Proteins of the Castor Bean—Their Preparation, Properties, and Utilization

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Summary

This article is a review of technical papers and information that are now available on the proteins of the castor bean. Included among the various aspects of the subject are the following: The chemical composition of castor bean seeds and castor cake; the preparation and properties of their proteins, including the toxic albumin ricin, its properties, physiological reactions and amino acid content; the allergenic properties of castor bean protein; the utilization of castor bean cake or pomace for feeding, fertilizer and industrial purposes. An extensive bibliography is appended.

THE castor bean, Ricinus communis, was well known to the Ancients and is said to have originated in India. It is now cultivated widely in nearly all of the temperate and tropical parts of the world. It is raised almost exclusively for its oil, which is extensively used for medicinal and industrial purposes. Because of its physical properties the oil is of special value as a lubricant.

Castor Seeds

The seeds are composed of about 25 per cent husk and 75 per cent kernel. The percentage of husk appears to vary in different varieties of seed. For example, three varieties of seed from Cyprus were found to vary from 35 to 48 per cent. The oil content of the seed varies from 45 to 55 per cent (1), and that of the kernel from 60 to 65 per cent. The following percentage composition of castor seed from Bombay is given by Halenke and Kling (2):

	Composition of seed	Water	Protein	Oil	Carbohydrate	Fiber	Ash
Whole seed	....	5.14	17.88	46.65	12.61	14.99	2.73
Kernel	70	3.60	23.43	66.02	4.01	0.70	2.24
Husk	30	8.76	4.76	0.98	32.92	48.69	3.89

The percentage composition and ash content of several varieties of the seed are also given by Heller (3), and by Dubard and Eberhardt (4).

Castor Cake

The best oil is obtained by use of the hydraulic press. Solvent extraction methods are unsuitable for the production of medicinal castor oil. Oil extracted at low temperature dissolves the least amount of coloring matter, and is of a high quality. Only traces of ricin pass into the oil in the cold. During the first pressing about 25 to 30 per cent of the oil is expressed. More oil is obtained from the residue by further pressing in the warm, such further oil being used for manufacturing and lubricating purposes. After hot expression the oil content of the cake is solvent-extracted, which process further reduces the oil content to about 1 per cent or less (1).

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The following composition of castor cakes is given by Omega (1):

	From whole seed	From decorticated seed	From castor meal extracted by solvents
	%	%	%
Moisture	9.85	10.38	11.73
Ash	15.02	10.50	6.05
Oil	5.25	8.75	1.10
Protein	20.44	46.37	30.62
Crude fiber } Carbohydrate }	49.44	24.00	36.52 / 36.96
Phosphoric acid	1.62	2.26	....

Proteins of the Castor Bean

The high content of protein in the castor bean and the presence of an extremely toxic substance, long believed to be intimately associated with the protein, early stimulated an unusually great interest in investigations of the proteins of this seed.

Globulins, Proteoses. Among the early workers on castor bean proteins, Ritthausen, in 1882 (5), was the first to obtain the castor bean globulin in crystalline form. It separated from a 10 per cent sodium chloride solution of the oil-free meal in the form of octahedra. He determined the composition of the crystals and found that they were soluble in glycerine, a property that was later confirmed by Osborne (6). Ritthausen (7) also studied other protein fractions of the castor bean, but, because of the fact that his studies were pioneer work, many of his findings are confusing with respect to yields and composition.

The extensive investigations of Osborne and associates conducted over a period of 10 years or more yielded most of the information we have today on the chemical properties, yields, and composition of the different proteins of the castor bean. By extracting oil-free castor bean meal with 10 per cent sodium chloride solution and dialyzing the filtered salt extract there was obtained a yield of 12.4 per cent of globulin based on the weight of the meal extracted (8, 9). When heated in 10 per cent sodium chloride solution a turbidity developed at 75°C., and a slight coagulum separated at 86°C. On continued heating, another coagulum separated in much larger quantity at 95°C. The different coagulation temperatures indicated the presence of two globulins.

There was also isolated, in addition to the globulin, a quantity of protein having the properties of a proteose. The yield was 3.85 per cent. This figure was reported as a minimal yield, and it was believed that the amount actually present in the seed was much greater.

Osborne and Harris (10) found that the globulin gave a strong positive test for tryptophane, but gave a negative test with Molisch reagent. It contained the following percentages of the amino acids: Arginine, 13.19; histidine, 2.74; and lysine, 1.54 (11).

The Albumin, Ricin. Most of the interest in the proteins of the castor bean has centered on the extreme toxicity of the albumin. The name, ricin, was given to this albumin by Stillmark in 1888 (12), who was working under Kobert's guidance.

Chemical Properties. Like all albumins, ricin is soluble in water, and remains in solution after all of the globulin has been separated from a saline extract of the meal. It coagulates in an aqueous solution on heating to 60° to 70°C., depending on the rate of heating. Ricin comprises about 1.5 per cent of the oil-free meal (13). It can be precipitated from a 10 per cent sodium chloride solution by saturation with sodium chloride or magnesium sulfate. Ricin contains no phosphorus. It has the following percentage composition: C 52.01; H 7.02; N 16.56; S 1.29. Karrer and associates (14) determined the specific rotation of three different preparations of ricin:

Dry (dessicator)............................ $[\alpha]D^{18} = -42.53°$
Adsorbed by $Al(OH)_3$.................. $[\alpha]D^{18} = -41.4°$
Another preparation in H_2O.......... $[\alpha]D^{19.5} = -40°$

Like other toxalbumins ricin is digested with difficulty by proteolytic enzymes. Karrer and associates (14) found that after 5 months' digestion with pancreatin only two-thirds of the protein was digested. The undigested portion still had the same degree of toxicity as the original material, and had the same chemical composition. The destruction of the protein and of the toxicity proceeded at a parallel rate. Other digestion studies, using a glycerol extract of swine pancreas, led to the same results. As a result of their findings the following conclusions were drawn: that the toxic group is dependent on the existence of the protein, and that only in this combination can it manifest its action. In this sense there can be no longer any doubt that the ricin-toxin complex stands in intimate relation to the protein.

The fact that toxalbumins produce such powerful physiological effects indicate that they differ in some pronounced manner from other proteins, and that their toxicity is a peculiar property of the protein itself (15).

The following percentages of amino acids in ricin were determined by Karrer and associates (14):

AMINO ACID CONTENT OF RICIN
[Karrer *et al.* (14)]

	%
Humin	1.8
Ammonia	2.8
Glycine	0.0
Alanine	1.0
Valine	2.0
Leucine + isoleucine	16.0
Phenylalanine	0.4
Tyrosine	2.7
Cystine	1.0
Serine	?
Proline	4.6
Hydroxyproline	0.0
Aspartic acid	2.0
Glutamic acid	20.0
Tryptophane	0.4
Arginine	11.7
Lysine	6.3
Histidine	0.0
Peptide anhydrides	2.0
Total identified amino acids + NH_3	74.7
Non-identified amino acids	3.3
Total	78.0

Toxicity and Physiological Properties of Ricin. Although the poisonous character of castor beans had been long known, Dixson, in 1887 (16), was the first to isolate a toxic protein from the beans. For several years subsequent to Dixson's investigations much of the work on the castor bean proteins was devoted to attempts to find out whether the toxicity of ricin was to be attributed to the protein itself or to some factor intimately associated with it. These studies involved much work on the separation of various fractions and testing their physiological action. Osborne, Mendel, and Harris (13) were the first to isolate ricin as the protein to which the toxic property of the seed is to be ascribed, although 11 years earlier Osborne (17) arrived at the conclusion that the albumin appears to be so intimately associated with the toxic substance that it is very certain that it is the toxic substance itself.

Investigations on the action of proteolytic enzymes toward ricin led several (18, 19, 20) to the erroneous conclusion that ricin can be digested without diminution of toxicity. They did not appreciate the resistance of this protein to digestion. On the other hand, that ricin is quite resistant to digestion was shown by Karrer and associates (14) and others (21, 10, 22), and that given enough time the toxicity diminishes with the extent of digestion.

The toxicity of ricin is destroyed when a water solution of the albumin is heated to boiling (23) or even to the coagulation point of the protein (24). Dry heat, on the other hand, does not destroy the toxicity of the protein (3). Pure, dry ricin preparations suffer no deterioration in their physiological activities after being kept for many months (25).

The physiological effects of ricin are similar to those of many bacterial toxins and the typical toxalbumins. It causes, after several hours, a paralysis of the respiratory and vasomotor systems. Ingestion by mouth causes diarrhea, general prostration and hemorrhagic condition of the intestines, renal congestion and hypermia of the spinal medulla and brain. It coagulates the red blood corpuscles. Ricin also affects the eyes causing inflammation and severe panophthalmitis (23, 26).

A characteristic effect of ricin, in common with other toxalbumins, is to agglutinize the red corpuscles of mammalian blood. This property is lost when the protein solutions are heated to coagulation. The agglutinization involves a combination of the toxin with the corpuscles. The ricin can be extracted from the red blood cells by dilute hydrochloric acid, indicating that the toxin and the agglutinin are one and the same substances (27, 28, 29, 18, 30, 16, 31, 22, 13, 32).

Reid (20) found that when the cells of dog's brains were washed with physiological salt solution and agglutinated by adding a solution of ricin, the ricin could be extracted from the precipitate by very dilute hydrochloric acid. The clear extract was found to promptly agglutinate a suspension of cat's red blood corpuscles, showing that not only the toxin but also the agglutinin was united with the brain cells, thus indicating that the two are one and the same substance. The cells of many other organs of the animal are agglutinated by ricin. A differentiation of ricin into a toxin and an agglutinin is not justified.

That animals can be immunized against the toxic effect of ricin was demonstrated by Ehrlich (23).

This discovery led to the recognition of the close relationship of ricin to toxalbumins from other plant sources and the numerous bacterial toxins. Ricin-immunized animals contain an anti-toxin (anti-ricin). The acquired immunity to ricin lasts, in the case of mice, for many months. Procedures for developing immunity in animals are described by Cornevin (33).

Ricin is extremely toxic to animals whether ingested by mouth or injected subcutaneously or intravenously. It manifests its greatest effect when given intravenously. The toxicity of ricin is greatly diminished when administered through the alimentary canal instead of intravenously or subcutaneously. Even after administration of quantities many times larger than the fatal dose, a "latent period" of at least 15 hours intervenes before the toxic symptoms become manifest (25).

Ricin is apparently toxic to all species of mammalian animals. It has been shown to be toxic to man, rabbits, sheep, cattle, horses, pigs, dogs, rats, mice, guinea pigs, ducks, and chickens. The toxicity varies with the species of animals, guinea pigs being the most susceptible.

Working with rabbits, Osborne, Mendel, and Harris (13, 34) found that 0.0005 mg. of ricin per kilogram of body weight was fatal at the end of 7 days when injected subcutaneously. Karrer and associates (14) later obtained practically the same results. Ehrlich (23) reported that a guinea pig weighing 385 gm. died in 11 days after injection of 0.7 cc. of a ricin solution having a concentration of 1:500,000.

According to Cornevin (33), the following quantities of the castor bean seeds per kilo of body weight are lethal: rabbit, 2 gm.; sheep, 2.5 gm.; ox, 3.0 gm.; horse, 3.0 gm.; pig, 3.5 gm.; dog, 5.6 gm.; cock, 40 gm.; duck, 40 gm. Apparently, fowls are more resistant than mammalians to the toxic action of the castor bean protein; 0.18 gm. of ricin is lethal to man, a quantity corresponding to about six castor seeds. An account is given (35) of five cases of poisoning by the seed. Two were fatal after eating only 2.5 and 5 seeds, respectively.

Allergenic Properties of Castor Bean

Alilaire (36) first described human hypersensitivity to castor bean. He believed the allergen of the castor bean to be identical with the toxalbumin, ricin. Ratner and Gruehl (37) showed that castor beans contained an anaphylactogenic agent in addition to ricin. Barnard (39) claimed to have prepared non-toxic, allergenic extracts from the castor bean. According to Barnard the allergen was water soluble, heat stable and non-dialyzable. Grabar and Koutseff (40) later separated a non-toxic allergenic fraction from castor beans which they called "ricinallergene." Their preparation was water soluble, heat stable, and dialyzable.

Recent studies by Allergen Investigations of the Bureau of Agricultural Chemistry and Engineering (64, 65, 66, 67) have resulted in the isolation from castor beans of a powerful, non-toxic protein-polysaccharidic fraction. This fraction, which represented 1.8 per cent of the defatted castor bean meal, was isolated by a procedure developed for isolating a similar allergenic fraction from cottonseed. Chemical properties and amino acid content of the castor bean allergen were remarkably similar to those of the previously characterized cottonseed allergen. Both fractions were characterized by relatively high proportions of arginine and cystine. The castor bean allergen was freed from the toxalbumin, ricin, as shown by tests on guinea pigs. The allergen was antigenic and was immunologically distinct from other antigens present in castor beans. Positive passive transfer reactions were induced in human subjects by *0.0001 microgram* of the allergen. It is remarkably stable toward heat and chemical manipulations.

The activity of this castor bean allergen is unaffected by prolonged boiling in water. Therefore, attempts to detoxify castor bean pomace by heating or steaming to inactivate the ricin would not be effective in destroying its powerful allergenic activity. Moreover, this potent allergen of the castor bean exhibits an unusual sensitizing capacity in human subjects exposed to inhalation of the dust from the pomace. Figley and Elrod (41) proved that the unusually high incidence of asthma in a Toledo school and in the neighborhood was caused by castor pomace dust expelled from ventilating stacks of a nearby linseed and castor bean mill. Peters is quoted as stating (42) that sensitivity to castor bean is frequent in Bridgeport, Connecticut, where there is a castor oil bean mill. Vaughan (43) found that castor bean allergen was the causative factor in several asthmatics who were exposed to a fertilizer containing castor bean pomace. Sensitization to castor bean allergen by inhalation of the dust was demonstrated experimentally by Ratner and Gruehl (37). Bernton (64) has recently reported a case of severe allergic attacks in a man which incapacitated him for 2 weeks at a time. It was conclusively demonstrated that castor bean protein was the sensitizing agent. The attacks followed mere contact with an apparently clean burlap sack which at one time undoubtedly had contained or been in contact with castor bean pomace.

Utilization of Castor Bean Cake or Pomace for Feeding

Only a few cases are recorded of the use of castor cake, or pomace, as a protein concentrate for use in the feeding of farm animals. Because of the known toxicity of ricin it is a foregone conclusion that it would be highly hazardous to use unprocessed castor cake as a feed for animal production. Were it possible to entirely remove the relatively small amount of ricin present in castor cake, there seems to be little or no reason to believe that the large amount of protein remaining, represented chiefly by the globulin, could not serve as a protein concentrate for feeding purposes. A detoxified castor cake is reported (3) to have the following coefficient of digestibility: fat, 77; N-free extract, 90; and fiber, 10.

The amino acid composition of the globulin as given on a preceding page shows that it contains most of the nutritionally essential amino acids, with the exception of histidine. The percentages for phenylalanine and tryptophane are low. It should be remembered, however, that these amino acid determinations were made many years ago when the methods of analyses were quite inadequate and that, therefore, the results given are probably much too low.

There are a few recorded cases of attempts made to feed castor cake to farm animals. Wooldridge (44) refers to the feeding to horses of a mixed feed containing castor seed. Five died, and the services of the others were lost for several weeks. It has been pointed out on a previous page that the toxicity of castor beans varies with the species of animals and that, according to Cornevin (33), fowls are more resistant than mammals. This observation is confirmed by later findings. Atkinson (45) states that a mixture of 1 part castor bean meal and 5 parts of a grain meal mixture was fed to cockerels in quantities of 4.5 to 5 ounces per bird per day. The food was eaten eagerly, but the birds steadily lost weight. At the end of the 36th day, 1 bird died, followed 4 days later by another. On the 40th day, the castor meal feed was discontinued and the four remaining birds fed ordinary meal. The birds recovered slowly, but at the end of 3 weeks they had put on but very little flesh. Experiments on the feeding of fowls by Vilner (46) indicated that castor oil cake could be safely used, provided it was given progressively and carefully in amounts not exceeding 30 per cent (and preferably not over 20 per cent).

That castor cake is generally regarded as highly dangerous as an admixture in feeds is indicated by the number of tests for its detection that have been published (47, 48, 49, 50).

It seems to be quite definitely established that the toxicity of the isolated protein, ricin, is destroyed when its aqueous solution is heated to boiling, or even up to its coagulation point. As far as the writer is aware, there is no record of any work done on detoxification of castor cake or meal by heating. Inasmuch as ricin itself is detoxified by heating with water, it would appear that castor bean meal might be made safe for feeding purposes by steaming or by some other suitable heat treatment. It is obvious, however, that safeguards would have to be established to ensure that the heat processing had been adequate in all cases before the product could be safely offered as a feed for farm animals. There remains also the question whether such a detoxified castor seed product would be palatable to animals because of the small quantities of the oil remaining. Even after solvent extraction the meal may contain as much as 1 per cent of oil. The physiological effects of castor oil even in such small quantities might have harmful effects when fed over extended periods.

Use as Fertilizer

Castor seed cake is reported to have a high value as a fertilizer. Mixed with bone meal it has been long used in India in the culture of sugar cane. In France it has been extensively and satisfactorily used as a fertilizer for potatoes and other farm products (4). Its fertilizing value depends largely on its high nitrogen content (6.37 per cent) and on its "phosphoric acid" (2.55 per cent) and potash values (0.96 per cent) (1). It also has a high nitrification value. Field and laboratory experiments conducted in Ceylon (51) with various organic fertilizers, including peanut cake, fish guano, crushed fish, dried blood, and castor cake, showed that the highest percentage of nitrogen nitrified was that for castor cake and crushed fish. At the Massachusetts Experiment Station (52) it was found that in 3 months 75 to 100 per cent of the nitrogen in castor pomace was nitrified. The high oil content of the castor pomace (10.24 per cent) slightly hindered, but did not prevent, nitrate accumulation. Other workers have found castor cake to be similarly effective as a fertilizer (53, 54, 55, 56). It has been found to be effective in the culture of sugar cane. It tends not only to increase the number of cane tillers, but also to hasten their formation and to favor uniform maturity of the cane. It also has a peculiarly favorable effect on the height of the primary shorts and tillers (57).

Castor pomace is a well recognized article of the fertilizer trade in the United States. Bailey (58) states that castor pomace is valuable chiefly as a fertilizer because of its nitrogen, but cautions that it is poisonous to farm animals and it always should be stored where they will not have access to it.

In addition to the precaution pointed out by Bailey with reference to the use of castor pomace for fertilizer because of the toxic property of its protein, ricin, attention should also be directed to the powerful allergenic property of castor bean protein. Reference has been made on a previous page of this report to the high incidence of asthma caused by exposure to castor pomace dust, both from a castor bean mill and from a fertilizer containing castor pomace. Even after detoxification of the ricin by heat processing the allergenic property of the castor pomace still remains.

Guarantees for castor pomace are generally placed at 4.50 per cent of nitrogen. The nitrogen content has remained fairly constant over a period of 4 years, varying between 4.75 and 5.10 per cent. The nitrogen content and sources of a large number of samples are tabulated (58).

Other Industrial Uses

The castor seed contains an enzyme, lipase, which hydrolyzes fats. Use has been made to some extent of castor cake in the manufacture of detergents (59). A method for the determination of the specificity and optimal conditions for determining the lipase was developed by Reichel and Reinmuth (60), which depends on the amount of oleic acid liberated from triolein under specified conditions. When X-rays and ultra-violet rays were thrown on dry, powdered cake there was no effect on the enzyme action of the lipase, but ozone caused a sharp drop in hydrolytic activity (61).

Castor bean cake has been used in Manchuria as a source of glutamic acid for use in the making of sauces and condiments (62). Crude castor cake was found to yield 11 to 12.5 per cent of glutamic acid, approximately the same as that yielded by soybean, peanut, and cottonseed cakes.

The manufacture of a product called "casein" is described (63). By treating oil-free cakes of castor beans with NaOH (1° Bé) at 40-45° for 2 hours and subsequently reprecipitating the proteins with 3.5 per cent HCl, a yield of 49.5 per cent (of the weight of the cake) of crude "casein" was obtained.

It has been reported (personal communication) that a textile fiber has been made on an experimental scale from castor seed protein which compares favorably with fibers similarly made of proteins from other sources, such as soybeans, peanuts, and casein.

Various claims for the effective use of the castor bean plant in combating insect pests led the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture (69, 70) to conduct toxicity studies on ricin and ricinine, the latter an alkaloid known to occur in the seeds and other parts of the castor bean plant. Ricin proved to be non-toxic to coddling moth larvae. Ricinine, however, proved highly toxic. Ricin and ricinine have been shown to have no effect on grasshoppers (71).

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Report of the Cellulose Yield Committee 1946-47

DURING the past year the committee sent out samples to 11 laboratories for check yields analyses. The average results for the laboratories are given below:

Lab. No.	No. Sets Samples Tested	Samples A Linters	Samples B Linters	Samples C Fiber	Overall Average for Year
1	4	78.0	72.1	69.1	73.1
2	4	78.4	72.3	69.9	73.5
3	4	77.6	71.5	68.8	72.6
4	4	78.4	71.9	70.2	73.5
5	4	77.9	72.0	69.2	73.0
6	4	78.2	72.1	69.1	73.1
7	4	78.4	72.1	69.4	73.3
9	4	78.1	72.1	69.2	73.1
11	4	78.5	72.1	69.3	73.3
12	4	78.8	72.7	69.8	73.8
Avg.		78.2	72.1	69.4	73.2

The agreements of all the laboratories for the past year were very good as seen from the above results.

Last year the committee recommended the use of a wetting agent to be used in the cooking solution. This was not approved by the Uniform Methods Committee since we had put it in as an optional method. This year we are including this suggestion again but we are making it mandatory instead of optional. Last year's report included the work which showed that the oil could be used without affecting the yield.

In our present method we have two ways of mixing lint samples, one by hand and another with a lint mixer. Practically all of the laboratories at the present time are equipped with the lint mixing machine so it has been decided to make the lint mixing equipment mandatory and cut out the hand mixing method as more uniform results are obtained with the lint mixing machine.

Recommendations: It is recommended that:

1. The method be changed to include the use of red oil in wetting out the lint before digesting in the amounts of ½ c.c. of red oil to each 525 c.c. of 1% caustic solution.

2. The mixing of linters by hand be taken out of the method and make the mixing of linters by the lint mixer mandatory.

3. Samples be sent out during the next season at least 4 times.

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