drogen chloride was passed into the solution for 0.5 hour. The solution was allowed to stand at room temperature for one hour. Sufficient ethyl ether was added to dissolve the products, and the ether solution was washed with 5% aqueous sodium carbonate and water. The symmetrical distearin was crystallized from the ether solution and was then purified by several recrystallizations from acetone.

The use of ethyl alcohol as a solvent for the recrystallizations was avoided because it was found that the ethyl ether of triphenyl carbinol (melting point 82°C.), produced through the reaction of the trityl compounds with ethyl alcohol, interfered with the separation of the pure diglycerides.

The same procedure was followed for the preparation of symmetrical diolein except that the trityl compounds formed by the acid hydrolysis were separated from the diolein by crystallization from petroleum ether (Skellysolve F) solution. The solution was then washed with 5% aqueous sodium carbonate and water and the petroleum ether removed under vacuum. The crude diolein was dissolved in acetone, refluxed with decolorizing carbon for 10 minutes, filtered, and purified by several crystallizations from acetone.

The yields of symmetrical distearin and diolein were 87 and 90% respectively and were considerably higher than the yields that were obtained in this laboratory by the methods previously referred to (5, 8).

Preparation of symmetrical mixed triglycerides. The procedure outlined by Daubert and co-workers (8) for the synthesis of symmetrical triglycerides was employed with a modification similar to that described for preparation of the symmetrical diglycerides. The modified procedure will be exemplified by describing the preparation of symmetrical oleodistearin.

Eighty-five grams of distearin were dissolved in 125 g. of pyridine and 365 ml. of chloroform. Forty-three grams of oleoyl chloride (5% excess over the stoichiometric proportion) were added slowly and the mixture was allowed to stand at room temperature for one hour, then refluxed for 12 hours, cooled to room temperature, and dissolved in ethyl ether. The ether solution was washed with water, 0.5 N sulphuric acid solution at 0°C., 5% potassium carbonate solution, water, and then dried over anhydrous sodium sulphate. The ether was removed under vacuum and the crude oleodistearin dissolved in acetone. Decolorizing charcoal was added, the solution was refluxed for 15 minutes, and then filtered. The oleodistearin was recrystallized from the clear solution and purified by further crystallizations from acetone. The yield of pure symmetrical oleodistearin was 107 g. (91% of the theoretical). Similar yields were obtained in the preparation of the other mixed triglycerides.

Table II compares some observed iodine values and melting points of the diglycerides and symmetrical mixed triglycerides with theoretical values or with values previously reported by others for the pure compounds.

TABLE II

Properties of Diglycerides and Triglycerides

Glyceride	Wijs iodine value		Melting point °C. capillary tube	
	Observed	Theoretical	Observed	Reported
1,3 distearin	0	0	79.0	79.6(8)
1,3 dipalmitin	0	0	72.0	72.5(8)
1,3 diolein	81.7	81.8	.....	.....
Oleodistearin	28.7	28.6	38.5, 42.5[a]	38.5, 42.5(8)
Oleodipalmitin	30.5	30.7	35.5	35.5-36.5(8)
Stearodiolein	57.1	57.2	23.5	.....
Palmitodistearin	0	0	67.7	68.0(10)
Stearodipalmitin	0	0	67.7	68.0(10)

[a] Oleodistearin melting at 38.5°C. when held in capillary tube at 40° C. for 48 hours, solidified and gave a m.p. 42.5°C.

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Protein Denaturation in Soybean Meal During Processing

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THE steady increase in industrial utilization of soybean proteinaceous materials during the last decade has focused attention on the effect of oil solvent extraction on the properties of the meal. Denaturation of the proteins in soybean meal is inherent in soybean processing because of the necessity of applying heat in the presence of moisture. The extent of denaturation determines the physical, chemical, and nutritional properties of the meal. These imposed characteristics govern to a large extent the ultimate industrial application of the processed meal. The manufacture of soybean meals with different degrees of heat treatment and the judicious application of such meals can result in increased revenue for the processor.

Protein denaturation has been defined by Neurath, Greenstein, Putnam, and Erickson (8) as "any non-

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proteolytic modification of the unique structure of native proteins, giving rise to definite changes in chemical, physical, or biological properties." This definition is an excellent one for theoretical investigations of proteins and indicates the broad scope of the subject, but our inability precisely to define and measure the structure of protein molecules leaves much to be desired. A simplified experimental procedure is necessary for the study of denaturation in soybean processing plants. For our present purpose we are using the changes in nitrogen dispersibility in water at its natural pH of about 6.6 as the measure of protein denaturation.

Soybean meal intended for stock feeds is deliberately steamed or toasted until the protein is nearly insoluble in water. The proper amount of steaming develops the optimum nutritional value of the meal (7). However for industrial applications undenatured types of meal are required. A test, by Davidson and Cagle (4), for evaluating technical soybean meal as an adhesive for the wallpaper industry suggests that only a limited amount of protein insolubility can be tolerated. Hayward (5) has outlined specifications which require substantially undenatured soybean meal flakes for use as a brewing adjunct. Boyer (3) has described a method for solvent extraction of soybean meal from which a special type of protein may be isolated for fiber spinning. In his process he maintains "a much lower temperature" than is normally encountered in processing. He pointed out that his technique enabled him to prepare solutions containing 20% protein whereas it is normally difficult to prepare solutions containing more than 12% solids. It is also well known that maximum yields of protein can be obtained only from soybean flakes that have received a minimum of heat treatment.

Beckel, Bull, and Hopper (2) investigated the progressive denaturation of protein in soybean meal as related to the factors affecting its dispersibility in water. However the results of their work are difficult to translate to actual operating conditions employed in a commercial solvent extraction plant. The present investigation was therefore conducted to evaluate the effect of commercial manufacturing conditions on the subsequent dispersibility of the protein in water following each processing step. Such information should serve as a standard for comparing the degree of protein denaturation occurring during various unit operations for both commercial and pilot solvent extraction plants.

Denaturation in the Laboratory

In order to establish a basis for appraisal of the denaturation occurring in processing plants, nitrogen dispersibilities were determined on two series of laboratory prepared soybean samples which had been denatured by steam treatment at atmospheric pressure (210°-212°F.). The steam treatment for one series of samples occurred after extraction of the oil and the other before. For series 1 the beans were cleaned, cracked, dehulled, conditioned to about 15% moisture, and flaked at room temperature. The flakes were allowed to come to moisture equilibrium with the air. The oil was removed in a Soxhlet extractor with a hexane-pentane mixture (b. p. 92°-135°F.). Samples of the fat and solvent-free flakes were then steamed in an autoclave for varying periods and ground in a hammer mill so that more than 90% passed through a 100-mesh screen. The samples for series 2 were prepared the same as series 1 except that the full fat flakes were steamed in the autoclave, followed by oil extraction and grinding.

We had intended to determine the dispersible nitrogen in the full fat meal but the results were not reproducible. Several extraction techniques were attempted but none proved satisfactory for the purpose. Consequently all full fat samples in series 2, as well as the full fat commercial samples to be described later, were extracted with commercial hexane-pentane mixture to less than 1%. The nitrogen dispersion values were determined by suspending 2.5 g. of meal in 100 ml. of water and shaking the meal-water mixture for 30 minutes in a Precision Scientific Company mechanical shaker. The dispersion is then clarified in a centrifuge at about 2,000 times gravity and an aliquot taken for Kjeldahl nitrogen analysis. Further details of the method for determining dispersible nitrogen have been outlined in the literature (9). All reported results are the average of two or more determinations.

The nitrogen dispersion results for the two specially prepared meals are shown in Figure 1, where curve 1 is for the meal steamed after removal of the oil and curve 2 for the meal steamed before removal of the oil. The small difference between the two curves cannot be attributed to the presence of oil in the meal for series 2 at the time of steaming. Although fatty acids are known to have some protective action against denaturation, the effect is small compared to the denaturing action of steam. In other experiments of a similar type in this laboratory, it has been found

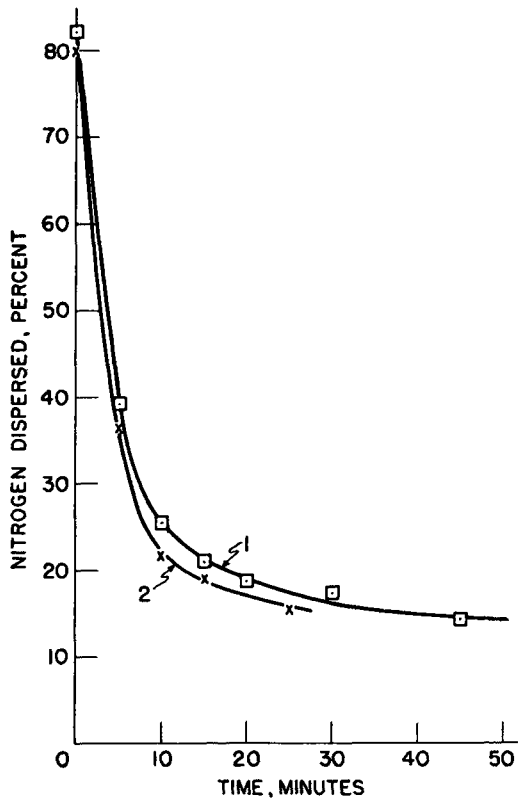

FIG. 1. Change in nitrogen dispersibility of soybean meal in water with increasing time of atmospheric steam treatment. Curve 1, the flakes steamed after solvent extraction of the oil, and Curve 2, the flakes steamed before solvent extraction.

rather difficult precisely to duplicate the denaturing effects of steam over short periods of time. The difference in curves 1 and 2 probably is due to experimental error.

These data show the sensitivity of soybean protein to denaturation with moist heat. The 15% of nitrogen remaining dispersible after 45 minutes of steam treatment includes soluble peptones and peptides as well as non-protein nitrogen. According to Becker, Milner, and Nagel (1) the non-protein nitrogen may vary between 2.88 and 7.80%. The nitrogen dispersibility of commercial soybean meal after heat treatment by the same test procedure is frequently as low as 7 or 8%.

Procurement and Treatment of Commercial Samples

The sequence of unit operations employed in a solvent extraction plant for soybean oil is as follows: a) weighing the beans, b) cracking, cleaning, and dehulling, c) conditioning the grits (heating to predetermined temperature and adjustment of the moisture content), d) flaking, e) extracting the oil, f) desolventizing, g) deodorizing and h) toasting or other heat treatment of the residual flakes. These manufacturing steps are logically divided into three groups: a) preparation of the bean for extraction, which involves the first four operations, b) extraction of the oil from the prepared flakes, the fifth operation, and c) recovery of the residual meal in a usable form which requires the three remaining steps. Recovery of the oil from the miscella by evaporation and steam stripping are operations not affecting the protein and will not be considered in this work. During each of the operations listed, the proteinaceous material undergoes various treatments with respect to time, temperature, and moisture content that might be expected more or less to denature the protein and change its dispersibility. It is those changes with which this investigation is concerned.

With the cooperation of various soybean processors samples were obtained from five commercial plants, operating with low boiling point solvents made up of hydrocarbon mixtures. The plants listed in Table I as 1, 3, and 5 were currently producing animal stock feed at the time of collection while plants 2 and 4 were producing meal for use as specialty products. Composite samples of approximately five pounds each were collected for evaluation from each of the important processing steps by combining smaller individual samples taken at hourly intervals. Warm samples and those containing solvent were cooled and air-dried immediately to minimize additional denaturation. Information regarding the processing conditions at the time of sampling was obtained and will be discussed later. For the dispersibility measurements aliquot samples were obtained from the large composites by sub-dividing in a Boerner sampler. The resultant small samples, approximately 25 grams, were ground through a $\frac{1}{64}$ screen in a hammer mill. The nitrogen dispersibility measurements were made as described for the laboratory prepared meals.

Operating Conditions

Operating conditions in a solvent extraction plant have been covered in a general manner in the literature (6). However in most instances the information is incomplete regarding the factors which cause denaturation of the protein, namely, time, temperature, and moisture content of the proteinaceous materials. For this reason a special effort was made to obtain data on the operating conditions during the interval of sampling, and these variables will be reviewed. It must be remembered that certain of these conditions are difficult to measure accurately in processing equipment, and therefore the data are estimates based on approximate determinations. This is especially true in the case of retention time for commercial equipment when the flow is not positive. The values reported are the maximum for distribution curves obtained by plotting quantity of test material recovered against time.

The successful operation of a solvent extraction plant is dependent on the proper preparation of the oleaginous material prior to extraction. The beans are transferred from storage, weighed, cracked, cleaned, and dehulled. The general average of moisture content during these steps was 10%. In only one plant, No. 4, was any heat treatment applied prior to the conditioning of the grits. At this plant the drying operation was reported to facilitate subsequent hull removal. In this particular process the beans were dried to a moisture of 9 to 9½% in a period of one hour with a stream of air heated to a temperature of 220°F. No actual temperature of the beans could be obtained under plant operating conditions. Conditioning or tempering of the cracked grits increases the plasticity of the protein, thereby improving their flaking characteristics. No standard type of conditioning equipment has been developed by the industry; therefore the variation between plants is in the mechanical equipment rather than in operating conditions. Consistent tempering results are obtained throughout the industry in that the grits are tempered to 9½ to 10½% moisture in a time interval of 15 minutes while being heated to 165°-175°F. No additional heat is added during the flaking operation since friction from the flaking rolls helps to maintain the conditioning temperature. The finished flakes are then transported to the extraction system, usually in another building. Sufficient time elapses in the interval, and the temperature is adequate to suggest some denaturation.

Extraction procedures are comparable between plants despite the wide variance in mechanical equipment used for this operation. The flakes containing the moisture added for flaking remain in contact with the hydrocarbon solvent for a period of 30 to 55 minutes and are held at a temperature of approximately 130°F. The amount of denaturation occurring at this point in the process will be a function of the chemical nature of the extractant as well as time, temperature, and moisture of the flakes.

The steps of desolventizing, deodorizing, and toasting, which are mainly responsible for the final characteristics of the meal, vary appreciably within the industry. Two systems are currently in use for the desolventizing step. Plants 2, 3, and 4 conduct this operation in equipment of the original "Schneckens" design while plants 1 and 5 remove the occluded solvent in the newer superheated vapor system.

The "Schneckens" type desolventizer consists of five or more horizontal, steam jacketed tubes, superimposed vertically and operated in series. The extracted flakes are conveyed through the series by a rotating ribbon type screw. The major part of the solvent is vaporized in the first three or four stages by application of indirect steam in the jackets and

hollow conveyer shafts. For final deodorization of the flakes, direct steam is usually introduced in the last stages or a separate drum. Further description of the "Schneckens" and variation in operation of this equipment will be found in the literature (6). The meal is usually in the "Schneckens" 10 to 15 minutes with a gradual increase in temperature to about 210°-212°F. just prior to being discharged, and during that time it is being conditioned to 10-11% moisture by condensation of the sparging steam.

The vapor desolventizing system is based on the principle of circulating a superheated vapor through the solvent saturated flakes, resulting in direct contact between the solvent to be vaporized and the heating medium. The principle used in this method of desolventizing shows promise of eventually replacing the older "Schneckens" type of equipment because of its economy and flexibility in operation. In this type of apparatus the residual meal is heated to 170°-174° F. during its 5 to 10 minutes' retention time. The deodorizing unit, which in this system is separate from the desolventizer, removes the last traces of solvent from the flakes by direct application of steam for an estimated period of 5 to 10 minutes. In the deodorizer the meal is heated to 208°-210°F.

In general, there are two different toasting procedures in practice, requiring different types of toasting equipment. Plants 1 and 5 toast the deodorized flakes under a pressure of 14-15 psig. for about 15 minutes while plants of type 3 toast the flakes for about 45 to 60 minutes at atmospheric pressure. As in some of the other operating steps the toasting equipment has not been standardized. The toasting operation is a critical processing step and the conditions have been adjusted to insure maximum nutritional value as previously mentioned. In the atmospheric system the meal reaches a temperature of 215°F. as compared to 240°F. in the pressure toasting. The meal is surrounded by an atmosphere of steam at all times in both processes and quickly approaches its equilibrium moisture content at the prevailing temperature. The rate of change of protein solubility for the atmospheric pressure method will be similar to that shown in the curves in Figure 1, but for the pressure systems the rate should be more rapid.

Discussion of Results

Interpretation of the dispersibility results given in Table I reveals several interesting facts not commonly appreciated in the industry. Values reported at station 1 indicate that the dispersibility of the protein from the original beans may vary considerably, a factor which must be recognized in the interpretation of the results of an investigation of this nature. The extent of this variability in dispersible nitrogen between beans is under further investigation at this laboratory. Dispersibility values for stations 2, 3, and 4 suggest that the protein is less sensitive to denaturation when the flakes still contain oil. However, up to the point of flaking, the internal cell structure of the bean is nearly intact, and some protection against change may be afforded by its natural condition. The nitrogen dispersion results are reasonably consistent from one plant to another and show that only a minor portion of the total denaturation effect in processing can be attributed to the preparation steps. In fact, more variation is evident between the original raw beans than the various preliminary operations. The low value obtained for the conditioner discharge of plant 2 is probably the result of a sampling error which can easily happen in a solvent extraction plant.

TABLE I

Effect of Solvent Extraction Processing on Protein Denaturation as Measured by Nitrogen Dispersibility in Water at pH 6.6

Station No.	Sample	Solvent Extraction Plant No.				
		1	2	3	4	5[1]
		Dispersible Nitrogen				
		%	%	%	%	%
I	Whole beans	84.6	81.4	87.9	87.0 84.5[2]	82.9
II	Dehuller discharge	84.9	79.3	87.1	84.1	79.3
III	Conditioning discharge	84.9	70.7	79.5	82.2	80.7
IV	Flaking roll discharge	84.0	78.6	74.2	80.7	77.9
V	Extractor discharge	87.9	78.4	.....	79.9	81.1
VI	Desolventizer discharge	85.3	.....	.....	.....	80.8
VII	Deodorizer discharge	43.7	46.6	48.0	51.6	65.4
VIII	Toaster discharge	7.2	.....	14.0	39.7	8.2

[1]No dehulling was practiced at this plant.
[2]After preheating to reduce moisture in beans.

Experience has indicated that, under conditions normally existing in solvent extraction plants, the extraction with hydrocarbon mixtures such as those used commercially does not appreciably denature the protein. The denaturing effect of the solvent on the protein is especially important in the manufacture of undenatured soybean meal products for industrial applications, and any new oil solvents proposed for the industry should be examined for their effect on the protein.

It is evident from this investigation that the major portion of the denaturation occurs in the final meal treating steps. As shown in Table I, the samples obtained from the desolventizer discharge from plants 1 and 5 have very high nitrogen dispersibility. If this type of meal is required for industrial use, the small amount of residual solvent can be removed by proper use of stripping gases without further significant denaturation. However in the systems using "Schneckens," it is not practical to remove flakes under like conditions and considerable denaturation normally occurs by the time the meal comes from the deodorizer discharge. For producing stock feed, with the vapor desolventizer system, the desolventizer is followed by a deodorizer that lowers the nitrogen dispersibility to about the same value as the meal from the deodorizer following the "Schneckens." In both systems the meal is given a final steam treatment (step VIII) to develop optimum nutritional value. For stock feed the nitrogen dispersion is usually in the range of 8-14%. The small amount of denaturation occurring in the vapor system has never been equalled in practice by the older method; however by careful regulation and control of conditions in the "Schneckens" the denaturation may be less than that reported in this publication. The vapor desolventizer has made possible the production of soybean flakes with a nitrogen dispersibility approaching that of the original beans.

Summary

The effects of soybean oil solvent extraction on the denaturation of the protein as measured by changes of dispersibility of the nitrogen in water has been studied. The results for five commercial plants were compared with denaturation changes produced by steam treatment of laboratory prepared flakes. The results show that by use of proper conditions and

equipment, flakes can be produced with a minimum of denaturation or, when desired, denaturation can be controlled within practical limits.

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Estimation of Vitamin A in Margarine. III. Migration of Lecithin From the Oil to the Aqueous Phase and Its Effect on the Spectrophotometric Blank

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IN the preceding paper (1) a simple and precise spectrophotometric procedure was described for the control of vitamin A fortification of margarine made in the plant. The reliability of the method was supported by physico-chemical and biological assay data. The method involves readings taken at 328 mμ of the whole margarine oil dissolved in cyclohexane after the spectrophotometer had been set at 100% transmission with the corresponding unfortified oil at the same concentration in cyclohexane.

Two control oils were indicated (1) as essential when assays are conducted on the margarine oil before and after emulsification with the milk phase, since lecithin was found to migrate from the oil to the aqueous phase in margarine manufacture. Inasmuch as lecithin absorbs light appreciably at 328 mμ, the blank unfortified oil in the spectrophotometric assay must contain this material in testing the fortified oil prior to churning while the blank in assays of the oil separated from margarine must be free of lecithin. In the present report spectrophotometric and fluorometric data are presented, supported by phosphorus analyses and oil stabilization studies, demonstrating unequivocally that lecithin migrates completely from the oil to the aqueous phase and that this phenomenon must be taken into consideration in spectrophotometric assays of margarine oils for vitamin A content.

Spectrophotometric and Fluorometric Findings

Three commercial lecithin preparations varying in color were added, each in 0.1625% concentration, as a supplement to a margarine oil (0.13% on the margarine basis). The lecithin samples alone were first analyzed with the results obtained given in Table I. Both the Lovibond Tintometer and the Beckmann Spectrophotometer were employed in the objective color measurements. Readings with the latter instrument were taken at 328 mμ since it is at this point that esterified vitamin A absorbs maximally. Fluorometric readings were taken, using a Pfaltz and Bauer model A fluorophotometer with a Corning red ultra No. 5840 filter for the irradiating light beam and two filters in series for the fluoroescent light. The latter were a straw yellow noviol shade A, No. 038, and a light shade blue-green, No. 428. A solution of quinine sulfate in 0.1 normal sulfuric acid, 0.200 microgram per cc., was used as the reference standard for the fluorometric analyses.

TABLE I

Ultra-Violet Light Absorption and Fluorescence of Lecithin Samples

Test	Sample A	Sample B	Sample C
Visual color observation	Light orange yellow color	Deep orange brown color	Dark brown
Flavor[a]	Good	Good	Good
F.F.A.(%)	9.3	10.2	9.3
Lovibond color (1% in mineral oil)	25Y-1.0R	30Y-1.4R	35Y-2.4R
	Samples as received		
E Value[b]	36	49	58
Fluorescence[c]	184	273	307
Ratio of fluorescence to E value	5.1	5.6	5.3
	Samples after 60 hrs. at 140°F.		
E Value[b]	45	61	67
Fluorescence[c]	282	432	448
Ratio of fluorescence to E value	6.3	7.1	6.7

[a] For flavor evaluation of the commercial lecithin a 1% concentration in USP mineral oil is employed.
[b] Absorbancy × 100 of a 1% solution in cyclohexane vs. cyclohexane at 328 mμ, using a cell of 1 cm. depth.
[c] Fluorescence in cyclohexane expressed as microgram equivalents quinine sulfate per gram of sample.

Attention is directed to the fact that all three lecithin preparations were good in flavor and any one of them could have been used, despite differences in color, as a margarine oil supplement. It may be noted that absorption at 328 mμ on the samples as received paralleled fluorescence, and this is apparent from the constancy of the ratio of fluorescence to light absorption. After the lecithin preparations were held at a moderately elevated temperature (140° F.) for 60 hours, both the absorbency and fluorescence values increased appreciably. The constancy of ratio of the latter to the former value was maintained, but the average ratio was greater in the heated samples (6.7 as compared to 5.3) due to a proportionately greater increase in the fluorometric values.

In Table II are given the results of similar spectrophotometric and fluorometric tests conducted on a given margarine oil not fortified with vitamin A but supplemented with each of the lecithin preparations in 0.1625% concentration. It will be noted in the tests with the freshly prepared samples that supple-