Castor Meal Antigen Deactivation— Pilot Plant Ammonia Process

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ABSTRACT

This is one paper of a series reporting on processes to deactivate the castor antigens. This report gives an analysis of ammonia process variables affecting antigenicity responses. The degree of destruction of antigen obtained by treatment with ammonia under relatively mild conditions of heat is comparable to that obtained with high pressure steam.

INTRODUCTION

Domestic castor bean acreage and production in the U.S. have not developed at the rate expected several years ago (1) and the bulk of the castor oil used here is still imported. Two reasons that have contributed to this lag are the competitive situation as regards other crops, and low foreign operating costs. A third factor is that there appears to be less concern abroad as to the effects of the allergenic proteins in the castor bean (2). The extremely potent allergens in castor beans and castor pomace are an important obstacle to the development of a domestic castor bean production program. Many industrial workers who process the beans for oil or handle fertilizer containing castor meal become sensitized to the allergens (3,4).

The pomace, or oilseed meal, represents about one-half the weight of the castor bean (5). It must be disposed of at a reasonable price in order to make production of the crop economically feasible. Destruction of the allergens could raise the current value of castor pomace used as a fertilizer from about $30/ton to approximately $50/ton or more based on its protein value in animal rations (4).

Steam and lime are two pilot plant processes known to deactivate castor allergens (6,7). In laboratory studies it has been shown possible to deactivate antigens and allergens using ammonium hydroxide (8). It is the purpose of this paper to present the results of studies on process variables of ammonia treatment and the development of a practical process for destruction of castor antigens using this reagent.

EXPERIMENTAL PROCEDURES

Four process variables considered likely to affect the deallergenation of castor meal are ammonia concentration, process temperature, quantity of liquid in the slurry and process time. A few preliminary experiments had been carried out to obtain information for a more complete factorial design.

A single lot of castor pomace (Plains Cooperative Oil Mill, Lubbock, Texas) was used for all preparations. The castor pomace as received was placed in a stream jacketed

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TABLE I

Process Experiments

Exp. no.	Process variables			
	Process temperature, C	Process time, hr	Liquid-solids ratio	Molarity
1	100	1.5	0.1:0.2	1, 6
2	80, 100, 120	1.5, 4.5	0.15:0.25	6
3	60, 70, 80	1.5	0.075:0.15	2, 4, 6
4	60, 80	1.5, 4.5	1.0:2.0	6
5	20, 50, 80	.75, 1.5	0.25:1.0	6, 10

TABLE II

Potencies for Effects Found Significant in Analyses of Variance

Experiment	Variable	LCL[a]	Potency[b]	UCL[a]
Number 1				
Liquid-solids ratio	R = 0.1	.0203	.144	1.02
	R = 0.2	.00295	.0275	.256
Molarity	M = 1	.0927	.379	1.55
	M = 2	.00182	.0196	.211
Number 2				
Time, hr	θ = 1.5	.00687	.0126	.0229
	θ = 4.5	.0108	.0159	.0233
Liquid-solids ratio	R = 0.15	.00427	.00821	.0158
	R = 0.25	.0148	.0207	.0289
Number 3				
Molarity	M = 2	.324	.512	.807
	M = 4	.237	.399	.673
	M = 6	.393	.729	1.35
Number 4				
Temperature, C	T = 60	.00184	.00405	.00891
	T = 80	.00168	.00474	.0133

[a]95% confidence level; LCL = lower confidence level; UCL = upper confidence level.

[b]μg CBWU/mg treated castor pomace.

TABLE III

Effect of Extraction Conditions on Apparent Potency of Untreated Castor Meal

	Time, hr								
	1.5			6			24		
Treatment	LCL[a]	Potency[b]	UCL[a]	LCL[a]	Potency[b]	UCL[a]	LCL[a]	Potency[b]	UCL[a]
1 M	13.2	25.0	47.4	35.1	106	320	11.5	26.2	59.3
2 M	15.2	45.4	136	46.9	123	324	23.8	42.6	76.2
4 M	16.8	58.2	201	21.7	47.0	102	19.7	52.0	137
6 M	9.14	19.7	42.6	19.8	42.3	90.7	21.5	38.1	67.5
15 M	18.8	337	3940	24.5	56.5	131	34.7	68.9	137
Hot water	15.0	31.9	67.8	19.1	47.4	118	12.7	26.6	55.6
Cold water	49.3	154	479	7.42	17.3	40.5	24.2	53.2	117

[a]95% confidence level.

[b]Potency expressed as μg CBWU/mg castor meal; each level equivalent to a 12.5 mm biological response. CBWU dose equivalent to 12.5 mm = 0.0163 μg.

reactor. The vessel was equipped with a double flight, ribbon type, variable drive agitator. The product temperature was monitored by a thermocouple imbedded in the mix. The meal from each batch was tray dried at 80 C to a residual moisture content of ca. 10%. The variables studied are shown in Table I.

A representative sample from each batch was collected. Ten (10) g of ground meal (Wiley mill, 40 mesh screen) were mixed with 200 ml of distilled water. After adjusting the pH to 5 (using HCl), the slurry was heated for 1.5 hr at 100 C. The extract was filtered through a Millipore filter (0.8μ). The liquid extract was used as whole extract or diluted with physiological saline solution.

The antigenicity of a test extract was determined by an abdominal intradermal injection using passively sensitized guinea pigs (9). Guinea pig response to a standard purified castor allergen preparation (CBWU) was used as a control to identify nonreactive pigs and effectiveness of the anti-castor serum (6). This preparation was also used as the standard for expressing potency of castor meals.

Analyses of variance were calculated for experiments 1-4 and potencies were calculated for the significant effects. Experiment 5 is discussed in more detail later. There are two reasons why most of the potencies do not show the significance exhibited in the analyses of variance (1). Potencies are calculated at a 12.5 mm response as opposed to an effect averaged over all dose levels (2). Analysis of variance allows calculation of a pooled error term over all data with removal of all sources of variation. the data are divided for calculation of individual regression to obtain potencies.

Referring to Table II and experiment 1, an increase in water and ammonia concentration seems to have resulted in greater antigen destruction. Yet the effect of increased water in experiment 2 shows increasing antigenicity. This same inconsistency with respect to ammonia concentration is shown in experiment 3 as compared to experiment 1.

These inconsistancies suggested that ammonia was doing more than simply catalyzing destruction of antigens. It had been assumed until now that all the antigens are water soluble. To determine whether some water insoluble antigens might be solubilized by ammonia treatment the following seven treatments were run. For the first five treatments fifteen 10 g samples of untreated castor meal were each treated with 200 ml NH_4OH of different molarities (1,2,4,6,15 M). The slurries were agitated at room temperature. The extraction times were 1.5, 6 and 24 hr.

The extracts of each of the above preparations were tested by the assay procedure described earlier (9). The dilutions ranged from 1:10 to 1:100,000 and two guinea pigs were used per dose.

Table III shows the potencies for each of the extracts. While most potencies of the ammonia extracts are greater than those of the hot (100 C) water extracts, the confidence limits are wide and the data do not present a consistent pattern. Also, the cold water extract potencies do not appear to differ from those of the ammonia extracts.

TABLE IV

Effect of Extraction Conditions on Purified Castor Antigen (CBWU)—Analysis of Variance

Source	df	mean square	F_{calc}	$F_{.05}$
Treatment	3	7.826	3.20	2.78
Log dose (linear)	1	1032.154		
Residual	55	2.444		

TABLE V

Effect of Extraction Conditions on Apparent Potency of Castor Antigen (CBWU)

Treatment	LCL[a]	Apparent potency[b,c]	UCL[a]
1.5 hr, 100 C	1.93	3.08	4.93
6 hr, 100 C	1.90	3.03	4.83
24 hr, 100 C	1.31	2.00	3.04
Room temperature	2.65	4.43	7.42

[a]95% confidence level.

[b]Assay: five guinea pigs per dose.

[c]μg CBWU/mg castor meal.

TABLE VI

Biological Responses (mm) for Experiment 5

Temperature, C	Liquid-solids	Dose[a]	$M_1(6)$ $\theta_1(1.5)$	$M_1(6)$ $\theta_2(.75)$	$M_2(10)$ $\theta_1(1.5)$	$M_2(10)$ $\theta_2(.75)$
		1:0	18.25	14.83	13.17	18.67
			14.50	15.83	16.33	16.75
	1:4	1:100	15.67	12.09	10.58	15.75
			14.00	15.75	13.25	15.83
		1:1000	12.67	7.42	8.75	4.50
			10.25	4.75	5.42	5.58
20						
		1:0	21.00	16.33	22.25	21.42
			20.58	15.92	18.50	22.25
	1:1	1:100	17.67	8.58	18.08	14.50
			16.67	10.00	9.25	12.67
		1:1000	10.08	9.42	9.33	7.50
			5.92	8.33	9.00	5.92
		1:0	20.83	18.50	14.83	19.58
			17.92	20.67	15.83	24.75
	1:4	1:100	15.83	15.67	13.42	12.08
			15.50	16.00	13.33	11.58
		1:1000	5.25	11.33	6.50	6.33
			10.42	8.50	3.75	6.17
50						
		1:0	14.75	16.33	19.25	19.83
			17.50	18.75	16.00	19.33
	1:1	1:100	4.50	15.75	8.00	9.17
			10.33	13.08	11.83	7.58
		1:1000	1.50	10.25	8.67	7.42
			0.0	7.75	7.08	8.83
		1:0	14.00	7.25	10.50	14.92
			12.58	8.00	14.92	13.75
	1:4	1:100	0.0	1.00	2.00	0.0
			0.0	0.67	2.42	0.0
		1:1000	0.0	0.0	0.0	0.0
			0.0	0.0	0.0	0.0
80						
		1:0	14.42	11.50	18.50	9.92
			14.00	11.00	16.25	13.92
	1:1	1:100	3.33	0.0	2.00	0.0
			0.0	0.0	0.67	0.0
		1:1000	0.0	0.0	0.0	0.0
			0.0	0.0	0.0	0.0

[a]Dose: Extract dilution in terms of castor pomace– 1:0 = 2500 μg, 1:100 = 25 μg, 1:1000 = 2.5 μg.

The wide range of confidence limits makes it difficult to demonstrate small differences, but this is not as troublesome in deactivation studies where the treatments reduce potencies up to .0001 or less of the original level. There is no evidence in this experiment that increased biological responses from more severe treatments can be explained by extraction differences. However, the assay extractions were made at room temperature as opposed to higher temperatures in the ammoniation process. The application of heat in the ammoniation process (Table I) may enhance peptide hydrolysis and produce components that are themselves biologically active.

To corroborate whether or not a hot water extraction changes the antigenicity content of a sample, four (14.8 mg) weighings of water soluble, highly potent CBWU lyophilizates were made. Each was dissolved in 200 ml distilled water. These were heated to 100 C for 1.5, 6 and 24 hr. The fourth, a control, was not heated. Three dose

TABLE VII

Analysis of Variance of Process Variables–Experiment 5

Source	df	mean square	F_{calc}	$F_{.05}$ req'd
Liquid-solids	1	0.01	0.00	3.93
Time	1	2.75	0.41	3.93
Temperature	2	1000.24	150.18	3.08
Molarity	1	0.60	0.09	3.93
Log dose (linear)	1	2955.52	443.75	3.93
Liq-sol x time	1	3.75	0.56	3.93
Liq-sol x temp.	2	30.54	4.59	3.08
Liq-sol x mol	1	11.36	1.71	3.93
Time x temp.	2	31.04	4.66	3.08
Time x mol	1	9.39	1.41	3.93
Temp x mol	2	15.40	2.31	3.08
Liq-sol x log dose (lin)	1	10.20	1.53	3.93
Time x log dose (lin)	1	0.25	0.04	3.93
Temp x log dose (lin)	2	19.88	2.99	3.08
Mol x log dose (lin)	1	38.01	5.71	3.93
Dose 2nd order interactions	9	11.27	1.69	1.96
Residual	114	6.66		

TABLE VIII

Potencies for Liquid-Solids x Temperature Subclasses—Experiment 5

	Liquid-Solids					
	.25			1.00		
Temperature, C	LCL[a]	Potency[b]	UCL[a]	LCL[a]	Potency[b]	UCL[a]
20	.159	.306	.591	.344	.681	1.35
50	.296	.581	1.14	.0953	.186	.362
80	.000611	.00224	.00819	.000880	.00303	.0105

[a]95% confidence level.

[b]μg CBWU/mg treated castor pomace.

TABLE IX

Potencies for Time x Temperature Subclasses—Experiment 5

	Time, hr					
	.75			1.5		
Temperature, C	LCL[a]	Potency[b]	UCL[a]	LCL[a]	Potency[b]	UCL[a]
20	.159	.306	.591	.344	.681	1.35
50	.277	.542	1.06	.102	.199	.387
80	.000472	.00180	.00689	.00114	.00376	.0124

[a]95% confidence level.

[b]μg CBWU/mg. Experiment 5 treatment.

TABLE X

Analysis of Variance of Response Data
From a Selected Ammonia Treatment With Batch Replication

Source	df	mean square	F_{calc}	$F_{.05}$ req'd
Batch	17	36.364	3.49	2.28
Dose	1	3016.889	289.42	4.45
Batch x dose	17	10.424	5.38	1.78
Pigs: batch x dose	72	1.938		

levels (1:10, 1:100, 1:1000) were tested using five guinea pigs per dose.

An analysis of variance of these data shows that the CBWU responses are indeed affected by the treatment (Table IV). A comparison of the potencies (Table V) shows that the hot water treatment for 1.5 hr as used in the routine procedure did not give significantly lower results than the room temperature extraction for 24 hr. However the longer the extract was heated at 100 C, the lower the potency. Thus the 24 hr, 100 C extract did give significantly lower potency results than the 24 hr, room temperature extract or the 1.5 hr, 100 C extract.

An advantage of the heat treatment during extraction is that if traces of ricin are present in the untreated meal it is readily detoxified by heat. Ricin would have an adverse effect on the response of the guinea pig. For this reason the use of the 1.5 hr, 100 C extraction conditions was continued as the routine procedure.

The assay responses obtained from experiment 5 of Table I are shown in Table VI. The analysis of variance is given in Table VII. The molarity x log dose (linear) interaction signifies nonparallelism of the dose response curves for the two molarities. There is no significant deviation from parallelism for the other process variables. The significant liquid-solids x temperature and time x temperature show that the effect of temperature is not expected to be constant for different liquid-solid ratios or different times. Examination of the potencies (Tables VIII and IX) shows the effect of these interactions to be negligible compared to the large temperature effect.

Based on the above results the following process was selected to prepare a sufficient quantity of deactivated meal for poultry and cattle feeding trials. Each batch, processed in the Patterson vessel, consisted of 9 kg of castor pomace as received and 2.25 liters of 6 M NH_4OH. The process temperature was set at 80 C for 45 min (see Table VI). Eighteen batch replicates were made. Each batch was assayed using the standard hot water extraction procedure with two guinea pigs per dose at three dose levels.

An analysis of variance of these data (Table X) shows a significant difference in batch variance. In terms of variance components shown in Table XI, 6.67% of the total variance is due to batch preparations. This value is almost twice that given for the assay error item, i.e., pigs within batch x dose. The implication is that process control can be improved to reduce batch variability. Until this variability is reduced it would be advisable to assay every batch. Comparative potency values for the untreated castor meal, an ammonia treated meal and a steam treated meal are given in Table XII. The potency estimate for the ammonia treatment was calculated from the same data used to prepare Table X and

TABLE XI

Variance Components for a Selected
Ammonia Treatment With Batch Relication

Source	Variance component	Per cent total variance
Batch	4.323	6.67
Dose	55.675	85.96
Batch x dose	2.829	4.37
Pigs: batch x dose	1.938	2.99
Total	64.765	

TABLE XII

Comparison of Steam and Ammonia Treatments for Deallergenation

Treatment	LCL[a]	Potency[b]	UCL[a]
Untreated castor	0.91	4.13	8.92
Ammonia treated castor[b]	0.0153	0.0177	0.0204
Steam treated castor[c]	0.00353	0.0192	0.105

[a]95% confidence level.

[b]Potency ratio expressed as μg CBWU/mg castor meal; each level equivalent to a 12.5 mm biological response. CBWU dose equivalent to 12.5 mm = 0.0163 μg.

[c]Process: ammonia, T = 80 C, R = .75, M = 6, θ = 45 min.

[d]Process: steam, P = 10 psig, R = 0.5, θ = 1 hr (data from Reference 9).

XI. The potency estimate for the steam treatment is from a favorable process reported earlier (6). It can be seen that there is good potency agreement between the ammonia and steam treatments.

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