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Investigation of the Physiological and Biochemical Changes by Developmental Stages of Orange Fruit (*Citrus sinensis*) Grown in Vietnam

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Abstract

The physiological and biochemical changes of Song Con oranges collected in Vietnam from formation to maturity were studied to determine the physiological ripening time of the fruit, which is crucial for collection and preservation. Fruits were collected at 8, 12, 18, 21, 24, 27, 29, 30 & 32 weeks after anthesis (WAA) and surveyed for size, pigment content, reducing sugar, starch, total organic acid, vitamin C, protein, and lipid. Results showed that oranges fruit reached the maximum size at week 30. The content of chlorophyll *a* and *b* increased gradually from fruit formation to week 12, then decreased rapidly until the fruit was fully ripe. Carotenoids increased gradually until the fruit ripened. Starch content and total organic acid content increased gradually and peaked at week 18, then gradually decreased. Reducing sugars and vitamin C content increased during the early stages and peaked at week 30, then decreased gradually. Protein content showed the same trend, as it declined gradually after peaking at 21 WAA. The lipid content gradually increased from 8 to 27 WAA and then decreased as the fruit proceeded toward ripening. These results show that the Song Con orange fruit should be harvested at physiological maturity (30 WAA) to ensure the high nutritional value and quality of the fruit during storage.

Keywords: biochemical characteristics, fruit developmental stage, orange fruit, physiological characteristics

Introduction

Orange (*Citrus sinensis*) is a member of the citrus family (Rutaceae) and was for many years known as *Citrus aurantium* var. *sinensis* L. (Snart et al., 2006). Orange is a citrus orchard that is grown quite popular in tropical and subtropical climates. Vietnam is one of the countries in the citrus growing center (Southeast Asian Center) so the citrus orchard has been planted for along time and is widely distributed from the North to the South (Phong et al., 1999). Oranges are an important source of nutrients for the human body like vitamins, sugar, mineral, etc. (Spiegel-Roy et al., 1996). Vitamin C is also necessary for the proper functioning of the immune system. Vitamin C is good for preventing colds, and coughs (Parle & Chaturvedi, 2012). The beneficial effect of citrus fruit consumption on human health is due to the presence of antioxidant and anti-radicle properties (Betoret et al., 2009).

One of the world's largest orange exporters is Vietnam, where Song Con oranges are widely grown and have high economic value (Thuan, 2004). In the production process, the stage of fruit collection and preservation is an important step to ensure the quality of the fruit. Therefore, it is necessary to investigate the physiological and biochemical characteristics of oranges from formation to ripeness to improve the quality to help consumers use and preserve Song Con oranges better.

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Materials and Methods

Research Materials

Song Con orange was harvested in Thanh Hoa, Vietnam (19°18′00″N and 104°22′00″E). We collected samples at 8, 12, 18, 21, 24, 27, 29, 30 & 32 WAA. Samples were collected from March to September. At each developmental stage of the orange fruit, we collected samples from all plants, with 05 fruits/plant.

Investigation of the Fruit Size

At 8 WAA, the size of the fruit was measured until the fruit was fully ripe. The mean values of fruit size are presented in centimeters.

Investigation of the Pigment Content in the Fruit Peels (Trong et al., 2022)

Orange peel samples were crushed, and 5 mg of the crushed sample and 100 µL of distilled water were placed in a test tube and allowed to stand for 10 min. Add 8 mL of 80% acetone and centrifuge to extract the chlorophyll, measuring optical density at 662 nm, 644 nm and 440.5 nm. The pigment content was calculated by spectrophotometry.

Investigation of the Reducing Sugar and Starch Content (Chau et al., 1998)

10 mL of the test solution was placed in a conical flask, then 10 mL of Fehling was added. The mixture was boiled for 3 min, and filtered to remove the precipitate. The Cu_2O precipitate was completely dissolved with $Fe_2(SO_4)_3$ (5 mL) in H_2SO_4. The resulting solution was titrated with $KMnO_4$ 1/30N. A control experiment was conducted at the same time as replacing the sugar solution with distilled water. Calculate the amount of $KMnO_4$ used for the titration and determine the equivalent amount of reducing sugar and starch in the sample.

Investigation of the Total Organic Acid Content (Ermakov et al., 1972)

10 mL of test solution was added to a 100 mL flask, adding a few drops of phenolphthalein reagent. Titrate with 0.1N NaOH until a persistent pink color appears. The total organic acid content was calculated based on the titration method.

Investigation of the Vitamin C Content (Arya et al., 2000)

Crush 5g of fresh orange with 5 mL of HCl 5%, put into a 100ml flask, add distilled water and mix well. Proceed to titrate the solution with liquid I_2 until a blue color appears. The vitamin C content was calculated based on the titration method.

Investigation of the Protein Content (Trong et al., 2022)

Add 0.5 mL of $CuSO_4$ 1% to 1 mL of the sample in a test tube, and stay at 30°C for 10 minutes. Add 0.5 mL of Folin 1N, and stay at 30°C for 30 minutes, The spectrophotometer was measured at 750 nm. Protein concentration was calculated based on a standard graph.

Investigation of the Lipid Content (Mui, 2001)

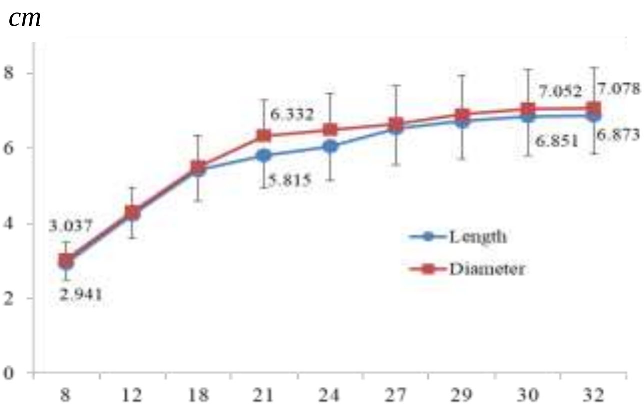
Add the ether to half the volume of the flask, which was placed in the beaker. Add ether to flood the bag of ingredients. A cooling tube was installed and the material was soaked in solvent for several hours. The Soxhlet extractor was placed inside the bain-marie so that the solvent condensation rate reached about 10-15 drops per hour. The lipid content in the samples was calculated based on drying the lipid container dried to a constant weight.

Methods of Data Analysis

Analysis of variance (ANOVA) was used to analyze the significance of differences between treatments using IRRISTAT software version 5.0, applying the statistical significance level of $P < 0.05$.

Results and Discussion

From 8 to 21 WAA, the length and diameter changed significantly (Figure 1), fruit diameter increased from 3.037 cm to 6.332 cm, while fruit length increased from 2.941 cm to 5.815 cm. The size increase between 8 WAA and 21 WAA was due to an increase in both the number and size of cells. From 21 to 32 WAA, the fruit reached the maximum value for the cultivar under the study conditions (at 30 WAA, the fruit diameter was 7.052 cm, fruit length was 6.851 cm). After 30 WAA, the fruit size increased very slowly and remained almost unchanged. During this study, we observed that at 30 WAA, the orange peel started to appear slightly yellow and the fruit stopped growing, so 30 WAA can be said to be the physiological maturity period of oranges.



Weeks after anthesis (WAA)

Figure 1. Changes in length and diameter of orange fruits

The content of chlorophyll a and chlorophyll b in orange peels was low during the first weeks, the chlorophyll a content was 0.046 mg/g, and the chlorophyll b was 0.317 mg/g at 8 WAA. From 8 to 12 WAA, the chlorophyll a and b content increased rapidly and reached the highest value at week 12. After 12 WAA, the contents of chlorophyll a and b decreased rapidly to week 29 because fruits began to move to the stage of ripening, at which time chlorophyll was decomposed and carotenoid pigments were synthesized rapidly. The maturation of some fruits related to chlorophyll resolution has been published in many studies; for example, during orange coloration, a visible degreening process associated with chlorophyll degradation has been reported (Lai et al., 2015). This result is consistent with several studies stating that chlorophyll breakdown is associated with the maturity of some kinds of fruits (Du et al., 2014; Wei et al., 2019).

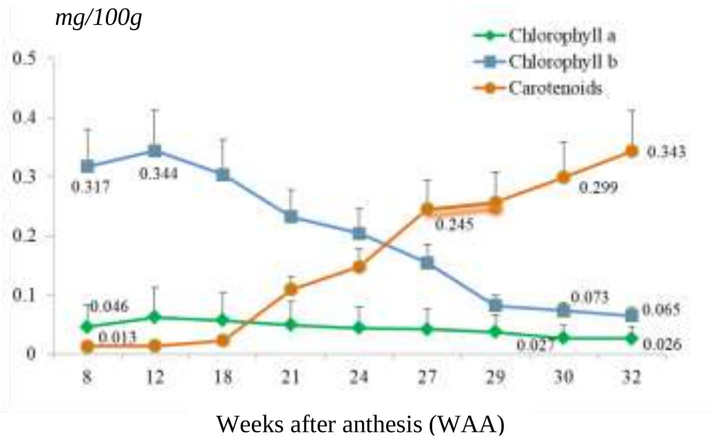


Figure 2. Changes in pigment contents in orange peels

The carotenoid content in orange peel was low in the first week (0.013 mg/g) (Figure 2). From 8 to 18 WAA, the carotenoid content increased slowly and then increased rapidly with the ripening stage of the fruit. At week 32, the carotenoid content reached 0.343 mg/g. In the early stages, the fruit is green because it contains a lot of chlorophyll; When entering the ripening process, the yellow color of the carotenoid becomes more obvious due to the decomposition of chlorophyll (Charoenchongsuk et al., 2015). This result is similar to previous reports that fruit ripening is associated with a significant increase in carotenoid content (Ramesh et al., 2017).

Figure 3 shows that at week 8, the reducing sugar content in oranges was low, reaching 2,188%. From 8 to 18 weeks, the reducing sugar content increased slowly and reached 2.683% when the fruit was at week 18. From 18 to 32 weeks, the reducing sugar content increased rapidly and reached 9.275% at week 30. This is consistent with Patel et al., 2013) announcement about the rapid increase in total sugar content in the fruit at the later development stage.

The starch content reached 0.405% at the time of fruit 8 WAA, then gradually increased and reached the highest value of 0.694% at 18 WAA (Figure 3). After 18 weeks, due to the strong metabolism in the fruit, including the breakdown of starch to provide materials for respiration, this causes the starch content in the fruit decreased gradually. At 32 WAA, the starch content decreased to 0.102%. During the ripening stage, the activity of α-amylase enzyme increases, and starch is converted into sugar needed for respiration, increasing the amount of reducing sugar to create sweetness in the fruit (Yashoda et al., 2005).

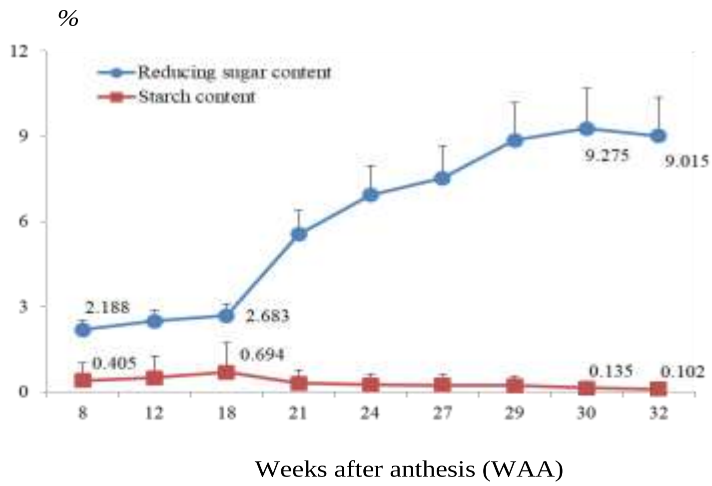


Figure 3. Contents of reducing sugar, and starch in orange fruits

The organic acids in fruit can be found in free form or combination, and these acids are important components that contribute to the assessment of fruit quality, flavor and acidity (Vallarino et al., 2019). The data in Figure 4 shows that in the early stage, the organic acid content was 55.012 mg/100g. During the fruit growth period from 8 to 18 WAA, the total organic acid content gradually increased and reached the highest value of 80.012 mg/100g when the fruit was at week 18. At this time, there was decomposition of substances such as proteins and lipids led to the production of many intermediate products such as amino acids, which increase the organic acid content. After 18 weeks, the organic acid content decreased because it was used more in respiration for energy (Brummell, 2013). On the other hand, the energy required for the biosynthesis of substances specific to the ripening process of fruits such as enzymes, esters for flavor and sugar synthesis for sweetness, leads to a decrease in organic acid content (Prasanna et al., 2007).

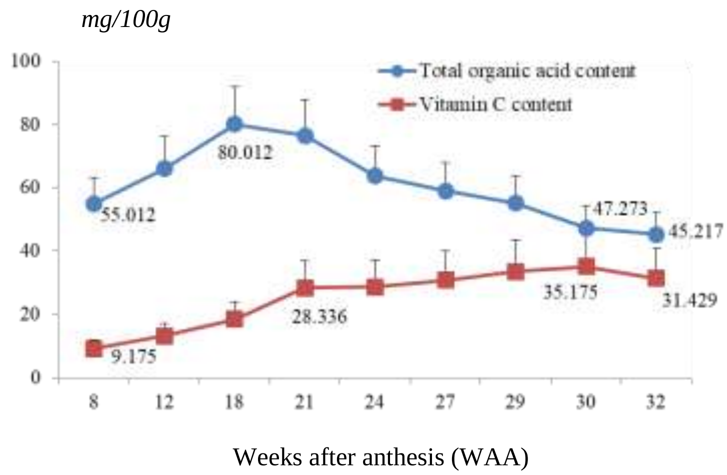


Figure 4. The content of total organic acid and vitamin C in orange fruits

Vitamin C content is an important indicator used to evaluate the nutritional value of the fruit. Vitamin C content at week 8 reached 9.175 mg/100g, from 8 to 21 WAA vitamin C content increased rapidly and reached 28.336 mg/100g at week 21. After 21 WAA, vitamin C content increased but at a slower rate and reached the highest value of 35.175 mg/100g at week 30. After that, the vitamin C content decreased. The variation in vitamin C content of oranges at different growth stages is related to different metabolic pathways in the fruit and enzymatic degradation activities (Singh et al., 2011).

Figure 5 shows that the protein content in oranges has a relatively high content at week 8 (4.050%) and increased sharply in the period from 8 to 21 WAA (from 4.050% to only 7.043%). After 21 weeks, the contents of protein gradually decreased to 32 WAA. This is the period when the fruit enters the physiological mature stage, at which time the degrading activity in the fruit predominates, in which the activity of the protease enzyme increases, which degrades the protein in the fruit.

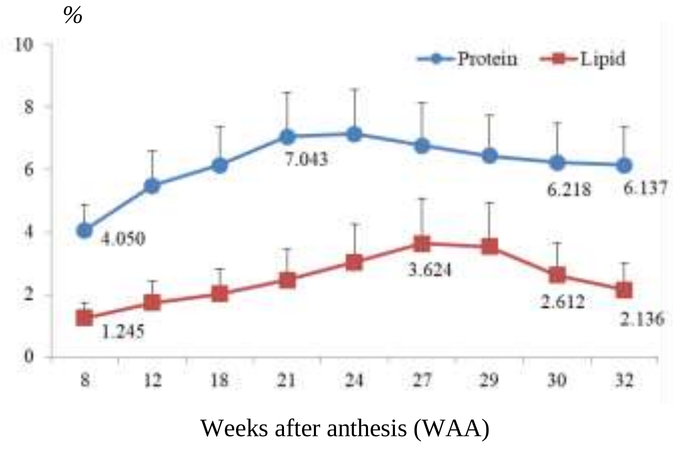


Figure 5. The content of protein and lipids in orange fruits

Lipid content in orange fruit has a relatively high content at 8 WAA (reached 1.245%), then increased rapidly according to the ripening of the fruit. The highest lipid content was 3.624% at 27 WAA (Figure 5). After 27 WAA, the fruit is physiologically mature, the metabolism in the fruit is stronger, and under the action of lipase, lipids are rapidly hydrolyzed to provide materials for the respiratory process, thereby reducing the lipid content in the fruit (Wills et al., 1998).

Conclusion

At week 30, the size of the orange fruit reached its maximum and remained almost unchanged, the rind turned pale yellow due to the increase in carotenoid content and the sharp decrease in chlorophyll. Some of the main nutritional components in oranges such as vitamin C, reducing sugars reach their highest values at week 30. Therefore, this is the most suitable time to harvest oranges for excellent quality.

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