1. INTRODUCTION

1.1. Background

CV. Sempurna Boga Makmur in Semarang, Central Java, is one of the companies which produce *nata de coco*. The company produce *nata de coco* using coconut milk instead of coconut water. Although it has been established for several years, the company is still having a problem with the less optimal quality of *nata de coco* produced. The company wants to achieve good quality *nata de coco* typified by good physical attributes i.e. thick, white, and soft.

In making *nata de coco*, there are two major processes, i.e. fermentation process and post-fermentation treatment (Gama *et al*., 2012). To get high quality of *nata de coco*, an optimization of both fermentation and post-fermentation must be done. Presently, most study about *nata de coco* is focused on optimizing the fermentation condition (Jagannath *et al*., 2008; Zakaria and Nazeri, 2012; Ramana *et al*., 2000; Chawla *et al*., 2009; Dobre *et al*., 2008; Hartati and Palennari, 2010). Meanwhile, there are still lacks of study about the post-fermentation condition of *nata de coco* (Gama *et al*., 2012; Somogyi *et al*., 1996; Palungkun, 1996). Therefore in this study, an investigation on the optimal post-fermentation condition of *nata de coco* was conducted in order to address the problem in the CV. Sempurna Boga Makmur. This study also becomes the first attempt on optimizing the post-fermentation condition of *nata de coco*.

In this study, the post-fermentation condition that is going to be optimized is the boiling process. Among the other post-fermentation treatment, boiling process is the most influential step that affects the texture, color, and thickness of *nata de coco* greatly. According to Gama *et al*. (2012) and Somogyi *et al*. (1996), boiling process of *nata* should be done for 5-10 minutes. This boiling process can be repeated if the *nata* is still having sour taste and smell (Palungkun, 1996) or it still not transparent enough (Gama *et al*., 2012). Moreover, the boiling process itself will be affected by the amount of the water used for boiling. In CV. Sempurna Boga Makmur, the boiling condition is suspected to be not optimal since there is no standardize of time and amount of water used in the boiling process. The boiling process itself is repeated for high number of

times (5-6 times). It is therefore important to determine the water ratio, boiling time, and boiling repetition at which optimal *nata de coco* quality is achieved.

The response surface methodology (RSM) is a statistical method that is useful for the optimization of chemical reactions and/or industrial processes (Azami *et al*., 2011). RSM is reported to satisfactorily predict and optimize *nata de coco* fermentation process (Jagannath *et al*., 2008; Zakaria and Nazeri, 2012). In this research, RSM is used to optimize the condition of boiling process such as water ratio, boiling time, and boiling repetition. By doing so, it could be expected that the boiling process can be optimized and will result in a better quality of *nata de coco*.

1.2. Literature Review

1.2.1. *Nata de coco*

Nata de coco is a white, smooth, jelly-like, chewy material that is usually used for making sweets and desserts. *Nata de coco* is highly regarded for its high content of dietary fiber and its low fat and cholesterol content. It promotes a healthy digestive system (Gama *et al*., 2012). The term of "*nata*" is originated from Spanish language which means "cream". Therefore *nata de coco* means "cream from coconut" (Palungkun, 1996). The name of the *nata* is in accordance with the substrate in which *Acetobacter xylinum* growth. Therefore, there are some term like *nata de coco* which is *nata* from coconut water, *nata* de pina from pineapple juice, *nata* de mango from mango juice, etc (Pambayun, 2002).

Nata de coco is native to the Philippines and was first developed locally in 1949 (Jagannath *et al*., 2008). The growth of *nata de coco* pellicle by *Gluconacetobacter xylinus* was first described in the 1880s by Brown, who identified this jelly-like product as chemically equivalent to cellulose. In 1991, *nata de coco* was introduced to Japan through its use in diet drinks, and it became very popular, especially among young girls (Gama *et al*., 2012). Over the years, *nata de coco* has become popular in other countries like Japan, Korea, USA, Europe, Thailand, Indonesia, China, Vietnam, and Malaysia (Jagannath *et al*., 2008; Gama *et al*., 2012).

Nata de coco was introduced to Indonesia in 1973 and it began to widely distributed in 1981 (Marina, 2012). Indonesia has many *nata de coco* producers at various business scales ranging from household and small to middle and large scale. Indonesia has great potential for *nata de coco* production because Indonesia is the top coconut producer in the world. In 2009, the production of coconut in Indonesia reached 21,565,700 metric ton (Gama *et al*., 2012).

1.2.2. *Nata de coco* Production

Coconut water and coconut milk can be used as raw materials for *nata de coco* (Gama *et al*., 2012). Coconut milk contains protein 0.7%, fat 35%, water 50%, and carbohydrate 2.8% while coconut water contains water 91.23%, protein 0.29%, fat 0.15%, carbohydrate 7.27%, and ash 1.06% (Palungkun, 1996). Although coconut milk is better in nutrition content, *nata de coco* production from coconut milk is rarely to be done compared to *nata de coco* production from coconut water. This is due to higher cost of using coconut milk. The benefit of using coconut milk to make *nata de coco* is to have a chewier texture and brighter color (Marina, 2012).

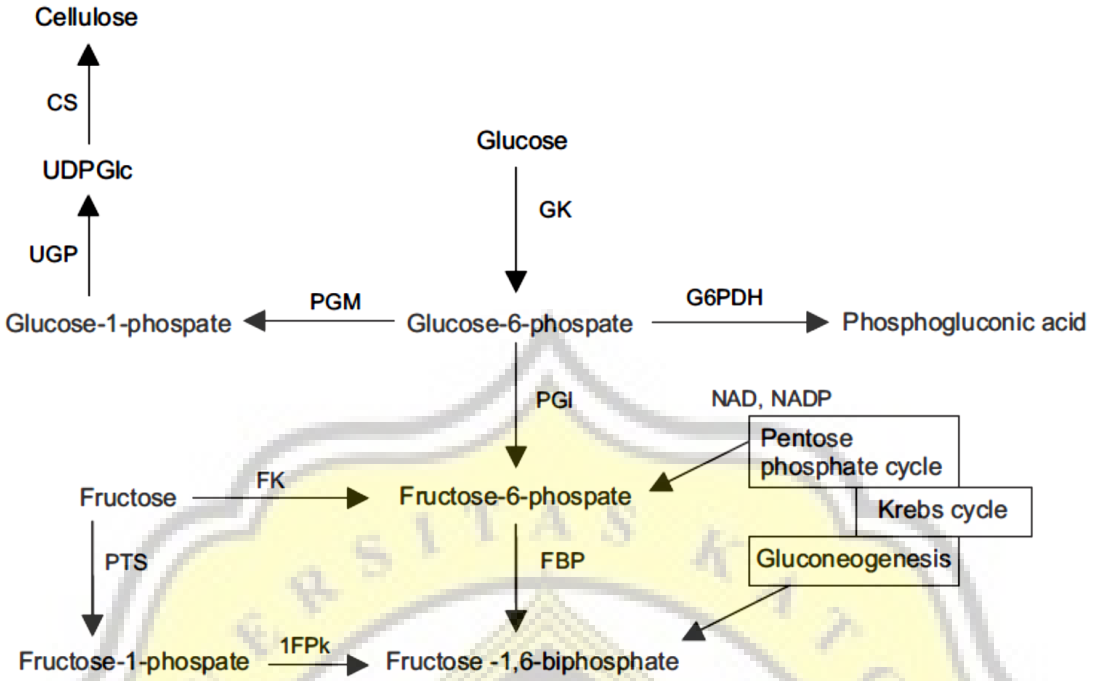
In making *nata de coco*, there are two major processes, i.e. fermentation process and post-fermentation treatment. The fermentation process consists of the preparation of ingredient, mixing the ingredient, inoculation of the culture starter, and incubation until *nata de coco* sheet was form. The post-fermentation treatment consists of scraping thin bacterial films off the *nata* surface, soaking the *nata* in water, cutting into small cube, boiling the *nata* in water, and cooked the *nata* in sugar syrup (Gama *et al*., 2012).

1.2.2.1. Fermentation Process

The ingredient needed for making the *nata* are coconut water or coconut milk or fruit juice, nitrogen source, sugar (sucrose), acetic acid 99.8%, and inoculum (Pambayun, 2002). The common process for the production of *nata de coco* uses coconut water or coconut milk as a fermentation medium with supplements of sucrose and ammonium sulfate at concentrations of 5%–10% and 0.5%–0.7% respectively. The initial pH of the fermentation medium is normally adjusted to 4.0–5.0 with diluted acetic acid. The medium is statically cultivated with 5%–10% of a stock culture in a plastic tray (or jar)

at a level of approximately 2–4 cm in height and covered with a piece of clean newspaper or similar material. The optimum temperature range for *nata* production is between 23°C and 32°C (Gama *et al*., 2012). According to Somogyi *et al*. (1996), the optimum conditions for the coconut water medium appears to be 6% sugar, pH of 3.5, and 30-35°C incubation temperature. While for the dilute coconut milk medium, the optimum conditions range from 32-35°C incubation temperature, a pH of 3.5-4.5, and 6-8% sugar. Under suitable conditions, good quality *nata de coco* that has a smooth surface and a soft, chewy texture is obtained after 7–14 days of incubation (Gama *et al*., 2012).

The most commonly used bacteria for *nata de coco* fermentation is *Acetobacter xylinium* (Gama *et al*., 2012). *Acetobacter xylinum* is a rod-shaped aerobic gram-negative bacterium which occurs as a contaminant in vinegar fermentation. The bacterium is known to produce cellulose in the form of a surface pellicle at the air-liquid medium interface at 30-40°C under static culture conditions (Ramana *et al*., 2000). According to Palungkun (1996), *nata de coco* is formed due to the uptake of glucose from sugar solution or from coconut water by *Acetobacter xylinum* cell. Glucose is then combined with amino acid and form precursor in cell membrane. This precursor then was excreted with enzyme which polymerizes glucose to cellulose outside the cell. The complete biochemical reactions of cellulose synthesis by *Acetobacter xylinum* can be seen in Figure 1.



CS cellulose synthase, GK glucokinase, FBP fructose-1,6-biphosphate phosphatase, FK fructokinase, 1FPk fructose-1-phosphate kinase, PGI phosphoglucoisomerase, PMG phosphoglucomutase, PTS system of phosphotransferases, UGP pyrophosphorylase uridine diphosphoglucose, UDPGlc uridine diphosphoglucose, G6PDH glucose-6-phosphate dehydrogenase, NAD nicotinamide adenine dinucleotide, NADP nicotinamide adenine dinucleotide phosphate

Figure 1. Biochemical Pathway of Cellulose synthesis by *Acetobacter xylinum* (Chawla *et al*., 2009)

There are a number of operating factors that affect the production of *nata de coco*. A culture medium with appropriate concentrations and types of nitrogen and carbon is required for the growth of *Acetobacter* bacteria and bacterial cellulose (BC) biosynthesis (Gama *et al*., 2012). Many strains of *Acetobacter xylinum* are capable of producing cellulose in varying amounts and growing on a wide variety of substrates like glucose, sucrose, fructose, invert sugar, ethanol and glycerol (Jagannath *et al*., 2008). Among the carbon sources, sucrose, glucose and mannitol were found to be suitable for optimum levels of cellulose production by *Acetobacter xylinum* (Ramana *et al*., 2000). Many producer of *nata de coco* chose table sugar (sucrose) as carbon source due to practical and economical consideration. The typical amount of table sugar added to the *nata de coco* solution is about 2-7.5%. The higher amount of table sugar results in the decrease of *nata de coco* hardness; however this will increase the cost (Sutarminingsih, 2004). *Acetobacter xylinum* was able to utilize a wide range of protein and nitrogen

sources such as peptone, soybean meal, glycine, casein hydrolysate, and glutamic acid for cellulose synthesis (Ramana *et al*., 2000). The effect of various nitrogen sources on the production of bacterial cellulose has been reported; casein hydrolyzate gave yield of 5 g/L, and peptone gave yield of 4.8 g/L of cellulose in *Acetobacter xylinum*. The addition of extra nitrogen favors the biomass production, but diminishes cellulose production (Chawla *et al*., 2009).

Other important factors on *nata de coco* production are pH, temperature, and dissolved oxygen (DO) (Gama *et al*., 2012). Temperature is a crucial parameter that affects both growth and cellulose production. In most of experiments, the maximal cellulose production was observed between 28 and 30°C (Chawla *et al*., 2009). The optimum pH of the culture medium for bacterial cellulose production is between 4.0 and 6.0, the yield of cellulose decreasing below pH 4. The pH decreases during fermentative production because of the accumulation of gluconic, acetic or lactic acids in the culture broth. Therefore, it is important to control the pH within the optimal range (Chawla *et al*., 2009). In static cultures, substrates have to be transported entirely by diffusion and as carbon sources are generally available, the oxygen availability might become the limiting factor for cell metabolism and could have a negative effect on cellulose production and quality of the cellulose (Chawla *et al*., 2009).

Cellulose production by *Acetobacter xylinum* is also known to be affected by the concentration of sugar, nitrogen source and pH. Maximum thickness of *nata* was obtained at pH 4.0 with 10% sucrose and 0.5% ammonium sulphate concentrations. These conditions also produced good quality *nata de coco* with a smooth surface, soft, and chewy texture (Jagannath *et al*., 2008).

According to Dobre *et al*. (2008), among the four factors (operating temperature, fructose concentration, ethanol concentration and air specific flow rate), the most important factors that influencing the synthesis of bacterial cellulose by *Acetobacter xylinum* in static conditions are operating temperature and fructose concentration. The quality and yield of *nata de coco* also depends on the maturity of the coconut. Ten to eleven-month-old nuts are recommended (Somogyi *et al*., 1996). The research of Hartati

and Palennari (2010) showed that starter age also influencing *nata de coco* production. Fermentation with starter age of 4 days gives the highest yield.

Beside the medium and environment, sanitation must be maintained during *nata de coco* fermentation. *Acetobacter xylinum* is very sensitive to the physical and chemical change in the environment. Therefore, all the equipment used during the production must be cleaned and dried (Sutarminingsih, 2004). During fermentation process, the *nata* tray must not be moved. A shake caused by moving the tray of *nata de coco* will result in layered *nata de coco* (Pambayun, 2002).

1.2.2.2. Post-fermentation Treatment

After the fermentation is completed, *nata de coco* sheet is harvested by separating the *nata* from the spent liquid medium. The cream or thin bacterial films adhering to the gel surface is removed with a blunt instrument. The *nata* is then cut into small, uniform cubes (approximately 1.5 cm × 1.5 cm × 1.5 cm) and washed by soaking the cubes (*nata*) for 1 or more days in several changes of water to remove the sour (acid) taste and smell (Gama *et al*., 2012). It is best to soak the *nata* for at least 3 days as it will remove the acid taste and smell completely (Palungkun, 1996).

Nata de coco cubes are then boiled for 5-10 minutes in water. Boiling process was conducted to soften the texture and removing excess sour taste and smell (Somogyi *et al*., 1996; Gama *et al*., 2012). Boiling process is able to remove the acid taste and smell due to the evaporation of acetic acid (Palungkun, 1996). This boiling process can be repeated if the *nata* is still having sour taste and smell (Palungkun, 1996) or it still not transparent enough (Gama *et al*., 2012). After that, *nata de coco* cubes are cooked in sugar syrup for food applications. The sweetened *nata* product is packed in sterilized pre-serving jars, cans, or plastic bags before using it in low-calorie desserts, salads, and high-fiber foods (Gama *et al*., 2012).

Boiling is a wet-thermal treatment. Heat is transferred through convection from hot water into *nata de coco*. One of the changes that may happen during boiling of *nata de coco* is the swelling of the cellulose fiber. *Nata de coco*, as bacterial cellulose, is

insoluble in most solvents due to its high crystalline nature and the existence of strong hydrogen bonding in its structure. However certain solvents like water can cause swelling, by affecting the amorphous and crystalline regions of the cellulose fibers. As the individual cellulose fiber swells, intermolecular bonds that binding the fibers together, break as a result of the internal stress produced by swelling. Due to this swelling, the degree of order within the fiber will be reduced and this may contribute to the reduction in mechanical properties (George *et al*., 2005).

The mechanisms of cellulose swelling are described on Figure 2. Fibers are swelling by ballooning with a helical structure around the balloons (1 to 3). The breakage of the helical structure and the unswollen sections between the balloons leads to a high swelling (4 to 6). The highly swollen sections are then tear into thin sections and finally into fragments (7 to 10) (Mantanis *et al*, 1995).

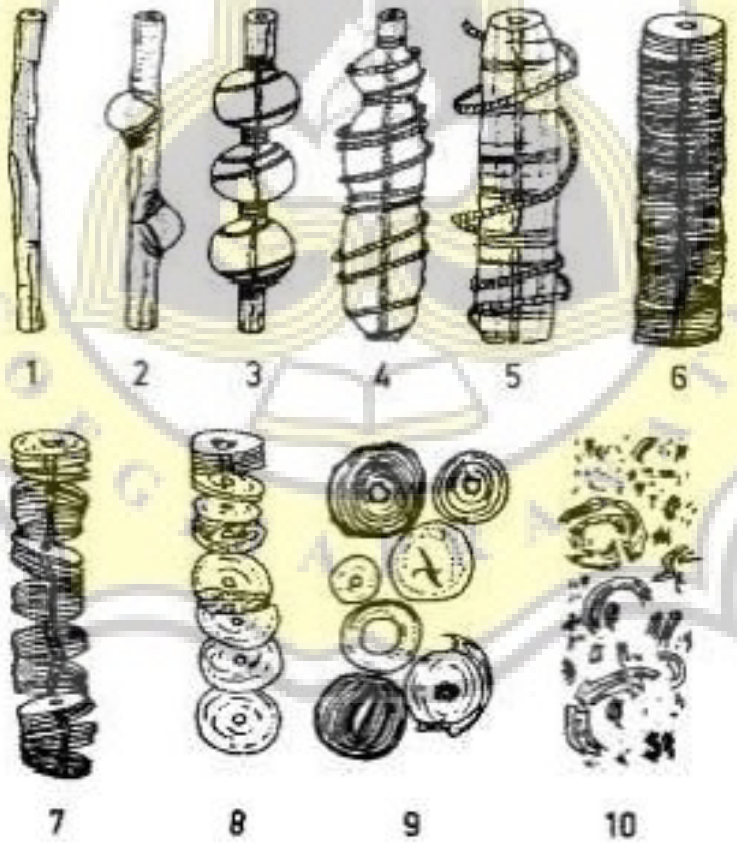


Figure 2. Mechanisms of Cellulose Fiber Swelling

Mantanis *et al* (1995) found that temperature had a significant influence on the swelling of cellulose gels. At a temperature of 80-140° C, there is known to be an occurrence of transformation related to the melting of the crystalline phase of cellulose. The melting of the crystalline phase of cellulose may cause reduction of hardness since the degree of crystallinity has a great influence on hardness. (Surma-Slusarska *et al*., 2008).

1.2.3. Response Surface Methodology (RSM)

Optimization refers to improvement of the performance of a system, a process, or a product in order to obtain the maximum benefit from it. The response surface methodology (RSM) is a statistical method that is useful for the optimization of chemical reactions and/or industrial processes and is widely used for experimental design. Whenever multiple system variables may influence the outputs, RSM can be utilized to assess the relationship between the dependent (response) and independent variables, as well as to optimize the relevant processes. The response surface methodology also was used to assemble a model in order to describe the way in which the variables are related and the way in which they influence the response (Azami *et al*., 2011).

Response surface methodology comprises a body of methods for exploring the optimum operating conditions through experimental methods. Typically, this involves several experiments and using the results of one experiment to provide direction for what to do next. The result of the experiment could lead on focusing the experiment around a different set of conditions, or collecting more data in the current experimental region in order to fit a higher order model or confirming what we seem to have found (Lenth, 2009).

The fundamental methods of response surface analysis involve fitting first order (linear) or second order (quadratic) functions of the predictors to one or more response variables, and then examining the characteristics of the fitted surface to decide what action is appropriate. It may seem like response surface analysis is simply a regression problem. However, there are several intricacies in this analysis and in how it is commonly used that are enough different from routine regression problems that some

special help is warranted. These intricacies include the common use (and importance) of coded predictor variables; the assessment of the fit; the different follow up analyses that are used depending on what type of model is fitted, as well as the outcome of the analysis; and the importance of visualizing the response surface (Lenth, 2009).

An important aspect of response surface analysis is using an appropriate coding transformation of the data. The way the data are coded affects the results of canonical analysis and steepest ascent analysis; for example, unless the scaling factors are all equal, the path of steepest ascent obtained by fitting a model to the raw predictor values will differ from the path obtained in the coded units, decoded to the original scale. Using a coding method that makes all coded variables in the experiment vary over the same range is a way of giving each predictor an equal share in potentially determining the steepest ascent path. Thus, coding is an important step in response surface analysis (Lenth, 2009).

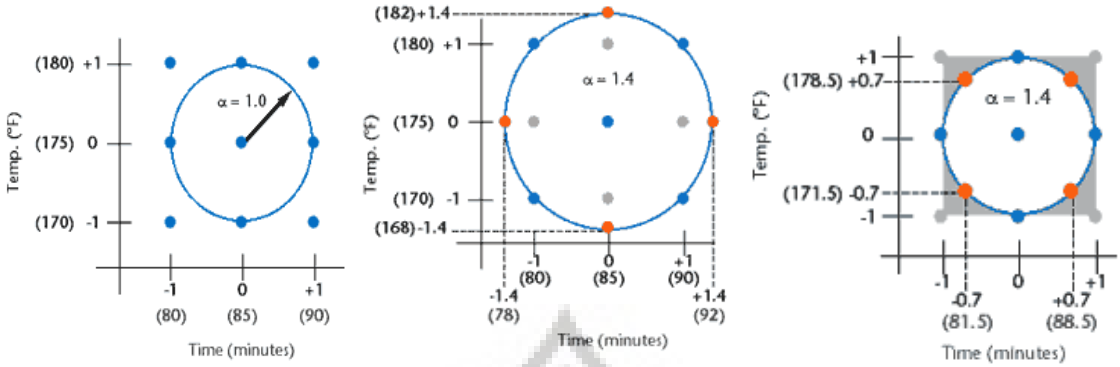
Most practitioners of RSM now generate their experiment designs and analyze their data using a statistical software program running on a personal computer. Many of these software programs can generate many classes of RSM designs and, in some cases, offer several varieties of each class. However, the central composite design (CCD) is the most popular of the many classes of RSM designs due to the following three properties:

- A CCD can be run sequentially. It can be naturally partitioned into two subsets of points; the first subset estimates linear and two-factor interaction effects while the second subset estimates curvature effects. The second subset need not be run when analysis of the data from the first subset points indicates the absence of significant curvature effects.
- CCDs are very efficient, providing much information on experiment variable effects and overall experimental error in a minimum number of required runs.
- CCDs are very flexible. The availability of several varieties of CCDs enables their use under different experimental regions of interest and operability.

(Verseput, 2000).

The blocks in a CCD are of two types one type, called a “cube” block, contains design points from a two level factorial or fractional factorial design, plus center points; the other type, called a “star” block, contains axis points plus center points. In the following discussion, the term “design points” refers to the non-center points in a block. The levels of the factors are coded, so that the cube blocks contain design points with coordinate values all equal to ±1, and center points at (0; 0; . . . ; 0). The design points in the star blocks are at positions of ±α along each coordinate axis. The value of α, and choices of replications of design points and center points, are often selected based on considerations of rotatability (i.e., the variance of the prediction depends only on the distance from the center) and orthogonality of blocks (so that the coefficients of the fitted response-surface equation are not correlated with block effects) (Lenth, 2009).

Three main varieties of CCD are available in most statistical software programs are face centered, rotatable and inscribed. In rotatable CCD, each experiment variable is represented at five levels with axial points (design points in the star blocks) of α. The α value is determined by $2^{k/4}$ which k is number of factor. The face centered CCD requires only three levels of each experiment variable since the axial points is α=1. This making it the simplest variety of CCD to carry out as well as the least prone to corruption due to sources of experimental error associated with setup and operation. The face centered CCD with extended axial points, five levels are required. The inscribed CCD also uses an α value of 1.4 to describe a circular geometric region. However, inscribing restricts the actual design region to the defined variable ranges by locating the axial points at the lower and upper bounds of the variable ranges. The factorial points are brought into the interior of the design space (inscribed) and set at a distance from the center point that preserves the proportional distance of the factorial points to the axial points (Verseput, 2000). The different between these three CCDs can be seen more clearly in Figure 3 below.



(a) (b) (c)

Figure 3. Two Variable Face Centered CCD (a), Rotatable CCD (b), Inscribed CCD (c) (Verseput, 2000).

1.3. Objective

The objective of this research is to figure out the optimal condition for boiling process of post-fermentation *nata de coco* in terms of water ratio, boiling time and boiling repetition by using response surface methodology (RSM).