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THE EFFECT OF NATURAL PRESERVATIVE EXTRACTS ON THE PHYSICOCHEMICAL AND ANTIOXIDANT ACTIVITY OF COCONUT SAP

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Abstract

Coconut sap produced from coconut trees has a short shelf life due to contamination and undergoes natural fermentation. One way to extend the shelf life of coconut sap is by adding preservatives. This research aims to determine the effect of adding natural preservative extracts on the antioxidant value, pH, sugar, and alcohol content of coconut sap by adding natural preservatives. There are five samples: pure sap (NM); sap with sugar lime (NK); sap with sugar lime and cinnamon extract (NKM); sap with sugar lime and mango leaf extract (NDM); sap with sugar lime and noni leaf extract (NKD). The study was conducted by extracting natural preservatives, namely cinnamon extract, mango leaves extract, and noni leaves extract, using the maceration method. These natural preservative extracts were mixed with $Ca(OH)_2$ in a 1:1 ratio and dissolved in distilled water. The mixture was then mixed with the sap. The results of the antioxidant test showed that the IC_{50} values of the NM, NK, NKM, NDM, and NKD samples were 52.911, 144.409, 26.043, 49.275, and 24.21 ppm, respectively. The highest pH value was found in the NK sample at 13.8, and the lowest in the NM sample at 3.8. The lowest sugar content was in the NM and NDK samples, with 10.6 and 14.6%. The highest alcohol content was found in the NK and NKD samples, with alcohol contents of 14.80% and 14.20%. This study shows that adding natural preservatives such as cinnamon, mango leaves, and noni leaves can increase the antioxidant activity of coconut sap, raise its pH, and maintain its sugar content to prevent it from being reduced to alcohol.

Keywords: Alcohol; Antioxidant; Coconut sap; IC_{50}; pH

1. INTRODUCTION

Coconut sugar is produced by processing sap from the flower part of the coconut tree. Coconut sap can also be developed into palm sugar, bee feed, and bioethanol (Mugiono et al., 2014). According to research by Asghar et al. (2020), coconut sap has higher nutritional and antioxidant content than other saps like palm sap and sugarcane sap as a source of natural sugar. Using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, coconut sap showed antioxidant content at 23.42%, compared to palm sap at 19.82% and sugarcane sap at 12.40% (Asghar et al., 2020). This highlights the potential of coconut sap to produce healthier sugar products than palm sugar and sugarcane sugar (Pathirana et al., 2023).

Coconut sap produced from coconut trees does not last long due to contamination and natural fermentation, causing it to turn acidic. The deterioration is attributed to the presence of free radicals causing rancidity and the presence of an invertase enzyme that contaminates the sap which

aids in the hydrolysis of sucrose, reducing sugar content in the sap (Jannah et al., 2024). One way to extend the shelf life of sap is by adding preservatives. In food preservation, preservatives containing antioxidants are beneficial for preserving food without reducing its nutritional value (Hermiati et al., 2013).

Antioxidants can slow down or prevent oxidative stress caused by free radicals (Chaudhary et al., 2023). In addition to serving as preservatives, antioxidants also increase antioxidant levels in coconut sap (Haryanti et al., 2018). Antioxidant production is divided into two types: synthetic and natural antioxidants. Synthetic antioxidants are synthetic supplements widely used in food products such as packaged foods or drinks. Natural antioxidants are those that form naturally within food. Natural antioxidants can be found in vegetables, fruits, and spices. The quality of natural antioxidants in food can quickly degrade physically and chemically (Fadhil et al., 2023). Therefore, additional antioxidants as preservatives are needed to protect food ingredients from oxidation reactions.

Sumbersari Mandah Village has an agro-industry of coconut sap (Djanatiya et al., 2010). This village's coconut sap collected by tappers is mixed with $Ca(OH)_2$ to prolong its shelf life. The more lime added, the longer the sap will last. However, this will affect the resulting sugar, making it darker and potentially lowering the quality and antioxidant content of the produced brown sugar. Good quality brown sugar has a distinctive aroma, a yellowish-brown color, a maximum water content of 10.0%, and a maximum sucrose content of 77% (Palma, 1995). Poorly preserved coconut sap will produce brown sugar that quickly becomes watery due to microbial activity, reducing the sugar content. This village has abundant resources, including cinnamon (Rosa et al., 2023), mango leaves (Rahmiyani et al., 2016), and noni leaves (Marbun et al., 2020), which can be used as antioxidant resources.

This study added cinnamon, mango, and noni leaf extract as preservatives for coconut sap from Sumbersari Mandah Village.

2. MATERIALS AND METHOD

2.1 Materials

Technical grade of calcium hydroxide and 96% ethanol acquired from the local chemical store, pure sap, cinnamon (*Cinnamomum verum*), mango (*Mangifera indica L*) leaves, noni (*Morinda citrifolia*) leaves from Mandah Village, South Lampung, analytical grade of methanol (Merck), and distilled water from the local market.

2.2 Preparation of Natural Preservative Extracts

The extracts were prepared using the maceration method. Fifty grams of each preservative powder were placed in vacuum jars, and 300 ml of ethanol solvent was added. The mixture was left for 48 hours and stirred every 12 hours. The macerated mixture was filtered to separate the solids using filter paper. The filtrate was evaporated using an IKA HB digital rotary evaporator at 60°C with a rotation speed of 54 rpm until a thick extract was obtained.

2.3 Preparation and Placement of Natural Preservatives

Five grams of the thick extract was mixed with $Ca(OH)_2$ in a 1:1 (m/m) ratio and dissolved in 20 mL of distilled water. The mixture was then placed in the sap collection container for tapping (Tanra et al., 2019). Tapping was carried out until the sap volume reached 3.5 litres.

2.4 Testing Coconut Sap with Added Preservatives

2.4.1 Testing sugar and alcohol content

This test used a sugar and alcohol refractometer with units in % brix. The sample solution was added to the refractometer's prism, and the lid was closed. Then, the device was held to a light source to look through the lens, and an internal scale was seen. The brix reading was where the light and dark areas met on the scale (Instrument Choice, 2020).

2.4.2 pH analysis

This test was conducted using a pH meter. The pH meter was turned on by pressing the on button. Then, the pH meter was inserted into a container that contained the samples to be tested. When dipped, the scale of the numbers would move randomly and wait until the numbers become fixed to obtain the pH value of the samples (Sumber Aneka Karya Abadi, 2023)

2.4.3 Testing antioxidants with the DPPH method

The DPPH Stock Solution was prepared by dissolving 5 mg of DPPH with 100 ml of PA methanol. Each sample is diluted to concentrations of 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm, and 0.5 ppm.

3 ml of the sample solution was put into a test tube, and 3 ml of the DPPH solution was added. The sample was incubated for 30 minutes in a dark place. It was tested using a Scientific GENESYS 150-type UV-Vis spectrophotometer at a wavelength of 517 nm. For the blank solution, 2 ml of PA methanol and 1 ml of DPPH solution were used instead (Oessoe et al., 2023).

2.4.4 *Calculating antioxidant activity and determining IC_{50}*

The antioxidant activity is determined using the following equation ((Jumina et al., 2019):

$$\frac{blankabsorbance - sample\ absorbance}{blank\ absorbance}\ x\ 100\% \qquad (1)$$

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity of Coconut Sap

The test was conducted for three consecutive days, and the absorbance of the samples was

Table 1. Results of coconut sap antioxidant activity test

Sample code	Sample Concentration (ppm)	1st day	2nd day	3rd day
		Antioxydant activity		
NM	0,5	0,489	0,517	0,616
	0,4	0,122	0,446	0,285
	0,3	-0,191	0,28	-0,136
	0,2	-0,639	0,005	-0,226
	0,1	-0,741	-0,266	-0,217
NK	0,5	0,791	0,688	0,84
	0,4	0,478	0,655	0,454
	0,3	0,283	0,286	0,214
	0,2	-0,703	-0,248	-0,867
	0,1	-1,774	-0,72	-1,671
NKM	0,5	0,894	0,915	0,805
	0,4	0,725	0,908	0,598
	0,3	0,479	0,793	0,517
	0,2	0,521	0,717	0,375
	0,1	0,134	0,503	0,288
NDM	0,5	0,796	0,956	1,095
	0,4	0,726	0,911	1,074
	0,3	0,633	0,908	1,033
	0,2	0,646	0,737	0,892
	0,1	0,527	0,638	0,842
NKD	0,5	0,502	1,052	0,479
	0,4	0,288	0,823	0,204
	0,3	0,051	0,655	-0,150*
	0,2	-0,063	0,43	-0,103*
	0,1	-0,312	0,229	1,188*

measured to calculate their antioxidant activity, as presented in Table 1.

The test at 2nd-day has been indicated as the best coconut sap antioxidant values obtained for samples treated with natural preservatives. Negative antioxidant activity values indicate that, at that concentration, the antioxidant activity is very weak or even absent (Tanra et al., 2019). The negative antioxidant activity value indicated that negative results were considered in the antioxidant assay. Few compounds work via the redox pathway, opposite to antioxidants (Olszowy-Tomczyk & Wianowska, 2023). To determine the highest antioxidant content, the IC_{50} values were calculated, as shown in Figure 1. NK obtained the highest IC50 value, while NKD obtained the lowest IC_{50} value. A low IC_{50} value indicates that the antioxidant contained is very potent (Andriani et al., 2019). The value for IC50 can be described in Table 2.

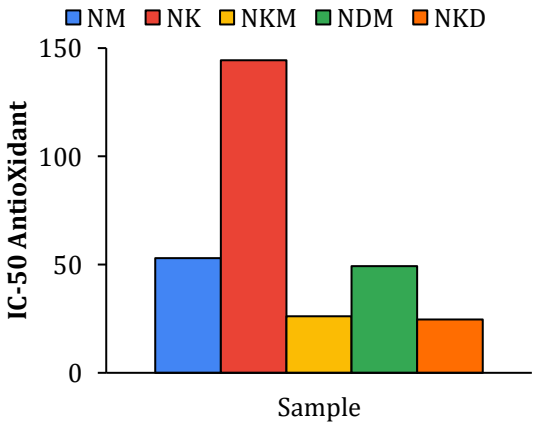

Figure 1. Effect of natural preservatives on antioxidant activity of coconut sap

Table 2. IC_{50} value information

IC_{50} Value	Information
< 50 ppm	Very strong
50 ppm < IC_{50} ≤ 100 ppm	Strong
100 ppm < IC_{50} ≤ 150 ppm	Moderate
150 ppm < IC_{50} ≤ 200 ppm	Weak
> 200 ppm	Very weak

3.2 pH Analysis of Coconut Sap

pH testing on the samples was conducted to observe the effect of natural preservatives on the pH of the coconut sap produced. The pH value of NM was used as a reference value in the study. The results of the coconut sap pH test can be seen in Figure 2.

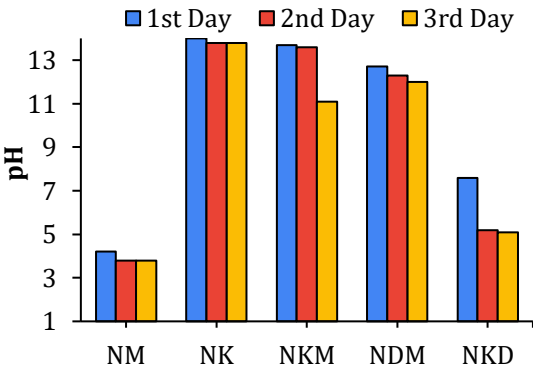

Figure 2. The effect of different natural preservatives on the pH of coconut sap

In the NK sample, the alkaline pH of the sap is influenced by the addition of lime, which is a strong base. Slaked lime ($Ca(OH)_2$) dissolved in water ionizes to form OH^- ions, which are basic and can neutralize acidic conditions (Suntoro & Suyatno, 2016).

The pH of the NKM, NDM, and NKD samples was higher than the NM sample. These are also influenced by the addition of lime in each use of natural preservative samples, which affects the pH of the natural extracts containing acids. In the NKD sample, the resulting pH remained acidic due to the high ascorbic acid content in noni leaves, which limited NKD's ability to prevent pH decline to just one day (Marbun et al., 2020). The pH value of the NKD sample also decreased from the first to the third days, which might be influenced by the endophytic bacteria of noni leaves. This was indicated by the sample becoming odorous and containing gas during physical observation, which significantly affected the chemical properties of the sap, including a notable decrease in pH. Based on the research of Yang et al. (2015), noni leaves have endophytic bacteria, and their distribution comprising Pseudomonas (35%), Acinetobacter (25%), Bacillus (10%), Burkholderia (5%), Gluconobacter (8%), Streptococcus (7%), Staphylococcus (4%), Propionibacterium (2%), and Sphingomonas (1%) (Yang et al., 2015). From these bacteria, the Streptococcus species and some Bacillus species can convert the sugars in the sap into lactic acid (Serna Cock & Rodríguez de Stouvenel, 2005). While gluconobacter bacteria is

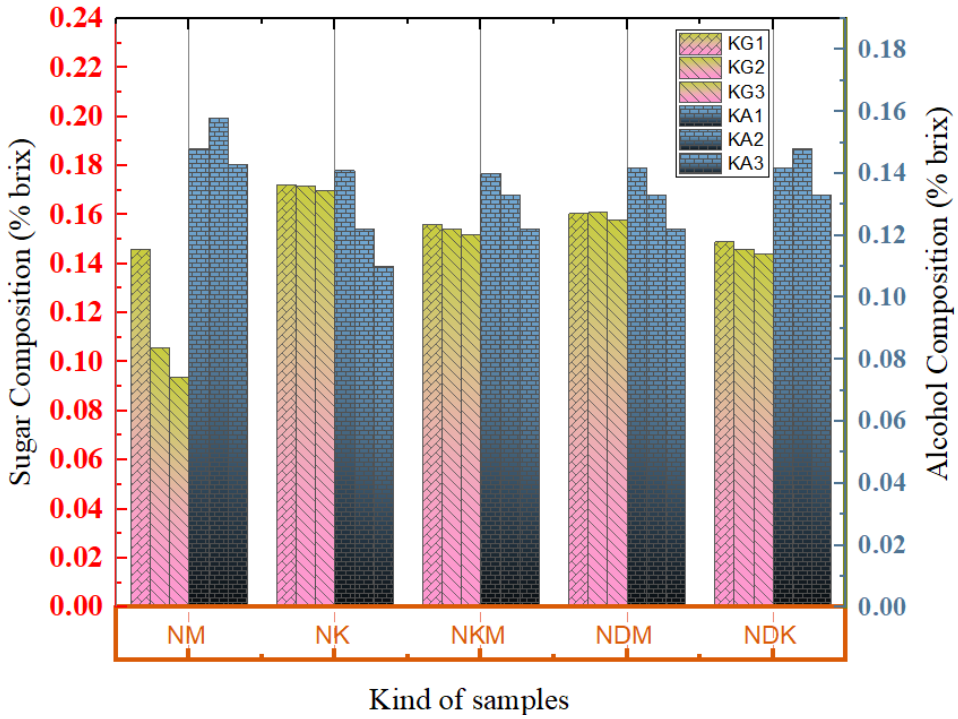

Figure 3. Effect of natural preservatives on coconut sap sugar and alcohol content on KG1=sugar composition 1st day, KG2=sugar composition 2nd day, KG3=sugar composition 3rd day, KA1 = alcohol composition 1st day, KA2 = alcohol composition 2nd day, KA3 = alcohol composition 3rd day 2 and 3 (1st day, 2nd day, 3rd day)

an acetic acid bacterium that is widely known to oxidize components such as sugars and alcohols into acetic acid (da Silva et al., 2022; Serna Cock & Rodríguez de Stouvenel, 2005).

3.3 Sugar and Alcohol Content of Coconut Sap

The sugar and alcohol content of the samples was tested to observe the effect of natural preservatives on the sugar and alcohol content of the coconut sap produced. The test results are shown in Figure 3.

The sugar and alcohol content of NM was used as the baseline for comparison in this test. Figure 3 shows that the highest sugar content was found in the NK sample, while the lowest was in the NM sample. The storage time of the sap affects its sugar content, as evidenced by the decrease in sugar content over the three-day test period. The average reduction in sugar content for NKM, NDM, and NDK was 0.2%, 1.57%, and 0.25%, respectively. The percentage decrease in sugar content indicates that adding natural preservatives can prevent the reduction in sap sugar content better than NM, which had a percentage decrease of 2.6%. The alcohol content test of sap was conducted based on the fermentation reaction of sugar into alcohol in the sap, as well as the presence of carbon dioxide gas in the sap sample, as shown by the following reaction:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (2)$$

Based on this equation, the relationship between sugar and alcohol content in the sap is inversely proportional. The higher the sugar content in coconut sap, the lower the alcohol content. This occurs because lime preservatives can prevent acidic conditions in coconut sap that can trigger the fermentation process of converting glucose into alcohol (Rizki & Lubis, 2021).

4. CONCLUSION

The addition of natural preservatives from cinnamon extract, mango leaf extract, and noni leaf extract can increase the antioxidant activity of coconut sap, and the highest antioxidant activity was found in the sap sample with noni leaf extract preservative (NKD) with an IC_{50} value of 24.521 ppm. The highest pH value of sap with a mixture of extract preservatives was obtained in coconut sap with sugar lime (NK), with the smallest average pH decrease of 0.1% over three consecutive days. Adding natural antioxidants slows the reduction in sugar content and improves the decrease of the alcohol content. The highest sugar and alcohol content was found in the NK and NM samples.

5. REFERENCES

Andriani, Y., Ramli, N. M., Syamsumir, D. F., Kassim, M. N. I., Jaafar, J., Aziz, N. A., Marlina, L., Musa, N. S., & Mohamad, H. (2019). Phytochemical analysis, antioxidant, antibacterial and cytotoxicity properties of keys and cores part of Pandanus tectorius fruits. Arabian Journal of Chemistry, 12(8), 3555–3564.

Asghar, M. T., Yusof, Y. A., Mokhtar, M. N., Ya'acob, M. E., Mohd. Ghazali, H., Chang, L. S., & Manaf, Y. N. (2020). Coconut (Cocos nucifera L.) sap as a potential source of sugar: Antioxidant and nutritional properties. Food science & nutrition, 8(4), 1777-1787.

Badan Standardisasi Nasional. (1995). Standar Nasional Indonesia Gula Palma.

Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Calina, D., & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. In Frontiers in Chemistry (Vol. 11). Frontiers Media S.A.

da Silva, G. A. R., Oliveira, S. S. de S., Lima, S. F., do Nascimento, R. P., Baptista, A. R. de S., & Fiaux, S. B. (2022). The industrial versatility of Gluconobacter oxydans: current applications and future perspectives. In World Journal of Microbiology and Biotechnology (Vol. 38, Issue 8). Springer Science and Business Media B.V.

Djanatiya, E., Hasyim, A. I., & Soelaiman, A. (2010). Analisis kelayakan finansial dan pemasaran agroindustri gula kelapa di kecamatan natar kabupaten lampung selatan.

Haryanti, P., Wiseso Marseno, D., & Santoso, U. (2018). Effects of different weather conditions and addition of mangosteen peel powder on chemical properties and antioxidant activity of coconut sap. Agritech, 3(38), 295–303.

Hermiati, Manalu, N., & Sinaga, M. (2013). Ekstrak daun sirih hijau dan merah sebagai aintioksidan pada minyak kelapa. Jurnal Teknik Kimia USU, 2, 37–43.

Instrument choice. (2020, October 6). What is a brix refractometer, and how do they work?

Jannah, Z. F., Suciati, S., Fakhrudin, I. A., Silvita, S., Prasetyo, O., & Rachman, H. T. (2024). Identification of scientific knowledge of the process making palm sugar as local wisdom of banyumas and its potential for empowering scientific thinking skills. Jurnal Pendidikan Sains Indonesia, 12(4), 935–960.

Jumina, J., Siswanta, D., Zulkarnain, K., Triono, S., Priatmoko, P., Yuanita, E., Fatmasari, N., & Nursalim, I. (2019). Development of C-arylcalix[4]resorcinarenes and C-arylcalix[4]pyrogallolarenes as antioxidant and UV-B protector. Indonesian Journal of Chemistry, 19, 273.

Fadhil, K. A., Suryati, T., & Jayanegara, A. (2023). Comparison between natural and synthetic antioxidants in beef products: A metaanalysis. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan, 11(1), 19-26.

Marbun, R., Sholahuddin, S., & Rahayuni, T. (2020). Pengaruh kombinasi suhu dan dehumidifikasi udara pengering terhadap aktivitas antioksidan

irisan buah mengkudu (Morinda citrifolia). Pro Food, 6, 560.

Mugiono, M., Marwanti, S., & Awami, S. N. (2014). Analisis pendapatan usaha gula merah kelapa (studi kasus di desa Medono kecamatan Kaliwiro kabupaten Wonosobo). Mediagro, 10(2).

Oessoe, Y. Y. E., Assa, J. R., Paat, F. J., Tangkeallo, S. C. T., Tooy, D., Koapaha, T., Tumbelaka, S., Mamuaja, C. F., & Latumakulita, L. A. (2023). Antioxidant activity on sarrabba is based on the proportion of red ginger extract (Zingiber officinale Roscoe) and Cinnamon extract (Cinnamomum verum J.Presl). Journal of Agriculture, 2(02), 205–211.

Olszowy-Tomczyk, M., & Wianowska, D. (2023). Antioxidant properties of selected flavonoids in binary mixtures—considerations on myricetin, kaempferol and quercetin. International Journal of Molecular Sciences, 24(12).

Pathirana, D., Wijesekara, I., Yalegama, L., & Jayasinghe, M. (2023). Collection methods to preserve nutritive and physicochemical properties of unfermented coconut (Cocos nucifera) sap. CORD, 39, 9–15.

Rahmiyani, I., & Nurdianti, L. (2016). Aktivitas antioksidan ekstrak daun mangga (Mangifera indica L. Var. Gedong) menggunakan metode DPPH. Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-ilmu Keperawatan, Analis Kesehatan dan Farmasi, 16(1), 17-23.

Rizki, S., & Lubis, S. (2021). Analisis total koloni dan uji kadar alkohol pada fermentasi air nira (Arenga pinnata). KENANGA Journal of Biological Sciences and Applied Biology, 1, 1–7.

Rosa, R., Usman, H., & Desra, A. (2023). Uji Aktivitas antioksidan ekstrak kayu manis (Cinnamomum burmanii). Jurnal Farmasi Sains Dan Obat Tradisional, 2(1), 151.

Serna Cock, L., & Rodríguez de Stouvenel, A. (2005). Producción de ácido láctico por una mezcla de Lactococcus lactis y Streptococcus salivarius en fermentaciones en discontinuo. Revista Colombiana de Biotecnología.

Sumber aneka karya abadi. (2023, May 8). pH meter.

Suntoro, A., & Suyatno. (2016). Mempelajari penambahan kapur sirih $Ca(OH)_2$ sebagai bahan penghambat kerusakan pada nira kelapa. Edible, 49–53.

Tanra, N., Syam, H., & Sukainah, A. (2019). Pengaruh penambahan pengawet alami terhadap kualitas gula aren (Arenga pinnata Merr.) yang dihasilkan. Jurnal Pendidikan Teknologi Pertanian, 5, 83.

Yang, L., Yinhu, L., Su, Y., Hui, W., Cao, Y., Jie, L., Bai, F., Qiu, C., Xin, F., Dai, W., & Chi, C. (2015). Diversity and distribution of endophytic bacterial community in the Noni (Morinda citrifolia L.) plant. African Journal of Microbiology Research, 9, 1649–1657.