



Detoxification of castor meal through reactive seed crushing

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ABSTRACT

Non-edible oil crops, such as castor or jatropha, contain several toxic components. Post-harvest treatments should be used to reduce the risks associated with the possible dispersion of toxic compounds in the environment. A new processing technology named reactive seed crushing was developed, which combines in a single process seed-crushing, solvent extraction, oil refining, transesterification and meal detoxification. When applied to castor seeds, it was demonstrated that the process produced a detoxified meal and a castor oil methyl ester of acceptable quality for downstream processing.

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1. Introduction

Castor (*Ricinus communis*) is a well established oilseed crop with many industrial applications. It is mainly cultivated in tropical climates with India, Brazil and China being the main producers. Castor seed contains about 48% oil, composed of more than 85% ricinoleic acid (12-hydroxy 9-octadecenoic acid) (Wang et al., 2011). Castor oil is mainly used for lubricants, but also for polymers such as polyurethanes. It has a history of more than 50 years in the production of Polyamide-11, also known as Rilsan-11. More recently, castor oil gained interest to produce sebacic acid (a 10 carbon atoms linear diacid), which can also be used as a monomer or to produce solvents, for example.

The annual worldwide production of castor beans is approximately one million tons (Olsnes, 2004). The residual castor meal that is left after extraction of the oil represents about one half of the weight of the castor bean (Robb et al., 1974) and it has a protein content of 34–36% (Anandan et al., 2005), which could be a good source of protein for animals. However, castor meal has not found a place as a protein supplement, mostly because the seed contains several toxic compounds. The oil itself has only a laxative effect, but the meal contains a highly toxic protein, ricin, a less toxic *R.*

communis agglutinin (RCA), a low molecular weight alkaloid, ricinine (MW = 164.2 g/mol), and an allergen compound named CB-1A. In its crude form, ricin is often found in association with RCA. Both Ricin and RCA are highly concentrated in the seed meal and they comprise up to 5% of the weight of the seed meal (Bradberry et al., 2003), which makes castor a possible threat and weapon for terrorist attacks. Ricin is also known as the poison used in the “Bulgarian umbrella” which killed a dissident many years ago. Antibody-based immunoassays are standard technologies for detection of ricin in castor meals (Griffiths, 2011), but one of the drawbacks is that they cannot differentiate different ricin isoforms and RCA due to the high homology of their sequence. The content and toxicity of ricin in castor meals reported in most literature were a mixture of different ricin isoforms and RCA. Although it was not mentioned that often, the allergen in castor seeds might be somewhat more critical, since the 8–20% of the population was allergic to castor based on the reports, with in some cases anaphylactic shock (ICOA, 1989; Audi et al., 2005). After oil extraction, these toxic compounds remain in the meal.

Various methods including physical, chemical and biological treatments have been employed to detoxify the castor meal (Barnes et al., 2009; De Oliverira et al., 2010), but the detoxification process is not always effective (Roels et al., 2010; Hong et al., 2011). For these reasons, castor meal cannot be used for animal feed and is most often used as a natural fertilizer since it is rich in nitrogen. So far, there is no international regulation limiting the amount of castor material in fertilizer. However, there are national regulations, e.g. Germany allows *R. communis*-residual material in fertilizer if no acute oral toxicity in rats is observed after uptake of 2000 mg material per kg body weight (Worbs et al., 2011).

Abbreviations: CFT, cell-free translation; cps, counts per second; HFA, hydroxyfatty acid; IALCTMS, immunoaffinity and liquid chromatography–tandem mass spectrometry; LLNA, local lymph node assay; LD₅₀, mean lethal dose; RSC, reactive seed crushing; RCA, *Ricinus communis* agglutinin; S.D., standard deviation.

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Table 1
Equipment and method used for flattening of castor seeds.

Equipment	1st flattening		2nd flattening	
	Rotational speed (rpm)	Inter-roller space (mm)	Rotational speed (rpm)	Inter-roller space (mm)
Flattener with rollers Ø 240 mm, flute pitch: 1 striation/cm	430	0.1	430	0.1
Average flake thickness	0.4–0.6 mm		0.3–0.5 mm	
Length	3–4 mm		2–3 mm	

In this study, we report a reactive seed crushing (RSC) method using methanol as a solvent for the extraction of the triglycerides, and at the same time as a reactant for the transesterification reaction. Methanol is used in a large excess to facilitate the extraction. Indirectly it facilitates the transesterification reaction. A basic catalyst, sodium hydroxide, is used in the process.

Hydroxyfatty acid (HFA) rich oils are more soluble in methanol than other oils such as soybean oil, rapeseed oil and jatropha oil. For this reason, in a biodiesel producing process, the reaction starts more easily with HFAs-containing oils, but as the reaction proceeds, glycerol is produced and it is also more soluble in HFA rich esters, and do not separate readily. The higher solubility of methanol in HFA rich oils might explain why the extraction of the castor oil proceeds smoothly.

2. Materials and methods

2.1. Raw material

Castor seeds were received from India. Samples of castor meal were obtained from Soabe in Madagascar. Immediately after oil expression by low temperature seed crushing, without adding any solvent, the seed meal samples were dried and sealed in plastic bags and then shipped. The seed meal samples were found to contain high level of free fatty acids, probably produced by hydrolysis during transportation.

2.2. Preparation of castor seed flakes

To give the seeds greater plasticity and therefore more effective crushing during flattening, the castor seeds were preheated at a temperature $\leq 100^\circ\text{C}$ and then flattened as it is (whole seed) using a serrated-roller flattener, according to a double-flattening method described in Table 1. This operation was carried out by passing seed through the flattener twice to obtain flakes as described. After flattening, the flakes were dried under a flow of hot air at 100°C for 16 h to achieve a residual moisture content of less than 2%. The residual moisture of the seed was determined by thermogravimetric analysis and expressed as percentage of the crude material.

2.3. Defatted flakes

The castor seed flakes were defatted by hexane extraction in a Soxhlet according to the standardized method NF ISO 659. The defatted flakes were subsequently desolvented under a stream of hot air in a ventilated oven at 60°C for 16 h. Their final water content was 1.3% by weight.

2.4. Preparation of castor seed cake by RSC method

The previously dried flakes were fed into a thermo-regulated percolation column fitted with a support grid (fixed bed). In a typical experiment, a pump fed the column with a mixture of sodium hydroxide (0.3% relative to the seed weight) and methanol (having a methanol/seed weight ratio of 2). The extraction and

transesterification reactions were carried out at 50°C for 30 min. The bed was drained for 15 min. The flake was then extracted and washed. For this, the column was fed with anhydrous methanol, which again diffuses by percolation without subsequent recirculation of the methanol. The required amount of solvent was injected for a period of 5 min, and the liquid was then drained for 15 min. Five washing cycles were carried out. The defatted cake soaked with alcohol was then dried in a ventilated oven at 120°C for 4 h.

The liquid phases obtained either after reaction or following the washing of the seed cake with anhydrous methanol, were combined in order to be purified. The methanol was first removed by vacuum distillation using a rotary evaporator (90°C in a final vacuum at 40 mbar). The fatty phase recovered was then centrifuged (4500 rpm) for 5 min in order to separate the ester from the crude glycerine. The ester was finally washed with demineralized water at 90°C , until the washing waters were neutral. Finally, the ester was dried at 90°C under a vacuum of 40 mbar.

The other product directly resulting from the process was the castor seed cake. The defatted seed cake soaked with methanol was dried at 120°C in a ventilated oven for 4 h. The aim of this drying step was to remove the remaining solvent (methanol) used during the extraction from the seed cake. In parallel, this drying completes the decomposition of the ricin and the CB-1A allergen remaining in the seed cake.

2.5. Measurement of functional ricin

To measure the content or activity of ricin in defatted castor seed flakes and seed cakes three different methods were used.

2.5.1. Immunoaffinity and liquid chromatography–tandem mass spectrometry (IALCTMS)

Castor seed flakes or seed cakes (250 mg) were ground to 1 mm particle size and soaked in 0.5 mL of Extraction Buffer (EB) composed of 100 mM of potassium phosphate (pH 7.4), 0.15 M of sodium chloride, 0.1% of bovine serum albumin (BSA) and 0.01% of sodium azide for 1 h with agitation every 10 min. The supernatant recovered by centrifugation for 15 min at 15,000 rpm was used for the quantitative determination of ricin as described previously (Becher et al., 2007). Briefly, magnetic beads with covalently bound protein G were incubated with antibody against the ricin B chain for 1 h at room temperature. Five microliters of beads with captured IgG was transferred to 500 μL of samples and incubated for 2 h at 37°C with gentle shaking. After removing nonspecific binding by washing, a 14-mer RNA substrate containing the GAGA sequence (3.55 nmol) in 60 μL of the depurination buffer (10 mM ammonium acetate, pH 4) was added to the samples and incubated for 4 h. After filtration using Nanosep 10 (Pall Corporation, Ann Arbor, MI), samples (40 μL) were injected and analyzed by LC/MS for adenine released. Ricin concentration in the sample was determined based on the amount of adenine released by ricin from a calibration curve. The limit of detection for this method was 0.1 ng/mL based on a signal-to-noise ratio of the adenine peak of at least 3.

2.5.2. Cell-based assay

HeLa cells (CCL-2) were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (Invitrogen) and maintained in a humidified incubator (37 °C, 5% CO₂). For cytotoxicity assays, the cells were trypsinized, adjusted to approximately 0.5×10^5 to 1.0×10^5 cells/mL, seeded (100 µL/well) onto white 96-well plates (Corning Inc., Corning, NY), and allowed to adhere overnight. HeLa cells in each well were then treated with 100 µL of pure ricin at different concentrations or 500 ng/mL total proteins extracted from castor samples (defatted seed and seed cake) for 1 h at 4 °C. The cells were washed to remove unabsorbed toxin and then incubated for 24 h at 37 °C. Cell viability was assessed using CellTiter-Glo reagent (Promega, Madison, WI) according to the manufacturer's instructions, except that the reagent was diluted 1:5 in phosphate-buffered saline (PBS) prior to use. Luminescence was measured as counts per second (cps) with a Victor 2 plate reader (Perkin Elmer, Shelton, CT). The viability of cells treated with medium only (without adding any ricin) was considered as 100%. Toxicity was presented as the percentage of cells killed by ricin in the samples and calculated as [(cps obtained from the medium control – cps from the sample)/cps from the medium control $\times$ 100]. All data represent the mean of three replicates from a representative experiment $\pm$ standard deviation (S.D.). Three individual experiments were performed.

2.5.3. Cell-free translation (CFT) assay

The activity of ricin in samples was assessed in a cell-free translation assay as described previously (He et al., 2008) with small modifications. The translation lysate mixture consisting of nuclease-treated rabbit reticulocyte lysate, amino acids, RNasin, nuclease-free water and luciferase mRNA was prepared in a ratio (v/v) of 35:1:1:33:2. After mixing, the lysate was distributed (15 µL/tube) to 1.5 mL micro tubes containing 3 µL PBS buffer (as a control) or castor extracts containing 50 ng/mL proteins. The CFT reaction was incubated at 30 °C for 90 min with gentle shaking (60 rpm). Aliquots of the reaction were then transferred to a black microtiter plate (5 µL/well). The Bright-Glo Luciferase Assay Buffer (100 µL) containing the Bright-Glo Luciferase Assay Substrate (Promega) was added to each well and luminescence was measured as cps in a Victor 2 plate reader (Perkin Elmer). Translation lysate with PBS in lieu of sample was used as a control. Toxicity was presented as the percentage of translational inhibition [(cps from PBS control – cps from castor meal sample)/cps from PBS control $\times$ 100]. All data represent the mean of three replicates from a representative experiment $\pm$ S.D. Three individual experiments were performed.

2.6. Allergenicity test/sensitizing capacity

The CB-1A allergen was assayed by means of the precipitin test as described (Spies and Bernton, 1962). The skin sensitizing capacity of the defatted flakes and seed cakes of castor seeds was evaluated according to the local lymph node assay (LLNA) test adapted from the OECD Guidelines No. 429, of April 24, 2002 and Council Regulation (EC) No. 440/2008, B. 42, of May 3, 2008. A good correlation of the allergenic capacity was found between the thresholds determined experimentally in mice by the LLNA method and those determined in humans by the Human Repeated Insult Patch Test method (HRIPT) (Lepoittevin, 2008).

The LLNA method was based on the application of the test substance to the ear of a group of mice on days 0, 1 and 2 and the injection of tritiated thymidine on day 5. The incorporation of radioactivity in the draining lymph nodes was measured to determine the cell proliferation following exposure to the substance tested.

Table 2

Effect of the RSC method on the extraction of transesterification of castor oil.

<i>Processing conditions</i>	
Weight of seed flakes	350 g
Weight of methanol	700 g
Weight of NaOH used	1.1 g
Methanol/flake ratio	2
Catalyst relative to flakes	0.30%
<i>Processability</i>	
Methyl ester yield	87.10%
Fat in the defatted seed cake	2.60%
Loss of esters ^a	10.30%
<i>Methyl esters recovered</i>	
Acid number (mg KOH/g)	0.46
MeC16 content	0.85%
MeC18:2 content	2.90%
MeC18:1 content	3.46%
MeC18:0 content	0.96%
Methyl ricinoleate content	90.65%
Monoglyceride content	1.19%
Diglyceride content	nd ^b
Triglyceride content	nd ^b

^a Loss of esters = (theoretical weight of esters) – (weight of esters produced) – (potential weight of esters in the reduced fat seed cake).

^b Not detected.

The female mice selected for the LLNA test were of the standardized species CBA/j. Their age on the first day of treatment was between 8 and 12 weeks and their weight was between 18 and 25 g. For each dose, three mice developing in the same cage were used.

The reactant used with a view to causing proliferation was [³H]methyl-thymidine (³H-TdR; GE Healthcare, Les Ulis, France). At least 3 days before the injections, the required amount of ³H-TdR was diluted with 0.9% NaCl (20 µCi of ³H-TdR in 250 µL and 0.9% NaCl per animal). The solution obtained was stored at 4 °C in the dark.

A preliminary test was carried out on each animal by measuring the thickness of the ear before each application and 72 h after the final application with a micrometer. The vehicle most suitable for evaluating the sensitizing capacity of the defatted flakes and seed cakes was a homogeneous suspension of these materials at 10% and 25% in propylene glycol.

2.7. Evaluation of acute toxicity

The acute toxicity of the defatted flakes and seed cakes of castor seeds was evaluated according to the OECD Guidelines No. 423, of December 17, 2001, and Council Regulation (EC) No. 440/2008, B.1 tris of May 3, 2008. The female rats selected for the acute toxicity test were of the Sprague-Dawley standardized species and 8 weeks old on day 1. The vehicle used during the evaluation of the defatted flakes and seed cakes was the methylcellulose powder (0.5%). The product was administered orally by gavage to randomly grouped rats from day 0 to day 8 and the administered daily doses of defatted flakes and seed cakes were 50, 300 and 2000 mg/kg of body weight. For each dose, a group of 3 female rats was used.

3. Results

3.1. Effect of the RSC method on extraction and transesterification of castor oil

Before the RSC process castor seeds were flattened and dried at 100 °C for 16 h to achieve a residual moisture content of less than 2%. Table 2 illustrates one typical scale of the RSC process, conditions, the process ability and quality of the methyl esters recovered after processing. When these flattened and dried seeds were brought into contact with a mixture of sodium hydroxide

Table 3
Measurement of functional ricin using three different methods.

Sample no.	Sample type	Seed lot no.	Process type	NaOH (%) ^a	Dry temp (°C)/duration (h)	Test method		
						IALCTMS Ricin (%)	CFT assay Toxicity (%) ^d	Cell assay Toxicity (%) ^d
1	Defatted seed	1	–	–	20/48	1.8	100 ± 0	97 ± 1.1
2	Defatted seed	2	–	–	20/48	21 ^c	100 ± 0	99 ± 0.4
3	Seed cake	1	Reactive (R), Meth	0.5	120/4	0.01	9 ± 1.5	9 ± 2.1
4	Seed cake	1	R, Meth	0.3	120/4 ^b	2.2×10^{-4}	3 ± 0.6	5 ± 0.1
5	Seed cake	2	R, Meth	0.3	120/4	5.4×10^{-4}	8 ± 4.5	6 ± 0.1
6	Seed cake	1	R, Meth	0.3	100/4	8.6×10^{-4}	5 ± 0.9	5 ± 0.8
7	Madagascar cake	–	R, Meth	0.3	100/4	0.026	25 ± 1.0	26 ± 0.3
8	Seed cake	1	R, Meth	0.3	50/4	0.034	52 ± 3.4	50 ± 2.3
9	Seed cake	1	R, 50Meth/50Hex	0.3	100/4	0.06	47 ± 2.0	39 ± 2.3
10	Seed cake	2	Non reactive (NR), Meth	0	100/4	0.14	64 ± 1.8	33 ± 3.0
11	Madagascar cake	–	NR, Meth	0	100/4	0.07	32 ± 3.6	46 ± 0.8
12	Seed cake	2	NR: 50Meth/50Hex	0	100/4	1.86	99 ± 0	94 ± 1.0

^a NaOH percentage vs flakes by weight.^b Plus final drying at 100 °C for 4 h.^c The value is over estimated due to an unknown component that may interfere with the method.^d Data represent the mean of three replicates from a representative experiment ± S.D.

(0.3% relative to flake by weight) and methanol (methanol/flake weight ratio of 2) for extraction and transesterification at 50 °C for 30 min, we obtained 87.1% methyl esters. Among them, more than 90% was the methyl ricinoleate and only 1.2% was monoglycerides (a good quality methyl ester should contain a low glyceride content). In the presence of 0.3% of catalyst, the esters obtained had very low residual acidity. It was noticed that this method was very robust, it allowed direct transesterification of oils from seeds having high acidities (>2 mg KOH/g) with appreciable yield (data not shown).

3.2. Reduction of functional ricin measured by IALCTMS, CFT assay, and cell-based assay

Defatted flakes of castor seed as prepared in Section 2.3 and the seed cake resulting from the RSC method in Section 2.4 were evaluated for the presence of ricin using three different assay methods. Results from Table 3 indicate that the amounts of ricin present in the defatted castor flakes (sample nos. 1 and 2) were at least 1.8% in weight when measured by the IALCTMS method and the toxicities measured by the CFT assay and cell-based assay were more than 97%. In contrast, the amounts of ricin in seed cakes processed through RSC method with 0.3–0.5% of sodium hydroxide, methanol and dry temperature ≥ 100 °C (sample nos. 3, 4, 5, 6) were less than 0.01% when measured by the IALCTMS method and the toxicities measured by the CFT assay and cell-based assay were less than 10%. The sample no. 7 was processed in the same way as the sample no. 6, but the decrease of toxicity after processing was less significant. This is probably due to the difference in the quality of the sample. This sample was obtained after pressing in Madagascar, which had to travel to France and probably had time to hydrolyze since the free fatty acid content was fairly high. These data demonstrate that the RSC process plays an important part on the detoxification of castor meal and more than 90% of ricin activity was lost after this

process. However, only less than 50% of ricin activity was lost if the final drying temperature was reduced to 50 °C for 4 h (sample no. 8). These results confirm that ricin is sensitive to temperatures ≥ 100 °C, but not thermally decomposable at 50 °C. The results also illustrate the detoxifying effect of NaOH and/or methanol used in RSC at higher temperatures.

The results from the CFT and cell-based assays showed a significant difference in toxicity between the sample no. 6 (5%) and sample no. 9 (39–47%), indicating that for the RSC process it is better to use methanol than the mixture of methanol and hexane. One of the explanations is that methanol in the process has a denaturing effect on proteins, and less methanol is engaged in the co-solvent process (mixture of methanol and hexane). The other possibility is that the hexane limits the access of methanol and sodium hydroxide to the proteins and the effect of sodium hydroxide might also be limited in the presence of hexane. Table 3 also demonstrates that the effect of sodium hydroxide is important for the detoxification of the seed meal, with a decrease of toxicity 7–13-fold more than a non reactive process (sample no. 10/sample no. 6). Methanol alone (sample no. 10) has a positive effect to reduce the active ricin content compared to the mixture of methanol and hexane (sample no. 12) in the absence of sodium hydroxide, but the effect is enhanced in the presence of the sodium hydroxide catalyst (sample no. 6).

3.3. Evaluation of the sensitizing capacity of the castor seed flakes and seed cakes produced by the RSC method

A batch of defatted castor seed flakes as prepared according to Section 2.3 and the seed cakes resulting from the reactive grinding method described in Section 2.4 were evaluated for their capacity of sensitizing allergic reactions using the LLNA method in Section 2.6. From the results given in Table 4, it was clear that the seed cakes no longer exhibited any sensitizing capacity, unlike the defatted flakes, which was highly sensitizing.

Table 4
Sensitizing capacity of castor defatted seed flakes and seed cake after RSC process.

Product	Maximum dose (%) ^a	Main observations	Conclusion
Defatted flakes	10	Lymphocyte hyper-proliferation attributed to contact hypersensitivity	Product is sensitizing
Seed cake	10	No lymphocyte hyperproliferation was noted	Product is not sensitizing

^a Dose defined during the preliminary test following the death of 2 animals out of 3 after application of the product formulated at 10% and 25%. The surviving 3rd animal did not, on the other hand, trigger any ear irritation at the dose of 10% (dose retained for the main test).

3.4. *In vivo* toxicity of the castor seed flake and seed cake

The *in vivo* toxicity of the castor seed flake and seed cake was determined for oral routes of intoxication in female rats. Three groups of rats were fed 50, 300, and 2000 mg/kg of body weight daily, respectively, for 8 days. Toxicity was measured as mean lethal dose (LD₅₀) values. The lower the units (mg material fed/kg of rat body weight), the higher the toxicity, as less material was required for lethality. In this study, the rat LD₅₀ for the defatted flake in the oral route of intoxication was between 300 and 2000 mg/kg; at the dose of 2000 mg/kg, 100% mortality was observed on day 2. On the other hand, the rat LD₅₀ for the seed cake was more than 2000 mg/kg; at the dose of 2000 mg/kg, no clinical sign, no mortality of the rats were observed for 8 days and a weight gain was even noted at this high dose. These data demonstrated that the seed cake was well detoxified at the end of the RSC method.

4. Discussion

The conventional method to prepare fatty acid methyl esters from oilseeds is in two steps, namely a step of oil extraction, with or without solvent, and a step of transesterification of the oil in the presence of alcohol and catalyst, producing an ester phase and a glycerine phase. The concept of RSC has been described in the patent literature (Lacaze et al., 1997; Hoang, 2001; Haas, 2003) aiming to develop a new technology to produce biodiesel that would avoid the costly seed crushing and oil refining operations. However, in these earlier works only conventional edible crops were addressed, such as rapeseed, soybean, and sunflower. In addition, ethanol was often the alcohol of choice, which is more expensive than methanol.

Compared to most oil crops, castor oil is unique. It comprises glyceryl triricinoleate as the main component. Ricinoleic acid is a HFA. No other known natural oil contains such a high proportion of HFA. It is this characteristic glycerides composition which distinguishes castor oil from any other vegetable oils and fats and it is this composition which gives it its notable physical and chemical properties. There are several reports on processes for the transesterification of castor oil (Hillion, 2000). One of them described was to process castor seeds by a batch reaction (stirred bed reaction) (Khalil, 2009). However, this method has many drawbacks: (1) additional production cost linked to the use of dehulling equipment; (2) generation of solid by-products with very low added value (shells); (3) poor mechanical strength of the seed cake; (4) formation of fines responsible for clogging of filters; and (5) in the case of castor seeds, toxic and allergenic risk due to the tendency of the dry fines to be dissipated in the ambient air. In addition, all our attempts to duplicate this process have led to failures.

The present paper relates to a method for the RSC of castor seeds, which starts from specifically processed castor seeds in the presence of a light alcohol and a basic catalyst. This process makes it possible to carry out the grinding and the reaction for transesterification of the triglycerides present in the castor oil in a single step, simultaneously producing a defatted cake, glycerol and esters of fatty acids, in particular ricinoleic acid. The esters produced are intended mainly for the production of 11-aminoundecanoic acid, the monomer of Rilsan® 11, which is a polyamide with unique physical properties, developed by Arkema but could also be used to produce biodiesel or aviation fuel. The other advantage of this method as compared with the conventional methods lies in the small amounts of water used. Our experimental results have also shown that the RSC method can be applied to castor seeds having a degree of acidity of less than 2 mgKOH/g. Most importantly, the RSC method also detoxifies and deallergenizes the seed cake, which is very important for the safety of human and animal

because castor meal is currently used as a natural fertilizer in the producing countries. Even if it cannot be used as animal feed, its current value in India is about one forth to one third of the soybean meal value. Castor meal is also sold in European countries as a natural fertilizer for organic farming.

It is worth to point that although methanol is toxic and used in a large excess as a solvent and reactant for extraction in the RSC process, the excess of solvent is recovered by evaporation and drying of the seed meal. The final heat treatment recovers the methanol and achieves the best detoxification. Therefore, the final meal is free of methanol. Also, the excess of methanol is recycled in the process. So basically only the amount of methanol needed to make an ester is consumed (stoichiometrically). This means that methanol consumption is about 10% of the oil weight in the RSC process and the current industrial methanol price is about \$350–400/ton.

In summary, the RSC method developed in this study is simple, rapid and inexpensive. It is capable of inactivating not only the ricin but also the powerful allergen CB-1A, reducing the risks to the health of the individuals who handle them and enable the use of castor seed cake in animal feed. This is particularly important for the economy of countries that are large producers of castor oil such as India, China, and Brazil. While castor oil has many industrial uses, castor seed cakes have not yet found a use on the industrial scale, in particular owing to the allergy problems.

5. Conclusion

We have developed a RSC method for treating castor seeds with methanol and sodium hydroxide. This process combines seed crushing, solvent extraction, oil refining, esterification and meal detoxification into a single step, thereby reducing the capital cost and increasing the economic value for processing castor seeds. The process allows destroying “in planta” the ricin toxin and the allergen present in the castor seeds at the same time, enabling the safe handle and use of castor seed cakes. However, the results reported here have been obtained from small scale pilot studies. For large commercialization of castor bean products, further investigations are needed. This processing method has the potential to be applied to other toxic crops, which are rich in hydroxyl fatty acids such as jatropha, after making slight modifications based on the oil composition, seed texture and the presence of the toxic compounds.

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