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Compositional and structural studies of the oils from two edible seeds: Tiger nut, *Cyperus esculentum*, and asiato, *Pachira insignis*, from Ghana

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ABSTRACT

A comprehensive lipid profiling has been carried out on the seed oils of tiger nut, *Cyperus esculentum,* and asiato, *Pachira insignis,* from Ghana in order to evaluate their potential uses for the region. Composition of FAs and phytosterols were determined using GC–MS, while tocol composition was determined with HPLC. TAG composition and regiochemistry were determined using ES-FTICR-MS and ^{13}C NMR, respectively. The major FA components in asiato seed oil were palmitic acid (56.58%) and sterculic and dihydrosterculic acids (20.06% combined). The major FA components in tiger nut oil were oleic (65.55%), palmitic (16. 32%), and linoleic (12.13%) acids. Asiato seed oil had 18 major TAG classes, dominated by, C51:1 (38.45%) and C50:1 (13.941%). Tiger nut oil had 7 major TAG classes, with C54:3 (29.00%) and C52:2 (27.82%) dominating. The *sn*-1/3 and *sn*-2 positions in the TAGS for asiato oil were predominantly occupied by saturated acyl chains (87.25%) and cyclic acyl chains (71.44%), respectively. Oleoyl chain primarily occupied both *sn*-1/3 (52.68%) and *sn*-2 (77.62%) positions in the tiger nut oil. Total tocol content in asiato oil was 200.31 μg/g, with γ-tocopherol (182.99 μg/g) dominating. Tiger nut oil had a total tocol content of 120.10 μg/g, dominated by α-tocopherol (86.73) and β-tocopherol (33.37 μg/g).Tiger nut oil had total 4-desmethylsterol content of 986 μg/g, dominated by β-sitosterol (517.25 μg/g) and stigmasterol (225.25 μg/g), while asiato oil had total desmethylsterol content of 590.24 μg/g, dominated by β-sitosterol (518.91 μg/g). The presence of cyclopropenoid FAs in asiato oil makes it unsuitable for food uses. Tiger nut oil can replace imported olive oil in food products in the West African region.

1. Introduction

There is increasing awareness of the importance of vegetable oils as sources of food, biofuel, health enhancing compounds, i.e., nutraceuticals, as feedstock for industrial polymers and for many other industrial products. Thus the world demand for vegetable oils is set to rise even more rapidly from year to year, and this trend will impact on the price levels of oils. It is therefore important that poor countries and communities which have non-conventional seed oils carry out research that can lead to commercial production of their seed oils to at least satisfy local demand. An important component of this search is to collect detailed scientific data that will inform on the potential uses of all such seed oils. In this regard we have been carrying out detailed studies of the physicochemical, compositional and structural studies of non-conventional seed oils that are found in the region of Africa south of the Sahara. In the current study we have attempted to carry out comprehensive lipid profiling of two seed oils from edible seeds, tiger nut, *Cyperus esculentum*, and asiato, *Pachira insignis*, which are grown in Ghana, and indeed in many parts of the West African region.

Tiger nut, *C. esculentum*, is a popular snack in Ghana and in other parts of West African. In Ghana tiger nut is eaten raw as a snack or crushed and the resulting white milk is cooked into a delicious porridge. The oil from the nut is extracted by traditional methods on a small scale for food uses. Tiger nut, *C. esculentum*, of the Cyperaceae family, is a plant which was cultivated in ancient Egypt (Zohary & Hopf, 2000). Tiger nut now grows widely in the West African region both as a cultivated and as a wild grass-like plant. Tiger nut is known in other parts of the world, especially in the Valencia region of Spain where it is commonly known as “chufa” and the oil from the nut is now produced on a commercial scale for the European market (Tigernuts Traders, 1997–2010).

Asiato is the common name among the communities in the Volta region of Ghana for the plant *P. insignis* of the Bombacaceae family. Also commonly known as “wild chestnut”, *P. insignis* is one of two species out of 24 of the genus *Pachira* which have edible seeds (Janick

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& Paull, 2008). In Ghana, the seeds of asiato are ground into paste and used as a thickener in stews and sauces, very much in the same way as groundnut paste is used in many parts of the West African region. Even though asiato seeds are rich in oil, this commodity is extracted only in a few rural communities for use in cooking.

There are reports in the literature about the FA composition of the seed oils from tiger nut, *C. esculentum* (Glew et al., 2006) and asiato, *P. insignis* (Berry, 1980). There are also some literature reports about the phytosterols and the vitamin E compounds (tocopherols and tocotrienol) in the Spanish tiger nut oil (Tigernuts Traders, 1997–2010). However, literature reports about the detailed composition of the principal components, such as triacylglycerols, and about the content of the minor components, such as the phytosterols and the vitamin E compounds, in the seed oils of tiger nut and asiato plants grown in the West African region are rather lacking. The aim of this study was therefore to collect comprehensive data on the physicochemical, compositional and structural properties of the seed oils from the seeds of asiato and tiger nut grown in Ghana, West Africa. Such data will inform on the nutritional value of the seeds and will also be useful in evaluating the two West African seed oils for other potential uses, such as biodiesel, biolubricants, sources of oleochemicals, etc., that will add extra value to these oils in the region.

2. Materials and methods

2.1. Materials

Tiger nuts, *C. esculentum,* and asiato seeds, *P. insignis*, were purchased from an open market in Accra, Ghana.

2.2. Methods

2.2.1. Extraction: Solvents and reagents

Solvents and reagents used in this work, unless otherwise stated, were all of analytical grade. Solvents used for high-resolution MS were of HPLC grade. Solvents were obtained from Rochelle Chemicals, South Africa, BDH (Merck Chemicals, Pty Ltd. UK), Riedel-de Haën (Sigma Aldrich, GmbH) or JT Baker Chemicals Co. (Phillipsburg, NJ, USA). The seeds and nuts were manually dehulled and after thorough cleaning were macerated in a Waring commercial blender (Gateshead, UK). The powders were extracted with a mixture of *n*-hexane/2-propanol (3:1, *v*/*v*) in a Soxhlet apparatus for 6 h.

2.2.2. Physicochemical properties

The bulk physical and chemical properties (Table 1) were determined according to standard IUPAC methods for the analysis of oils and fats (Dieffenbacher & Pocklington, 1987). All experiments, unless otherwise stated, were conducted in triplicate.

2.2.2.1. Composition of lipid classes. Analysis of the lipid classes (Table 2) in each oil sample was carried out by adsorption column chromatography, using florisil (adjusted to 7% water *w*/*w*, Saarchem Pty Ltd., Muldersdrift, Republic of South Africa) and gradient elution using *n*-hexane, mixtures of *n*-hexane/diethyl ether, diethyl ether, methanol and acetone (Semporé & Bézard, 1996).

2.2.3. Separation of acylglycerols

Triacylglycerols (TAG), diacylglycerols with free fatty acids (DAG + FFA) and monoacylglycerols (MAG) in the oil samples were further separated by gradient elution on silica gel (adjusted to 5% H_2O, Saarchem Pty Ltd.) using benzene (100%), benzene: diethyl ether (9:1) and diethyl ether (100%) respectively (Semporé & Bézard, 1996).

2.2.4. Fatty acid composition

2.2.4.1. Preparation of FAMEs. The oil samples (2 g each) were transesterified by refluxing in dry methanol that contained ethanoyl chloride to yield fatty acid methyl esters (FAMEs) (Christie, 1982). These were stored under nitrogen and used for GC–MS and ^{1}H NMR analyses.

2.2.4.2. Preparation of picolinyl esters. The method of Destaillats and Angers (2002) was adopted in this preparation. Portions of the oil samples were dissolved in dry dichloromethane and allowed to react with a mixture of potassium *tert*-butoxide in tetrahydrofuran and 3-hydroxymethylpyridine at room temperature for 2 min. Sodium bicarbonate solution was then added and the organic layer was extracted, dried with anhydrous sodium sulphate and stored under nitrogen for GC–MS analysis.

2.2.4.3. Instrumentation and separation conditions. FAMEs and picolinyl esters in dichloromethane were analysed in a ThermoQuest Voyager GC–MS coupled to ThermoQuest Trace GC 2000 SERIES (San Jose, California, USA). Separation was effected on a DB-5MS capillary column (0.25 μm × 0.25 mm × 30 m; J & W Scientific, California, USA) consisting of 5% phenyl-methylpolysiloxane stationary phase. UHP Helium was used as carrier gas at a flow rate of 1 mL/min. Injection temperature was 220 °C, while interface temperature was 300 °C. Initial temperature was 60 °C, held for 1 min and then ramped to 200 °C at the rate of 15 °C per minute. It was then held for 1 min before the second ramp at the rate of 5 °C per minute to 300 °C. This was then held isothermally for 25 min.

2.2.4.4. Nuclear magnetic resonance analysis. Proton NMR spectra of the FAMEs, dissolved in $CDCl_3$, were acquired at 300 MHz using a Bruker Avance DPX 300 spectrometer. The relative compositions of the saturated, monounsaturated and diunsaturated fatty acids together with their average chain lengths were determined from the relative sizes of the integrals of the signals for the allylic, diallylic and methyl protons using Holmback's equations, Table 4 (Holmback, 2000; Yeboah, Motshegwe, & Holmback, 1998).

2.2.5. Analysis of triacylglycerols (TAGs)

2.2.5.1. High resolution mass spectrometric analysis. TAG extracts (~0.2.5 mg) were dissolved in methanol and the methanolic solutions were introduced continuously via a syringe pump into an electrospray ionisation source (APPOLO) on a high resolution FTICR mass spectrometer (Bruker Daltonics Apex III) at a flow rate of 2 μL min^{-1}. All data were acquired with 512 K data points and zero-filled to 2048 K by averaging 32 scans and applied to Fourier transform and magnitude calculation. After acquisition, mass spectral

Table 1
Physical and chemical properties of the oils from tiger nut and asiato seeds.

Sample	% yield (w/w)	RI at 25 °C	Density g/cm^3 at 20 °C	SV (mg KOH/g)	IV (Wij's)	AV (mg KOH/g)	PV (mEq/kg)	p-AV	UM (% w/w)
Tiger nut	15.9	1.471 ± 0.001^f	0.912	180.24 ± 1.99	91.31 ± 0.07	1.38 ± 0.15	5.54 ± 0.62	−2.93 ± 0.16	0.60 ± 0.07
Asiato	38.9	1.470 ± 0.001^f	0.913	189.49 ± 0.55	46.99 ± 3.50	4.44 ± 0.14	4.04 ± 0.27	3.01 ± 0.08	0.41 ± 0.05

Key: RI = refractive index; SV = saponification value; IV = iodine value; AV = acid value; PV = peroxide value; and p-AV = *p*-anisidine value.
f = RI was determined at 23.5 °C.

Table 2
Percentage composition of lipid classes in the seed oils of tiger nut and asiato seeds as estimated from adsorption column chromatography[a].

Sample	Hydrocarbons	Triacylglycerol + FFA	Sterol esters	Free sterols	DAGS	MAGS	Glycolipids	Phospholipids
Tiger nut	11.99	65.94	3.84	2.62	2.02	2.74	5.56	1.38
Asiato	10.01	56.84	7.79	6.52	1.44	3.22	0.50	3.16

Key: FFA = free fatty acids; DAG = diacylglycerols; and MAG = monoacylglycerols.
[a] Values are means of two determinations.

raw data were post-processed using Bruker XMASS acquisition and processing Software (Version 6.12) according to full width half maximum (FWHM) criteria and taken as input data for calculations. Molecular formulas were assigned by use of the software above. Molecular formulas were limited to 200 ^{12}C, 300 ^{1}H, 30 ^{16}O and 1 ^{23}Na atoms.

2.2.5.2. ^{13}Carbon NMR analysis. Standard compounds (tri-palmitin, tri-olein, and tri-linolein) were obtained from Fluka and used without further purification. About 200 mg of sample was dissolved in deuterated chloroform ($CDCl_3$) and the solution (700 μL) was placed in a 5 mm (diameter) NMR tube. ^{13}Carbon NMR spectra were recorded on a Varian Mercury 400 spectrometer operating at 100.6 MHz. ^{13}Carbon NMR spectra for quantitative analyses were recorded with a spectral width of 1300 Hz, 64 K data points, a pulse repetition time of 60 s, a 90° flip angle and full proton decoupling. Two hundred scans were accumulated per spectrum. The FID's were zero-filled to 128 K prior to Fourier transform resulting in a digital resolution of 0.02 Hz/point. A line broadening of 0.05 Hz was used for the exponential weighting. Peaks were assigned by spiking the standard compounds into the real test samples.

2.2.6. Analysis of tocopherols and tocotrienols

2.2.6.1. Saponification. Oil samples for the analysis of tocopherols and tocotrienols were saponified according to the method reported by Panfili, Alessandra, and Mario (2003). Oil sample (2.0 g) in an amber screw capped bottle was flushed with nitrogen and 10 M potassium hydroxide (2.0 mL) was added. Absolute ethanol (2.0 mL) and 0.2 M sodium chloride solution (2.0 mL) were then added and the mixture flushed with nitrogen again. Ethanolic pyrogallol (0.5 M, 5.0 mL) was finally added as antioxidant and then flushed with nitrogen. The bottle was placed in a 70 °C water bath and mixed after every 5 min for 45 min, after which the bottle was cooled in ice and 0.2 M sodium chloride solution (15.0 mL) was added. The suspension was extracted twice with 9:1 (v/v) *n*-hexane-ethyl acetate (15.0 mL). The combined organic layer was evaporated to dryness. The dry residue was dissolved in *n*-hexane-isopropanol (99:1) and passed through silica Chromabond™ SPE cartridge. The filtrate was dried and re-dissolved in *n*-hexane (10.0 mL). This was then appropriately diluted prior to HPLC–FLD analysis.

2.2.6.2. HPLC–FLD analysis. A Merck HPLC system (Darmstadt, Germany) that consisted of a Merck-Hitachi HPLC L-7100 Intelligent pump, a Rheodyne injector fitted with a 5 μL loop and a Merck L-7480 fluorescence detector was used for the tocopherol and tocotrienol analysis. The analysis was achieved using normal-phase HPLC on Nucleosil 100-5 column (5 μm × 4 mm × 25 cm) from Macherey-Nagel, (Düren, Germany). The mobile phase was *n*-hexane-isopropanol (99.7:0.3 v/v) at a flow rate of 1.0 mL/min. Fluorometric detection of all peaks was performed at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Tocopherol peaks were identified and quantified against authentic tocopherols used as external standards. For each extract quantitative analysis was performed in triplicate. Tocotrienol peaks were confirmed by GC–MS and quantified against the corresponding tocopherols.

2.2.7. Analysis of phytosterols

2.2.7.1. Saponification of oil samples. 0.5 M Ethanolic potassium hydroxide solution (25.0 mL) was added to oil sample (2.5 g) in 250-mL round-bottomed flask and the mixture was refluxed for 1 h. Water (100.0 mL) was then added down the condenser and the mixture was extracted with diethyl ether (100 mL × 3) in a 500-mL separatory funnel. The combined ether solution was washed with water (40.0 mL × 3), and then washed successively with 0.5 M aqueous potassium hydroxide (40.0 mL), distilled water (40.0 mL) and repeatedly with aqueous 0.5 M KOH (40.0 mL) before finally washing with more 40.0 mL portions of distilled water until the washings were neutral to phenolphthalein indicator. The ether solution was then dried under anhydrous magnesium sulphate, and the solvent evaporated by distillation on a water bath to release the unsaponifiable matter.

2.2.7.2. Acetylation. The whole unsaponifiable matter (UM) was acetylated according to the method described by Wilson, Kodicek, and Booth (1962). The UM (2.0 mg) was dissolved in 2:1 (v/v) pyridine-acetic anhydride solution (600 μL) and the reaction was allowed to proceed at room temperature over night. The excess reagents were then removed with a slight warming under a stream of nitrogen gas. The mixture was re-dissolved twice in dichloromethane and the solvent removed in a stream of nitrogen. The acetylated products were transferred into a sample vial and dissolved again with dichloromethane, flushed with nitrogen and stored at 4 °C for GC–MS analysis.

2.2.7.3. Analysis by GC–MS. The acetylated lipids in dichloromethane were analysed in a ThermoQuest Voyager GC–MS coupled to ThermoQuest Trace GC 2000 SERIES (San Jose, California, USA). A DB-5MS capillary GC column (0.25 μm × 0.25 mm × 30 m) from J & W Scientific (California, USA) was used for separation and UHP helium was used as carrier gas at a flow rate of 1 mL/min. The injection temperature was 220 °C, while the interface temperature was 300 °C. The initial temperature was 60 °C held for 1 min and then ramped to 200 °C at the rate of 15 °C per minute. It was then held for 1 min before the second ramp at the rate of 5 °C per minute to 300 °C. This was then held isothermally for 25 min.

2.2.7.4. SPE fractionation of sterols. The SPE method of Damirchi and Dutta (2006) was modified for this determination. A 500 mg, 3 mL silica solid phase extraction (SPE) cartridge from Macherey-Nagel (Düren, Germany), was used per sample. The cartridge was attached to a Macherey-Nagel vacuum manifold (Düren, Germany). Waste tubes were positioned to collect the conditioning solvent. The cartridges were conditioned by passing *n*-hexane (5.0 mL) through them. After conditioning, the *n*-hexane was discarded and a clean test tube was positioned to collect the phytosterol fraction. The UM (2.0 mg) sample aliquots in 2 mL *n*-hexane were then loaded onto the cartridges. The UM was then washed with *n*-hexane-ethyl acetate (10.0 mL), 99:1(v/v), to remove any non-sterol compounds. Vacuum was applied at 5 mm Hg for each elution. This fraction was discarded after checking with analytical TLC that it did not contain compounds of interest. 4-Methylsterols were then eluted with *n*-hexane-ethyl

acetate (10.0 mL), 99:1(v/v), followed by 14.0 mL of 97:3 (*v/v*) *n*-hexane-ethyl acetate. Prior to eluting pure 4-desmethylsterols, additional *n*-hexane-ethyl acetate (2.0 mL), 97:3 (*v/v*), was used to wash the cartridges. 4-Desmethyl sterols were then eluted with *n*-hexane-ethyl acetate (25.0 mL), 97:3 (v/v). The fractions were dried, spiked with 10 μg of 5α-cholestane as internal standard and acetylated prior to GC–MS analysis. The components were quantified against 5α-cholestane.

2.2.8. Data analysis

Experiments for the determination of the physicochemical parameters, the GC–MS analysis of fatty acid composition and, experiments for the determination of tocopherols, tocotrienols and phytosterols by HPLC and GC–MS analyses were all carried out in triplicate and results are expressed as mean values ± SD. NMR and FTICR-MS results are expressed as values for single runs.

3. Results and discussion

3.1. Oil yield

Tiger nuts, *C. esculentum*, and asiato seeds, *P. insignis* are widely distributed in the West African region. The plants grow wild and are also cultivated for their seeds and nuts, respectively. Tiger nut in particular is available all year round in the whole region as a snack food. The oil yield from tiger nut obtained in this study, as shown in Table 1, was 15.9% *(w/w)*, which is lower than that of soybean oil (18–22%) but much higher than corn oil yield (about 6%) (Bockisch, 1998). Thus when grown on large scale like corn, tiger nut can yield a lot of valuable oil. Table 1 shows the oil yield from asiato seed as 38.9% *(w/w)*, which makes it attractive for large scale production. While this level of oil yield is lower than sunflower (55%), it is higher than safflower (32%) and linseed oil (34%), both produced on commercial scale (Bockisch, M, 1998). However as we shall learn later, asiato seed oil is recommended mainly for non-food uses.

3.2. Physicochemical properties

Preliminary characterization of the seed oils from tiger nut, *C. esculentum,* and asiato, *P. insignis* was carried out by collecting data (Table 1) on some bulk physicochemical properties of the oils. Refractive index and relative density generally shed light on structural properties such as average molecular mass and degree of unsaturation of the fatty acids in oils and fats. Thus the similar refractive index and density values for tiger nut (1.471 and 0.912) and asiato (1.470 and 0.913), given in Table 1, would tend to suggest that the two seed oil samples had similar average FA chain lengths and degrees of unsaturation. Also the comparable saponification values (SV) of 180.24 for tiger nut and 189. 49 mg KOH/g for asiato seed oils also pointed in the direction of similar average chain lengths for the FAs in the two oils. However, the FA compositions of the two oil samples, as estimated by GC–MS and by proton NMR estimation of the fatty acid classes, given in Table 3, clearly show that the degree of unsaturation in the tiger nut oil was much higher than that in the asiato oil. Indeed the iodine value (IV) for tiger nut oil, 91.31 (Wij's) and that for asiato oil, 46.99 (Wij's) agreed with the GC–MS and proton NMR estimation of the degrees of unsaturation in the two seed oils. It would appear however that the presence of substantial amounts of cyclopropenoid/cyclopropanoid fatty acids (Table 3) in asiato seed oil might have exerted similar effect to that of unsaturation on the texture of asiato oil, and hence the similar refractive index and density of asiato oil to those of tiger nut oil.

The tiger nuts and the asiato seeds used in this investigation were several months old before the extraction of the oils. Thus the parameters for oxidative and hydrolytic rancidity/stability, given in Table 1, i.e. acid value (AV), peroxide value (PV) and *p*-anisidine value (*p*-AV): 1.38 mg KOH/g, 5.54 mEq/kg and −2.93, respectively, for tiger nut, and 4.44 mg KOH/g, 4.04 (mEq/kg) and 3.01, respectively, for asiato, are quite reasonable as they compare very favourably with Codex recommended values for virgin olive oil (Kirk & Sawyer, 1991). Thus these parameters indicate that both tiger nut and asiato seed oils should have good keeping-capacity.

Table 3
Percentage FA compositions by capillary GC–MS and fatty acid classes from integrals of ^{1}H NMR signals of the FAMEs from test oils.

Fatty acid	Asiato	Tiger nut
12:0	ND	ND
14:0	0.17 ± 0.01	ND
9-16:1	0.31 ± 0.01	ND
16:0	56.58 ± 0.17	16.32 ± 0.06
17:0	0.20 ± 0.00	ND
9,12,15-18:3	ND	ND
9,12-18:2	6.58 ± 0.11	12.13 ± 0.12
9-18:1	9.33 ± 0.09	65.55 ± 0.08
11-18:1	0.79 ± 0.05	ND
18:0	4.72 ± 0.01	5.33 ± 0.04
20:0	1.26 ± 0.00	0.68 ± 0.01
Cyclic fatty acids	20.06 ± 0.15	ND
Total unsaturated	17.01	77.68
Total saturated	62.87	22.33
Fatty acid classes	**Estimated from proton NMR integrals (%mol)**	
18:3,ω3	ND	ND
Diunsaturated	ND	10.4
Monounsaturated	29.8	64.0
Total unsaturated	29.8	74.4
Total saturated	70.2	25.6
Average carbon chain	15.9	17.1

Key; ND = not detected.

3.3. Lipid classes

Given in Table 2 is the percent composition of the lipid classes in the two seed oils as determined by adsorption column chromatography (Semporé & Bézard, 1996). The results of the analysis essentially gave general information about the relative amounts of neutral lipids (hydrocarbons, triacylglycerols, sterol esters and free sterols) and polar lipids (diacylglycerols, monoacylglycerols, glycolipids and phospholipids) present in the seed oils. Table 2 shows that neutral lipids, dominated by triacylglycerols, constituted the bulk of the seed oils from tiger nut and asiato seeds. It should be noted that the percent compositions of hydrocarbons, sterol esters and free sterol shown in Table 2 were rather exaggerated as TLC showed that elution of triacylglycerols spanned a wide portion of the neutral lipid range, overlapping with the hydrocarbons, sterol esters and free sterols in the gradient elution. The relatively low values for diacylglycerols (DAG) and monoacylglycerols (MAG) gave some support to the low acid values given in Table 1, as they indicated limited lipase hydrolysis of the oils. On the whole the low content of the polar lipids would suggest that mechanically pressed oils from tiger nut and asiato seeds could be used as virgin oils, without much refinement, as in the case of olive oil.

3.4. Fatty acid composition

As inferred above, triacylglycerols (TAG) constituted the bulk of the seed oils from tiger nut and asiato seed and hence profiling the triacylglycerols and their constituent fatty acids (FA) was crucial for the detailed characterization of the two oil samples. In profiling the principal components we first carried out the determination of the FA compositions of the test seed oils by GC–MS, which was complemented with proton NMR estimation of the FA classes (Holmback, 2000; Yeboah et al., 1998). The results of these determinations are presented in Table 3. The GC–MS determination of the FA compositions was carried out by analysing both FA methyl esters (FAME) and FA picolinyl esters of the oils. The FAME

analysis provided data on the molecular weight as well as retention times of the respective compounds. GC–MS analysis of the FA picolinyl esters on the other hand provided distinct diagnostic ions in the mass spectra that facilitated the location of the positions of double bonds and cyclic rings in the acyl chains (Wretensjö, Svensson, & Christie, 1990). Thus from the mass spectra of the FA picolinyl esters, prepared from the asiato seed oil, it was established that asiato oil contained FAs with cyclopropene and cyclopropane rings at C9 and C10, hence indicating the presence of sterculic acid, 9,10-methylene-octadecenoic acid (cp9,10-18:1) and dihydrosterculic acid-9,10-methylene-octadecanoic acid (cp9,10-18:0), in the asiato oil. The occurrence of these two cyclic fatty acids in the seed oil of asiato, *P. insignis*, is consistent with the reported presence of the same two cyclic FAs in *Pachira aquatica* (Bohannon & Kleiman, 1978).

The dominant FA component in asiato seed oil, as given in Table 3, was palmitic acid, 16:0, (56.58%), followed by the combined content of sterculic acid (cp9,10-18:1) and dihydrosterculic acid (cp9,10-18:0) at a level of 20.06%. Indeed the total saturation in the asiato seed oil was 62.87%, excluding the cyclic FAs as they could not be sufficiently resolved in the GC–MS experiment. Asiato seed oil is indeed solid at room temperature (about 23–24 °C) and turns liquid at about 29–30 °C. Other fatty acids present were 14:0 (0.17%), 16:1 (0.31%), 17:0 (0.20%), 18:0 (4.72%), 18:1 (9.33%), 18:2 (6.58%) and 20:0 (1.26%). Asiato seed, in paste form, is used to thicken stews in some communities in Ghana. The seeds are also eaten raw or roasted and are said to taste like cashew nuts. However the edibility of the asiato seed oil, and indeed the whole seed, is here called into question because of the presence of the cyclopropenoid FA in the oil. It has been reported by Aitzetmüller (1996) and also by Berry (1980), that cyclopropenoid FAs are toxic to higher animals and also could be co-carcinogenic. However, Aitzetmüller in the same paper reported that some cyclopropenoid FAs are destroyed at high temperatures. This may be the mitigating factor which explains why no negative effects associated with the consumption of asiato seeds have so far been reported. The oil yield of 38.9% (w/w) from asiato seeds is good and so the oil can be considered as a candidate for commercial exploitation. Asiato seed oil should be stable to oxidation on account of its high saturation content and therefore should be suitable for deep frying purposes, assuming the cyclopropenoid FAs are destroyed at high temperatures. However in order to avoid any possible health hazards, it is reasonable to recommend that asiato seed oil should not be used for food purposes and that it is more suitable for non-food uses like soap making, biofuel, biolubricants and such like applications.

The FA profile of the seed oil from tiger nut, shown in Table 3, closely resembles the FA profile for olive oil; with FA composition of oleic acid, 18:1, (65.55%), palmitic acid, 16:0, (16.32%), linoleic acid, 18:2, (12.13%), stearic acid, 18:0 (5.33%) and arachidic acid, 20:0, (0.68%), the tiger nut oil sample was virtually like some variety of olive oil, whose major FA content is given as oleic acid (55–83), palmitic acid (7.5–20), linoleic acid (3.5–21), stearic acid (0.5–5.0) and arachidic acid (0–0.8%) (Firestone, 2006). This favourable FA content of tiger nut oil, combined with its nutty flavour, should make tiger nut oil a very attractive vegetable oil which can be used in a number of food products in the West African region in place of imported olive oil. Even though the oil yield obtained in this work was 15.9% (*w/w*), tiger nut, already grown on large scale, can be grown on commercial scale in the West African region for exploitation of its oil as it is currently being done in Spain.

3.5. Composition and regiochemistry of triacylglycerols

FA composition of oils and fats furnishes information on the structural components of the triacylglycerols (TAGs) which in fact constitute the bulk of oils and fats. The regiochemical distribution of the FA groups on the glycerol backbone has been shown to have important physiological and nutritional effects on humans (Hunter, 2001; Summers et al., 1999), and in addition the composition of intact TAGs can serve as fingerprint in the authentication of oils (El-Hamdy & El-Fizga, 1995). The composition of intact TAGs and their regiochemistry were, in this work, studied by a high resolution mass spectrometric technique, ESI-FTICR-MS, and ^{13}C NMR, respectively. Table 4 shows the assignment of major mass peaks of TAG classes (*Cx*:*n*) (Stoll, Schmidt, & Thurow, 2006) in the positive ion ESI-FTICR mass spectra of oil samples from tiger nut and asiato seeds.

The regiospecific distribution of the FAs on the glycerol backbone was determined from ^{13}C NMR spectroscopy. Table 5 shows the percent positional distribution of the acyl chains on the glycerol backbone. From the positive ion ESI-FTICR spectrum 18 major TAG classes were assigned for asiato, of which the five most abundant were C51:2 (38.45%), C50:1 (13.94%), C50:2 (9.58%), C54:4 (8.15%) and C53:3 (6.12%). The occurrence of substantial amounts of TAGs with odd numbered total carbon such as C51:2 and C53:3 was consistent with the large amounts of cyclic FAs detected in the GC–MS analysis of the FAME from asiato seed oil. As shown in Table 5, the occupancy of the *sn*-1/3 positions in the glycerol backbone was 87.25% saturated acyl chains, which would be mainly palmitic acid in accordance with the FA composition of the asiato seed oil, Table 3. On the other hand occupancy of the *sn*-2 position was 28.56% oleoyl and about 71.44% unknown (uk) acyl chain, which was so labelled because of the unavailability of authentic standards for identification. However, from the FA composition data of the asiato seed oil, the unknown (uk) acyl chain can safely be attributed to the cyclopropenoid/cyclopropanoid FAs (20.06%) present in the oil. Shown below in Scheme 1, are five possible dominant TAG molecules in the seed oil of asiato as predicted from a combination of the FA composition, Table 3, assignment of major peaks of TAGs in the positive ESI-FTICR spectrum, Table 4, and the positional distribution of the FAs on the glycerol backbone, Table 5. It should be noted here that cp-18:1 refers to sterculic acid (cp9,10-18:1) where the cyclopropenoid ring represents two double bond equivalents (DBE). It is interesting to note that even though palmitic acid content (56.58%) was so high in the asiato seed oil the presence of intact tripalmitin (TAG, C48:0) was very small (0.71%), which was in agreement with the regiospecific distribution of the acyl chains obtained from the ^{13}C NMR analysis, Table 5.

Table 4
Assignment of major mass peaks of triacylglycerols in the positive ion ESI-FTICR mass spectra of oil fractions from tiger nut and asiato seeds.

Observed mass (*m/z*)	Mass ion $[M+Na]^+$	Theoretical mass	TAG CN:DB	Relative intensity.	% composition
Asiato					
825.6973	$[C_{51}H_{94}O_6Na]^+$	825.6943	C48:2	0.0109	0.42
827.7111	$[C_{51}H_{96}O_6Na]^+$	827.7099	C48:1	0.0233	0.90
829.7261	$[C_{51}H_{98}O_6Na]^+$	829.7256	C48:0	0.0203	0.78
839.7111	$[C_{52}H_{96}O_6Na]+$	839.7099	C49:2	0.0205	0.79
853.7254	$[C_{53}H_{98}O_6Na]^+$	853.7256	C50:2	0.2491	9.58
855.7409	$[C_{53}H_{100}O_6Na]^+$	855.7412	C50:1	0.3627	13.94
867.7411	$[C_{54}H_{100}O_6Na]+$	867.7412	C51:2	1.0000	38.45
877.7231	$[C_{55}H_{98}O_6Na]^+$	877.7256	C52:4	0.0364	1.40
879.7409	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.0701	2.70
881.7575	$[C_{55}H_{102}O_6Na]^+$	881.7569	C52:2	0.0836	3.21
891.7418	$[C_{56}H_{100}O_6Na]^+$	891.7412	C53:4	0.1289	4.96
893.7569	$[C_{56}H_{102}O_6Na]^+$	893.7569	C53:3	0.1592	6.12
895.7708	$[C_{56}H_{104}O_6Na]^+$	895.7725	C53:2	0.0953	3.66
901.7266	$[C_{57}H_{98}O_6Na]^+$	901.7256	C54:6	0.022	0.85
903.7387	$[C_{57}H_{100}O_6Na]^+$	903.7412	C54:5	0.0251	0.96
905.7565	$[C_{57}H_{102}O_6Na]^+$	905.7569	C54:4	0.2121	8.15
907.7702	$[C_{57}H_{104}O_6Na]^+$	907.7725	C54:3	0.0627	2.41
919.7752	$[C_{58}H_{104}O_6Na]^+$	919.7725	C55:4	0.0188	0.72
Tiger nut					
855.7396	$[C_{53}H_{100}O_6Na]^+$	855.7412	C50:1	0.2253	6.53
877.724	$[C_{55}H_{98}O_6Na]^+$	877.7256	C52:4	0.1394	4.04
879.7407	$[C_{55}H_{100}O_6Na]^+$	879.7412	C52:3	0.4897	12.20
881.7557	$[C_{55}H_{102}O_6Na]^+$	881.7569	C52:2	0.9431	27.35
903.7408	$[C_{57}H_{100}O_6Na]^+$	903.7412	C54:5	0.1705	4.94
905.7552	$[C_{57}H_{102}O_6Na]^+$	905.7569	C54:4	0.4797	13.91
907.7711	$[C_{57}H_{104}O_6Na]^+$	907.7725	C54:3	1.0000	29.00

Table 5
Positional distribution of fatty acyl chains on the glycerol backbone of triacylglycerols in tiger nut and asiato seed oil samples.

Sample	*sn*-1,3 composition					*sn*-2 composition				
	Saturated	Oleoyl	Linoleoyl	uk[1]	uk[2]	Saturated	Oleoyl	Linoleoyl	uk[3]	uk[4]
Tiger nut	35.36	52.68	11.96	ND	ND	ND	77.62	22.38	ND	ND
Asiato	87.25	4.4	ND	8.35	ND	ND	28.56	ND	ND	ND

Key: uk = unknown; ND = not detected.

Table 4 shows seven major TAG classes for tiger nut oil, of which the dominant classes were C54:3 (29.00%), C52:2 (27.35%), and C54:4 (12.20%). It is quite evident from the FA composition of the tiger nut oil that the C54:3 TAG class would be mainly triolein, in which oleoyl acyl group occupied all the three positions on the glycerol backbone. This is very much supported by the fatty acyl distribution on the glycerol backbone given in Table 5, which shows that the oleoyl chain predominantly occupied both the *sn*-1/3 and the *sn*-2 positions at 52.68% and 77.62%, respectively. Again, by combining the results shown in Tables 3, 4 and 5, we predict the four major TAG molecules, shown in Scheme 1, for tiger nut oil. It is worth noting that occupancy of the *sn*-2 position in the TAGs of the tiger nut oil was 100% unsaturated, a structural feature which is consistent with most vegetable oils (Gunstone, 1979). This structural feature makes tiger nut oil particularly nutritive as it ensures easy absorption of unsaturated FAs into the blood stream.

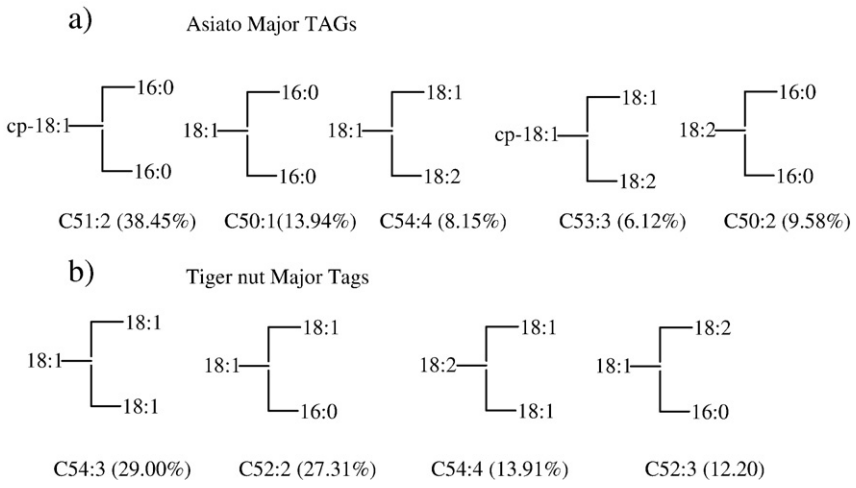
3.6. Major components of the unsaponifiable matter

The compositional and structural studies of the principal components of the seed oils of asiato, *P. insignis,* and tiger nut, *C. esculentum*, were extended to the major components of the unsaponifiable matter (MU), i.e., the phytosterols, tocopherols and tocotrienols. Quantitative and qualitative information about these minor constituents in oils and fats has been shown to be crucial in authenticating the identity of oils and fats (Damirchi, Savage, & Dutta, 2005). Furthermore, tocols are antioxidants which protect the integrity of biomolecules against oxidative attack, and hence efficacious to health. Also, certain phytosterols like β-sitosterol have been shown to have important bioactive properties such as cancer prevention and lowering of total plasma cholesterol (Awad & Fink, 2000; Määttä, et al., 1999). Thus phytosterols and tocols are the subject of intense search by the food processing industry for incorporating them into functional foods and for use as nutraceuticals.

3.6.1. Tocopherols and tocotrienols

The determination of the tocopherols and tocotrienols, the vitamin E compounds, was carried out, as described in the Materials and methods section, by carefully saponifying portions of the oils and extracting the unsaponifiable matter. Normal-phase HPLC with fluorescence detection (HPLC–FLD) was used to determine the tocopherols and tocotrienols, which were then quantified against external standards. Normal-phase HPLC was chosen for the separation of the tocols as it is capable of resolving α- and β-tocopherols and tocotrienols. The tocopherols and tocotrienols were eluted in order of increasing polarity, which corresponded with the decreasing order of methylation on the chromanol ring of the tocopherols and the tocotrienols. Thus the order of elution was the α-homologue followed by β-, γ- and δ-homologues. This order of elution was consistent with an earlier report (Eitenmiller & Lee, 2004). The quantitative analysis was performed with calibration curves. A linearity test was carried out over the concentration ranges 1 ppm to 5 ppm from which regression analysis of the plot of area response versus concentration for each isomer gave an excellent relationship with correlation coefficients of 0.9997 for α-tocopherol, and 1.0000 each, for β-, γ- and δ-tocopherols.

Results of the determination of the tocopherols and tocotrienols are given in Table 6, which shows that asiato seed oil contained α-tocopherol (α-T, 13.14), γ-tocopherol (γ-T, 182.99) and δ-tocopherol (δ-T, 10.18 μg/g), making a total tocol content of 206.3 μg/g in the asiato oil. This total tocol content just fell within the typical total tocol range of 200–800 μg/g for vegetable oils. On the other hand the total tocol content in the highly unsaturated tiger nut oil was only 120.1 μg/g, made up of α-T (86.73) and β-tocopherol (33.37 μg/g). However, the peroxide value (PV) for the tiger nut oil, which was extracted from nuts of several months old, was 5.54 mEq/kg (Table 1). This was well within the Codex recommended PV of 10 mEq/kg for unrefined vegetable oils (Kirk & Sawyer, 1991). Thus in spite of the rather low tocol content, oxidative stability in the tiger nut oil was



Scheme 1. Possible major TAGs for a) asiato seed oil and b) tiger nut oil predicted from Tables 3, 4 and 5.

Table 6
Tocopherol and tocotrienol contents (μg/g mean ± SD) in tiger nut and asiato seed oils obtained by HPLC–FLD.

Sample	α-T	β-T	γ-T	δ-T	α-T3	Total tocol
Asiato	13.14 ± 0.50	ND	182.99 ± 5.32	10.18 ± 0.69	ND	206.3 ± 4.8
Tiger nut	86.73 ± 5.31	33.37 ± 1.57	ND	ND	ND	120.1 ± 5.7

Key: ND = not detected.

reasonably good as the oil was far from being rancid. This analysis did not show any obvious correlation between degree of unsaturation and total tocol content, which once again tends to uphold an earlier observation that total vitamin E content in a seed oil may not be a foolproof indicator of the stability of the oil, as stability of vegetable oils can be influenced by natural anti-oxidants and synergists other than vitamin E (Eitenmiller & Lee, 2004).

3.6.2. Phytosterols

Two sets of data, both given in Table 7, were collected for the compositional analysis of the phytosterols in the unsaponifiable matter from asiato seed and tiger nut oils. The first set of data was obtained from the determination of the relative percent composition of all phytosterols in the acetylated total unsaponifiable matter from the seed oils by GC–MS. The components in the region where phytosterols eluted were selected and identification of the sterols was based on comparison of retention times and reference mass spectra of authentic standards. The relative percent composition of each sterol, shown in Table 7, was calculated as a ratio of its peak area to the total area of all identifiable sterol peaks in each oil sample. It can be noted in Table 7 that the sterol content in the oil from asiato, *P insignis*, consisted solely of 4-desmethylsterols and tiger nut oil consisted of about 90% of 4-desmethylsterols. Thus the second set of data was obtained from the determination of the absolute amount of each 4-desmethylsterol in the test oil samples. This was achieved by carrying out a modified Damirchi's SPE method for pre-fractionating the unsaponifiable matter prior to acetylation and subsequent GC–MS analysis, using 5α-cholestane as internal standard. The absolute amount of each identifiable 4-desmethylsterol was calculated as μg/g of the test oils by using the relationship, $4\text{-desmethylsterol} = (A_z \cdot m_{is})/(A_{is} \cdot m)$, where A_z = start peak area, A_{is} = internal standard peak area, m_{is} = mass (μg) of internal and m = mass of oil in g weighed for the analysis (Reina, White, & Jahngen, 1997).

The two sets of results for the relative percent composition of phytosterols and the absolute amounts of 4-desmethylsterols in the asiato and tiger nut oils, shown in Table 7, corroborate each other. The predominant phytosterol in the asiato seed oil was β-sitosterol at 87.72%, which translated into 518.91 μg/g of oil. This was followed by campesterol (5.26%, 34.85), Δ^5-avenasterol (4.52%, 25.65) and stigmasterol (1.76%, 10.83 μg/g), making a total of 590.24 μg/g of phytosterols in the asiato seed oil. This level of phytosterol content in the asiato oil fell within the total phytosterol range reported for coconut oil (470–1140 μg/g). Table 7 shows that in addition to 4-desmethylsterols the seed oil from tiger nut also contained two 4,4-dimethylsterols in small amounts: 24-methylenecycloartanol (4.17%) and cycloartenol (1.43% overall). The absolute amounts of the 4,4-dimethylsterols could not be determined due to lack of authentic standards. The 4-desmethylsterol content in tiger nut oil was once again dominated by β-sitosterol at 50.37%, which translated into 517.25 μg/g of oil. The other common vegetable oil phytosterols like stigmasterol (20.62%, 225.25) and campesterol (15.33%, 161.35) were present in larger amounts than in asiato oil. Other minor 4-desmethylsterols in tiger nut oil were Δ^5-avenasterol (3.75%, 37.57) and Δ^7-avenasterol (1.73%, 17.04 μg/g) which together made a total 4-desmethylsterol content of 986.49 μg/g of tiger nut oil, falling within the upper end of the coconut oil phytosterol range (470–1140 μg/g). This moderately high content of phytosterols further enriches the quality and value of the tiger nut oil as food source. In fact we learn from this work that tiger nut oil is much richer in phytosterols than olive oil (100 μg/g total) (Firestone, 2006), and hence it is the phytosterol profile of tiger nut oil that truly distinguishes it from olive oil.

4. Conclusions

As far as we are aware, this is the first time the composition and the regiodistribution of the acyl chains in the glycerol backbone of the triacylglycerols in the seed oils of asiato, *P. insignis*, and tiger nut, *C. esculentum*, have been reported. We are also not aware of any reports of the profile of the phytosterols and the tocols in the asiato seed oil. The results of this study have provided much justification for the use of tiger nut oil in food products. The high content of oleic acid (65.55%) and the 100% occupancy of the *sn*-2 position of the triacylglycerols together with the presence of a good amount of phytosterols (986.49 μg/g), especially

Table 7
Relative percent composition (mean ± SD) of phytosterols and absolute amount of 4-desmethylsterol in tiger nut and asiato seed oils.

	Asiato		Tiger nut	
Compound	Relative % comp.	Absolute 4-desmethylsterol (μg/g)	Relative % comp	Absolute 4-desmethylsterol (μg/g)
Cholesterol	ND	ND	ND	ND
22-Dehydrocholesterol	ND	ND	ND	ND
24-Me-cholesterol	ND	ND	ND	ND
Campesterol	5.26 ± 0.06	34.85	15.33 ± 0.10	161.35
Campestanol	ND	ND	ND	ND
Stigmasterol	1.76 ± 0.08	10.83	20.62 ± 0.15	225.25
Stigmastanol	ND	ND	ND	ND
Sitosterol	87.72 ± 0.04	518.91	50.37 ± 0.24	517.25
β-Amyrin	ND	NA	ND	NA
Δ5-Avenasterol	4.52 ± 0.07	25.65	3.75 ± 0.03	37.57
Sitostanol/Δ5-avenasterol	ND	ND	ND	ND
24-methyllophenol	ND	NA	ND	NA
Cycloartenol	ND	NA	1.43 ± 0.02	NA
Lupeol	ND	NA	ND	NA
22-Dihydrospinasterol	ND	ND	2.59 ± 0.03	28.03
Δ7-Avenasterol	ND	ND	1.73 ± 0.01	17.04
24-Me-cycloartanol	ND	NA	4.17 ± 0.28	NA
Citrostadienol	ND	NA	ND	NA
Others	0.75 ± 0.02	Total 590.24	ND	Total 986.49

β-sitosterol (517.25 μg/g) make tiger nut oil a very nutritious and health enhancing oil. Thus tiger nut oil should be developed into a commercial product in the West African region for use in food products in place of imported olive oil. In contrast, asiato seed oil, *P. insignis*, is not recommended for use in food products on account of the reported toxicity of the cyclopropenoid FA, which was found in significant amount in the oil. Furthermore the occupancy of about 71.44% at the *sn*-2 position of the triacylglycerols makes asiato oil unsuitable for human consumption as the cyclopropenoid fatty acid will readily be absorbed into the blood stream. However the high oil yield (38.9%) in the *P. insignis* seed makes the seed a good candidate for commercial exploitation to produce oil for extraction of its moderate content of phytosterols (590.24 μg/g) for the functional food industry, and also for non-food uses such as soap/detergent manufacture, biodiesel, biolubricants and the like.

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