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Sterols of sunflower seeds from different locations in Nigeria

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ABSTRACT

Sunflower (*Helianthus species)* is cultivated for oil production. Analysis of sterol components of sunflower seeds from different locations in Nigeria is presented. Sterols were separated from unsaponifiable fraction by Thin-layer Chromatography and analyzed with gas chromatograph. The result shows the oil and the unsaponifiable oil contents by location, Benue (37.32±0.07;0.82±0.01), Kano(34.57±0.10;0.72±0.01), Bida(34.06±0.09;0.70±0.00), Kaduna (33.92±0.08; 0.68±0.00) and Minna(31.91±0.05;0.64±0.04) in descending order. There was no significant difference in the oil content of the seed samples but significant difference occurs in the percentage of unsaponifiable oil content. Cholesterol (71.50) was predominant in the leaves surface while β-sitosterol (30.40) was the most abundant sterol in the intracellular portion of the leaves. The combined concentration of unknown sterols varied between 9.4 and 12.03 for the free sterol and between 2.84 and 5.81 for the sterol ester. Cholesterol/stigmasterol and cholesterol/ β-sitosterol ratios were found to be agronomically and geographically related. Significant difference occurred in unknown sterol esters/known sterols esters ratio. This study showed that the chemistry of sunflower seeds is not agronomic and geographically dependent and that the stigmasterol, campesterol and β-sitosterol are significant phytosterols in sunflower. Unidentified sterols component is abundant enough to influence the health benefits of the sunflower oil positively or negatively.

Keywords: Sunflower seeds, cholesterol, stigmasterol, β-sitosterol, campesterols

INTRODUCTION

Phytosterols occur naturally in plants and in many processed foods. Many vegetable oils such as olive, soybean and peanut have a high amount of plant sterols. Oil seeds are primarily cultivated to obtain edible oil for consumption (1). No study has established the levels of intake of plant sterol in Nigeria but average dietary intake of plant sterols in western diets are between 200-400mg/day with vegetarians having higher intake than those on mixed diets. Some authors have confirmed the efficacy of phytosterols in lowering blood cholesterol levels (2, 3, 4, 5, 6, 7), membrane fluidity and permeability (8), and embryogenesis (9) but the practical application in many developing countries including Nigeria is largely unknown due to high level of ignorance and poor economic accessibility to phytosterols-based commercial products. Phytosterols differ structurally from cholesterol by a methyl or ethyl group in their side chains and are not synthesized in the human body. Ologunde *et al.* (10) found appreciable levels of

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these sterols in certain food products in Nigeria including *Vernonia galamensis*, *Amaranthus cruentus* A. *caudatus*, *A hybridus* and *A hypochondriacus*.

Sunflower seed (*Helianthus species*) is a green herbaceous weed plant with a wide spread in Nigeria and is used for manufacturing of oil used in the production of margarine and some other oil-based products. There are generally two commercially grown types; the oil seed type and the confectionery or non-oil sunflower. Sunflower has found various applications including improving soil fertility, poultry feeds, enriched margarines (11), paper production, and oil production for human consumption. Sunflower oil is nutritionally advantaged to be so used because it contains sterols and sterol esters which lower the concentration of cholesterol in the body (12, 13, 14). It provides fat-soluble vitamins, mono-unsaturated fatty acids and polyunsaturated fatty acids to promote optimal health, strengthen immune system, growth and good eyesight. Moreover, increased consumption of sunflower can promote food security and economic empowerment. Also, there is increasing interest in phytosterols usage as neutraceuticals and functional foods to inhibit the uptake of cholesterols from the diet. This study evaluated the concentration of total sterol and various sterols components in oil obtained from some hybrid of sunflower seeds cultivated in different states of Nigeria.

MATERIALS AND METHODS

Collection of samples

A quantity of 1.0 kg each of five different samples of the same hybrid of sunflower seeds were collected from Kaduna (NYSC farm), Bida (NYSC farm), Kano (NYSC farm), Zaria (Leventis Foundation farm) and Benue Yandev Agricultural Development Project farm. These farms cut across two agro-ecological zones in Nigeria. Extraction of oil from ground seed sample was by soxhlet method using Petroleum spirit (40-60°C) as solvent. The solvent was recovered from the oil solvent mixture by simple distillation.

Separation of sterols from unsaponifiable fraction was by thin-layer chromatography (15) and sterol fractions were extracted (15) and analyzed with a Perkin Elmer GC-5A gas chromatograph equipped with a flame ionization detector. Detector temperature was 280°C. All analyses were done in quadruplicate. Simple descriptive statistic was used; the results are presented as the mean and standard deviation.

Procedure

Extraction of oil from ground seed sample was by soxhlet method. Petroleum spirit (40-60°C) was used as solvent. A quantity of 100g dry ground seeds was placed in the extractor. Extractor solvent, few crystal of butylated hydroxyl volume (BHT) and anti-gumps were added into a round bottom flank connected to the extractor and condenser. Gentle heating was carried out for five hours. The solvent was recovered from the oil solvent mixture by simple distillation. The oil-solvent mixture left after distillation was oven dried at 105°C for 24hours. The oil was subsequently cooled in desiccators to constant weight and oil content determined and recorded. The extracted oil was suspended in known amount of n-hexane, stored in a deep freezer until needed for use.

Separation of sterols from unsaponifiable fraction

This was carried out by Thin-layer Chromatography (TLC). Plate was divided into two parts. One part was spotted with a concentrated solution of unsaponifiables while the other was spotted with a concentrated mixture of free sterols in chloroform. The plate was transferred to a saturated tank containing a solution mixture of petroleum ether, diethyl ether, and acetic acid (90:10:1, v/v), and a filter paper lines.

The tank was covered and then sealed with tape. Development was stopped when the solvent front reached the stop line located 1.5cm from the top of the plate. The plate was dried in drying tank under the flow of nitrogen. After the plate was dried and the samples were covered with a piece of glass, the ensemble was placed in an iodine tank until the yellow spots from standard on the side of plate became visible. The plate was removed from

Preparation of sterols butyrate

N-hexane was evaporated from a solution of free sterol obtained above. After complete dryness, 6cm³ of freshly prepared butyric anhyride: pyridine (2:1, v/v) was added at room temperature and transferred to a steam bath, and the solutions evaporated to near dryness using a flow of nitrogen. Each semi-dry residue was transferred to 5cm³ volumetric flasks with n-hexane, diluted to volume and stored 10°C. Butyrate of sterol standards were prepared and analyzed along with samples.

Gas liquid chromatography (GLC) of sterols ester

Sterol fractions were analyzed with a Perkin Elmer GC-5A gas chromatograph equipped with a flame ionization detector. The chromatograph was fitted with a 2m glass column, 3mm internal diameter, packed with gas chrom-z, 80-100 mesh, and coated with 1.5% OV-17. The column was operated at 250°C with nitrogen at 50cm³/min as carrier gas. Detector temperature was 280°C. Under these conditions the retention time for â-sitosterol was one minute.

Statistical analysis

All analyses were done in quadruplicate. Simple descriptive statistic was used; the results are presented as the mean and standard deviation.

RESULTS AND DISCUSSION

The relative oil content and the relative percentage of the unsaponifiable components of oil as extracted from the seed samples from various sampled farms are presented in Table 1. The Benue sample had the highest oil content (37.32±0.07%) when compared to Kano sample (34.57±0.10%), Bida sample (34.06±0.09%), Kaduna sample (33.92±0.08%) and Minna sample (31.91±0.05) in descending order of value. The unsaponifiable component of the oil content varies with the collection farm: Benue sample had the highest (0.82±0.01%), followed by Kano sample (0.72±0.01%), Bida sample (0.70±0.00%), Kaduna sample (0.68±0.00%) and Minna sample(0.64±0.04) in descending order. There was no significant difference in the oil content of the seed samples but there was significant difference in the percentage of unsaponifiable fraction of oil. This suggests the retention of certain specie characteristics irrespective of the cultivation environment.

Table 1. oil content, percentage unsaponifiable sterol fraction of oil samples

Sample locations	Oil content	% Unsaponifiable
Kaduna	33.92 ± 0.08	0.68 ± 0.00
Kano	34.57 ± 0.10	0.72 ± 0.01
Minna	31.91 ± 0.05	0.64 ± 0.04
Bida	34.06 ± 0.09	0.70 ± 0.00
Yander Benue	37.32 ± 0.07	0.82 ± 0.01

Values are average of four determinations ± SD

The comparative sterol composition of the seed lipids by free ester and sterol ester are presented in Table 2. The result shows cholesterol as the predominant sterol ester followed by stigmasterol in all samples. The free sterol and sterol ester vary with different locations in this order, cholesterol stigmasterol campesterol β-sitosterol. The unknown sterols were found in all the varieties at relatively low concentrations. The combined concentration of unknown sterols varied between 9.4 and 12.03 for the free sterol and between 2.84 and 5.81 for the sterol ester. The various samples possess similar distribution of the sterols with little differences and low percentage of β-sitosterol in all samples. Benue variety had the lowest content of campesterol (6.16±0.12%).

The comparative values of known and unknown free ester and sterol ester from different locations are shown in Table 3. The relative sterol ester composition, cholesterol to stigmasterol ratio obtained for each of the samples ranged between 5.0 in Bida sample and 6.7 in Minna sample. There were significant differences among the cholesterol to stigmasterol ratio from different collection farms although only a little difference was found between Kaduna sample (5.39) and Kano (5.46) varieties. The cholesterol to stigmasterol values are found to be agronomically and geographically related. No

Table 2. Comparative values of free ester and sterol ester from different locations

Major Sterols	GLC Relative Retention time	TLC Rf	Free Ester	Sterol Ester	Free Ester	Sterol Ester	Free Ester	Sterol Ester	Free Ester	Sterol Ester	Free Ester	Sterol Ester
Cholesterol	1.00	0.48	66.10 ±2.64	74.40 ±1.37	61.64 ±0.96	70.26 ±1.28	61.08 ±0.76	68.68 ±0.28	60.50 ±2.64	71.28 ±1.64	61.60 ±1.29	76.84 ±1.28
Unknown I	1.05	0.48	5.30 ±0.37	1.60 ±0.02	4.80 ±0.13	0.50 ±0.00	5.28 ±0.42	1.29 ±0.00	4.80 ±0.72	1.52 ±0.01	5.21 ±0.01	0.52 ±0.00
Unknown II	1.07	0.48	2.00 ±0.31	2.70 ±0.16	2.48 ±0.00	2.22 ±0.10	3.49 ±0.10	4.22 ±0.14	3.24 ±0.05	3.16 ±0.34	2.56 ±0.01	2.46 ±0.04
Campesterol	1.18	0.48	7.70 ±0.02	6.60 ±0.82	10.27 ±0.26	6.52 ±0.21	10.28 ±0.18	7.28 ±0.11	10.46 ±0.47	7.68 ±1.28	10.07 ±0.78	6.16 ±0.12
Unknown III	1.21	0.48	2.10 ±0.10	0.20 ±0.01	3.68 ±0.09	0.12 ±0.00	3.26 ±0.22	0.30 ±0.18	2.16 ±0.22	0.20 ±0.00	1.90 ±0.12	0.22 ±0.00
Stigmasterol	1.24	0.48	8.10 ±0.28	13.80 ±0.43	8.40 ±0.18	12.86 ±0.62	8.72 ±0.13	10.26 ±0.22	9.20 ±0.18	14.26 ±1.26	9.56 ±0.92	13.00 ±0.01
β-Sitosterol	1.36	0.48	8.70 ±0.36	0.70 ±0.00	9.13 ±0.16	7.52 ±0.21	7.89 ±0.14	7.97 ±0.12	9.70 ±0.42	1.90 ±0.06	9.10 ±0.80	0.80 ±0.00

Values are average of four determinations ± SD

Table 3. Comparative values of known and unknown free ester and sterol ester from different locations

Parameters	Kaduna		Kano		Minna		Bida		Benue	
	Free Ester	Sterol Ester	Free Ester	Sterol Ester	Free Ester	Sterol Ester	Free Ester	Sterol Ester	Free Ester	Sterol Ester
Unknown Total	9.4	4.5	10.96	2.84	12.03	5.81	10.2	4.88	9.67	3.2
Known Total	90.60	95.5	89.44	97.16	87.97	94.19	89.86	95.12	90.33	96.8
Cholesterol/β-Sitosterol ratio	7.60	106.29	6.75	9.34	7.74	8.62	6.24	37.52	6.77	96.05
Cholesterol/Stigmasterol		5.39		5.46		6.7		5.0		5.90
Known/Unknown ratio	9.64	21.22	8.16	34.21	7.31	16.21	8.81	19.49	9.34	30.25

correlation was found between Minna and Bida varieties despite agroecological and geographical proximities of the two locations.

There was no significant difference among the free cholesterol to β-sitosterol ratio from the various collection farms but significant difference existed in the cholesterol to â-sitosterol ratio. The cholesterol/β-sitosterol ratio was found to be agronomically and geographically related; Kaduna sample had cholesterol/β-sitosterol ratio of 106.29, followed by Benue sample (96.05) and Bida sample (37.52) while Kano and Minna sample had 9.34 and 8.62 respectively.

Similarly there was a significant difference in the relative composition of the total unknown sterol components of the samples from different collection farms but the total known sterol components were not significantly different. Statistical analysis showed a significant difference in the proportion of unknown sterol esters to known sterols esters but no significant difference between unknown free esters components in various samples.

The sterol composition of leaf surface lipids and the intracellular portion of the leaves from Kaduna location is presented in Table 4. The trend obtained in both the surface and intracellular parts of the leaves do not resemble that of the seeds. The cholesterol (71.50) is the predominant sterol in the leaves surface with its value closely followed by stigmasterol (9.30) and campesterol (8.70). Conversely, β-sitosterol (30.40) was found to be the most abundant sterol in the intracellular portion of the leaves followed by stigmasterol (19.20).

Table 4. Distribution of lipids in Kaduna sample leaves and seeds

Sterol	RRT*	Leaves	
		Surface	Intracellular
Cholesterol	1.00	71.50	14.90
Unknown I	1.05	5.90	7.70
Unknown II	1.07	4.00	12.00
Campesterol	1.18	8.70	10.10
Unknown III	1.21	Trace	3.90
Stigmasterol	1.24	9.30	19.20
β-Sitosterol	1.36	0.60	30.40
Cholesterol/ Stigmasterol		7.69	0.78

* Relative Retention Time. Values are average of four determinations ± SD

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Cholesterol	1.00	0.48	66.10 ±2.64	74.40 ±1.37	61.64 ±0.96	70.26 ±1.28	61.08 ±0.76	68.68 ±0.28	60.50 ±2.64	71.28 ±1.64	61.60 ±1.29	76.84 ±1.28
Unknown I	1.05	0.48	5.30 ±0.37	1.60 ±0.02	4.80 ±0.13	0.50 ±0.00	5.28 ±0.42	1.29 ±0.00	4.80 ±0.72	1.52 ±0.01	5.21 ±0.01	0.52 ±0.00
Unknown II	1.07	0.48	2.00 ±0.31	2.70 ±0.16	2.48 ±0.00	2.22 ±0.10	3.49 ±0.10	4.22 ±0.14	3.24 ±0.03	3.16 ±0.34	2.56 ±0.01	2.46 ±0.04
Campesterol	1.18	0.48	7.70 ±0.02	6.60 ±0.82	10.27 ±0.26	6.52 ±0.21	10.28 ±0.18	7.28 ±0.11	10.46 ±0.47	7.68 ±1.28	10.07 ±0.78	6.16 ±0.12
Unknown III	1.21	0.48	2.10 ±0.10	0.20 ±0.01	3.68 ±0.09	0.12 ±0.00	3.26 ±0.22	0.30 ±0.18	2.16 ±0.22	0.20 ±0.00	1.90 ±0.12	0.22 ±0.00
Stigmasterol	1.24	0.48	8.10 ±0.28	13.80 ±0.43	8.40 ±0.18	12.86 ±0.62	8.72 ±0.13	10.26 ±0.22	9.20 ±0.18	14.26 ±1.26	9.56 ±0.92	13.00 ±0.01
β-Sitosterol	1.36	0.48	8.70 ±0.36	0.70 ±0.00	9.13 ±0.16	7.52 ±0.21	7.89 ±0.14	7.97 ±0.12	9.70 ±0.42	1.90 ±0.06	9.10 ±0.80	0.80 ±0.00

Values are average of four determinations ± SD

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Unknown Total	9.4	4.5	10.96	2.84	12.03	5.81	10.2	4.88	9.67	3.2
Known Total	90.60	95.5	89.44	97.16	87.97	94.19	89.86	95.12	90.33	96.8
Cholesterol/β-Sitosterol ratio	7.60	106.29	6.75	9.34	7.74	8.62	6.24	37.52	6.77	96.05
Cholesterol/Stigmasterol		5.39		5.46		6.7		5.0		5.90
Known/Unknown ratio	9.64	21.22	8.16	34.21	7.31	16.21	8.81	19.49	9.34	30.25

correlation was found between Minna and Bida varieties despite agroecological and geographical proximities of the two locations.

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Stigmasterol	1.24	9.30	19.20
β-Sitosterol	1.36	0.60	30.40
Cholesterol/ Stigmasterol		7.69	0.78

* Relative Retention Time. Values are average of four determinations ± SD

CONCLUSION

This study showed that the chemistry of sunflower seeds is not linked to agronomic and geographical factors and the oil content of is not agronomically or geographically dependent. It further shows that that cholesterol is a significant component of sterols in sunflower seeds thus negating the assumption that cholesterol content in several plants is insignificant and shows. The proportion of cholesterol was lower in the leaf intracellular lipids than in the surface lipids.

Phytosterols found in appreciable quantity are stigmasterol, campesterol and β-sitosterol in order of abundance. Unidentified sterols component is abundant enough to influence the health benefits of the sunflower oil positively or negatively. This study therefore identifies sunflower oil as a good source of phytosterols of great health benefits in human nutrition. Further study is however necessary to identify the unidentified sterol components of the sunflower oil and the influence of processing on the various sterol components.

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