

FAQ's on Ricin presence in Castor oil

1. What is Ricin

Ricin is a toxic glycoprotein (Toxalbumin – a carbohydrate binding protein) found in castor beans. Chemically it is a glycoprotein composed of two polypeptide chains – A and B, linked by a disulfide bond. Ricin functions by disrupting the protein synthesis mechanism of the cell. The A-chain of ricin is the toxic portion of the protein. The A-chain functions by depurinating the 28S subunit of the infected cell's ribosomes. The B-chain of ricin is responsible for getting the A-chain into the target cell. The B-chain consists of a galactose-binding region capable of binding a target cell's membrane and initiating cell-entry. Without the B-chain's functionality, the A-chain cannot enter the cell. However, once it is in the cell, the A-chain can disrupt up to 1500 ribosomes per minute. This unique action makes ricin highly toxic, with an LD50 (lethal dose for 50% of a population) of 3–5µg/kg when inhaled and 1–20 mg/kg when ingested.

In addition to the ricin (RCA₆₀), castor seeds also contain another lectin - Ricinus Communis agglutinin (RCA₁₂₀). Ricin and RCA₁₂₀ are almost identical in their protein sequence. However, ricin is a heterodimer consisting of the A-chain disulphide bound to the B-chain, whereas the agglutinin is a tetramer consisting of two A-chains bound to two B-chains. The differences between the two proteins are evident in their relative toxicities. Ricin is a highly toxic protein whereas RCA₁₂₀ agglutinin is a weak toxin.

2. Is there a risk to get Ricin in castor oil?

According to the International Journal of Toxicology's Final Report on Castor Oil,¹ it is said that, during extraction process, ricin does not partition into the oil¹. In addition, there have also been a few other studies on presence of ricin in castor oil and all these studies have shown the ricin is not detectable in the castor oil⁴⁸. Further, heating during the oil extraction and refining process denatures and inactivates the toxic protein, if any. This guarantees the oil is free from ricin and can be used for medicinal and cosmetic purposes and it assures that the oil doesn't contain any ricin.

3. Is there a risk to get Ricin in a product made out of castor oil?

Please refer the answer to question number 2 above. It is reported in the literature¹ that ricin is insoluble in castor oil and doesn't get partitioned into the oil during extraction process. Moreover, the oil obtained is thermally/ steam treated which assures the deactivation of ricin present, if any.

Ricin is susceptible to high heat (80 °C for 10 minutes or 50°C for 1 hour) and to chlorine (100 mg/L free available chlorine in 20 minutes). Temperature above 80 °C destroys/inactivates it.^{2,4,5} According to the thesis submitted to the University of Minnesota, USA³, heat inactivation of ricin followed first-order kinetics. The half-life ($t_{1/2}$) of ricin at 72, 80, 85 and 90 °C were 72.6, 9.0, 2.0 and 0.5 min, respectively.

Castor oil and products made out of it are safe to use and do not contain ricin. The Cosmetics Ingredient Review expert panel reviewed the safety test data and concluded castor oil and its derivatives as safe ingredients and have no side/ill effects.¹

4. [Did poisoning event happen with Castor Oil \(human being or animals\)? / What is the castor oil toxicity \(for human beings, animals, and environment\)?](#)

After intensive clinical studies, castor oil is classified by US Food and Drug Administration as Generally Recognized as Safe (GRAS) and effective for internal use as a stimulant laxative⁶. This assures the non-toxicity of the castor oil. However, there are indeed regulations on the daily intake of the castor oil. The Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives established an acceptable daily castor oil intake (for man) of up to 0.7 mg/kg body weight.

In another study conducted, in which people ingested castor oil at dietary concentrations as high as 10% for 90 days and were closely monitored. They did not experience any ill side effects.

In animals, the acute toxicity data demonstrated that Castor oil is not harmful by ingestion according to regulation EU 1272/2002 for the classification and labelling of substances.

The LD50 oral (rat) is mentioned in various safety datasheets (MSDS) & in references^{50, 51} as being more than 5000mg/kg. As per these same references, when tested on eye and skin of rabbit, castor oil evoked only mild response. On human skin in the 48hour dermal patch test castor oil was found non-irritating. No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC. (Under REACH castor oil is exempted as per Annex II Article 2(7)(b), S.No.9 of Commission Regulation (EC) 987/2008).

Environment: It is mentioned as being readily biodegradable in various safety data sheets (MSDS), with minimum biodegradability 60% BSB40. The acute fish toxicity is mentioned as LC50>100mg/l and Bacteria toxicity as EC50>50mg/l.

5. Ricin is a part of the castor cake: what is the castor cake composition?

Castor cake is a main by-product of the castor oil production. In general, processing one ton of castor seeds for oil extraction generates around 550 Kg of cake, however, the actual value varies with seed oil content and the process adapted. The castor cake is composed of proteins (28-43%), globulins (60%), albumins (16%), proteoses (4%), glutelins (20%), conjugated proteins and non-protein nitrogen compounds^{7,8}. The ricin content in castor beans varies in the range 1-5%⁹.

Table 17. Proximate composition and mineral content of the deoiled castor cake

Components	Content (%)	Minerals	Content (%) ^a
Moisture	10	Iron	0.1909 ± 0.0004
Crude protein	30	Zinc	0.012 ± 0.0003
Lipids	<1	Manganese	0.0073 ± 0.0006
Ash	5	Copper	0.0037 ± 0.0005
Crude fiber ^a	28.84 ± 0.83	Sodium	0.029 ± 0.004
NDF ^a	49.44 ± 1.09	Magnesium	0.46 ± 0.01
ADF ^a	40.58 ± 0.43	Potassium	0.75 ± 0.04
Lignin (H ₂ SO ₄) ^a	23.54 ± 0.38	Calcium	1.54 ± 0.06
Cellulose ^a	10.45 ± 0.25	Phosphorus	0.55 ± 0.02
Hemicellulose		9.51 ± 1.06	
NDF-N		0.86 ± 0.13	
ADF-N		1.40 ± 0.10	
NPN		1.80 ± 0.19	
pH		5.91 ± 0.00	
Acidity Index ^b		0.95 ± 0.00	

a: Values expressed on dry basis, except moisture. b: Value expressed in mg of NaOH/g of cake. NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, NDF - N = Nitrogen in NDF, ADF - N = Nitrogen in ADF, NPN = Non-Protein Nitrogen.

6. What is the Ricin included in the castor cake becoming in each application?

The ricin present in the castor cake is to a large extent denatured during the process of Solvent Extraction and Desolventizing & Toasting. This is referenced in the ICOA Bulletin⁵⁴.

7. Did poisoning event happen with the ricin contained in the castor cake?

EFSA (European Food Safety Authority) reported Human cases of intoxication with the seeds not with the cake : 700 cases of intoxication from intake of castor beans have been reported, a few fatalities have been described. The fatal oral dose in human has been estimated to range from 1 (about 5 to 10 castor beans) to 20 mg of ricin/kg b.w. ^{52, 53}.

There are some reports on poisoning events with the ricin contained in the castor cake in cattle and dogs^{10,11}. In one study, the fatality rate has been estimated, i.e. from 98 cases of dog poisoning, the deduced fatality rate was about 7%¹².

However, these events have happened very rarely and where it did occur, the castor cake had not been properly treated to denature the ricin^{10,11}. Overall, with modern supportive care, the fatality rate is low, except in suicide cases where a ricin containing extract is injected.

8. What are the castor cake usages?

Castor cake is known as a high source of protein and has numerous usages.

- The castor cake contains nitrogen (up to 5%), phosphorus (2%) and potassium (around 1%), which makes it suitable as a fertilizer or as an organic manure.^{10,17,18} It has found its utility as organic fertilizer in various crops e.g. sugarcane, grapes, cotton, rice, wheat, potato, tomato, tea, coffee, tobacco, horticulture, lawns, etc.
- The castor cake is also used as a fungicide and in plant-parasitic nematode control.¹⁹
- The castor cake has also been referenced for use as a filler to obtain composite materials in various polymers, such as polystyrene, low density polyethylene, polypropylene, poly(hydroxyalkanoates), etc.^{7,20}. The inclusion of castor cake is reported to elevate the mechanical properties and biodegradable characteristics in low density polyethylene^{7,20-23}.
- The protein isolated from the castor cake also has potential to serve as a premium raw material to manufacture surfactants, fibers, plastics, etc.

- There are also reports that, after a proper detoxification, in a mix with other feed materials at 10-20% level, it has been used as a supplement feed, particularly for sheep, cattle, dogs chicken, fish, etc.¹³⁻¹⁶. Infact, the detoxified castor cake presents a better animal feed owing to its high protein value.
- The calorific value of deoiled castor cake is around 4000kcal per kg and it is useful as a substitute of fossil fuels e.g. coal for generating energy. It is a clean fuel and generates very little ash.

9. What are the existing technologies to destroy the ricin in the Castor cake?

Due to the its high protein content and multifarious applications, the castor cake remains the center of research among the scientific community. The prime limiting factor in restricting the use of castor cake and even the cultivation of castor plant is the occurrence of toxic ricin..

Ricin has several ways for its inactivation. Following are the summary of options developed for the detoxification of the castor cake.

- Heating the castor cake in autoclave for 1 h in steam at 15 psi can destroy the ricin and remove the allergen²⁴.
- Heating the castor cake in autoclave at 125°C for 15 min, completely eliminates the ricin with minimum changes in the cake²⁵.
- Ethanol has also been used to detoxify the castor cake. The repeated extraction of castor cake with ethanol has resulted in the detoxification of castor cake²⁶.
- The alkali treatment is one of the most efficient treatments found to inactivate the ricin in castor cake. The use of alkali reagents such as Ca(OH)₂ (0.5%), NaHCO₃ (0.5%) and autoclaving reduces ricin to non-detectable limits²⁷.

Recent detoxification techniques –

- The effective methods include autoclaving, boiling for 2 h and alkali treatment with sodium or calcium hydroxide²⁸⁻³⁰.
- In another method, the reactive seed crushing using alkaline methanol is reported as an efficient way to detoxify the castor cake. The seeds were flattened and treated with alkaline methanol to get esters directly and the amount of ricin in the remaining cake was less than 0.01% as analysed by Immunoaffinity and liquid chromatography-tandem mass spectrometry (IALCTMS) method³⁴.
- According to a recent study, where the relation of castor cake detoxification in reference to the pH has been studied, it is said that the cytotoxic activity of ricin when heated under basic condition lowers dramatically (The measured half-life for

ricin added to yogurt fruit drink at 90°C was only 0.24 ± 0.04 min at pH 6.5 but it increased 30-fold to 7.3 ± 1.1 min when tested at pH 4.5)²⁹. It is further mentioned that, the half-life for the ricin inactivation at 75°C is high in lactose-rich foods²⁹.

- A thorough study of detoxification using thermal and chemical means has been carried out by Anandan et al.²⁸. The castor cake was systematically treated either physically or chemically and analysed for ricin content at each point of treatment.

Table 1: Analysis of physical and chemical treatment of castor cake²⁸

Sr. No.	Treatments		Ricin (mg/Kg)	% Decrease
	Untreated sample		388	
1.	Soaking (h) in water	3	136	65
		6	55	86
		12	62	84
2.	Steaming (min)	30	103	73
		60	57	85
3.	Boiling (min)	30	38	90
		60	37	91
4.	Autoclaving	15 psi, 30 min	58	85
		15 psi, 60 min	-	100
5.	Heating	100 °C, 30 min	187	52
		120 °C, 25 min	193	50
Chemical treatment				
6.	Ammonia (mL/Kg)	7.5	191	51
		12.5	158	59
7.	Formaldehyde (g/Kg)	5	236	39
		10	75	81
8.	Lime (g/Kg)	10	129	67
		20	144	63
		40	-	100
9.	Sodium chloride (g/Kg)	5	78	82
		10	85	86
		20	70	91
10.	Tannic acid (g/Kg)	5	180	54
		10	282	27
11.	Sodium hydroxide (g/Kg)	2.5	69	82
		5	53	86
		10	36	91

- In another attempt, it is described that the toxicity of the meal is reduced by heating a solution of the meal in water to boiling, or even as low as 70°C; the temperature at which the ricin protein irreversibly unfolds^{32,33}.
- The effect of calcium hydroxide on castor meal has been studied in detail and it says that the calcium hydroxide during boiling with castor meal for 10 min does have effect on the denaturation of ricin. The denaturation causes a physical change in the conformation of the protein³³.

In the same article, the ricin A-peptide chain in ricin which is responsible for toxicity has been analysed in cold pressed untreated castor cake, hot pressed cake, whole seed and milled seed, followed by its inactivation and compared.

Table 2: Detection of Ricin A-Chain and its inactivation³³

Initial treatment	Secondary treatment	Duration (min)						
		0	10	20	30	40	50	60
Cold pressed	Boiled	+	-	-	-	-	-	-
	Autoclaved	+	-	-	-	-	-	-
	Urea	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	+
	Guanidine	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	+
Hot pressed	None	-	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Whole seed	Boiled	+	+	-	-	-	-	-
	Autoclaved	+	+	+	-	-	-	-
	Urea	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	+
	Guanidine	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	+
Milled seed	Boiled	+	+	+	+	+	+	+
	Autoclaved	+	-	-	-	-	-	-

+: presence; -: absence; n.t.: not tested.

10. Castor seeds contain a powerful allergen: CB1A - What is it? Is CB1A toxic?

1. Castor bean allergen-1 (CB1A) is the principal allergen of the castor bean. It is virtually non-toxic^{46, 49}, does not cause death though may cause allergic reactions. It is a polysaccharidic protein factor. While the ricin is heat-labile, the CB1A is a very stable allergen and a point of concern for the castor bean industry⁴⁰. CB1A is among one of the most heat-stable proteins found. In most normal heating conditions, the CB1A is known to retain its native antigenic structure, immune precipitating and allergenic properties.⁴¹ In general, CB1A is a group of low molecular weight micro heterogeneous proteins⁴². It consist of low molecular weight albumin storage proteins from the castor endosperm⁴³. The CB1A component compares physiochemically with the 2S storage protein of seeds. The amino acid composition of CB1A consists of relatively high arginine, cysteine and glutamic acid content. It doesn't contain tryptophan^{44,45}. The CB1A is considered as a nontoxic fraction⁴⁶, however, it has capacity to sensitize the people who have hypersensitivity³⁵⁻³⁹.

The potent allergen CB1A in castor seed and cake has been a matter of concern and as a result deallergenation of castor cake has been proposed by many researchers²⁴⁻³⁴. The work⁴⁷ carried out by the Food Protein Research and Development Center, concluded that in the order of preference, sodium hydroxide-sodium hypochlorite mixture, calcium hydroxide, sodium bicarbonate, sodium hydroxide and sodium hypochlorite are very effective in destroying CB-1A when used in combination with proper heat treatment. Further, it was concluded that the detoxified and deallergenated castor meal is safe for use as animal feeds as demonstrated by chick and swine feeding studies.

11. Is there a risk to find CB1A in castor oil?

During the processing of castor beans to obtain castor oil, the toxic ricin and allergens such as CB1A are retained in the castor cake only and don't get partitioned in the castor

cake⁴⁹. Moreover, the castor oil is steam/ heat treated, ensuring that it is free from any unwanted toxic containments. As already mentioned, after a stringent inspection, Food and Drug Administration (FDA) and Cosmetics Ingredient Review expert panel classified castor oil and its derivatives as safe ingredients for using it as a laxative (medicinal) and in food and cosmetic applications.

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