ent cottonseed pigment gland samples caused greater body-weight depressions than did three samples of pure gossypol. Since all of the 10 diets varied only to the extent that the small amounts of cottonseed piigment glands or pure gossypol were incorporated into the standard stock diet, these differences in body-weight performance were obtained with diets whose protein and fiber levels were constant. The efficiencies of feed utilization were less for the groups fed cottonseed pigment glands than for the groups fed samples of pure gossypol.

If the free (or bound, or total) gossypol were the sole factor(s) involved in cottonseed toxicity, it would appear to be a simple matter indeed to determine by well-controlled experimentation whether a true statistical correlation exists between growth and the free (or bound, or total) gossypol level in the diet. Gallup (5) had stated in 1928 however that the chemical methods available at that time for determination of gossypol and its related compounds did not suffice as a measure of the toxicity of heated cottonseed products. In 1948 Boatner *et al.* (6) reported that cottonseed pigment glands, distinct morphological entities containing the toxic components of cottonseed, added to the diets of chicks produced marked retardation of growth and a high incidence of deaths whereas addition of an equivalent amount of pure gossypol to the diet caused little or no retardation in growth. They likewise found that no correlation was apparent between the nutritive value for chicks of various cottonseed products and their relative contents of gossypol and gossypurpurin. Eagle *et al.* (7, 8, 10) showed that the different acute oral toxicity (LD 50) values for these pigment glands in various animal species bore no relation to their gossypol content.

Reports of feeding tests, in which various levels of pure gossypol were incorporated into the diets of rats, showed that while the body weight depression caused by pure gossypol is proportional to the amount added to the diet, the greater mortality and body-weight effects caused by adding various levels of cottonseed pigment glands to the diet cannot be attributed to their analyzed gossypol content alone (10). Feeding tests on rats likewise have shown that the residual toxicity of cottonseed meals could not be explained on the basis of their analyzed free gossypol content (11, 12). These conclusions are confirmed by the results from the nine experiments on more than 300 rats and 1100 chicks reported in the present paper.

Summary

Twenty-four cottonseed meals fed to chicks in practical feeding rations for eight weeks led to good growth performance and favorable feed efficiency in many cases, despite the unusually high free gossypol content of the rations.

The results from replicated, protein-quality evaluations in chicks fed for eight weeks at the 15% protein level were closely parallel to those from similar experiments in rats fed at the 9% protein level and showed that the percent nitrogen solubility in 0.02 N sodium hydroxide of cottonseed meals is a poor indicator of protein quality.

Constant gossypol levels of 0.1%, supplied by additions of different amounts of cottonseed pigment glands, caused greater body-weight depressions than did the same gossypol level supplied by pure gossypol.

The toxicity of cottonseed pigment glands and of cottonseed meals cannot be accounted for solely on the basis of analyzed gossypol content.

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Studies on Guar Seed Oil (Cymmopsis Psoralioides)

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PARIKH (1) and Ramakrishnan (2) have studied in detail the nutrient composition of guar seed, a cheap legume available in abundance in certain parts of India, and have found that the seed contains a good percentage of high quality protein along with about 4% of fat. The oil was extracted from dried seeds, and its properties were studied as reported in this paper.

Experimental

The fat was extracted from crushed seeds by petroleum ether (b.p. 40°–60°C.). The characteristics as determined by standard procedures (3) are given in Table I.

The mixed fatty acids (excluding the unsaponifiable fraction) were analyzed spectrophotometrically by the method of Hilditch, Morton, and Riley (4)

TABLE I

Characteristics of Guar Seed Fat

Oil in dry seeds	4.2%
Lovibond color of oil	29.9Y–6.1R–3.1B
Specific gravity of the oil at 38°C.	0.9217
Free fatty acid as oleic	0.33%
Butyro refractometer value at 40°C.	64.30
Saponification value	196.80
Iodine value (Wijs 30 min.)	88.00
Refractive index at 35°C.	1.4680
R. M. value	0.7
Polenske value	0.4
Unsaponifiable matter	3.52%

for polyethenoid acids. Results of the analysis are given in Table II.

In order to assess the *in vitro* digestibility of guar oil (both fresh and rancid) the hydrolysis of guar oil by guar, pancreatic, peanut, and mold lipases was carried out. The hydrolysis of peanut oil by these lipases was also carried out for comparison. Guar, peanut, and *Aspergillus Niger* mold lipases were prepared according to Ramakrishnan's method (5, 6), and pancreatic lipase was prepared according to Willstatter's method (7).

TABLE II

Analysis of the Mixed Fatty Acids of Guar

Saponification equivalent	261.6
Iodine value (Wijs)	88.1
$E^{1\%}_{1\,cm.}$ 234 mμ (180°/60 min.)	168.69
$E^{1\%}_{1\,cm.}$ 268 mμ (170°/15 min.)	1.68
Saturated acids	16.4%
Oleic acid	64.0%
Linoleic acid	19.1%
Linolenic acid	0.4%

Each set of the experiments consisted of 1 ml. of oil, 4.5 ml. of water, 2 ml. of phosphate buffer, 0.1 g. of lipase, 0.5 ml. of 10% gum arabic, and a few drops of toluene in a conical flask incubated for 24 hrs. at 37°C. A continuous shaking arrangement was provided throughout the experiment. A blank accompanied the samples and consisted of all except the enzymes. After the period of incubation the contents were taken out and titrated against N/10 NaOH after adding 25 ml. of neutral alcohol and warming for some time. Phenolphthalein was used as the indicator. Necessary precautions were taken to carry out the experiments under sterile conditions. From the difference in ml. of N/10 NaOH between the sample and the blank, the activity of the lipase was calculated in terms of the percentage of F.F.A. produced. The results are given in Table III.

Ramamoorthy and Bannerjee (8) have found that the unsaponifiable portion of coconut oil, if added to fresh peanut oil, stabilizes the oil to some extent. Since the unsaponifiable fraction of guar oil comes to 4%, experiments were carried out to study the effect of this fraction on the stabilization of guar and

TABLE III

Digestibility of Guar and Peanut Oil by Different Lipases

	Hydrolysis after 24 hrs. in terms of % of F.F.A. produced			
	Fresh guar oil	Rancid guar oil F.F.A. (2.4%)	Fresh peanut oil F.F.A. (.05%)	Rancid peanut oil F.F.A. (3.1%)
Guar lipase[a]	15.9	0.6	12.6	0.30
Pancreatic lipase[b]	65.4	1.5	95.6	2.4
Peanut lipase[c]	30.9	0.6	35.4	0.6
Aspergillus Niger lipase[d]	47.4	1.2	56.4	1.5

[a] Disodium phosphate-citric acid buffer of pH 5.5 was used for the experiment.
[b] Disodium phosphate-citric acid buffer of pH 7.2 was used for the experiment.
[c] Disodium phosphate-citric acid buffer of pH 5.2 was used for the experiment.
[d] Disodium phosphate-citric acid buffer of pH 6.2 was used for the experiment.

peanut oils with and without the addition of an antioxidant like hydroquinone.

Guar oil, peanut oil, and the unsaponifiable fraction of guar oil were prepared, both fresh and used. Ten ml. of the oil, 30 μg unsaponifiable fraction, and 30 μg hydroquinone were used for the experiment. The mixture in a test tube, corked and sealed with paraffin wax, was kept inside a cupboard at room temperature. At different intervals of time 1.0 ml. of the oil was tested for free fatty acid, peroxide (9), and Kreis values (10). The average of several experiments is given in Table IV.

Results

Table I shows that the saponification value and the iodine value of the guar oil fall within the range of the values for edible oils.

From Table II it can be seen that linolenic and linoleic acid are apparently present in guar oil.

Table III shows that fresh guar oil can be digested by pancreatic lipase even though the rate of hydrolysis is not as high as in the case of peanut oil. The hydrolysis of guar oil by other lipases is similar to that of other oils. Rancid guar oil is hydrolyzed to a negligible extent by different lipases. Guar oil, being easily hydrolyzed by pancreatic lipase, may be satisfactory for edible purposes.

Table IV shows that the unsaponified fraction of guar oil added to peanut and guar oil stabilizes the oil to a certain extent. Experiments carried out with the hydroquinone alone added to the oil shows that, after 60 days, the stabilizing power of hydroquinone goes down and the addition of unsaponifiable matter protects hydroquinone to some extent. Since the unsaponifiable fraction, if added alone, protects the oil to some extent from becoming rancid, attempts will be made to fractionate it further and study the effect

TABLE IV

Effect of the Unsaponified Fraction of Guar Oil on the Stability of Guar and Peanut Oil

	0 day			30 days			60 days			90 days		
	F.F.A. %	Kreis value Δ 540/ g. per ml.	P.V. me./kg.	F.F.A. (%)	Kreis value Δ 540/ g. per ml.	P.V. me./kg.	F.F.A. (%)	Kreis value Δ 540/ g. per ml.	P.V. me./kg.	F.F.A. (%)	Kreis value Δ 540/ g. per ml.	P.V. me./kg.
1. Guar oil alone	0.33	1.9	0.5	0.60	3.3	2.1	1.6	3.8	2.6	2.4	12.0	4.8
2. Guar oil + hydroquinone	0.35	2.0	0.5	0.40	2.4	1.9	0.42	2.6	2.2	0.8	8.0	4.0
3. Guar oil + unsap. fraction	0.33	1.9	0.5	0.37	2.2	1.8	0.40	2.2	2.0	0.44	3.8	3.1
4. Guar oil + unsap. fraction + hydroquinone	0.38	2.0	0.5	0.40	2.4	1.9	0.40	2.4	2.1	0.40	4.2	3.2
5. Peanut oil alone	0.06	1.0	0.1	0.07	1.3	0.5	0.10	1.8	0.61	0.37	3.0	1.0
6. Peanut oil + hydroquinone	0.08	1.1	0.1	0.08	1.4	0.5	0.09	1.4	0.62	0.18	2.0	1.0
7. Peanut oil + unsap. fraction	0.06	1.0	0.1	0.06	1.2	0.5	0.08	1.4	0.60	0.10	1.8	0.6
8. Peanut oil + unsap. fraction + hydroquinone	0.09	1.1	0.1	0.09	1.2	0.5	0.09	1.4	0.62	0.09	1.4	1.0

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.

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

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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.

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