

Total phenolic content, antioxidant activity and organoleptic test of *Nata de pina* between various parts of honey pineapple variety (*Ananas comosus* [L.] Merr. Var. Queen)

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Abstract

This study was aimed to analyze the total phenolic content, antioxidant activity and organoleptic properties of *Nata de pina* between various parts of honey pineapple variety (*Ananas comosus* [L.] Merr. Var. Queen). This study was a two - factors randomized experimental study with variations of *Nata de pina* from fruit peel, fruit flesh, fruit core, and fermentation processing. Folin-Ciocalteu reagent was used to analyze the total phenolic content and 2,2'-diphenyl-1-Picrylhydrazyl (DPPH) assay was used to determine the antioxidant activity. The total phenolic content of *Nata de pina* ranged from 102.37-249.99 mg of GAE/100 g of samples while the IC₅₀ DPPH ranged from 101.71-555.45 ppm. There was a difference of the total phenolic content and antioxidant activity of the juice and *Nata de pina* produced from different parts of the fruit. Fruit core *Nata de pina* presented the highest content of the total phenolic content and antioxidant activity. There were significant differences in the organoleptic tests *viz* color (p=0.030), flavor (p<0.001), texture (p<0.001). Fruit flesh *Nata de pina* had the highest organoleptic score. Overall, based on result of this study, fruit core *Nata de pina* was recommended as the best product based on the high total phenolic content (139.88 mg GAE/100 g), antioxidant activity (263.38 ppm) and well-liked panelists based on the acceptance of aroma and color.

1. Introduction

Cardiovascular disease is a disease caused by impaired cardiac and vascular function. Most cardiovascular diseases are accompanied by increased oxidative stress in the body. Oxidative stress caused by excessive production of free radicals or lack of antioxidants. Free radicals are molecules that contain one or more electrons not paired in their outer layers, so one of them can be stabilized by antioxidant by completing the lack of electrons. If the number of free radicals are not sufficient, then the body will utilize antioxidants from food (Go *et al.*, 2014; Kehrer and Klotz, 2015; Cervantes Gracia *et al.*, 2017). Many foods in Indonesia contain antioxidants especially fruits. Pineapple (*Ananas comosus* [L.] Merr.) is one of Indonesia's local fruits with increasing production. The *Queen* variety is widely found in Indonesia and the Honey pineapple variety (*Ananas comosus* [L.] Merr. Var. Queen), a subspecies of the *Queen* variety, contains natural antioxidants in every parts of its fruit such as phenolic compounds, flavonoids, vitamin C, vitamin E, and others.

Antioxidant activity between various parts of honey pineapple variety such as flesh, peel, bract, and core ranged from 0.13 to 68.17 µg/mL (Fidrianny *et al.*, 2018). In addition, the total phenolic content differs in each part of the fruit such as P-coumaric acid, ferulic acid, caffeic acid, sinapic acid, P-coumaroyl quinic acid, P-hydroxybenzoic acid, and P-hydroxy benzoic aldehydes in the fruit skin, while catechins, epicatechin, gallic acid, and ferulic acid found in the fruit peel. The total phenolic content ranged from 71.07 mg of gallic acid/100 g to 126.95 mg gallic acid/100 g (Hassan *et al.*, 2011; Ferreira *et al.*, 2016).

Honey pineapple variety has the potential to be used as a raw material for *Nata de pina* production. *Nata* is a cellulose formed by *Acetobacter xylinum*. Generally, *Nata* is made from fermented coconut water, but the juice of various parts of honey pineapple variety such as peel, core, and fruit flesh can also be used as fermentation medium in making *nata*. These parts are known to contain organic acids and minerals that can accelerate the growth of *A. xylinum*. Besides, producing

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Nata de pina is one of the ways to utilize the peel and other underutilized parts of the fruit that is seldom or not consumed by people (Almeida *et al.*, 2013). Utilization of the various fruit parts of honey pineapple variety may increase the nutrient content such as total phenolic content, antioxidants activity, and improved appearance. However, the heating process in processing *Nata de pina* is known to affect the total phenolic content and antioxidant activity (Nayak *et al.*, 2015). Therefore, this study was conducted to analyze the total phenolic content, antioxidant activity and organoleptic test in *Nata de pina* made from the various fruit parts of honey pineapple variety as an alternative food with good quality and flavor.

2. Materials and methods

2.1 Sample

Juice of the various parts of the honey pineapple variety (peel, flesh and core) and *Nata de pina* prepared using the juice of the respective fruit parts were analyzed as samples. The juice represents the pre-fermentation product while the *Nata de pina* represents the fermented product. Honey pineapple were obtained from the district of Belik, the regency of Pematang, Central Java, Indonesia. The characteristics of the fruits used are 5 to 6 months, large and round, open fruit crown, wrinkle stem, flatter bract fruit with yellow base and a distinctive smell of pineapple.

2.2 *Nata de pina* preparation

The juices from the peel, flesh and core are each mixed with water at the ratio of fruit juice: water = 1: 2 was prepared as the fermentation media. The mixture was heated and added with 7.5% sucrose and 0.5% urea. Then, glacial acetic acid was added to adjust the pH to 4 and stirred slowly until the temperature reached 100°C. The fermentation medium was poured onto a 20 x 40 x 5 cm plastic pan and covered to allow to cool to room temperature (28-31°C) for 12 hrs. Aseptically, 10% starter culture of *A. xylinum* was inoculated into the fermentation medium. The fermentation process was carried out for 8 days in a dark room at room temperature.

After 8 days, the formed *Nata de pina* was cut and soaked for 2 days in marinated water which is replaced twice a day. Next, the soaked *Nata de pina* was boiled for 15 mins in a sugar solution (5 g/L) (Sutanto, 2012). The *Nata de pina* was then freeze-dried at -50°C, 0.03 hPa using a freeze dryer (Power Dry LL 1500) until the moisture content was below 5% (Pa'e *et al.*, 2014) to retain the content of *Nata* nutrients.

2.3 Determination of total phenolic content (TPC)

TPC using the Folin Ciocalteu assay was carried out according to existing protocols in the laboratory as described by Musci *et al.* (2017). The Folin–Ciocalteu solution was prepared by diluting the 2M Folin–Ciocalteu reagent (Sigma-Aldrich, Milan, Italy) with distilled water at the ratio of 1:10 (v/v). A 10% (w/v) Na₂CO₃ solution was prepared by diluting pure Na₂CO₃ (Sigma-Aldrich, Milan, Italy) with distilled water. In the reaction tubes, Na₂CO₃ (1 mL) was added to each *Nata de pina* sample (250 µL). After 5 mins, Folin Ciocalteu reagent (1 mL) was added. After 120 mins incubation at 25°C, protected from light, the absorbance was measured at 765 nm using a UV-Vis spectrophotometric. The analysis was carried out in triplicates. The total phenolic contents in all samples was calculated the using the formula: $C = c \times V/m$ where, C = total phenolic content mg GAE/ 100 g sample, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume measured at the spectrophotometer in mL, m = mass of sample in g.

Results were expressed in milligram gallic acid equivalents per 100 g of sample (mg GAE/ 100 g sample). Working standard solutions of gallic acid at eight different concentration levels (0, 2, 4, 6, 8, 10, 12 and 14 µg/mL) were prepared by diluting the stock solution and measured the absorbance to build a calibration curve. All the standard solutions were stored at 4°C until analysis (Musci and Yao, 2017).

2.4 Determination of antioxidant activity

The antioxidant capacity of each sample was determined using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay according to Sami *et al.* (2015). DPPH radical solution (0.4 mM in methanol) of 1 mL was added to the sample. After 30 mins incubation in darkness at 30°C, the absorbance was measured at 517 nm by UV-Vis spectrophotometer. The analysis was carried out in triplicates. Methanol was used as blank and DPPH 50 µg/mL as control. Antioxidant activity was presented as inhibitory concentration 50% (IC₅₀) of DPPH scavenging activity by calculating IC₅₀ of each extract using a calibration curve of five different concentration levels (80, 100, 120, 140 and 160 ppm) (Sami and Rahimah, 2015).

2.5 Determination of organoleptic test

The organoleptic test of *Nata de pina* using hedonic tests including color, aroma, flavor, and texture were carried out on twenty-five semi-trained panelists with 5-point hedonic scale, i.e. 1 = very dislike to 5 = very fond. The best-recommended product selected based on the

effectiveness index using the modified De Garmo method by Susrini (2005). Determination of the best-recommended product starts by assigning a variable weight (BV) of 0–1 to each parameter based on its importance. The normal weight (BN) is determined on each parameter from the division of the variable weight per treatment by the total variable weights of all treatments. The value of effectiveness (NE) is obtained by comparing the difference in the value of treatment (NP) and the worst value with the difference of the best and worst value. Yield value (NH) is determined by the multiplication value of the effectiveness with the normal weight then sum. The highest total yield value treatment was considered the best-recommended product in this study (Susrini, 2005).

2.6 Statistical analysis

Data were presented as mean with standard deviation. The normality of data distribution was tested by the Shapiro-Wilk method. The total phenolic content and antioxidant activity before and after the fermentation was analyzed using the T-test in pairs (Pair T-tests), while the treatment was analyzed using the One way ANOVA test for normal distribution data and test Kruskal-Wallis for irregular distribution data is continued with Tukey's advanced test to find out the real difference between treatment. Organoleptic Test Data was analyzed using the Kruskal-Wallis test and continued with the Mann-Whitney advanced test to determine the apparent difference between treatment (Sugiyono, 2015).

3. Results and discussion

3.1 Total phenolic content (TPC) and antioxidant activity

The total phenolic content (TPC) in Table 1 presented statistically significant differences between the different fruit parts' juices ($p < 0.001$) and in the samples of *Nata de pina* ($p = 0.016$). However, the TPC showed significant reductions in samples of *Nata de pina* from the juice. The highest TPC value obtained was from the fruit core whether in juice (249.99 mg GAE/100 g) or *Nata de pina* (139.88 mg GAE/100 g) (Hassan et al., 2011; Ferreira et al., 2016). The significant difference in the fruit parts' juices and *Nata de pina* was due to the presence of other sources of natural antioxidants that are different in each part of the fruit. It is known that the core of pineapple honey had a higher content of oxalic acid, another source of antioxidant. Oxalic acid is a common constituent of plants and some species. Oxalic acid is known to have a strong chelating ability with cations. Previous studies had explained that oxalic acid can inhibit the formation of hydroxyl radicals and lipid peroxidation, as well as reduced the rate of ascorbic

oxidation through the process of compression by the presence of hydrogen peroxide and $\text{Cu}^{(2+)}$. These results indicated that oxalic acid can be a natural antioxidant, but the availability of antioxidant capacity in oxalic acid is unclear (Kayashima and Katayama, 2002).

Table 1. Total phenolic content (TPC)

Fruit Parts	Total Phenolic Content		$P^{\#}$
	Juice Mean±SD (mg GAE/100 g samples)	<i>Nata</i> Mean±SD (mg GAE/100 g samples)	
Peel	153.82±2.48 ^b	102.37±5.38 ^b	0.001 ^{x*}
Flesh	198.12±6.14 ^c	115.13±5.20 ^c	0.006 ^{x*}
Core	249.99±7.23 ^d	139.88±2.51 ^d	0.003 ^{x*}
p	<0.001 ^{y*}	0.016 ^{z*}	

[#]Analyzed via Paired T-test, ^yAnalyzed via one-way ANOVA, ^zAnalyzed via Kruskal Wallis Test. Different superscript letters in the same column showed significant differences ($p < 0.05$).

The results of antioxidant activity measured by the DPPH assay was presented in Table 2. Statistically significant difference in the group of fruit part's juices ($p < 0.001$) and *Nata de pina* ($p < 0.001$). The highest antioxidant activity was found in the fruit core where the juice and *Nata de pina* had IC_{50} 101.71 and 263.28 ppm, respectively. An increase in the antioxidant activities was observed when *Nata de pina* was produced from the juice of the fruit parts. The decrease in the content was related to the processing of *Nata de pina* where thermal treatment was involved to kill pathogenic bacteria and other contaminants before fermentation and subsequently the produced *Nata de pina* was re-heated to make the texture supple (Sutanto, 2012).

Table 2. Antioxidant activity

Fruit Parts	Antioxidant activity		p^x
	Juice Mean±SD (ppm)	<i>Nata de pina</i> Mean±SD (ppm)	
Peel	197.22±7.08 ^b	555.45±30.87 ^b	0.003*
Flesh	126.70±9.22 ^c	441.89±26.78 ^c	0.001*
Core	101.71±1.00 ^d	263.38±9.02 ^d	0.001*
p^y	<0.001*	<0.001*	

^xAnalyzed via paired T-test, ^yAnalyzed via one-way ANOVA. Different superscript letters in the same column showed significant differences ($p < 0.05$).

Fermentation process is known to increase the TPC and antioxidant activity. During the fermentation process, *A. xylinum* thrives in the acidic environment in the media by converting glucose into gluconate acid and fructose into acetic acid. This causes the concentration of organic acids contained in the media to increase, thereby increasing the medium phenolic compounds and

eventually increases the antioxidant activity. The fermentation process could have increased the TPC and antioxidant activity slightly (Pothakos *et al.*, 2016), but might be counter-destroyed during the thermal process as shown in the results of this study.

Some research showed different results regarding temperature and prolonged heating on the TPC. In the previous studies, the temperature and long-cooking high during processing can essentially increase the TPC. The increase in TPC is caused by the release of the phenol compounds on the cell wall. However, the TPC began to decrease at a temperature of 104-105°C from several varieties of cherries and plums. In this research, the water temperature was not measured during the heating process. The indicator used were only boiling water at a temperature of 100°C at the beginning of heating of *Nata de pina* heating as well as time. Based on the process, the significant reduction could have started when the *Nata de pina* was heated due to the heating factor and the solubility of phenol in boiling water. The decrease of the TPC during cooking can occur by two processes through dissolving in the processing fluid and through oxidation. The TPC can be oxidized by the activity of the polyphenol oxidase enzyme thereby forming a reactive-semiquinone radical ortho. Due to the direct heating in boiling water, the cell walls and plasma membrane suffered quicker damage allowing water to insert into the cell wall and vacuoles and dissolving the phenol compounds into the processing fluid (Srinivas *et al.*, 2010; Aisyah *et al.*, 2015).

Antioxidant activities decreased after the heating process when making the product, this caused the value of IC₅₀ to increase in the samples of *Nata de Pina*. The heating process during processing can cause the degradation of natural antioxidants contained in honey pineapple variety fruit thereby accelerating the oxidation of antioxidant compounds. The oxidation process can be accelerated by the presence of heat, rays, alkali, enzymes, oxidizing agents, by copper and iron catalysts. The heating process with a temperature of 60°C for 30 mins can decrease 10% antioxidant capacity in some types of fruits. In this study, the TPC and antioxidant activity were relatively low after the processing of *Nata de pina*.

3.2 Organoleptic test

The results of organoleptic test in Table 3 presented statistically significant difference in the color acceptance ($p=0,030$), flavor ($p<0.001$) and texture ($p<0.001$), but not significant in the aroma ($p=0.955$). Fruit peel *Nata de pina* and fruit flesh *Nata de pina* indicated neutral or almost liked appearance by the panelist. Fruit core *Nata de pina* presented neutral or almost liked by the panelist in the color and aroma assessment but was less liked in the flavor and texture. The color of *Nata de pina* showed almost the same appearance that is slightly yellowish-white. The white color is produced due to of *A. xylinum*. The bacteria produce a compliable enzyme into a chain of sugar or cellulose fibers from the millions of biomass planted on the substrate, then will produce millions of cellulose yarn pieces and produce a solid white-looking coating to transparent (Nugroho and Aji, 2015).

The aroma of *Nata de pina* showed insignificant difference between all the samples of *Nata de pina*. This is due to the similarity of the distinctive aroma of the *Nata de pina*, which is a typical acid-scented fermentation. During fermentation, the precursor to flavor and aroma develops and the pigment is degraded by enzymes such as invertases, glycosidase, protease, and polyphenol oxidase. Fermentation affects the decline of compounds such as sugar, peptides, and amino acids, which are converted into flavor and smell profiles (Anal, 2019). The immersion process in the *Nata de pina* production is known to reduce the acid smell of *Nata de pina* produced. This process can eliminate the effects of acetic acid added to the fermentation medium during the processing and does not affect the flavour (Asri *et al.*, 2018).

The indicator of flavor showed a significantly difference, but only in the fruit core *Nata de pina*. According to panelists, fruit core *Nata de pina* had a very bland flavor. Fruit flesh *Nata de pina* had the highest acceptance. The variation of the resulting flavor arose from the acidic flavor due to the fermentation process. Texture indicators showed some variations from easily chewed and swallowed until hard to chew and swallow. The variation is influenced by the processing of basic materials and the fermentation process by *A. xylinum*. *A. xylinum* produce cellulose which will make

Table 3. Organoleptic test

Fruit Parts	Color		Aroma		Flavor		Texture	
	Mean±SD	Category	Mean±SD	Category	Mean±SD	Category	Mean±SD	Category
Peel	3.44±1.39 ^b	Neutral	3.20±0.71	Neutral	3.68±0.99 ^a	Neutral	3.72±0.89 ^{ab}	Neutral
Flesh	3.76±0.97 ^{ab}	Neutral	3.08±0.86	Neutral	3.92±1.00 ^a	Neutral	3.48±1.39 ^b	Neutral
Core	3.96±0.98 ^{ab}	Neutral	3.20±0.65	Neutral	2.76±1.13 ^b	Dislike	2.96±1.46 ^b	Dislike
<i>p</i> *	0.03		0.955		<0.001		<0.001	

*Analyzed via Kruskal Wallis Test. Different superscript letters in the same column showed significant differences ($p<0.05$)

the smooth surface of *Nata de pina*. A texture that has the highest acceptance is the fruit peel *Nata de pina* (Phong et al., 2017).

3.3 The best-recommended product

The results of the best-recommended product in Table 4 had confirmed that fruit core *Nata de pina* had the highest value (Index effectiveness = 0.696) compared to other parts of the fruit. Fruit peel *Nata de pina* had the lowest value. The best-recommended product had the highest TPC (139.88 mg GAE/100 g), antioxidant activity (263.38 ppm) and well-liked panelists based on the acceptance of aroma, color despite less liked for the flavors and textures. Product evaluation considers all variables to determine the product quality including the TPC, antioxidant activity and organoleptic tests. The flavor can be improved with the addition of low-calorie natural sweeteners in the syrup when packing the product.

Table 4. The best-recommended product

Samples	Index effectiveness
Peel	0.493
Flesh	0.512
Core	0.696

4. Conclusion

Nata de pina produced of the various fruit parts of honey pineapple variety had a difference in the total phenolic content and antioxidant activity. The waste of the fruit such as the peel and the core of honey pineapple variety can be utilized to produce *Nata de pina*. *Nata de pina* produced of the various fruit parts of honey pineapple variety gave a significant difference to the level of acceptance of color, flavor, and texture. The fruit core *Nata de pina* is the best product recommended based on the results of the analysis of the total phenolic content, antioxidant activities as well as organoleptically.

Conflict of interest

The author declares no conflict of interest. The authors alone are responsible for the content and writing of this article.

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