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Gallic acid content in sapodilla fruit and seed (*Manilkara zapota*) and the correlation with germination control in recalcitrant seed

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Abstract. *Putri KF, Solichatun, Pitoyo A. 2021. Gallic acid content in sapodilla fruit and seed (*Manilkara zapota*) and the correlation with germination control in recalcitrant seed. Cell Biol Dev 5: 7-16.* Sawo or sapodilla (*Manilkara zapota* (L.) P. Royen) is a tropical fruit with many benefits. Sapodilla seed is classified as a recalcitrant but has a low storage capacity. The storability of seeds is influenced by moisture content and accumulation of germination inhibitor compounds present in the seeds. One of the inhibitor compounds is gallic acid. This study aims to determine the content of gallic acid in sapodilla fruit and seeds and its relation to the control of seed germination. There were two parts to the research; the first was to assess the level of gallic acid at different stages of sapodilla fruit development. The second part examined the effect of sapodilla seeds' ripening and storing period on gallic acid content and seed viability. The data were taken from the morphological characteristics of sapodilla fruit and seeds during the development period. The preparation of seed anatomy, measurement of seed moisture content, tetrazolium test, germination test, and analysis of gallic acid content in sapodilla fruit and seeds were also carried out. The content of gallic acid was measured using UV-vis spectrophotometry. Data were analyzed with the One Way ANOVA test, and if there was a significant difference, the DMRT test (Duncan Multiple Range Test) was continued with a test level of 5%. The Independent Sample T-Test analyzed data from seed germination and viability tests. This research revealed that the fruit parts, seed coats, cotyledons, and sapodilla seed embryos all contain gallic acid inhibitor compounds. The highest gallic acid content in sapodilla seeds was in the fruit, while the lowest was in the cotyledons and embryos. The gallic acid content in sapodilla fruit and seeds decreased during the development process and storage treatment. The seed storage treatment decreased the germination percentage of sapodilla seeds. That indicates that gallic acid plays a role in controlling sapodilla seed germination.

Keywords: Fruit, gallic acid, germination inhibitor, recalcitrant seed, sapodilla

INTRODUCTION

Sapodilla (*Manilkara zapota* (L.) P. Royen) is a famous Indonesian tropical fruit from the Sapotaceae family and is included in the group of eleven superior national fruits (Trubus Online 2017). Apart from being consumed as fresh fruit, sapodilla has other benefits, namely as a source of antioxidants and a source of biodiesel. Based on the research conducted by Chanda and Nagani (2010), sapodilla leaf acetone extract has a fairly high antioxidant content (IC50 = 140 µg / mL). Another study by Shui et al. (2004) compared the antioxidant content of raw sapodilla fruit, and after storing treatment, the most antioxidant content was found in raw sapodilla fruit. Sapodilla seeds have an oil content of 23-3. The triglyceride content in sapodilla seed oil can be processed into a source of biodiesel (Kumar et al., 2015).

Sapodilla fruit is classified as a single true fruit; it means the fruit developed from the ovaries. Single fruit also means the fruit is formed from one flower with one ovary (Tjitrosomo 1983). It is short-stemmed fruit, round to ovate or elliptical, and reddish-brown to yellowish. Sapodilla fruit has thin skin and soft flesh with a reddish-brown to yellowish color. Sapodilla fruit taste is generally sweet and watery. Up to 12 seeds can be found in one fruit, but most are less than 6. Sapodilla seeds are oval, flat, black, or shiny brown, about 2 cm long, and the chip seeds (cotyledons) are waxy white (Morton 1987).

Sapodilla cultivation can be done in the generative method with seeds and the vegetative method with grafts (Srivastava et al., 2017). Sapodilla propagation by seed takes 30 days for seeds to germinate; it generally contains more than 1 seed of varying sizes, and the best seeds are the largest. Sapodilla seeds naturally germinate in 4-6 weeks, and the germination success reaches 80% with the epigeal type. Sapodilla is a slow-growing tree species. Sapodilla trees that come from seed need 5-8 years after planting for the plants to start reproducing. Vegetative propagation of sapodilla takes 2-4 years after planting to start reproducing (Morton 1987). The generative propagation of sapodilla, even though it is slow to bear fruit, has advantages, namely the ability to bear longer fruit and stronger roots compared to vegetative propagation. However, seed generative seed propagation faces problems regarding seed availability.

Based on the physiological responses of seeds during storage, seeds were categorized into orthodox, intermediate, and recalcitrant seeds (Hong et al. 1998). Orthodox seeds are seeds that can be stored for a long time and can remain viable with a moisture content of below 10%. In addition, orthodox seeds can be stored at low temperatures and humidity. On the other hand, recalcitrant

seeds cannot be stored for a long time, cannot stand or die if stored at cold temperatures, and cannot stand being stored when their water content drops below the critical water content (Hasanah and Rusmin 2006). Recalcitrant seeds are interesting to be studied due to their physiological behavior, which has not been fully understood yet (Fu et al. 1993).

Recalcitrant seeds have relatively high water content but cannot survive an intensive drying process. Recalcitrant seeds usually come from tropical or subtropical tree species. Recalcitrant seeds can only be stored in a humid medium with a relatively warm temperature to avoid desiccation damage because most seeds are sensitive to cold temperatures (chilling) (King and Roberts 1979). Most recalcitrant seeds are covered with a fleshy, watery pulp structure and impermeable seed coat. This structure keeps the seeds in a humid environment. Recalcitrant seed preserves the moisture content of the seeds to remain high (> 30-50%) and is sensitive to drying. That is inversely proportional to orthodox seeds, which can maintain the viability of the seeds with a very low moisture content below 10%. Recalcitrant seeds are generally larger and heavier than orthodox seeds, related to the water content (Chin et al. 1987).

According to research conducted by Shivashankar et al. (2015), sapodilla seeds experience a process of losing seed moisture content very rapidly during the first ten days of storage. Loss of moisture content decreases the ability of seeds to germinate. Sapodilla seeds are classified as recalcitrant seeds, decreasing their viability if they experience a decrease in water content (Farnsworth 2000). A decrease in seed moisture content is common during the storage period, which causes the need for seed storage management so that it can be germinated at the desired time. A good understanding of the recalcitrant properties of sapodilla seed can improve the successful management of recalcitrant seed storage.

Many factors play a role in the aspect of seed germination. One of which is the germination inhibitor. One of the germination inhibitor compounds comes from a group of phenolic compounds, namely gallic acid. Seeds synthesize inhibitor compounds to prevent the germination process. However, the phenolic compound content in each species is different (Yukiko et al. 2001).

This research was conducted to see gallic acid content in the sapodilla fruit and seeds, both freshly picked and those that have undergone ripening/storage. Research on the content of gallic acid in sapodilla fruit and seed samples representing the development process and seed storage treatment is important to know the factors that cause recalcitrant properties of sapodilla seeds. In addition, research on sap germination and viability of sapodilla seeds after storage treatment was also carried out to support research on recalcitrant properties of sapodilla seeds. Furthermore, research on the physiology of sapodilla seed development is possible because in one tree and at one time, fruit samples can be taken of different ages and sizes (Hossain et al., 2015). Therefore, this research is expected to provide an understanding of recalcitrant seeds, especially in the physiological aspects of the gallic acid content in seeds, regarding the content of gallic acid as an inhibitor of seed germination and can provide information regarding the development of recalcitrant seed storage methods which, so far, have encountered many obstacles.

MATERIALS AND METHOD

The research was conducted for 5 months in 2018 at the Biology Laboratory and Integrated Laboratory of Science (FMIPA), Sebelas Maret University, Surakarta, Indonesia.

Sampling

Sapodilla (*Manilkara zapota* (L.) P. Royen) is taken from the Manyaran Village, Wonogiri, Central Java, Indonesia, which comes from one sapodilla tree (Figure 1). The selected sapodilla fruits represent the four stages of fruit development. The sample size was taken based on references from Rastegar (2015). Samples of sapodilla fruit were added at the 4th developmental stage, which has undergone a ripening process, and samples of the 4th developmental stage of sapodilla seeds were stored for 10 days at room temperature.

Characterization of sapodilla fruit and seeds

Sapodilla fruit was characterized based on the size referred by Rastegar (2015) and added with the sapodilla fruit sample for the 4th developmental stage, which has undergone a ripening process, and the sapodilla seed sample for the 4th development stage stored for 10 days at room temperature (Table 1). Fruit characterization includes fruit color, length diameter (cm), width diameter (cm), and fruit weight (grams). Each was labeled or marked. Then sapodilla seeds are separated from the fruit, and seed characterization is carried out at each development stage, including length diameter (cm), width diameter (cm), seed weight, and color.

Figure 1. Sapodilla. A. Leaves, B. Flower, C. Fruit

Table 1. The sample size of sapodilla fruit (Rastegar, 2015)

Type of fruit samples	Length diameter (cm)	Width diameter (cm)	Weight (g)	Seed morphology
1st development stage	3.9 ± 0.3	3.4 ± 0.3	24 ± 2	The color of the seed coat is half white
2nd development stage	4.7 ± 0.4	4.0 ±0.2	37 ± 4	The color of the seed coat is a quarter of the white part
3rd development stage	5.5 ± 0.2	4.5 ± 0.3	60 ± 7	The color of the seed coat is brownish-black with a hint of white
4th development stage	6.8 ± 0.4	5.4 ± 0.3	115 ± 9	The color of the seed coat is brownish-black and has hardened

Fruit ripening and seed storage treatment

The sapodilla fruit sample at the 4th developmental stage was stored for 5 days. Storage was done by placing sapodilla fruit into a paper bag, closed tightly, and placed at room temperature for 5 days. Sapodilla was then separated from the fruit and seeds. As many as 70 sapodilla seeds were stored at room temperature (27-30°C) for 10 days for later tetrazolium test, gallic acid content measurement, seed moisture content, and seed germination test (Kusumiyati et al. 2017).

Making anatomical preparation of sapodilla seeds

The seed samples from the four stages of sapodilla development were each made into anatomical preparations using the paraffin embedding method with single safranin staining. First, making permanent preparations using the paraffin embedding method following the procedure of Soerodikosoemo (1987).

Observation of the anatomical structure of the sapodilla

Observation of the anatomical structure of sapodilla seeds was carried out in two ways: observation of seed anatomical preparations made by the paraffin embedding method and observation of fresh sapodilla seed preparations. Observation of seed anatomical preparations using the paraffin embedding method using a digital microscope with a magnification of 100x. The observable parameters were the sapodilla seed embryo structure and documented during development.

Observation of fresh seed preparations was carried out by cross-cutting the sapodilla seed into two parts, partially soaked with 0.1% tetrazolium salt to color the structure of the sapodilla seed parts with red color and the rest was soaked with distilled water to be observed as a fresh sapodilla seed preparation. Next, observations were made using a stereo microscope with a magnification of 40x. The observable parameters were embryo development during the development process, which was then documented.

Measuring the moisture content of sapodilla seeds

The seed sample was weighed as much as 5 grams, then chopped and put into a petri dish, then dried in an oven at 105°C for 3 hours until a stable weight was obtained. Drying the seeds was carried out 3 times. The seeds are then weighed in a stable dry weight and then put into the formula (Kamil 1982):

$$KA = \frac{wetweight - dryweight}{wetweight} x100\%$$

Tetrazolium test for sapodilla

The sapodilla seeds are soaked in water for 24 hours, and then the seeds are split longitudinally until the embryo is clearly visible. Next, the split seeds were immersed in 0.1% tetrazolium solution for 24 hours. Observation of seed viability was based on a red staining pattern in the sapodilla seed embryo area (Subantoro and Prabowo 2013).

Sapodilla seed germination test

The germination test was carried out using samples of sapodilla seeds from the 4th developmental stage of sapodilla fruit that had been brooded for 4-5 days and the 4th development stage sapodilla seeds that had been stored for 10 days, 5 seeds of each with 4 replications were germinated in polybags for 40 days. On the 40th day, the germination percentage was calculated, and the root length and height of the sprouts were measured.

Analysis of gallic acid content in sapodilla fruits and seeds

The modified Folin-Ciocalteu procedure determined the total phenol content according to Chaovanalikit and Wrolstad (2004). First, one-half gram of dry sample was extracted with 20 mL of 70% methanol. The mixture was left for 1.5 hours, then filtered with Whatman No. 1 filter paper. Next, 0.5 mL sample extract was reacted with 0.5 mL of Folin-Ciocalteu reagent 50% in a test tube and homogenized using a vortex. Next, the sample was added with 2 mL of Na2CO3 2% solution, then incubated for 30 minutes at room temperature. Then the absorbance of the extract was read with a spectrophotometer at a wavelength of 750 nm. The levels of gallic acid compounds are determined based on the standard gallic acid curve.

Data analysis

The germination test results and seed viability were statistically analyzed using the Independent Sample T-Test. In addition, the moisture content of the seeds and the gallic acid content in sapodilla fruit and seeds were statistically analyzed using the One Way ANOVA test. Finally, if there was a significant difference, the DMRT test (Duncan Multiple Range Test) was carried out with a test level of 5%.

RESULTS AND DISCUSSION

The results of observations of sapodilla fruit development are presented in Table 2 and Figure 2. The observed morphological characters were weight, length diameter, width diameter, and fruit color.

The observations included the fruit's color, size, and weight (Table 2). The morphological data can be used to determine the age of the fruit. According to Rastegar (2015), the estimated age of sapodilla fruit at the 1st developmental stage is 2 months after the flower blooms, the sapodilla fruit for the 2nd developmental stage is 3 months after the flower blooms, the sapodilla fruit for the 3rd development stage is 4 months after the flower blooms, and sapodilla fruit development stage 4 is 5 months after the flowers bloom. Therefore, the older the fruit, the more the fruit size will be based on the obtained morphological data. Sapodilla fruit has increased weight during development (Figures 2 and 3). Mature sapodilla fruit (sapodilla development stage 4) has increased in weight up to five times the initial weight (sapodilla development stage 1). This increase in weight is because, in the developmental stage, the fruit wall originating from the ovary wall (pericarpium) experiences thickening, and the cells divide to form several layers of tissue (Tjitrosomo 1983).

Figure 2 shows morphological changes during the sapodilla fruit development stage, including the size, weight, and color of the exocarpium (outer layer of the fruit) from green to yellowish-brown. The hormone ethylene makes the color changes that occur due to fruit ripening. In this process, chlorophyll degradation and carotenoid formation occur, which causes the color of the fruit to change (Gong et al., 2015).

Apart from the development of sapodilla fruit, the development of the seeds is also observed. The morphological data of sapodilla seeds are shown in Table 3 and Figure 4. Each stage of sapodilla seed development has different seed sizes and weights. During the development process, the sapodilla seed coat changes color from predominantly white (1st development stage) to blackish brown to dark black (4th development stage), which is caused by the sapodilla seed coat that undergoes deposition of phenolic compounds. The accumulation of phenolic compounds in the seed coat causes the structure of the seed coat to become hard and compact. This structure protects the seeds from fungal and pathogenic attacks (Debeaujon et al., 2000).

The seed coat contains several specialized areas, one of which is the hilum. Hilum of sapodilla seeds are on the edges, are round, and tend to be oval. The position of the hilum is concave and brownish-white (Figure 5). The hilum is a scar formed when the funiculus is released from the seed when the seed matures. The hilum is important in germination; it functions as a hole for water to enter the seeds (Hanna 1984). The result is that the perfect hilum development is seen in the sapodilla seed sample at the 4th stage of development.

Table 2. Morphology of sapodilla fruit development

Fruit sample	Weight (grams)	Length diameter (cm)	Width diameter (cm)	Color of fruit
1st development stage	18-30	3.40-4.60	2.80-3.60	The skin of the fruit is predominantly green with a slightly brownish tinge
2nd development stage	33-43	4.60-5.40	3.60-4.00	The skin of the fruit is predominantly brown with a slightly greenish tinge
3rd development stage	52-68	5.10-6.90	3.90-4.70	The skin of the fruit is bright yellowish brown
4th development stage	104-130	6.50-7.90	5.40-6.00	The fruit skin is predominantly dark brown with a slightly yellowish tinge

A B C D

Figure 2. Morphology of sapodilla fruit development. Note: A. 1st development stage of sapodilla, B. 2nd development stage of sapodilla, C. 3rd development stage of sapodilla, D. 4th development stage of sapodilla. Bar = 5 cm

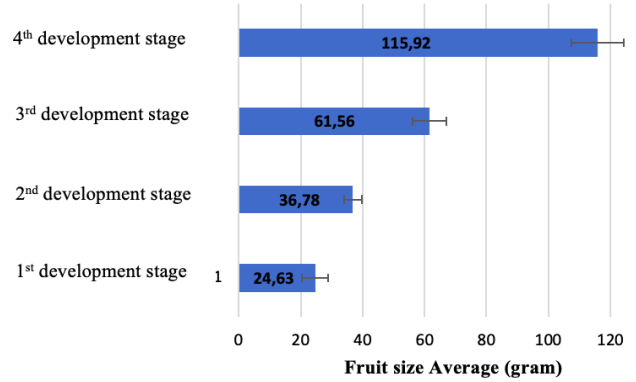

Figure 3. Increased weight of sapodilla fruit during development

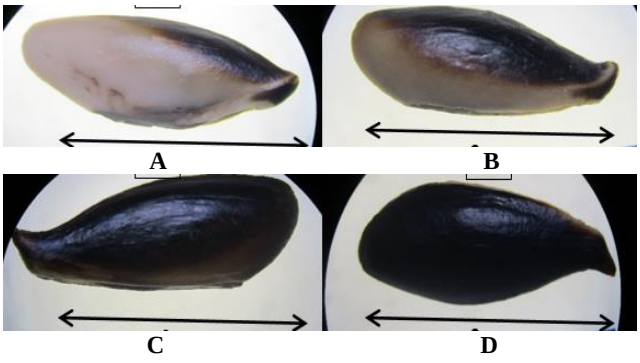

Figure 4. Morphological observations of sapodilla seeds using a 40x magnification stereo microscope at each stage of seed development. Note: A. 1st development stage of sapodilla, B. 2nd development stage of sapodilla, C. 3rd development stage of sapodilla, D. 4th development stage of sapodilla. Bar = 2 cm

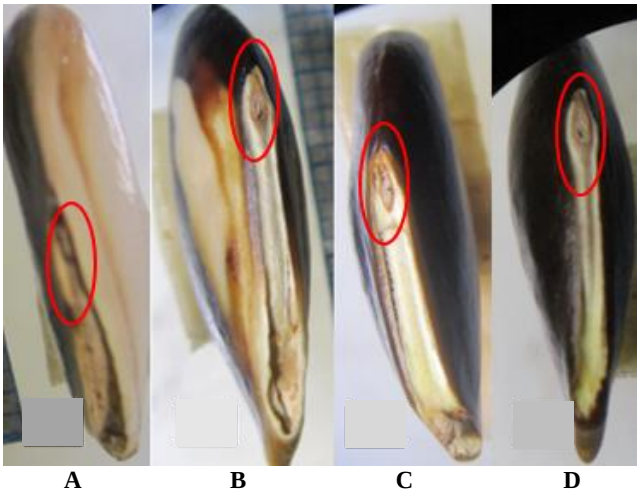

Figure 5. Morphological observations of sapodilla seeds using a stereo microscope with a 40x magnification at each stage of seed development. Note: A. 1st development stage of sapodilla, B. 2nd development stage of sapodilla, C. 3rd development stage of sapodilla, D. 4th development stage of sapodilla

Figure 6 shows a sapodilla seed whose coat has been removed, showing the sapodilla cotyledons protected by a thin brown membrane. It also shows the sapodilla seed embryo when it is being split. The sapodilla seed embryo is waxy white. The parts of the sapodilla seed include the seed coat, cotyledons, and embryos. The cotyledons take up almost the entire seed space and provide food reserves for sprouting growth. The embryonic axis consists of the root and shoot meristem, forming the mature plant after germination. The cotyledons are the last differentiated organ system that will age after germination and are responsible for synthesizing and storing food reserves for germination (Borisjuk et al., 2003).

The embryo in sapodilla plants shows three main parts of the plant body: the radicula, caulicula, and plumula (Figure 7). Roots of embryo or prospective roots (*radicula*) will grow into hypocotyl, at the end of which will grow primary roots. The root system is the taproot system in dicot plants such as sapodilla trees. The embryo stem (*caulicula*) is a candidate for the stem. Finally, *plumula* is a candidate for the first leaf (Ningsih 2006).

The embryo development of sapodilla seeds can be seen in Figure 8. In the first development stage of sapodilla seeds, the embryo is not fully formed yet (plumules have not been formed yet). At the second stage of development of sapodilla seeds, the embryo begins to form completely, although the parts are still unclear. At the 3rd stage of development of sapodilla seeds, the embryonic axis appears, and the parts that will develop into the radicula, caulicula, and plumula appear. Finally, at the 4th stage of development of sapodilla seeds, the differentiation process is complete, and the embryonic axis is clearly visible.

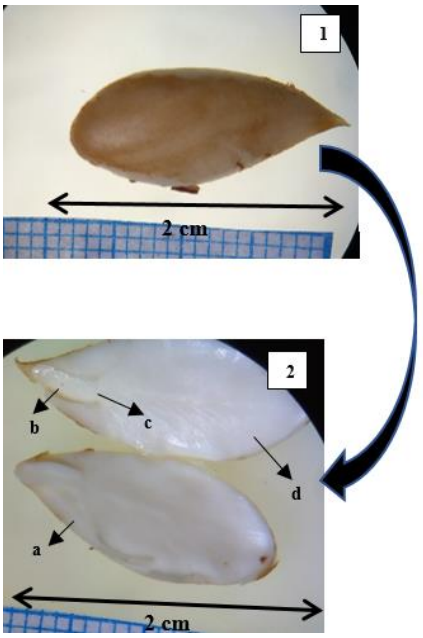

Figure 6. Sapodilla seed. Note: 1. The sapodilla seed whose coat has been removed, 2. Sapodilla seed embryo, a. Cotyledons, b. Radicula, c. Caulicula, d. Plumula

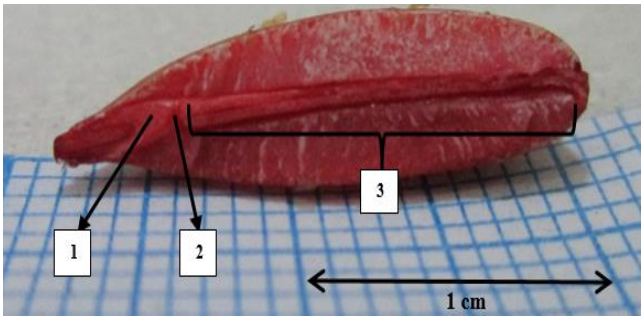

Figure 7. Cross-section of tetrazolium test results for sapodilla seed. Note: 1. Radicula, 2. Caulicula, 3. Plumula. Bar = 1 cm

Sapodilla fruit development stage

In this study, the selected fruit samples were sapodilla fruit from four stages of development. This sample selection aims to determine the physiology of sapodilla seed development, especially regarding the content of gallic acid inhibitor compounds and seed moisture content that experiences change during the development process.

The statistical analysis of the gallic acid content in sapodilla fruit and seeds during the development stage, done with One Way ANOVA, showed a significance value of less than 0.05, meaning that the results were significantly different between samples or treatments. Table 4 shows that all fruit parts, seed coats, cotyledons, and sapodilla seed embryos contain gallic acid inhibitor compounds. Generally, the highest gallic acid content in sapodilla seeds was in the fruit, while the lowest was in the cotyledons and embryos. However, the gallic acid content in sapodilla fruit and seeds during development decreased. The most significant decrease occurred in the seed coat because gallic acid content in the seed coat functions as an antioxidant compound that protects seeds from oxidative damage and an inhibitor of germination (Pammenter and Berjak 2014).

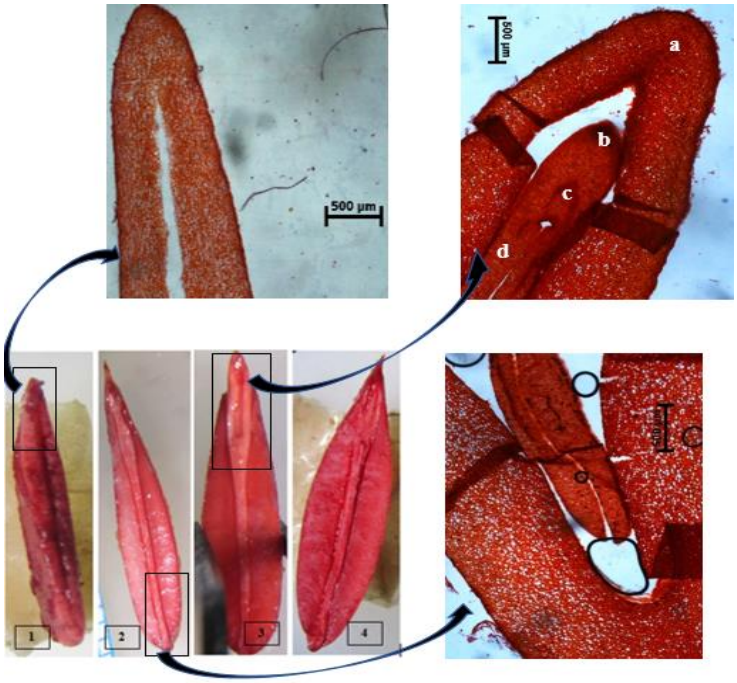

Figure 8. Cross-section of the tetrazolium test result and paraffin embedding of sapodilla seed. Note: 1. 1st stage of sapodilla seed development, 2. 2nd stage of Sapodilla seed development, 3. 3rd stage of Sapodilla seed development, 4. 4th stage of sapodilla seed development, a. Cotyledons, b. Radicula, c. Caulicula, d. Plumula. Color: Safranin. Microscope magnification: 40x.

Table 3. The morphology of sapodilla seed

Seed sample	Weight (grams)	Length diameter (cm)	Width diameter (cm)	Color of seed
1st development stage	0.40-0.60	1.90-2.50	1.00-1.20	Three-quarters of the seed coat is white, and the remaining quarter is blackish-brown
2nd development stage	0.50-0.70	2.20-2.60	0.70-1.30	Half of the seed coat is white, and the remaining half is blackish-brown
3rd development stage	0.51-0.79	2.10-2.50	0.80-1.40	One-fourth of the seed coat is white, and three-fourths of the remaining part is blackish-brown
4th development stage	0.60-0.80	1.90-2.30	1.10-1.30	All parts of the seed coat are black

Table 4. Gallic acid content in sapodilla fruit and seeds during the development stage

Types of fruit samples	Content of gallic acid (ppm)		
	Seed coat	Cotyledon and embryo	Fruit
1st development stage	0.6533[d]	0.6486[d]	0.6605[d]
2nd development stage	0.6520[c]	0.6422[c]	0.6570[c]
3rd development stage	0.6517[c]	0.6390[b]	0.6567[c]
4th development stage	0.6425[b]	0.6366[a]	0.6566[c]

Note: numbers followed by different letters in the same column and row in the above table are significantly different according to DMRT at the 95% confidence level

The accumulation of gallic acid and tannic acid compounds in seeds is closely related to self-defense mechanisms and dormancy control (Debeaujon et al. 2000). The skin, as the outer part of the seeds, acts as a protector of the seeds so that fungi or other microorganisms do not easily attack them. According to Inacio et al. (2013), phenolic compounds in Palicourea rigida seeds are responsible for seed dormancy. Storage of seeds at a low temperature of 5ºC for 12 months resulted in the degradation of phenolic compounds in P. rigida seeds, resulting at the end of dormancy.

Based on the research results, sapodilla seeds contain the highest gallic acid compound in the fruit. That is because the fruit has a role as a seed protector, therefore to support this role, the sapodilla fruit, which is fleshy and juicy, contains high gallic acid to protect the seeds from mechanical damage and microorganisms attack (Debeaujon et al. 2000). During the development period, the content of gallic acid in sapodilla fruit and seeds decreases. That is related to the nature of recalcitrant seeds that do not have dormancy. Therefore, during their development stage, there is a decrease in gallic acid content to condition the seeds to germinate immediately after being released from the parent plant (Pammenter and Berjak 2014).

The moisture content of seeds is important because the seed damage is influenced by their moisture content (Sutopo 1985). Based on Table 5, mature sapodilla seeds (4th stage of development) have a relatively high moisture content of 34.84%. Mature seeds with high water content caused low seed storage capacity (Chin et al. 1989). That is very different when compared to orthodox seeds. Orthodox seeds generally have a moisture content below 10% (Debeaujon et al. 2000).

Fruit ripening and seed storage

In this study, fruit ripening is carried out because cultivators in the field generally harvest sapodilla fruit in raw condition and then do the ripening for 3-5 days. Furthermore, Sapodilla fruit is classified as a climacteric fruit in which a surge in ethylene synthesis and respiration happens after the fruit is harvested (Agustiningrum et al. 2014). Therefore, fruit ripening treatment is carried out to determine the effect of ripening on the content of gallic acid inhibitor compounds in sapodilla fruit and seeds.

The statistical analysis of the gallic acid content in sapodilla fruit and seeds during fruit ripening using One Way ANOVA showed a significance value of less than 0.05, meaning that the results were significantly different between samples or treatments. In Table 6, it is known that the fruit parts, seed coat, cotyledons, and sapodilla seed embryos all experience a decrease in the content of gallic acid during ripening. In general, the highest content of gallic acid in sapodilla is in the fruit, while the lowest is in the cotyledons and embryos. The most significant decrease occurred in the fruit because, in the fruit ripening process, there is a change in the sapodilla fruit structure, which was originally hard and gummy and has undergone a degradation process to become soft and watery (Kusmiyati et al. 2017). The soft and watery flesh condition is thought to dissolve the gallic acid content. In the seed section, the most significant reduction in gallic acid occurred in the seed coat, which is thought to be due to the location of the seed coat on the outermost layer of the seed and directly adjacent to the pulp. In addition, the high water content in the ripe sapodilla fruit is thought to dissolve the gallic acid in the seed coat.

The seeds used for propagation often encounter problems, one of which is that they are not resistant to storage. Seed storage aims to maintain seeds' availability so they can be germinated at the desired time. Based on this background, in this study, sapodilla seeds were stored to determine their durability in storage.

Table 5. The moisture content of sapodilla seed during the development stage

Seed sample	Moisture content (%)
1st development stage	55.60[a]
2nd development stage	47.60[b]
3rd development stage	43.89[c]
4th development stage	34.84[d]

Note: numbers followed by different letters in the same column and row in the above table are significantly different according to DMRT at the 95% confidence level

Table 6. Gallic acid content in sapodilla fruit and seeds during fruit ripening

Types of fruit samples	Content of gallic acid (ppm)		
	Seed coat	Cotyledon and embryo	Fruit
Mature sapodilla in raw condition	0.6425b	0.6366a	0.6566c
Mature sapodilla which is ripe through a ripening process	0.6421b	0.6365a	0.6532a

Note: numbers followed by different letters in the same column and row in the table above are significantly different according to DMRT at the 95% confidence level

The statistical analysis of gallic acid content in sapodilla seeds for 10 days of storage using One Way ANOVA showed a significance value of less than 0.05, meaning that the results significantly differed between samples or treatments. Table 7 shows that the gallic acid content in fresh sapodilla seeds is higher than that in stored seeds. That is because the sapodilla seed in its development has been conditioned so that it will germinate immediately after being separated from the parent plant. Furthermore, this is evidenced by the decrease in gallic acid germination inhibitor content during the development and storage stages.

Table 8 shows that sapodilla seed moisture content decreased significantly during the 10 days of storage at room temperature. That is because the storage treatment creates environmental conditions with low humidity or a tendency to dry out, which causes a decrease in seed moisture content and decreases the seeds' viability. Recalcitrant seeds are very sensitive to dry environmental conditions (Shivashankar et al., 2015). The reduced viability of sapodilla seeds can be proven by the tetrazolium test of sapodilla seeds that has been carried out, and the results showed a decrease in seed viability by 45% after 10 days of storage (Table 9).

The viability of sapodilla seeds was statistically analyzed using the independent sample t-test. The independent-sample t-test table shows a significance value of 0.04. This value is <0.05, so according to the basis of decision making in the independent sample t-test, it can be concluded that there is a significant difference between the average percentage of viability of control sapodilla seeds and after 10 days of storage treatment. Therefore, the storage treatment that was carried out had a significant effect on the percentage of seed viability.

The tetrazolium test results of sapodilla seeds in Figure 9 show that the viable sapodilla seeds (control), the cotyledons, and the embryos are evenly red. In contrast, the sapodilla seeds with storage treatment are blackish red, which shows the cells have experienced death. The seeds generally can no longer germinate; if it germinates, the seedlings will grow slowly and abnormally, and even such seedling growth often ends in death (Subantoro and Prabowo 2013).

The tissue damage or death that occurs will affect the ability of the seeds to germinate; therefore, the sapodilla seed germination test was carried out to strengthen the seed viability data. Unfortunately, the tetrazolium test cannot detect seed abnormalities but only can detect live and dead seeds (Subantoro and Prabowo 2013). That is due to many factors such as the presence of dormancy, disease, and damage due to the chemicals used that affect the growth and development of seeds so that the test for the ability of seed germination cannot be done by the tetrazolium test (Subantoro and Prabowo 2013). Based on this, the sapodilla seed germination test was carried out in a polybag with soil media.

The results of the sapodilla seed germination test are statistically analyzed using the independent sample t-test. The independent sample test table shows a significance value > 0.05, both data on the percentage of germination, root length, and height of sprouts. So, according to the basis of decision making in the independent sample t-test, it can be concluded that the germination test results between the sapodilla seeds as control and after 10 days of storage treatment are not significantly different. Therefore, the seed storage treatment has no significant effect on sapodilla seed germination.

Based on Table 10, the percentage value of germination, root length, and height of sprouts decreases, but it is not significant based on the results of statistical data analysis using the independent sample t-test.

Figure 9. Color absorption results in the tetrazolium test of sapodilla seed. Note: 1. Control and 2. 10 days storage treatment. Bar = 1 cm

Table 7. Gallic acid content in sapodilla seeds for 10 days of storage

Type of seed sample	Content of gallic acid (ppm)	
	Seed coat	Cotyledon and embryo
Fresh sapodilla seeds (control)	0.6421b	0.6365a
Stored sapodilla seeds	0.6404a	0.6365a

Note: numbers followed by different letters in the same column and row in the table above are significantly different at the 95% confidence level according to DMRT

Table 8. The moisture content of sapodilla seed after 10 days of storage

Seed sample	Moisture content (%)
Fresh sapodilla seeds (control)	32.51a
Saved sapodilla seeds	16.38b

Note: numbers followed by different letters in the same column and row in the table above are significantly different according to DMRT at the 95% confidence level

Table 9. Viability of sapodilla seed by tetrazolium test

Treatment	Viability (%)	Sig.
Fresh sapodilla seeds (control)	100	0.04
Sapodilla seeds that have been stored for 10 days	55	

Note: Significance is tested by an independent sample t-test

Table 10. The results of the sapodilla seed germination test

Treatment	Germination (%)	Root length (cm)	Sprouts' height (cm)
Fresh sapodilla seeds (control)	100	3.98	3.79
Sapodilla seeds that have been stored for 10 days	80	3.37	2.28
Sig.	0.141	0.192	0.255

Note: Significance is tested by an independent sample t-test

Figure 10. Germination stage of sapodilla seed. Note: 1. Day 0, 2. Day 40. Bar = 2 cm

Figure 11. Sprouts of sapodilla. Note: 1. Plumula, 2. Cotyledons

The germination percentage value of fresh sapodilla seeds planted immediately after being separated from the fruit is 100%, meaning all seeds can germinate completely. However, the percentage value of germination decreased by 20% on sapodilla seed which was given storage treatment for 10 days. It means that not all planted seeds germinate successfully. Some have failed to germinate because of rotting. Failure in germination is thought to occur because, during the storage process, the sapodilla seed cannot withstand environmental conditions, namely low humidity levels resulting in mechanical damage to the seeds (Sutopo 1998).

Table 10 also compares the parameters of the root length and height of the sprouts. The sprouts of the control sapodilla seed have longer roots and higher sprouts than sprouts from seeds stored for 10 days. Based on these results, it can be concluded that the vigor of sapodilla seed sprouts in the storage treatment for 10 days is lower than the sprouts of control sapodilla seed. Germination vigor includes physiological aspects during germination and sprout development (Sutopo 1998).

Figures 10 and 11 show the germination process of sapodilla seeds for 40 days. First, there is a change in the sapodilla seed, which was originally hard, to become soft after imbibition, and the radicle appears. Then, the sprouts continue to develop; the plumules start to appear; at first, they are close and eventually open; the sapodilla type of germination is the epigeal type, in which the cotyledons are raised above the soil surface during growth periods. The uplift of these cotyledons to the soil surface is caused by the growth and extension of the hypocotyl (Kamil 1982). According to Sutopo (1985), the seed germination process is a complex series of morphological, physiological, and biochemical changes. The first stage of seed germination begins with the absorption of water by the seeds, softening the seed coat, and the hydration of the protoplasm. The second stage begins with cell and enzyme activities and an increase in the respiration rate of the seeds. The third stage is where substances such as carbohydrates, fats, and proteins are broken down into dissolved forms and localized to growth points. The fourth stage is the assimilation of the materials described earlier in the meristematic region to produce energy for the activities of component formation and growth of new cells. Finally, the fifth stage is the sprouts' growth through cell division, enlargement, and division at growing points. While the leaves cannot function as photosynthetic organs, the growth of sprouts is very dependent on the food supply in the seeds.

Sapodilla is classified as recalcitrant seed. The process of development evidences this to maturity. Sapodilla seeds have a high moisture content above 30% and cause low seed storage capacity (Chin et al. 1989). The storage treatment causes the seed moisture content to decrease, consequently affecting the seeds' viability. Sukarman and Rusmin (2000) stated that the decrease in moisture content in recalcitrant seeds resulted in damage, so the seeds' ability to germinate decreased.

Sapodilla seeds experience a decrease in gallic acid content during the development process. Gallic acid acts as a seed germination inhibitor. Lower germination inhibitor content means a higher chance of the seeds germinating. Recalcitrant seeds are highly able to germinate, meaning that these seeds do not experience dormancy (Pammenter and Berjak 2014). The 10 days of storage treatment on seed at room temperature results in a decrease in water content and gallic acid content. Gallic acid is a group of phenolic compounds acting as a germination inhibitor and a

protector of seeds from mechanical and microbiological damage. Decreasing the gallic acid content in the seeds increases the risk of mechanical damage to the seeds.

In conclusion, all fruit, seed coat, cotyledons, and sapodilla (*Manilkara zapota* (L.) P. Royen) seed embryos contain gallic acid inhibitor compounds. The highest gallic acid content in sapodilla is in the fruit, then the seed coat, while the lowest is in the cotyledons and embryos. That is because the fruit and seed coat structure protects the sapodilla seed. The gallic acid content inhibits the seeds from germinating and protects them from oxidative damage during storage. The gallic acid content in sapodilla fruit and seeds decreases during development. The gallic acid content in sapodilla fruit and seeds decreases during development and storage treatment. The seed storage treatment causes a decrease in the germination percentage of sapodilla seeds. That indicates that gallic acid plays a role in controlling sapodilla seed germination.

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