CHAPTER 3
GENERAL MATERIALS AND METHODS

Sources of Samples

The primary source of samples for this study was from local *Jatropha curcas* Linn Luanti accession harvested from a jatropha pilot project conducted by the Institute of Agro-Biotechnology (ABI) Malaysia located at Luanti Baru Village in Keningau, Sabah, Malaysia. Another ten different accessions were also observed for fruit maturity uniformity according to different accession study. The ten selected accessions are located at Tenom Research Station, Sabah and at Binakan Sook, Keningau, Sabah, Malaysia. *Jatropha curcas* Linn Tanzania accession and IP1 accession were used for preliminary experiments and for respiration study. The Tanzania accession samples were brought from Bogor University Farm and IP1 accession were brought from Jatropha Plantation at Serang, Banten, Indonesia.

The jatropha pilot project belonging to the ABI is on Acrisol soil type. Sabah, Malaysia is characterized by a humid tropical climate which is moist and wet throughout the year with heavy rainfall (2,500 to 5,000 mm p.a.), average daily temperatures of 21-32°C and humidity averaging about 85%. Due to small seasonal variations in incoming solar radiation, the annual difference in day length is only 2 minutes along the equator and 49 minutes in the northern regions, giving a day length of 12.30 hours all year round (Nieuwolt 1982). Rainfall is affected by the North – East (November – March) and South – West (June-August) monsoons which result in heavy rainfall. For the months April-May and September-October, less rain is experienced because of changes in monsoonal winds.

The plot was previously cropped with hill paddy but has been left idle for more than a year. A one hectare plot size was prepared by manual land clearing followed by minor open burning after the weeds were dried within two weeks of slashing. The seedlings were obtained from wild jatropha trees growing around the village. Only yellow and black fruits were collected, separated from fruit coat and
dried under shade for three days. The seeds were directly planted onto the plot with a planting distance of 2 x 2 meters with two seeds per hole. No fertilizer was applied along the observation plot but weeds were controlled chemically with glyphosate (N-(phosphonomethyl)glycine) and hand weeding as needed, especially for weeds around the crop stem.

**Experimental Design and Statistical Analysis**

The predetermined targeted sample characteristics were collected randomly through all destructive and non-destructive measurable variables throughout the study. Minimum of three and maximum of 30 replications were performed to increase probability to 95% that the changes on the measurable variables were due to the selected modified variables. The data was analyzed using ANOVA and the differences between means calculated.

**Measurement of Physical Variables**

**Measurement of fruit maturity uniformity**

The jatropha ripening index was first established by modifying the guava colour index (Silip 2003) wherein Index 1 = immature fruit or small dark green fruit, Index 2 = full-sized green fruit or mature green, Index 3 = more green than yellow, Index 4 = more yellow than green, Index 5 = yellow fruit, Index 6 = more yellow than black, Index 7 = more black than yellow, Index 8 = black fruit and Index 9 = dry fruit. The established fruit colour index can be generalized into five indexes. Those are Index 1 = immature fruit or small dark green fruit, Index 2 = full-sized green fruit or mature green, Index 3 = yellow fruit, Index 4 = back wet fruit and Index 5 = dry fruit. The appearances and description of both nine and five fruit colour maturity indexes is presented in the Figure 1. The black and dry fruit are considered as
senescent fruits. Uniformity of fruits according to the established fruit maturity index was assessed by calculating the percentage number of fruits of similar maturity or similar ripening from the total number of fruits in a group such as per tree or bunch. The formula for this calculation is as follows:

\[
\text{Fruits} \left( \% \right) = \left[ \left( \frac{\text{St.y}}{\text{At1}} \right) \right] \times 100
\]

Where: \(\text{At1}\) = the total number of fruits in the observation and \(\text{St.y}\) = the total number of fruits of a specific index.

**Measurement of total number of fruit bunches**

Total fruit bunches was obtained by counting the number of fruit bunches on each jatropha tree.

**Measurement of total fruits**

Total fruits were obtained by simply counting the number of fruits per bunch and number of fruits per tree.

**Measurement of fruit length, thickness and circumference size**

Fruit length size was determined by measuring the outer curve of individual fruits with a tape from the distal end to the point at the proximal end where the fruit coat was judged to terminate. Fruit thickness was determined by measuring the thickness at the equatorial region of each individual fruit using vernier caliper. Fruit circumference size was determined by measuring each individual fruit with a tape at the widest midpoint of each fruit.
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<td><img src="immature_small_dark_green_fruit.png" alt="Image 1" /></td>
<td>Index 1 Immature, small dark green fruit</td>
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<td>Index 1 Immature, small dark green fruit</td>
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<td>Index 2 Full-sized mature green fruit</td>
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<td>Index 3 More green than yellow</td>
<td><img src="more_green_than_yellow.png" alt="Image 3" /></td>
<td>Index 3 Fully ripe yellow fruit</td>
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<td>Index 5 Fully ripe yellow fruit</td>
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<td>Index 8 Black wet fruit</td>
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<tr>
<td><img src="dry_fruit.png" alt="Image 9" /></td>
<td>Index 9 Dry fruit</td>
<td><img src="dry_fruit.png" alt="Image 9" /></td>
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</tbody>
</table>

1 The appearances and description of both nine and five fruit colour maturity indexes for measurement of *J. curcas* L. fruit maturity uniformity.
Measurement of color

Colour of each sample was measured at a single point in the equatorial region using a Konica Minolta Colour Reader (CR-100, Minolta Corp. Japan). The measurement was expressed in L*, C* and h° colour values. The measurements were made under constant light-source of fluorescent bulb at room temperature (26±2 °C). The values of L* (lightness), a* (red-green axis) and b* (yellow-blue axis) which represent coordinates in the colour chart indirectly reflected Chroma (C*) and hue angles (h°). L* formed the vertical axis with values ranging from 0= black to 100= white. Values of a* and b* were used to compute chroma 

\[ C^* = (a^*^2 + b^*^2)^{1/2} \]

which represents the hypotenuse of a right angle and hue (h° = tan⁻¹ b*/a*). It is defined as beginning at the +a* axis and is expressed in degrees where 0° would be +a* (red), 90° would be +b* (yellow), 180° would be −a* (green) and 270° would be -b* (blue).

Measurement of firmness

An indication of firmness was obtained by the force necessary to cause penetration of a standard probe within a specified distance into the product. A stand mounted penetrometer or the penetrometer in combination with a drill press was used for measuring coat and seed firmness. Force required to penetrate 1 cm into coats and seeds were measured using a 6 mm diameter cylindrical probe mounted on a bench-top firmness tester. Coat and seed firmness is usually reported in kilogram force (kgf) or newtons (N) (1 kgf=9.80665N)
Measurement of Chemical Variables

Extraction of CJO using Modified Hydraulic Presser

Oil extraction was carried out by mechanical pressing using screw pressing. Prototype of a modified hydraulic presser (MHP) was developed based on modifications of a similar presser developed by Situmorang (2009). The MHP was made with thermostat, mold compartment and 5 ton hydraulic jack (Appendix 11).

Extraction of CJO by chemical solvent

The soxhlet technique was used for chemical extraction with hexane solvent (boiling point of 40 – 60 °C). The extracted lipid was obtained by filtrating the solvent using a rotary evaporator apparatus at 40 °C followed by heating in an oven at 105 °C for three hours to evaporate any remaining solvent and water.

Measurement of extracted oil yield

The extracted oil yield was measured by dividing the amount of extracted oil by the weight of sample before extraction.

Measurement of total soluble solid concentration

The SSC of coat and seed was determined using a hand refractometer (Model N1, Atago) modified by Dadzie and Orchard (1997). A total of 30 g sample tissue in distilled water was blended using a kitchen blender for 2 min and filtered through a filter paper. A drop of the filtrate was placed on the prism glass of the refractometer. The refractometer was pointed towards a light source and readings of SSC (%) were recorded. The recorded values were multiplied by a factor of three (because the initial tissue sample was diluted three times with distilled water) and the readings were corrected to a standard temperature of 20 °C according to Bourne (1997).
Measurement of pH value

The remaining juice from the SSC determination was used to measure juice pH using a glass electrode pH meter (Crison Micro pH 2000). The pH meter was calibrated using a buffer of pH 4.0 and pH 7.0 before use.

Measurement of free fatty acid

Acid value of kernel oil was determined according to the American Organization of Chemical Scientist Official Method Cd 3a-63 and the percentage of free fatty acid was calculated using oleic acid as a factor.