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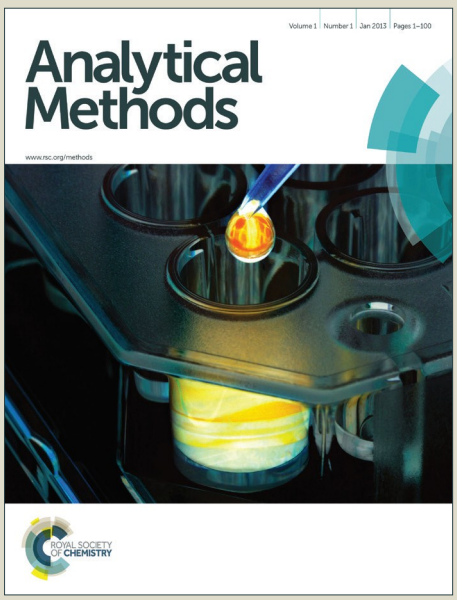

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Immediate Differentiation of Unusual Seed Oils by Easy Ambient Sonic-Spray Ionization Mass Spectrometry and Chemometric Analysis

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Vegetable oils have gained continuous economic importance being increasingly used as renewable raw materials by the food, cosmetics and biofuels industries. As an alternative to the traditional sources of oils, unusual vegetable oils from *Jatropha curcas*, *Bombacopsis glabra*, *Capparis flexuosa*, *Siparuna guianensis*, *Moringa oleifera*, *Hibiscus tiliaceus*, *Virola bicuhyba*, *Pouteria caimito* and *Syagrus coronata* seeds are used. We describe herein the immediate as well as direct, fast and low cost characterization of seed oils via triacylglycerol (TAG) and free fatty acids (FFA) profiles by easy ambient sonic-spray ionization mass spectrometry (EASI-MS) and chemometric analysis. The oils are shown to display indeed typical and unique chemical profiles of triacylglycerol (TAG) and free fatty acids (FFA) with contrasting carbon lengths, degree of unsaturation or with presence of other chemical functions on the alkyl chain. *V. bicuhyba* and *S. coronata* seed oils were found to be constituted of relatively short chain TAG making them potential raw materials for obtaining biogasolines or biokeresones used as aviation fuels. The TAG profiles of *H. tiliaceus* were very similar to soybean, *P. caimito* similar to andiroba oil and TAGs of *C. flexuosa* and *S. guianensis* similar to palm oil. FFA composition from *H. tiliaceus, P. caimito, C. flexuosa* and *S. guianensis* are rich in oleic or linoleic acids, which is an important requirement of feedstock for biodiesel production. Some polyphenolics compounds determined by EASI-MS in some of these oils are also known to provide important nutritional and therapeutic characteristics to human health. EASI-MS can therefore offer immediate characterization of such oils and help in quality monitoring and control of adulteration and to guide their application in food, cosmetics and the biofuels industries.

1. Introduction

Vegetable oils have gained growing economic importance worldwide because they are renewable raw materials used widely by the food and cosmetics industries, as well as for production of biofuels, especially biodiesel. There are several traditional sources of vegetable oils such as palm kernel, soybean, castor bean, cotton seed, sunflower seed, besides typical amazonian oils as babassu (*Orbignya* spp.), Brazil nut (*Bertholletia excelsa*), andiroba (*Carapa guianensis*), cupuaçu (*Theobroma grandflorum*), murumuru (*Astrocaryium murumuru*), buriti (*Mauritia flexuosa*), passion fruit (*Passiflora* spp.) and ucuúba (*Virola sebifera*).[1,2] Increasing

demands for vegetable oils have however encouraged the use of alternative sources of oilseed crops.[3] *Jatropha curcas, Bombacopsis glabra, Capparis flexuosa, Siparuna guianensis, Moringa oleifera, Hibiscus tiliaceus, Virola bicuhyba, Pouteria caimito* and *Syagrus coronata* are oilseeds crops known to produce high amount of oils, with potential use in various applications.[4-16] Knowledge of the chemical composition of unusual seed oils is an important parameter to predict their properties and quality. Such composition which includes mainly esters of oleic, linoleic or linolenic acids greatly influences biodiesel oxidative stability.[1,17] Characterization and typification of different vegetable oils are also essential for their differentiation, quality monitoring and control of adulteration.

Gas (GC-MS) or liquid (LC-MS) chromatography coupled to mass spectrometry[18] have been the main techniques applied to characterize vegetable oils but they require substantial sample preparation and time-consuming separation protocols. MS-only protocols using different ionization techniques have also been applied for the immediate analysis of oils providing comprehensive TAG and FFA profiles in a direct and rapid fashion.[2,19-24] MALDI-MS has also been a main technique used for direct MS analysis of oils, and has been already applied to typification of amazonian oils (*Brazil nut, buriti, andiroba, passion fruit, murumuru, ucuúba and cupuaçu*). But MALDI-MS also requires sample preparation and the use of a matrix.[2] Electrospray ionization mass spectrometry (ESI-MS) with direct infusion of analyte solutions has also been widely used to characterize vegetable oils after dilution of the sample typically with methanol–water (1:1) or methanol–chloroform (1:1), and has provided typical TAG and FFA profiles for the oils,[2] but faster analysis of the intact sample has also been performed by applying direct desorption/ionization MS techniques.[25]

Easy ambient sonic-spray mass spectrometry (EASI-MS) is one desorption/ionization technique that has been widely applied for oil characterization by TAG and FFA profiles.[19-23] For oil typification EASI-MS provides a direct and fast protocol that require very little sample manipulation/preparation. Simply, just a single drop (2 μl) of the crude oil is sufficient for analysis and sample "preparation" is limited to just dropping such droplet on a paper surface. The MS data is obtained in few seconds and in both the positive and negative ion modes enabling high analytical frequency and comprehensive TAG [for EASI (+)] and FFA [for EASI (-)] chemical profiles. For EASI-MS, the mass analyzer may be selected among the most compact, simplest and least expensive such as a single quadrupole.[24] EASI(±)-MS has indeed been applied with great success for oil analysis being able to properly characterize vegetable oils via their TAG and FFA profiles,[21] helping quality control and adulteration screening as demonstrated for the typification of diverse vegetables oils such as soybean, palm, castor, andiroba, buriti and jatropha;[20-24, 26] to monitor oxidation;[19, 27] and to characterize fresh and frying soybean oil for biodiesel production.[28]

DESI is another ambient MS technique that has been often applied to oil analysis[29-31] with similar results as compared to those of EASI-MS.[31] However EASI-MS is a dual-mode[32] simpler voltage-free technique that avoids any electrical or discharge interferences, have display fewer molecular species largely concentrated on

[TAG + Na]$^+$ ions which simplifies data analysis, superior S/N ratios and much less fragmentation of TAG ions to DAG or MAG ions.[31]

Here we show that EASI(±)-MS offers indeed a powerful immediate tool able to characterize and differentiate unusual seed oils obtained from *Jatropha curcas*, *Bombacopsis glabra*, *Capparis flexuosa*, *Siparuna guianensis*, *Moringa oleifera*, *Hibiscus tiliaceus*, *Virola bicuhyba*, *Pouteria caimito* and *Syagrus coronata*. The oils were found to display quite contrasting TAG and FFA profiles, which provide important information for their quality control and potential applications.

2. Material and methods

2.1. Materials

Fresh seeds of *Jatropha curcas, Bombacopsis glabra, Capparis flexuosa, Siparuna guianensis, Moringa oleifera, Hibiscus tiliaceus, Virola bicuhyba, Pouteria caimito and Syagrus coronata* were collected in rural areas of Bahia State, Brazil. All solvents were of HPLC quality, and all chemicals were analytical grade (>99%), being the chloroform purchased Sigma-Aldrich (Missouri, USA), methanol purchase from Merck SA (Rio de Janeiro, Brazil) and petroleum ether purchased from Vetec (Rio de Janeiro, Brazil). Sodium methoxide (0.5 mol L^{-1}) was purchased from Sigma–Aldrich (St. Louis, USA). Boron trifluoride-methanol complex was purchased from Merck (Darmstadt, Germany). A 37-component fatty acid methyl ester (FAME) mix was purchased from Sigma-Aldrich (Bellefonte, USA).

2.2. Extraction of oil from seeds

The seeds were first dried in an oven at 50°C for 2 days and then crushed using a mill (Tecnal, TE-625). The oils of the seeds were extracted by the Soxhlet method from 5g of ground seeds using 230 mL of ethyl ether heated at 65°C for 4 h. After that, the solvent was removed by means of rotary evaporation at 50°C.[33] The resulting oil was transparent with a yellowish color typical of vegetable oils.

2.3. EASI(±)-MS analysis

Data was collected on a single quadrupole mass spectrometer LCMS-2010 EV (Shimadzu, Japan) coupled with an in house-fabricated EASI source[34,35] operating in either the positive or negative ion modes. The source was operated with methanol flow rate of 20 μL min^{-1} and 3 L min^{-1} for the nebulizing gas (N_2). The surface-entrance angle was 30°. The crude samples (2 μL) were dropped on a paper surface (brown Kraft envelope paper), and EASI-MS data were collected for over 30 s, initially scanning over the range of *m/z* 200-1200. Since EASI is based on sonic spray ionization, one of the softest ionization techniques, no TAG and FFA fragments were observed.

2.4. Analysis of Fatty Acid Composition by GC-MS

The oil was weighed (75 mg) in a screw-capped glass tube. The tube was placed in a water bath (45ºC) to obtain the fatty acid methyl esters (FAME) using a two-step methylation procedure. Firstly, 3.5 mL of sodium methoxide (0.5 mol L^{-1}) was added, after 10 min, 6.0 mL of boron trifluoride–methanol complex (12%). The reaction was run for 40 min. The FAME mixture was then analyzed by GC-MS (QP-2010 Plus - Shimadzu, Japan) using a DB-23 MS capillary column (60m; 0.25 mm i.d., 0.25 μm film thickness; Agilent Corp.). The carrier gas was helium in a flow rate of 1 mL min^{-1} with a split ratio of 1:10. Analyses were performed under the following temperature oven program: 50 to 175ºC at a rate of 25ºC min^{-1}, to 230 at 4 ºC min^{-1} and held for 5 min. The ion source temperature was 230 ºC and transfer line of 250 ºC. A scan time of 1s and mass range of *m/z* 50-500 were used. FAME were identified by comparison of their retention times with those of standards using a commercial FAME standard mixture. All oil samples were analyzed in triplicate.

2.5. Multivariate data analysis

Multivariate analysis was performed with data from profiles of oils obtained from the analysis by EASI-MS in triplicate. Mass spectral data were acquired, accumulated over 60 s and processed using the LCMS solution v.3.70 software. The data were aligned and preprocessed by autoscaling (data mean-centering followed by variance scaling) to generate a final data matrix of 27 samples and 55 ions (variables) ranging from *m/z* 800 to 1000 (a range that contained all ions TAG). To discriminate the oil samples after EASI-MS fingerprinting, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) was performed on the data using the Piroutte version 4.0 program (Infometrix, Seattle, WA).

3. Results and discussion

Fig. 1 shown pictures of the seeds/nut of *J. curcas, B. glabra, C. flexuosa, S. guianensis, M. oleifera, H. tiliaceus, V. bicuhyba, P. caimito* and *S. coronata.* Table 1 shows the oil content of the different seeds, which ranges from 25% to 58% (w/w). Among the nine seeds, *V. bicuhyba (58%)* and *B. glabra* (57%), *J. curcas* (51%) and *S. guianensis* (51%) display the highest oil contents. The other five seeds display substantially lower oil contents ranging from 41% to 25%, which anyway are higher than those of the commonest soybeans which display an oil content as low as 18%.[11] Note however that the oil contents reported for such seeds vary widely, since different extraction methods, solvents, time of extraction and temperature are used. For example, the oil content of *J. curcas* seeds was reported to range from 30% to 34%[6] whereas another study conducted with various varieties of *J. curcas* reported an oil content ranging from 28% to 38%.[6,36] *B. glabra* seeds oil content was found to range from 35% to 47%,[7] but another study reported an oil content ranging from 40 to 50%.[6] *M. oleifera* seeds was reported to display an oil content from 35% to 40%,[6,37] in a range that fits the 36.5 % obtained

in this study. The oil content of *H. tiliaceus* seeds extracted with hexane in a Soxhlet apparatus was reported to be only 2.2%, but the extraction time was omitted.[38] The oil content in the *V. bicuhyba* seed was found to be 57%[13] whereas the oil content of *P. caimito* was reported to be as low as 13%.[39] The licury palm (*S. coronata*) seed was reported to display an oil content of 49%.[40] Finally, we were unable to find previous reports on the oil content of *C. flexuosa* and *S. guianensis*.

Table 1 lists also some other important characteristics of such oils such as harvesting requirements and seed preprocessing before oil extraction that prospects for large scale cultivation as needed for their use in the biofuels, cosmetics and food industry. Although it is important for a seed to be commercially viable to display high oil contents, the knowledge of the oil composition in terms of carbon length and unsaturation levels, for instance, is crucial to guide its applications.

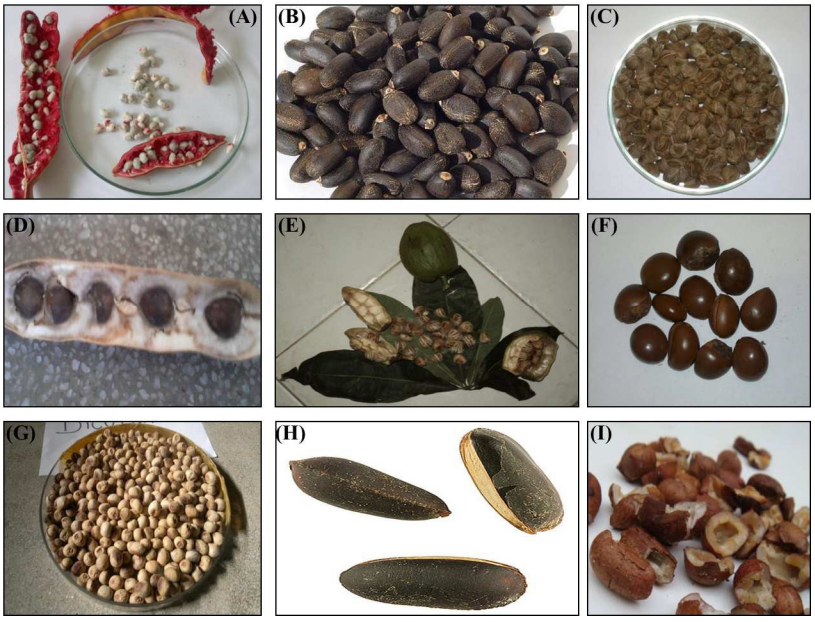

Fig. 1 Pictures of the seeds/nuts of *C. flexuosa* (A), *J. curcas* (B), *H. tiliaceus* (C), *M. oleifera* (D), *B. glabra* (E), *S. guianensis* (F), *V. bicuhyba* (G), *P. caimito* (H) *and S. coronata* (I).

Table 1 Overall characteristics for oil production of *J. curcas, B. glabra, C. flexuosa, S. guianensis, M. oleifera, H. tiliaceus, V. bicuhyba, P. caimito and S. coronata* seeds.

Plant name	Oil % (w/w) Exp*	Oil % (w/w) Lit**	Harvesting requirements	Preprocessing for oil extraction	
				Fruit	Seed/nut
J. curcas	51.2	28 – 38	Manual or mechanized harvesting	Mechanical or manual threshing	Cracking - soft shell
B. glabra	57.0	35 – 50	Manual or mechanized harvesting	Manual threshing	None
C. flexuosa	36.2	NA	Pods manual picking	Manual threshing/simple dehulling	None

S. guianensis	51.2	NA	Manual or mechanized harvesting	Mechanical threshing/crack a hard shell	None
M. oleifera	35.6	35 – 40	Pods manual picking	Manual threshing/simple dehulling	None
H. tiliaceus	25.1	2.2	Pods manual picking	Manual threshing	None
V. bicuhyba	58.3	57	Manual or mechanized harvesting	Mechanical threshing/crack a hard shell	Cracking - hard nut
P. caimito	31.6	13	Fruits fall to ground/ Manual picking	Manual dehulling	Cracking - soft shell
S. coronate	41.3	49	Manual or mechanized harvesting	Mechanical or manual dehulling	Cracking - hard nut

*oil content obtained in this study; **oil content reported in another study; NA = not available

3.2. EASI(+)-MS analysis

Fig. 2 shows all the EASI(+)-MS profiles of the vegetable oils acquired in the *m/z* 200-1200 range. Note that the TAG ions are detected in the *m/z* 800-1000 range whereas minor DAG ions are detected in the *m/z* 600-800 range. The only exceptions in regard to these ranges were observed from the *V. bicuhyba* and *S. coronata* seed oils in which TAG ions were detected in the *m/z* 650-850 and *m/z* 600-1000 ranges (Fig. 2H and 2I). As typical for EASI, TAGs were detected mainly as sodium adducts $[TAG + Na]^+$ whereas $[TAG + K]^+$ and $[TAG + Li]^+$ ions were also present but with much reduced abundances. This beneficial feature of EASI, as noted, greatly facilitates data analysis. Table 2 shows the TAG compositions revealed by such $[TAG + Na]^+$ profiles. Note that all oils display predominance of TAG composed by palmitic (P), oleic (O), stearic (S) and linoleic (L) acids. But two clear exceptions were found for the *V. bicuhyba* and *S. coronata* seed oils, which are composed mainly by TAG containing lauric acid (La). The EASI(+)-MS TAG profiles of the *V. bicuhyba* and *S. coronata* seed oils were the most distinct among the set of vegetable oils analyzed. Note that for the *V. bicuhyba* seed oil (Fig. 2H), the major $[TAG + Na]^+$ ion were those of *m/z* 689 (LaLaM), 717 (LaLaP), 743 (LaLaO) and 745 (LaLaS). The EASI(+)-MS TAG profile of the *V. bicuhyba* seed oil is therefore found to be quite similar to that of *ucuúba* oil as revealed by MALDI(+)-MS.[2] For the *S. coronata* seed oils (Fig. 2I), the major $[TAG + Na]^+$ ions were those of *m/z* 605 (LaCaCa), 633 (LaLaCa), 689 (LaLaM), 717 (LaLaP), 743 (LaLaO) and 745 (LaLaS). Both of these oils from *V. bicuhyba* and *S. coronata* seeds are therefore composed of relatively light TAG and could serve as feedstocks to produce lighter biofuels such as biokerosenes, biogasolines and aviation fuels.

Fig. 2A, 2F and 2G display the TAG profiles for the *C. flexuosa*, *S. guianensis* and *P. caimito* seed oils, which were all similar showing the same set of major ions with different relative abundances. These profiles reveals the presence of mainly of P, S, O and L acids, such as the ions of *m/z* 881 (POO ou PSL), 853 (PPL), 855 (PPO) e 907 (OOO). The $[TAG + K]^+$ ions of *m/z* 869 (PPL) and $[TAG + Li]^+$ ion of *m/z* 617 (LaLaCa) were also detected in all three seed oils. Interestingly, the TAG profile of the *P. caimito* seed oil was found to be very similar to that of *andiroba* oil.[18] Since *andiroba* and *P. caimito* are from the Amazonian region and since

andiroba oil has attracted great interest from the cosmetics and pharmaceutical industries,[26] the immediate and effective characterization of the TAG and FFA should be important for their quality control and adulteration if they indeed become similarly explored.

The TAG profiles of the *C. flexuosa*, *S. guianensis* seed oils dominated by the $[TAG + Na]^+$ ions of *m/z* 905 (OOL ou SLL) and 881 (POO) were also very similar to that of palm oil[21] and the TAG profile of *J. curcas* seed oil (Fig. 2B) was also quite similar to that of Brazil nut oil[23] and from the oils obtained from immature seeds.[22] Also interestingly was to note that the *J. curcas* oil has been proposed as a proper feedstock for the production of aviation biokerosene[34,41] since it is rich in oleic and linoleic acids.

As Fig. 2C shows, the TAG profile of *H. tiliaceus* seed oil revealed, via the major ions such as $[TAG + Na]^+$ of *m/z* 877 (PLL/POLn), 853 (PPL), 901 (LLL) and $[TAG + K]^+$ of *m/z* 869 (PPL), the predominance of palmitic, oleic and linoleic acids. Note that the EASI(+)-MS TAG profile of soybean oil also presents major ions of *m/z* 877 and 901.[18,28,42,43] It can be expected therefore that biodiesel from the *H. tiliaceus* seed oil should display similar chemical properties as that from soybean oil. This feedstock, which is not a food source, seems to offer a non-edible competitive alternative for biofuel production. Currently in Brazil, ca. 75% of biodiesel is derived from the edible soybean oil.[44]

As Fig. 2D shows, the TAG profile from the *M. oleifera* seed oil reveals mainly the presence of oleic acid, via the $[TAG + Na]^+$ ions of *m/z* 881 (POO) and *m/z* 907 (OOO/SLO). The main TAG ions of *M. oleifera* oil were also detected in olive and buriti oils, which are commercialized worldwide and highly appreciated due to its improved organoleptic properties and health benefits.[21] The TAG profile of the *B. glabra* shows mainly palmitic, oleic and linoleic acid via the $[TAG + Na]^+$ ions of *m/z* 853 (PPL), 855 (PPO) and 883 (PSO); $[TAG + K]^+$ ions of *m/z* 869 (PPL) and $[TAG + Li]^+$ ions of *m/z* 617 (LaLaCa) (Fig. 2E).

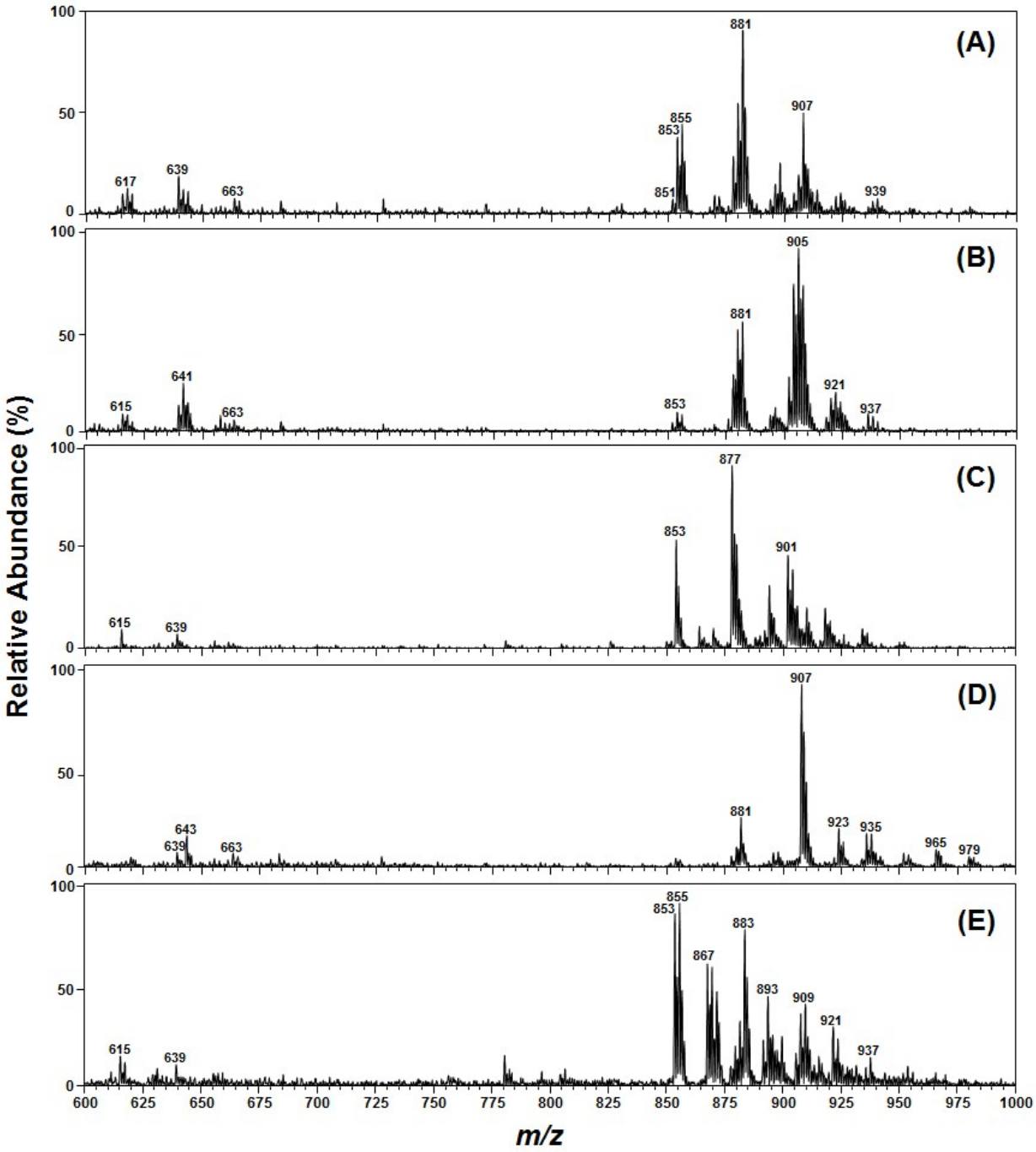

Fig. 2 EASI(+)-MS of the vegetable oils (A) *C. flexuosa,* (B) *J. curcas*, (C) *H. tiliaceus*, (D) *M. oleifera*, (E) *B. glabra*, (F) *S. guianensis*, (G) *P. caimito*, (H) *V. bicuhyba* and (I) *S. coronata.*

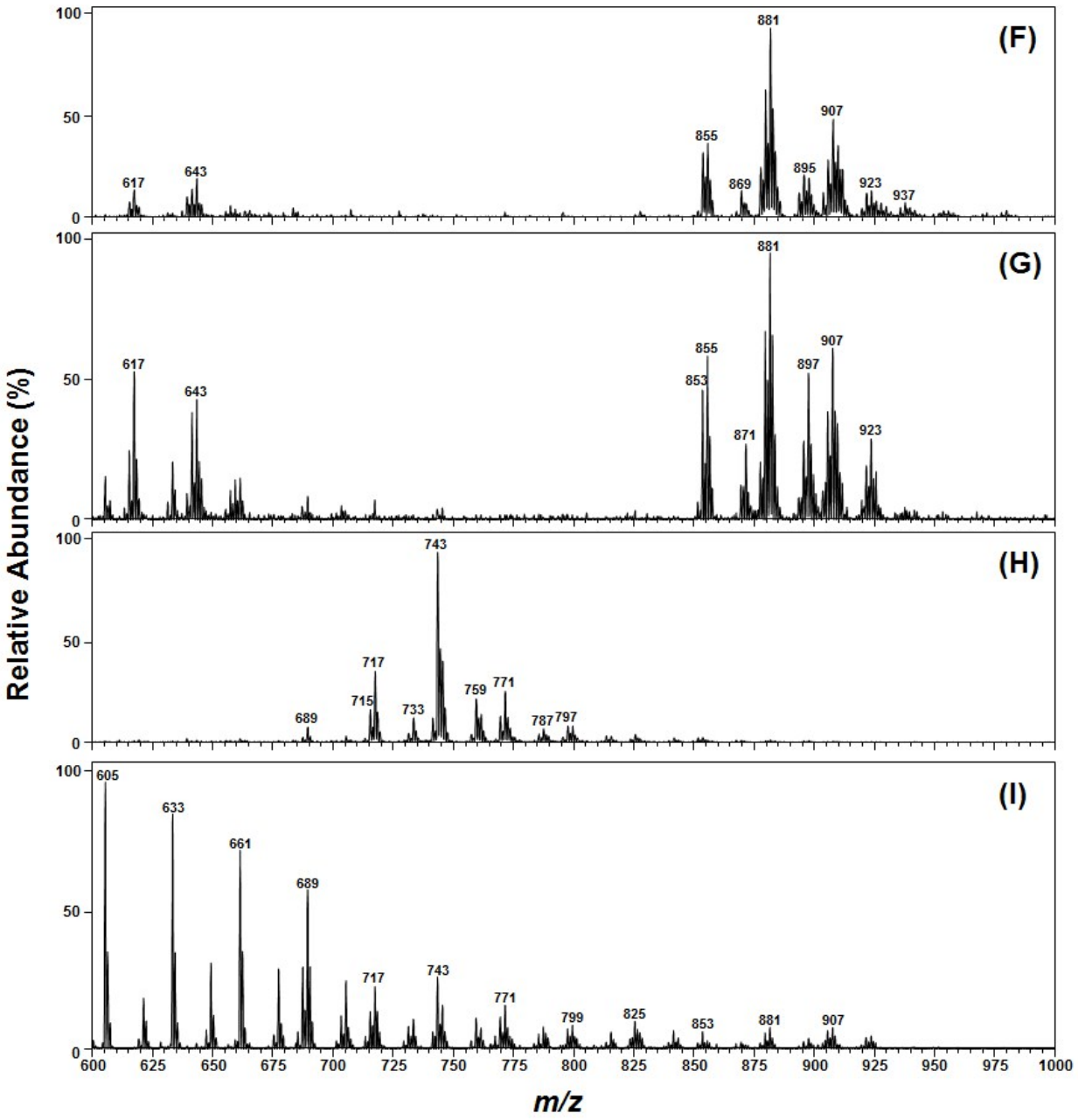

Fig. 2 EASI(+)-MS fingerprinting of vegetable oils *C. flexuosa* (A), *J. curcas* (B), *H. tiliaceus* (C), *M. oleifera* (D), *B. glabra* (E), *S. guianensis* (F), *P. caimito* (G), *V. bicuhyba* (H) and *S. coronata* (I).

Table 2 [TAG + Na]$^+$ ions and relative abundance (%) detected via EASI(+)-MS for the oils from *C. flexuosa* (CF), *J. curcas* (JC), *H. tiliaceus* (HT), *M. oleifera* (MO), *B. glabra* (BG), *S. guianensis* (SG), *P. caimito* (PC) *V. bicuhyba* (VB) and *S. coronata* (SC)

[TAG + Na]$^+$ m/z	TAG	CF	JC	HT	MO	BG	SG	PC	VB	SC
605	LaCaCa	-	-	-	-	-	-	-	-	**100**
633	LaLaCa	-	-	-	-	-	-	-	-	93.1
689	LaLaM	-	-	-	-	-	-	-	6.6	66.8
717	LaLaP	-	-	-	-	-	-	-	40.9	26.1
743	LaLaO	-	-	-	-	-	-	-	**100**	28.7
745	LaLaS	-	-	-	-	-	-	-	47.9	17.4
771	MMPo	-	-	-	-	-	-	-	33.3	17.6
773	MMP	-	-	-	-	-	-	-	10.2	5.5
799	MMO	-	-	-	-	-	-	-	9.9	10.2
825	PPoPo	-	-	-	-	-	-	-	5.8	11.4
829	PPP	5.7	-	-	-	-	-	-	-	-
853	PPL	43.6	-	55.1	-	80.8	48.3	30.8	-	6.3
855	PPO	55.4	8.2	12.9	-	**100**	53.4	53.3	-	-
877	PLL/POLn	32.6	41.4	**100**	-	-	35.6	12.8	-	-
879	PLO/PSLn	53.2	54.2	67.4	12.1	13.3	71.1	49.2	-	5.8
881	POO/PSL	**100**	43.4	18.6	24.9	32.4	**100**	**100**	-	9.1
883	PSO	33.0	13.8	-	10.1	75.0	40.8	26.8	-	-
901	LLL/OLLn	-	28.8	42.5	-	-	-	7.2	-	-
903	OLL/OOLn	-	94.7	46.5	-	-	12.6	9.9	-	-
905	OOL/SLL	25.4	**100**	37.8	-	12.4	35.1	34.9	-	7.5
907	OOO/SLO	45.1	94.6	-	**100**	27.9	57.7	55.3	-	8.5
909	SOO/SSL	25.5	44.9	-	44.4	38.4	50.7	31.3	-	-
911	SSO	-	-	-	9.5	15.6	17.7	7.5	-	-
935	ALO	-	10.1	9.1	18.1	-	-	-	-	-
937	SAL/AOO	-	9.5	-	19.0	12.5	10.4	-	-	-
939	SAO	11.4	5.25	-	-	-	-	-	-	-
965	AAL	-	-	-	11.0	-	-	-	-	-
967	AAO	-	-	-	6.0	-	-	-	-	-
969	AAS	-	-	-	-	-	-	-	-	-

La=Lauric Acid; M = Myristic Acid; P = Palmitic Acid; Po = Palmitoleic Acid; S = Stearic Acid; O = Oleic Acid; L = Linoleic Acid; Ln = Linolenic Acid; A = Arachidic Acid; Ca = Capric Acid.

3.3. Differentiation of vegetable oils for multivariate analysis

EASI-MS data of all vegetable oils were subtracted from solvent-generated background data. The peak most intense in the spectrum is assigned as 100% and the others present peaks are assigned with decreasing relative abundances in relation to the peak most intense. Peak picking and relative abundances, peak selection

and *m/z* alignments were all performed in data pre-treatment. The consistent variables (ion peak intensity information) were obtained by filtering peaks with 80% missing values. To remove the offsets and adjust the importance of high and low abundance peaks to an equal level, the data was pre-processed by both mean-centering and variance-scaling prior to multivariate analysis. The resulting scaled datasets comprised of the *m/z* of the ion peaks and their relative intensities were further subjected to multivariate data analysis. To evaluate the performance of the direct EASI(+)-MS technique to discriminate and classify the different types of vegetable oils in terms of their TAG profiles (multivariate data), principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied. ~~Data comprised of the *m/z* of the ions and the relative abundance of the ion peaks was used in such evaluation.~~ Fig. 3 shows PCA plot of the first three main components. When the nine types of oils are compared, three oils, that is, *B. glabra*, *V. bicuhyba* and *S. coronata* displayed the most distinct features, most particularly for the *S.coronata* seed oil, and therefore their samples were placed each in well defined and quite distant cluster whereas the other six oils were placed all together in a quite close proximity. Removing from the multivariate analysis the three most distinct oils, differentiation of the other six types of oils was however also possible (Fig. 4). The variances of PC1, PC2 and PC3 were 34.86%, 28.32% and 14.32% respectively, representing 77.50% of the total TAG variability of data.

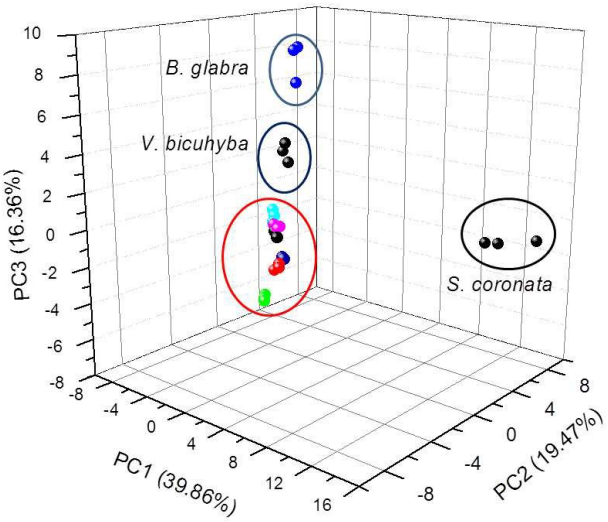

Fig. 3 PCA (score plot) of TAG profiles from vegetable oils *C. flexuosa*, *J. curcas*, *H. tiliaceus*, *M. oleifera*, *B. glabra*, *S. guianensis, P. caimito, V. bicuhyba* and *S. coronata.*

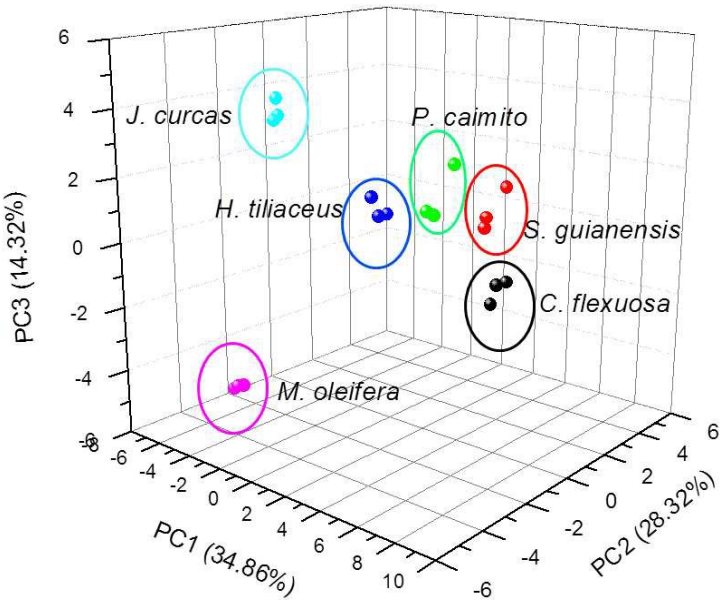

Fig. 4 PCA (score plot) of TAG profiles from vegetable oils *C. flexuosa*, *J. curcas*, *H. tiliaceus*, *M. oleifera*, *S. guianensis* and *P. caimito.*

Although the PCA score plot provides visualization of clusters of samples, it provides no information on the closeness between groups and between single samples. Also, only three PCs can be graphically shown, and in this case, they together comprise less than 80% of the data variation. Hierarchical clustering analysis (HCA) was therefore applied for most similar oils and Fig. 5 shows the resulting dendrogram. Note in this dendogram the six well-defined clusters, in which samples are grouped in clusters in terms of their nearness or similarity. Three groups of samples *M. oleifera* (MO), *H. tiliaceus* (HT) and *J. curcas* (JC) are clearly discernible. *H. tiliaceus* (HT) consist of those samples with highest levels of TAG PLL/POLn. *M. oleifera* (MO) and *J. curcas* (JC) oils are also shown to have similar TAG profiles with highest levels of OOO/SLO and OOL/SLL, OOL/OOLn, OOO/SLO, respectively. *S. guianensis* (SG), *P. caimito* (PC) and *C. flexuosa* (CF) are the most similar clusters in terms of TAG composition, so they are closer together.

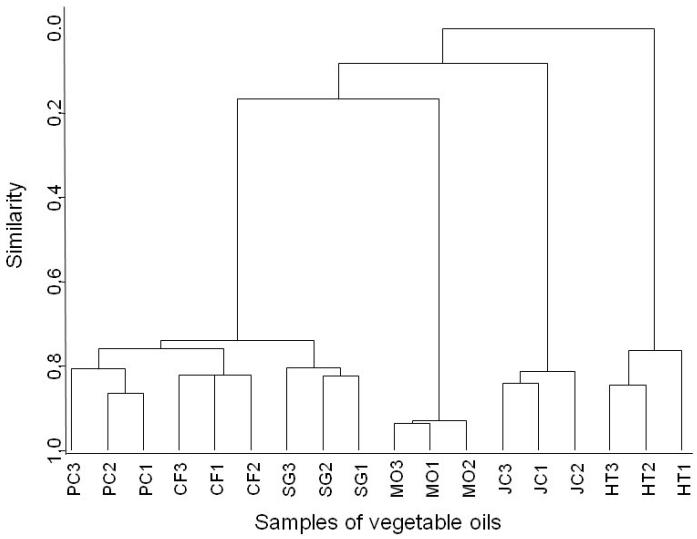

Fig. 5 Dendrogram of HCA analysis of vegetable oils *C. flexuosa* (CF), *J. curcas* (JC), *H. tiliaceus* (HT), *M. oleifera* (MO), *S. guianensis* (SG) and *P. caimito* (PC).

3.4. EASI(-)-MS analysis

Fig. 6 shows that these unusual seed oils can also be properly characterized and their properties inferred by their typical FFA profiles as revealed by EASI(-)-MS immediate analysis. Note that the bipolar nature of the EASI droplets allows concomitant formation of both gaseous $[TAG + Na]^+$ and $[FA - H]^-$ ions from the same spray plume. For FFA, the EASI(-)-MS display mainly $[FFA - H]^-$ ions from palmitic (*m/z* 255), linolenic (*m/z* 277), linoleic (*m/z* 279), oleic (*m/z* 281), stearic (*m/z* 283), gondoic (*m/z* 309), arachnidic (*m/z* 311), heneicosylic (*m/z* 325), behenic (*m/z* 339) and octacosenoic (*m/z* 421) acids. The most diverse and hence characteristic FFA profile was displayed by the *V. bicuhyba* seed oil in which myristic acid (*m/z* 227) and cetoleic acid (*m/z* 337) predominate. *S. coronata* seed oil also showed a very distinct and characteristic FFA profile, as for the EASI(+)-MS profile of TAG and DAG ions. The *B. glabra* seed oil also displayed a unique feature in its FFA profile, that is, the gondoic acid (*m/z* 309) appears as the most abundant FFA.

C. flexuosa, *J. curcas*, *M. oleifera*, *S. guianensis* and *P. caimito* seed oils show predominant $[FFA - H]^-$ ions from oleic acid (*m/z* 281) as also observed for the andiroba oil.[21] In these oils, linoleic acid (*m/z* 279) is also a major FFA constituent as also found for their *H. tiliaceus* seed and Brazil nut oils.[21] Palmitic, linoleic and oleic acids are the main constituents of oils of olive, hazelnut, soybean, grape seed, canola, butter and lard oils as revealed by EASI(-)-MS.[42] Note the predominance of mono-unsaturated FAs in most of the oils studied, such as oleic acid, which is beneficial for biodiesel production since low percentage of poly-unsaturated FA is a key requirement for a high quality biodiesel.[45] Note that the soybean biodiesel has only ca. 23% of methyl ester from oleic acid in its composition.[46]

Besides the FFA, other important bioactive components in these seed oils were also detected by EASI(-)-MS (Table 3) of FFA, but also includes more acidic components such as phenolic constituents including 1-

acetoxypinorenisol (*m/z* 415) in *H. tiliaceus* and *V. bicuhyba* seed oils, chlorogenic acid (*m/z* 353) in *C. flexuosa*, *H. tiliaceus*, *M. oleifera*, *B. glabra*, *S. guianensis*, *V. bicuhyba* and 3,4-DHPEA-EDA (*m/z* 377) in *J. curcas*, *H. tiliaceus*, *B. glabra*, *V. bicuhyba*, *S. coronata*. These polyphenols have been proposed as markers of olive oils,[27] chia seeds and its oil.[47] Polyphenolics are known to confer to the oil important nutritional and therapeutic characteristics which are beneficial to human health,[48] and their detection in the vegetables oils herein studied seems to make them promising sources of food oil.

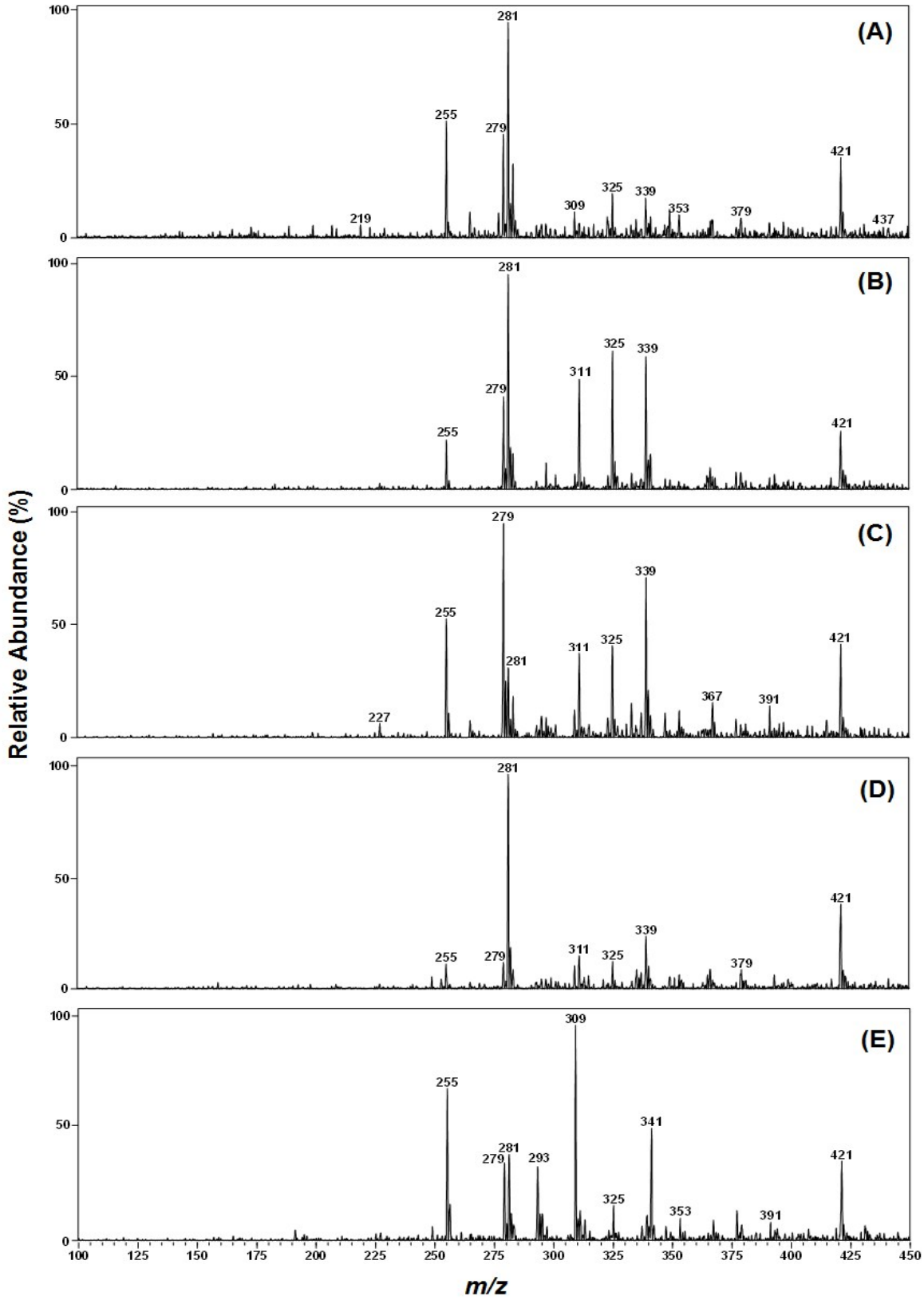

Fig. 6 EASI(-)-MS fingerprinting of FFA of the vegetable oils (A) *C. flexuosa*, (B) *J. curcas*, (C) *H. tiliaceus*, (D) *M. oleifera*, (E) *B. glabra*, (F) *S. guianensis*, (G) *P. caimito*, (H) *V. bicuhyba* and (I) *S. coronata*.

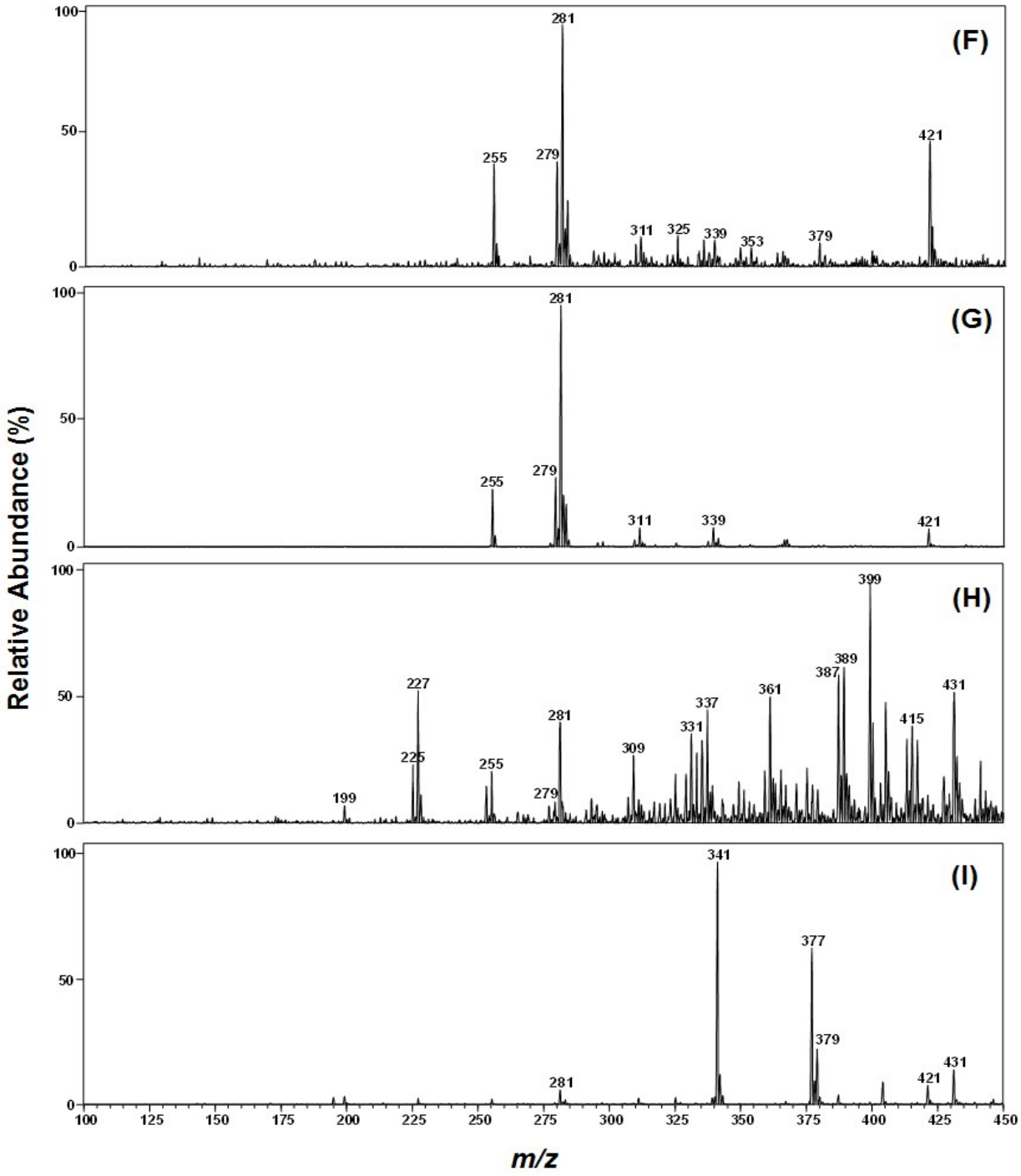

Fig. 6 EASI(-)-MS fingerprinting of FFA of the vegetable oils (A) *C. flexuosa*, (B) *J. curcas*, (C) *H. tiliaceus*, (D) *M. oleifera*, (E) *B. glabra*, (F) *S. guianensis*, (G) *P. caimito*, (H) *V. bicuhyba* and (I) *S. coronata*.

Table 3 FFA detected as $[FFA - H]^-$ ions by EASI(-)-MS and their relative abundances (%) in the vegetable oils *C. flexuosa* (CF), *J. curcas* (JC), *H. tiliaceus* (HT), *M. oleifera* (MO), *B. glabra* (BG), *S. guianensis* (SG), *P. caimito* (PC), *V. bicuhyba* (VB) and *S. coronata* (SC).

$[M - H]^-$ *m/z*	Common Name IUPAC Name	CN:DB*	Relative abundance (%)								
			CF	JC	HT	MO	BG	SG	PC	VB	SC
199	Lauric Acid Dodecanoic acid	12:0	-	-	-	-	-	-	-	6.5	-
225	Myristoleic acid Tetradecenoic acid	14:1	-	-	-	-	-	-	-	20.3	-
227	Myristic Acid Tetradecanoic Acid	14:0	-	-	6.6	-	-	-	-	46.3	-
255	Palmitic Acid Hexadecanoic Acid	16:0	54.4	20.2	60.9	11.5	77.5	40.6	22.3	15.5	-
277	Linolenic Acid Octadecatrienoic Acid	18:3	11.1	-	-	-	-	-	-	6.7	-
279	Linoleic Acid Octadecadienoic Acid	18:2	43.1	12.7	**100**	12.3	41.6	41.2	27.5	8.9	-
281	Oleic Acid Octadecenoic Acid	18:1	**100**	**100**	33.3	**100**	44.4	**100**	**100**	45.3	5.5
283	Stearic Acid Octadecanoic Acid	18:0	33.2	16.3	15.5	10.0	11.6	27.0	16.5	-	-
309	Gondoic Acid Eicosenoic Acid	20:1	11.6	6.6	14.1	11.2	**100**	7.4	-	26.7	-
311	Arachidic Acid Icosanoic Acid	20:0	9.8	51.7	39.0	17.0	15.5	13.0	6.7	7.9	-
337	Cetoleic acid Docosenoic acid	22:1	5.3	7.0	11.9	6.9	6.3	7.4		40.1	-
339	Behenic Acid Docosanoic Acid	22:0	17.6	63.9	72.7	26.0	16.2	13.3	8.0	14.9	-
367	Lignoceric Acid Tetracosanoic Acid	24:0	12.1	6.5	19.3	-	10.0	-	-	15.4	-
391	- Hexacosdisenoic Acid	26:2	6.3	-	12.3	-	6.8	-		16.6	-
421	- Octacosenoic Acid	28:1	44.1	43.5	44.7	48.8	41.9	63.4	7.6	10.4	8.6
423	Montanic Acid Octacosanoic Acid	28:0	-	8.1	5.5	7.3	-	8.3		8.3	-

* CN = carbon number and DB = number of double bound

3.5. GC-MS analysis of FAMEs

To investigate whether the FA profiles as revealed by direct EASI(-)-MS analysis would indeed correspond to the actual composition in the oil, such profiles were also characterized as FAMEs by GC-MS. The identification of FAMEs was performed by comparison of retention times with a standard FAME mixture (Table 4). Indeed the FAME profiles for all oils as obtained by classical protocol of GC-MS were similar to those obtained by EASI-MS where they were detected directly as FFA with no derivatization, reducing sample manipulation as well as derivatization bias and artifacts.

Table 4 FAME as detected by GC-MS and their relative concentration (%) in the vegetable oils *C. flexuosa* (CF), *J. curcas* (JC), *H. tiliaceus* (HT), *M. oleifera* (MO), *B. glabra* (BG), *S. guianensis* (SG) and *V. bicuhyba* (VB).

FAME	CN:DB	JC	BG	CF	SG	MO	HT	VB
Methyl laurate	C12:0	0.02	0.01	0.01	-	-	0.17	18.42
Methyl myristate	C14:0	0.23	0.38	0.42	0.87	0.13	2.27	30.72
Methyl myristoleate	C14:1	0.01	-	-	-	-	0.16	22.44
Methyl palmitate	C16:0	14.53	43.86	27.75	24.72	7.25	27.48	11.82
Methyl palmitoleate	C16:1	1.34	1.24	1.65	0.08	1.67	0.51	5.21
Methyl cis-10-heptadecenoate	C17:1	0.07	0.12	0.05	-	0.04	0.85	-
Methyl stearate	C18:0	10.59	7.68	7.74	18.13	8.63	4.55	1.11
Methyl oleate	C18:1	42.85	27.38	42.06	37.93	56.20	18.25	8.73
Methyl linoleate	C18:2	29.45	15.38	15.58	17.06	1.49	42.97	0.82
Methyl linolenate	C18:3	0.34	0.17	2.33	0.30	0.25	0.11	0.09
Methyl arachidate	C20:0	0.39	0.83	0.91	0.64	6.25	0.64	0.04
Methyl gadoleate	C20:1	0.09	2.42	0.18	0.16	4.08	1.62	0.47
Methyl behenate	C22:0	0.04	0.39	0.80	0.06	12.15	0.24	0.04
Methyl lignocerate	C24:0	0.04	0.14	0.53	0.04	1.87	018	0.10

* CN = carbon number and DB = number of double bound

4. Conclusions

As demonstrated herein by the oils from the seeds of *V. bicuhyba, B. glabra, J. curcas, S. guianensis, C. flexuosa*, *M. oleifera*, *H. tiliaceus*, *P. caimito* and *S. coronata,* quite unique EASI(+)-MS profiles of TAG and EASI(-)-MS profiles of FFA as well as other natural acids and phenolic bioactive constituents can be obtained in a quite direct and rapid fashion. These profiles provide, in a fully direct, rapid and simple way, immediate typification and differentiation of oils as well as comprehensive information on their chemical that helps to predict main properties from a single oil droplet. The sample preparation-free protocol of EASI(±)-MS can be therefore applied for quality monitoring, control of adulteration and as a guide for the potential applications of the large diversity of vegetable oils available all around the world.

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Notes

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Abbreviations: BG - *Bombacopsis glabra,* CF - *Capparis flexuosa,* EASI - easy ambient sonic-spray ionization mass spectrometry, ESI-MS - electrospray ionization mass spectrometry, FFA – free fatty acids, GC-MS - gas chromatography mass spectrometry, HCA - hierarchical cluster analysis, HT - *Hibiscus tiliaceus,* JC - *Jatropha curcas,* LC-MS - liquid chromatography mass spectrometry, MALDI – Matrix assisted laser desorption/ionization, MO - *Moringa oleifera,* PC - *Pouteria caimito,* PCA – Principal Component Analysis, SG - *Siparuna guianensis,* SC - *Syagrus coronata*, TAG – triacylglycerol, VB - *Virola bicuhyba.*

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