

Effect of Storage Time Duration on the Quality of Immersion Oil from Kepuh Vegetable Material (*Sterculia foetida* L) on Imaging of Plant Tissue of Shallot Cell

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ABSTRACT

The dependence of optical microscopes on one of the consumables, especially at high magnification, so that the imaging results are clearly visible or not blurry, is immersion oil. In an effort to answer this need, a study has been conducted to find an alternative immersion oil by utilizing vegetable oil from the kepuh plant (*Sterculia Foetida* L) from Alor Regency. This study tested the quality of kepuh seed meat oil from soxhlet extraction and purified using the Deguming, Neutralization, Bleaching, Stearin, Centrifugation methods, and stored in glass bottles, wrapped in aluminum foil, and stored at room temperature (25-280C) from 2016 to 2024 (8 years). The test used three parameters, namely first; comparison of physical and chemical properties parameters, namely density, viscosity, refractive index, acid number and Aperture Value for kepuh oil data in 2016 and 2024, were recorded to have changed, but the imaging photographs still provided the same brightness as standard oil. Second; the GC-MS identification parameters showed that, even though there were 32 peaks in the kepuh oil spectrum, and the nomination of two compounds was identified, namely linoleic acid 34.58%, stearic acid 6.98%, the imaging effect was still clearly visible. Third, a comparison of the imaging results of standard immersion oil and immersion oil from kepuh for oil conditions in 2024 showed imaging results that were not significantly different.

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Introduction

Indonesia has 554 specialized hospitals, 3,623 inpatient health centers, 6,370 non-inpatient health centers, and 7,917 primary care clinics, as well as 2,268 general hospital units, (Ministry of Health of the Republic of Indonesia, 2018) [1]. These hospitals in their operation, quite rely on the use of optical microscopes, because they can be used to observe and identify microscopic objects that cannot be seen directly with the eye while the analyst is working. The operation of optical microscopes is highly dependent on the use of immersion oil which functions to clarify the object observed at a high magnification of 100x, because without the use of immersion oil at this

magnification, the resulting photograph will look blurry or unclear (Widiastuti, 2012) [2]. For example, standard immersion oils are used to detect malignant skin cancers such as *Melanoma Akral* (Chen *et al.*, 2014) [3], detect the development or stage of malaria parasites in the blood (Kusriastuti, 2011) [4], identify the development of worm larval formation in *microfluidic minilens-based* white blood cells (Gulari *et al.*, 2012) [5], also identified malaria bacteria and parasites in the blood (Siddig Ibrahim & Khamis Kafi, 2014) [6], and identified *E. coli* bacteria (Maharani *et al.*, 2020) [7]. Meanwhile, Immersion oil from canary vegetable material (*Canarium commune*/C. *Avenue*) has been used to identify malaria parasites in human red blood cells

(Mautuka *et al.*, 2018) [8], Immersion oil from ketapang vegetable material (*Terminalia Catapa*) has also been used to identify malaria parasites in human red blood cells (Mautuka, 2017) [9]. Immersion oils from vegetable ingredients can last a long time, if processed with the right method, this can be seen from the results of research by (Mautuka *et al.*, 2022) [10], that the immersion oil from kesambi vegetable ingredients that has been stored for 5 years, is still clear as a result of its imaging in identifying onion cells.

Immersion oil is an oil that has specific physical and chemical properties, namely containing a Carbonyl group C=O, a hydrocarbon compound C-H, and containing a hydroxyl group -OH, having a viscosity, density, refractive index, acid number, and a certain Aperture Value (NA), (Mautuka, 2016) [11]. There are two types of immersion oils, namely First; Standard immersion oils that are currently commercialized, are made by synthesis with high technology and have chemical content with a combination of several compound components including halogenated paraffin, liquid monoolefin polymer, ester compounds, liquid saturated hydrocarbon compounds, and saturated aliphatic alcohol (Toshiaki Tanaka, 1987) [12]. Second; oil extracted from various types of vegetable materials, including those used as samples in this study, namely *Kepuh seed meat oil* (*Sterculia foetida* L) as a result of previous research by (Mautuka, 2016) [11]. This plant comes from Alor-NTT Regency, known as a traditional medicinal plant that also has strong antioxidant activity (Kurnia Dewi, 2024) [13], with a refractive index of 1.4662 (Ketaren, 1986) [14].

From the data on the availability of very large health laboratories, which is around 20,732 in Indonesia (Ministry of Health of the Republic of Indonesia, 2018) [1], of course, the need for the use of immersion oil is also quite high. On the other hand, the results of previous studies show the potential of vegetable fat oils that can be used as an alternative to immersion oil, so this study aims to determine the extent of durability or quality of oil from *Kepuh* seed meat after being stored for approximately 8 years in glass bottle containers wrapped in

aluminum foil and stored at room temperature (Figure 2). reviewed from physical and chemical parameters, namely refractive index, viscosity, density, acid number, NA, and confirmed by the results of GC-MS analysis and imaging test results on onion cells.



Figure 1. *Kepuh* Immersion Oil, (200 mL) is stored in a glass bottle container and wrapped in aluminum foil. Archived since October 2016. Analysis of the storage time of immersion oil from *Kepuh* seed meat is very important because vegetable oils and fats certainly have limited resistance to damage. In addition, the author's experience has also found that standard (commercialized) immersion oil has been damaged (the imaging results are unclear/blurry), and it is not known how long the oil has been produced, so this study is expected to provide us with information, how long is the storage time of immersion oil from *Kepuh* seed meat.

The use of immersion oil in the preparation glass of an optical microscope for brightness analysis, can be done by calculating the Aperture Value (NA). The Aperture value is a measure of the separation power of an objective lens that will determine the separation power of the specimen, so that it is able to show adjacent substructures as two separate objects (Titta Novianti, S. Si., 2020) [15] and can be calculated using the following equation:

$$NA = n \cdot \sin \alpha, \quad (1)$$

$$\sin \alpha = b/c, \quad (2)$$

with c obtained from the use of the pitagoras formula, namely $c = \sqrt{a^2 + b^2}$ (3)

Where:

NA = Aperture Value

n = Oil Bias Index

a = Distance from the center point of the lens with Specimens

b = Lens radius

c = Wide-angle distance of the lens to the

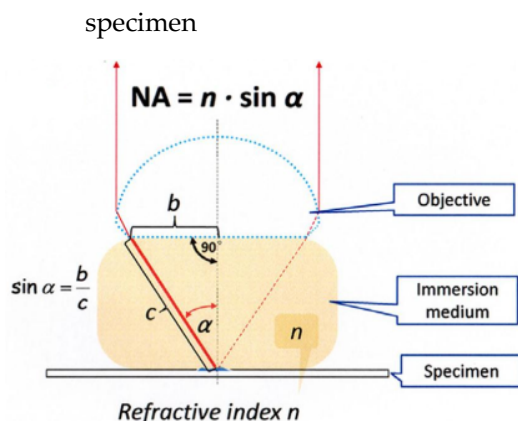


Figure 2. Illustration of the Use of Immersion Oil in Optical Microscopes (Rottenfusser, 2013) [16].

Materials and Methods

Materials and Equipment

The ingredients used in this study are oil from Kepuh seed meat that has been stored for 8 years, pure n-hexane, 96% alcohol, KOH, pp indicator, and onion cells. Glass equipment, thermometer, ABA8 centrifuge, Abbe refractometer, Ostwald Viscometer, picnometer, Magnius MLX brand Binocular Optical Microscope, the lamp used is xenon at λ max 200-700 nm, and lens refractive index 1.25, lancet, auto klic, caliper, and GC-MS brand Shimadzu QP 2010, using FID and MS detectors, the column used is HP-5MS UI, setpoint 1 mL/min, post run 1 mL/min, The phase of motion used is helium.

Working Procedure

Two types of immersion oil are prepared, namely standard immersion oil and immersion oil from Kepuh seed pulp, 100 ml each, test the density, viscosity, refractive index, acid number (through titration), record and store the results, then carry out imaging tests on an optical microscope by; first, prepare a sample of onion cells on a prepartate glass, observe the onion cells without dripping with immersion oil, a photograph of the imaging results was taken, second; Drip enough standard immersion oil on the sample, set the lens while making observations and then take a photograph of the imaging results, then measure the distance between the objective lens and the onion cell sample with transparent plastic paper of different thickness measured with a caliper, then record the number to be used as a number to calculate the aperture

value (NA) (Mautuka, 2016) [11]third; use the second procedure above for the immersion oil from the flesh of Kepuh seeds. Furthermore, GC-MS analysis using FID and MS detectors, the column used is HP-5MS UI, setpoint 1 mL/min, post run 1 mL/min, the motion phase used is helium.

All data obtained are then interpreted according to the function and purpose of using immersion oil, namely as a light-transmitting medium.

Results and Discussion

Standard Immersion Oil

The standard immersion oil used is an immersion oil purchased in 2016, and stored in the same container as the kepuh oil container, and has been analyzed GC-MS and identified to contain 49.68% benzyl benzoic acid, (Mautuka *et al.* 2022) [10].

Immersion oils from vegetable ingredients

The Immersion Oil from the vegetable ingredients used is oil from the flesh of kepuh seeds as a result of previous research by the author in 2016, which was extracted by the socleation method, and purified by the Deguming, Neutralization, Bleaching, Stearin, and Sentrifization methods, filled in transparent glass bottle containers and covered with a dense elastic cap and wrapped in aluminum foil on all parts of the bottle. then stored in an indoor cabinet with an average temperature of 25-28°C, (Figure 1).

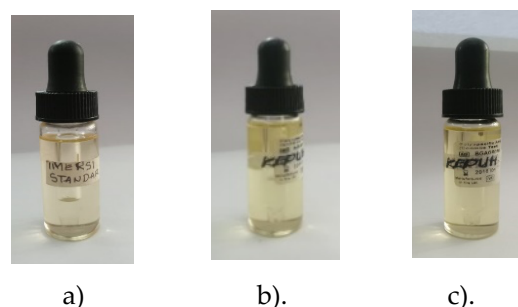


Figure 3. a). Standard Immersion Oil, photographed in 2024, b) Kepuh Immersion Oil, photographed in 2016 c). Kepuh immersion oil photographed in 2024.

Based on Figure 3, it can be interpreted that kepuh oil, which was stored from October

2016 to 2024, did not have any noticeable physical changes such as its color and brightness. This is also confirmed by the results of the GC-MS analysis which shows that there are only a few peaks of the spectrum of low-intensity non-fatty acid derivative compounds identified, and the photograph of the results of the Kepuh oil imaging test on onion cells is still clearly visible.

Results of Physical and Chemical Parameter Test of Two Types of Immersion Oil

Table 1. Test results of Physical and Chemical parameters of Standard oil and Kepuh oil (25°C) in 2016 and 2024

N O	Liquid Type	Density (g/mL) (25°)	Viscosity (cP) (25°)	Bias Index (25°)	Bilang an Sour (mg KOH /gram)	NA Average
Data on the physical and chemical condition of immersion oil in 2016 (Mautuka, 2016)						
1	Standard	1,019	59,432	1,515	0,126	1,13
2	Kepuh Oil	0,825	72,079	1,466	0,560	1,06
Data on the physical and chemical condition of immersion oil in 2024						
1	Standard	1,022	59,210	1,510	0,130	1,17
2	Kepuh Oil	0,830	72,019	1,460	0,565	1,12

Table 1 shows the differences in physical and chemical properties of the two types of immersion oils for 2016 and 2024 (8 years of storage), namely in the parameters of density, viscosity, refractive index, acid number and average NA.

Oil that has been stored for a long time undergoes chemical changes or oxidizes so that the oil experiences an increase in density. This is due to the entry of oxygen into the fat molecules (e.g., the formation of hydroperoxide or other oxidation products) during the transfer of oil from the parent container to the storage container, thereby slightly increasing the molecular mass.

Meanwhile, the decrease in viscosity value, generally caused by the oxidation and/or

hydrolysis process, is more specifically due to the decomposition process of the two immersion oils, even though standard oils are made by synthesis with high technology and kepuh oils are made by separation methods and the application of various stages of purification in layers carefully.

The decrease in the refractive index value is due to the oxidation process, especially when exposed to air and light (during the transfer of oil from the parent container to the storage container), which can cause changes in its molecular structure.

Meanwhile, the storage time of immersion oil can also affect the higher the number of acids. This happens because oil, when transferred from one container to another, has the potential to be exposed to water sourced from the air so that the oil undergoes a hydrolysis process and the breakdown of ester bonds in triglycerides (the main component of oil), resulting in free fatty acids.

The higher the density, the decreasing the viscosity value and refractive index, as well as the higher the acid number value, automatically affect the average value of NA.

There is no obvious difference in the photographic results of the two immersion oils, influenced by two factors, first; Aperture Value (NA). The higher the NA, the clearer the imaging photograph, as illustrated in Figure 2 (Rottenfusser, 2013) [16]. The second factor; That is, the high or low number of acids, which explains the amount of free fatty acids in both types of oils, because if the acid number of an oil is high, then the oil is considered to still contain various impurities if the purpose of using the oil is to transmit light as illustrated in Figure 2.

Although there are changes in the physical and chemical properties of standard immersion oil and immersion oil from Kepuh for 2016 and 2024 (Table 1), it does not have a significant effect on the imaging results of the photograph on the onion cell sample (Figure 4), because the optical properties of Kepuh oil in this condition still have good light conductivity. so it can be ensured that the two immersion oils with a storage time of 8 years in transparent glass bottle containers, wrapped in aluminum foil and stored in room temperature of 25-28°C, are

still suitable for use as an alternative to immersion oil.

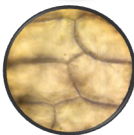
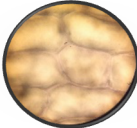
NO	Imaging Photography Results	
	Treatment	Test Results
1	Oil-Free	
2	With Standard <i>Immersion Oil</i>	
3	With Immersion Oil from Kepuh	

Figure 4. Photograph of the results of the shallot cell imaging test using Standard Immersion Oil and Immersion Oil from Kepuh Plant Materials.

Standard Immersion Oil Compound Identification Results

In this journal, the authors used data from the identification of GC-MS of standard immersion oils by (Mautuka et al., 2022) [10], namely a retention time of 12.14 minutes, a compound presentation of 49.68%, and containing molecular ions at m/z 212, allowing the type of benzoic acid compound to be used.

Identification of Kepuh Immersion Oil Compounds.

The results of the identification of kepuh immersion oil compounds are in accordance with figure 5, namely the sample analyzed using GC-MS in August 2024, showing that there are 32 spectrum of wavelengths read on the kepuh oil GC chromatogram. However, not all of them show the spectrum of fatty acids of kepuh oil, but it is suspected that there are chemical residues from the extraction of sorclets such as n-hexane and other foreign elements as impurities. This is confirmed by the results of physical and chemical property tests (Table 1) which show the oxidation and/or hydrolysis process in kepuh oil. While the fatty

acids derived from kepuh oil identified based on Table 2, there are 8 maximum peaks that indicate the presence of linoleic acid and stearic acid compounds, which can provide the identity of retention time (Rt), percent area, and relative molecular time (Mr) typical of vegetable oils and fats.

Based on Figures 5, 6, 7, 8, 9, 10, 11, and Table 2, it shows the presence of compounds with specific molecular types one after another in the oil, but the photograph of the imaging test results is not significantly different from the standard oil (Figure 4). This condition shows that even though there are two dominant compounds with more than one maximum peak in kepuh oil and also the presence of non-fatty acid-derived peaks from vegetable oils and fats produced from the use of solvents during extraction and oxidation and hydrolysis of kepuh oil, the light transmittance or optical properties of kepuh oil are not significantly different from standard oils, And these two types of oil can still be used even though the shelf life is quite long, which is 8 years.

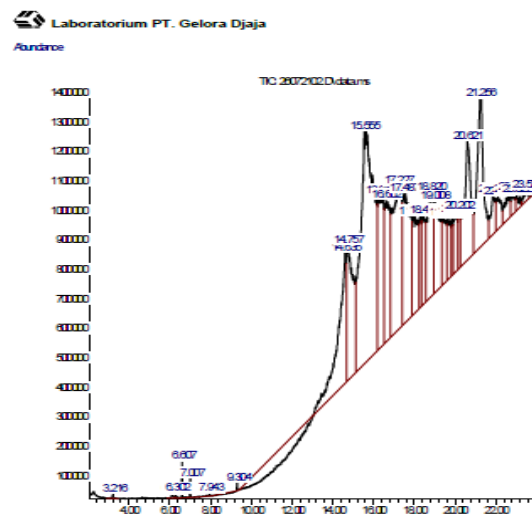


Figure 5. GC Chromatogram of Kepuh Oil

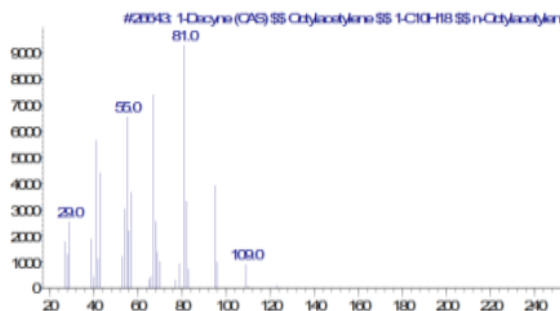


Figure 6. Peak Mass Spectra Number 1, Octylacetylene or 1-octyne compound

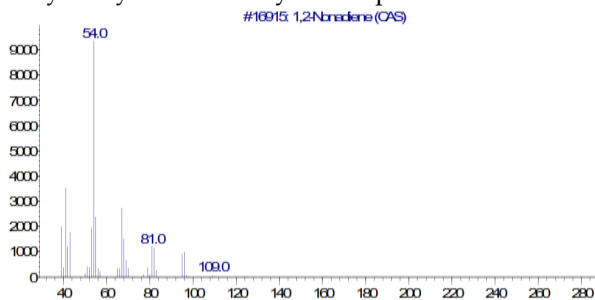


Figure 7. Peak Mass Spectra Number 2, compound 1,2-Nonadiene or nonadiol

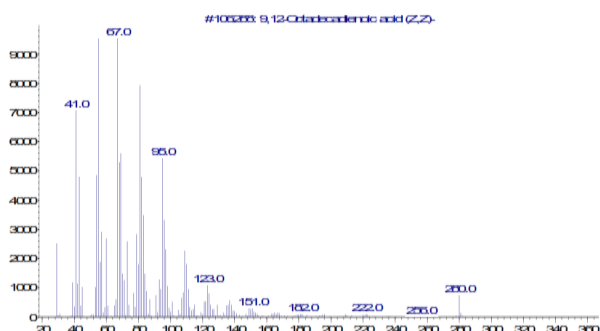


Figure 8. Peak Mass Spectra Number 3, 9,12-Octadecadienoic Acid or linoleic acid

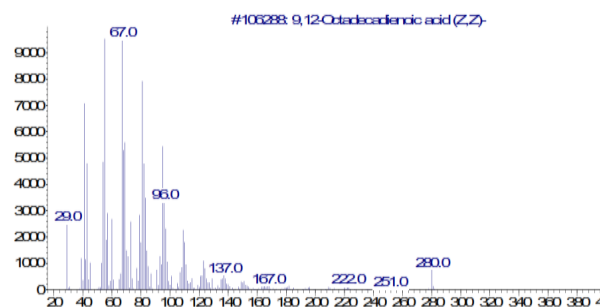


Figure 9. Peak Mass Spectra Number 4, compound 9,12-Octadecadienoic Acid or linoleic acid

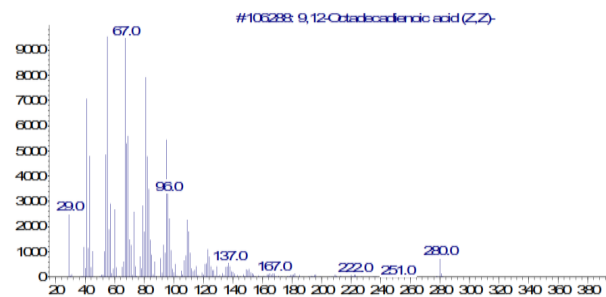


Figure 10. Peak Mass Spectra Number 5, compound 9,12-Octadecadienoic Acid or linoleic acid

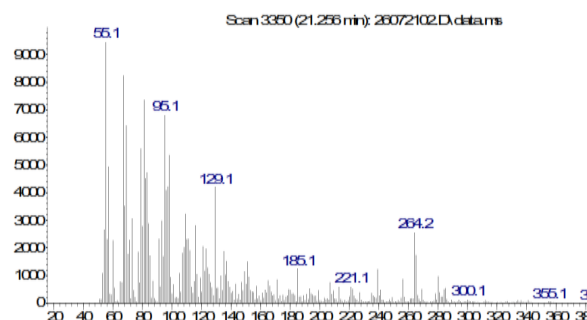


Figure 11. Peak Mass Spectra Number 6, Octadecanoic acid compound or stearic acid

Table 2. Peak Number Data, Retention Time, Percent Area, Molecular Weight, and Type of Kepuh Oil Compound

No	Rt (min)	% Area	Mr (g/mol)	Compound name
1/9	14,755	6,42	280	9,12-Octadeca Dienoic Acid or linoleic acid
2/10	15,555	22,4 8	280	9,12-Octadeca Dienoic Acid or linoleic acid
3/11	16,316	6,11	280	9,12-Octadeca Dienoic Acid or linoleic acid
4/12	16,602	5,22	280	9,12-Octadeca Dienoic Acid or linoleic acid
5/13	17,227	9,73	280	9,12-Octadeca Dienoic Acid or linoleic

				acid
6/14	17,487	7,02	280	9,12-Octadeca Dienoic Acid or linoleic acid
7/25	20,621	5,68	280	9,12-Octadeca Dienoic Acid or linoleic acid
8/26	21,256	6,98	264	Octadecanoic acid or asam stearate

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Conclusion

Based on the results of the discussion, it can be concluded that the quality of kepuh oil that has been made in 2016 by extracting by the socleation method, purified by the Deguming, Neutralization, Bleaching, Stearin, Sentrifugasi method, and stored in glass bottle containers, wrapped in aluminum foil, and stored at room temperature (25-260C) for 8 years, but the results of imaging tests on onion cells in 2024 still look clear and not undergo significant changes.

There are three parameters used to ensure the quality of immersion oil from kepuh, namely **first**; physical and chemical property parameters such as density, viscosity, refractive index, acid number and Aperture Value (NA) of the two types of oil in 2016 and 2024 have changed, but the photograph of the imaging results still provides clear brightness (figure 4). **Second**; The parameters of the GC-MS identification results showed that, even though there were 32 peaks of the kepuh oil spectrum, and different compounds were identified, namely linoleic acid 34.58%, stearic acid 6.98% contained in kepuh oil, the imaging effect was

still clearly visible.

Third, the comparative parameters of the imaging results of standard immersion oil and immersion oil from Kepuh for oil conditions in 2024 show that the imaging results are not significantly different

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