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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (6): 1001-1005, 2004
ISSN 1028-8880

Effect of Processing Method on Phorbol Esters Concentration, Total Phenolics, Trypsin Inhibitor Activity and the Proximate Composition of the Zimbabwean *Jatropha curcas* Provenance: A Potential Livestock Feed

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Abstract: In a study to detoxify *Jatropha curcas* seed four potential detoxification methods were tested for their effectiveness. The first method entailed oil expulsion from unshelled *Jatropha curcas* seed using a Sunhdra industrial oil expeller. The second method involved laboratory-based petroleum-ether solvent extraction of shelled and minced kernels. Thirdly, shelled kernels were subjected to industrial detoxification that involved double solvent extraction with the hexane-ethanol system accompanied by moist-heat treatment at 90°C for 30 min. The fourth method was an extension of the third whereby the meal generated was wet extruded (126°C, 2 atmospheres, 10 min contact time) followed by re-extraction with hexane and moist heat treatment (121°C for 30 min). The detoxification methods had significant ($P<0.0001$) effect on both the anti-nutritional factors (ANFs) and the proximate composition of the meals. Oil expulsion left the phorbol esters (PEs) of the unshelled seed at 0.70 mg g^{-1}, laboratory petroleum-ether extraction reduced the PEs content by 67.69% from 6.5 mg g^{-1} in the raw shelled kernels to 2.10 mg g^{-1}, double solvent extraction followed by moist-heat treatment reduced PEs by 70.77% to 1.90 mg g^{-1}. Double solvent extraction accompanied with wet extrusion, re-extraction with hexane and moist-heat treatment reduced PEs content to 0.80 mg g^{-1}, an 87.69% decrease. All the methods except laboratory petroleum-ether solvent extraction managed to completely inactivate trypsin inhibitors in the meals. Total phenolics (TPs) content was lowest ($P<0.0001$) in the oil-expulsion produced meal (8.50 g Kg^{-1}) while the laboratory petroleum-ether produced meal contained 64.57 g Kg^{-1} TPs. Oil-expulsion and double solvent extraction accompanied with wet extrusion, re-extraction and moist-heat treatment completely inactivated lectins in the meals. The high chemical nutrient potential of *Jatropha curcas* seed (crude protein, calcium and phosphorus) is only realised with shelling and extraction of most of the oil. None of the tried methods managed to completely detoxify the *Jatropha curcas* seed. The two most 'effective methods' reduced the PEs to 0.70 and 0.80 mg g^{-1}, respectively compared to the non-toxic variety with 0.11 mg g^{-1} PEs.

Key words: Physic nut, anti-nutritional factors, detoxification

INTRODUCTION

The Physic nut (*Jatropha curcas* Linn) a multipurpose tree (*Euphorbicaea* family) was introduced in Zimbabwe from Mozambique during the pre-colonial era[1]. Live fencing of vegetable gardens and homesteads have been the traditional uses of the plant in Zimbabwe. Recently both smallholder and commercial farmers have developed interest in the plant due to the high oil and crude protein (CP) content of its kernels. Mutoko rural communities are exploiting *Jatropha* oil in soap production. The Plant Oil Producers Association (POPA) is investigating ways of making *Jatropha curcas* residue from oil extraction suitable for livestock feeding.

The plant is well adapted to grow in marginal areas with low (480 mm) rainfall and poor soils. In such areas it grows without competing for space with food crops [2,3]. *Jatropha curcas* seed meal (10–20 g Kg^{-1} residual oil)

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has a CP content ranging from 580 – 640 g Kg^{-1} of which 90% is true protein[4,5]. The plant's ability to thrive in marginal areas and its high CP makes it an attractive complement and or substitute to soyabean meal (SBM) as a protein source in livestock feeds in Zimbabwe. A combination of unreliable rainfall and a plethora of pests and diseases limit soyabean production particularly in the smallholder-farming sector of Zimbabwe where resources are limiting. The use of *Jatropha curcas* will reduce the competition between Man and livestock for soyabean that is currently prevailing since soyabean is used in both livestock and human feeds. Despite its intrinsic advantages, *Jatropha curcas* seed like soyabean seed has the problem of anti-nutritional (ANFs). In addition to thermo-labile lectins and trypsin inhibitors, *Jatropha curcas* contains toxic lipo-soluble but thermo-stable phorbol esters (PEs)[3,5]. The PEs have to be removed or lowered to levels that do not elicit a toxic response from animals for the *Jatropha curcas* seed meal to be used as an ingredient in livestock feeds. Makkar and Becker[5] report PEs to be highly soluble in ethanol giving some possibility of detoxification of the meal.

The purpose of the study was to investigate the effectiveness of oil-expulsion, solvent extraction, double solvent extraction combined with moist-heat treatment and double solvent extraction combined with wet extrusion and moist-heat treatment as methods in detoxifying *Jatropha curcas*. The effect of the detoxification method on chemical nutrient composition was also studied.

MATERIALS AND METHODS

Processing of materials: *Jatropha curcas* seed was procured from Agri-Seed Services Zimbabwe. Part of the unshelled *Jatropha curcas* seed was passed through a Sunhdra industrial oil expeller to generate *Jatropha curcas* cake (JC). The JC was milled through a 1 mm screen. The milled JC coded Unshelled *Jatropha Curcas* Meal (UJM) had samples kept for laboratory analyses. The bulk of the *Jatropha* seed was shelled with a motorised groundnut sheller. Samples of the shelled kernels were minced in the laboratory and subjected to soxhlet petroleum ether extraction. The resultant dried residue coded Laboratory Processed *Jatropha* Meal (LPJM) was milled through a 1 mm screen and had samples kept for laboratory analyses.

Industrial detoxification procedure: The bulk of the shelled kernels were industrially processed at Pymarc Private Limited and Speciality Animals Feeds Company (SAFCO) all of Zimbabwe. The kernels were passed through an industrial mincer to break them up in preparation for the solvent extraction. The minced kernels were soaked in hexane for 8 h followed by 3 cycles of solvent extraction at 30°C, each of 45 min duration. The dried hexane-extracted kernels were soaked in 95% ethanol for 6 h after which each batch was subjected to 3 cycles of ethanol extraction at 35°C each of 45 min duration. After ethanol extraction each batch (still in the extraction pot) was heated at 90°C for 30 min to distil off and recover the ethanol. The resultant product was dried in the shade and coded Stage 1 Industrially Processed *Jatropha* Meal (IPJM1). Samples of IPJM1 milled through a 1 mm screen were kept for laboratory analyses.

The bulk of the dried IPJM1 was wet extruded (20% moisture) at 126°C under 2 atmospheres with a 10 min contact time at SAFCO. The extruded meal was re-extracted with hexane as before. The re-extracted meal while still in the extraction pots was heated with pressurised steam at 121°C for 30 min for each batch that was re-extracted. The heating served two purposes: primarily to inactivate lectins and trypsin inhibitors in the meal as suggested by Makkar and Becker[5] and secondly to distil off and recover all the hexane in the meal. The resultant meal was dried and its samples coded as Stage 2 Industrially Processed *Jatropha* Meal (IPJM2) were milled through a 1 mm screen and kept for analyses.

Determination of anti-nutritional factors

Phorbol esters estimation: Samples of the *Jatropha curcas* meals (UJM, LPJM, IPJM1 and IPJM2) had their PEs concentration estimation done at the Proyecto Biomasa Laboratory in Austria. The estimation was done as described by Aderibigbe *et al.*[6]. Phorbol-12-myristate-13-acetate was used as the standard during the determination of PEs concentration.

Trypsin inhibitor activity assay: Trypsin inhibitor activity (TIA) was determined as outlined by Gaborit *et al.*[7]

Lectin assay: Lectin activity (LA) was determined using the haemagglutination test as described by Gordon and Marquardt[8]. Modifications to the procedure included using rabbit, sheep and pig erythrocytes in place bovine (cattle) erythrocytes and the use of both bromelain (protease) treated and untreated erythrocytes. The end point of haemagglutination was estimated visually as the dilution still showing agglutination. The titre value was expressed as the reciprocal of the lowest dilution still showing visible agglutination of erythrocytes.

Total phenolics determination: Precipitation with trivalent ytterbium as described by Reed *et al.*[9] was used to determine total phenolics (TPs).

Chemical nutrient analysis

Proximate analyses: All samples were analysed for dry matter (DM), CP, ether extract (EE), ash, calcium (Ca^{2+}) and phosphorus (P) content using the Official Methods of Analysis of the Analytical Chemists[10]. Determination of neutral and acid detergent fibres (NDF and ADF, respectively) was done as described by Van Soest *et al.*[11]. Gross energy value of the meals was done on a CP400 adiabatic bomb calorimeter. Each of the analysis was done in triplicate.

Statistical analysis: The effect of different processing methods on PEs concentration, TIA, TPs and proximate composition of the differently processed *Jatropha curcas* meals was tested using the MSTAT-C statistical package. Means were separated using the Least Significant Difference procedure.

RESULTS AND DISCUSSION

The PEs concentration, TPs and TIA (ANFs) and the proximate composition of the *Jatropha* meals are presented in Table 1 and 2, respectively. Phyto-haemagglutinating activity of the samples is presented in Table 3. Detoxification method had significant effect (P<0.0001) on both the ANFs and proximate composition of the differently processed *Jatropha* meals.

The PEs concentration was highest (P<0.0001) in LPJM and lowest in UJM followed by IPJM2 (Table 1). Results indicated the proportionality between PEs concentration and EE particularly Table 1 and 2) when considering meals IPJM1 and IPJM2: as the EE fraction decreased so did the PEs concentration. This is in conformity with the findings of Heller[3] and Makkar and Becker[4] that stated the lipo-soluble nature of PEs. The seemingly mismatch between the Pes concentration of LPJM and IPJM1 relative to their EE fractions (Table 1 and 2) can be ascribed to the solubility of PEs in different organic solvents. Results seem to point that petroleum ether is a poor solvent of the PEs compared to the hexane/ethanol solvent system used in the generation of IPJM1 (Table 1). The lipo-soluble nature of PEs dictates that UJM (176.53 g Kg^{-1} EE and 0.70 mg g^{-1} PEs) would have a higher PEs concentration compared to IPJM2 (30.20 g Kg^{-1} EE and 0.80 mg g^{-1} PEs). This 'anomaly' seems to point out that either most of the PEs are in the kernel and not in the shell (UJM had all the shell material and the shell constitutes half the mass of the seed, then the low PEs in UJM is probably due to the reduced mass of the material with the PEs fraction in UJM) or that there might be a substance, probably, some polyphenol in the shell fraction that complexes with PEs reducing their extractability. Chivandi[12] reported a PEs concentration of 6.50 mg g^{-1} in raw *Jatropha curcas* kernels indicating that each of the processing methods managed to remove varying concentrations of the toxic PEs.

Heat generated during the oil expulsion process (UJM) and the moist heating of IPJM1 and IPJM2 during the detoxification procedure was enough to

Table 1: Anti-nutritional factors composition (phorbol esters concentration, trypsin inhibitor activity, and total phenolics content) on dry matter basis of the differently processed *Jatropha* meals

	Parameter		
	PEs (mg g^{-1})	TIA (TUI mg^{-1})	TPs (g Kg^{-1})
Meal			
UJM	0.70^{b}	0.00^{b}	8.50^{b}
LPJM	2.10^{a}	22.06^{a}	64.57^{a}
IPJM1	1.90^{a}	0.00^{b}	34.67^{b}
IPJM2	0.80^{b}	0.00^{b}	36.67^{b}
Grand mean	1.38	5.51	36.10
Sig. level	***	***	***
SE	0.11	0.29	3.06
CV(%)	13.75	9.12	14.68
LSD	0.47	1.31	13.77

^{ab}Within column means with different superscripts are significantly different at P<0.0001; ***Significant at P<0.0001.

Table 2: Proximate composition–crude protein (CP g Kg^{-1}), ether extract (EE g Kg^{-1}), ash (g Kg^{-1}), calcium (Ca^{2+}g Kg^{-1}), phosphorus (P g Kg^{-1}), neutral detergent fibre (NDF g Kg^{-1}), acid detergent fibre (ADF g Kg^{-1}) and gross energy (GE MJ Kg^{-1}) of the differently processed *Jatropha curcas* meals

	Parameter								
	DM	CP	EE	Ash	Ca^{2+}	P	NDF	ADF	GE
Meal									
UJM	917.12^{a}	284.67^{c}	176.53^{b}	81.47^{d}	8.11^{c}	9.27^{c}	405.26^{a}	344.09^{a}	20.50^{b}
LPJM	910.29^{b}	588.09^{a}	102.53^{c}	104.33^{b}	11.14^{ab}	21.33^{b}	168.44^{b}	84.22^{b}	18.31^{c}
IPJM1	919.90^{a}	442.35^{b}	221.57^{a}	92.77^{c}	10.18^{b}	20.58^{b}	195.67^{b}	101.46^{b}	21.21^{a}
IPJM2	883.39^{c}	576.99^{a}	30.20^{d}	119.70^{a}	12.38^{a}	22.26^{a}	177.35^{b}	101.88^{b}	17.35^{d}
Grand mean	907.68	473.02	132.71	99.57	10.45	18.36	236.68	157.91	19.34
Sig. level	***	***	***	***	***	***	***	***	***
SE	0.99	3.10	7.03	0.86	0.33	0.18	6.67	6.01	0.12
CV(%)	0.19	1.14	9.17	1.45	5.41	1.66	4.88	6.59	1.09
LSD	4.45	13.98	31.62	3.76	1.47	0.79	30.00	27.05	0.55

^{abcd}Within column means with different superscripts are significantly different at P<0.0001; ***Significant at P<0.0001

Table 3: Haemagglutination titre values of *Jatropha curcas* meals

	Titre value					
	Rabbit		Sheep		Pig	
	U	T	U	T	U	T
Meal						
UJM	0	0	0	0	0	0
LPJM	32	1024+	0	1024+	0	1024+
IPJM1	32	1024+	0	1024+	0	1024+
IPJM2	0	0	0	0	0	0

U–Untreated (undigested) erythrocytes, T–Treated (Protease digested) erythrocytes

inactivate thermo-labile trypsin inhibitors. The 22.06 TUI mg^{-1} in LPJM (Table 1) indicated that the laboratory petroleum ether based solvent extraction failed to inactivate trypsin inhibitors in the meal. However the 22.06 TUI mg^{-1} in LPJM reported in this study is much lower than the 51.60 TUI mg^{-1} in raw soyabean reported by Gaborit *et al.*[7]. TPs were highest ($P<0.0001$) in LPJM while IPJM1 and IPJM2 had statistically similar ($P>0.05$) TPs levels (Table 1). The seemingly low TPs in UJM could be an indicator that most of the TPs are in the kernels and not in shells and or that there is a substance in the shell fraction that interferes with complete extraction of TPs from the meal. Extraction solvents seem to have had an effect on the TPs levels of the meals. Meals IPJM1 and IPJM2 had about half the amount of TPs in LPJM (Table 1) pointing to a possibility of higher solubility of TPs in the hexane/ethanol solvent system (lower TPs levels) compared to the petroleum ether system (higher TPs in the meal).

Sheep and pig erythrocytes caused agglutination of meals LPJM and IPJM1 (Table 3) after digestion with bromelain, a protease. Only rabbit erythrocytes caused agglutination without protease treatment. The high titre values with meals LPJM and IPJM1 indicate presence of active phytohaemagglutinins in them while the zero titre values in the meals (UJM and IPJM2) point to complete inactivation of the haemagglutinins (Table 3). Agglutination is a reversible binding reaction between multivalent haemagglutinins and carbohydrates on the erythrocyte cell membranes[13]. Results of Table 3 indicated that rabbit erythrocytes present more binding sites for agglutination to occur. This means that rabbit erythrocytes are more sensitive to haemagglutinins compared to sheep and pig erythrocytes by possibly having more receptor sites for the haemagglutinins on their cell membranes. Protease treatment of the erythrocytes from sheep and pig blood seem to 'unmask' their haemagglutinin receptor sites as witnessed by the high titre values both sheep and pig erythrocytes exhibit after the treatment. Increment of the titre value from 32 to 1024+ with rabbit erythrocytes upon protease treatment indicated that protease treatment increases sensitivity of erythrocytes to haemagglutinins. Furthermore it clear from results that reliance on untreated erythrocytes can lead to erroneous decisions as they may indicate absence of haemagglitinins when in fact they are present in the potential animal feed ingredient (Table 3). Haemolysis was not observed in all the differently processed meals suggesting that saponins, that are known to cause haemolysis of erythrocytes[14], were absent from the meals.

The chemical nutrient potential of *Jatropha curcas* meal, with particular reference to its high protein potential, only surpasses that of solvent extraction (hexane) produced soyabean meal on two conditions. The *Jatropha curcas* seed has to be shelled and then solvent extracted to remove the shell (fibres) and ether extract fractions in the seed, respectively (Table 2). Besides masking of the high protein potential of the *Jatropha* meal (UJM with 284.67 g Kg^{-1} CP, 405.26 g Kg^{-1} NDF and 344.09 g Kg^{-1} ADF versus IPJM2 with 576.99 g Kg^{-1} CP, 177.35 g Kg^{-1} NDF and 101.88 g Kg^{-1} ADF) production of *Jatropha* meals without first removing the shells results in very high NDF and ADF levels (Table 2) that cannot be tolerated by monogastrics such pigs and poultry.

Double solvent extraction (hexane/ethanol system) coupled with moist-heat treatment managed to completely inactivate lectins and trypsin inhibitors in the resultant meal (IPJM1) but still left a high concentration (1.90 mg g^{-1}) of residual PEs that is much higher than the 0.11 mg g^{-1} PEs reported by Makkar and Becker[4] in the non-toxic Mexican variety. Double solvent extraction accompanied with wet extrusion and re-extraction with hexane and moist-heat treatment, in addition to completely inactivating lectins and trypsin inhibitors in the meal (IPJM2), reduced the PEs concentration to 0.80 mg g^{-1}, an 87.69% reduction and produced a meal with higher CP, Ca^{2+} and P compared to hexane-extracted soyabean meal. The 0.80 mg g^{-1} PEs left when a combination of double solvent extraction (hexane/ethanol system), wet extrusion, re-extraction and moist-heat treatment used in the detoxification of shelled *Jatropha* kernels means that there is scope to fine tune and or research further on the methodology for it to be able to lower the PEs concentration to levels comparable to those in non-toxic varieties before conducting feeding trials.

ACKNOWLEDGMENTS

The authors wish to express their sincere gratitude to the University of Zimbabwe Biochemistry Department that availed its laboratory facilities for most of the laboratory analyses. The GTZ/SACCAR is thanked for funding the study.

REFERENCES

1. Mapako, M., 1998. Background information on Makosa Ward, Mutoko District. In: Foidl, N and Kashyap, A. Edn., pp: 52-53, Exploring the potential of *Jatropha curcas* in Rural Development and Environmental Protection. Scientific and Industrial Research Development Centre, Harare, Zimbabwe.
2. Gaydou, A.M., L. Menet, G. Ravelojaona and P. Geneste, 1982. Vegetable energy sources in Madagascar: ethyl alcohol and oil seeds. Oleagineux, 37: 135-141.
3. Heller, J., 1996. Physic nut, *Jatropha curcas* L., IPGRI, Rome, Italy.
4. Makkar, H.P.S., K. Becker, F. Sporer and M. Wink, 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. J. Agri. Food Chem., 45: 3152-3157.
5. Makkar, H.P.S. and K. Becker, 1997. Potential of *Jatropha curcas* seed meal as a protein supplement to livestock feed, constraints to its utilization and possible strategies to overcome constraints. In: Biofuels and industrial products from *Jatropha curcas*, '*Jatropha* 97', Managua, Nicaragua.
6. Aderibigbe, O.A., C.O.L.E. Johnson, H.P.S. Makkar, K. Becker and N. Foidl, 1997. Chemical composition and effect of heat on organic matter and nitrogen-degradability and some anti-nutritional components of *Jatropha* meal. J. Ani. Feed Sci. Technol., 67: 223-243.
7. Gaborit, T., L. Quillien and J. Gueguen, 1993. Determination of trypsin inhibitor activity in seeds by microtitre plate method. In: Recent advances of research of anti-nutritional factors in legume seeds, 1-3 December, Wageningen, The Netherlands.
8. Gordon, J.A. and M.D. Marquardt, 1974. Factors affecting haemagglutination by concanavalin A and soyabean agglutinin. Acta Biochimica et Biophysica, 332: 136-144.
9. Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Animal Sci., 73: 1516-1528.
10. Association of Official Analytical Chemists (AOAC), 1990. Official Methods of Analysis. (15th Edn.) Washington DC, USA.
11. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
12. Chivandi, E., 2000. Effect of feeding *Jatropha curcas* on the performance of weaned fattening pigs. MSc Thesis, Bunda College of Agriculture, University of Malawi, Lilongwe, Malawi.
13. Gabius, H.J. and S. Gabius, 1993. Lectins and glycobiology. Springer-Verlag, Heildelberg, Germany.
14. Price, K.R., I.T. Johnson and G.R. Fenwick, 1987.The chemistry and biological significance of saponins in food and feedstuffs. CRC Critical Review. Food Sci. Nutr., 26: 27-35.