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Reduction in Trypsin Inhibitor Activity in Jatropha cake by Chemical, Thermal and Radiation Treatment

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Abstract

The trypsin inhibitor present in jatropha cake is one of the major antinutrients, which prohibits its use as animal feed. In this study, attempts were made to remove trypsin inhibitor using chemical, thermal and radiation treatment methods. The observations revealed that thermal treatment is the most effective technique for complete removal of trypsin inhibitor, whereas chemical treatment is able to reduce it to 26%. The radiation treatment shows no effect on reducing trypsin inhibitors in jatropha cake.

Keywords: Jatropha cake, trypsin inhibitor, chemical, thermal and radiation.

Introduction

Jatropha cake, a by-product of oil extraction process from jatropha seeds, is rich in crude proteins (50-58% depending upon the oil extraction efficiency of the process) and thus may serve as highly nutritious protein supplement in animal feed[1]. However, the presence of high level of antinutrients, trypsin inhibitor, phytates, phorbol esters, curcin etc. in jatropha cake prevents to do so[2,3]. Amongst these, the trypsin inhibitor is the major antinutrients found in the jatropha cake which prevents its use as protein supplement in animal feed.

Trypsin inhibitors are chemicals that reduce the availability of biologically active trypsin, an enzyme essential for nutrition in many animals, including humans[4]. The inhibitor interferes the physiological process of digestion where the normal functioning of pancreatic proteolytic enzymes is prohibited in non-ruminants, leading to severe growth depression. It is referred that the anti nutrient effect of trypsin inhibitors is mainly due to their direct interaction with pancreatic proteolytic enzymes and a corresponding reduction in the digestibility of the proteins of the diet[5].

Trypsin inhibitors are known to be heat labile and can be inactivated by exposing it to high temperature[6,7]. Many methods have been mentioned in the literature for detoxifying jatropha cake. Based on their nature, the detoxification process can be categorized into three groups i.e. thermal, chemical, and radiation treatment methods[8].

In the present study, an effort was made to subject jatropha cake towards chemical, thermal and radiation treatment technique and the reduction in trypsin inhibitor activity in treated jatropha cake was estimated to ascertain the effectiveness of the treatment processes.

Material and Methods

Materials: Jatropha seed, (Jatropha curcas species) were procured from M/s SVM Exports, Thani, Tamil Nadu. Trypsin, Tris (hydroxymethyl) methyl amine and N- Benzoyl - L-Arginine 4- Nitroanilide hydrochloride (BAPNA) were procured from Sigma Aldrich. Sodium hydroxide (NaOH), hydrochloric acid (HCl), Dimethyl sulphoxide (DMSO) and acetic acid (AcOH) were procured from sdFine Chem Ltd and used as received.

Preparation of jatropha cake: The jatropha cake was obtained after oil extraction from jatropha seed kernel by solvent extraction method using n-hexane. Approx 100gm of ground seed kernels were taken and were placed in a soxhlet apparatus. The extraction cycles were then run for 6 hours for extracting oil from jatropha seed kernel. The n-hexane was then distilled out from the extract in a rotary evaporator to recover jatropha oil. The residual wet cake was then dried in oven while maintaining it at 70°C for 2hrs to obtain the dried jatropha cake.The percentage of cake left behind was calculated as below:

$$\% \text{ of cake} = \frac{\text{Wt of cake obtained in gm} \times 100}{\text{Wt of ground seed kernel taken in gm}}$$

Chemical treatment of jatropha cake: The dried jatropha cake was soaked in 95% ethanol for 6 hrs after which it was subjected to three cycles of ethanol extraction at 35°C each of 45 min. duration. After ethanol extraction, it was kept in extraction pot and heated at 90°C for 30 min to distil off and recover ethanol. The resultant cake was dried in the shade and designated as industrially treated Jatropha cake, which was used for estimating trypsin inhibitor.

Thermal treatment of jatropha cake: The dried jatropha cake was autoclaved at 121°C for 30 mins, 60 mins and 90 mins duration. After autoclaving, the cake samples were dried in an

air circulatory oven at 25°C for 4 -5 hours. The dried cake was then used for estimating trypsin inhibitor.

Radiation treatment of jatropha cake: The gamma irradiation of the dried jatropha cake was performed at Shriram Applied Radiation Centre, Delhi using cobalt source having 470 kilocurie strength. The jatropha cake was exposed to various doses of radiation i.e. 50 kGy, 70 kGy, 100 kGy and 125 kGy. The exposed cake was then used for estimating trypsin inhibitor.

Method to estimate trypsin inhibitor: Trypsin inhibitor in jatropha cake was estimated indirectly by inhibiting the activity of trypsin as per the method described by Makkar and Becker et al[9]. The method involves extraction of the inhibitors in 0.01 M NaOH solution from the cake sample while keeping the pH of the suspension between 9.5 to 9.8. The unfiltered suspension was then mixed with known volume of bovine trypsin solution and BAPNA solution. The BAPNA was subjected to hydrolysis by trypsin to produce yellow coloured p-nitroanilide. The degree of inhibition of yellow colour product by the cake extract was recorded at 410 nm using UV-spectrophotometer (Agilent Technologies). The trypsin inhibitor activity was then expressed as mg trypsin per g sample weighed. The reagents prepared and the procedure followed was described below:

Reagents: i. Tris buffer: Tris (hydroxymethyl) methyl amine (1.512g) and $CaCl_2.2H_2O$ (0.735g) were dissolved in 225 ml distilled water. The pH was adjusted to 8.2 with HCl and the volume was made up to 250 ml with distilled water. ii. BAPNA solution: BAPNA (0.04g) was completely dissolved in 1ml DMSO and diluted to 100 ml with tris buffer, pre-warmed to 37°C. This reagent was kept at 37°C while in use and freshly prepared each day. iii. Standard trypsin solution: Crystalline bovine trypsin (0.005 g) was dissolved in 250 ml of 0.001M HCl solution. The solution was stored in refrigerator, which is stable for 2-3 weeks without appreciable loss in activity. 2 ml of this solution (40µg trypsin) was used with each aliquots and the absorbance of the supernatant at 410 nm was measured against a reagent blank. iv. 0.01 M NaOH solution: 400 mg of NaOH was dissolved in 900 ml of distilled water and diluted to 1 lit. with distilled water, v. 0.001 M HCl: .09 ml of hydrochloric acid (37% weight per volume, w/v) was diluted to 1 lit. with distilled water.

Procedure: Preparation of sample extract: Dried jatropha cake samples, treated and untreated were finely powdered in a ball mill so as to pass through a 100 mesh sieve. A sample equivalent to 1 g dried materials was weighed out. The sample was agitated with 50 ml of 0.01M NaOH solution on a magnetic stirrer for 3 hours. The pH of the resulting slurry was maintained between 9.5-9.8 with 1M NaOH or 1M HCl.

Determination of Trypsin Inhibitor: The following solutions were pipette into a series of 10ml tubes: i. Reagent blank: 2 ml deionized or distilled water. ii. Standard (40 µg trypsin): 2 ml trypsin solution and 2 ml distilled water. iii. Sample blank (s): 1 ml diluted sample extract and 1 ml distilled water. iv. Sample (s): 1 ml diluted sample extract, 1 ml distilled water and 2 ml trypsin solution.

After mixing and preheating to 37°C, for 10 minutes, pipetted 5 ml of BAPNA solution (pre-warmed to 37°C) into each tube and mixed. After exactly 10 min incubation at 37°C, added 1 ml of acetic acid (30% v/v) to each tube to terminate the reaction. Then added the trypsin solution (2 ml) to the reagent blank (a) and sample blank (c) tubes. After centrifugation (at 3000g for 10 min at room temperature), the absorbance of the clear solution was measured at 410 nm. The color should be stable for several hours.

Calculation: Change in absorbance $(A_I) = (A_b - A_a) - (A_d - A_c)$, (due to trypsin inhibitor / ml diluted sample extract), Percentage inhibition in each sample tube = $A_I / (A_b - A_a)$.

If this percent inhibition value is less than 40% or greater than 60%, the assay must be repeated making a more suitable dilution of the sample suspension. Since 1µg pure trypsin would give an absorbance of 0.019, the weight of pure trypsin inhibited / ml diluted sample extract is $A_I / 0.019$ µg (i.e. 50 A_I/19 mg per 50 ml). From this value, the trypsin inhibitor activity (TIA) is calculated in terms of milligrams of pure trypsin / g sample as weighed (mg / g).

TIA = (22.632 DAI)/ S

Where D is the dilution factor (the factor by which original plant extract (1g in 50ml) was diluted so as to obtain an inhibition between 40% and 60% by 1ml of the diluted extract) and S is the sample weight (g).

Results and Discussion

The trypsin inhibitor activity in chemically treated, thermally treated and radiation treated jatropha cake was determined by the above mentioned method.

Effect of chemical treatment on trypsin inhibitor: The effect of chemical treatment on trypsin inhibitor of jatropha cake was tabulated in table 1. It was observed that chemical treatment reduce the trypsin inhibitor activity by 26%. The untreated cake has trypsin inhibitor activity of 7.84 mg trypsin / per g sample which goes down to 5.8 mg trypsin / per g sample by chemical treatment.

Table - 1
Trypsin inhibitor activity (TIA) in chemically treated jatropha cake

SN	Methods	TIA (mg of trypsin per g sample	% Reduction
1	As such	7.84	-
2	Chemically treated cake	5.8	26%

Effect of thermal treatment on trypsin inhibitor: The jatropha cake was autoclaved at 121°C for 30 mins, 60 mins and 90 mins. The trypsin inhibitor activity after autoclaving was tabulated in table 2. It can be seed that after autoclaving, the jatropha cake shows no trypsin inhibitor activity. This was in accordance with the literature available which shows that trypsin inhibitor was heat sensitive.

Table-2
Trypsin inhibitor activity (TIA) in thermally treated jatropha cake

SN	Sample details	TIA (mg of trypsin per g sample	% Reduction
1	As such	7.84	Nil
2	30mins	Nil	100
3	60mins	Nil	100
4	90mins	Nil	100

Effect of radiation treatment on trypsin inhibitor: The trypsin inhibitor activity in jatropha cake after gamma irradiation was given in table 3. It can be seen from the table that irradiation of the cake even upto 125 kGy does not reduce the trypsin inhibitor activity.

Table - 3
Trypsin inhibitor activity (TIA) in irradiated jatropha cake

SN	Radiation dose	TIA (mg of trypsin per g sample)	% Reduction
1	As such	7.84	Nil
2	50 kGy	7.84	0
2	70 kGy	7.84	0
4	100 kGy	7.84	0
3	125 kGy	7.84	0

Conclusion

From the above study, it comes out that trypsin inhibitors present in jatropha cake can be removed by thermal treatment i.e. autoclaving the cake at 121°C for 30mins. Chemical treatment of jatropha cake proves effective in reducing trypsin inhibitor activity by 26% whereas no effect of radiation was seen on trypsin inhibitors.

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Reference

1. Pradhan S., Sahoo N.K., Naik S.N. and Sahoo P.K., Estimation of toxic phorbol ester in jatropha curcas oil, cake and biodiesel, *Asian Journal of Chemistry***, 22(5)**, 4069-4074 **(2010)**

2. Donlaporn S. and Worapot S., Toxic compound, anti nutritional factors and functional properties of protein isolated from detoxified jatropha curcas seed cake, *Int. J. Mol. Sci*, **12**, 66-77(**2011)**

3. Martizez- Herrera J., Siddhuraju P., Francis G., Davila-Ortiz G. and Becker K., Chemical composition, toxic antimetabolite constituents and effects of different treatmemts on their levels, in four / provenances of jatropha curcas L. from Mexico, *Food Chemistry*, **96** , 80-89 **(2006)**

4. Igwenyi I.O., Ezeifedika O.M., Agbafor K.N., Offor C.E., Ibiam U.A. and Aja P.M., Anti-nutritional factors in Afzelia afrcana and Citrullus colocynthis and Effects of the Seeds on Liver Enzyme Activities of Albino Rats, *Indian Journal of Science and Technology,* **6(7)**, 5008-5012 July **(2013)**

5. Azzaz Nabil A.E., El-Nisr Neveen A., Elsharkawy Eman E. and Elmotleb Eman A., Chemical and pathological evaluation of jatropha curcas seed meal toxicity with or without heat and chemical treatment, *Australian Journal of Basic and Applied Sciences*, **5(12),** 49-59 (**2011)**

6. Azza A. Abou –Arab. and Ferial M. Abu-Salem., Nutritional quality of jatropha curcas seeds and effect of some physical and chemical treatments on their anti-nutritional factor, *African Journal of Food Science,* **4(3)**, 93-103, March (**2010)**

7. Makkar Harinder P.S. and Becker K., Jatropha curcas, a promising crop for the generation of biodiesel and value added coproducts*, Eur. J. Lipid Sci. Technol*, **111**, 773-787 **(2009)**

8. Udomsap P., Duangmance T. and Chollacoop N., Investigation of phorbol ester content in high quality biodiesel production process, *Utility Exhibition on Power and Energy Systems: Issues & Prospects for Asia (ICUE), 2011 International Conference and*, **28-30 Sept**, 1-4 **(2011)**

9. Makkar Harinder P.S., Siddhuraju P. and Becker K., *Methods in Molecular Biology TM 393. Plant Secondary Metabolites,* Editor. John M. Walker, Human Press, 1-6 **(2007)**