mulls of the isolated nonionics, fatty alcohols from hydrolyzed alkyl sulfates, and alkylaryl sulfonates.

Discussion

Table VI lists data (in duplicate) for quantitative analysis of the actives. It is evident that the method has given good results compared to theory for neutral oils, nonionics, sodium dodecylbenzene sulfonate, and sodium xylene sulfonate contents found. Neutral oils from Detergents B and D were contaminated with fatty amides and therefore were saponified with alcoholic NaOH. The purified neutral oils were recovered, following petroleum ether extraction. Detergent C neutral oil was contaminated with fatty alcohol-EO condensate. In this case the purified neutral oils were extracted with petroleum ether, following precipitation of the nonionic with phosphotungstic acid. Amounts of contaminants removed from the neutral oils were added to the nonionics recovered from the ion exchange effluent.

Although good values were obtained for nonionic contents, in some cases the residue from the ion exchange effluent appeared to be slightly contaminated with resin. Dissolving the effluent residue in isopropyl alcohol, filtering, and evaporating the alcohol appeared to correct this error. Alkyl sulfate content was determined by difference: total anionics less the sum of alkylaryl sulfonate and hydrotrope. Good results are shown for the alkyl sulfate in Detergent B, but values for Detergent D are high. It is possible that the sample may have been contaminated with ion exchange resin at this point. Calculating the alkyl sulfate content by loss of anionic weight before and directly after hydrolysis may have provided better results. No significant amounts of carboxylates (fatty acids) were found.

Fatty alcohols from the hydrolysis were analyzed for total active hydrogen content. They are in agreement within limits of test precision with values for alcohols from the original samples. Low recovery for fatty alcohol from Detergent B may result from volatilization during drying of the extract.

Combining weights of the sodium dodecylbenzene sulfonates recovered are in good agreement with theory except those for Detergent C, which are a little low.

Infrared absorption spectra of the nonionics, sodium dodecylbenzene sulfonates, and fatty alcohols from hydrolysis of alkyl sulfates were generally comparable to spectra for the original samples of actives.

The scheme presented will provide suitable commercial analytical data for active ingredients from detergent compositions. Actives composed of mixed anionics and nonionics can be quantitatively analyzed. In case a mixture of fatty alkanolamide and ether type nonionic are recovered in the ion exchange effluent, they can be separated following saponification of the amide and extraction of the fatty acid. On the other hand, two ether type nonionics would be difficult to separate. Fatty isethionate, for example, would not be recovered intact by this scheme since it is reported to hydrolyze during elution from the anion exchange resin (3). Soaps however could be analyzed by this method.

The total elapsed time for analysis of a surfactant mixture by this scheme is about 40–60 working hours. For certain products this amount of time can be justified to obtain a complete active characterization. Once this is done, analysis of the anionic portion of similar samples could be run by cationic titration before and after acid hydrolysis to check for sulfonate and sulfate contents. Running this on the original samples of built detergents saves a considerable amount of time. The active would need to be extracted with isopropyl alcohol only for subsequent nonionic analysis by ion exchange.

Summary

A scheme has been presented for the separation of active ingredients from built detergent compositions and subsequent analysis of active components. Active components investigated are those commonly encountered in practice. Data obtained are in good agreement with expected values. The main difficulty encountered was the characterization of a fatty alcohol-EO sulfate which gave higher than expected values for its analyzed content. It was also difficult completely to characterize this fatty alcohol-EO sulfate.

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Detoxification and Deallergenization of Castor Beans[1]

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DURING THE PAST FOUR YEARS acreage planted to castor beans has increased almost five-fold, from 5.1 to 24.0 thousand acres, and the annual domestic production has increased almost fifteen-fold, from 3.4 to 49.9 million pounds (1). This is attributed to the development of high-yielding bean varieties and improved harvesting machines. These improvements have made production of this crop more attractive to farmers as a replacement for crops in surplus supply or with acreage restrictions.

A very important economic factor affecting the further development of castor beans is that the meal

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or pomace, produced by present commercial extraction processes, has value only as a fertilizer. It can be used neither as animal feed nor in the preparation of industrial products. The pomace contains three undesirable constituents (12): a violently poisonous heat-labile protein called ricin; a toxic alkaloid component called ricinine; and a powerful and very stable allergen, known as CB-1A (11). The ricin, which is present in relatively large amounts, can be detoxified in normal practice by treating or cooking the beans or pomace with steam (2, 3). The ricinine is present in small amounts and, because it is not seriously toxic, it is not considered particularly detrimental (3). The allergen, a powerful, nontoxic, protein polysaccharidic fraction, is the most troublesome constituent. CB-1A represents about 12.5% of the weight of the pomace, as determined by the antigen-dilution precipitin test.

With the anticipated increase in castor production and the appearance of larger amounts of the pomace in the fertilizer market, growers, handlers, processors, and public health officials have expressed deep concern over the risk that farmers and farm stock may become seriously sensitized to the potent castor bean allergen. This also applies to operating personnel in castor bean-extraction plants and to residents in the vicinity of such installations (2).

It is the consensus among castor bean processors that the industry needs a practical and inexpensive method for detoxifying the ricin and completely destroying or inactivating the allergenic factor without affecting the quality of the oil product and, if possible, without seriously degrading the meal product for industrial uses.

In work at this laboratory which demonstrated that castor beans can be directly solvent-extracted successfully on a bench-scale by the filtration-extraction process (4), the addition of sodium hydroxide in the moist cooking of flaked castor bean meats had been explored as a possible means of improving the color and reducing the fatty acid content of the oil product. Assays of the pomaces so produced indicated that the ricin could be completely detoxified and that the allergen content could be reduced by as much as 98% as a direct result of the the alkali treatment. It was on the basis of these promising results that the present research was undertaken.

This paper will report the results of probing experiments in which were investigated a number of types of treatments for the detoxification and deallergenization of castor beans and/or pomaces, with the anticipation that one or more selected conditions would point up potential avenues for further research. The treatments were mainly of the practical type, which could be readily integrated or combined with conventional processes of solvent extraction. They included cooking of the castor bean meats and pomaces under various conditions of moisture and temperature; dry-heat treatment at elevated temperatures; treatments with chemicals such as sodium hydroxide, hydrochloric acid, anhydrous ammonia, sodium hypochlorite, sodium chloride, ammonium sulfate, potassium permanganate, formaldehyde, urea, and combinations of the above; and biological treatments, including aerobic fermentation and enzymatic digestion. The results indicate that all of the treatments were effective in destroying or inactivating the allergenic factor to varying degrees. Some yielded pomaces of practically zero allergen content, as measured by the assay procedures described herein for the determination of allergen content.

Raw Materials and Equipment

The castor beans used in this investigation were obtained from commercial processors and were representative of current oil mill receipts. Of the four lots of beans, three were domestically produced, and one was of Brazilian origin. Varietal information was not obtainable. Each lot had been thoroughly cleaned and partially decorticated. Each was well mixed to insure uniformity and stored under refrigeration at 40°F. while awaiting processing. Pertinent data on the lots of beans are tabulated below:

Lot No.	A	B	C	D
Source	Domestic	Foreign	Domestic	Domestic
Moisture, %	4.4	4.8	4.4	4.5
Hull, coat, %	5	8	16	2

Flaking was carried out with a set of 12-in.-in-diam., one-pair-high smooth rolls. For cooking the prepared flakes, the following bench- or pilot-plant units were employed: a 15-lb. capacity vessel (5) equipped with agitator, reflux condenser, and spray injector for addition of steam, water, or chemical solutions; a 1-lb. capacity vessel (6), similarly equipped; and a 12-qt. household type of pressure cooker. The ammoniation experiments were conducted in an electrically-heated pilot-plant-size pressure reactor. For heat-treatment studies a forced-draft, electrically-heated cabinet dryer was used. The mixing and slurrying operations were conducted with a Waring Blendor or a 3-speed Hobart mixer equipped with a steam-jacketed bowl.

Solvent extraction of the treated materials was carried out with the 5¼-in.-in-diam. (500-g. capacity) bench-scale filter test unit used for evaluation of oil-bearing materials for filtration-extraction, previously described (7); or with a 1½-in.-in-diam. (120-g. capacity) unit.

Experimental Treatments

The treatments reported have been classified into Groups I through IV and are itemized in Tables I through IV, respectively. Groups I and IV comprised chemical and biochemical treatments of flaked castor bean meats and/or pomaces; Group II, treatments of pomaces with ammonia at elevated pressures; and Group III, heat treatment of pomaces at elevated temperatures.

In order to facilitate efficient extraction of the oil and to insure thorough detoxification of the ricin, it was decided at the outset to employ as a standard procedure, in most of the experiments, the initial steps, as described below, of moist-cooking the flaked raw meats, followed by hexane-extraction to recover the pomace.

The moist-cooking operation comprised the following sequence of steps: preheating the flaked castor bean meats, of 0.010-in. thickness, to about 170°F.; adjustment of moisture content to about 16% by the addition of water with or without chemicals; heating to 212–215°F.; maintaining this temperature by continuous heating under reflux conditions over a 12- to 15-min. period; and drying the cooked flakes to 7–9% moisture content in 10–20 min.

Solvent-extraction of the cooked flaked meats was carried out with the standard bench-scale filter test

TABLE I

Allergen Reduction by Alkaline and Nonalkaline Treatments of Castor Bean Meats and Pomaces

Experiment Group I

Exp. No.	Bean	Treatment				CB-1A in Pomaces		
		Meats			Pomace, alkali	Ring test	Precipitin test	Schultz-Dale test
		Moist cooking	Alkali	Solvent-extraction				
		%			%	%	%	%
1a	C	No	No	Isopropyl alcohol	No	12.5	12.5	.....
b	C	Yes	No	Isopropyl alcohol	No	12.5	6.3	.....
c	C	No	No	Hexane	No	12.5	12.5	.....
d	C	Yes	No	Hexane	No	6.3	3.1	.....
e	C	Yes	NaOH(1%)	Hexane	No	0.05	0.4	.....
f	C	Yes	NaOH(1%)[a]	Hexane	No	0.05	0.4	.....
2a	B	Yes	No	Hexane	No	1.6	6.3	.....
b	B	Yes	NaOH(1%)	Hexane	No	0.025	0.2	.....
3a	A	Yes	No	Hexane	No	3.1	3.1	.....
b	A	Yes	NaOH(1%)	Hexane	No	0.05	0.4	.....
4a	D	Yes	No	Hexane	NaOH(2%)[b]	0.0	1.6	.....
b	D	Yes	No	Hexane	NaOH(2%)[c]	1.6	6.3	.....
c	D	Yes	NaOH(2%)[d]	Hexane	No	0.0	0.0	.....
						0.0[e]	0.0[e]	.....[f]
d	D	Yes	KOH(2%)[d]	Hexane	No	0.8	3.1	.....
e	D	Yes	$Ca(OH)_2$(2%)[d]	Hexane	No	0.4	6.3	.....

[a] Extended cooking period (60 min.). [b] Solvent-damp pomace moistened to 20% H_2O and autoclaved with NaOH at 20 psig. [c] Solvent-damp pomace moistened to 20% H_2O and heated with NaOH at 0 psig. [d] Meats autoclaved at 20 psig. [e] Duplicated assay by Allergens Laboratory. [f] Not determinable. Doses reactive with sensitized tissue were of the same order as doses with the nonsensitized tissue.

unit, using hot hexane (130°F.) washes, and a solvent-meats ratio of 2:1 unless otherwise specified. The solvent-damp extracted pomace was then air-desolventized.

In the treatments of raw meats the chemicals were added during the cooking operation. In other experiments the pomaces so produced were subjected to various further treatments. In all cases the extent of detoxification and deallergenization achieved was determined by assay of the final pomaces by the test methods described herein for percentage of ricin and percentage of allergen (CB-1A).

The various experimental treatments are described below and in Tables I through IV.

GROUP I. ALKALINE AND NONALKALINE TREATMENTS OF CASTOR MEATS AND POMACES

Table I gives the conditions for alkaline treatments employing NaOH, KOH, or $Ca(OH)_2$ in the moist-cooking operation and for heat treatments of the pomace with NaOH. Alkaline and nonalkaline (control) treatments of flaked meats from four different lots of beans were studied.

GROUP II. AMMONIATION OF POMACES PRODUCED BY HEXANE-EXTRACTION OF ALKALI-COOKED CASTOR MEATS

Pomaces were ammoniated in the pressure reactor in a series of eight tests, based on statistical design (Latin Square technique). The experiments were conducted by using two selected conditions for each of the important variables, *i.e.*, moisture content, temperature, ammonia pressure, and treatment time (Table II).

GROUP III. HEAT TREATMENT OF POMACES PRODUCED BY HEXANE-EXTRACTION OF ALKALI- AND NONALKALI-COOKED CASTOR MEATS

Table III gives the conditions for the heat treatment of pomaces prepared by both nonalkaline and alkaline treatments of flaked meats from two lots of beans. For the second lot (D) the effect of time-temperature for the heat treatments was studied. The pomaces were placed in covered aluminum containers equipped with thermocouples and were heated in a dryer capable of developing temperatures up to 500°F.

TABLE II

Allergen Reduction by Ammoniation of Pomace Produced by Hexane-Extraction of Alkali-Cooked Castor Meats [a]

Experiment Group II

Experiment No.	Bean	Treatment				CB-1A in Pomace	
		Moisture	Temp. °F.	Ammonia pressure psig.	Time in min.	Ring test	Precipitin test
		%				%	%
1a[b]	B	8.5	.....	.....	.....	0.4	0.8
b	B	16.9	90	40	45	0.1	0.8
c	B	16.9	90	100	15	0.05	0.8
d	B	16.9	175	100	45	0.025	1.6
						0.05[c]	0.2[c]
e	B	16.9	175	40	15	0.1	1.6
f	B	6.4	90	100	45	0.05	0.8
g	B	6.4	90	40	15	0.05	0.8
						0.10[c]	0.4[c]
h	B	6.4	175	40	45	0.05	0.8
i	B	6.4	175	100	15	0.2	1.6

[a] Flaked meats moist-cooked with 1% NaOH. [b] Control. [c] Sample retested after 1½ months' storage with residual ammonia in a sealed container.

GROUP IV. CHEMICAL AND BIOLOGICAL TREATMENTS OF CASTOR MEATS AND POMACES

Table IV lists the conditions for these exploratory treatments. Pertinent data not included in the table are as follows.

Expt. 1a (NaOCl). Flaked meats were agitated in a Hobart mixer at 170°F. with 16.7% by weight of a 5.25% solution of NaOCl[3] for 15 min. This caused oil flow and agglomeration of the mixture (pH 6.3) into small ball-like masses. After pressure-cooking the mixture was washed with hexane, pulverized, and extracted with hexane.

Expt. 1b (NaOCl). Pomace was agitated in the Hobart mixer at 170°F. with 21% by weight of a 5.25% solution of NaOCl for 15 min. Treated mixture was brought to dryness on a steam bath.

Expt. 1c (NaOCl). Same as 1b except that the flaked meats had been cooked with 1% added NaOH.

Expt. 2a (NaOH, HCHO). Flaked meats were preheated to 170°F. in the small laboratory cooker with 1% by weight of NaOH dissolved in 10% by weight of water. After mixing for 2 min., 8% by weight of formalin (37.4% HCHO, 12.3% methanol, and 50.3% H_2O) was added, and mixing was continued for 2 min.

Expt. 2b (HCl, HCHO). Same procedure as 2a

[3] Clorox is manufactured by the Clorox Company, Oakland, Calif.

TABLE III
Allergen Reduction by Heat Treatment of Pomaces Produced by Hexane-Extraction of Alkali- and Nonalkali-Cooked Castor Bean Meats
Experiment Group III

Experiment No.	Bean	Meats, NaOH	Pomace		CB-1A in Pomace		
			Temp. reached, °F.	Time elapsed, min.	Ring test	Precipitin test	Schultz-Dale test
		%			%	%	%
1a	B	1.0	Control	Control	0.024	0.20	....
b	B	1.0	401[b]	95	0.0	0.006	0
2a	B	0	Control	Control	1.6	6.2	....
b	B	0	401[b]	95	0.0	0.006	0
3a	D	1.0	Control	Control	0.4	1.6	....
b	D	1.0	257	52	0.4	1.6	....
c	D	1.0	307	58	0.025	0.8	....
d	D	1.0	349[a]	85	0.006	0.8	....
e	D	1.0	405[b]	113	0.0	0.0	....
4a	D	0	Control	Control	3.1	6.2	....
b	D	0	264	52	1.6	6.2	....
c	D	0	302	58	3.1	6.2	....
d	D	0	347[a]	90	1.6	3.1	....
e	D	0	405[b]	125	0.0	0.0	....

[a] Pomaces had light-brown, scorched appearance. [b] Final pomaces had dark-brown, charred appearance.

except that 2.5% hydrochloric acid (36.9% HCl) was used in place of NaOH.

Expt. 2c (NaOH, HCHO). The alkaline pomace was agitated in the Hobart mixer for 15 min. at 170°F. with 27% by weight of formalin and 25% by weight of additional water. This mixture was dried to 6–8%.

Expt. 2d (NaOH, HCHO, NaOCl). The alkaline pomace was agitated in the Hobart mixer at 170°F. with 27% by weight of formalin for 5 min. Then 28.4% by weight of a 5.25% solution of NaOCl was added, and mixing was continued for 10 min. Mixture was dried to 6–8% on steam bath.

Expt. 4 (Urea). Pomace was mixed in a Hobart mixer at 170°F. for 15 min. with 5% by weight of urea dissolved in 180% by weight of water. Treated mixture was dried on a hot plate to 8–10% moisture.

Expt. 5 ($KMnO_4$). Pomace was mixed in a Hobart mixer for 45 min. with 10% by weight of potassium permanganate dissolved in 370% by weight of water. Slurry was heated to 175°F. to accelerate oxidation and was agitated at maximum mixer speed for 3 hrs. After standing over-night, the partially oxidized pomace (black in color) was dried on a hot plate to 5–6% moisture content.

Expt. 6 (Aerobic Fermentation). Pomace was mixed with 200% of tap water and allowed to stand uncovered for 72 hrs. at approximately 80°F. Then the slurry was incubated at 98.6°F. for 100 hrs. Considerable mold formation was observed along with a strong, sweetish odor. Pomace was recovered by drying (hot plate) to 5–6% moisture content.

Expt. 7 (Trypsin). Pomace (pH 8.3) was agitated in a Hobart mixer at 100°F. with 50% by weight of water and 6% by weight of trypsin, an amount equivalent to about 10% by weight of the protein in the pomace. The mixture was stirred vigorously, allowed to stand for 60 min. at an adjusted pH of 8.3, and incubated at 98.6°F. for 90 min. Then it was oven-heated at 212°F. for 90 min. to arrest enzymatic digestion and further dried to 5–6% moisture content.

Analytical Test Procedures

The product pomaces, obtained from the various physical, chemical, and biochemical treatments of the castor beans and pomaces, were assayed for allergen (CB-1A) content by a qualitative antigen-dilution precipitin method with modifications suggested by E. J. Coulson of the Allergens Laboratory, Eastern Utilization Research and Development Division (9), and by the Schultz-Dale test (10). Some of the pomaces were assayed for comparative ricin content by a blood corpuscle agglutination test recommended by the Baker Castor Oil Company (8). Brief descriptions of the test procedures are as follows.

BLOOD CORPUSCLE AGGLUTINATION TEST FOR RICIN

The red blood corpuscle solution was prepared by removing the plasma from citrated whole human blood (citrated guinea pig blood may be substituted) by centrifugation and pipetting, washing the corpuscles three times with a physiological saline solution (0.85% sodium chloride in demineralized water), and then diluting them to 10 times their volume with saline solution. Pomace to be tested is placed in solution by mixing a ground 0.5-g. sample in 49.5 g. of physiological saline solution, allowing it to stand 1 hr. and filtering. It is then stored under refrigeration until used.

The test procedure is as follows. Test tubes are placed in a rack, and 0.9 ml. of saline solution is pipetted into the first tube and 0.5 ml. in each of

TABLE IV
Allergen Reduction by Moist-Cooking of Castor Bean Meats with Chemicals and by Chemical and Biochemical Treatments of Pomaces
Experiment Group IV

Experiment No.	Bean	Treatment		CB-1A in Pomace		
		Meats,[a] chemical	Pomace, chemical or biochemical	Ring test	Precipitin test	Schultz-Dale test
		%	%	%	%	%
1a	D	NaOCl(0.9%)[b]	No	6.3	6.3	.....
b	D	No	NaOCl(1%)	6.3	6.3	.....
c	D	NaOH(1%)	NaOCl(1%)	0.8	0.8	.....
2a	D	NaOH(1%), HCHO(3%)	No	0.0	0.0	0.03
b	D	HCl(0.9%), HCHO(3%)	No	0.0	0.0	0.003
c	D	NaOH(2%)	HCHO(10%)	0.0	0.0	<0.003
d	D	NaOH(2%)	HCHO(10%), NaOCl(1.5%)	0.0	0.1	.....
3a	D	NaCl(4%)	No	1.6	6.3	.....
b	D	$(NH_4)_2SO_4$(3%)	No	6.3	6.3	.....
4	D	No	Urea(5%)	1.6	6.3	.....
5	D	No	$KMnO_4$(10%)	1.6	6.3	.....
6	D	No	Aerobic fermentation	1.6	3.1	.....
7	D	NaOH(0.7%)	Trypsin(6%)	0.0	0.05	0.15

[a] All treated meats defatted by hexane-extraction. [b] Autoclaved at 20 psig. pressure.

the remaining tubes. To the first tube 0.1 ml. of the pomace solution is added and mixed, using a small glass rod. One-half or 0.5 ml. of this solution is transferred to the second tube and mixed; a like amount is transferred to the third tube, etc., down to tube No. 10. Then, to each tube, 0.5 ml. of red blood corpuscle solution is added and thoroughly mixed. Thus the dilutions of the pomace solutions in the 10 tubes are 1:10, 1:20, 1:40, etc., and 1:5120, respectively. After standing for 5 min., the tubes are centrifuged exactly 2 min. Observations are then made relative to the appearance and characteristics of the red blood agglutination. The tubes are then shaken gently to redisperse the corpuscles, and the following ratings are made:

4+ complete agglutination with no dispersion upon shaking
3+ complete agglutination with some breaking up of blood upon shaking
2+ agglutination, but complete breaking up of blood in the agglutinated particles
1+ agglutination, easily dispersed to visible agglutinated particles
± upon redispersion, a magnifying glass is required to see the agglutinated particles

The highest final dilution showing a 1+ is called the titre. The titre values are in direct proportion to the amounts of ricin contained in the pomace sample.

ANTIGEN-DILUTION TEST FOR CASTOR BEAN ALLERGEN (CB-1A)

Chemical and Biological Reagents

Buffered Saline Solution. 0.50 g. $NaH_2PO_4 \cdot H_2O$, 2.60 g. $Na_2HPO_4 \cdot 12H_2O$, 8.8 g. NaCl (biological), and 0.1 g. merthiolate are dissolved in distilled water and diluted to one liter. This solution is then stored at refrigerator temperature.

Anti-CB-1A and Normal Rabbit Sera. These sera are stored at −4°F. Before use they are thawed and centrifuged clear at 36–41°F. They are not to be refrozen. The thawed sera are stable for 4–6 weeks at refrigerator temperature. Both were furnished and shipped under dry ice by the Allergens Laboratory.

CB-1A Stock Solution. To 5 mg. of powdered CB-1A (furnished by the Allergens Laboratory) were added 6.40 ml. of buffered saline. This is a 1:1280 dilution of CB-1A, equivalent to No. 8 serial solution.

Preparation of Extracts. The pomace samples to be tested are ground to pass a 40-mesh sieve. Extracts of the samples are prepared in the proportion of 2.0 g. of sample to 20 ml. of buffered saline (1:10 dilution). The extraction period is three days in a refrigerator at 43°F. with frequent shaking of the suspension. The suspension is then centrifuged in a refrigerated centrifuge at 36–41°F. The supernatant solution is clarified by filtration through a No. 50 Whatman filter paper. On storage in the refrigerator some of the castor extracts become cloudy. These should be clarified before use, by recentrifugation at 36–41°F.

Preparation of 2-Fold Serial Dilutions. Buffered saline is used for preparing the 2-fold serial dilutions of the extracts. For convenience the dilutions are designated by number. For example, the original 1:10 extract is placed in a 20-ml. tube labelled No. 1. For preparation of Dilution No. 2, 2 ml. of Dilution No. 1 are transferred into Tube No. 2, containing 2 ml. of buffered saline, giving a dilution of 1:20, etc.

For setting up the CB-1A reference standard, serial Dilutions Nos. 11 to 18, inclusive, should be prepared. It is not necessary to use the normal rabbit serum control with the CB-1A dilutions.

For raw castor meal and regular-cook castor meal, Dilutions Nos. 5 through 16 should be prepared for use with the antiserum. Dilutions Nos. 5 through 8 usually suffice for control tests with normal rabbit serum. Dilutions required for treated castor meal should include Dilutions Nos. 1 through 12. Reading of the "ring" test (see below) will indicate whether or not higher dilutions are needed. Control tests should be set up with normal rabbit serum in Dilutions Nos. 1 through 5.

Procedure. A series of new 45 mm. × 5 mm. precipitin tubes, each containing 0.15 ml. of CB-1A antiserum, is set up in a precipitation-tube rack. The required number of precipitin tubes containing 0.15 ml. of normal rabbit serum for control tests are also set up. Beginning with the highest dilution, 0.15 ml. of the castor extract and of the CB-1A stock solution are then carefully layered over the antiserum and the appropriate control serum, respectively. The tubes are then incubated 30 min. at 98.6°F. The reaction ("ring" test) at the interface of the two liquids in each tube is then observed and recorded. (If the "ring" test with the highest dilution of extract is markedly positive, additional dilutions of antiserum may be set up at this time.) The contents of each tube are then mixed with a small stirring rod, beginning with the most dilute and ending with the most concentrated. The tubes are again incubated 30–60 min., then stored in the refrigerator for 48 hrs. The precipitates formed are then observed and recorded.

Estimation of CB-1A Content in Pomace. To estimate the CB-1A content of a pomace by the "ring" or the "precipitin" test, the tube containing the highest dilution of extract that yields a barely perceptible precipitate is compared with the highest dilution of stock Cb-1A solution that yields a barely perceptible precipitate. To illustrate, if the stock CB-1A solution yielded a perceptible precipitate with the antiserum with dilution No. 16 (equivalent to 0.00305 mg. CB-1A) and if the sample castor extract yielded a perceptible precipitate with Dilution No. 8 (equivalent to 0.781 mg. CB-1A), it can be calculated that the pomace would contain 0.00305/0.781 times 100 or 0.39% of CB-1A. The method has been found to give reproducible results, particularly in the range of low contents of CB-1A. However it must be pointed out that because it utilizes serial dilutions, an error of one tube in a reading is equivalent to a 100% error.

SCHULTZ-DALE TEST

The Schultz-Dale technique (10) for determination of percentage of castor bean allergen (CB-1A) consists of measuring the degree of contraction of uterine muscles of a sensitized virgin female guinea pig immersed in a bath of oxygenated tyrode solution. The Schultz-Dale procedure was employed in this study for further assay (by the Allergens Laboratory) of certain of the treated pomace products which tested exceptionally low in CB-1A content by the precipitin test. No final tests were made on naturally sensitive human subjects.

Discussions of Results

Tables I through IV list for each of the experiments in Groups I through IV, respectively, the esti-

mated percentages by weight of castor bean allergen (CB-1A) contained in the resulting pomaces, as determined by the modified antigen-dilution screening test and by the semiquantitative anaphylactic method (Schultz-Dale technique).

The over-all results indicate, in general, that the different treatments of meats and/or pomace were effective in reducing the content of allergen to varying degrees from 0 to 100% as discussed below.

Group I. Alkaline Treatments. Table I shows that for the three lots of beans, A, B, and C, moist-cooking with 1% added NaOH (Expts. 1e, 2b, and 3b) was effective in reducing the CB-1A content to 0.2–0.4%, a reduction of 97 to 98.4%, compared to only 50 to 75% for the controls (Expts. 1d, 2a, and 3a). In Expt. 4c cooking with 2% NaOH at 20 psig. pressure yielded a completely deallergenized pomace, as measured by the precipitin test. However, when assayed by the Schultz-Dale method, the zero reading was not determinable, *i.e.,* it was difficult to conclude that the sample had absolutely no CB-1A since a limiting value for CB-1A could not be established because sample doses reacting with sensitized tissue were of the same order as doses reacting with nonsensitized tissue. Expts. 4d and 4e demonstrated that the use of KOH or $Ca(OH)_2$ in moist-cooking at 20 psig. pressure was less effective than NaOH (Expt. 4c) in reducing allergen content.

In carrying out the antigen-dilution tests, it was observed that, in general, extracts of all of the pomaces produced by alkaline or other chemical treatments gave atypical or aberrant precipitin reactions and were characterized by large differences in the results of the "ring" test and the "precipitin" test. It may be postulated that such meals contain relatively large proportions of partially altered allergens.

Extracts of the pomaces prepared by simple solvent extraction of raw castor bean flakes (1a, 1c) and of flakes cooked without added chemicals gave typical precipitin reactions. All contained high concentrations of CB-1A. Preparations 1a and 1c produced copious precipitates with normal rabbit serum, which is typical of raw uncooked castor meal and can be attributed to the fact that ricin precipitates serum protein. However typical precipitin reactions occurred with the antiserum in the range where no precipitates were produced with normal serum. Extracts of pomaces from all other treatments in this study, where moist-cooking without added chemicals was employed, did not yield any precipitates in the normal serum, regardless of CB-1A content. Cooking apparently inactivates the ricin components.

Treatments 4a, 4b, and 4c show that when castor bean pomace prepared from moist-cooked flakes was cooked at atmospheric or at 20 psig. pressure with 2% NaOH in the presence of added moisture, the CB-1A content was not reduced as completely as by autoclaving the raw flakes at 20 psig. with NaOH and added moisture.

In experiments not listed in the table, acid treatments were tried, in which flaked raw meats were intimately mixed for 20 min. at 160°F. with 2.0% of hydrochloric acid and aqueous acetone, followed by acetone-extraction. The resultant pomaces (pH 3.9) showed practically no reduction in allergen content.

Group II. Ammoniation Treatments. Table II lists the results of the precipitin tests made on the eight samples of pomace which had been subjected to ammoniation under the various conditions of moisture, temperature, ammonia pressure, and treatment time. The purpose was to explore the use of ammonia as a means of further reducing the CB-1A content of pomace which had already been partially deallergenized to about 0.8% CB-1A content by alkali-cooking of raw flakes, followed by hexane-extraction. Data show no apparent reduction over control; however a previous experiment had shown that the allergen content of ammoniated pomace decreases with extended storage. Accordingly the least and the most severely treated pomaces from Expts. 1d and 1g, respectively, were hermetically stored and were retested after 45 days to ascertain the effect of prolonged contact with residual ammonia. The results indicated reductions down to 0.2% CB-1A, a result which was sufficiently promising to warrant additional storage. However, upon retesting after 150 days, no further reductions were noted.

Group III. Heat Treatment. Results in Table III indicate that partially deallergenized pomaces produced from Beans B and D by hexane-extraction of alkali- or nonalkali-cooked flakes were completely deallergenized by simple heating to about 405°F. in an elapsed period of about 125 min. However the resulting meals had a dark brown, charred appearance, indicative of considerable heat damage. The meals produced from Bean B (Expts. 1b, 2b) tested 0% CB-1A by both the precipitin and the Schultz-Dale methods. It is significant to note in the data for Bean D that temperatures of heating up to 347°F. were ineffective in reducing the CB-1A content to very low levels. It can be postulated that temperatures higher than 347°F. are necessary to destroy or inactivate the allergen by the combined effects of accelerated oxidation and chemical breakdown.

Group IV. Chemical and Biochemical Treatments. In this series (Table IV) the best results were obtained by the treatments with formaldehyde (Expt. 2) and by trypsin digestion of the pomace (Expt. 7).

In the formaldehyde treatments the pomaces produced in Expts. 2a, 2b, and 2c tested 0% CB-1A by the precipitin method and practically 0% by the Schultz-Dale method; that produced from Expt. 2d tested 0.1% CB-1A. The treatments differed from each other as shown in Table IV. All employed moist-cooking of flaked meats with different chemicals as noted, followed by hexane-extraction to produce the pomaces. In Expt. 2a, NaOH and formaldehyde were used; in 2b, HCl and formaldehyde; and in 2c and 2d, NaOH only. In the latter two experiments the alkaline pomaces produced were treated with formaldehyde without and with added NaOCl, respectively. The results demonstrate that formaldehyde definitely reacts with the allergenic component in some unexplained way (possibly by polymerization or reduction) to render it undetectable by both test methods.

In Expt. 7 deallergenization to 0.2% CB-1A (98.4% reduction) was achieved by trypsin digestion in an aqueous alkaline medium of partially deallergenized pomace that had been prepared from alkali-cooked flaked meats.

In Expts. 1a and 1b moist-cooking of castor bean flakes with NaOCl and treatment with NaOCl of pomace prepared from moist-cooked flakes yielded reductions in CB-1A content of only 50%. In Expt. 1c treatment of alkaline pomace with NaOCl did not further reduce the allergen content.

All of the other treatments listed in Table IV were essentially ineffective in reducing the contents of CB-1A by more than 50 to 75%, which may be considered negligible.

Summary

Results are reported of a series of experimental treatments of flaked castor bean meats and pomaces directed toward total detoxification of the ricin and total destruction or inactivation of the allergenic property.

The treatments comprised cooking of the flaked raw meats or pomaces under various conditions of moisture and temperature, with chemicals such as sodium hydroxide, hydrochloric acid, sodium hypochlorite, formaldehyde, ammonia, ammonium sulfate, potassium permanganate, and urea, and with selected combinations of these. Also tried were aerobic fermentation, enzymatic digestion, and simple heating of pomaces at elevated temperatures.

The five most promising deallergenization treatments and the corresponding percentage reductions in allergen content as measured by the precipitin test were as follows: dry heating of pomace to 401°F., 100%; moist-cooking of flaked meats with 2% NaOH and 10% HCHO, possibly 100%; moist-cooking with 0.9% HCl and 3% HCHO, possibly 100%; moist-cooking with 2% NaOH at 20 psig. pressure, possibly 100%; moist-cooking with 1% NaOH, 98.4%. For the first four treatments the Schultz-Dale test indicated possible reductions of 100, 99.9, 99.9, and 100%, respectively. While the latter method is generally considered to be reasonably accurate, final tests would have to be conducted with naturally sensitive human subjects.

The data also showed that the ricin component can be completely detoxified by a mild moist-cooking of the flaked meats, either with or without added alkali.

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The Formation of Polarographically Reducible Substances in Autoxidizing Lard[1]

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THE FIRST APPLICATION of polarography to autoxidizing fat by Lewis and Quackenbush (7) served to demonstrate that more than one type of "peroxide" is included in the iodimetric determination of peroxide value. Subsequent studies by Swern *et al.* (12) were made with hydroperoxides and related compounds thought to occur in autoxidizing fats, and a method was presented by Willits *et al.* (13) and Ricciuti *et al.* (9) for the quantitative determination of hydroperoxides in oleate and linoleate. Evidence for distinct nonhydroperoxide type compounds has been found by Swern *et al.* (10) in methyl oleate and by Kalbag *et al.* (4) in soybean oil. In our laboratory it was observed that the addition of ethyl cellulose to the solvent greatly improved polarographic differentiation of the peroxidic compounds in a fat.

The present paper extends the investigation of reducible autoxidation products in lard and the effects of temperature, pro-oxidants, and antioxidants on their formation.

Experimental

Apparatus. A Sargent Model XXI recording polarograph was used with a water-jacketed cell (30 ± 0.2°C.), which had an internal mercury pool anode with the approximate surface area of 6.6 sq. cm. The capillary in the solvent system with open circuit had a drop time t of 3.80 seconds. The value M was 1.85 mg./second; therefore $m^{2/3}\ t^{1/6}$ (5) was 1.88 $mg.^{2/3}\ sec.^{-1/2}$.

For the autoxidizing apparatus a bottomless Erlenmeyer flask (1 liter) was ground to fit tightly to a heavy glass plate. The sample was placed in a 100-mm. Petri dish on the plate and a Pyrex gas dispersion tube with coarse fritted disc (30 mm. in diameter) stoppered into the neck just above the sample. This apparatus was placed in a forced-air oven with

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