



PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING GROWTH AND DEVELOPMENT OF PERSIMMON FRUIT (*DIOSPYROS KAKI* L.) GROWN IN VIETNAM

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Abstract

Some biochemical changes according to the age of persimmon fruit grown in Vietnam were studied. The content of chlorophyll in persimmon peel reaches the highest value at 12 weeks old and decreases rapidly when the fruit is 22 weeks old, the content of carotenoids increase until the fruit is fully ripening. The content of vitamin C and reducing sugars increased continuously and reached the maximum value at 21 weeks old and then decreased slightly. Starch content gradually increases from the early stages and reaches its maximum at 15 weeks old, then decreases. Lipid content gradually increases from the early stages and reaches its maximum at 18 weeks old, then decreases. The content of protein decreased continuously from formation to fruit ripening. Based on the results of the research, we found that at week 21 the nutritional value of the fruit is the best, so persimmon fruit should be harvested at this time to ensure the nutritional value during storage.

Key words: persimmon fruit, biochemical indicators; physiological indicators, ripening.

Introduction

Persimmon (*Diospyros kaki* L.) is tropical fruits belonging to Ebenaceae family, a plant genus that includes over 500 species that are distributed worldwide and is one of the largest angiosperm genera (Duangjai *et al.*, 2006). Among 500 species, *Diospyros kaki*, *Diospyros virginiana*, *Diospyros oleifera* and *Diospyros lotus* (Bibi *et al.*, 2007) are of significant importance, of which *Diospyros kaki* is the most promising specie (Rahman *et al.*, 2002; Zheng *et al.*, 2006). It is commonly cultivated in warm regions of the world including China, Korea, Japan, Brazil, Turkey, and Italy (Itamura *et al.*, 2005; Yokozawa *et al.*, 2007). The persimmon fruits are rich in various nutrients and phytochemicals, such as carbohydrates, organic acids, vitamins, tannins, polyphenols, dietary fiber, triterpenoids, and carotenoids, which contribute significantly to their taste, color, and nutritive and medicinal value (Altuntas *et al.*, 2011). The persimmon is not so popular in European communities but its demand is increasing owing to consumer's awareness regarding its medicinal value. Persimmon is

one of the nutritious fruits bestowed with strong antioxidant activity (Jung *et al.*, 2005; Igual *et al.*, 2008). Persimmons have a high antioxidant potential that may have beneficial effects against oxidative damage in humans. Additionally, they are effectual in soothing lifestyle related disparities e.g. cardiovascular disorders and diabetes mellitus (Masood *et al.*, 2015). Persimmon fruits have size from 1.5 cm to 9 cm in diameter and the varieties may be spherical, acorn, or pumpkin shaped. Its color varies in different cultivars from yellow and orange to deep red depending on the species and variety.

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

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Materials and Methods

Research materials

Research materials: Nhan Hau persimmon was harvested in Bac Giang, Vietnam (21°16'29" N and 106°12'06" E). Analytical experiments of physiological and biochemical criteria were analyzed at the Hanoi National University of Education and Hong Duc University.

Sample collection method

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 care conditions are quite even. When the fruit has just been formed, we conducted the fruit marking on the experimental trees, recording data by day and month. Each stage of the study we collected samples from all plants: 10 fruits per tree. The collected samples are 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 is stored at -80°C to analyze other indicators.

Determination of length and diameter fruit: The length and diameter of the fruit were measured by palme calipers and accurate to mm.

Determination of pigment content in the peel by spectral method (Ma *et al.*, 2013): 2g of fresh leaves are chopped into a porcelain mortar, crushed with a small amount of acetone 80% and puree, add acetone and filter through a funnel Buchner into the extraction vessel, we get a mixture of green pigments. Measure the filtrate on the spectrophotometer at the corresponding wavelengths. Chlorophyll content was calculated by the formula: C_a (mg/L) = $9.784 \times E_{662} - 0.990 \times E_{644}$. C_b (mg/L) = $21.426 \times E_{644} - 4.650 \times E_{662}$. Carotenoids content was calculated by the formula: $C_{\text{carotenoids}}$ (mg/L) = $4.695 \times E_{440.5} - 0.268 \times C_{(a+b)}$. Then the pigment content per 1g of fresh fruit peel is calculated.

Determination of reducing sugar content, starch by Bertrand method (Mui, 2001): 10 mL experimental solution was put in a 1000 mL conical flask, 10mL Fehling was then added. The mixture was boiled for 3 minutes. Precipitate appeared and was filtered into a Buchner vacuum filter. The flask and the filter funnel were cleaned with hot distilled water for 3-4 times. The resulting sediment of Cu_2O in the Buchner filter was completely dissolved by using $\text{Fe}_2(\text{SO}_4)_3$ (5mL) in H_2SO_4 and

carefully stirred with a glass rod. The flask and the filter funnel were rinsed and placed in the conical flask. The resulting solution was titrated with KMnO_4 1/30N until a light pink color appeared within 20-30 seconds. Calculated the amount of KMnO_4 using for titration, looked up the table for an equivalent amount of reducing sugar in the sample. A control experiment was conducted at the same time in which the sugar solution was replaced with distilled water, from that determine the content of reducing sugar and starch in the sample.

Determination of vitamin C content by titration method (Arya *et al.*, 2000): 5g of sample was triturated with 5mL of 5% HCl in a ceramic bowl. It was grinded and put in a volumetric flask where distilled water was then added to 50 mL mark and well stirred. 20 mL of the solution was put in a 100 mL conical flask and titrated by I_2 solution with starch as a color indicator until blue color appeared, thereby determining the vitamin C content.

Determination of tannin content by titrimetric method (AOAC, 1980): Quantitative estimation of tannin was performed by titrating the extract with standard potassium permanganate solution following the method of AOAC. 5 ml aliquot of the extract was mixed with 12.5 ml of indigo-carmin solution and 375 ml of distilled water. This mixture was titrated against the KMnO_4 solution. The concentration of tannin was estimated using the following relationship: 1 ml of standard KMnO_4 solution = 0.595 ml of 0.1N Oxalic acid 1 ml of 0.1 N Oxalic acid = 0.0042g of tannin.

Determination of protein content by Lowry method (Chau *et al.*, 1998): 5mL of C (including a 0.5 mL of 1% CuSO_4 solution) was added to 1 mL of the sample in the test tube, mixed thoroughly and incubated at room temperature for 10 minutes. The solution was mixed with 0.5 ml of D (1N Folin), incubated for 30 minutes and measured by colorimeter at a wavelength of 750 nm. Protein concentration was calculated by standard graph. Determination of lipid content by Soxhlet method (Chau *et al.*, 1998): A flask was put on a bain-marie, the amount of ether added equals to half of the volume of the flask. The material bag was put in the extraction thimble which is connected with the flask. The solvent was added to submerge the material bag and above the upper part of the siphon arm of the thimble. Cooling tube was installed, the material was soaked in the solvent for a few hours. Soxhlet extractor was put inside the bain-marie so that the condensation rate for the solvent should be set at about 10-15 drops per hour. After the extraction, the flask was removed, a welding tube was installed to distill the ether. The lipid container is dried to determine its content.

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) for Windows computers.

Statistical analysis

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Results and Discussion

Changes in length, diameter, pigment content of persimmon fruit

Table 1 shows that both the length and diameter increase according to the growth and development of the fruit. From the period of 2 weeks to 22 weeks of age, fruit length increased from 0.425 cm to 5.872 cm while fruit diameter increased from 0.467 cm to 5.414 cm. From 2 to 18 weeks old, the size of fruit increased rapidly, after 18 weeks old, the size of fruit increased slowly. This is due to an increase in both the number and size of cells in the persimmon fruit. During the study, we observed that at 21 and 22 weeks of age, the size the fruit reached the maximum value and almost unchanged. Therefore, it can be said that the time of 21 weeks of age is physiological maturity stage of persimmon Fig. 6.

The color of the fruit peel is an important factor of persimmon maturation indices and quality, which changes from green to orange, yellow or red flush, depending on the type of cultivar. In persimmon fruit, the green pigmentation is attributed to the presence of chlorophylls (Sudhakar *et al.*, 2016). The data from Table 1 shows

that, in the first weeks, the content of chlorophyll in persimmon peel is low. The content of chlorophyll *a* is 0.365 mg/g fresh peel, chlorophyll *b* is 0.453 mg/g fresh peel at 2 weeks old. From 2 to 12 weeks old, the content of chlorophyll *a* and chlorophyll *b* increased and reaches the highest value at 12 weeks old (Chlorophyll *a* is 0.604 mg/g fresh peel, chlorophyll *b* is 0.677 mg/g fresh peel). After 12 weeks old, the content of chlorophyll *a*, chlorophyll *b* gradually decreases and decreases rapidly to 22 weeks old, this is because fruits begin to move to the stage of ripening, decomposed chlorophyll pigment and carotenoid pigment are synthesized.

Carotenoids content in persimmon peel increases with age of fruit development. In the first weeks of persimmon, low carotenoids content reached 0.062 mg/g fresh peel at 2 weeks old. From 2 to 22 weeks old, the content of carotenoids increase rapidly according to the ripening of the fruit. At 22 weeks old, persimmon fruit is rich in carotenoid compounds, the content of carotenoids reached 0.711 mg/g fresh peel. These molecules are lipid-soluble stains contributing to yellow-orange colors of persimmon fruit and red colors when persimmon 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).

Changes in reducing sugar, starch, tannin in persimmons content

Reducing sugar is considered an important indicator of fruit quality. The content of reducing sugar in the early period of persimmon fruit at 2 weeks is relatively low, reaching 1.254% weight of fresh fruit. From 2 to 21 weeks old, the content of reducing sugar increased rapidly and reached 13.916% when fruit was 21 weeks old. This

Table 1: Changes in length, diameter, pigment content of persimmon fruit at different maturation stages.

Age of fruit development	Length (cm)	Diameter (cm)	Chlorophyll <i>a</i> (mg/g fresh peel)	Chlorophyll <i>b</i> (mg/g fresh peel)	Carotenoids content (mg/g fresh peel)
2 weeks	0.425 ^c ±0.035	0.467 ^d ±0.033	0.365 ^{bc} ±0.001	0.453 ^{ab} ±0.003	0.062 ^f ±0.001
4 weeks	1.472 ^d ±0.041	1.642 ^d ±0.045	0.413 ^b ±0.003	0.588 ^a ±0.004	0.126 ^e ±0.001
8 weeks	3.451 ^d ±0.046	3.741 ^c ±0.091	0.575 ^a ±0.002	0.602 ^a ±0.002	0.225 ^d ±0.001
12 weeks	4.635 ^c ±0.071	4.590 ^b ±0.067	0.604 ^a ±0.002	0.677 ^a ±0.001	0.415 ^e ±0.003
15 weeks	5.152 ^b ±0.093	4.815 ^b ±0.073	0.428 ^{ab} ±0.001	0.435 ^{ab} ±0.005	0.447 ^e ±0.001
18 weeks	5.619 ^{ab} ±0.135	5.165 ^{ab} ±0.104	0.275 ^c ±0.001	0.302 ^b ±0.001	0.492 ^e ±0.002
20 weeks	5.804 ^a ±0.123	5.308 ^a ±0.095	0.159 ^c ±0.002	0.182 ^c ±0.002	0.567 ^e ±0.001
21 weeks	5.865 ^a ±0.087	5.397 ^a ±0.112	0.105 ^c ±0.003	0.114 ^c ±0.001	0.686 ^e ±0.002
22 weeks	5.872 ^a ±0.128	5.414 ^a ±0.069	0.094 ^d ±0.001	0.082 ^c ±0.003	0.711 ^a ±0.004

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

research results are consistent with the research about total sugar which increases rapidly in the later stages of fruit development increased rapidly (Patel *et al.*, 2013). At 22 weeks old, the content of reducing sugar decreased to 12.821% weight of fresh fruit so the quality of the fruit decreased.

When the fruit has just formed, low starch content only reaches 1.350% weight of fresh fruit at 2 weeks old. The highest starch content was 7.138% at 15 weeks old. This is the time when fruits tend to accumulate nutrients in preparation for ripening process. After 15 weeks old, the content of starch in the fruit decreases due to the strong metabolism in the fruit. At 22 weeks old, the content of starch decreased to 1.237% weight of fresh fruit. During this period, the activity of α -amylase enzyme also increased, under the action of α -amylase enzyme, starch converts into sugar as a material for energy-generating respiration. From unripe to ripe stage, the starch content came down (Yashoda *et al.*, 2005). During ripening, starch is hydrolyzed to glucose, thus, glucose, fructose and sucrose generally increase (Bernardes *et al.*, 2008). When fruit enters the ripening period, starch decomposes into sugar to increase the amount of reducing sugar to create sweetness for the fruit.

Tannins are one of the important categories of bioactive molecules present in the persimmon meat (Ahn *et al.*, 2002) with molecular weight up to 1.12×10^4 da (Bae *et al.*, 2000; Wu and Hwang, 2002; Jo *et al.*, 2003). Tannin in persimmons fruit has a relatively high content at 2 weeks old reached 1.508%. The content of tannin is

Table 2: Content of reducing sugar, starch, tannin in persimmons at different maturation stages.

Age of fruit development	Reducing sugar content (% weight of fresh fruit)	Starch content (% weight of fresh fruit)	Tannin content (% weight of fresh fruit)
2 weeks	1.254 ^f ±0.021	1.350 ^e ±0.074	1.508 ^b ±0.023
4 weeks	3.497 ^e ±0.022	2.945 ^d ±0.042	1.702 ^a ±0.050
8 weeks	4.245 ^e ±0.024	3.681 ^{cd} ±0.038	1.835 ^a ±0.044
12 weeks	6.412 ^d ±0.015	5.231 ^{ab} ±0.094	1.926 ^a ±0.028
15 weeks	7.115 ^d ±0.034	7.138 ^a ±0.048	1.774 ^a ±0.013
18 weeks	9.286 ^c ±0.026	6.015 ^{ab} ±0.023	1.561 ^b ±0.018
20 weeks	11.054 ^b ±0.038	4.216 ^c ±0.025	1.231 ^b ±0.024
21 weeks	13.916 ^a ±0.012	2.549 ^d ±0.036	0.976 ^{bc} ±0.009
22 weeks	12.821 ^a ±0.031	1.237 ^e ±0.043	0.605 ^c ±0.013

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

high in the early period of making persimmons fruit acrid and pungent. The tannin content in persimmons increased rapidly and reached 1.926% when fruit was 12 weeks old. After 15 weeks old, the content of tannin in the fruit decreases and rapidly decreases in the period of 18 to 22 weeks old makes persimmons ripen soft, not acrid.

Changes in protein, lipid, vitamin C content

Vitamin C content is one of the important indicators to assess the nutritional value of many fruits. The content of vitamin C from 2 to 18 weeks old increases rapidly, this is a period of strong flesh fruit development and the accumulation of vitamin C along with other nutrients in the fruit. After 18 weeks, vitamin C content continued to increase, but at a slower rate, the highest value reached 51.215 mg/100g fresh fruit on the 21th week, then vitamin C content decreases. 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 protein, lipid, vitamin C in persimmons at different maturation stages

Age of fruit development	Protein content (% weight of dried fruit)	Lipid content (% weight of dried fruit)	Vitamin C content (mg/100g fresh fruit)
2 weeks	3.861a±0.972	1.015c±0.652	22.214f±0.652
4 weeks	3.665a±1.261	1.396bc±0.931	28.076e±0.931
8 weeks	2.518b±2.467	1.635b±1.347	33.515d±1.347
12 weeks	2.075b±2.138	1.817ab±2.125	39.735c±2.125
15 weeks	1.829bc±1.575	2.105a±1.628	42.328c±1.628
18 weeks	1.756bc±0.937	2.168a±1.015	46.175b±1.015
20 weeks	1.438bc±1.208	1.409b±2.437	48.431b±2.437
21 weeks	1.272c±1.025	1.204bc±2.015	51.215a±2.015
22 weeks	0.954c±0.874	1.012c±1.108	50.856a±1.108

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

The content of vitamin C from 2 to 16 weeks old increases rapidly, this is a period of strong flesh fruit development and the accumulation of vitamin C along with other nutrients in the fruit, the highest value reached 46.610 mg/100g fresh fruit flesh on the 16th week, after 16 weeks, vitamin C content decreases.

The results of the data in table 3 shows that the content of protein in persimmons fruit has a relatively high content from 2 weeks old and decreased sharply in the period from 2 to 22 weeks old (from 3.861% to only 0.954%). This is a period of ripe fruit, a strong decrease

in protein content during this period due to the increase in the activity of protease enzyme that has dissolved protein.

Lipid content in persimmon fruit has a relatively high content from 2 weeks old (reached 1.015%), then increase rapidly according to the ripening of the fruit. The highest lipid content was 2.168% at 18 weeks old. After 18 weeks old, the content of lipid in the fruit decreases due to the strong metabolism in the fruit. Under the action of lipase enzyme, the lipid hydrolyzes rapidly when fruit enters the ripening period.

Conclusion

Over the course of the study, we found that persimmons reached physical maturity at 21 weeks of age, at which time the fruit size was maximum and almost unchanged and persimmons fruit achieved the best quality. Therefore, this is the time to harvest the most appropriate. If harvested earlier or later, the quality of the persimmon fruit will be significantly reduced.

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