Matrix-Assisted Laser Desorption Ionization time of flight mass spectrometric analysis of some curcurbita oils for triacylglycerol composition

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Four varieties of curcurbita plant seed oils viz, Bottle gourd (*Lagenaria vulgaris*), Snake gourd (*Tricosanthes cucumerina*), Sponge gourd (*Luffa cylindrica*) and Flat Calabash gourd (*Cucurbita maxima*) were analyzed by matrix assisted laser desorption ionization (MALDI) time of flight (TOF) mass spectrometry (MS) using α-cyano-4 hydroxicinnamic acid dissolved in acetonitrile as matrix. The Bottle gourd oil contained four major triacylglycerols (TAGs) which include trilinolein (LLL), palmitoyldilinoleoyl glycerol (PLL), oleoyldilinoleoyl glycerol (OLL) and dioleyollinoleoyl glycerol (OOL). Snake gourd oil showed three major TAGs as tripucinin ($P_nP_nP_n$), palmitoyldilinoleoyl glycerol (PLL) and distearoyloleoyl glycerol (SSO). Sponge gourd oil was found to consist of four TAGs, dipalmitoyloleoyl glycerol (PPO), palmitoyldilinoleoyl glycerol (PLL), palmitoylstearoyllinoleyol glycerol (PSL) and distearoyloleoyl glycerol (SSO). Five major TAGs viz; palmitoyldilinoleoyl (PLL), trilinolenin ($L_nL_nL_n$), trilinolein (LLL), stearoyldilinoleyol glycerol (SLL) and distearoyloleyol glycerol (SSO) were identified in flat calabash seed oil. The TAGs compositions were consistent with the fatty acid profiles obtained for the different oils by gas chromatographic technique.

ANALISI PER SPETTROMETRIA DI MASSA MALDI-TOF DELLA COMPOSIZIONE TRIGLICERIDICA DI ALCUNI OLI DI CUCURBITACEE

Quattro varietà di oli di semi di cucurbitacee, *Lagenaria vulgaris*, *Tricosanthes cucumerina*, *Luffa cilindrica* e *Cucurbita maxima*, sono state analizzate con la tecnica di spettrometria di massa MALDI-TOF usando come matrice acido α-ciano-4-idrossicinnamico dissolto in acetonitrile. *Lagenaria vulgaris* contiene quattro maggiori trigliceridi (TAG): trilinoleina (LLL), palmitoildilinoleil glicerolo (PLL), oleildilinoleil glicerolo (OLL) e dioleillinoleil glicerolo (OOL).
Tricosanthes cucumerina contiene tre trigliceridi maggiori: tripucinina ($P_nP_nP_n$), palmitoildilinoleil glicerolo (PLL) e distearoleil glicerolo (SSO). *Luffa cilindrica* è costituita da 4 TAG maggiori: dipalmitiloleil glicerolo (PPO), palmitildilinoleil glicerolo (PLL), palmitilstearillinoleil glicerolo (PSL) e distearoleil glicerolo (SSO). In *Cucurbita maxima* sono stati identificati cinque principali TAG: palmitildilinoleil glicerolo (PLL), trilinolenina (L_n L_n L_n), trilinoleina (LLL), stearildilinoleil glicerolo (SLL) e distearoleil glicerolo (SSO). Il profilo in acidi grassi dei vari oli è stato ottenuto per analisi gascromatografica.

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INTRODUCTION

Traditional analysis of triacylglycerols (TAGs) of plant oils had generally relied on saponification and then methylation to form fatty acid methyl esters which are subsequently detected by gas chromatography or gas chromatography mass spectrometry [1]. These methods however cannot detect the actual TAGs composition. For TAG compositional analysis, methods like (I) thin layer chromatography (TLC), both the argentation [2] and reverse phase [3], (II) supercritical fluid chromatography [4] and high performance liquid chromatography [5] have been developed.

TLC methods rely on the retention factor and can lead to false identification while the other methods rely on the retention time for the identification which is difficult for partially separated peaks. The methods are also time consuming and require reference standards for unambiguous identification.

Matrix-assisted laser desorption ionization (MALDI) time of flight (TOF) mass spectrometry (MS) is an emerging powerful tool that enables the analysis of complex mixtures of bimolecules such as proteins, peptide and carbohydrate, synthetic polymers and oligomers [6]. MALDI-TOF MS has also been found to be very good for TAGs compositional analysis of relevant oils [1,7]. However it has not been widely applied to lipid analysis for TAGs composition despite its numerous advantages as enumerated by Schiller and Arnold [8] and the fact that the preparation of samples for MALDI-TOF MS analysis is fast and easy.

In this paper the MALDI-TOF MS has been applied to determine the TAGs composition of oils derived from some members of cucurbitacea family using α-cyano-4-hydroxycinnamic acid as matrix.

EXPERIMENTAL

The seeds of Bottle gourd (*Lagenaria vulgaris*), Sponge gourd (*Luffa cylindrica*), Snake gourd (*Tricosanthes cucumerina*) and Flat calabash gourd (*Cucurbita maxima*) were obtained from a local farm in Ayedun Ekiti, Ekiti state, Nigeria.

The seeds were oven dried (40°C), bad seeds removed by handpicking and the good seeds were ground. Oil was extracted from the milled powder with n-hexane using Soxhlet method. α-cyano-4-hydroxycinnamic acid (CHCA), acetonitrile, tetrahydrofuran (THF), tetra methyl ammonium hydroxide (TMAH), Toluene and n-pentane were purchased from Fluka (Seelze, Germany). 2,5 dihydroxy benzoic acid (DHB) and dithranol (1,8 dihydroxy-9 [10H] anthracenone) were obtained from Sigma (St Louis. MO)

MALDI spectra were acquired on a PerSeptive Biosystems (Framingham, MA, USA) Voyager DE-Pro instrument time of flight spectrometer. The instrument used an N_2 laser (337 nm, 3 ns pulse width, 20 Hz repetition rate). In order to enhance the spectral resolutions, all spectra were measured in the reflector mode, positive ion MALDI spectra (150 summed acquisitions) with delayed extractions and accelerating voltage at 20,000V and grid voltage at 75%. A minimum of 20 experiments were performed on each oil in order to confirm reproducibility of the MALDI-TOF spectra. All solutions were prepared in 2 ml Eppendorf microcentrifuge tubes and vortexed for about 15 s prior to use.

The fatty acid composition of the oils were determined as follows. Fatty acid methyl esters of the oils (FAMES) were prepared by shaking 50 mg of sample dissolved in 10 ml of toluene with 0.2 ml of TMAH. 4 ml of water was added, reaction mixture allowed to settle and 1μl of the toluene layer injected into a Hewlett Packard GC (model CP 9000) equipped with split/splitless injection port and flame ionization detector and fitted with a J&W scientific fused silica column DB5, 25x0.25 mm, μ_f = 0.25 μm. The injection port and detector temperatures were 250°C and 280°C respectively. The oven temperature was programmed as follows: 120°C for 3 min, then temperature increased at 4°C/min till 260°C and at 260°C for 5 min. The identification of the peaks was done on a Hewlett Packard HP 5973 Mass Spectrometer (mass selective detector) interfaced with a HP 6890 series GC (Agilent Technologies, Waldron, GmbH). The mass spectrometer parameters used were electron impact energy 70eV, transfer line 270°C, source temperature 250°C, emission current 0.5 mA, cycle time 0.6 sec/scan from 45 to 400 a.m.u, electron multiplier voltage 1.7kV and pre-amp sensitivity 10^{-7} Amp/V.

Preparation of matrix solution

α-Cyano-4-hydroxycinnamic acid (CHCA) (1.0 mg, 5.29 μmol) was dissolved in 1 ml of CH_3 CN/THF (7:1V/V), DHB was prepared in 50% water: 50% CH_3CN with 0.25% trifluoroacetic acid while dithranol was prepared in chloroform solution and were used as just prepared.

Preparation of analyte solution

Each oil sample (1.0 mg) was dissolved in 1ml of n-pentane and used as needed

Preparation of sample solution for MALDI analysis

Matrix solution (90 μml) was added to analyte solution (10 μml). About 1μml of each sample solution was deposited on a 100 well sample plate. The deposited samples crystallized within 30s upon evaporation of the solvent. The sample plate was loaded into the MALDI ion source.

RESULTS AND DISCUSSION

Fatty acid composition

The fatty acid composition of the different varieties of cucurbita is presented in Table I. *Lagenaria vulgaris*, *Luffa cylindrica* and *Cucurbita maxima* exhibit similar trend in their fatty acid composition. They comprise mainly palmitic, stearic, oleic and linoleic acids, with linoleic acid being the most abundant and stearic acid the least. However *Tricosanthes cucumerina* display a totally different fatty acid composition. It has punnicic acid and palmitic acid as the most and least abundant fatty acids respectively.

Table I - Fatty acid composition (%) of some cucurbita species seed oils

Component	A	B	C	D
Palmitic	11.6	6.1	11.1	11.5
Stearic	3.3	8.4	9.7	7.1
Oleic	16.3	14.1	18.4	8.5
Linoleic	65.0	18.2	53.7	69.0
Linolenic	-	-	-	1.6
Punnicic	-	47.8	-	-
Others	3.8	5.3	7.1	2.3

A - Bottle gourd (*Lagenaria vulgaris*), B - Snake gourd (*Tricosanthes cucumerina*), C - Sponge gourd (*Luffa cylindrica*), D - Flat calabash gourd (*Cucurbita maxima*)

MALDI-TOF mass spectra

Preliminary experiments were carried out using different matrices viz, α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxy benzoic acid (DHB) and dithranol (1,8-dihydroxy-9 [10H] anthracenone) for the MALDI analysis of the Bottle gourd oil. The use of the three matrices did lead to some sodiated molecules but the shot to shot reproducibility and the detection limits were poor for both DHB and dithranol but good for CHCA. Hence CHCA was adopted as matrix for further analysis of the oil samples for triacylglycerol composition.

Bottle gourd (*Lagenaria vulgaris*)

The fatty acid profile consists of the MALDI-TOF mass spectral data (Table II, Fig 1) in which most of the major sodiated TAG molecules contain linoleic acid. A total of four TAGs were identified among which trilinolein ((LLL) and palmitodilinoleoyl glycerol (PLL) were the most abundant. The MALDI-TOF also indicated two ions at m/z 892 and 917. Eventhough the nature of these two ions is unknown, it may be attributed to oxidative products from PLL and LLL respectively. The very low intensity of these peaks shows that they have low concentration. However further studies need to be done to ascertain this conclusion. No TAG with stearic acid was identified in the oil despite its presence as indicated by the fatty acid composition. This is because, as suggested by Ayorinde et al., [7] TAGs with a stearic moiety can be detected by MALDI-TOF MS only when the oil contains significant levels of stearic acid (>5%). Percentage of stearic acid in Bottle gourd oil is only 3.3% so the absence of stearic acid TAG in the mass spectrum can be explained by its low amount as well as the low affinity of TAGs with stearic acid for the CHCA surface.

Sponge gourd (*Luffa cylindrica*)

Figure 2 presents the MALDI-TOF mass spectra of Sponge gourd. Based on the fatty acid profiles in Table I, one would expect Sponge gourd to have similar TAGs molecular distribution to Bottle gourd. However this is not the case. The MALDI-TOF MS of Sponge gourd shows four sodiated TAGs. The major TAGs are palmitoleyoldilinoleoyl glycerol (PLL), dipalmitoyloleoyl glycerol (PPO), palmitoylstearoyllinoleoyl glycerol (PSL) and distearoyloleyol glycerol (SSO). The ion at m/z 861 may be attributed to the matrix as earlier reported [7]. Two other ions at m/z 893 and 895 were identified by the MALDI-TOF MS spectra. Based on the fatty acid composition of the oils, they may at best be attributed to oxidation products from PLL.

Flat calabash gourd (*Cucurbita maxima*)

Five major TAGs were identified (Fig. 3, Table II) as palmitoyldilinoleoyl glycerol(PLL), trilinolenin ($L_nL_nL_n$), trilinolein (LLL), stearoyldilinoleoyl glycerol(SLL) and distearoyloleoyl glycerol (SSO). The TAGs composition consists of the fatty acid composition of the oil with linoleic acid (69%) being the most abundant fatty acid.

Table II - Sodiated TAG molecules from MALDI –TOF mass spectra of Bottle gourd, Snake gourd, Sponge gourd and Flat calabash gourd oils

Sample	Masses (m/z) of sodiated TAGs	Sodiated triacylglycerols
Bottle gourd	877.77	PLL
	892.99	Oxidation products of PLL
	901.77	LLL
	903.78	OLL
	905.79	OLL
	917.84	Oxidation products of LLL
Snake gourd	861.20	Matrix
	877.09	PLL
	895.11	$P_nP_nP_n$
	910.95	SSO
Sponge gourd	855.71	PPO
	861.66	Matrix
	877.10	PLL
	881.77	PSL
	893.04	Oxidation products of PLL
	895.09	Oxidation products of PLL
	911.95	SSO
Flat calabash gourd	877.66	PLL
	894.92	$L_nL_nL_n$
	901.69	LLL
	905.68	SLL
	910.98	SSO

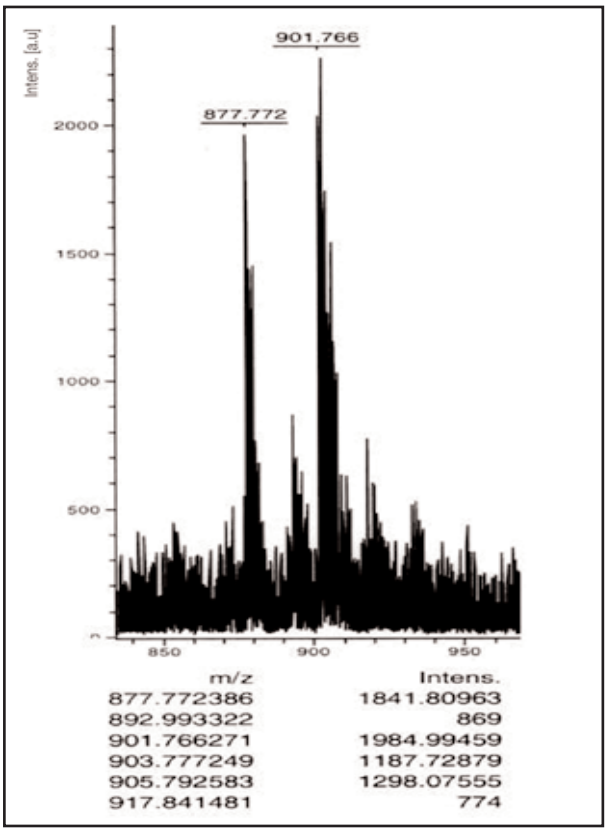

Figure 1 – Partial MALDI-TOF mass spectrum of Bottle gourd oil showing sodiated TAG molecules from a range (m/z 830-960)

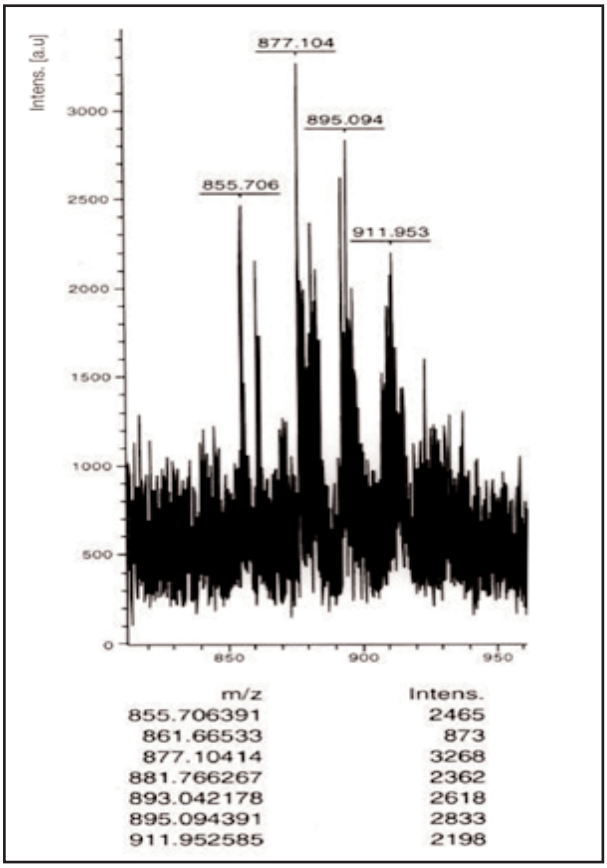

Figure 2 - Partial MALDI-TOF mass spectrum of Sponge gourd oil showing sodiated TAG molecules from a range (m/z 830-960)

Snake gourd (*Tricosanthes cucumerina*)

The MALDI-TOF MS gave a simple distribution of three sodiated TAGs with tripunnicic ($P_nP_nP_n$) being the most abundant. The other two ions are palmitoyldilinoleoyl glycerol (PLL) and distearoylo-leyol glycerol (SSO). An ion with m/z 861 may be attributed to the α-cyano-4-hydroxycinnamic acid used as a matrix.

CONCLUSION

This study further confirms the simplicity of MALDI-TOF MS in the characterization of the triacylglycerols of oils. The method provided the molecular distribution of TAGs in the cucurbita oils and confirmed linoleic acid as the major fatty acid in Bottle gourd (*Lagenaria vulgaris*), Sponge gourd (*Luffa cylindrica*), and Flat calabash gourd (*Cucurbita maxima*) while punnicic acid is the major fatty acid in Snake gourd (*Tricosanthes cucumerina*)

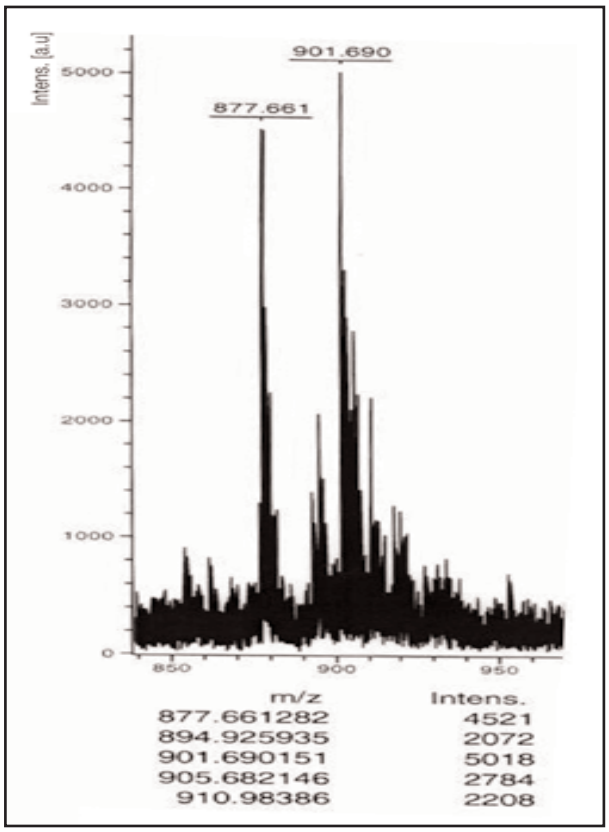

Figure 3 - Partial MALDI-TOF mass spectrum of Flat Calabash gourd oil showing sodiated TAG molecules from a range (m/z 830-960)

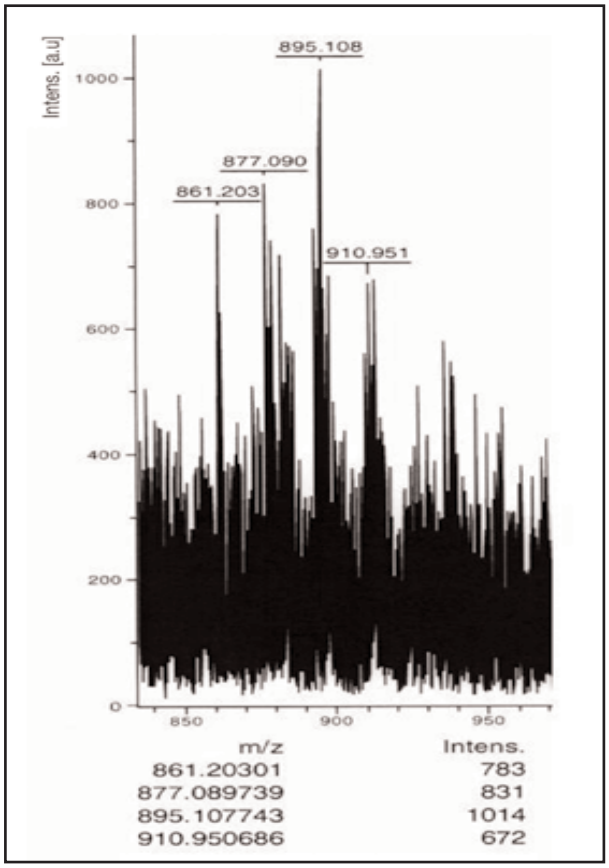

Figure 4 - Partial MALDI-TOF mass spectrum of Bottle snake gourd oil showing sodiated TAG molecules from a range (m/z 830-960)

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