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*Original Research Article*

# Characterisation and Quality Assessment of Phospholipid from Sesame Seeds (*Sesamum indicum*)

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ABSTRACT

Phospholipids are amphiphilic molecules that can be used as basic materials for drug delivery systems. Phospholipids can be obtained from natural sources such as sesame seeds. This study investigated the chemical properties of a phospholipid and the total lipids extracted from sesame seeds. The phospholipid extract, with a yield of 0.475%, was brownish-yellow. It has a crude fat content of 96% comprising of saturated fatty acids (Methyl, 14-methyl-Pentadecanoic acid) and two unsaturated fatty acids (9,12-Octadecadienoic acid and 11-Octadecenoic acid). FTIR analysis of the phospholipid revealed the presence of double bonds and a characteristic choline absorption. The antioxidant screening using the DPPH free radical scavenging method showed 34.16% and 30.98 % antioxidant activity on days 7 and 14, respectively. A peroxide number test revealed that it maintained a low peroxide value of 3.1988 mg $O_2$/g even after 14 days in storage. The study concluded that the phospholipid extract of sesame seed has good antioxidant activity, chemical properties, and quality and could be used as a pharmaceutical excipient in drug delivery.

***Keywords***: Sesame, phospholipid, peroxide number, antioxidant activity, fatty acid

## Introduction

Sesame (*Sesamum indicum*) belongs to the Pedaliaceae family, which contains natural antioxidants and phospholipids that are beneficial for various applications, such as emulsifiers in food products and as drug delivery agents for active substances.[1] Studies have been conducted on sesame phospholipids, specifically concerning the identification and isolation of the sesame phospholipid group as well as its use in beta carotene and vitamin C encapsulation.[2] Phospholipid was isolated from Sesame in the research conducted by Hudiyanti et al.[3] Sesame phospholipid is comprised of cephalin groups with hydrophilic groups in the form of ethanolamine, consisting of C18:2 fatty acids and C18:0 fatty acids.[3] The literature search lacked information on the chemical properties, antioxidant activity, and storage quality of sesame phospholipids.

This study aimed to characterise (using GC-MS and FTIR spectrophotometric methods), investigate its antioxidant capacity, and determine the quality of sesame phospholipid to provide guidelines concerning the quality of sesame phospholipid and its potential application. The study further compared the quality of sesame seeds' phospholipids and the total lipid extract.

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## Materials and Methods

*Solvents and reagents*

Chloroform, methanol, filter paper, sodium chloride, ethanol, hexane, and aquades as pro-analyst materials were obtained from E. Merck.

*Plant material*

Sesame seeds (*Sesamum indicum* L.syn) were purchased from a supermarket in Semarang, Indonesia, in May 2022. The seeds were in good condition, not mouldy, and not rancid.

*Preparation and extraction of plant material*

The isolation and purification of the sesame phospholipid were conducted according to the method Hudiyanti *et al*.[3] The dried sesame seeds were ground to powder using a blender. About 100 g of the powdered sesame seed sample was homogenised in a mixture of chloroform and methanol (2:1) in a separating funnel to give two phases, A (upper phase) and B (lower phase), and filtered using a Whatman no. 1 filter paper.

*Isolation of Total Lipid Extracts*

The chloroform filtrate was taken and washed with 0.9% NaCl saline solution in a ratio of 5:1. The chloroform phase was taken and dried with a vacuum evaporator at 40°C to obtain the total lipid extract.

*Purification of The Phospholipid Extract*

The total lipid extract (10 g) was dissolved in 45 mL chloroform and 15 mL of methanol in a separation funnel. The mixture was shaken for two minutes and left to separate. The ethanol extract (15 mL) was fed into a second separation funnel containing 45 mL chloroform. The mixture was shaken for two minutes. The lower phase was collected in an evaporation flask. The procedure was repeated four to six times. The lower phase (chloroform layer) was evaporated to produce the phospholipid extract.

*Chemical Property Analysis of the Total Lipids and Phospholipids Extracted from Sesame Seeds*

The gravimetric method described in AOCS (2005) was used to test the water content of 1 g of the sample[6], while the Kjedahl method was utilized to determine the protein content of 0.05 g of the sample. Also, the ash and crude fibre contents were determined using the AOCS

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method referenced above. The fat content analysis was done using the Soxhlet method with a sample weight of 1 g.[4,5]

*Peroxide Number Analysis of the Total Lipids and Phospholipids Extracted from Sesame Seeds*

The peroxide number test was conducted following the AOCS (2009) method.[7] Five grams of the sample was added to 30 mL of acetic acid and chloroform in a ratio of 3:2. The mixture was subjected to iodometric titration. The analysis was conducted on the first, seventh, and fourteenth days of storage.[8]

*Antioxidant Activity Analysis of the Total Lipids and Phospholipids Extracted from Sesame Seeds*

The antioxidant activity was analysed using DPPH (1,1-diphenyl-2-picrylhydrazyl) with 0.5 mg of the sample and 4 mL of a 20-mg/L DPPH solution in ethanol. The mixture was incubated for 30 minutes. The antioxidant activity was indicated by a change of colour from purple to yellow. The solution absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.[9,10]

## Results and Discussion

The extraction, using chloroform and methanol solvent at a ratio of 2:1, yielded 300 mL of total lipid from 942 g of sesame seeds. Methanol and chloroform were used to enhance the effectiveness of the extraction. Methanol is a polar solvent that can maximise the extraction of total lipids, including in polar compounds. Chloroform is a nonpolar solvent that can extract nonpolar lipids from Sesame. The total lipid extract obtained consisted of polar and nonpolar lipids. Among the molecules obtained using this solvent was that of a phospholipid. The phospholipid was a semi-polar lipid containing phosphate groups esterified at the sn-3 position of the glycerol skeleton.[11]

The purification of the phospholipid extract from the total lipid using fractionation resulted in both polar and nonpolar lipids. The purification produced a polar lipid that contained a phospholipid with a yield of 0.475%. The sesame phospholipid extract obtained appeared like a bright yellow gel (Figure 1) that dissolved in ethanol and became browner over time due to oxidation. The result complied with that of the sesame lecithin previously reported.[3,12] Furthermore, our investigation into the chemical properties was carried out according to the parameters listed in Table 1. The durability of the ingredients in foodstuffs that affect their physical content, such as their texture and taste, is reflected in their water content.[4] The water content of the sesame seeds, which was below the maximum value of 10% regulated by the Indonesian National Standard (SNI), showed that the sesame seeds were of good quality.[13] Table 1 shows that the water content of the phospholipid produced was higher than that of soy lecithin, based on the SNI of soy lecithin, which has a maximum of 0.9%.[14] This result suggested that the phospholipid extract still needed further evaporation to obtain a lower water content in accordance with the SNI. The metal content in the phospholipid extract (Table 1) was below the limits determined by the SNI.[4,14] Heavy metal contamination was negligible in the phospholipid extracted from Sesame.

An FTIR spectroscopy of the sesame phospholipid was carried out in the absorption area of 4000 to 450 $cm^{-1}$ with spectral results as shown in Figure 2. The FTIR spectra were compared with the spectra of sesame phospholipids from previous studies and revealed specific phospholipid groups, as indicated in Table 2. The absorption area showed that the strongest absorption at wavenumber 2923-2858.39 $cm^{-1}$ was for $CH_2$ vibrations with asymmetrical and symmetrical stretching modes. The vibrations for C=O esters were presented in the 1750-1700 cm-1 area, and the vibrational absorption region for $PO_2$ was at 1100-1000 $cm^{-1}$. These results indicated the specific uptake of choline in the sesame phospholipid and the presence of double bonds.[15-17]

A GC-MS analysis was performed after the phospholipid esterification process using Na-methanolic to obtain the fatty acid esters. The GC-MS analysis resulted in a chromatogram, as presented in Figure 3. The peaks with more than 3% abundance were chosen for analysis, and the results were compared to the database spectra (NIST. LIB and WILEY. LIB).[12]

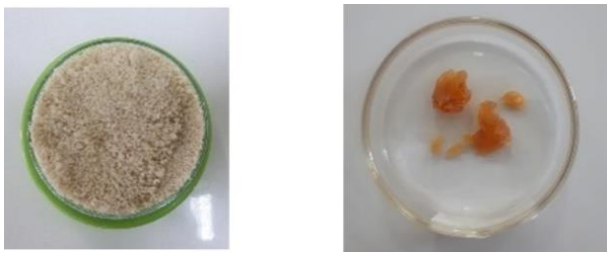

**Figure 1**: Sesame and Sesame Phospholipid

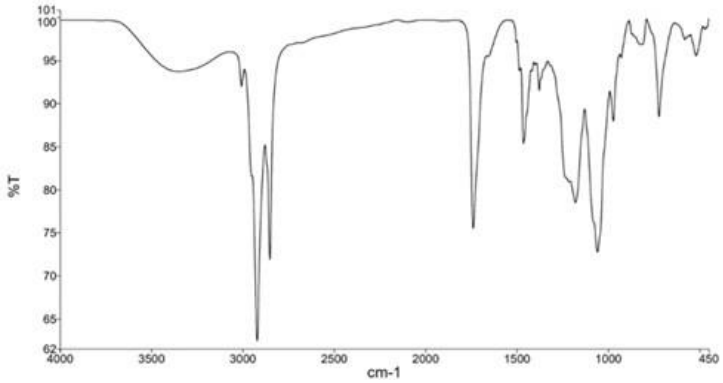


**Figure 2:** FTIR spectrum of sesame phospholipid showing absorption bands

**Table 1:** Proximate analysis data on Sesame and phospholipid compared to Soy Lecithin

| Parameters | Sesame | Phospholipid | Soy Lecithin |
|---|---|---|---|
| Water content | 2.228% | 1.360% | 0.9 % |
| Ash content | 2.909% | - | |
| Crude fiber content | 73.152% | - | |
| Crude fat content | 7.509% | 96 % | |
| Carbohydrate content | 3.162% | - | |
| Protein content | 11.040% | 2.729% | |
| Pb metal contamination | 0.258 mg/L | 0.2758 mg/L | 2 mg/kg |
| Cu metal contamination | 0 | 0 | 30 mg/kg |
| Zn metal contamination | 0.024 mg/L | 0 | 40 mg/kg |

Table 3 shows three peaks with the highest percentage of abundance, namely peak numbers 4, 5, and 6. From the database, it was found that the fatty acids belonging to these peaks were Methyl, 14-methyl-pentadecanoic acid, 9,12-octadecadienoic acid, and 11-Octadecenoic acid. The molecular fragmentation based on the MS spectrum is presented in Table 4.

The fragmentation pattern at the fourth peak was that of Methyl, 14-methyl-pentadecanoic acid, with a molecular weight of 270. The base peak at m/z 43 and ions at m/z 239 showed the loss of a methoxy group. The fragmentation showed the loss of the methylene group, represented at m/z 87, 101, 115, 129, 143, 171, 185, and 227. The $C_3H_5^+$ ion was shown at m/z 41, and the $C_4H_9^+$ ion at m/z 57. The fragmentation pattern started with the loss of a methoxy group in linoleic acid, as indicated at m/z 263. A missing carboxyl group was shown at m/z 164 and 150, while at m/z 67, 81, 95, 109, 121 and the base peak at m/z 41, a double bond was indicated in the compound. M/z 41 and 55 were homologous to the $CnH_{2n-1}^+$ ion. The loss of a methoxy group and a hydrogen atom from methyl 11-octadecanoic acid at m/z 264 indicated that the fragmentation pattern at the sixth peak indicated 11-Octadecenoic acid. The loss of McLafferty ions (m/z 74) was shown at m/z 222. The loss of a carboxyl group was shown at m/z 180, and homologous formulae $[CnH_2n-3]^+$ were shown at m/z 137 and 123. The presence of a double bond was shown at m/z 41, 55, and 69.[18-20] Figure 4 illustrates the fragmentation pattern at peak 5, which indicated that the molecule was probably that of 9,12-Octadecadienoic acid. The GC-MS showed that the sesame phospholipid consisted of saturated fatty acids, i.e., Methyl, 14-methyl-Pentadecanoic acid, and two unsaturated fatty acids, i.e., 9,12-Octadecadienoic acid, and 11-Octadecenoic acid.

**Table 2:** Sesame phospholipid absorption spectra

| **Bond type** | **Phospholipid absorbance bands ($^{cm-1}$)** | |
|---|---|---|
| | **Sesame[11] (Comparative)** | **Sesame** |
| =C-H stretching | 3008.7 | 3008.79 |
| $CH_2$ asymmetric stretching | 2923.9 | 2923 |
| | 2916.37 | |
| $CH_2$ symmetrical stretching | 2854.5 | 2858.39 |
| | 2846.93 | |
| C=O stretching | 1743.5 | 1739.89 |
| | 1728.22 | |
| C=C | 1651 | - |
| $(CH_3)_3N+$ asymmetrical bending | 1489.05 | - |
| $CH_3$ asymmetrical bending | 1458.1 | 1464.9 |
| | 1450.47 | |
| $CH_3$ symmetrical bending | 1365.60 | 1377.67 |
| $PO_2$- asymmetric stretching | 1200 | - |
| CO-O-C asymmetric stretching | 1242.16 | 1179.25 |
| | 1164.9 | |
| | 1180.44 | |
| $PO_2$- symmetrical stretching | 1095.5 | |
| | 1087.85 | |
| C-O-P stretching | 1041.56 | 1058.84 |
| $(CH_3)_3N+$ asymmetric stretching | 972.12 | 971.91 |
| PO- asymmetric stretching | 810.10 | 817.74 |
| $CH_2$ wagging | 717.52 | 721.73 |

**Table 3:** Sesame phospholipid chromatogram

| **Peak No.** | **Retention Time** | **% Abundance** | **Compound Estimates** |
|---|---|---|---|
| 4 | 35.610 | 13.25 | Methyl, 14-methyl-Pentadecanoic acid |
| 5 | 39.100 | 44.34 | 9,12-Octadecadienoic acid |
| 6 | 39.231 | 30.56 | 11-Octadecenoic acid |

**Table 4:** Mass/Charge (m/z) Data of Fatty Acid Ester Mass Spectra of Sesame Phospholipid Extracts

| **Peak number** | **% Abundance** | **Fragmentation** | **Approximate compounds** |
|---|---|---|---|
| 4 | 13,25 | 28,41,43(*base peak*),57,74,87,101,115,129,143,171,185,227,239,270 | Methyl, 14-methyl-Pentadecanoic acid |
| 5 | 44,34 | 28,41(*base peak*),55,67,81,95,109,121,135,150,164,263 | 9,12-Octadecadienoic acid |
| 6 | 30,56 | 28,41(*base peak*),43,55,69,74,97,123,137,180,222,264 | 11-Octadecenoic acid |

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The antioxidant activity of the phospholipid extract and the total lipids of sesame seeds are presented in Figure 5. The antioxidant activity analysis was performed on the first, seventh, and fourteenth day of storage under 4°C. The phospholipid extract showed a significant increase in antioxidant activity from 14.95-34%. This increased to 160% on the seventh day but decreased to 30.98% on the fourteenth day. On the seventh and fourteenth days, the sesame sample and total lipid extract increased from 5.47-25.64 % and 26.14-28.56%, respectively. The results showed that the sesame phospholipid could be utilised as a natural antioxidant as it provides the highest activity compared to Sesame and the total lipid extract. This result was in line with a previous study that suggested that sesame phospholipid had antioxidant activity.[12]

The research conducted by Sugino *et al*. showed that the antioxidant activity of phospholipid from egg yolks in the total lipid extract enriched with docosahexaenoic acid demonstrated a potent antioxidant activity with higher phospholipid concentrations. The antioxidant activity decreased with increasing degrees of saturated fatty acid chains in the phospholipid.[21] Lecithin from rapeseed showed strong antioxidant activity because the phospholipid contained unsaturated fatty acids and phenolic compounds with the potential to provide synergistic effects.[22]

The phospholipid extract, total lipid, and sesame seeds were analysed on the first, seventh, and fourteenth days of storage. The peroxide numbers of the Sesame, total lipid, and phospholipid obtained on the first day were 0.0538, 0.0378, and 0.712 $mgO_2/g$, respectively; the results on the seventh day were 0.0705, 0.0405, and 1.706 $mgO_2/g$, respectively; while the results on the fourteenth day showed a significant increase in the peroxide numbers to 0.1784, 0.1675, and 3.1988 $mgO_2/g$, respectively. These results showed an oxidation reaction in the phospholipid extract, where the unsaturated fatty acid residues were possibly oxidised to form peroxide compounds.[23] The peroxide number of the phospholipid extract obtained was compared with the SNI of soy lecithin, which was 5 $mgO_2/g$. The comparison showed that the peroxide number of the phospholipid extract was still below the maximum peroxide number.[14]

## Conclusion

Based on its chemical properties, the phospholipid extract was shown to be of good quality. It exhibited the highest antioxidant activity compared to the total lipid and sesame seed extract. The phospholipid extract also exhibited good antioxidant activity of 34.16% on the seventh day of storage. The study concludes that different phytochemicals in sesame seeds, especially phospholipids, make it a potential source of natural antioxidants and could be applied as an excipient in drug formulation.

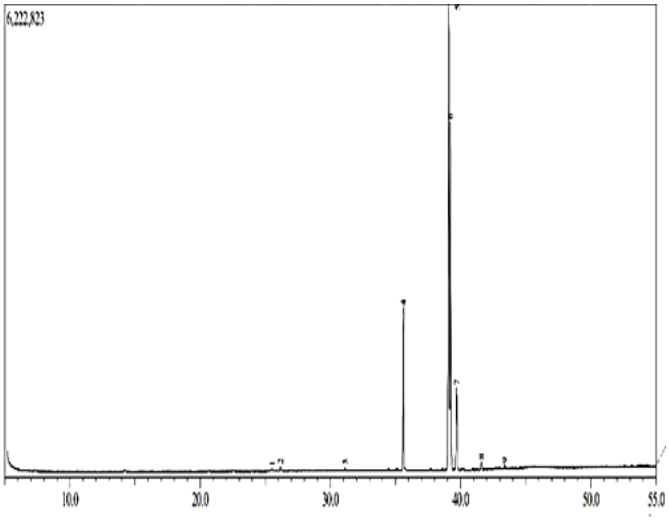


**Figure 3:** Sesame Phospholipid Chromatogram

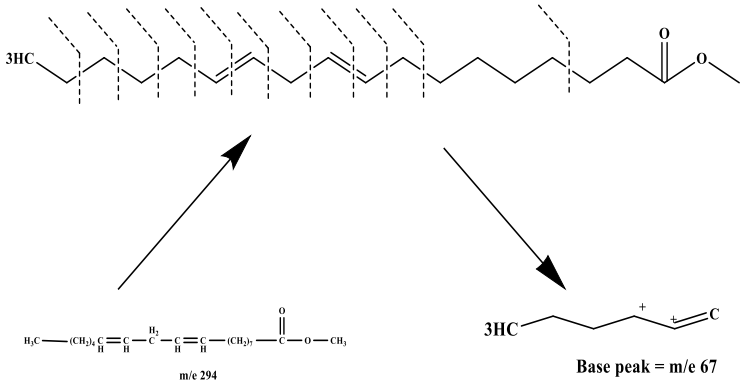


**Figure 4:** Fragmentation patterns of octadecadinoic acid

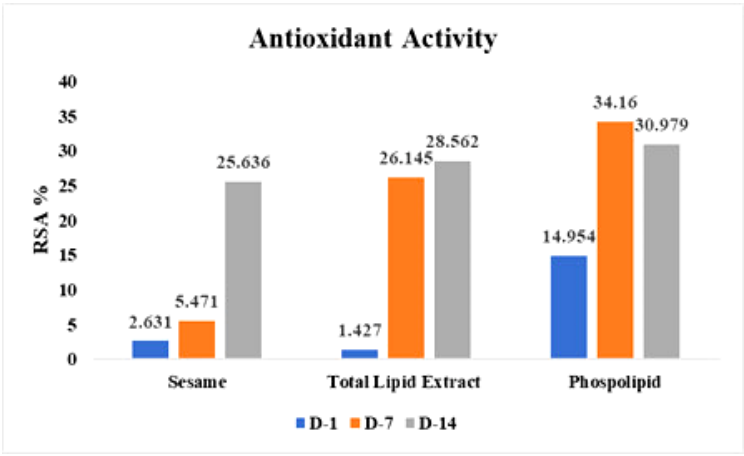


**Figure 5:** Antioxidant activity of Sesame, total lipid extract, and Phospholipid.

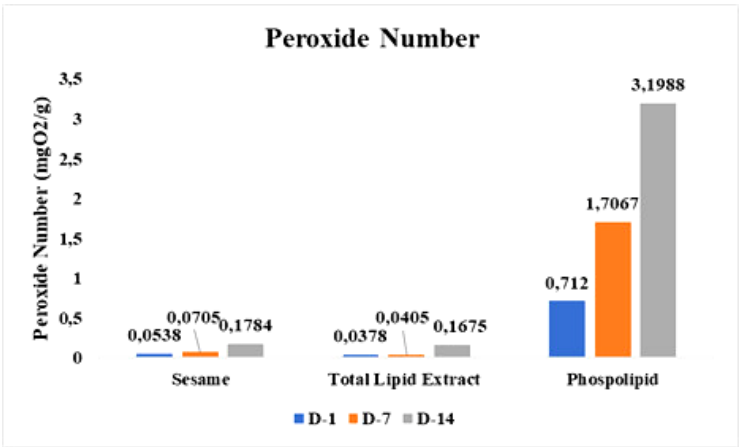


**Figure 6:** Peroxide number of Sesame, total lipid extract, and phospholipid.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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