Macroscopic and Microscopic Analysis of Development Mature Fruit 
Bruguiera gymnorrhiza (L.) Lamk

S. Handayani, S. Setyoyuowo, Aulani’am, E. Suprayitno

1 Faculty of Agriculture Technology, Brawijaya University, East Java, Indonesia
2 Department of Biochemistry, Brawijaya University, East Java, Indonesia
3 Faculty of Fisheries and Marine Science, Brawijaya University, East Java, Indonesia

Abstract
The development of mature fruit Bruguiera gymnorrhiza (large mangrove leaf) are green color change (age 70 Daf), green-purple (age 80 Daf) and ended up being a full purple (90 Daf). Fruit is also experiencing accretion diameter, except for the tip (tip: 1.14 to 1.13 cm; middle: 1.63 to 1.69 cm; base: 1.23 to 1.25 cm), lengthy (16.51 to 18.81 cm) and reduction weight (30.08 to 27.14). Increasingly fruit age, the harder texture (173.50 to 191.50 N). it was distinctive fruit flavors and slightly bitter tannins, so the fruit can not be consumed directly. The fruit (tip, middle and base) that have cell development is endocarp and mesocarp. Cells in the endocarp shape are a hexagon (tetrahedron) irregular, in Mesocarp cells undergo renewal and there is also a prism shape. It is evenly distributed throughout the cell wall thickening section (lacunar). Types include parenchyma and collenchyma tissue. At the same exocarp, endocarp cell shape, with tight cell structure, composed of epidermal cells and hypodermic. Cells of the epidermis have stomata.

Keywords: B. gymnorrhiza, macroscopic, mature fruit, microscopic

Introduction
Large-leafed mangrove (Bruguiera gymnorrhiza) is one of the most important and widespread mangrove species in the Pacific. B. gymnorrhiza thrives under a broad range of intertidal conditions, including salinity levels from near freshwater to full strength seawater, and tolerates a range of flooding and other soil types. Typically, it is most common in the middle and upper intertidal zones, rather than in the lower intertidal zone or along the seaward edge of mangrove stands [1].

Flowering and “fruiting” occur continuously throughout the year, although distinctly seasonal peaks of hypocotyl production were reported for July–August in northern parts of the range and January–February in southern parts. Trees have notable and long periods of reproductive development, taking 1–2 years from first emergence of flower buds until maturation and drop [2].

B. gymnorrhiza fruit is viviparous, means that the species produces seeds that germinate on the parent plant. The dispersal unit, a viviparous seedling, is called a hypocotyl. There is no apparent fruit stage. Instead, a hypocotyl emerges singly from an attached mature calyx. Mature hypocotyls with attached calyx bodies are located at the third to fifth nodes below the apical shoot. The hypocotyl is cylindrical, elongate, stocky, dark green, coriaceous, with longitudinal ribbing giving an angular appearance, the root tip bluntly pointed, mature dimensions 15–25 cm (6–10 in) long and about 2 cm (0.8 in) wide. Expended calyces often remain attached after mature propagules fall from parent trees. “Fruiting,” when mature hypocotyls fall, occurs January to February in the southern hemisphere, and July to August in the northern hemisphere [1].

In Indonesia, a number of plants are used as food, for example: soft stems of A. marina, young roots of R. stylosa dan N. Fruticans and hypocotyls of B. gymnorrhiza [3]. B. gymnorrhiza is used as food by coastal communities during times of food crisis. The fruit of this plant contain energy and carbohydrate high enough [4]. The fruit (hypocotile) is reportedly “eaten cooked, after scraping or grating, washing, and drying (to remove tannins) and sometimes mixed with coconut [2]. This fruits is potential as alternative food sources due to the mangrove is a tropical forest that is easy growing, on the other hand are rich mangrove steroid compounds, saponins, flavonoids and tannins (condensed and hydrolysable tannins).

Other compound are anthocyanins, carbohydrates, carotenoids, catechins, diterpenes, gibberellins, fatty acids, hydrocarbons, inorganic salts, lipids, flavans and flavan polymers, minerals, phenolic compounds, procyanidins, proteins, steroids, carboxylic acids, triterpenes, (B, Fr, L, R, S) [5-14].
which in this case has not been widely explored. Chemical aspects of mangrove plants is very important because of its potential as a functional food development, agrochemical and medically valuable compounds.

**Purpose**

The research aims to give an information on the mature tissues and part of the fruit is very necessary as a knowledge base for utilizing the fruit for processing.

**Material and Method**

**Raw Materials**

Fruits that used in this study are actually a mature hypocotile. Fruits obtained from Sawohan village, Sidoarjo, East Java, Indonesia. Fruits obtained from research, washed, sorted by size, maturity level and disability, vacuum packed, then taken to a laboratory to be analyzed macroscopically and microscopically.

**Materials and tools**

Material analysis include fruit *B. gymnorrhiza*, FAA (Formal Acetil Dehyd = Formalin: Acetic Acid Glacial: Alcohol), distilled water, 1% safranin, reagent Iodine, glycerin, nail polish, and paper label. Analysis tools include the ruler, calipers, tape color, sliding mikrotome, blade, cutter, glass slides, cover glass, light microscope olympus CX21, objective micrometer, micrometer eyepiece, nikon digital cameras and pencils.

**Making Preparations Semi-Permanent**

Fruit *B. gymnorrhiza*, was measured to determine the parts to be cut to make preparations mikroskopinya. Take part in include: 2 cm base, middle, 2 cm tip. Each piece is cut into pieces of 2 parts vertically, and than incorporated into FAA fixative solution for 24 hours. Sliced using Sliding Microtome with thickness 15-20 microns. Washed distilled water for 5 minutes. Soaked in a solution of 1% safranin for 1 minute. Washed distilled water for 5 minutes. Placed on a glass slide, was given a drop of glycerine, then covered with a cover glass. The edge cover glass were nail polish. Observed under a light microscope with a magnification of 40x and 100x. Measured the thickness of the endocarp, mesocarp and eksokarp using ocular micrometer. Photographed using a digital camera.

**Method**

Observations age fruit *B. gymnorrhiza* made at growing plants once every 2 days to see the fruit skin discoloration. Plants were observed growing along the banks of the river and embankment ponds. Macroscopic analysis included age *B. gymnorrhiza* fruit pieces, fruit size (diameter and length), fruit hardness, weight, and organoleptic. Microscopic analysis includes the thickness of the fruit (endocarp, Mesocarp and eksokarp), cell type, the space between cells, and the tissue type that has given color. Measurements and observations using light microscopy.

**Result and Discussion**

**Macroscopic analysis age of fruit**

The fruit that comes from mature hypocotile characterized by dark green leather, and than gradually changed color to purple at the edges, and ends with a full hypocotile color purple. During the development of the fruit begins to mature hypocotile flowering takes 60 days (dark green fruit color), 70 days (purple-dark green fruit color) and 90 days (full purple fruit color). After this period hypocotile experienced abscission and fell from the parent tree.

**Organoleptic mature fruit**

For mature fruit color development was initially dominated hypocotile by pigment chlorophyl. Then the chlorophyl degraded so pigment antocyanin appear to undergo abscission.
Distinctive fruit flavors and a slightly bitter tannins. Flavor of fruit *B. gymnorrhiza* caused by secondary metabolites (example: tannins, alkaloids and saponins) are abundant in mangrove plants. These compounds included in the class of phenol and soluble in organic solvents such as water, methanol and ethanol [15]. The more aged fruit grow stronger bitter taste.

During the development of mature fruit shape hypocotile cylindrical shaped, elongate tapering ends, slightly bent and stocky. Looks purple fruit shape slimmer than green fruit. This is because the older, long growing fruit, while fruit weight decreases (Table 1). Fruit weight due to the development of an increasingly thickened endocarp cells, which is often found in the aerenchyma cells. Increasing fruit diameter at the base and center of the fruit, while the end of the decline. This gives a more pointed shape at the end of the fruit mature period. This allows the fruit shapes like stuck in the mud when it fell, making it possible to grow into new plants. Based on this analysis suggests that the rapid cell growth occurs in the middle and the base of the fruit.

The texture of the fruit indicated by harsh violence (Newton). Violence fruit ranged from 173.50 to 191.50 Newton. Increasing age of the fruit (dark green to purple color fruit), fruit growing violence. This is because a change in a cell type parenchym colenchym cell components rich in starch, cellulose, pectin and lignin that gives the plasticity [16].

| Table 1. Macroscopic Analysis of Mature *B. gymnorrhiza* Fruits |
|--------------------------------|--------------------------------|----------------------------------|
| Parameter                     | Dark green fruit color         | Dark green to purple fruit color |
| Picture                        |                                | Purple fruit color               |
| Age fruit (daf)                | 60 days after flowering        | 70 days after flowering          | 90 days after flowering          |
| Organoleptic :                |                                | Purple of the tip and light green of base fruits |
| fruit color                    | Dark green of the tip and light green of base fruits (50 percent) |
| fruit flavors                  | Taste tannin and slightly bitter |
| fruit shape                    | cylindrical, elongate tapering ends and stocky |
|                                | cylindrical, elongate tapering ends and stocky |
|                                | cylindrical, elongate tapering ends and stocky | purple on all parts of fruits |
| Lengthy (cm)                  | 16.51 ± 2.97                  | 16.90 ± 1.92                     | 18.81 ± 3.05                     |
| Weight (gr)                   | 30.08 ± 5.56                  | 29.88 ± 7.27                     | 27.14 ± 4.88                     |
| Diameter (cm)                 |                                |                                  |                                |
| - tip of fruit                | 1.14 ± 0.09                   | 1.23 ± 0.09                      | 1.13 ± 0.09                      |
| - middle of fruit             | 1.63 ± 0.06                   | 1.66 ± 0.08                      | 1.69 ± 0.07                      |
| - base of fruit               | 1.23 ± 0.08                   | 1.24 ± 0.07                      | 1.25 ± 0.06                      |
| Hardnes (N)                   | 173.50 ± 0.06                 | 186.50 ± 0.00                    | 191.50 ± 0.09                    |

**Microscopic analysis**

**Cell type and tissue type**

In this study, each sample was cut into three parts, i.e 2 cm from the tip and base, and in the middle hypocotile. Measuring the thickness of the endocarp, and eksokarp Mesocarp using micrometer ocular. Documentation to see the shape of the cell, the space between the cell and tissue types do with 200x and 400x magnification. Results from cross-sectional pieces *B. gymnorrhiza* changes with 3 different colors (dark green, dark green-purple and purple) are presented in figures 1, 2 and 3.

The observation suggests that part endocarp cells form a hexagon (polygon) with thin cell walls. Some cells undergo cell wall thickening evenly (lacunar), many containing aerenchyma cells and the
space between cells. In this section dominates parenchyma tissue. Between the endocarp and there mesocarp bulkhead (border), which consists of two layers of cells (endodermis) containing cells aeranchyma and have regular patterns (Fig. 1a and 2a). Part mesocarp, cell shape varied, namely polygon shapes (tetrahedron) are elongated (an outgrowth of the inner cell endocarp, Fig. 1a), triangular (prism), square (beam) irregular (Fig. 2b and 3b) and getting closer eksocarp cells tend to form rounded. There are many aeranchyma cells and the space between cells. Cells undergoing cell wall thickening likely uneven cell section (lacunar), which characterizes the type of network collenchyma. Part eksokarp, cell shape similar to the cell endocarp. Consists of several layers of cells (epidermis and hypodermic) and the epidermis are stomata [17]. The composition of the cell is very tight and solid so hardly found space between cells (Fig.2b and 3a). The presence of space between cells in the endocarp and mesocarp and make fruit aeranchyma cell B. gymnorrhiza floating in the water.

Figure 1a. Micrograph of endocarp (End) and mesocarp (Me) of the tip of the cross-sectional of dark green fruit color.

Figure 1b. Micrograph of mesocarp (Me) and eksocarp (Eks) of the middle of the cross-sectional of dark green fruit color.

Figure 1c. Micrograph of endokarp (End), mesocarp (Me) and eksocarp (Eks) of the base of the cross-sectional of dark green fruit color.

Figure 2a. Micrograph of endocarp (End) and mesocarp (Me) of the tip of the cross-sectional of dark green-purple fruit color.

Figure 2b. Micrograph of mesocarp (Me) and eksocarp (Eks) of the middle of the cross-sectional of dark green-purple fruit color.

Figure 2c. Micrograph of endocarp (End), mesocarp (Me) and eksocarp (Eks) of the base of the cross-sectional of dark green-purple fruit color.
The thickness of the endocarp, mesocarp and eksokarp fruit

The observation of the thickness of the endocarp, mesocarp and eksokarp of 3 kinds of fruit color (dark green, dark green-purple and purple color) in the image served 4a, 4b and 4c. Each section endocarp, meso and eksokarp measured at the tip, middle and base of the fruit. Measuring the thickness of the tip and the base is 2 cm from the outer portion.

Eksokarp and endocarp thickness, the tip is the same during fruit development (endocarp is 4,50 mm and eksokarp is 0,25 mm), while the thickness mesocarp with increasing age of the fruit (dark green to purple color) which is 3,17 mm thinner into 3,00 mm.
Endocarp thickness and mesocarp the middle during fruit development experience. Endocarp thickness increases arising after the purple rind, while the mesocarp in full purple fruit (8.00 mm to 8.33 mm). Part eksokarp still not having the thickness (0.50 mm).

Endocarp thickness of the base of the fruit during fruit development had added (dark green: 6.10 mm; green-purple; purple: 6.12 mm and 6.32 mm). Part mesocarp contrary, the purple fruit thickness decreases. Part eksokarp remain thickened.

Based on the analysis of observations of the thickness of the endocarp, mesocarp and eksokarp during fruit development (at mature fruit) showed that the multiplication of cells occurs in endocarp and mesocarp. Decrease in thickness at the base mesocarp purple fruit that is because at the base of the fruit (fruit color purple) started to decompose and dry out. Furthermore, the fruit will fall to the ground, loose flower petals and leaves will grows.

**Conclusion**

During the period of mature green fruit changes color to purple (70-90 day after flowering), increase of diameter, elongation and decrease of weight. The longer life of the fruit, the harder texture. Distinctive fruit flavors and a slightly bitter tannins.

The fruit (tip, middle and base) who have cell development is endocarp and mesocarp. Cells in the endocarp shape is a hexagon (tetrahedron) irregular, in Mesocarp cells undergo renewal and there is also a prism shape. Evenly distributed throughout the cell wall thickening section (lacunar). Types include parenchyma and collenchyma tissue. At the same eksokarp endocarp cell shape, with tight cell structure, composed of epidermal cells and hypodermic. Cells of the epidermis has stomata.
References


