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ORIGINAL ARTICLE

TAG Profiles of *Jatropha curcas* L. Seed Oil by Easy Ambient Sonic-Spray Ionization Mass Spectrometry

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Abstract Easy ambient sonic-spray ionization mass spectrometry (EASI–MS) was used to follow the maturation of *Jatropha curcas* L. seeds via the monitoring of the triacylglycerides (TAG) profile of the oil. Results show that TAG composition is significantly modified during seed development but remains nearly unchanged during storage. The EASI–MS oil analysis performed herein is simple, requires just a tiny droplet of the oil and is performed without any pre-separation or chemical manipulation. The oil from *Jatropha gossypifolia* L. was also evaluated, and a very different TAG profile was obtained.

Keywords *Jatropha curcas* L. · Oil profile · Fatty acids · Triacylglycerides · Ambient mass spectrometry

Introduction

Jatropha curcas L. (Euphorbiaceae) is a perennial oilseed crop with high inedible oil content (up to 50% of seed weight) [1] that has attained economical importance in the tropics due to its potential as a renewable vegetable oil source for biodiesel production [2, 3, 4, 5]. *Jatropha* is a small tree (3–6 m height) originating from Central and South America, drought-resistant, and can be cultivated on non-agricultural lands, diminishing competition with food crops for biofuels. The plant is, however, still classified as wild with high variability in growth and yield parameters. The major fatty acids of the *Jatropha* oil are oleic acid (18:1; 34.3–45.8%), linoleic acid (18:2; 29.0–44.2%), palmitic acid (16:0; 14.1–15.3%) and stearic acid (18:0; 3.7–9.8%) [6].

In oilseeds, the reserve mobilization for germination and seedling establishment are mainly stored in the form of lipids, found mostly as triacylglycerides (TAG) that are stored in small spherical organelles called oil bodies [1]. Many of the genes involved in *Jatropha curcas* L. lipid metabolism have been identified [7], but little is known about the oil profile of germination and maturation of *Jatropha* seed. Knowledge of the synthesis of storage oil is essential to improve seed cultivation and harvest. To help bridge this gap we report herein oil characterization of different seed stages of *Jatropha curcas* L. performed by easy ambient sonic-spray ionization mass spectrometry (EASI–MS). For direct MS oil analysis (no hydrolysis or derivatization), three ionization techniques, namely electrospray ionization (ESI) [8, 9], matrix-assisted laser desorption (MALDI) [10, 11] and EASI [12] have been applied. But ESI and MALDI still require some sort of sample preparation. EASI is, however, an ambient ionization technique [13] which requires no sample handling at all, providing the fastest MS analysis (a few seconds) of the fully undisturbed sample in the open atmosphere. EASI(+)–MS of tiny single oil droplet placed on paper surfaces at ambient conditions has recently been shown to provide characteristic TAG profiles for different types of

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vegetable oils with proper qualitative responses [12]. The results help to increase our understanding of the differential basis of oil accumulation in seeds. *Jatropha gossypifolia* L. was also evaluated, and its TAG profile was compared to the parent species.

Experimental Procedures

Biological Material

Jatropha curcas L. fruits [7] were harvested, dissected and the seeds were collected for immediate oil extraction. The seeds were separated into four samples: young and fresh seeds (Fig. 1a), intermediate development seeds (Fig. 1b), total development seeds (Fig. 1c) and dry seeds (seeds stored more than 1 month) (Fig. 1d). For *Jatropha gossypifolia* L. only dry seeds (seeds stored more the 1 month) were used (Fig. 2). All seeds were washed with 70% ethanol.

Oil Extraction

The oil was extracted from twenty seeds of each sample of *Jatropha curcas* L. and sixty seeds of *Jatropha gossypifolia* L. The seeds were macerated, petroleum ether (50 mL) was added, and the samples were shaken for 24 h. After filtering, the organic layer was evaporated and the oil was obtained.

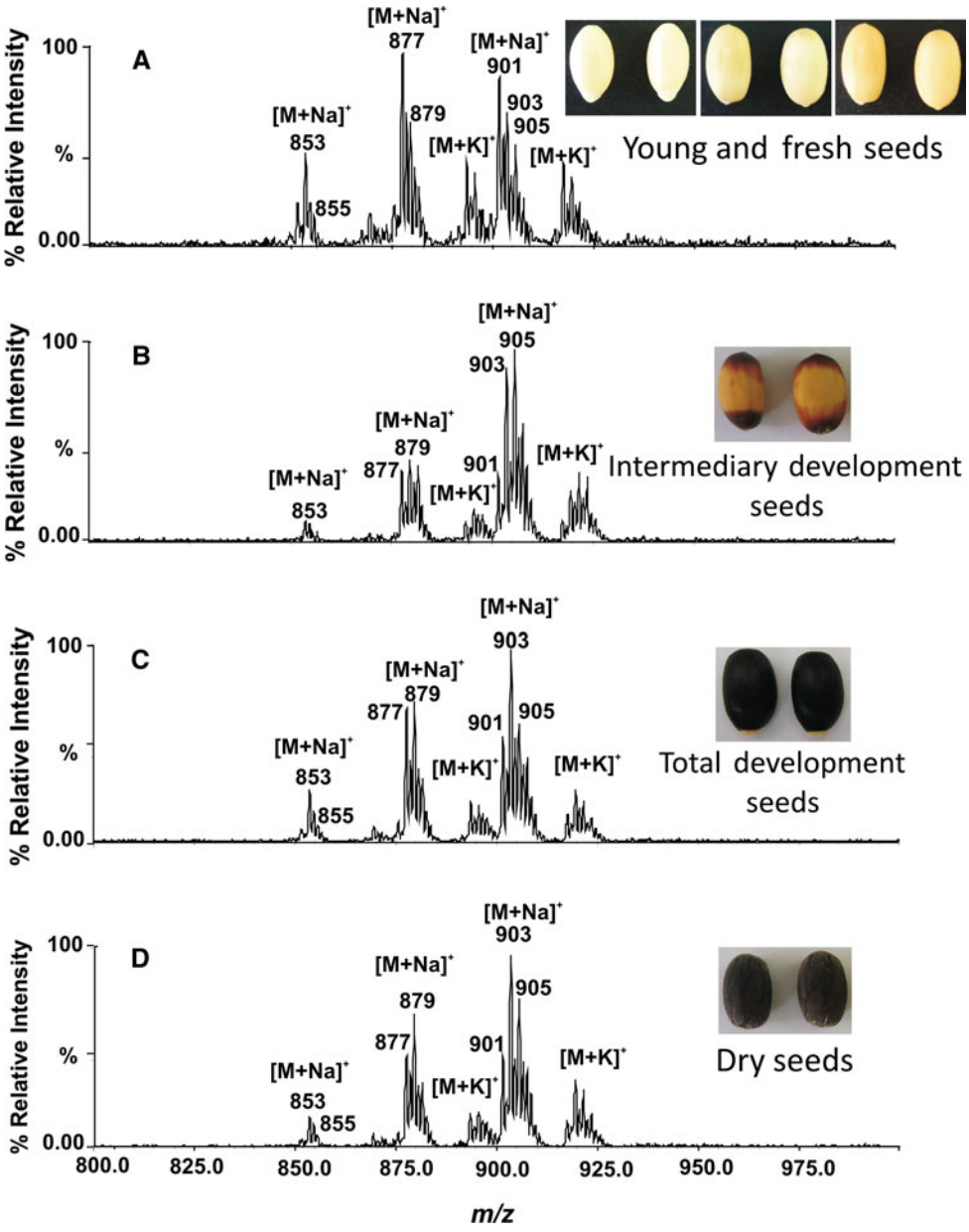

Fig. 1 TAG profiles obtained by EASI(+)–MS of oil extracted from **a** young and fresh seeds, **b** intermediate development seeds, **c** total development seeds and **d** dry seeds of *Jatropha curcas* L

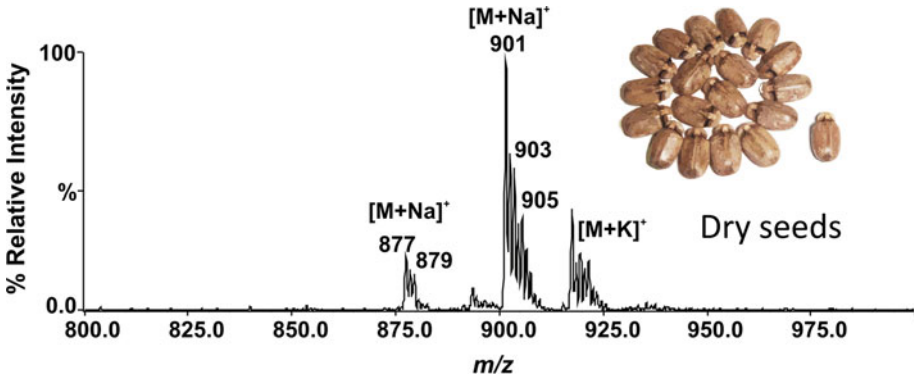

Fig. 2 TAG profile obtained by EASI(+)–MS the oil extracted from dry seeds of *Jatropha gossypifolia* L., a genetically modified version of *Jatropha curcas* L

Easy Ambient Sonic-Spray Ionization Mass Spectrometry

Spectra from *Jatropha curcas* L. after different seed stages were obtained in the positive ion mode, using a single-quadrupole mass spectrometer (Shimadzu LCMS 2010, Shimadzu Corp., Kyoto, Japan) equipped with a homemade EASI source, which is described in detail elsewhere [14, 15]. Typical EASI–MS conditions were as follows: a N_2 nebulizing gas 3 L min^{-1}, surface angle of ca. 30°, and methanol flow rate of 20 μL min^{-1}. The oil sample (2 μL) was placed directly onto the paper surface (brown Kraft paper) and the mass spectra accumulated over 60 s and scanned in the 50–1,000 *m/z* range. Thus, the EASI–MS analyses were performed for the crude oil sample without any sample preparation.

Fourier Transform–Ion Cyclotron Resonance–Mass Spectrometry

Twenty milligrams of the oil extracted from *Jatropha curcas* L. seeds were dissolved in 20 mL of chloroform:methanol (1:1) solution. Then 100 μL was diluted with one milliliter of methanol. The solution was spiked with 10 μL of 5 mmol L^{-1} CH_3COOLi. Mass analysis was performed with a FT–ICR–MS equipment. Mass spectra were collected in the positive ion mode in a LTQ FT Ultra mass spectrometer (ThermoScientific, Bremen, Germany). Samples were infused at a flow rate of 5 μL/min. Mass spectra were accumulated over 5 min, scanned over the 800–1,000 *m/z* range, centered and aligned using the Xcalibur 2.0 software (ThermoScientific, Bremen, Germany). The high mass accuracy obtained using FT–ICR–MS (typically 0.1 ppm) allows unequivocal attribution of TAG molecular composition.

Results and Discussion

Figure 1 shows the TAG profiles obtained by EASI(+)–MS from the oil of the seeds of *Jatropha curcas* L. in different development phases. TAG were detected mainly as $[TAG + Na]^+$ and $[TAG + K]^+$ ions in the range of *m/z* 850–925. TAG composition measured by EASI–MS corresponds closely to the composition of fatty acids of *Jatropha curcas* L. oil as determined by other methods [6]. Figure 1a shows oil TAG profile from of young and fresh seeds of *Jatropha curcas* L. The most abundant $[TAG + Na]^+$ ion is that of *m/z* 877 corresponding to PLL. Oils from intermediate development seeds showed a substantially modified TAG profile (Fig. 1b). The most abundant $[TAG + Na]^+$ ions in this stage were those of *m/z* 903 and *m/z* 905, corresponding to LLO and LOO (and/or SLL), respectively. Moreover, the relative abundance of the ions of *m/z* 853, 855, 877, 879 and 881 decreased when compared to that for the young and fresh seeds (Fig. 1a). The EASI–MS for oil obtained from total development seeds (Fig. 1c) shows a lower abundance of the ion of *m/z* 905 (LOO and/or SLL), in addition to the ion of *m/z* 903 (LLO), which is now the most abundant. This trend indicates that young and fresh seeds present higher proportions of palmitic acid in their TAG molecules as compared to total development seeds, for which oleic acid predominates. This result is also in accordance with the high amounts of oleic and linoleic acid known for *Jatropha curcas* L. seeds, followed by palmitic and stearic acids [1]. Figure 1d shows the spectrum of dry seeds which is found to be very similar to that of total development seeds (Fig. 1c). Interestingly, the seed oil TAG profile of *Jatropha curcas* L. remains nearly unchanged during storage. Table 1 shows the major ions detected in the positive ion mode as confirmed by FT–ICR–MS (Table 2).

Figure 2 shows the TAG profile of the dry seeds of *Jatropha gossypifolia* L. The most abundant $[TAG + Na]^+$ ion is that of *m/z* 901 corresponding to LLL, whereas the ions of *m/z* 877 (PLL), 879 (PLO), 903 (LLO) and 905 (LOO) are also abundant. Interestingly, the genetic modification significantly alters the TAG profile as compared to the parent seed (Fig. 1d), and can therefore be used to distinguish between the two species. We note also that the TAG profile of the oil from *Jatropha gossypifolia* L. is quite similar to that of soybean oil [12].

Table 1 Assignment of the ions detected by EASI(+)–MS of oil extracted from *Jatropha curcas* L. seed

MM Da	$[TAG + Na]^+$ *m/z*	$[TAG + K]^+$ *m/z*	Assignment[a]	Elemental composition[b]	TAG[c]
830	853	869	50:2	$C_{53}H_{98}O_6$	PPL
832	855	871	50:1	$C_{53}H_{100}O_6$	PPO
854	877	893	52:4	$C_{55}H_{98}O_6$	PLL
856	879	895	52:3	$C_{55}H_{100}O_6$	PLO
858	881	897	52:2	$C_{55}H_{102}O_6$	POO
878	901	917	54:6	$C_{57}H_{98}O_6$	LLL
880	903	919	54:5	$C_{57}H_{100}O_6$	LLO
882	905	921	54:4	$C_{57}H_{102}O_6$	LOO or SLL
884	907	923	54:3	$C_{57}H_{104}O_6$	OOO or SLO

[a] Carbon number followed by the number of unsaturated bonds

[b] Identified by FT–ICR–MS

[c] *O* Oleic acid, *L* linoleic acid, *Ln* linolenic acid, *P* palmitic acid and *S* stearic acid

Table 2 Assignment of the ions detected by FT–ICR–MS of oil extracted from *Jatropha curcas* L. seed

Theoretical MM Da	Theoretical $[TAG + Li]^+$ *m/z*	Experimental $[TAG + Li]^+$ *m/z*	Error (ppm)	Elemental composition	TAG[a]
830.73579	837.75180	837.75198	0.218	$C_{53}H_{98}O_6Li$	PPL
832.75144	839.76745	839.76780	0.420	$C_{53}H_{100}O_6Li$	PPO
854.73579	861.75180	861.75190	0.119	$C_{55}H_{98}O_6Li$	PLL
856.75144	863.76745	863.76777	0.373	$C_{55}H_{100}O_6Li$	PLO
858.76709	865.78310	865.78366	0.649	$C_{55}H_{102}O_6Li$	POO
878.73579	885.75180	885.75190	0.116	$C_{57}H_{98}O_6Li$	LLL
880.75144	887.76745	887.76767	0.250	$C_{57}H_{100}O_6Li$	LLO
882.76709	889.78310	889.78377	0.756	$C_{57}H_{102}O_6Li$	LOO or SLL
884.78274	891.79875	891.79899	0.272	$C_{57}H_{104}O_6Li$	OOO or SLO

[a] *O* Oleic acid, *L* linoleic acid, *Ln* linolenic acid, *P* palmitic acid and *S* stearic acid

Conclusion

In conclusion, the spectra summarized in Fig. 1 show that EASI–MS, performed on a tiny single droplet of the sample placed on an inert surface at ambient conditions, functions as a simple, direct and easy way to follow seed development of *Jatropha curcas* L. by displaying characteristic TAG "signatures". These TAG profiles are shown to change during maturation and drying but remain nearly unaltered during storage. A very distinct TAG profile was revealed for *Jatropha gossypifolia* L. with nearly unknown properties. The knowledge of the TAG profile of an oil is useful to direct its proper use by the chemical, food and pharmaceutical industries and for biodiesel production [3, 6].

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