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# Physicochemical properties and fatty acids content of Lard

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## Abstract

**The study was conducted to evaluate the physicochemical properties and fatty acids content of lard. The qualitative determination of fatty acids of using GC-MS analysis revealed that the higher concentrations of fatty acids 9- octadecenoic acid methyl ester Hexadecenoic acid, methyl stearate and 9,12-Octadecadienoic acid (Z,Z)- with different concentrations. Many physical and chemical properties of lard were determined in the study including the density, viscosity, refractive index. Peroxide value, acid value, iodine value and saponification value. Also, we determine rancidity of lard.**

## Keywords

**Lard, GC.ms, phytochemicals, Fatty acid.**

## INTRODUCTION

Lipids are naturally occurring compounds soluble in organic solvents such as benzene and chloroform form (Hart et al., 2011). However, some lipids are often more soluble in water than organic solvents (Chrtie, 2003). A more accurate definition suggested by Chrtie (2003) "that lipids are FAs and their naturally occurring derivatives (esters or amides), together with compounds closely related through biosynthetic pathways e.g., proteinoids and alcohols" (Chrtie, 2003). Fatty acid analysis in terms of its single composition and functionality has continually gained recognition due to its multiple roles in humans and other organs.

Fatty acids are defined as compounds that contain an aliphatic chain with a carboxylic acid group. They greatly influence both the physical and chemical properties of the glycerides because of their preponderant weight in the glyceride molecule which contributes to 94-96% of the total weight of the triacylglycerol molecule (Clements et al, 2009).

Lard, one of the derivatives of pigs, obtained from the rendering of pork fat twe. In some countries lard is cheapest edible fat (Che Man and Sadzili, 2010). From a religious point of view, food is not allowed to contain lard. For this reason, several analytical methods, either physically or chemically based, have been developed to identify lard (Rohman and Che Man, 2010).

Animal fats and vegetable oils, including lard, were composed of triacylglycerols (TAGs), diacylglycerols (DAGs), free fatty acids, and other minor constituents such as phospholipids, sterols, tocopherols, carotenoids, and fat-soluble vitamins. (Gunston, 2004). Chemanet al. (2011) used Fourier transform infrared spectra in combination with principal component analysis and cluster analysis chemometrics to distinguish lard from other animal fats (Rohmanet al., 2012).

Lard or industrially processed lard can be effectively blended with other vegetable oils to produce shortening, margarine, and other edible oils. In addition to complications and health risks, there are restrictions on the use of animal products in the food industry from an Islamic perspective (Rohman et al. 2012). Fraud is on the rise in the food industry as the prices of organic products continue to rise and availability is limited by high, to reassure both consumer confidence and fair-trade practices., a reconsideration of procedures for certification is required.

Lard contains significant proportions of palmitic, stearic, oleic and linoleic acids. Small amounts of palmitoleic acid and trace amounts of linoleic, arachidonic, and mystic acids (Heena et al, 2013). Using physical properties such as refractive index, viscosity, melting point, saponification and iodine number is no longer practical for detecting contamination as more modern and sophisticated procedures and approaches are available.

However, each fat has specific constituents at known levels, the presence and amount of which should be considered a detection tool. Sensitive, sophisticated and sophisticated methods for detecting and quantifying foreign material contamination should therefore be given due consideration. DNA-based polymerase chain reaction (PCR), Fourier transform infrared spectroscopy (FTIR), electronic nose technology (e-nose), differential scanning calorimetry (DSC) and chromatography to detect lard in food and foods. Several methods have been adopted for this purpose. Base technique. (MarlianaAzir et al., 2017)

Meager studies in Sudan were conducted to assess the physical and chemical characteristics and fatty acids composition of lard which affects the quality of foods. The present investigation aimed to evaluate the physicochemical properties and fatty acids content of lard.

## MATERIALS AND METHODS

### *Collection of lard*

The lard sample was collected for a butcher responsible from pig breeding in his house in Omdurman.

### *Sample Preparation*

Lard was extracted during the process of rendering pork fat. Lard was prepared according to Cheman by rendering the corresponding animal fat as follows: The twe was cut into small pieces, mixed and baked in an oven (Memmert, Germany) for 2 h at 90 - Dissolved with 100 C0. The melted fat was filtered through triple-folded muslin cloth, dried by adding anhydrous sodium sulfate ($Na_2 SO_4$), and then centrifuged at 3000 rpm (1500×g) for 20 minutes.

The fat layer was decanted, shaken well, centrifuged again, and filtered through Whatman filter paper No. 2 containing anhydrous $Na_2SO_4$ to remove traces of water. Filtered samples were used for further analysis. Sampling was done in triplicate and consisted of 3 pig samples. Rendering was done in an oven at 90-100oC for 2 hours. The melted fat was collected, filtered through glass wool, dried over anhydrous $Na_2SO_4$, and centrifuged at 3,000 rpm for 20 minutes. The fat was dried over anhydrous sodium sulfate and stored in a freezer (-20°C) until further analysis.

## *Physiochemical properties*

### Physical properties:

**Specific gravity**

The specific gravity of oil was determined by AOAC (1990). The dry pycnometer is filled with a bubble-free prepared sample after removing the sidearm cap. A stop was inserted into the pycnometer which was immediately immersed in a water bath of 30.0±0.2 and held for 30 minutes. The oil from the capillary opening of the pycnometer stopper was carefully wiped off. Remove the bottle from the bath, wash it and dry it thoroughly. I uncapped the sidearm and quickly weighed the bottle so that the temperature did not drop below 30°C.

**Specific gravity at** 30 $^0$C/ 30 $^0$C = $\frac{A-B}{C-B}$

***Where:***
A: weight in gm of specific gravity bottle with oil at 30 $^0$C.
B: weight in gm of specific gravity bottle at 30 $^0$C.
C: weight in gm of specific gravity bottle with water 30 $^0$C.

**Refractive index**

The refractive index of the oils was determined by AOAC (1990). The refract meter was first adjusted at 1.3330 at 20$^0$ C with pure distilled water as a blank reading. A drop of the oil was placed in the instrument and the telescope was adjusted so that the cross-hairs were distinct and in focus. The adjustment of the knob was rotated until the lower part of the field was dark and the upper part was light and a clear definite boundary appeared. The coarse adjustment knob was moved first and then the fine adjustment knob until the boundary line coincided with the intersection of the cross hair in the telescope. The instrument was read when temperature was stable.

**Determination of color**

Color was determined according to AOAC (1990). The sample liquid and filtered through a filter paper to remove any impurities and traces of moisture till sure that the sample was evident and free from turbidity. The glass cell of desired size cleaned with carbon tetrachloride and allowed to dry. The cell filled with the oil and placed in position in the tintometer. The colors matched with sliding red, yellow and blue color.
Report the color of the oil in terms of Lovibond units as follows:

**Color reading = (a Y + 5 b R) or (a Y + 10 b R).**

***Where,***

a = sum total of the various yellow slides (Y) used
b = sum total of the various red (R) slides used
Y + 5R the mode of expressing the color of colored oils
Y + 10 R for the dark-colored oils.

**Viscosity**

Viscosity was determined according to AOAC (1990). The absolute viscosities of different vegetable oils were determined using a Lamy viscometer RM100 (Lamy, France), a rotary viscometer with a coaxial cylinder. About 25 mL of oil was put into the Tube DIN 1 barrel and the Bobs MK Din-9 was inserted. Tube Radius (Ra) 16.25 mm, Bob Radius (Ri) 15.5 mm. Bob length 54 mm. The correct mode was set for the appropriate measurement system (MS 19) and the measurement time was fixed at 60 seconds. The torque of each sample at different temperatures was recorded over a range of shear rates (Y) from 64.5 to 4835 s−1. All viscometrical measurements of samples were performed in triplicate. All replicates were run twice. Average torque values of two runs were recorded for each iteration at a given shear rate. Shear stress was obtained from.

## Chemical properties:

### Acid value

Acid numbers were determined according to AOAC (1990). The oil was thoroughly mixed before weighing. Approximately five chilled oil samples were accurately weighed into a 250 ml Erlenmeyer flask and 50 ml was added to 100 ml hot freshly neutralized ethyl alcohol and approximately 1 ml phenolphthalein indicator solution. The mixture was boiled for about 5 minutes and titrated hot against standard sodium hydroxide with vigorous shaking during the titration. The weight of oil used for estimation and the strength of alkali used for titration should not exceed 10ml of alkali required for titration. Calculations were performed according to the following formula:

$$\textbf{Acid value} = \frac{56.1VN}{W}$$

***Where:***

V = Volume in ml of standard sodium hydroxide used
N = Normality of the Sodium hydroxide solution
W = Weight in g of the sample

The acidity frequently expressed as free fatty acid for which calculation shall be.

$$\text{Free fatty acids as oleic acid percent by weight} = \frac{28.2VN}{W}$$

Acid value = Percent fatty acid (as oleic) x 1.99

### Saponification value

Saponification numbers were determined according to AOAC (1990). Approximately 1.5 to 2.0 g of sample was transferred to a 200 ml Erlenmeyer flask. Add 30 mL of 0.5 N potassium hydroxide ethanol and secure the condenser to the flask. The flask was gently heated and shaken occasionally while controlling the heat so that the backflow of ethanol did not reach the top of the cooling pipe. After heating for 1 hour, it was immediately cooled and titrated with 0.5N HCl before the test liquid solidified. Perform a blank test three times and obtain the average value of the titration amount of 0.5N hydrochloric acid. The saponification was calculated as followed:

$$\boldsymbol{Saponification\ value\ (mg/g) = (BL1 - EP1) \times TF\ \times C1 \times K1/SIZE}$$

***Where,***

$\boldsymbol{EP1}$ : Titration volume (mL)
$\boldsymbol{BL1}$ : Blank level (25.029mL)
$\boldsymbol{TF}$ : Reagent (HCl) factor (1.006)
$\boldsymbol{C1}$ : concentration conversion coefficient (28.05 mg/mL)
(Potassium hydroxide in Eq.:56.11×0.5)
$\boldsymbol{K1}$ : Unit conversion coefficient (1)
$\boldsymbol{Size}$ : Sample size (g).

### Unsaponification value

Unsaponification matters were determined according to AOAC (1990). Accurately 5g of well-mixed oil/fat sample weighed into a 250ml conical flask. Add 30ml of alcoholic potassium hydroxide solution. The content boiled under reflux air condenser for one hour or until saponification complete (complete saponification gives a homogeneous and transparent medium). Take care to avoid loss of ethyl alcohol during the saponification. The condenser washed with about 10 ml of ethyl alcohol. The saponified mixture was transferred while still warm to a separating funnel. The saponification flask

washed first with some ethyl alcohol and then with cold water, using a total of 50 ml of water to rinse the flask. Cool to 20 to 25℃. Fifty ml of petroleum ether were added to the flask, shacked vigorously, and allowed the layers to separate. The lower soap layer transferred into another separating funnel and repeats the ether extraction for another three times using 50 ml portions of petroleum ether. The combined ether extract was washed three times with 25 ml portions of aqueous alcohol followed by washing with 25 ml portions of distilled water to ensure ether extract free of alkali (washing are no longer alkaline to phenolphthalein). The solution transferred to 250 ml beaker, rinse separator with ether, added rinsing to leading solution. Evaporated to about 5ml and transferred quantitatively using several portions.

**Peroxide value**

Peroxide value was determined according to AOAC (1990). Five grams of the sample were delivered into a conical flask with the stopper. About 25 mL of solvent (15 ml acetic acid+10 ml choloroform) were added and gently shacked to dissolve the sample completely. The air inside flask gently replaces with nitrogen to remove reing oxygen. One ml of saturated potassium iodide was added and immediately seals the flask and gently shaken it for one minute. The flask left at room temperature 15 to 20℃ in a dark room. Thirty mL of pure water were added, and the flask sealed and stirred. Titration with 0.01mol/L sodium thiosulphate was performed to measure peroxide value.
The peroxide value was measured as follows:

$$\boldsymbol{Peroxide\ value\ (meq/kg) = (EP1 - BL1) \times TF \times R\ /\ SIZE}$$

***Where,***

$\boldsymbol{EP1}$ : Titration volume (mL)
$\boldsymbol{BL1}$ : Blank level (0.00mL)
$\boldsymbol{TF}$ : Factor of reagent (1.006)
$\boldsymbol{R}$ : Constant (10)
$\boldsymbol{SIZE}$ : Sample size ( g )

**Iodine value**

Iodine value was determined according to AOAC (1990). To 300 ml conical flask with ground-in stopper 0.1g sample was added. Twenty ml of carbon tetrachloride were added and the flask was sealed. Twenty-five mL Hans solution also added and the flask also sealed. The flask content shacked for one minute. And kept sealed and left in a dark room (about 20°C) for 30 minutes with continuous shaking every 5 minutes. Ten m of 15% potassium iodide and 100 ml of water were added, and the flask sealed and shacked for 30 seconds. The flask content titrated with 0.1mol/L sodium thiosulfate to obtain iodine value. Like we, perform blank test to obtain blank level.

The Iodine value was calculated as follow:

$$\boldsymbol{Iodine\ value\ (cg/g) = (BL1 - EP1) \times TF \times C1 \times K1/SIZE}$$

***Where:***

$\boldsymbol{BL1}$ : Titration volume (mL)

$\boldsymbol{EP1}$ : Blank level (47.074mL)

$\boldsymbol{TF}$ : Factor of titrant (1.006)

$\boldsymbol{C1}$ : Concentration conversion coefficient (1.269)

(Atomic mass of iodine: 126.9/100)

$\boldsymbol{K1}$ : Unit conversion coefficient (1)

***SIZE*** : Sample size ( g )

## *Sample methylation*

2ml of the sample was mixed thoroughly with 7ml of alcoholic sodium hydroxide (Noah) that was prepared by dsolving 2 g in 100 ml methanol. 7 ml from alcoholic sulfuric acid (1ml $H_2SO_4$ to 100 ml methanol) was then added. The mixture was then shacked for 5 minutes. The content of the test tube was left to stand overnight.1 ml of supersaturated sodium chloride (NaCl) was then added and the contents were shaken. 2ml of normal hexane was added and the contents were shacked thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was taken using deposable syringe. 5 µl from the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filtered through syringe filter 0.45 µm and dried with 1g of anhydrous sodium sulfate as drying agent and 1µl of the diluted sample was injected in the GC.MS instrument.

## *GC-MS parameters*

The qualitative and quantitative analysis of the sample was carried out by using GCMS technique model (GC/MS-QP2010-Ultra) from Japan's Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C/min to 300°C as final temperature degree with three minutes hold time. The injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NT) results were recorded.

# RESULTS AND DISCUSSION

## *Phocemical properties of lard*

The physical properties of lard density, viscosity, refractive index, melting point and color were shown in Table (1) below. The density was 0.8990 the in agreement with previous study reported by (Codex Alimentations, 1999) (0.894-0.904), the viscosity reported (70.883), the result showed that refractive index (1.448) the result was in range by reported by (Codex Alimentarius, 1999) (1.448-1.460)$^{th}$ indicated the purity and identity of substance. Our result indicated that the melting point of lard was (41.1900) resembles the finding reported by (Budavari, 1989) (36-42). The melting point of a solid is the temperature at which changes state from solid to liquid. At the melting point the solid and liquid phase exists in equilibrium.

| Samples | Physical tests | | | | | | |
|---|---|---|---|---|---|---|---|
| | Density | Viscosity | Refractive indent | Milting point | Color | | |
| | | | | | R | Y | B |
| Lard | 0.899±0.0015 | 70.883±0.3148 | 1.4417±.0005 | 41.190±0216 | 1.7±0.1414 | 6.233±0.1247 | 0.0 |

**Table (1):** Physical properties of lard

The present study investigated the chemical properties of lard Table (2) peroxide value, acid value, iodine value and saponification value. The result showed that the peroxide value reported (5.5100) th result depended of the time of storage because the Fresh fats have a peroxide value of 1-2, whereas rancid fats have a peroxide value of 15- 20. Rancidity caused by oxidation and hydrolysis. while acid value was (0.8433). In the study, saponification value (SV) 'and iodine value reported (195.9667, and 55.2333) It indicates the degree of unsaturation i.e., the number of double bonds present in the length of the chain. The iodine value low for animal fats and high for vegetable oils. The higher the iodine value, the lower becomes the melting point

respectively th result was agreement with previous study by (Codex alimentarius,1999) (192-203 and 55-65). Compared with the data of (Shahidi et al. 2005) the presence of chemical parameters (Table 2.) is in good correlation.

| Samples | Chemical tests | | | | |
|---|---|---|---|---|---|
| | Acid Value | Peroxide Value | Saponification Value | Iodine Value | An wonted Matters |
| Lard | .8433±.1268 | 5.5100±.0.108 | 195.9667±.494 | 55.2333±.4.6427 | 7.3567±.1.5789 |

**Table (2):** chemical properties of lard

According to the table (3) below result the peroxide value of lard sample was increase with increasing of the time and that mean that lard sample be rancidity so that the peroxide value of lard was influence by storage and time, Th test used to determine the rancidity of tallow. If the peroxide value low, th normally suggests that fat has not become rancid and has good stability. Fresh fats have a peroxide value of 1-2, whereas rancid fats have a peroxide value of 15- 20. Rancidity caused by oxidation and hydrolys.

| Months | First month | Second month | Third month | Four month |
|---|---|---|---|---|
| Peroxide value | 2 | 5 | 8 | 10 |

Table (3): The effect of the storage of the lard sample in peroxide values

## *Fatty acid content of lard*

The GC-MS result of lard oil showed that the oil contain high concentrations of 9- Octadecanoic acid methyl ester (Oleic acid) (37.88%) and Hexadecenoic acid, methyl ester palmitic (24.46%) then Methyl stearate (Stearic acid)(16.52%) and 9,12 octadecanoic (Z,Z) methyl ester (Linoleic acid) (6.98%) (Table 4). Similar fatty acids content of lard with different ration was reported by Koman and Danilova (1974) that means our results were found the lard possess higher percentage of unsaturated fatty acids than saturated fatty acids, that was accordance with some of the data on fatty acid compositions of animal fats were possess higher percentage of unsaturated fatty acids than saturated fatty acids and accordance with the findings reported previously (Marikkar 2015; Rohman & Che Man 2010).

| ID# | Name | Ret.Time | Area | Area% |
|---|---|---|---|---|
| 1. | Decanoic acid, methyl ester | 9.034 | 431849 | 0.08 |
| 2. | Dodecanoic acid, methyl ester | 11.738 | 1627079 | 0.31 |
| 3. | Methyl myrtoleate | 14.061 | 121048 | 0.02 |
| 4. | Methyl tetra decanoate | 14.187 | 15106647 | 2.87 |
| 5. | Pentadecanoic acid, methyl ester | 15.320 | 541970 | 0.10 |
| 6. | 7-Hexadecenoic acid, methyl ester, (Z)- | 16.161 | 2884136 | 0.55 |
| 7. | 9-Hexadecenoic acid, methyl ester, (Z)- | 16.211 | 22460153 | 4.27 |
| 8. | Hexadecenoic acid, methyl ester | 16.463 | 128674706 | 24.46 |
| 9. | Hexadecenoic acid, 14-methyl-, methyl ester | 17.058 | 396848 | 0.08 |
| 10. | Hexadecenoic acid, 15-methyl-, methyl ester | 17.153 | 598618 | 0.11 |
| 11. | c-10-Heptadecenoic acid, methyl ester | 17.222 | 1719206 | 0.33 |
| 12. | Heptadecanoic acid, methyl ester | 17.435 | 2703210 | 0.51 |
| 13. | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 18.185 | 36714300 | 6.98 |
| 14. | 9-Octadecenoic acid (Z)-, methyl ester | 18.289 | 199210086 | 37.88 |
| 15. | Methyl stearate | 18.472 | 86920985 | 16.52 |
| 16. | 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- | 19.743 | 938006 | 0.18 |
| 17. | c-11,14-Eicosadienoic acid, methyl ester | 20.050 | 3918483 | 0.74 |
| 18. | c-11-Eicosenoic acid, methyl ester | 20.072 | 13246554 | 2.52 |
| 19. | Eicosanoid acid, methyl ester | 20.271 | 4381024 | 0.83 |
| 20. | Ethyl 5,8,11,14,17-icosapentaenoate | 21.567 | 564431 | 0.11 |
| 21. | 13-Docosenoic acid, methyl ester, (Z)- | 21.793 | 319182 | 0.06 |

| | | | | |
|---|---|---|---|---|
| 22. | Docosanoic acid, methyl ester | 21.973 | 1816266 | 0.35 |
| 23. | Tetracosanoic acid, methyl ester | 23.549 | 742771 | 0.14 |

**Table (4):** Fatty acid composition of Lard

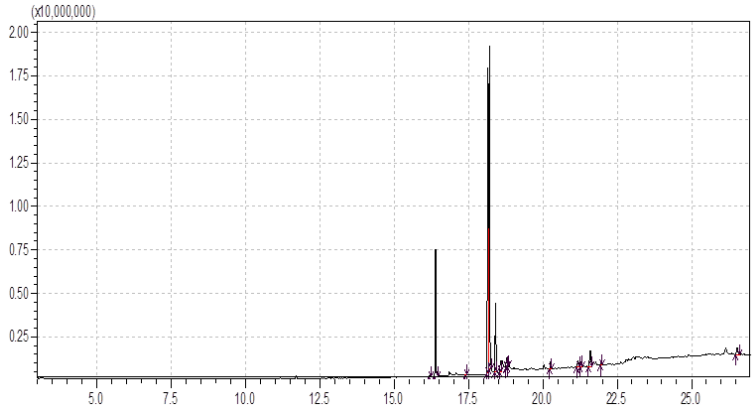


**Figure 1:** Lard possesses higher percentage of unsaturated fatty acids than saturated fatty acids

## CONCLUSION

In the study physiochemical properties of lard were determined by using AOAC methods and the fatty acids composition were obtained by using the (GC-MS-QP-2010) after methylation the lard the obtained fatty acids were compared with the compounds in NT library. The results were good when we compared by the previous study.

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