Castor Meal Antigen Deactivation—Pilot Plant Steam Process

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ABSTRACT

A series of pilot plant batches of castor meal have been prepared using steam as the deactivating medium. Treatments consisted of 12 combinations of 10, 20, 40 and 80 psig steam pressure for 15, 30 and 60 min each. A multiple regression equation derived from the above data relates the guinea pig response to the process variables. This equation is also presented as a two-dimensional surface contour. The antigenicity levels of untreated castor meals were found to be significantly different. This difference was not retained after treatment. Because of the uncertainty of the antigenicity of the pomace before treatment, an alcohol extract from castor beans (CBWU) has been adopted as a standard for potency evaluations. Amino acid analyses were performed on selected treatments. Considerable antigen deactivation has been achieved using mild steam treatments, e.g., 10 psig for 60 min. This process has a mild effect upon the essential amino acid, lysine.

INTRODUCTION

Castor beans contain a number of harmful components: a violently poisonous protein called ricin, a mildly toxic alkaloid component called ricinine and a powerful but very stable group of allergens known as CB-1A (1). The ricin, although extremely poisonous, is quite easily detoxified by cooking the beans with steam. The small quantity of ricinine present is not considered particularly detrimental, but the very powerful allergen fraction, a protein polysaccharide referred to as CB-1A (1) is very stable.

Although the oil itself is cathartic it is nonallergenic and nonpoisonous. It is the oil cake residue, sometimes referred to as castor meal or pomace, that contains these harmful constituents. It is because of these allergens that the potential use of the meal as a feed ingredient or even as a fertilizer has been severely restricted. It is the purpose of this paper to describe one method that can be used to deactivate the antigens of this meal.

One of the objects of this series of experiments was to determine whether the ID assay technique (2) would indicate castor antigen deactivation using low pressure steam. If deactivation was indicated, as suggested by Jenkins (3), a study of the relative effect of different levels of pressure and time would be of interest. Our earlier experience with steam processing based upon the intravenous biological assay technique seemed to suggest that high pressures would be required (2,4).

EXPERIMENTAL PROCEDURES

A single lot of castor meal was used to prepare 12 experimental batches by steaming the meal for 15, 30 or 60 min at 10, 20, 40 or 80 psig. In each case, 3 kg of the castor meal "as received" was placed in a 10 gal pressure vessel. Then 1½ liters of water was added forming a 1:2 liquids-solids slurry. The vessel was sealed and mildly agitated using an anchor type mixer. Steam was admitted to the jacket to preheat the slurry. Dry steam (100% quality) was fed directly to the product through a sparge coil. The 12 process conditions or treatments are shown in Table I. After each experiment the meal was tray-dried at 80 C to a final moisture level of about 10%.

Sample Preparation

A representative sample of each batch was collected. After grinding in a Wiley mill, 10 g of the meal was mixed with 200 ml distilled water. The pH was adjusted to 5 using HCl. The slurry was then heated to 100 C for 1½ hr, cooled and filtered through 0.8 μ millipore filter.

Biological Assay

White guinea pigs were passively sensitized with an intradermal injection (.05 cc) of rabbit anticastor serum (2). After a latent period of approximately 2½ hr, a 0.5% solution of Evans blue dye in physiological saline solution was injected into the cephalic vein. Then the experimental extract was intradermally injected at the anticastor serum

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TABLE I

Average Dimensions of Diffused Dye, mm[a]

Process time, min.	Dilution	Dose, μg[b]	Steam pressure, psig 10	20	40	80
15	1:0	2500	18.92	20.88	21.96	13.29
	1:10	250	16.88	18.34	18.05	11.21
	1:100	25	16.09	16.09	12.96	9.25
	1:1000	2.5	11.67	11.09	7.42	2.46
	1:10000	.25	4.59	2.21	2.34	---
30	1:0	2500	17.88	20.88	22.05	13.67
	1:10	250	9.09	18.34	20.96	7.84
	1:100	25	9.09	14.00	12.29	2.21
	1:1000	2.5	4.50	8.34	7.50	---
	1:10000	.25	3.09	1.09	2.21	---
60	1:0	2500	16.67	18.42	15.09	14.50
	1:10	250	9.88	12.38	8.83	9.42
	1:100	25	3.42	8.00	2.17	1.92
	1:1000	2.5	5.25	2.63	---	---
	1:10000	.25	1.00	1.25	---	---

[a]Six sites per pig averaged using two pigs per dose (mm).
[b]Equivalent meal (μg).

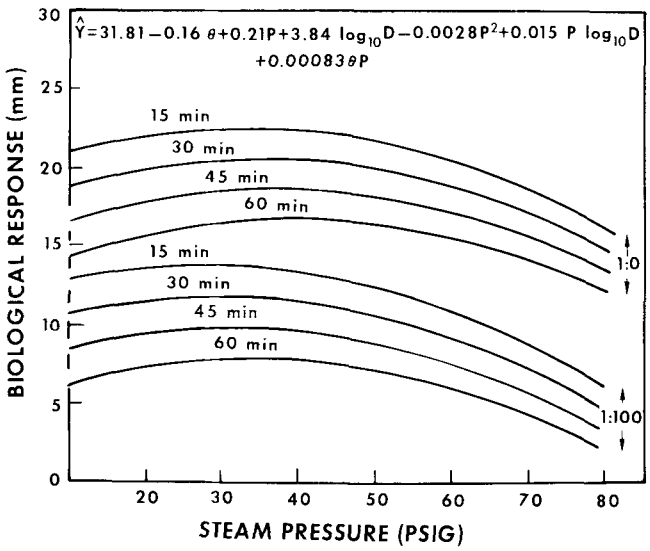

FIG. 1. Process response profiles.

site.

One milliliter of extract solution was equivalent to 0.05 g of the original sample meal. Since all injections were 0.05 cc, whole extract (1:0) was equivalent to 0.0025 g of meal. Tests were made with whole extract and serial dilutions of it in physiological saline. The dimension of the area showing the diffused dye at the antibody-antigen site was the measure of response (2). The test results are shown in Table I.

RESULTS AND DISCUSSION

An analysis of variance of the steam data (Table I) (2) showed, as expected, the process variables time and pressure had pronounced effects on the biological response. The analysis also gave evidence for significant time and pressure X log dose interactions.

A step-wise least squares regression analysis (5) of the data led to the following equation:

$$\hat{Y} = 31.81 - 0.16\ \theta + 0.21\ P + 3.84 \log_{10} D - 0.0028\ P^2 + 0.015\ P \log_{10} D + 0.00083\ \theta\ P$$

in which $\hat{Y}$ is the predicted biological response (mm), θ, the process time (min), P, the steam pressure (psig), and D, the castor meal preparation (μg equivalent castor meal dose). Variables have been deleted when the F value for inclusion was less than one. The multiple correlation coefficient is 0.917. A two-dimensional surface contour from this equation using the IBM 1800 computer and the 1627 plotter is shown in Figure 1.

As expected, the overall response levels decreased with greater dilutions and longer times. It was somewhat surprising to notice a slight increase in biological response as one increases the process steam pressure from 10 to

TABLE II

CBWU[a] Regression Analysis

Source of variance	df	Mean Square	$F_{calc.}$
Log_{10} dose, Linear	1	60,746.640	18,286.2
Log_{10} dose, Quadratic	1	2.997	0.9
Residual	1882	3.322	

[a]Alcohol extract of castor beans.

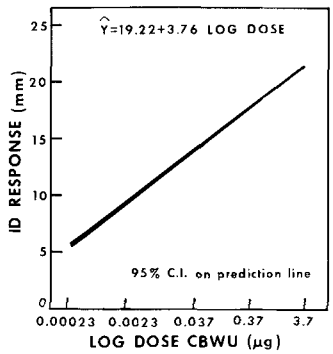

FIG. 2. CBWU regression and 95% confidence intervals.

approximately 30 psig. This seemingly paradoxical response may be the result of two opposing reaction rates. The process may cause greater antigen extraction than destruction at these lower pressures.

The fact that pressure shows a greater effect at 1:100 than 1:0 dose illustrates the significant pressure X log dose interaction. The presence of this interaction makes it essential to use several dose levels in determining the effect of the process variables.

We have, in the past, been using two test sites on each guinea pig as an animal control (2). One site has been intradermally injected with a low titre alcohol extract of castor beans (CBWU) to test the activity of the anticastor serum. A high titre dose was intradermally injected in the other site to make certain that the response of the animal was not inhibited. An analysis of variance of some 1800 CBWU data points shows strong evidence of log-dose response linearity ($F_{calc.}$)(Table II). The regression equation and the confidence limits on the predictions and on a single observation are shown in Figures 2 and 3, respectively. Any single observation falling outside the confidence band shown in Figure 3 was cause for rejection of the data obtained from that animal. To avoid any possible influence of CBWU on the responses of the test extracts, the CBWU controls were injected last.

Since the adoption of the more sensitive intradermal assay technique (2) to determine the level of residual antigens in the meal, the question of uniform antigenicity of untreated pomaces was raised. A number of lots of untreated castor meal obtained from a single source were assayed. The log dose response from two of these meals, typical of the group, is shown in Figure 4. At dilutions of 1:10 to 1:10,000, the confidence limits of lot A predictions do not include the predictions of lot B, and vice versa. We can conclude the antigenicities of these lots are significantly different at these dose levels. Lot B was the original pomace used for this steam series.

In retrospect, the differences in antigenicity of the

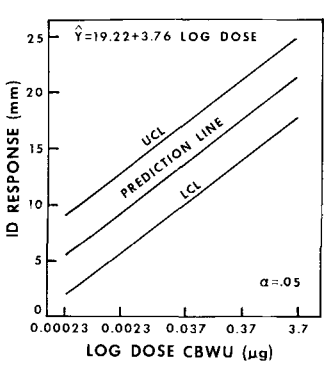

FIG. 3. CBWU control chart.

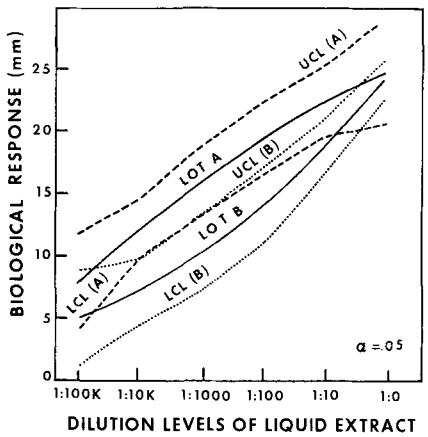

FIG. 4. Antigenicity comparison of two untreated castor meals.

TABLE III

Antigencity Difference After Treatment of Castor Lots A and B (1:10 Dilution)

Source of variation	df	ms	$F_{calc.}$	$F_{.05}$
Lot A vs. Lot B	1	3.5364	0.76	4.4
Pigs, lots A and B	18	4.6692	---	---

pomace is not surprising. The last unit operation in oil recovery from the pomace is solvent extraction (6,7). The residual solvent in the pomace is recovered by steam distillation. Earlier experiments using steam to deactivate the antigens in the meal were reported by the writer (4). The intravenous antigen assay method used then, however, was not sensitive enough to detect small changes in antigenicity using mild steam treatments (2,4,8).

TABLE IV

Potency Ratios of Steam-Treated Castor Meals

Treatment No.	P, psig	θ, min	Dose (μg) equivalent 12.5 mm response	95% LCL[a]	Potency ratio[b]	95% UCL[a]
Lot A	—	—	0.347	21.6	46.9	102.0
Lot B[c]	Control	Control	3.94	1.91	4.13	8.92
0	—	—				
1	10	15	11.6	.503	1.41	3.95
2	10	30	307	.0167	.0531	.168
3	10	60	848	.00353	.0192	.105
4	20	15	13.3	.543	1.22	2.75
5	20	30	24.6	.410	.662	1.07
6	20	60	198	.0463	.0820	.145
7	40	15	23.2	.389	.702	1.27
8	40	30	17.5	.464	.928	1.85
9	40	60	971	.0140	.0168	.0201
10	80	15	794	.00763	.0205	.0551
11	80	30	1590	.00348	.0103	.0303
12	80	60	1040	.00814	.0157	.0303

[a]Abbreviations: LCL, lower control limit; UCL, upper control limit.

[b]Potency ratio expressed as μg CBWU (alcohol extract of castor bean) per milligram of castor meal. Each level equivalent to a 12.5 mm biological response. CBWU dose equivalent to 12.5 mm = 0.0163 μg.

[c]Original untreated castor meal.

TABLE V

Amino Acid Analysis of Steam-Treated Castor Meals (g AA/16 g N)

		Treatment No.			
		1	3	10	12
Amino acid	Lot B, untreated	10 psig 15 min	10 psig 60 min	80 psig 15 min	80 psig 60 min
Lysine[a]	2.96	2.69	2.56	2.06	1.72
Histine[a]	2.08	1.97	1.92	2.14	1.94
Ammonia	2.24	2.20	2.22	2.24	2.14
Arginine[a]	10.91	10.11	9.71	9.20	6.59
Ornithine	0.07	0.20	0.30	0.18	0.24
Aspartic	9.46	9.49	8.85	8.89	8.22
Threonine[a]	3.47	3.49	3.36	3.28	3.13
Serine	5.61	5.62	5.38	5.10	4.84
Glutamic	18.30	18.01	17.40	17.27	17.40
Proline	3.43	3.27	3.33	3.23	3.42
Glycine[a]	4.25	4.20	4.05	4.05	3.92
Alanine	4.34	4.32	4.24	4.18	4.21
Valine[a]	5.86	5.73	5.72	5.76	5.76
Isoleucine[a]	4.87	4.79	4.64	4.61	4.59
Leucine[a]	6.27	6.23	5.98	6.04	5.93
Tyrosine	2.22	2.07	1.89	1.96	2.12
Phenylalanine[a]	3.87	3.81	3.71	3.64	3.59
Methionine[a]	1.75	1.73	1.67	1.64	1.54
Cystine	2.12	1.99	1.83	1.04	0.62
Nitrogen (dry basis)	5.93	5.02	4.70	4.59	5.21

[a]Essential amino acids for chicks.

The antigenicity differences of the untreated meals raised the question of whether or not these differences would be retained after treatment. To help answer this question pomace from lots A and B was treated with steam at 80 psig for 1 hr. The extracts (1:10) from each lot were tested on 10 guinea pigs. These meals, before treatment, were significantly different at this dose level (Fig. 4). The analysis of variance shows this difference was not retained after treatment ($F_{calc.} < F_{.05}$)(Table III).

Since differences in untreated meals may not be retained after treatment, CBWU was selected as a reference standard for potency evaluation. The potencies relative to CBWU of the untreated castor meals together with the treated meals are given in Table IV. Lower potency indicates more deactivation. Lot B was the original untreated meal used for the treatments reported in this paper.

Amino acid compositions of several meals were determined by the use of a modified Phoenix Amino Acid Analyzer using the hydrolysis procedure and correction factors developed by Kohler and Palter (9). The basic column was developed using conditions which separated ornithine from lysine since base treatments are known to convert part of the arginine into ornithine. The results are listed in Table V.

Steam pressure, or its resultant temperature, has significantly reduced the lysine content of the meal. Approximately 40% of this essential amino acid was lost when the pomace was processed at 80 psig for 60 min. The loss at 10 psig for the same time period was 13%. Arginine was also reduced but is still as good as that found in soybean meal.

The selection of a process treatment for industrial applications will depend upon product utilization. Greatest economic returns would be from a meal suitable as a feed supplement for poultry or swine. Hence, protein quality is of importance. Pomace treated with steam at 10 psig for 60 min decreased the antigen content 200-fold, but caused a 13% drop in lysine. This lysine deficit could be made up by greater use in the ration of a high lysine material such as fish meal.

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