

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1. Material

D-Fructose and D-Glucose were obtained from Kagawa Rare Sugar Research Center, Japan. Lysine (with the purity index 99%) was obtained from Cheil Jedang Indonesia, Co. Ltd. ABTS or 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) was purchased from AppliChem, Germany (Lot No.2X001714). While, the instrument needed were digital balance, beaker glass, micropipette, microtubes, dry oven, vortex, refrigerator, pH meter, spectrophotometer and digital colorimeter.

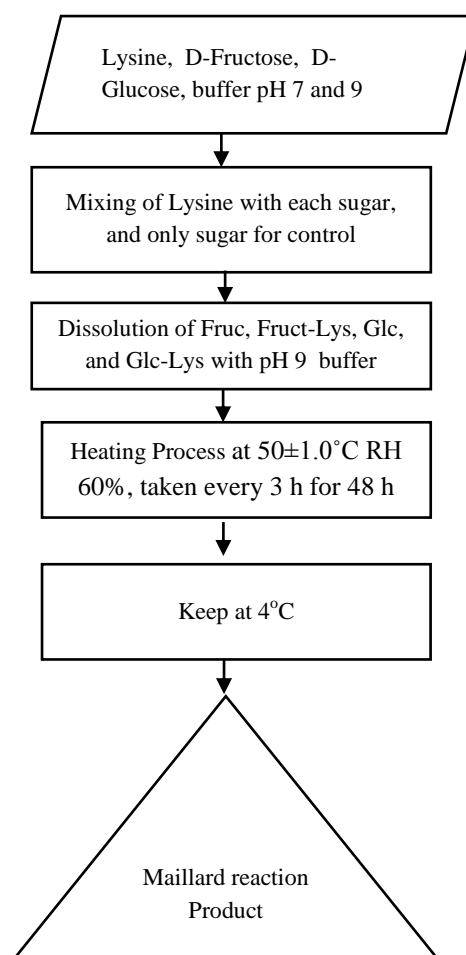
#### 3.2. Method

The method on this present study is consist of MRPs model preparation, physical and chemical analysis, which are browning intensity, color development, spectroscopic measurements, and ABTS radical scavenging activity. Then, the correlation between browning intensity and scavenging activity was studied.

##### 3.2.1. Preparation of MRPs Model

Preparation of MRPs Fructose–Lysine (Fruc–Lys) model system has been adopted from Yu et al. (2012) with some modification. There were two model systems; Fruc–Lys and Glucose–Lysine (Glc–Lys) with ratio 1:1 (5 g) were dissolved in 100 ml of 10 mM Carbonate buffer (pH 9). As much as 200 µL of each solution then transferred to 1.5 ml microtube which has a resistance at high

temperature. The sample was heated at temperature 50°C and RH 60% for 48 h using control drying oven. The samples were taken after heated in every three hours. Every model systems were prepared in triplicates. After heating treatment, the samples were cooled at room temperature for 1 minute and then kept in 4°C to stop the reaction. Prior to measurement, the samples were diluted until 200  $\mu$ L of phosphate buffer (pH 7). The flow diagram of MRPs model preparation is shown in Figure 6.



**Figure 6.** The flow chart of generating Maillard reaction products from amino-sugar model system

### 3.2.2. Browning Intensity

The browning intensity of Fruc, Fruc–Lys, Glc, and Glc–Lys were measured based on the method of Phisut and Jiraporn (2013). MRPs sample after heating were diluted with phosphate buffer until 200  $\mu$ L. The browning intensity were determined by monitoring absorbance at 420 nm using spectrophotometer (UV-1280; Shimadzu, Kyoto, Japan).

### 3.2.3 Color Development

The MRPs sample after heating were diluted with phosphate buffer until 200  $\mu$ L. Color development of samples were determined by digital colorimeter TES-135 to obtain values  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) then calculate the browning index or browning development from this equation (2) (Alvarenga et al., 2014):

$$x = \frac{a + 1.75(L)}{5.645L + a - 3.012(b)} \quad (1)$$

$$BI = \frac{100(x - 0.31)}{0.172} \quad (2)$$

$L$ ,  $a$ , and  $b$  are the values from digital colorimeter,  $x$  is the value obtained from equation (1), and  $BI$  is the browning index.

### 3.2.4. Spectroscopic Measurements

The MRPs samples of Fruc, Fruc–Lys, Glc, and Glc–Lys after heating were diluted with phosphate buffer until 200  $\mu$ L in microtube. The samples were measured for emission spectrum (190–750 nm). This spectroscopic measurements method has been adopted from Jing and Kitts (2004).

### 3.2.5. ABTS Radical Scavenging Activity

The 7 mM ABTS was diluted with 10 mM phosphate buffer (pH 7.4). Then 5 ml of MRPs solution was reacted with 88  $\mu$ L of 140 mM potassium persulfate ( $K_2S_2O_8$ ) and store in a dark room for 16 h. Before use, approximately 100  $\mu$ L of this solution diluted first with 4.9 ml of ethanol until it reach an absorbance  $7.00 \pm 0.020$  at 734 nm by using spectrophotometer. 100  $\mu$ L of ethanol was reacted with 9 ml of ABTS solution then loaded in spectrophotometer as absorbance of blanko ( $A_0$ ). 100  $\mu$ L of sample was reacted with 9 ml of ABTS solution then loaded in spectrophotometer as absorbance of sample ( $A_1$ ). The inhibition percentage of ABTS radical was calculate using formula on Equation (3):

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100.$$

$A_0$  is the absorbance with blank and  $A_1$  is the absorbance with the sample (Dimitrova et al., 2010).

### 3.2.6. Correlation between Browning Intensity and Scavenging Activity

Correlation between browning intensity and scavenging activity was conducted using Graphpad Prism analysis. Browning intensity value and scavenging activity value were analyzed for regression value ( $R^2$ ). Value of  $R^2$  should be in a range 0 – 1, 1 indicates the closeness of the greater value, and positive correlation obtained when the  $R^2$  value more than 0,6 Alvarenga et al. (2014).

### **3.3. Data Analysis**

The results were reported in figures and the figures were generated from Appendix. The scavenging activity and physical phenomena of MRPs were analyzed using descriptive analysis, then the significance of correlation between browning intensity and scavenging activity was analyzed using Graphpad Prism version 6.0. This study was adopted from Nilsson et al. (2004) and Aon and Colaneri (2001), who has stated that the significant correlation should be if P value  $<0.0001$ .