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# Lecithin Isolated from Melon Seed Oil: Antioxidant Activity

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author OUN designed the study, author DCO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PEA managed the analyses of the study and author JAO managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Context:** A number of antioxidants have been proposed for certain products; however, little information is available about the efficacy of vegetable oil lecithin as antioxidant in food products.
**Aim:** The study was designed to extract and study oil from melon seed, then isolate and determine the physicochemical and antioxidant property of crude lecithin from the oil.
**Methodology:** Oil was extracted from the melon seed according to Association of Official Analytical Chemists (AOAC) standard method and characterized. Lecithin was also isolated from the melon seed oil and its physicochemical properties determined. Then, the antioxidant property of the extracted lecithin was evaluated.
**Results:** The percentage oil yield was 48.25 ± 2.0%. It had a yellow colour, specific gravity of 0.93±0.01 and viscosity of 66.79±2.0% $mm^2$/sec. Its acid, peroxide, saponification and iodine values were respectively 3.36±0.02 mg KOH $g^{-1}$, Nil, 140.25±2.5 mg KOH $g^{-1}$ and 126.90±1.0 mg iodine $g^{-1}$. The percentage yield of lecithin was 0.20±0.01%. It was found to be dark yellow, fairly

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soluble in acetone, petroleum ether and chloroform. The antioxidant property test shows that there was a decrease in peroxide values as the concentrations of lecithin increased.
**Conclusion:** The result suggests that the lecithin has antioxidant activity.

*Keywords: Lecithin; melon seed oil; antioxidant.*

## 1. INTRODUCTION

Melon is an annual monoecious plant with a non-climbing habit. Melon, known as "Egusi", among the yoruba populace of the South Western Region of Nigeria (*Colocynthis citrullus* L) belongs to the family *Cucurbitaceae* [1]. There is confusion in the nomenclature of Egusi melon seed. It is referred to as *Citrullus vulgaris* by some researchers [2], *Citrullus lanatus* by others [3,4]. Some researchers take it to be *Citrullus colocynthis Schrad. Citrullus colocynthis* Schrad is a wild species of the *cucurbitaceae* growing in Morocco, Algeria, the Sahara desert and India and differs from *Colocynthis citrullus* [5]. In order to do away with this confusion it was recommended that *Colocynthis citrullus* together with the vernacular name “Egusi” is used for the crop [6]. In some parts of Africa, egusi melon seeds are common component of daily meals. They are used in ground form to thicken stews [7].

Lecithin is a glycerophospholipid in which the two fatty acids are attached, in an ester linkage, to the first and second carbon atoms of glycerol and a highly polar group (Choline) is attached through the phosphodiester linkage to the third carbon [8]. According to Szuhaj [9], commercial lecithin is a complex mixture of phosphatides, triglycerides, phytosterols, tocopherols and fatty acids. Lecithin plays an important role in human cell function. The linoleic and linolenic acids part of lecithin protect the heart against coronary disease [10,11]. Lecithin is used as lubricants and softening agents in textile industry [12]. In food, lecithin is normally added to such food products as baked foods, chocolate, confectionery, washing peanut, butter, shortening, margarines, power mixes etc.

A number of antioxidants have been proposed for certain products [13]. However, only a few of the substances can be used in food products because of the toxic nature of the inhibitors. It has been shown that vegetable lecithin is an effective non- toxic antioxidant in food products [13]. Melon seed is popular because of its use in soup preparation.

This study was therefore designed to extract oil from melon seed, then isolate, characterize and determine the antioxidant property of lecithin from the oil.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Melon Seed for Extraction

The melon seeds, obtained from Enugu, South Eastern Part of Nigeria, were washed first with distilled water and again with 7% sodium chloride solution. This was followed by dehaulling using electrical dehauling machine (MyJoe2011). The shell-free melon seeds were dried in the sun for about 8 hours within which a constant weight was achieved. The seeds were thereafter put in air-tight container and stored in a cool dry place till used.

### 2.2 Extraction of Melon Seed Oil

Extraction of melon seed oil was carried out using the standard methods of AOAC [14]. This method employs soxhlet extraction technique, with n-hexane as solvent. 100 g of the ground melon seed was loaded into soxhlet apparatus and extraction was carried out for 8hours for maximum oil yield.

### 2.3 Determination of the Chemical Properties of the Oil

#### 2.3.1 Determination of acid value

The acid value was determined using hot ethanol solution which was neutralized with potassium hydroxide (0.1 N) using phenolphthalein indicator. The neutralized ethanol solution (20 ml) was mixed with oil (2 g). The resulting solution was titrated with potassium hydroxide (0.1 N) until the colourless solution turned pink. The acid value was calculated according to AOAC standard formula (Equation 1), [15].

$$A = \frac{5.61 \times T}{W} \quad (1)$$

Where; A = acid value, T = volume of KOH in milliliter, W = weight of oil in grams

### 2.3.2 Determination of iodine value

The iodine value was determined by weighing the oil (5 g) into a conical flask containing 3:2 v/v glacial acetic acid – chloroform mixture (30 ml). Saturated potassium iodide solution (0.5 ml) was added and the solution swirled in the dark for one minute followed by the addition of 30 ml distilled water. The mixture was titrated with sodium thiosulphate (0.01 M) with vigorous shaking until virtually all the yellow color has disappeared. Starch indicator (0.5 ml) was then added and titration continued until all the blue color has disappeared. The titer value was recorded. The iodine value was calculated according to AOAC standard formula (Equation 2), [15].

$$I = \frac{(B-S)\times 1.1269 \times 100}{W} \quad (2)$$

Where; I=iodine value, B =volume of sodium thiosulphate used in blank titration, S = volume of sodium thiosulphate used in test titration, W=weight of oil in grams

### 2.3.3 Determination of saponification value

The saponification value was determined by adding oil (2 g) to 0.5 M alcoholic KOH (25 ml). The mixture was refluxed for 30 minutes before it was titrated with 0.5 M tetraoxosulphate (vi) acid using phenolphthalein indicator. Blank was run in which no oil was added and there was no refluxing. The titer values of both the sample and blank were recorded. The saponification value was calculated according to AOAC standard formula (Equation 3), [15].

$$SV = \frac{(B-S)\times N \times 56.1}{W} \quad (3)$$

Where; SV=saponification value, B=volume in milliliter of standard tetraoxosulphate(vi)acid used for blank titration, S=volume in milliliter of standard tetraoxosulphate(vi) used for test titration, N= normality of KOH, W=concentration of oil in grams

### 2.3.4 Determination of peroxide value

The peroxide value was determined as described by the Association of Official Analytical Chemists [16]. The oil was dissolved in a chloroform-acetic acid mixture (1:1) and added to a saturated solution of potassium iodide. The peroxides present oxidize the iodide to iodine and the iodine is then titrated to a colorimetric endpoint using sodium thiosulfate with starch as an indicator. The amount of iodine produced is directly proportional to the peroxide value (Scheme 1).

$$2I^- + H_2O + ROOH \rightarrow ROH + 2OH^- + I_2 \quad \text{(Scheme 1)}$$

The iodine produced is titrated with sodium thiosulphate (Scheme 2)

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^- \quad \text{(Scheme 2)}$$

The peroxide value was calculated according to AOAC standard formula (Equation 4), [15]

$$P = \frac{T\times M\times 100}{W} \quad (4)$$

Where; P=peroxide value, T=volume of sodium thiosulphate used, M=molarity of sodium thiosulphate, W=weight of oil

## 2.4 Determination of the Physical Properties of the Oil

### 2.4.1 Determination of color and viscosity

The color was determined by visual method. The viscosity was determined using viscometer. Oil was poured into the broad arm of the viscometer until its lobe is almost filled up. The oil was sucked through the thin arm of the viscometer until it got to the upper mark on a small lobe of the thin arm. The oil was allowed to sink back with timing until it reached the lower mark on the small lobe of the thin arm. The same process was carried out with ordinary water and calculated using equation 5

$$V = \frac{FT_S \times 1.0038}{FT_W} \quad (5)$$

Where; V = viscosity, $FT_w$ = flow time of water, *FTs* = flow time of oil

### 2.4.2 Determination of relative density

The relative density of the oil was determined using relative density bottle. The bottle was first weighed empty, weighed when filled with water and finally weighed when filled with the oil. The weight of water and oil were determined by taking the difference between the weight of empty bottle and weight of bottle filled with water and oil respectively. This was used to calculate the relative density (Equation 6)

$$R.D = \frac{W_{oil}}{W_{water}} \quad (6)$$

Where; R.D = Relative density, $W_{oil}$ = weight of oil, $W_{water}$ = weight of water.

## 2.5 Extraction of Lecithin from the Oil

The lecithin was extracted by degumming process. Distilled water (2 ml) was added to the oil (100 ml) and agitated slowly at 70℃ to hydrate the lecithin for an hour. This is called degumming. The hydrated gums were removed by continuous centrifugation. The resulting wet gums were dried over filter paper.

## 2.6 Characterization of Lecithin

### 2.6.1 Phosphate test

Carrying out the phosphate test on the lecithin, saturated solution of $CaCl_2$ was added to the emulsion of lecithin in water to precipitate the phospholipids. The solution (3 ml) was saponified with 1% $Na_2CO_3$ (5 ml) for 10 minutes. On addition of diluted HCl to the soap, fatty acids were precipitated. Finally, the mixture was filtered and tested for phosphate by adding two drops of 5% ammonium molybdate to the filterate.

### 2.6.2 Color and solubility test

Color of lecithin was determined by visual method. Solubility test on lecithin was carried out with chloroform, petroleum ether and methanol on both the crude lecithin and soy bean lecithin used as standard. This was done by adding 0.01 g of the lecithin to 2 ml of the solvents, followed by vigorous shaking.

## 2.7 Determination of Antioxidant Property of Lecithin

The antioxidant activity in the melon seed oil of lecithin was determined according to the modified procedure described by Duh and yen [17]. Different masses of lecithin extract (0.01 – 0.05 g) were weighed separately into different test tubes containing 0.08 g oil each. The tubes were fitted with oxygen pump. Each treatment was placed in an oven at 70℃ and supplied with oxygen for 1 hour. Peroxide values were determined for each of the treatment as described in section 2.2.4 and represented graphically. Control was also carried out in which there were no lecithins added.

## 2.8 Statistical Analysis

Descriptive statistics and percentages were used to analyze the data. The results were presented as Mean ± standard error of the mean (SEM) in tables, words and graph.

# 3. RESULTS

## 3.1 Percent Yield of Oil

The percentage yield of oil was 48.20±2.0%.

## 3.2 Chemical Properties of the Oil

Chemical properties used to evaluate melon oil include acid, iodine, saponification and peroxide values and are as shown on the table below:

**Table 1. The average chemical properties of melon seed oil**

| Chemical properties | Values |
|---|---|
| Acid value (mg KOH $g^{-1}$) | 3.36 ± 0.02 |
| Iodine value (mg iodine $g^{-1}$) | 126.90 ± 1.0 |
| Saponification value (mg KOH $g^{-1}$) | 140.25 ± 2.5 |
| Peroxide value (mEq/1000 g) | Not detected |

## 3.3 Physical Properties of the Oil

Color, viscosity and relative density are the physical properties used to evaluate melon seed oil. The result is shown in table below:

**Table 2. The physical properties of melon seed oil**

| Physical properties | Values |
|---|---|
| Color | Yellow |
| Viscosity ($mm^2$/sec) | 66.79 ± 2.0 |
| Relative density | 0.93 ± 0.01 |

## 3.4 Percentage Yield of Lecithin

The percentage yield of the extracted lecithin was 0.2±0.01%.

## 3.5 Phosphate Test on Lecithin

The phosphate test solution turned yellow which confirmed the presence of lecithin.

## 3.6 Solubility Test on Lecithin

The solvents used to test the solubility of melon seed oil and the results are shown below:

**Table 3. Shows the solubility test result on the lecithin extract**

| Lecithin | Acetone | Chloroform | Petroleum ether |
|---|---|---|---|
| Melon seed lecithin extract | ++ | ++ | ++ |
| Soy bean lecithin standard (Non-GMO Lecithin Granules) (http://www.swansonvitamins.com) | +++ | +++ | +++ |

*++ = fairly soluble, +++ = completely soluble*

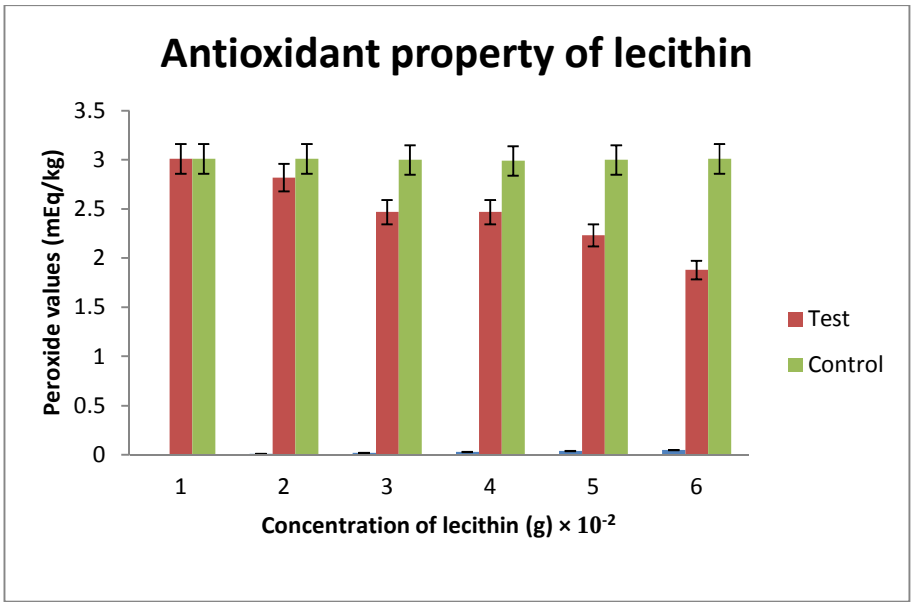


**Fig. 1. Antioxidant property of lecithin**

### 3.7 Color of Lecithin

The extracted lecithin was dark yellow in color.

### 3.8 Antioxidant Property of Lecithin

The antioxidant property of lecithin is presented in Fig. 1 above.

## 4. DISCUSSION

The percentage oil yield of the egusi melon seed (48.20±2.0%) is quite high, thus classifying the melon seed as a good source of dietary oil. The value is close to 49.70% obtained from egusi melon by Uddoh [18], 47.50% obtained from groundnuts by Oyenuga [19], 47.00% obtained from fluted pumpkin seed by Asiegbu [20]. In a similar work in Cameroon, Oloefe et al. [21] and Achu et al. [22] obtained oil yield of 50% and 44-53% respectively.

The measured physicochemical properties of the extracted oil also show that the oil has properties comparable to other vegetable oils. The peroxide value of the oil was zero, signifying that the oil has not undergone deterioration. Peroxide value of oil is a measure of the extent to which rancidity has occurred when exposed to atmospheric oxygen [23]. The zero peroxide value of this oil, suggests that the melon seed oil may contain an active antioxidant that resists the action of atmospheric oxidation of the unsaturated fatty acid component of the seed [23].

The acid value (3.36±0.02 mg KOH $g^{-1}$) is relatively low and compares well with those of oils classified as good for diet. It is similar to 3.5 mg KOH $g^{-1}$ obtained from fluted pumpkin, but lower than 7.6 mg KOH $g^{-1}$ obtained from tropical almond by Christian [24]. Acid value is the total amount of potassium hydroxide in milligrams that is needed to neutralize the acid in one gram of oil [25]. High acid value is an indication of high amount of acid in the oil. This is not ideal to health. Thus, the low acid value of the oil shows that it is good dietary oil [25].

The iodine value of the oil (126.9 ± 1.0 mg iodine $g^{-1}$) is higher than 14.0 – 17.0 mg iodine $g^{-1}$ obtained from palm kernel, 86.107 mg iodine $g^{-1}$

obtained from peanut, but close to 118.0 -145.0 mg iodine $g^{-1}$ obtained from sunflower by Aremu et al. [26]. Iodine value is a measure of the amount of unsaturated fatty acid present in the oil. The high level of iodine value of this oil suggests that it has high level of unsaturated fatty acids. It also suggests that the oil is ideal in protecting the body against coronary heart disease [11].

The saponification value (140.25 ± 2.5 mg KOH $g^{-1}$) is close to 143.3 mg KOH $g^{-1}$ obtained from melon seed oil by Oluba et al. [7]. However, it is lower than 188-196 mg KOH $g^{-1}$ obtained from some vegetable oils by Pearson [27]. Saponification value is a measure of the average molecular weight (or chain length) of all the fatty acids present in a sample. Oil with high saponification value contains high proportion of lower fatty acids. Thus, lower value obtained in the work shows that it contains high amounts of higher fatty acids [7,27].

The color of the oil is yellow and this suggests it may contain carotenoid pigments. Carotenoid pigments are responsible for most of the colors in plant seed oils. The oil has relative density of 0.93 and viscosity of 66.79 $mm^2$/sec indicating that it is less dense but more viscous than water. It will float on top when mixed with water. The percentage yield of lecithin (by weight) is as low as 0.2±0.01%. This low value could be attributed to either the light nature of lecithin or the method of extraction used.

The final mixture during phosphate test turned yellow which indicates the presence of phosphate group in the test compound. This confirms the presence of lecithin as lecithin is the phosphate-containing compound in oils.

In the solubility test on the lecithin extract, soy bean lecithin (standard) was soluble in acetone, chloroform and petroleum ether as against the extract that was fairly soluble in the solvents. Impurities in the crude extract (e.g. oil) could have possibly interfered with the crude extract solubility in the solvents. This result could be compared with the study by Orthoefer [28], which indicated that crude lecithin contains about 30-35% crude oil.

The result of the antioxidant property of lecithin is presented in Fig. 1. From the result, lecithin shows a possible antioxidant activity as the peroxide values decreased for each 0.01 g increase in the quantities of lecithin oxidized in oil at 70℃ for one hour. Lecithin extract may have possibly interfered with the chain reaction that causes rancidity. A theory of antioxidants suggests their interference with a chain reaction due to a free radical mechanism. The antioxidants acts by stopping the chain reaction that causes oil rancidity, at its first stage by supplying a hydrogen atom and therefore destroying the free radical and preventing it from being converted to peroxide [29].

## 5. CONCLUSION

It was concluded that the lecithin extracted from melon seed oil exhibited antioxidant activity.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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