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Utilisation of whey waste as a substrate for making nata de whey

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KEYWORDS

Acetobacter xylinum
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ABSTRACT

Increasing in demand and imports of cheese is parallel to an increase in cheese production annually. High cheese production is directly proportional to generation of whey. Whey is considered as wastewater and usually directly disposed to environment causing a detrimental impact such as water pollution. Whey contains 55% of dairy protein and is potential to be used for nata seed growth. Therefore, it is necessary to valorise whey into a high value-added product. This research aimed to use nutrient-rich whey wastewater as a medium of development for *Acetobacter xylinum* bacteria in the production of nata de whey, as well as to investigate the effect of of sucrose addition to the characteristics of nata de whey. The research design used was Randomized Block Design (RBD) with 2 factors of *Acetobacter xylinum* (inoculum) concentration (i.e. 5, 10, and 15%) and sucrose concentration (i.e. 3, 4, and 5%). The results showed that the treatment with addition of 10% inoculum and 4% sucrose produced the nata de whey with superior quality. The resulted nata de whey has pH of 3.2 ± 0.13, total sugar of 4.24 ± 1.11%, total acid of 1.67 ± 0.08%, yield of 84.41 ± 7.27%, thickness of 1.345 ± 0.18 mm, and moisture content of 83.4 ± 1.97%, respectively.

Introduction

Annually, demand for cheese is increasing (Kamarudin et al., 2013), therefore cheese production has also continued to increase to 126.77 tonnes per year since 2009 (Nurul, 2009). However, the production of cheese generates about 800 tonnes of whey (Kongruang, 2008). Whey is a non-clotting liquid produced from cheese processing and has high volume than curd with the ratio of 1:1 (curd: whey) (Kongruang, 2008; Bono et al., 2009). Whey, as wastewater, is usually directly disposed to environment, thus causing water pollution (Kamarudin et al., 2013). This is because, whey contains biochemical oxygen demand (BOD) value of 30,000-50,000 mg/g (Coban and Biyik, 2011). Whey also contains 6.5% solids, composed of 4.8% lactose, 0.6% protein, 0.6% mineral, 0.1% lactic acid, 0.2% non-protein soil, and 0.1% fat (Kongruang, 2008); beneficial for microorganism growth (Kamarudin et al., 2013); and nata seedlings development (Surma-Ślusarska et al., 2008).

Nata is produced by substrate fermentation at pH 4-4.5 through the addition of sucrose as a carbon source for bacterium *Acetobacter xylinum* (Upadhyay et al., 2010; Coban and Biyik, 2011). This fermentation results in the white cellulose layer floating on the surface of the liquid substrate media known as nata (Sumiyati, 2009). Nata is a high-fiber, low-calorie food with 98% water (Kamarudin et al., 2013). In making nata de whey, some sucrose is converted into nata and some portion is degraded into acetic acid, causing the pH to decrease until reaches the optimum pH of the substrate for the development of *Acetobacter xylinum* (Suwannapinunt et al., 2007; Tomita and Kondo, 2009) to create nata (Surma-Ślusarska et al., 2008). Because whey contains about 55% of complete dairy consumption (Kongruang, 2008); it is anticipated that nata de whey can provide economically balanced dietary intake for lower to middle class society.

Several factors are necessary in producing nata de whey include the addition of *Acetobacter xylinum* and inoculum saccharose. The inoculum is added as an *Acetobacter xylinum* starter. Starters are microbial populations prepared to be inoculated on fermentation media (Upadhyay et al., 2010). The fresh starter can be used up to 6 days after inoculation, but it cannot be used after more than 9 days of inoculation. In making nata, the volume of

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starter added should be more than 5% of the volume of media (Tomita and Kondo, 2009). While, sucrose is one of the most potential types of sugar for fermentation and used as a carbon source by *Acetobacter xylinum* for cellulose production (Pae et al., 2011). The addition of excess sucrose causes bacterial cell plasmolysis and drastically lowers pH (Upadhyay et al., 2010). While, lack of sucrose can inhibit the normal growth of bacteria, thus limiting the production of nata. The addition of sucrose in fermentation should be in the range of 5-10% (Suwannapinunt et al., 2007; Pae et al., 2011).

This research aimed to use nutrient-rich whey wastewater as a medium of development for *Acetobacter xylinum* bacteria in the production of nata de whey, as well as to investigate the effect of of sucrose addition to the characteristics of nata de whey. The use of whey wastewater can increase the economic value of whey and to reduce the wastewater pollution induced by whey.

Research Methods

Materials

Materials used in this study include *Acetobacter xylinum*, sucrose, yeast extract, K_2HPO_4, $(NH_4)_2SO_4$, $MgSO_4$, agar, mozzarella cheese whey waste (Junrejo sub-district KUD Batu City), nata starter (obtained from Muhammadiyah Malang University), and acetic acid 25 % to set pH to 3-4.

Experimental set-up

The research design used was Randomized Block Design (RBD) arranged with 2 factors. Factor I was *Acetobacter xylinum* concentration (later indicated as inoculum), which consisted of 3 levels (i.e. 5, 10, and 15%). Factor II was sucrose concentration consisted of 3 levels (i.e. 3, 4, and 5%). All treatment combinations were carried out in triplicate.

Fermentation

The procedures for fermentation was based on Suwannapinunt et al. (2007). A total of 100 mL whey was mixed with various sucrose concentration as determined above, 0.5% of K_2HPO_4, 0.05% of $(NH_4)_2SO_4$, 0,05% of $MgSO_4$, and 2% of acetic acid. The solution was then heated to a temperature of 80°C and continued to cool down until reached temperature of 30°C. The fermentation process was carried out by mixing whey medium with beads containing cells of *Acetobacter xylinum* at determined concentrations. The fermentation was done in a closed container for 14 days.

Physical characteristics analysis

Thickness analysis was carried out based on Upadhyay et al. (2010), in which nata was drained for 5 minutes, following the measurement using a caliper. Yield analysis was based on Suwannapinunt et al. (2007), in which cellulose pellicle was drained for 10 minutes, then weighed. The yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{cellulose\ (g)}{medium\ (g)} \text{ x } 100\% \quad \text{..... (1)}$$

Elasticity analysis was based on the procedures described in Nurul (2009). The elasticity is expressed in units of gram force (gf). The values obtained were the average value of five different measurement. The calculation was based on the formula (2), as follows:

$$\text{Elasticity} = \frac{\text{average measurement result x}\frac{1}{10}\,(mm)}{\text{load weight (g) x test time (sec)}} \quad \text{...(2)}$$

Moisture content (MC) analysis was carried out based on AOAC (1995). The calculation of moisture content is using the following formula:

$$MC\ (\%) = \frac{(initial\ weight - final\ weight)}{sample\ weight}\ x\ 100\% \text{ .....(3)}$$

Chemical characteristics analysis

pH value was measured using the pH meter, in which the pH probe was previously calibrated before use (AOAC, 1995). Total sugar was measured using a spectrophotometry method, using wave length of 630 nm (AOAC, 1995). Total titrated acid analysis was carried out based on the titrimetric method described in Tomita and Kondo (2009). The total acid calculated using formula (4), as follows:

$$\text{Total titrated acid} = \frac{V1\ x\ N\ x\ B}{V2\ x\ 1000} \text{ x } 100\% \text{ x D .....(4)}$$

Where V1 is the volume of NaOH (in mL), V2 is volume of the sample (in mL), N is normality of NaOH, B is molecular weight of acetic acid (60) and D is dilution factor of the sample.

Hedonic Analysis

The preference of the nata was carried out using Hedonic scoring method, modified from Upadhyay et al. (2010). In this test, 25 semi-trained panelists were participated. Each panelist was required to evaluate appearance, taste, color and aroma of nata de whey. The score used was a score of 1-7, where 1 (very disliked) and 7 (very liked). The results from Hedonic test was then statistically analysed.

Data Analysis

The statistical analysis in this study was using variant analysis (ANOVA) followed by a real difference test known as Least Significance Different (LSD) at confidence level of 0.01% and 0.05% and Duncan Multiple Range Test (DMRT) at confidence level of 0.01% and 0.05%. Organoleptic test (i.e. Hedonic test) was analysed using Friedman test both at confidence level of $\alpha = 0.05$ and $\alpha = 0.01$.

Results and Discussion

Physical characteristics of nata de whey

a. Yield

Based on Table 1, the highest yield was obtained from treatment combination of 10% inoculum and 4% sucrose, with the value of 84.41%. According to Upadhyay et al. (2010), an increase in cellulose production is strongly linked to *Acetobacter xylinum* activity. However, Novianti (2008) claimed that addition of sucrose in the fermentation medium affected the development of *Acetobacter xylinum.* Kamarudin et al. (2013) stated that adding sucrose at the limit of the optimum concentration may maximise the output generated. In this study, it is therefore, the concentration of 10% inoculum and 4% sucrose was selected as ideal treatment due to its highest cellulose production.

A surface area is another factor that impacts the yield in fermentation (Tomita and Kondo, 2009). *Acetobacter xylinum,* as facultative aerobic bacteria, grows on the surface of the medium to get the oxygen. The ANOVA analysis indicated that the concentration of inoculum was statistically significant at $\alpha = 0.01$, while the concentration of sucrose was statistically significant at $\alpha = 0.05$ on the yield of nata de whey.

b. Thickness

Table 1 also shows that treatment with 10% inoculum and 4% sucrose generated nata de whey with the highest thicknes. According to Surma-Ślusarska et al. (2008), nata density is the quantity of sucrose converted into cellulose by *Acetobacter xylinum.* The ideal addition of sucrose lead to the highest thickness of nata. This was potentially due to during the conversion of sucrose to cellulose by *Acetobacter xylinum*, networks of cellulose threads with polysaccharide was continuously formed, thus thickening the nata layer (Sumiyati, 2009).

In line with this study, Suwannapinunt et al. (2007) stated that concentration of sucrose that are too high or too low may inhibit activity of *Acetobacter xylinum* in forming the cellulose. The size of the nata generated during fermentation is directly proportional to the yield. In this case, an increase in the yield of nata causing an increase in the nata's thickness. The ANOVA results showed that the concentration of inoculum had a very significant effect ($\alpha = 0.01$), while the concentration of sucrose had a significant effect ($\alpha = 0.05$) on the thickness of the nata de whey.

Table 1. Yield, thickness, elasticity and moisture content of nata de whey (in average values)

Sample	Yield (%)	Thickness (mm)	Elasticity (gf)	Moisture Content (%)
Control	64.42±0.02	0.81±0.01	20.7±0.02	89.78±0.07
Inoculum 5%, sucrose 3%	58.90±0.02	1.01±0.01	33.0±0.06	87.55±0.04
Inoculum 5%, sucrose 4%	67.73±0.03	1.22±0.03	32.73±0.07	85.88±0.07
Inoculum 5%, sucrose 5%	55.33±0.02	0.75±0.01	15.33±0.02	88.38±0.06
Inoculum 10%, sucrose 3%	72.84±0.04	1.10±0.02	24.63±0.05	88.51±0.08
Inoculum 10%, sucrose 4%	84.41±0 .04	1.34±0.01	49.30±0.07	87.86±0.04
Inoculum 10%, sucrose 5%	74.25±0.05	1.07±0.03	26.43±0.05	89.47±0.07
Inoculum 15%, sucrose 3%	75.10±0.04	1.22±0.02	37.8±0.06	88.62±0.04
Inoculum 15%, sucrose 4%	70.03±0.05	1.15±0.01	36.7±0.07	83.42±0.06
Inoculum 15%, sucrose 5%	67.36±0.03	1.32±0.03	34.7±0.08	85.13±0.07

Notes: Values in average ± standard deviation from 3 replications

c. Elasticity

Table 1 shows that the highest elasticity was also obtained from treatment with 10% inoculum and 4% sucrose, giving the value of 49.3 gf. According to Pae et al. (2011), the elasticity of nata is influenced by the density of the nata, the compactness between the nata layers, and the formation of cellulose. The elasticity generated increases as an increase in the thickness of the cellulose formed. This demonstrates that the chewy texture of the nata is possibly due to the tightness of the cellulose matrix, thus preventing water to enter the cavities of the pellicle, as well as making the texture of the nata to be more elastic.

Nurul (2009) strengthens this result by stating that increasing the concentration of sucrose and the fermentation duration causes an increase in the elasticity of the nata. A high texture value indicates a smooth texture of the product, while a low texture value shows a rough texture of the product. The ANOVA results also indicates that the inoculum concentration had a substantial impact ($\alpha = 0.05$), while the concentration of sucrose had a very important impact ($\alpha = 0.01$) on the elasticity of the nata de whey.

d. Moisture content

The highest moisture content of the nata de whey, based on Table 1, was obtained from treatment with 10% inoculum and 4% sucrose. According to Tomita and Kondo (2009), nata's moisture content is influenced by the density of the nata, the compactness between nata's layers, and the cellulose structure formed. Cellulose's comparatively elevated moisture content is due to the cluster of hydroxyl cellulose that may bind to groups of water hydrogen groups (Coban and Biyik, 2011). In addition, increasing the sucrose concentration correlates with increasing the thickness of the nata, as well as increasing the water content trapped in the nata. The ANOVA results demonstrates that the concentration of inoculum and sucrose did not influence the moisture content of nata de whey ($\alpha= 0.05$).

Chemical characteristics of nata de whey

a. PH value

The pH measurement before and after fermentation was to evaluate pH modifications during fermentation (Sumiyati, 2009). The optimum pH value was observed at treatment with 10% inoculum and 4% sucrose (Table 2). According to Bono et al. (2009) and Novianti (2008), the enzyme activity generated by *Acetobacter xylinum* bacteria functions optimally at pH 3.5-5.5, in which the optimum pH for nata formation is 4. Reduction in pH was due to the formation of acetic acid by acetic acid bacteria during fermentation. The release of H^+ ions from acetic acid and other organic acids contribute to a decrease in the pH value. According to Upadhyay et al. (2010), during fermentation, not only cellulose but also acetic acid is produced by *Acetobacter xylinum*.

Acetic acid accumulation reduces the pH of the medium. In the catabolic phase of *Acetobacter xylinum*, acid accumulation promotes glycolysis and krebs cycles, thus oxidation and fermentation can operate optimally. Such condition also promotes the development of Adenosine triphosphate (ATP) as an energy in cellulose synthesis chains formation (Coban and Biyik, 2011). In nata formation, the mechanism of transforming sucrose to glucose and fructose using invertase enzymes also requires acidic circumstances. The ANOVA results shows that that both inoculum and sucrose concentration had no significant effect on the pH of nata de whey ($\alpha= 0.05$).

Table 2. Total sugar, pH, and total titrated acid before and after fermentation (in average values)

Sample	Total Sugar (%)		pH		Total Titrated Acid (%)	
	Before	**After**	**Before**	**After**	**Before**	**After**
Control	6.91±0.05	0.72±0.02	3.26±0.04	2.58±0.03	0.99±0.02	0.57±0.01
Inoculum 5%, sucrose 3%	8.54±0.05	4.40±0.04	3.81±0.04	3.67±0.05	0.96±0.01	1.67±0.05
Inoculum 5%, sucrose 4%	8.04±0.04	5.31±0.04	3.83±0.04	3.35±0.01	0.92±0.04	1.68±0.03
Inoculum 5%, sucrose 5%	9.04±0.03	4.24±0.05	3.87±0.03	3.z35±0.05	0.96±0.02	1.49±0.01
Inoculum 10%, sucrose 3%	6.78±0.05	5.22±0.03	3.82±0.04	3.37±0.02	1.18±0.02	1.54±0.05
Inoculum 10%, sucrose 4%	8.74±0.05	6.32±0.05	3.80±0.04	3.20±0.04	1.27±0.02	1.47±0.04
Inoculum 10%, sucrose 5%	8.66±0.05	5.89±0.03	3.80±0.03	3.25±0.04	1.34±0.02	1.48±0.03
Inoculum 15%, sucrose 3%	8.44±0.03	6.35±0.04	3.78±0.02	3.31±0.02	1.21±0.01	1.54±0.04
Inoculum 15%, sucrose 4%	7.86±0.05	7.19±0.03	3.77±0.05	3.33±0.03	1.02±0.04	1.56±0.03
Inoculum 15%, sucrose 5%	7.23±0.02	6.01±0.03	3.75±0.03	3.42±0.02	1.08±0.03	1.58±0.04

Notes: Values in average ± standard deviation from 3 replications

b. Total Titrated Acid

Measurement of total titrated acid before and after fermentation was to evaluate changes in the total acid value during fermentation (Sumiyati, 2009). Based on Table 2, the total titrated acid after fermentation was higher than that of before fermentation. An increase in the concentration of inoculum and sucrose, causing an increase in the total acid concentration. Bono et al. (2009) and Sumiyati (2009) stated that sucrose is hydrolysed to pyruvic acid by bacteria, which is then synthesised into acetic acid. Therefore, increasing the sucrose concentration

may results higher total acid. Furthermore, Kongruang (2008) added that a more optimum ratio of the bacterial inoculum with sucrose concentration increases the acetic acid produced, thus higher the total acid concentration was generated.

However, the concentration of the total titrated acid from the selected treatment was not parallel to the pH value. This study was in agreement with the finding from previous study (Habibillah, 2009). The measured pH value is the concentration of H^+ ions in the form of dissociated acids, while the total titrated acid measures total acid components both in dissociated or not dissociated froms. The ANOVA results indicated that both inoculum and sucrose concentration have no significantly influence on the total titrated acid of nata de whey ($\alpha = 0.05$).

c. Total sugar

Total sugar measurement before and after fermentation was to assess the presence of and the use of sugar by *Acetobacter xylinum* during fermentation (Bono et al., 2009). Based on Table 2, increasing the concentration of inoculum and sucrose increases the average total sugar. This study is in line with Novianti (2008) who reported that total sugar increases with an increase in the concentration of inoculum. The findings also indicated that an increase in concentration of inoculum was inversely proportional to an increase in bacterial activity which hydrolyses glucose and fructose to sucrose. Acetic acid bacteria, however, have an optimal limitation on sugar consumption as a carbon source during the fermentation process. Thus, all added sucrose was not completely converted to acetic acid. The remaining sugar was considered as total sugar.

The total sugar concentration after fermentation tends to be lower than the initial total sugar. This indicates that *Acetobacter xylinum* consumed most of the sucrose added to produce nata cellulose (Nurul, 2009). Furthermore, the concentration of sucrose also affected the total sugar. This is possibly due to *Acetobacter xylinum* polymerises glucose from a sugar solution into cellulose on outside the cell. Thus, addition of sucrose increases the amount of cellulose layers (fibre) formed by *Acetobacter xylinum* and forms additional oxidation of oxidising acetic acid to CO_2 and H_2O (Nurul, 2009; Bono et al., 2009). The ANOVA result shows that both inoculum and sucrose concentration had a very significant effect on total sugar of nata de whey ($\alpha = 0.01$).

Hedonic test results

a. Flavour

The flavour of food is frequently affected by the ratio of sugars and acids added (Sumiyati, 2009). In addition, organic acids are formed in the milk fermentation process which can strengthen flavour. Figure 1 shows that there was no obvious difference in the acceptance of product flavour between treatments. However, the panelists preferred flavour was from data de whey produced from fermentation with 10% inoculum and 4% sucrose.This is because variations in supplying the concentration of inoculum produce uniform cellulose nata, thus the acceptance of the product's flavour tends to be the same. While, nata with an addition of 4% sucrose is preferred by panelists because the level of sweetness is acceptable. This finding is in accordance with Sumiyati's statement (2009) that panelists prefer nata products with not acidic and neutral flavour. Therefore, it is necessary to handle nata products before consumption by soaking, boiling, adding sugar water, or adding flavors (e.g. vanilla, cinnamon, etc.). The ANOVA results also showed that both inoculum and sucrose concentration did not considerably influence the flavor acceptance of nata de whey ($\alpha = 0.05$).

b. Color

Color is the first parameter to determine product's level of customer acceptance. The more interesting color of a food product causes a greater customer's acceptance level. Figure 1 shows that there was no obvious difference in the acceptance of product color from all treatments. This is because all products produced tend to have identical colors. Such condition was possibly due to the variation in the inoculum added during fermentation contributes to the production of standardised metabolites by *Acetobacter xylinum*.

The addition of sucrose at different concentrations did not influence the color of nata de whey. Sucrose was used only as a source of carbon to produce organic acids. Generally, cellulose in nata has a white gelatinous appearance, and the color changes to be more transparent after immersion treatment of boiling in sugar water. All panelists preferred the white colour of the nata, therefore, no color deviation was observed from nata de whey. The ANOVA results showed that inoculum and sucrose had no significant effect on the colour acceptance of nata de whey ($\alpha = 0.05$).

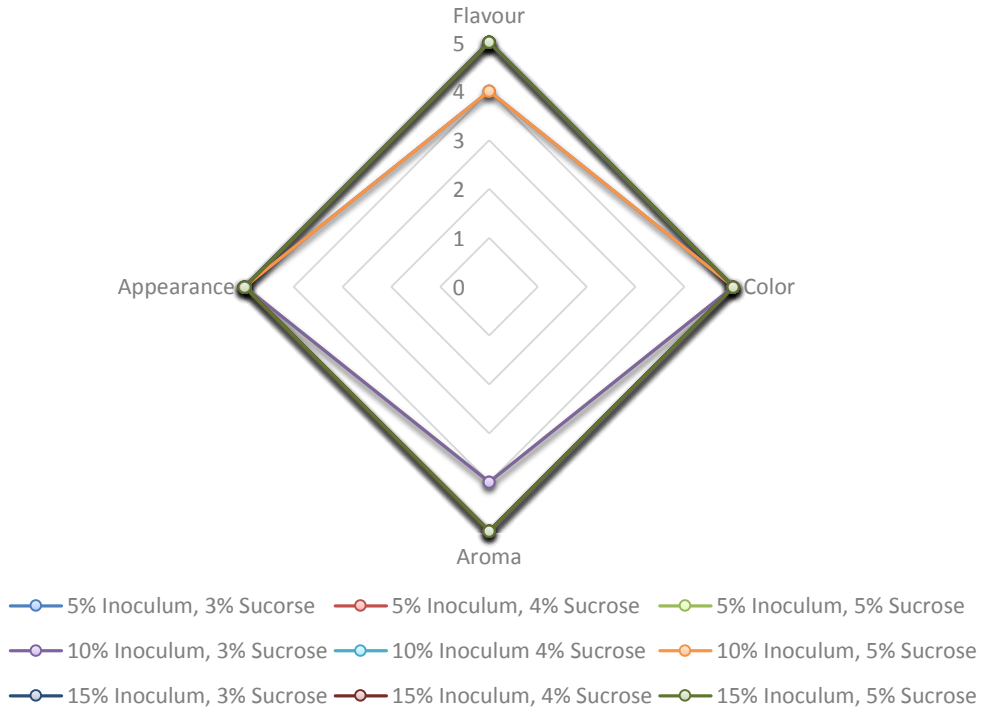

Figure 1. Spider chart of hedonic analysis

c. *Aroma*

Aroma is a parameter that influences the quality of any processed products. The aroma of food affects the perception of the food's delicacy. Figure 1 indicates that with the addition of higher inoculum and sucrose concentration, the panellists were tended to prefer the nata product. This was due to a correlation between taste and aroma of food. Increasing sucrose concentration also enhances the product's aroma.

The findings also demonstrated that an increase in sweetness of products could offer aroma preferred by panellists. The typical sour aroma of nata de whey is produced during fermentation in the form of volatile compounds and acetic acid (Surma-Ślusarska et al., 2008). Acetic acid also provides a unique aroma of nata de whey along with its role in the formation of cellulose nata.

d. Appearance

Appearance is one of the primary attributes that customers can recognize. The objective of this appearance analysis is to determine the acceptance of the panelists evaluated by the appearance of the nata de whey product surface, integrity, texture, and shape. Figure 1 shows that there was no obvious difference from all treatments in accepting the product appearance. This is because the nata produced tend to have similar attributes each other. The appearance of the expected nata de whey is square blocks with a distinctive elasticity and a small amount of fibrous texture. In this study, the panellists were able to distinguish the texture of nata based on elasticity, as previous study revealed that panellists prefered the thick nata texture (Coban and Biyik, 2011).

The texture of nata is depended on the MC value, in which increasing MC increases the texture value. This is in accordance with Surma-Ślusarska et al. (2008), who stated that nata is an *Acetobacter xylinum* fermented food product in the form of a mixture of cellulose-based biomass and has a white gelatinous appearance. The ANOVA showed that both inoculum and sucrose concentration did not significantly influence the appearance acceptance of nata de whey (α= 0.05).

Conclusions

Concentrations of inoculum and sucrose had a significant effect on yield and thickness of nata (α= 0.05) and had a very significant effect on total sugar, yield, thickness, and elasticity of nata (α= 0.01). Treatment with addition 10% inoculum and 4% sucrose was selected as best treatment due to superior quality of nata de whey. The best treatment has characteristics of pH (3.2 ± 0.13), total sugar (4.24 ± 1.11%), total acid (1.67 ± 0.08%), yield (84.41 ± 7.27%), thickness (1.345 ± 0.18 mm), and moisture content (83.4 ± 1.97%), respectively.

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Conflict of interest

Authors declare that there is no conflict of interest.

References

AOAC (Analysis of The Association of Analytical Chemists). (1995) *Official Methods of Analysis of The Associationof Analytical Chemists*, Washington D.C

Bono, A., Ying, P.H., Yan, F.Y., Muei, C.L., Sarbatly, R., and Krishnaiah, D. (2009) 'Synthesis and characterization of carboxymethyl cellulose from palm kernal cake', *Advances in Natural and Applied Sciences*, 3(1), pp. 5-11

Coban, E.P., and Biyik, H. (2011) 'Effect of various carbon and nitrogen sources on BC synthesis by *Acetobacter iovaniensis* HBB5', *African Journal Biotechnology*, 10, pp. 5346-5354

Kamarudin, S., Kalil, M.S., Takriff, M.S., Wan Yussof, W.M., Dayang Radiah A.B., and Norhasliza, H. (2013) 'Different media formulation of biocellulose production by *Acetobacter xylinum* (0416)', *Pertanika Journal Science and Technology*, 21, pp. 29-36

Kongruang, S. (2008) 'Bacterial BC production by *Acetobacter xylinum* strains from agricultural waste products', *Applied Biochemical Biotechnology*, 148, pp. 245-256

Novianti, M.M. (2008) 'Kualiatas mikrobiologis *granul effervescent whey* bubuk yang diperkaya sinbiotik dengan penambahan *effervescent mix* yang berbeda selama penyimpanan', *Undergraduate Thesis*, Institut Pertanian Bogor, Bogor.[In Indonesian]

Nurul. (2009) 'Studi pembuatan *nata de coco* dari tiga jenis air kelapa dengan tiga jenis gula terhadap produksi *nata de coco*', *Undergraduate Thesis*, Universitas Andalas, Padang [In Indonesian]

Pae, N., Zahan, K.A., and Muhamad,. II. (2011) 'Production of biopolymer from *Acetobacter xylinum* using different fermentation methods', *International Journal Engineering Technology*, 11, pp. 90-98Sumiyati. (2009) 'Kualitas nata de cassava limbah cair tapioka dengan penambahan gula pasir dan lama fermentasi yang berbeda', *Undergraduate Thesis*, Universitas Muhammadiyah Surakarta. [In Indonesian]

Surma-Ślusarska, B., Presler, S., and Danielewicz, D. (2008) 'Characteristics of bacterial cellulose obtained from *Acetobacter xylinum* culture for application in papermaking', *Fibres and Textile in Eastern Europe*, 16(69), pp. 108-111

Suwannapinunt, N., Burakorn, J., and Thaenthanee, S. (2007.) 'Effect of culture conditions on bacterial BC (BC) production from *Acetobacter xylinum* TISTR976 and physical properties of BC parchment paper', *Journal Science and Technology*, 14, pp. 357-365

Tomita, Y., and Kondo, T. (2009) 'Influential factors to enhance the moving rate of *Acetobacter xylinum* due to its nanofiber secretion on oriented templates', *Carbohydrate Polymers*, 77, pp. 754-759

Upadhyay, A, Lama, J.P., and Tawata, S. (2010) 'Utilization of pineapple waste: a review', *Journal Food Science Technology Nepal*, 6, pp. 10-18