2. MATERIALS AND METHODS

The analyses were conducted at Food Science Laboratory (for *nata de coco* production and *nata* quality measurement) and Food Engineering Laboratory (for post-fermentation treatment) of Food Technology Department Soegijapranata Catholic University, January 2013 to February 2013.

2.1. Materials

The equipments and instruments used for making *nata de coco* include tray, weight balance (Great Scale), measuring cylinder, stirrer, and newspaper. The equipment and instrument used for boiling process and analysis are Lloyd texture analyzer, chroma meter (CR-400 Konica Minolta), vernier caliper (Tricle 150 x 0.055 mm), weight balance (Great Scale), sauce pan, gas stove (Rinnai RI-602 A), and stopwatch. Materials used in this experiment are grated coconut flesh, sugar (sucrose), acetic acid 99%, inoculum culture (taken from the previous production batch of the factory), and distilled water.

2.2. Methods

2.2.1. *Nata de coco* Production

2.2.1.1. Fermentation Process

Nata de coco is produced with the same procedure that the factory used with modification on amount of sugar, water, and starter added. Modification of tap water to distilled water was done in this experiment to minimize the variance of result that may occur. The procedure to produce *nata de coco* is described in the Figure 4 below.

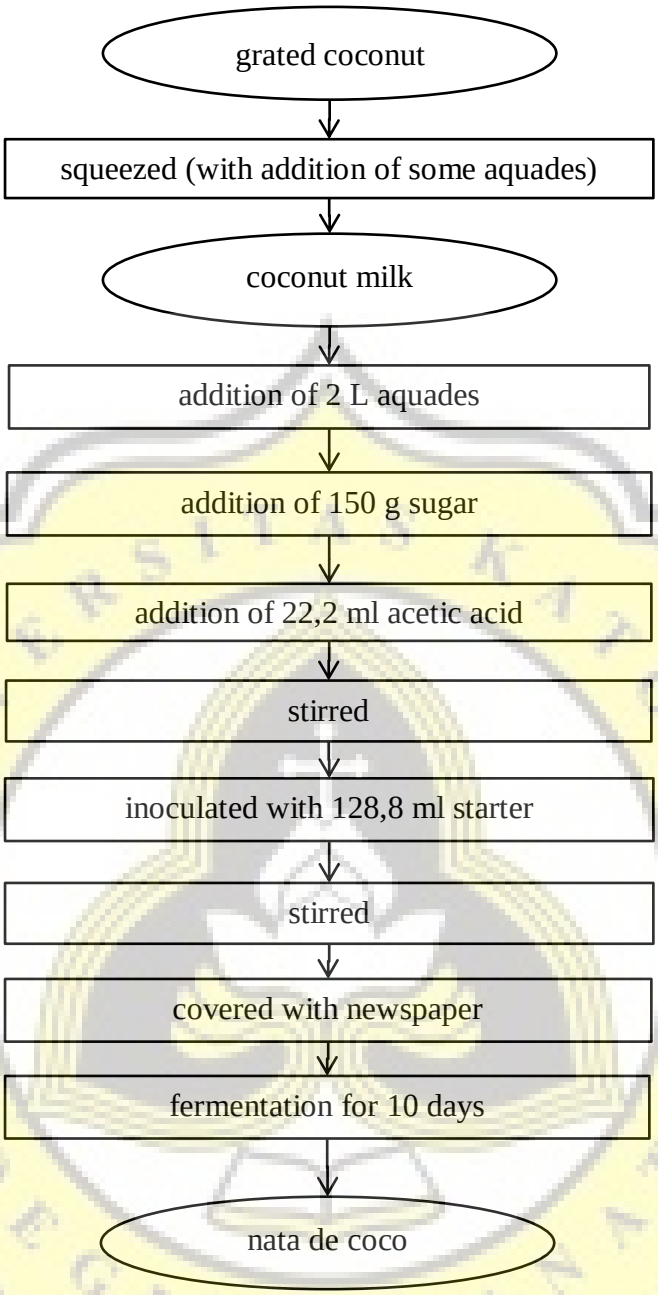

Figure 4. Flow Diagram of *Nata de coco* Production

2.2.1.2. Post-fermentation Treatment

Nata de coco sheet was harvested by separating the *nata* from the spent liquid medium. Thin bacterial films were scrapped off the *nata* surface using blunt instrument. *Nata de coco* sheet then was measured for initial hardness, color, and texture. After that, the *nata* was cut into small cubes approximately 1.5 cm × 1.5 cm (Figure 5). The *nata* cubes were then washed by soaking them in water for 10 days. The water for soaking was changed daily. The *nata* cubes then were boiled with cold water of different treatments

i.e. water ratio, boiling time, and boiling repetition (see section 2.2.2). All *nata* cubes were boiled over with the same heat. For every treatment and replication, the flame was kept to be approximately the same. The flame level used for all the processing can be seen in Figure 6. After the boiling, *nata de coco* hardness, color, and texture were measured to see the effect of boiling.

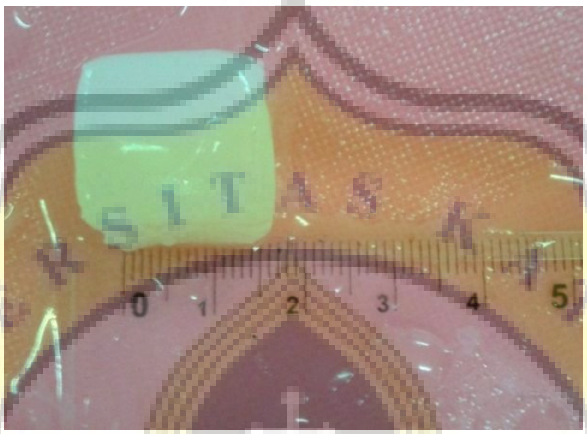

Figure 5. *Nata de coco* Cube

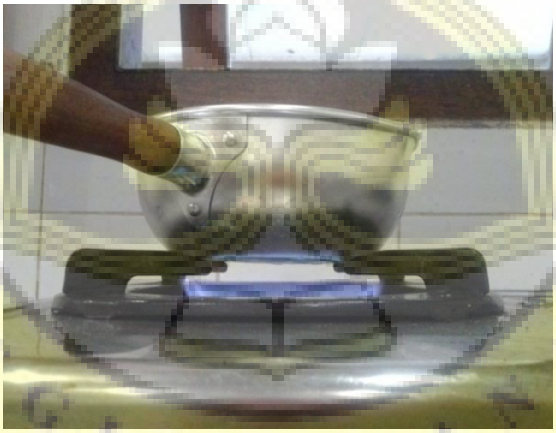

Figure 6. The Flame Used for Boiling

2.2.2. Experimental Design

The response surface methodology (RSM) was applied to find the optimum boiling condition (water ratio, boiling time and boiling repetition) that affect the responses (thickness, hardness, and color). For each independent variable, 5 levels were defined. The three independent variables and the coded values of the five levels of each variable are shown in Table 1.

Table 1. Coded Values and Corresponding Actual Values of Independent Variables

Independent Variables	Code and Actual Value				
	–2	–1	0	+1	+2
Nata and water ratio	1:3	1:4	1:5	1:6	1:7
Boiling time (min)*	3	5	7	9	11
Boiling repetition**	1	2	3	4	5

* Boiling time started to count when the flame is on
** The water was changed for next boiling repetition

The experimental design of RSM for boiling condition of *nata de coco* is shown in Table 2. The experimental design was generated by Design of Experiments Wizard from SYSTAT 12 (Systat Software Inc., Chicago, USA) software. The design used in the research is central composite design (CCD) for 3 factors. The cube and star portion was not separated by block. Two replications of the center point were used in the experiment. In SYSTAT, the experimental design was automatically generated in rotatable CCD with α of ±1,682 for 3 factors. However, in this research, the type of CCD used is the face centered CCD with extended axial points (α ± 2). Further info about how to generate an experimental design using SYSTAT is explained in the Appendix 1.

Table 2. Experimental Design of RSM for Boiling Condition of *Nata de Coco*

Run	Code			Actual Value		
	X_1	X_2	X_3	X_1	X_2	X_3
1	-1	-1	-1	1:4	5	2
2	+1	-1	-1	1:6	5	2
3	-1	+1	-1	1:4	9	2
4	+1	+1	-1	1:6	9	2
5	-1	-1	+1	1:4	5	4
6	+1	-1	+1	1:6	5	4
7	-1	+1	+1	1:4	9	4
8	+1	+1	+1	1:6	9	4
9	-2	0	0	1:3	7	3
10	+2	0	0	1:7	7	3
11	0	-2	0	1:5	3	3
12	0	2	0	1:5	11	3
13	0	0	-2	1:5	7	1
14	0	0	2	1:5	7	5
15	0	0	0	1:5	7	3
16	0	0	0	1:5	7	3

X_1 : water ratio, X_2 : boiling time, X_3 : boiling repetition

2.2.3. Quality Measurement of *Nata de coco*

2.2.3.1. Thickness Analysis

Thickness of *nata de coco* was measured by using vernier caliper. Thickness was measured for every treatment for 20 times replication.

2.2.3.2. Color Analysis

The color of *nata de coco* was measured by using a chroma meter that has been calibrated. The color is measured in L*, a*, and b* value. Color was measured for every treatment for 20 times replication.

2.2.3.3. Hardness Analysis

The hardness of the *nata de coco* was measured by a Lloyd texture analyzer equipped with a 1 KN load cell. Texture profile analysis test was done by using cylindrical probe. Each treatment was measured with test speed 5 mm/s; trigger 5 gf; nominal length 10 mm; and sample compressed 50% with 20 times replication.

2.2.4. Data Analysis

The experimental results were inputted in SYSTAT data sheet (extension .syz). The data sheet was consist of 11 columns which are run, batch, analysis replication, water ratio, boiling time, boiling repetition, thickness, hardness, L*, a*, and b*. Estimation of the model for each response then was conducted with RSM in SYSTAT 12. The result of estimation model consist of three parts which are estimations of the regression coefficients, analysis of variance (ANOVA) result, and lack of fit test result. The test criteria used for acceptable model was $p \geq 0.01$ for lack of fit test. Surface plots of each response for pairs of factors were also produced with SYSTAT. Canonical analysis was conducted to determines the stationary point and the optimal response for each response separately, and its nature (maximum or minimum or saddle point). Further info about how to estimate model, conducting canonical analysis, and generating surface plot in SYSTAT are explained in the Appendix.