the highest value at 10 weeks old (Chlorophyll *a* was 0.3526 mg/g fresh peel). The content of chlorophyll *b* decreased. After 10 weeks old, the content of chlorophyll *a* gradually decreased and decreased rapidly at 19 and 20 weeks old, this is because fruit began to move to the stage of ripening, decomposed chlorophyll pigment and carotenoid pigment were synthesized²².

Carotenoid content in longan peel increased with the age of the fruit. In the first week of longan, low carotenoid content reached 0.001 mg/g fresh peel at 1 week old. From 1 to 12 weeks old, the content of carotenoids increased slowly, then increased rapidly according to the ripening of the fruit. At 20 weeks old, the content of carotenoids reached 0.336 mg/g of the fresh peel.

Changes in reducing sugar content, starch content and total organic acid content of longan fruit

The content of reducing sugar in the early period of longan fruit (8 weeks) was relatively low, reaching 2.798% weight of fresh fruit flesh. From 8 to 12 weeks old, the content of reducing sugar increased slowly and reached 2.939% when the fruit was 12 weeks old (Table 2). After this period, the flesh increased rapidly, the cells continue to grow and expand, thus increasing the synthesis of energy and the components that make up the cell. In the fruit period from 12 to 19 weeks old, the content of reducing sugar increased rapidly and reached 9.290% when the fruit was 19 weeks old. At this time, some organic acids and starches were converted into sugars. This is the time when longan has a characteristic taste and aroma; longan harvest at this stage is most appropriate. If harvested earlier, it will reduce the quality of the fruit. At 20 weeks old, the content of reducing sugar decreased to 8.070% weight of fresh fruit flesh so the quality of the fruit decreased.

The longan fruit had low starch content when the fruit has just formed, until the fruit was 8 weeks old, the starch content reached 0.328% weight of fresh fruit flesh (Table 2). After that, saccharose from leaves and peels gets transferred into the fruit to provide materials for the synthesis of starch. Hence, starch content in the fruit increased gradually. The highest starch content was 1.320% at 16 weeks old. After 16 weeks old, the content of starch in the fruit decreased due to the strong metabolism in the fruit. Under the action of α -amylase enzyme, starch converted into sugar as a material for energy-generating respiration. When the fruit enters the ripening period, starch decomposed into sugar to increase the amount of reducing sugar to create sweetness for the fruit¹⁰. During this period, the activity of α -amylase enzyme also increased.

At the stage when fruit starts to form, the accumulation of large organic matter amounted to 38.750 mg/100 g of the fresh fruit flesh. In the 8 to 12 weeks old fruit, the total organic acid content increased gradually and reached the highest value of 53.125 mg/100 g in the fresh fruit flesh at 12 weeks old (Table 2). This is because protein exchange processes, hydrocarbon exchange, lipids take place strongly in the fruit, creating intermediate products such as amino acids, xetoaxit, etc., increasing the content of organic acids²².

In the 12 to 20 weeks old fruits, organic acid content decreased due to its consumption in respiration to provide energy for starch synthesis processes. On the other hand, energy is needed for the biosynthesis of fruit-specific ripening substances such as enzymes for hydrolysis, esters to create an aroma for fruit in the ripening period and synthesis of sugar to create sweetness for fruits, resulting in a decrease in total acid content²³.

Changes in vitamin C content, protein content and lipid content of longan fruit

The content of vitamin C from 8 to 15 weeks old increased rapidly, this is a period of strong flesh fruit development and the accumulation of vitamin C along with other nutrients in the fruit. After 15 weeks, vitamin C content continued to increase, but at a slower rate, the highest value reached 57.261 mg/100g fresh fruit flesh on the 19th week, then vitamin C content decreased (Table 3). This result may be related to the activity of certain groups of enzymes involved in ascorbic acid degradation such as ascorbate oxidase, phenolase, cytochrome oxidase, ascorbate peroxidase. Especially, unlike the other antioxidant enzymes, the ascorbate peroxidase activity in the pulp increased continuously during ripening.

The content of protein in longan fruit had a relatively high content from 8 weeks old and decreased sharply in the period from 8 to 20 weeks old (from 9.243% to only 1.040%). This is a period of ripe fruit, a strong decrease in protein content during this period due to the increase in the activity of the protease enzyme that has dissolved protein.

Lipid in longan fruit had a relatively high content from 8 weeks old (reached 0.251%), then increased rapidly according to the ripening of the fruit. The highest lipid content was 0.452% at 16 weeks old (Table 3). After 16 weeks old, the content of lipids in the fruit decreased due to the strong metabolism in the fruit. Under the action of the lipase enzyme, the lipid hydrolyzes rapidly when fruit enters the ripening period.

Changes in the activity of enzymes α -amylase, catalase, peroxidase of longan fruit

In the 8 weeks old fruit, α -amylase enzyme activity was low (reached 0.166 UI/g/h) but increased slowly from 8 to 15 weeks during which accumulation of starch reserves was increased in the fruit (Table 4).

Table 3 — Content of vitamin C, protein and lipid in longan fruit

Age of fruit (Week)	Vitamin C content (mg/100 g fresh fruit flesh)	Protein content (% dried fruit weight)	Lipid content (% dried fruit weight)
8	8.019 ^g ±0.055	9.243 ^a ±0.024	0.251 ^c ±0.005
10	20.039 ^f ±0.038	9.150 ^a ±0.046	0.319 ^b ±0.130
12	32.681 ^e ±0.216	8.250 ^a ±0.053	0.367 ^b ±0.032
15	43.017 ^d ±0.191	5.890 ^b ±0.195	0.431 ^a ±0.025
16	48.920 ^c ±0.073	2.894 ^c ±0.137	0.452 ^a ±0.018
18	52.825 ^b ±0.039	2.054 ^{cd} ±0.027	0.337 ^b ±0.021
19	57.261 ^a ±0.520	1.210 ^d ±0.094	0.230 ^c ±0.011
20	53.520 ^b ±0.504	1.040 ^d ±0.058	0.211 ^c ±0.040

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant differences ($\alpha = 0.05$) by Tukey's test.

Table 4 — Activity of enzymes α -amylase, catalase, peroxidase in longan fruit

Age of fruit (Week)	α -amylase activity (UI/g/h)	Catalase activity ($\mu\text{M H}_2\text{O}_2/\text{g}/\text{min}$)	Peroxidase activity (UI/g/sec)
8	0.166 ^d ±0.020	13.056 ^e ±0.023	0.241 ^d ±0.003
10	0.186 ^d ±0.017	14.875 ^e ±0.016	0.351 ^d ±0.026
12	0.248 ^d ±0.022	21.542 ^d ±0.091	0.380 ^d ±0.063
15	0.449 ^c ±0.016	31.005 ^a ±0.030	0.617 ^c ±0.018
16	0.898 ^b ±0.025	28.325 ^b ±0.011	0.840 ^c ±0.040
18	1.109 ^a ±0.114	25.208 ^c ±0.027	1.993 ^b ±0.051
19	1.026 ^a ±0.021	11.844 ^f ±0.041	2.738 ^a ±0.019
20	1.041 ^a ±0.016	10.043 ^f ±0.019	3.201 ^a ±0.024

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent significant differences ($\alpha = 0.05$) by Tukey's test.

From 15 weeks onwards, α -amylase enzyme activity in fruit increased rapidly and reached a peak at 18 weeks old (1.109 UI/g/h). 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 will gradually decrease²⁴. After 18 weeks, the enzyme amylase activity decreased.

Since the fruit was just formed, the catalase activity was very high reaching 13.056 $\mu\text{M H}_2\text{O}_2/\text{g}/\text{min}$ at 8 weeks. Catalase activity increased gradually from 8 to 15 weeks, reaching the highest value at 15 weeks with 31.005 $\mu\text{M H}_2\text{O}_2/\text{g}/\text{min}$. During this period, the metabolism took place strongly, resulting in a rapid increase in mass, strong oxidation reactions, H_2O_2 created a lot. High catalase activity, enhance H_2O_2 resolution, detoxify cells. In the period from 15 to 20 weeks, catalase activity decreased, the result was the accumulation of sugar, starch, water, oxidation reactions slowed down, H_2O_2 produced less.

From 8 to 16 weeks, the activity of the peroxidase enzyme was low and increased slowly (from 0.241 to 0.840 UI/g/sec). Because at this time, the oxidation process of substances is strong, which forms a large amount of H_2O_2 , at this stage, the enzyme catalase has a major role in breaking down H_2O_2 to detoxify the cell. From 16 to 20 weeks, peroxidase enzyme activity increased rapidly from 0.840 to 3.201 UI/g/sec (Table 4). This is due to the oxidation of reduced substances, lower H_2O_2 concentration in the fruit, H_2O_2 resolution 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 many ring compounds. Besides, this enzyme catalyzes the metabolic reactions of ring compounds, indole, and amines. On the other hand, it is also involved in the production of ethylene, the hormone that stimulates ripening²⁵.

Conclusion

The longan fruit at 19 weeks gradually changed from green to light yellow due to decreased chlorophyll and increased carotenoid content. At the 19th week, the fruit reached the maximum value of reducing sugar, vitamin C, and high protein and lipid content. Ingredients such as starch, total organic acids, α -amylase activity, catalase activity, and peroxidase activity changed with fruit growth and development. For the study, it was found that longan fruit reached physiological maturity at 19 weeks, this is the most appropriate time to harvest. If harvested earlier or later, the quality of longan fruit will decrease significantly.

Conflict of interest

The authors declared that the present study was performed in absence of any conflict of interest.

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