3 MATERIALS AND METHODS

3.1 Overview

A schematic structure of the whole process flow has been constructed and is illustrated in Figure 3.1. As the starting point, the banana stem was collected and prepared for fermentation process. Then, inoculums were prepared in term of probiotic milk drink. The range for factors selected was decided beforehand using Response Surface Methodology (RSM) in Design-Expert® software. The experimental runs were done by Central Composite Design (CCD). The effects of fermentation time and temperature on lactic acid fermentation were investigated. After fermentation process, the samples were collected and were analyzed by High Performance Liquid Chromatography (HPLC). The last step was data analysis by using Design Expert.

![Flow chart process of experiment.](image-url)
3.2 Substrate Preparation

Banana plants were collected from Gambang, Pahang. Each banana stem was washed and cut into parts. The banana stems were pressed using sugarcane presser machine to get the juice. The fresh banana stem sap was stored in a refrigerator at 4°C to ensure its freshness (Hamid, 2013). To avoid any fermentation, the experiment using this banana stem sap was carried out on the same day. Figure 3.2 shows the step to get the juice from banana stem.

Banana stem was cut into shorter length.

The banana stem was cut into small pieces.
Sugarcane machine was used to press the banana stem.

The waste of banana stem after pressed with sugarcane presser.