4. DISCUSSION

4.1. Effect of Boiling to Quality of *Nata de Coco*

Nata de coco quality (thickness, hardness, and color) was measured before and after the boiling process. The initial thickness of *nata de coco* was about 1.452 cm and after the boiling process, the thickness was in the region of 1.8 - 2.2 cm. This result showed that boiling process is able to increase the thickness of *nata de coco*. The increase of *nata de coco* thickness after boiling is due to the swelling properties of *nata de coco*. According to George *et al*. (2005), *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, in this case is water, can cause swelling by affecting the amorphous and crystalline regions of the cellulose fibers.

The hardness of *nata de coco* is also affected by the boiling process. The result showed that after boiling, hardness of *nata de coco* was reduced from the initial hardness of 565.702 gf to 370-520 gf. The reduction of hardness after boiling was related to the swelling of *nata de coco*. As the individual cellulose fiber swells, intermolecular bonds that binding the fibers together, break as a result of the internal stress produced by swelling. This may contribute to the reduction in mechanical properties (George *et al*., 2005). The heat from boiling process may also contribute to the reduction in hardness of *nata de coco*. According to Surma-Slusarska *et al*. (2008), at a temperature of 80-140°C, the melting of the crystalline phase of cellulose takes place. In this experiment, the temperature of boiling process for all the combination of boiling condition was about 95-96°C. Therefore, it is suspected that melting of the crystalline phase of cellulose was also occurred in this experiment and may have caused reduction of hardness since the degree of crystallinity has a great influence on hardness.

The result of run 9-16 shown that the L* value, which is the function of lightness i.e., the apparent proportion of incident light reflected by an object was found to increase from 54.494 to around 61-63 after boiling. In the other boiling condition (run 1-8), the L* value is almost the same with the initial L* value. The degree of redness in *nata de coco* was indicated by a* value, while b* value gave the degree of yellowness. The initial *nata de coco* was associated with higher a* and b* values, due to the higher amount of red and yellow color materials in the membranes. The a* values of *nata de coco* were lower in all boiling process. The same trend with L* value is observed for the b* value. The boiling combination in run 1-8 didn't change the b* value, but the boiling combination in run 9-16 was found to slightly decrease b* value. This indicates that an optimal boiling condition is able to improve the quality of *nata de coco*, as it makes the *nata* color become lighter. According to George *et al*. (2005), the change of *nata de coco* color into white and glistening probably due to removal of coloured debris and impurities. As we known, the microbial cellulose obtained after fermentation is not pure; it contains some impurities like cells and/or the medium components (Chawla *et al*., 2009).

Different combination of water ratio, boiling time, and boiling repetition gives various responses. The highest thickness and hardness was obtained in boiling condition of *nata* and water ratio of 1:3, boiling time 7 minutes, and boiling repeated 3 times, while the lowest and hardness thickness was obtained in boiling condition of *nata* and water ratio of 1:4, boiling time 5 minutes, and boiling repeated 2 times. The highest L* value was reached in boiling condition of *nata* and water ratio of 1:5, boiling time 7 minutes, and boiling repeated 3 times, while lowest L* value was reached in boiling condition of *nata* and water ratio of 1:6, boiling time 5 minutes, and boiling repeated 4 times. Boiling condition of *nata* and water ratio of 1:5, boiling time 11 minutes, and boiling repeated 3 times resulted on the highest a* value, while boiling condition of *nata* and water ratio of 1:4, boiling time 5 minutes, and boiling repeated 4 times resulted on the lowest a* value. Boiling condition of *nata* and water ratio of 1:4, boiling time 5 minutes, and boiling repeated 4 times result on the highest b* value, while boiling condition of *nata* and water ratio of 1:5, boiling time 7 minutes, and boiling repeated 1 times resulted on the lowest b* value. Figure 8-11 exhibit that run 13-16 have a tendency in better *nata de coco* quality. This was in accordance with the result of the canonical analysis, in which critical value obtained was close to experimental range in run 13 and 15.

4.2. Model Estimation for Thickness of *Nata de Coco*

Table 5 shows that the regression for thickness was significant, which is desirable as it indicates that the terms in the equation model have a significant relationship on the response. Independent variable of boiling time (X_2) and boiling repetition (X_3), the squared variables of water ratio (X_1^2) and interaction of water ratio and boiling time (X_1X_2) are the significant model terms (shown in Table 4). The other model terms can be said to be not significant. Therefore, the empirical function for thickness (Y_1) can be described as the equation below.

$$Y_1 = 1.796 + 0.067x_2 + 0.102x_3 + 0.028{x_1}^2 - 0.016x_1x_2$$

The quadratic regression equation for the thickness of *nata de coco* yielded an R^2 of 0.153. The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The R^2 value always lied between 0 and 1. The closer R^2 value to 1.00, the stronger the model was and the better it predicted the response (Zakaria and Nazeri, 2012). This indicated that only 15.3% sample variation for thickness of *nata de coco* was attributed to the independent variables and 84.7% of the total variation cannot be explained by the model. This R^2 result is in accordance with the lack of fit test result. The lack of fit test result indicated that the thickness model is significantly lack of fit. Significant lack of fit indicates that there might be contributions in the regressor–response relationship that are not explained of by the model (Noordin *et al*., 2004).

4.3. Model Estimation for Color of *Nata de Coco*

P value in Table 5 indicates that L*, a*, and b* regression is not significant. This indicates that there are no functional relationship between L*, a*, and b* and water ratio, boiling time, and boiling repetition. The regression equation yielded an R^2 of 0.015 for L* value, an R^2 of 0.031 for a* value, and an R^2 of 0.038 for b* value. This indicated that almost 99% of the total variation cannot be explained by the L*, a* and b* model. The lack of fit test result indicated that the L*, a* and b* model is significantly lack of fit. The R^2 value and lack of fit test result emphasize that water ratio, boiling time, and boiling repetition doesn't have a significant relationship on L*, a* and b* value.

4.4. Model Estimation for Hardness of *Nata de Coco*

Table 5 shows that the regression for hardness was significant. This indicates that there are functional relationship between the terms in the equation model and the response. The squared variables of water ratio (X_1^2) and boiling time (X_2^2) are the significant model terms (shown in Table 4). The other model terms can be said to be not significant. Therefore, the empirical function for hardness (Y_5) can be described as the equation below.

$$Y_5 = 1{,}443.823 + 32.749{x_1}^2 + 5.058{x_2}^2$$

The quadratic regression equation for the hardness of *nata de coco* yielded an R^2 of 0.105. The model was not significantly lack of fit ($P > 0.1$). This means that 10.5% of the change of hardness was governed by the equation.

4.5. Response Surface Plot

The 3D response surface plot is a graphical representation of the regression equation. It is plotted to understand the interaction of the variables and locate the optimal level of each variable for maximal or minimal response. Each response surface plotted represents the different combinations of two test variables at one time while maintaining the other variable at the zero level. The convex response surfaces suggest that there are well-defined optimal variables. If the surfaces are rather symmetrical and flat, the optimized values may not vary widely from the single variable conditions (de Lima *et al.*, 2009). The surface plots of thickness, L* and b* take shape of twisted flat surface (Appendix 10-Appendix 12). This indicate that the range of the study was not able to find maximum or minimum thickness, L* and b* value. The surface plots of a* and hardness (Figure 12-Figure 17) take a convex shape. Therefore minimum a* value and hardness can be well-defined within the range of the study. This result was in accordance to the characteristic of the stationary points in the canonical analysis. The characteristic of stationary points for thickness, L*, and b* were a saddle point in which neither a maximum nor a minimum can be obtained, while the stationary points for hardness and a* were showing a minimum characteristic.

4.6. Selection of Optimal Boiling Condition

Canonical analysis result (Table 6) shows that there are different optimum boiling conditions for each response. The optimal hardness of *nata de coco* is predicted to be 2.085 for *nata* and water ratio of ≈1:7, boiling time ≈13 minutes, and boiling repeated ≈5 times. The optimal L*, a*, and b* value of *nata de coco* is predicted to be obtainable at *nata* and water ratio of ≈1:5, boiling time ≈7 minutes, and boiling repeated ≈3 times. The optimal hardness of *nata de coco* is predicted to be 367.849 gf for *nata* and water ratio of ≈1:5, boiling time ≈6.5 minutes, and boiling repeated ≈1 times.

The critical value of water ratio and boiling time obtained in this research is giving an improvement for the company in setting standardize of time and amount of water used in the boiling process. The critical value of boiling repetition in this research suggest that boiling process only need to be repeated 1 times for optimum hardness, 3 times for optimum color, and 5 times for optimum thickness. According to Palungkun (1996), the boiling process can be repeated if the nata is still having sour taste and smell. The repetition might not be needed if the soaking process was able to remove the sour taste and smell completely. According to the common practice in the company, the boiling process is repeated for 5-6 times until the pH is neutral. This indicates that the company actually can reduce the boiling repetition used from 5-6 times up to 1.

The optimal boiling conditions for all response that predicted in this research are possible to be implemented by the company. However, the optimal boiling condition should be picked by compromising the adequacy of the model. Therefore, the best model among the five response models is selected as the optimum condition for boiling of *nata de coco*. A good model must have a significant regression and the lack of fit must be insignificant. The various coefficient of determination, R^2 values should be close to 1 (Noordin *et al*., 2004).

In this research, the R^2 value for all responses is very low. Lack of fit test indicates that all the responses were significantly lack of fit at $P < 0.05$. Hardness was not significantly lack of fit at $P < 0.1$. The low R^2 value and significant lack of fit in this experiment may be occurred due to minimum number of center points used. When generating the experimental design, SYSTAT program asked how many center points desired with the criteria of minimum center point at 2. In this research, the center point was chosen at minimum level with the consideration of time and high number of run that must be done. According to Carlson (1992), the choice of number of center points depends entirely on desired properties of the design. Orthogonal designs minimize the average variance of prediction of the response surface equation. However, in some cases, it is more important to have the variance of predictions be nearly constant throughout most of the experimental region, even if the overall variance of predictions is increased somewhat. In such situations, the designs in which the variance of predictions is the same at the center of the design as it is at any point one unit distant from the center is preferable. This property of equal variance between the center of the design and points one unit from the center is called uniform precision. To achieve orthogonal designs, 9 replicates of center point should be used for 3 factors. If uniform precision want to be achieved, 6 replicates of center point should be used for 3 factors. Therefore, the minimum number of center point used in this experiment maybe is enough to generate a model by using SYSTAT but it is still insufficient to generate a good model.

In addition, it is important to note that R^2 is reflecting variation solely obtained from the sampled data. If the data exhibit an inflated error that does not represent the true level of variation present in the overall population (e.g. due to incorrect sampling techniques), R^2 can be low, while meaningful relationships may still exist (Colton and Bowe, 2002). *Nata de coco* sheet used in this experiment has uneven surface. According to Sanchez (2008), large fermentation trays did not provide the right tension to hold the *nata* firmly along the side of the trays. The middle portions of pellicle formed tended to sink, thus becoming thinner than the portion attached to the sides of the tray. Uneven initial thickness may affect the model of boiling effect. Therefore, an incorrect sampling technique due to the using of uneven initial thickness of *nata* might be also contributing in the low R^2 value.

Colton and Bower (2002) stated that the R^2 statistic can be small, yet one or more of the regression coefficient p-values can be statistically significant. Such a relationship between predictors and the response may be very important, even though it may not explain a large amount of variation in the response. Therefore, low R^2 value in all responses model was considered to be fine as long as the model gives a significant regression. From all of responses model, thickness and hardness model were the one that fulfilling those requirement. However, since all of the response models except for hardness were lack of fit, it was decided to select the optimum boiling conditions for optimizing hardness. Hardness of *nata de coco* was predicted to be 367.849 gf for *nata* and water ratio of ≈1:5, boiling time ≈6.5 minutes, and boiling repeated ≈1 times.