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FULL PAPER

Total lactic acid bacteria, fiber content, and physical properties of *Nata de pina* between various parts of honey pineapple variety (*Ananas comosus* [L.] Merr. Var. Queen)

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Abstract

Fiber is one of the nutrients that are important for health. Adequate fiber intake prevents several diseases such as stroke, colorectal cancer, heart disease, and diabetes mellitus. One of the foods that have high fiber content is Nata. This study is aimed to analyze the total lactic acid bacteria, fiber content, and physical properties of *Nata de pina* between various parts of honey pineapple variety. This study was a one-factor randomized experimental study with variations of *Nata de pina* from flesh, peel, and pineapple fruit core. Analysis of total lactic acid bacteria using Total Plate Count (TPC) methods and fiber content using enzymatic-gravimetric methods. The data described the physical properties by thickness using calipers, yield using scales, texture using texture analyzer, and color analysis using digital colorimetry. There were significant differences in the total lactic acid bacteria between the treatment group of Nata fermented water 12th hour ($p<0.001$), 2nd day ($p<0.001$), 4th day ($p<0.001$), 6th day ($p=0.007$), and 8th day ($p=0.047$). The highest total soluble and insoluble fiber of *Nata de pina* from the pineapple peel (1.92% and 0.049%). There were differences in physical properties test that include thickness, yield ($p<0.001$), and texture. There were differences in a color analysis that include L* ($p=0.005$), a* ($p=0.012$), and b* ($p=0.002$). Various parts of honey pineapple affect total lactic acid bacteria, fiber content, thickness, yield, texture, and color of *Nata de pina*.

1. Introduction

Fiber is one component of nutrition that is beneficial to health. Dietary fiber is a part of plants that cannot be digested by the digestive tract. But it can prevent several diseases such as stroke, heart disease, and diabetes mellitus (Li and Komarek, 2017; Hapsari *et al*., 2018). Dietary fiber can be divided into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Generally, soluble dietary fibers include pectin, gum, and glucans. Whereas included in insoluble fiber are cellulose, hemicellulose, and lignin. About one-third of the Total Dietary Fiber (TDF) is soluble dietary fiber, while the largest group is insoluble fiber. (Dai *et al*., 2017; Yang *et al*., 2017).

Consumption of enough fiber based on the recommended amount can reduce the risk of stroke, colorectal cancer, cardiovascular disease, and type 2 diabetes (Li *et al*., 2017). A diet high in grains, fruits, and vegetables can prevent cancer because of the high fiber content. Fiber provides a protective effect from cancer cells by increasing the fecal period and frequency of bowel movements, thereby reducing contact between carcinogens and colonic lumen. In the intestinal lumen, the fiber will ferment and produce fatty acids which will stimulate the growth of the colonic mucosa and induce apoptosis (Hapsari *et al*., 2018).

One of the fruits that become Indonesia's main commodity is pineapple. Based on Fixed figures states that in 2014 the pineapple production reached 1.84 million tons. (Pusat Data dan Sistem Informasi Pertanian Kementrian Pertanian., 2015). Pineapple which has the Latin name *Ananas Comosus L. Merr* is one type of fruit that is much in demand by the public. Honey pineapple is the most popular grown in Indonesia. The nutritional content found in pineapple is calcium, potassium, vitamin C, carbohydrates, fiber, water, and various other minerals (Hossain *et al*., 2015). Pineapple can be

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consumed directly or in the form of preparations. One of the processed foods made from pineapple is Nata (Department of Health and Ageing Office of the Gene Technology Regulatory, 2008).

Nata is a fermented product by the *Acetobacter xylinum* which is grown on media containing carbohydrates, vitamins, and inorganic acids that are needed (Almeida *et al*., 2013). Nata is a low-calorie food product with 2.5% fiber content and 98% water (Nugroho *et al*., 2015; Rizal *et al*., 2016). The fiber contained in Nata is classified as insoluble fiber that cannot reduce cholesterol levels in the blood but can prevent the occurrence of colon cancer, difficulty removing large water, obesity, and diabetes mellitus (Mirmiran *et al*., 2016). The fiber and water content affects the texture of Nata. The higher the fiber content in Nata, the lower the level of elasticity (Ratnaningsih *et al*., 2017). Nata made from pineapple is called *Nata de pina*. *Nata de pina* is not only made from the flesh of the fruit. Pineapple waste such as peel and fruit core also has the potential to be made into *Nata de pina* (Rizal *et al*., 2016) The results of the study indicated that pineapple made from pineapple peel has the largest thickness of 1.11 cm while pineapple made from pineapple flesh and pineapple fruit core respectively - each has a thickness of 0.2 cm and 0.74 cm (Hamad *et al*., 2017).

In the fermentation process in making Nata, it is known that there is an activity of lactic acid bacteria (Nugroho *et al*., 2015). Bacteria are the most common microorganisms found in foods that are fermented naturally or using a starter. Lactic acid bacteria are one of the bacteria found in fermented foods or drinks that are acidic (Tamang *et al*., 2015). In general, lactic acid bacteria are a group of gram-positive bacteria that can convert glucose, fructose, and other sugars into lactic acid. This bacterium is beneficial for health, especially in the human digestive system. Research conducted by Jiachao Zhang *et al*. (2017) regarding the microorganisms in *Nata de coco* during fermentation, it was stated that there were the Lactobacillus bacteria which is one of the genera of lactic acid bacteria. The results of this study also state that the content of acetic acid and lactic acid increases during fermentation (Anna *et al*., 2010). Lactic acid bacteria produce exopolysaccharides which affect the texture and viscosity of fermented products (Zamfir *et al*., 2014; Gupta *et al*., 2018). Therefore, this work is aimed to identify total lactic acid bacteria, fiber content, and physical properties of *Nata de pina* between various parts of honey pineapple variety.

2. Materials and methods

2.1 Materials

Honey pineapple was collected from Pemalang, Indonesia. Coconut water was obtained at Semarang, Indonesia. While coconut water, sugar, vinegar, etc were purchased from local market.

2.2 Preparation making Nata de coco

Coconut water used for making Nata de coco as control. Coconut water was heated at 100^{o}C in a water bath for 10-15 mins. During the process, 7.5% sucrose and 0.5% urea were added into the media. Glacial acetic acid was added to adjust the pH to 4. After heating, each media was put into the trays that had previously been sterilized. Each tray was closed using newspaper and glued with rubber to minimize contamination. The tray then placed at room temperature (28-31^{o}C) for 12 hrs and 10% of *A. xylinum* starter was added in media. After the starter was added, the tray was closed again and fermented for 8 days.

2.3 Preparation of pineapple juices and making of Nata de pina

The peel, flesh, and pineapple fruit core used for fermentation media. Each part of pineapple fruit was mixed with water in the ratio of 1:2. The juices were heated at 100^{o}C in a water bath for 10-15 mins. During the process, 7.5% sucrose and 0.5% urea were added into the media. Glacial acetic acid was added to adjust the pH to 4. After heating, each media was put into the trays that had previously been sterilized. Each tray was closed using newspaper and glued with rubber to minimize contamination. The tray then placed at room temperature (28-31^{o}C) for 12 hrs and 10% of *A. xylinum* starter was added in media. After the starter was added, the tray were closed again and fermented for 8 days. (Sutanto, 2012)

2.4 Determination of total lactic acid bacteria

Analysis of total lactic acid bacteria on fermented media for 12th hour, 2nd day, 4th day, 6th day, and 8th day. Nata fermented water was use for the analysis of total lactic acid via Total Plate Count (TPC).

2.5 Determination of fiber content

The test used for fiber contents determination were enzymatic-gravimetric methods. (Prosky *et al*., 1985; Prosky *et al*., 1994) Fiber contents analysis were carried out at the laboratory of Saraswanti Indo Genetech.

2.6 Determination of physical properties

The physical properties were evaluated by thickness,

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yield, texture, and color measurement. The thickness determination of Nata samples was used calipers in millimeter. Determination of yield used scales in the ratio between the weight of Nata produced and the weight of media during the Nata making process. The texture determination which is the hardness of Nata samples were used texture analyzer in kg/m^2. The color of Nata samples was determined using digital colorimetry according to the *L*a*b** value with two repetitions. *L** (lightness/darkness that ranges from 0 to 100), *a** (yellowness/greenness that ranges from -120 to 120) and *b** (yellowness/blueness that range from -120 to 120) were measured (Salehi and Kashaninejed *et al*., 2014).

2.7 Statistical analysis

Data analysis was performed using SPSS v.16.0 that had previously been tested for normality using the *Shapiro-Wilk* test. The total lactic acid bacteria were analyzed using the One way ANOVA test if the data were normally distributed and the *Kruskal-Wallis* test if the data were abnormally distributed followed by the Tukey test to find out the real difference between treatments. Physical properties were analyzed using the *Kruskal-Wallis* test and continued with the *Mann Whitney* test to find out the real difference.

3. Results and discussion

3.1 Total lactic acid bacteria

Based on Table 1, there were significant differences in total lactic acid bacteria between the treatment group of Nata fermented water 12th hour ($p<0.001$), 2nd day ($p<0.001$), 4th day ($p<0.001$), 6th day ($p=0.007$), and 8th day ($p=0.047$). Figure 1 shows *Nata de pina* from pineapple peel is the highest total lactic acid bacteria while Nata de pina from fruit core is the lowest total lactic acid bacteria. The acidic conditions in the fermentation process caused fermented water contains lactic acid bacteria (Zhang *et al*., 2017). Lactic acid bacteria are gram-positive bacteria that can grow on low pH sugar-containing media (Anna *et al*., 2010). The highest total LAB is required in Nata fermented water made from coconut water. The presence of natural LAB and more energy sources such as calcium, fructose, and sucrose in coconut water increases bacterial growth (Tan *et al*., 2014). Fermentation of pineapple can increase the growth of lactic acid bacteria. Besides, lactic acid bacteria are also contained in pineapple. Pineapple fermented water on the fruit peel contains more lactic acid bacteria compared to pineapple fermented water made from flesh and fruit core. The difference in lactic acid bacteria is caused by the fermentation of pineapple waste containing more lactic acid bacteria (Di Cagno *et al*., 2010; Niederreiter *et al*., 2018). Increasing the total lactic acid bacteria during the fermentation process produces acid so that can inhibit the growth of bacteria. Based on statistics, the total lactic acid bacteria in Nata fermented water does not improve the texture of the Nata produced because Nata was not the end product of lactic acid bacteria fermentation. The texture of Nata was affected by the water and cellulose content. (Ratnaningsih *et al*., 2017)

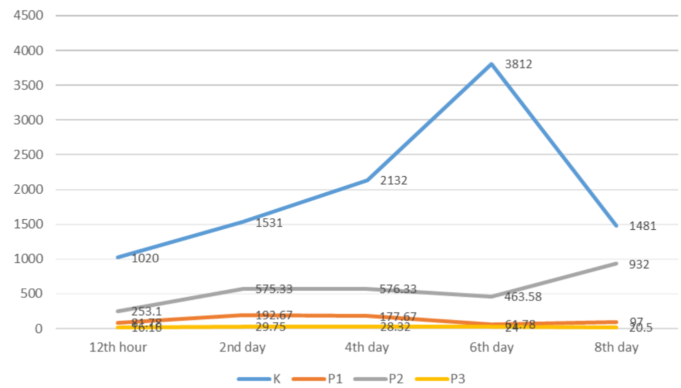

Figure 1. Total lactic bacteria

3.2 Fiber content

The result in Table 2 shows that Nata de pina from pineapple fruit had the highest soluble fiber (1.92%) and insoluble fiber (0.049%). The pineapple fruit peel has the potential to be used for Nata. The study showed that the pineapple fruit peel contains higher carbohydrates than other pineapple parts. *A. xylinum* is a bacterium from the family of acetic acid bacteria can convert carbohydrates into acids acetate (Hamad *et al*., 2017). In this study, *Nata de coco* had a higher soluble fiber compared to *Nata de pina*. Coconut water contains more sugar and

Table 1. Total lactic bacteria

Sample	12th hour (10^{12} CFU/mL)	2nd day (10^{12} CFU/mL)	4th day (10^{12} CFU/mL)	6th day (10^{12} CFU/mL)	8th day (10^{12} CFU/mL)
K	1020±187.38^{b}	1531±368.95^{c}	2132±324.36^{c}	3812±2.15^{b}	1481±1.1
P1	81.78±24.44^{a}	192.67±91.21^{ab}	177.67±99.97^{ab}	61.78±54.47^{a}	97±57.98
P2	253.1±134.68^{a}	575.33±90.27^{b}	576.33±101.75^{b}	463.58±303.19^{a}	932±620.21
P3	16.16±2.35^{a}	29.75±3.08^{a}	28.32±4.77^{a}	24±0.03^{a}	20.5±0.58
p*	0	0	0	0.007	0.047

*Using One way anova Test. Equal letters (same column) indicate that there is significant difference among the values ($p<0.05$).
K = control/Nata de coco; P1 = Nata from fruit flesh; P2 = Nata from fruit peel; P3 = Nata from fruit core

nitrogen for the growth of the *A. xylinum*. *A. xylinum* is a cellulose-producing bacteria. This bacterium works to convert glucose into gluconic acid and stimulate organic acids. *A. xylinum* can grow and work on media that contain enough nutrients such as carbon, nitrogen and other minerals. In this study, pineapple peel which is a waste can be potentially made into Nata de pina as it is the most nutritious part of the pineapple (Lestari, 2014; Hamad *et al*., 2017).

Table 2. Fiber content

Sample	Soluble fibre (%)	Insoluble fibre (%)
K	2.22±0.014	0.0098±0.0007
P1	0.305±0.007	0.0195±0.0007
P2	1.92±0.014	0.049±0.0014
P3	0.195±0.007	0.0055±0.0002

K = control/Nata de coco; P1 = Nata from fruit flesh; P2 = Nata from fruit peel; P3 = Nata from fruit core

3.3 Physical properties

3.3.1 Thickness

According to the study, Nata de coco showed the highest thickness (0.97 cm). The thickness *Nata de pina* showed that Nata from pineapple fruit peel had the highest thickness (0.39 cm) compared to *Nata de pina* from flesh (0.27 cm) and core (0.15 cm). Nata thickness is one of the parameters of making Nata (Sahin *et al*., 2006) The results of the study showed that *Nata de coco* has a higher thickness than *Nata de pina*. Coconut water contains more nutrients than pineapple such as glucose, fructose, and sucrose (Tan *et al*., 2014; Hamad *et al*., 2017). The main sugar content in coconut water is fructose. Coconut water contains 32.5 mg/mL of fructose, while the fructose content of pineapple is 10 mg/mL (Hong *et al*., 2013; Manivannan *et al*., 2018). Fructose is the main carbon source for bacterial growth to produce cellulose (Zeng *et al*., 2011). *Nata de pina* produced a lower thickness than Nata de coco. Pineapple contains polysaccharide that causes pineapple juices to become turbid. Turbid media increases the level of viscosity so that oxygen supply is obstructed (Ernawati, 2012)

Nata from pineapple peel produces a higher thickness than other fruit parts. The highest fructose content is found in the pineapple peel (Nadzirah *et al*., 2013). Nata from pineapple core produces the lowest thickness due to the oxalate content in pineapple. It is known that the highest oxalate content is found in pineapple core. Oxalate is an organic acid that can inhibit the formation of cellulose (Siener *et al*., 2015; Lu *et al*., 2016). In the Nata making process, sugar as a carbon source is important. The availability of sugar in the media will affect bacterial growth. *A. xylinum* needs sugar as a source of carbon for cell metabolism. Media that contains more sugar will produce higher Nata (Lestari *et al*., 2014; Hamad *et al*., 2017).

3.3.2 Yield

The result of yield anaylsis showed that there were significant differences between the mean scores for the sample ($p<0.05$). Also, it is obvious from the results that *Nata de pina* from pineapple fruit peel (12.02%) had the highest yield compare to *Nata de pina* from flesh (8.86%) and core (5.53%) of pineapple fruit. This study also showed that *Nata de coco* produced higher yield compared to *Nata de pina.* Coconut water contains more glucose, fructose, and sucrose than pineapple (Tan *et al*., 2014; Hamad *et al*., 2017). The yield is the ratio between the weight of Nata produced and the weight of media during the Nata making process. Nata yield calculation is performed to determine the percentage of Nata biomass obtained from the fermentation process by the *A. xylinum* (Sahin *et al*., 2006). The yield of *Nata de pina* from pineapple fruit peel is higher than other parts of the fruit. The yield of Nata can be affected by the thickness of the Nata so that the thicker the Nata formed, the higher the yield (Talenta, 2018). This study showed that the highest Nata thickness is found in Nata de coco and the lowest in Nata which made from pineapple fruit core. The yield value is directly proportional to the thickness of the Nata produced.

3.3.3 Texture

The results of texture analysis are shown in Table 3. The lowest texture was obtained on *Nata de pina* from pineapple peel (38.5 kg/m^2) followed by *Nata de pina* from pineapple core (57.75 kg/m^2) and pineapple flesh (81.25 kg/m^2). The texture is an important component in food that is related to product acceptance by consumers. Texture can be affected by cellulose formed during the fermentation process. Cellulose is the main product of Nata, which is classified as insoluble fiber. High levels of insoluble fibre affect the high content of insoluble fiber which then will affect the texture of Nata to be lower (Sahin *et al*., 2006; Ratnaningsih *et al*., 2017).

3.3.4 Color

The result showed that there are significant differences between the lightness ($p=0.005$), redness ($p=0.012$), and yellowness ($p=0.002$) value. The lowest *L* (lightness) value of Nata de pina is produced from pineapple peel and the highest value from pineapple flesh. Since the major color of pineapple flesh is yellow, while the major color of pineapple peel is greenish. The value of *L* (lightness) is the level of brightness that ranges from zero to one hundred on the chromameter. *L* value of close to 100 shows that the analyzed sample has a high brightness (Nugrahani., 2014). The lowest *a* (redness) value of *Nata de pina* is produced from flesh

Table 3. Physical properties

Sample	Thickness (cm)	Yield (%)	Texture (kg/mm^2)	*L**	*a**	*b**
K	0.97±0.014	60.1±1.15[b]	87±2.4	64 (64-65)[a]	-18 (-19 – (-17))[ab]	37 (36-37)[d]
P1	0.27±0.007	8.86±0.53[a]	81.25±1.1	69 (67-69)[b]	-19 (-20 – (-19))[a]	21 (21-22)[b]
P2	0.39±0.014	12.02±1.22[a]	38.5±5.65	63.5 (62-64)[a]	-17 (-17 – (-16))[b]	26 (25-26)[c]
P3	0.15±0.007	5.53±0.3[a]	57.75±1.87	68 (67-69)[b]	-19 (-20 – (-17))[a]	18.5 (17-20)[a]
p		0.000[xz]		0.005[yz]	0.012[yz]	0.002[yz]

[x]Using One way anova Test; [y]Kruskal-Wallis; [z]Post-hoc Mann Whitney Test. Equal letters (same column) indicate that there is significant difference among the values ($p<0.05$). K = control/Nata de coco; P1 = Nata from fruit flesh; P2 = Nata from fruit peel; P3 = Nata from fruit core

and pineapple core while the highest value from pineapple peel. The value of *a* (redness) obtained in this study is negative, which indicates that the Nata has a greenish color. This study also showed that the lowest *b* (yellowness) value from pineapple core and the highest *b* from Nata de coco. The value of *b* (yellowness) obtained in this study is positive, which shows that the Nata had a yellowish color. Yellowish color that forms on *Nata de pina* because of the basic color of pineapple. While the yellowish color of *Nata de coco* affected by the sugar that heated in the process of heating the media (Rizal *et al*., 2013; Nugrahani, 2014).

4. Conclusion

Various parts of honey pineapple affect the total lactic acid bacteria, fiber content, thickness, yield, texture, and color of *Nata de pina*. Besides, lactic acid bacteria do not show some effect in terms of texture. The physical properties analysis such as thickness, yield and color have differences. *Nata de pina* from pineapple fruit peel contained the highest soluble and insoluble fiber that means it can be alternate food for adequate fiber intake and prevents several noncommunicable disease.

Conflict of Interest

The authors declare no conflict of interest.

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