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Analytical Methods Section

Determination of the triglyceride, phospholipid and unsaponifiable fractions of yellow nutsedge tuber oil

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Lipid classes, the fatty-acid distribution in triglycerides and the composition of the triglyceride, phospholipid and unsaponifiable fractions of the tuber oil of yellow nutsedge (*Cyperus esculentus*) were studied. Oleic acid was the dominant fatty acid in the whole tuber oil, the triglycerides and 2-monoglycerides obtained from pancreatic lipase treatment and the phospholipids. Phospholipids in the tuber oil accounted for 5·4 ± 0·5% of the total, with a phosphorus content of 0·004%. Ethanolamine glycerophospholipid (22·0 ± 1·1%), serine glycerophospholipid (1·7 ± 0·1%), choline glycerophospholipid (35·0 ± 2·3%) and inositol glycerophospholipid (26·0 ± 1·4%) were detected and quantified. Hydrocarbons (20·57 ± 3·3%), waxes (2·01 ± 0·4%), sterol esters (3·89 ± 0·3%), higher aliphatic alcohols (3·51 ± 0·2%), triterpenoid alcohols (1·88 ± 0·3%) and sterols (14·56 ± 1·1%) were the constituents of the unsaponifiable fraction. Quantifications of the sterols and alcohols of the unsaponifiable fraction were carried out; β-sitosterol was found to be the major sterol, while cycloartenol was the major terpene alcohol.

INTRODUCTION

Properties of yellow nutsedge (*Cyperus esculentus*) tuber oil grown in Nigeria have been highlighted earlier (Oderinde & Tairu, 1988). However, studies on the lipid classes and the distribution and composition of triglyceride, phospholipid and unsaponifiable fractions of the tuber oil have been lacking. Information from these analyses could serve as a means of proper chemical and taxonomic classification of the tuber within the Cyperaceae family, as well as forming a basis for industrial utilisation of the tuber oil.

In the continued effort to bring into focus the many available lesser-known agricultural/industrial raw materials of considerable potential (Oderinde & Tairu, 1989; Oderinde *et al.*, 1989, 1990*a*, *b*), the tuber oil of yellow nutsedge has been examined for its triglycerides and other lipid classes, including phospholipid and unsaponifiable fractions.

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MATERIALS AND METHODS

Extraction of tuber oil

Fresh samples of yellow nutsedge tubers obtained from the greenhouse of the Botany Department, University of Ibadan, were broken up into large pieces, pulverised and exhaustively extracted with chloroform/methanol (2:1, v/v).

Analysis of triglycerides

Lipids of yellow nutsedge tuber oil were separated by preparative thin-layer chromatography (TLC) (Sanders, 1980) and identified, using authentic samples, after spraying with specific reagents (Beiss, 1964; Dittmer & Lester, 1964). The composition of fatty acids at the 2-position of the triglycerides was determined by lipolysis with pancreatic lipase according to Tan *et al.* (1981), while hydrolysis products were separated using the procedure of Luddy *et al.* (1964). Whole oil, triglycerides and 2-monoglycerides were converted to their corresponding methyl esters by the acid-catalysed methanolysis of

Urakami *et al.* (1976), with modifications, and subsequent analysis of the esters on a Pye-Unicam Series 304 gas chromatograph with a glass capillary column and equipped with a flame ionisation detector. The triglyceride fraction was calculated from the fatty acids (mol%) of the original triglycerides and corresponding 2-monoglycerides (Van der Wal, 1960; Coleman, 1961).

Determination of phospholipids

Phospholipids were separated using a combination of column chromatography and TLC. Separated phospholipid was determined (as dioleyl lecithin) colorimetrically (Stewart, 1980). Further separation of the phospholipid was achieved by a combination of column chromatography and two-dimensional TLC using various solvent systems (Olcott *et al.*, 1964; Burkhardt, 1970; Rouser *et al.*, 1970). Isolated phospholipids were converted to their corresponding methyl esters by base-catalysed transmethylation (Luddy *et al.*, 1970), before being injected into the gas chromatograph for analysis of the fatty-acid compositions.

Determination of unsaponifiable fraction

The unsaponifiable fraction was extracted by ether from a solution of the oil in 2 M methanolic potassium hydroxide. Column chromatography was used to elute various constituents (Capella *et al.*, 1960), which were spotted individually on TLC to ascertain their purity; their infrared spectra were recorded by a Perkin Elmer double-beam spectrophotometer. Sterols were further separated by a TLC technique (Gaydou *et al.*, 1983), in which rhodamine-B identified the sterol fraction when the unsaponifiable mixture was run in a solvent mixture of ether and chloroform (10:90, v/v). Sterol bands were scraped off, eluted with carbon disulphide and injected into a Pye-Unicam Series 304 chromatograph using appropriate column materials and conditions. Relative retention times (RRT) were expressed against cholesterol–TMS. Terpene alcohols were also isolated on TLC and analysed by GLC; relative retention times (RRT) obtained were expressed against cycloartenol.

Table 1. Lipid classes of yellow nutsedge tuber oil

Class	Composition (%)
Hydrocarbons/sterol-esters	1·36 ± 0·01
Triglycerides	87·14 ± 0·30
Free fatty acids	4·29 ± 0·11
Sterols	0·81 ± 0·09
Phospholipids	5·60 ± 0·13
Triterpene alcohols	0·80 ± 0·06

RESULTS AND DISCUSSION

Tables 1 and 2 show the lipids and fatty-acid compositions, as well as the fatty-acid composition of triglycerides and 2-monoglycerides derived from them by lipolysis. Triglycerides were the most dominant lipid species (87·14%) of the whole oil; phospholipid constituted about 6%. Fatty acids in the triglycerides were similar to those in the whole oil. However, palmitic and oleic acids decreased by 3·0 and 0·5 mol%, respectively, while linoleic acid increased by 1·7 mol%. Oleic acid was the major fatty acid in the derived 2-monoglycerides; its relative amount in this position was higher than that observed in the whole oil or the triglycerides. In contrast, the relative amounts of other fatty acids in the 2-position were lower than those found in the triglycerides, except for linolenic acid, which increased by 2·10 mol% relative to the triglycerides and 2·50 mol% relative to the whole oil.

Under strict random distribution, the experimentally determined proportions of fatty acids at the 2-position, or any other position, in the triglycerides would be 33⅓ mol%. None of the fatty acids at the 2-position of yellow nutsedge triglycerides satisfied this condition. Thus, they do not follow a random distribution. The experimentally determined proportion of oleic acid (38 mol%) at the 2-position was higher than the 33⅓

Table 2. Fatty-acid compositions of yellow nutsedge tuber oil and its derived tryglycerides and 2-monoglycerides

	Fatty-acid composition					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Whole tuber oil (mol%)	12·50 ± 0·14	3·10 ± 0·06	77·20 ± 2·20	9·80 ± 0·17	0·20 ± 0·07	0·30 ± 0·03
Triglycerides (mol%)	9·40 ± 0·11	1·50 ± 0·01	76·70 ± 2·50	11·50 ± 0·10	0·60 ± 0·02	0·30 ± 0·03
2-Monoglyceride (mol%)[a]	1·30 ± 0·03	0·60 ± 0·01	86·90 ± 4·10	8·40 ± 0·10	2·70 ± 0·04	0·10 ± 0·06
Proportion in 2-position (%)[b]	4·60	13·30	37·80	24·30	51·0	11·10
Proportion in 1, 3-position (%)[c]	95·40	86·70	62·20	75·70	49·0	88·90

[a] Derived from triglycerides by pancreatin lipolysis of triglycerides.

[b] Proportion in 2-position of triglycerides = $\frac{\text{mol\% fatty acid in 2-position} \times 100}{\text{mol\% fatty acid in triglyceride} \times 3}$.

[c] Proportion in 1, 3-position of triglycerides = 100 minus proportion in 2-position.

Table 3. Phospholipid classes of yellow nutsedge tuber oil

Class	Composition (%)
Ethanolamine glycerophospholipid	22·0 ± 1·1
Inositol glycerophospholipid	16·0 ± 1·4
Serine glycerophospholipid	1·7 ± 0·4
Choline glycerophospholipid	35·0 ± 2·3

mol% predicted by random theory, indicating a specificity of oleic acid for the 2-position of yellow nutsedge tuber triglycerides; linolenic acid also showed specificity for the 2-position. Other fatty acids exhibited specificity for the combined 1,3-positions; thus, the yellow nutsedge tuber oil was characterised by moderately low 16:0 and 18:2 and low 18:0, 18:3 and 20:0 fatty acids.

The glyceride types of yellow nutsedge tuber oil calculated (Van der Wal, 1960) consists of GS_3 (0·05 mol%); GS_2U (3·0 mol%); GSU_2 (27·5 mol%); and GU_3 (69·5 mol%). Prominent glycerides are triolein (44·7 mol%), dioleolinolein (21·4 mol%), monosaturated diolein (20·9 mol%), saturated oleolinolein (6·4 mol%), oleodilinolein (3·4 mol%) and monosaturated dilinolein (0·5 mol%). Results obtained were in agreement with the hypothesis of Gunstone (1962) that the 2-position of a triglyceride is preferentially esterified by C_{18}-unsaturated acids. Oleic acid, in this case, showed a preference over linoleic and linolenic acids for the 2-position; however, it was observed that linolenic acid showed, to a lesser extent, specificity for the 2-position.

Considerable difference was noticed between the fatty-acid compositions and distribution in the triglycerides and those found in the individual fractions of phospholipids. Whereas arachidonic acid was not found in the neutral oil fraction of the yellow nutsedge tuber oil (Oderinde & Tairu, 1988), trace amounts of the acid occurred in the phospholipid fractions. Separated phospholipids, which include ethanolamine glycerophospholipid, contained 0·81 ± 0·01% of the $C_{20:1}$ (Tables 3 and 4), while inositol glycerophospholipid had 0·43 ± 0·03%; serine glycerophospholipid and choline glycerophospholipid had 0·66 ± 0·10% and 0·09 ± 0·01%, respectively. Prominent fatty acids in the phospholipid fractions were oleic and linoleic acids, and their amounts ranged from 45 to 56% and 30 to 38%, respectively. No significant difference was observed in the composition of palmitic, stearic, linolenic and arachidonic acids for the phospholipid fractions when compared with the whole oil's fatty-acid contents. The major phospholipids separated are shown in Table 3; the figures are in general agreement with previous data obtained for other vegetable oil phospholipid contents.

Table 5. Constituents of the unsaponifiable fraction of yellow nutsedge tuber oil

Constituent	Composition (%)
Hydrocarbons	20·57 ± 3·30
Waxes	2·01 ± 0·40
Sterol esters	3·89 ± 0·30
Higher aliphatic alcohols	3·51 ± 0·20
Triterpene alcohols	1·88 ± 0·30
Sterols	14·50 ± 1·10

Table 5 lists the various constituents of the unsaponifiable fraction separated through a combination of column chromatography and TLC techniques; the amount quoted is based on the isolated unsaponifiable fraction. Infrared spectra revealed only CH_2 and CH_3 groups for the hydrocarbons. Esterified acids in the sterol esters are being further investigated. Sterols were further investigated after purification, using an OV17 glass capillary column. RRTs were expressed against cholesterol–TMS, and the composition of the sterol fraction is given in Table 6. Tentatively, by comparison of RRTs with standard sterol mixtures, four sterols have been identified. The most important of these sterols is β-sitosterol, followed by campesterol. Stigmasterol and cholesterol were detected in minute amounts in the sample. The sterol composition of yellow nutsedge tuber oil is quite similar to that found in most vegetable oils, especially olive oil (Fedeli & Jacini, 1971). TLC separation of triterpene alcohols and subsequent analysis on gas chromatography gave seven peaks, two of which were not identified (Table 7). Of the five identified peaks, cycloartenol was the highest component, followed by 24-methylcycloartenol.

Tuber characteristics (personal observation) and tuber-oil characteristics indicate that the yellow nutsedge tuber has potential as a commercial source of high-oleic-acid vegetable oil and a high-carbohydrate tuber cake. The tuber oil could be exploited in much the same way as olive oil, i.e. for high-quality salad oil and

Table 4. Fatty-acid compositions of the phospholipids of yellow nutsedge tuber oil

Class	Fatty-acid composition (%)							
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{20:4}$
Ethanolamine glycerophospholipid	9·13 ± 0·11	2·20 ± 0·10	53·40 ± 1·30	32·70 ± 3·10	1·70 ± 0·10	0·03	0·81 ± 0·01	—
Inositol glycerophospholipid	11·30 ± 0·10	5·80 ± 0·30	46·41 ± 4·16	35·00 ± 1·41	1·06 ± 0·01	trace	0·43 ± 0·03	trace
Serine glycerophospholipid	10·04 ± 0·10	3·50 ± 0·13	47·40 ± 1·30	38·10 ± 2·21	0·30 ± 0·01	trace	0·66 ± 0·10	trace
Choline glycerophospholipid	7·50 ± 0·20	5·20 ± 0·10	56·00 ± 4·30	30·10 ± 1·10	1·10 ± 0·03	trace	0·09 ± 0·01	—

Table 6. Sterol composition of yellow nutsedge tuber oil

Sterol	RRT	Composition (%)
Cholesterol	1·00	0·10 ± 0·01
β-Sitosterol	1·67	88·70 ± 6·20
Stigmasterol	1·43	1·02 ± 0·04
Campesterol	1·34	10·20 ± 1·20

as a source of medium-chain fatty acids for oleochemicals. The sweet tuber has been used in fermentation products (Oderinde & Esuoso, 1989); further work in this area could enhance the industrial use of the tuber. Improvement of the use of the tuber oil is envisaged through the analytical data thus provided on the glyceride, phospholipid and unsaponifiable fractions. Further improvements of tuber yield and characteristics after agronomic studies, coupled with the exploitation of its tuber carbohydrate resource, could serve to enhance the economic position of yellow nutsedge.

Table 7. Terpene-alcohol composition of yellow nutsedge tuber oil

Terpene alcohol	RRT	Composition (%)
β-Amyrin	0·80	0·30 ± 0·03
Butyrospermol	0·90	0·10 ± 0·01
α-Amyrin	0·93	1·90 ± 0·10
Unidentified peak	0·94	1·20 ± 0·11
Cycloartenol	1·00	72·30 ± 3·20
24-Methylcycloartenol	1·18	24·10 ± 2·20
Unidentified peak	1·32	0·03

REFERENCES

Beiss, U. (1964). Paper chromatographic separation of plant lipids. *J. Chromatogr.*, **13**, 104–10.

Burkhardt, H. J. (1970). The phosphatides of safflower seeds involved in colour formation occurring in extracted and heated crude oils. *J. Am. Oil Chem. Soc.*, **47**, 69–72.

Capella, P., Zotti, G. de, Ricca, G. S., Valentini, A. F. & Jacini, G. (1960). Chromatography on silicic acid of the unsaponifiable matter of fats. *J. Am. Oil Chem. Soc.*, **37**, 564–7.

Coleman, M. H. (1961). Further studies on the pancreatic hydrolysis of some natural fats. *J. Am. Oil Chem. Soc.*, **38**, 685–8.

Dittmer, J. C. & Lester, I. L. (1964). A simple specific spray for the detection of phospholipids on thin layer chromatograms. *J. Lipid Res.*, **5**, 126–7.

Fedeli, E. & Jacini, G. (1971). Lipid composition of vegetable oils. *Adv. Lipid Res.*, **9**, 335–69.

Gaydou, E. M., Ralaimanarivo, A. & Bianchini, J. P. (1983). Fatty acid and sterol compositions of seed oil from the Australian baobab: *Adansorica gregorii*. *Riv. It. Sost. Grasse.*, **60**, 747–51.

Gunstone, F. D. (1962). Distribution of fatty acids in natural glycerides of vegetable origin. *Chem. Ind.*, 1214–23.

Luddy, F. E., Barlord, R. A., Harb, S. F., Magidam, P. & Reimenschneider, R. W. (1964). Pancreatic lipase hydrolysis of triglycerides by semi-micro technique. *J. Am. Oil Chem. Soc.*, **41**, 693–6.

Luddy, F. E., Herb, S. F., Magidman, P., Spinelli, A. M. & Wasserman, A. E. (1970). Colour and the lipid composition of pork muscles. *J. Am. Oil Chem. Soc.*, **47**, 65–8.

Oderinde, R. A. & Esuoso, K. O. (1989). Hydrolysis and ethyl alcohol production from *C. esculentus* 'imumu'. *Ghana J. Chem.*, **1**, 48–53.

Oderinde, R. A. & Tairu, A. O. (1988). Evaluation of the properties of yellow nutsedge (*Cyperus esculentus*) tuber oil. *Food Chem.*, **28**, 233–7.

Oderinde, R. A. & Tairu, A. O. (1989). Utilisation of yellow nutsedge tuber for composite flour. *Pak. Sci. Ind. Res.*, **32**, 570–3.

Oderinde, R. A., Tairu, A. O. & Atinsola, F. M. (1989). Chemical investigation of Cyperaceae family 1: the proximate analysis of *Cyperus rotundus* tuber (Choqui). *Riv. It. Sost. Grasse.*, **66**, 211–13.

Oderinde, R., Tairu, O., Awofala, F. & Ayediran, D. (1990*a*). A study of the chemical composition of some members of Cucurbitaceae family. *Riv. It. Sost. Grasse.*, **67**, 259–61.

Oderinde, R. A., Tairu, A. O., Dawodu, F. A. & Bamiro, F. O. (1990*b*). Chemical investigation of Cyperaceae family. 2. Preliminary report on *Cyperus tuberosus* tuber composition. *Riv. It. Sost. Grasse.*, **67**, 301–3.

Olcott, H. S., Shuster, C. Y. & Froines, J. R. (1964). Phospholipids of tuna white muscle. *J. Am. Oil Chem. Soc.*, **41**, 36–41.

Rouser, G., Fleischer, S. & Yamamoto, A. (1970). Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids*, **5**, 494–7.

Sanders, T. H. (1980). Effects of variety and maturity on lipid classes composition of peanut oil. *J. Am. Oil Chem. Soc.*, **57**, 8–11.

Stewart, J. M. C. (1980). Colorimetric determination of phospholipids with ammonium ferrothiocyemate. *Anal. Biochem.*, **104**, 10–14.

Tan, B. K., Hamilton, R. J. & Berger, K. G. (1981). Glyceride analysis of palm oil after solvent fractionation. *J. Am. Oil Chem. Soc.*, **58**, 1–5.

Urakami, C., Oko, S. & Han, J. S. (1976). Composition of the neutral and phospholipid fraction from ginlego nuts and fatty acid composition of individual classes. *J. Am. Oil Chem. Soc.*, **53**, 525–9.

Van der Wal, R. J. (1960). Calculation of the distribution of the saturated and unsaturated acyl groups in fats, from pancreatic lipase hydrolysis data. *J. Am. Oil Chem. Soc.*, **37**, 18–20.