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Effect of starter sources and old fermentation on making nata de whey towards chemical quality

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Abstract. Whey cheese is a by-product of traditional or modern cheese-making processes. The yield of each cheesemaking reaches around 83% of the volume of milk used.whey can be processed into nata de whey by adding *Acetobacterxylinum*. The purpose of this study were to determine the effect of fermentation duration and differences in starter sources on the chemical quality produced by nata de whey. All samples were analyzed for fat, protein, cellulose and reducing sugars. this use study of two factorial randomized block design. The first factor is the difference in the starter (commercial and pineapple fruit extract) and the second factor is the length of fermentation with three replications. The results showed that the effect of fermentation time and the difference in starter sources on nata de whey had a very significant impact (P <0.01) on the calculation of fat content in the nata the whey. The duration of fermentation and differences in the source of the stater on nata de whey had no significant effect (P> 0.05) on the content of protein, reducing sugar and cellulose content produced.

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1. Introduction

Whey cheese is a by-product of traditional or modern cheese making. Whey cheese is generally in the form of a clear liquid that is greenish yellow (1). Whey cheese is obtained from the screening process and pressing curd during the cheese making process. Whey produced in each cheesemaking reaches around 83% of the volume of milk used. Whey cheese produced when the company provides 1 kilogram of cheese from 10 liters of milk will yield about 8-9 liters of whey (2)

Whey in Indonesia is generally used as animal feed and as food additives for food products. Whey was used as an additive to food products such as making sweets, ready-to-drink drinks, smoothies, substitutes for flour, cereals, ice cream, bread, and other products by utilizing the functional properties of the whey cheese (3). The use of whey is not only used for food additives, but also other bioactive components can also be taken. Several multifunctional bioactive ingredients in whey fermentation such as organic acids (lactic acid), active peptides and proteins that act as antimicrobials, antioxidants, and immune system boosters (4). Another use of whey cheese is to make whey cheese into a fermented product, for example, the addition of *Acetobacterxylinum* with functional food products, namely nata de whey.

Nata de whey is a type of fermented food by the bacterium *Acetobacterxylinum*. The bacterium *Acetobacterxylinum* is grown in liquid media containing sugar, it will produce acetic acid and a white layer floating on the surface of the liquid media (5). The media for making nata de whey must contain sugar as a

source of carbon and the source of N as the substrate for the growth of the bacterium *Acetobacterxylinum* (6). Starter nata de whey can not only be obtained commercially but can also be achieved naturally, for example, the making of starter seeds of *Acetobacterxylinum* from pineapple. Pineapple can be used as a medium for the growth of *Acetobacterxylinum* because it has water, carbohydrates and minerals in the substrate as nutrients and not all nutrients in the substrate can be fulfilled, so additional nutrients are given in the form of sucrose (carbon) and urea (nitrogen) (7). The addition of inorganic or organic nitrogen sources will increase the activity of *Acetobacterxylinum*in producing nata de whey. The formation of nata can be influenced by the length of fermentation, where the optimum range of fermentation will provide the best quality (8). Nata de whey is very well consumed by people who have a low-calorie diet or high fiber diet and high water content in nata can also function to facilitate the body's metabolic processes because the fiber nata de whey in the human body will bind all the remaining residual combustion elements that are not absorbed by the body, then thrown through the anus in the form of feces or bolus (9).

This study uses raw materials produced from cheese waste (whey). the manufacturing of nata de whey is assisted by the commercial bacteria Acetobacter xylinum starter and the starter is pineapple juice. besides using two different source sources, timefermentation can be divided into 3 namely 8 days, 11 days and 14 days, this is to see the best treatment for making nata de whey on chemical quality.

2. Material and methods

Are whey, *Acetobacterxylinum*, food grade NPK, sugar, glacial acetic acid, 0.1N NaOH, concentrated H_2SO_4, phenolphthalein indicator, aquades, Pb acetate, $Na_2S_2O_3$, concentrated H_2SO_4, distilled water, and buffer solution. The experimental design used was a factorial two completely randomized design (CRD). The research parameters are the content of fat, protein, reducing sugars and cellulose in nata. will be analyzed using factorial completely randomized design with three replications.

3. Results and Discussion

The physicochemicalquality observed in this study included protein, fat, production sugar and cellulose content. Different fermentation treatments and starter sources in nata de whey had no significant effect (Fhit> Ft. 5%) on the testing of protein, reducing sugar and cellulose in each treatment and were significantly different (Fhit>Ftab 0.05%) in the assay fat. The average chemical quality of nata de whey can be seen in the Table 1.

Table 1. Average Physicochemical Quality of Nata De Whey

Variabel	Media	Fermentation		
		W8	W11	W14
Protein	P_0	2,54±013	2,70±0,55	2,70±0,10
	P_1	2,26±0,10	2,41±0,04	2,42±0,06
Fat	P_0	$0,44±0,02^{a}$	$0,41±0,02^{ab}$	$0,41±0,00^{ab}$
	P_1	$0,39±0,00^{b}$	$0,42±0,02^{ab}$	$0,41±0,00^{ab}$
Reducing Sugar	P_0	0,58±0,00	0,56±0,00	0,54±0,00
	P_1	0,54±0,00	0,53±0,00	0,52±0,00
Cellulose	P_0	0,34±0,00	0,36±0,00	0,35±0,00
	P_1	0,41±0,00	0,41±0,00	0,42±0,00
		0,38±0,04	0,38±0,03	0,38±0,01

Note: Different superscript on the same line show statistically significant in the test level of 1%. P0 = 15% commercial whey + *Acetobacterxylinum*, P1 = whey + *Acetobacterxylinum* 15% from pineapple juice), W8 = 8 days storage, W11 = 11 days storage time and W14 = 14 days long storage time

3.1 Protein Levels

The results of the analysis of variance showed different fermentation time and source of starter in nata de whey had no significant effect (P> 0.05%) on the protein content of nata de whey. The duration of fermentation is having a significant impact on the treatment of different starter sources, while the interaction between the length of fermentation and various sources of nata has a significant effect as shown in the table. The treatment of P0 (15% commercial whey + *Acetobacterxylinum*) differs very significantly or higher compared to treatment P1 (whey + *Acetobacterxylinum* 15% from pineapple juice) because protein is a vital food substance for the body which is composed of amino acids and contains elements main elements such as C, O, H, and N. The amount of C content is 52.40%, O is 21-23.50%, H is 6.90-7.30%, N is 15.30-18%, besides that, the protein contains P, Fe, and Cu. Thus, one of the most important and quite specific ways to determine the quantity of protein quantitatively is to determine the N content in food ingredients or other ingredients such as nata de whey.

The treatments carried out in this study have a different protein content, both from treatment and repetition. The content of organic chemicals contained in the media making different nata de whey is one factor in the difference in the amount of protein content in nata de whey. The number of N sources in the starter growth medium of the bacterium *Acetobacterxylinum* affects the amount, content, and protein content of nata de whey (10). The average protein content in treatment P0 is 2.54 to 2.70 while in treatment P1 is 2.26 to 2.42.It is due to the number of organic chemicals contained in the media used, both cheese liquid waste and starter (commercial and pineapple juice) are not homogeneous. From the average protein content that is owned by nata de whey shows the amount of substance of organic chemicals found in the media of making nata de whey is in the treatment P0 and P1.The highest average level of nata de whey protein is the duration of fermentation and the start of commercial *Acetobacterxylinum* on the 14^{th} and 11^{th} days of 2.70%, while the average protein content at the 8th day of fermentation is 2, 54 and vice versa with the length of fermentation and the starter of pineapple juice on the 8^{th} day of 2.26%, while the average crude fiber content in the 11th day of fermentation was 2.41% and the duration of fermentation on the 14^{th} day amounting to 2.42%.

3.2 Fat level

The results of the analysis of variance showed different fermentation time and source of starter in nata de whey had a significant effect (P <0.05%) on protein levels of nata de whey. The duration of fermentation does not have a significant impact on the treatment of different starter sources, while the interaction between the period of fermentation and various sources of nata does not have a significant effect. The treatment of P1 (15% whey + *Acetobacterxylinum* from pineapple juice) was significantly different or higher compared to P0 (15% commercial whey + *Acetobacterxylinum*) due to the high growth of *Acetobacterxylinum* influenced by oxygen availability, medium pH, nitrogen source and long fermentation was affected by nutrient availability and bacterial growth (11). The highest average level of nata de whey protein with the duration of fermentation and the start of commercial *Acetobacterxylinum* on the 8^{th} day was 0.44%, while the average protein content at the 11^{th} and 14^{th} days of fermentation was 0.41 % and vice versa with the length of fermentation and the starter of pineapple juice on the 8th day of 0.39%, while the average crude fiber content at the fermentation time of the 11^{th} day was 0.42% and the duration of fermentation on the 14^{th} day amounting to 0.41%. Different starters and different fermentation times affect fat content because the fat content in nata de whey is influenced by the ingredients used such as whey, pineapple juice, and sugar. Nata has a crude fiber content of 2.75%; protein 1.5 - 2.8%; 0.3% fat and the rest is water (12). Judging from its composition, whey contains nutrients including 6.5% solids consisting of 4.8% lactose, 0.6% protein, 0.6% mineral, 0.15% lactic acid, 0.25% non-protein nitrogen and 0.1% fat (13). The average value of fat content in the addition of starter types decreases, because the fat contained in the media is used for metabolism in the formation of nata. Reduced fat in the media will affect fat content in nata, because the fat contained in the nata is fat that is trapped by the nata matrix. The average value of fat levels in the treatment incubation period tends to increase, presumably because of the lysis of bacterial cells *Acetobacterxylinum* at the death phase. The death phase usually occurs after the 8^{th} to 15^{th} day; cells undergo lysis and release the components contained therein (14).

3.3 Reducing Sugar

The length of fermentation and the different sources of starter in nata de whey had no significant effect (P> 0.05%) on levels and coarse nata de whey. The duration of fermentation has a non-significant impact on the treatment of different starter sources, while the interaction between fermentation and various sources of nata does not have a real effect. The treatment of P0 (15% commercial whey + *Acetobacterxylinum*) differs very significantly or higher compared to treatment P1 because the high growth of *Acetobacterxylinum* is influenced by the availability of oxygen, medium pH, nitrogen source and duration of fermentation (11), as well as decreasing crude fiber content is influenced by nutrient availability and bacterial growth.

The highest average reducing sugar content of nata de whey is the fermentation and starter of commercial *Acetobacterxylinum* on the 8^{th} day of 0.58%, while the average content at the 11^{th} day of fermentation is 0.56% and the length of fermentation day to day -14 of 0.54% and vice versa with the period of fermentation and the starter of pineapple juice on the 8^{th} day was 0.54%, while the average, reducing sugar content at the 11th day of fermentation was 0.53% and the 14^{th} day of fermentation is 0.52%.

This shows that the longer the fermentation time, the reduced sugar content produced is lower because it uses the raw material of pineapple juice. This indicates that the liquid media sugar content in the treatment was decomposed by the larger *Acetobacterxylinum* bacteria. Bacteria *Acetobacterxylinum* if grown in liquid media containing sugar, will produce vinegar or acetic acid and white solids floating on the surface of liquid media known as nata. The formation of nata occurs because of the process of taking glucose from a solution of media, sugar or a medium containing glucose by *Acetobacterxylinum* cells. Then the glucose is combined with fatty acids to form precursors on cell membranes. These precursors are then excreted, and together the enzyme polymerizes glucose into cellulose outside the cell so that it can be seen that the sugar content that is converted into cellulose is the highest in the use of fruit flesh and a mixture of fruit and pineapple waste while the lowest is pineapple waste (12).

Reducing sugar is a pure sugar produced by hydrolysis of complex carbohydrates (sucrose) needed by *Acetobacterxylinum* bacteria in the formation of nata. Reducing sugar which is fructose and glucose serves to provide monosaccharides which are ready to be used by *Acetobacterxylinum* bacteria in their metabolic processes, making it faster to form cellulose fibers (15). The measurement of reducing sugar levels in the remaining incubation medium nata de whey was carried out to determine the residual sugar which was not utilized by *Acetobacterxylinum* to synthesize cellulose. Table 1 shows that the lowest reducing sugar content was obtained by nata de whey in treatment P1 (whey + *Acetobacterxylinum* 15% from pineapple juice) that is equal to 0.52%. The high level of reducing sugar remaining in the treatment medium P0 (whey + *Acetobacterxylinum* 15% commercial) is caused by inhibition of bacterial growth and activity in forming cellulose, this is due to the amount of sugar contained in high medium and available to *Acetobacterxylinum* bacteria but the amount of N (ammonium sulfate) is limited.

This is because some of the sugar in the medium has been used to form cellulose and will stimulate *Acetobacterxylinum* to synthesize more cellulose by absorbing the sugar content in the medium, while the amount of sugar in the medium is only small so that the reducing sugar content is left in the medium it becomes lower (16). Shows the medium with the amount of sugar that causes the growth and activity of *Acetobacterxylinum* bacteria for nata synthesis is getting better and increasing so that the reducing sugar content remaining in the medium is low. The better the growth of nata bacteria, the more sugar can be converted into nata so that the higher the percentage decrease in reducing sugar in the medium after incubation. Sucrose contains nutrients (carbon sources) that are needed by *Acetobacterxylinum* bacteria for growth and activity. Sucrose will be converted into cellulose or fiber which absorbs the sugar content in the media, and the formation of increased cellulose causes the fiber structure to be tight so that the reducing sugar content remaining in the medium after fermentation becomes small (17).

3.4 Cellulose

The duration of fermentation and the different sources of starter in nata de whey had no significant effect ($P>0.05\%$) on cellulose nata de whey. The duration of fermentation has a significant effect on the treatment of different starter sources, while the interaction between the period of fermentation and various sources of nata is having a significant impact. The treatment of P0 (15% commercial whey + *Acetobacterxylinum*) differed very significantly or lower compared to treatment P1 (whey + *Acetobacterxylinum* 15% from pineapple juice) due to the high growth of *Acetobacterxylinum* influenced by oxygen availability, medium pH, nitrogen source and long fermentation (11), so does the decrease in cellulose affected by nutrient availability and bacterial growth.

The highest average nata de whey cellulose is the length of fermentation and starter of *Acetobacterxylinum* from pineapple juice on the 14th day by 0.42%, while the ordinary cellulose at the fermentation time of day 8 and day 11 is 0, 41% and vice versa with the fermentation time and commercial starter on the 8^{th} day of 0.41%, while the average cellulose at the 11^{th} day of fermentation was 0.36% and the fermentation time of day 14 was 0, 35%.

Cellulose formation has been seen in incubation to 24 hours, where a thin layer on the surface of the fermentation media has been seen. Increasing the time of fermentation, the bottom area of the media that is initially cloudy becomes clear. This is because the nutrients contained in the media have been used by the bacteria *Acetobacterxylinum*. The resulting cellulose thickens with the length of fermentation time. Cellulose is located on the surface of the media (floating) because of the presence of CO_2 gas that drives cellulose so it is on the surface. CO_2 gas is a primary metabolite produced by bacteria during the fermentation process (18). Each bacterial cell secretes cellulose yarns which bind to one another with one another which will form a strong thread. In this case a calm condition is needed so that the threads of the thread are united in a solid manner. In the process of cellulose formation, *Acetobacterxylinum* also forms primary metabolites. The primary metabolites produced are acetic acid, water and energy that are reused in the metabolic cycle. Acetic acid is utilized by *Acetobacterxylinum* to create optimum growth conditions. In addition, acetic acid can be oxidized to CO_2 and H_2O if the nutrients in the media have run out. The acid formed will affect the pH so that pH tends to decrease.The type of sugar, sugar concentration, nitrogen source and pH value of the media influence the productivity of cellulose. The type of sugar that provides the highest product is fructose followed by a combination of fructos, glucose, lactose and sucrose on media containing sucrose. Nitrogen sources that can be used are sources of inorganic nitrogen (ammonium sulfate ammonium, phosphate and potassium, nitrate) and organic nitrogen sources (tripton peptone, yeast extract and urea) and a combination of these two nitrogen sources.

4. Conclusion

The effect of fermentation time and various sources of starter on nata de whey has no significant effect on protein, reducing sugar, and cellulose produced. Conversely, the importance of the fermentation time and the difference in the source of the starter on nata de whey has a very significant effect on fat content. fermentation has been proven to reduce fat content in nata de whey, the lowest fat content in the 14-day fermentation treatment

References

1. F. Fatma, S. Soeparno, N. Nurliyani, C. Hidayat, M. Taufik. Karakteristik whey limbah dangke dan potensinya sebagai produk minuman dengan menggunakan Lactobacillus acidophilus FNCC 0051. Agritech. **32**, 4 (2012).
2. D. Ariyanti, H. Hadiyanto. Pembuatan Bioetanol Dari Limbah Keju (Whey) Melalui Proses Fermentasi Fed-batch Dengan Kluyveromyces Marxianus. J Teknol Kim dan Ind.**2** (2):155–62 (2013)
3. De Witt JN. Lecture's Handbook on Whey and Whey Product. Eur Whey Prod Assoc Brussels, Belgium.(2001)
4. Deeth HC, Bansal N. Whey proteins: from milk to medicine. Academic Press.(2018)
5. S. Usmiati, A. Bakar. Teknologi pengolahan susu. Balai Besar Penelit dan Pengemb Pascapanen Pertan Press Bogor. (2009)
6. A. Jagannath, S.S. Manjunatha, N. Ravi, P.S. Raju. The effect of different substrates and processing conditions on the textural characteristics of bacterial cellulose (nata) produced by Acetobacter xylinum. J Food Process Eng. **34** (3):593–608 (2011)
7. M.F.F. Amir. Effect of Different Additives on the Production of Bacterial Cellulose from Pineapple Waste. UMP (2013)
8. J. Majesty, B.D. Argo, W.A. Nugroho. Pengaruh penambahan sukrosa dan lama fermentasi terhadap kadar serat nata dari sari nanas (nata de pina). J Keteknikan Pertan Trop dan Biosist. **3** (1):80–5 (2014)
9. Y. Kiswanto, S. Saryanto. Pengaruh Suhu dan Lama Penyimpanan Air Kelapa Terhadap Produksi Nata de Coco. (2010).
10. A. Khairul. Produksi Nata de Coco. Bogor: ITB; (2010)
11. M.S. Bethan, H.N. Fadillah. Pembuatan Nata De Pina Dari Limbah Kulit Nanas (Ananas Comusus L. Merr) Dengan Proses Fermentai Menggunakan Bakteri Acetobacter Xylinum= Making Nata De Pina from Pineapple Skin Waste (Ananas Comusus L. Merr) with Fermentation Process Using Bacteria Acetob. Institut Teknologi Sepuluh Nopember. (2018)
12. A. Sutanto. Pineapple liquid waste as nata de pina raw material. Makara J Technol. **16** (1):63–7 (2012)
13. D. Dwianto. Kajian Sifat Fisik, Kimia dan Tingkat Kesukaan Nata de Whey. Universitas Mercu Buana Yogyakarta. (2018)
14. Z. Mahmud, Y. Ferry. Prospek pengolahan hasil samping buah kelapa. Perspektif. **4** (2): 55–63 (2015)
15. U.S.P. Suripto. Identifikasi Mutu Pasca Panen Nata de Coco Berdasarkan Lama Perendaman dan Perebusan. Inov Agroindustri **1** (1):29–37 (2018).
16. Keshk SMAS. Bacterial cellulose production and its industrial applications. J Bioprocess Biotech. **4** (2):1 (2014).
17. S. Djajati, U. Sarofa. Pembuatan nata de manggo (kajian. konsentrasi sukrosa dan lama fermentasi). J Teknol Pangan. **3**, 2 (2018).
18. R. Melliawati, N. Nuryati, L. Magfiroh. The treatment of fruit-rind waste into cellulose by Acetobacter sp. RMG-2 bacteria. In: Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia. p. 300–5 (2015).