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Lipid Composition of Three Types of Vegetables: *Corchorus olitorius, Telferia occidentalis* and *Amaranthus hybridus*, Consumed In Nigeria

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Abstract

The levels of fatty acids, phospholipids and phytosterols were determined in three common vegetables consumed in Nigeria (*Corchorus olitorius, Telferia occidentalis* and Ama*ranthus hybridus*) on dry weight basis. Results showed that crude fat varied from 0.250-4.18 g/100g; SFA ranged from 15.3–23.0% of the total fatty acids, total monounsaturated fatty acids varied from 12.4-23.4% and PUFA ranged from 53.5–72.4%. Linoleic acid (C18:2 *cis*- 9,12) was the highest PUFA in all the three samples with a range of 28.2–31.5%. Among the phytosterols, sitosterol was highest in all the samples with a range of 0.126-0.202 mg/100g. The samples were genrally low in phospholipids, however phosphatidylcholine was significantly high in *Corchorus olitorius* with a value of 0.391 mg/100g. Statistical analysis showed that there were significant differences in LA/ALA, n-3 and n-6/n-3 values.

Keywords: *Fatty Acids; Phospholipids; Phytosterols; Vegetables*

List of Abbreviations

Eng : English; Sp : Spain; Nig: Nigeria; Fr: France; West Afr: West Africa; Syn: Synonyms; Ind: India; Philipp: Philippines; E1: *Corchorus olitorius*; U2: *Telfaria occidentalis*; AR3: *Amaranthus hybridus*; FAME: Fatty acid methyl esters; GC: Gas chromatography; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; CLA: Conjugated linoleic acid; LA: Linoleic acid; ALA: Alpha-linolenic acid; TFAs: Total Fatty acids; SCFAs: Short chain fatty acids; MCFAs: Medium chain fatty acids; VLCFAs: Very-long chain fatty acids; TC: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; CHD: Coronary Heart Disease; NIDDM: Non-insulin-dependent diabetes mellitus; PPARγ: Peroxisome proliferator-activated receptor gamma; GLA: Gamma linoleic acid; AHA: American Heart Association; EPSI: Essential PUFA status index; Lp(a): Lipoprotein(a);

Introduction

Proteins occur in animal as well as vegetable products in important quantities. In developed countries, people obtain much of their protein from animal products. In most parts of the developing world, the major portion of dietary protein is derived from plant products. Green vegetable has long been recognized [1,2] as the cheapest and most abundant potential source of protein because of its ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as sunlight, water, carbon dioxide, atmospheric nitrogen (as in legumes). Cassava leaves, a by-product of cassava root harvest are rich in protein (14-40% dry matter), minerals, vitamins B1, B2, C and carotenes [3,4]. Similarly, leaves of several tropical vegetables and leguminous browse plants such as *Glyricidia sepium* and *Leucaena leucocephala* which abound in most sub-Saharan Africa are rich in protein and minerals while the pro-vitamin A content is among the highest in plant species [5,6].

Three different types of vegetables are involved in this study. *Telfairia occidentalis* Hook. f. Common names are fluted pumpkin, fluted gourd, telfairia nut (Eng.); calabaza costillada (Sp.); iroko (Nig.); krobonko (Ghana); oroko, pondokoko, gonugbe (Sierra Leone). Centre of origin and distribution are tropical West Africa, not widely known in other parts of the tropics. Areas of cultivation are West Africa (Nigeria, Ghana, and Sierra Leone) [7]. The young vigorous shoot of the female plant are used as cooked vegetable. Seeds are ground for use in soups or boiled and eaten as a nut. Seeds contain 30% protein and a high percentage of non-drying oil. Leaf concentrate of *Telfairia occidentalis* and *Amaranthus hybridus* had earlier been prepared to determine their amino acid profiles [8]. *Corchorus olitorius* .L. Common names are long–fruited jute, tossa jute, jute mallow, Jews mallow, bush okra, West African sorrel (Eng.); corette potagère (Fr.); yute (Sp.); krin-krin (West Afr.); ewedu, oyo, eyo (Nig.). Centre of origin and distribution are probably south China, now found growing wild in parts of tropical Asia (India) and tropical Africa; naturalized in many tropical countries. Leaves and young shoots contain a high proportion of mucilage and are used as a cooked vegetable, in a similar manner to spinach greens. The protein content of young leaves is approximately 1.5%; that of older leaves varies from 5-6%. Harvested leaves may be dried and stored for significant periods [8]. *Amaranthus hybridus* subsp. *Cruentus* (syn.). Common names are African spinach, Indian spinach, spinach, amaranth, bush greens (Eng.); amarante (Fr.); bledo (Sp.); badi chauli, chota, chaulai (Ind.); kulitis (Philipp.); tete arowojeja (Nig.) [9]. Centre of origin and distribution are: many species probably originated in the Andean region of South America or Mexico and are now widely distributed throughout most tropical areas. An Indian centre of diversity has also been accepted by some authorities. The nutritional content of the leaves of various species of *Amaranthus* varies but, in general, the leaves of plants of most species contain a high level of vitamin A, calcium and potassium [7]; the nutritional contents of the leaves of several species of *Amaranthus* have also been reported by Grubben [10]. The seeds of *Amaranthus* species are used as food in some tropical areas and a protein content of up to 15% has been reported. High contents of lysine (6.2% of the total protein) and methionine (2.3%) have been recorded [7]. In the present research, the lipid profiles (fatty acids, phospholipids and phytosterols) of the dry leaves of *Corchorus olitorius* (E1), *Telfaria occidentalis* (U2) and *Amaranthus hybridus* (AR3) were reported.

Materials and Methods

Collection and treatment of samples

The vegetable samples [*Corchorus olitorius* (E1), *Telfaria occidentalis* (U2) and *Amaranthus hybridus* (AR3)] were collected from Iworoko Ekiti market, Ekiti State, Nigeria. The tender leaves were carefully plucked and air-dried, after which the dried leaves were ground into fine powder using pestle and mortar and stored in screw capped plastic containers prior to analysis.

Extraction of Lipid

Each sample (0.25g) was weighed into the extraction thimble. A volume of 200 mL of petroleum ether (40-60°C boiling range) was measured and added to the dried 250 mL capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled [11]. The lipid was extracted for 5 h. The extraction flask was removed from the heating mantle arrangement when it was almost free of petroleum ether. The extraction flask with the crude oil was oven dried at 105°C for the period of 1h. The flask containing the dried oil was cooled in the desicator and the weight of the cooled flask with the dried oil was taken.

Preparation of Methyl Esters and Analysis

The extracted fat (50 mg) was saponified for 5 min at 95°C with 34 mL of 0.5 M KOH in dry methanol The mixture was neutralized by 0.7 M HCl. A volume of 3 mL of 14 % boron trifluoride (BF_3) in methanol (14% v/v; Supelco Inc Bellefonte PA USA) was added [11]. The mixture was heated for 5 min at 90°C to achieve complete methylation process. All the fatty acid methyl esters (FAME) were extracted into redistilled n-hexane (2 x 3 mL). The content was concentrated to 1 mL for analysis and 1µL was injected into the injection pot of the GC. The FAME were analysed using a gas chromatograph (GC; HP 5890 Series II auto sampler 7673 powered with HP 3365 ChemStation; Hewlett-Packard Co Avondale PA USA) fitted with a flame ionization detector. Nitrogen was used as the carrier gas. The oven programme was: initial temperature at 60°C first ramping at 10°C/min for 20 min maintained for 10 min. The injection temperature was 250°C whilst the detector temperature was 320°C A polar (HP INNOWAX) capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness)

Bellefonte PA USA) was used to separate the esters Split injection was used having a split ratio of 20:1. The peaks were identified by comparison of their retention times with authentic standards of FAME.

Sterol analysis

The sterol analysis was as described by AOAC. (2005) [11]. Known weights of the aliquots of the extracted fat were added to the screw-capped test tubes. Each sample was saponified at 95°C for 30 min, using 3 mL of 10% KOH in ethanol, to which 0.20 mL of benzene had been added to ensure miscibility. Deionised water (3 mL) was added and 2 mL of hexane was used in extracting the non-saponifiable materials. Three extractions, each with 2 mL of hexane, were carried out for 1h, 30 min and 30 min respectively, to achieve complete extraction of the sterols. The hexane was concentrated to 1 mL in the vial for gas chromatographic analysis and 1 µL was injected into the injection pot of GC. The peaks were identified by comparison with standard sterols. The sterols were analysed using similar conditions as for fatty acid methyl ester analyses.

Phospholipid Analysis

Modified method of Raheja., *et al*. [12] was employed in the analysis of phospholipids. A weight of 0.01g of the extracted fat was added to each test tube. To ensure complete dryness of the oil for phospholipids analysis, the solvent was completely removed by passing a stream of nitrogen gas on the oil. A volume of 0.40 mL of chloroform was added to the tube followed by the addition of 0.10 mL of chromogenic solution. The tube was heated at 100°C in water bath for about 1 min and 20 sec. The content was allowed to cool to the laboratory temperature and 5 mL of hexane was added and the tube shaken gently several times. The solvent and the aqueous layers were allowed to separate. The hexane layer was recovered and concentrated to 1.0 mL for analysis. The phospholipids were analysed using an HP 5890 powered with HP gas chromatograph (HP 5890 powered with HP ChemStation rev. AO9.01 [1206] software [GMI, Inc, Minnesota, USA]) fitted with a pulse flame photometric detector. Nitrogen was used as the carrier gas with a flow rate of 20-60 mL/min. The oven programme was: initial temperature at 50°C, first ramping at 10°C/min for 20 min, maintained for 4 min, second ramping at 15°C/min for 4 min and maintained for 5 min. The injection temperature was 250°C whilst the detector temperature was 320°C. A polar (HP5) capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) was used to separate the phospholipids. Split injection type was used having a split ratio of 20:1. The peaks were identified by comparison with standard phospholipids.

Quality Assurance

Standard chromatograms were prepared for phytosterols, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for the standard mixtures and correlation coefficient was determined for each fatty acid, phytosterol and phospholipid. Correlation coefficient ≥ 0.95 was considered acceptable.

Calculation of Fatty Acid as Food per 100 G in Sample

At the data source and reference database levels, values for individual fatty acids are usually expressed as percentages of total fatty acids. At the user database level, values per 100g of food are required. A conversion factor derived from the proportion of the total lipid present as fatty acids is required for converting the percentages of total fatty acids to fatty acids per 100 g of food. Total lipid level (crude fat) was multiplied by conversion factor of 0.8. For fatty acids, precision is best limited to 0.1g/100g of fatty acids [13].

Statistical analysis

Statistical analysis [14] was carried out to determine the mean, standard deviation, coefficient of variation in per cent. Also calculated were the chi-square (X^2). The X^2 values were subjected to the Table (critical) value at $\alpha = 0.05$ to see if significant differences existed in the values of fatty acids, phytosterols and phospholipids between the vegetable samples.

Results and Discussion

Results

In Table 1 are shown the crude fat levels of the samples (dry weight). The range was 0.250-4. 18g/100g with E1 being the highest (4.18g/100g) and AR3 being the lowest (0.250g/100g). The variation was high between the three samples with the coefficient of

variation (CV%) being 121. The chi square (X^2) values showed no significant differences. The calculated total fatty acids (crude fat x 0.80) followed the trend as shown in the table while the calculated total energy followed similar CV% as in the crude fat whereas X^2 values of energy showed significant differences.

Parameter	E1	U2	AR3	Mean	SD	CV%	X^2	Remark
Crude fat (g/100g)	4.18	0.820	0.250	1.75	2.12	121	5.31	NS
Total Fatty acid (g/100g)[a]	3.34	0.656	0.200	1.40	1.70	121	4.11	NS
Energy (kJ/100g)	124	24.3	7.40	51.8	62.8	121	154	S

Table 1: *Crude fat and total fatty acid levels of the vegetable samples (g/100g dry weight). a = Crude fat x 0.8, SD = standard deviation, CV% = coefficient of variation, X^2 = Chi square S = significant, NS = not significant, E1 = Corchorus olitorius, U2 = Telfaria occidentalis, AR3 = Amaranthus hybridus.*

In Table 2, fatty acid profiles of the samples in % total fatty acids are shown. While C2:0, C3:0, C6:0 and C8:0 recorded not detected in all the samples, C5:0 recorded 0.00% in all the three samples whereas C10:0 recorded 0.00% only in E1 and AR3. The highest SFA came from C16:0 having a range of 11.0-14.9% with variation (CV%) of 15.1 whereas total SFA range was 15.3-23.0% and CV% of 26.4. This meant that the SFA values were generally low and close as shown by the low CV%. The MUFA (cis) levels were also low at 4.80-7.83% in the samples, these values were close and they have a CV% of 30.7. The MUFA (trans) levels were fairly higher than the levels reported for the cis, the levels being 7.61-15.6% with a CV% of 42.6. The C18:2 (cis-9, 12) had fairly high values in all the three samples with a range of 28.2-31.5% and a CV% of 5.53. This CV% indicated that the values were very close. Also C18:2 (cis-9, trans-11) (conjugated linoleic acid or CLA) were also of fairly high values that are closer to the levels of C18:2 (cis-9, 12), the range being 21.9-31.1% with a CV% of 17.3. All other PUFA values were very low in the samples contributing less than 1.00% with the exception of C18:3 (cis-6, 9, 12) and C18:3 (cis-9, 12, 15) in E1 and AR3 samples contributing between 4.36 and 6.80%; actually total PUFA ranged as 53.5 - 72.4% and CV% of 19.9.

Fatty Acids	E1	U2	AR3	Mean	SD	CV%
C2:0	-	-	-	-	-	-
C3:0	-	-	-	-	-	-
C4:0	4.91e-4	1.27e-2	0.00	4.40e-3	7.19e-3	164
C5:0	0.00	0.00	0.00	0.00	0.00	0.00
C6:0	-	-	-	-	-	-
C8:0	-	-	-	-	-	-
C10:0	0.00	5.04e-3	0.00	1.68e-3	2.91e-3	173
C12:0	3.89e-3	0.00	0.00	1.30e-3	2.25e-3	173
C14:0	4.28e-3	8.41e-3	3.38e-3	5.36e-3	2.68e-3	50.1
C16:0	14.9	12.9	11.0	12.9	1.95	15.1
C18:0	5.16	7.85	4.19	5.73	1.90	33.1
C20:0	2.03e-2	0.639	2.41e-2	0.228	0.356	156
C22:0	2.45e-2	1.38	3.19e-2	0.479	0.781	163
C24:0	4.57e-3	0.239	5.78e-3	8.31e-2	0.135	162
SFA total	20.1	23.0	15.3	19.5	5.13	26.4

C14:1 (*cis*-9)	3.61e-4	1.06e-2	5.29e-4	3.83e-3	5.86e-3	153
C16:1 (*cis*- 9)	1.39-4	3.02e-3	2.04e-4	1.12e-3	1.64e-3	147
C18:1 (*cis*-6)	0.658	3.52	0.579	1.59	1.68	106
C18:1 (*cis*-9)	4.47	4.29	4.22	4.33	0.129	2.98
C20:1 (*cis*-11)	2.30e-4	4.99e-3	3.37e-4	1.85e-3	2.72-3	147
C22:1 (*Cis*-13)	0.00	0.00	0.00	0.00	0.00	0.00
C24:1 (*cis*- 15)	0.00	0.00	0.00	0.00	0.00	0.00
MUFA (*cis*)	5.13	7.83	4.80	5.92	1.82	30.7
C18:1 (*trans*-6)	5.05	7.43	4.26	5.58	1.65	29.6
C18:1 (*trans*-9)	1.39	5.60	1.36	2.78	2.44	87.6
C18:1 (*trans*-11)	1.87	2.59	1.99	2.15	0.386	17.9
MUFA (*trans*)	8.31	15.6	7.61	10.5	4.48	42.6
C18:2 (*cis*-9,12)	28.2	31.5	30.1	29.9	1.66	5.53
C18:2 (*trans*-9,11)	27.1	21.9	31.1	26.7	4.61	17.3
C18:3 (*cis*-6, 9,12)	4.36	4.28e-2	5.48	3.29	2.87	87.2
C18:3 (*cis*-9,12,15)	6.80	7.25e-2	5.68	4.18	3.61	86.2
C20:2 (*cis*-11,14)	2.30e-4	4.99e-3	3.37e-4	1.85-3	2.72e-3	147
C20:3 (*cis*-8,11,14)	0.00	0.00	0.00	0.00	0.00	0.00
C20:3 (*cis*-11,14,17)	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 (*cis*- 5,8,11,14)	0.00	0.00	0.00	0.00	0.00	0.00
C22:2 (*cis*-13,16)	0.00	0.00	0.00	0.00	0.00	0.00
C20:5 (*cis*-5,8,11,14,17)	0.00	0.00	0.00	0.00	0.00	0.00
C22:6 (*cis*- 4,7,10,13,16,19)	-	0.00	0.00	0.00	0.00	0.00
PUFA total	66.5	53.5	72.4	64.1	12.9	19.9

Table 2: *Fatty acids composition of the three vegetable samples (% total fatty acids).*

In Table 3, the fatty acids calculated as food lipids sources are shown. Categories of fatty acids of significance in lipid food composition were: C16:0 (0.500 g/100 g) (only in E1), C18:0 (0.172 g/100g), C18:2 (cis- 9, 12), (0.942 g/100g) only in E1, and C18:2 (cis-9, trans-11) (0.905 g) (only in E1); total SFA (3.05e-2-0.672 g/100g); MUFA cis (9.60 e-3 -0:171g/100g); MUFA trans (1.52e-2 -0.278g/100g) and total PUFA (0.145-2.22 g/100g).

Fatty Acids	E1	U2	AR3	Mean	SD	CV%
C2:0	-	-	-	-	-	-
C3:0	-	-	-	-	-	-
C4:0	1.64e-5	8.33e-5	0.00	3.32e-5	4.41e-5	133
C5:0	0.00	0.00	0.00	0.00	0.00	0.00
C6:0	0.00	0.00	0.00	0.00	0.00	0.00
C8:0	0.00	0.00	0.00	0.00	0.00	0.00
C10:0	0.00	3.31e-5	0.00	1.10e-5	1.91e-5	173
C12:0	1.30e-4	0.00	0.00	4.33e-5	7.50e-5	173

C14:0	1.43e-4	5.52e-5	6.76e-6	6.83e-5	6.90e-5	101
C16:0	0.500	8.46e-2	2.20e-2	0.201	0.258	128
C18:0	0.172	5.15e-2	8.38e-3	7.74e-2	8.50e-2	110
C20:0	6.78e-4	4.19e-3	4.82e-5	1.64e-3	2.23e-3	136
C22:0	8.18e-4	9.05e-3	6.38e-5	3.31e-3	4.99e-3	151
C24:0	1.53e-4	1.57e-3	1.16e-5	5.77e-4	8.61e-4	149
SFA total	0.672	0.151	3.05e-2	0.285	0.352	124
C14:1 (*cis*-9)	1.21e-5	6.95e-5	1.06e-6	2.76e-5	3.68e-5	134
C16:1 (*cis*- 9)	4.64e-6	1.98e-5	4.08e-7	8.29e-6	1.02e-5	123
C18:1 (*cis*-6)	2.20e-2	2.31e-2	1.16e-3	1.54e-2	1.24e-2	80.2
C18:1 (*cis*-9)	0.149	2.81e-2	8.44e-3	6.20e-2	7.63e-2	123
C20:1 (*cis*-11)	7.68e-6	3.27e-5	6.74e-7	1.37e-5	1.69e-5	123
C22:1 (*Cis*-13)	0.00	0.00	0.00	0.00	0.00	0.00
C24:1 (*cis*- 15)	0.00	0.00	0.00	0.00	0.00	0.00
MUFA (*cis*)	0.171	5.14e-2	9.60e-3	7.74e-2	8.87e-2	115
C18:1 (*trans*-6)	0.169	4.87e-2	8.52e-3	7.53e-2	8.33e-2	111
C18:1 (*trans*-9)	4.64e-2	3.67e-2	2.72e-3	2.86e-2	2.30e-2	80.2
C18:1 (*trans*-11)	6.25e-2	1.70e-2	3.98e-3	2.78e-2	3.07e-2	110
MUFA (*trans*)	0.278	0.102	1.52e-2	0.132	0.137	104
C18:2 (*cis*-9,12)	0.942	0.207	6.02e-2	0.403	0.472	117
C18:2 (*trans*-9,11)	0.905	0.144	6.22e-2	0.370	0.465	126
C18:3 (*cis*-6, 9,12)	0.146	2.81e-4	1.10e-2	5.23e-2	8.10e-2	155
C18:3 (*cis*-9,12,15)	0.227	4.76e-4	1.14e-2	7.97e-2	0.128	161
C20:2 (*cis*-11,14)	7.68e-6	3.27e-5	6.74e-7	1.37e-5	1.69e-5	123
C20:3 (*cis*-8,11,14)	0.00	0.00	0.00	0.00	0.00	0.00
C20:3 (*cis*-11,14,17)	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 (*cis*- 5,8,11,14)	0.00	0.00	0.00	0.00	0.00	0.00
C22:2 (*cis*-13,16)	0.00	0.00	0.00	0.00	0.00	0.00
C20:5 (*cis*-5,8,11,14,17)	0.00	0.00	0.00	0.00	0.00	0.00
C22:6 (*cis*- 4,7,10,13,16,19)	-	0.00	0.00	0.00	0.00	0.00
PUFA total	2.22	0.351	0.145	0.905	1.15	127

Table 3: *Fatty acid (g/100 g) of vegetable samples as food.*

The energy contribution of the fatty acids in the vegetable samples is shown in Table 4. The energy contributions were as varied as the fatty acids distribution. The contributions were total SFA (1.13-5.52 kJ/100g); MUFA cis (0.355-6.34 kJ/100g); MUFA trans (0.563-10.3 kJ/100g) and total PUFA (5.35-82.1kJ/100g) whereas contribution in PUFA due to LA was (41.7–58.8%).

Fatty Acids	E1	U2	AR3	Mean	SD	CV%
C2:0	-	-	-	-	-	-
C3:0	-	-	-	-	-	-
C4:0	6.07e-4	3.08e-3	0.00	1.23e-3	1.63e-3	133
C5:0	0.00	0.00	0.00	0.00	0.00	0.00
C6:0	0.00	0.00	0.00	0.00	0.00	0.00
C8:0	0.00	0.00	0.00	0.00	0.00	0.00
C10:0	0.00	1.22e-3	0.00	4.08e-4	7.06e-4	173
C12:0	4.81e-3	0.00	0.00	1.60e-3	2.78e-3	173
C14:0	5.29e-3	2.04e-3	2.50e-4	2.53e-3	2.55e-3	101
C16:0	18.4	3.13	0.814	7.45	9.56	128
C18:0	6.38	1.91	0.310	2.86	3.14	110
C20:0	2.51e-2	0.155	1.78e-3	6.07e-2	8.26e-2	136
C22:0	3.03e-2	0.335	2.36e-3	0.123	0.185	151
C24:0	5.65e-3	5.80e-2	4.28e-4	2.14e-2	3.18e-2	149
SFA total	24.9	5.59	1.13	10.5	12.6	120
C14:1 (*cis*-9)	4.46e-4	2.57e-3	3.91e-5	1.02e-3	1.36e-3	133
C16:1 (*cis*- 9)	1.72e-4	7.33e-4	1.51e-5	3.07e-4	3.77e-4	123
C18:1 (*cis*-6)	0.813	0.854	4.28e-2	0.570	0.457	80.2
C18:1 (*cis*-9)	5.52	1.04	0.312	2.29	2.82	123
C20:1 (*cis*-11)	2.84e-4	1.21e-3	2.49e-5	5.07e-4	6.24e-4	123
C22:1 (*Cis*-13)	0.00	0.00	0.00	0.00	0.00	0.00
C24:1 (*cis*- 15)	0.00	0.00	0.00	0.00	0.00	0.00
MUFA (*cis*)	6.34	1.90	0.355	2.86	3.11	108
C18:1 (*trans*-6)	6.24	1.80	0.315	2.79	3.08	111
C18:1 (*trans*-9)	1.72	1.36	0.101	1.06	0.849	80.2
C18:1 (*trans*-11)	2.31	0.629	0.147	1.03	1.14	110
MUFA (*trans*)	10.3	3.79	0.563	4.87	4.94	101
C18:2 (*cis*-9,12)	34.9	7.65	2.23	14.9	17.5	117
C18:2 (*trans*-9,11)	33.5	5.32	2.30	13.7	17.2	126
C18:3 (*cis*-6, 9,12)	5.39	1.04e-2	0.406	1.93	3.00	155
C18:3 (*cis*-9,12,15)	8.40	1.76e-2	0.420	2.95	4.73	160
C20:2 (*cis*-11,14)	2.84e-4	1.21e-3	2.49e-5	5.07e-4	6.24e-4	123
C20:3 (*cis*-8,11,14)	0.00	0.00	0.00	0.00	0.00	0.00
C20:3 (*cis*-11,14,17)	0.00	0.00	0.00	0.00	0.00	0.00
C20:4 (*cis*- 5,8,11,14)	0.00	0.00	0.00	0.00	0.00	0.00
C22:2 (*cis*-13,16)	0.00	0.00	0.00	0.00	0.00	0.00
C20:5 (*cis*-5,8,11,14,17)	0.00	0.00	0.00	0.00	0.00	0.00
C22:6 (*cis*- 4,7,10,13,16,19)	0.00	0.00	0.00	0.00	0.00	0.00
PUFA total	82.1	13.0	5.35	33.5	42.4	127

Table 4: *Energy contribution (kJ/100g) of the fatty acids in the vegetable samples.*

Some calculated quality parameters from Table 2 are shown in Table 5. These calculated parameters predicted the nutritional qualities of the vegetables in their lipid compositions. The X^2 analyses of the parameters showed some of them to be significantly different at $\alpha = 0.05$.

Parameter	E1	U2	AR3	Mean	SD	CV%	X^2	Remark
SFA	20.1	23	15.3	19.5	3.89	20.0	1.55	NS
$MUFA_{cis}$	5.13	7.83	4.8	5.92	1.662	28.1	0.934	NS
$MUFA_{trans}$	8.31	15.6	7.61	10.5	4.43	42.1	3.73	NS
MUFA total	13.4	23.4	12.4	16.4	6.08	37.09	4.51	NS
PUFA	66.5	53.5	72.4	64.1	9.67	15.08	2.92	NS
LA/ALA	4.15	434	5.3	148	247.8	168	830	S
n-6	59.7	53.4	66.7	59.9	6.65	11.1	1.48	NS
n-3	6.80	7.25e-2	5.68	4.18	3.61	86.2	6.22	S
n-6/n-3	8.78	737	11.7	253	420	166	1392	S
PUFA/SFA	3.31	2.33	4.73	3.46	1.21	34.9	0.842	NS
MUFA/SFA	0.669	1.02	0.811	0.833	0.177	21.2	0.0748	NS
EPSI	4.96	2.29	0.584	2.61	2.21	84.5	3.73	NS

EPSI = Essential PUFA status index, S = significant, NS = not significant, X^2 = Chi square.

Table 5: *Some calculated parameters on fatty acids in the vegetable samples.*

The cholesterol level was in trace amount in each sample as shown in Table 6 (2.52 e-3-8.87-3 mg/100g), stigmasterol (6.25e-3-9.79e-3 mg/100g). Sitosterol had values higher than 0.100 mg/100g (0.126-0.200 mg/100g) while ergosterol, campesterol and 5-avenasterol all have a value of 0.00 mg/100 g each in the samples. These values were widely varied with a CV % of 22.2-173.

Sterols	E1	U2	AR3	Mean	SD	CV%
Cholesterol	6.07e-3	2.52e-3	8.87e-3	5.82e-3	3.18e-3	54.7
Cholestanol	0.00	8.78e-3	1.89e-2	9.23e-3	9.46e-3	103
Ergosterol	0.00	0.00	1.43e-2	4.77e-3	8.26	173
Campesterol	0.00	0.00	1.42e-2	4.73e-3	8.20e-3	173
Stigmasterol	6.25e-3	9.79e-3	8.91e-3	8.32e-3	1.84e-3	22.2
5- Avenasterol	0.00	0.00	0.00	0.00	0.00	0.00
Sitosterol	0.126	0.202	0.138	0.155	4.09e-2	26.3
Total	0.138	0.223	0.203	0.188	4.44e-2	23.6

Table 6: *Phytosterols Levels of the vegetable samples (mg/100g).*

Phospholipids levels in the samples are depicted in Table 7. All samples have low levels of phospholipids having values of 1.29 e-3 - 0.391 mg/100g. The values were also highly differently distributed as shown in the CV % of 36.3-132.

Phospholipids	E1	U2	AR3	Mean	SD	CV%
Phosphatidylethanolamine	1.50e-3	1.29e-2	7.95e-3	7.45e-3	5.72e-3	76.7
Phosphatidylcholine	0.391	3.90e-2	3.53e-2	0.155	0.204	132
Phosphatidylserine	1.34e-2	1.21e-2	6.20e-2	1.06e-2	3.84e-3	36.3
Lysophosphatidylcholine	3.88e-2	2.72e-2	8.03e-3	2.47e-2	1.56e-2	63.0
Phosphatidylinositol	1.95e-2	5.03e-3	1.68e-3	8.74e-3	9.47e-3	108
Total	0.477	9.61e-2	5.91e-2	0.211	0.231	110

Table 7: *Phospholipids levels of the vegetable samples (mg/100g).*

Discussion

The levels of crude fat had the value in E1 (*Corchorus olitorius*) better than the value reported for sorghum grain (1.83g/100g) and in other cereals: millet (1.10g/100g), maize (1.72g/100g) and rice (0.63g/100g) [15]; *Ficus asperifolia* (2.78g/100g, *Ficus sycomorus*) (3.00g/100g), [16], *Sesamum indicum* (1.66g/100g), *Balanites aegyptiaca* (2.90g/100g) [17]. Generally the values were also comparably lower than those reported for co-fermented wheat/ cowpea (6.35g/100g) and wheat (5.61g/100g) [18]. Since the crude fat levels were relatively low the vegetables could not be said to be major sources of dietary fat. The total fatty acid (TFA) profiles showed that E1 had the highest level of (TFA) (3.34g/100g) and lowest in AR3 with a value of 0.200g/100g dry weight. Both crude fat and total fatty acids were not significantly different in their group when subjected to X^2 analysis at $\alpha = 0.05$ whereas there was significant difference in the calculated energy levels based on X^2 analysis.

The most abundant fatty acid in nature is usually the palmitic acid (C16:0) and it is found in appreciable amounts in the lipids of animals, plants and lower organisms. It is present in amounts that vary from 10-40% in seed oils [19]; C16:0 was the highest concentrated SFA in the samples and varied from 11.0-14.9%. The values of 15.3-23.0% (total SFA) were close to the values of 16.6-18.3% in the grains of treated sorghum bicolor [20] and 14.8-19.3% in the seeds of three types of chilies [19]. Stearic acid (C18:0) was the second most abundant SFA in nature, and again it is found in the lipids of most living organisms; this observation supports our present report in which C18:0 value ranged as 4.19-7.85% as the second most concentrated SFA in the samples. Fatty acids of 4-6 carbon atoms are usually called short chain fatty acids (SCFAs); in all the samples, while C4:0 levels were in trace, C5:0 had 0.00 % in all the samples and C6:0 was not detected in any of the samples. Usually, fatty acids of between 8–12 carbon atoms are often regarded as medium chain fatty acids (MCFAs) and are found mostly in butterfat and the tropical oils [21].While C8:0 was not detected in the samples, negligible levels were recorded for C10:0 and C12:0, the values ranged between 0.00-3.89e-3%. Long-chain fatty acids have from 14-18 carbon atoms and can either be SFA, MUFA or PUFA, for example stearic acid is an 18 – carbon atom (a SFA), and oleic acid is an 18 – carbon atom MUFA. Another MUFA is the 16–carbon atom palmitoleic acid which has strong antimicrobial properties (in the present samples it ranged from 1.39e-4-3.02e-3%). Very-long-chain fatty acids (VLCFAs) have 20-24 carbon atoms. In the present samples, fatty acids within this bracket recorded 0.00% in all the samples. Some people can make these fatty acids from EFAs, but others, particularly those whose ancestors ate a lot of fish, lack enzymes to produce them. These "obligate carnivores" must obtain them from animal food such as organ meats, egg yolks, butter and fish oils [21,22].

Some benefits have been attributed to SFA. They include: SFA constitute at least 50% of cell membranes, they are what give our cells necessary stiffness and rigidity; they play a vital role in the health of our bones, for calcium to be effectively incorporated into the skeletal structure, at least 50% of the dietary fats should be saturated [23]; they lower Lp(a), a substance in the blood that indicates proneness to heart disease [24], they protect the liver from alcohol and other toxins, such as Tylenol [21]; they enhance the immune system [25]; they are needed for the proper utilization of EFAs. Elongated omega-3 FAs are better retained in the tissue when the diet is rich in SFA [19,20], C16:0 and C18:0 are the preferred foods for the heart, which is why the fat around the heart muscle is highly saturated [24,26], the heart draws on this reserve of fat in times of stress. Short and medium-chain SFAs have important antimicrobial

properties; they protect us against harmful microorganisms in the digestive tract. The much maligned SFA may not be the cause of our modern diseases [20]. As opined by Adeyeye., *et al*. [19], the concept of 'a balance' among classes of SFA, MUFA, and PUFA is the issue of which specific SFA or PUFA are best. Many studies have suggested that SFA raise TC, LDL, and that PUFA lowers them. But certain SFA (as consumed in our daily diets) are better than others in terms of their impact on the LDL/HDL ratio. Fats rich in C12:0 and C14:0 (e.g. milk fat, coconut oil and palm kernel oil) raise LDL the most. Stearic acid (C18:0) is not very prevalent in saturated fats, but it is neural in its effect on blood cholesterol when consumed in natural fats. The most common SFA is palmitic acid (C16:0), so-named because it represents the major SFA in palm oil. The C16:0 SFA is present to some degree in essentially all fats and is by far the most prevalent SFA in our diets. Considering the influence on the lipoprotein profile, palmitic acid is intermediate, i.e. it can be neutral when placed on a triglyceride molecule with MUFA, PUFA, or stearic acid, or cholesterol-raising when attached along with C12:0 and C14:0. In high amounts, palmitic acid can even raise the TC and LDL when substituted for C18:0, MUFA or PUFA in people who already have elevated TC or eat large amount of cholesterol [22,27]. Accordingly, the general advice has been to remove as much as SFA from the diet as possible. But this is not practical because the manufacture of many food products requires SFA and extreme removal of dietary SFA is not prudent because their exclusion from the diet surprisingly exerts an adverse effect on the LDL/HDL ratio [22]. It is therefore an advantage to note that C12:0 had approximately 0.00% in all the samples, C14:0 had values that ranged from 3.38e-3–8.41e-3% whereas C16:0 had values between 11.0-14.9%.

Oleic acid is by far the most abundant monoenoic fatty acid in plant and animal tissues, both in structural lipids and in depot fats. For example, it can comprise 30-40% of the total fatty acids in adipose fats of animals, and 20-80% of the seed oils of commerce. Olive oil contains up to 78% of oleic acid, and it is believed to have especially valuable nutritional properties as part of the Mediterranean diet. Indeed, it has a number of important biological properties both in the free and esterified form. Oleic acid is the biosynthetic precursor of a family of fatty acids with the (n-9) terminal structure and with chain-lengths of 20-24 or more.

The oleic acid constituted a level of 54.8-87.9% among the MUFA *cis* in all the samples. According to Kris-Ethaton. [28], there is epidemiological evidence that dietary MUFA have a beneficial effect on the risk of CHD. Moreover, evidence from controlled clinical studies has shown that MUFAs favourably affect a number of risk factors for CHD, including plasma lipids and lipoproteins, factors related to thrombogenesis, *in vitro* LDL oxidative susceptibility (even when compared with PUFA), and insulin sensitivity. Compared with SFA, MUFAs lower total and LDL cholesterol levels and decrease plasma triglyceride levels. However, additional research is required in humans and appropriate animal models to get a better and clear understanding of the effects of high-MUFA diets on atherogenesis [28,29]. A diet high in MUFA (in a close comparison with high-carbohydrate diet) improves glycemic control in individuals with NIDDM who maintains a normal body weight. It was then the submission of Kris-Ethaton. [28] that individuals with elevated triglycerides or insulin may also benefit from a high-MUFA diet.

In the present report petroselinic acid occupied the second position with values range of 0.579-3.52% of total fatty acids in the samples .Studies in vitro by Weber., *et al*. [30] revealed that triacylglycerols containing petroselinoyl [18:1 (n-12)] moieties are hydrolysed by pancreatic lipase at much lower rates than other triacylglycerols. Consumption of coriander (*Coriandrum sativum*) oil compared with other oils, led to significantly greater liver weights. No significant differences were observed among the groups fed various levels of oleic acid in body weight, the weights of heart, liver, kidneys, spleen or testes, lipid content of heart, or total cholesterol, HDL cholesterol and triacylglycerols concentration of blood plasma. Ingestion of coriander oil led to the incorporation of 18:1 (n-12) into heart, liver and blood lipids and to a significant reduction in the concentration of arachidonic acid in the lipids of the heart, liver and blood with a concomitant increase in the concentration of linoleic acid compared with the results for the other groups. The data show that petroselinic acid from dietary triacylglycerols is absorbed by rats as readily as oleic acid, but the former reduces the concentration of arachidonic acid in tissue lipids suggests [in view of the earlier studies] [31], petroselinic acid- mediated inhibition of arachidonic acid synthesis.

The total MUFA (*trans*) levels in the vegetable samples ranged as 7.61-15.6% which is almost twice the levels of MUFA cis isomers. *Trans* fatty acids are generated when vegetable oils are partially hardened by hydrogenation to replace naturally occurring SFAs in the diet. Because trans fatty acids typically are monounsaturated, it is thought that they exerted a neutral effect on cholesterol metabolism and other biological functions. However, more recent data have revealed a negative influence on lipoproteins and possibly other functions as well. To examine this point more directly, *trans* 18:1 n-9 (elaidic acid) was compared head-to-head with the most cholesterol-raising saturated fat and the neutral cis 18:1 n-9 (oleic acid) in humans [32]. Diets enriched in C18:3 n-3 or C22:6 n-3 have been shown to exert a significant anti-CHD effect in humans both in clinical and epidemiological studies [22]. While 18:2 n-6 was moderately high in the samples (28.2-31.5%), 18:3 n-3 was in traces or very low (4.28e-2-5.48%).

The term conjugated linoleic acid (CLA) refers to a group of constitutional and stereoisomers of linoleic acid (LA, cis -9, cis-12 octadecanoic acid). The two bonds of CLA may be in the 7,9;8,10;9,11;10,12; or 11,13 positions [33]. These double bonds may occur in every geometric configuration. Naturally occurring dietary CLA is composed primarily of the cis-9, trans-11 isomer [34], also known as rumenic acid [35], with trans-10, cis- 12 CLA comprising a smaller portion and other isomers present in trace amounts.

The majority of CLA in the human diet comes from the meat and milk products of ruminant animals, especially beef and products from cow's milk such as cheese and butter. Most CLA is produced in the rumen by hydrogenation of LA to cis-9, trans-11-CLA [36]. A small portion of cis-9, trans-11-CLA is then directly absorbed into surrounding tissue [37], while most is hydrogenated at the 9-position to yield vaccenic (trans-11- octadecanoic) acid [38]. Vaccenic acid is then absorbed into tissues, where it can be turned back into cis-9, trans-11-CLA by the Δ-9-desaturase enzyme. One study found that this conversion also occurs in human tissues, with an average of 19% of dietary vaccenic acid being converted to cis-9, trans-11-CLA [39]. The findings of many recent, isomer-specific experiments strongly suggest that some effects of CLA are isomer-specific or may result from synergistic action of multiple, independent isomer-specific mechanisms [40].

The CLA values in the present results ranged from 21.9 to 31.1% whereas LA values ranged from 28.2-31.5%; the values for these two fatty acids were very close and CLA formed the second largest values of all n-6 fatty acids in the samples. Vaccenic acid (CLA precursor) values were also appreciable in the samples with range value of 1.87-2.59%. Ruminants and human beings eating these vegetables would have good supply of both vaccenic acid and CLA from them.

The human health effects of CLA became the subject of widespread inquiry following a study in which Ha., *et al*. [41] first reported its inhibitory effects on mouse epidermal neoplasia. Since that time, a large body of *in vitro* and animal research has accumulated to suggest that CLA promotes various aspects of human health [42]. Popular attention to these findings has led to the marketing of CLA as dietary supplement. Some benefits of CLA as a general dietary supplement are listed bellow [43].

1. CLA shows beneficial effects in animal models of atherosclerosis, but its effects on cholesterol ratios appear to vary by isomer.
2. CLA may reduce markers of diabetes through a PPARγ-dependent pathway in animals, but some animal and human studies have shown deleterious effects.
3. CLA usually reduces fat depot mass in various animal models, possibly by inhibiting lipoprotein lipase-mediated lipid uptake into adipocytes.
4. CLA inhibits initiation and growth of breast, colorectal, prostate and skin cancers in animal models, but findings from studies of human CLA intake and cancer incidence are inconclusive.

Another important long-chain fatty acid is gamma-linolenic acid (GLA) (C18:3 cis-6, 9, 12). It formed a level of 4.36% (E1), 5.48% (AR3) but trace level of 4.28 e-2% (U2). It formed the third largest group of the n-6 fatty acids. It is found in evening primrose, borage and black currant oils. The body makes GLA out of omega-6 linoleic acid and uses it in the production of substances called prostaglandins, localized tissue hormones that regulate many processes at cellular level.

The samples as food sources revealed the level of fatty acids when a particular quantity (g/100g) of vegetable oil is consumed as food. The information is necessary to be able to calculate the energy contribution by each type of fatty acid. As expected, the variation in the concentrations of fatty acids as food went as (g/100g): SFA (3.05e-2–0.672) > MUFA cis (9.60e-3–0.171) < PUFA (0.145–2.22). The energy contributions from the fatty acids in the samples were generally low. Summary of the energy contributions in kJ/100g were as follows: SFA (1.13 -24.9) (15.3 – 23.0%); MUFA *cis* (0.355–6.34) (4.79–15.6%) and PUFA (5.35–82.1) (53.4–72.3%). In the samples total energy intake from these samples as contributed from SFA was greater than 10% energy whereas the recommended range of ADMR (acceptable micronutrient distribution range) for PUFA is 6.11% energy [44] which is less than the values obtained in our results, the implication of this is that results from our samples could lead to the replacement of SFA with PUFA in the diet.

The original American Heart Association (AHA) step I fat recommendation recognizes the significance of the fatty acid balance at approximately 1:1:1 for SFA: MUFA: PUFA. There are several reports from the literatures which actually support the importance of this balance for generating the best LDL/HDL ratio. As observed by Adeyeye., *et al*. [19], it would appear that the balance is critical at any level of fat intake if one wishes to avoid adversely affecting the lipoprotein profile, the view that was also supported by Hayes. [22]. The best dietary fat would contain an ideal balance (7:1) of n-6 linoleic to n-3 linolenic acids. This balance is not available in partially hydrogenated margarines in which most of the n-3 linolenic acid has been destroyed by processing, and is also unlike most vegetable oils that contain only a small amount of this important fatty acid (22). The results obtained for LA/ALA in the present report were in the range of 4.15 - 434 (i.e. 4.15 for E1, 434 for U2 and 5.3 for AR3), while results for E1 and AR3 were in agreement with the recommended ratio (7:1), 434:1for U2 was at sharp variance with the recommended ratio. The reason had been due to high level of LA and low level of ALA in the sample. The ratio of PUFA/SFA (P/S) is important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by SFA and PUFA fats [45,29]. The ratio of PUFA to SFA in the present report varied between 2.33 and 4.73. One can opine that these proportions are positive towards PUFA as compared with the SFAs. The EPSI values ranged between 0.584-4.96. This has further demonstrated the good nutritional status of the fatty acids.

The animal sterols were significantly very low, the range being 0.00–9.79e-3 mg/100g for cholesterol, cholestanol, ergosterol and 5-avenasterol. 0.00 mg/100g was recorded for campesterol in E1 and U2 and trace amount in AR3, values ranging between 6.25e-3-9.79e-3 mg/100g were recorded for stigmasterol in all the samples while only sitosterol had a value higher than 0.10mg/100g in all the samples, the range being 0.126–0.202 mg/100g.

Plant sterols are C-28 or C-29 sterols, differing from cholesterol (C-27) by the presence of an extra methyl or ethyl group on the cholesterol side chain. Cholesterol is an essential component of cell membranes in higher animal species. Plant sterols play an analogous role in plants; their content is highest in edible oils, seeds and nuts [46]. The major dietary sterols are sitosterol (C-29), campesterol (C-28) and stigmasterol (C-29). These represent < 50% of the total intake of sterols in the Western diets; the remainder is cholesterol [47]; this is an exact opposite of the situation in Nigeria where more vegetable food sources are consumed. The most common dietary plant stanol, sitostanol, is a saturated derivative of sitosterol. It occurs naturally in wood pulp, tallow oil and, in lesser amounts, in soybean oil. The Western daily diet contains 100-300 mg plant sterols and 20-50 mg plant stanols [48]. Only 1.5-5% of sitosterol is absorbed when typical amounts of sterols are consumed (240-320 mg) [49]. Cholesterol absorption is much more efficient, with between 20 and 80% of dietary cholesterol absorbed. Differential absorption rates among plant sterols are related to the length of the side chain. The longer the side chain of the sterol, the less is absorbed because of its increased hydrophobicity [50]. This wide range in a normal population suggests considerable individual variability in the handling of various plant sterols. Due to some health reasons, the lowest serum levels of sterols are desirable [51]. Thus, Lees and Lees. [52] suggested that plant sterol preparations that contain more absorbable sterols such as campesterol should not be recommended for therapeutic use.

Plant sterols interfere with the uptake of both dietary and biliary cholesterol from the intestinal tract in humans [50]. The reason for this is not fully understood; however, plant sterols appear to decrease the solubility of cholesterol in the oil and micellar phases,

thus displacing cholesterol from bile salt micelles and interfering with its absorption [53]. In humans, intestinal infusion of sitostanol was more efficient in reducing cholesterol absorption than infusion of sitosterol (-85% and -50%, respectively) [50]. In addition, Becker., *et al.* [54] showed that 1.5 g/d of sitostanol increased fecal secretion of neutral and acid steroids more effectively (88 %) than did 6 g/d of sitosterol (45%).

The levels of phospholipids in the samples were very low, the range being: 1.68e-3–0.391 mg/100g. With these levels, they could not have been thought to contribute much to the nutritional qualities of the vegetables.

Conclusion

The samples were generally low in crude fat but very good in PUFA > SFA > MUFA. The following quality parameters were very good: n-6/n-3 particularly for E1 and AR3, PUFA/SFA, MUFA/SFA and EPSI. The samples were virtually devoid of sterols and phospholipids. Samples will therefore be good for people with heart diseases.

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