Original Paper

Ricin Extraction and Detoxification of Castor Cake

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ABSTRACT

Castor is cultivated for its unique oil, which has widespread industrial applications. But the utilization of the press-cake is limited due to the presence of ricin, the most toxic constituent in castor. Its presence hampers the use of the cake as animal feed despite its high nutritive value.

In this study, ricin was extracted by precipitation, ultrafiltration and then analysed after purification. The ricin content that was estimated varied from 1.04 to 1.65%. For detoxification, a combination of physical and chemical treatments was used namely: alkalization, soaking, heating and autoclaving.

Ricin content in the detoxified castor cake was subsequently analysed as per ricin estimation procedure, and it was found that the ricin content was only in trace quantity.

Key Words: Animal feed; Castor cake; Ricin

INTRODUCTION

Castor (*Ricinus communis* L.) is an important non-edible, industrial oilseed crop being cultivated since long in the arid and semi-arid regions of the world. Castor seeds contain about 42.5–55.0% of castor oil, which is unique in nature, in that ricinoleic acid comprises roughly 90% of its total fatty acids. Castor oil is considered as a technical grade of triricinolein, in which the hydroxyl groups of ricinoleic acid give the castor oil properties of high viscosity and unusual chemical reactivity of high industrial applications. Therefore, demand of castor oil *vis-a-vis* castor cultivation is directly related to industrial growth. India is the leading country in the world in castor production, accounting for about 80% of total production.

Castor cake and residue are left after extraction of castor oil, representing about one-half of the weight of castor bean, with high protein content (32–36%). After decortication, the protein content of castor cake can be further increased up to 60%.[1] In adition, the cake also contains a high concentration of starch, which could be used as a good source of animal feed.

Unfortunately, despite its easy availability and high protein content, castor cake has not found a place as protein supplement in animal feed due to toxicity, and is mostly used as manure. Of the toxins present in the castor cake, ricin is the most lethal. The ricin content in castor cake is reported to be up to the extent of 1.5%.[2,3] Ricin, the naturally occurring cytotoxic protein found in castor plants is one of the most potent and deadly plant toxins known to man.[4] Ricin, if inhaled, injected or ingested, is almost equally toxic and has an LD_{50} of approximately 1 mcg/kg body weight for mice, rats and dogs, and is ten times more potent against rabbits.[5] The toxic dose for humans is also likely to be in the mcg/kg range, and ranks among the most toxic substances known.

Ricin, a 66 kDa protein, consists of an A and B chain. The B chain binds to cell surfaces allowing intracellular incorporation of the A chain, which enzymatically inactivates ribosomes, therefore shutting down protein synthesis, leading first to death at the cellular level, then to tissue damage and finally to multiorgan dysfunction and organism death. The three other toxins present (ricinine,

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ricinus communis agglutinin and allergen CB-1A) are of little consequence with regard to the feeding value in animals, either due to presence in lower concentrations, or insignificant toxic effects. Allergen is a matter of concern for people handling the cake, while animals are not affected by it.[6]

In order to use castor cake as animal feed suitably, it is imperative to eliminate the toxic principles, either by removing them as such, or by converting them by decomposition to non-toxic and harmless substances. In this study, an attempt has been made to detoxify the castor cake collected from various industries from North Gujarat through a series of physical and chemical treatment methods.

MATERIALS AND METHODS

Collection of Castor Cake: Eight samples of solvent-extracted and toasted castor cake obtained from different oil mills of North Gujarat, India, were used for the study, and the same cakes were also analysed for some basic properties by standard methods.[7]

Estimation of Ricin

1. Extraction of Ricin: Ricin from castor cake was isolated as per procedure suggested by Funatsu et al (1971) with slight modifications.[8] The de-fatted, powdered castor cake was suspended in double distilled water (1:5) and adjusted to pH 4.0 using HCl. The suspension was agitated for 8 hrs. The solution was then filtered under vacuum. The yellow coloured supernatant was collected and the residue was decanted. The residues were treated with distilled water and again filtered under vacuum. The supernatant was precipitated by adding solid NaCl till its saturation.
2. Purification of Ricin: The precipitate was dissolved in deionized distilled water. It was re-precipitated at pH 8.0 by saturation with $(NH_4)_2SO_4$. After repeating re-precipitation with the same salt, it was dissolved in deionized water and dialyzed at 4ºC for a period of 72 hrs against tris-buffer adjusted to pH 6.8. The buffer was changed once in 2 hrs for the first 12 hrs, and subsequently once in 6 hrs for the remaining period. After 72 hrs, it was centrifuged at 4000 rpm for 10 min to separate the insoluble matter from the clear solution containing ricin. The solution was concentrated under vacuum and the amount of solution was measured.
3. Estimation of Ricin: Ricin was estimated as the quantity of protein present in the dialysate as per the method of Lowry et al (1951).[9] Crude ricin was separated and made into grind cake (to 40 mesh) and a slurry was prepared with CCl_4, which was allowed to settle and the ricin was then skimmed off, dried and weighed.[10]
4. Detoxification of Castor Cake: For detoxification, combinations of physical and chemical treatments were used, namely, alkalization, soaking, heating and autoclaving. First of all, 10 g of various DOC sample was weighed into flasks, and added to 20 mL of 0.5 M NaOH with 0.5 M $Ca(OH)_2$ as equivalent to number of moles of NaOH. This was soaked for 3 hrs, and then placed in a water bath at 120°C for an hour. The resulting sediment was washed several times with water and dried after autoclaving at 15 psi for 60 min. Ricin content was analysed as per the procedure mentioned above.

RESULTS AND DISCUSSION

Basic Properties of Castor Cake: Some basic properties of castor cakes collected were analysed using standard methods (**Table 1**). The percentage acid insoluble cake ranged from 0.48 to 0.56, bulk density (g/cc) from 0.61 to 0.67, moisture percentage from 10.12 to 13.45, percentage organic carbon from 18.23 to 23.04, nitrogen percentage from 4.41 to 5.13, phosphorus percentage from 1.84 to 2.08, potash percentage from 0.93 to 1.40, and C/N ratio from 4.01 to 5.74.

Ricin Content in the Castor Cake Samples: Ricin content in the castor samples varied from 1.04 to 1.65%, with a mean value of 1.4 among the samples (**Table 2**). It was found that DOC-4 sample showed lowest percent ricin content (1.04), while DOC-3 had highest percent ricin content (1.65). These values are in agreement with the findings of other investigators.[11] Ricin is a highly stable protein and can remain for months without deteriorating. Although there were claims that normal extraction and desolventization processes for meals are capable of total destruction of ricin, the presence of ricin in the castor cake indicated that the normal processing methods were not capable of destroying the toxin totally, and there was a need to detoxify ricin for further use as livestock feed.[12]

Detoxification of Castor Cake: This study revealed that after the specified sequence of treatments to detoxify the cakes, only traces of ricin remained. Quantification of ricin has been used to assess the efficacy of ricin (**Table 2**). It was observed that the ricin detoxification

Table 1 Basic Properties of Castor Cakes Samples in the Study

Parameters	Sample name								Mean
	DOC-1	DOC-2	DOC-3	DOC-4	DOC-5	DOC-6	DOC 7	DOC 8	
Acid insoluble (%)	0.55	0.53	0.56	0.54	0.55	0.51	0.52	0.51	0.53
Bulk density (g/cc)	0.64	0.66	0.63	0.67	0.66	0.62	0.61	0.64	0.64
Moisture (%)	11.45	10.12	11.12	10.80	13.05	13.45	11.75	10.83	11.57
O.C. (%)	21.16	22. 24	20.25	19.98	20.86	19.63	23.04	21.56	20.93
N (%)	4.84	4. 41	5.02	4.98	4.58	4.60	5.13	5.04	4.88
P_2O_5 (%)	1.92	1.84	1.88	1.98	1.87	2.00	1.90	1.86	1.91
K_2O (%)	1.40	0.93	1.22	1.30	0.99	1.08	1.11	1.17	1.15
C:N ratio	4.37	5.04	4.03	4.01	4.55	4.26	4.49	4.28	4.38

Table 2 Analysis of Ricin in Castor Cakes and its Detoxification

Sr. no.	Sample name	Ricin content in castor cake (%)		Detoxification efficacy (%)
		Before detoxification	After detoxification	
1	DOC-1	1.09	Trace	100
2	DOC-2	1.44	Trace	100
3	DOC-3	1.65	Trace	100
4	DOC-4	1.04	Trace	100
5	DOC-5	1.14	Trace	100
6	DOC-6	1.58	Trace	100
7	DOC-7	1.29	Trace	100
8	DOC-8	1.30	Trace	100

efficacy achieved by the detoxification process under study was 100%. The toxic protein present in the castor cake is a heterodimer consisting of two chains of 32 and 34 kDa connected with a disulphide bond.[13,14] There are reports that several isoforms of ricin exist,[15] and the molecular weights of two chains are reported to be 30 kDa in each, according to some investigators,[16] while others put it at 32 kDa in each.[17]

Ricin is water soluble and it can be removed from the cake by merely washing with hot water. But this method was found unsatisfactory, since ricin, as with other types of proteins, clings to or is enmeshed or occluded with other proteins so that it cannot be removed solely by washing. This indicates that ricin might be composed of more hydrophilic rather than hydrophobic aminoacids. The first stage in detoxification was to soak the cake in an alkaline solution by adding sufficient alkali to solubilize the water soluble proteins, and a large proportion of alkali soluble proteins. The alkali has a number of other functions. Its presence retards or prevents the tendency of the cake to agglomerate into lumps when mixed with water. During the alkali treatment, it was found that ricin could be dispersed to a high degree in the dispersed phase. Under these conditions, ricin might be converted to harmless substances, merely by heating the dispersion to a relatively low temperature, which would not seriously

affect the properties of the proteins. It aids in the opening up of the cellular structure and freeing the proteins. It also controls and retards the coagulation of proteins under the temperatures involved.

Dispersion of proteins in water tended to coagulate when heated to temperatures above 55°C, and these coagulates were not easily re-despersed in alkaline solutions. As the pH value of the aqueous dispersion of castor protein increased above 7.0, the temperature at which the coagulation takes place rises. Occasional stirring was done to obtain an adequate dispersion of the proteins. Anandan et al (2005) found that lime and sodium hydroxide treatments were more effective among several alkalis tested.[18] Lime being a weaker alkali, at lower levels the efficacy was not optimum and only at higher levels of 4 g/kg optimum efficacy could be achieved. Sodium hydroxide being a strong alkali, was effective even at smaller levels. About 2.5 g/kg NaOH could reduce the toxin by about 82% and when the concentration was increased to 10 g/kg, the reduction was upto 91%. Increasing the concentration of sodium hydroxide could increase the elimination of the toxin showing that the toxin is highly susceptible to strong alkali.

The dispersion was then heated to a temperature of 120°C for 1 hrs to effect the detoxification. This condition would have little or no degradating effect on the proteins. Washing several times would allow the removal of solubilised proteinaceous material. Subsequently, during the process of autoclaving, and heating under pressure, ricin and ricinine were also destroyed. Autoclaving at 15 psi can decrease the ricin content to trace levels.[19,20] Combination of chemical treatments together with heat treatment can detoxify castor meal by inactivating the ricin and allergen.

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