INVESTIGATION OF PHYSICAL AND CHEMICAL PROPERTIES OF CITRONELLA OIL FOR DETERMINING THE CONCENTRATION OF CITRONELLAL COMPOUND

Andry Tiawan[1], Tutun Nugraha[2], Niken T. Listyorini[3]

[1, 2, 3]Chemical Engineering, IULI University,
Associate Tower Intermark, BSD City, 15310
e-mail: [1]andrytiawan18@gmail.com, [2]tutun.nugraha@iuli.ac.id, [3]niken.listyorini@iuli.ac.id

ABSTRACT. This journal explores the physical and chemical properties of citronella oils extracted from *Cymbopogon* species, known for their insect-repellent qualities, essential oils and cosmetic applications. The aim is to determine the relation between concentration of citronellal and its properties for further research. The study analyzes density, refractive index, color, solubility, and utilizes GC-MS analysis. Different citronella oil samples with varying citronellal compositions are examined. The results show variations in properties among samples with different concentrations. Density and refractive index had the potential for determining citronellal concentration. GC-MS analysis identifies and quantifies components, with a focus on citronellal. The refractive index is inversely proportional to the concentration. The relation between the value of refractive index and citronella concentration is ($y = -0.0002x + 1.4773$) with $R^2 = 0.9934$. Refractive index analysis has the strongest potential to be used as field method of measurement due to its higher resolution nature and ease of it use by untrained persons.

Keywords: Citronella, Citronellal, Geraniol, Physicochemical Properties, Density, Refractive Index

1. INTRODUCTION

Indonesia, as a tropical country, possesses a rich biodiversity of essential oil plants, making it a significant player in the global essential oil industry. The demand for essential oils has been steadily increasing, driven by the growth of industries such as perfume, cosmetics, food, pharmaceuticals, aromatherapy, and more. In 2020, Indonesia had 189 essential oil exporters across all provinces, with a total export value of USD 215.81 million. West Java emerged as the leading contributor, accounting for approximately 31.9% of the country's essential oil exports, followed by Central Java and North Sumatra. DKI Jakarta, West Java, and East Java had the highest numbers of essential oil exporters (Anggriyani, 2022).

Essential oils, also known as volatile or flying oils, are derived from plants and contain various chemical components. Indonesia is home to around 40 out of 80 aromatic plant species traded globally for essential oils. One such plant is citronella, which possesses oil cells in its parenchyma tissue and is highly valued for its economic potential. The main chemical constituents of citronella oil are citronellal, citronellol, and geraniol. These components can be further utilized to create compounds with increased economic value, emphasizing the importance of accurate testing and standardization. Distillation of citronella leaves for approximately five hours yields oil with maximum geraniol content of 85% and a minimum citronellal content of 35% according to the Indonesian National Standard (SNI). Distillation beyond 4.5 hours does not significantly enhance the levels of these two components, and the amount of plant leaves used in distillation affects the specific gravity of the oil (Mawardi, Indi R, Yuniar, Khoirunnisa, & Oktaviani, 2019).

West Kalimantan, located in Indonesia, is abundant in tropical plants, including citronella. The citronella essential oil industry in Indonesia faces challenges related to inconsistent product quality and fluctuating prices. Obtaining raw materials, farmer responses, outdated technology, and production processes contribute to these issues. The competition with Sri Lanka for raw material production further complicates the business. Most

Indonesian farmers cultivate wet-type citronella, leading to lower-quality essential oil, impacting its reputation in the global market (Mawardi, Indi R, Yuniar, Khoirunnisa, & Oktaviani, 2019). Overcoming these challenges necessitates adopting advanced technologies, such as modern distillation and isolation processes, to improve the quality of citronella essential oil.

To address these concerns, the research aims to identify accurate techniques and portable tools for determining the concentration of citronella essential oils. This involves investigating the relationships between physical and chemical properties in accordance with the Indonesian National Standard (SNI) (Omarta, Jayuska, & H.Silalahi, 2020).

2.1. Basic Principle

There are several different types of samples that will be used to undergo the research. The methods referring to the SNI include colour determination, density, solubility, refractive index ,and gas chromatography.

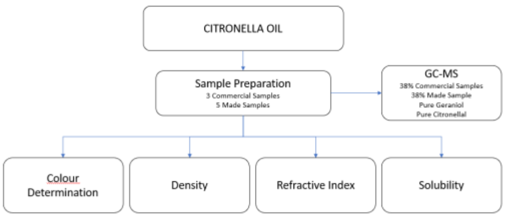

Figure 1. Research Methods

Density determination using a pycnometer. To ensure precise measurements, the pycnometer, a specialized container, underwent a meticulous cleansing procedure. It was thoroughly washed and rinsed with ethanol to eliminate any potential impurities. Subsequently, the pycnometer was carefully dried and weighed using an accurate analytical balance. Distilled water was then added to the pycnometer, surpassing the designated calibration mark, and securely sealed to prevent any loss of content. The external surface of the pycnometer was diligently dried to remove any remaining moisture. After allowing sufficient time for the contents to settle, the pycnometer was weighed again. This entire process was repeated twice to ensure the reliability and precision of the measurements.

Throughout the experiment, utmost attention was given to maintaining measurement accuracy. The pycnometer underwent a rigorous series of steps, starting with thorough cleaning and ethanol rinsing to ensure the removal of potential contaminants. The pycnometer's weight was meticulously recorded using a highly precise analytical balance after it was completely dried. The volume measurement involved filling the pycnometer with distilled water beyond the calibration mark and securely sealing it. Care was taken to eliminate any moisture present on the outer surface of the pycnometer. After an appropriate settling period, the pycnometer was weighed again, repeating the entire process twice for the sample to ensure consistent and dependable measurements.

Colour determination is also included in the SNI parameter. The color assessment was conducted visually by comparing the observed color with the color range specified in the Indonesian National Standard (SNI). The SNI guidelines indicate that the acceptable color for citronella oil falls within the range of pale yellow to yellowish-brown. This visual observation approach ensured adherence to the established standard and allowed for subjective interpretation of color perception.

The Indonesian National Standard (SNI) for citronella oil is a technical standard set by the National Standardization Agency of Indonesia (BSN) (Agustina & Jamilah, 2021). It outlines the requirements and specifications that must be met by citronella oil produced or sold in Indonesia. The SNI covers various aspects, including extraction methods, chemical composition, physical and sensory qualities (such as color, aroma, and clarity), and other relevant parameters to ensure product quality and safety. It is recommended to refer to official documents issued by the BSN Indonesia or contact them directly for detailed and up-to-date information on the specific requirements outlined in the SNI for citronella oil.

Solubility is also included in the SNI parameter. To assess the solubility of the substance, a 1 mL sample is carefully placed into a 10 mL graduated cylinder. Gradually, 0.5 mL increments of 80% ethanol solution are added from a burette to the cylinder. After each addition, the solution is vigorously shaken to ensure thorough mixing and achieve a uniform consistency.

The solubility characteristics of the solution are observed visually after each 0.5 mL addition of the ethanol solution. The observer examines whether the solution appears clear or turbid. Clear solutions indicate effective solubility, while turbid solutions suggest limited solubility or the formation of precipitates. The process continues until a maximum of 10 mL of ethanol solution has been added.

The interpretation of solubility results follows the guidance provided by the SNI standard. If the solution remains clear at a 1:2 ratio, where the volume of ethanol added is twice the volume of the sample, subsequent ratios using the same procedure are likely to yield clear solutions as well. This observation indicates good solubility of the substance in the 80% ethanol solution, making further solubility tests beyond the 1:2 ratio unnecessary.

Refractive index is also included in the SNI parameter. The determination of the refractive index values (nD20 and nD), the Hanna Instrument HI96816 refractometer is used. Start by calibrating the refractometer as per the manufacturer's instructions. Clean the prism thoroughly to remove any dirt or residue. Power on the refractometer and wait for it to reach a stable temperature. Once ready, ensure the prism is completely dry before proceeding. Apply a small amount of the liquid sample onto the prism, ensuring full coverage. Close the prism, ensuring there are no trapped air bubbles. Read the nD20 refractive index value displayed on the screen and take note of the measurement temperature. To determine the nD refractive index, refer to the available scale or mode and follow the instructions provided by the manufacturer. By following these steps, accurate refractive index values can be obtained using the Hanna Instrument HI96816 refractometer.

GC-MS analysis was conducted in collaboration with Ditek Jaya services, employing a straightforward method. The samples were prepared using appropriate techniques. The GC-MS instrument was set up with an RTX-5MS column. The oven temperature program was configured to start at 70°C and increase by 20°C per minute, with a maximum temperature of 250°C. The instrument was calibrated, and quality control samples were run. The sample injection temperature was set to 20°C. The samples were loaded into the autosampler tray, and the injection method was defined. The analysis run was initiated following the instructions provided by the software. The process was monitored to ensure proper separation and detection. Data processing was carried out using suitable software to identify peaks. The results were analyzed based on the research objectives, and the method and parameters were documented accordingly.

2.2. Sample Feed Oil

The graph and table display the findings of the analysis conducted on pure samples of citronellal and geraniol using Gas Chromatography Mass Spectrometry (GCMS).

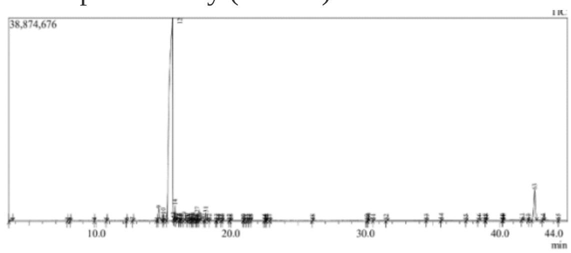

Figure 2. GCMS Graph Geraniol

GCMS	GERANIOL	TOTAL
GERANIOL	87.95	87.95
CITRONELLAL	0.05	0.05
CITRONELLOL	0.02	0.02
Squalene		*6.11*

Table 1. Geraniol Sample Composition

Based on the given data, peak number 12 in the graph corresponds to geraniol, which was expected as the sample was believed to be a pure solution of geraniol. The graph indicates that geraniol constitutes the highest proportion in the sample, with a quantity of 87.95%. However, there is an additional significant component observed in peak number 63, identified as squalene, accounting for 6.11% of the sample.

It is important to note that the GC-MS analysis has certain limitations. In order to convert the percentage area into percentage concentration, a calibration curve is typically necessary. However, the calibration curve was not provided, which is acknowledged as a limitation. Moreover, the assumption was made that the percentage area is directly proportional to the percentage concentration of the sample.

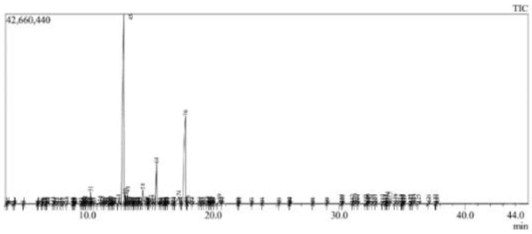

Figure 3. GCMS Graph Citronellal

Table 2. Citronellal Sample Composition

CITRONELLAL	TOTAL
GERANIOL	0
CITRONELLAL	53.26
CITRONELLOL	0.51
1,3-Diacetin	*25.68*
3-Heptadecanol	*6.33*

Based on the provided data, peak number 45 in the graph represents citronellal, which was expected as the sample was believed to be a pure solution of citronellal. The graph indicates that citronellal comprises the largest proportion in the sample, with an amount of 52.42%. However, there is also a distribution of citronellal observed in other peaks, contributing to a total accumulation of 53.26% of citronellal in the sample. Additionally, the graph displays the presence of other components in the sample, such as 1,3-Diacetin in peak 76, accounting for 25.68% of the sample, and 3-Heptadecanol in peak 64, with an amount of 6.33%.

One of the commercial samples we selected is the 38% commercial sample for further verification of its concentration using GCMS. Among the other samples, we chose this particular sample because it appeared clearer and had the highest claimed concentration.

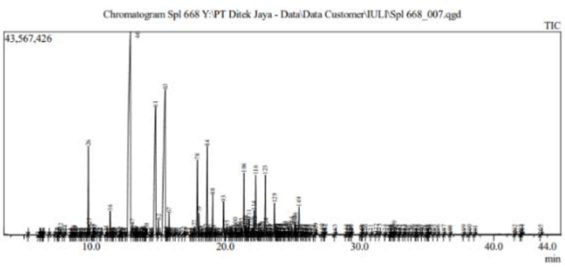

Figure 4. GCMS Commercial Sample Graph

Table 3. Commercial Sample Composition

GCMS	TOTAL
GERANIOL	19.96
CITRONELLAL	27.8
CITRONELLOL	11.31
Geranyl acetate	*4.18*
D-Limonene	*3.05*
6-Octen-1-ol, 3,7-dimethyl-, acetate	*3.31*

Based on the provided data, the commercial sample exhibits several significant peaks, specifically peaks numbered 46, 62, and 63. The sample is claimed to contain 38% citronellal. The graph illustrates that citronellal constitutes the highest quantity in the sample, with peak 46 containing 27.70% of citronellal. The second largest peak, number 63, corresponds to geraniol, accounting for 19.94% of geraniol in the sample. The third prominent peak, number 61, represents citronellol, with an amount of 11.31% in the sample. However, there are also combined peaks representing citronellal and geraniol, contributing to a total accumulation of 27.8% citronellal and 19.96% geraniol in the sample. Furthermore, the graph displays the presence of other components in the sample, including geranyl acetate in peak 84 with 4.18%, 6-Octen-1-ol, 3,7-dimethyl-, acetate in peak 78 with 3.31%, and D-Limonene in peak 26 with 3.05%. Overall, there is a discrepancy in the amount of citronellal compared to the Certificate of Analysis (CoA).

2.3. Standar Nasional Indonesia (SNI)

To evaluate the quality of citronella oil, it is necessary to refer to the characteristics and properties outlined in the SNI number 8835:2019. The table provided below displays the relevant parameters specified by the standard for assessing the quality of citronella oil (Agustina & Jamilah, 2021).

Table 4. SNI Citronella

Parameter	SNI 8835:2019
Tampilan	Cairan Jernih
Warna	Kuning pucat hingga coklat kekuningan
Bau	Bau daun segar, khas aroma sitral kuat
Bobot Jenis relatif pada suhu 20°	0,869 - 0,907

Indeks Bias pada suhu 20°	1,4800 - 1,4930
Putaran optic pada suhu 20°	-6° dan +1°
Kelarutan dalam alkohol 70%	Tidak lebih dari 3 bagian volume etanol 70%

To determine the quality of the citronella oil based on the parameters indicated in the graph, the sample needs to undergo testing in accordance with the SNI table provided, excluding the optical rotation parameter.

Three commercial samples of citronella oil were tested and evaluated for their clarity and color, which represent the first parameter in assessing the quality of the oil.

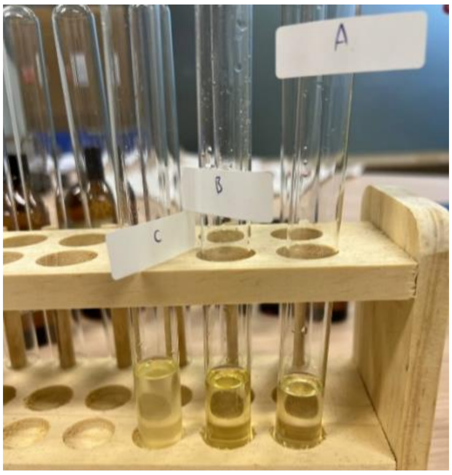

Figure 4. Commercial Sample

Table 5. Colour Determination Sample

Sample	Clarity	Colour
Comm A	Slightly Clear	Less Yellow
Comm B	Clearest	Yellow
Comm C	Blurry	Blurry Yellow

According to the data in the table, the color assessment of the citronella oil aligns with the SNI criteria. Sample Comm B exhibits the highest level of clarity, while sample Comm C appears to be the least clear or blurry.

Five different samples of citronella oils, labeled as A, B, C, D, and E, were prepared based on variations in the concentrations of citronellal and geraniol.

Table 6. Density Sample

	50/50	40/60	35/65	30/70	20/80
C (mL)	2.5	2	1.75	1.5	1
G (mL)	2.5	3	3.25	3.5	4
m0 (g)	12.074	12.078	12.18	12.2	12.194
m1 (g)	16.688	16.658	16.69	16.7	16.658
m2 (g)	16.734	16.618	16.76	16.6	16.602
Density 1 (g/mL)	0.9228	0.916	0.902	0.89	0.8928
Density 2 (g/mL)	0.932	0.908	0.915	0.88	0.8816
Average	0.9274	0.912	0.909	0.89	0.8872

The objective of this experiment was to investigate the relationship and correlation between the concentration of citronellal and the density value. It was observed that variations in the concentration of citronellal had an impact on the density value of the oil.

Three commercial samples of citronella oils, labeled as Comm A, Comm B, and Comm C, were selected based on variations in the concentration of citronellal as indicated in their respective Certificate of Analysis (CoA).

Table 7. Density Commercial

	Comm A	Comm B	Comm C
	37.45%	38%	37.70%
V (mL)	5	5	5
m1 (g)	4.0110	4.1100	4.1100
m2 (g)	4.0390	4.1130	4.0490
Density 1 (g/mL)	0.8022	0.8220	0.8220
Density 2 (g/mL)	0.8078	0.8226	0.8098
Average	0.8050	0.8223	0.8159

In the commercial market, citronella oil samples need to have a citronellal concentration of at least 35% to meet the market acceptance criteria outlined in the SNI. Hence, the available concentration of citronellal in the commercial citronella oil sample is approximately 38%. It is evident that even slight differences in concentration can impact the density value of the oil.

Five citronella oil samples, designated as A, B, C, D, and E, were prepared based on variations in the concentrations of citronellal and geraniol.

Table 8. Refractive Index Sample

	50/50	40/60	35/65	30/70	20/80

ND20	1.4666	1.4686	1.4694	1.4708	1.4731
ND20	1.4665	1.4685	1.4693	1.4708	1.4731
ND	1.4661	1.4683	1.4689	1.4703	1.4725
ND	1.4662	1.4681	1.4688	1.4703	1.4726
Average					
ND20	1.4666	1.4686	1.4694	1.4708	1.4731
ND	1.4662	1.4682	1.4689	1.4703	1.4726

The objective of this experiment was to examine the relationship and correlation between the concentration of citronellal and the refractive index value. It was observed that changes in the concentration of citronellal had an impact on the refractive index value of the oil. The sample with the lowest concentration of citronellal exhibited the highest refractive index value.

Three commercial citronella oil samples, identified as Comm A, Comm B, and Comm C, were selected based on variations in the concentration of citronellal as indicated in their respective Certificates of Analysis (CoA).

Table 9. Refractive Index Commercial

	Comm A	Comm B	Comm C
ND20	1.4664	1.4686	1.4708
ND20	1.4664	1.4686	1.4711
ND	1.4661	1.4683	1.4705
ND	1.4661	1.4683	1.4707
Average			
ND20	1.4664	1.4686	1.4710
ND	1.4661	1.4683	1.4706

The table data reveals the refractive index values of the commercial samples, indicating that the refractive index ranges from approximately 1.46 to 1.47.

Three commercial citronella oil samples, designated as Comm A, Comm B, and Comm C, were selected based on variations in the concentration of citronellal according to their respective Certificates of Analysis (CoA). The solubility of the citronella oil was assessed by adding 0.5 mL of the oil for 20 iterations, labeled from A1 to A20, and identifying the state at which the oil appeared most opaque.

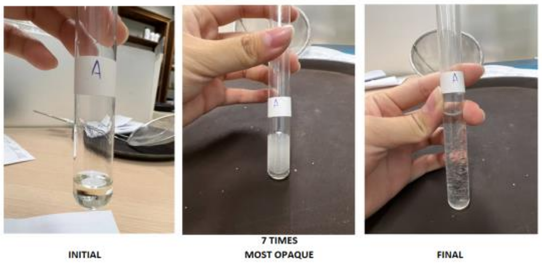

Figure 5. Sample A Commercial Solubility

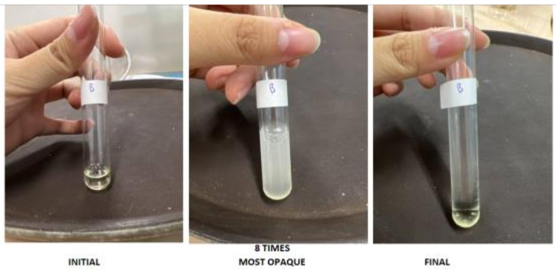

Figure 6. Sample B Commercial Solubility

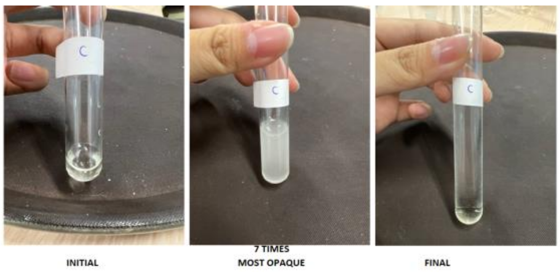

Figure 7. Sample C Commercial Solubility

Based on the observations, the provided image demonstrates that the clarity of the solution gradually decreases from A1 to A7 and then improves from A8 to A20, with sample A7 being the blurriest and sample A20 being the clearest.

Similarly, according to the data, the solution becomes progressively blurrier from B1 to B8 and subsequently becomes clearer from B9 to B20, with sample B8 being the blurriest and sample B20 being the clearest.

Furthermore, as per the data, the solution gradually becomes blurrier from C1 to C7 and then starts to become clearer from C8 to C20, with sample C7 being the blurriest and sample C20 being the clearest.

2.4. Result Discussion

The graph shown below is the data from the value of density of the commercial sample.

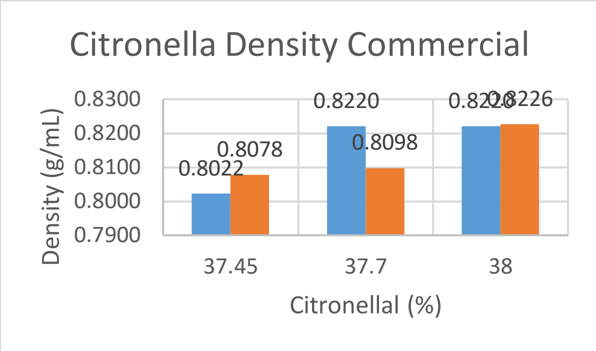

Figure 8. Citronella Commercial Density

The graph of the commercial sample indicates a direct relationship between the density and the concentration of citronellal. As the concentration of citronellal increases, the density also increases. Notably, the data for the 38% citronellal concentration exhibits the highest density value among the recorded samples.

The density prepared sample data is shown in the graph below;

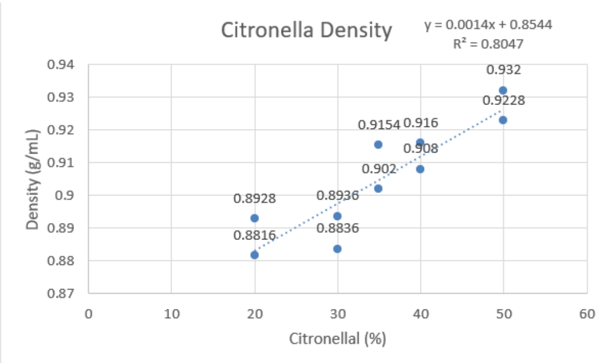

Figure 9. Citronella Sample Density

Based on the data depicted in the graph, there is a clear correlation between the density and the concentration of citronellal in the essential oil. The graph demonstrates that as the concentration of citronellal increases, the density also increases. The trendline consistently rises from the lowest concentration of citronellal to the highest concentration, indicating a linear relationship. This suggests that density can be utilized as a reliable indicator for determining the concentration of citronellal in the essential oil.

In the graph, the relationship between density and citronellal concentration is evident. The trendline shows a consistent increase from the lowest to the highest concentration of citronellal. Although the overall trendline appears to rise linearly, the data points are slightly more scattered, suggesting some variability in the measurements.

The refractive index commercial sample data is shown in the graph below

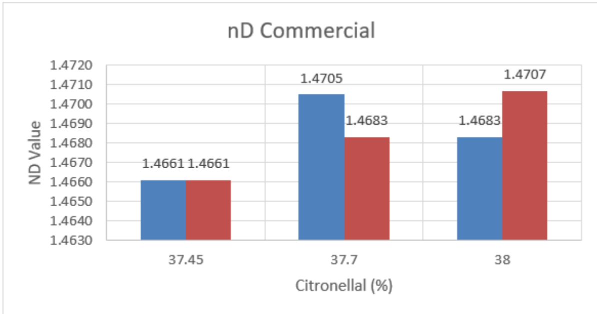

Figure 10. ND Refractive Index Commercial Sample

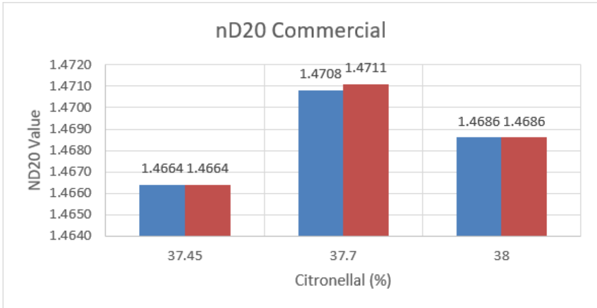

Figure 11. ND20 Refractive Index Commercial Sample

According to the graph of commercial sample, there is a correlation between the refractive index and the concentration of citronellal in the sample. The graph illustrates that as the concentration of citronellal increases, the refractive index also increases. However, it is important to note that the data points appear to be widely dispersed, indicating a high degree of variability. Specifically, the data for the 37.7% citronellal concentration shows significant spread or dispersion.

The graph provided displays the refractive index data of the prepared samples.

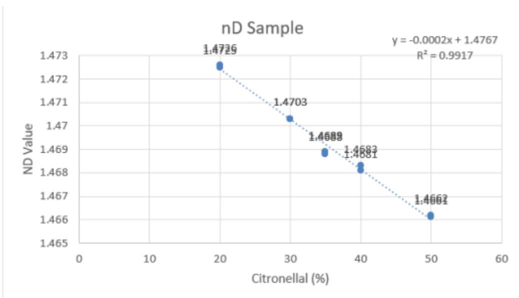

Figure 22. ND Refractive Index Sample

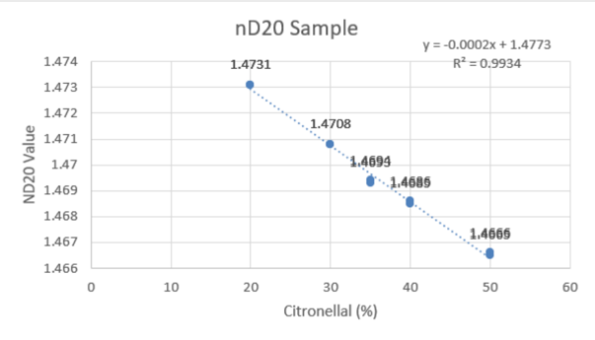

Figure 33. ND20 Refractive Index Sample

The graphs depict a downward trend in the prepared samples. The data demonstrates that higher density corresponds to lower refractive index values, while lower density corresponds to higher refractive index values. The overall trendline exhibits a linear downtrend, and the data points appear to be relatively tightly clustered without significant dispersion.

A mixture of pure citronellal and geraniol samples was prepared with the aim of achieving a 38% citronellal composition. The samples were then analyzed using Gas Chromatography-Mass Spectrometry (GCMS). The results of the analysis were presented in the form of a graph and a corresponding table.

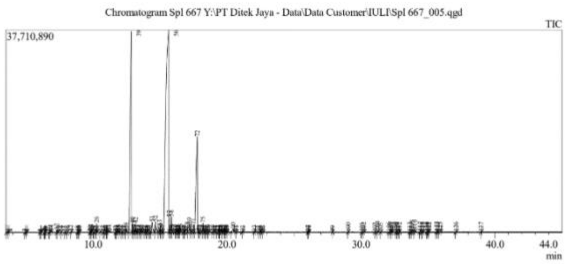

Figure 44. GCMS Graph Sample 38%

Table 50. 38% Citronellal Sample Composition

LARUTAN 38%	TOTAL
GERANIOL	53.55
CITRONELLAL	24.51
CITRONELLOL	0.06

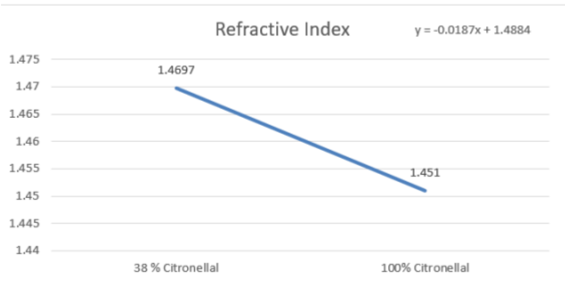

Figure 65. Sample and Pure Graph

The refractive index value of the pure sample was obtained from the Certificate of Analysis (CoA), indicating a value of 1.4697. By inputting a concentration of 38 for citronellal into the linear equation (y = -0.0002x + 1.4773) derived from figure 13, the resulting value obtained was 1.4697, which matches the CoA value.

Based on the refractive index data, the graph of the refractive index exhibits a linear downward trend due to the negative gradient of the linear equation. This indicates that an increase in the concentration of citronellal will have an impact on reducing the value of the refractive index.

To assess the relationship between concentration and refractive index data, a one-way ANOVA was conducted to test the hypothesis. The results of the analysis are described below.

ANOVA

Ref Index

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	4	.000	105.245	.000
Within Groups	.000	5	.000		
Total	.000	9			

Figure 76. ANOVA Test

The null hypothesis (H0) stated that there is no difference in the refractive index resulting from variations in citronella concentration. The alternative hypothesis (H1) proposed that there is indeed a difference in the refractive index due to changes in citronella concentration.

The obtained p-value (0.000, which is less than the significance level of 0.05) led to the acceptance of H1. Therefore, it can be concluded that there is a significant difference in the refractive index resulting from variations in citronella concentration.

3. CONCLUSION

Based on the researcher's findings, there is a direct correlation between the concentration of citronellal and both density and refractive index. The writer's research suggests that accurate tools can be developed to determine citronellal concentrations by utilizing these physical property parameters. The density increases as the concentration of citronellal increases, as evidenced by the upward trendline observed from 20% to 50% concentration. On the other hand, the refractive index decreases with an increase in citronellal concentration, indicating an inverse relationship between the two. Refractive index analysis has the strongest potential to be used as field method of measurement due to its higher resolution nature and ease of it use by untrained persons.

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