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ORIGINAL PAPER

Removal and Degradation of Phorbol Esters during Pre-treatment and Transesterification of *Jatropha curcas* Oil

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Abstract Phorbol esters present in *Jatropha curcas* oil are toxic when consumed and are co-carcinogens. These could be a potential constraint in the widespread acceptance of *Jatropha* oil as a source of biodiesel. Phorbol esters were quantified in the fractions obtained at different stages of oil pre-treatment and biodiesel production. During degumming some phorbol esters were removed in the acid gums and wash water. This implies that the use of these acid gums in animal feed is not possible and care should be taken when disposing the wash water into the environment. Silica treatment did not decrease the phorbol esters, while stripping/deodorization at 260 °C at 3 mbar pressure with 1% steam injection completely degraded phorbol esters. Phorbol esters were not detected in stripped oil, fatty acid distillate, transesterified oil (biodiesel) and glycerine. The presence of possibly toxic phorbol ester degradation products in these fractions could not be ruled out.

Keywords Phorbol esters · Transesterification · Biodiesel · Biofuel · *Jatropha*

Introduction

At present, nearly 90% of the world's energy demand is met by the combustion of non-renewable fossil fuels. Due to the increased energy demand throughout the world, the oil reserves are expected to last many years less than projections made earlier. This coupled with the contribution of fossil fuel combustion to global warming and acid rain and increasing mineral oil prices have given momentum to the exploitation of renewal sources of energy, the production of biodiesel through esterification of vegetable oils and animal fats being one of them. The use of edible oils for biodiesel production could be detrimental to food availability. A recent study by Azam et al. [1] on the evaluation of 75 non-edible oils as a source of biodiesel identified *Jatropha curcas* as one of the most promising plants.

Jatropha curcas belongs to the Euphorbiaceae family. It is considered to be native to Central and South America and is widely present throughout Central America, Africa and Asia. *Jatropha* is a vigorous, drought- and pest-resistant plant, and can grow under a wide range of rainfall regimes ranging from 200 mm to over 1,500 mm per annum. It survives also on barren, eroded lands, and under harsh climatic conditions [2]. Its seeds contain 30–35% oil and the plant has a productive life of 40–50 years. Traditionally, *Jatropha* has been used for its oil and its other plant parts and derivatives for medicinal purposes and for soap production.

A potential major constraint in the widespread acceptance of *Jatropha* as a source of biodiesel is the presence of phorbol esters, which, when consumed by man and animal, are toxic and are also co-carcinogens [3]. This makes the oil unsuitable for food and feed applications. In view of the current debate of 'oil for food' versus 'oil for fuel'; however, this toxicity is a potential advantage for *Jatropha*. *Jatropha* oil can be seen as a 'technical oil', and therefore does not compete directly with the food markets. At the same time this can also be a disadvantage. Due to the

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toxicity of the plant and oil, some special precautions need to be exercised during the processing of *J. curcas* seeds and oils. By-products of the vegetable oil pre-treatment and biodiesel production process, such as fatty acid distillate (FAD), gums and glycerine have several applications in the food and feed industries and the presence of phorbol esters could render it unfit for nutritional purposes. The aim of this study was to follow the flow of phorbol esters during various stages of pre-treatment and biodiesel production from *Jatropha* oil.

Materials and Methods

Seeds and Oils Used

Three different types of *J. curcas* oils were used for this study. Two samples of *Jatropha* oil were from the toxic genotypes from India and one from the non-toxic genotype. The non-toxic genotype is present only in Mexico [2] and the seeds were collected from Papantla (LN 20′15°, LO 97′15°; altitude, 80 m above mean sea level; annual rainfall, 1,500 mm; soil type, calcaric regosol), Veracruz State, Mexico. Humans consume the roasted seeds of the non-toxic genotypes (as roasted nuts in many parts of the world) during Christmas time in Mexico. The kernels are also added to delicacies and their paste is consumed with meals. Earlier studies [4–6] have shown that the non-toxic or edible genotypes are either free of phorbol esters or have very low levels. The oil from the non-toxic genotype used in the present study was free of phorbol esters.

The oil used from the non-toxic genotype was solvent extracted using petroleum ether. The two oil samples from the toxic genotypes were derived from cracked and partially dehulled *Jatropha* seeds by mechanical pressing, followed by solvent extraction of the press cake with *n*-hexane. The seeds were cracked with a 'diamond head' cracking mill and then sent through a vibrating sieve with an opening of 12 and 1 mm to separate the entire seeds and fines from the cracked seeds. Partial dehulling was then accomplished in a self-constructed fluidized bed hull separator, which reduced the hull content of the cracked seeds from 40 to 25%. The mechanical pressing of the seeds was done in a mini 100 press from Desmet Rosedowns Ltd. (Hull, UK). The press cake was then percolated 6 times (total duration of percolation was approximately 2 h) with *n*-hexane in a glass percolation column at 60 °C. The ratio of total solvent over press cake was 1:1 (w:w). The ratio of the pressed oil and the extracted oil obtained was 2:1 (w:w). The extraction procedure used in this study simulates the usual industrial application where the oil yields are maximized by sequential pre-pressing followed by solvent extraction.

Pre-treatment Steps Prior to Transesterification

During acid degumming 0.85 kg/ton H_3PO_4 was added as a 40% (w:w) solution to the pre-heated crude oil in a beaker at 80 °C. The oil was then high-shear mixed for 2 min at 16,000 rpm using a T25 ULTRA-TURRAX® from IKA®Werke GmbH & Co (Staufen, Germany), then stirred at 80 °C and 120 rpm for 5 min using a Eurostar mechanical blade agitator from IKA®Werke GmbH & Co (Staufen, Germany) and cooled down to 60 °C. Thereafter 0.77 kg/ton NaOH was added as a 14% (w:w) solution and the mixture was high-shear mixed again for 2 min. The amount of caustic soda added was just enough to neutralize the phosphoric acid, but was too low to neutralize the free fatty acids (FFA) present. Due to the higher phosphorous content, the solvent extracted oil was degummed with more acid (1.50 kg/ton H_3PO_4); hence more caustic soda was used for neutralization (1.35 kg/ton NaOH). Extra water was added to the oil in order to bring the total water content to 3% (w:w). The mixture was then stirred for 15 min at 120 rpm using a mechanical blade agitator at 60 °C, centrifuged for 15 min at 2,000*g* and decanted. The degummed oil was then washed with 3% (w:w) fresh water, centrifuged (2,000*g*, 15 min) and decanted.

The degummed oil was silica-treated at 80 °C in a rotary evaporator. The oil was heated to 80 °C and citric acid was added as a 30% (w:w) solution to obtain a final concentration of 0.09% (w:w). The acidified oil was high-shear mixed for 2 min at 16,000 rpm and left to maturate for 10 min in a rotary evaporator at 100 rpm and atmospheric pressure. Then 0.3% (w:w) Trisyl 300 (W.R. Grace Inc., South Pittsburgh, TN) was added as a slurry in the oil. Simultaneously 0.5% water was added to the mixture. After 30 min reaction at 80 °C and atmospheric pressure, the temperature was increased to 95 °C and the pressure reduced to 50 mbar in order to dry the mixture. After 30 min vacuum drying, the oil was filtered through a Whatman No. 1 filter paper.

Stripping or deodorization was done in self-made lab-deodorizer equipment at 260 °C and 3 mbar for 1 h and with 1% steam injection. The lab-deodorizer consisted of a glass reactor (capacity approx. 400 ml), containing the oil, which was placed inside a closed oven adjusted at 260 °C. Steam was injected through a sintered tube immersed in the oil. The amount of injected steam was regulated with a peristaltic pump, connected to a burette filled with water. The vapors escaping from the oil were condensed in a double-jacketed water cooler, which was connected to the vacuum pump.

Transesterification Process for Biodiesel Production

In a temperature-controlled and mechanically stirred (120 rpm) glass multi-neck reactor, 500 g of pre-treated oil was pre-heated in a thermostatic bath at 60–62 °C. To the oil a pre-heated mixture (55 °C) of 101.5 g methanol and 10.0 g $NaOCH_3$ solution (30% w:w in methanol) was added to obtain 21.7% methanol and 0.6% $NaOCH_3$ in the oil. After addition of the methanol-catalyst mixture, the agitation speed was increased to 450 rpm. After a 2-h reaction at 60–62 °C, the agitation was stopped and the reaction mixture was transferred to a thermostatic (60 °C) separation funnel. After settling for 1 h, the glycerine layer was drained and the fatty acid methyl ester (FAME)-layer was transferred to a glass beaker. Citric acid in water (30 w:w) and pure water were added to the FAME-layer to bring the level of citric acid to 750 ppm and of water to 3% (w:w). This mixture was then high-shear mixed for 2 min at 16,000 rpm, stirred for 15 min at 120 rpm and 55 °C, centrifuged (15 min, 2,000*g*) and the biodiesel decanted. Finally, the washed biodiesel was vacuum dried in a rotary evaporator at 120 °C and 50 mbar for 30 min.

Analytical Determinations

Free fatty acid content (FFA), acid value (AV), oxidative stability, iodine value (IV) and water content were determined according to AOCS Official Methods (7).

Cloud Point

The cloud point of the biodiesel samples was measured in a Mettler Toledo FP90 Central Processor, connected to a FP81 HT MBC Cell, at a cooling rate of −1 °C/min after heating the sample to 130 °C.

Element Analysis

Elements (P, Ca, Mg, Na, K and Fe) were determined by Inductive Coupled Plasma (ICP) spectrometry according to AOCS Official Method Ca 20-99 [7], using the Thermo Scientific iCAP 6000 ICP Spectrometer.

Fatty Acid Composition

Preparation of fatty acid methyl esters was done according to AOCS Official Method Ce 2-66 (alternate method for fats and oils with acid value <2) [7]. The FAMEs were separated on a 6,890 N gas chromatograph from Agilent Technologies, equipped with a flame ionization detector and a BPX 70 capillary column (60 m × 0.10 mm id) (Supelco, Bellefonte, PN, USA). The column-temperature was elevated at rates of 10 °C/min from 60 to 150 °C, 5 °C/min from 150 to 175 °C and held for 45 min at 175 °C. The detector and injector temperatures were at 250 °C. Helium was used as the carrier gas at a flow rate of 0.3 ml/min. The flow rates of hydrogen and air were respectively 30 and 400 ml/min. Injection volume was 0.5 µl.

Free and Total Glycerine

The free and total glycerine and mono-, di- and triglycerides in biodiesel were determined according to the EN14105 official method [8]. The compounds were separated on a 5890 Series II Plus Hewlett Packard gas chromatograph, equipped with an on-column injection system, a flame ionization detector and a DB-5HT capillary column (15 m × 0.32 mm id, 0.10 µm film thickness) from Agilent Technologies.

Phorbol Ester Content

Phorbol esters were determined at least in duplicate according to Makkar et al. [9], based on the method of Makkar et al. [4]. Briefly, 0.5 g of the sample was extracted four times with methanol. A suitable aliquot was loaded into a high-performance liquid chromatography (HPLC) reverse-phase C18 LiChrospher 100, 5 µm (250 × 4 mm id, from Merck (Darmstadt, Germany). The column was protected with a head column containing the same material. The separation was performed at room temperature (23 °C) and the flow rate was 1.3 ml/min using a gradient elution [9]. The four phorbol ester compound peaks were detected at 280 nm and appeared between 25.5 and 30.5 min. The spectra were taken using Merck-Hitachi L-7450 photodiode array detector. Phorbol-12-myristate 13-acetate (Sigma, St. Louis, MO) was used as an external standard (appeared between 31 and 32 min). The area of the four phorbol ester peaks was summed and converted to phorbol-12-myristate 13-acetate equivalent by taking its peak area and concentration.

Results and Discussion

Evaluation of Phorbol Ester Determination

The main purpose of taking oil from the non-toxic genotype was to rule out interference, if any, of other compounds in the determination of phorbol esters, especially for the fractions generated during biodiesel production. No typical phorbol ester peak was present for the non-toxic oil, although four minor peaks appeared between 25.5 and 30 min but their spectra differed from those of phorbol esters (Figs. 1, 2). Therefore, content of

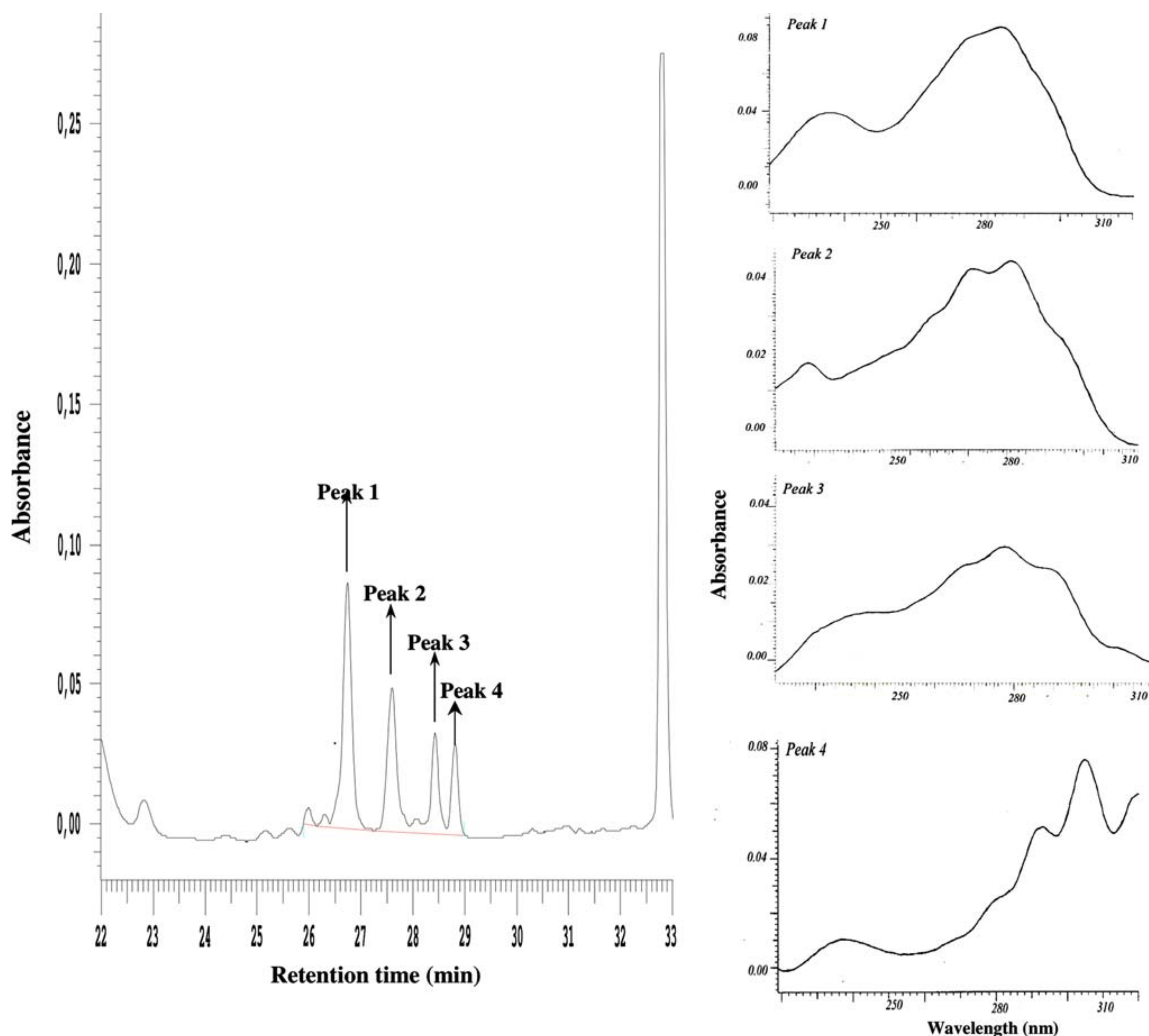

Fig. 1 HPLC chromatogram and absorption spectra of toxic Jatropha oil

phorbol esters in the oil from the non-toxic (edible) genotype was regarded as nil. The presence of some innocuous compounds related to phorbol esters in the oil from the non-toxic genotype cannot be ruled out. The inclusion of non-toxic oil in the study also provided a comparative assessment of characteristics of the toxic and non-toxic oil and of the corresponding biodiesel.

General Characteristics of Oil and Biodiesel

Various characteristics of the oil and biodiesel are given in Tables 1 and 2. The hexane-extracted oil contained higher levels of FFA, P and Ca than cold pressed oil. The non-toxic oil had the lowest FFA-content and intermediate levels of Ca and P. During degumming the levels of P were reduced to below 20 ppm in all three oil samples, and even reduced further to below 10 ppm after silica filtration (results not shown). During these pre-treatment steps the FFA-content remained nearly unaffected. The free fatty acids were efficiently removed during stripping to below 0.05%.

The fatty acid composition of toxic and non-toxic oil samples used in this study was similar to that obtained in an earlier study [2]. The two studies were conducted using different provenances of toxic and non-toxic seeds, and the data suggest that fatty acid composition is relatively

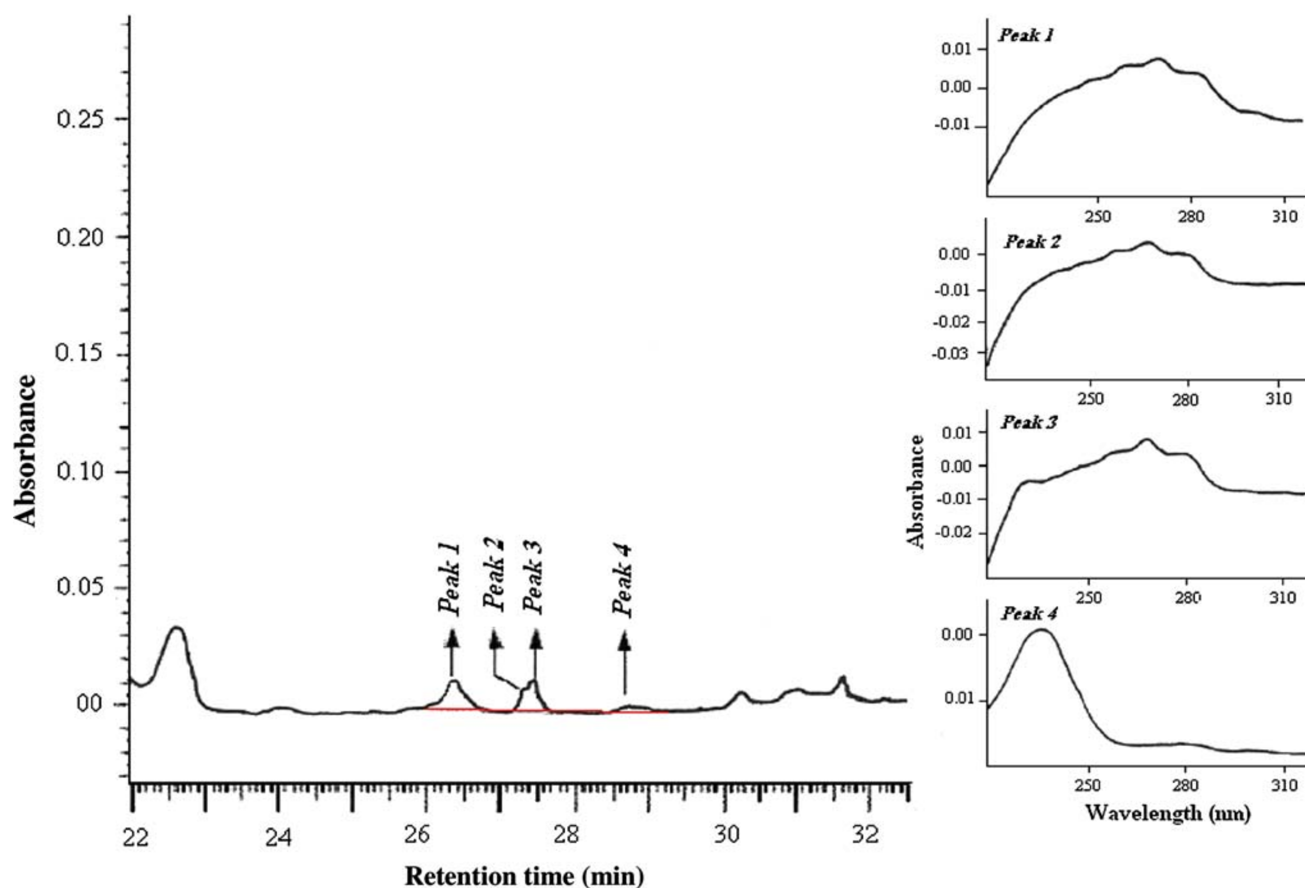

Fig. 2 HPLC chromatogram and absorption spectra of non-toxic Jatropha oil

constant. However, there are considerable differences in the levels of oleic and linoleic acids between the toxic and non-toxic oils. Oleic acid is higher in the toxic oil and linoleic acid in the non-toxic oil. These differences appear to be genetically controlled. Non-toxic oil has a potential to be used as an edible oil and the higher level of linoleic acid (polyunsaturated fatty acid) could be considered advantageous for human health. On the other hand, a higher level of this fatty acid in the non-toxic oil is expected to decrease oxidative stability of biodiesel produced from this oil, although oxidative stability did not differ substantially for the biodiesel produced from the toxic and non-toxic oil (Table 2). The cloud point of biodiesel from the non-toxic oil was lower (0.6 °C) compared to that from the toxic oil (1.9 and 2.2 °C). The difference in cloud point can be directly related to the lower stearic acid content of the non-toxic oil. According to Imahara et al. [10] the cloud point of biodiesel can be predicted from the amount of saturated fatty acid methyl esters, regardless of the composition of the unsaturates. Water content, acid value, element content (P, Ca + Mg and Na + K) and free and total glycerol of all biodiesel samples were within the European EN14214 specification.

Phorbol Ester Levels in Different Fractions

To determine the repeatability of the method the phorbol ester content was analyzed 11 times on an oil sample spiked with approximately 2.5 mg/g phorbol esters. The average measured phorbol ester content was 2.55 mg/g with a standard deviation of 0.07 mg/g and a relative deviation of 2.75%.

The concentration of phorbol esters in the toxic oils was 3.10 mg/g for the solvent extracted oil and 3.77 mg/g for the cold pressed oil (Table 1). The difference in phorbol ester content could be ascribed to the oil extraction procedures.

The differences in phorbol ester content after each refining step were tested on their significance ($\alpha = 0.95$) using the Student's t test for two sets of independent measurements. For the toxic oils, the effect of degumming on the phorbol ester content was not consistent. Degumming decreased the concentration of phorbol esters significantly to 2.48 mg/g in the solvent extracted oil, corresponding to a relative decrease of 20%. During degumming only a slight significant decrease of 4% to 3.62 mg/g could be observed for the pressed oil. Haas and

Table 1 General composition of the different crude *Jatropha* oil samples

Parameters	Solvent extracted	Cold pressed	Non-toxic
Phorbol esters (mg/g)	3.10	3.77	N.D.
Water (ppm)	197	731	735
Free fatty acids (% as C18:1)	6.87	5.34	3.00
Elements (ppm)			
P	87.9	35.5	54.9
Ca	51.1	21.6	32.8
Mg	23.9	23.0	N.D.
Na	13.3	6.44	1.48
K	15.3	28.7	6.57
Fe	8.31	0.29	0.07
Fatty acid composition (% w:w)			
C14:0	0.1	0.1	0.2
C16:0	15.8	15.3	12.0
C16:1	0.9	0.9	0.6
C17:0	0.1	0.1	0.1
C17:1	0.0	0.1	0.1
C18:0	6.7	6.8	6.4
C18:1*t*	0.0	0.1	0.1
C18:1*c*	42.1	42.0	36.7
C18:2*t*	0.0	0.1	0.1
C18:2*c*	34.1	34.4	43.5
C18:3*t*	0.0	0.0	0.0
C18:3*c*	0.2	0.2	0.1
C20:0	0.0	0.1	0.1
Total saturated	22.8	22.3	18.8
Total mono-unsaturated	42.9	43.1	37.5
Total Poly-unsaturated	34.3	34.6	43.7
Iodine value (–)	96.5	97.1	108.1

Values are average of two values

ND Not detectable

Table 2 General quality parameters of the Jatropha biodiesel samples produced

Parameters	EN14214	Solvent extracted	Cold pressed	Non-toxic
Phorbol esters (ppm)	–	ND	ND	ND
Water (ppm)	Max. 500	290	<50	216
Acid Value (mg KOH/g)	Max. 0.50	0.16	0.11	0.08
Cloud point (°C)	–	1.9	2.2	0.6
Oxidative stability at 110 °C (h)	Min. 6	5.9	8.7	7.9
Elements (ppm)				
P	Max. 10	1.0	0.07	0.03
Ca + Mg	Max. 5	ND	ND	ND
Na + K	Max. 5	0.05	ND	ND
Free and total glycerol (%)				
Free glycerol	Max. 0.02	0.005	0.008	0.005
Monoglycerides	Max. 0.80	0.72	0.62	0.73
Diglycerides	Max. 0.20	0.21	0.16	0.17
Triglycerides	Max. 0.20	0.06	0.09	0.06

Values are the average of two values

ND Not detectable; *Max*. Maximum; *Min*. Minimum

Table 3 Phorbol ester content (expressed in mg/g ± SD, $n = 3$) of the different fractions obtained during pre-treatment and transesterification of three different *Jatropha* oil samples

Parameters	Solvent extracted	Pressed	Non-toxic
Crude oil	3.10 ± 0.25	3.77 ± 0.03	ND
Degummed oil	2.48 ± 0.24	3.62 ± 0.19	ND
Acid gums	2.02 ± 0.07	3.35 ± 0.00	ND
Wash water	2.72 ± 0.01	2.08 ± 0.48	ND
Silica-treated oil	2.51 ± 0.33	3.76 ± 0.50	ND
Spent silica	NA	NA	NA
Stripped oil	ND	ND	ND
Fatty acid distillate	ND	ND	ND
Biodiesel	ND	ND	ND
Crude glycerine	ND	ND	ND
Biodiesel wash water	ND	ND	ND

ND Not detectable; *NA* Not analyzed

Mittelbach [11] observed no decrease in phorbol ester concentration in the degummed oil but a decrease of approximately 29% was found when the degummed oil was neutralized with caustic soda. In the applied degumming process in this study, just enough caustic soda was added to neutralize the phosphoric acid, without neutralization of FFA. Silica treatment decreased the phorbol ester content in the solvent extracted oil by 8% to 2.29 mg/g. In the press oil the concentration did not change (Table 3).

Phorbol esters were not detected in any of the samples after the 'stripping' (or deodorization) treatment. Phorbol esters were also not detected in the fatty acid distillate, the side-stream obtained during the stripping treatment. These results suggest that high temperature applied during stripping destroyed the phorbol esters present in the oil samples. Haas and Mittelbach [11] reported no loss of phorbol esters during deodorization. It may be noted that these authors deodorized the oil at 200 °C for 2 h under atmospheric pressure. These mild conditions could be applied because the FFA was already adequately removed in a chemical neutralization step and stripping was mainly done to improve odor and color. In the current study stripping was done at 260 °C and low pressure (3 mbar) in order to remove FFA. It seems that at high temperature the phorbol esters get degraded, while at 200 °C they remain unaffected.

The biodiesel and glycerine produced from the oil from the toxic genotypes were free of phorbol esters. However, four peaks appeared in the elution range of 25.5–30 min from the HPLC for the biodiesel samples, including the one from the non-toxic genotype. These four peaks were similar in terms of their absorption spectra (Figs. 3, 4) but were completely different from those of the phorbol esters (Fig. 1). These observations and the absence of phorbol esters in the oil after the stripping process suggest that these peaks are not of phorbol esters. This also highlights the need to take spectra of the peaks appearing in the elution range of phorbol esters and to match them with those of the phorbol esters, to ascertain the presence of phorbol esters in the biodiesel. This is particularly important for those studies wherein only biodiesel is taken for quantification of phorbol esters.

Although phorbol esters were not detected in the biodiesel and glycerine samples in the present study, phorbol esters (mg/g) were detected in biodiesel (Firm 1:0.41−1.32; Firm 2:1.19) and glycerine (Firm 1:0.58−0.97; Firm 2:0.061) samples obtained from industrial plants. For Firm one higher level of phorbol esters in biodiesel and glycerol were for the Jatropha oil containing higher level of phorbol esters (4.42 mg/g) and lower for the oil containing lower level (2.17 mg/g), suggesting that the initial concentration of phorbol esters is one of the factors determining the residual levels of phorbol esters in biodiesel and glycerol. No detailed information on the pre-treatment and transesterification procedures adopted for production of these samples is available. However, it is clear that different oil pre-treatment conditions could affect the presence of phorbol esters in biodiesel or glycerine produced from toxic *Jatropha* oil.

To monitor what happens with the phorbol esters during transesterification, a phorbol ester free stripped Jatropha oil sample was spiked with 2.49 mg/g phorbol esters isolated from the Jatropha oil and transesterified on lab-scale. The washed and dried biodiesel contained 0.27 mg/g phorbol

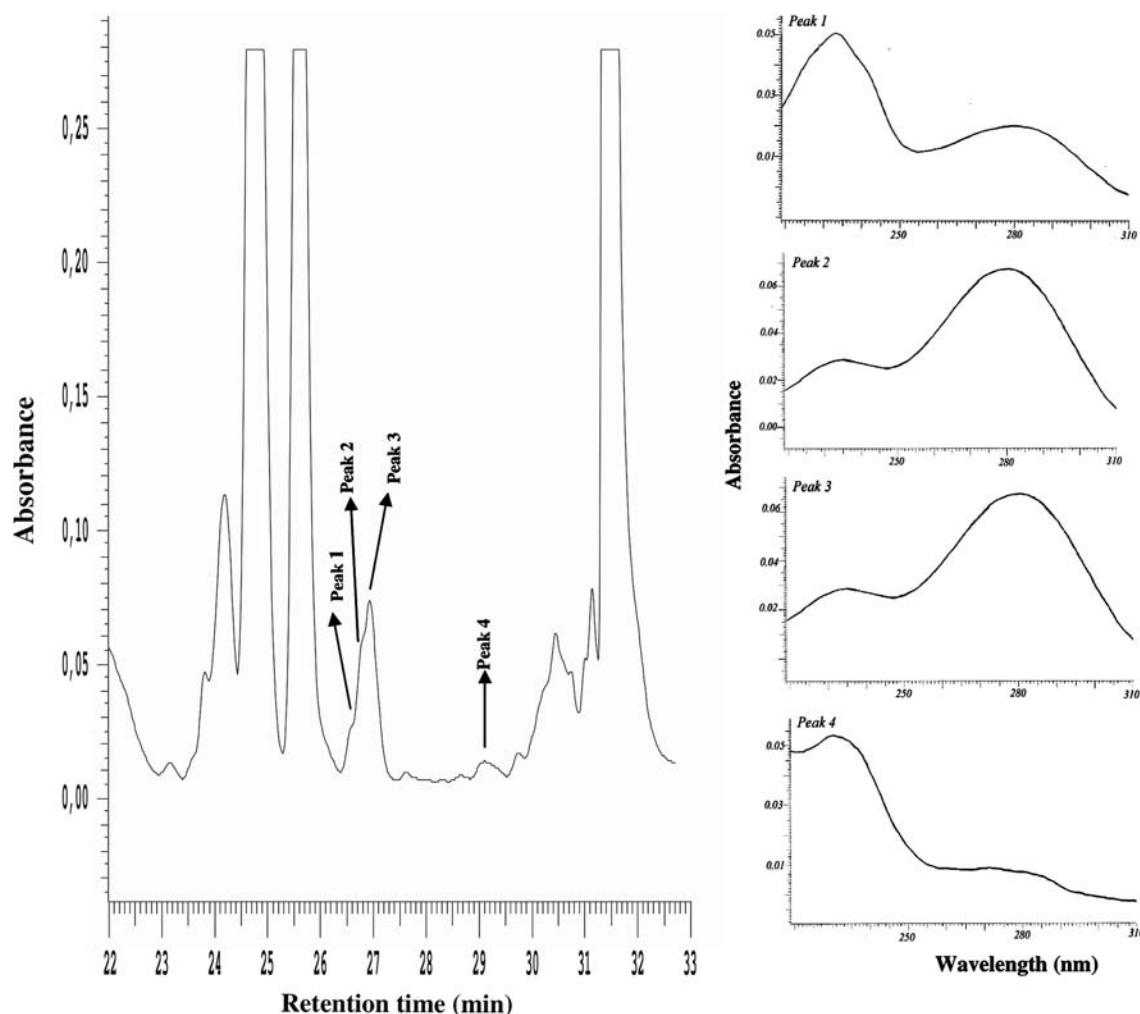

Fig. 3 HPLC chromatogram and absorption spectra of biodiesel from toxic Jatropha oil

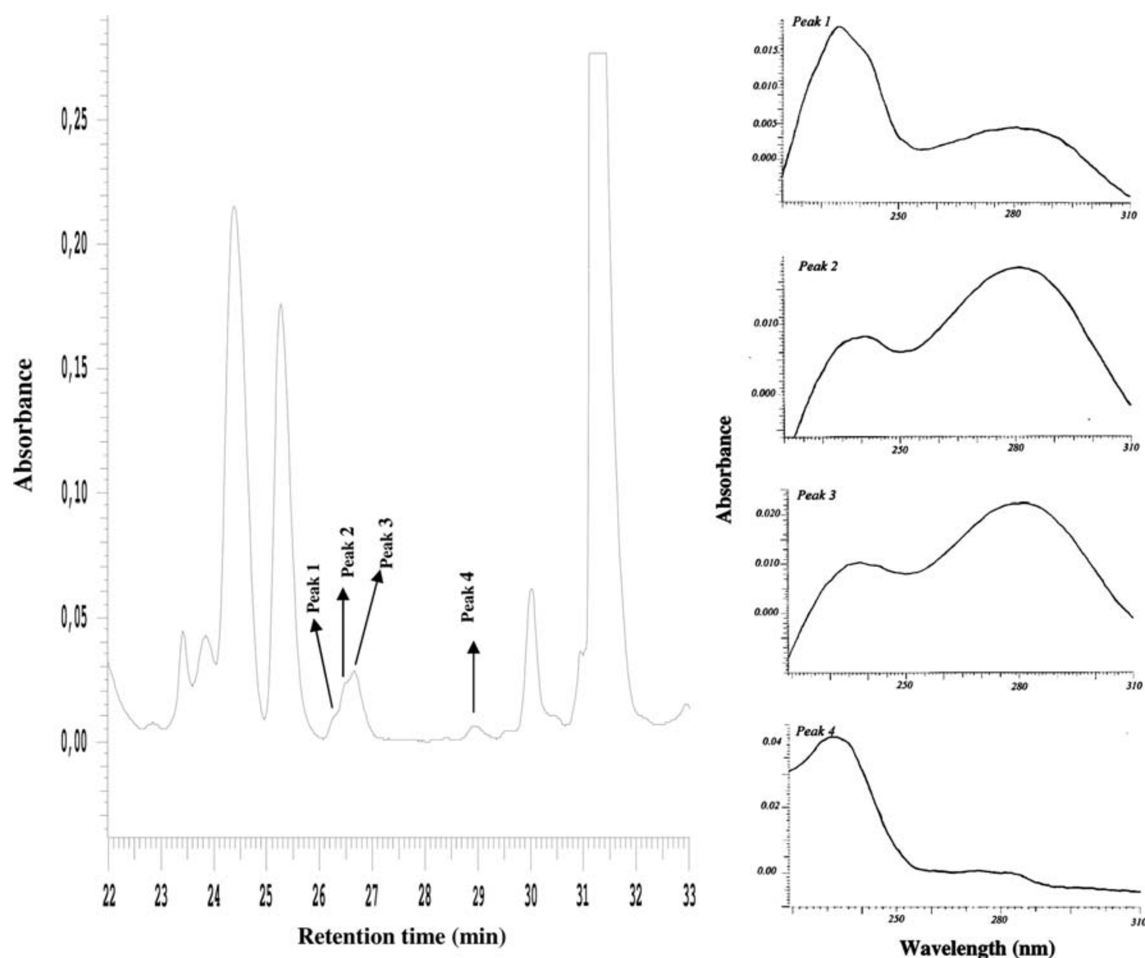

Fig. 4 HPLC chromatogram and absorption spectra of biodiesel from non-toxic Jatropha oil

esters, while the toxin content in the glycerine fraction was only 0.043 mg/g. This indicates that during transesterification most of the phorbol esters are destroyed or removed with the wash water. Unfortunately the wash water fraction was too small to perform toxin analysis.

Presence of Phorbol Esters in Side-Streams from Oil-Refining and Biodiesel Production

The acid gums obtained during the degumming stage were rich in phorbol esters and therefore unsuitable for animal feed applications. Also the wash water was contaminated and one has to be careful with the disposal of this fraction into the environment. Although, no concrete data are available on the fate of phorbol esters in the environment, they are considered to be degraded completely in soil in 6 days [12]. The regulations demand proper treatment of the industrial effluents before they are channeled into the environment, soil or water channels. The aerobic digesters used by industries are expected to degrade phorbol esters present in the washings. However, it is vital that the phorbol esters should be monitored in the digesters and in the effluents before they are disposed of into the environment.

No phorbol esters were detected in the fatty acid distillate and glycerine fractions. It is however possible that some phorbol ester degradation products are present, rendering these side-streams unsuitable for food and feed applications. The chemical structure and toxicity of these degradation products were not explored in this study.

Conclusions

The characteristics of toxic and non-toxic oil and biodiesel produced from these oils are similar. The phorbol esters are destroyed by the stripping process (under the conditions used in this study) during the process of biodiesel production. In the chemical refining of the oil (degumming, neutralization, silica/bleaching, mild deodorization/stripping) the temperature is low and phorbol esters are unaffected. In physical refining (degumming, silica/bleaching, deodorization/stripping at 240–260 °C and

under vacuum) the deodorization conditions are much more severe, leading to phorbol ester degradation. The biodiesel and glycerol produced through the process outlined in this study were free of phorbol esters; however, these could contain phorbol ester degradation products. At present no information is available on the nature or toxicity of the degraded products. The toxicity of the stripped oil (free of phorbol esters) which goes to the biodiesel and glycerol production is being investigated in our laboratory using fish as a model, to understand the role of phorbol esters degraded products in eliciting toxicity, if any. The presence of phorbol esters in the acid gums renders this fraction unsuitable for use in animal feed. The washings obtained during the degumming process are rich in phorbol esters and their disposal into the environment needs due care.

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References

1. Azam MM, Waris A, Nahar NM (2005) Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. Biomass Bioenergy 29:293–302
2. Becker K, Makkar HPS (2008) *Jatropha curcas*: a potential source for tomorrow's oil and biodiesel. Lipid Technol 20:104–107
3. Goel G, Makkar HPS, Francis G, Becker K (2007) Phorbol esters: structure, occurrence and biological activity. Int J Toxicol 26:279–288
4. Makkar HPS, Becker K, Sporer F, Wink M (1997) Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. J Agric Food Chem 45:3152–3157
5. Makkar HPS, Becker K, Schmook B (1998) Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. Plant Foods Human Nutr 52:31–36
6. Makkar HPS, Aderibigbe AO, Becker K (1998) Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chem 62:207–215
7. AOCS (1990) Official methods and recommended practices of the American oil chemists' society, 4th edn. AOCS Press, Champaign
8. EN 14105:2003, Fat and oil derivatives—Fatty acid methyl esters (FAME)—determination of free and total glycerol and mono-, di- and triglyceride content (reference method)
9. Makkar HPS, Siddhuraju P, Becker K (2007) A laboratory manual on quantification of plant secondary metabolites, human press. Totowa, New Jersey, p 130
10. Imahara H, Minami E, Saka S (2006) Thermodynamic study on cloud point of biodiesel with its fatty acid composition. Fuel 85:1666–1670
11. Haas W, Mittelbach M (2000) Detoxification experiments with the seed oil from *Jatropha curcas* L. Industrial Crops Products 12:111–118
12. Rug M, Ruppel A (2000) Toxic activities of the plant *Jatropha curcas* against intermediate snails and larvae of schistosomes. Tropical Med Int Health 5:423–430