of different fractions on the stability of fresh edible oils.

Summary

The properties of guar seed oil (*Cyammopsis Psoralioides*) were studied, and it was found that it may be satisfactory for edible purposes. An interesting observation has been made that the unsaponifiable fraction of guar oil may be used as a stabilizer for oils.

Acknowledgment

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Toxic Protein from Trichloroethylene-Extracted Soybean Oil Meal[1,2]

L. L. McKINNEY, F. B. WEAKLEY, R. E. CAMPBELL, A. C. ELDRIDGE, and J. C. COWAN, Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois; J. C. PICKEN JR., Veterinary Medical Research Institute, and N. L. JACOBSON, Agricultural Experiment Station, Iowa State College, Ames, Iowa

LITERATURE REVIEWS on the toxicity of trichloroethylene-extracted soybean oil meal (TESOM) have appeared in recent papers (15, 21). It has been repeatedly shown that TESOM contains a toxic component capable of producing a refractory, hemorrhagic aplastic anemia when fed to cattle. However the toxic principle in TESOM has not been characterized or identified.

One hypothesis suggested that during processing trichloroethylene might undergo autoxidation in the extraction plant and the toxicity might be associated with one or more of the autoxidation products or develop from their reaction with some component of the soybeans. Investigation of this hypothesis has been reported. The autoxidation products were carefully determined (15), and their reaction products with soybeans, defatted soybeans, soybean protein, and casein were assayed for symptoms found to be characteristic of TESOM toxicity (19). The assay results did not appear to support the hypothesis that the autoxidation products of trichloroethylene were either directly or indirectly involved in the formation of the toxic entity.

The present paper reports studies on the fractionation of toxic TESOM to determine which component of the meal is associated with the toxicity and to obtain information on the stability of the toxic principle.

Toxic Meal Used

Because of the lack of information on the stability of the toxic factor to heat, acid, or alkali it was considered necessary initially to attempt fractionation at a neutral pH and at temperatures of less than 50°C. A previous preliminary extraction of a toxic commercial TESOM with water had shown that the major portion of the toxicity remained with the water-insoluble fraction, suggesting the possibility of the association of the toxic factor with the protein or insoluble carbohydrates (18). Since the normal processing of solvent-extracted soybean oil meals for feed use includes a heat treatment step (cooking or toasting) to insure destruction of antinutritional factors known to be present in the raw meals, analyses were made for water-soluble total nitrogen on several commercial TESOMs known to be highly toxic. Results ranging from 7 to 25% total nitrogen soluble in water indicated a marked denaturation of the protein in these meals.

It came to our attention in the fall of 1952 that one of the trichloroethylene extractors was producing a TESOM for industrial use that assayed in the range of 70% total water-soluble nitrogen. Arrange-

TABLE I

Analyses of Undenatured Toxic TESOM, Moisture-Free Basis

Component	Method used	Control [a]	Toxic TESOM
Moisture	2 hrs. at 130°C.	....	8%
Total N	Kjeldahl	....	8.5%
H_2O sol. N	5.0 g. meal/100 ml. H_2O	....	70% of total
Nonprotein N	0.8 *N* TCA	....	3.5% of total
Protein (calcd.)	Total N less N.P.N. × 6.0	....	49.2%
Total P	Colorimetric (14)	....	0.71%
Inorganic P	Earle and Milner (6)	....	0.04%
Nucleic acid	Spectrophotometric (5)	....	1.12%
Phytic acid	.......[b]	....	2.3%
Ash	Ignition, 550°C.	....	6.3%
Total Cl	Optical density, AgCl	40–50 p.p.m.	100–200 p.p.m.
Total Fe	Colorimetric	103 p.p.m.[c]	258 p.p.m.
Sulfhydryl	Amperometric (9, 13)	7.6 $M/10^6$ g.	7.1 $M/10^6$ g.
Thiamin	Thiochrome	15.5 p.p.m.	12.0 p.p.m.
Thiamin	*L. fermenti*	15.2 p.p.m.	11.9 p.p.m.
Niacin	*L. arabinosus*	32.7 p.p.m.	32.5 p.p.m.
Pantothenic acid	*L. arabinosus*	13.8 p.p.m.	13.4 p.p.m.
Pyridoxine	*S. carlsbergensus*	6.5 p.p.m.[d]	5.6 p.p.m.
Ascorbic acid	Indophenol [e]	0	0

[a] Hexane-extracted meal prepared in a Soxhlet from the same beans used to produce the TESOM.
[b] Phytic acid = total P less (inorganic P plus nucleic acid) × 3.55.
[c] Fe in 6 samples of commercial untoasted hexane-extracted soybean meal ranged from 80 to 137 p.p.m. One sample of commercial toasted meal gave 165 p.p.m.
[d] Control meal from different beans.
[e] Extraction with xylene and interference of sulphydryl groups inhibited with p-chloromercuribenzoic acid.

[1] Presented at the fall meeting, American Oil Chemists' Society, Chicago, Ill., September 24–26, 1956.
[2] Investigation conducted jointly by the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture and the Veterinary Medical Research Institute and Agricultural Experiment Station, Iowa State College. Journal Paper J-3054, Iowa Agricultural Experiment Station, Ames, Project 814. A report of work done, in part, under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. Contract supervised by Northern Utilization Research and Development Division, Agricultural Research Service.

ments were made to procure a supply of this meal to be prepared under our observation. Essentially the normal processing (24) was modified by "speeding" the flakes through the desolventizers so as to impart the least amount of heat denaturation to the bean proteins and yet remove the residual solvent. The normal heat treatment or toasting step was eliminated entirely. The meal was prepared in November 1952 from recently harvested beans. Use of the new crop of beans was considered desirable because earlier studies have shown that recently harvested beans more consistently produced highly toxic TESOM (18, 20). Calf assay of this meal indicated a high level of toxicity, typical of commercial TESOMs prepared from new beans for feed purposes.

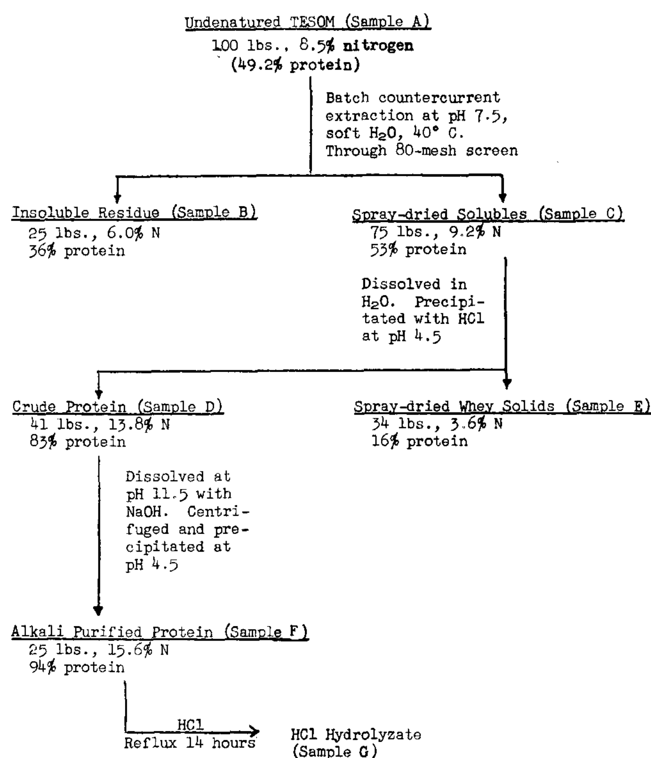

FIG. 1. Fractionation of toxic TESOM (moisture-free basis)

Analysis of the meal is shown in Table I. Since 70% of the total nitrogen was soluble in water, it appeared to offer excellent possibilities for fractionation studies. Both the chlorine and iron contents were about 100 p.p.m. greater than found in commercial hexane-extracted soyben oil meals, which is in agreement with our analyses on other samples of toxic TESOMs (15). The Fujiwara test (8) was positive (5 to 10 p.p.m. based on trichloroethylene) but negative after steaming the meal. This test is still weakly positive after 3 year's storage of the meal. Amperometric measurements of sulfhydryl groups with the rotating platinum electrode apparatus (13), using p-chloromercuribenzoate (9), gave 0.5 mole per 10^6 g. of meal less than that obtained from hexane-extracted meal. The possibility that sulfhydryl groups are involved in the toxicity is being studied. The thiamin content of these meals is known to be very sensitive to heat treatment, and the difference noted between the laboratory-prepared control and the TESOM sample was expected. There appeared to be no appreciable discrepancies in the vitamin contents which were determined. Also the TESOM did not appear to be toxic to the microorganisms used for vitamin assay.

Fractionation Studies

The fractionation scheme is shown in Figure 1 where yields are based on 100 lbs. of TESOM, moisture-free basis whereas a total of 400 lbs. was actually fractionated. Separation into pH 7.5 insolubles (Sample B) and pH 7.5 spray-dried solubles (Sample C) was carried out in a commercial plant which was equipped with a batch countercurrent extraction system and a large spray dryer. The remainder of the fractionation was carried out in the laboratory as required. The crude protein (Sample D) was contaminated by meal fines and contained about 83% protein (N x 6.0). The factor 6.0 is used throughout this report to convert protein nitrogen (total N less N.P.N.) to protein content because purification of the protein extracted at pH 7.5 by previously described methods (14, 22) yielded a product containing 16.6–16.8% nitrogen. Non-protein nitrogen (N.P.N.), as measured by the amount of nitrogen soluble in 0.8 *N* trichloroacetic acid, remained in the water-soluble fractions.

The samples were assayed for toxicity by feeding to young calves as previously described (19). For the initial screening, assay levels which would give definitive results were selected. Experience in this laboratory and elsewhere (16) has repeatedly shown that the variation in response of young calves to very toxic preparations (meals) is comparatively small when the level of meal administration is sufficiently high, *i.e.,* 0.25 lb. meal/100 lbs. body weight of calf/day. In view of this experience, fractions were fed, where possible, at protein levels equivalent to at least 0.25 lb. of original meal/100 lbs. body weight of calf/day. Results are shown in Table II. With the exception of the first assay all samples were adjusted to 20% moisture and autoclaved for 15 min. at 15 p.s.i. and then dried to 9% moisture content before feeding. As observed in previous experiments (19), the feeding of the raw meal (Sample A) resulted in digestive disturbances, diarrhea, unpalatability, and poor digestion, and the results of the assay were not satisfactory. A valid assay was obtained after the raw TESOM was autoclaved (Sample A-1). It is evident from the data presented in Table II that in the initial fractionation the major portion of the toxic entity appeared in the water-soluble fraction (Sample C). Subsequent fractionation of the water solubles into pH 4.5 insolubles (Sample D) and whey solids (Sample E), followed by assay of each, revealed that Sample D was very toxic in comparison to Sample E. Though Sample E was not well tolerated by the assay calf, the consumption of 33.5 lb. of this sample without clinical, hematologic, or postmortem signs of TESOM toxicity in the calf indicated that the toxic component was not present in appreciable amounts in this fraction.

The data in Table II indicate that the toxic entity is associated with the protein. Comparison of Samples A-1 and D, on the basis of protein content, lends additional evidence to this contention.

TABLE II

Toxicity Assays of Fractions

Sample No.	Description of sample	Amount of sample consumed and days to:[a]					Lbs. protein (calc'd)[b]		
		Blood symptoms[c]		Death					
		Days	Lbs.[d]	Days	Lbs.	Lbs./day	To symptoms	To death	Fed/day/ cwt.
A	Raw TESOM	34	24.4	67	48.1	0.72	12.0	23.7	0.35
A-1, autoclaved	Toasted TESOM	27	8.6	44	12.7	0.29	4.2	6.2	0.14
B, autoclaved	H_2O insolubles	63	46.8	(111)[e]	(56.1)[e]	0.69[e]	16.8	(20.2)[e]	0.24
C, autoclaved	H_2O solubles								
	Calf A	25	17.0	28	19.7	0.70	9.0	10.4	0.37
	Calf B	27	18.5	30	19.7	0.66	9.8	10.4	0.35
D, autoclaved	Crude protein	27	6.9	32	7.8	0.25	5.7	6.5	0.21
E, autoclaved	Whey solids	....	....	(85)[f]	(25.1)[f]	0.30[f]	....	(4.0)[f]	0.048

[a] All samples calculated to 9% moisture; all sample data calculated to lbs./100 lbs. body wt. of calf.
[b] Protein = total N − N.P.N. × 6.0, corrected to 9% moisture.
[c] Definite shift toward leucopenia and/or relative lymphocytosis.
[d] $\text{Lbs./100 lbs. calf} = \frac{\text{Total sample consumed}}{\text{Initial calf wt.} + \text{calf wt. at symptoms or death}} \times 200.$
[e] Feeding of sample stopped on 81st day; calf sacrificed on 111th day, no gross lesions.
[f] Calf remained normal during 85-day assay period; calf sacrificed on 85th day, no gross lesions.

Toxic Protein Studies

Since the results of fractionation studies indicated that the globulin proteins extracted at pH 7.5 and precipitated at pH 4.5 (Sample D) contained appreciable amounts of the toxic entity, further studies on the protein were undertaken. The procedure used for producing a supply of standardized isolated protein (Sample H), extracted at pH 7.5 and precipitated at pH 4.5, from undenatured TESOM (Sample A) is outlined in Figure 2. The pilot plant used

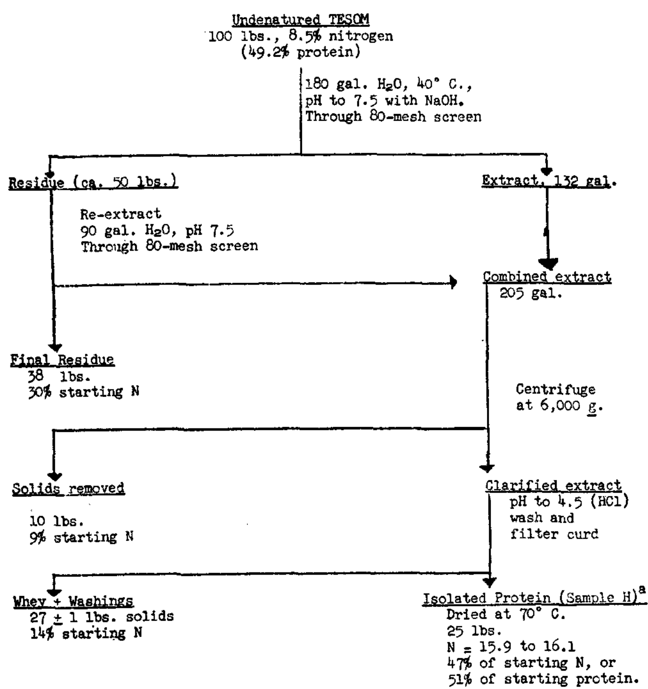

FIG. 2. Pilot-plant preparation of TESOM protein (moisture-free basis)

for the preparation of this protein has been described (3). The nitrogen content of the protein ranged from 15.9 to 16.1% in different preparations, and the yield represented 51% of the total protein in the meal. Analyses of the protein (Sample H) are shown in Table III. Neither the iron nor the chloride contents appeared to differ a great deal from those of protein prepared from hexane-extracted soybeans. Most of the iron (80%) was present as ferrous oxide and chloride, which indicated that it stemmed from corrosion. If the toxic protein contains organically bound chlorine, the quantity must be small. From numerous determinations it was concluded that the chlorine content of the toxic protein was perhaps 25 p.p.m. greater than that of non-toxic protein. The greatest source of error in determining these small amounts of organically bound chlorine stemmed from the relatively high and inconsistent values obtained for blanks.

Extended dialysis of this protein against 10% sodium chloride or sodium acetate to remove phytin and nucleic acids (14) increased the nitrogen content to 16.5 and reduced the phosphorus and ash contents to 0.036 and 0.4%, respectively. The results of this purification correlated with the nonprotein components reported in Table III indicate that the protein preparation (Sample H) was 96% pure protein.

During the course of these investigations an additional criterion of toxicity capable of extending the quantitative aspects of the assay was included in the hematologic evaluation of the assay calves. This feature of the bioassay, the correlation of blood platelet (thrombocyte) decline with toxicity, was developed at the University of Minnesota and was kindly made available by M. O. Schultze in advance of publication (16). The combined assay relates the decrease in blood platelets, development of leucopenia, and relative lymphocytosis with the levels of toxic TESOM fed to assay calves over a period of approximately 60 days. The results of this combined assay with the toxic protein (Sample H) are shown in Table IV along with that for the autoclaved TESOM (Sample A-1) calculated to equivalent protein basis.

TABLE III

Analyses of Toxic Protein (Sample H), Moisture-Free Basis

Component	Method used	Results
Total N	Kjeldahl	16.0 ± 0.1%
Protein	N × 6.0	96%
Total P	Colorimetric (14)	0.52%
Nucleic acid	Spectrophotometric (5)	0.75%
Phytic acid	Total P less N.A.P. × 3.55	1.60%
Cl, after dialysis	Optical density, AgCl	Approx. 55 p.p.m.[a]
Fe, after dialysis	Colorimetric, A.O.A.C.	253 p.p.m.[b]
Ash	Ignition, 550°C.	0.9%
Carbohydrates	Molisch	Positive

[a] Cl in protein from hexane-extracted flakes varied from 5 to 50 p.p.m. on different determinations with an average of 30 p.p.m.
[b] Fe in protein from hexane-extracted flakes = 236 p.p.m.

TABLE IV

Toxicity Assays of Pilot-Plant TESOM Protein

Sample No.	Amount of sample consumed and days to:[a]							Remarks
	Platelet decrease		Leucopenia [b]		Death			
	Days	Lbs.[c]	Days	Lbs.[c]	Days	Lbs.[c]	Lbs./day	
A-1[d]	....	....	27	4.4	44	6.5	0.15	Moribund; sacrificed; typical severe lesions.
H	....	....	24	9.8	26	10.3	0.40	Died; typical severe lesions.
H	25	4.1	32	5.2	35	5.5	0.16	Moribund; sacrificed; typical severe lesions.
H	25	3.2	39	4.8	43	5.1	0.12	Moribund; sacrificed; typical severe lesions.
H	26	1.3	36–40[e]	1.8	(69)[f]	(3.2)[f]	0.047[f]	Good condition; sacrificed; few mild lesions.

[a] All samples calculated to 9% moisture; all sample data calculated to lbs./100 lbs. body wt. of calf.
[b] Definite shift toward leucopenia and/or relative lymphocytosis.
[c] $\text{Lbs./100 lbs. calf} = \frac{\text{Total sample consumed}}{\text{Initial calf wt.} + \text{calf wt. at symptoms or death}} \times 200.$
[d] Assay reported in Table II. Calculated to equivalent protein content, *i.e.*, 96% protein.
[e] Transitory shift to leucopenia and relative lymphocytosis. Duration of 4 days.
[f] Feeding of sample stopped on 69th day, calf sacrificed on 78th day.

This protein was not autoclaved and was well tolerated by the calves. Other work has shown that unheated isolated soybean protein may be free of the natural antinutritional factors which are present in raw soybean oil meal, when fed to poultry (7) and swine (2).

Four assays at different levels of intake were conducted with Sample H to establish the response of assay calves to graded dosages of the toxic protein. The responses obtained were typical in magnitude and severity with those observed when very toxic whole meals are fed at levels approximately twice those of the purified protein, *i.e.*, at equivalent protein contents (16). Although a response was obtained with as little as 0.05 lb. per day per hundred pounds of calf weight, the level chosen for a good assay response to the toxicity was 0.125 lb. per day per cwt. The results obtained with the whole meal (Sample A-1) were similar to those obtained with the isolated protein (Sample H) when both were fed at the same level on a protein basis.

Since only 17% of the total nucleic acid or phytic acid present in the TESOM remained with the isolated protein, it appears unlikely that the toxicity is associated with these components. Since the analysis accounted for 99% of the composition of the isolated protein, it appears doubtful that an unknown impurity could be concentrated in this fraction.

Quantitative colorimetric analyses were made to determine whether the alpha positions or hydroxy group of tyrosine, the alpha position of the nitrogen in the indole nucleus of tryptophan, the benzene ring of phenylalanine, or the alpha-amino nitrogen were blocked off in the toxic protein. Ultraviolet absorption spectra of an alkaline dispersion of the pilot-plant protein revealed that about one-half of the absorption was caused by impurities in the protein. To obtain suitable proteins for colorimetric analyses TESOM, and flakes made from the same beans from which the TESOM was made, were extracted with alcohol, which is known to remove most of the natural pigments (1). Protein was prepared from these meals by the method used for preparing the pilot-plant protein except that the curd was dialyzed 6 days against 5% salt solution, followed by dialysis against distilled water to remove the salt. The yield was 18% of the starting meal weight, and both proteins contained 16.85% nitrogen, moisture-free basis. The ultraviolet and infrared spectra of these two proteins were identical. The colorimetric analyses of these two proteins are compared in Table V. No appreciable difference was found between the proteins prepared from toxic and nontoxic meals. It appeared therefore that these functional groups were not involved. However it is possible that they were set free by the test conditions.

Stability of the Toxic Factor

Comparisons of the assays, based on the protein, of Samples C and D, Table II, with the assay of Sample H, Table IV, when fed at about the same levels indicate that there is no marked change in toxicity on autoclaving the moist protein. The relative stability of the toxic factor to autoclaving is also evidenced by assay results obtained before and after autoclaving the raw TESOM (Samples A and A-1, Table II).

When the crude protein (Sample D, Figure 1), containing 83% protein, was assayed, the possibility existed that the toxicity resided in the impurities carried along with the protein. In our first attempt to prepare a purified protein, Sample D was dispersed at pH 11.5 at 60°C. and the dispersion was clarified with a Sharples centrifuge at 13,000 *g*. During this operation the protein was exposed to the alkaline pH for 4 hrs. After clarification of the dispersion the protein was precipitated at pH 4.5 with hydrochloric acid, and the curd was dried at 70°C. The resulting protein preparation was designated as Sample F (Figure 1). The toxicity assay of this protein is shown in Table VI. The data indicate that Sample F was less toxic than Sample D (Table II), from which it was prepared. Furthermore the degree of toxicity of this "alkali-purified" protein was less than that of the whole meal, calculated to protein content (Sample A-1, Table IV). The question arose as to whether this apparent loss in toxicity resulted from the alkali treatment or from removal of impurities. The answer to this question was indicated when the toxicity assay for Sample H was obtained. Sample H was of greater purity (96% protein) than Sample F (94%), yet about one-half as much was required to kill the calf in about one-half of the time. The lower toxicity of Sample F indicates that the toxic principle is somewhat labile to mild alkali at 60°C.

TABLE V

Colorimetric Analyses of Protein from Toxic TESOM Compared with Control Protein from the Same Beans

Method used	Treatment of protein	Amino acid tested for	Relative optical densities	
			Control protein	Toxic protein
Millon (10)	H_2SO_4 hydrol.	Tyrosine	0.171	0.169
Thomas (25)	Enzyme hydrol.	Tyrosine	0.361	0.364
Folin (11)	None	Tyrosine + trypt.	0.235	0.235
Spies (23)	None	Tryptophan	0.556	0.554
Block (4)	Enzyme hydrol.	Phenylalanine	0.327	0.327
Ninhydrin (12)	None	Alpha amino group	0.162	0.163

TABLE VI

Toxicity Assays of Protein Hydrolyzates

Sample No.	Description of sample	Amount of sample consumed and days-to:[a]							Remarks
		Platelet decrease		Leucopenia [b]		End of sample feeding			
		Days	Lbs.[c]	Days	Lbs.[c]	Days	Lbs.[c]	Lbs./day	
F	Control for G[d]	....	....	35	7.1	67	12.9	0.19	Moribund 67th day—sacrificed—severe lesions.
G	HCl hydrolyzate of F	....	....	....[e]	....	45	14.9	0.33	Sacrificed 65th day—no typical lesions.
I	Control for J[f]	26	3.3	33	4.1	62	7.1	0.11	Sacrificed 64th day—no typical lesions.
J	Enzymatic hydrolyzate of H	25[g]	3.0	....[h]	....	54	6.5	0.12	Sacrificed 58th day—no typical lesions.

[a] All samples calculated to 9% moisture; all sample data calculated to lbs./100 lbs. body weight of calf, based on original unhydrolyzed protein.
[b] Definite shift toward leucopenia and/or relative lymphocytosis.
[c] $\text{Lbs./100 lbs. calf} = \dfrac{\text{Total sample consumed}}{\text{Initial calf weight} + \text{calf weight at symptoms or end of feeding}} \times 200.$
[d] Protein was dispersed in alkali at pH 11–12 and held for 4 hrs., centrifuged, and then precipitated with HCl at pH 4.5.
[e] No leucopenia or relative lymphocytosis observed. Blood platelets not determined.
[f] Control for enzymatic hydrolyzate: Sample H dispersed at pH 7.5, autoclaved 1 hr. at 15 p.s.i., and held at 40° for 48 hrs. with pH at 10 for 1 hr., at pH 9 for 1½ hrs,, and then to pH 7.3.
[g] A progressive but atypical platelet decline was observed from the start. A level of 200,000/mm.[3] was observed by the 25th day. A minimum level of 64,000/mm.[3] was observed on the 46th day.
[h] A mild but transitory degree of relative lymphocytosis was observed during the period of the 28th to 48th day. Leucopenia was not observed.

Sample F was hydrolyzed with hydrochloric acid to produce Sample G, as indicated in Figure 1. The acid hydrolysis was carried out by refluxing the protein with 1.25 equivalents of 28% hydrochloric acid, based on nitrogen, for 14 hrs. The amino-nitrogen of the hydrolyzate was 75% of the total nitrogen, indicating complete hydrolysis. The hydrolyzate was concentrated under vacuum to a sirup containing 78.5% solids and then neutralized to pH 6.5 with sodium hydroxide for toxicity assay. The results of the assay for this hydrolyzate are shown in Table VI (Sample G). Sample F served as a control for the unhydrolyzed protein. Since no toxic symptoms were obtained with Sample G, it becomes evident that the toxic principle is labile to prolonged acid hydrolyses.

In a further attempt to obtain a hydrolyzate of the toxic protein so as to isolate the toxic principle, enzymatic hydrolysis was carried out. For this hydrolysis the undried protein curd from Sample H (Figure 2) was dispersed at pH 7.5 to give a protein concentration of 6% and autoclaved for 1 hr. at 15 p.s.i., to sterilize. After cooling, the dispersion was raised to pH 10 with sodium hydroxide because laboratory experiments had shown that this starting pH gave maximum hydrolysis in the absence of buffers. Crude, 1:110, trypsin (30 mg. per gram of dry protein) was slurried in water at pH 4.3 and passed through a sterile filter before adding to the protein dispersion. Filtering this crude trypsin resulted in a loss of 27% of its weight and about 10% of its proteolytic activity. This sterile procedure was considered necessary because toluene is known to cause aplastic anemia in some species, and its use as a preservative was avoided. The sterile protein mixture was incubated for 48 hrs. at 40°C., at which time the pH had dropped to 7.2 and the amino nitrogen was 23% of the total nitrogen. The hydrolyzate was then spray-dried and designated as Sample J. Since the stability of the toxic factor to autoclaving a neutral dispersion of protein, followed by incubation at an alkaline pH, was unknown, a control was prepared in which the protein curd from Sample H was carried through the same process without the addition of the enzyme. During incubation of the control the pH was adjusted to 9 after 1 hr. and to 7.3 after 2½ hrs. in accordance with the pH drop observed during the enzymatic hydrolysis. This control was designated as Sample I. The results of the assay of these two preparations are shown in Table VI.

Both Samples I (Enzymatic Control) and J (Enzymatic Hydrolyzate) showed marked and essentially equal reductions in toxicity when compared to the assay of the basic toxic protein fed at comparable levels (Sample H at 0.125 lb./100 lb. body weight of calf/day). Therefore it was not possible to assess the effect of the tryptic digestion upon the toxicity of the basic protein. The nature of the destruction of toxicity in the control (Sample I) is the subject of further investigation.

Further work is being directed toward hydrolysis of the protein without appreciable loss of toxicity in the hope of isolating and identifying the toxic factor.

Summary

Fractionation studies have been carried out on a specially prepared undenatured toxic trichloroethylene-extracted soybean oil meal to determine which component of the meal is associated with the toxicity. Calf-assay of the samples indicated that the toxic factor is associated with the protein.

The toxic factor associated with the protein is labile to strong acid hydrolysis. Measurable destruction of the toxic factors occurs when the protein is treated with alkali at pH 11–12 at 60°C. Because destruction of the toxic factor occurred when the neutral protein dispersion was autoclaved for 1 hr. and was followed by an alkaline treatment simulating the pH conditions encountered in tryptic digestion, it was not possible to evaluate the effect of tryptic digestion upon the toxic factor.

Analyses of the toxic protein failed to indicate the nature of the reaction of trichloroethylene with the protein to produce the toxicity.

Acknowledgment

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Possible Mechanisms in Thermal Polymerization of Vegetable Oils. II. Polymer Formation[1,2]

R. P. A. SIMS, Chemistry Division, Science Service, Department of Agriculture, Ottawa, Canada

THE RESEMBLANCE between thermal polymerization of vegetable oils and polycondensation reactions has been pointed out by many workers (1, 4, 8, 13). However, in some cases, the extent of reaction was estimated from bulk viscosity measurements that, except during the early stages of bodying, were shown to bear a nonlinear relation to monomer disappearance (14) or from iodine number determinations that lose reliability with an increasing extent of reaction. In the present paper, polymer formation was followed, using a molecular still in which monomeric glycerides were separated from polymer and trimeric acyl groups from esters of higher complexity. Disappearance of unsaturation was followed by hydrogenation in dilute solution. Reaction temperature has been shown to affect polymer formation (3, 9, 13, 14, 16). Therefore an attempt has been made to assess the influence of temperature on polymerization mechanism.

The kinetics of the polymerization of methyl esters of unsaturated fatty acids have been studied by Paschke and Wheeler (8, 9) and by Rushman and Simpson (10). Paschke and Wheeler developed the idea that initially nonconjugated dienoic and trienoic acyl groups polymerize by way of a Diels-Alder type of reaction that is preceded by isomerization of the double bonds to the conjugated position. Nonconjugated acyl groups were shown to react with conjugated material. The latter workers have shown that polymerization of methyl linoleate is kinetically of the second order and proceeds more readily than diene conjugation. Second-order kinetics were also found by Sims (12) in a study of dilution polymerization of linseed oil. Further work is reported here in which the behavior of tung and oiticica oils, containing large amounts of conjugated trienoic acids in the *trans* configuration, is contrasted with linseed and safflower oils where the unsaturation is initially *cis* and nonconjugated.

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Materials and Methods

Linseed, safflower, tung, and oiticica oils were polymerized in an all-glass apparatus kept under constant positive pressure of oxygen-free nitrogen (12). The oil was degassed before being added to the polymerization vessel that was swept with nitrogen throughout the reaction. Samples were collected in an evacuated receiver, cooled under nitrogen, and kept under refrigeration in nitrogen-filled bottles. The nonconjugated oils were alkali-refined and bleached before heating whereas the conjugated oils were polymerized without prior purification.

Some samples of heated oils were hydrogenated at atmospheric pressure in dilute cyclohexane solution, using a platinum on silica catalyst (15). Details of the preparation of esters from glycerides, their hydrogenation, and their distillation have been reported elsewhere (14).

Content of polymeric glycerides and acyl groups was determined by micromolecular distillation (11); concentration of dimer was expressed in weight percentage, identical with base mole percentage, the molar percentage of monomer units that have dimerized. Where necessary, apparent concentration of monomeric and polymeric glycerides and acyl groups was corrected for the presence of thermal decomposition products. With oiticica oil as much as 16% of a sample distilled in the mono- and di-glyceride range. The results were therefore calculated on a true glyceride basis. With the other oils the differences were of the order of 3% or less and were not applied to the results.