Effect of Postharvest Maturation Storage at Different Age Fruit on Chemical Characters Fruit and Seed of Wood-apple (Feronia limonia (L.) Swingle)

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Abstract. Wood-apple fruits generally processed and consumed are those of the fallen mature fruits which are then stored in postharvest ripening for 3 to 6 days. Seeds are obtained from the waste of processed wood-apple fruits. The seeds are not directly extracted but they had experienced a postharvest maturation storage prior to planting. During postharvest maturation storage, there are changes in the chemical characteristics of wood-apple fruit flesh and seeds. The research objective was to determine the effect of postharvest maturation storage on the different fruit ages on the chemical character of fruit and seed of wood-apples. The results showed that postharvest maturation storage did not affect the total sugar content of the fruit, but it affected the content of tannins and phenols, meanwhile fruit age affected the total sugar content, tannin and phenol fruit. Postharvest maturation storage did not affect the dry weight, moisture content and protein content of the seed, but it affected electrical conductivity and respiration rate of seeds. Meanwhile fruit age affected the dry weight, moisture content, protein content, electrical conductivity and the respiration rate of seeds.

1. Introduction
Wood-apple (Feronia limonia (L.) Swingle) is a fruit plant of the Rutaceae family with many benefits. Wood-apple fruit flesh tastes sweet slightly acidic, aromatic, generally eaten directly or made syrup. Research by Joshi & Jain reported that wood-apple mature fruit contained 70% of aromatic pulp-containing protein (2.2%), carbohydrates (22.1%), fat (3.3%), ash (1.3%) and produce 127 kcal /100 g flesh fruit with vitamin C (15.9 mg), Ca (3.5 mg), Fe (8.5 mg), Na (46.5 mg), P (386.3 mg), Zn (0.8 mg), Cu (0.5 mg), Mn (0.7 mg), total soluble solids from 15.0 to 18.40 Brix, acidity of 1.7 to 4.6%, and total sugar content of 5.1 to 14.3% [1].

Wood-apple fruits generally processed and consumed are those of the fallen mature fruits which are stored in a postharvest maturation for 3 to 6 days. The results of preliminary observations showed that during the postharvest maturation storage, there was a change in flesh color and texture from white and hard into brown and soft one with unique smell indicated that during postharvest maturation storage, there was an increased maturity level. Based on these observations, wood-apple is classified into a climacteric fruit, because ripening process keep taking place after it is separated from the tree. Fantastico [2] stated that, based on changes in respiration rate, fruits are divided into two groups, i.e. climacteric and non climacteric. A climacteric fruit shows a clear increase in respiration activity during cell maturation.
stage, whereas non climateric fruit does not have a pattern like fruit climateric respiration and it is only able to be ripe when the fruit is still on the tree.

Wood-apple seeds that have been planted are those from waste of processed wood-apples. The seeds are not directly extracted after the fruits are ripe and then fell out of the tree. They experienced a postharvest maturation storage before planting. Zanzibar et al. stated that postharvest maturation storage is one activity that can be done in temporary storage or in a seed conservation period [3]. According to Schmidt [4], temporary storage or conservation period is intended to condition the seed to achieve an optimum potential prior to further handling. Sadjad [5] stated that the period of conservation is a temporary storage period before the seed is stored, during harvesting and processing or after the storage period before planting. This period is usually short or in the period of seed during transport, waiting for further processing and the time of planting.

Murniati et al. [6] suggested that the postharvest maturation storage fruit is similar to priming treatment on the seeds inside fruit since in her research of papaya, fruits harvested with 30-40% of yellow color followed by postharvest maturation storage for 4 days significantly have maximum growth potential, germination, rate of germination and first count is increased and the same as the control, the fruits harvested with 80-90% of yellow color. While Sanchez et al. [7] found that there were significant differences between seed peppers which are kept dry with the seeds stored in the fruit harvested before physiological maturity (30 days after anthesis), so it is thought that in situ priming on the seed occurred in the fruits during postharvest.

During postharvest maturation storage it is thought that changes which affect the seeds occur. The aim of the study was to investigate changes in the chemical characteristics of wood-apple fruit and seed during postharvest maturation storage at the different age of fruits.

2. Materials and methods
The research was conducted from March 2014 to January 2015 in Laboratory of Seed Technology, Laboratory of Plant Science and Greenhouse of Agriculture Faculty and Laboratory of Food Chemistry and Biochemistry of Agricultural Technology Faculty, Gadjah Mada University (UGM). The wood-apples fruit used comes from Dasun Village, District Lasem, Rembang, Central Java, Indonesia.

The study was conducted on wood-apple fruit from the age of 6 months after anthesis until mature fruit exactly detached from the tree. The experimental design used was a completely randomized design (CRD) of two factors, i.e. the duration of postharvest maturation storage and fruit age. The duration of postharvest maturation storage consists of three levels, i.e. 0 days of postharvest maturation storage (DPM), 3 DPM and 6 DPM. Fruit age consists of 4 levels, i.e. 6 months after anthesis (MAA), 7 MAA, 8 MAA and mature fruit exactly detached from the tree. Each combination of treatment was repeated three times. Postharvest maturation storage was conducted at room temperature (27-28 ºC), at the end of postharvest maturation storage seeds were extracted from each fruit by washing it with water until clean and air drying for 12 hours [8].

Chemical characteristic of fruit was observed for the total sugar content by using Anthrone Method [9]. Tannins content was observed by using Permanganometry Method [10] and phenol content by using the Folin-Ciocalteu Method [11].

Seed observation consists of moisture content, dry weight, electrical conductivity, respiration rate and seed protein content. Seed moisture content was measured by the weight of the seeds after 12 hours drying with oven method at 105 °C for 16 hours [12]. Seed dry weight (g) was measured by weighing seeds that have been put into oven at 60 °C for 3 x 24 hours, a total of 25 seeds of each fruit age. Electrical conductivity (mS) was measured by soaking as many as 25 seeds in 100 ml aquades for 20 hours at room temperature. The electrical conductivity of the seed soaking water was measured by using the conductivity meter HI 8819. Seed respiration rate (mg CO2/g seed/h) was measured by using Titration Methods, while the seed protein content is measured by the Kjeldahl method [13].

Data were analyzed by using analysis of variance and then to determine the differences among the treatments, Duncan's Multiple Range Test (DMRT) of 5% [14] is used. The software used to analyze the data was the R version 3.1.1.
3. Results and Discussion

3.1. Effect of Postharvest Maturation Storage at Different Age fruit on Chemical Characters Wood-apple Fruit

The results showed that postharvest maturation storage did not affect the total sugar content, but affected the content of tannins and phenol of the fruit. There was no interaction between postharvest maturation storage and age on total sugar content of the fruit. While between postharvest maturation storage and tannin content showed an interaction, as well the phenol content of fruit (Table 1).

Table 1. Effect of postharvest maturation storage at different ages fruit on total sugar content of wood-apple fruit

| Fruit age (months after anthesis/MAA) | Total sugar content (%) | Mean   |
|--------------------------------------|-------------------------|--------|
|                                      | 0 days of postharvest    | 3 days of postharvest | 6 days of postharvest |          |
|                                      | maturation storage      | maturation storage  | maturation storage  |          |
| 6                                    | 6.475a                  | 8.148   | 7.863   | 7.495 c   |
| 7                                    | 8.173                   | 7.170   | 9.243   | 8.195 c   |
| 8                                    | 10.435                  | 11.338  | 11.493  | 11.089 b  |
| Fallen mature fruit                  | 13.786                  | 12.788  | 13.393  | 13.322 a  |
| Mean                                 | 9.717 p                 | 9.861 p  | 10.498 p | (-) b     |

*aNumber followed by same letter on row or column indicates no significant differences with DMRT 5%
*bIndicates no interactions between the duration of postharvest maturation storage and fruit age

Total sugar content of fruit increased significantly with increasing age and reached a maximum when mature fruit exactly detached from the tree or called fallen mature fruit at the age of from 8.25 to 8.75 months after anthesis (MAA). This was in line with research by Murrinie et al. [15] which found that the total sugar content of the fruit flesh wood-apple increased in line with increasing age and reached a maximum when the ripe fruit exactly detached from the tree. Ropiah [16] in her research on mangosteen found that increased levels of total sugar content was in line with the increasing age of the fruit. Total sugar content of the mangosteen fruit has risen sharply since the first observations at the age of 90 days after anthesis (DAA) to the last observation on the age of 115 DAA. Forazi cit. Maryayah et al. [17] said that during the development of the fruit until harvest, total sugar content would increase. The increase in total sugar content of which coincided with the increasing age of the fruit which was caused by the hydrolysis of starch into maltose and hydrolysis of the disaccharide (maltose and sucrose) into glucose and fructose. Starch is the main carbohydrate that is stored in most plants. With the increasing age of the fruit, the starch content in the fruit as sinks will also increase. Nagy & Shaw cit. Ropiah [16] states that the hydrolysis of starch and disaccharides lead to an increase in the sugar content of bananas from 1% to 20%. Maryayah et al. [17] found that during the ripening process of bananas, the carbohydrate content decreased from 34-35% to 14-16%, while the sugar content increased from 1-2% to around 18-19%.

Postharvest maturation storage did not affect the total sugar content of fruit. This was due on the fruit had been separated from the plant, either manually harvested or already mature and then fell. The supply assimilate towards fruit stops, including the supply of starch as the main carbohydrate forming sugar content. Therefore, the sugar content did not change even though the fruit has postharvest maturation stored.

Besides the total sugar content in fruit development, there were also changes in other chemical properties such as tannins and phenols. Observations showed that the fruit postharvest maturation storage and the age of fruit affected tannin content of wood-apple fruit (Table 2).
Table 2. Effect of postharvest maturation storage at different ages fruit on tannin content of wood-apple fruit.

| Fruit age (months after anthesis/MAA) | Tannin content (%) |
|---------------------------------------|---------------------|
|                                       | 0 days of postharvest maturation storage | 3 days of postharvest maturation storage | 6 days of postharvest maturation storage | Mean |
| 6                                     | 2.577 e            | 2.011 cd          | 1.875 c            | 2.154 |
| 7                                     | 2.370 de          | 2.006 cd          | 1.055 b            | 1.810 |
| 8                                     | 2.127 cd          | 1.967 c           | 0.791 b            | 1.628 |
| Fallen mature fruit                   | 0.272 a           | 0.204 a           | 0.190 a            | 0.222 |
| Mean                                  | 1.837             | 1.547             | 0.978 (+)          |       |

*Number followed by same letter on row and column indicates no significant differences with DMRT 5%
*Indicates an interactions between the duration of postharvest maturation storage and fruit age

Tannin content decreased with increasing age of the fruit and the duration of postharvest maturation storage. The highest tannin content obtained in the fruit ages of 6 and 7 MAA without postharvest maturation storage and lowest in fallen mature fruit either without or with postharvest maturation storage. In the fruit ages of 6, 7 and 8 MAA, postharvest maturation storage caused a decrease in tannin content of the fruit flesh, while on the fallen mature fruit postharvest maturation storage did not cause a decrease in tannin content, because tannin contain on fallen mature fruit had reached the minimum. According to Raharjo [18] tannins were present in large amounts in the leaves, stems and immature fruit.

Tannins are secondary metabolites that are known to have several benefits including astringent, anti-diarrhea, anti-bacterial and antioxidant. Tannins give astringency on the fruit. Tannin content which decreases with increasing age of the plant is in accordance with the statement of Amelia [10] that the young fruit contains tannin higher than the old one. Pansera *cit. Zahroyana* [19] also stated that the tannin content reaches a maximum when the fruit is young or during growth and development and decreased during fruit ripening. The decrease in tannin content of the fruit is most likely due to the presence of tannins degraded or tannin present in fruits that are not able to precipitate the protein any longer [20].

Postharvest maturation storage and age fruit also affected the phenol content of wood-apple fruit. Phenol content decreased with increasing age of the fruit and the length of postharvest maturation storage. Table 3 shows that the lowest phenol content was found in fallen mature fruit. According to Matto *et al.* [21] in the process of fruit maturity there was an increase of simple sugars that presented a sweet taste, a decrease in organic acids and phenolic compounds that reduced astringency and sourness, and the increase of volatile substances that gave flavor to the fruit. Robards *et al.* *cit.* Dungir *et al.* [22] stated that the total phenol content could be produced from a number of simple molecules such as phenolic compounds up to complex molecules such as tannins. The observations showed a decrease tannin content was in line with the increasing age of the plant and increasing the duration of postharvest maturation storage (Table 2), thus the phenol content also decreased. Research findings by Putri *et al.* [23] showed that the total phenol content of fruit roselle purple sepals was five times higher than the red sepals due to the high content of tannins in fruit purple sepals.

Table 3. Effect of postharvest maturation storage at different ages fruit on phenol content of wood-apple fruit.

| Phenol content (%) | Mean |
|--------------------|------|
Fruit age (months after anthesis/MAA)  | 0 days of postharvest maturation storage | 3 days of postharvest maturation storage | 6 days of postharvest maturation storage |
--- | --- | --- | ---
6 | 1.760 d | 1.146 c | 0.698 b | 1.201 |
7 | 2.351 e | 1.214 c | 0.353 a | 1.306 |
8 | 1.684 d | 1.129 c | 0.349 a | 1.054 |
Fallen mature fruit | 0.324 a | 0.364 a | 0.282 a | 0.323 |
Mean | 1.530 | 0.963 | 0.421 (+) |

*Number followed by same letter on row and column indicates no significant differences with DMRT 5%*  
*Indicates an interactions between the duration of postharvest maturation storage and fruit age*

3.2. Effect of Postharvest Maturation Storage at Different Age fruit on Dry Weight and Chemical Characters Wood-apple Seed

Dry weight, moisture content and protein content of the seed were not affected by postharvest maturation storage, but are influenced by the age of fruit (Table 4). Seed dry weight increased with increasing age of fruit and reached a maximum at the age of 8 MAA, and tend to remain until fallen mature fruit. This is in line with research conducted by Murrinie et al. [24] on wood-apple which shows the dry weight of the seeds increased and reached a maximum at the age of 8 MAA.

Seed protein content also showed the same trend as the dry weight of the seed, but still increased until fallen mature fruit. Seeds were part of the fruit that also acts as a sink, an increase in dry weight showed a growth in the seeds because of the division and cell enlargement due to the flow of assimilate into the seed to be stored and synthesized into a new compound.

Table 4. Effect of postharvest maturation storage at different fruit ages on dry weight, moisture content and protein content of wood-apple seed.

| Treatment | Seed dry weight (g) | Seed moisture content (%) | Seed protein content (%) |
|-----------|---------------------|--------------------------|-------------------------|
| Duration of postharvest maturation storage | | | |
| 0 day | 0.0266 p | 23.38 p | 21.41 p |
| 3 days | 0.0261 p | 23.51 p | 21.32 p |
| 6 days | 0.0263 p | 23.15 p | 21.59 p |
| Fruit age (months after anthesis) | | | |
| 6 | 0.0206 c | 38.07 c | 21.01 b |
| 7 | 0.0238 b | 25.90 b | 20.91 b |
| 8 | 0.0306 a | 16.36 a | 21.72 ab |
| Fallen mature fruit | 0.0303 a | 13.05 a | 22.12 a |

*Number followed by same letter on same column indicates no significant differences with DMRT 5%*  
*Indicates no interactions between the duration of postharvest maturation storage and fruit age*

In contrast to the dry weight of the seed, seed moisture content decreased with increasing the age of fruit. The minimum seed moisture content was achieved when the mature fruit falls, although it is not significantly different from the seed of fruit 8 MAA. This suggested that the process of accumulation of assimilates in the seeds had stopped at the age of 8 MAA, then the seed entered the drying phase until mature fruit exactly detached from the tree or called the fallen mature fruit (Figure 1).
The dry weight and moisture content suggested that the seeds began to enter a period of maturation at 8 MAA and continued until it reached physiological maturity at the time of mature fruit exactly detached from the tree or called the fallen mature fruit at age 8.25 to 8.75 MAA. Research by Blay et al. [25] in two cultivars of Solanum Gilo indicated that the 1000 grain weight of seed increased with increasing levels of maturity. Furthermore Tresniawati et al. [26] suggested that physiological maturity seed Kemiri Sunan of Garut provenances achieved at 28 weeks after flowering with morphological criteria of brownish green fruit color, soft fruit leather, brown seed coat with the dry weight of the maximum and minimum moisture content.

Byrd [27] suggested that seed maturity generally occurred along with fruit maturity. He also argued that after physiological maturity is reached, the translocation of nutrients that was stored into the seeds stopped, so it did not happen again and increased the size of the growth process, in other words the attributes of seeds had reached the maximum. It was estimated that after the kawista fruit reached the age of 8 MAA, the flow of water and assimilates through peduncle and pedicel stopped and the tissues were disconnected. This was in line with the statement of Schmidt [4] that when the seeds and fruit are mature, the water and the assimilate flow through the peduncle and pedicel into the fruit, and then to the seed through funicle would stop and the tissues were disconnected. Simultaneously the abscission area grows and seed or fruit finally detached from the tree.

Postharvest maturation storage did not affect dry weight, moisture content and protein content of wood-apple seed because after being separated from the plant, it had no longer supplied the assimilate into fruit and finally to the seeds, as well as the synthesis of food reserves in the seed did not occur again. This was in line with research by Sanchez et al. [7] which found that postharvest storage on pepper had no effect on the dry weight of the seed. Similarly, moisture content the seed was not affected by postharvest maturation storage since wood-apple seeds enclosed in fruit flesh surrounded rind very hard so it did not change moisture content of the seeds.

Observation of the electrical conductivity of seeds showed that there were interactions between postharvest maturation storage and fruit age. Electrical conductivity of seed decreased with increasing age of the fruit and the duration of postharvest maturation storage (Table 5). Postharvest maturation storage at fruit age of 6, 7 and 8 MAA caused a decrease in electrical conductivity values, indicating that postharvest maturation storage reduces cell membrane damage of seeds from fruits that had not reached full maturity.
Table 5. Effect of postharvest maturation storage at different ages fruit on electrical conductivity of wood-apple seed.

| Fruit age (months after anthesis/MAA) | Electrical conductivity (mS) | Mean |
|---------------------------------------|-------------------------------|------|
|                                       | 0 days of postharvest maturation storage | 3 days of postharvest maturation storage | 6 days of postharvest maturation storage |
| 6                                     | 18.48 f<sup>a</sup>           | 6.96 e            | 2.06 b           | 9.17   |
| 7                                     | 6.58 e                         | 3.87 d            | 1.68 ab           | 4.04   |
| 8                                     | 3.80 d                         | 2.80 e            | 1.42 a            | 2.67   |
| Fallen mature fruit                   | 1.16 a                         | 1.23 a            | 1.22 a            | 1.20   |
| Mean                                  | 7.51                           | 3.72              | 1.60              | (+)<sup>b</sup> |

<sup>a</sup> Number followed by same letter on row and column indicates no significant differences with DMRT 5%

<sup>b</sup> Indicates an interaction between the duration of postharvest maturation storage and fruit age

Postharvest maturation storage did not affect the electrical conductivity in seeds from mature fruit exactly detached from the tree or called the fallen mature fruit, because the seed had reached physiological maturity so that the cell membrane had been fully formed. Adjisir [28] found that cocoa seeds harvested young showed a rudimentary formation of cell membranes, while the seeds harvested too ripe would experience greater metabolic leakage due to membrane damage was greater.

Observation of the respiration rate of seeds obtained an interaction between postharvest maturation storage with age fruit (Table 6). It appeared that the increased age and increased duration of postharvest maturation storage causes a decrease in respiration rate. Respiration in seeds influenced by external factors such as temperature, humidity and oxygen as well as internal factors such as moisture content and food reserves in the seed. High seed moisture content would increase the respiration rate of seeds. According to Tatipata [29] seed moisture content level had an important role in seed storage due to moisture content was associated with seed respiration rate. High seed moisture content plays a role in activating an enzyme in the process of decomposition of food reserves, such as protease enzymes that broke down proteins, lipase enzymes that broke down fat and amylase enzymes that broke down carbohydrates. Food reserves in the seed were broken down into simpler compounds through the process of respiration [30]. Sutopo [12] also stated that increasing the moisture content the seeds with increased enzyme activity would accelerate the process of respiration.

Table 6. Effect of postharvest maturation storage at different ages fruit on respiration rate of wood-apple seed.

| Fruit age (months after anthesis/MAA) | Electrical conductivity (mS) | Mean |
|---------------------------------------|-------------------------------|------|
|                                       | 0 days of postharvest maturation storage | 3 days of postharvest maturation storage | 6 days of postharvest maturation storage |
| 6                                     | 1.09 d<sup>a</sup>           | 1.82 b            | 1.61 c           | 1.507   |
| 7                                     | 2.00 a                         | 2.01 a           | 1.10 d           | 1.703   |
| 8                                     | 0.09 e                         | 0.08 e           | 0.07 e           | 0.080   |
| Fallen mature fruit                   | 0.05 e                         | 0.05 e           | 0.06 e           | 0.053   |
| Mean                                  | 0.990                          | 0.938            | 0.580            | (+)<sup>b</sup> |

<sup>a</sup> Number followed by same letter on row and column indicates no significant differences with DMRT 5%

<sup>b</sup> Indicates an interactions between the duration of postharvest maturation storage and fruit age
Observation of the seed moisture content wood-apple in Table 4 show that it decreased with the increasing age of the fruit. The seeds of the young fruit had a higher moisture content, so that the higher respiration rate. At the age of 6 and 7 MAA, the longer the postharvest maturation storage, the respiration process would continue at a decreasing rate as a result of reduced substrates food reserves in the seed. On 8 MAA fruit and fallen mature fruit, moisture content had reached the minimum because the seed has undergone a drying phase, so it did not affect the food enzyme decomposer reserves in the seed. Therefore, postharvest maturation storage does not affect the respiration rate of seeds in the fruit 8 MAA and fallen mature fruit.

4. Conclusion
Postharvest maturation storage did not affect the total sugar content, but affected the content of tannins and phenolic of wood-apple fruit, meanwhile age fruit affected the total sugar content, tannin and phenolic of fruit. Total sugar content of fruit increased with increasing age and reaches a maximum (13.3%) at the time of mature fruit exactly detached from the tree or called the fallen mature fruit at the age of from 8.25 to 8.75 months after anthesis (MAA). Tannin and phenol content of fruit flesh decreased with increasing age of the fruit and the longer the duration of postharvest maturation storage. Postharvest maturation storage reduced the content of tannins and phenols fruit flesh aged 6, 7 and 8 MAA, but it did not affect the fallen mature fruit, because the content of tannins and phenols had reached a minimum when the mature fruit was exactly detached from the tree, respectively 0.27% and 0.32%.

Postharvest maturation storage did not affect dry weight, moisture content and protein content of the seed, but affected the electrical conductivity and the respiration rate of seeds. While age fruit affects the seed dry weight, moisture content, protein content, electrical conductivity and the respiration rate of seeds. Dry weight of the seeds increased in line with increasing age and the fruit had reached the maximum from 8 MAA which is 0.0306 g. Seed moisture content decreased with increasing age and reaches a minimum when the mature fruit has fallen, that was 13.05%. Seed protein content increased along with increasing age and reached a maximum in the fallen mature fruit that is 22.12%. Electrical conductivity and seed respiration rate decreased in line with increasing age of the fruit and the length of postharvest maturation storage fruit. Seed from the fallen mature fruit showed the lowest rate of electrical conductivity and the respiration rate.

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