Jurnal Belantara Vol. 7, No. 2, Agustus 2024 (197-208) E-ISSN 2614-3453
DOI: 10.29303/jbl.v7i2.1055 P-ISSN 2614-7238

EFFECT OF CAJUPUT LEAF STORAGE TIME (*Melaleuca leucadendron* Linn) ON OIL QUALITY (*Case Study In Rote Ndao District*)

Melintong Tamonob[1], Luisa M. Manek*[1], Adrin[1]

[1]Department of Forestry, State Agricultural Polytechnic of Kupang
Jl. Prof. Dr. Herman Yohanes Lasiana Kupang 85011 Indonesia

*Email: mooj15manek@gmail.com

Received: 01/07/2024, Revised: 31/07/2024, Approved: 02/08/2024

ABSTRACT

Rote Ndao Regency, East Nusa Tenggara Province is one of the areas producing cajuput oil. Cajuput oil is one of the non-timber forest products produced by distilling cajuput leaves of the *Melaleuca leucadendron* L. species. Several factors affect the quality of cajuput oil, namely filling the leaves in the kettle, tree varieties, where cajuput grows, leaf storage, distillation techniques, distillation temperature, and leaf age. The purpose of this study was to determine the effect of the length of time of storage of raw materials on the yield, refractive index, and sineol content of cajuput oil and to determine the best time of storage of raw materials before distillation. Cajuput oil leaves are then stored with variations in the length of time ranging from, 3 days, 4 days, and 5 days and the distillation method used is the boiled method. The results showed that the highest average per parameter was obtained in storage for 5 days with a yield value of 2.43%, refractive index of 1.45%, and sineol content of 56.67%. The average values of sineol content and refractive index are included in the Indonesian National Standard (SNI 06-03954- 2014).

Keywords: Length of storage; distillation method; cajuput oil quality; Rote Ndao Regency

INTRODUCTION

Cajuput (*Melaleuca leucadendrin* Linn) is a native Indonesian plant found in Eastern Indonesia and is one of the NTFPs that has high economic value (Tata et al., 2019). The utilization of non-timber forest products (NTFPs) has recently increased because it is used by the community as a source of livelihood (Palmolina, 2014); Wahyu Safitri et al., 2023), have strategic value, provide comparative benefits because are directly related to the community around the forest (Kartila et al., 2018). Cajuput plants can produce cajuput oil through the process of refining their leaves with the principle of distillation (Tata et al., 2019). Cajuput oil is generally used as a raw material for medicines and has been utilized by the Indonesian people for a long time (Tata et al., 2019). The main component of cajuput oil is 1,8-cineole, this component has long been used empirically to treat infections and respiratory disorders. The greater the content of sineol, the better the quality of cajuput oil (Aryani et al., 2020). The component that has a large enough content in cajuput oil, namely sineol, is 50% to 65% (Khabibi, 2011; Aryani et al., 2020).

The use of sineol in inhaled cajuput leaf derivatives can be used to treat pharyngitis, bronchitis, sinusitis, asthma, and *Chronic Obstructive Pulmonary Disease* (COPD) (Cermelli et al., 2008; Sadlon and Lamson, 2010; Aryani et al., 2020). Warm water vapor inhalation therapy mixed with cajuput oil in children with Acute Respiratory Tract Infection (ARI), can improve airway hygiene with marked improvement in vital signs (decreased pulse and respiratory rate), decreased cough intensity, and decreased ronchi sound (minimalist) (Arini & Syarli, 2022). *In vivo* inhalation of sineol compounds has the potential as a slimming aromatherapy (can provide a slimming effect), without reducing feed consumption (Batubara et al., 2016). The Central Bureau of Statistics (BPS) noted that Indonesia's cajuput oil production reached 25.06 million liters in 2020, an increase of 229.5% from 7.6 million liters in the previous year. The increase is thought to be due to the high public demand for cajuput oil during the COVID-19 coronavirus pandemic. This is because cajuput oil can relieve COVID-19 symptoms such as headaches, nasal congestion, and stomach pain (Rizaty, 2021).

Cajuput plants also have good prospects for development (Alam et al., 2019). Cajuput development has long been carried out on Java Island and in Maluku and Buru Islands are natural stands which are generally old stands (Rimbawanto et al., 2017). However, the demand for *cajuput* oil on a national scale cannot be met, as a result, Indonesia must import oil from *Cajuput* leaves to be mixed with cajuput oil with a level of 1.8 cineole does not reduce the quality of cajuput oil but the distinctive odor of the oil is no longer prominent (Rimbawanto et al., 2017). According to (Guenter, (2011); (Smith & Idrus, (2018), factors that are thought to affect the quality of cajuput oil (yield and quality) include filling the leaves in the kettle, tree varieties, where cajuput grows, leaf storage, distillation techniques, distillation temperature, and leaf age. In addition, according to (Smith & Idrus, 2018), other factors that also affect the quality of cajuput oil cajuput is the place where cajuput grows, this is because the soil fertility and climate of each place are different. Besides influencing the growth rate of cajuput plants (Wedhana et al., 2018), it also affects the yield and properties of essential oils (Zuzani et al., (2015); Kartiko et al., 2021). Other factors are plant conditions, growing environment, harvesting age, sufficient sunlight and rainfall or water conditions, and fertile soil (Zuzani et al., (2015); Kartiko et al., 2021). In addition to these factors, crop patterns with an agroforestry system on former mining land with a spacing of 0.5 m x 0.5 m can produce a fairly high cajuput oil yield of 2.73% compared to monoculture cropping patterns (Juliarti et al., 2022). Silvicultural treatments such as selection of cajuput oil leaf size, fertilization treatment, distillation techniques (pure leaves and dirty leaves), cajuput intercropping cultivation techniques, proper site selection, and the use of superior seeds were able to increase cajuput oil yield (Tata et al., 2019).

Judging from the location where it grows, one of the locations for the development of cajuput oil on a *home* industry scale in East Nusa Tenggara is in Ingguinak Village, Northwest Rote Subdistrict, Rote Ndao Regency. This distillation house conducts 2 distillations a week and 4 distillations a month with a 150 kg boiler capacity. The problem is that the distillation process is done directly after the leaves are picked. The oil produced in one distillation is 1 liter, so the yield produced for 1 distillation is 0.6%. When compared to the amount of raw materials needed for 1 time distillation, the resulting yield is still very low. The purpose of the study was to determine the effect of the length of time of storage of distillation raw materials on yield, refractive index, and sineol content of cajuput oil (*Melaleuca leucadendron* Linn).

METHODS

Research Time and Location

This research was conducted from November 7, 2022, to February 6, 2023, at the Bioscience Laboratory of Nusa Cendana University Kupang.

Research Materials and Tools

Materials used in this study include cajuput leaves (*Melaleuca leucadendron* L.); distillation results in the form of cajuput oil; 1 liter of distilled water for each replication, used to help evaporation during distillation; Label paper, to give information on each sample; methanol, as an HPLC solvent; tissue, to clean the refractometer lens, and Eppendorf sample bottles.
The tools used in this study, among others; a set of cajuput oil distillation equipment with a capacity of 150 grams of raw material; HPLC (*High-Performance Liquid Chromatography*) to determine the content of cineol content in cajuput oil; a Refractometer to determine the refractive index value; hot Plate to heat the distillation flask; Eppendorf to move micro-scale samples; eyela CCA-1111, as a cooling condenser; micro-scale syringe to inject cajuput oil into HPLC; Analytical scales to determine the weight of cajuput oil and the weight of distillation raw materials; sample bottle as a container for each sample of distillation results; Blender to smooth the distilation raw materials; calculator to calculate yield; cell phone for documentation; stationery; Microsoft Excel 2010 and SPSS Statictiks 29.02.0, to process data.

Research Design

Cajuput leaves were treated by drying them indoors for 3 days, 4 days, and 5 days. After drying, the raw materials were blended and then weighed each with a weight of 150 grams. Then it was distilled with a duration of 2 hours using the boil method. Each treatment level was carried out 3 times so that in total there were 9 treatment combinations. This study used a completely randomized design (CRD) to determine whether there was an effect of the length of time the raw material was stored on the quality of cajuput oil. Cajuput oil quality test according to the Indonesian National Standard (SNI 06-03954-2014) (Indonesian Standardization Agency, 2014). The parameters tested in this study were yield, refractive index, and cineol content. The yield value was calculated using the formula (Haris, 1998; Aryani, 2020):

$$\text{Yield} = \frac{\text{ouput}}{\text{input}} \text{x} 100\%$$

Description:
Output = weight of essential oil produced (kg)
Input = weight of raw materials (kg)

Data Analysis

Data analysis using the RAL linear equation model to determine the effect of the length of time the raw material is stored on the quality of cajuput oil (*Melaleuca leucadendron* Linn), with the following linear equation:

Linear RAL Equation: $Yij = \mu + \tau i + \varepsilon ij$ Where: i

= 1,2,3,....,n; j = 1,2,3, ,n

Description:
1. Yijk = Observation on the i-th treatment and j-th replication.
2. μ = Population mean.
3. τi = i-th treatment effect.
4. εijk = the effect of experimental error of the Ith treatment of the jth replication.

If the analysis of variance (ANOVA) shows a significant effect, further tests are carried out using

BNT (Least Real Difference). Analysis of RAL, Anova, and BNT using the SPSS software version
29.0.2 (Aqil & Efendi, 2015).

Research Hypothesis

The conclusion criteria for hypothesis testing in the analysis of variance are as follows (Nuryadi et al., 2017):

1. If the value of F count> F table (0.05) then H0 is rejected and H1 is accepted.

2. If the calculated F value < F table (0.05) then H0 is accepted and H1 is rejected.

The hypothesis or temporary answer in this study is as follows (Tersiana, 2018):

H0 : There was no significant effect of raw material storage time on the yield, refractive index and sineol content of cajuput oil.

H1 : There is a significant effect of raw material storage time on the yield, refractive index, and sineol content of cajuput oil.

RESULTS AND DISCUSSION

I. Effect of Storage of Raw Materials on the Yield of Cajuput Oil (*Melaleuca leucadendron* Linn)

The yield of cajuput oil produced from the distillation process is expressed through the percentage of the oil produced based on the weight of the leaves during the distillation process (Tata et al., 2019). The results of the analysis *of variance* (ANOVA) of cajuput oil yield can be seen in Table 1.

Table 1: Analysis of Yield Variance

SK	DB	JK	KT	Fhit	Ftab		Notation
					0,05	0,01	
Treatment	2	3,1338	1,5669	18,8783	5,143253	10,92477	**
Error	6	0,498	0,083				
Total	8	3,6318					

Source (*Resources*): Primary Data 2023

*Description: **: Very significant influence*

The significant value of the effect of the length of time the raw material is stored for distillation on cajuput oil yield with a calculated F value of 18.87> F table value of 5.14 (0.05) so that H1 is accepted and H0 is rejected. Because the value of F count> F table means that there is an authentic influence between the length of time the raw material is stored on cajuput oil yield.

These results were further tested using BNT (Least Significant Difference) to determine the most influential treatment (Table 2).

Table 2: Results of Least Significant Difference Test for Yield

Treatment	Average	BNT Value
H_3	1,11 a	
H_4	1,26 a	0,5756
H_5	2,43 b	

Resources: Primary Data 2023
Notes: Mean values followed by the same notation mean are not significantly different.
Description: Average values followed by the same notation are not significantly different

The results of further tests of the Least Significant Difference (BNT), day 5 treatment is significantly different from the treatment of day 3 and day 4. Thus, the best storage treatment to increase cajuput oil yield is for 5 days. The average yield value produced continues to increase in each treatment, namely 1.1% (H3), 1.26% (H5) and 2.43% (H5). This is in line with the results of research (Aulia et al., (2022); (Manek et al., (2023), that fresh cajuput leaves produce lower oil yields (0.307%) compared to withered leaves for 2 days (0.522%) and 4 days (0.617%). This is because fresh leaves still contain a lot of water so that water vapor is more difficult to penetrate the cell wall, which causes the distillation process to be less than optimal. According to (Siarudin & Widiyanto, (2014), the high-water content in fresh leaves will cause the distillation process to be long and require more energy to evaporate the water in the leaves. Therefore, fresh cajuput leaves are usually stored for some time so that the water content evaporates, thus reducing the water content in the leaves. The average yield value of each treatment can be seen in Figure 1.

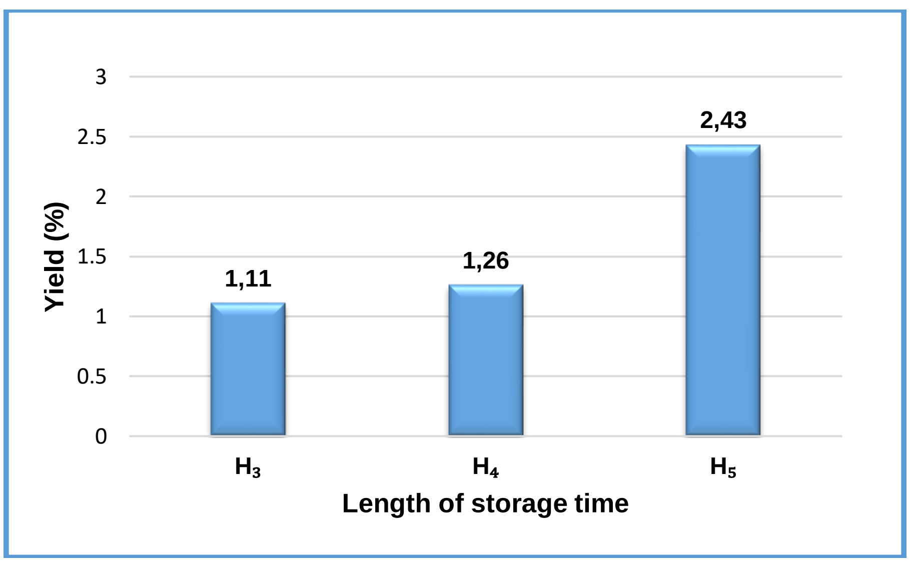


Figure 1. Histogram of Average Yield Value

One of the factors that affect the percentage yield and quality of cajuput oil is the treatment before distillation and the method of distillation (Nujana, 2006; Ethadpur and Tavasolian, 2019: Kartiko et al., 2021). The *Hydrodistillation* method in quantity produces a lower percent yield, namely (25ml/10kg cajuput leaves) than *Steam distillation (*31ml/10kg cajuput leaves) (Mbaru et al., 2018). This is due to variables such as distillation time, compressibility of leaves in the distillers, and condensate water rate. The higher the compressibility of the leaves in the distillers, the smaller the cajuput oil yield, and the slower the condensed water rate. Based on the results of research (Muyassoh, 2016) distillation by steam method on young leaves, withered and dry leaves with operating pressure variations of 0.5; 1; 1.5; 2; 2.5 kg/cm^2, the results showed that in dry leaf variables with a pressure of 2kg/cm^2, resulting in a yield of 0.83%. The variety and time of taking cajuput leaf raw materials are thought to affect the yield of cajuput oil in this study. Distillation of cajuput leaves using the steam distillation method with raw materials aerated for 48 hours produced yields of 1.43% from Maluku and 1.84% from West Nusa Tenggara. The difference in yield can be caused by differences in growing locations (altitude), where cajuput leaves from Maluku are at an altitude of 1086 masl and from West Nusa Tenggara are at 225 masl (Rienoviar et al., 2023). Supporting the opinion (Satriadi et al., (2020); Rienoviar et al., 2023) that cajuput plants growing in lowland areas have higher oil content than plants growing in highland areas.

II. Effect of Storage of Distilled Raw Materials on the Bias Index of Cajuput Oil (*Melaleuca leucadendron* Linn)

The refractive index parameter is used to test the purity and quality of essential oils using a refractometer (Rimbawanto et al., 2017). According to (Raningsing et al., 2018); Kartiko et al., 2021), the refractive index is a measure that shows the refraction of oil and air light. Whether there is an effect of storage of distilled raw materials on the refractive index of cajuput oil can be tested using ANOVA (Table 3).

Table 3. Analysis of variance of the refractive index

SK	DB	JK	KT	Fhit	Ftab		Notation
					0,05	0,01	
Treatment	2	0,00482	0,00241	15,5	5,1433	10,9248	**
Error	6	0,00093	0,00015				
Total	8	0,00575					

Source (*Resources*): Primary Data
*Description: **: Very significant influence*

The results of variance showed a significant value between the length of time of storage of distillation raw materials on refractive index. The value of F count 15.5 > F table 5.14 (0.05) so that H1 is accepted and H0 is rejected, because the value of F count > F table means that there is a real influence between the length of time storage of distillation raw materials on the refractive index of cajuput oil. Based on the bias test, each treatment gave a different response (Table 4).

Table 4. Results of Least Significant Difference Test for refractive index

Treatment	Average	BNT value
H_3	1,39 a	
H_4	1,42 b	0,0249
H_5	1,45 c	

Resources: Primary Data
Notes: Mean values followed by the same notation mean are not significantly different.
Description: Average values followed by the same notation are not significantly different

Based on Table 4, the Least Significant Difference (BNT) further test shows that the treatment of day 3 is significantly different from day 4, the treatment of day 4 is significantly different from day 5. The difference in the value of the BNT further test is indicated by an increase in the refractive index value in all treatments with consecutive values, namely 1.39 (H3), 1.42 (H4), and 1.54 (H5). When viewed from the highest average value between each treatment, the 5th-day treatment is the best for the refractive index value of cajuput oil (Figure 2).

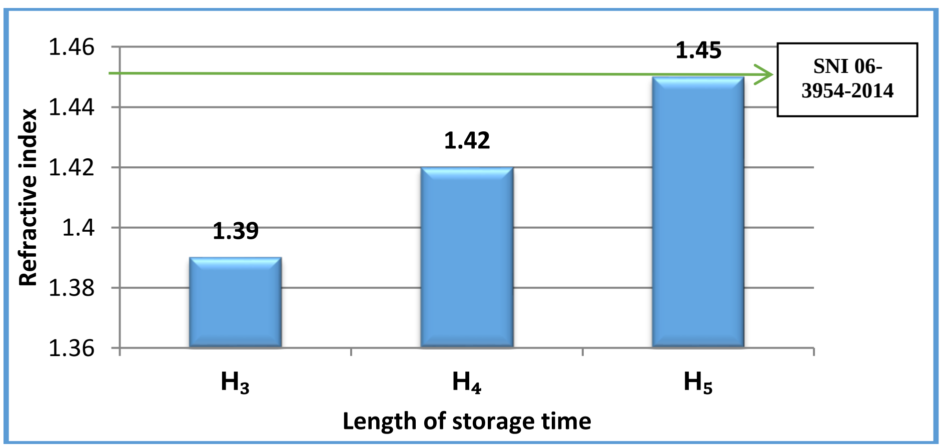


Figure 2. Histogram of Average refractive index value

The refractive index value in the raw material storage treatment for 5 days (H5) meets the Indonesian National Standard with a value of 1.45. This value is as required in the Indonesian National Standard (06-3954-2014) which is 1.450-1.470. Based on the results of research (Kartiko et al., 2021), the leaves of *M. leucadendron* L. from 4 different locations with raw material treatment were chopped, producing a variety of refractive index values, namely between 1.429 and 1.450. The results of cajuput oil distillation from four districts in Maluku also have various refractive index values, namely between 1.4605 and 1.4646 (Smith & Idrus, 2018). The refractive index of cajuput oil obtained from the *Hydrodistillation* method is 1.4585, while the *Steam distillation* method is 1.4602 at 20°C (Mbaru et al., 2018). The refractive index value of essential oils is strongly influenced by long-chain components, such as oxygen or sesquiterpene group components, and the number of carbon chains contained in essential oils.

The more long-chain components, the oil density will increase which causes the speed of light in essential oils to be lower so that the refractive index value is high (Langenau, 1955; Rimbawanto et al., 2017).

III. Effect of Storage of Raw Materials on the Sineol Content of Cajuput Oil (*Melaleuca leucadendron* Linn)

The length of storage of raw materials is thought to affect the levels of sineol in cajuput oil. Therefore, to determine whether or not there is an effect of storage of distilled raw materials on *cajuput* oil (*M. leucadendron* Linn) cineol content, it was tested using ANOVA (Table 5).

Table 5. Analysis of variance of the cineole content

SK	DB	JK	KT	Fhit	Ftab		Notation
					0,05	0,01	
Treatment	2	35,4203	17,7101	14,8146	5,1433	10,9247	**
Error	6	7,1727	1,1955				
Total	8	42,5930					

Source (*Resources*): Primary Data 2023
*Description: **: Very significant influence*

The results of the analysis of variance showed the value of F count 14.81 > F table 5.14 (0.05) so that H1 was accepted and H0 was rejected. Thus, the value of F count> F table, there is an authentic influence between the length of time the raw material is distilled to the level of cajuput oil sineol so it is necessary to do a further test of the Least Real Difference or BNT (Table 6).

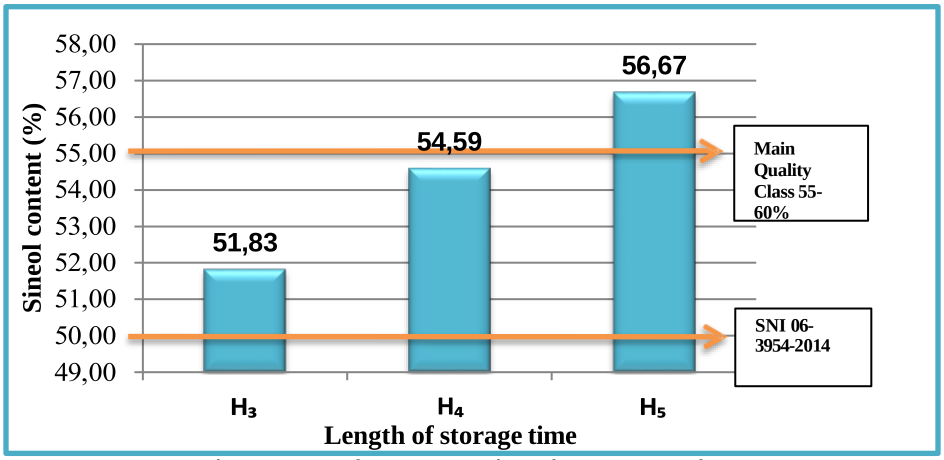
Table 6. Results of Least Significant Difference Test for cineole content

Treatment	Average	BNT value (0.05)
H_3	51,8300 a	
H_4	54,5933 b	2,1844
H_5	56,6733 b	

Resources: Primary Data
Notes: Mean values followed by the same notation mean are not significantly different.
Description: Average values followed by the same notation are not significantly different

A further test of the Least Significant Difference (BNT) showed that the 3rd-day treatment was significantly different from the 4th day treatment and the 5th day treatment, while the 4th day treatment was the same as the 5th day treatment. The highest average value is found on the

5th day of treatment, so it can be concluded that the longest storage time for raw materials to increase the value of cajuput oil sineol content is 5 days (Figure 3).



Histogram of average cineol content values

Figure 3. Histogram of Average cineole content value

The sineol content of *cajuput* oil (*M. leucadendron* Linn) from Rote Ndao Regency ranged from 51.83% to 56.57%. The quality class of sineol content required in the Indonesian National Standard (06-3954-2014) is Super >60%, Main 55-60%, and First 50-<55%. The highest average value of sineol content was obtained in the 5-day storage treatment which was 56.67% and entered into the main quality class of cajuput oil. The treatment of raw materials on the 3rd and 4th days fell into the first quality class. Based on the results of this study, it is known that all raw material treatments fall into the Indonesian National Standard (06-3954-2014).

According to (Khabibi, 2011; Smith & Idrus, 2018), states that the difference in the value of sineol content obtained between each treatment is caused by differences in water content in the leaves. The more water content in the leaves will cause the conditions in the distilled bed to be more saturated, resulting in an increase in the hydrolysis rate. The higher the hydrolysis rate, the lower the sineol content obtained. According to (Guenther, 2011; Smith & Idrus, 2018), that the hydrolysis process can convert termen into alcoholic acids. Sineol is one of the ester groups that is expected to turn into acids and alcohols when the hydrolysis process occurs in the distilled bed. The difference in distillation techniques carried out by (Mbaru et al., 2018) obtained the results that distilling cajuput leaves with the *steam distillation* method produced 59.77% lower sineol content compared to the *Hydrodistillation* method which is 68.22%. The results of distillation with the steam method on young leaves, withered and dry leaves with operating pressure variations of 0.5; 1; 1.5; 2; 2.5 kg / cm^2, obtained the results that the best quality of cajuput oil in dry leaf variables with a pressure of 2kg / cm^2, producing cineol levels of 91.50% (Muyassoh, 2016).

The place where cajuput grows also affects the quality of cajuput oil, this is because the soil fertility and climate of each place are different (Smith and Idrus, 2018). According to (Aryani et al., 2020), several factors are thought to affect the yield and quality of cajuput oil produced in Indonesian cajuput factories, namely: leaf filling, cajuput tree variety, leaf storage, distillation technique, and leaf age. Judging from the place of growth and age of the plant, the results of research (Juliarti et al., 2022) reported that 2-year-old cajuput developed on ex-mining land with an agroforestry pattern produced 57.18% sineol content and was classified as the main quality. Analysis of the quality of cajuput oil from distilling the leaves of 1-year-old cajuput plants in

KHDTK Kemampo produced a cineol content of 72.3% and was included in the super quality class (Muslimin et al., 2017; Tata et al., 2019). According (Rimbawanto et al., 2017), the content of 1,8 cineole in cajuput oil varies, usually controlled by genetic factors that are passed down from the parent tree to its offspring. In addition to these genetic factors, the content of 1,8 cineole in the oil will tend to decrease as the plant ages (Pujiarti et al., 2011; Rimbawanto et al., 2017).

CONCLUSION

The treatment of *cajuput* leaves (*M. leucadendron* Linn) from Ingguinak Village, Northwest Rote Subdistrict, Rote Ndao Regency, produced a yield value of 2.43%, a refractive index value of 1.45 and a cineol content of 56.67%, with a raw material storage time of 5 days. Refractive index parameters and cineol content meet the Indonesian National Standard (06-03954-2014) and sineol content is in the main quality class.

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