Pilot Plant Deactivation of Castor Meal Antigen

Lime Process

A. C. Mottola, A. P. Hendrickson, D. E. O'Connell, Rhoda Palter, and G. O. Kohler

Castor beans contain a powerful allergenic protein fraction which severely limits the usefulness of the meal after oil extraction. The solvent-extracted meal or pomace cannot be handled safely by people subject to allergic sensitization. The allergen can be destroyed by treatment with lime. A factorial experiment under pilot plant conditions was carried out to determine the process factors that affect the deactivation of these antigens, and a regression equation correlating these factors to a guinea pig bioassay response was developed. A process for deactivating the castor meal antigens consists of treating the meal with three times its weight of water and 8% of its weight with lime. The mixture is heated to 140° C. for 60 minutes and then acidified with H_3PO_4 to pH 5 prior to drying.

Castor beans contain a number of harmful components that can severely limit the usefulness of the residual meal left after the oil is extracted. These include a violently poisonous but heat-labile protein, ricin; a mildly toxic alkaloid, ricinine, present only in small quantities; and a powerful allergen. The allergen fraction ranges from 6 to 9% of the weight of the pomace (Coulson *et al.*, 1960) and is relatively stable and difficult to deactivate. The ricin component ordinarily is destroyed during desolventizing, and too little ricinine is usually present to present serious difficulties.

The castor bean meal must be made safe for people handling it before it can be used widely as a feed ingredient or industrial intermediate. Efforts have been made to deactivate the allergenic factor. Gardner *et al.* (1960) describe the following treatments as promising: dry-heating the pomace to 400° F. for 125 minutes or cooking under various conditions with alkali or acid, with or without added formaldehyde.

Spies *et al.* (1963) described a procedure to inactivate ricin and the castor bean allergens using $Ca(OH)_2$. Layton (1966) developed a procedure using ammonium hydroxide. Jenkins (1963) described a steam-cooking process. All of the reported results have been based on laboratory investigations using small quantities of materials with no assurance that the findings could be applied directly to commercial-scale operations, and in some cases the assay procedures lacked the sensitivity of the method used in this work. The work reported here was carried out to bridge the gap between small-scale laboratory studies and commercial application.

The primary objective has been to develop an industrial antigen-deactivation process, which, it is hoped, will retain or enhance the feed value of the meal. Castor bean meal contains about 35% protein; with decortication it can be increased to over 60%.

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Albany, Calif. 94710

The use of lime has certain inherent advantages. Calcium phosphate produced in the neutralization step can supply the calcium and phosphorus requirements of livestock.

EXPERIMENTAL

Design of Experiment. In an attempt to define the process variables, a factorial experiment was designed to determine the effect of time (θ), temperature (T), and lime concentration (C) on antigen deactivation. Since this is a heterogeneous system, the ratio (R) of liquids to solids was also considered.

To determine the effect of each of these variables, three levels of time, temperature, and lime concentration were selected with two levels for the liquid-solids ratio. Figure 1 shows the factorial design. Each cell, numbered 1 to 54, represents an experiment using a combination of all four process variables.

Processing of Meal. The vessel available for these experiments was a steam-jacketed Patterson reactor (13-gallon capacity), equipped with a horizontal, variable-drive, ribbon-type agitator. Product temperature was controlled manually in accordance with the response of an iron-constantan thermocouple embedded in the product mix. The batch weight for each run was constant at 3.35 kg. Lime slurries of 4, 6, or 8% were prepared, using enough water to form a liquid–pomace solids ratio of 2 to 1 or 3 to 1. The lime percentage were based upon the weight of dry meal.

After the required 15-, 30-, or 60-minute process time had elapsed, the jacket steam pressure was released, and a bleed valve to the vapor space within the vessel was opened. Each batch was cooled to ambient temperature and then acidified with $5M$ H_3PO_4 to a final pH of 5. The product was tray-dried at 80° C. to a residual moisture level of about 10%. A total of 54 batches was made.

Assay for Antigenicity. A number of methods are available for determining the presence of protein antigens–e.g., the Schultz-Dale technique, ring test, and precipitin test (Gardner *et al.*, 1960). The effectiveness of the

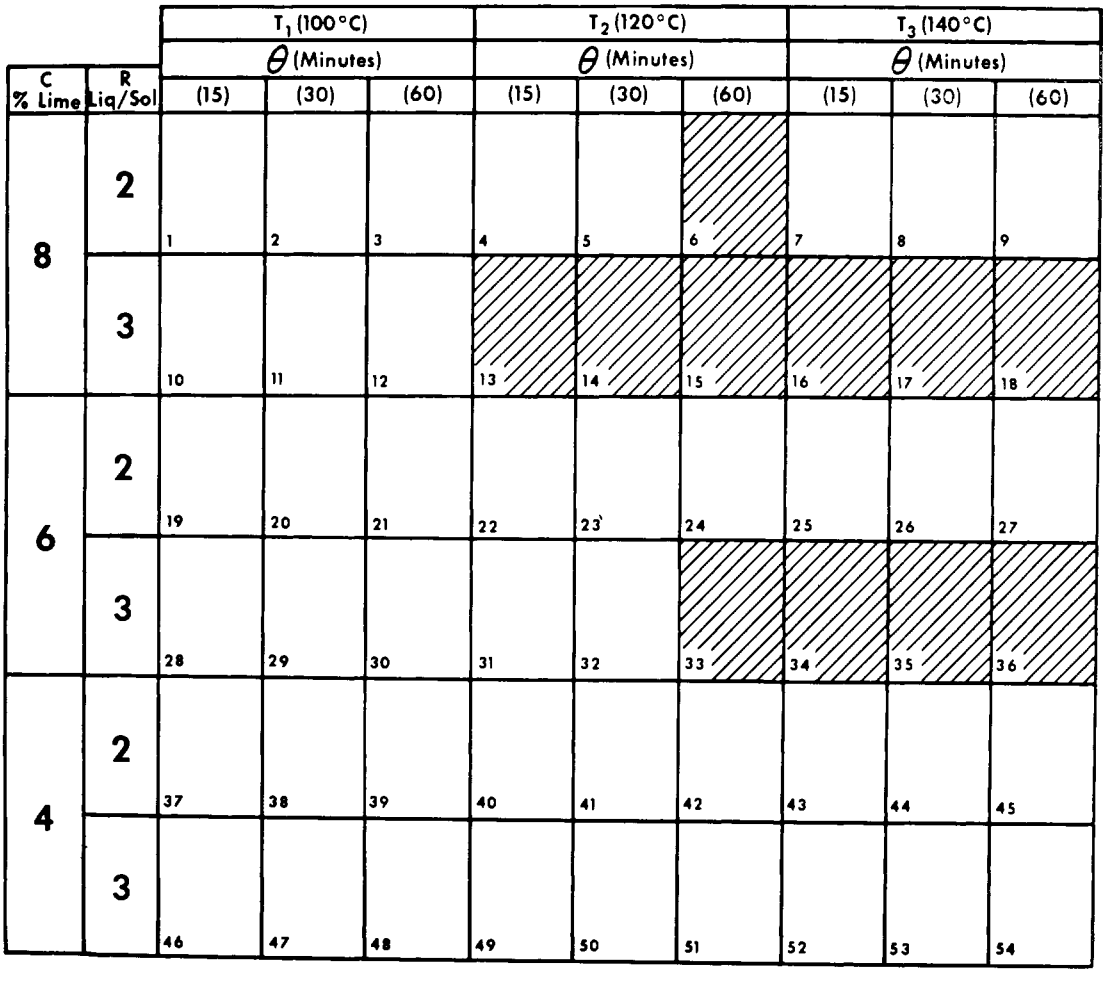

Figure 1. Factorial design for lime-treated castor meal

antigen deactivation treatments in our work was evaluated exclusively by a bioassay technique referred to as the passive cutaneous anaphylaxis (PCA) test (Layton *et al.*, 1961; Ovary, 1958).

A representative sample from each batch was ground through a 2-mm. screen using a Wiley mill. Two hundred milliliters of distilled water was added to 10 grams of this meal. The slurry was thoroughly mixed, carefully adjusted to a final pH of 5 by the addition of HCl, then heated to 100° C. with constant agitation for 90 minutes. Under these conditions, Spies *et al.* (1960) report that more than 32 hours are required to effect the precipitin reaction. After cooling, the slurry was filtered through an 0.8-micron Millipore filter to remove nonspecific particulate material that hindered interpretation of subsequent guinea pig bioassay observations. Unless the extract was filtered, many of the test animals died from anaphylactoid shock. Approximately 160 ml. of filtrate was lyophilized for antigen assay.

The relative destruction of the antigens in the treated meal was determined by a modified passive cutaneous anaphylaxis method (Layton *et al.*, 1961) as follows.

Young male or female albino guinea pigs weighing from 275 to 325 grams were selected for the test. The abdominal hair of the test animal was removed with electric clippers so that the skin was clearly visible. The abdomen was marked with a red wax pencil into four quadrants.

Two diagonal sites were intradermally injected with 0.05 ml. of a standardized rabbit anticastor serum (Layton *et al.*, 1961). The remaining sites were injected with serum from a normal nonsensitized control rabbit. After a waiting period of $2\frac{1}{2}$ to 3 hours, the animal was placed on a small operating table. The hair was clipped from the foreleg just above the wrist to locate the cephalic vein.

One hundred milligrams of the lyophilizate, prepared as described above, was dissolved in 1 ml. of isotonic solution, and centrifuged to remove trace colloidal particles. An intravenous injection of 1 ml. of 0.5% Evans blue dye (Merck Index, 1952) in normal saline solution was made. Then, 0.2 ml. of the above prepared isotonic saline solution containing 20 mg. of the lyophilized castor meal extract was injected into the same vein.

A positive reaction for PCA was indicated within 2 to 5 minutes by the bluing of the skin at the sites injected with rabbit anticastor serum. The depth of the color is proportional to the antigen content (Layton *et al.*, 1961; Ovary, 1958). The control sites injected with serum from nonsensitized rabbits remained uncolored.

Table I. Bioassay Data

Cell No.	Process Parameters				Analytical				
	C	R	T, °C.	θ, Min.	No. of assays	Hi PCA	Lo PCA	Av. PCA	$\hat{Y}$[a]
1	8	2	100	15	2	2	0.5	1.3	2.58
2				30	5	4	0	1	2.23
3				60	4	4	1	2	1.51
4	8	2	120	15	2	3	2	2.5	1.24
5				30	3	2	1	1.7	1.06
6				60	4	2	1	1.3	0.71
7	8	2	140	15	3	1	0.5	0.7	1.05
8				30	4	3	1	1.5	1.05
9				60	2	0.5	0	0.3	1.05
10	8	3	100	15	2	4	2	3	2.07
11				30	2	2	1	1.5	1.71
12				60	2	1	1	1	1.00
13	8	3	120	15	2	1	0.5	0.8	0.73
14				30	5	3	0	0.7	0.55
15				60	2	0.5	0	0.3	0.19
16	8	3	140	15	2	0	0	0	0.53
17				30	2	0.5	0	0.3	0.53
18				60	2	2	1	1	0.54
19	6	2	100	15	2	4	3	3.5	3.17
20				30	2	4	3	3.5	2.81
21				60	2	3	2	2.7	2.10
22	6	2	120	15	3	3	0.5	2.2	1.44
23				30	6	4	0	1.3	1.27
24				60	2	0	0	0	0
25	6	2	140	15	5	4	0	1	0.87
26				30	2	0	0	0	0.87
27				60	2	1	0.5	0.8	0.87
28	6	3	100	15	2	4	3	3.5	3.00
29				30	3	4	3	3.3	2.65
30				60	2	2	0	1	0.86
31	6	3	120	15	2	1	0	0.5	2.97
32				30	2	0.5	0	0.3	1.10
33				60	2	1	0.5	0.8	0.73
34	6	3	140	15	2	1	0	0.5	0.70
35				30	2	0.5	0.5	0.5	0.70
36				60	2	1	0	0.5	0.71
37	4	2	100	15	2	4	3	3.5	3.75
38				30	2	3	3	3	3.40
39				60	2	4	3	3.5	2.69
40	4	2	120	15	2	0.5	0.5	0.5	1.65
41				30	2	0.5	0	0.3	1.47
42				60	2	1	1	1	1.11
43	4	2	140	15	3	1	0.5	0.8	0.69
44				30	4	3	1	1.5	0.69
45				60	2	1	0	0.5	0.69
46	4	3	100	15	2	4	4	4	3.94
47				30	2	4	4	4	3.58
48				60	2	4	1	2.5	2.87
49	4	3	120	15	3	4	2	3	1.83
50				30	4	4	0.5	1.6	1.65
51				60	3	1	0	0.3	1.29
52	4	3	140	15	2	1	0	0.5	0.87
53				30	5	1	0.5	0.6	0.87
54				60	4	3	0	1.6	0.87

[a] $\hat{Y}$ = Predicted response values using regression equation.

Table II. Least Squares Analysis of Variance

Source of Variance	Sum of Squared Deviations	Degrees of Freedom	Mean Squared Deviations	F_{calcd}[a]	$F_{0.05}$[b]
Main					
Temperature, T	18.3817	1	18.3817	16.005	3.92
Temperature, T^2	10.7985	1	10.7985	9.403	
Time, θ	5.6341	1	5.6341	4.906	
Concentration, C	3.9566	1	3.9566	3.445	
Liquid–solids ratio, R	1.9211	1	1.9211	1.673	
Interaction					
Concn. × temp., CT	9.2298	1	9.2298	8.037	
Temp. × time, $T\theta$	4.2611	1	4.2611	3.710	
Concn. × ratio, CR	2.9314	1	2.9314	2.552	
Mean	296.1267	1	296.1267	257.847	
Reduction	406.2080	9	45.1342	39.300	
Error	155.0420	135	1.1485	...	
Total	561.25	144	...	...	

[a] Calculated variance ratio.
[b] Tabular variance ratio at 95% confidence level.

Table III. Process Cells Indicating a PCA of 1 or Less

Cell No.	C	T	R	θ	$\hat{Y}$[a]	$S_{\hat{Y}}$[b]	$\hat{Y}_{bi}$[c]
6	8	120	2	60	0.69	0.32	1.22
13	8	120	3	15	0.71	0.35	1.28
14	8	120	3	30	0.53	0.28	0.99
15	8	120	3	60	0.18	0.37	0.79
16	8	140	3	15	0.52	0.42	1.21
17	8	140	3	30	0.52	0.36	1.11
18	8	140	3	60	0.52	0.46	1.28
33	6	120	3	60	0.73	0.30	1.23
34	6	140	3	15	0.68	0.35	1.25
35	6	140	3	30	0.68	0.30	1.18
36	6	140	3	60	0.69	0.36	1.29

[a] Predicted mean PCA.
[b] Estimate of standard error.
[c] $Y + t_\alpha S_y$ = upper fiducial limit where:
$T\alpha(n - P - 1) = t_{0.05}(135) = 1.65$;
n = 144 assay observations (based on entire experiment of 54 treatments with unequal assay numbers);
$p = \delta_{\equiv}$ number of equation coefficients.

RESULTS AND DISCUSSION

Castor meal antigen deactivation was evaluated in terms of a dilution factor related to the untreated castor meal. Antigenicity of the untreated castor meal was determined by a dilution sequence, whose end point was the lowest concentration of extract eliciting a PCA response of 1+. Figure 2 shows the PCA response to raw castor meal as a function of the amount of castor meal extract injected. For a PCA response of 1+, 0.0023 mg. of untreated castor solids was required. If the treated meal extract, injected at the 20-mg. level, yielded a response of 1+ or less, this was equivalent to an antigen content of 0.0001 or less of the original meal.

Because of assay replication of a few selected runs, which resulted in an unbalanced experimental design, the least squares analysis of data with unequal subclass numbers was used (Harvey, 1960).

The bioassay responses of 144 guinea pigs are summarized in Table I, where C is per cent $Ca(OH)_2$; R is liquid to solids ratio; T is process temperature, °C.; θ is process time, minutes; PCA is guinea pig response; and cell no. refers to the factorial chart (Figure 1). The analysis of variance of these data (Table II) indicated the following experimental factors to be significant (α = 0.05) for antigen deactivation.

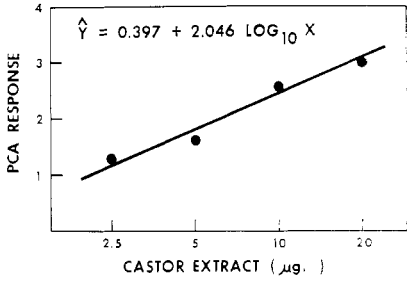

Figure 2. Potency of untreated castor meal

Main Effects	Interactions
Temperature, °C., T	Concn. × temp. (CT)
Time, minutes, θ	

A quantitative relationship relating all of the process parameters to the dependent guinea pig bioassay response ($\hat{y}$) was developed (Snedecor, 1962), leading to the following equation:

$$\hat{y} = 36.091863 - 0.906199C + 0.882094R$$

$$- 0.469852T + 0.00144T^2 - 0.083324\theta -$$

$$0.174674CR + 0.009620CT + 0.000596T\theta$$

with a multiple correlation coefficient 0.65 ($P < 0.001$).

By substituting the various process conditions used in the original experiment in this equation, PCA value was calculated for each cell of the factorial design. Cells and conditions that showed calculated values of 1+ or less are listed in Table III, which also shows the standard errors of estimates and the upper PCA limit at 95% confidence level.

The analysis of the data suggests 11 different process combinations that should give mean PCA's of less than 1+. The shaded areas in Figure 1 are in the range of generally satisfactory responses. Cell 18 at 140° C. for 60 minutes using 8% lime at 3-to-1 liquid-solids ratio is the most conservative choice. Using the process conditions shown for cell 18, five replicate batches were made and tested. The average PCA response was 0.8, with a standard deviation of 0.45.

The effect of the process treatment upon the amino acids of treated castor meal (cell 18) was evaluated by a liquid column chromatographic procedure developed by Moore *et al.* (1958). Cystine and methionine were determined by a performic acid oxidation method (Moore, 1963). The amino acid composition of the untreated castor meal and the treated meal (cells 13, 14, and 18) is shown in Table IV. Cystine, serine, threonine, arginine, and lysine are adversely affected by the process. The loss of these essential amino acids precludes the use of this meal in rations for poultry and swine. The product apparently would be most useful in ruminant rations, since cattle and sheep do not have the essential amino acid requirements of monogastric animals. The total nitrogen content is decreased by the process because of loss of ammonia and dilution with calcium phosphate. Further work on the nutritional aspects of allergen-deactivated meals is in progress.

It cannot be stated unequivocally that a product which gives a PCA test of 1+ or less under the conditions of the test would be safe for human handling under all conditions. Panzani and Layton (1963) and Spies *et al.* (1960) have made a few clinical studies on human subjects. Further work is necessary to relate antigenicity as measured by the guinea pig PCA tests with allergenicity in human subjects.

Table IV. Partial Amino Acid Analysis
(Grams of amino acid per 16 grams of N)

	Raw Castor	Cell[a]		
		13	14	18
Lysine[b]	2.85	1.65	1.73	1.72
Histidine[b]	1.89	1.59	1.68	1.63
Ammonia	2.04	2.21	2.37	2.35
Arginine[b]	7.17	5.12	5.07	4.34
Aspartic acid	8.50	8.29	8.52	8.50
Threonine[b]	3.18	1.09	0.95	0.73
Serine	5.30	1.38	1.16	0.79
Glutamic acid	17.82	16.44	17.26	17.81
Proline	3.39	3.12	3.35	3.42
Glycine[b]	4.13	4.66	5.00	5.13
Alanine	3.92	4.00	4.35	4.60
Cystine	2.14	0.18	0.25	0.33
Valine[b]	5.47	5.23	5.65	5.73
Methionine[b]	1.67	1.43	1.66	1.63
Isoleucine[b]	4.66	4.29	4.46	4.38
Leucine[b]	5.80	5.68	6.01	6.05
Tyrosine	1.82	1.94	2.20	1.76
Phenylalanine[b]	3.41	3.36	3.55	3.37
% N recovered	84.20	70.67	73.90	72.30
% N sample (dry)	6.27	3.37	3.98	4.17

[a] Process conditions described in Figure 1.
[b] Essential for normal chick growth.

ACKNOWLEDGMENT

Appreciation is expressed to Kenneth Smith for his work in preparing the batches for this experiment and to Frank Naughton, Development Department, Baker Oil Co., for furnishing the raw materials.

LITERATURE CITED

Coulson, E. J., Spies, J. R., Stevens, H., *J. Am. Oil Chemists' Soc.* **37,** 657 (1960).
Gardner, H. K., Jr., D'Aquin, E. L., Koltun, S. P., McCourtney, E. J., Vix, H. L. E., Gastrock, E. A., *J. Am. Oil Chemists' Soc.* **37,** 142 (1960).
Harvey, W. R., "Least Squares Analysis of Data with Unequal Subclass Numbers," U. S. Dept. Agr. **ARS-20-8** (July 1960).
Jenkins, F. P., *J. Sci. Food Agr.* **14,** 773 (1963).
Layton, L. L., U. S. Patent **3,294,776** (Dec. 27, 1966).
Layton, L. L., Lee, S., Dante, B. T., DeEds, F., *J. Am. Oil Chemists' Soc.* **38,** 597 (1961).
Merck Index of Chemicals and Drugs, 6th ed., p. 422, Merck and Co., Rahway, N. J., 1952.
Moore, S., *J. Biol. Chem.* **238,** 235–7 (1963).
Moore, S., Spackman, D. H., Stein, W. H., *Anal. Chem.* **30,** 1185 (1958).
Ovary, Z., *Progr. Allergy* **5,** 459 (1958).
Panzani, R., Layton, L. L., *Intern. Arch. Allergy* **22,** 350 (1963).
Snedecor, G. W., "Statistical Methods," Chap. 14, "Multiple Regression and Covariance," pp. 413–45, Iowa State College Press, Ames, Iowa, 1962.
Spies, J. R., Coulson, E. J., Bernton, H. S., Stevens, H., Strauss, A. A., *Ann. Allergy* **18,** 393 (1960).
Spies, J. R., Coulson, E. J., Wells, P. A., U. S. Patent **3,101,266** (Aug. 20, 1963).

Received for review December 11, 1967. Accepted June 24 1968. Division of Agricultural and Food Chemistry, 155th Meeting, ACS, San Francisco, Calif., March 1968. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.