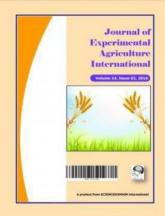

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Isolation and Characterization of Lecithin from Selected Nigerian Varieties of Soybean (*Glycine max*)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lecithin is an important by-product of vegetable oil processing that has foremost functions in health, food manufacturing and processing, industrial product development and pharmaceutical formulation. The importation of lecithin to Nigeria for Industrial use can be reduced through sourcing of lecithin from Nigerian varieties of oil bearing seeds such as soybean. Following careful selection of seeds, sorting and cleaning; oil was extracted from four varieties of Nigerian nationally released varieties of soybean using soxlet procedure. Lecithin was subsequently isolated from the extracted oil through the process known as degumming. Quality parameters such as Iodine Values (I.V), Peroxide Values (P.V), Saponification Value (Sap.V), Acid Values (A.V) and Free Fatty Acid (FFA) values were evaluated for all oil and lecithin samples extracted. Percentage yield of lecithin isolated from the selected varieties of soybean namely: TGX 1904-6F, TGX 1987-62F, TGX 1448-2E and TGX 1978-.10F are 2.71%, 2.5% and 2.07% respectively. Value of some quality indices such as the Jodine values, peroxide values, Acid Values and Free Fatty Acid obtained from this

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study were also found to be within the FAO/WHO recommended specifications. This study has provided detailed information from the characteristics stand point and quality index of oil extracted from the selected varieties of soybean as well as lecithin isolated from their oils; such data is however desirable so as to channel each of the selected soybean varieties to appropriate utilization and enhanced value addition necessary to improve the soybean value chain in Nigeria. This study therefore concludes that quality lecithin may be sourced from some Nigeria varieties of soybean to meet various industrial purposes.

Keywords: Soybean; phosphotidylcholine; quality index; oil characteristics; oil degumming.

1. INTRODUCTION

Lecithin is an important by-product of vegetable oil processing industries which has several applications in health, agriculture and food industries [1]. The compound is a mixture of glycerol-phospholipids from animal, vegetable and microbial sources. It contained varying amount of triacylglycerols, fatty acids glycolipids, sterols and sphingolipid [2]. The major source of commercial lecithin is soybean oil and it is called 1,2-diacylglucerrro-3-phosphoryl chlorine [3].

Lecithin has diverse role in human nerve activities especially in the control of nerve activities and breathing [2]. Lecithin is extensively used in agriculture, one of its major applications is its incorporation into animal feeds where it supplies essential ingredients needed in animal ration [4]. Lecithin also improves feed processing and adds to physicochemical characteristics required for feed palatability to animals. As a source of choline, lecithin serves as antioxidants for the highly unsaturated oils in animal feeds as well as in fertilizer production, lecithin serves as conditioning and spreading agent. It is also incorporated into pesticides where it is used for adhesion, as an antioxidant, biodegrading and dispersing agent, as an emulsifier, stabilizer, viscosity modifier and penetrating agent [5].

Lecithin is both lipophilic and hydrophilic hence its ability to attract both-water and fat characteristic which made it act as a bridge between water and oil [6].

Industrially, lecithin plays diverse roles as a result of hydrophilic and lipophilic properties and these includes but not limited to roles play in several industrial sectors as an emulsifying agent, dispersing, wetting, conditioning agents. Also, antioxidant properties of lecithin made the compound essential as an ingredient in production of items such as; chocolate foods, chewing gum, edible oil, ice cream, instant foods, insecticides, ink, leather, macaroni and noodles etc [1].

Other industrial sectors where lecithin is required for production also includes; pharmaceutical and drug manufacturing, cosmetics, Self Emulsifying Drug Delivery Systems (SEDDS), beverage manufacturing and paint manufacturing [7].

Production and quality of lecithin could however, be affected by crude oil storage, soil type, nutrient availability, climate changes, drying process and handling manner [8].

Soybean (*Glycine max (L*)) belongs to the leguminosae family, a plant which originated from Eastern Asia [9]. Nigeria is one of the largest producers of soybean in the world with an annual production output of 600,000 MT/annum [10]. The crop consists of approximately 40% protein, 23% carbohydrate, 20% oil, 5% mineral, 4% fibre and 8% moisture [11]. In 2009, soybean accounted for 365million tons of vegetable oil in the world far ahead of other oil crops [9].

There are numerous varieties and ecotypes of soybean adapted to various ecological conditions [12]. However, the National Cereal Research Institute – a Nigerian Government research and development organization has developed and released nine (9) different varieties of soybean adapted to various ecological and agronomic conditions of Nigeria [10].

The Nigerian Industrial sector imports approximately 1500 tons of lecithin annually for use in the agriculture, healthcare, pharmaceutical manufacturing, food processing, instant food production, cosmetics formulation, paint manufacture etc [13].

Meanwhile, the importation of such large amount of lecithin into the country constitutes a lot of pressure on the value of the Nigerian currency (Naira) as the importation involves huge amount of foreign exchange. Therefore, there is a growing pressure on local manufacturers to source raw materials locally, hence the need to conduct research towards the exploration of raw materials of oil- bearing seeds such as soybean

which is being cultivated abundantly across most of the agricultural belt of the country.

Lack of significant information and data in terms of biochemical components such as lecithin as well as the unavailability information from the nutritional standpoints and oil characteristics have been discovered to be a major limiting factor to the development of a robust oilseed value chain in Nigeria [14].

Therefore, the evaluation of lecithin content of some Nigeria varieties of soybean is essential to the improvement of oilseed value chain in Nigeria.

The aim of this study was to however extract and characterize lecithin from oil of selected varieties of Nigeria soybean for industrial application.

2. MATERIALS AND METHODS

The four cultivars of soybean namely; TGX 1904-6F, TGX 1957- 62F, TGX 1443- 2E and TGX1987-10F were obtained from the Soybean Research Programme of the National Cereals Research Institute, Badeggi in Niger State, Nigeria. The seeds were subsequently, subjected to the following experimental procedures:

2.1 Seed Preparation

500 g each of the four varieties were manually washed with distilled water, sorted and then dried at room temperature for two weeks after which they were pulverized using electronic blending machine and kept at 4°C until use.

2.2 Proximate Analysis

The protein and ash contents were determined using AOAC [15] number: 923.03 and IACST [16] methods, Nitrogen conversion factors 6.235 was used to calculate the crude protein of the soybean samples while oil contents and moisture contents of the samples were evaluated according to IUPAC [17].

2.3 Extraction of Seed Oil

The extraction of oil from the soybean seed varieties was carried out in accordance to the AOAC method [15] which involves the use of soxhlet apparatus using petroleum ether as the extraction solvent which was added to the extracting flask in 2:1 volume ratio and each variety of the soybean sample was made to extract for between 5-7 hours after which the solvent and oil mixture was evaporated and the oil recovered from the solvent.

2.4 Production of Lecithin from Soybean Oil

70 ml each of soybean oil was measured into a cleaned and dried conical flask and heated up to 70°C, 2% of water was added at this temperature, six (6) drops of Hydrogen peroxide was then added and stirred with a magnetic stirrer for 1 hour as described by Donatus et al. [18]. This method is called degumming of oil. Lecithin was then isolated by adding 20 ml of acetone to the mixture because lecithin is insoluble in acetone [18,19].

2.5 Characterization of Extracted Oil and Isolated Lecithin

The AOAC [15] and IUPAC [17] methods were used to determine the Acid value, (A.V) peroxide value (P.V), Saponification value (SAPV) and the Refractive Index (RI) while the Free Fatty Acid (FFA) of both oil and lecithin were determined following the IACST method [16].

2.6 Statistical Analysis

Values generated from triplicate laboratory trials were analyzed using Statistical Packages for Social Science (SPSS) version 16 (SPSS Inc. USA) and presented herewith as means and Standard Error of Means (SEM). Comparisons between different groups were determined by one way Analysis Of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT). The level of significance was set at $P<0.05$. Values presented in tables are means and SEM of three (3) determinations. Values along the same row with different superscripts are significantly different ($P<0.05$).

3. RESULTS AND DISCUSSION

As shown in Table 1: The proximate composition of selected soybean varieties; the moisture content ranged between 3.04 ± 0.14 – 3.5 ± 0.55%, going by the moisture content which gives a level of information about the actual content of the dry matter and the storability of plant material, all the four evaluated soybean varieties can be subjected to the same storage conditions and values that require moisture because there are no statistically ($P>0.05$) in the

values of their moisture content yet their moisture content conforms with acceptable moisture content of soybean put in the range of 2 80 to 4.8% [20]. The carbohydrate content of the evaluated soybean varieties can be applied in food processing and formulation requiring such range of carbohydrate contents as there is no statistically (P>0.05) significant difference in their proximate carbohydrate contents. The carbohydrate content ranged between 14.66± 0.34 – 15.94± 1.36, this values fall within ranges reported for soybean varieties of other tropical Sub Sahara African countries [7].

The result of the proximate composition of the evaluated soybean varieties (Table 1) however, shows that there are significant difference (P>0.05) in the values of the most economic proximate parameters of ash, protein fats and fibre in all the four soybean varieties.

However, the benefit of the statistically (P>0.05) significant difference observed in these parameters (Ash, protein, fat and fibre) is that each of the varieties can be subjected to different utility, product formulation and vale addition on the respective strength of data from these significantly different proximate stand point. This is in fact, a tool required to extend the values of these various of soybean [14].

For instance, the proximate Ash content help to determine the amount and the type of mineral embedded in each variety while the proximate, protein content is a requirement for industrial formulation of animal feeds. The fat content is helpful in understanding the potential of each seed varieties as sources of lipid base industrial raw materials such as lecithin. Proximate values observed for all the evaluate varieties however fall within ranges previously reported for soybean [20].

The percentage oil yield of the evaluated soybean samples are presented in Table 2. The oil yield was higher in TGX 1987 -10F(32.23 ± 0.13 %) when compared to the yields from TGX 1904-6F (28.46 ± 0.30 %),TGX 1448 -2E (30.56 ± 0.43 %) and TGX 1987 -62F (28.26 ± 0.23 %) which had the lowest oil yield. From the result shown in Table 2, each of the soybean varieties used in this study can be said to posses appreciable inherent oil yield potential capable of making them useful as sources of vegetable oil through which raw materials such as lecithin can be produced. This is however so, as oil content of soybean is expected to range between 25-35 % depending on a number of factors such as varietal morphological characteristics, agronomic conditions, environment etc [20].

As presented in Table 3, the physicochemical characteristics of oils extracted from the selected Nigerian varieties of soybean revealed no statistically significant difference (P<0.05) in the values of the Refractive Index (R.I),Specific Gravity (S.G) and the Iodine Value (I.V) amongst all the varieties evaluated and these parameters ranged between 1.45±0.10 – 1.46±0.03, 0.94±0.01 – 0.94±0.02 and 123.04±1.42 – 125.20±2.14 respectively while there are statistical significant difference (P<0.05) in the values of Peroxide Value (P.V), Saponification Value (Sap. V) Acid value (A.V) and the free fatty Acid (FFA), an indication that each of the varieties can be subjected to different utilization necessary to create demand for each of the evaluated soybean varieties.

Table 1. Proximate composition of selected Nigerian soybean varieties

Variety	Moisture	Ash	Protein	Fat	Carbonhydrate	Fibre
TGX 1904-6F	3.38±0.10^{a}	6.48±0.35^{b}	38.79±0.32^{a}	28.72±0.60^{b}	14.66±0.34^{a}	6.94±0.26^{ab}
TGX 1987-62F	3.57±0.55^{a}	8.13±0.55^{c}	37.07±1.39^{a}	26.93±0.18^{ab}	17.94±0.26^{b}	6.33±0.21^{a}
TGX 1448-2E	3.04±0.14^{a}	5.09±0.19^{a}	44.00±0.28^{b}	24.24±0.76^{a}	15.94±1.36^{a}	6.00±0.71^{a}
TGX 1987-10F	3.29±0.17^{a}	11.21±0.95^{d}	40.02±0.39^{b}	21.37±0.34^{a}	15.56±0.35^{a}	7.16±0.14^{b}

Values are mean ± SEM of 3 determinations. Values along the same row with different superscript are significantly different (P < 0.05)

Table 2. Percentage oil yield of selected Nigerian Soybean varieties

S/N	Sample description	Oil yield %
1	TGX 1904-6F	28.46±0.30
2	TGX 1987-62F	28.26±0.23
3	TGX 1448-2E	30.56±0.48
4	TGX 1987-10F	32.23±0.28

Table 3. Physicochemical characteristics of oil extracted from selected Nigerian soybean varieties

Sample	Refractive Index	S.G	I.V 1_2/100 g	P.V mEq/1000 g	Sap. V (mgKOH/g)	A.V (mgKOH/g)	FFA (%)
TGX 1904-6F	1.46±0.03^{a}	0.94±0.02^{a}	123.09±2.04^{a}	24.00±0.23^{ab}	219.35±5.46^{a}	4.76±0.32^{b}	2.38±0.23^{b}
TGX 1987-62F	1.46±0.03^{a}	0.94±0.02^{a}	124.60±1.42a	20.60±0.45^{a}	228.33±4.61^{a}	4.31±0.33^{b}	2.15±0.34^{b}
TGX 1448-2E	1.46±0.02^{a}	0.92±0.01^{a}	125.20±2.14a	23.52±0.13^{ab}	339.60±3.47^{b}	3.08±0.41^{a}	1.54±0.24^{a}
TGX 1987-10F	1.46±0.10^{a}	0.93±0.01^{a}	123.04±4.02a	28.00±0.45^{b}	252.45±4.57^{a}	4.31±0.22^{b}	2.15±0.46^{b}

Values are mean ± SEM of 3 determinations. Values along the same row with different superscript are significantly different (p< 0.05). S.G=Specific Gravity, I.V=Iodine Value, P.V=Peroxide Value, Sap.V=Saponification Value, A.V=Acid Value, FFA=Free Fatty Acid.

Table 4. Percentage Yield of Lecithin Isolated from Selected Nigerian Soybean

S/N	Sample description	Yield %
1	TGX 1904-6F	2.71±0.05
2	TGX 1987-62F	2.57±0.12
3	TGX 1448-2E	2.28±0.04
4	TGX 1987-10F	2.07±0.04

Table 5. Physiochemical Characteristic of lecithin isolated from selected Nigerian Varieties of Soybean

	Refractive index	S.G	I.V 1_2/100g	P.V mEq/1000g	Sap. V (mgKOH/g)	A.V (mgKOH/g	FFA (%)
TGX 1904-6F	1.46±0.03^{a}	0.92±0.01^{a}	79.51±0.03^{a}	13.93±0.06^{b}	241.48±0.03^{b}	1.950±0.01^{a}	0.97±0.00^{a}
TGX 1987-62F	1.46±0.00^{a}	0.92±0.03^{a}	80.01±0.03^{a}	10.60±0.00^{a}	237.02±0.00^{b}	2.52±0.00^{ab}	1.27±0.01^{b}
TGX 1448-2E	1.46±0.02^{a}	0.92±0.03^{a}	78.29±0.02^{a}	13.49±0.02^{b}	171.61±0.49^{a}	2.89±0.02^{ab}	1.45±0.01^{b}
TGX 1987-10F	1.46±0.00^{a}	0.93±0.00^{a}	78.61±0.01^{a}	16.97±0.02^{c}	187.80±0.046^{a}	3.06±0.02^{b}	1.53±0.01^{b}
ALCOLEC $^{(C)}$Soybean lecithin standard (Non GMO Lecithin liquid) (www.americanlecithincompany.org	NA	NA	80	6	124	29	14.5
FAO/WHO recommendation	NA	NA	85 max	10 max	NA	36 max	18 max

Values are mean ± SEM of 3 determinations. Values along the same row with different superscript are significantly different (P < 0.05).S.G=Specific Gravity, I.V=Iodine Value, P.V=Peroxide Value, Sap.V=Saponification Value, A.V=Acid Value, FFA=Free Fatty Acid

Lecithin was isolated from oil of each of the evaluated Nigerian varieties of soybeans, as presented in Table 4, lecithin yields of (2.71 %, 2.57 %, 2.28 % and 2.07 %) were obtained from TGX 1904-6F, TGX 1987 – 62F, TGX 1448 -2E and TGX 1987-10F respectively. Lecithin yield from oils of the evaluated soybean seeds are higher than yield previously reported for Nigerian varieties of melon seeds (0.72-1.17%) [6]. The higher yield of lecithin form the evaluated soybean varieties as compared to other oil seed is an indication that oils from these soybean varieties can serve as potential sources of industrial lecithin for commercial purposes.

As presented in Table 5, the saponification value of lecithin isolated from the evaluated soybean varieties was found to range between 171.61±0.49 – 241.48±0.03 mgKOH/g, these values are however, higher than the saponification values reported for the standard non-GMO lecithin; Alcolec ^{R} being imported into Nigeria, the Saponification values as presented in Table 5 also fall within the FAO/WHO recommendation [21]. Meanwhile, Saponification value is directly related to the chain length of fatty acids, higher saponification value therefore indicates the presence of low molecular weight fatty acid in triglyceride which is well desirable for the industrial formulation of products such as soap and paints.

The iodine values of isolated lecithin from all evaluated varieties were also measured and also reported in Table 5, iodine values obtained in this study ranges between 78.61± 0.01 – 80.01 ± 0.03 (1_2/ 100 g), these values are however lower than 89.26 (1_2 / 100 g) reported for lecithin isolated from boil [19]. Though, higher than the iodine value of the standard lecithin adopted for this study; the iodine values generated from this study still fall within the recommended range of 85 (1_2/100g) which is an indication of their potential use in iodine value dependent industrial formulation such as paints production.

Acid value as presented in Table 5, was found to range between 1.950±0.01 – 2.06±0.02 %, the acid value is a quality index of lecithin and a measure of its acidity, the acid values of lecithin obtained in this study were much lower than the value of 33.10 % reported for lecithin extracted from squid viscera oil [19]. The lower acid value of lecithin obtained in this study however, indicates higher quality of lecithin from the evaluated soybean varieties.

Peroxide value is also a major quality index of lecithin as it describes the extent of spoilage which may have occurred due to rancidity occasioned by handling and processing. Peroxide value of lecithin obtained in this study ranged between 10.60 ± 0.00 – 16.97 ± 0.02 mEq/100 g (Table 5). However, the peroxide value of TGX 19.87 - 62F was found to be within the recommended range of 10 mEg/100 g, it is also within the range reported for the standard lecithin (Alcolec) brand which was reported to be 6 mEg/100g. Peroxide values of lecithin extracted from the other three soybean varieties were however, higher than that of the standard lecithin and also do not fall within the recommended range, a situation which may be as a result of genetic variation, handling, processing and environmental factors.

Meanwhile, the FAO. WHO [21] recommended a peroxide value of 10 mEq/100g for all food grade lecithin.

4. CONCLUSION AND RECOMMENDA-TION

The study has shown that the evaluated soybean oils contain appreciable quantities of lecithin. The isolated lecithin also demonstrated some industrial qualities sequel to the evaluated physiochemical characteristics which fall within certain recommendations of the FAO/WHO as well as earlier reported ranges.

A major importance of lecithin analysis is to find the quality index of lecithin as it is necessary for effective and proper utilization. It is therefore safe to conclude that the evaluated Nigerian varieties of soybean are likely potential sources of lecithin which may be used as raw material for cottage, industrial and commercial purposes.

This is however, a development necessary for effective utilization of the evaluated Nigerian Soybean varieties capable of leading to increased cultivation and its attendant effects on job creation which may result to a robust soybean value chain in Nigeria, a feat which is required to maximize the contribution of soybean to the Agricultural GDP of the Nigerian economy.

A research into the enzymatic and Molecular modification of lecithin content of the evaluated soybean varieties may however aid the adoption of Nigeria varieties of soybean as a reliable source of lecithin for industrial use in Nigeria.

ETHICAL APPROVAL

The research was approved by the National Cereals Research Institute, Badeggi, Nigeria and the Federal University of Technology, Minna, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dreon DM, Vranizan KM, Krauss RM, Austin MA, Wood PD. The effects of polyunsaturated fats versus mono-unsaturated fat on plasma lipoproteins. Journal of American Medical Association. 2010;263(18):2462–2466.
2. Gordon MW. Contemporary nutrition issues and insight. 4th Edition. Boston McGraw-Hill Publishers. 2000;23-45.
3. Dashiell A. American Oil Chemist Society (AOCS). Central Soya Coy. Fort Wayne. Indiana, 46818 USA. 2003;443.
4. Nahoski EE. Lecithin and health; brain nutrient – Phosphatidylcholine. NOHA News. 2015;6(1):10–12.
5. Callins AO. Functional properties of world's most important oil seeds. International Journal of Agriculture. 2010;42:878–894.
6. Olisa NM. Extraction, characterization and industrial uses of lecithin from three varieties of *Cucumis melo* (melon seed) oil. M.Sc. Thesis in the Department of Biochemistry, University of Nigeria, Nsukka, Nigeria. 2000;58.
7. Lisa K. Karr-Lilienthal, Laura L. Bauer, George C. Fahey. Chemical composition and nutritional quality of soybean meals prepared by extruder processing for use in poultry diets. Journal of Agricultural and Food Chemistry. 2016;54(21):8108–8114.
8. Renfree C. Lecithin chemical and supplementation. American Oil Chemists Society. Indiana, Fortwayne Publications. 2005;97.
9. Singh G, Shivakumar BG. The role of soybean in agriculture, soybean: Botany, production and uses. Wallingford, UK, Cabi Publishing. 2010;24-47.
10. NCRI. Beniseed research programme report. Annual research evaluation and monitoring workshop. National Cereals Research Institute, Badeggi, Nigeria; 2016.
11. Njoku U, Ifeoma I. Evaluation of the nutritional potentials of beniseed. Nigerian Journal of Biochemistry and Molecular Biology. 2015;23(1):39-41.
12. Ismaila U, Gana AS, Tswanya NM, Dogara D. Cereals production in Nigeria: Problems, constraints and opportunities for betterment. African Journal of Agricultural Research. 2010; 5(12):1341-1350.
13. NBS. Raw material commodity survey, 3rd quarter report. Nigerian Bureau of Statistics, Abuja, Nigeria; 2011.
14. RMRDC. Survey report of ten selected agro raw materials in Nigeria. Raw Materials Research and Development Council, Abuja, Nigeria. 2004;89.
15. AOAC. Official methods of analysis, 13th edition. Association of Official Analytical Chemist. Washington D.C; 1990.
16. IACST. Official methods of analysis, 6th edition. International Association for Cereals Science and Technology. Newyork; 1986.
    Available:http/www.icc.org
17. IUPAC. Official methods of analysis, 2.301. International Union of Pure and Applied Chemistry; 1988.
    Available:http/www.iupac.org
18. Donatus CO, Obioma UN, Patrick EA, Jonas AO. Extraction and characterization of lecithin from Nigerian Melon. Journal of Advances in Biology and Biotechnology. 2016;10(1):264–272.
19. Uddin MS, Kishimura H, Chun BS. Isolation and characterization of lecithin from squid (*Todarodes pacificus*) viscera deoiled by supercritical carbon dioxide extraction. Journal of Food Science. 2011; 76(2):350-354.

20. IPGRI. Descriptors for soybean (*Glycine max*). The International Plant Genetic Resources Institute, Rome, Italy; 2004.
21. FAO/WHO. Specification of identity and purity of sweetening agents, emulsifying agents, flavouring agents and other food additives. Food and Agricultural Organisation / World Health Organization; 1980.
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