



Effect of Detoxification Methods on Anti-nutritional Factors and Proximate Composition of Defatted *Jatropha curcas* Kernel Meal

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ABSTRACT

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A study was conducted to evaluate the effect of chemical treatment and solid state fermentation on the anti-nutritional factors (ANFs) and proximate composition of defatted *Jatropha* kernel meal (DJKM). The DJKM samples were treated with alkaline methanol (90% methanol containing 0.2M NaOH; CT-1), alkaline ethanol (80% ethanol containing 0.1M NaOH; CT-2), highly alkaline methanol (90% methanol and 3% NaOH; CT-3), modified ethanol (4-times extraction using 80% ethanol; CT-4) and modified methanol (4-times extraction using 90% methanol; CT-5), in combination with moist heat treatment in all cases. Another sample was treated by solid state fermentation (SSF) using *Aspergillus niger* fungus (FT). The untreated sample was considered as control (J0). In comparison to control and the chemical treated samples (CT-1, CT-2, CT-3, CT-4 and CT-5), SSF treated sample contained significantly ($P < 0.05$) lower levels of moisture and crude fibre, and higher levels of ether extract and total ash. However, these parameters did not vary ($P > 0.05$) among the control and chemically treated samples. Though the CP content of FT sample was significantly higher than the control, it was found comparable to all the chemically treated samples. The moisture and ether extract contents of control and treated samples exhibited a reverse trend. Significantly lower and higher GE values were observed in CT-2 and CT-3, respectively; however, it was not affected much due to other treatments. Results indicated that any of the chemical treatments could not result in significant ($P > 0.05$) removal of phytic acid and tannin, whereas FT was able to significantly ($P < 0.05$) remove 100% phytic acid and 65.79% tannin from DJKM. However, all the treatments were equally effective in removal (100%) of trypsin inhibitor from DJKM. It is concluded that among the different treatment methods used, solid state fermentation was found to be the most effective method for removal of ANFs from DJKM.

Keywords: Anti-nutritional factors, Chemical treatment, *Jatropha*, Solid state fermentation.

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INTRODUCTION

Aquaculture is currently the fastest developing sector compared to all other terrestrial farmed animal production systems. Future fish demand for the ever-increasing human population can be met through technological interventions by implementing intensified feed-based aquaculture system with increasing productivity but the shortage of feed ingredients and increasing cost of conventional feed resources is posing a great challenge to the sustainability of aquaculture sector (Fawole *et al.*, 2018). The feed cost typically varies between 50 and 60% of production cost in intensified aquaculture system (Miles and Chapman, 2011; FAO, 2012); therefore, cost effective, eco-friendly and quality feed are the key to sustain the feed-based intensified aquaculture (Sanap *et al.*, 2018). These factors have stimulated researchers to explore the unconventional plant-based feed ingredients which are not only of low cost, and easily available but also not competing with human food and terrestrial animal feed sectors (FAO, 2008; Fawole *et al.*, 2018). Soybean meal is the most widely used plant-based feed ingredients in aquafeed but its increasing price and competition with terrestrial animals and human beings for food and feed have coerced several studies to find out suitable alternatives (Harter *et al.*, 2011; Kumar *et al.*, 2012; Fawole *et al.*, 2016; Phulia *et al.*, 2017a,b; Shamna *et al.*, 2015; Shamna *et al.*, 2017; Sanap *et al.*, 2018).

Jatropha curcas is a multipurpose and drought-resistant small perennial tree/large shrub, widely spread throughout the tropics and subtropics. Its seeds and kernels have been reported to be containing 27-40% and 60% oil, respectively (Makkar *et al.*, 1998b). This oil can be trans-esterified into biodiesel meeting the European and American biodiesel standards (Azam *et al.*, 2005). The kernel meal generated after oil extraction of *J. curcas* is a rich source of CP (58-62%; Makkar *et al.*, 2007; Makkar *et al.*, 2008) and reported to be of superior amino acids composition than soybean meal (except lysine) making it a potential protein ingredient in aquafeeds. However, its direct utilization in animal diets largely remain limited owing to the presence of highly toxic components - phorbol esters (PEs) and other anti-nutritional factors such as phytic acid, trypsin inhibitor, tannin and lectin, etc. (Makkar and Becker, 1997; Angel *et al.*, 2015; Shamna *et al.*, 2015). Therefore, it is imperative to detoxify the kernel meal prior to its utilization in animal feeds. Though heat labile anti-nutritional factors such as lectin and trypsin inhibitor can be easily deactivated by moist heating/autoclaving, it is very difficult to degrade PEs and phytic acid by heat treatment even at higher temperature (Phulia *et al.*, 2017a). The PEs exhibit their adverse effects through activation of protein kinase C (PKC) pathway, which is involved in synthesis of phospholipid, protein and DNA, regulation of enzyme activities through phosphorylation and dephosphorylation of protein, cell differentiation and gene expression and thus responsible for carcinogenic and skin irritant effects (Wink *et al.*, 1997; Goel *et al.*, 2007; Makkar and Becker, 2009; Devappa *et al.*, 2010). Raw or defatted seeds of *Jatropha* when force-fed to fish, chicks, pigs, goat, mice and rats caused severe toxicity symptoms before death (Adam, 1974; Adam and Magzoub, 1975; Liberalino *et al.*, 1988; Chivandi *et al.*, 2000; Gadir *et al.*, 2003; Angel *et al.*, 2015).

The adverse effects of phytic acid can be overcome through supplementation of mineral mixture or microbial phytase enzyme in the diet (Sardar *et al.*, 2007). Over the last two decades, several approaches have been evaluated for detoxifying *Jatropha* by-products (kernel meal, seed cake, protein concentrate and protein isolate). These approaches include a range of physical (Makkar *et al.*, 1998a), chemical (Makkar and Becker, 1997; Aregheore *et al.*, 2003; Martinez-Herrera *et al.*, 2006; Chivandi *et al.*, 2006; Rakshit *et al.*, 2008; Baocai *et al.*, 2014; Li *et al.*, 2015; Nokkaew and Punsuvon, 2015), biological (Belewu and Sam, 2010; Joshi *et al.*, 2011; Hassaan *et al.*, 2016; Shamna *et al.*, 2015; Phulia *et al.*, 2017a,b) and radiation treatments (Diwani *et al.*, 2011; Gogoi *et al.*, 2014; Sanap *et al.*, 2018). But except for few studies, most of them have not yielded satisfactory results. Thus, several alternative efforts are still undergoing to develop economic, user-friendly and eco-friendly methods to produce completely detoxified products. With this backdrop, the purpose of this study was to investigate effect of various detoxification methods on proximate composition and anti-nutritional factors of defatted *Jatropha* kernel meal (DJKM).

MATERIALS AND METHODS

Preparation of Jatropha kernel meal

J. curcas seeds procured from Genius Herbs, Coimbatore, India were deshelled manually to obtain kernels. The kernels were ground with the help of a kitchen grinder and defatted using diethyl ether (boiling point 40-60°C) in a soxhlet extraction apparatus (Socslus, Pelican equipments, Chennai, India) to obtain defatted *Jatropha* kernel meal (DJKM). The DJKM was dried in oven at low temperature (50°C) and later packed in air-tight polyethylene bags and stored at -20°C until further use.

Detoxification of DJKM

The DJKM samples were detoxified using three different chemical methods along with solid state fermentation using *Aspergillus niger* as detailed below:

Alkaline methanol/ethanol plus moist heat treatment: This treatment was carried out according to Makkar and Becker (2010) with some modifications. Exactly, 150 ml of 90% methanol containing 0.2M NaOH was added to 10g of DJKM (15:1 v/w) in round bottom flask of refluxing unit of a solvent extraction apparatus at 65°C for 30 min. After extraction was over, the mixture was cooled and filtered through Buchner funnel with suction arrangements and using Whatman filter paper No. 1 to obtain the residue. While filtering in Buchner funnel, the residue was washed with 120 ml of 90% methanol without NaOH. The residue was then re-treated with 150 ml 90% methanol solvent (without NaOH) through the same refluxing method. Finally, the residue was given hot water washings for complete removal of both the solvent and the alkali. The residue was then given moist heat treatment through autoclaving (121°C, 30 min) followed by freeze drying using lyophilizer. The dried samples were designated as CT-1. The same procedure was adopted using ethanol; however, the extraction was carried out at 78°C using 80% ethanol containing 0.1M NaOH. The treated sample was designated as CT-2.

Highly alkaline methanol plus moist heat treatment: Three steps chemical treatment with 90% methanol and 3% NaOH was employed in this treatment. In this detoxification approach, 10g DJKM was mixed with 30 ml 90% methanol (1:3, w/v) in a 150 ml beaker. The mixture was sonicated for 5 min and then incubated at 70°C for 15 min in a water bath. Then the mixture was centrifuged ($4500 \times g$; 15 min) and the supernatant was carefully discarded. The meal was retreated with 30 ml 90% methanol following the same procedure. The meal was freeze-dried using lyophilizer and 8g dried meal was mixed with 8 ml 3% NaOH solution (1:1, w/v) in a 100 ml beaker and incubated at 70°C for 5 min in a water bath. The alkali treated meal was further autoclaved (121°C, 30 min) and washed with hot water in muslin cloth to make it alkali free. The sample was then lyophilized and the dried sample was stored at -20°C for further analysis. This treatment was designated as CT-3.

Modified ethanol/methanol plus heat treatment: In this modified ethanol/ methanol mediated detoxification approach, 10g DJKM was mixed with 50 ml of 80% aqueous ethanol or 90% aqueous methanol (1:5, w/v) in a 150 ml beaker. The mixture was heated on a magnetic stirrer at 78°C or 65°C for 5 min for the methanol and ethanol treatments, respectively. After heating, the mixture was filtered using Whatman filter paper No. 1. The filtered residue was retreated (three times) in the same way. The residue was then given moist heat treatment through autoclaving (121°C, 30 min) followed by freeze drying using lyophilizer. The treated samples were designated as CT-4 and CT-5.

Solid state fermentation (SSF): The defatted meal was detoxified by SSF, as suggested by Belewu and Sam (2010) using *Aspergillus niger* (MTCC 281) procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. The fungus was sub-cultured in Czapek Yeast Extract Agar (CYA). The fungal spores were harvested in Tween-80 solution and diluted to achieve 10^7 - 10^8 spores/mL by serial dilution. Fermentation of dried, cooled and autoclaved DJKM was done aerobically at 30°C using *A. niger* for a period of three weeks. The fermented product was then autoclaved to stop the fungal growth further. The fermented meal was then cooled and lyophilized to obtain dried fermented Jatropa kernel meal (FT).

Analysis of proximate composition

Proximate composition of the detoxified DJKM samples was determined as per AOAC (1995). The gross energy (GE) was calculated as per the formula given by Smith (1981):

$$\text{Gross energy (kcal/100g)} = [5.64 \times \text{CP (g/100g)} + 9.44 \times \text{EE (g/100g)} + 4.11 \times \text{TC (g/100g)}]$$

Determination of anti-nutritional factors

Phytate or phytic acid estimation was carried out following the spectrophotometric procedure of Vaintraub and Lapteva (1998) and phytate was expressed as % phytic acid equivalent. The method used for the estimation of trypsin inhibitor (TI) was a modified method of Makkar *et al.* (2007) and the activity was expressed in trypsin inhibitor units (TIU, mg/g). Total tannin was estimated using spectrophotometric method as described

by Makkar *et al.* (2007). The reagent used was Folin-Ciocalteu reagent and the results were expressed as percent tannic acid equivalent.

Statistical analysis

All the data were statistically analyzed using ANOVA in SPSS package version 16.0 and the significant difference between the means was determined by Duncan's multiple range tests. Comparisons were made at the 5% probability level.

RESULTS AND DISCUSSION

Results for the proximate composition of control and treated samples are given in Table 1. The moisture content of the detoxified samples was significantly ($P < 0.05$) lower in FT group compared to all chemical treated groups (CT-1 to CT-5) and control (J0). The decline in moisture content due to solid state fermentation of DJKM (FT) in the present study was in agreement with earlier report of Belewu and Sam (2010). Xiao *et al.* (2011) also did not found any variation in moisture/DM content in DJKM treated with 90% methanol or ethanol. The result indicated an inverse relationship between ether extract (EE) and moisture content in all treatment groups. The FT group exhibited significantly highest ($P < 0.05$) EE compared to all other treatments including control (J0). The increased EE in FT group might be attributed to more lipogenic than lipolytic abilities of fungus (Belewu *et al.*, 2010).

In the present study, the chemically treated DJKM samples (CT-1 to CT-5) did not show significant variation in terms of their crude protein (CP) contents. On the contrary, an increase in the CP content with the chemically treated samples was observed by Aregheore *et al.* (2003), Martinez-Herrera *et al.* (2006) and Xiao *et al.* (2011) which

Table 1. Effect of detoxification methods on proximate compositions (%) and gross energy (kcal/100g) of DJKM

Treatments [†]	Moisture	CP	EE	TA	CF	NFE	GE
J0	7.86 ^a	57.83 ^b	0.62 ^b	9.65 ^{bc}	9.12 ^a	22.77 ^{ab}	463.13 ^{ab}
CT-1	8.17 ^a	57.95 ^{ab}	0.60 ^b	9.68 ^{bc}	9.16 ^a	22.59 ^b	463.08 ^{ab}
CT-2	8.14 ^a	57.97 ^{ab}	0.61 ^b	9.73 ^b	9.15 ^a	22.53 ^b	462.94 ^b
CT-3	8.04 ^a	57.96 ^{ab}	0.61 ^b	9.62 ^c	9.16 ^a	22.63 ^b	463.41 ^a
CT-4	8.18 ^a	57.94 ^{ab}	0.60 ^b	9.66 ^{bc}	9.16 ^a	22.62 ^b	463.23 ^{ab}
CT-5	8.04 ^a	57.95 ^{ab}	0.61 ^b	9.64 ^{bc}	9.15 ^a	22.64 ^b	463.28 ^{ab}
FT	7.04 ^b	58.0 ^a	0.73 ^a	9.85 ^a	8.50 ^b	22.91 ^a	463.15 ^{ab}
SEM	0.092	0.017	0.011	0.019	0.051	0.036	0.048

[†]The treatments included: J0, Untreated defatted *Jatropha* kernel meal (DJKM); CT-1, 90% methanol with 0.2M NaOH plus autoclaving; CT-2, 80% ethanol with 0.1 M NaOH plus autoclaving; CT-3, 90% methanol & 3% NaOH plus autoclaving; CT-4, 80% ethanol plus autoclaving; CT-5, 90% methanol plus autoclaving; FT, solid state fermentation by fungus.

^{ab}Mean values in the same column with different superscripts differ significantly ($P < 0.05$).

has been attributed to a proportionately higher loss of non-protein components (Aregheore *et al.*, 2003). Our results are in corroboration with earlier findings of Belewu *et al.* (2010), Belewu and Sam (2010) and Oseni and Akindahunsi (2011), wherein they reported increased CP resulted from fermentation of sample. The TA content was significantly higher ($P < 0.05$) in FT group compared to control (J0) and chemical treated samples (CT-1 to CT-5). The TA content in control and treated samples varied between 9.62 and 9.85%, which was well within the reported value of Makkar *et al.* (1998b). The increased CP and TA contents in fermented sample could be attributed to microbial source (Martinez-Herrera *et al.*, 2006). Significantly higher ($P < 0.05$) CP, EE and TA contents in FT group in the present investigation are in congruent with Hassaan *et al.* (2016) who reported increases in these nutrients in *Jatropha* seed meal fermented by *Bacillus licheniformis* and *Bacillus pumilus* compared to control.

The knowledge of dietary fibre is important in food analysis, as they are responsible for the viscosity of the gastrointestinal contents, the intestinal transit time, absorption of glucose and starch digestion (Adolfo Lutz Institute, 2005). The CF content was significantly lowest ($P < 0.05$) for FT group compared to all other treatments including control. In agreement with the present finding, Hassaan *et al.* (2016) reported reduction in CF in *Jatropha* seed meal fermented by *B. licheniformis* and *B. pumilus* which could be indicative of CF degradation by fungal fibrolytic enzymes (Jacqueline and Visser, 1996). In contrary to present observation, Martinez-Herrera *et al.* (2006) reported increased CF in *Jatropha* kernel meal treated by 90% ethanol and 90% ethanol plus NaHCO_3 followed by autoclaving (121°C , 25 min). Though significantly ($P < 0.05$) lower and higher gross energy values were observed in CT-2 and CT-3, respectively, it was not affected much due to other treatments.

The effect of various detoxification methods on anti-nutritional factors of DJKM is given in Table 2. The phytic acid content did not vary significantly ($P > 0.05$) between control and any of the chemical treatment groups, whereas it was not detected in FT group indicating 100% removal. Similar to our results, Aderibigbe *et al.* (1997), Makkar *et al.* (1997) and Martinez-Herrera *et al.* (2006) also did not observe any reduction in phytic acid level by chemical treatment of *Jatropha* meal. Xiao *et al.* (2011), however, reported a significant reduction of phytic acid in *Jatropha* kernel meal treated by 90% methanol and 90% ethanol. Phytic acid (8.61%) in the control group was found similar to the reported value of 8.9% by Makkar and Becker (2009) and the values in all chemically processed samples were very close to those reported by Aderibigbe *et al.* (1997). However, in the present study, solid state fermentation of DJKM by *A. niger* could able to remove phytic acid completely which might be mediated through phytate-degrading fungal enzymes (Jacqueline and Visser, 1996). The high level of phytic acid present in *J. curcas* meal might decrease the bioavailability of minerals (especially P, Ca, Mg, Mn, Zn and Fe) by forming insoluble complexes. Phytic acid also decrease protein digestibility through formation of undegradable complexes with protein and also by reducing the activities of digesting enzymes such as trypsin and pepsin (Reddy and

Table 2. Effect of detoxification methods on anti-nutritional factors of DJKM

Treatments [†]	Phytate (% phytic acid equivalent)	Tannin (% tannic acid equivalent)	Trypsin inhibitor (mg/g)
J0	8.61 ^a	0.38 ^a	21.27 ^a
CT-1	8.25 ^a	0.34 ^a	ND [‡]
CT-2	8.33 ^a	0.37 ^a	ND
CT-3	8.37 ^a	0.34 ^a	ND
CT-4	8.33 ^a	0.36 ^a	ND
CT-5	8.28 ^a	0.35 ^a	ND
FT	ND	0.13 ^b	ND
SEM	0.660	0.019	1.664

[†]The treatments included: J0, Untreated *Jatropha* kernel meal (DJKM); CT-1, 90% methanol with 0.2M NaOH plus autoclaving; CT-2, 80% ethanol with 0.1 M NaOH plus autoclaving; CT-3, 90% methanol & 3% NaOH plus autoclaving; CT-4, 80% ethanol plus autoclaving; CT-5, 90% methanol plus autoclaving; FT, solid state fermentation.

^{a,b}Mean values in the same column with different superscripts differ significantly ($P < 0.05$).

[‡]Not detected.

Pierson, 1994; Shamna *et al.*, 2015). In corroboration to our finding, phytic acid in plant tubers (*Ipomoea batatas*) was potentially removed by *Saccharomyces cerevisiae* fermentation (Antai and Nkwelang, 1998). In addition, phytic acid in soybean, cowpea and ground bean was effectively removed by *R. oligosporus* (Egounlety and Aworh, 2003). Hassaan *et al.* (2016) reported 85.27 and 91.29% reduction phytate level in *Jatropha* seed meal fermented by *B. licheniformis* and *B. pumilus* for a period of 9d, respectively.

In this study, the trypsin inhibitor content (21.27 mg/g) in the control group was found to be within the reported range of 18.40-26.85 mg/g (Makkar *et al.*, 1998b). However, all the detoxification methods employed in the present study were able to completely remove trypsin inhibitor from the DJKM. Trypsin inhibitors in JKM are heat-labile and are completely removed by heat treatment at 121°C for 30 min (Aregheore *et al.*, 2003; Makkar and Becker, 2009). Contrary to our results, Aderbigbe *et al.* (1997) found that autoclaving could inactivate the trypsin inhibitor levels by 83-99%. However, Hassaan *et al.* (2016) reported 92.59 and 95.09% reduction of trypsin inhibitor in *Jatropha* seed meal fermented by *B. licheniformis* and *B. pumilus* for a period of 9d, respectively. Trypsin inhibitors interfere with normal functioning of pancreatic proteolytic enzymes in monogastric animals including fish, leading to severe growth depression (White *et al.*, 1989; Hajos *et al.*, 1995).

There was no effect noticed on tannin content in DJKM detoxified by chemical treatments, however, the FT group exhibited significant ($P < 0.05$) removal of tannin by 65.79% which might have been mediated through tannin degrading fungal enzymes (Jacqueline and Visser, 1996). Our findings corroborated the observation of Xiao *et al.* (2011) and Ameen *et al.* (2011).

In conclusion, solid state fermentation of DJKM using *A. niger* was found to be a more effective method than the chemical treatments in terms of removal of antinutritional factors (ANFs) and improving the proximate composition of DJKM.

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