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The Effect of Storage Time on the Quality of Immersion Oil Made from Kesambi *(Scheichera Oleosa)* in the Image of Onion Cell Plant

Zakarias A. Mautuka*[*1], Martasiana Karbeka[1], Monika Molina[1], Suratno Suratno[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Tribuana Kalabahi, Nusa Tenggara Timur, Indonesia
[2]Department of Pharmacognosy, Semmelweis University, Budapest, Hungary

*Corresponding author: mautukazakarias@gmail.com

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Abstract

The need for immersion oil becomes very important in carrying out various laboratory analyses with a microscope to clarify the image of objects with sharp imaging. To answer this need, research has been carried out to find an alternative to immersion oil by utilizing one of the typical Alor plants that grow a lot in dry areas, namely Kesambi. In this study, the results of the immersion oil test from Kesambi which was made in 2016 were compared with the results of the retest of the same oil in 2021 and compared with standard immersion oil to determine the quality of the immersion oil. In the existing stock of Kesambi immersion oil, physical properties analysis, GC-MS, and imaging of onion cells were carried out. The results showed that the comparison of the physical properties of Kesambi immersion oil which was analyzed in 2016 and the results of the re-analysis in 2021 showed that there was no significant difference based on the parameters of density, viscosity, refractive index, acid number, and aperture value. Results GC-MS in standard immersion oil only contained 49.68% benzyl benzoic acid and reanalyzed Kesambi immersion oil found palmitic acid 12.26%, oleic acid 46.46%, stearic acid 5.26% arachidic acid 13, 84%, and other spectra were detected as impurities. Although there were impurities and differences in fatty acid content between the standard immersion oil and the immersion oil from Kesambi that had been stored for five years, there was no difference in the imaging results of onion cells.

Keywords: *Oil immersion; storage time; kesambi oil; physical and chemical properties*

Introduction

Indonesia has 2.268 general hospitals and 554 specialized hospitals, 3.623 public health centers, inpatient care, and 6.370 non-inpatient and various other professional laboratories, according to data released by the Indonesian Central Agency on Statistics in 2018 (Kemenkes RI, 2018). The use of immersion oil to clarify the item being examined at high magnification is critical to the microscope's functionality. The photographic results of special imaging at a high magnification of 100 times will appear hazy or indistinct if immersion oil is not used (Tim Pengampu Mata Kuliah Praktikum Biologi, 2020). Standard immersion oil, for example, was used to detect the development of worm larvae formation in white blood cells by using microfluidic lenses (Gulari, Tripathi and Chronis, 2012), to detect malignant skin cancers like Melanoma Akral (Chen *et al.*,

2014), and to identify bacteria and malaria parasites in the blood (Siddig Ibrahim and Khamis Kafi, 2014). Meanwhile, malaria parasites in human red blood cells were identified by using immersion oil from Walnut *(Canarium commune/C. Avenue)* ingredients (Mautuka *et al.*, 2017).

Immersion oil is a type of oil with specific physical and chemical characteristics. It has density, viscosity, refractive index, acid number, and NA values, as well as a hydroxyl group (OH), a carbonyl group (C=O), and a hydrocarbon compound (C-H) (Mautuka, 2016). Standard immersion oil, which is currently commercialized and created synthetically with high technology and cost, is one sort of this oil (Toshiaki Tanaka, 1987). The second type is made from a variety of plant components, one of which is the flesh of the Kesambi seed *(Scheichera oleosa)*, which grows abundantly in the Alor district – Nusa Tenggara Timur.

Because of the importance of immersion oil's function and the high demand in Indonesia, this study aims to determine the extent to which the oil from Kesambi *(S oleosa)* seed flesh has been stored for approximately 5 years in a glass bottle. It is in terms of physical properties and chemistry, such as refractive index, viscosity, density, and acid number, and collaborated with GC-MS analysis and tested through the identification of onion cells.

Because of the author's experience working in the laboratory, finding standard immersion oils that have been damaged (the imaging results are blurry or unclear), and it is unknown how long the oil has been produced and stored, the analysis of the durability or quality of the oil from the seed flesh of the Kesambi *(S oleosa)* is very important. Furthermore, because every vegetable oil and fat is readily oxidized, this study should provide us with information on how long immersion oil made from Kesambi (Soleosa) seed flesh has a shelf life.

The aperture value of the microscope plays a significant role in the application of immersion oil on preparations on glass slides for analysis with a microscope. An aperture value is a measure of an objective lens's refractive power that determines the specimen's separation power, allowing neighboring microscopic structures to be seen as two independent objects (Tim Pengampu Mata Kuliah Praktikum Biologi, 2020). It may be computed using the following equation:

$NA = n.sin$ …….…….. (1)

$\text{Sin}\,\alpha = b/c$ …….. (2)

Where c is calculated using the Pythagorean formula, namely

$$c = \sqrt{a^2 + b^2} \quad \text{……..(3)}$$

Where:

NA = Aperture Value.

N = Refractive Oil Index

a = Distance between the specimen and the lens's center

b = Radius of the lens

c = wide-angle distance from the lens to the specimen.

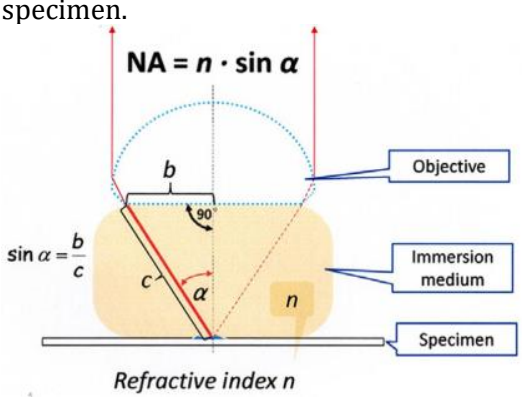

Figure 1. Illustration of the Use of Immersion Oil in Optical Microscopes

Research Methodology

Materials

The materials used in this research are oil from Kesambi seed flesh that has been stored for 5 years, pure n-hexane, 96% alcohol, KOH, pp indicator, and onion's cells, Glassware, thermometer, ABA8 centrifuge, Abbe refractometer, Ostwald viscometer, pycnometer, Binocular Optical Microscope, Magnius MLX brand. The lamp used is xenon at λ max 200-700 nm, and the refractive index of the lens is 1.25, lancet, auto click, caliper, and GC-MS brand Shimadzu QP 2010, using FID and MS detectors. The HP-5MS UI column was utilized, with a set point of 1 mL/min and

a post-run of 1 mL/min, using helium as the mobile phase.

Research Procedure

Two types of immersion oil, conventional immersion oil and immersion oil from Kesambi seed flesh, are made, each with a capacity of 100 ml, and then tested for density, viscosity, refractive index, and acid number (by titration). By the way, on an optical microscope, prepare a sample of onion cells on prepared glass, observe the onion cells without adding immersion oil, and take a photograph, then drop enough standard immersion oil on the sample, adjust the lens while making observations, and take a photograph. Finally, use transparent plastic paper with different thicknesses that have been measured with a caliper to measure the distance between the objective lens and the onion cell sample.

Then, record the numbers to be used as numbers to calculate the value aperture (NA) (Mautuka, 2016); third, use the second procedure above for oil immersion from the flesh of Kesambi *(S oleosa)* seeds. Furthermore, FID and MS detectors, with the HP-5 MS UI column, setpoint 1 mL/min, post-run 1 mL/min, and helium as the mobile phase, carried out GC-MS evaluation. All of the data collected is then analyzed in light of the oil's role as a light-conducting medium.

Results and Discussion

Immersion Oil made from Vegetable Ingredients. Immersion oil made from vegetable ingredients used is from the flesh of the Kesambi *(S oleosa)* as the previous research result in 2016. It was filled in a transparent glass bottle, covered with a rubber cap, and wrapped in aluminum foil on the surface of the bottle, then stored in an ordinary aluminum cabinet in a room with an average temperature of 24–270C, as shown in Figure 2.

a). b).

Figure 2: Immersion oil of Kesambi *(S oleosa)* in glass bottles. a). a photograph was taken in 2016. b). a photograph was taken in 2021.

Figure 2 illustrates that Kesambi oil held from July 2016 to 2021 shows no significant physical changes in color or brightness. This is also supported by the GC-MS study, which indicates that there is not a lot of spectrum. In the image, unidentified compounds are detected and confirmed. On onion cell imaging, the results of the Kesambi oil test are still visible. Results of GC-MS Characterization on Standard Oil. Results of GC-MS Characterization on Standard Oil can be seen in Figure 3.

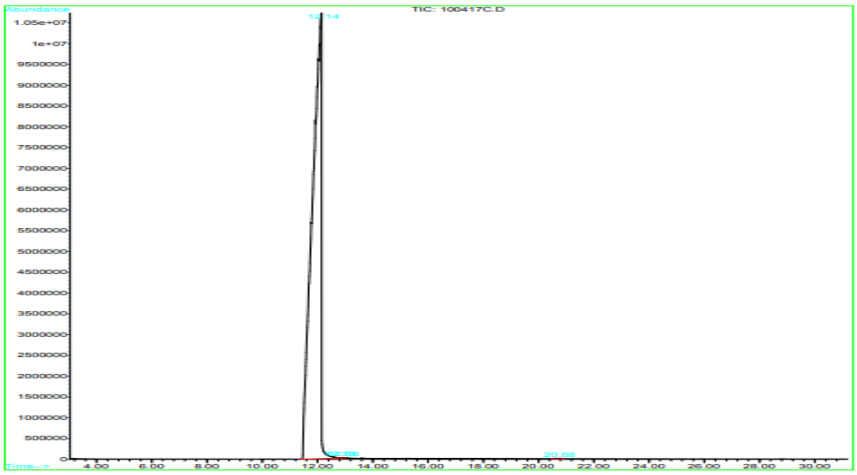

Figure 3: GC Chromatogram of Standard Oil

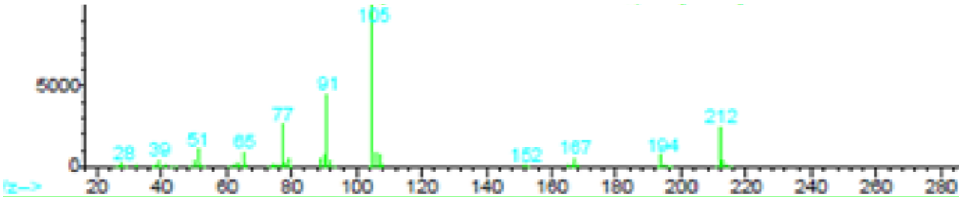

Figure 4: Standard Oil Mass Spectra for Benzyl Benzoic Acid

Figure 3 confirmed that only one peak was identified in the GC chromatogram of standard oil, with a retention time of 12.14 minutes and a compound presentation of 49.68%. The mass spectra of standard oil identified the peak of a molecular ion at m/z 212, allowing the compound's molecular formula to be $C_{14}H_{12}O_2$, which is derived from benzyl benzoic acid, as shown in Figure 4.

According to Figures 3 and 4, the standard oil has a high level of clarity because it only has one spectrum that represents one identifiable compound (Mautuka *et al.*, 2017). This is most likely due to the synthetic nature of the standard oil. When comparing this condition to the findings of the acid number analysis of the two oils, the standard oil is assessed to be smaller, both in 2016 and 2021.

GC-MS Characterization of Kesambi Oil (*S. oleose*)

Figure 5 shows the findings of the GC-MS analysis, which show that Kesambi oil has 23 peaks, but only five of them have the highest percent area, implying that chemicals may influence photographic imaging results and the effect of brightness.

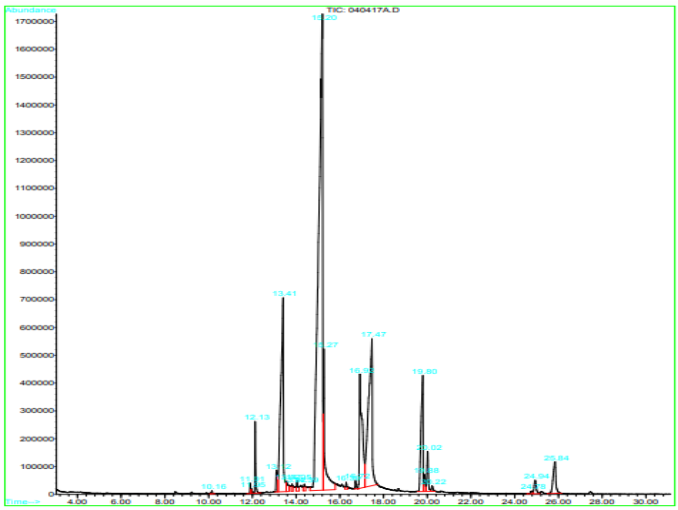

Figure 5. GC Chromatogram of Kesambi Oil

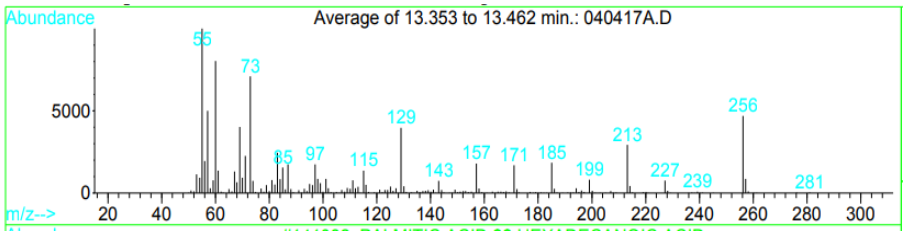

Figure 6. Peak Mass Spectra No. 6, Palmitic Acid

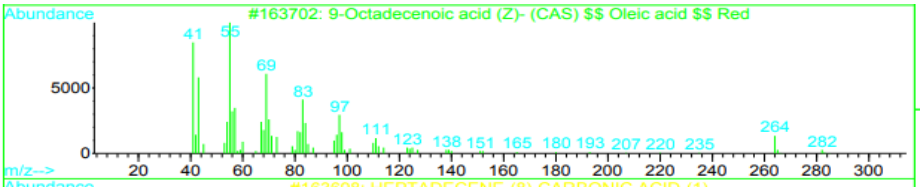

Figure 7. Peak Mass Spectra No. 11, Oleic Acid

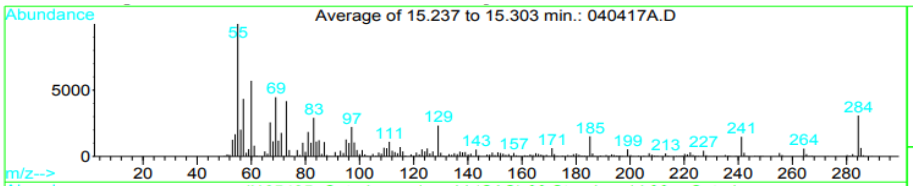

Figure 8. Peak Mass Spectra No. 12, Stearic Acid

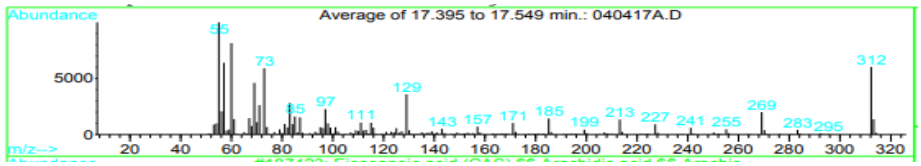

Figure 9. Peak Mass Spectra No. 16, Arachidic Acid

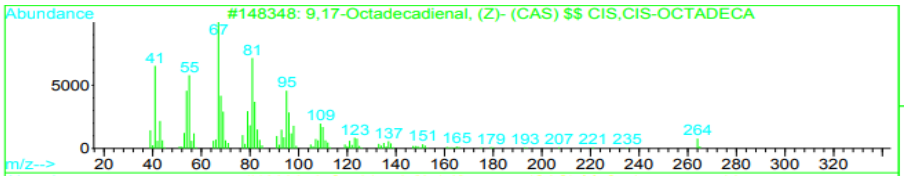

Figure 10. Peak Mass Spectra No. 17, Octadecadienal

Table 1. Data Peak Number, Retention Time, Percentage Area, Molecular Weight, Type of Compound, and Structure

No	Tr	% Area	Mr	Compound Type Molecular Formula
6	13.41	12.26	256	Palmitic Acid $C_{16}H_{32}O_2$
11	15.20	46.46	282	Oleic acid/cis-9-octadecenoic acid $C_{18}H_{34}O_2$
12	15.27	5.26	284	Stearic acid/octadecanoic acid ($C_{18}H_{36}O_2$). $CH_3 (CH_2)_{16}COOH$
16	17.46	13.84	312	Arachidic acid $C_{20}H_{40}O_2$
17	19.79	5.53	264	(Z) 9, 17-Octadecadienal/$C_{18}H_{32}O$

Other peaks in Figure 5 indicate the presence of other compounds. They are not analyzed and considered to have no significant effect because their concentration is so small The five notable peaks may be observed in detail in the mass spectra images in Figures 6, 7, 8, 9, and 10 as well as in Table 1.

The number of compounds with specified molecular formulas in each other in Kesambi oil is shown in Figures 5, 6, 7, 8, 9, 10, and Table 1. However, the photographic test results are not significantly different from the standard oil. This condition shows that despite the presence of several compounds in Kesambi oil, its light conductivity, and optical properties are similar to those of standard oil.

Results of Physical and Chemical Parameter Test for Both Types of Immersion Oil

Table 2. The results of physical and chemical parameter tests for Standard Oil and Kesambi Oil (27^0C) in 2016 and 2021.

No	Liquid Types	Density (g/mL) (25^0)	Viscosity (cP) (second) (25°)	Bias Index (25°)	Acid Number (mg KOH /gram)	NA Average
Data on the physical properties of immersion oil in 2016 (Mautuka, 2016)						
1	Immersion Oil	1.019	59.432	1.515	0.126	1.07
2	Kesambi Oil	0.809	45.697	1.467	0.499	1.13
Data on the physical properties of immersion oil in 2021						
1	Immersion Oil	1.019	59.432	1.515	0.126	1.09
2	Kesambi Oil	0.809	44.797	1.469	0.505	1.15

Based on Table 2, the physical and chemical parameters of the two oils, in this case, density, viscosity, refractive index, acid number, and average NA, did not differ considerably between the oil conditions in 2016 and the oil conditions after five years of storage (2021). Researchers have been focusing their attention on the free fatty acid content in Kesambi oil, which increased from oil conditions in 2016 to 2021, as shown in Table 2. But, this rise had no significant impact on photographic imaging outcomes, as shown in Figure 11. In the sense that, Kesambi oil's optical characteristics still have strong light conductivity. Free fatty acids, on the other hand, are fatty acids that are not bonded as triglycerides because of the hydrolysis and oxidation processes. As a result, if the content is high in an oil whose role is to pass light, the free fatty acids will block the light. The photographic results of the imaging test of the two oils do not differ significantly, and they are influenced by several factors, the first of which is aperture value (NA). The larger the NA, the clearer the imaging results, as illustrated in the calculation in Figure 1 (Rottenfusser, 2013).

However, the imaging results are not only determined by the size of the NA but also by the second factor, namely the large or small number of acids, which explains the amount of free fatty acid content in both types of oil. If the acid number of oil is high, then the oil is considered to still contain various impurities if the oil functions to transmit light as illustrated in Figure 11. Therefore, it can be understood that the physical and chemical conditions of the two oils in Table 2 have no significant effect on the photographic imaging results.

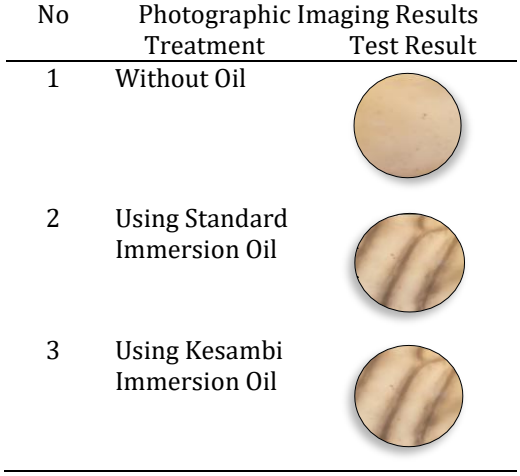

Figure 11. Photograph of Onion Cell Imaging Test Results Using Standard Immersion Oil and Immersion Oil from Kesambi

Conclusions

The quality of Kesambi oil created in 2016, extracted using the soxhletation method, and stored in a glass container at room temperature (24–27℃) in the results of retesting in 2021 did not indicate any obvious changes. There are three conducted parameters to reanalyze the quality of Kesambi Oil. First, the GC-MS analysis revealed that the standard immersion oil contained 49.68 % benzyl benzoic acid, while

the reanalyzed Kesambi oil contained 12.26% palmitic acid, 46.46% oleic acid, 5.26 % stearic acid, 13.84 % racadinic acid, and other spectra were detected as impurities. Although there is a difference in fatty acid content, the image produced by standard immersion oil and Kesambi immersion oil is not considerably different. Second, based on the criteria of density, viscosity, refractive index, acid number, and aperture value, there was no noticeable difference in the quality of the physical properties of Kesambi immersion oil between 2016 and 2021. Third, imaging results from standard immersion oil and Kesambi immersion oil for oil conditions in 2021 show that the image results are not considerably different.

Suggestion

It is necessary to conduct further research on the length of storage time needed to determine the expiration time of immersion oil made from Kesambi oil.

Acknowledgment

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