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Study of some physiological and biochemical metabolism of sour apple fruit (*Ziziphus mauritiana* Lam.) grown in Vietnam

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Abstract

Research to determine the ripening time of the fruit is the scientific basis for better harvesting and preservation. The results showed that sour apple fruit reached its maximum size 11 to 12 weeks after anthesis (WAA). At this time, indicators such as length, diameter, fresh weight, and volume changed little. The ratio of dry matter content and the ratio of seeds and pods decreased until fruit ripening, while the water content and the ratio of pulps increased gradually from fruit formation to fruit ripening. The starch content gradually increased and reached its maximum at 6 WAA, then decreased gradually and then decreased gradually. Reducing sugars increased during the early stages and peaked at 12 WAA, then decreased gradually. The total organic acid content reached its maximum when the fruit was 10 WAA and then decreased gradually. This result shows that sour apple fruit should be harvested at physiological maturity (11-12 WAA) to ensure the highest nutritional value and quality of the fruit.

Keywords: Biochemical indexes, physiological indexes, physiological maturity, sour apple fruit

Introduction

Ziziphus mauritiana is a tropical fruit tree belonging to the Apple family (Rhamnaceae). The species is believed to have originated in the Indo-Malaysian region of South-East Asia (Janick, et al., 2008). It is now widely naturalized throughout the old world tropics from Southern Africa through the Middle East to the Indian Subcontinent and China, Indomalaya, and into Australasia and the Pacific Islands. The fruit contains a lot of water and has a sweet and sour taste. The fruits ripen at different intervals even when on just one tree and are pale green when green and pale yellow when ripe (Tran et al., 2001). The size and shape of the fruit depth end on different varieties in the wild as well as the type that is grown. The fruit is eaten when ripe, soaked in wine, or used to make drinks. It is a nutrient-rich fruit and contains a lot of vitamin C. The fruit is used to treat stomach pain, anemia, hypertension, neurasthenia and memory loss. Besides, the seeds of the sour apples are also ground into powder to make medicine. Apple juice has a pleasant taste and high concentration of phenolic

physiological and biochemical changes of apples. The medical importance of *Ziziphus mauritiana* was reported by Rathore, et al. (2012), the plant fruit compounds were extracted with

compounds, vitamins, minerals, dietary fiber, and antioxidant capacity (Kalinowska et al., 2014; Liaudanskas et al., 2015).

In the world, there have been many studies on the

al. (2012), the plant fruit compounds were extracted with petroleum ether, chloroform, methanol, 95% ethanol, and distilled water for 48 hours help of cold percolation method and found different types of secondary metabolites as flavonoids, glycosides, saponins, phenols, lignins, sterols and tannins were presented. Unripeapple juice made from apples harvested in July/august can be used to produce Sour apple concentrate a food ingredient that provides acidity and flavor and as an alternative to verjuice and pomegranate sauce in salad dressings and other applications where it scored higher than both. (Ossama et al., 2019). Many secondary metabolites present inside *Z. mauritiana* such as alkaloid, flavonoid, glycoside, phenol, saponin, steroid, sterol, tannin and terpenoid but for the left part only the absence of saponin

detected was reported by Saidatul, et al. (2017). The study aimed to evaluate the effects of fruit thinning and fruit wrapping materials on fruit size, fruit quality traits and fruit loss rate due to insects and diseases infecting jujube fruit variety 05 was reported by Phuong et al. (2020).

During the production process, fruit collection and preservation steps are crucial and may have direct effects on consumers (Pacheco et al., 2019). Therefore, it is necessary to understand the physiological and biochemical indicators of fruit from formation to fruit ripening to improve the quality and storage of the fruit. This research aims to determine the physiological ripening time of sour apples to help consumers better use and preserve sour apple fruit.

Materials and methods

Research materials

Sour apple fruits were obtained from five-year-old plants located in an orchard in Thach Thanh District (Thanh Hoa province, Vietnam) from September to December 2022. A total of 20 plants, selected based on the visual uniformity of fully opened flowers in the middle part, were marked on a single day at flowering time. The fruit samples from marked inflorescences were harvested at ten development stages, specifically, 2, 4, 6, 8, 10, 11, 12, and 13 weeks after anthesis (WAA). At each stage, ten of the previously marked trees were sampled and two fruits were collected from each tree according to the method described previously (Nguyen et al., 2021). The fruits were hand-picked in the morning and immediately transported in a refrigerated container to the Laboratory of Biology, Faculty of Natural Sciences, Hong Duc University (Thanh Hoa, Vietnam).

Determination of fruit length and diameter

Fruit length and diameter were measured from randomly selected fruit during the study. The length from the base of the fruit to the base of the calyx and the diameter at the largest bulge of fruit from both sides were measured with the help of Palme calipers. The mean values of length and diameter are presented in centimeters.

Determination of the volume of the fruit

The volume is determined by measuring instruments: 5ml, 10ml, and 20ml depending on the time. Fill the cylinder with water to a certain level, then drop the fruit in, the volume of the flask increases to reflect the actual volume of the fruit.

Determination of fresh weight, dry matter and water content of fruit

Use an electronic scale to weigh each fruit, each time weighing 10 numbered balls, taking the average data.

Determination of the ratio of seeds, pods, and pulps in sour apple

Weigh the 10 fruits, then separate the pods, seeds, and pulps. The ratio of pods weight, seeds weight, and pulps was determined by electronic balance with an accuracy of 10^{-4} g.

Determination of reducing sugar and starch contents (Pham et al., 1996; Nguyen, 2001) The experimental solution was put in a 500 ml conical flask, and 10 ml of Fehling was then added. The mixture was boiled for 3 minutes. The precipitate was filtered into a Buchner vacuum filter. The resulting sediment of Cu_2O in the Buchner filter was completely dissolved by using $Fe_2(SO_4)_3$ (5 ml) in H₂SO₄. The resulting solution was titrated with KMnO₄1/30N until a light pink color appeared within 20-30 seconds. A control experiment was conducted at the same time in which the sugar solution was replaced with distilled water. Calculated the amount of KMnO₄ used for titration, and looked up the table for an equivalent amount of reducing sugar and starch in the sample.

Determination of the total organic acid content (Sinclair et al., 1945) Five grams of pulp was crushed in a ceramic mortar and the sample was transferred into a 250 ml Erlenmeyer flask. Distilled water was added to increase the volume to approximately 150 ml and the flask was heated in a water bath at 80°C for 15 min. The solution was cooled and then it was transferred to a 250 ml volumetric flask, topped with water, shaken vigorously and allowed to settle. A volume of 25 ml of the filtrate was pipetted into a 100 ml Erlenmeyer flask, 3 drops of 0.1% phenolphthalein were added and the solution was titrated with 0.1 mol·l-1 NaOH until a faint pink color persisted for 30 s. The total organic acids content was determined by the titration value and calculated as previously described.

Methods of data processing

Data were processed and analyzed by ANOVA with the software IRRISTAT 5.0.

Results and discussion

Changes in length and diameter of sour apple fruit

Table 1 data showed that at week 2 the fruit length was 2.41 cm and the diameter was 1.58 cm. In the period from 2 to 10 WAA, the fruit length changes markedly, showing a significant difference through statistical analysis. At this stage, the fruit has a fast growth rate due to the strong division and elongation of the cells (Heller et al., 1995). After that, the length and diameter of the fruit continued to increase but at a slower rate, when the fruit was 11 WAA, the length of the fruit reached 3.51 cm and the diameter of the fruit reached 3.09 cm with little change. When the fruit was 13 WAA, the fruit length still increased but not significantly, at this time the values did not show a statistically significant difference. Thus, the growth in length and diameter of sour apples are closely

related, this relationship is controlled by metabolic processes plus the regulation and control of complex endogenous hormones in the cell (Quinet et al., 2019).

Age of fruit	Length (cm)	Diameter (cm)
(WAA)		
2	$2.41^{\text{e}}\pm0.02$	$1.58^{\text{d}} \pm 0.01$
4	$2.97^{\text{d}} \pm 0.03$	$2.36^{\circ} \pm 0.01$
6	$3.17^{\text{d}}\pm0.05$	$2.53^{bc} \pm 0.02$
8	$3.35^{\circ} \pm 0.01$	$2.76^{\rm b}\pm0.01$
10	$3.42^{bc} \pm 0.05$	$2.85^{\text{b}}\pm0.04$
11	$3.51^{b} \pm 0.02$	$3.09^{a} \pm 0.05$
12	$3.62^a \pm 0.02$	$3.12^{a} \pm 0.02$
13	$3.65^{\mathrm{a}} \pm 0.05$	$3.13^{a}\pm0.01$

Table 1. Changes in length and diameter of sour apple fruit

Note: In the same data column, values with similar letters represent non-significant differences, and values with different letters represent significant differences (p < 0.05).

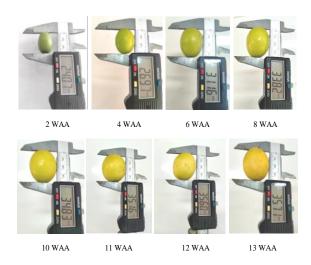


Fig. 1 The growth and development of sour apple fruit

Change in fresh weight and volume in sour apple fruit

Corresponding to the growth in length and diameter, the volume of sour apples increased from 2 to 13 WAA, which increased rapidly from 2 to 10 WAA, when the fruit was 10 WAA, the volume of the fruit reached 16.11 ml, this is the period of intense cell growth. Cells increase water uptake due to rapidly increasing vacuole volume accompanied by primary wall dilation. This wall expansion is due to the action of H⁺-ATPase to break the hydrogen bonds between cellulose microfibrils, this process is involved in the regulation of phytohormone auxin (Nguyen et al., 2013). After 10 WAA the volume of the fruit continued to increase but at a slow rate and the fruit reached its peak volume at 11 to 13 WAA, at this time the volume of the fruit increased but very slowly and the results did not show any meaningful difference.

The results of Table 2 showed that the fresh weight of fruit varies proportionally with the development of the fruit and this change is consistent with the change in the volume, length, and diameter of the fruit during the growth process. At week 2, the fruit weight reached 3.25 g, then the fruit weight increased rapidly and reached 17.13g at 11 WAA, and at week 13 the weight reached 17.36 g.

11 ult			
Age of fruit (WAA)	Fresh weight of fruit (g)	The volume of the fruit (ml)	
2	$3.25^{f} \pm 0.11$	3.01 ^e ± 0.06	
4	$7.95^{e} \pm 0.19$	$7.80^{d} \pm 0.09$	
6	$11.31^{d} \pm 0.12$	$10.12^{c} \pm 0.14$	
8	$13.05^{\circ} \pm 0.15$	$14.21^{b}\pm 0.15$	
10	$15.17^{b} \pm 0.14$	16.11 ^{ab} ± 0.05	
11	$17.13^{a}\pm0.25$	$17.25^{a} \pm 0.12$	

Table 2. Change in fresh	weight and	volume in sour apple
	fruit	

Note: In the same data column, values with similar letters represent non-significant differences, and values with different letters represent significant differences (p < 0.05).

 $17.65^{a} \pm 0.11$

 $17.28^{a} \pm 0.14$

 $17.36^{a} \pm 0.17$

12

13

Changes in the contents of water and dry matter in sour apple fruits

From the data of Table 3, it can be seen that the sour apple fruit from the early stages contains a relatively large amount of water, accounting for 70.46% (2 WAA). From 2 to 6 WAA, the water content in the fruit increased rapidly from 70.46% to 86.47% and showed a statistically significant difference. The water content continued to increase until 11 WAA, reaching 87.68%, after 11 WAA the water content increased slowly and reached 87.79% at 13 WAA, at which time the difference was not significant compared to the 11 WAA. In contrast to the increase in water content, the dry matter content decreased gradually. The amount of dry matter accounted for the highest percentage at the time of 2 WAA and reached 29.54%, then gradually decreased and decreased the most at the period from 2 to 6 WAA from 29.54% to 13.53%. The growth of cells causes the water content in the fruit to increase rapidly from 2 to 6 WAA, leading to a decrease in the amount of dry matter and by 13 WAA, the amount of dry matter is only 12.21%.

 Table 3. Changes in the contents of water and dry matter in sour apple fruit

Age of fruit (WAA)	Water content (%)	Dry matter content (%)
2	$70.46^{\rm f}\pm0.24$	$29.54^a\pm0.21$
4	$81.76^{\rm e}\pm0.26$	$18.24^b\pm0.15$
6	$86.47^d\pm0.18$	$13.53^{\circ} \pm 0.12$
8	$87.13^{\circ} \pm 0.35$	$12.87^{dc} \pm 0.16$
10	87.54 ^{bc} ± 0.14	$12.46^d\pm0.11$
11	87.68 ^{ab} ± 0.17	$12.32^d\pm0.09$
12	$87.73^{a} \pm 0.21$	$12.27^d\pm0.06$
13	$87.79^{a} \pm 0.25$	$12.21^{d} \pm 0.12$

Note: In the same data column, values with similar letters represent non-significant differences, and values with different letters represent significant differences (p < 0.05).

The change in the ratio of seeds, pods and pulps in sour apple fruit

Data from Table 4 showed that, at 2 WAA, the ratio of seeds accounted for 27.08%. This is the time when the new fruit is formed, so the fruit size is still small, the flesh of the fruit is small while the seeds have a relatively large weight. The ratio of seeds decreased with the growth and development of the fruit, which decreased sharply from the period of 2 to 6 WAA, this is because according to the growth of the fruit, the ratio of fruit pulp increased, accounting for a large proportion, leading to a rapid decrease in the ratio of seeds. The ratio of seeds was 7.52% at 12 WAA and 7.49% at 13 WAA. At this point the difference is not statistically significant, the fruit stops growing and enters the ripening stage.

According to the change in rind thickness, the ratio of pods and the ratio of pulps also changed. At week 2, the ratio of pods reached 24.01% while the ratio of pulp accounted for 48.92%, this time due to the newly formed fruit, the ratio of pulp was low. After 2 WAA, the pulp cells grew rapidly leading to a high ratio of pulps and reaching 79.40% at 6 WAA, while the ratio of pods gradually decreased and reached 10.96% at 6 WAA. From 6 to 13 WAA, the ratio of pulps continued to increase and the ratio of pods decreased, but at a slower rate, by 13 WAA the ratio of pulps reached its maximum and accounted for 84.39%, while the ratio of pods reached the lowest value is 8.12%.

Table 4. The change in the ratio of seeds, pods, and pulps in
sour apple fruit

Age of fruit	Ratio of seeds	Ratio of pods	Ratio of pulps
(WAA)	(%)	(%)	(%)
2	$27.08^a\pm0.26$	24.01 ^a ± 0.24	$48.92^d\pm0.18$
4	$13.21^{\text{b}}\pm0.18$	$14.84^{b}\pm 0.21$	$71.95^{\text{c}}\pm0.16$
6	$9.64^{\circ}\pm0.12$	$10.96^{\circ} \pm 0.14$	$79.40^b\pm0.09$
8	$9.58^{\rm c}\pm0.16$	9.73 ^{cd} ± 0.11	$80.69^{\rm b}\pm0.11$
10	$8.37^{cd} \pm 0.05$	$8.70^{\rm d}\pm0.05$	82.93 ^{ab} ± 0.17
11	$7.53^{d}\pm0.07$	$8.17^{\rm d}\pm0.09$	$84.30^a\pm0.13$
12	$7.52^{d}\pm0.09$	$8.16^{\rm d}\pm0.07$	$84.32^a\pm0.22$
13	$7.49^{\rm d}\pm0.05$	$8.12^{\text{d}} \pm 0.02$	$84.39^a\pm0.27$

Note: In the same data column, values with similar letters represent non-significant differences, and values with different letters represent significant differences (p < 0.05).

Changes in the contents of reducing sugar, starch, and total organic acid in sour apple fruit

The results in Table 5 showed that in the early period of sour apple fruit development (first 2 weeks), the reducing sugar content was relatively low, reaching 1.13% of the weight of fresh fruit. From 2 to 6 WAA, the content of reducing sugars increased slowly and reached 1.47% when the fruit was 6 WAA. During the fruit development period from 6 to 12 WAA, the content of reducing sugar increased rapidly and reached 6.14% when the fruit was 12 WAA. These research results are consistent with research on total sugar, which increases rapidly in the later stages of fruit development (Patel et al., 2011). At week 13, the reducing sugar content decreased to 6.12% of the fresh fruit weight, indicating a decrease in fruit quality.

Age of fruit (WAA)	Reducing sugar content (%)	Starch content (%)	The total organic acid content (mg/100g fresh fruit)
2	$1.13^{\text{e}} \pm 0.05$	$5.12^{\circ} \pm 0.09$	$18.17^{ m g} \pm 0.11$
4	$1.25^{\mathrm{e}} \pm 0.07$	$6.28^{b} \pm 0.17$	$22.35^{\rm f} \pm 0.24$
6	$1.47^{\rm e}\pm0.02$	$7.25^{a} \pm 0.12$	$34.21^{e} \pm 0.18$
8	$2.05^{\text{d}} \pm 0.04$	$6.42^{b} \pm 0.21$	$55.29^{\circ} \pm 0.41$
10	$3.18^{\circ}\pm0.05$	$5.34^{\circ} \pm 0.25$	$67.32^{a} \pm 0.34$
11	$5.02^{b} \pm 0.11$	$5.09^{\circ} \pm 0.19$	$60.62^{b} \pm 0.15$
12	$6.14^{a} \pm 0.09$	$4.36^{d} \pm 0.14$	$58.93^{b} \pm 0.18$
13	$6.12^a \pm 0.12$	$3.16^{e} \pm 0.15$	52.41°±0.36

 Table 5. Changes in the contents of reducing sugar, starch, and total organic acid in sour apple fruit

Note: In the same data column, values with similar letters represent non-significant differences, and values with different letters represent significant differences (p < 0.05).

When the fruit had just formed, the starch content reached 5.12% of the weight of fresh fruit (2 WAA). The highest starch content was 7.25% at 6 WAA (Table 5). This is the time when fruits tend to accumulate nutrients in preparation for the ripening process. After 6 WAA, the content of starch in the fruit decreased due to the strong metabolism in the fruit. At week 13, the content of starch decreased to 3.16% of the weight of fresh fruit. During this period, the activity of the α -

amylase enzyme also increased. Due to increased α -amylase enzyme activity, starch converts into the sugar required for respiration. From the unripe stage to the ripe stage, the starch content declined. When the fruit enters the ripening period, starch decomposes into sugar to increase the amount of reducing sugar to create sweetness in the fruit (Yashoda et al., 2005).

Organic acids are of increasing interest because of their role in plant physiology as co-factors, buffers and mediators of the most important metabolic pathways of carbohydrates, lipids and proteins (Poonam et al., 2021). Therefore, organic acids play a role in maintaining the quality and nutritional value of the fruit. At 2 WAA, fruit accumulated a large amount of total organic acids of 18.17 (mg/100g) (Table 5). From 2 to 10 WAA, the total organic acids content gradually increased, reaching a maximum of 67.32 (mg/100g) at 10 WAA. From 10 to 13 WAA, the total organic acids content decreased, presumably because organic acids are used in respiration to provide energy for starch synthesis (Brummell, 2013). During ripening, energy continues to be needed for the biosynthesis of substances specific to the ripening period of the fruit, such as hydrolytic enzymes that give the fruit a sweet taste, leading to a gradual decrease in the content of total organic acids (Prasanna et al., 2007).

Conclusion

Sour apples at 11 to 12 WAA reach almost maximum size in terms of length, diameter, fresh weight, and volume. At this time, the fruit skin is thinner, and the fruit has a great value in some parameters such as the ratio of pulps, water content, and reducing sugars, while other components such as the dry matter, starch content, and total organic acids vary with fruit growth and ripening. After 12 WAA, some important components of the fruit such as reducing sugars decreased. Therefore, the time of 11 to 12 WAA is the most appropriate time to harvest, if picking earlier or later will affect the quality of the fruit.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

References

- Brummell, D. A. (2013). Chapter 11 Fruit growth, ripening and post-harvest physiology. In: *Plant in Action. Plant and Food Research.*
- Heller, R., Esnault, R., & Lance, C. (1995). Physiologie végétale, Développement, 15e e'dition. Masson II. Paris Milan Bacelone. French.

- Janick, J., Paull, R. E., (2008). The encyclopedia of fruit & nuts. Cabi Publishing. pp. 615-619.
- Kalinowska, M. (2014), Apples: content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. *Plant Physiol Biochem*, 84, 169-188.
- Liaudanskas, M. (2015), A Comparative Study of Phenolic Content in Apple Fruits. International Journal of Food Properties, 2015, 18(5), 945-953.
- Nguyen, K. N. and Le, T. V. (2021). Study on some of the biochemical indexes under the stage of development of longan fruit (*Euphoria longan* Lamk.) grown in Quang Ninh, *Vietnam J. Agri. Sci, 19*(1), 1-7.
- Nguyen, M. V. (2001). The practice of biochemistry, Ha Noi, Vietnam: National University Publishing House.
- Nguyen, N. K. and Nguyen, V. D. (2013), Textbook of plant growth regulators, Education Publishing House. Hanoi.
- Nguyen, T. P., Nguyen, H. V., Vu, T. H., Nguyen, T. T. H. and Nguyen, T. H. X. (2020), Evaluation of the effect of pruning methods and fruit wrapping materials on the size and quality of apple varieties 05 (*Ziziphus mauritiana* Lamk.). *Vietnam Journal of Agricultural Science*, 18(1), 14-23.
- Ossama, D., Ghenwa, A. H., Raymond, A. and Mohamed R., (2019), Sour Apple Concentrate: processing essentials and organoleptic properties, *International Journal of Environment, Agriculture and Biotechnology* (*IJEAB*), 4(6), 1738-1744.
- Pacheco, R. M., Verón, R. & Cáceres, S. (2019). Efecto del raleo de flores y estado de madurez de cosecha sobre el rendimiento y calidad de fruto de pimiento. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 51(1), 19-28.
- Patel, P. R., Gol, N. B. & Rao, T. V. R. (2011). Physiochemical changes in sunberry (*Physalis minima* L.) fruit during growth and ripening. *Fruits*, 66(1), 37-46.
- Pham, C. T. T., Nguyen, H. T. and Phung, T. G. (1996). Practicing biochemistry, Ha Noi, Vietnam: Education Publishing House.
- Poonam, P., Anthony, J. and Jitender, G., (2021), Organic acids: versatile stress-response roles in plants. *Journal of Experimental Botany*, 72(11), 4038-4052.
- Prasanna, V., Prabha, T. N. and Tharanathan, R. N. (2007). Fruit ripening phenomena-an overview. *Crit. Rev. Fd. Sci. Nutr.* 47, 1-19.
- Quinet, M., Angosto, T., Yuste-Lisbona, F. J., Blanchard-Gros, R., Bigot, S., Martinez, J. P., & Lutts, S. (2019). Tomato fruit development and metabolism. *Frontiers in plant science*, 10, 1554.
- Saidatul, A. M. Y. & Rohaiza, S. (2017), Phytochemical analysis and bioactivity studies of *ziziphus mauritiana* (twigs and leaves). Journal of Academia UiTM Negeri Sembilan 5, 17-26.
- Sinclair, W. B., Bartholomew, E. T., & Ramsey, R. C. (1945). Analysis of the organic acids of orange juice. *Plant Physiology*, 20, 3-18.
- Surendra, K. R., Shashank, B., Suresh, D., & Aanchal, J. (2012), Preliminary phytochemical screening of medicinal plant Ziziphus mauritiana Lam. Fruits. *International Journal of Current Pharmaceutical Research*, 4(3), 160-162.
- Tran, T. T., & Pham, V. C. (2001). Apple tree (Zizyphus mauritiana L.) and planting techniques, Labor and Social Publishing House, Hanoi, Vietnam.
- Yashoda, H. M.;, Prabha, T. N., & Tharanathan, R. N., (2005). Mango ripening: changes in cell wall constituents in relation to textural softening. J. Sci. Fd. Agr, 86, 713-721.

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