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Green Production and Preliminary Evaluation of Some Physic-Chemical Properties of Lecithin from Locally-Sourced Soybean (*Glycine max*) in Nigeria

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Abstract: *Background*: Lecithin is a very important pharmaceutical and food excipient. Despite its wide applications, most, if not all, of the lecithin used in Nigeria are imported from other countries. Moreover, their extraction usually involve use of organic solvents. *Objectives*: The aim of the study was to extract and characterize lecithin from "locally-sourced" soybean using green approach. *Materials and methods:* The soybean nuts were cracked, milled and sieved to obtain the flour. Then lecithin was extracted from the flour using aqueous method. The extract was subjected to purification in order to reduce neutral oil from the crude lecithin. The powdered crude lecithin obtained was packaged in sealed nylon bags in screw-capped containers and the yield determined. The extract was characterized for moisture content, free fatty acid (FFA) content, acid value (AV), iodine value (IV), saponification value (SV), peroxide value (PV), and some particle properties such as bulk, tap and true densities, flow rate and angle of repose, following standard procedures. An industrial lecithin sample, (lipoid S 75®, Lipoid, Germany), was used as reference. *Results*: The percentage yield of the extracted lecithin ranged from 21.0 ± 0.40 to 25.0 ± 0.41. The moisture content of the reference lecithin was 0.09 ± 0.07, while that of the extracted lecithin was 0.76 ± 0.21%. The FFA of the reference obtained was 3.38 ± 0.14, while that of the extracted was 7.52 ± 0.29%. The AV of the reference was 10.9 ± 0.28, while that of the extracted was 14.5 ±0.32 mgKOH/g, the IV of the reference was 124.7 ± 1.07, as against that of the extracted with 114.1 ± 0.65 mg I/g oil. The AV and FFA were within the WHO recommended specifications. *Conclusions*: Locally-sourced soybean is a potential source of lecithin with comparable qualities to those of the standard reference. Therefore, the green extraction approach could be exploited for industrial applications in Nigeria.

Keywords: Lecithin, Soybean, Extraction, Physiochemical Properties

1. Introduction

Lecithin is a very important pharmaceutical and food excipient employed as emulsifier, solubilizer, suspending agent, lubricator, vesicle former, wetting agent and viscosity modifier. Despite its wide applications, most, if not all, the lecithins used in Nigeria are imported. Therefore, local production of lecithin from soybean (*Glycine max*), which is cultivated in large quantities in Nigeria, would save foreign exchange and impact the economy. Glycerol is one of the major components of lecithin [1]. Other components include: triacylglycerol, fatty acid glycolipids, sterols and sphingolipid [2]. Soybean oil has been identified as the major source of lecithin [3]. In humans, lecithin plays a diverse role in the control of nerve activities and breathing [2]. In

agriculture, it is incorporated into animal feeds in order to provide the essential ingredients needed in the ration [4]. It is also incorporated into pesticides where it is used for adhesion, anti-oxidant, biodegrading and as dispersant, emulsifier, stabilizer, viscosity modifier and penetrating agent [5]. Lecithin has both lipophilic and hydrophilic properties [6]. Due to its anti-oxidant properties, lecithin is used in the production of insecticides, leather, ink and noodles [7]. Its usefulness is extended in the pharmaceutical, cosmetics, beverage and paint manufacturing industries [8]. Soybean (*Glycine max) Linn*, originated from Eastern Asia and belongs to the leguminosae family. *Glycine max* seed consists of 40% protein, 23% carbohydrate, 20% oil, 5% mineral, 4% fiber and 8% moisture [9].

The National Cereal Research Institute has developed nine (9) different varieties of soybean that can be grown in Nigeria [10]. This is due to the large tons of lecithin imported by industrial sector for use in agriculture, healthcare, pharmaceutical and food processing (NBS, 2011). Over a long period, it puts pressure on the value of our currency due to the huge amount of foreign exchange involved in the importation. Our local manufacturers now have to explore various ways to source raw materials locally, hence the need to explore oil-bearing seeds such as soybean that is cultivated abundantly across many of the states in Nigeria. Commercial lecithin is mainly derived from soybean and egg [11], as well as other plant sources such as sunflower. Soy lecithin is preferred due to the relatively low cost of production and is also produced as a by-product of edible oil processing from the water degumming step [12]. The most common method of extraction of lecithin is the use of organic solvents. However, the preferred trend in the production of materials for human consumption, especially food and pharmaceutical agents and additives, is the application of green chemistry tools, such as the use of the greenest solvent, water. This approach is safer, more economical and sustainable.

The aim of the study was to extract lecithin from locally-sourced soybean by aqueous method and carry out preliminary characterization of some of the physic-chemical properties of the product.

2. Materials and Methods

2.1. Materials

Soybean was obtained from Nsukka main market, Enugu State Nigeria. Lecithin reference sample (Lipoid S 75®), was obtained as a kind gift from Lipoid GmbH, Ludwigshafen, Germany. Distilled water was procured from the STC, University of Nigeria, Nsukka (UNN). Acetone (MPB, England). All other reagents and solvents used were analytical grade.

2.2. Methods

2.2.1. Authentication of Soybean Seed Sample

The soybean seeds were identified and authenticated by a taxonomist, Mr. Felix Nwafor, of the Department of Pharmacognosy and Environmental Medicine, UNN. A herbarium voucher number of PCG/UNN/0313 *Glycine max* was assigned.

2.2.2. Preparation of Soy Flour

The soybean seeds were cracked, and grinded (PM and T grinding machine, Japan) [8]. The resulting flour was sieved (Sieve no. 4, 4.75 mm, V8SF, Gilson Company Inc. USA).

2.2.3. Extraction

Lecithin extraction was carried out following the aqueous degumming technique [8], with slight modifications. A 10.0 g quantity of the powder (flour) was placed in a beaker and water (three times the amount by weight of powder) added, and heated on a water bath at 55°C for 30 min (SM800B-Uniscope, England). The oil and water phase were separated by centrifugation at 3000 rpm (SM800B, Uniscope Surgifriend Medicals, England) for 30 min. The gum (crude lecithin) which was formed in the lower layer (subnatant) was then dried in vacuum oven for 1 h at 40°C [8]. The process was repeated 5 times to get sufficient quantities of lecithin.

2.2.4. Purification of the Lecithin Extract

The extract was subjected to purification to reduce neutral oil from the crude lecithin [13]. This was done by treating the oven-dried crude lecithin with acetone in the ratio of 1:6 (w/v) and the mixture stirred for 1 h. The solvent and lecithin were separated by decantation and the treatment was repeated until the solvent became colourless. The acetone was then removed by heating at low temperature of 40°C. The product was pulverized and packaged into sealed nylon bags in screw-capped containers until further use.

2.2.5. Characterization of Some Physic-Chemical Properties

The colour was observed with the naked eye, while the odour was observed with the nose when the container was opened to detect the smell. The taste was observed by placing a little portion of the lecithin on the tongue, while the appearance was assessed by observing and feeling a little portion of the sample placed on the fingers.

2.2.6. Determination of Surface Morphology Using Optical Microscope

The morphology of the reference and extracted lecithin was analyzed using an optical microscope (Hund Wettzlar, Germany) attached with a moticam camera (Moticam 2.0 MP, CMOS, China) after drying of the extracted lecithin at room temperature. A concentration of 0.1% w/v of the dispersion was prepared and dispersed in distilled water and a drop of the dispersion smeared on a microscope slide using a glass rod. The smear was viewed on the microscope at x 100 magnification and images of the micrograph captured using the moticam camera [14].

2.2.7. Determination of Surface Morphology Using Scanning Electron Microscope (SEM)

Shape and surface morphology of the extracted and

reference lecithin were studied using scanning electron microscopy (SEM) (Jeol USA, JSM-7900F). For shape and surface morphology, the lecithin was mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument in order to neutralize the charging effects before scanning in SEM with an acceleration voltage of 20 KV [14].

2.2.8. Determination of Moisture Content

The moisture content was determined using the Mettler Toledo moisturizer (DJ-388IE, Hangzhou-Shuji, Japan). It is a computerized machine that consists of a scale, oven, time and a printout screen. The moisturizer was set at 105°C for 30 min. To a tarred aluminum dish in the moisturizer, weighed quantity (5 g) of the lecithin extract was placed and the machine closed to start automatically. After 30 min, the result was read off the printout screen as it appeared in percentage. The moisture content of the reference lecithin sample was similarly determined [14].

2.3. Determination of Free Fatty Acid (FFA)

The extracted lecithin (10 g) was weighed into 250 cm^3 flask followed by the addition of alcohol. The mixture was boiled on a hot plate until all the oil dissolved completely and the phenolphthalein added (3 drops). The solution was titrated with 0.1 M sodium hydroxide until a faint pink end point was observed and the titer value (T) recorded [15]. The percentage FFA of the samples was calculated using the equation:

$$\% \text{ FFA (as oleic acid)} = \frac{\text{T x M x 28.2}}{W} \quad (1)$$

2.3.1. Determination of Acid Value (AV)

The same procedure was repeated as that of the FFA, but the equation used was thus:

$$\text{AV} = \frac{\text{56.1 x M x T}}{W} \quad (2)$$

AV = Acid value, T = Volume of Sodium hydroxide used, M = Molarity of Sodium hydroxide used, W = Weight of sample used, 56.1 = Molecular weight of Potassium hydroxide (Milwidsky, 1982).

2.3.2. Determination of Saponification Value (SV)

The extracted lecithin (4.0 g) was weighed into a flask and to this was added 0.5 M alcoholic KOH. The mixture was then heated to saponify the fat or oil. The unreacted KOH was then back titrated with 0.5 M HCl using phenolphthalein as indicator. A blank sample was also prepared and back titrated accordingly. The sample and blank titers (V_1 and V_2) were recorded. The saponification values of the samples were then calculated using equation [15]:

$$\text{SV} = \frac{[(\text{V2} - \text{V1}) \text{ x M x } 56.1]}{W} \quad (3)$$

SV: Saponification value, V_1: volume of HCl used for the sample, V_2: volume of HCl used for the blank, M: molarity of HCl, W: weight of sample used, 56.1: molecular weight of KOH.

2.3.3. Determination of Peroxide Value (PV)

To a weighed sample (1.0 g) in a flask was added powdered potassium iodide (1.0 g) and solvent mixture (2:1 glacial acetic acid: chloroform v/v). The resulting solution was then placed on a water bath to dissolve properly and 5% potassium iodide (20 cm^3) was then added. The sample solution was then titrated with 0.002 N sodium thiosulphate using starch as indicator. The peroxide values of the samples were calculated using equation [16]:

$$\text{PV} = 2 \text{ x V} \quad (4)$$

PV; peroxide value, V: volume of sodium thiosulphate used N: Normality of sodium thiosulphate used, W: weight of samples used.

2.3.4. Determination of Iodine Value (IV)

A weighed quantity (0.1 g) of the sample was added to a 300 ml conical flask with ground in stopper. A 20.0 ml carbon tetrachloride was added and sealed. A 25.0 ml Hanus solution (a mixture of iodine monobromide in glacial acetic acid) was added and sealed, then shook for 1 min. It was sealed and left in a dark room (about 200°C for 30 min). A 10.0 ml of 15% potassium iodide and 100 ml water was added and sealed, and then agitated for 30 sec. It was then titrated with 0.1 mol/l sodium thiosulphate to obtain iodine value. Likewise blank test was obtained [17]:

$$\text{Iodine value (g/g)} = \frac{[\text{BLI} - \text{EPI}] \text{ x TF x Cl x Kl}}{size} \quad (5)$$

EPI: Titration volume (ml), BLI: Blank level (47.074 ml), TF: factor of titrant (1.006), Cl: concentration conversion factor (1.269), W: unit conversion coefficient (1).

2.3.5. Determination of Flow Rate

A plastic funnel was placed 7.5 cm above platform in ring supported by a retort stand and a sheet of paper was placed below the funnel assembly. A meter rule was placed below the funnel orifice making sure that it completely closed as 30 g of the pulverized lecithin extract was transferred into the funnel. The ruler was drawn away and the timer simultaneously started. The timer was stopped when all of the sample had passed through the funnel. With the aid of a meter rule, the height of the heap was measured with a pencil and the contour of the base of the powder heap was outlined. The angle of the conical heap formed was determined using $\tan \Theta = h/r$ as the angle of repose (AR). The granule were turned to the funnel and the experiment was repeated twice and results were recorded [18]:

$$\text{Flow rate (g/s)} = \frac{\text{mass}}{\text{time}} \quad (6)$$

2.3.6. Determination of Angle of Repose

The weighed balance was zeroed. A 30 g of the lecithin sample was weighed out and transferred to the cylinder which was placed on a cup base. The cup base was removed gradually and the height of the lecithin sample was recorded. The experiment was repeated twice [19]:

$$\text{Tan } \Theta = \frac{\text{height of powder heap,(h)}}{\text{radius of powder heap,(r)}} \quad (7)$$

2.3.7. Determination of Bulk Density

A weighed quantity (20 g) was placed in a 100 ml graduated cylinder. The cylinder was dropped onto a wooden surface three times from a height of one inch at 2 sec. interval. The volume assumed after the treatment was taken as the bulk volume [20]:

$$\text{Bulk density (g/ml)} = \frac{mass}{bulk\ volume} \quad (8)$$

2.3.8. Determination of Tapped Density

A known quantity (20 g) of the powder was placed in a 100 ml graduated cylinder. The cylinder was tapped up to 500 times on the wooden surface or to a constant volume. The final volume attained represented the tapped volume [20]:

$$\text{Tapped density (g/ml)} = \frac{mass}{tapped\ volume} \quad (9)$$

2.3.9. Determination of Particle Density (True Density)

The particle density of the lecithin powder was determined by the fluid displacement method using the pycnometer [21]. The empty pycnometer was weighed and recorded as W_1. It was then filled with an organic non-solvent. The outside of the pycnometer was wiped with tissue paper ensuring that no trace of the solvent was left on the bottle. The pycnometer containing the organic non-solvent was weighed and recorded as W_2. A 1.0 g quantity of the powder was weighed out and denoted as W_4. A small portion of the non-solvent (5 ml) was removed from the pycnometer. The weighed powder were then transferred to the pycnometer and shaken vigorously. The pycnometer was then filled with the powder to the 50 ml mark with a portion of the 5 ml that was removed. The weight of the pycnometer, the powder and the non-solvent was recorded as W_3 (ASTM, 2014):

$$\text{Particle density} = \frac{W_2 - W_1}{(W_2 - W_1) - (W_3 - W_4)} \quad (10)$$

where W_1 = weight of empty pycnometer, W_2 = weight of pycnometer + non-solvent W_3 = weight of the pycnometer + powder + non-solvent, W_4 = weight of pycnometer filled with powder.

2.3.10. Determination of Hausner's Ratio

The Hausner's ratio (HR), defined as the ratio between tapped and bulk density [21], is a common technique widely used to describe the packing behavior of powders when they are subjected to tapping.

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \quad (11)$$

2.4. Data and Statistical Analysis

All the measurements were repeated at least thrice and the data obtained analyzed by Student *t*-test and One-Way Analysis of Variance (ANOVA) using Statistical Product and Services Solution software (SPSS, version 22.0 Inc., Chicago IL, USA) and Excel Microsoft Office version 2012. The results were presented as mean ± SD, and statistical differences between means considered significant at ($p < 0.05$).

3. Results and Discussion

3.1. The Percentage Yield of the Extracted Lecithin

The percentage yield of the extracted lecithin is presented in Table 1. From the results obtained, the extracted lecithin can be said to possess appreciable inherent lecithin yield potential capable of making it useful as a source of lecithin via which raw materials such as lecithin can be produced. This is in line with the work done by [22], were the extracted oil yield ranged from 28.46 ± 0.30 to 32.23 ± 0.28.

3.2. Organoleptic Properties of the Extracted and Reference Lecithin

Table 2 shows the organoleptic properties of the extracted and reference lecithin. The extracted lecithin had a brownish-yellow colour and an appearance of fine to granular powder. This could be as a result of the method of extraction used, while the reference lecithin was yellow in colour and sticky in nature. One of the challenges we encountered was inability to ascertain why the reference sample was gummy, while the final extract obtained after our processes was particulate and powdered. This opens a window for further research.

3.3. Morphology Using Optical Microscope and Scanning Electron Microscope.

Figures 1 and 2 show the surface morphology of the extracted and reference lecithin that was carried out using the moticam camera. They had spherical shapes in different sizes, smooth surfaces and presence of vesicles. Figures 3 and 4, show the detailed morphological features of the extracted and reference lecithin as obtained from the scanning electron microscope. The micrographs showed that they were spherically shaped with a smooth surface, and spherical vesicles were also present [23].

3.4. Physic-Chemical Properties of the Reference and Extracted Lecithin

3.4.1. Angle of Repose

As shown in Table 3, the values for the angle of repose of the extracted lecithin were found to range from 18.11° to 18.13°. This indicated excellent good flow properties of powder [24]. The angle of repose of the reference could not be determined due to the gummy nature of the lecithin.

3.4.2. Compressibility Index

The results of the CI ranged from 17.5 to 17.7% (Table 3). Generally, CI values up to 15% result in good to excellent flow properties, thus the powder showed good flow properties. All these results, indicated that the powder possessed satisfactory flow properties. These parameters, provide the quality index of the extracted lecithin.

3.4.3. Moisture Content

The moisture content gives a vital information concerning the actual content of the dry matter and its storage conditions. The maximum recommended limit for soybean moisture content is 4.8% [25]. The moisture content of the reference lecithin was lower (0.09 ± 0.07), compared to that of the extracted lecithin (0.7 ± 0.2). The extracted lecithin showed a higher significant difference than the reference ($p < 0.05$). This is in agreement with the works done by Mumeen and colleagues [11], were the recorded moisture content was between the stipulated limit of 2.80 to 4.80% for lecithin. A high percentage of FAA (> 1.5%) is a determination of unsuitability of the oil for edible purpose.

3.4.4. Free Fatty Acid (FFA)

The FFA value of the extracted lecithin (7.52 ± 0.29) was higher than that of the reference (3.38 ± 0.14). This is not in agreement with the works of Mumeen and colleagues [11], were the reference lecithin recorded a higher value for FFA than the extracted lecithin. This could be as a result of environmental factors during processing and packaging. According to WHO, the maximum limit for FAA is 18%, thus both the reference and extracted lecithin were found to be within the stipulated limit [25]. The extracted lecithin showed a higher significance difference than the reference lecithin ($p < 0.05$).

3.4.5. Iodine Value (IV)

The IV is used to determine the rancidity by oxidation of the oil [19]. The higher the iodine value, the greater is the liability of the oil or fat to become rancid by oxidation. It is also used to determine the relative amounts of unsaturated fatty acids in the triglyceride molecules. The reference lecithin recorded a higher IV (124.7 ± 1.07) compared to the extracted form (114.1 ± 0.65). According to WHO, the maximum limit for lecithin is 85 mg I/g oil, thus the extracted and reference lecithin were found not to be within the range.

3.4.6. Saponification Value (SV)

High SV is used to indicate the presence of low proportion of lower fatty acids (Nielsen, 2002). The extracted lecithin recorded a higher degree of SV when compared to that of the reference lecithin. Saponification value has a direct correlation to the chain length of fatty acid. High SV indicates the presence of low molecular weight fatty acid in triglycerides [26].

3.4.7. Acid Value (AV)

The AV is the measurement of free fatty acids present in the oil or fat [26]. The extracted lecithin had a higher AV (14.5 ± 0.32) compared to that of the reference (10.9 ± 0.28 mg KOH/g) without a significant difference ($p > 0.05$). AV is a known quality index of lecithin. In this study, the AV obtained was lower than 33.10% which was reported by Uddim and co-workers (2011) in the extraction of lecithin from squid viscera oil [28]. Although the lower the acid value, the higher the quality of lecithin obtained (IPGRI, 2004). The AV obtained was found to be within the range of 18 mg KOH/g.

3.4.8. Peroxide Value (PV)

The PV is another important quality index of lecithin. It is used to ascertain the extent of spoilage that may occur as a result of rancidity during handling, processing and storage. PV obtained from extracted lecithin was 13.3 ± 0.31, while that of the reference was 9.6 ± 0.40. The values obtained, showed that the reference lecithin was found within the range of 10 mEq/100 g as stipulated by WHO (WHO, 1980), while the extracted was found not to be within the range. This could be as a result of some environmental factors, processing and handling (WHO, 1980). It is also used as a measurement of rancidity which occurs by auto-oxidation [28].

3.4.9. Bulk Density (BD)

It is used to determine the actual quantity of the powder that can be fitted into a capsule with a specified volume [27]. It is also useful in tablet production were the actual quantity that should be fitted into a blender or a hopper on a tablet press is determined. The BD of the extracted lecithin was 0.76 ± 0.21, while that of the reference could not be determined, due to the gummy nature of the lecithin.

Table 1. *Percentage yield of the extracted lecithin (mean ± SD).*

No. of times extracted	Yield (g)	Yield (%)
1	2.50	25.0 ± 0.41
2	2.30	23.0 ± 0.40
3	2.40	24.0 ± 0.41
4	2.10	21.0 ± 0.40
5	2.20	22.0 ± 0.41

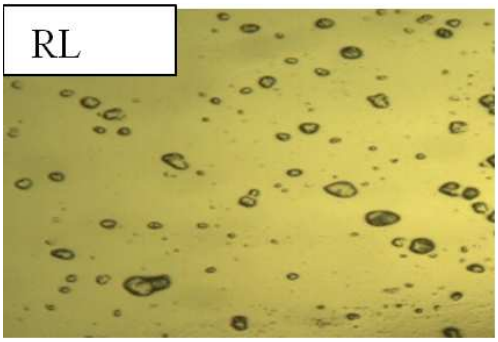

Figure 1. *Optical photomicrograph of the RL (x1000).*

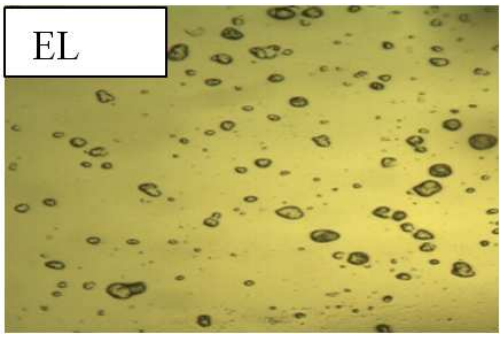

Figure 2. *Optical photomicrograph of the EL (x1000).*

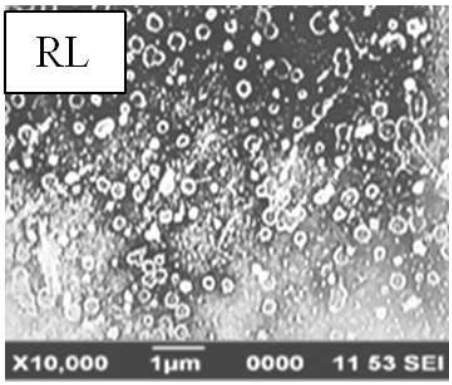

Figure 3. *SEM photomicrograph of the RL (x10,000).*

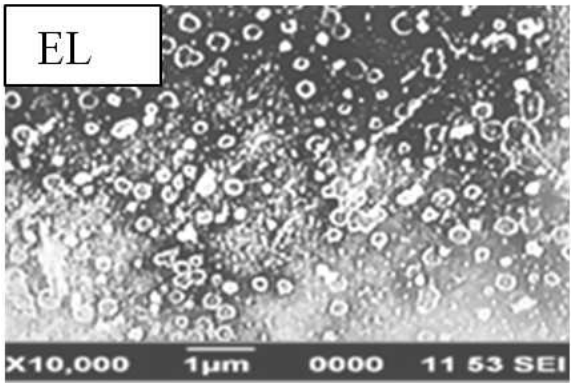

Figure 4. *SEM photomicrograph of the EL (x10,000).*

Table 2. *Results of the organoleptic properties of the extracted and reference lecithin.*

Property	Extracted lecithin	Reference lecithin (Lipoid® S 75)
Colour	Brownish-yellow	Yellow
Odour	Odourless	Odourless
Taste	Tasteless	Tasteless
Appearance	Fine to granular powder	Gummy

Table 3. *Summary of the results of the physic-chemical properties of the reference and extracted lecithin samples. Value obtained (Mean ± SD).*

Property	Reference Lecithin	Extracted Lecithin
Moisture content (%)	0.09 ± 0.07	0.76 ± 0.21
*Free fatty acid (%)	3.38 ± 0.14	7.52 ± 0.29
Saponification value (mg KOH/g)	42.3 ± 7.05	85.8 ± 7.34
*Acid value (mg KOH/g)	10.9 ± 0.28	14.5±0.32
*Peroxide value (mEq O_2/kg)	9.6 ± 0.40	13.3 ± 0.31
*Iodine value (mg I/g oil)	124.7 ± 1.07	114.1 ± 0.65
Bulk density (g/ml)	NA	0.76 ± 0.21
Tapped density (g/ml)	NA	0.95 ± 0.02
True density (g/ml)	NA	0.96 ± 0.19
flow rate (g/sec)	NA	1.65 ± 0.40
Angle of repose (degrees)	NA	18.12 ± 0.01
Compressibility index (%)	NA	17.5 ± 30.6
Hausner's ratio	NA	1.2 ± 1.4

*WHO Recommendation [27]: The maximum value for FFA, AV, PA and IV is 18%, 36 mg KOH/g, 10 mEq O_2/kg and 85 mg I/g oil respectively. NA: Not available due to the gummy nature of the reference lecithin.

4. Conclusion

This study has shown that the extracted lecithin from locally-sourced soybean exhibited comparable qualities with the reference (Lipoid S 75®). The yield obtained from the extracted lecithin was significantly ($p < 0.05$) higher than reported methods. The evaluated physic-chemical properties also demonstrated that majority of the characteristics were within the WHO recommendations. Locally-sourced soybean is a potential source of lecithin for industrial applications in Nigeria.

Due to the higher costs of imported goods, the government should create enabling environment for an effective utilization of locally produced soybeans various uses, including lecithin production, which would lead to value addition and increased job creation and improvement of the gross domestic product (GDP) of the economy.

Declaration of Interest

The authors declare that they have no competing interests.

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