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## Research Article

# Morphological and Physiological Changes During Growth and Development of Wood-apple (*Feronia limonia* (L.) Swingle) Fruit

<sup>1</sup>Endang Dewi Murrinie, <sup>2</sup>Prpto Yudono, <sup>2</sup>Azis Purwanto and <sup>2</sup>Endang Sulistyaningsih

<sup>1</sup>Faculty of Agriculture, Muria Kudus University, Kudus, Indonesia

<sup>2</sup>Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia

## Abstract

**Background and Objective:** Wood-apple is a fruit plant of many benefits, i.e., as ingredients in beverage and food, medication, rootstock for citrus plant and building material. Unlike fruit in general, fruit maturity of wood-apple is not followed by a visual color change as an indicator of maturity. The research objective was to study the morphological and physiological changes during the growth and development of wood-apple fruit, so it can be used to determine the harvesting time of the fruit to obtain high quality.

**Materials and Methods:** The study was conducted by observing the growth and fruit development wood-apple, beginning from anthesis until the fruit mature. The experimental design used was a completely randomized design consisting of nine fruit ages of 1, 2, 3, 4, 5, 6, 7, 8 months after anthesis and mature fruit exactly detached from the tree, hereinafter called the fallen mature fruit. Data were analyzed by one-way analysis of variance (ANOVA) and followed by DMRT. **Results:** Weights and fruit diameter increased during growth and fruit development, from anthesis until 6 months after anthesis and stopped increasing until the mature fruit fell off the tree. Similarly rind thickness and weight of the rind and flesh of fruit and seed weight also increased and reached a maximum at the age of 6 months after anthesis. Total sugar content and total soluble solids of fruit flesh increased in line with the increasing age of the fruit and reached a maximum when the mature fruit detached from the tree. In contrast phenolic content decreased with increasing age of the fruit and reached a minimum at the time of fallen mature fruit. **Conclusion:** Wood-apple fruit maturity occurred when the mature fruit exactly detached from the tree or called the fallen mature fruit at age 8.25-8.75 months after anthesis.

**Key words:** Fruit size, phenol, total soluble solids, total sugar content, wood-apple

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**Corresponding Author:** Endang Dewi Murrinie, Faculty of Agriculture, Muria Kudus University, Gondangmanis, P.O. Box 53 Bae, Kudus, Indonesia

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Wood-apple (*Feronia limonia* (L.) Swingle) (Syn. *Limonia acidissima* L.) is a fruit plant belongs to the family *Rutaceae*. Wood-apple plants have many uses, among other as a raw material of drinks and food, medicine, rootstock for citrus crops, as well as building materials<sup>1-7</sup>. It origins from Southern India then spreads to Southeast Asia, including Indonesia<sup>8</sup>. The wood-apple tree prefers a monsoon climate with a distinct dry season. The tree grows up to an elevation of 450 m in the Western Himalayas<sup>5</sup>. Wood-apple is a deciduous, slow-growing, erect tree with a few upward-reaching branches bending outward near the summit where they are subdivided into slender branchlets. The bark is ridged, fissured and scaly with the sharp spines of 2-5 cm long on some of the zig-zag twigs. The leaves alternate, 7.5-12.5 cm long, dark-green, leathery, often minutely toothed, blunt or notched at the apex, dotted with oil glands and slightly lemon-scented when crushed. Flowers look dull-red or greenish, to 1.25 cm wide, borne in small, loose, terminal or lateral panicles. The fruit is round, 5-12.5 cm wide, with a hard, woody, greyish-white, scurfy rind about 6 mm thick, pulp brown, mealy, odorous, resinous, astringent, acid or sweetish, with numerous small, white seeds scattered through it<sup>5</sup>.

Wood-apple fruit flesh tastes sweet slightly acidic with flavorful specialties, usually eaten directly or used as a syrup as found in Rembang, Central Java, Indonesia. Preliminary observation in Dasun Village, Rembang, Jawa Tengah, Indonesia showed that mature wood-apple fruits contain aromatic fruit flesh (75.9% of the fruit weight) with 1.17% protein, 22.36% carbohydrate, 0.05% fat, 1.35% ash, 14.5 mg of vitamin C, 13.1-16.6% sugar content and 87 kcal/100 g. While the study by Ghosh *et al.*<sup>9</sup> showed that wood-apple mature fruit contains 40.4-60.6% aromatic fruit flesh with 5.1-14.3% sugar content, 15.0-18.4% Brix total soluble solid, 1.7-4.6% acidity and 2.7-13.0 mg of vitamin C.

Preliminary observations indicated that growth and development of wood-apple fruit took approximately 8 months after anthesis. Nitsch<sup>10</sup> described the three stages of fruit growth, i.e. (1) pre-anthesis: Ovary growth, especially with the multiplication of cells, (2) Anthesis: Pollination and fertilization of the ovule, stimulate the growth of ovary, flowers are not fertilized fall or loss and (3) Post-fertilization: The size of the fruit increased, mainly occurs due to cell enlargement. During the growth and fruit development will occur mobilization and transport of the vegetative part to the development of fruits and seeds, resulting in morphological and physiological changes.

Unlike fruit in general, fruit maturity of wood-apple is not followed by a visual color change that can be used as an indicator of maturity. At the beginning of its formation, the fruit is green, then turns into a greyish white until reaching maturity and falling and makes it difficult to distinguish the maturity of the fruit by its color change. The research objective was to study the morphological and physiological changes during wood-apple fruit growth and development. Morphological changes observed were fresh weight (g), fruit diameter (cm), the thickness of the rind (mm), the weight of the rind (g) the weight of the fruit flesh and seeds (g). Whereas the physiological changes consist of a total sugar content, total soluble solids and phenolic content of fruit flesh. By knowing the morphological and physiological changes that occur during growth and development wood-apple fruit, an appropriate harvesting time with high quality can be determined.

## MATERIALS AND METHODS

The research was conducted in September, 2013-December, 2014 in the village of Dasun, District Lasem, Rembang, Central Java, Indonesia, which has an alluvial soil type with an elevation of 5 m above sea level<sup>11</sup>, an average daily temperature of 29.5°C with the maximum and minimum temperature of 33.7 and 26.1°C, respectively. The maximum and minimum relative humidity is of 75.7 and 43.2%. The relative humidity in September and October was lower than any other months, while the highest was in January. The annual rainfall during the study was of 1.180 mm. Based on the classification of the type of climate according to Schmidt and Ferguson, the location of the research belongs to type C climate<sup>12</sup>. The chemical analysis was conducted in the Laboratory of Seed Technology of the Faculty of Agricultural and Laboratory Chemistry and Food Biochemistry of the Faculty of Agricultural Technology, Gadjah Mada University.

The study was conducted by observing the growth and fruit development wood-apple, beginning from anthesis until the fruit mature. The experiment was administered in a completely randomized design (CRD) with nine variations of the age of the fruit, i.e. 1, 2, 3, 4, 5, 6, 7 and 8 months after anthesis (MAA) and mature fruit exactly detached from the tree, hereinafter called the fallen mature fruit. The number of samples for each of the age of 20 pieces. Morphological observation was made to (1) Fruit weight (g): By weighing each sample pieces using digital scales, (2) Fruit diameter (cm): measured using calipers, (3) The thickness of the rind (mm): measured using calipers, (4) The weight of the rind (g): Weigh

rind that has been cleaned of flesh using digital scales, (5) The weight of the fruit flesh together with the seeds (g): Separate the seeds and their flesh from the rind and then weighed with digital scales.

The observations of physiological changes were carried out on (1) The total sugar content of the fruit flesh was done using methods of Apriyantono *et al.*<sup>13</sup>, (2) The total soluble solids were determined using a hand refractometer, by crushing the fruit flesh wood-apple, then fluid from the flesh of the fruit were dropped on the surface of the hand refractometer prism and read the numbers on the scale of hand refractometer and (3) The phenolic content of fruit flesh were analyzed by Folin-Ciocalteu Method<sup>14</sup>.

**Statistical analysis:** The data were analyzed by one-way analysis of variance (ANOVA) and then to determine the differences among the treatments, Duncan's multiple range test (DMRT) with 5% significance level<sup>15</sup> was used. Software used to analyze the data is R version 3.1.1. by R Core Team, Vienna, Austria<sup>16</sup>.

## RESULTS AND DISCUSSION

Wood-apple fruit growth and development began with the flowering process. Flowers wood-apple formed compound inflorescences with a length of 8-20 mm. Wood-apple flower is a complete flower that consists of 4-6 pink sepals, 4-6 white petals with a red tip, 7-12 red anthers with white filament, green carpel and light green stigma. Thus wood-apple flower is bisexual, it is in accordance with statement of Orwa *et al.*<sup>5</sup> that its flowers are bisexual.

Wood-apple flowering process from flower bud initiation until anthesis took 20-26 days. After anthesis, pollination occurred and was followed by fertilization and subsequently formed fruit. Wood-apple fruit is round with a grayish white hard rind and white flesh fruit with many seeds in it. Wood-apple fruit flesh are usually consumed and processed after the post-harvest maturation fruit storage of 3-6 days. Mature fruit that had not been stored showed a hard and white flesh texture, then after stored texture it became soft and the color changed to brown and flavorful, showing that fruit ripening remained after separate from the plant. Based on these observations wood-apple was included in the group of climacteric fruit<sup>17</sup>.

The results showed that the growth and development of wood-apple fruit from anthesis until the mature fruit exactly detached from the tree or called the fallen mature fruit

takes  $238 \pm 7$  days after anthesis or 8.25-8.75 months after anthesis (MAA). Unlike fruits in general, the fruit maturity of wood-apple is not followed by a visual color change that can be used as an indicator of maturity. At the beginning of its formation, the fruit is green, then turns into a greyish white at 4 MAA. The rind color of wood-apple fruit did not change any longer after the wood-apples reach maximum size at age 6 MAA and up to mature fruit at age 8,25-8,75 MAA. Meanwhile the result of research by Ropiah<sup>18</sup>, on the mangosteen (*Garcinia mangostana* L.) showed changes in the color of the fruit skin during the growth and development of fruit. Mangosteen rind changes color from green to reddish brown, reddish purple and finally it became blackish purple when the fruit reaches maturity, i.e., at age 115-120 days after anthesis. A research by Ngitung and Bahri<sup>19</sup>, on Noni fruit (*Morinda citrifolia* L.) also showed the occurrence of color change during fruit development. Noni fruit was green at the time of formation up to the maximal fruit (14-18 weeks after the anthesis of the first interest), in the next 1-2 weeks it turned yellowish white and 1-2 weeks later turned into transparent white, that is, when the fruit reached its maturity. During the growth and development of fruit wood-apple experienced changes in morphology and physiology. Morphological changes as measured by the weight and diameter of the fruits showed an increase in line with the increasing age of the fruit. Growing fruit weight wood-apple formed a sigmoid curve (Fig. 1). It was slow at the age of 1-2 MAA, then entering a phase of rapid growth at 2-6 MAA and was then likely to stabilize at age 6 MAA until fallen mature fruit. Weight of the fruit from the age of 6 MAA until the fallen mature fruit was not significantly different, ranging from 249.94-264.43 g, showed that at the age of 6 MAA fruit weight of wood-apple already tended to be stable.

In line with the weight of fruit, observations of fruit diameter showed an increase in line with increasing age of fruit and reached a maximum at the age of 6 MAA (Table 1). As sinks, wood-apple fruit growth and development underwent the size increases in line with the age of the fruit. The increase in fruit size wood-apple that occurred during the process of growth and development of the fruit was caused by division and cell enlargement. According to Lodh and Pantastico<sup>20</sup>, the growth began in the form of cell division and enlargement, in which cell division is a major factor in the enlargement and continuing for the fruit on the tree. This is in line with the statement Hidayat<sup>21</sup>, that there are two processes that lead to an increase in the size of the fruit, i.e., cell division and enlargement, respectively. The cells divided themselves actively and enlargement of meristematic tissue took place. The process was supported by fotosintat supply from leaves as the source to the fruit that served as a sink.

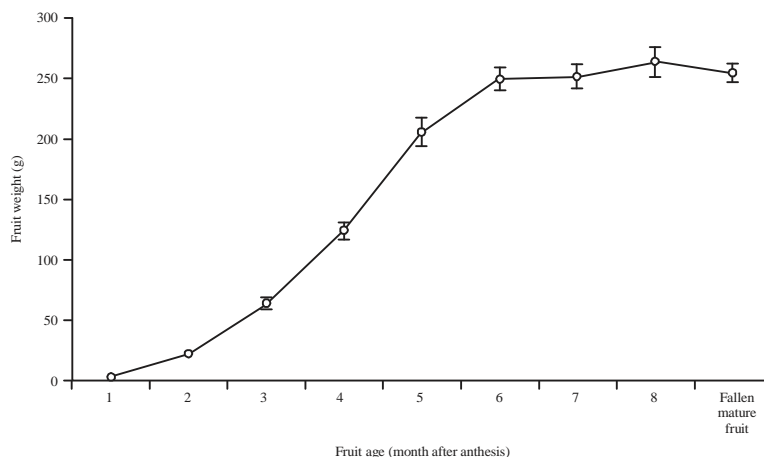


Fig. 1: Curve of wood-apple fruit weight at different ages  
Mean ± SEM

Table 1: Diameter, thickness and weight of the rind, flesh and seed weight and total sugar content of the fruit flesh of wood-apple fruit at different ages

Fruit age (month after anthesis)	Fruit diameter (cm)	Thickness of rind (mm)	Weight of rind (g)	Fruit flesh and seed weight(g)	Total sugar content of fruit flesh (%)
1	1.31 <sup>f</sup>	1.47 <sup>e</sup>	1.41 <sup>e</sup>	1.39 <sup>e</sup>	0.31 <sup>g</sup>
2	3.40 <sup>e</sup>	1.80 <sup>d</sup>	7.39 <sup>e</sup>	14.82 <sup>e</sup>	1.04 <sup>fg</sup>
3	4.98 <sup>d</sup>	2.11 <sup>c</sup>	17.12 <sup>d</sup>	46.72 <sup>d</sup>	1.93 <sup>f</sup>
4	6.39 <sup>c</sup>	2.23 <sup>c</sup>	30.73 <sup>c</sup>	93.47 <sup>c</sup>	2.09 <sup>f</sup>
5	7.42 <sup>b</sup>	2.38 <sup>b</sup>	46.56 <sup>b</sup>	159.63 <sup>b</sup>	4.32 <sup>e</sup>
6	7.91 <sup>a</sup>	2.56 <sup>a</sup>	58.92 <sup>a</sup>	191.01 <sup>ab</sup>	6.48 <sup>d</sup>
7	8.03 <sup>a</sup>	2.65 <sup>a</sup>	61.09 <sup>a</sup>	190.97 <sup>ab</sup>	8.17 <sup>c</sup>
8	8.05 <sup>a</sup>	2.67 <sup>a</sup>	60.78 <sup>a</sup>	203.65 <sup>a</sup>	10.44 <sup>b</sup>
Fallen mature fruit	8.05 <sup>a</sup>	2.68 <sup>a</sup>	59.00 <sup>a</sup>	190.43 <sup>ab</sup>	13.79 <sup>a</sup>

\*Number in the same column followed by the same letter are not significantly different shows based DMRT 5%

Along with the increase in the size of the fresh fruit, the rind thickness and weight improved and tended to remain from the age of 6 MAA. Similarly, the weight of the fruit flesh and seeds also increased and reached a maximum at the age of 6 months (Table 1). Rind weight ratio with the weight of the fruit at the beginning of high growth, then declined and relatively fixed after a 4 month old fruit, in contrast with fruit flesh and seeds weight ratio and the weight of fruit to be increasing and relatively fixed at the age of 4 months (Fig. 2).

Fruit growth required sufficient amount of nutrients, causing the mobilization and transportation from the vegetative part to the development of fruits and seeds. According Lodh and Pantastico<sup>20</sup>, the weight of the fruit flesh and rind increased during the growth and development of the fruit. The weight of the fruit flesh at the beginning of the development of the fruit was very low, while the rind was very high.

Observation of the total sugar content of the fruit flesh wood-apple showed an increase in line with increasing age and reached a maximum when the ripe fruit lost (Table 1). The increase in total sugar content of fruit wood-apple was due to

decomposition of starch into glucose, moreover according to Pantastico<sup>17</sup> pectin and cellulose are carbohydrate reserves which can also serve as a potential source for the formation of sugar. Furthermore Muchtadi *et al.*<sup>22</sup> stated that in case of hydrolysis of starch, glucose will be formed so that the sugar in fruit will increase. Starch is the main carbohydrate that is stored on most plants. In the storage organs such as fruit, carbohydrates accumulate in amiloplast formed as the result of translocation of sucrose or other carbohydrates from leaves. The formation of starch mainly occurs through a process involving the contribution of recurring units of glucose from sugar nucleotide, i.e., adenosine diphosphoglucose (ADPG). Formation of ADPG takes place using ATP and glucose-1-phosphate dichloroplast.

Research by Ropiah<sup>18</sup>, on the mangosteen fruit showed total sugar content of fruit increased significantly until to the age of 105 days after anthesis (DAA) and then tend to remain until the age of 115 DAA which showed that the total sugar content of the mangosteen fruit is stable at the age of 105 DAA, the stability of the total sugar content can be used as a guide determine the maturity level of the mangosteen fruit.

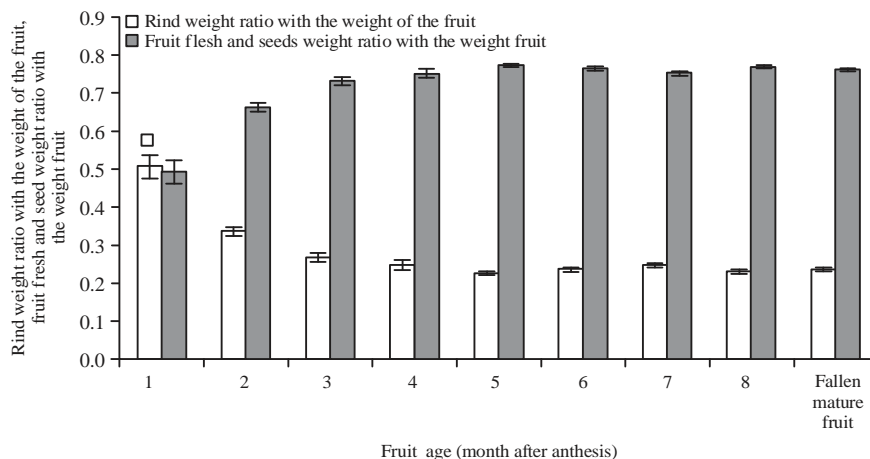


Fig. 2: Curve of ratio rind weight and flesh fruit weight with the weight of wood-apple fruit at different ages  
Mean ± SEM

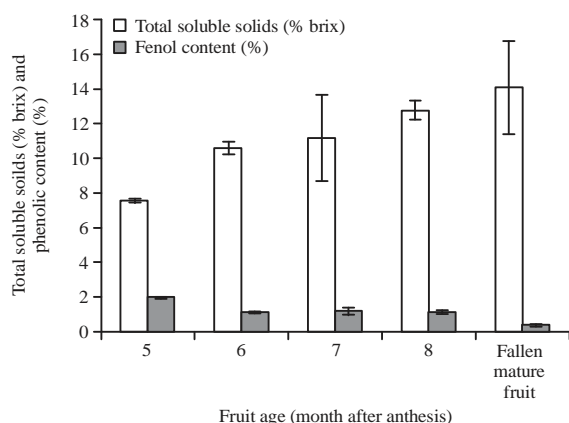


Fig. 3: Total soluble solids and phenolic content of wood-apple at different age fruit  
Mean ± SEM

Increasing the sugar content of the fruit flesh also followed by increased total soluble solids content and reaches a maximum at the fallen mature fruit i.e., 4.12% Brix (Fig. 3). Increased total soluble solids along with the increasing age of the fruit is caused by the breakdown of complex materials such as carbohydrates, proteins and fats into sucrose, glucose and fructose<sup>18</sup>. Suketi<sup>23</sup>, stated that sugar is the main component of soluble solids. During the maturation process, total soluble solids fruit increased due to a breakdown of carbohydrate polymers, in particular starch changes to sugar so that the sugar content as a component of dissolved solids in the fruit flesh generally increases.

Total soluble solids the fruit flesh can be used as an indicator of fruit maturity. Research by Suketi<sup>23</sup>, showed that the total soluble solids content of papaya genotypes IPB 10A

females and hermaphrodites, PB 174 female genotypes, genotypes 1 x IPB IPB 10A females, IPB genotype 1 x PB 174 females influenced by fruit maturity stage. Furthermore, Chan<sup>24</sup>, stated that the total soluble solids content is used as an indicator of the quality of fruit and papaya fruit maturity. According Suketi *et al.*<sup>25</sup>, the chemical character from the fruit affecting the level of preference is the total soluble solids content of fruit.

The increased sugar content and total dissolved solids in the fruit flesh with the increasing age was followed by a decrease in phenol content (Fig. 3). Lowest phenol content was obtained from fallen mature fruit, i.e., 0.364%, it is emphasized that wood-apple fruit maturity occurs when the mature fruit exactly detached from the tree or called the fallen mature fruit.

Sugar content and total soluble solids which were significantly highest and phenol content fruit flesh which were significantly lowest in fallen mature fruit showed that the highest fruit quality and fruit maturity wood-apple occurred in fallen mature fruit. This is in accordance with the statement of Matto *et al.*<sup>26</sup>, that in the process of fruit maturation there is an increase of simple sugars that gives and a sweet taste, a decrease in organic acids and phenolic compounds that reduces astringent and sour flavor and the increase of volatile substances give the specific aroma of the fruit.

## CONCLUSION

Growth and development of wood-apple fruit from anthesis until the mature fruit exactly detached from the tree or called the fallen mature fruit took 238 ± 7 days after anthesis or 8.25-8.75 months after anthesis (MAA). Fruit size

increased during growth and fruit development, from anthesis until 6 months and did not increase any longer until the fallen mature fruit. Total sugar content and total soluble solids of fruit flesh increased in line with increasing age of the fruit and reached a maximum when fallen mature fruit. In contrast phenol content decreased with increasing age of the fruit and reached a minimum at the time of fallen mature fruit. Wood-apple fruit maturity occurred when the mature fruit exactly detached from the tree or called the fallen mature fruit.

### SIGNIFICANCE STATEMENTS

This study discovers the following. Firstly, growth and development of wood-apple fruit from anthesis until the mature fruit exactly detached from the tree or called the fallen mature fruit. Secondly, fruit size increased during growth and fruit development. Finally, total sugar content and total soluble solids of fruit flesh increased in line with increasing age of the fruit and reached a maximum when fallen mature fruit. The findings can be beneficial for researchers and farmers to provide the best quality fruits by providing information on accurate harvesting time. This study help the researches to uncover the critical areas of fruit maturity that many researchers were not able to explore. Through this research it was found that wood-apple fruit maturity occurred when the mature fruit exactly detached from the tree or called the fallen mature fruit. Thus a new theory on fruit maturity of wood-apple may be arrived at.

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