Castor Meal Antigen Deactivation: Pilot Plant Lime Process

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ABSTRACT

Castor meals treated with $Ca(OH)_2$ to reduce their allergen content have been re-evaluated using a more sensitive biological assay procedure. Results indicate that treatment with 4% lime at 120 C for 15 min should produce optimal deallergenization. Amino acid analysis of treated products shows that steam and lime deallergenization procedures tend to destroy more labile amino acids than ammonia treatment does.

INTRODUCTION

The residue remaining after the oil has been expelled from castor beans is called pomace or castor meal. This meal is an excellent source of protein and carbohydrate. It could become an important cattle and poultry feed supplement were it not for the presence of the highly potent castor antigens (1). These low molecular weight protein-carbohydrate complexes have acted as specific irritants to hypersensitive individuals causing fatal or near fatal allergic reactions (2).

A process developed at this laboratory using $Ca(OH)_2$ to reduce castor antigenicity was described previously (3). The biological assay using an iv antigen challenge resulted in such variable responses that an optimum process could not be determined (3). An intradermal procedure has since been described that is more sensitive and much less variable than the iv challenge (4). The original work reported in Reference 3 was re-evaluated using the ID assay technique.

It is the purpose of this paper to describe a pilot plant process using $Ca(OH)_2$ to deactivate these castor meal antigens.

EXPERIMENTAL PROCEDURES

Castor Meal Treatment

The meal "as received" was essentially free of ricin due to destruction of this toxin during the desolventizing process. It was placed in a steam-jacketed Patterson reactor. A horizontal double acting helical flight provided the required agitation. The product temperature was monitored by a thermocouple imbedded in the mix. The pomace weight of each run was constant at 3.35 kg. Lime slurries of 4, 6 or 8% based upon the weight of the dry meal were prepared. Using water, liquid-solids ratios of 2:1 and 3:1 were investigated. Three levels of time (15, 30 and 60 min) and three levels of temperature (100, 120 and 140 C) were studied.

After processing, each batch was cooled to ambient temperature and then acidified (5M H_3PO_4) to a final pH of 5. The material was tray-dried at 80 C to a moisture content of ca. 10%.

Assay Sample Preparation

The residual antigens were obtained from an extraction

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TABLE I

Analysis of Variance

Source of variation	df	Mean square deviation	$F_{calculated}$[a]	$F_{.05}$[b]
Concentration (C)	2	75.5592	20.64	3.03
Liquid/solid ratio (R)	1	10.6241	2.90	3.88
Temperature (T)	2	15.6418	4.27	3.03
Time (θ)	2	9.5495	2.61	3.03
Log dose linear (D_ℓ)	1	9758.92	2665.52	3.88
C x R	2	10.5528	2.88	3.03
C x T	4	3.3815	0.92	2.40
C x θ	4	14.0559	3.84	2.40
R x T	2	8.4592	2.31	3.03
R x θ	2	0.1379	0.04	3.03
T x θ	4	6.7319	1.84	2.40
C x D_ℓ	2	9.1859	2.51	3.03
R x D_ℓ	1	11.5324	3.15	3.88
T x D_ℓ	2	1.1265	0.35	3.03
O x D_ℓ	2	3.8525	1.05	3.03
Residual	290	3.6612	—	—

[a]Calculated variance ratio.

[b]Tabulated variance ratio at 5% significance.

TABLE III

Potencies for Temperature Subclasses

Process temperature, C	Dose, μg[a]	95% LCL[a]	Potency[a]	95% UCL[a]
100	867	0.016	.019	0.022
120	1011	0.014	.016	0.019
140	782	0.018	.021	0.024

[a]See Table II for definitions.

TABLE II

Potency for (C x θ) Interaction Subclasses

θ, Min	C, % $Ca(OH)_2$	Dose, μg[a]	95% LCL[b]	Potency[c]	95% UCL[b]
15	4	1234	0.010	0.013	0.018
	6	1106	0.011	0.015	0.020
	8	650	0.020	0.025	0.032
30	4	1294	0.009	0.013	0.017
	6	597	0.021	0.027	0.035
	8	633	0.020	0.026	0.033
60	4	1061	0.012	0.015	0.020
	6	948	0.013	0.017	0.023
	8	741	0.017	0.022	0.028

[a]Dose (μg) equivalent to a 12.5 mm biological response.

[b]Lower and upper confidence limits.

[c]Potency - μg CBWU/mg treated meal.

TABLE IV

Proximate Analysis of Castor Meals

	Total solids,[a] %	N, %	Crude fat, %	Crude fiber, %	Ash, %	Ca, %	PO_4, %
Untreated	90.4	5.9	4.34	23.8	7.8	.63	—
Lime-treated	94.3	5.35	4.39	25.1	13.9	2.81	2.53
Steam-treated	92.5	5.25	1.52	30.3	8.5	.54	.75
Ammonia-treated	93.6	6.39	2.22	25.1	9.2	.59	.84

[a]All values except total solids are reported as percentages on a moisture-free basis.

TABLE V

Amino Acid Composition of Castor Meal Samples, Grams Amino Acid per 100 g Meal

Amino acid	Cell 37[a]		Cell 39[a]		Cell 40[a]		Ammonia[b]		Steam[c]		Untreated castor meal	
	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$	$\bar{x}$	$S\bar{x}$
LYS	.698[d]	.021	.760[d]	.016	.736[d]	.030	.867[d]	.030	.752[d]	.042	.999	.030
HIS	.599[d]	.017	.652	.013	.658	.024	.775	.024	.563[d]	.034	.677	.024
AMM	.720[d]	.010	.735	.008	.765	.015	1.250	.015	.652[d]	.021	.756	.015
ARG	2.674[d]	.062	3.040[d]	.047	2.647[d]	.087	3.889	.087	2.854[d]	.124	3.736	.087
ORTH	.216	.008	.103	.008	.258	.011	.123	.011	.088	.015	e	e
ASP	3.033[d]	.034	3.071	.025	2.995[d]	.047	3.369	.047	2.637[d]	.067	3.141	.047
THR	.733[d]	.012	1.044[d]	.009	.656[d]	.018	1.228	.018	.972[d]	.025	1.151	.018
SER	1.036[d]	.018	1.643[d]	.014	.948[d]	.026	1.987	.026	1.544[d]	.037	1.884	.026
GLU	6.337[d]	.055	6.431	.042	6.231[d]	.078	6.595	.078	5.050[d]	.110	6.562	.078
PRO	1.084[d]	.039	1.049[d]	.029	1.077[d]	.055	1.225	.055	.980[d]	.077	1.275	.055
GLY	1.589	.021	1.512	.016	1.599	.029	1.499	.029	1.180[d]	.041	1.495	.029
ALA	1.430	.023	1.427	.017	1.400	.032	1.496	.032	1.216[d]	.045	1.437	.032
CYS	.212	.009	.183	.009	.192	f	.383	f	.537	f	.713	f
VAL	1.946	.041	1.960	.031	1.848	.059	2.012	.059	1.685[d]	.083	1.955	.059
MET	.563	.003	.592	.003	.534	f	.656	f	.491	f	.617	f
ILE	1.578[d]	.019	1.631	.014	1.540[d]	.026	1.695	.026	1.363[d]	.037	1.673	.026
LEU	2.121[d]	.022	2.132[d]	.017	2.070[d]	.032	2.222	.032	1.752[d]	.045	2.201	.032
TYR	.775	.039	.838	.029	.832	f	.835	f	.547	f	.815	f
PHE	1.194[d]	.025	1.272	.019	1.216[d]	.036	1.407	.036	1.079[d]	.050	1.314	.036

[a]See Figure 1 of Reference 3: cell 37 4% lime, 100 C, 15 min, 2:1 water-meal ratio; cell 39 4% lime, 100 C, 60 min, 2:1 water-meal ratio; cell 40 4% lime, 120 C, 15 min, 2:1 water-meal ratio.

[b]Ammonia treatment: 1 part 6M NH_4OH to 4 parts meal, 80 C, 0.75 hr.

[c]Steam treatment: 80 psig, 60 min, 1:2 water-meal ratio.

[d]These mean values are significantly different from the values for the untreated castor meal at the 5% level.

[e]Not run.

[f]No replication.

of a 10 g batch sample in 200 ml distilled water (4). The extract was acidified to pH 5 using HCl, then boiled for 1 hr, 30 min. The slurry was filtered through an 0.8μ Millipore filter. The liquid extract or dilutions of it in physiological saline solutions were used for the biological assay. In the original work, the extract was lyophilized before testing (3).

Biological Assay

White guinea pigs were sensitized essentially by the procedure reported by Mottola et al. (4). In the previous work (3), the antigen challenge was systemic via the cephalic vein using a single dose level (20 mg lyophilized extract). The present assay is based on an intradermal antigen challenge at the site previously sensitized by anticastor serum. Three liquid dose levels (1:0, 1:10 and 1:100) were used to test for residual antigenicity in each batch processed. Whole extract (1:0) is equivalent to 2.5 mg castor meal. An iv injection of Evan's blue dye is required to make the antigen-antibody reaction site visible. The average dimensions of the diffused dye are used as the response variable.

Amino acid analyses were carried out by the ion exchange method of Spackman et al. (5). Details of the protein hydrolysis and amino acid procedures are described under procedure B by Kohler and Palter (6).

RESULTS

An analysis of variance of the experimental data is given in Table I. Of the five main effects, neither θ (process time) nor R (liquid solids ratio) has an apparent overall effect upon the biological response, at least not over the range tested. There are no significant interactions between D_0 (the log dose linear response) and the main effects, i.e., C, R, T and θ. The implication here is that there is no significant deviation from parallelism for the dose response lines of the different treatments. This is contrary to results for our steam process (7). The main effect of lime concentration is significant. Also the interaction of concentration with time is significant. Potencies (μg CBWU standard per mg meal) are shown in Table II. In every case, the potency at 4% lime concentration is lower than that at 6 or 8%. Since the potency at the 4%, 15 min process time is not significantly different from that at 4%, 30 min, the shorter process time is preferable, i.e., 15 min. The significant temperature effect is exhibited by potencies in Table III. Although a linear response was expected, these data suggest a quadratic with a minimum potency (maximum destruction) near the 120 C temperature level. If this effect should prove to be a real one it could be due to production of antigens at a rate greater than destruction. Such an effect was noted in the case of ammonia treatments described earlier (7). Without further confirmation, the process temperature was tentatively selected at the 120 C level.

Since the primary objective of castor deallergenization is to prepare a safe-to-handle nutritious feedstuff, typical proximate analyses of treated and untreated meals are

shown in Table IV. The amino acid content of several types of deallergenized meals are compared in Table V. Castor meals deallergenized by the lime process were compared with samples deallergenized by steam alone (7) and steam and ammonia (8). The data are adjusted to g/100 g material and the lime data corrected for extra ash.

The results show marked variations in degree of destruction of specific amino acids by the various processes. With respect to cystine, destruction was greatest in the case of the lime process and least in the case of the steam process. As for the hydroxy amino acids, serine and threonine, the lime treatment was the most destructive while the ammonia treatment was the least. Lime treatment caused conversion of a significant amount of arginine into ornithine, but this did not completely account for arginine losses either during lime or steam processing. Both lime and steam treatments destroyed significant amounts of methionine while the ammonia process did not. From the loss figures it is clear that the nutritive value of the protein for poultry and swine is decreased by all procedures. This suggests that ruminant feeds are perhaps a more promising outlet for deallergenized castor meal. However its use in the rations of monogastric animals is not precluded and will depend on the relative costs of other amino acid and energy sources.

It will be noted that the treatments we are now considering to be close to optimum are much milder than those suggested in our earlier paper (3). This shift is based on the use of the more sensitive and reproducible assay used in the present work (4).

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REFERENCES

1. Ridlehuber, J., Oil Mill Gazeteer 1968:62.
2. Aspen, E.M., Jr., W.C. Cooper, R.J.M. Horton and L.D. Scheel, "Health Aspects of Castor Bean Dust," USDHEW publication no. 999-AP-36, 1967, p. 28.
3. Mottola, A.C., A.P. Hendrickson, D.E. O'Connell, R. Palter and G.O. Kohler, Agr. Food Chem. 16:725 (1968).
4. Mottola, A.C., L. Eldridge, V. Herring and G.O. Kohler, JAOCS 47:458 (1970).
5. Spackman, D.H., W.H. Stein and S. Moore, Anal. Chem. 30:1190 (1958).
6. Kohler, G.O., and R. Palter, Cereal Chem. 44:512 (1967).
7. Mottola, A.C., B. Mackey and V. Herring, JAOCS 48:510 (1971).
8. Mottola, A.C., B. Mackey, V. Herring and G.O. Kohler, Ibid. 49:101 (1971).

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