meal temperatures of 111.1 C. Run III was given a 10-min toast treatment at constant temperature with the vent closed and exhaust fan off before toast-drying, whereas for run IV, toast-drying was started immediately at the end-of-sparge. Although the urease activity declined rapidly in run III, the urease activity for the final meal product for both runs III and IV were at similar low levels, and the meals contained ca. 3 mg of trypsin inhibitor/g (92% trypsin inhibitor inactivation). The additional toasting period given for run III required to dry the meal to 12% moisture appeared to have little effect on final meal product quality. Sipos and Witte (4) also reported that toasting time at constant moisture was not critical to meal quality.

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Effect of Succinylation of Cottonseed Flour during Protein Extraction on the Yield and Some of the Properties of Protein Isolates

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ABSTRACT

A new method has been developed to improve extraction and recovery of protein isolates, which are highly water-soluble, low in sensitivity to calcium precipitation and light-colored, from defatted cottonseed flour. Optimal conditions include extraction of flour with acidified *n*-butanol (pH 4.5) to remove chromophores, succinylation of proteins at pH 8.5 using succinic anhydride at a concentration of 30% on protein basis, and precipitation of protein at pH 4.5. The resulting succinylated isolates contain more hydrophobic and neutral amino acids than untreated isolates. Gel filtration chromatography and polyacrylamide gel electrophoresis of protein isolates (water-, salt [4% NaCl]- and alkali [0.2% NaOH]-soluble), sequentially fractionated from succinylated and untreated flours, suggest that succinylation converts much of the salt- and alkali-soluble proteins to water-soluble forms. Succinylation increases emulsion stability of isolates. The 3 succinylated isolates showed similar chromatographic patterns on Sephadex G-100 columns and electrophoretic mobilities in polyacrylamide gels, whereas the corresponding 3 isolates from untreated flour had different gel patterns and mobilities. Mobilities of major protein components of the 3 isolates were increased by succinylation.

INTRODUCTION

Chemical modification of proteins induced by acylation with succinic, acetic and 3,3-dimethyl-glutaric anhydrides has been shown effective in improving properties of proteins in certain formulated foods (1-5). Reactions of proteins with these chemicals result in amino groups with different electrostatic charges than the original proteins. Succinic anhydride reacts with unprotonated free amino groups at high pH and changes distribution of charges in protein molecules (6). Moderate succinylation results in unfolding, expansion and increased negative charges in protein molecules, but extensive succinylation may cause the protein to dissociate into subunits (4,7). These types of molecular changes alter chemical and functional properties of proteins (8).

Acetic or succinic anhydride acylation is reported to alter functionality of glandless cottonseed flour (9,10). Succinylation of peanut flour increases nitrogen solubility at neutral pH, water absorption and retention capacities, emulsion capacity and viscosity (4). Final characteristics depend on the ratio of succinic anhydride to protein in the reaction mixture. Acetylation of soy protein isolate decreases water binding capacity and gel strength, but increases solubility in the pH range of 4.5 to 7.0 (11). Franzen and Kinsella (12) reported that succinylation of more than 90% of available amino groups shifts the isoelectric point of soy protein, and increases emulsifying and foaming capacities and stabilities. However, acetylation has little effect on functional properties of soy isolate.

Developments of glandless cottonseed varieties (13) and liquid cyclone processing techniques have resulted in cottonseed protein ingredients with very low gossypol contents (14), and have increased opportunities to incorporate cottonseed protein ingredients into, e.g., baked goods and meat analogs. The objectives of this research were to convert water-insoluble globular proteins of glandless cottonseed to water-soluble forms by succinylation, and to evaluate effects of succinylation on protein extractability, and on functional and physicochemical properties.

EXPERIMENTAL PROCEDURES

Materials

Glandless cottonseed flour defatted by hexane extraction at the Food Protein Research and Development Center at

Texas A&M University was used as the raw material. Protein content of defatted flour was 57.6% (N × 6.25) on a dry wt basis. Reagent-grade chemicals and glass-distilled water were used unless noted otherwise.

Effect of Succinylation of Cottonseed Flour on Extractability of Protein and Its Recovery at Various pH Values

Protein extractability. Cottonseed flour was suspended in water (1:10, w/v) and then adjusted to pH 8.5 with 4 N NaOH solution. Succinic anhydride (in ratio of 0-100% to protein content of the flour) was added during a 10-min period with constant stirring while maintaining pH between 8.0-8.5 with 4 N NaOH. Supernatants were separated by centrifugation at 6,900 × g at 10 C for 20 min using a Sorvall RC-2B refrigerated centrifuge. Protein contents of supernatants were determined by the micro-Kjeldahl method.

Protein recovery. Supernatants were adjusted to pH range of 3.0 to 8.0 with 1 N HCl, and were separated by centrifugation. Protein content was determined by the micro-biuret method.

Fractionation of Proteins from Succinylated and Untreated Cottonseed Flour

Succinylated flour. (a) Butanol extraction: cottonseed flour was suspended in *n*-butanol (pH 4.5) acidified with 6 N HCl (flour-to-solvent ratio of 1:5, w/v), and agitated by shaker at room temperature for 30 min. Residue was separated by centrifugation. (b) Succinylation: residue from the butanol extraction was suspended in water (1:10, w/v) and then succinylated using succinic anhydride at 30% of total protein by the procedure described previously under protein extractability. (c) Fractionation of proteins—water-soluble isolate: The succinylated suspension was centrifuged at 10 C and residue was saved for extraction of salt-soluble protein. Supernatant was slowly adjusted to pH 6.0 with succinic anhydride, and then adjusted to 4.5 with 3 N HCl. The protein isolate separated by centrifugation was suspended in water, adjusted to pH 7.0 with 4 N NaOH, dialyzed with water for 48 hr, and freeze-dried.

Salt-soluble isolate: the residue of the succinylated suspension was extracted with 4% NaCl (ratio 1:10, w/v) by shaking at room temperature for 30 min and centrifuging. Residue was used for isolation of alkali-soluble (0.2% NaOH) isolate. Extraction was repeated twice, and supernatants were combined for precipitation of protein. Protein in the supernatant was recovered by the water-soluble protein isolation procedure just described.

Alkali-soluble (0.2% NaOH) isolate: residue from the salt extraction step was extracted twice with 0.2% NaOH solution.

Untreated cottonseed flour. The same procedures were followed for preparation of protein isolate from untreated cottonseed flour as for fractionation of succinylated flour. Cottonseed flour was successively extracted with water, 4% NaCl, and 0.2% NaOH (1:10, w/v). Protein in the supernatants was precipitated with 3 N HCl at pH 4.5.

Gel Filtration and Disc Gel Electrophoresis

The isolates were studied by gel filtration chromatography using a reverse-flow Sephadex G-100 column (2.5 × 90 cm) and elution with 0.2% NaOH containing 4% NaCl. Absorbance of eluent was monitored at 280 nm by a LKB Model Uvicord II absorptiometer.

Protein isolates were separated by electrophoresis on 8% polyacrylamide disc gels with 0.04 M $NaHCO_3$ buffer (pH 9.4) using the modified procedure outlined by Cherry et al. (15).

Protein Solubility, Calcium Precipitability and Emulsion Stability of Water-Soluble Isolates of Succinylated and Untreated Flours

Protein solubility in water (pH 6.8-7.0) was determined by the micro-Kjeldahl method before and after centrifugation of 2% protein suspension at 7,710 × g for 10 min.

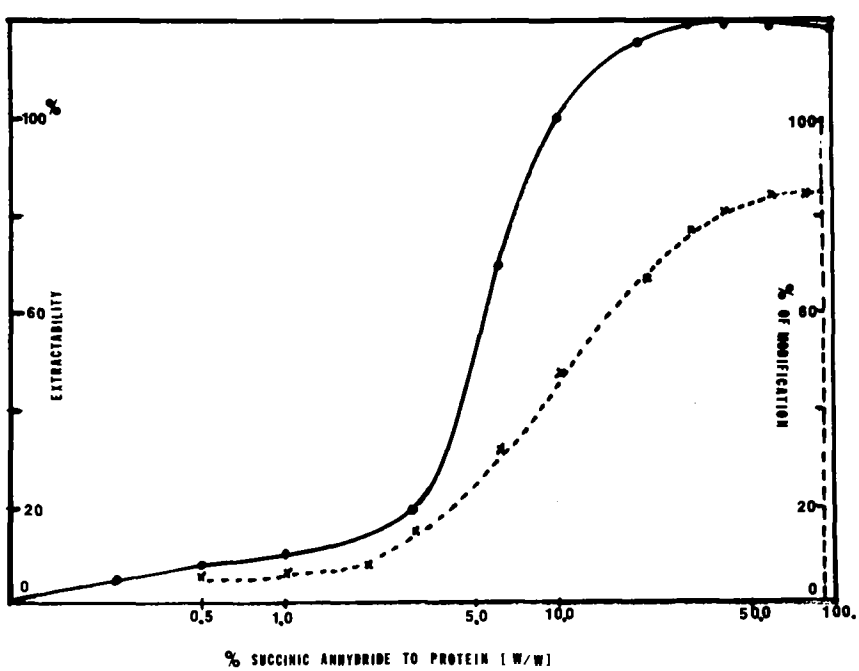

FIG. 1. Effect of succinic anhydride concentration during succinylation of cottonseed flour on degree of succinylation and extractability of resulting protein: solid line indicates protein extractability as an increased percentage ratio to the value obtained from untreated flour by water extraction. Dotted line indicates percentage succinylation of protein. Data are the average of 3 experiments with 2 assays each. Standard deviations are less than 2%.

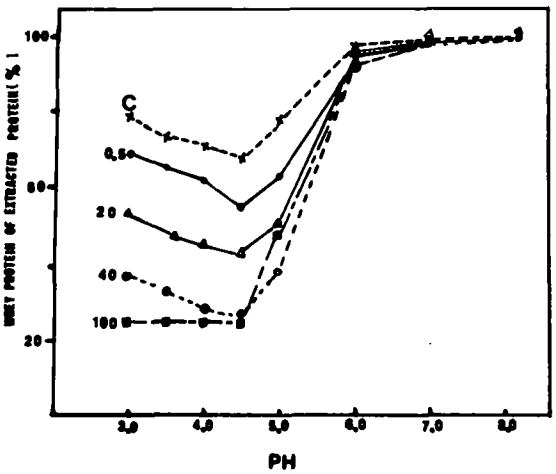

FIG. 2. Recoveries of protein at various pH levels from extracts at various concentrations of succinic anhydride; numbers represent percentage succinic anhydride concentration. C = extract from untreated flour. Data are the average of 3 experiments with 3 assays each. Standards deviations are less than 3%.

Supernatant from the solubility test was used for the Ca^{++}-precipitability test. Calcium phosphate was added to supernatant to a total $CaHPO_4$ concentration of 18 mM. After centrifugation, protein content in supernatant was determined by the micro-Kjeldahl method. Precipitated protein was calculated as a percentage of total protein in the suspension.

To determine emulsion stability, 0.5 g of water-, salt- or alkali-soluble isolate were suspended with 12 mL sunflower seed oil and 38 mL 0.01 M citrate phosphate buffer (pH 7.0). The suspension was mixed in a Waring blender at top speed for 2 min, and poured into a graduated cylinder. Volumes of emulsion, oil and water phases were observed at various time intervals.

To determine solubility profiles of water-soluble isolates at various pH levels, water suspensions of 0.5% succinylated and 0.2% control isolates were adjusted to the desired pH with 1 N HCl or 1 N NaOH, and were then centrifuged at 5,900 x g for 20 min. Suspension of more than 0.2% control isolates showed poor solubility at neutral pH. The protein content of the supernatant was determined by the biuret method (16).

Chemical Analysis

The extent of succinylation was determined by the trinitrobenzene sulfonic acid method described by Hall et al. (17). Percentage of modification was estimated from the amount of unreacted lysine in unsuccinylated and succinylated cottonseed proteins. Neutral sugar by the phenol-sulfuric acid method (18), phosphorus by the Fiske-Subbarow method (19) and total amino acids (20) were also determined.

RESULTS AND DISCUSSION

The water-soluble proteins of cottonseed are the predominant functional proteins of the cytoplasm, and represent 25-30% of total nitrogen of defatted flour. The water-insoluble proteins are essentially the storage proteins located in the protein bodies and represent ca. 60-65% of total nitrogen (21). Water-soluble protein isolates, obtained by the selective extraction procedure (22), represent 11% of the weight and 15% of the nitrogen of the flour; storage protein isolates comprise 30% of weight and 53% of the nitrogen.

Treatment of a flour-water suspension with solid succinic anhydride at concentrations greater than 5% increased extractability of protein (Fig. 1). At very low succinic anhydride concentrations, extractability of protein was similar to that of water extraction alone. However, protein extractability was improved by increasing the amounts of succinic anhydride in the reaction mixture. Maximal extraction of protein occurred at a final concentration of 20% succinic anhydride, based on flour protein content. At this concentration, the protein was ca. 64% succinylated. However, succinylation increased further at higher concentrations of succinic anhydride to a maximal level of 85% when using 50% succinic anhydride. The presence and reactivity of other materials, such as carbohydrates, some phenolic compounds and imidazole groups, also may have affected the extent of succinylation.

Sodium succinate, formed from succinic anhydride and sodium hydroxide during succinylation, increases the ionic strength of the flour suspension and osmotic pressure across the protein body membranes. These reactions may have induced rupture of protein bodies and increased release of storage proteins into the extracting medium. Also increased protein extractability after succinylation may have resulted from solubilization of globular proteins by increased concentrations of sodium succinate. Conversion of amino groups from positive to negative charge by succinylation (23) may also be responsible for increased water solubility of proteins. Martinez et al. (22) reported that increases in ionic strength were necessary to rupture the membranes of protein bodies and to solubilize enclosed proteins. Also, it has been reported that extraction of yeast cell proteins is facilitated by succinylation after cell disruption (24).

In the classical selective precipitation procedure for preparation of cottonseed isolates (22), the alkali extract is acidified to pH 7.0 to obtain storage protein, and then to pH 4.0 to obtain nonstorage protein. Maximal precipitation of succinylated proteins was obtained at pH 4.5 (Fig. 2).

TABLE I

Effects of Succinylation of Defatted Cottonseed Flour on Extractability and Precipitability of Proteins

Succinic anhydride concentration (%)	Protein extractable (%)	HCL-precipitable protein (pH 4.5)[a] (as % of extractable protein)	HCL nonprecipitable protein (pH 4.5)[b] (as % of extractable protein)	Succinic anhydride precipitable protein (pH 4.5)[a] (as % of extractable protein)	Succinic anhydride nonprecipitable protein[a] (pH 4.5)[b] (as % of extractable protein)
Control (0%)	28 ± 2.0	35.9 ± 1.5	17	32 ± 2.5	18.5
10%	55 ± 4.5	63 ± 3.3	20.1	63 ± 3.8	20
30%	65 ± 3.7	69 ± 5.0	20	70 ± 2.7	18.9

[a]To precipitate protein with succinic anhydride, pH was adjusted to 6.0 with succinic anhydride followed by HCl to 4.5. Data are the average of 3 experiments, with duplicate assays for each experiment. Values are given as mean ± standard deviation.

[b]Values are calculated from determination of total extracted protein and precipitable protein at pH 4.5 No significant difference ($p>0.05$).

TABLE II

Composition and Yield of Protein Isolates

Treatment	Isolate	Composition of isolate[a]			Yield[b] of dry matter (%)	Protein recovery[b] (%)	Free amino group[c] (%)	Hunter color value		
		Protein (%)	Sugar (%)	Phosphorus (%)				L	a	b
Succinylated[d]	Water-soluble	86.1	9.4	0.3	31.9	48.1	48	85.4	-3.6	9.5
	Salt-soluble	89.2	6.7	0.23	10.6	16.6	43	89	-4.0	8.2
	Alkali-soluble	90.2	4.3	0.2	4.9	7.8	11	80.4	-3.4	7.2
Untreated	Water-soluble	81.5	7.0	1.7	7.5	10.7	100	81.4	-1.0	3.5
	Salt-soluble	91.3	2.4	0.3	27.3	43.6	50	75	-2.0	2.0
	Alkali-soluble	84.7	5.3	0.8	8.0	12.5	60	70.1	-5.4	10.0

[a]Data represent means of 2 experiments with 3 determinations. Protein content of defatted cottonseed flour was 57% on a dry wt basis. Flour (100 g) was used for each experiment.
[b]Data are the average of 2 preparations. Coefficients of variation ranged from 1.0 to 2.6%.
[c]Percentage of untreated water-soluble isolate.
[d]Succinylated at 30 succinic anhydride on protein basis.

Protein precipitability at this pH increased as succinic anhydride concentration increased to 40%. About 30% of untreated cottonseed flour protein was precipitable when extracted with water alone, and was 70% precipitable when the concentration of succinic anhydride reached 30% or higher (Fig. 2 and Table I). Thus, extractability and recovery of water-soluble proteins were increased by succinylation of cottonseed flour prior to isolation and precipitation of proteins at pH 4.5. No appreciable difference was observed in isoelectric precipitation of protein from the extract with either HCl or succinic anhydride.

Table I shows that ratios of cottonseed whey protein (nonprecipitable at pH 4.5) to total protein were almost the same for succinylated and untreated samples. Evidently, this fraction was not altered by succinylation, and increases in protein extractability were probably due to release and solubilization of protein from protein bodies and membrane-bound protein. Therefore, sequential, 3-step extractions were used to determine changes in proportions of the water-, 4% NaCl (pH 6.5)-, and 0.2% NaOH (pH 11.5)-soluble protein fractions after succinylation of flour protein at 30% succinic anhydride concentrations. In untreated cottonseed flour, a large portion of the protein (43.6% of total protein) was recovered as salt-soluble isolate. Recoveries of water- and salt-soluble isolates were 10.7 and 12.5%, respectively (Table II). Amounts of proteins recovered from salt and alkali extracts was markedly decreased by succinylation of cottonseed flour at 30% succinic anhydride, indicating their conversion to water-soluble forms (representing 48% of total protein). Yields of salt- and alkali-soluble isolates were 16.6 and 7.8%, respectively. The converted water-soluble protein isolate contained 86% protein, 0.3% phosphorus and 9.4% neutral sugar on a dry basis. Neutral sugar content was slightly higher in the succinylated isolates than in untreated fractions.

Isolates prepared from butanol extraction were lighter in color at neutral pH than those from untreated flours, as shown by Hunter color values in Table II. Whiteness values (L value) of isolates were markedly increased by butanol

TABLE III

Amino Acid Composition of Water-Soluble Isolates from Untreated and Succinylated Cottonseed Flours

	Water-soluble isolate from succinylated flour	Water-soluble isolate from untreated flour
	g amino acid/100 g protein	
Lysine	4.12	6.67
Histidine	2.68	2.42
Ammonia	1.91	2.09
Arginine	11.49	12.92
Aspartic acid	8.63	6.85
Threonine	2.97	2.32
Serine	4.23	2.75
Glutamic acid	20.62	28.03
Proline	3.71	3.41
Glycine	4.03	2.97
Alanine	3.90	2.40
Valine	4.36	2.75
Methionine	1.27	1.44
Isoleucine	2.97	2.03
Leucine	5.79	3.77
Tyrosine	3.11	3.72
Phenylalanine	5.44	3.07
Total	90.93	89.91
Classified distribution of amino acids:		
1. Basic	18.29] 47.54	22.01] 56.89
2. Acidic	29.25	34.88
3. Uncharged polar	10.31	8.79
4. Hydrophobic group	31.47	21.84

extraction of flours.

Alkali-soluble isolates from butanol-extracted and untreated flours were more yellow-brown than the water- and salt-soluble isolates. But alkali-soluble isolate from butanol-extracted flour was much lighter in color than that from untreated flour.

Amino acid profiles of water-soluble isolates from succinylated and untreated flours are shown in Table III. Succinylated isolates contained more uncharged polar and hydrophobic amino acids, and less acidic and basic amino acids and lysine than untreated isolates.

Succinylated isolate was ca. 100% water soluble, and untreated isolate was 41% soluble (in 2% protein suspension, pH 6.8-7.0, room temperature). About 63% of the solubilized protein of untreated isolates was precipitated at neutral pH by addition of $CaHPO_4$, whereas only 7.7% of succinylated isolate protein was precipitated (Table IV). Succinylation of cottonseed flour during protein extraction increases yields of water-soluble proteins and reduces calcium-precipitability of proteins.

Figure 3 shows solubility patterns of water-soluble protein isolates prepared from succinylated and untreated cottonseed flour at various pH values. Both isolates were almost 100% soluble at pH 10. However, solubility of untreated isolate decreased rapidly as pH was lowered, whereas succinylated isolate remained 100% soluble until it was acidified to pH 6. Both isolates show minimal solubility around pH 4.0. The isolate from succinylated flour was also highly soluble at pH lower than pH 3.0.

Emulsion stabilities of citrate buffer (pH 7.0) suspensions of protein isolated from succinylated and untreated flours were determined at room temperature. Suspensions prepared with isolates from untreated flour separated into oil and aqueous phases within 30 min after homogenization. However, succinylated isolate-oil suspensions did not separate during the 24-hr time period (Table V). Succinylation also increased emulsion stability of oil-protein suspensions.

To obtain information on how protein association and formation of complexes are affected by succinylation of

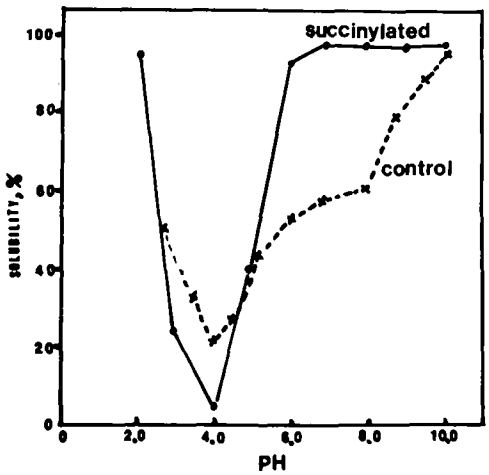

FIG. 3. Solubility profile of succinylated and untreated protein isolates at various pH levels. Succinylated isolate was obtained as a "water-soluble" fraction from cottonseed flour suspension succinylated with 30% succinic anhydride. Untreated isolate is a water-soluble isolate from untreated flour. Data are the average of 3 assays on 1 sample which is a mixture of 2 preparations. Standard deviations are less than 0.8%.

flour, protein isolates were separated on Sephadex G-100 columns. Chromatograms of protein isolates of succinylated and untreated cottonseed flours (separated with water, salt and alkali solutions) are presented in Figure 4. Water-soluble isolate from untreated flour showed 3 peaks, including a major peak that eluted at 350 mL. Major peaks of salt- and alkali-soluble isolates had elution volumes of 300 mL and 200 mL, respectively. Isolates from succinylated flour showed a large peak at an elution volume of 200 mL, which coincides with the void volume. Earlier elution indicates that isolates from succinylated flour are larger in molecular size than those from untreated flour. It is suggested that increases of negative charges on protein molecules by attachment of succinic ions result in increasing intramolecular electrostatic repulsive forces among peptide chains of protein, and that these forces may have resulted in increased molecular size of proteins by swelling and unfolding.

Similar phenomena have been noted by Grant (8). Succinylated derivatives of wheat protein eluted much earlier in gel filtration chromatography than untreated protein. However, addition of excess succinic anhydride to wheat flour suspensions enhanced dissociation of protein subunits due to repulsions between negatively charged proteins.

Samples for gel filtration chromatography were succinylated in 30% succinic anhydride for this study. Succinylation of $E\text{-}NH_2$ groups of lysine was ca. 62% complete. It

TABLE IV

Solubility and Ca^{++}-Precipitability of Water-Soluble Protein Isolates from Succinylated (30% Succinic Anhydride) and Untreated Cottonseed Flour

	Succinylated isolate (%)	Untreated isolate (%)
Solubility at pH 7.0	100	41.2
Ca^{++}-precipitability	7.7	62.9

Data are the average of 4 assays of samples which are a mixture of 2 preparations. Standard deviation is less than 2.5%.

TABLE V

Emulsion Stabilities of Protein Isolates

Treatment	Isolate	1/2 hour			4 hr			16 hr			24 hr		
		F[a]	A	T	F	A	T	F	A	T	F	A	T
Untreated	Water-soluble	20	20	50	0	28	50	0	28	50	0	29	50
	Salt-soluble	4	12	50	4	26	49	4	27	49	4	28	49
	NaOH-soluble	9	2	55	5	17	53	5	22	53	5	23	53
Succinylated	Water-soluble	10		53	9		53	9		53	9		53
	NaCl-soluble	2		49	1.5		49	1.5		49	1.5		49
	NaOH-soluble	10		53	8		53	8		53	8		53

[a]F: foam, A: aqueous phase, T: total volume (mL). Data are the average of 10 replicates of sample isolate which is a mixture of 2 preparations. Coefficients of variation for 10 replicates ranged from 1.2-2.5%.

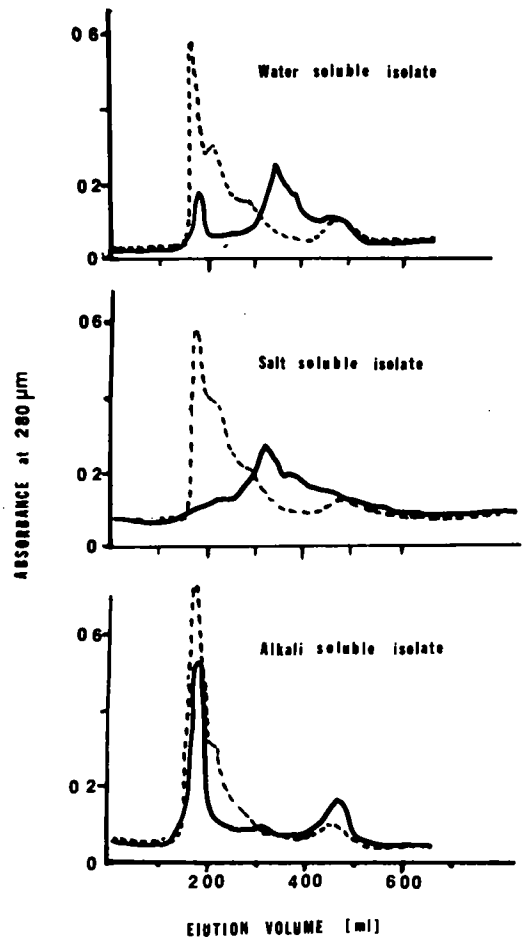

FIG. 4. Gel filtration chromatograms of cottonseed protein isolates extracted sequentially with water, salt (4% NaCl) and alkali (0.2% NaOH) from succinylated and untreated cottonseed flours. The solid line represents a chromatogram of isolates from untreated flour. The dotted line represents isolates from the flour succinylated with 30% succinic anhydride. Sample was eluted on Sephadex G-100 column with 0.2% NaOH containing 4% NaCl.

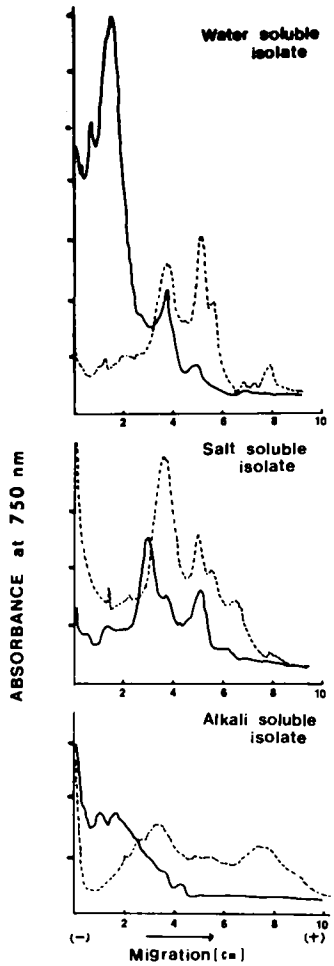

FIG. 5. Polyacrylamide disc gel electrophoretic pattern of cottonseed protein isolates sequentially extracted with water, salt (4% NaCl) and alkali (0.2% NaOH) from succinylated (at 30% succinic anhydride) and untreated flours. The solid line represents isolates from untreated flour. The dotted line represents succinylated samples.

is currently unknown whether degree of association of protein subunits is linear in relation to level modification.

Polyacrylamide disc gel electrophoretic patterns of protein isolates from succinylated and untreated flour were scanned, and are shown in Figure 5. Major bands of water-soluble and alkali-soluble protein isolates from untreated flour are mainly located in the 1.0-2.0 cm region, whereas those of the salt-soluble isolate are in the 3.0-4.0 cm region. Gel patterns of the 3 isolates are quite different from each other, indicating that the component proteins of isolates might be heterogenous.

Mobilities of proteins in the 3 isolates from succinylated flour were relatively greater than those of untreated samples, and show major bands mainly in region 3.0-4.0 cm. Similarity of gel patterns of these isolates suggests that protein components of the 3 succinylated isolates might be homogenous. The same phenomenon was also observed after Sephadex-gel chromatography of these 3 isolates. Succinylation may have resulted in shifting of mobilities by increasing the negatively charged groups of protein molecules.

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Phospholipid-Phospholipid Interaction in Soybean Oil

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ABSTRACT

Phospholipid-phospholipid interaction in soybean oil is described. Phosphatidylcholine was effectively removed from soybean oil by degumming (water hydration), whereas phosphatidylethanolamine and phosphatidic acid were hardly hydratable. However, the degree of their hydration increased in the presence of phosphatidylcholine. The spectrophotometric assay based on charge transfer interaction between 7,7,8,8-tetracyanoquinodimethane and phospholipids at 480 nm was used to determine the formation of phospholipid micelles in soybean oil. The critical micelle concentrations were 0.085, 0.84 and 2.6 mM for phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid, respectively. Phosphatidylcholine interacted with phosphatidylethanolamine or phosphatidic acid to form mixed micelles. The critical micelle concentrations of phosphatidylcholine-phosphatidylethanolamine mixture and phosphatidylcholine-phosphatidic acid mixture were 0.16 and 1.3 mM, respectively. The degree of hydration of phospholipids was related to their critical micelle concentrations. Interaction of phosphatidylcholine with phosphatidylethanolamine or phosphatidic acid was confirmed by determining the changes in the chemical shifts of ^{31}P NMR spectra.

INTRODUCTION

The soybean phospholipids in crude soybean oil which consist of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) (1,2) are normally hydratable with water, i.e., they swell, form gels which then precipitate from the oil and are easily separated by centrifugation. This processing step forms the basis for manufacture of food-grade lecithin, and worldwide production is estimated at 100,000 tons (3). Water hydration normally removes about 80-95% of the phospholipids as shown by elemental phosphorus (4). The residual phospholipids are subsequently removed from soybean oil by alkali refining (5).

However, under certain conditions of bean storage, handling or processing, presumably enzymatic (2,6) processes degrade soybean phospholipids and render them nonhydratable (2,7). The nonhydratable soybean phospholipids are strong emulsifying agents and their presence during refining can entrain considerable amounts of acid in the soupstock, thereby increasing the refining loss.

In this paper, the behavior of phospholipids and phospholipid-phospholipid interaction in soybean oil are studied by ^{31}P NMR spectroscopy, determination of critical micelle concentration (cmc) and the way they respond to the degumming method.

EXPERIMENTAL PROCEDURES

Materials

CM52 carboxy cellulose was used (Whatman Ltd., Springfield, England). Alumina Woelm N-Super I was from Woelm Pharma GmbH & Co., Eschwege, West Germany. Thin layer plates were products of E. Merck (Art 5721), Darmstadt, West Germany. Phospholipase D was obtained from Boehringer Mannheim Gmbh, Mannheim, West Germany, and 7,7,8,8-tetracyanoquinodimethane (TCNQ) was purchased from Aldrich Chemical Co., Milwaukee, WI. All other reagents were analytical grade.

Isolation and Purification of Phospholipids

PC and PE were isolated from commercial soybean lecithin by alumina column chromatography (8). The PE fraction was further purified by carboxy methyl cellulose column chromatography according to Comfurius and Zwaal (9). Phosphatidic acid was prepared from PC by phospholipase D treatment (10). The phosphatidic acid preparation contained Ca^{2+} (mol ratio of phosphatidic acid/Ca^{2+} = 2). The purity of the phospholipid preparations was checked by thin layer chromatography (TLC) (11).

TABLE I

Hydration of Phospholipids in Oil

Degumming	Phospholipids in oil (μg/g oil)				
	PC[a]	PA	PE	PA+PC	PE+PC
Before	1,700	2,000	1,900	1,900+2,490	2,100+2,100
After	trace	1,300	470	770+1,080	trace+trace

[a]PC, phosphatidylcholine; PA, phosphatidic acid; PE, phosphatidylethanolamine.