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Reduction of phorbol ester content in jatropha cake using high energy gamma radiation

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ABSTRACT

In this paper, an attempt has been made to remove phorbol ester present in jatropha cake by exposing it to gamma radiation. A sensitizer was also used to accelerate the degradation of phorbol esters. The phorbol ester content in the cake was estimated by high performance liquid chromatography (HPLC). It was observed that gamma irradiation of the jatropha seed cake was effective in reducing the phorbol ester content. Originally, the phorbol ester content in the cake was found as 0.29 mg/g, which on exposure to radiation was reduced by 33.4% and 96% with radiation dose of 30 and 125 kGy respectively. The presence of a sensitizer was found to enhances the susceptibility of phorbol esters degradation by oxidative degradation on exposure to ionizing radiation.

1. Introduction

Jatropha curcas, a tropical plant belonging to the family of Euphorbiaceae is commonly known as physic nut. In India, jatropha is also known as Ratanjyot, Jamalgota, Chandrajyot etc. It is a seed bearing tree containing 40–60% oil with a fatty acid composition similar to that of oil used for human consumption (Donlaporn & Suntornsuk, 2010; Makkar, Becker, Sporer, & Wink, 1997; Pradhan, Sahoo, Naik, & Sahoo, 2010). Since its kernels contain a high amount of oil (40–60%, w/w), the seeds serve as a potential source of biodiesel being currently promoted in India, Thailand and other South East Asian countries.

On oil extraction from jatropha seed, considerable quantity (approx 45%) of seed cake is generated as a by-product. The Jatropha cake is rich in crude proteins (50–58%) and has the potential to be used as a livestock meal for an alternative to mustard and soya cake. However, it is presently used only as a low value manure inspite of having all amino acids comparable with the FAO reference protein except lysine (Apiwatanapiwat, Vaithanomsat, Somkliang, & Malapant, 2009; Makkar, Francis, & Becker, 2008;). Amino acid composition of Jatropha and Soybean meal revealed an almost similar pattern of essential amino acids, except for lysine and sulphur amino acids, lysine being lower and sulphur amino acid higher in Jatropha meal. But the presence of various antinutrients in the seed cake prevents its use as highly nutritious

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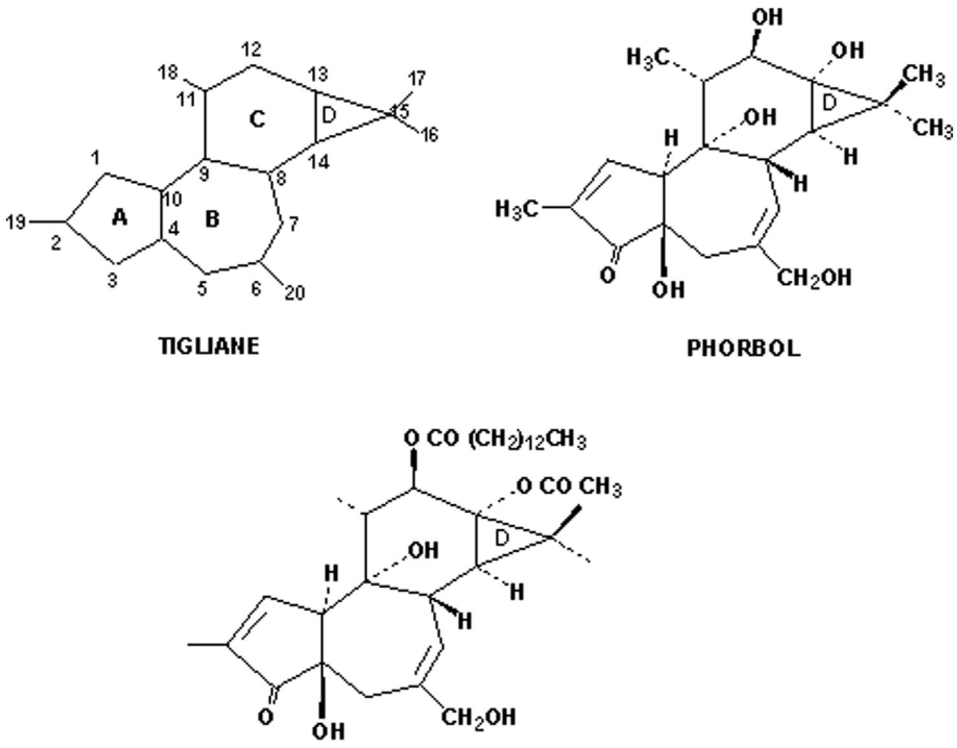

Fig. 1 – Structure of Phorbol 12-myristate 13-acetate (PMA).

protein supplement in animal feed. The anti-nutrients present in the seed cakes are trypsin inhibitor, lectin, saponin, phytic acid and toxic compounds called phorbol esters (Chivandi, Mtimuni, Read, & Makuza, 2004; Herrera, Siddhuraju, Francis, Davila-Ortiz, & Becker, 2006; Saetae & Suntornsuk, 2010; Saetae & Suntornsuk, 2011). Thus it could be an excellent protein source once it is detoxified. To make the seed cake suitable for use as livestock feed, the anti-nutrients must be removed. The trypsin inhibitor and lectins are heat labile, and can be inactivated by heat treatments, but phorbol esters and phytates remain unchanged. Phytates constitute a major single anti-nutritive component of Jatropha meals which is not heat labile and can have adverse effects especially mediated by decrease in bioavailability of minerals particularly calcium, zinc and iron, however, the adverse effect of phytate can be mitigated by addition of minerals in diet.

Phorbol esters (PEs) are the major impediment to the wide commercial use of jatropha meal as a feedstock. During extraction of oil from jatropha seeds, 70–75% of PEs goes with the oil, and 25–30% remains strongly bounded to the matrix of seed meal. Due to the high toxicity of PEs, seed cake cannot be used as animal feeds without detoxification. The phorbol esters have been found to be responsible for skin-irritant effects and tumour promotion since they stimulate the protein, kinase C (PKC) (Wink, Koschmieder, Sauerwein, & Sporer, 1997). The most studied jatropha species, *J. curcas*, contains PEs of about 1–3 mg/g in jatropha meal and 3–6 mg/g in oil.

The term phorbol refers to a group of compounds known as tigliane belonging to the closely related families of diterpenes with polycyclic structure as shown in Fig. 1 (Devappa, Makkar, & Becker, 2010; Goel, Makkar, Francis, & Becker, 2007; Kodekalra, 2012; Roach, Devappa, Makkar, & Becker, 2012).

The structure of the phorbol esters is dependent on the tetracyclic diterpene carbon skeleton know as tigliane. Tigliane is the fundamental alcohol moiety in the phorbol esters. Tigliane contains four rings designated as A, B, C, and D. Hydroxylation of this basic structure at different positions and then ester bonding to various acid moieties results in formation of large varieties of phorbol ester compounds. The phorbol, the parent diterpene of phorbol esters, contains five hydroxyl groups with different reactivity towards acylation. Ring A is on the left and trans linked to the 7-member ring B. Ring C is 6-membered and cis linked to the cyclopentane D ring. The two categories of phorbols, α and β, differ in their OH group in ring C. The placement of OH group makes the phorbol an active (β) or inactive (α) type, which results in spatial arrangement of ring D and precludes activation of PKC and other structurally similar phorbol ester receptors. The inactive α phorbol ester have similar lipophilicity and physicochemical properties as the active β phorbols, but are unable to activate PKC due to conformational shifts.

Haas, Sterk, and Mittelbach (2002) reported six types phorbol esters from J. curcas seed oil, where all compounds possess the same diterpene moiety. The acetylation of phorbols at various positions to various acid moieties by ester bonding results into the different types of PE. However the widely prevalent phorbol is TPA (4 β-12-O-tetradecanoyl-phorbol-13-acetate).

The phorbol esters are insensitive to heat even at 160 C. Therefore heat treatment is not effective to detoxify kernel