SYMPOSIUM ON SYNTHETICS AND SUBSTITUTES FOR THE FOOD INDUSTRY

Introduction

The purpose of this symposium is to review the recent advances in synthetics and substitutes of interest to the food industry. Synthetic substances are recognized as those prepared by synthesis rather than natural growth. A substitute is an artificial product used to replace a natural substance. The scope of the symposium will be limited primarily to the chemical and physical properties of synthetics and substitutes. It has not been possible to include all research areas, so the topics of this Symposium are some of those with current research interest and activity.

A greater opportunity exists today for using synthetics and substitutes in food product formulation. The increasing demand for convenience and designed consumer foods has created more food processing operations and business opportunities. In addition, the use of synthetics and substitutes is playing an increasingly important position in obtaining and holding technological advantages. Many times the use of synthetics and substitutes gives better or equal quality in a food product at lower cost. Other reasons for using synthetics and substitutes may be for purposes of obtaining unique physical properties, elimination of toxic substances, enhanced nutritional qualities, replacement of a disappearing natural product, or improved product stability.

The consumer generally sees food as grocery store items such as bread, bacon, eggs, milk, corn flakes, potatoes, steaks, and TV dinners. Many grocery store products have frequently undergone considerable processing and manipulation of their character to give better acceptance or consumer protection. Improved acceptance involves flavor, texture, color, and utility, while protection generally is based on nutrition, toxicology, and microbiology.

A chemist sees food products as materials composed of carbohydrates, fats, proteins, minerals, and vitamins that are eaten by man to sustain growth, repair, and to provide energy. It is to be expected that many of the synthetics and substitutes considered by chemists would be replacements for these food constituents. In practice, some of the synthetics and substitutes used by the food industry are such things as plant proteins, starch derivatives, synthetic colorings, flavors, amino acids, and vitamins. Many of these substances are obtaining increasingly larger markets.

Some dietary and technological trends that give increased usage to synthetics and substitutes are illustrated by the following use of these materials in foods. Soybean protein products are finding increasingly larger markets in the meat industry. Such products as soy flour, soy protein concentrate, and isolated soy protein are used in various luncheon meats, frankfurters, and canned meats. Artificial meats are being constructed from various soy protein products to make such things as beeflike, chickenlike, and baconlike products. Synthetic and substitute flavorings are finding increasing applications in new consumer products. Increased uses of flavor enhancers, vitamins, colorings, and starch derivatives are apparent by increasing yearly quantities of products being sold to the food processing industries. The trend towards dietetic foods and the lower cost per sweetness pound are the principle reasons for greater uses of synthetic sweeteners. These products are found in the diet colas, candies, and the retail synthetic sweeteners for home use.

These are only a few uses that synthetics and substitutes are finding in the food industry. Many more will be covered in the symposium. It will be apparent that these materials have made and are continuing to make a considerable impact on our food processing industries and consumer goods.

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Cottonseed Protein Products—Composition and Functionality

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Very early in the processing history of cottonseed, it was recognized that cottonseed could be a multiple source of edible products—both protein and oil (Richardson, 1917). However, the presence of the chemically reactive, antinutritional factor—gossypol (Berardi and Goldblatt, 1969)—curtailed any rapid development of edible protein products. Cottonseed processing became a triangular compromise between oil quality, gossypol inhibition, and protein quality. The economically important oil and nutritionally important gossypol were given major emphasis. As a result, the full potential of the quality and functionality of the protein of the cottonseed was seldom evident in commercially produced meals (Altschul *et al.*, 1958; Bailey, 1948). Two developments—the elimination of gossypol through breeding (McMichael, 1959; Miravalle, 1969) and the development of the Liquid Cyclone method (Gastrock *et al.*, 1969) for processing glanded cottonseed—have recently provided renewed interest in the potential of edible cottonseed protein products.

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STRUCTURE

The seeds of cereals such as rice, wheat, and barley are composed primarily of endosperm tissue with a very small

Cottonseed, when essentially completely dehulled and defatted (lipid—1% or less) will provide a product which contains 60% protein (N × 6.25) and 3% or less fiber. Concentration to 70% protein can be accomplished by alcohol or dilute salt extraction and by air or liquid classification. Further concentration to isolates requires collective or selective extraction of the proteins. Such isolates can differ in solubility characteristics, number of molecular species, average molecular weight, amino acid composition, and functionality. An isolate of the low molecular weight proteins is rich in lysine and sulfur amino acids, and has minimum solubility at pH 4 and maximum at pH 8.5. It has poor baking characteristics in bread, but good whippability at acid pH. An isolate of the high molecular weight proteins is low in lysine and sulfur amino acids, and has a minimum solubility at pH 7 with maxima at pH 4 and 9. It has excellent baking characteristics in bread, even at 15% substitution of the flour.

embryo. These seeds store starch as the major energy source. Conversely, oilseeds, specifically cottonseed, are composed primarily of embryo tissue with a very thin layer of residual endosperm. These seeds, as the name suggests, store oil as the major energy source. The cotyledons of the cottonseed embryo typically contain three major classes of cells: epidermal, palisade, and spongy mesophyll (Figure 1) (Wall, 1965). In addition to these cell types, glanded cottonseed varieties contain intercellular structures called pigment glands which are the deposition sites of gossypol pigments (Figure 2). These glands, distributed throughout the cotyledons and the periphery of the axial tissue, range in size from 100 to 400 μ (Boatner, 1948). The presence of these glands and their gossypol contents is genetically controlled. Through breeding, the glands and the gossypol can be eliminated. The gland-free or glandless character has been introduced into all major varieties of cotton (Cottonseed Qual. Res. Conf., 1962; Miravalle, 1969).

The intracellular structure of the cottonseed is a highly departmentalized organization. Lipid, protein, and phosphorus are neatly packaged and embedded in the cytoplasm, in addition to the other basic cell structures (Figures 3 and 4) (Engleman, 1966; Yatsu, 1965). This natural compartmentalization of the cell is extremely important to the processing of the cottonseed for edible products.

FLOUR

Cottonseed flour is the finely ground material produced from dehulled and defatted seed. The lipid particles or spherosomes in the cells of the seed are each surrounded by a membrane. This membrane is not a classical unit membrane, but has a rather coarse irregular configuration (Figure 4). Hexane permeates but does not necessarily dissociate this membrane during fat extraction. In commercial operations to assure efficient oil extraction, the membrane is usually disrupted by the addition of heat, moisture, and mechanical pressure. Solvent extraction operations also use heat and moisture in the desolventization procedure. The heat, moisture, and pressure used in the process of oil removal also affect the protein constituents of the seed. Only through a judicial use of these variables can the cottonseed be defatted without denaturation of the proteins. Excessive use of heat and moisture will produce a dark colored, denatured flour even in the absence of the yellow pigment, gossypol. Glandless cottonseed properly dehulled and hexane extracted with vapor phase desolventization or its equivalent can provide an essentially white, bland, undenatured flour (Martinez, 1969c; Vix, 1968).

The composition of cottonseed flours prepared on a pilot plant scale from different varieties of glandless cottonseed is given in Table I. As the efficiency of the defatting and dehulling operations decreases, the lipid and crude fiber contents increase and, consequently, the nitrogen and ash contents decrease. Cottonseed hull particles can contribute heavily

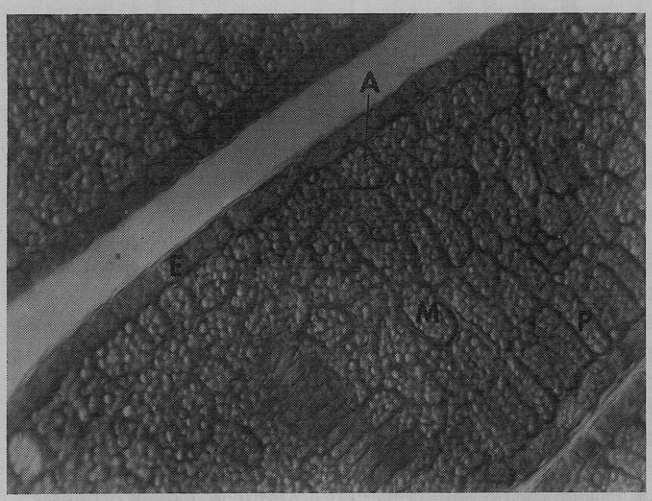

Figure 1. Section of cotyledon of cotton embryo showing epidermal (E), palisade (P), and spongy mesophyll (M) cells, filled with aleurone grains (A)

Formal-calcium fixation, Chlorazol Black E stain, taken at 1100X. Courtesy of Ines deGruy

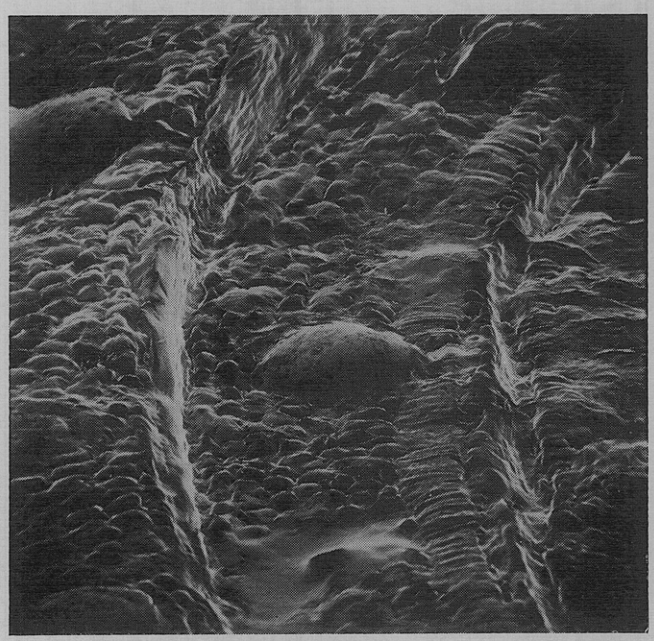

Figure 2. Scanning electronmicrograph of cross section of hydrated glanded cotton embryo

Large bulbous shapes are pigment glands. Courtesy of Wilton R. Goynes, Jr.

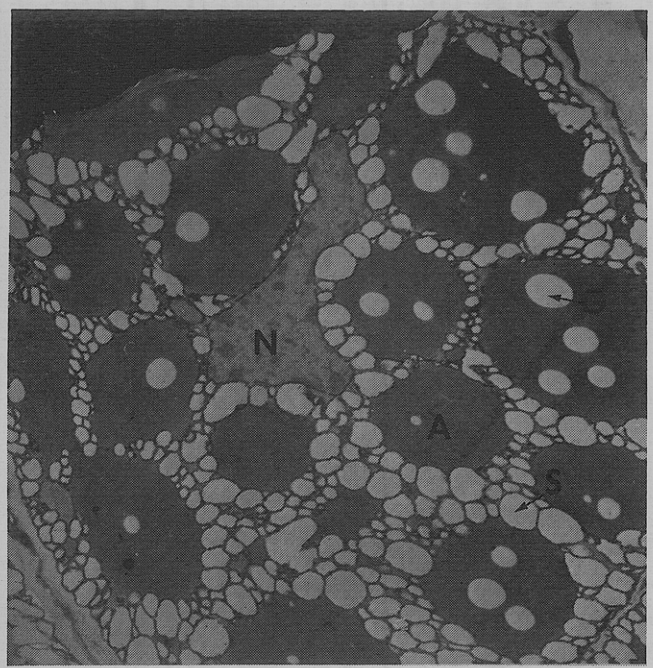

Figure 3. Spongy mesophyll cell from cotyledon of dry cotton embryo, aleurone grains–protein bodies (A), spherosomes (S), globoids (G), nucleus (N)

Lithium permanganate fixation. Taken at 7000X. Courtesy of Lawrence Y. Yatsu

to the crude fiber content of a flour. But, even in the total absence of hull particles, a defatted (0.9% lipid) Acala glandless flour had a crude fiber content of 2.2% (dry weight basis). The total sugar content of the flours, as determined by the Munson and Walker procedure (Association of Official Agricultural Chemists, 1965), appears to vary with the variety of seed, or possibly with the climatic conditions of the growth year, or both.

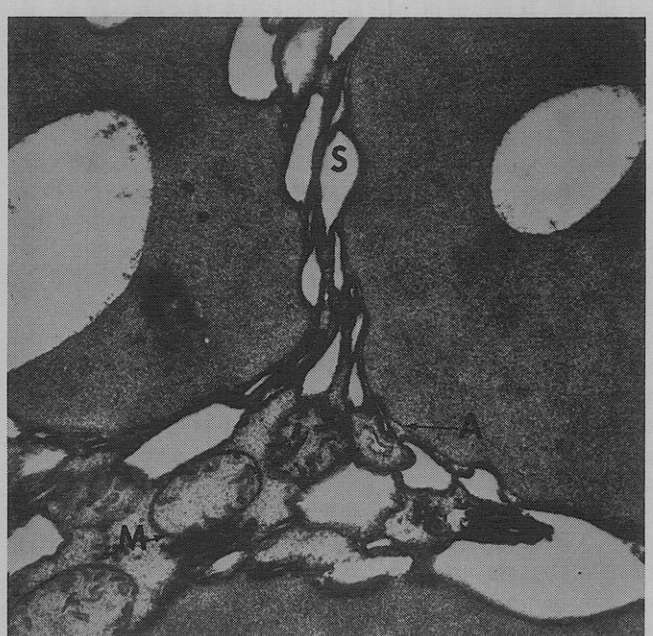

Figure 4. A higher magnification of a spongy mesophyll cell showing the base cytoplasm of the cell containing mitochondria (M)

Note heavy membrane surrounding spherosomes (S) and unit membrane of the aleurone grains–protein bodies (A). Globoids do not appear to have a membrane. Courtesy of Lawrence Y. Yatsu

Table I. Glandless Cottonseed Flour[a]—Composition

Variety	Acala (1966)	Gregg 25-V (1966)	Watson GI-16 (1968)
Composition—%[b]			
Protein	65.9	63.6	58.8
Nitrogen	10.54	10.18	9.41
Lipid	1.2	1.2	1.3
Crude fiber	2.6	3.5	2.8
Ash	8.6	7.1	9.3
Phosphorus	2.3	2.4	2.5
Calcium	0.1	0.1	0.2
Total sugar	5.1	3.2	6.0

[a] Prepared by Engineering and Development Laboratory, SURDD. [b] Dry weight.

Denaturation effects due to the defatting operation can be adequately estimated by the nitrogen solubility of the flour. The standard procedures developed for soy protein products, however, are not applicable to cottonseed protein products. Unlike soy proteins, cottonseed proteins are not readily dispersible in water. A totally undenatured cottonseed flour would have a Protein Dispersibility Index (American Association of Cereal Chemists, 1962) no greater than 30. Alkali is needed to solubilize the major proteins of the cottonseed. The nitrogen solubility index for cottonseed, as proposed by Lyman (Lyman *et al.*, 1953) is relatively insensitive in the area of major interest for food use—that is, between 70 and 100. Recent work in our laboratory suggests that a ratio of 0.4 mequiv of base rather than 2.0 mequiv of base per g of flour would provide a more discriminating measure of protein denaturation in defatted flour (Berardi *et al.*, 1967).

CONCENTRATES

Further concentration of the proteins of the cottonseed can be accomplished by either wet or dry processing of the defatted flour. A prerequisite for any of these procedures is a properly defatted cottonseed flour in which the protein bodies are not glued in place by denatured cytoplasmic proteins, but are free to separate from the other cellular particulates when the cell is ruptured by grinding.

The composition and yield of cottonseed protein concentrates obtained by various methods are given in Table II. The first two methods are wet operations. Ninety percent ethanol gives optimum extraction of residual lipid and sugars with minimum removal of nitrogen (Berardi *et al.*, 1968). Ethanol extraction increased the protein content from 66 to 72%, decreased the lipid content from 1.2 to 0.1%, and decreased the sugar content from 5.1 to 2.1%. Little shift in the amino acid composition of the product from that of the original flour is to be expected because of the minimal loss in nitrogen.

Aqueous extraction at essentially neutral pH (pH 6.3 to 6.8) is another wet procedure for the preparation of cottonseed protein concentrates (Martinez, 1969d). Water or very dilute divalent cationic salt solutions such as 0.008*M* $CaCl_2$ can be used to extract the defatted flour (Table II). The increase in protein content depends upon both the solubility characteristics of the constituents of the flour and the particular extraction system used. Though this procedure produces an increase in fiber content, it also provides maximum removal of the total sugar. The selective removal of the water-soluble, low molecular weight proteins and the sugars results in a significant reduction in total weight and total nitrogen and, consequently, a marked change in the average

Table II. Composition and Yield of Cottonseed Protein Concentrates Prepared by Various Procedures

Procedure	Variety	Composition %[a] Protein	Nitrogen	Lipid	Crude Fiber	Ash	Phosphorus	Calcium	Total Sugar	Yield[b] % of Total Weight	% of Total Nitrogen
Extraction	Glandless										
90% Ethanol	Acala	71.9	11.50	0.1	2.7	8.7	2.3	...	2.1	84	93
0.008M $CaCl_2$-Water[c]	Acala	75.9	12.15	2.6	3.7	8.5	3.0	0.6	0.3	60	77
	Watson	69.5	11.12	2.2	4.1	11.4	3.5	0.5	0.4	67	81
Water-water	Watson	70.4	11.27	1.5	4.7	10.5	2.9	0.2	0.8	61	73
Air Classification											
	Glandless										
	Acala	73.7	11.79	0.7	1.6	9.4	2.6	0.1	4.7	56	63
	Gregg	69.3	11.08	0.7	2.3	7.9	...	...	2.4	60	65
Liquid Cyclone	Glanded	69.0	11.05	0.7	2.6	8.6	2.1	0.1	4.7	60	65

[a] Dry weight basis. [b] As is basis. [c] Successive extractions.

Table III. Pertinent Characteristics of Air-Classified Glandless Cottonseed Flour

Sample	MMD[a] μ	Yield %	Composition (%) Dry Weight Nitrogen	Lipid	Ash	Phosphorus	Crude Fiber	Sugar Total
Flour	15	100	10.73	0.88	7.84	2.16	2.2	7.33
Fraction								
1	8	31.9	12.10	0.48	9.03	2.67	1.5	4.09
2	11	19.2	11.38	0.37	8.46	2.33	1.9	4.42
3	16	26.7	10.22	0.54	7.41	2.02	2.7	8.84
4	19	10.2	9.56	0.75	6.75	1.78	3.1	9.48
5	56	12.0	7.50	1.84	5.35	1.24	3.5	13.14

[a] Mass median diameter, the diameter at which 50% of the weight of the sample is undersize.

molecular weight and amino acid composition of the final product. In this water extraction process, the protein body proteins are retained in their original structures and concentrated at the expense of the low molecular weight cytoplasmic proteins of the cell.

Wet operations suffer from two major difficulties: the processing of the extract, the byproduct, in an economically feasible manner, and the drying of the major product. The first difficulty involves low-yield product recovery, solvent recovery, and pollution problems. The second difficulty involves denaturation of the major product during drying. This difficulty is usually more severe when alcohol is part of the extracting solvent system.

The dry procedure of air classification has neither of these difficulties (Martinez, 1969a; Martinez *et al.*, 1967). A cottonseed meal with a high nitrogen solubility can be ground in a pin mill and air classified to give two products, one for food and one for feed without further processing. In this procedure of separation of particles, clusters of unruptured cells, cell wall fragments with adhering residual cytoplasm, and residual spherosome membranes are separated from free, intact protein bodies. This mechanical separation results in an increase in nitrogen at the expense of the lipid, sugar, and crude fiber contents of the resulting concentrate. The parallel increase of ash content with nitrogen content evident in most of these concentrates is consistent with the particulate nature of the intracellular constituents. The major constituents of the seed which analyze as ash are the salts of phytic acid—the hexaphosphate of inositol. Since the phytin of the cottonseed is stored in the globoids, which in turn are embedded in the protein matrix of the protein bodies (Lui and Altschul, 1967), any concentration of the intact protein bodies will result in a corresponding increase in the ash content.

The yield and composition of the fractions obtained from a four-cut air classification profile are given in Table III. The parent flour was made from a hexane-extracted, laboratory-prepared, glandless cottonseed meal which was totally free of hull particles. The air-dried meal was ground in a pin mill and air classified in a fine cut classifier. A four-cut analytical profile is produced by removing the smallest flour particles at the finest cut point of the classifier and successively reclassifying the residue at increasingly coarse cutpoints. The nitrogen, ash, and phosphorus contents are highest in the finest fractions. As the size of the "average particle" (MMD) increases, the lipid, crude fiber, and sugar contents increase, and the nitrogen, phosphorus, and ash contents decrease. Such a fractionation can only be accomplished with selective grinding. Grinding to a narrow range or single particle size would make nitrogen concentration by air separation possible.

In commercial production, only a single cut is made at the point which provides the most advantageous balance between yield and nitrogen content. The results given for the Gregg flour in Table II were obtained by such a single-cut procedure with commercial pilot plant equipment. The protein content increased from 64 to 69% in the concentrate and decreased to 49% in the residue. This residue would, therefore, meet the nitrogen requirements of a feed ingredient. It would also provide a cottonseed protein product with maximum nutritive quality.

Since the major shift in nitrogen in the air classification process is brought about principally by the removal of nonprotein constituents, the protein characteristics of the concentrate do not differ appreciably from those of the proteins of the parent flour.

The nonaqueous, liquid classification procedure called the Liquid Cyclone Procedure was developed primarily for removal of pigment glands (Gastrock *et al.*, 1969). Since this procedure removes hull particles and cell-wall fragments as well as pigment glands, the final product is very similar in

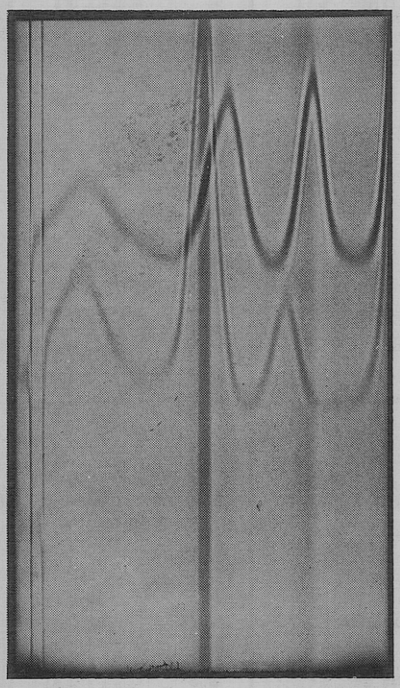

Figure 5. Ultracentrifuge pattern of alkaline extracts (1 g to 15 ml of 0.027*M* NaOH) of defatted soybean and cottonseed flours

Upper—soybean extract (0.027*M* NaOH, 1 g:15 ml) dialyzed against standard phosphate buffer pH 7.6, 0.5 ionic strength, 0.01*M* mercaptoethanol. Lower—cottonseed extract (0.027*M* NaOH, 1 g:15 ml) dialyzed against 0.2*M* Na_2CO_3–$NaHCO_3$ buffer pH 10.5. Analytical rotor at 59,780 rpm with standard and wedge quartz cells, 60 min past top speed. Sedimentation from left to right

composition to the glandless air-classified concentrate. Appropriately defatted flakes are ground in hexane to remove the cellular tissue from the pigment gland. The pigment glands are then separated from the flour particles in a liquid centrifuge called a Liquid Cyclone. The final product, a light yellow, bland concentrate with a high nitrogen solubility, contains 0.04% or lower free gossypol and less than 0.3% total gossypol.

ISOLATES

Further concentration of the proteins of the defatted cottonseed to an isolate requires extraction. An alkaline extract of the total proteins of the cottonseed has an ultracentrifuge pattern similar to that of an alkaline extract of the total proteins of soy, with one major exception. In American varieties of the soybean, the 11S component is present in similar (Figure 5) or greater proportion (Wolf and Smith, 1961) than the 7S component. This difference in protein distribution suggests that the average molecular weight in alkali of the total proteins of soy is greater than that of cottonseed.

The composition of the solvent used to extract the proteins of seed materials is dictated by a number of variables. The location of the protein, the amino acid composition and sequence of the protein, and the nonprotein constituents, which are coextracted, all influence the solvent requirements for extraction. Alkaline extraction of the total proteins of the cottonseed requires about 0.4 mequiv of base per g of flour containing about 60% protein. If the flour is extracted with water prior to the alkaline extraction, only 0.23 mequiv of base are required per g of flour. If the flour is doubly extracted with water, the mequiv of base needed to extract the major portion of the protein of the cottonseed are even

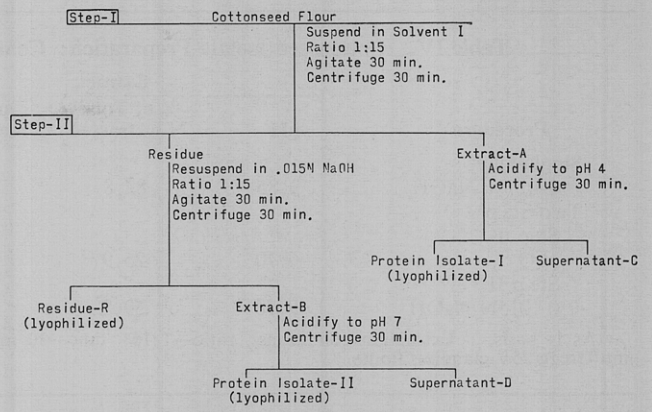

Figure 6. Flow diagram of the two-step, selective extraction procedure for the preparation of cottonseed isolates

lower. This decrease in mequiv requirements is due to the removal of certain protein and nonprotein constituents extracted by water. There is, however, a definitive requirement of ions which are necessary to rupture or solubilize the membrane surrounding the protein body and to solubilize the enclosed proteins. This requirement will vary with the pH of the solution, the type of ions in solution, and the concentration of protein-body proteins in the particular sample. But the proteins solubilized by the appropriate mequiv of base or salt are essentially the same (Martinez, 1969b). Microscopic and extraction studies have shown that water and solvents of low salt and alkali concentration, such as 0.008*M* $CaCl_2$, 0.02*M* NaCl, and 0.005*M* NaOH, extract only the nonprotein-body proteins even after three successive extractions. These nonprotein-body proteins—the 2S component in the ultracentrifuge pattern (Figure 5)—represent about 30% of the total nitrogen, 65% of which is precipitable at pH 4. These proteins are many in number, low in molecular weight, and high in the nutritionally important amino acids—lysine, cystine or cysteine, and methionine.

Reextraction of the water or dilute salt extracted flour with the appropriate mequiv of base or salt will solubilize the protein body proteins—the 7S and 12S proteins of the seed (Figure 5). These proteins represent about 60% of the total nitrogen, 94% of which is precipitable at pH 7. They are few in number, high in molecular weight, and relatively low in lysine and the sulfur amino acids.

Based on these solubility characteristics, a two-step extraction procedure (water or dilute salt followed by dilute alkali) for the preparation of cottonseed protein isolates was devised (Figure 6) (Berardi *et al.*, 1969a). A comparison of the results obtained by this procedure and a single-step extraction procedure is given in Table IV. These results show that the major isolate from the two-step procedure is higher in nitrogen and lower in phosphorus than that obtained by a single-step procedure. Recoveries of total nitrogen and total weight are similar with a slightly higher recovery with the two-step procedure. Of major importance to the pollution aspect of this process is the fact that the nitrogen content of the whey from each of the two precipitation steps is less than half that of the whey from a single-step procedure.

The two-step procedure was transferred to the pilot plant without technical difficulties. Curds were well formed and separated clearly. Modifications in the extraction procedure were necessary, however, because of the use of a desludging type centrifuge. A double water extraction was necessary to ensure a clean separation of the water-soluble and water-insoluble proteins.

Table IV. Cottonseed Isolate Preparation: Comparison of Single and Two-Step Extraction Procedures

Procedure	Extract pH	Extract % of Total N Extracted	Extract pH of Precipitation	Isolate[a] N %	Isolate[a] P %	Isolate[a] % of Total N	Isolate[a] % of Total Wt
Single-Step[b,c]							
0.027N NaOH	9.85	82.8	5.0	14.90	0.90	62.8	39.2
Two-Ste p[b,c]							
Step I							
H_2O	6.70	25.9	4.0	13.38	3.47	16.1	11.2
Ste p II							
0 .015N NaOH	9.78	59.8	7.0	16.05	0.47	52.6	29.8

[a] As is basis. [b] Extraction conditions: ratio—1:15; time—30 min; temperature—25° C; glandless flour. [c] Average of trials from Acala and Gregg 25V glandless flours.

The two isolates obtained by this procedure differ in polyacrylamide gel electrophoretic pattern (Figure 7), in amino acid composition (Table V), and in solubility characteristics (Figure 8). The solubility curve of the isolate from the single-step extraction (Curve C—Figure 8) is obviously modified by the presence of both groups of proteins. The major advantage of this selective, two-step extraction procedure would, therefore, appear to be the separation achieved between two classes of proteins and the preparation of two isolates with widely different characteristics and potentially widely different uses.

The unusual acid solubility of the major isolate of cottonseed is probably best understood in terms of the association-dissociation phenomena of the proteins. The ultracentrifuge patterns in Figure 9 show the relationship between the molecular size of the proteins of the major isolate in acid and alkaline mediums. The 7S and 12S proteins dissociate to very low molecular weight monomers. These monomers will reassociate and dissolve in alkaline solution, but do not necessarily assume their original configuration.

The 7S and 12S proteins also undergo association-dissociation phenomena with changes in ionic strength of alkaline buffer. The sedimentation constant increases with a decrease in ionic strength. However, this increase is not due to interaction between the two proteins. The sedimentation constant of each protein increases in the presence or absence of the other. These proteins also are not affected by reducing agents. Ultracentrifugal, electrophoretic, and chromatographic (Sephadex G-200) characterization of the proteins in Isolate-II was unaffected by the presence of dithiothreitol. In view of the low cystine-cysteine content of these proteins, this is not unexpected. Characteristics such as these, which are governed by the composition and structure of the protein,

Table V. Amino Acid Composition of Cottonseed Flour and Isolates

Amino Acid	Glandless Flour (Acala) g/16gN	Isolate-I g/16gN	Isolate-II g/16gN
Lysine	4.3	6.0	3.0
Histidine	2.8	2.6	3.0
Arginine	12.1	10.4	11.3
Aspartic	8.8	6.7	8.4
Threonine	3.0	2.9	2.7
Serine	4.0	3.4	4.5
Glutamic	19.8	21.8	18.9
Proline	3.5	3.1	3.1
Glycine	4.0	3.2	3.7
Alanine	3.6	3.2	3.5
Valine	4.5	3.3	4.4
½ Cystine	...	2.6	0.3
Methionine	1.2	1.7	1.0
Isoleucine	3.3	2.6	3.1
Leucine	5.6	5.1	5.8
Tyrosine	3.0	3.3	2.6
Phenylalanine	5.3	3.7	6.3

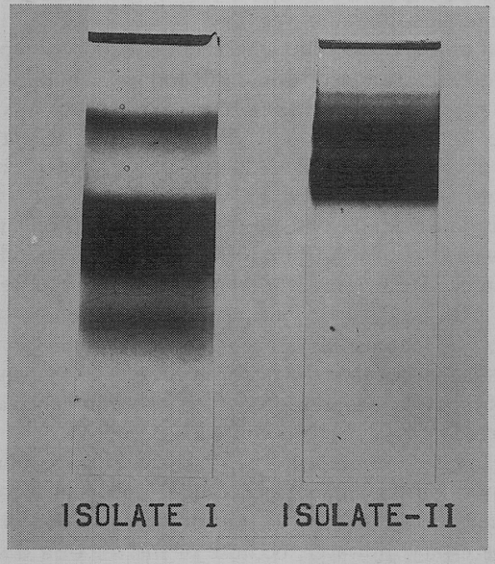

Figure 7. Vertical polyacrylamide gel electrophoretic patterns of isolates from the two-step extraction process, 0.037*M* Na_2CO_3–$NaHCO_3$ buffer pH 10.5 in gel, 0.025*M* buffer in reservoir. Gel stained with Nigrosine

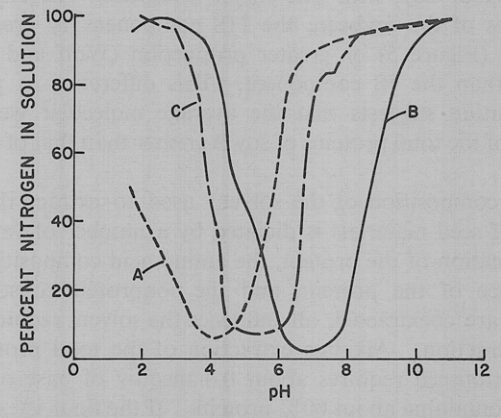

Figure 8. Nitrogen precipitation curves of 1% solutions (pH 10.5) of isolates from the two-step and single-step extraction procedures

Curve A—Isolate-I, two-step extraction; Curve B—Isolate-II, two-step extraction; Curve C—Isolate from single-step extraction. pH Values taken after centrifugation

should provide a basis for understanding the functionality of the protein products and the environmental effects of other food constituents.

COLOR, FLAVOR, NUTRITIONAL VALUE AND FUNCTIONALITY

In addition to functionality, color, flavor, and nutritive quality, all influence the end use of protein products. Glandless cottonseed flours and air-classified concentrates, when properly processed, are light cream in color, with a low moderate flavor profile free of any soy-type note. In three separate trials with three different glandless flours, using the standard AOAC procedure for PER evaluation, the cottonseed flours were equal in nutritive value to the casein control (Hopkins, 1967). Lysine is considered to be the first limiting amino acid in cottonseed. Therefore, the air-classified concentrates, which were slightly lower in lysine than the parent flours, were significantly lower in PER, 2.3 rather than 2.5 (casein set at 2.5). However, the residues from the air classification process were equal in PER to the parent flours.

The color, flavor, and nutritive value of concentrates produced by wet operations would, in large measure, depend upon the method of drying used in the commercialized process. Potentially, the alcohol extracted product should have good flavor and color and equal nutritive value. The water extracted concentrate should also have good flavor and color, but will probably be lower in nutritive value due to loss of the low molecular weight proteins.

The lyophilized isolates are light cream in color. Spray dried isolates are significantly darker—a medium tan color. The major isolate, Isolate-II, has a low moderate flavor profile, but the minor isolate, Isolate-I, has a decidedly "green" flavor note. This material on autoclaving develops a rather interesting cheese like flavor (Hepburn, 1969).

The purity of Isolate-I can be improved by washing the isolate at pH 4.8 rather than pH 4 prior to neutralization. This procedure reduces the phosphorus constituents by 25%. This wash procedure also eliminates the heat coagulability of water solutions of the isolate. Sixty-five percent of the total nitrogen in a 5% suspension of Isolate-I (washed at pH 4.8 and neutralized) remained in solution after centrifugation at 9000xg. The nitrogen in solution is unaffected by heating at 100° C for 30 min. Isolate-I also has a very interesting whippability at acid pH. The foam volume is nine times that produced by sodium caseinate at pH 4, and 75% of that produced by sodium caseinate at pH 7 (Berardi *et al.*, 1969b).

The acid solubility of the major isolate, Isolate-II, immediately suggests use in citric acid based beverages. Pale yellow solutions as high as 6% protein in 0.2 *M* citric acid have been prepared. Such solutions, which are low in viscosity, can be boiled without producing precipitation.

The full range of cottonseed products from flours to isolates have been tested by the American Institute of Baking in both sponge, dough, and continuous bread formulations (Hepburn, 1969; Martinez *et al.*, 1969). With slightly reduced mixing times, 2.5 *vs.* 4 min and 30 ppm additional bromate, the flour and air-classified concentrate at the 3% and 2.6% levels, respectively, produced bread comparable, generally in all characteristics, to the control containing 3% nonfat dry milk (NFDM). The bread, supplemented with the air-classified concentrate, had a slightly reduced break and shred, and firmer texture. The major isolate, substituted at the 1.6% level, produced bread comparable in all characteristics to the control and increased absorption from 66 to 68.

The same cottonseed products were also substituted at the 10% level. The flour and concentrate gave typical high protein breads with good flavor and general characteristics. However, 60 ppm additional bromate was necessary for optimum loaf characteristics, and machinability was marginal. The bread prepared with the isolate at the 10% level still had all the general characteristics of white bread. At the 15% level of substitution, still without additional bromate, the loaf assumed high protein bread characteristics of excellent quality.

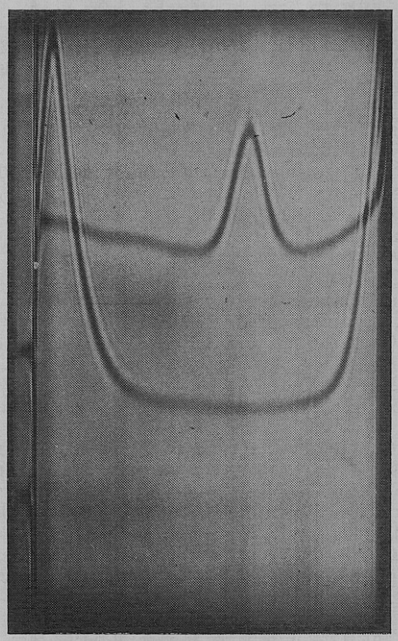

Figure 9. Ultracentrifuge pattern of Isolate-II in acid and alkaline medium, analytical rotor at 59,780 rpm with standard and wedge quartz cells, 66 min past top speed, protein concentration 1.5%

Lower pattern—Isolate-II in 0.2*M* citric acid. Upper pattern—Isolate-II in 0.2*M* Na_2CO_3-$NaHCO_3$ buffer pH 10.5. Sedimentation from left to right

Neither the flour nor the air-classified concentrate performed satisfactorily in continuous bread formulation. Volume, bread characteristics, and shock resistance were all lacking. However, the major isolate again performed excellently. At the 3% level the general loaf characteristics were better than the soy and NFDM controls.

Cottonseed and oilseeds in general are excellent sources of concentrated protein. Oilseeds are also a versatile source of products of varying protein and nonprotein composition. Though the classes of constituents are similar, the various oilseed products will differ markedly in the characteristics of the individual constituents, protein and nonprotein. Each oilseed protein product must, therefore, be examined for its basic functional characteristics to determine the most appropriate use in food products.

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