Research Article

Isolation and Characterization of Oil from Different Parts of Fluted Pumpkin Seeds

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ABSTRACT

Isolation and characterization of oil from different parts of fluted pumpkin seeds were studied using different extractions method, column and gas-liquid chromatography methods for the characterization of the oil obtained. The results showed that the oil from the seed was 29.5 - 37±0.3 and seed coat 0.5- 25.0±0.1. The oil consists of phospholipids 40-58%, glycolipids 26-36%, free fatty acids 1-6% free sterols 1-2% neutral lipids 10-24% and sterylesters 0.80-4%. The fatty acid profiles showed more than 60% unsaturated fatty acids comprising soley (18 fatty acids. The seed coat oil has very significant degree of saturated fatty acids accounting for more than 70% mainly C16 and C18 fatty acids. The oil generally contains trace amount of (18:3 fatty acids).

Keywords: fluted pumpkin seeds, fatty acid profiles, oil extracts, isolation, characterization.

INTRODUCTION

Fluted pumpkin (*Telferia occidentalis hook*) is a dioceious perennial vine plant of belongs to the family of Cucurbitaea and a specie of(*Telferia occidentalis hook*) (Asoegwu 1988). It is a vegetable crop that grow mostly in the tropics of west Africa countries of Ghana, Chad, Togo, Cameroun and Nigeria it is a drought tolerant crop but grow best at lower attitude under medium rainfall in a sandy loam soil (Green soil 1968). The yield of fluted pupkin crops are significantly enhanced in a well drained soil. For commercial production, the use of fertilizers and irrigation may be used for the productive life span. The leaves and roots of fluted pumpkin are used as herbal medicine (Asiegbu 1987, Longe *et al 1983).* A fluted pumpkin seed contains 47% oil, 31% crude protein, significant amount of vitamins minerals and carbohydrates (Asiegbu 1987, Asoegwu 1988). The isolation and characterization the oil from different parts of the fluted pumpkin in seeds is the subject matter of the work.

MATERIALS AND METHODS

Sample collection

Fluted pumpkin seed pods were produced from the University of Nigeria Nsukka, demonstration from Enugu state. The seed pods were indentified and authenticated by Dr. E. U, Onugbo, of Crop science department of university of Nigeria Nsukka. The voucher species of the seeds pods deposited at the Herbarium of Michael Okpara University of Agriculture, Umudike. All chemicals reagents used in this work are analytical grade reagents supplied and used neat by May and beaker chemicals Ltd. London.

Sample Preparation

The procured seed pods were manually harvested and the seeds removed by separating them from their pods, washed clean. Each seed was thereafter separated from the seed using domestic knifes. The separated seeds and seed coats each pooled together to form composite seeds and composite seed coats respectively. The seeds are the endocarps while the seed coats are epicarps. The two different parts of the seeds are grouped into two portions the wet and dry one extraction respectively. Each portions of the seeds and seed coats for dry oil extraction were each air- dried for 72 hrs by spreading them in thin-film layers on a wooden slab in the laboratory for complete drying. Both the dry and seeds coats were reduced into small sizes and then milled into powder using a ball and hammer millers. Each coarse powder further reduced into fine powder using electric blender (biatone-model). Each powder materials, then sieved into ultra- fine powder of seeds and seed coat (dry and wet) were transferred into clean labeled plastic containers until required for analysis.

Sox let Extraction

Fine powder of seed and seed coats (wet and dry), 40g each was weighed out into labeled sox let apparatus, and extraction carried out using 100ml each of petroleum ether for 21/2 hr. after each extraction duration of 21/2 hr. each extract was concentrated to remove the

petroleum ether solvent using a rotary evaporator at 45^0c using a hot air circulating oven to obtain a concentrated oil extract from bath seed and seed coats (wet and dry) respectively. Each oil extract was weighed and the weight of each oil extract was recorded.

Folch *et al* Extraction Method

Ten grams each of the seed and seed coats fine powder (wet and dry) was each weighed out and transferred into each labeled conical flask, each containing 30ml of chloroform-methanol mixture (2:1) volume by volume. Each conical flask and its content(s) homogenized for five minutes each and then filtered using no. 40 filters paper. Each oil extracts concentrated as in the soxhet extraction procedure described above. Each residues of seed and seed coats (wet and dry) was re-homogenised using 30ml petroleum ether and 30ml methanol singly. The oil extracts of each sample was added to already extracts oil from each sample and then used for the chemical characterization carried out. Each oil sample was dried using anhydrous sodium carbonate before characterizing them.

Identification of components of the oil extracts: each oil sample extracts was analyzed using a thin-layer chromatographic method to identify each components of the oil, using different solvent mixtures, and each spot developed using iodine vapour in a chromatographic tank.

Different Solvent Mixture Used

For neutral oil components; petroleum ether: glacial acetic acid (90:10:1) were used, for polar components, Chloroform; methanol,; glacial acetic acid ; water (80:20:1) were used. Various Rf valves were calculated after each thin layer chromatographic assay from the developed spots and material matched with standard RF values of Known oil components.

Column Chromatographic Analysis

column chromatographic analysis of each oil extract were carried out and eluted with specific eluting solvent systems to separate the oil extracts into various lipids classes. Solvent systems used include 30ml each of 2.5: 50 ether in R- hexane 30ml of each of 5:10:30, methanol in chloroform and 30ml each of pure methanol each eluted fraction namely neutral lipids, glycolipids and phospholipids respectively were qualified using UV-visible spectrophotometer at specific wavelength of absorption. Neutral lipids absorbs at 570mm where chromotrophic acid reagents was used to develop colour. The phospholipids absorbs at 600mm where trichloride reagents was added to devlope colour and the glycolipids absorbs at 560mm when ferric chloroacetic acid reagent was added to develop colour, (Christie 1982, C. Offery *et al*., 1973, Zak 1957)

Gas liquid chromatographic analysis: the fatty acid profiles of each oil sample extracts were carried out using GLC (model Beckman), with helium gas as carrier gas at a flow rate of soul per minute, and column temperature of 180^0c each peak areas arising from the chromatograms were measured using an electronic digital integrator. The confirmation of each fatty acid methyl ether was made by comparison with known fatty acid methyl ester standards. The concentration of each fatty acid. The sample seeds and seed coats (wet and dry) were calculated from each peak area of chromatogram by triangulation and expressed as percentage weight of the total fatty acid methyl ether (Odoemena *et al*., 1988)

RESULTS AND DISCUSSION

The results of the isolation and characterizations of oil extracts from different parts of fluted pumpkin seeds are presented the table (1-5) below.

From Table it was observed that the seeds contain more moisture than the seed coats. This is significant and enables the seed to be viable for use replanting seeds. Table 2 contains the various percent yield of oil from different parts of the seeds. The wet seeds contain most oil yield (39.50%) while the seed coats dried yield the least oil (0.50%). The soxhet method of extraction yield more oil than Folch *et al.,* method of extraction. From table 3, the various oil components were identified from the different parts of the seeds. These include monoglycerides, diglycerides, cholesterols, and glycerosterates, among many other components (table 3). From the identified lipids from the oil extracts, the oil many rich source of biofuels and cosmetic oils

From table 4, the detailed percent yield of the various components of the oil extracts from different parts of the seeds were presented. It was observed that soxhet method of extraction and seeds yield more oil than the Folch *et al* method of extraction and seed coats

The phospholipids components are dominant components of the oil from both seeds (55%) and seed coats (45%) respectively). The rich phospholipids are a source of Lecithin. This bioactive substance when hydrolysed by snake venom enzymes yields lysolecithin and lyso cephalin which are used in both herbal and orthodox medicine a haemolycytic – actions including substances in both seeds and seed coats of fluted pumpkins are

potential source of raw materials for food and pharmaceutical industries. For table 5, the detailed fatty acids profiles were presented. It is observed that the seeds and the seed coats contain significant degree of unsaturation (67%). This makes the oil, good oil for patient with heart diseases. The oil from different part of fluted pumpkin seeds contain essential oil component like cerebrosides, cephalin and lecithin. These oil components may be the scientific basis for the use of fluted pumpkin seed as herbal medicine.

Therefore, the fluted pumpkin seed oils from both seeds and seed coats should exploited as raw materials for oil for use in cosmetics, pharmaceutical and other industrial where unsaturated oils are needed.

Table1: Moisture content of fluted pumpkin seeds

Part of seeds	Moisture contents obtained	% Moisture contents
Whole seed (seeds to seed coats)	17.70 ± 0.1	69.60
Seeds	11.10 ± 0.12	29.50
Seed coats	7.30 ± 0.30	12.50

Values are means of four determinations ± standard deviations

Table 2: Oil content of different parts of fluted pumpkinseeds

Part of seeds	Condition of the seed	Method of extraction used	% yield
Seeds	Fresh	soxhlet	39.50 ±0.10
seeds	dried	Soxhlet	29.00 ± 0.20
Seed coats	fresh	Soxhlet	12.50 ±0.20
Seed coats	Dried	soxhlet	0.50 ± 0.10
Seed	fresh	tolch et al	37.00 ± 0.20
Seed	dried	tolch et al	30.50 ±0.10
Seed coats	fresh	tolch et al	25.02 ±0.10
Seed coats	dried	tolch et al	7.50 ± 0.10

Values are means of four determinations ± standard deviations

Table 3: Oil extracts from different parts of fluted pumpkin seeds

Part of seeds	Condition of the seed	Eluating solvent system used	Rf value obtained	Component of the oil identification
Seed	Dry	Petroleum ether: diethyether glacial actic acid	0.14 , 0.24 ,0.29,0.45,0.85	0.14 (monogly cerides),0.24(diglycarides)0.29 (cholester ol), 0.45(gly caroltistearate)
Seed	Wet	Petroleum ether: diethyether glacial actic acid	and 0.97	0.97 (cholester ol stearate),
Seed coat	Dry	Petroleum ether: diethyether glacial actic acid	0.24,0.29,0.45,0.85, and 0.97	The same as above
Seed coat	Wet	Petroleum ether: diethyether glacial actic acid	0.24,0.29,0.45,0.85 and 0.79	0.62 (phosphatidy in sitol)
Seed coat	Dry	Chloroform : m ethanol	0.62	0.62 (phosphatidy in ositol),0.23(sterol)
Seed coat	Wet	Chloroform : m ethanol	0.62	0.62 (phosphatidy in ositol),0.23(sterol)
Seed	Dry	n- heptane: diethyl ether glacial actic acid	0.62 0.23	0.62 (phosphatidy in ositol),0.23(sterol)
Seed	Wet	n- heptane: diethyl ether glacial actic acid	0.00 0.23	0.00 (phosphatidy in ositol),0.23(sterol)
Seed coat	Dry	n- heptane: diethyl ether glacial actic acid	0.00 0.23	Same as above
Seed coat	wet	n- heptane: diethyl ether glacial actic acid	0.00 0.23	Same as above

Values are means of four determinations

Table 4: Oil extracts from different parts of fluted pumpkin seeds using gas liquid chromatographic method

Components	Percentage of components by total oil extracts							
	Dry seed	Wet seed	Dry coat method	Seed coat (wet)	Dry seed modules	Wet seed	Seed coat (dry) et al	Seed coat (wet)method of extraction
	Soxhlet extraction							
Phospholipids	55	50	45	40	58	48	42	40
Lysophotidy/cholnic	25	35	25	20	30	25	20	20
Photidy/cholnic	20	10	15	10	12	18	12	10
Photidy/inositol	10	5	5	10	8	5	12	10
Glycolipids	35	30	35	36	26	32	34	36
monogalactosylai glycaride	15	10	15	16	8	14	16	16
digalaltosylar glycoside	10	10	10	6	4	10	10	10
sterylglycoside	8	8	8	4	2	5	6	6
unidontified	2	2	2	24	16	3	2	4
Neutrol lipids	10	20	20	24	16	20	24	24
Trigly caride	4	4	4	2	6	4	2	2
diglycaride	2	4	4	2	2	4	2	2
monogly coride	0.8	4.5	2.8	8	0.8	4.5	8	8
steryl estems	0.8	1.0	2.8	4	1.2	1.0	4	4
free stirols	1.4	1.0	1.4	2	1.0	1.0	2	2
frave fatty acids	1.0	5.5	4.0	6	5	5.5	6	6

Values are means of four determinations

Table 5: Fatty acid profile of oil extracts from different parts of fluted pumpkin seeds

Parts of seeds	Condition of seeds	Methods of extraction used	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	% Unsaturation
Seed	Dry	Soxhlet methods	Trace	14.30	18.00	33.00	33.00	Trace	67.14
Seed	Wet	"	"	12.00	20.00	33.00	33.00	Trace	67.34
Seed coat	Dry	"	8.00	30.00	32.00	15.00	10.00	Trace	26.32
Seed coat	Wet	"	Trace	30.00	30.00	15.00	10.00	Trace	26.32
Seed	Dry	Modified to/ch et al	10.00	16.00	20.00	30.00	30.00	Trace	62.50
Seed	Wet	"	"	20.00	20.00	35.00	20.00	Trace	57.89
Seed coat	Dry	"	12.00	35	33.00	10.00	8.00	Trace	18.37
Seed coat	wet	"	18.00	30.00	27.00	12.00	10.00	Trace	22.68

Values are means of four determinations

REFERENCES

1. Asiegbel JC. Some biochemical evalueation of fluted pumpkin seeds. J Sci Food Agric. 1987; 5:231-235.
2. Asoegwu SS. effect of irrigation on the leaf and pod production of fluted pumpkin (Telferia Occidentelis) in south eastern Nigeria scientia Horticulture. 1988;34:161-168.
3. Christe WW. Lipid analysis, isolation, separation, identification and structural analusis of lipids. 2nd Ed. Pergamon Press. Oxford London, 1982;61- 62.
4. Gofrerried SP and Rosenberg B. Improved manual of spectrophotometric procedures for the determination of serum triglyceride. Clinical chemistry. 1973;19:1077–1080.
5. Grensil TW. A guide for gardeners, green vegetables. Evans Brothers Publishers Ltd., London. 1982;78-80.
6. Henly J and Zak B. Clinical Biochemistry. Heimann Publishers, London. 1977;367-376.
7. Kimball JW. Biolosyn 4th Feb. Third World Students Series, North Caroline. U.S.A. 1957;43-46.
8. Longe OG, Fermince OG and Fetuga BI. Nutritional Values of Fluted

Pumpkin (Telferia Occidentalis). J Agric Food Chem. 1983;31:989–994.
9. Ngoddy PO and Ihekeronye AI. Integrated Food Science and Technology for the Tropics. Macmillian Education Ltd. London, 1985;60-68.
10. Odoemena CS and Oneyeneke EC. Lipids proce. African Conf. Biochem. Nsukka. 1988; 80-93.
11. Zak B. Simple rapid Microtechnique for serum total cholesterol. Am J Med. 1957;27:58-589.