Technical News Feature

Characteristics and Uses of Glandless Cottonseed Food Protein Ingredients

The following article is Part II of a special technical news feature on glandless cottonseed, prepared by E. W. Lusas of the Food Protein Research and Development Center, Texas A&M University, College Station, Texas, and G. M. Jividen of Cotton Inc., Raleigh, North Carolina. It was presented in part at the World Conference on Emerging Technologies in the Fats and Oils Industry, in Cannes, France, in November 1985. Part I was published in the June 1987 issue of JAOCS.

Composition

Compositions of glandless cottonseed kernels, flour, concentrate and isolates, made by conventional alkali solubilization acid precipitation procedures, are shown in Table 1 (1). Cherry and Berardi (2) have reported typical assays for protein, lipid, fiber and ash of 93.4, 1.1, 0.5 and 3.4%, respectively, in classical protein isolates; 86.2, 2.8, 0.2 and 5.9% in nonstorage protein isolates; and 98.0, 0.8, 0.1 and 1.4% in storage protein isolates; however, pilot plant results have varied among researchers.

Compositions of concentrates and isolates prepared by aqueous extraction and industrial membrane processes are presented in Table 2 (3,4). Essential amino acid profiles of selected products are shown in Table 3 (5,6). Numerous composition and functionality characteristics of glanded and glandless cottonseed products also have been summarized (2). Compositions of food proteins are affected by conditions of extraction and the types and degrees of modification (7). Coprecipitated concentrates and isolates of glandless cottonseed flour and cheese whey also have been prepared by Thompson (8).

Lawhon and Cater (9) showed that the extent of prior heat treatment of the extracted meal (flour) and the pHs selected for extraction and reprecipitation of glandless cottonseed protein significantly affect solubility of the resulting isolate and its functionality in products. Nitrogen solubility profiles of isolates precipitated from essentially nonstorage protein (Extract I) and storage protein (Extract II) at different pHs are shown in Figure 1. Extracts were made from meals receiving three types of heat treatment. Meal 1 was derived from

TABLE 1

Percent Composition of Glandless Cottonseed Kernels, Flour and Flour Derivatives[a]

Analysis	Raw kernels	Roasted kernels	Flour	Conc	Isolates SP	Isolates NSP
				Dry weight basis		
Moisture	7.0	1.5				
Protein (N × 6.25)	36.2	38.1	62.6	73.5	95.1	75.2
Fat	36.9	40.0	0.8	1.5	0.2	0.2
Fiber	1.6	1.6	2.8	3.0	1.2	1.3
Ash	—	4.2	7.8	11.8	6.3	10.7

Conc, protein concentrate; SP, storage protein isolate; NSP, nonstorage protein isolate.
[a]Lusas et al. (1).

TABLE 2

Percent Composition of Aqueous and Membrane Processed Glandless Cottonseed Protein Concentrates and Isolates (Dry Weight Basis)

Analysis	Aqueous-processed[a] Concentrate	Aqueous-processed[a] Isolate	Membrane-processed isolate[b] Concentrate	Membrane-processed isolate[b] NSP	Membrane-processed isolate[b] SP
Protein (N × 6.25)	67.0	90.8	71.1	80.2	92.0
Oil	7.2	5.1	4.2	2.8	0.3
Crude fiber	1.8	1.3	—	1.4	0.2
Ash	4.9	3.7	5.0	8.2	5.4
Total sugars	19.1	4.2	5.5	7.9	5.9

[a]Lusas et al. (3).
[b]Lawhon et al. (4).

TABLE 3

Amino Acid Composition of Glandless Cottonseed Flour, Concentrate and Isolate

Amino acid	Glandless C/S flour	Protein concentrate	UF isolates		Acid-ppt'd isolates		FAO/WHO reference[b]
			NSP	SP	NSP	SP	
Lysine	4.0	4.0	6.7	3.3	6.2	2.9	5.5
Tryptophan	1.5	1.5	1.7	1.6	1.6	1.0	1.0
Threonine	3.2	3.1	3.0	3.0	3.4	2.6	4.0
Valine	4.5	4.9	4.1	4.7	4.6	4.6	5.0
Cystine[c]	2.4	2.0	3.9	1.0	3.1	1.3	—
Methionine	1.4	1.5	1.7	1.6	1.9	1.1	—
Methioninet cystine	—	—	—	—	—	—	3.5
Isoleucine	3.1	3.2	3.0	3.1	3.4	3.0	4.0
Leucine	6.0	6.2	5.6	5.9	6.4	5.6	7.0
Phenylalanine	6.0	6.2	3.9	5.9	4.1	6.3	—
Phenylalaninet tyrostine	—	—	—	—	—	—	6.0
Available lysine	3.8	3.1	3.1	2.9	5.8	2.8	—

[a]Lusas et al. (5).

[b]FAO/WHO (6).

[c]Cystine as cysteic acid.

unheated glandless cottonseed flakes, extracted with hexane at ambient temperature and desolventized by flowing warm air. Meal 2 was prepared by preconditioning flakes from 7.5–12% moisture, cooking to a temperature of 225 F (107 C), extracting with solvent, and desolventizing with indirect heat at 190 F (87 C). Meal 3 was prepared by cooking flakes as for Meal 2, prepressing, and then extracting with solvent and desolventizing as for Meal 2. Extracts I were obtained by leaching meals at pH 6.5, and Extracts II by re-extracting the insoluble residues remaining from preparation of Extracts I at pH 10. Protein isolates were then precipitated from Extracts I at pH 3, 4 and 5, and from Extracts II at pH 6, 7 and 8. Recoveries of the total protein present in the seed varied with the pHs of extraction and precipitation.

Evolution of cottonseed protein research techniques has been summarized (10). Proteins have been characterized as albumins or globulins on the basis of water, or salt and alkali solubility, respectively. Various numbers of subunits with differing molecular weights have been reported, depending upon the analysis technique used (11). Food protein technologists generally have adopted the storage protein/nonstorage protein criterion from the two-step extraction procedure of Berardi et al. (12). In this process, "storage protein" is the isolate fraction precipitated after acidifying an alkaline extract (pH 10) of cottonseed to pH 7, and "nonstorage protein" is the fraction that precipitates at pH 4. In relating basic protein studies to these definitions, it is sometimes overlooked that (a) the criterion for an "albumin" is solubility in neutral (pH 7) water; and (b) some proteins may not be soluble at pH 10, while others still may be precipitated at pH 4.

Although definitions may not be synonymous among authors, approximately one-third of total cottonseed proteins consist of albumin nonstorage 2S fraction types (10,13). This fraction also contains the cottonseed allergens, and attracts the yellow pigments in the seed. The remaining "storage protein" globulin fraction consists of at least the two subfractions "acalin A" and "acalin B," designated as 7S and 12S (or 11S), respectively, by Dieckert and coworkers (14–16), but also identified as 5S and 9S by Youle and Huang (13). Approximate molecular weights for fractions obtained from cottonseed globulin proteins are 22,000 for the 2S fraction, and 130,000 and 240,000–300,000 for acalin A and acalin B, respectively, with various polypeptide subunits found in acalin A and acalin B, depending upon the method of analysis (10,11).

The traditional model has been that storage proteins occur mainly in seed cell protein bodies, which require alkali or salt to rupture the membrane and dissolve the storage globulins, and that the water-soluble proteins are predominantly the functional protein of the seed cytoplasm (10). However, evidence exists that 2S proteins also are present in the protein bodies, and degrade rapidly during germination to provide peptide structures for the seedling, in effect functioning as "storage proteins." The names "storage" and "nonstorage" may have a limited physiological basis in the chemistry of the seed, but are likely to continue in use by food protein scientists as a convenient means of describing two easily separable cottonseed protein fractions, each with different amino acid profiles and functional characteristics.

Although protein content and purity increases as cottonseed is converted into flour, concentrate or isolates, the color also darkens surprisingly often. The yellow pigments in biscuits containing liquid cyclone-processed (glandless) cottonseed flour are flavonoids, and the brown components are bound gossypol and gossypol-like pigments (17). Blouin et al. (18) found seven major flavonoids (tentatively identified as isoquercitin, rutin, quercitin 3-0-robinoside, quercetin 3-0-neohesperidoside, kaemferol 3-0-new-hesperidoside, quercetin 3-0-glucoside, and kaemferol 3-0-glucoglucoside) in hexane-defatted glandless cottonseed flour. Biscuits containing cottonseed

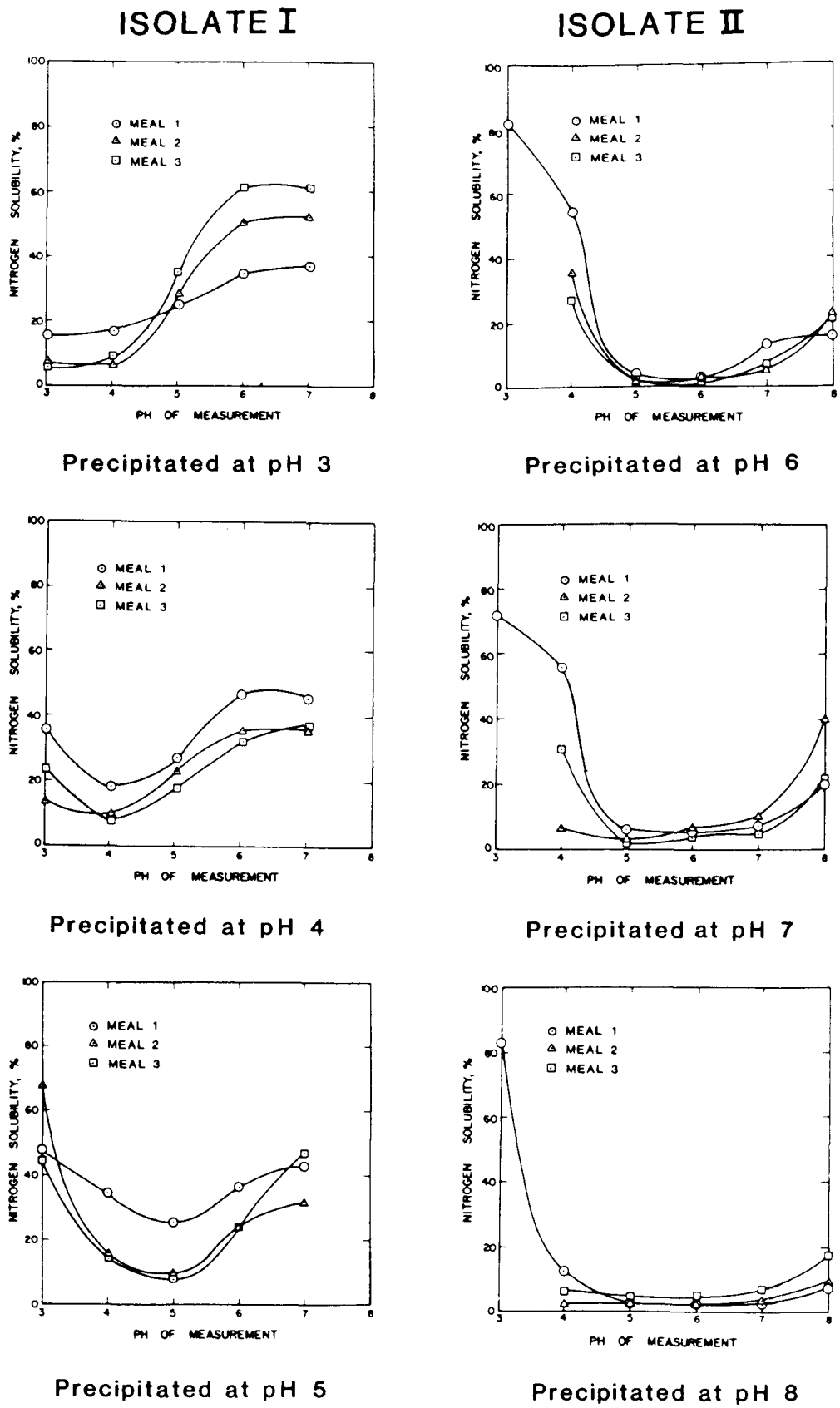

FIG. 1. Nitrogen solubilities of Extract I (nonstorage) and Extract II (storage) protein isolates precipitated at various pH's (9).

flours free of flavonoids were brown, those with extracted non-flavonoid fractions were tan, and those to which extracted non-flavonoid fractions were added were yellow. Choi et al. (19) extracted yellow pigments mainly in water-soluble isolates from defatted glandless cottonseed flour, but most of the dark brown pigments and phosphorous were extracted in alkali-soluble isolates. The yellow pigments were preferentially bound to small molecular weight proteins, but the dark pigments were bound to large protein molecules. In a later study, it was determined that light-colored protein isolates could be produced from aqueous protein extracts (pH 9.0) of defatted flour by filtering through an ultra-filtration membrane with a 100,000 molecular weight cut off (MWCO) pore size, and then spray-drying the retentate (20). Yields of these proteins were approximately 68% of the extracted solids. A process for producing light-colored protein isolates from glandless cottonseed was patented by Lawhon (21).

Lawhon et al. (22) found sugars in cottonseed flours from 16 varieties to average 13.5%, with a range of 11.4–16.9%; no statistical differences were found between glanded and glandless varieties. The composition of 16.1% total sugars in glandless cottonseed flour consisted of 11.95% raffinose, 2.62% sucrose and 0.68% stachyose, with only a trace of glucose (23).

Wozenski and Woodburn (24) found phytic acid contents (as myoinositol hexaphosphate) of 4.25, 3.94 and 2.49% in defatted glandless cottonseed flour, air-classified glandless flour and toasted kernels, respectively. Phytate contents of 2.30, 1.03, 1.51, 2.84 and 2.49%, respectively, were reported for defatted glandless cottonseed, soybean, peanut, sunflower and sesame flours, of which about 70% was present in water-soluble form at pH 4–6 (25). Comparison of relative solubilities of protein and phytates in defatted cottonseed flour (Fig. 2) showed that the greatest solubility difference occurs at approximately pH 4.0, where nearly 75% of the phytate is soluble, compared to 18% of the cottonseed protein. The two-step selective precipitation technique for making glandless cottonseed protein isolates has the inherent property of leaving most of the phytate in the whey at the normal nonstorage protein precipitation pH of 4.0, a property unique in preparation of oilseed protein isolates.

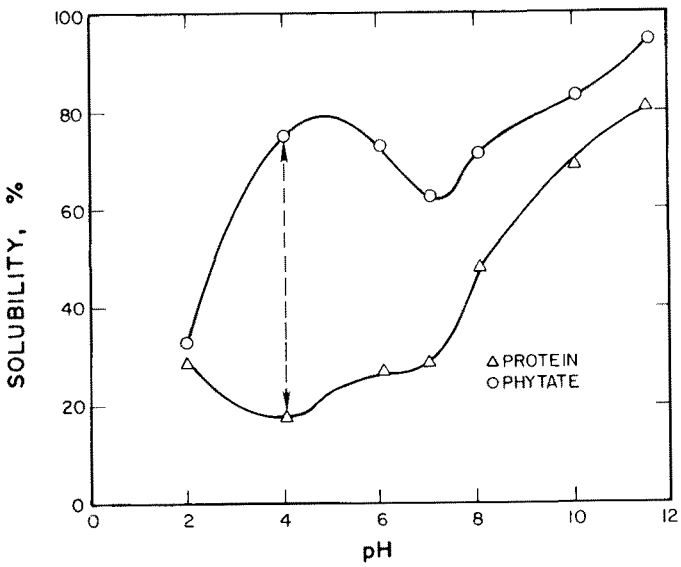

FIG. 2. Effect of pH on solubilities of defatted cottonseed flour protein and phytate (25).

Protein quality

Martinez and Hopkins (26), in summarizing nearly 15 years of studies at the U.S. Department of Agriculture's Southern Regional Research Center on processing effects on cottonseed protein quality, have noted that chemical scores calculated from amino acid composition have been generally unsatisfactory for estimating protein efficiency ratios (PER) except in very broad terms. The PER of glanded cottonseed flakes, when extracted with butanol and air-desolventized, is approximately 2.13 (all values corrected to 2.5 for casein reference), and the lysine content (g lysine/16 g N) is 4.3. Both decrease with heat treatment, with an accompanying reduction in free gossypol. For example, commercially processed glanded cottonseed meals had the following PER and lysine analyses, respectively: solvent-extracted, 1.82, 3.9%; prepress-solvent, 1.74, 4.0%; screw press—low speed, 1.26, 3.6%; and screw press—high speed, 0.88, 3.4%. When glandless cottonseed meats were cooked in the absence of added free moisture to temperatures as high as 108.9 C, PER decreased only from 2.34 to 2.30, and the ε-amino free lysine ("EAF available lysine") decreased only from 3.82% to 3.76%. When extracted flakes were autoclaved (suggestive of heat treatment as in commercial desolventization), EAF lysine decreased from 4.1% to 3.1%. However, autoclaving of defatted flakes, from which the sugars also had been subsequently extracted, decreased EAF lysine only from 4.6% to 4.1%, suggesting an additional browning reaction effect from the presence of sugars during commercial desolventization.

Reber et al. (27) determined the following PER (adjusted to casein at 2.5) for glandless cottonseed whole kernels: raw, 1.93; cooked (by steaming), 2.10; and dry roasted, 1.77. Relative protein values (RPV) indicated utilization of 91%, 96% and 91% of the protein in raw, cooked and roasted kernels, respectively. Supplementation of roasted cottonseed with 0, 0.2, 0.4, 0.6 and 0.8% L-lysine indicated a peak PER response at 0.45%.

Typically, PER of defatted glandless cottonseed flours have been in the 2.1–2.3 range. Glandless cottonseed storage protein isolates are lower in lysine and sulfur amino acids contents, and have lower PER than the nonstorage protein isolates, whose PER typically are higher than that of ANRC casein (2.5). However, PER of isolates are grossly affected by their relative purity, resulting from the method of extraction. "Classical" glandless cottonseed isolates (prepared by alkali extraction of all proteins and precipitation in one step) have PER of 2.0—2.2, typically slightly less than the PER of the protein in the native whole seed. When prepared by the "selective" extraction procedure, PER of storage and nonstorage proteins are about 1.4 and 2.3, respectively (26).

Safety

Factors considered in assessing safety of glandless cot-

tonseed include potential natural antinutrients (such as residual gossypol and the cyclopropenoid fatty acids) and contaminants (such as aflatoxins and agricultural chemicals residues). U.S. Food and Drug Administration (FDA) regulations limit the tolerance for arsenic to a maximum background level of 0.2 ppm total arsenic calculated as As, thus addressing the potential problem of carryover of arsenical desiccants. Pesticide and herbicide limitations for food products apply to glandless cottonseed, and chemical producers are required to show evidence that no residuals remain in the food and feed fractions when applying to the Environmental Protection Agency for a label permit.

The general maximum tolerance of 20 parts per billion (ppb) aflatoxin for food products also applies to glandless cottonseed products. Although excessive aflatoxin levels have been experienced in glanded cottonseed at certain Arizona and California locations, these also have been regions with other aflatoxin-associated problems, including specific insects and high humidity. Domestic glandless cottonseed produced commercially thus far has been grown in the High Plains and other relatively low humidity areas of Texas, where aflatoxin has not been a problem. The glandless cottonseed crop is checked for aflatoxin content, but a continuous surveillance program, as is in place for peanuts, may be warranted as growing of glandless cottonseed for food uses expands to other localities. A rapid analytical method for aflatoxins in cottonseed products has been reported by McKinney (28).

The requirement that glanded cottonseed kernels must be roasted to attain a temperature of at least 121 C for not less than 5 min before sale, or must be used in hard candy where kernel temperature during cooking will exceed 121 C for not less than 5 min, helps control potential spread of insects, destroys vegetable microbial cells, potentially inactivates heat-sensitive antinutritional factors and binds some of the free gossypol in the seed.

Although FDA regulations specify that free gossypol content in glandless cottonseed kernels and cottonseed flour for human food use shall not exceed 450 parts per million (ppm), this generally has been interpreted as total gossypol in the goals of the glandless cottonseed industry. Establishment of grades by the National Cottonseed Products Association (NCPA) has encouraged development of analytical procedures with increased sensitivity. Current Association of Official Analytical Chemists and American Oil Chemists' Society procedures have detection limits of approximately 40–50 ppm gossypol and are not sensitive enough for practical quantification of gossypol at concentrations less than 100 ppm. High pressure liquid chromatographic (29,30) and fluorimetric (31) methods are being developed to be more specific to actual gossypol and to detect levels as low as 5 ppm.

Inquiries continue about the safety of free and bound gossypol, even at levels as low as those in glandless cottonseed products. Conferences have been held to review the safety of gossypol in animal feeds when inactivated by iron (32), and in glanded cottonseed food protein ingredients when bound by moist heat (33). Comprehensive reviews of the physiological effects of gossypol in laboratory and domestic animals have been prepared (34,35). No reports of gossypol toxicity in humans who have consumed cottonseed products have been found in the technical literature (36). Since direct toxicity studies are not conducted on humans, conclusions about safety must be inferred from laboratory animal studies. Of course, in contrast to those of domesticated animals, human diets are heterogenous, and ingested cottonseed products would be expected to be more diluted with other foodstuffs.

Bakery goods containing glanded cottonseed flour were sold for 18 mo in the Oklahoma A&M College food store and cafeteria with no reported instance of dietary or other disturbances due to gossypol (37). No observable effects of gossypol have been found in developing countries where gossypol-bound glanded cottonseed products have been used. In Institute of Nutrition for Central America and Panama (INCAP) studies, the amount of gossypol excreted by children consuming cottonseed meal was essentially identical with that ingested, and nitrogen balance was not affected (38).

No effects from gossypol were observed in children fed Incaparina containing 38% cottonseed flour with up to 0.057% free gossypol and 0.88% total gossypol (39). Liver biopsies in children fed cottonseed protein concentrates showed no damage. Close clinical surveillance, extending over six months in Peru and two years in Guatemala, showed no evidence of gossypol toxicity in children fed foods containing cottonseed flour. No difficulties were experienced in Guatemala in families that used Incaparina for four years. No evidence of toxicity was reported in feeding children various foods containing Liquid Cyclone Process cottonseed flour (40) or in women on liquid diets containing the flour over extended periods (41).

Binding of gossypol with amino acids during processing appreciably decreases dietary efficiency of glanded cottonseed proteins. Smith (42) reported that increasing the bound gossypol content in glanded meals from 0.45 to 1.3% required at least 25% more protein to produce the same weight gain in growth studies of weanling rats. Addition of bound gossypol to glandless cottonseed meal in rat-feeding diets decreased weight gains, and these losses were not regained by supplementation with various essential amino acids (43). Thus, significant improvements in protein nutrition efficiency should be expected from using glandless cottonseed food products over glanded flours in which gossypol has been bound.

The potential role of cyclopropenoid fatty acids (CPFA) in glandless cottonseed products has received considerable attention and has been reviewed (44). Two compounds, malvalic and sterculic acid, are involved; they have similar chemical structures, but sterculic acid has one more carbon atom between the cyclopropene ring and the carboxyl end. These compounds, reacted with sulfur from carbon disulfide, give the pink-colored positive Halphen reaction used for many years to detect the presence of cottonseed oil in mixed oils and foods (44).

Pandey and Suri (45) found a range of 0.66 to 1.15% CPFAs in raw oils of 30 varieties of Indian cottonseed, with mean values differing among species: 0.67%, *G. barbadense*; 0.83%, *G. hirsutum* (American upland types); 1.14%, *G. herbaceum*, and 1.40%, *G. arboreum*. No correlation was observed between iodine value and CPFA content. Cyclopropenoid fatty acids are not stored in

specialized structures or localities in cottonseed, but occur in the spherosomes with the other oil that is extracted (46).

A new, more sensitive HPLC method has been developed (47) that allows the separation and quantification of malvalic, sterculic and dihydrosterculic acids in cottonseed oils. This method also has been used to determine that the tyledon is essentially free of CPFA, while the hypocotyl (root portion) contains the major portion of these compounds.

Concerns about cyclopropenoid fatty acids come from historical observations of hardening of body fat in animals fed cottonseed meal (48) (conversely, animal fats soften with the feeding of oilseeds with high levels of polyunsaturated oils). Also, development of a pink tint in whites of stored eggs from hens fed high levels of cottonseed meal containing appreciable residual lipids has been noted (49). In an extensive review, Phelps (50) concluded that cyclopropenoid compounds cause elevated stearic acid levels (at the expense of oleic acid) in yolk, heart, plasma, liver and ovary fat of hens, and also in the body fat of hens and swine. In later reviews, it was concluded that more than 0.1 to 0.2% cottonseed lipid in the diet of laying hens could cause potential problems—a level in agreement with general industry recommendations (44).

Malvalic acid is the predominant CPFA in cottonseed oil. It is known to be less physiologically active than sterculic acid, and occurs at a ratio of 2.5–2.75:1 to sterculic acid (51,52). Yet, interestingly, the major part of CPFA studies have been conducted with oil from the Java olive *(Sterculia foetida)*, its purified extracts, or derived methyl sterculate. CPFAs are 60 times more concentrated in *Sterculia foetida* oil than in cottonseed oil and account for about 50% of its weight. Also, the ratios of malvalic acid to sterculic acid are reversed in *S. foetida* oil and are approximately 1 to 10 (53). *S. foetida* oil is synonymous with CPFA in earlier physiological studies, and scientists are well-advised to forego reviews and examine the original reports to determine the actual materials used. In addition to *S. foetida* oils containing a seven-fold increase in the ratio of the more active sterculic acid, potential effects of other components where crude *S. foetida* oils were used also may warrant consideration.

A literature review in 1965 (50) indicated effects on humans and domesticated animals need more research, including development of improved analytical methods and agreement on reliable laboratory animal industry mean of about 0.25% CPFA in edible refined and deodorized cottonseed oil. Since then, additional techniques have been developed to further reduce CPFA in oil during deodorization (54,55) and elimination of the Halphen test response by hydrogenation (56,57).

The specific concerns raised are that the CPFA may have health implications to consumers of glandless cottonseed kernels because they are not extracted with the oil in glandless cottonseed kernels. As an example, Hendricks et al. (58) reported hepatocellular carcinomas in rainbow trout fed glandless cottonseed kernels or a lightly processed cottonseed oil for one year. In earlier studies, the same laboratory had concluded that sterculic and malvalic acids, in combination with aflatoxin B_1 and its metabolites, are synergists in increasing the incidence and severity of rainbow trout liver cancer, and also are primary hepatocarcinogens. However, neither synergistic nor carcinogenic properties of sterculic acid have been unequivocally demonstrated in mammals.

FDA considered the objections regarding CPFA presented in 1978 and decided not to amend or rescind existing regulations regarding sale of glandless cottonseed products (59).

Pyke (60) found no differences in growth or organ tissues of rats fed diets containing 20% glandless cottonseed kernels over two generation cycles. Reber and Pyke (61) fed glandless cottonseed kernels as raw, cooked or roasted ground flours. The flours were tested as 20% substitutions in laboratory chow, against 94% laboratory chow plus 6% cottonseed oil. Sexually mature rats (F_0) were fed for two weeks before being bred and through lactation. From their offspring (F_1), 50 males and 50 females were selected from each group and were fed the diets from weaning until 24 weeks of age. At 13 weeks, the rats were bred, and their offspring (F_2) were raised to weaning. There were no statistically significant differences due to treatment in the number of litters born, litter size, or weights of the young of the F_0 and F_1 females. Growth and food consumption were similar for F_1 rats in all treatments. No detrimental effects were found due to feeding glandless cottonseed kernels. Among the groups, however, growing females utilized cooked or roasted cottonseed more efficiently than raw cottonseed or the control diet (62). The percentages of pups alive at birth surviving to 4 days were significantly higher for rats fed raw or cooked cottonseed than for roasted cottonseed.

Nutrition

The majority of human nutrition studies on glandless cottonseed products have been conducted at Texas Woman's University, Denton, TX. Studies on nutritional value of glandless cottonseed protein in diets of older subjects in nursing homes indicated good potential for use of cottonseed protein products in a variety of foods (40). Working with young adult women, Onley and Alford (63) and Alford and Onley (64) determined that nitrogen intake required from glandless cottonseed protein to maintain nitrogen equilibrium was 0.0106 g N per kg body weight. Later studies with glandless cottonseed flour confirmed the nitrogen equilibrium intake at 6.3 g nitrogen per day for the reference 58 kg woman (65–67). In studies on amino acid fortification of defatted glandless cottonseed flour in rat diets, it was determined by chemical analysis that lysine, methionine and isoleucine were the three most deficient amino acids compared to those of casein (68,69). Fortification with these amino acids did not improve either the PER or the biological value (BV) of cottonseed protein. However, the mean fasting serum threonine concentration was significantly lower in rats fed the fortified glandless cottonseed flour diets than in those fed the casein diet.

A study to determine effects of a glandless cottonseed flour diet on the calcium and phosphorus status of young women found that the subjects came into negative calcium balance at an intake of 668 mg total calcium/day, but phosphorus still remained positive at the lowest intake of 918 mg/day (70,71). Apparent digestibilities were

53% and 50% for phosphorous and calcium intakes, respectively. Positive calcium retention without separate calcium supplementation would require at least 728 mg Ca/day in the diet from cottonseed flour (equivalent to 14.6 g N/day). Studies on effects of various dietary proteins and amino acids on serum lipid metabolism in rats found that diets containing protein from animal sources induced greater serum and high-density lipoprotein (HDL)-cholesterol concentrations as well as increased lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) activities than those containing plant protein sources (72). Animals fed an arginine-supplemented casein diet (to simulate the arginine-to-lysine ratio of cottonseed protein) showed decreases in both serum and HDL-cholesterol compared to the casein control group, whereas addition of lysine to the cottonseed protein diet (to simulate the arginine-to-lysine ratio of casein) caused an increase in these cholesterol fractions.

In feeding a gallstone-producing diet to examine effects of dietary proteins on gallstone formation, casein produced 100% gallstones in test animals (male hamsters), whereas soybean and cottonseed proteins induced only 32% and 0% gallstones, respectively. Casein produced a four-fold increase in biliary cholesterol, whereas soybean and cottonseed produced three- and two-fold increases, respectively, compared to the commercial laboratory diet. Serum cholesterol was reduced by substituting dietary vegetable protein for animal protein (73). Later work by Raymond et al. (74) verified that 20% casein pelleted and powdered diets produced more gallstones (63% and 90%, respectively), than 20% cottonseed protein pelleted diets (0%). Cholesterol was the only bile constituent that was significantly higher in absolute concentration in casein-fed animals, compared to animals on cottonseed protein diets. Relative bile acid concentrations were found to be significantly higher in animals fed the cottonseed protein diet. Sullivan et al. (75) extended the studies to include casein, bovine albumin and egg albumin animal proteins, and soy, cottonseed and peanut vegetable proteins. Gallstone incidence was higher among hamsters fed animal proteins, with the exception of egg albumin. Bile acid concentrations within the vegetable protein diet groups were significantly higher than within the animal protein diet groups. Hamsters fed the animal protein diet showed significantly higher percentages of biliary cholesterol in the bile fluid.

Studies evaluating quality and supplementary value of several protein sources found net protein utilization (NU) values of 58%, 65.8% and 78.6% for glandless cottonseed flour, soybean oil meal and casein, respectively, in diets fed to rats at the 10% protein level; NPU were 59.3%, 63.2% and 69.1%, respectively, in diets containing 20% protein (76). In yeast breads containing 20% glandless cottonseed flour substituted for wheat, the resulting textures were not as desirable as for all-wheat breads. Food efficiency (weight gain/food intake) was 0.42 for breads containing glandless cottonseed flour, compared to 0.07 for all-wheat flour breads, when fed to rats as the exclusive food source for 10 days. High protein cookies containing glandless cottonseed flour, with or without lysine supplementation, demonstrated losses of lysine during baking proportional to the lysine content of the dough (77). No significant differences in lysine losses were found between baking in microwave or convection ovens.

Rhee and Rhee (78) complexed defatted flours and isolates of glandless cottonseed, peanut or soybean by mixing 5% suspensions of protein ingredient with an equal amount of 5% solution of glucose or sucrose and freeze drying. The complex was then heated, equilibrated to 52% relative humidity, tightly sealed, and heated at 100 C for 0, 2 or 6 hrs. Relatively minor changes occurred in the protein-sucrose mixtures upon heating. However, the protein-glucose mixtures showed major increases in browning index when heated for 6 hrs. Approximately 83% of the available lysine in the cottonseed flour-glucose mixture and 79% in the cottonseed isolate-glucose complex were lost after 2 hrs heating. This coincides with 63% and 79% decreases in C-PER (calculated PER) for these complexes, respectively. These studies indicate that in protein enrichment programs, emphasis should be placed on the ultimate availability of the supplementary proteins to the user after cooking, rather than on merely increasing the protein content of the food source alone.

El-Sayed et al. (79), using in vitro enzyme digestibility methods, reported that Egyptian glandless cottonseed flour is 90.7% digestible by pepsin-pancreatin, compared to 100.6% for casein and 73.7% for wheat flour.

Progress on human nutrition studies in Africa by the Institut de Recherches du Coton et des Textiles Exotiques (IRCT-France) has been summarized (80–82). Consumption of glanded cottonseed meal has been common practice for a long time in certain African populations, usually to bridge the gap between food crop harvests or during times of scarcity. In the Moundang populations of North Cameroon, cottonseed flour is a common part of the diet. Although cases of edema have been observed, most native consumers know how to partially inactivate the gossypol, by adding potassium in the form of soda ash during cooking. Studies were conducted in Senegal, Chad, Mali and Dakar between 1967 and 1974 using gossypol-deactivated glanded cotton flours as protein sources for normal children, and a mixture of cottonseed flour and powdered skim milk was used successfully to treat kwashiorkor. In 1972, Cornu, Delpech and Favier, working in Chad, found glandless cottonseed flour well accepted when cooked with mixtures of millet or sorghum as porridge, fritters and dumplings, or as a component of sauces. Glandless cottonseed kernels were immediately accepted and introduced into the daily diet, with no problems attributable to CPFA observed. Since 1975, research in Mali on use of glandless cottonseed in food has been assumed by CARE-MALI. With development of glandless cottonseed varieties for Ivory Coast, processing trials have been conducted and costs of production estimated.

Functionality

Studies have been done on nutritional and functional supplement roles of cottonseed proteins (83,84). Functionality is affected by the natural ratios of storage to nonstorage proteins in glandless cottonseed flour, and the resulting ratios in prepared concentrates and isolates (9,85). Texture responses in products are affected by pH; the presence of water; types and quantities of salts, carbohydrates, fat, fiber and other proteins; and processing,

including heat, shear and texturization. Properties which often are evaluated in laboratory tests include nitrogen solubility; protein dispersibility; viscosity; gel strength; water absorption; oil absorption; emulsion capacity; foaming (whipping) properties, including volume and stability; and color. Proteins also are evaluated in model food systems, including (a) bakery goods, where loaf volume, crumb texture and color in breads, and spread characteristics in cookie doughs are considered; (b) meat products, where moisture absorption and fat retention of frankfurters during cooking, drip loss (shrinkage) of meat loaves during cooking, and flavor stability during storage are evaluated; and (c) frozen desserts, where texture, overrun and flavor are considered.

Nitrogen solubility profiles of six unheated oilseed flours at different pHs are shown in Figure 3. Heating, as typically occurs during seed conditioning, solvent extraction, and desolventizing, would greatly affect these curves.

Lawhon et al. (86) prepared two glandless cottonseed protein concentrates, one by extraction of glandless cottonseed flour at pH 4.5, followed by spray-drying the residue, and the other by raising the pH of the residue to pH 6.8 (to make a "sodium proteinate") before spray-drying. Nitrogen solubility profiles of the resulting concentrates were similar to those of the parent flour, except for pHs of 9 or higher. However, the concentrate spray-dried at pH 4.5 produced substantially lower loaf volumes, coarser crumb, and lower crumb reflectance than either the neutralized (pH 6.8) concentrate or the parent flour.

Bound water content of dry glandless cottonseed protein isolate, equilibrated at 84% relative humidity, was reported at 22.4 g water/16 g N for nonstorage protein isolate and 16.0 g water/16 g N for storage protein isolate (87). While percentage of protein solubility varied considerably for storage protein isolate (20%, 5% and 30% at pHs 4.5, 6.0 and 7.5, respectively), bound water of dry storage protein isolate, equilibrated at 84% relative humidity, showed only little change with pH (15.6%, 16.0% and 16.4%, respectively, for pHs 4.5, 6.0 and 7.5).

In studies relating effects of processing heat treatments and pHs of nonstorage and storage protein precipitations, addition of sugar increased whip viscosities of unheated nonstorage proteins, but decreased whip viscosities of heated nonstorage proteins, and generally decreased whip viscosities of storage proteins (9). Addition of sugar reduced volume increases of protein solutions on whipping, except for unheated nonstorage protein at pHs 4 and 5, and of storage protein at pHs 6 and 7. Unheated storage proteins made the strongest gels when their solutions were acidified (pH 3.5). Additional studies extending these conditions were reported later (88).

Comparisons of functional properties of soy, peanut and glandless cottonseed storage and nonstorage proteins prepared by ultrafiltration have been reported (89). Comparisons of emulsification capacities of soy and glandless cottonseed flours, bovine hemoglobin and low-heat nonfat dry milk solids are reported (90). Generally, glandless cottonseed flour proteins function similarly to soy protein isolate near neutral pHs, but (probably because of the two distinct "storage" and "nonstorage" fractions) deviate considerably from soy away from neutrality.

Methanolic extracts of defatted glandless cottonseed flours, concentrates and isolates had higher total phenolic contents and displayed greater antioxidant activities against linoleate oxidation catalyzed by metmyoglobin, Fe^{++}-EDTA and fresh beef homogenates, and against autoxidation of safflower oil than extracts from counterpart peanut and soybean products (91). A later study reported that glandless cottonseed flour was effective in retarding lipid oxidation and discoloration of raw ground beef patties containing from 0 to 3% salt (92).

Various techniques have been evaluated for modifying the functionality of glandless cottonseed proteins. When mechanically dehulled seed was germinated for up to 5 days, three-fold increase in free fatty acid content resulted, but no differences were found in relative concentrations of the 2S, 7S and 12S peaks (93). Cunningham et al. (94,95) modified cottonseed storage protein by using proteolytic enzymes contained in a semi-permeable membrane reactor, and were able to hydrolyze and permeate up to 80% of storage proteins through hollow fiber membranes.

Glandless cottonseed flour was acylated with succinic and acetic anhydrides (96). Specific viscosities and water-holding, oil-holding, and emulsifying capacities were increased from 1.2- to 10-fold over nonacylated flour. Partial succinylation of cottonseed flour increased the yield of protein isolate precipitated at pH 4.5 (97). Succinylated isolates were more water-soluble, less heat-coagulable and lighter in color than conventional isolates. They showed higher oil absorption, emulsification capacity, gel strength, water hydration, water retention and viscosity. Also, bulk density was decreased, and fluffy isolates were produced. Further research (98) showed that, in addition to improved extraction and recovery, succinylated protein isolates were low in sensitivity to calcium precipitation. Studies have suggested that succinylation converts much of the salt- and alkali-soluble proteins to water-soluble forms. Maleylation, succinylation, dimethylglutarylation and sodium sulfite treatment of cottonseed flour increased protein extraction and precipitated more protein at pH 4.0, compared to extracts of unmodified flour (99). However, acetylation decreased protein extraction and precipitation at pH 4.0. Proteins isolated from succinylated, maleylated and dimethylglutarylated flours

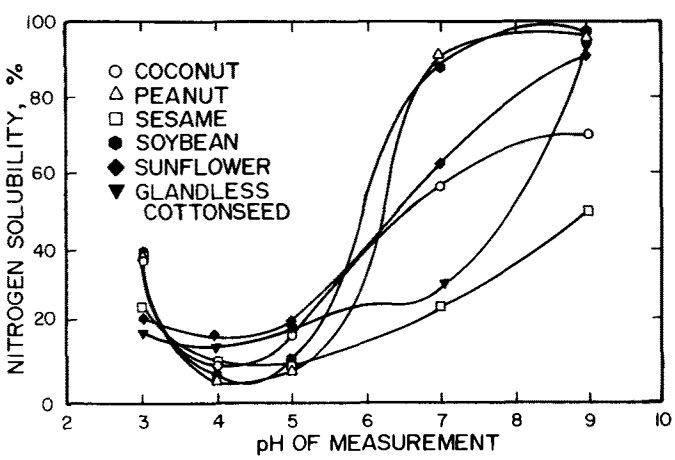

FIG. 3. Nitrogen solubility profiles of six oilseed flours.

were highly water-soluble and did not coagulate on heating. Acetylation decreased heat coagulability of the resulting protein isolate, but did not affect water solubility of the isolate. Sensitivity of protein isolates to calcium ions was not affected by acetylation or sodium sulfite treatment of the flour. Additional studies (100) showed that succinylation follows first order kinetics in respect to concentrations of succinic anhydride present. Emulsion capacity and oil absorption capacity were not significantly changed at less than 60% succinylation, but increased markedly above that level. Emulsion capacity had a positive, but not linear, correlation with water solubility of proteins.

Texturized products

Taranto et al. (101) evaluated the operating characteristics of a Wenger X-5 laboratory extruder in texturizing defatted glandless cottonseed meal. Generally, texturization results were erratic. Of 48 equilibrium extruder runs, 22 produced products did not disintegrate during retorting. On the basis of later morphological and ultrastructural evaluations, including photomicrography, it was postulated that working and kneading by the extrusion screw is not a prerequisite for formation of striated texture (102). Generally, photomicrographs of cottonseed products (which are more difficult to texturize) showed rough, pitted structures, while soy products possessed smooth, continuous structures (103). Uniformity of the protein matrix and distribution of insoluble carbohydrates within that matrix were found to influence morphological and rheological properties of extrusion-textured cottonseed flour. In general, the more uniform the protein matrix, the greater the stress; the more evenly dispersed the soluble carbohydrates, the greater the resilience. Nonextrusion texturization, using a Korean "texturizing" snack food hand press, also was studied (104). During nonextrusion texturization in this unit, the protein bodies are ruptured and fused under heat and pressure to form a fibrous protein-insoluble carbohydrate matrix.

Several techniques have been studied for extraction of extruder-texturized glandless cottonseed and soy flours to remove off-flavors, which often occur in these products (105–107). Textured products with protein contents of approximately 80% (moisture-free basis) were obtained. Extracted extrudates had greater water-absorption capacities and water-holding capacities than the original textured proteins, but the converse was found for oil emulsification capacities. Taranto et al. (102,103) have reported on basic factors affecting texturization (production of muscle-like fibrils) in glandless cottonseed and soy flours by extrusion and non-extrusion.

A nonextrusion method has been developed for producing textured, chewy particles of gelled cottonseed proteins by stirring water suspensions of storage protein isolates at pH 4.5–9.0 while heating to 90 C (108). Isolates containing both storage and nonstorage proteins required suspension in 0.3% NaCl solution between pH 4.0 and 9.0 while texturizing. Texturized products can be dehydrated for storage and rehydrated as needed. Meat products containing 10–20% of this rehydrated texturized cottonseed protein had acceptable texture, color, flavor and chemical properties, but meat products containing 30% of the product were considered too bland.

Usage

The potential for food uses of glandless cottonseed products was recognized early (109–111) and has been reviewed on several occasions (40,112). Also, guidelines regarding their use have been suggested (83,113). Kernels have been the major glandless cottonseed ingredients used commercially in food products, although considerable research has been published on functional and nutritional effects of flours, concentrates and isolates. These ingredients are attractive for their functionality, bland flavor and high protein content, which is almost nutritionally equivalent to soy. Among the oilseed proteins, glandless cottonseed products are more bland than soy, but should cost less than peanut proteins when produced in large quantities.

Kernel uses. Uses of glandless cottonseed kernels or full-fat products have led development of the glandless cottonseed industry, since they provide immediate markets without investment in the additional processing facilities necessary to produce defatted flours, protein concentrates and isolates. Kernels are used commercially in the U.S. as alternatives to nuts, in confectionery products, toppings, ice cream specialties, snack foods and bakery products. Kurzius (114) obtained a domestic patent for preparation of yeast-raised bakery products using cracked glandless cottonseed, and ProTeina® bread, enriched in protein content by 60%, has been sold in selected regional markets (115). Particle-size variations of glandless cottonseed kernels, including whole and cracked kernels, flakes, and full-fat flours, have been evaluated in various commercial and experimental food products (116), and a glandless cottonseed "butter" has been marketed.

Combinations of long grain rice, wild rice, bulgur wheat and white corn grits cooked with glandless cottonseed kernels have been prepared as side dishes and found attractive. Such combinations have the advantage of supplementing cereal proteins (usually low in lysine) with oilseed proteins (typically high in methionine) to produce foods with improved amino acid profiles. Incorporation of up to 18% glandless cottonseed kernels resulted in tortillas as organoleptically acceptable as all-corn control tortillas, although a darker color was noticed when glandless cottonseed kernels were incorporated at levels above 12% (117). Incorporation of 18% kernels increased the protein content of tortillas by 62% (from 11.1% to 18.0%) and appreciably improved the PER. Tortillas fortified with glandless cottonseed kernels were preferred over soy-fortified tortillas at all levels. Full-fat and defatted glandless cottonseed flours were evaluated as substitutes for cowpeas in deep-fried Nigerian foods (akara, chin-chin and puff-puff) (118). Taste panel and protein quality evaluations indicated that both ingredients could be used to increase the quantity and quality of proteins in traditional Nigerian foods.

A patented process has been developed to produce Tamucurd®, a tofu-like product from glandless cottonseed kernels (119,120). The resulting curd product is bland and can be used as a cream cheese substitute in cooking and

in preparation of a link-sausage type meat substitute product. Also, it may be spray-dried for later reconstitution and use.

Early cottonseed food proteins. Commercial efforts to develop defatted cottonseed food products go back at least to 1876, when Texan J. W. Allison placed a cottonseed flour on the market, especially recommended for use in human diets requiring a low starch content. This product was not produced in large quantities. A later version, "Baumgarten Process Allison Flour," was developed by Gus A. Baumgarten at the Schulenberg, Texas, Oil Mill in the early 1880s and was produced until about 1950. Proflo® flour was introduced by the Traders Oil Mill Company in Fort Worth, Texas, in 1939. This product contained approximately 55–60% protein and 4.5% fat. It was promoted for use as a non-allergenic dietary protein source; for its functional properties in bakery-type products, including increasing water absorption of cookie doughs without affecting spread, reducing stickiness, assisting machinability and pan release of baked goods, and reduction of fat absorption in deep-fried doughnuts; and for use as an emulsifier, natural antioxidant and flavor and color source. Because of a limited market, sales of Proflo® for human use were suspended in 1975, although the product continued to be made for industrial uses (121). The potential exists for producing similar products from glandless cottonseed.

Defatted glanded cottonseed meal became widely known as a potential source of nutrients for humans during the late 1950s and the 1960s, with development of "Incaparina" at the Institute of Nutrition for Central America and Panama (INCAP) by Scrimshaw, Bressani and co-workers. This low-cost food, also known as "INCAP Vegetable Mixture 9," contained 38% cottonseed flour. The flour was produced in Central American cottonseed oil mills, using expellers or prepress-solvent extraction techniques, and contained about 0.05% free gossypol (122). The mixture was later fortified with lysine to offset losses resulting from gossypol binding during processing. This development helped catalyze extensive worldwide research into low-cost vegetable protein sources, including degossypolized meals from traditional cottonseed varieties, and development of glandless cottonseed as a food protein source.

Bakery products. The concepts of glandless cottonseed flours, concentrates and isolates are acceptable in the bakery products industry, where compounding of various ingredients is commonplace (123). Early research documented that all oilseed protein flours increase absorption and usually decrease mixing tolerance of doughs as the replacement level of wheat flour increases (124). This results in decreased loaf volumes and a serious deterioration of crumb grain, texture and color. While heat pretreatment of the flour or kernels may decrease absorption, and changes can be made in liquid addition and mixing times, generally the maximum successful wheat flour replacement level has been approximately 10% (113). Breads fortified with oilseed flours to increase protein by 30%, baked by the short-time dough procedure, had greater loaf volumes and crumb color and grain scores than those prepared by the straight dough procedure (125). However, crust color was darker for breads prepared by the short-time procedure. The addition of 1.5% sodium stearoyl 2-lactylate in the short-time dough process formula was effective in recovering some of the loaf volume lost by the addition of cottonseed flour.

Breads incorporating air-classified glandless cottonseed protein concentrate were essentially equal to the wheat flour control in loaf volume, specific loaf volume and crumb reflectance (86). Wet-processed glandless cottonseed protein concentrate, dried at pH 6.8, gave nearly 50% greater volume than concentrate dried at pH 4.5 and was essentially equivalent to glandless cottonseed flour in loaf volume, specific loaf volume and crumb reflectance. The presence of cottonseed flours or concentrates in doughs increased water absorption in all instances. Glandless cottonseed protein concentrates behaved similarly to glandless cottonseed flour when used to fortify bread by 30% protein (126). Concentrates prepared at low pHs adversely affected the baking properties of breads, and calcium ions at any pH level reduced loaf volume significantly. Storage protein concentrates produced by ultrafiltration gave higher loaf volumes than glandless cottonseed flour, while the nonstorage proteins gave lower loaf volumes than flour in breads formulated to increase protein content by 28.3% (127). Breads containing storage protein were equivalent in loaf volume to the 100% wheat flour breads. In all cases, addition of sodium stearoyl 2-lactylate at 1.5% substantially increased loaf volumes. Later studies found no differences in loaf volume, grain and texture between breads formulated with 8% nonstorage protein isolate and those with membrane-processed storage protein isolate (128).

In studies in Egypt, higher levels of glandless cottonseed flour increased water absorption and mixing tolerance index and decreased mixing time, stability and valorimeter values (129). Dough strength, extensibility and elasticity were reduced with increasing levels of cottonseed flour. Defatted glandless cottonseed flour was processed by the two-step extraction procedure of Berardi et al. (7) to produce storage and nonstorage protein fractions (130). When evaluated in breads, all fractions increased water absorption, but mixing properties and extensibility were affected to various degrees by the different fractions. Panned and balady ("pocket") breads and biscuits were prepared from hard and soft wheat flours with three levels of cottonseed flours (131). Generally, hard wheat flour bread had larger volumes than breads made from soft wheat flour, and addition of cottonseed flour reduced loaf volume in panned breads. A 5% replacement of wheat flour by cottonseed flour increased bread protein by approximately 10%. However, more than 5% replacement lowered loaf volume and specific volume by more than 20%. Panned breads with 5% cottonseed flour had the best total score. Incorporation of 0.4% calcium stearoyl 2-lactylate in the formula improved organoleptic characteristics of breads and made them softer. In balady bread, loaf volume and specific loaf volume of bread made with 5% cottonseed flour were higher than those of breads made with 0, 10, and 15% cottonseed flour, and bread color was better accepted. Loaf weight of breads increased with increasing levels of cottonseed flour. Biscuits containing 5% cottonseed flour were scored as more tender and having better flavor than all-wheat flour biscuits.

In other studies, sugar cookies formulated with 6%

100-mesh cottonseed flour were highly acceptable, and preferred over all-wheat flour controls (132). The replacement guideline for glandless cottonseed flour in quick breads, such as biscuits, muffins, coffee cakes and nut breads, is considered to be approximately 25% of the wheat flour (113). Chemically leavened deep-fried doughnuts contain approximately 7.5% protein. For doughnuts fortified to levels of 12, 14.5 and 15.5% protein with glandless cottonseed flour, sensory panel scores for flavor, texture and overall acceptability for fortified doughnuts were slightly less than for all-wheat control doughnuts (133). Doughnuts fortified with glandless cottonseed flour were judged statistically equivalent to doughnuts fortified with high-solubility soy flour.

Meat products. Glandless cottonseed food protein ingredients have been evaluated as processing aids in meat products, and also as meat extenders and replacements in their textured forms.

"High nitrogen solubility" glandless cottonseed flour (15.6% soluble protein) and "low nitrogen solubility" glandless cottonseed flour (5.8% soluble protein) were evaluated in comparison with soy flours, soy protein concentrates, soy protein isolates, nonfat dry milk solids and fish protein concentrate (134). A high nitrogen solubility index (NSI) was not clearly indicative of ability to form a stable emulsion, and functional property tests were not necessarily indicative of performance of the respective ingredients in finished frankfurters. Glandless cottonseed flours performed well compared to the other ingredients evaluated with regard to processing shrinkage, cooking yield, color score and peelability score.

Meat loaves containing 25% partially rehydrated wet-processed cottonseed protein concentrate had 55% less loss of cook-out juices than control loaves (86). Molonon and Bowers (135) replaced 0, 15 and 30% ground beef in meat patties with hydrated texturized cottonseed flour and found no difference among treatments in cooking loss. PER of the beef-texturized cottonseed flour blends changed little with addition of texturized cottonseed flours. Frankfurter-type sausages were prepared in which 5, 10 or 15% of the meat was replaced with cottonseed flour or cottonseed storage protein isolate (136). Compared to all-meat controls, frankfurters made with increasing levels of cottonseed proteins generally had higher pH values, less cured color, less firmness of skin, softer texture, and were less desirable as judged by sensory levels. (However, levels of cottonseed product evaluated were considerably higher than the 3% processing aid normally used.) Within each level of use, frankfurters containing storage protein isolate were more preferred than those containing glandless cottonseed flour. Ziprin et al. (137) evaluated stabilities of beef patties containing glandless cottonseed, peanut, soy and textured soy flours; glandless cottonseed, peanut and soy concentrates; and glandless cottonseed (classical), peanut and soy isolates. All oilseed protein ingredients retarded development of oxidative rancidity in cooked refrigerated patties, with the highest antioxidant effects potential shown by the cottonseed protein ingredients.

Meat loaves were prepared with 0, 10, 20, and 30% rehydrated textured glandless cottonseed storage protein isolate (138). The taste panel did not detect significant differences in initial flavor, aftertaste or overall flavor of meat loaves with 0 or 10% storage protein isolates. Meat loaves with 20% storage protein isolate were judged to be acceptable. Seventeen volatile compounds were identified in meat loaf flavor by combined direct gas chromatography–mass spectrometry. Natural logarithms of hexanal and hexanol, and of ratios of hexanal and hexanol to chloroform, correlated well with taste panel scores for aroma, initial flavor, aftertaste, and overall flavor.

Dairy products and beverages. Approximately 20% of protein in the domestic diet is supplied by dairy products. The trend has been to increasing cheese production, but decreasing consumption of fluid milk solids, milk fat, nonfat dry milk and butter (139). Production of milk is an expensive operation in the U.S., and around the world, whose overall population is increasing more rapidly. Also, a significant portion of the global population is intolerant to the lactose present in milk. For such reasons, interest is high in potentially plentiful, nutritious, low-cost beverages.

All oilseed proteins are sensitive to precipitation by calcium, resulting in problems if direct supplementation of the beverage is required to achieve equivalency to milk in calcium content. Research on decreasing the calcium precipitability of cottonseed protein by acylation is summarized elsewhere in this review. Chakrabarty and Randolph (140) achieved 86% stabilization of glandless cottonseed nonstorage protein by addition of *k*-carrageenan at a stabilizer:protein ratio of 0.2.

Good taste panel acceptance was reported for orange-flavored "ade" beverages and orange-flavored fruit drinks containing glandless cottonseed protein isolate at levels up to 3% (127). The storage protein fraction is especially interesting in potential protein-fortified beverage applications, since it resolubilizes at the low pHs of soda pop and citrus juices.

Highly acceptable Egyptian processed cheese products were obtained by incorporating up to 5.4% cottonseed flour, although undesirable darkening was experienced with increasing levels of cottonseed (141). Glandless cottonseed classical protein isolate was as preferred as soybean isolate at levels up to 40% replacement for sodium caseinate in imitation mozzarella cheese analogs (142). However, the product became appreciably darker when extended with cottonseed protein isolate.

Frozen desserts. Simmons et al. (143) evaluated soft-serve frozen desserts containing glandless cottonseed flour and storage protein isolate, and soy flour, protein concentrate and isolate as replacements of milk solids by protein-lactose blends. No statistical differences in overall acceptance were found up to 20% replacement by glandless cottonseed flour, and 60% replacement by storage protein isolate. In later work, using ultrafiltration membrane-processed ingredients, Lawhon et al. (144) achieved successful replacement of nonfat dry milk solids by 20 to 40% glandless cottonseed storage protein isolate. El-Deeb (145) successfully substituted 10% milk solids nonfat with defatted glandless cottonseed flour in Egyptian vanilla-flavored ice cream and 15% glandless cottonseed flour in chocolate-flavored ice cream.

Miscellaneous. El-Sayed et al. (146) successfully used 25% glandless cottonseed flour in Egyptian baby foods, replaced 10% meal with cotton flour in sausages, and also compared cottonseed flour soup with lentil soup. Eighty

percent of the non-water ingredients in the soup were cotton flour. Cotton flour soup was less acceptable than lentil soup, but the protein content was 80% higher than for lentil soup.

Tortillas were fortified with glandless cottonseed flour and high solubility soy flour, to achieve 11%, 13%, and 15% protein in the blends (147). This is equivalent to 18%, 40%, and 61% increases, respectively, in protein contents over the traditional corn tortilla. Tortillas fortified with glandless cottonseed flour were darker than those with the same substitution levels of soy flour, but were slightly preferred over the soy flour-fortified tortillas.

Spadaro et al. (148) extruded defatted peanut and glandless cottonseed flours with brown- and white-rice grits and with yellow corn. Generally, brown-rice grit products were preferred over white-rice or corn grit products, and peanut flour was preferred over glandless cottonseed flour. Products consisting of brown-rice grits and 10% and 20% glandless cottonseed flour had good flavor and texture characteristics, but were not rated as highly by the taste panel as those with peanut flour. White-rice grit products with 20% glandless cottonseed flour were considered acceptable, although bland in flavor compared to those with brown-rice grits. Products consisting of yellow corn grits with 20% glandless cottonseed flour did not receive acceptable flavor ratings.

Powdered shortenings (75% fat) using glandless cottonseed storage protein isolate as the encapsulating agent were more flowable than those encapsulated with sodium caseinate (149). Encapsulated shortenings produced cake batters with aeration, but with cake volumes and crumb textures equivalent to cakes made with plastic shortening (150). Glandless cottonseed protein isolates prepared by classical and aqueous extraction processes were poor ingredients for production of coffee whiteners (151) and showed poor whitening capacity, separation of proteins through sedimentation, and separation of fat as cream layer on coffee. Succinylated cottonseed proteins were better encapsulating agents, resulting in markedly improved characteristics as coffee whiteners. Fifty percent replacement of sodium caseinate with succinylated classical cottonseed isolate did not affect the quality of whiteners compared to the 100% sodium caseinate-based control whitener.

Wu and Bates (152,153) reported on making of "yuba-type" (rolled lipid-protein films) from glandless cottonseed kernels, and their use as alternatives to meat. Film formation rate and protein content were improved by the addition of soybean protein isolate.

Considerable progress has been made in breeding, growing and processing glandless cottonseed during the past quarter century. Collectively, much is known about utilization and nutritional characteristics of glandless cottonseed kernels, flours, and protein concentrates and isolates. Some persons, originally highly enthusiastic about glandless cottonseed, have lost hope that it will ever be commercialized in a significant way; others are still skeptical that remaining problems can be resolved. Considered as a new variety, glandless cottonseed already may have taken too long to achieve acceptance; considered as a new crop, accomplishments thus far have been extraordinary. For example, soybeans were planted in the U.S. in 1765 (154) but were first grown for oil just prior to World War I (155). However, it was not until the development of continuous solvent extractors in the early 1930s that their oil could be efficiently extracted. Sales of glandless cottonseed kernels and flour for food uses have been authorized only since 1976. Although more voluminous in research publications, the American experience with glandless cottonseed is very similar to that of the French in Africa. To paraphrase Buffet (82), technical practicality and interest in food consumption of glandless cottonseed products already have been demonstrated by research, but manufacture and use of these products still await development of appropriate industrial infrastructures.

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