International Food Research Journal 24(2): 696-702 (April 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Fatty acid characterisation, sterol composition and spectroscopic analysis of selected Cucurbitaceae seed oils

[1*]Ogunbusola, E. M., [2]Fagbemi, T. N. and [2]Osundahunsi, O. F.

[1]*Department of Food Science and Technology, Federal University Oye-Ekiti, Ekiti State, Nigeria*
[2]*Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria*

Article history

Received: 5 October 2015
Received in revised form: 8 April 2016
Accepted: 14 April 2016

Keywords

Lagenaria siceraria
Cucumeropsis mannii
Fatty acid composition
Phytosterol
Spectroscopic analysis

Abstract

The need to exploit more plant food for value addition and food security necessitated investigation into three species of Cucurbitaceae for fatty acid, sterol and spectroscopic analysis. The seeds of two varieties of *Lagenaria siceraria* (calabash and bottle gourd) and white melon *(Cucumeropsis mannii*) were processed into flour, defatted and the oils extracted from the seed flours were analyzed for fatty acid, sterol composition, proton and ^{13}C NMR and Infra Red spectroscopy. The predominant fatty acid group in the seed oils was unsaturated fatty acid. The Cis-linoleic acid was found to be the most abundant fatty acid in the seed oils (59.47 – 60.40 %). Phytosterols identified in the seed oils were campesterol (12.20 – 50.58 mg/100g), stigmasterol (4.47 – 5.98 mg/100g) and β-sitosterol (205.81 – 300.04 mg/100g) while cholesterols were not detected in the seed oils. The proportions of monounsaturated, polyunsaturated and saturated acyl groups were predicted from the frequency of some IR bands and these were in agreement with the values obtained using GC. The FTIR spectra of the oils showed that the wave numbers of many of the bands were dependent on the nature of the seed oils. The $^{+}$H NMR spectra of the seed oils confirmed that *Cucumeropsis mannii* seed oil contain less polyunsaturated fatty acid than the two varieties of *Lagenaria siceraria* and that the seed oils contain insignificant amount of linolenic acid. *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils have potential for both domestic and industrial applications.

Introduction

Vegetable oils are essential components of the diets, they are constituents of many manufactured foods such as ice cream, cookies, margarine and are also used for cooking. Oils differ in their composition and this factor significantly affected the organoleptic properties, stability and influence on consumer health. Therefore edible oil characterization is a very important task which has been carried out by different chemical and chromatographic methods (Milovanovic and Picuric-Jovanovic, 2005; Stevenson et al., 2007; Chinyere *et al.*, 2009; Aboidun and Adeleke, 2010; Rezig *et al*., 2012). Numerous methods have been developed to analyze fatty acid composition of lipids. Gas chromatography has long remained the routine method for the identification of oils and fatty acid profile determination despite their tediousness.

Spectroscopic techniques have been used extensively for identification of oils. The technique is fast, usually requiring little time and cost, and provide a great deal of information with only one test (Che Man and Rohman, 2013). Fourier Transform Infrared FTIR spectroscopy has been used to characterise some tropical vegetable oils (Akintayo *et al*., 2002; Aremu *et al*., 2006; Oyewusi *et al*., 2007), to detect and quantify palm kernel oil (PKO) (Manaf, 2007), palm oil, corn and sunflower oils (Rohman and Che Man, 2011), to assess olive oil adulteration (Poiana *et al*., 2012), and for detection of milk adulteration (Nicolaou *et al.*, 2010).

Vegetable oils are utilized globally for many food and other industrial purposes. Despite the vast range of sources for vegetable oils, world consumption is dominated by soybean, palm, rapeseeds and sunflower oils (Stevension *et al.*, 2007). Due to increasing demand for oil both as domestic and industrial functional ingredients, it is needful to exploit the potentials of some less known underutilized cucurbit oil seeds. *Lagenaria siceraria* (Calabash and bottle gourd) and *Cucumeropsis mannii* (White melon) belong to the Cucurbitaceae family. They grow as creeper or climber in wet humid climate particularly in the South western Nigeria. Currently, *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils are not used commercially even though they have

*Corresponding author.
Email: *eunice.ogunbusola@fuoye.edu.ng*

characteristics that are well suited for industrial applications and can contribute to healthy human diets. They are grown in Nigeria for their oil bearing seeds which are sometimes fermented to yield a product known as 'ogiri' – a local condiment used in a variety of local foods or used as thickeners in local soups. Studies on Cucurbitaceae oils have been limited to those of *Colocynthis citrullus, Citrullus vulgaris*, and pumpkin (Stevension *et al*., 2007; Rezig *et al*., 2012). There is need to exploit the potentials of these underutilized oil seeds that are almost going into extinction particularly in South Western Nigeria. This will necessitate analysis of their oils and residual cake to ascertain their nutritional benefits and to promote their utilization in food industries. Our previous research on these seeds was focused on their protein fractions, functional properties of their protein isolates and utilization of the isolates in food product formulation (Ogunbusola *et al*., 2010, 2012, and 2013). To the best of our knowledge, studies on the phytosterols and spectroscopic analysis of many curcubit oils have not been reported especially those of *Lagenaria siceraria* and *Cucumeropsis mannii* oils. The aims of this study were to determine the fatty acid profile, sterol composition and in addition the spectroscopic characterisation and identification of oils from *Lagenaria siceraria* and *Cucumeropsis mannii.*

Materials and Methods

Preparation of samples

Seeds of two varieties of *Lagenaria siceraria* (calabash gourd seed; coded LS_1 and bottle gourd seed; coded LS2) and Cucumeropsis mannii (White melon seed; coded CM) were bought from Anibaba farm in Irele Ekiti, Ekiti State, South Western Nigeria. The seeds were shelled, washed, dried in a Hot air oven at 50^0C and pulverized using a Brabender blender. Oil was extracted from the seed flours in a Soxhlet type apparatus using n-hexane as solvent.

Fatty acid analysis

Fatty acid methyl esters of the oil samples were prepared using the method of Bannon *et al.* (1982). The fatty acids were analyzed by injecting the fatty acid methyl ester into the Gas liquid Chromatograph (GLC) using a Perkin Elmer Autosystem XL Gas Chromatography. Column: SGE BPX70 (Phenomenex Cat No. CG0-5512), 30 m length, 0.25 mm interior diameter, 0.25 μm film thickness. Carrier gas: Helium at 20 Psi (1.85 mL/min). Detector: Flame ionization (FID) at 265^0C. Hydrogen flow (45 mL/min) and air flow (450 mL/min). Injector: Split, packed at 265^0C, Spilt flow ratio 40:1 (76.9 mL/min). Temperature Program: 60^0C for 2 min, increase to 180^0C at 10 0C/min. increase to 235^0C at 4^0C/min. Total runtime is 27.7 min.

Determination of sterol composition of oils

The method reported by Dutta (1997) was used in the analysis of sterols in Lagenaria siceraria and Cucumeropsis mannii seed oils. One (1 g) gram of oil sample was weighed into 50 mL screw topped glass test tube. About 10 mL of alcoholic KOH solution was added, the tube was capped and vortex to mix and allowed to stand overnight in the dark to saponify. Distilled water (10 mL) was added to the tube, capped and vortex to mix completely. Ten milliliters (10 mL) of methylene chloride was added, recapped, vortex for at least 1 minute to extract. The tubes were allowed to stand at room temperature until layers are formed, the top layer was removed by vacuum suction. Then, 10 mL of distilled water was added to the test tube to wash the solvent layer, recapped and vortex until it was thoroughly mixed and centrifuged at 1000 rpm for 10 min. The top layer was later removed by suction. One milliliter (1 mL) of the solvent was added to a clean test tube, dried under nitrogen stream and the residue obtained was dissolved in 900 μL of n-hexane. About 100 mL of cholestane internal standard solution was added to the tube and transferred to GC vial. Then, 100 mL pyridine and 100 mL of Regisil(R) were added to the vial, capped tightly and vortex mix. The vial was heated for one hour using a heating block at 50^0C and later injected into the GC (Perkin Elmer Autosystem XL) with standard solution (Plant Sterol Standard Cat No. 1119, Matreya).

Fourier transform infrared (FTIR) spectra analysis

A film of small amount of each oil samples (approximately 2 ml) was deposited between two KBr discs while avoiding the presence of air. IR spectra were recorded with a Fourier Transform Infrared Spectrometer (FT/IR-430, Jasco Corporation, Japan). The frequency and intensity of each band was obtained automatically by using the "Find Peaks" command of the instrument software (Akintayo *et al.*, 2002).

Nuclear magnetic resonance (NMR) spectroscopy of oils

NMR spectra were recorded from deuterichloroform solutions with a Bruker model AC - 250 Spectrometer. Chemical shifts were measured in ppm downfield from internal tetramethylsilane. In order to ensure consistent spectra, the following

Table 1. Fatty acid composition of *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils

Fatty acid (%)	LS_1	LS_2	CM
Palmitic acid (C16:0)	12.11 ± 0.13$_b$	10.84 ± 0.18$_c$	13.69 ± 0.12$_a$
Stearic acid (C18:0)	8.49 ± 0.12$_b$	6.71 ± 0.14$_c$	12.17 ± 0.12$_a$
cis-Oleic acid (18:1)	17.86 ± 0.12$_b$	20.62 ± 0.16$_a$	13.36 ± 0.07$_c$
cis-Linoleic acid (C18:2)	60.15 ± 0.08$_a$	60.40 ± 0.17$_a$	59.47 ± 0.33$_b$
cis-Linolenic acid (C18:3)	0.12 ± 0.00$_b$	0.10 ±0.01$_c$	0.16 ± 0.01$_a$
Arachidic acid (C20:0)	0.40 ± 0.02	0.43 ± 0.04	0.37 ± 0.02
Behenic acid (C22:0)	0.10 ± 0.03	0.10 ± 0.01	0.08 ± 0.01
Unknowns	0.11 ± 0.09	0 05± 0.08	0 11 ± 0.03
Total unsaturated fatty acid (%)	78.34 ± 0.06$_b$	81.31 ± 0.06$_a$	73.17 ± 0.29$_c$
Total saturated fatty acid (%)	21.56 ± 0.05$_b$	18.64 ± 0.09$_c$	26.72 ± 0.26$_a$
Essential fatty acid	60.27 ± 0.08$_a$	60.50 ± 0.17$_a$	59.63 ± 0.34$_b$
Saturated/unsaturated	0.28 ± 0.01$_b$	0.22 ± 0.01$_c$	0.37 ± 0.01$_a$
Oleic/Linoleic	0.30 ± 0.01$_b$	0.34 ± 0.01$_a$	0.22 ± 0.01$_c$

Values with different subscripts on the same row are significant (P ≤0 05), LS1 = *Lagenaria siceraria* variety 1, LS_2 = *Lagenaria siceraria* variety 2, CM = *Cucumeropsis mannii*

instrumental parameters were applied; spectrum width, 5000 Hz; acquisition time, 3.2 77 s; delay time, 1 s and pulse width, 7 µsec (Akintayo and Bayer, 2002).

Statistical analysis

All determinations were carried out in triplicates, errors were recorded as standard deviation from the mean. Data were subjected to analysis of variance using SPSS 16 computer programme, while means were separated using New Duncan Multiple Range Test (NDMRT). Significance was accepted at 5% level of probability.

Results and Discussion

Fatty acid composition of Lagenaria siceraria *and* Cucumeropsis mannii *seed oils*

The results of the fatty acid composition of the seed oils are shown on Table 1. The predominant fatty acid group in *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils is the unsaturated fatty acid. The percentage unsaturated fatty acid in the seed oils ranged from 73.17 to 81.31%, consisting mainly linoleic (59.47 to 60.40%) and oleic (13.36 to 20.62%) and the saturated fatty acid in the seed oils ranged from 18.64 to 26.72%. *Lagenaria siceraria* oils (calabash LS_1 and bottle gourd LS_2) are significantly higher in oleic and linoleic acid than *Cucumeropsis mannii* seed oil. Linoleic acid was observed to be the principal fatty acid in the seed oils followed by oleic acid. Shinas *et al.* (2009) reported linoleic acid as the predominant fatty acid in pumpkin seed oil. Linoleic acid has been reported as the most important essential fatty acid required for growth, physiological functions and body maintenance (Salunkhe *et al.*, 1985).

The essential fatty acid (EFA) of *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils ranged from 59.63 to 60.50%. The range of percentage unsaturated fatty acid (73.17 to 81.31 %) of *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils compared favourably to the range 64.1% to 81.6% reported for some selected oils (Mistra *et al.*, 2009; Nyam *et al.*, 2009; Rezig *et al.*, 2012). High levels of blood cholesterol are associated with high intake of saturated fatty acids (Bender, 1992). It has been concluded that relative to carbohydrate, the saturated fatty acids elevate serum cholesterol; while the polyunsaturated fatty acids lower serum cholesterol (Hegsted *et al.*, 1993). High intake of *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils with high levels of unsaturated fatty acid may not lead to risk of increased blood cholesterol in the body.

Sterol composition of Lagenaria siceraria *and* Cucumeropsis mannii *seed oils*

Table 2 shows the sterol composition of Lagenaria siceraria and *Cucumeropsis mannii* seed oils. Cholesterol and brassicasterol were not detected in all the oil samples analysed making them heart friendly oils. Cholesterol is injurious to health as they block the arteries of the heart leading to hypertension. Stigmasterol content of the seed oils ranged from 4.47 to 5.98 mg/100g and β-sitosterol was the principal phytosterols detected in all the oil samples with the range of values from 205.81 to 300.04 mg/100g. Phytosterols have cholesterol lowering properties and impact on health. The levels of phytosterols in vegetable oils have been used for the identification of oils, oil derivatives and for the determination of oil quality (De-Blas and De-Valle, 1996).

Table 2. Sterol composition of *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils.

Sterols (mg/100g)	LS_1	LS_2	CM
Cholesterol	Nd	Nd	Nd
Brassicasterol	Nd	Nd	Nd
Campesterol	$39.60 \pm 0.51_b$	$50.58 \pm 0.59_a$	$12.20 \pm 0.22_c$
Stigmasterol	$4.85 \pm 0.04_b$	$4.47 \pm 0.09_c$	$5.98 \pm 0.19_a$
β-Sitosterol	$228.8 \pm 0.75_b$	$205.81 \pm 2.52_c$	$300.04 \pm 2.80_a$

Nd = Not detected. Values with different subscripts on the same row are significant ($P \leq 0.05$), LS_1 = *Lagenaria siceraria* variety 1, LS_2 = *Lagenaria siceraria* variety 2, CM = *Cucumeropsis mannii*

The sterol composition of *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils compared favourably to earlier researchers reports on vegetable oils. Nyam *et al.* (2009) reported no detection of cholesterol in Bittermelon, Kalahari melon, kenaf and pumpkin seed oils. The percent β-Sitosterol component of the total phytosterols in the seed oils ranged from 78.9 to 94.3%. Sitosterol was also the predominant sterol reported in pumpkin *(Cucurbita maxima)* (Rezig *et al*., 2012) and in five vegetable seed oils (Nyam *et al*., 2009). Sitosterol has been reported to have many beneficial effects on man and has been proven to lower blood LDL cholesterol by 10-15% (Yang *et al*., 2001). The commonly consumed plant sterols are sitosterol, stigmasterol and campesterol which are predominantly detected in the seed oils studied. The nutritional benefits derived from sterol include their antioxidative properties and ability to lower plasma cholesterol and LDL cholesterol (Pironen *et al*., 2000; Gordon and Magos, 2003).

FTIR spectra of Lagenaria siceraria *and* Cucumeropsis mannii *seed oils*

The IR spectra of *Lagenaria siceraria* (LS_1 and LS_2) and *Cucumeropsis mannii* seed oils (CM) are shown in Figure 1. The vibrational motion expected from fatty acids and triacyl glycerides are mainly C-H stretching, methylene asymmetrical stretching, C-C stretching and olefinic C=CH stretching which were shown in the seed oils spectra. The IR spectra of *Lagenaria siceraria* (LS_1 and LS_2) and *Cucumeropsis mannii* seed oils is in accordance with similar observations reported by other workers on vegetable oils (Rohman *et al*., 2011; Biswal and Bhadouriya, 2012; Rohman *et al*., 2012)).

Many of the bands showed frequencies vibration that could be used to classify the oil sample into groups. The frequencies of the bands at approximately 3008 cm^{-1} increase in the order CM > LS_1 and LS_2, and only CM shows peak at 1098 cm^{-1}. Based on the fatty acid composition of the oil samples, the

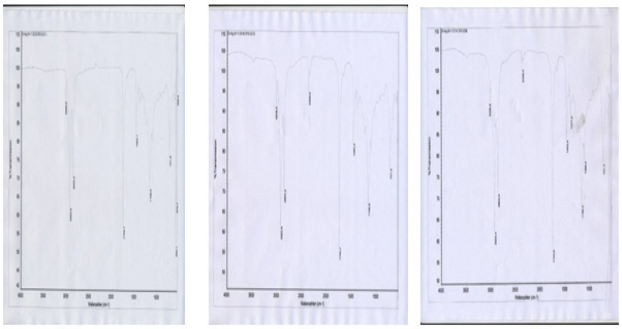
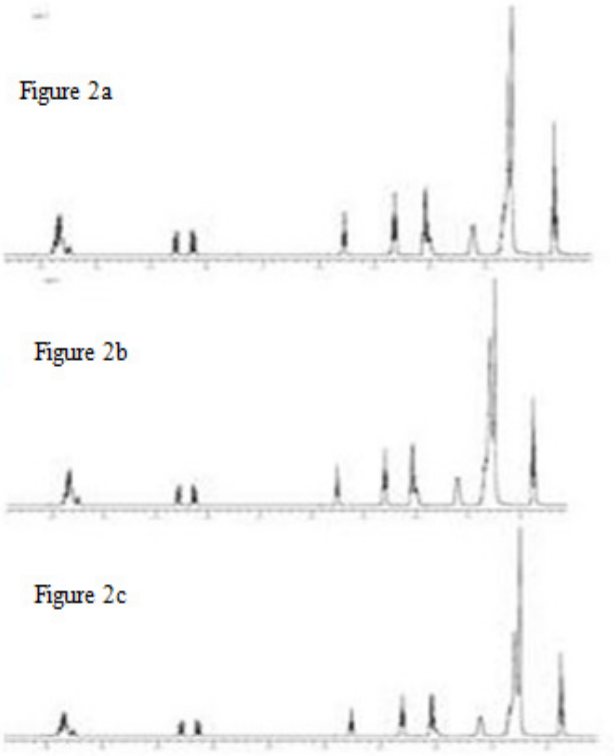


Figure 1. Fourier transform infra red spectrum of Lagenaria siceraria (LS_1 and LS_2) and *Cucumeropsis mannii* (CM) seed oils

trend (at peak approximately 3008 cm^{-1}) show that the frequency variation of bands are related to the proportion of mono and polyunsaturated acyl groups in the oil samples. Oils with the highest proportion of monounsaturated acyl groups show the smallest values of these frequencies. This corroborates the result of the fatty acid composition (Table 1) that *Lagenaria siceraria* seed oils contain higher unsaturated fatty acid than *Cucumeropsis mannii* seed oil. The absence of peaks at approximately 3025 cm^{-1} in all the oil spectra rules out the presence of trans olefins double bonds. Intake of trans fat have been reported to increase the level of LDL cholesterol and decrease HDL cholesterol.

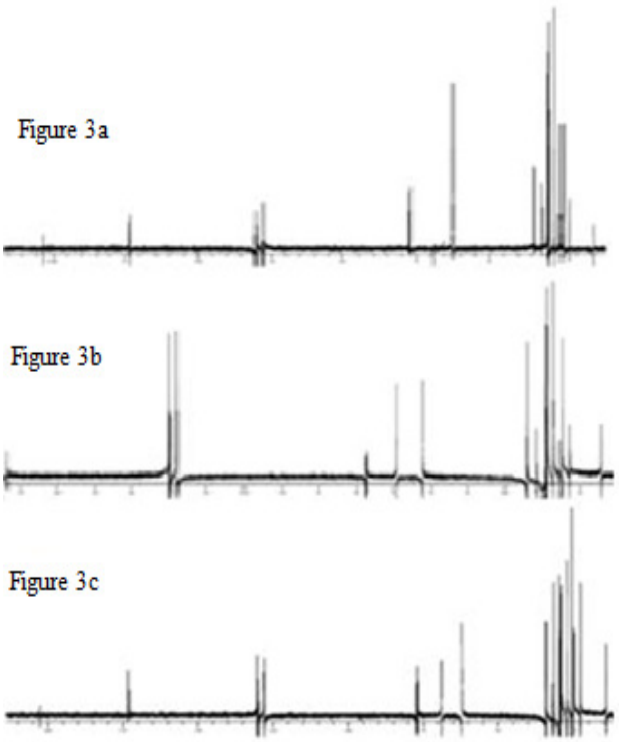
^1H and ^{13}C NMR spectra of Lagenaria siceraria *and* Cucumeropsis mannii *seed oils*

The ^1H NMR Spectra of *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils (δ ppm) is shown in figure 2. Some big and intense peaks were observed at δ1.20 – 1.40 ppm indicating the presence of –CH2– group. Peaks were also observed δ 1.95-2.08 ppm revealing the presence of $–CH_2C=C$ (methylene group attached to double bonded carbon atoms), indicating the presence of unsaturation in the oil samples. Peaks in the region δ 2.25 – 2.35 ppm indicate the presence of methylene groups next to carbon group ($–CH_2COO–$),



2a - *Lagenaria siceraria* (LS_1), 2b - *Lagenaria siceraria* (LS_2) and 2c - *Cucumeropsis mannii* (CM)

Figure 2. ^{1}H NMR spectra of *Lagenaria siceraria* (LS_1 and LS_2) and *Cucumeropsis mannii* (CM) seed oils



3a - *Lagenaria siceraria* (LS_1), 3b - *Lagenaria siceraria* (LS_2) and 3c - *Cucumeropsis mannii* (CM)

Figure 3. ^{13}C NMR spectra of *Lagenaria siceraria* (LS_1 and LS_2) and *Cucumeropsis mannii* (CM) seed oils

this is in agreement with the observations of Akintayo and Bayer (2002) and Oyewusi *et al.* (2007). Peaks at δ 2.72 – 2.80 ppm correspond to methylene groups surrounded by double bonded carbon atoms C=CCH2C=. Saturated and monounsaturated acids do not contain these peaks. This indicates that the seed oils contain polyunsaturated fatty acids. The peaks at δ 4.10 – 4.18 ppm arise from $–CH_2OCO–$ of glycerol. The peaks at δ 5.25 – 5.40 ppm arise from hydrogen (protons) on double bonded carbon atoms –CH=. The absence of series of peaks in the range δ 5.50 – 6.40 ppm indicates absence of conjugation in *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils (Rybicky, 1979). The relative areas and intensities of the $–CH_2$ and $=CCH_2C=$ peaks differ in the oil samples analysed. The $=CCH_2C=$ peak at δ 2.73 – 2.80 ppm is smaller in CM compared to LS_1 and LS_2 which may be an indication that CM does not contain much of diunsaturated fatty acids. Only polyunsaturated fatty acids would give signals at δ 2.78 ppm which corresponds to the chemical shifts of the double allylic methylene protons. The signal intensity of this region provided a good estimation of the total PUFA in the oils. From figure 2, the intensity of CM is smaller compared to LS_1 and LS_2 which showed that CM oil contains less PUFA than LS_1 and LS_2, this confirmed the result of the gas chromatography analysis of the oil samples (Table 1). The ^{1}H NMR spectra of all the seed oils do not show any peak at 0.97 ppm.

The important signals in the ^{13}C NMR spectra of *Lagenaria Siceraria* and *Cucumeropsis mannii* (CM) seed oils are shown in Figure 3. The ^{13}C NMR results are discussed based on the four important regions in the spectrum that provide essential information regarding the structure of the acyl groups and their distribution on the glycerol backbone. These regions are C – 1 carbon shift region, C – 2 carbon shift region, the C – 3, allylic, ω1 – ω3 carbon shift regions, and Olefinic carbon shift region, peaks were observed in these regions in the oil spectra.

Conclusion

The study has showed that oils extracted from the seed flours of *Lagenaria siceraria* and *Cucumeropsis mannii* are abundant in essential fatty acids, they contain health beneficial phytosterols with no cholesterol nor trans fatty acid detected. The seed oils have potentials for both domestic and industrial applications. The spectroscopy revealed the presence of both saturated and unsaturated triacylgrycerides in the oil samples, and higher percentage of unsaturation in the *Lagenaria siceraria* seed oils than

Cucumeropsis mannii.

Acknowledgement

The Authors acknowledge the Institute of Organic Chemistry, Karlsruhe Institute of Technology, Karlsruhe, Germany, where the spectroscopic analysis of the seed oils was carried out.

References

Abiodun, O.A. and Adeleke, R.O. 2010. Comparative studies on nutritional composition of four melon seeds varieties. Pakistan Journal of Nutrition 9(9): 905-908.

Akintayo, E.A., Olaofe, O., Adefemi, S.O. and Akintayo, C.O. 2002. Potentials of fourier transform infrared spectroscopy for characterizing vegetable oils. International Journal of Chemistry 12(2): 151-156.

Akintayo, E.T. and Bayer, E. 2002. Identification of oils by NMR spectroscopy. La Rivista Italiana Delle Sostanze Grasse 79: 207-210

Aremu, M.O., Olaofe, O., Akintayo, E.T. and Lajide, L. 2006. Characteristics of legume seed oils by fourier transform infrared spectroscopy. Biosciences and Biotechnology Research Asia 3(1a): 01-06

Bannon, C., Craske, J.D., Hai, N.T., Harper, N.L. and O'Rourke, K.L. 1982. Analysis of fatty acid methyl esters with high accuracy and reliability II. Methylation of oils and fats with Boron trifluoride – methanol. Journal of Chromatography 247: 63-70.

Bender, A. 1992. Meat and Meat products in human nutrition in developing Countries. FAO Food and Nutrition paper 53. Rome.

Biswal, P.K. and Bhadouriya, A.S. 2012. Use of Fourier Infrared Spectroscopy to cooking Oils. International Journal of Pharmacy, Chemistry and Biological Sciences 2(4): 670-680.

Che Man, Y.B. and Rohman, A. (2013). Analysis of Canola oil in Virgin coconut oil using FTIR Spectroscopic and Chemometrics. Journal of Food and Pharmaceutical Sciences 1: 5-9

Chinyere, C.G., Akubugwo, E.I., Chinyere, N.I. and Ugbogu, A.E. 2009. Nutritive values of *Lageneria sphaerica* seed (wild bottle gourds) from South – Eastern Nigeria. Pakistan Journal of Nutrition 8(3): 284-287.

De-Blas, O.J. and Del-Valle, G.A. 1996. Determination of Sterols by capillary column gas chromatography differentiation among different types of Olive oil: Virgin, refined and solvent extracted. Journal of American Oil Chemists Society 73: 1685-1689.

Dutta, P.C. 1997. Studies on phytosterol oxides II: Content in some vegetable oils and in French fried prepared in these oils. Journal of American Oil Chemists Society 74: 659-666.

Gordon, M.H. and Magos, P. 2003. The Effect of Sterols on the oxidation of edible oils. Journal of Food Chemistry 10(2): 141-147

Hegsted, D.M., Ausmna, L.M., Johnson, J.A. and Dallal, G.E. 1993. Dietary fat and serum lipids: An evaluation of the experimental data. American Journal of Clinical Nutrition 57(6): 875-883.

Knothe, G. and Kenar, J.A. 2004. Determination of the fatty acid profile by 1H-NMR Spectroscopy. European Journal of Lipid Science and Technology 106: 88-96

Manaf, M.A., Che Man, Y.B., Hamid, N.S.A., Ismail, A., and Syahariza, Z.A. 2007. Analysis of adulteration of virgin coconut oil by palm kernel olein using Fourier transform Infrared spectroscopy. Journal of Food Lipids 14: 111-121.

Milovanovic, M. and Picuric-Jovanovic, K. 2005. Characteristics and composition of melon seed oil. Journal of Agricultural Science 50(1): 41-47.

Mistra, P., Ramaswamy, H.S. and Chang, K.S. 2009. Pumpkin *(Cucurbita maxima)* seed oil extraction using supercritical carbon dioxide and physicochemical properties of the oil. Journal of Food Engineering 95: 208-213.

Nocolaou, N., Xu, Y. and Goodacre, R. 2010. Fourier Transform Infrared Spectroscopy and Multivariate analysis for the detection and quantification of different milk species. Journal of Dairy Science 94: 5651-5660.

Nyam, K.L., Tan, C.T., Lai, O.M., Long, K. and Che Man Y.B. 2009. Physicochemical Properties and bioactive compounds of selected seed oils. Journal of Food Science Technology 42: 1396-1403.

Ogunbusola, E.M, Fagbemi, T.N, and Osundahunsi, O.F. 2010. Amino acid composition of *Lagenaria siceraria* seed flour and protein fractions. Journal of Food Science Technology 47(6): 656-661.

Ogunbusola, E.M., Fagbemi, T.N. and Osundahunsi, O.F. 2012. Chemical and functional properties of full fat and defatted white melon *(Cucumeropsis mannii)* seed flour. Journal of Food and Engineering 2: 691-696.

Ogunbusola, E.M., Fagbemi, T.N. and Osundahunsi, O.F. 2013. In vitro protein digestibility, amino acid profile, functional properties and utilization of white melon *(Cucumeropsis mannii)* protein isolates. African Journal of Food Science Technology 4(7): 153-159.

Oyewusi, P.A., Olaofe, O. and Akintayo, E.T. 2007. Gas Chromatographic and Spectroscopic Analysis of Oils of wild Rubber *(Funtumia elastica)* seeds. Pakistan Journal of Science Industrial Research 50(6): 364-369.

Pironen, V., Lindsay, D.G., Mettinen, T.A., Toivo, J. and Lampi, A. 2000. Plant sterols: Biosynthesis, Biological function and importance to human nutrition.

Poiana, A., Mousalis, G., Alexa, E., Moigradean, D., Negrea, M. and Mateescu, C. 2012. Application of FT-IR Spectroscopy to assess the Olive oil adulteration. Journal of Agroalimentary Process Technology 18(4): 277-282.

Rezig, L., Chouaibi, M., Msaada, K. and Hamdi, S. 2012. Chemical Composition and Profile Characterization of Pumpkin *(Cucurbita maxima)* seed oil. Industrial Crops and products 37: 82-87.

Rohman, A., Che Man, Y.B., Ismail, A. and Puziah, H. 2011. FTIR Spectroscopy combined with Chemometrics for

analysis of Lard adulteration in some vegetable oils. Cyta Journal of Food 9(2): 96-101.

Rohman, A., Kuwat, T., Retno, S., Sismindari, Yuny, E. and Tridjoko, W. 2012. Fourier Tranform Infrared Spectroscopy applied for rapid analysis of Lard in palm oil. International Food Research Journal 19(3): 1161-1165.

Rohman, A. and Che Man, Y.B. 2011. The use of Fourier transform mid infrared (FT-MIR) spectroscopy for detection and quantification of adulteration in virgin coconut oil. Food Chemistry 129(2): 583-588.

Rybicky, J. 1979. Fast determination of fatty acids in oil utilizing resins by NMR Spectroscopy. Journal of Applied Polymer Science 23: 25-38.

Salunkhe, D.K., Kadam, S.S. and Chavan, J.K. 1985. Post harvest Biotechnology of Food Legumes. Boca Raton, FL. CRC Press.

Shinas, P., Karavalakis, G., Davaris,, C., Anastopoulos, G., Karonis, D., Zannikos, F., Stournas, S. and Lois, E. 2009. Pumpkin (*Cucurbita pepo* L.) seed oil as an alternate feedstock for the production of biodiesel in Greece. Biomass and Bioenergy 33: 44-49.

Stevenson, D.G., Eller, F.J., Wang, L., Jane, J., Wang, T. and Inglett, G.E. 2007. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. Journal of Agricultural and Food Chemistry 55: 4005-4013.

Yang. B., Karlsson, R.M., Oksman and Kallio, H.P. 2001. Phytosterols in sea buck-thorn (*Hippophae rhamnoides* L.) berries: Identification and effects of different origins and harvesting times. Journal of Agricultural and Food Chemistry 49: 5620-5629.