

CHAPTER 3

METHODOLOGY

3.1 RAW MATERIAL

In this study, OPT sap was obtained from OPT (*tenera* species), approximately 30 years old from the Federal Land Development Authority (FELDA) Jengka 14, Pahang in October 2012. A 10 cm thick disc was taken from the middle part of the trunk, which ranged in length from 9-13 m. The discs (33-42 cm in diameter) were cut into 4-6 pieces and squeezed through sugarcane press machine to collect the sap. All the squeezing process was done within 12 hour after the tree was cut down. The OPT sap was filtered using coffee filter and collected into a big container. It was mixed well before allocated into 5 L bottles and stored in a -20 °C freezer. Prior to use, the sap was centrifuged at 6,000 rpm for 15 min at 4 °C (Eppendorf, Centrifuge 5810 R, Germany) and the supernatant was used in the fermentation. Figure 3.1 shows the overall process in the preparation of OPT sap.



Figure 3.1: OPT sap preparation. (a) Oil palm tree was cut down (b) The trunk was cut into pieces (c) The trunk bark was removed (d) The middle part of the trunk was cut into smaller pieces (e) The trunk pieces was squeezed through the sugarcane machine (f) The sap obtained was filtered and stored.

3.2 CHARACTERISATION OF OPT SAP

The initial OPT sap sugar composition was checked for sugar composition analysis using HPLC as described in Chapter 3.10.1. The differences on composition of fresh OPT sap collected within 12 hour oil palm tree was felled and after 12 months of storage in -20 °C freezer were analyzed.

3.3 CHEMICAL REAGENTS

The analytical grade chemicals used in this study were ammonium sulphate, magnesium sulphate and calcium chloride from Fisher Scientific (U.K), whereas di-ammonium hydrogen phosphate, β -alanine and potassium dihydrogen phosphate were obtained from Merck (Germany). Medium component such as yeast extract were purchased from Fisher Scientific (France), peptone and tryptone from Sigma-Aldrich (USA) and agar powder by Sigma-Aldrich (Spain). Other chemicals like sulphuric acid and sodium hydroxide were bought from Fisher Scientific (U.K). HPLC grade sucrose were bought from Sigma-Aldrich (Switzerland), glucose and fructose were sourced from Merck (Germany), while ethanol and acetonitrile from Fisher Scientific (U.K).

3.4 MEDIUM PREPARATION

Two types of medium were used throughout this research namely, YPD agar and YPD broth. YPD agar was prepared by mixing 20 g of agar, 20 g of peptone and 10 g of yeast extract in 900 mL of distilled water in 1 L Schott bottle. Both flask were then covered with aluminium foil and was sterilized in an autoclave (Hirayama HVE-50, Japan) for 20 min at 121 °C. After autoclave, 100 mL of filter-sterilized 20% w/v glucose was added in the agar to avoid Maillard reaction. Then, agar was poured into sterilized Petri dish after the temperature has dropped to 60 °C. The agar was left solidified and all plates were sealed before being kept in the refrigerator at 4 °C until further use. To make YPD broth, the procedure was the same except no agar powder was added.