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Lipid Composition of Germinated and Non-Germinated Sorghum (*Sorghum bicolor*) Found in Nasarawa State, Nigeria

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Abstract: Sorghum (*Sorghum bicolor*) is an underutilized oil-bearing seed found in Nigeria. The fatty acid, phospholipids and phytosterols composition of germinated and non-germinated seed of *Sorghum bicolor* were evaluated using standard analytical techniques. The result showed that the most concentrated fatty acids (%) found in the geminated and non-germinated oils were linoleic acid (41.16, 59.45), oleic acid (33.80, 23.05), palmitic acid (18.20, 10.68) and stearic acid (2.35, 1.72). The fatty acids composition of the germinated and non-germinated oils contained a healthy mixture of all the types of saturated and unsaturated fatty acids. The polyunsaturated/saturated index (P/S) was 2.12 % and 3.82 % for germinated and non-germinated oils, respectively. The most prominent phospholipids (mg/100 g) found in the germinated seed oil was phosphatidylcholine (23.86) followed by phosphatidylethanolamine (9.22) and phosphatidylinositol (9.08) while the most prominent in the non-germinated seed oil was phosphatidylcholine (32.39) followed by phosphatidylethanolamine (13.03) and lysophosphatidylcholine (13.07). The high value of phosphatidylcholine showed that *Sorghum bicolor* may help in protecting the liver from disease and hepatitis. The total phytosterols for germinated and non-germinated were (45.93 mg/100 g and 56.69 mg/100 g), respectively. This suggests that *Sorghum bicolor* lipid is a good source of food supplement or dietary and health benefits to human.

Keywords: Fatty Acids, Phospholipids, Phytosterols, Germinated, Non-Germinated, *Sorghum bicolor*

Introduction

Seeds and fruits are the major source of vegetable oil and protein in the world [1]. Oil is a naturally occurring substance with fatty acids as its main constituent. The fatty acids present in oil include saturated, monounsaturated and polyunsaturated fatty acids that contribute to human physiology in different ways. Polyunsaturated fatty acids are present as component of membrane phospholipid in specific tissue or precursor of hormone like prostaglandins [2]. Phospholipids which are also called phosphatides consist of a glycerol combined with fatty acids and a phosphate ester. The majority of the phosphatides is removed from oil during the degumming and refining operations and is important source of natural emulsifiers [3]. Phytosterols are vegetable oils and plant sterols which have been shown to reduce both serum and LDL cholesterol [3].

Sorghum (*Sorghum bicolor*) is one of the most important weaning foods in low income and high-income countries [4] and ranks fifth among the world cereals, following wheat, maize, rice and barley in production area and total production. Sorghum is an extremely important crop in Asia, African and other semi-arid regions of the world [1] and is commonly used as food, feed and industrial crop [5]. The nutrient composition of *Sorghum bicolor* indicates that it is a good source of energy, proteins, carbohydrates, vitamins and minerals [6]. Those that contain good quality protein are those that are readily digestible and contain the essential amino acids in quantities that correspond to human requirements [4]. *Sorghum bicolor* is characterized by a relatively high concentration of fatty acids, which exceed that of other competing cereals like barley, wheat and millet [7].

Recently, interest has been on the search for lesser known underutilized oil-bearing seeds that can serve as alternative to the well-known conventional seed oils in market which are expensive and not within the reach of the ordinary man. To also address the problem of food insecurity and malnutrition which would contribute to the actualization of 2030 Sustainable Development Goals (SDGs) which was adopted by the United Nations and all Member States, the present study evaluated the fatty acid, phospholipids and phytosterols of germinated and non-germinated sorghum (*Sorghum bicolor*) obtained from Nasarawa State, Nigeria.

Materials and Methods

Collection of Samples

The seeds of sorghum (*Sorghum bicolor*) (about 4 kg) were purchased from a local market in Tudum Amba located in Lafia, the capital city of Nasarawa State, Nigeria.

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Preparation and Treatment of Samples

The seeds were carefully sorted out to remove the bad ones and stones, after which the seeds were divided into two equal parts and labeled F and G. The F portion of the seeds was soaked in fresh water for 3 h at 32^{o}C ambient temperature. It was separated from the water and transferred into a container and covered for three days (72 h) for germination, 1-4 cm sprout was developed. The second portion (G) was washed with distilled water to remove impurities and labeled non-germinated. The samples (germinated and non-germinated *Sorghum bicolor* seeds) were oven dried separately for 100 – 105^{o}C. The dried samples were separately ground using mortar and pestle but not to very fine particles in order to allow the free flow of solvent during extraction.

Extraction of Oil

An aliquot (4.5 g) of each sample was weighed into an extraction thimble and 200 ml of petroleum ether (40^{0}C – 60^{0}C boiling range) was added. The covered porous thimble containing the sample was extracted for 5 h using a Soxhlet extractor. The extraction flask was removed from the heating mantle when it was almost free of petroleum ether, oven dried at 105^{0}C for 1 h, cooled in a desiccator and the weight of dried oil was determined.

Determination of Fatty Acids

The oil extracted from each sorghum sample was converted to methyl ester using the method described by Adeyeye & Adesina [8] and Aremu *et al.* [9]. A 50 ml of an aliquot of the dried oil was saponified for 5 min at 90^{0}C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralized by 0.7 M HCl and 3 ml of 14% boron trifluoride in methanol was added and the mixture was heated for 5 min at 90^{0}C to achieve complete methylation. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane and concentrated to 1 ml for analysis. The fatty acid was analyzed using an HP5890 gas chromatography (Gnl, Inc., Minnesota, USA) fitted with a flame ionization detector and using chemstation software, Nitrogen was used as the carrier gas with a flow rate of 20 – 60 ml/min. The oven program was 60^{0}C initial temperature, ramping at 15^{0}C/min for 20 min held for 4 min with a second ramping at 15^{0}C/min for 4 min and held for 10 min. The injection temperature was 250^{0}C and the detector temperature was 320^{0}C. A polar (HP INNO Wax) capillary column (30 m x 0.53 × 0.25 µm) was used to separate the esters. A split ratio of relative retention time compared with known standards.

Determination of Phospholipids

The phospholipids content of *Sorghum bicolor* sample was determined by gas chromatography (GC) as described by Aremu *et al*. [9]. The dried oil (0.01 g) was put in test tubes and a stream of nitrogen was passed over the oil to remove any remaining solvent in the oil. Then, 0.40 ml of chloroform was added, followed by 0.10 ml of the chromo–genic solution. The tube was heated to 100^{0}C in water bath for 1 min 20 s, cooled to room temperature, 5 ml of hexane was added and the tube was shaken gently several times. After separation of the solvent, the hexane layer was removed and concentrated to 1.0 ml and analysis was carried out using a gas chromatography with a polar (HPS) capillary column (30 m x 0.53 × 0.25 µm). The oven program was; initially at 50^{0}C, ramping at 15^{0}C/min for 4 min and held for 5 min. The Injection temperature was 250^{0}C and the detector temperature was 320^{0}C. A split injection type was used having a split ratio of 2:0:1. The peak was identified by comparison with the known standard.

Determination of Phytosterols

Phytosterol was determined by the method described by AOAC [10] and supported by Aremu *et al.* [9]. Aliquot of the dried oil was added to screw-capped test tubes. The sample was saponified at 95^{0}C for 30 min, using 3 ml of KOH in ethanol, to which 0.20 ml of benzene was added to ensure miscibility. De–ionized water (3 ml) was added and 2 ml of hexane was used in extracting the non-specifiable materials. Three extractions, each with 2 ml of hexane, were carried out for 1 h and 30 min respectively to achieve complete extraction of the phytosterols. Hexane was concentrated to 1 ml for gas chromatographic analysis.

Statistical Analysis

Standard chromatography was prepared for phytosterols, phospholipids and fatty acid and compared with respective analytical result; calibration curves were prepared for the standard mixture and correlation coefficient was determined for each fatty acid, phytosterol and phospholipid. Correlation coefficient greater than 0.9 was considered acceptable. The statistical calculation included percentage value, grand mean, standard deviation and coefficient of variation.

Results and Discussion

The fatty acid composition of germinated and non-germinated *Sorghum bicolor* seed oil is presented in Table 1. The same fatty acids were presented in both the germinated and non-germinated samples with different concentrations. The most prominent fatty acid in the geminated and non-germinated samples was linoleic acid with concentrations (%) of 41.16 and 59.45 respectively. This was followed by oleic acid with concentrations (%) of 33.80 and 23.05 for the germinated and non-germinated samples respectively and palmitic acid with concentrations of 10.68 % for the germinated and 18.20 % for the non-

germinated. Linoleic and oleic acids were the major fatty acids observed in peanut, soy bean, and lentil according to Olaofe *et al.* [11]. The amount of linoleic acid (41.16%) and (59.45%) in germinated and non- germinated oil of *Sorghum bicolor* is comparable to that values reported by Aremu *et al.* [12] for *Daucus carota* and *Cucumis sativus* (54.04 and 57.62%). It has been reported that an insufficient intake of omega–6 fatty acid such as linoleic causes growth retardation in children, heart attack risk and skin ailments [12]. The germinated oil has higher concentration of oleic acid (33.80 %) compared with non–germinated. Various studies indicate that a diet rich in oleic acid decreases the development of atherosclerosis and lower serum cholesterol by diminishing oxidative stress and inflammatory mediators while promoting antioxidant defenses [13]. Stearic and linolenic acid for the germinated and non-germinated samples ranged between 1.39 to 2.35%. The low concentration of linolenic acid suggests that *Sorghum bicolor* seed oil may be stable to oxidative deterioration. However, alpha– Linoleic acid plays a significant role in the skin. In dry skin, it strengthens the lipid barrier of epidermis, protects against trans–epidermal loss of water and normalized the skin metabolism. Myristic, palmitoleic, margaric, arachidic, arachidonic, behenic, erucic and lignoceric acids were detected but with values less than 1 %. The coefficient of variation (%) ranged from 7.12 to 81.82 (Table 1).

Table 1: Fatty acid composition (%) of germinated and non-germinated *Sorghum bicolor*

Fatty Acid (%)	F	G	Mean	SD	CV (%)	F-G
Capylic Acid (C8:0)	0.00	0.00	0.00	0.00	0.00	
Capric Acid (C10:0)	0.00	0.00	0.00	0.00	0.00	
Lauric Acid (C12:0)	0.00	0.00	0.00	0.00	0.00	
Myristic Acid (C14:0)	0.62	0.71	0.67	0.06	8.96	-0.09
Palmitic Acid (C16:0)	18.20	10.68	14.44	5.32	36.84	7.52
Palmitoleic Acid (C16:0)		0.67	0.41	0.54	0.18	33.30	0.26
Margaric Acid (C17:0)		0.03	0.05	0.04	0.01	25.00	-0.02
Stearic Acid (C18:0)	2.35	1.72	2.04	0.45	22.06	0.63	
Oleic Acid (C18:1)	33.80	23.05	28.43	7.60	26.73	10.75	
Linoleic Acid (C18:2)	41.16	59.45	50.31	12.93	25.70	-18.29	
Linolenic Acid (18:3)	1.62	1.39	1.51	0.16	10.60	0.23	
Arachidic Acid (C20:0)		0.32	0.24	0.28	0.03	10.70	0.08
Arachidonic Acid (C20:4)		0.05	0.09	0.07	0.30	42.57	-0.04
Behenic Acid (C22:0)	0.27	0.50	0.27	0.60	22.22	-0.23	
Erucic Acid (C22:1)	0.19	0.36	0.28	0.02	7.14	-0.17	
Lignoceric Acid (C24:0)		0.73	1.36	0.55	0.45	81.82	-0.63

SD = Standard deviation, F = Germinated, G = Non-germinated, CV = Coefficient of variation.

Table 2 shows that the total saturated fatty acid values (%) for the germinated and non-germinated *Sorghum* seed oils were 20.17 and 15.26, respectively. These values are lower than that of raw and boiled tigernut (24.80 % and 20.50 %), dehulled African yam bean (54.51 %), Bambara groundnut (34.68 %), ripe mango (25.91 %), African locust bean (40.20 %) and mesquite bean (43.00 %) as reported by Aremu *et al.* [14], Adeyeye *et al.* [15], Aremu *et al.* [16), Ortutu & Aremu [17] and Aremu *et al.* [18], respectively. However, the values are higher than that of groundnut (12.3%) [19], soybean (15.2%) [20] and processed pinto bean (9.12%) [21]. The Total UFA values of 77.49 % for the germinated and 84.75% for the non-germinated samples are higher than that of raw (75.20 %) and boiled (79.50 %) tigernuts [14], ripe mango fruit pulp (74.10 %) [17] and mesquite bean (56.90 %) [16]. The result showed that oils from germinated and non-germinated *Sorghum bicolor* consist mainly of unsaturated fatty acids. Unsaturated fatty acids have been reported to improve blood cholesterol levels, ease inflammation and stabilize heart rhythms [17]. The high percentage of TUFA in germinated and non-germinated *Sorghum bicolor* suggests that they are important for this function. The ratios of MUFA/SFA in the germinated and non-germinated samples were 1.72% and 1.56% respectively, while the ratio of PUFA/SFA was 2.12% for the germinated and 3.83% for the non-germinated samples. These ratios are important in the determination of the detrimental effect of dietary fats. The higher the PUF/SFA ratio, the more nutritionally useful is the oil [15]. It has been reported that the severity of disease condition such as atherosclerosis is closely associated with the proportion of total energy supplied by PUFA and SFA [22]. The ratio of oleic/linoleic (O/L) has been associated with high stability and potentiality of the oil for deep frying [18]. The O/L levels were 0.82 for germinated and 0.39 for non-germinated sample. These values are lower than that of *Vitellaria paradoxa C.F. Gaertn* kernel (2.27 %) and pulp (1.67%) as reported by Aremu *et al.* [23]. Hence germinated and non-germinated oils of *Sorghum bicolor* may be useful as frying oil.

Table 2: Quality parameters of (*Sorghum bicolor*) on germinated and non-germinated.

Parameters	F	G	Mean	SD	CV%	F-G
Total SFA	20.17	15.26	17.72	3.47	19.58	4.91
Total MUFA	34.66	23.82	29.24	7.67	26.23	10.84
Total PUFA	42.83	60.93	51.88	12.80	24.67	-18.29
DUFA	41.16	59.45	50.31	12.93	25.70	-18.29
Total UFA	77.49	84.75	81.12	5.13	6.32	-7.26
MUFA/SFA	1.72	1,56	1,64	0.11	6.71	0.16
PUFA/SFA	2.12	3.83	2.98	1.21	40.60	1.71
Total EFA	42.78	60.84	51.81	12.77	24.65	-18.84
O/L	0.82	0.39	0.61	0.30	49.18	+0.43

SD = Standard deviation, F = Germinated, G = Non-germinated, CV = Coefficient of variation, SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, DUFA = Diunsaturated fatty acid, UFA = Unsaturated fatty acid, MUFA/SFA = Ratio of monounsaturated to saturated fatty acid, PUFA/SFA = Ratio of polyunsaturated to saturated fatty acid, EFA = Essential fatty acid, O/L = Ratio of oleic to linoleic acid.

Table 3: Phospholipids composition of *Sorghum bicolor* (germinated and non-germinated)

Phospholipid	F	G	Mean	SD	CV%	F-G
PE	9.22	14.34	11.78	3.62	30.73	-5.12
PS	2.12	4.19	3.16	1.46	46.20	-2.07
PC	23.86	32.39	28.13	6.03	21.44	-8.53
LC	7.15	13.07	10.11	4.19	41.44	-5.92
PL	9.08	2.79	5.94	4.45	74.92	+6.29
PA	1.17	8.95	5.06	5.50	108.7	-7.78
Total	52.60	75.73				

PE: Phosphatidylethanolamine, PS: Phosphoserine, PC: Phosphatidylcholine, LC: Lysophosphatidylcholine; PL: Phosphatidylinositol, PA: Phosphatidic acid

The result of the phospholipid composition of germinated and non-germinated sorghum oils is presented in Table 3. The phospholipid composition (mg/100 g) of the non-germinated (75.73) sample was higher that of germinated (52.60). Phosphatidylcholine had the highest concentration with values of 23.86 mg/100 g and 32.39 mg/100 g for the germinated and non-germinated samples, respectively. These observations agree with Wirtz *et al.* [24] who reported that phosphatidylcholine is among the abundant phospholipids in animals and plants, often amounting to almost 40% to 45% of the total, and as such contribute to the building of the membrane bilayer. The phosphatidylcholine is found in all living cell; although in human physiology, it is found particularly in nervous tissue such as the white mater of brain, nerves, neutral tissue and spinal cord [25]. Phosphatidylethanolamine and phosphatidylcholine with values of 14.34 mg/100 g) and 32.39 mg/100 g in the non-germinated sample showed greater concentrations compared to the values (mg/100 g) of 9.22 and 23.86 recorded for the germinated sample, respectively. Phosphatidic and lysophosphatidylcholine values (8.95 mg/100 g and 13.07 mg/100 g) in non-germinated sample are greater than the values recorded for the germinated sample (1.17 mg/100 g and 7.15 mg/100 g). The minor phospholipid in both samples is phosphatidylserine with values of 2.12 mg/100 g and 4.19 mg/100 g for the germinated and non-germinated samples, respectively. These values are higher than that of unripe (1.73 mg/100g), about to ripe (1.77 mg/100 g) and ripe (1.79 mg/100 g) mango fruit pulp as reported by Ortutu and Aremu [17]. Phosphatidylinositol is high in germinated sample but low in non-germinated sample while phosphatidic acid is high in non-germinated but low in germinated sample. The US food and drug Administration (USFDA) have stated that consumption of phosphatidylserine (PS} may reduce the rate of dementia and cognitive dysfunction in the elderly people, in young people it reduces mental stress and increases mental accuracy and stress resistance [26]. PS supplementation promotes a desirable hormonal balance for athletes and might reduce the physiological deteriorations that accompanies over training and/or overstretching [25]. Phosphatidic mediates cellular functions through different mode of action such as membrane tethering, modulation of enzymatic activities and structural effects on cell membranes. Processes in which phosphatidic plays a role include; signaling pathways in cell growth, proliferation, reproduction and responses hormones in biotic and abiotic stress [25]. The result showed that consumption of germinated and non-germinated *Sorghum bicolor* may participate well in these functions, most especially non-germinated *Sorghum bicolor*. The CV% varied from 1.46 in phosphoserine to 6.03 in phosphatidylcholine (Table 3).

Table 4: Phytosterols Composition of (*Sorghum bicolor)* on germinated and non-germinated

Phytosterol	F	G	Mean	SD	CV%	F-G
Cholesterol	1.15	2.14	1.65	0.70	42.42	-0.99
Cholestanol	1.16	1.15	1.16	0.01	0.86	0.01
Ergosterol	2.66	8.87	5.77	4.39	76.08	-6.21
Stig-masterol	3.56	3.71	3.64	0.11	3.02	-0.15
Savenasterol	2.11	1.73	1,92	0.27	14.06	+0.38
Sitosterol	32.23	35.52	33.88	2.33	6.88	-3.29
Campestrol	3.06	3.57	3.32	0.36	10.84	-0.51
Total	45.93	56.69				

SD: Standard deviation; F: Germinated; G: Non-germinated; CV: Coefficient variation.

The result of phytosterols in germinated and non-germinated *Sorghum bicolor* is presented in Table 4. The total phytosterol concentrations (mg/100 g) were 45.93 and 56.69 for the germinated and non-germinated oils, respectively. The most abundant phytosterol in the samples is sitosterol with concentrations (mg/100 g) of 32.23 for the germinated and 35.52 for the non-germinated. This is followed by stigmasterol (3.56 mg/100 g) for the germinated sample and ergosterol (8.87 mg/100 g) for the non-germinated sample. This observation agrees with Moreau *et al.* [27] who reported that the most abundant plant phytosterol are sitosterol, camp sterol and stigmasterol. The non-germinated sample was more concentrated in most of the sterol than the germinated sample with standard deviation (SD) of 0.11 to 4.39. The daily dietary intake of plant sterols differs among populations, but is typically between 160-400 mg and those eating vegetarian diet may eat up to 750 mg/day, which would provide some degree of cholesterol lowering [28]. Therefore, oil extracted from the non-germinated sample will be a very good source of dietary phytosterols. In addition to their cholesterol lowering properties, phytosterols posse's anti-cancer, anti-inflammatory, anti-atherogenicity, and anti-oxidation activities, and should thus be of clinical importance, even for those individuals without elevated LDL cholesterol [29].

Conclusion

The study carried out on the fatty acid, phospholipids and phytosterols composition of germinated and non-germinated *Sorghum bicolor* showed that, the total UFA was higher in both the germinated and non-germinated samples than that of oil rich foods like groundnut, soybean and pinto bean, thereby making the seeds fat good for human health. The quality parameters such as MUFA/SFA, PUFA/SFA and O/L were all observed to be good. Germinated and non-germinated *Sorghum bicolor* are good sources of phospholipids and phytosterols with the non-germinated sample showing to be more nutritive than the germinated sample.

Conflict of Interest

The authors declare that there is no conflict of interest reported in this study.

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