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Ozone for Phorbol Esters Removal from Egyptian Jatropha Oil Seed Cake

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ABSTRACT

Egyptian Jatropha oil seed cake, a main by-product from biodiesel production is nutritionally promising because of high nutrition content and low antinutrient level. The effect of some chemical treatments (sodium bicarbonate $NaHCO_3$, ozonation, ethanol extraction) and physical treatments (heat treatment and ɣ -irradiation) were subjected to the meal for inactivating the phorbol esters (phorbol 12-myrestate 13-acetate). The treatment methods were moistting equal portions of the meal with $NaHCO_3$ (0.1- 0.5 M) for 30 minutes, accompanied by ozone treatment (2-6 minutes), heat treatment at (90 °C -121 °C) for 30 minutes, ethanol extraction, ɣ-irradiation 50kGy for 30 minutes. The results showed best phorbol ester removal 75.26 % with $NaHCO_3$ moist combined with 3 minutes ozone treatment at ozone dose 50 mg/L. The ɣ –irradiation achieved 71.35 % successful phorbol esters removal at 50 kGy for 30 minutes irradiation. The $NaHCO_3$ moistting combined with heat treatment 90°C for 30 minutes detoxified the meal 55.95% from phorbol esters. While $NaHCO_3$ moist combined with heat treatment and water wash removed 76.48 % of the phorbol esters. The $NaHCO_3$ moist treatment alone detoxified the meal by 44.54 % from phorbol esters. The isolated phorbol esters were analyzed and confirmed on High Performance Liquid Chromatography (HPLC) system. The phorbol esters were easy eliminated with $NaHCO_3$ moist combined with heat treatment and water wash > $NaHCO_3$ moist combined with ozone treatment 3 minutes > ɣ– irradiation > $NaHCO_3$ moist combined with heat treatment.

Key words: detoxification, phorbol esters, chemical treatment, heat treatment, ɣ– irradiation.

INTRODUCTION

Jatropha has been paid a special attention as various parts/products of the plant hold potential for use as biofuel, animal feed, and inclusion in medicinal preparations. Jatropha plants have been mainly investigated as a source of oil. The seed kernel of the plant contains about 60% oil that can be converted into biodiesel and used as a substitute for diesel fuel. The seed cake remaining after oil extraction is an excellent source of plant nutrients [1]. However, the presence of high levels of anti nutrient prevents its use in animal feeding. Phorbol esters (phorbol-12-myristate 13-acetate) have been identified as the major toxic principle in Jatropha [2]. The main toxin present in Jatropha curcas and other Jatropha species is phorbol esters, and the antinutritional

factors present are trypsin inhibitor, lectins and phytate [3]. These along with other antinutritional factors, were determined in the platypylla kernel meal.

Phorbol esters are double – edged swords; besides having lots of negative effects on human and livestock, these also possess some beneficial effects. Some naturally occurring phorbols are tumor inhibitors, inhibit human immunodeficiency virus (HIV) replication, and possess anti-leukemia activity [4]. The jatropha oil and cake contains traces of toxins. The components associated with toxicity can be denatured or inactivated by heat treatment or the combination of heat and chemical (NaOH and NaOCl) treatments. Extraction with 80 – 90% ethanol also holds promise in detoxification.

Now Jatropha production is being scaled up as a biofuel crop, the toxicity of the seeds may become a hazard for the people who have to harvest and process the seeds[5]. Many researchers have been studying on the planting and testing of jatropha and biodiesel derived from its oil, as well as how to remove the toxic substances from the meal. The only problem is the detoxification step that has to be developed. The toxic substance in jatropha, phorbol ester, is also found to be valuable. It is being used in cancer research. The biological activities of the phorbol esters are highly structure specific. The phorbol esters, even at very low concentrations, show toxicological manifestation in animal feed diets containing them. Phorbol esters (phorbol - 12-myristate 13- acetate) have been identified as the major toxic principle in Jatropha [2], [6]. Phorbol esters are toxic and carcinogenic. They are rapidly absorbed through the skin and probably the intestinal tract. They may cause severe irritation of tissues (skin, eyes, mucous membranes and lungs) and induce sensitivity. Avoid formation and breathing of aerosols. Complete removal of the toxins is therefore necessary before Jatropha oil can be used in industrial application or in human medicine, the oil must be shown to be completely imocuous before it is used commercially. Diets containing Jatropha meal with 1.5 to 2 mg/g of phorbol esters have been found to cause suppression of feeding, lesions on the skin, weight loss and death in both fish and rats. Toxicity of Jatropha seeds has been studied extensively in different animal models like goats, sheep, mice, rats and fish when feed with phorbol ester containing feeds [7]. Chivandi et al., 2006 studied the dietary effects of industrially detoxified Jatropha curcas meal for pigs [8], [9]. They concluded that the dietary caused sever adverse effects in pigs. This indicated that the detoxification procedure failed to completely remove and or neutralize the toxic anti-nutritional factors in Jatropha curcas meal. They observed some of the toxicity ascribed to the residual phorbol esters in meal. Becker and Makker 1998 were not able to destroy phorbol esters by heat treatment [6], but were able to extract phorbol esters using 92% aqueous methanol, and the meal after extraction was not – toxic to rates. They added that carp (fish) were more sensitive to phorbol esters than rats, as the meal obtained after extraction with 92% aqueous methanol for removal of phorbol esters resulted in lower growth performance in carp. Areghe et al., 2003 studied the detoxification of Jatropha curcas using heat and chemical treatments [10]. They concluded that phorbol esters were reduced to a level of 0.09 mg/g when jatropha curcas meal was heated-treated and washed 4 times with 92% methanol. The meal derived from this treatment had a crude protein content of most oil seed meal e.g. soybean (45.7%, crude protein). A preliminary industrial evaluation was studied in rats. They also added that treatment at 121 ºC, 30 min (with 66% moisture) followed by 4 times washing with 92% methanol seams a better means of detoxifying jatropha curcas meal. They concluded that treatment was promising, but in economic terms it was expensive to produce Jatropha curcas meal from it. With exploitation by small – scale industry the price could be reduced if the methanol was recovered. Martinez – Herrera et al. 2006 studied the nutritional quality and the effect of various treatments (hydrothermal processing techniques, solvent extraction, solvent extraction plus treatment with $NaHCO_3$ meal of toxic and non – toxic varieties from different

regions of Mexico [11]. Chivandi et al., 2006 subjected shelled kernels to industrial detoxification [8]. They studied double solvent extraction (hexane / ethanol system) coupled with moist – heat treatment to inactivate lectins and trypsin inhibitors in the resultant meal. This treatment left high concentration (91.9 mg / g) of residual phorbol esters. They added that double solvent extraction with hexane and moist – heat treatment, reduced the phorbol esters concentration to 0.80 mg / g, and 87.69% reduction in meal. A complete project begins on 2004 and ended on 2007 with sponsor mark (BEXYZ) aimed to define feasible and economical processes for the detoxification of jatropha seed cake [12], [13]. They studied degradability of the phorbol esters by rumen micro organisms and they concluded that rumen microbes were found to be unable to degrade phorbol esters. *Hyles euphorbiae* larvae were reported to detoxify TPA(4β-phorbol-12,13-dibutyrate) when administered orally; these larvae were able to metabolize nearly 70% to 90% of the phorbol ester and about 10% to 30% was retained and recovered in the feces. A potential detoxifying strategy was suggested where the larvae first store the toxic compounds in an inert compartment and then by specific metabolism and detoxification, they excrete the toxin and its metabolites [14]. Chetna Joshi et al., 2011 studied the complete degradation of phorbol esters by *Pseudomonas aeruginosa PseA* strain during solid state fermentation (SSF) of deoiled Jatropha curcas seed cake [15]. Phorbol esters were completely degraded in nine days under the optimized SSF conditions viz. deoiled cake 5.0 g; moistened with 5.0 ml distilled water; inoculum 1.5 ml of overnight grown *P. aeruginosa*; incubation at temperature 30 _C, pH 7.0 and RH 65%.

Andrew et al., 2009 studied the toxic components of Jatropha curcas seeds, the potential for plants breeding to generate improved varieties and the suitability of jatropha curcas oil as a feed stock and biodiesel [16]. They concluded that meal obtained after extraction of oil cannot be used as a source of animal feed. Kumar et al., 2008 studied the detoxification of jatropha curcas seed meal and its utilization as a protein source in fish diet [17]. They concluded that performance of 75% detoxified Jatropha seed meal group was comparable to 75% soybean meal group and was lower than fish meal group; whereas performance of 50% detoxified fed group was better than that of 50% and 75% fed groups and similar to that of fish meal group.

More than 40 years of multispecies and, multigenerational animal studies have shown that there are no toxic effects from eating irradiated foods. P. Siddhuraju et al., (2002) stated that irradiation is one possible alternative and additional processing technique for reducing both heat-stable and heat labile ant nutrients [18]. Irradiation can be used to reduce several carcinogenic agents and antinutritional compounds [19], [20]. Irradiation has the advantage over other methods that few steps are involved compared with, for example solvent extraction. Also, the chance of degrading nutrient or the use of strong solvents, such as HCl / NaOH. Free radical ingestion does not create any toxicological or other harmful effects. This has been confirmed by a long-term laboratory study in which animal were fed a very dry milk powder irradiation at 45 kGy. No mutagenic effects were noted and no tumors were formed and no toxic effects were apparent in the animals over nine successive generations [21]. Soybean seeds were irradiated at dose levels of 0, 1, 5, 10, 20, 40, 60, 80, and 100 kGy using cobalt-60 source. Inhibition of 25.4% typsin inhibitor activities and 16.7% chemotropism inhibitor activities was found when the soybean seeds were irradiated at 100 kGy [22]. Sattar and Neelofar (1990) investigated the irradiation and germination effects on phytate (212.0 mg/100 g) to (190.0 – 205.0 mg/100 g depending on dose level [23]. The degradation of protease inhibitors on exposure to γ-irradiation, were 22 and 16% respectively [24]. Farag (1999) reported that a combination of autoclaving for 10 min plus irradiation up to 20 kGy reduced the level of chlorogenic acid in sunflower meal by 87% more than other processing method[25].

Phorbol esters are the tetra acyclic diterpenoids generally known for their tumor promoting activity. They occur naturally in many plants. The biological activities of phorbol esters are highly structure specific. The phorbol esters, even at very low concentrations, show toxicological manifestation in animals fed diets containing them [26], [27]. Therefore, various chemical and physical treatments have been evaluated to extract or inactivate phorbol esters so that seed meal rich in proteins could be used as feed resources. The mollusicidal and insecticidal properties of phorbol esters indicate its potential to be used as an effective biopesticide and insecticide. But before using them in agriculture (As biopesticides or insecticide) or health control (antimicrobial or antitumor), the fate of phorbol esters in water, soil, and plants and the potential environmental risks should be assessed [28].

In this study different physical and chemical treatment were attempted on the seed meal samples to decrease or eliminate phorbol esters present in cake before and after hexane extraction. Treatment with ozone, air, $NaHCO_3$, ethanol extraction and Gamma irradiation were studied. Thermal treatment, solvent extraction and water extraction followed the $NaHCO_3$ treatment was evaluated too. Series of ozone trends and series with $NaHCO_3$ trends were chosen as inexpensive treatments to be optimized.

MATERIALS AND METHODS

2.1. MATERIAL

2.1.1 Preparation of seed cake:

Seeds of Jatropa curcas were removed from the kernel manually. Then the seeds were dried 2 hours at 60 ˚C in an oven, ground using mechanical grinder, sieved up to (-2 mesh). This was stored in an airtight polyethylene bags and kept in a refrigerator prior to study.

Table (1) Chemical composition of (dry matter) and some nutritional parameters of extracted meal from Egyptian Jatropha

component	% content
Moisture	5.6
Ash	5.15
Fat	6.87
Nitrogen	2.13
Phosphorus	4.45
Potassium	1.55
Organic matter	>90
Phorbol ester	0.85 g/g

Table (2) Important antinutrient in seed meal of toxic and non toxic variety Jatropha curcas

Component	Toxic variety	Non-toxic variety
Phorbolesters (mg/g kernel)	2.79	0.11
Total phenols(% tannic acid equivalent)	0.36	0.22
Tannins (% tannic acid equivalent)	0.04	0.02
Phytates (% dry matter)	9.40	8.90
Saponins (% diosgenin equivalent)	2.60	3.40
Trypsin inhibitor (mg trypsin inhibited per g sample)	21.3	26.5
Lactins (1 mg of meal that produced haeimagglutination per ml of assay medium).	102	51

All data are on dry matter basis,
Source : Makkar et al., 1998 [30].

The chemical composition of Egyptian Jatropha curcas seed are good source of protein (32.88%), oil (27.36%) and carbohydrates (30.11%). The seeds are rich in various micro-elements, that is Mn, Fe, and Zn which recorded 28.37, 0.38 and 47.13 mg /kg respectively as well as macro – elements, that is K, Ca, Na, Mg, and P, which recorded 103, 13, 34.21, 8.44, 109.89 and 185.17 mg/kg respectively. The seeds contain 52.59 mg/1000g, 25.58 mg/g, 39.95 mg/100g and 1.51 g/100g phytic acid, trypsin inhibitor activity, total phenols and saponins, respectively [29].

2.1.3 Preparation of grinded Seeds:
After taking the (-2mesh) ground Jatropha seeds meal out of soxhelt apparatus it was evenly spread on a tray lined with aluminum foil. The tray was kept overnight in a fume cupboard to get rid of any remaining hexane and for the meal to get dry.

2.1.4 The ɣ-irradiation was from a cobalt source for 30 minutes, and was done at Middel Fastern Regional Radioisotope Center for the Arab Countries, Doki, Egypt, using GAM- MA CELL 220 (Atomic Energy of Canada Ltd., Activity 14190 Curie, Canada Ontario, Model CG 220, Type B (U). The dose was till Saturation (50 kGy).

2.1.5 High Performance Liquid Chromatography was performed with (HPLC) equipped with model 600 delivering system, model (Waters 486) UV detector set at 230 – 400 nm and the data recorded by Millennium chromatography. Manganese software 2010(Waters, Milford MA 01757). Reverse phase C_{18}, column 250 XYum in) 10 µm (Mecherey – Nagyel. CO, Germany) was used. Mobile phase isocratic program, flow rate 1 ml/ min. Mobile phase: (trichloro methane: Methanol) (99: 1). Detector photodiode array. For analysis, aliquots of prepared standards and tested solutions were transformed to HPLC vials and analysed immediately. The method was adapted from [31].The phorbol esters peak appeared between RT =15 – 20 min were identified and integrated at 230 – 400 nm. The results are expressed as equivalent to a standard, phorbol-12-myristate13-acetate, which appeared between 15-20 min.

2.1.6 The other Method adapted from [2] where the phorbol esters peaks appeared between 55 – 61 min. The results are expressed as equivalent to a standard, phorbol-12-myristate 13-acetate at 45 – 60 min.

2.2 METHODS:
2.2.1 Screening Methods:
A stock solution of $NaHCO_3$ was prepared up to 0.075%. Several portions of the prepared seed cake of (10g and 50 g) were soaked in 50 ml $NaHCO_3$ 0.075% for 30 minutes.

2.2.2 Emulsions were subjected singly to 3 minutes (bubbling) for, ozone pass with dose 50mg/L, subjected to gamma irradiation at 50 kGy and samples were treated with $NaHCO_3$ and/or followed by washing with ethanol.

2.2.3 Emulsions were subjected singly to 3 minutes (air bubbling) using air compressor with air flow rate 680 ml /min and 220 volt, for aerating the moistted samples.

2.2.4 Preparation of detoxified seed cake:
The treated samples (standards and samples) were extracted with methanol : water (9:1) (w/v). Extraction was repeated three times. The combined extracts were concentrated in a rotary evaporator and dispersed in diethyl ether, transferred into a separating funnel and then washed

with water. The water lower layer was discarded. Washing was repeated three times. The ether layer was concentrated and subjected to HPLC determination.

2.2.5 Treatment of oil seed cake for phorbol Esters detoxification by ozone gas:
50 g $NaHCO_3$ of 0.075% were added to serial portions of 10 g prepared seed cake as in (2.1) and (3.1.1). The mixtures were singly ozonated for (2,3,6,9 and 12) minutes at dose 50 mg/L and 300 m A.

The samples were then filtered and residues were dried in oven at 60 ˚C for 2 hours. The dried residues were extracted by methanol (9:1) w/v three times for each sample and extraction was completed as in (3.2) method.

2.2.6 Treatment of oil seed cake for phorbol esters detoxification by $NaHCO_3$ and thermal treatment:
A series of different concentrations for $NaHCO_3$ (0.1M – 0.5M) were prepared. Samples were soaked in different concentrations for 30 minutes each and then filtered and subjected to thermal treatment at 121˚C for 30 minutes then cooled at room temperature.

Samples were divided three portions:

a- 100 ml Sample + 100 ml H_2O and left unwashed.
b-100 ml Sample + 100 ml H_2O (washed with water) (1:5) (w:v) and kept moisted 1 h.
c- 100 ml Sample + 100 ml ethanol (washed with ethanol) and kept moisted 1 h.

The three portions of sample were dried at room temperature or at 90 ˚C.
- Methanol extraction (9:1) w/v was repeated three times.
- The combined extracts were concentrated in rotary evaporator and residues were dispersed in diethyl ether.
- The extracts were then transferred into separating funnels and washed with H_2O.

The water (lower layer) was discarded and washing was repeated three times. The ether layer was concentrated and used for identification of phorbol esters by HPLC.

2.2.7 HPLC Method for phorbol ester Determination in seedcake:
The phorbol esters were removed from jatopha curcas seed cake by methanol method (3.1) and (3.5) and analyzed by HPLC according to two methods: 1- Sa SaM et al.(1996) where phorbol peaks appears at RT =15-20 min Fig (2), and 2- Makkar,H.P.S. and Becker,K. (1997) where the peak of phorbol esters appears at RT=45-60 min Fig (1). The peak was integrated and the results were expressed as equivalent to phorbol 12- myrestate 13-acetate.

RESULTS AND DISCUSSION

The results indicated that the percentage of phorbol esters in oil – cake were found to be in the range of 0.3766 mg/g and 1.193 mg/g with an average value of 0.637 mg/g, Fig (1) shows the HPLC chromatogram of standard phorbol esters (phorbol-12-myristate 13-acetate) where the peak appears at RT= 55.730, using method(2) and achieved concentration of 0.3766 µg/ml. Fig (2) shows the HPLC chromatogram of standard phorbol esters (phorbol-12-myristate13-acetate0 where its peak appears at RT=15.899 min using method(1) and achieved peak area 6469 Au= 0.123 µg/ml.

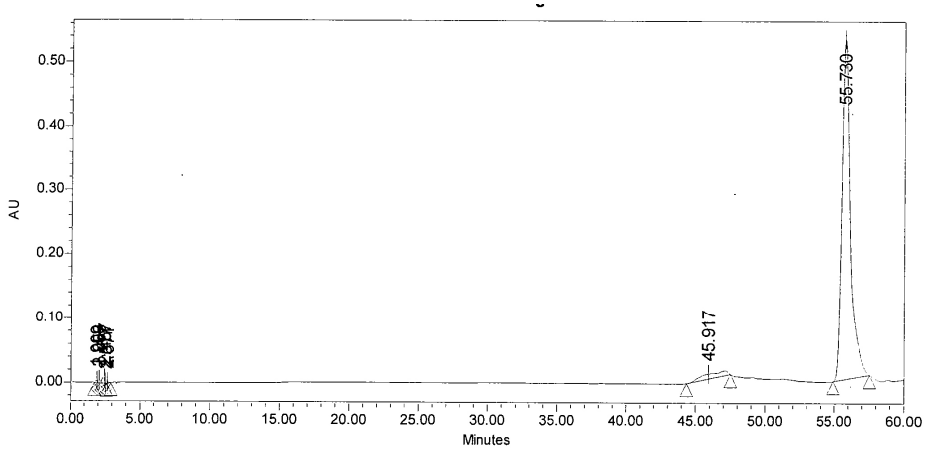

Fig (1)HPLC Chromatogram of the standard phorbol ester (phorbol-12-myristate 13-acetate) where the peak area of (56595 to AU) appears at RT= 55. 730.

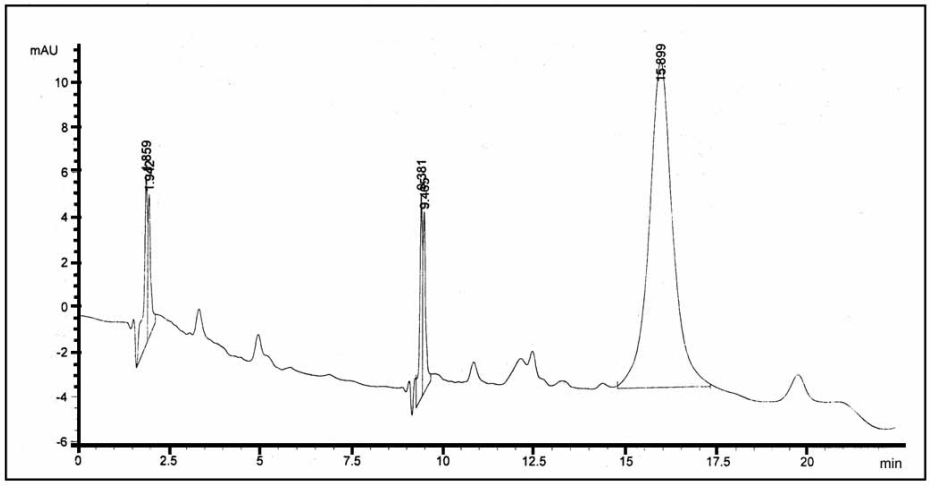

Fig (2)HPLC Chromatogram of the standard phorbol ester (phorbol-12-myristate 13-acetate) where the peak area of (646.9 AU) appears at RT= 15. 899.

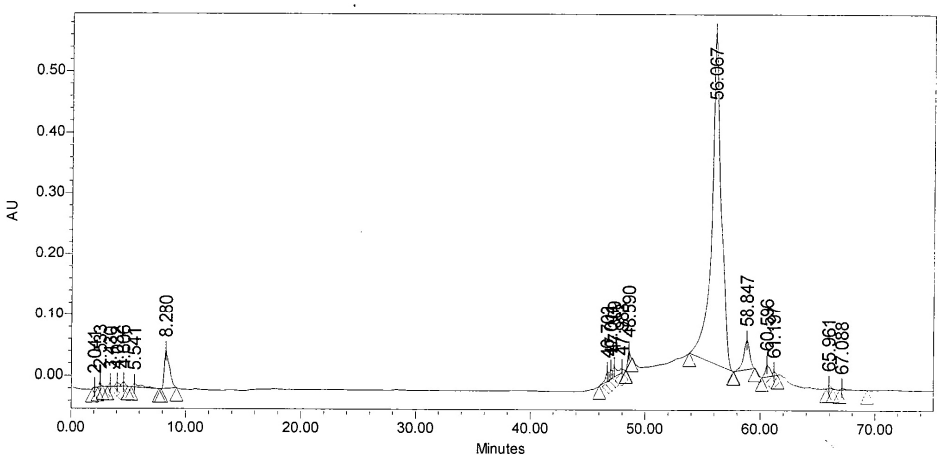

Fig (3) HPLC Chromatogram showing the phorbol ester of peak area decrease from (56595 to 31386 AU) appears at RT= 56.067 for original Jatropha seed cake after $NaHCO_3$ treatment achieved 44.45% phorbol esters removal .

Heat treatment 90˚C -121˚C for 30 minutes primarily inactivate lectins and trypsin inhibitors (Hivandi et al., 2006). Makkar et al., 1997 added that extraction of oil from meal reduces the oil content to (1-2%) reduces the concentration of phorbol esters.

The Original oil seed cake moist with 0.2N $NaHCO_3$ 30 min treatment showed 44.54% phorbol ester removal indicated by HPLC peak area decrease from (56595 AU to 31386 AU) Fig (3).

Heat treatment in combination with chemical treatment of sodium – carbonate has been studied to decrease the phorbol ester level in Jatropha. Our results achieved 55.95% phorbol esters removal indicated by decrease in HPLC peak area from (370 mAU to 264 mAU) with sodium bicarbonate 0.4N chemical treatment Fig (4).

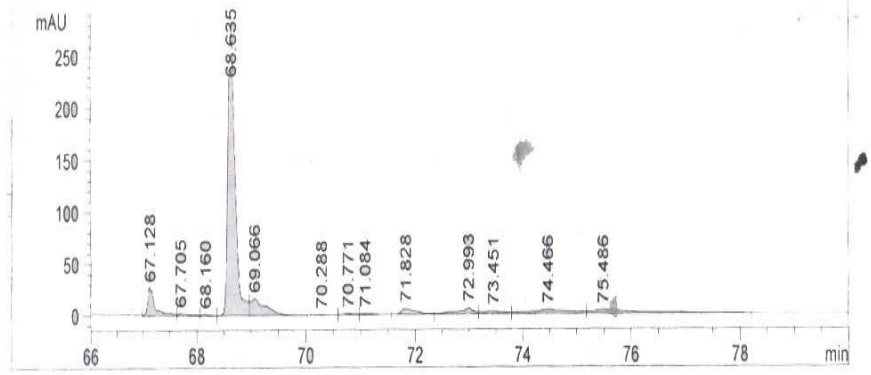

Fig (4) HPLC Chromatogram showing the phorbol ester of peak area decrease from (370 to 245mAU) at RT=68.635 for Jatropha seed cake ethanol wash only achieved 33.78% phorbol esters removal after $NaHCO_3$ 0.2N treatment combined with heat treatment.

Our results agreed with Aregheore et al., 2003. They used Na OH for washing phorbol esters. We used chemical treatment with Na H CO_3 0.2N combined with heat treatment and ethanol wash that removed phorbol esters up to 33.78% only indicated by decrease in HPLC peak area from(370 mAU to 245mAU). The thermal treatment combined with water wash increased phorbol ester removal up to 76.48% indicated by the decrease in HPLC peak area from (370 mAU to 141 mAU) Fig (5).

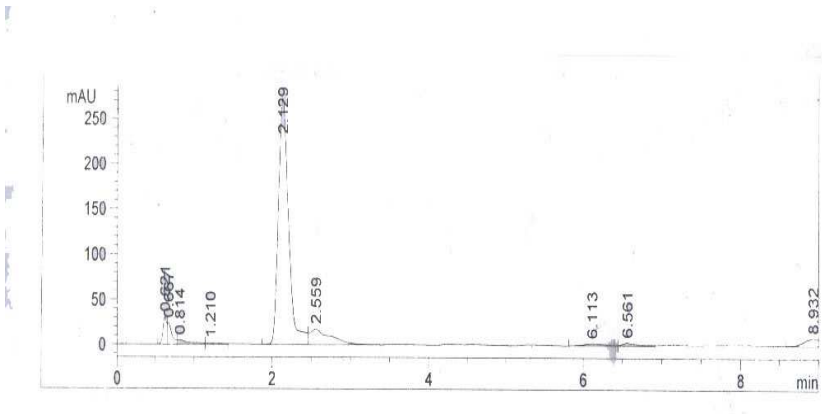

Fig (5) HPLC Chromatogram showing the phorbol ester of peak area decrease from (370 to141 mAU) at RT= 1.129 for Jatropha seed cake after $NaHCO_3$ 0.2N treatment combined with heat treatment and water wash (Sa Sam et al., method (1)) achieved 76.48%phorbol removal.

That is to say that Na H CO_3 moist removes only 34.32% but, Na H CO_3 combined with heat treatment removed phorbol esters 76.48%. So heat treatment and moisting increased phorbol esters removal by 42.16%. These treatments improve the nutritional value of oil seed cake. Our results agreed with (Purushothan et al., 2007).

Chemical oxidation by ozone gas combined with Na H CO_3 0.2N moistting showed its best results at ozonation 2 and 3 minutes of ozone dose 50 mg/ L at 300 mA. The significant phorbol esters 75.26% removal was confirmed by HPLC chromatogram, where peak area decreased from (56595 AU to 14000 AU) Fig (6). Air bubbling 3 minutes showed 78.53% of phorbol ester removal indicated by decrease in peak area from (56595 Au to 12150 AU), but the detoxified cake was spoilt i.e. air treatment needs autoclaving the samples before and after air treatment Fig (7).

Phorbol esters were present in oil seed cake by 0.3766 mg/g and were particularly affected by ɣ-irradiation dose of 50 kGy and showed significant decrease for phorbol esters up to 71.35% indicated by decrease in peak area from (56595 AU to 16210 AU) Fig (8).

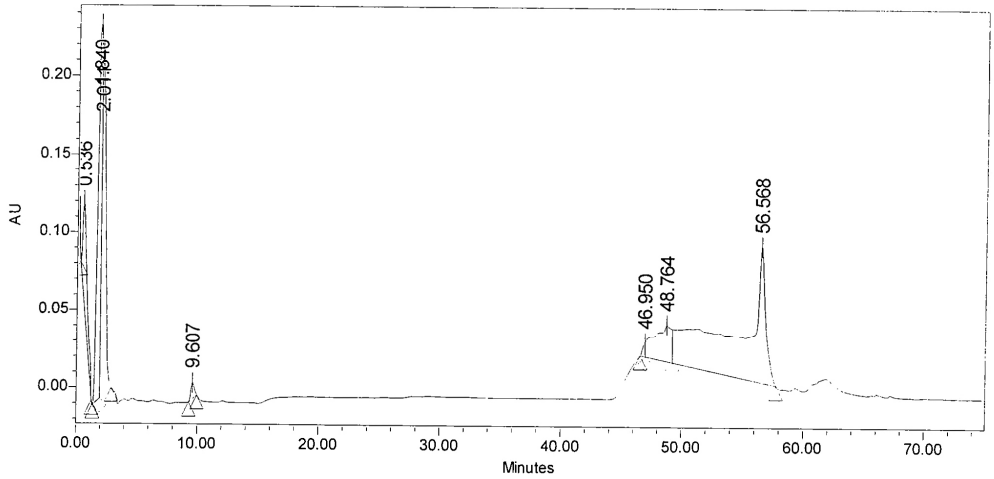

Fig (6) HPLC Chromatogram showing the phorbol ester 75.26% removal of peak area decrease from (56595to14000 AU) appeared at RT= 56.568 for Jatropha seed cake after $NaHCO_3$ treatment combined with ozone oxidation 3 minutes.

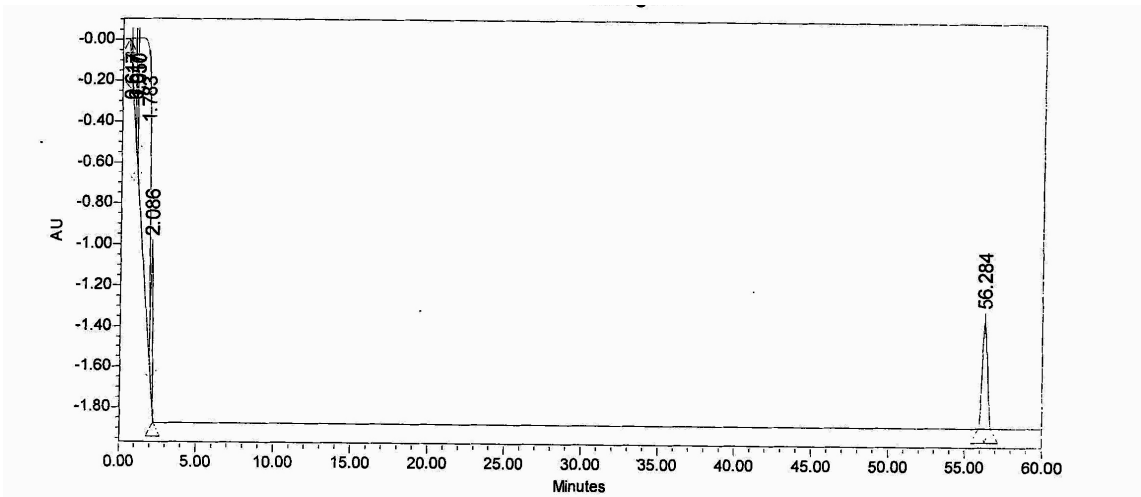

Fig (7) HPLC Chromatogram showing the porbol ester 78.53% removal indicated by decrease in peak area from (56595 to 12150 AU) at RT= 56.284 for Jatropha seed cake after $NaHCO_3$ treatment combined with air bubbling 3 minutes.

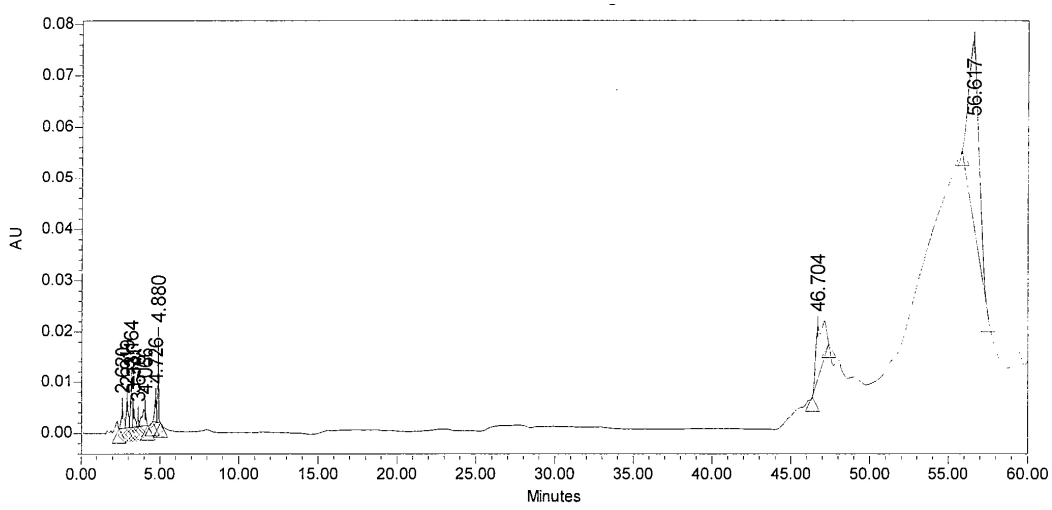

Fig (8) HPLC Chromatogram showing the phorbol ester 71.35% removal indicated by reduction in peak area from (56595 to 16210 AU) at RT= 56.517 for Jatropha seed cake after γ- irradiation dose of 50 kGy.

The above treatments eliminated the phorbol esters and showed promise for nearly complete detoxifying the oil cake. This would improve the way for their use as protein-rich ingredients in livestock feeds. But it is necessary to conduct feeding trays to evaluate the toxicity and nutritive value of the processed Jatropha oil cake.

CONCLUSION

The chemical oxidation by ozone or by air stripping for the jatropha seed cake meal gave a nearly clean nutrient as animal feed. This can increase the added ratio of Jatropha seed cake more than 20% - 50%. Thus the combination treatment of Na HCO_3 used in low normality of 0.2, which is a chemical that does not affect the animal stomach, but helps in food digestion achieved 44.54% phorbol ester removal. The advantage of the physical treatment (γ-irradiation) not only gives the 71.35% of phorbol detoxification as nearly ozone treatment achieved 75.26% removal, but also with less time consuming and less chemicals needed. The disadvantage of using aeration is the need of autoclaving before and after treatment, although this treatment showed highest phorbol ester removal 78.53%. So, we can conclude that ozone treatment can be the best and the less expensive method for phorbol esters removal from seed cake of jatropha compared to other tested methods.

Of course, as Khan and Islam [32] explained sustainability is not achieving only by minimizing risks and remediating phorbol engendered by the introduction of a given process or technology, but visualizing future potential problems, that is, having the time as main variable.

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REFERENCES

[1] Foial, N.,H.P.S. Makar, and K. Becker **2001**. *J. fuglie,* 45–76. Wageningen, The Netherlands; CAT publications.
[2] Makkar,H.P.S. and Becker,K. *J Agric.Food.Chem*. ,**1997**,45, 3152 – 3157.
[3] Makkar, H.P.S., and Becker, R. *European Journal of Lipid Science and Technology*. ,**2009**, 111. 773 – 787.
[4] Kupchan, S.M., Sigal, C.W. and Matz,M.J. *J.Am Chem. Soc*.,**1970**, 92 , 4476 – 4477.
[5] Animesh Agarual and Manish Saxena. *Der Chemica Sinica* ,**2011**, (2) ,issue (2), 172 – 176.
[6] Becker, K. and Makkar,H.P.S., *Human Toxicol*. ,**1998**, 40, 82 – 86.
[7] Goel,G.,Makker,H.P.S.,Francis,G. and Becker,K. *Int. J. Toxicol*. ,**2007**, 26, 279 -288.
[8] Chivandi E., Erlwanger K.H., Makuza S.M., Read J.S. and Mtimuni J.P. *Research Journal of Animal and Veterinary science*. ,**2006**, 1 (1) : 18 -24.
[9] Linn Vahl, Donatus Ebere Okwu, Offioug N. Ohenhen. Der Chemica Sinia ,**2010**, (1) issue (2) 6 – 14.
[10] Aregheore E.M., Becker K., Maker H.P.S. *J. Nat.Sci*., **2003**, 21, 50-56.
[11] Martinez–Herrea,J.,Siddhuraju,P.,Francis,G.,Davila-ortiz, Becker,K. Food Chem. ,**2006**,96, 80 – 89.
[12] Klaus Becker and Zohar Kerem (BEXYZ) project; **2004-2007.**
[13] Opasol A.O. and Adewoye S.O. *Avances in Applied Science*. ,**2010**, (1), issue (1) 182 -188.
[14] Hundsoberfer,A.K., J.N. Tshibangul, B.Wetterauer, and T. Wiuk. *Chemoecology* **2005**, 15: 261 – 267.
[15] Chetna Joshi, Priyanka Mathur, S.K. Khare .*Bioresource Technology* ,**2011,** 102, 4815–4819.
[16] Andrew J. King, Wei He, Jesus A. Cuevas, Mark Freudenberger, Daniete Ramiaramanana and Ian A. Graham. *Journal of Experimental Botany*. ,**2009**, (6), (10) 2897 – 2905.
[17] Kumar V., Makker H.P.S., Becker K., *J. Comparative Biochemistry and Physiology*. ,**2008**, 05.025.
[18] Siddhuraju P., Makkar H.P.S., Becker K., *Food Chemistry* ,**2002**, 78, 187 – 205.
[19] Ahn, H.J.Kim, JO J. H., Lee C., Yook, J.W., and Byun, M.W, *Food Control*,. **,2004$_b$,** 15, 197 – 203.
[20] Fan, X. and Mastorska, K., *Journal of Agriculture and food Chemistry***, 2006**, 54, 8266 – 8070.
[21] Ionnis S. Arvanitoyannis and Alexandros Ch. Srtrakos. Potential Uses of Irradiation. CHATER 16, Irradiation of food Commodities. Copyright@ **2010** Elsivier Inc. All rights of reproduction, in any form, reserved.
[22] Hafez, Y.S., Mohamed, A.I., Singh, G. and Hewedy, E.M. *Journal of Food Science*,**1985**, 50 (5), 1271 – 1274.
[23] Sattar, A. and Neelofar, A.M.A., *Plant Food for Human Nutrition*, **1990**, 40, 185 – 194.
[24] Rattansi,R. and Dikshit, M. *Journal of the American oil chemists` Society*,**1997,** 74(9), 1161 – 1164.
[25] Farag, M.D.E.H, *Journal of the Science of food and Agriculture*, **1999**, 79, 1565 – 1570.
[26] Belauer M.A. and Ogunsola F.O., *Journal of Agricultural Biotechnology and Sustainable Development*, **2010**, 2 (3) 35 -38.
[27] Aziz Uynnisa, B.Hemalatha, N.V. Cheuchu Lakshmi and D.Sninivas. *Der Chemica Sinica*, **2011**, 2 (1) 15-20.
[28] Premanand D.C., K.L. Senthikumar, B.Senthilkumar, M.Sara van Kumar, R. Thirumuty. *Der Chemica Sinica*, **2010**, 1, (2) 1-5.
[29] Azza A. Abou-Arab and Ferial M. Abu-Salem, *African Journal of Food Science*, **,2010**, 4 (3) 93 – 103.

[30] Maker, H.P.S., Aderibigbe, A.O., Becker, K., *Food chem.*,**1998**, 62, 207 – 215.
[31] Sa SaM. Dimitri jevic`. Vrsula Humer. Mayadah Shehadeh, W*Journal of Pharmaceutical and Biomedical analysis*.,**1996,** (15) 393-401.
[32] Khan, M.I.; Islam, M.R. True Sustainability in Technology Development and Natural Resource management; Nova Science Publishers; **2007**, New York, NY, USA; pp.2-9.