Glandless Cottonseed: A Review of the First 25 Years of Processing and Utilization Research[1]

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Enough cottonseed protein is produced in conjunction with the world's cotton crop to supply the protein needs of 260 million to 350 million people. Because of indigenous gossypol, a green-yellow pigment toxic to man and monogastric animals, the feeding of press cake, meal or whole seed has been limited primarily to cattle and other ruminants. Numerous innovative processes have been developed to produce cottonseed food protein ingredients by deactivation or extraction of gossypol or by mechanical separation of intact gossypol glands. Only a small amount of glanded cottonseed flour is used in human foods. This article reviews the first quarter century of glandless cottonseed processing and utilization research.

During this period, processes have been developed to prepare glandless cottonseed kernels, flour, protein concentrates and isolates. Composition and functionality characteristics of ingredients have been determined, potential food applications demonstrated, and nutritional qualities of consumer products evaluated. Processing and utilization research in Egypt, French-speaking Africa, India and the U.S. is summarized. In the U.S., sales of glandless cottonseed kernel (for snack and confection uses) and cottonseed flour, both containing less than 450 ppm free gossypol, are permitted by the Food and Drug Administration (FDA).

FOOD AND FEED PROTEIN RESOURCE

Many of the world's hungry are located in warm climates where cotton (*Gossypium hirsutum* L., *aboreum* L., *barbadense* L. or *herbaceum* L.) is grown. The weight of cottonseed in a dry mature boll exceeds that of the fiber. For every standard 227-kg (500-lb) bale of cotton produced (actually containing 218 kg, or 480 lb, of fiber), about 363.6 kg (800 lb) of cottonseed are separated at the gin. This seed contains approximately 22.5% protein, which is of relatively good nutritional quality compared to that of the other major oilseeds. The world's crop of approximately 66 million bales is grown on 32 million hectares (ha) (79 million acres), and produces approximately 27 million metric tons (MT) of cottonseed (1,2). The U.S. produces approximately 14 million bales of cotton, grown on 5.3 million ha (13 million acres), with an annual production of approximately 5.2 million MT of cottonseed. Allowing 5% of the seed for planting, the remaining cottonseed represents a global feed and food protein pool of approximately 5.7 million MT annually. Cottonseed potentially could provide the protein needs for 350 million persons annually at the 45 g/day rate, or of 240 million persons at 65 g/day (3).

However, the traditional varieties of cottonseed contain gossypol, a yellow-green polyphenolic compound toxic to man and monogastric animals. Whole cottonseed is fed mainly to cattle, and meals from oil milling are fed primarily to ruminants, with restricted quantities fed to poultry, swine or horses, or used as fertilizers.

Gossypol is not uniformly dispersed throughout the seed, but is deposited in scattered structures called "glands," which can be seen as black specks in the stems, leaves and green bolls of the plant, and in the seed. Glands in the seed are ovoid structures containing 35–50% gossypol. They constitute about 2.4–4.8% of the weight of dehulled cottonseed kernels, and are 0.025–0.178 mm in diameter. The gland walls are tough and resilient; they resist mechanical damage under dry conditions, but are ruptured by water and polar solvents to release the gossypol (4).

Literature on the chemistry, toxicity and analysis of gossypol has been summarized by Cherry and Leffler (5). Raw cottonseed kernels may contain from 0.6–2.0% free gossypol. However, FDA's limit for free gossypol in human food products and ingredients is 450 ppm, and the Protein Advisory Group of the United Nations Food and Agriculture and World Health organizations (FAO/WHO) has set maximum guidelines of 600 ppm free gossypol and 12,000 ppm total gossypol. Hence, various attempts have been made to deactivate (blind) gossypol during processing or to remove it by extraction with polar solvents or by mechanical separation of the gossypol-containing glands. Feed industry guidelines for free gossypol levels in poultry diets are 100 ppm maximum for broilers and 40 ppm for laying hens (6). These levels have been achieved by addition of iron salts, such as ferrous sulfate, which bind the gossypol in feeds and render it biologically inactive. It is thought that the gossypol binds to the free ε-amino group of lysine and possibly to arginine and cystine during heating (7). Application of moist heat during processing of cottonseed reduces free gossypol, but also decreases protein solubility and lysine bioavailability. Proflo®, a food grade cottonseed flour, was introduced in the U.S. in 1939 and marketed until 1975 as a cookie and doughnut dough conditioner and a color and flavor source. The product continues to be made for fermentation media and other industrial uses (8). Bound-gossypol flour has been used in nutrition intervention feeding products, such as Incaparina in South America (9,10).

Typically, commercial hexane extraction of cottonseed removes a relatively small portion of the gossypol with the oil. Processes to remove gossypol by extraction with polar solvents have included use of aqueous acetone (11),

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a mixture of acetone, hexane and water (12); sequential extraction with hexane, aqueous acetone and anhydrous acetone (7); butanol-hydrochloric acid solution (13); methylene chloride (14), and hexane-acetic acid (15).

Techniques also have been developed to mill glandless cottonseed in the presence of hexane, then separate the intact, heavier gossypol glands by the liquid cyclone process (LCP) (16–19). Also, an air classification process has been developed to separate intact gossypol glands from solvent-extracted ground flour (20–22). Both processes rely on the principle of conditioning and extraction of oil under anhydrous conditions to prevent rupture of glands and release of gossypol. Besides requiring considerable additional capital investment, LCP and air classification processes have the disadvantages that yields of the high-gossypol fraction are as much as or more than those of the low-gossypol fraction. Currently, the only known glanded cottonseed flour used for food is produced by Milouot Haifa Bay Settlement's Development Company Ltd., Israel, employing a combination of techniques including binding free gossypol by moist heat.

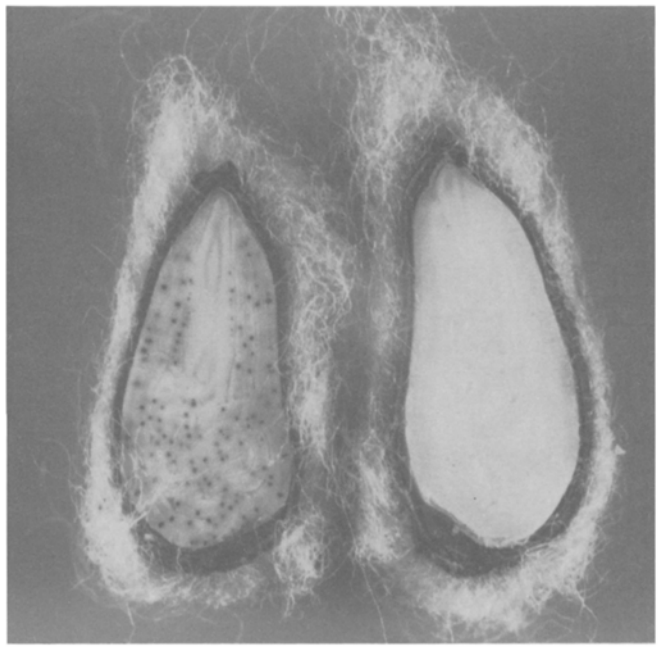

FIG. 1. Cross sections of glanded (left) and glandless (right) cottonseed.

EARLY DEVELOPMENTS

Breeding and plant development. Early genetic development of glandless cottonseed has been summarized (23–25). In the late 1940s, McMichael found that selection of plants from the "Hopi Moencopi" variety (*G. hirsutum* var *punctatum*) could result in almost complete elimination of pigment glands from leaves and bolls (26). A recessive gene, gl_1, when combined in the homozygous form (gl_1gl_1), was later shown to produce plants with gossypol-free bolls, hypocotyls, stems and petioles, but with leaves and seed containing the usual numbers of glands. When McMichael crossed Hopi Moencopi with certain upland cotton varieties (*G. hirsutum*), he found glandless seed appearing in later segregating generations (27,28). Two genes, designated gl_2 and gl_3, were found to control the presence of pigment glands in the seed. When present in the homozygous recessive condition (gl_2gl_2-gl_3gl_3), all parts of the plant above ground have no pigment glands and the seed contains essentially no gossypol. Later studies (29) showed that the presence of the dominant gene Gl_2 produced about 2.5 times more gossypol in the seed than Gl_3. In the expression of the gl_2 and gl_3 genes, 16 genotypes are possible, with only the single homozygous recessive form being completely free of pigment glands. The presence of pigmented glands can be determined visually by cross-sectioning a seed with a sharp blade and looking for black specks (Fig. 1). Lee (30) described gland distribution patterns in cotyledons carrying various combinations of the active alleles Gl_2 and Gl_3, and also identified the existence of two additional alleles, Gl_4 and Gl_5 in upland cotton, which are relatively weak in expression and have only slight effects on presence of gossypol in seeds.

Although McMichael first observed the glandless seed characteristic in Hopi cotton in 1953, he did not officially report his discovery and development of glandless cottonseed until 1959 (27). The winter of 1959–1960 marked the real beginning of breeding glandless cottons, when McMichael's glandless genetic lines were first crossed with a wide array of commercial type cottons at the winter cotton breeding nursery in Iguala, Mexico (31).

Although cottonseed comprises nearly two-thirds the weight of clean unginned cotton, its commercial value typically has been approximately 15% of the crop. Cottonseed often has been left at the gin in exchange for ginning and baling services. Although the concept of gossypol-free cotton as a more valuable food and feed protein source was readily understood, cotton producers also wanted to know whether the gossypol-free characteristic affects yields and quality of the commercially more valuable fiber. Contradicting claims were made in the late 1960s and early 1970s regarding yield and quality of fiber from the initial glandless cotton releases compared to traditional "glanded" varieties. Concurrent progress in cotton breeding was at an all-time high. Many plant morphology, disease and insect resistance, and local adaptation factors were being evaluated simultaneously, and earlier varieties were being replaced by improved lines. Eventually, it was shown that the glandless factor in itself does not decrease fiber yield or quality, if other factors are held constant.

As gossypol and associated terpenoids generally are regarded to be natural insecticides, research was conducted to determine whether glandless cotton is more susceptible to insect and rodent damage than glanded varieties. This interest was further intensified by observations that single test rows of glandless cotton appeared to attract insects from other nearby glanded varieties, and that field mice and other rodents would selectively eat glandless seed while ignoring the glanded seed.

Reasoning showed that rodent problems with glandless cottonseed should be no more serious than for other field grains and are relatively incidental when proper care is taken during storage. However, the possibility that glandless cottonseed plants might be more susceptible

to insect infestation has received considerably more attention. Some concerns were raised by entomology studies showing the cotton boll worm (*Heliothis zea* Buddie), tobacco budworm (*H. virescens* F.), pink bollworm (*Pectinophua gossyprella* Saunders) and lygus insects prefer glandless cotton, if given a choice (32). Indian researchers also have shown that spotted bollworm larvae (*Earias vittella* F.) survive and grow better when reared on glandless cottonseed leaves than on either glanded leaves or glandless leaves treated with 1.0% gossypol (33).

Grower reports at a 1977 industry conference indicated that insect control requirements under field conditions were not appreciably different for glandless varieties than for glanded varieties (34). Leading cotton breeders have felt that breeding in morphological characteristics such as okra leaf, frego bract, glabrousness and the nectarless characteristic would reduce disease and insect susceptibility of all types of cotton (35). Significant useful variability for decreased susceptibility to both the tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois) and tobacco budworm has been shown in glandless cottons (36). Experiment station trials for three successive years in Louisiana found that bollworm-budworm problems were no more serious on glandless strains than on the normal glanded checks (37). In summary, although glandless varieties may require closer supervision to intercept and control insect infestations, this does not appear to be a serious problem in practice.The possibility of developing a variety of cotton in which the plant forms glands after seed germination has been known at least since 1976 (23). This characteristic exists in *Gossypium bickii,* a diploid species of wild cotton, but has not been incorporated into any glandless cottonseed varieties released.

Glandless upland cotton planting seed was offered commercially in 1980 in Texas under the labels of Gregg, Lambright (Northstar), Lockett (Pioneer), Rogers and Paymaster (Acco) (38), and in Louisiana under the labels of Deltapine and Stoneville (39). In addition, Cooper continued McMichael's work (3) by developing several glandless strains in the Acala-type cottons being considered for establishment of a glandless cottonseed food and feed protein industry in California. As of early 1985, domestic glandless cotton breeding programs included those of (a) Bird at Texas A&M University, on use of the multi-adversity resistance (MAR) genetic improvement system to develop cold-tolerant, early maturing stripper-type glandless varieties resistant to major diseases and insects; (b) Owens at the Texas Agricultural Experiment Station at Halfway, TX, on development of hybrid glandless cottons for the High Plains region; (c) Caldwell at the Red River Agricultural Experiment Station, Bossier City, LA, on development of glandless open boll picker type cottons; (d) Bush of the Rogers Cottonseed Company, Waco, TX, on several stripper-type glandless cotton varieties; and (e) Cooper, on development of glandless Acala-type cottons for the San Joaquin Valley of California (3).

Initial utilization research and industry development. Characterization studies of glandless cottonseed began at USDA's Southern Regional Research Center (SRRC) as soon as quantities of experimental seed became available. Nutrition studies of glandless cottonseed meal in poultry and swine started in 1959 at Louisiana State University under the direction of Watts. Processing research began at Texas A&M University in late 1961 under the leadership of Wamble and Lawhon; this group later was renamed the Food Protein Research and Development Center. Alford and Milner initiated human nutrition studies at Texas Woman's University in 1973. A review on processing and utilization (40) reported that by 1963, glandless cottonseed already had been processed into oil and meal, and evaluated in poultry broiler and layer rations. Glandless cottonseed meal was found nearly as effective as soybean meal in achieving broiler gains and did not produce the green yolk discoloration in eggs commonly experienced with feeding glanded cottonseed meal.

The first commercial glandless cotton variety was Gregg 25V, planted in 1966 in the Lubbock, TX, area. This was a storm-proof boll-type, suited to mechanical stripper harvesting. The suppliers, the Gregg Seed farm, Plainview, TX, did not emphasize the glandless character of their new variety, but sold it on the basis of improved tolerance to Verticillium wilt, with glandlessness being incidental. The second glandless variety, Watson GL-16, was developed by the Ferris-Watson Seed Co., Garland, TX, and marketed by the Rogers Delinted Cottonseed Co., Waco, TX. This also was a storm-proof, boll-type cotton. It was evaluated in farm-scale trials and made available for commercial planting in 1969 (41).

In 1971, the Rogers Delinted Cottonseed Co. contracted with farmers in the Texas High Plains area to plant about 15,000 acres to the Watson GL-16 variety. The harvested cotton was "block ginned" (the gin was carefully cleaned to remove all other seed) in 50-bale lots at 18 gins. The seed was hulled, sized, sorted and bagged in 22.7-kg (50-lb) sacks by the Levelland Vegetable Oil Co., Levelland, TX, and stored at 2 C (42). Upon obtained FDA approval, the Rogers Delinted Cottonseed Co. began selling glandless cottonseed kernels under the name of "Cot-N-Nuts®," and subsequently built a modern kernel processing and roasting plant.

Sale of glandless cottonseed kernels was authorized in 1976 under Title 21 (Food and Drugs) of the Code of Federal Regulations (43). Sale of glandless cottonseed kernels and cottonseed flour as food additives is permitted with the restrictions that the free gossypol content not exceed 450 ppm, and that arsenic not exceed 0.2 ppm. Sale of *n*-hexane-extracted "partially defatted, cooked cottonseed flour" and "defatted cottonseed flour" is permitted, provided that no more than 60 ppm solvent residue remains in the extracted flour, and that the fat content of "defatted cottonseed flour" does not exceed 1% by weight. Glandless cottonseed kernels can be sold for use as snack foods, in baked goods and in soft candy provided they are roasted at 112 C for 5 min or more. Raw, glandless cottonseed kernels also may be used in making hard candy when kernel temperature during cooking exceeds 121 C for at least 5 min (43,44).

The National Cottonseed Products Association (NCPA), representing the domestic cottonseed processing industry, established the following grades of glandless cottonseed products in 1978: Class A, to contain not more than 400 ppm of total gossypol; Class AA, to contain not more than 100 ppm total gossypol; and Class AAA, to

contain not more than 10 ppm total gossypol (45).

Unlike the cereal grains, the entire kernel of cottonseed, consisting of two folded leaves and a rootlet, is the embryo of the future plant. Fertilization of a flower on a completely glandless plant with pollen of a glanded plant results in seeds with gossypol glands. Technically, the names "glandless" and "gossypol-free" for this type of seed are inaccurate. Growing large acreages of glandless cottonseed, without cross fertilization by windblown or insect-carried pollen of glanded varieties, will not be feasible as long as substantial acreages of glanded types continue to be grown in the same area. Therefore, other names such as "low-gossypol" and "white" cottonseed have been suggested.

Varying levels of gossypol can exist among individual seeds from the same plant, depending upon the combinations of Gl_2, gl_2, Gl_3, and gl_3 genes present. Mathematically, contamination of an otherwise glandless seed lot with more than 3.75% glanded seed, containing a typical level of 1.2% gossypol, would bring the total gossypol content in kernels to the maximum legally permissible level of 450 ppm. Also, as gossypol is only slightly soluble in hexane, the effect of solvent extraction of oil is to concentrate the gossypol in the remaining meal. In theory, a seed lot with an initial 450 ppm content of total gossypol will result in flour containing approximately 714 total ppm gossypol. Stated another way, the initial supply of kernels must not contain more than 270 ppm gossypol if a flour with less than 450 ppm total gossypol is sought. In practice, some gossypol is bound during processing, and the free gossypol content of defatted meals and flours is less than the total gossypol content.

For these reasons, maintenance of varietal and seed lot purity is essential in the production of glandless cottonseed products (34). In addition to cross fertilization from cotton plants in nearby fields, the glandless variety lot itself will tend to revert to the glanded condition during successive plantings due to fertilization between heterozygous plants. To produce products usable as human food and monogastric animal feed, it is necessary to segregate handling of glandless cottonseed in gins and oil mills, a procedure costing a premium over standard practices. Large cottonseed processors have been hesitant to handle glandless cottonseed until sufficient quantities become available to warrant dedicated processing facilities. Therefore, seedsmen have been the entrepreneurs in establishing glandless cottonseed processing facilities, probably because they are the most familiar with handling segregated seed lots.

All aspects of glandless cottonseed breeding, production, pest control, processing and utilization were reviewed comprehensively at a conference at Dallas, TX, in December 1977, sponsored by the ARS, USDA and the NCPA. The proceedings, *Glandless Cotton: Its Significance, Status and Prospects,* has become a standard reference (34). Use of glandless cottonseed kernels as a food ingredient has been popularized by a home-style cookbook, *Cottonseed Cookery* (46). A useful reference on nutrition and uses of glandless cottonseed kernels and flour, *Cottonseed—the New Staff of Life,* has been developed (47). Glandless cottonseed utilization research at Texas A&M University and Texas Woman's University has been sponsored by the Natural Fibers and Food Protein Commission of Texas, USDA and Cotton Incorporated. Glandless cottonseed kernels are now available commercially, and free samples of glandless cottonseed food proteins (defatted flour, concentrate and isolates) are available for experimental evaluation by food processors from the Food Protein Research and Development Center.

Yazaki USA Inc. purchased the Rogers Cottonseed Co. of Waco, TX, in 1984 as a wholly owned subsidiary, and rededicated its program exclusively to the development of glandless cotton varieties and production of food-grade kernels and flakes. Yazaki also has acquired many of the glandless cottonseed lines of earlier Texas seedsmen.

The best documented glandless cottonseed development program outside the U.S. has been led by IRCT France (Institut de Recherches du Coton et des Textiles Exotiques) through its network of collaborating research stations in French-speaking Africa. Development of glandless cotton as a food protein source started at Bebedjia, Chad, in 1958, and led to the realization that considerable cross-breeding with local cotton varieties and selection were required for adaptation in Africa (48). Two varieties were developed, "Bulk A glandless" for Mali in 1969 and "Bulk B glandless" for Chad in 1970. Trials in the 1972/73 and 1973/74 seasons showed that insects were not a problem and that the quality of the fiber was good; however, fiber yields were lower than those of traditional varieties. Nutrition studies using glandless cottonseed-enriched diets conducted with children in Dakar, Senegal and Mali are reported by Roux (49,50). Studies in Chad showed a 56% protein glandless cottonseed flour blends well with millet of sorghum flour in preparing the main meals of Chadian cooking (51). Plans to expand cultivation and processing of glandless cottonseed in Chad were abandoned with the local revolution in 1979, and IRCT's efforts then shifted mainly to Mali and the Ivory Coast, with additional research in Cameroon and Paraguay. Glandless cottonseed cultivation and oil mill processing studies in the Ivory Coast during 1980 through 1983 led to the conclusions that cultivation is economically viable in that country, and that conditions may be better for introducing glandless cotton in African nations than in larger industrialized countries like the U.S., where other competitive oilseed proteins are readily available (52). An additional advantage in Africa is that large, isolated land areas can be dedicated to this crop.

Research in Egypt was initiated in the late 1950s at the former Bahtim Agricultural Experiment Station, with glandless cottonseed obtained by an entirely different route. In 1959, dry seed of Giza 45, an Egyptian extra-long staple variety, was treated by soaking it in radioactive phosphorous (^{32}P) solution, and then planting it in pots and later transplanting to the field. A few plants morphologically different from Giza 45 were found. After selection for four years, one of the lines was found to have about 1% gossypol-free seed.

Stability of the hand-selected, gland-free seeds was demonstrated for four generations, and the resulting glandless line was named "Bahtim 110." It was later concluded that glandlessness in Bahtim 110 is a simple, partially dominant character that depends on one pair of genes, and that the dominant homozygous condition controls the complete absence of gossypol and gossypol

derivatives in all parts of the plant. Oil and protein contents of Bahtim 100 cottonseed were comparable to those of other commercial Egyptian varieties, but the yield of lint was extremely low and attempts to improve yield and quality were not successful. Limited evaluations were made of Bahtim 100 seed for food and feed protein use (53). More recently, interest has again returned to development of a local glandless cottonseed crop (54). Breeding research is in progress, and several papers have been published on utilization of glandless cottonseed products in Egyptian foods.

Glandless characteristics were introduced into Indian cotton varieties in the early 1970s for potential food uses, and analyses were performed on the kernels (55). Development of this crop proceeded slowly, because most processing was by screwpress using undehulled seed, except where the presscake was intended for export. However, new interest in developing glandless cottonseed has been shown recently in India. Also, plans were announced in 1974 to initiate a program to develop glandless cottonseed varieties in Pakistan by mutation breeding using ionizing radiation (56), but progress reports have not been found in the literature. Oral reports and private communications also have indicated that the following countries are either investigating, or have investigated, glandless cottonseed: Colombia, Israel, Mexico, the People's Republic of China, Peru and the Soviet Union.

OIL MILL EXTRACTION

Several hundred thousand tons of glandless cottonseed have been produced in the U.S. With the exception of seed used for planting, research or processing into edible kernels, the glandless seed generally has been intermixed with glanded seed and extracted. Results of only a few segregated extraction trials of glandless seed are publicly available.

The major differences noticed between glanded and glandless seed have been reduction in gossypol content, and yields of lighter colored meal and crude oil from the latter. Based on domestic industry 5-yr average values for glanded cottonseed, 909 kg (1 ton) of glandless cottonseed would be expected to yield approximately 145 kg crude oil, 235 kg hulls, 419 kg of 41% protein meal, 75 kg linters and 35 kg "waste" (loss) during processing (57). Evaluations of eight varieties each of glanded and glandless cottonseed grown in Texas for possible use are summarized in Table 1. Mean assay values generally were similar for whole seed, kernels and solvent-extracted oil for glanded and glandless varieties, except that glandless cottonseed kernels averaged 2% more oil content than glanded kernels. However, this may have resulted because the specific glandless seeds were smaller. Amino acid profiles were similar for glanded and glandless seed, with about 4.0 g(%) available lysine/100 g each (58). However, it is not uncommon for composition ranges within varieties grown at several locations and climates to be broader than between varieties, as shown in trials of four national variety test cultivars grown at eight different locations (59).

The storage and processing characteristics of glandless and glanded cottonseed are essentially identical. Lawhon and Wamble (60) found that the two types of seed responded similarly during storage, with development of free fatty acids accelerating with increased moisture levels, and refining losses in the crude oils proportional to the final free fatty acid contents.

Interest in oil milling of glandless cottonseed has been mainly in direct solvent extraction, since binding of gossypol (as by cooking and hard press or prepress) is not required and processing objectives usually have been to make the lightest colored oil and most soluble meal protein possible. Initial solvent batch extraction and hard press trials were conducted at Texas A&M in 1961. Both types of seeds were delinted to 3% linters content; equilibrated to 9% moisture before decorticating; flaked to 0.250 mm thickness; and, after preheating to 82 C, moistened to 12% and cooked 20 min to a temperature of 170 C, batch extracted with hexane, and desolventized for 12 min at 93 C. Flakes for hard press trials were prepared in the same manner as for solvent extraction, but were dried more in the cooker to lower the moisture content for pressing. In all cases, protein solubility of processed glandless cottonseed flakes or presscake was higher than that of glanded seed (89.6% compared to 84.8% for extracted flakes, and 91.1% compared to 59.3% for presscake) (60). This tendency for glandless cottonseed protein to be more soluble than glanded protein was again observed by Lawhon et al. (58) in the nitrogen solubility profiles of cottonseed flours made for food use from eight glanded and glandless varieties.

The first recorded processing of glandless cottonseed in a direct hexane extraction commercial facility was a 40-ton trial of Acala 4-42-77 seed at the Leland Oil Works (a 136 MT/day facility), Leland, MS, in 1965. The second major trial was of 600 tons of Gregg 25V at the Plains Cooperative Oil Mill, Lubbock, TX, with a processing rate of 182-204 MT/day on a line which normally handled 409 MT/day. Problems in resetting the equipment to produce high solubility glandless cottonseed meals were encountered on both lines. Meals with 80-90% nitrogen solubility and up to 4% available lysine (inversely related to the degree of heating) were made in both trials. Also, unexpected emulsification problems were encountered in refining when the amount of alkali added was selected on the basis of previous known relations to color of raw cottonseed oil. This indicated that another factor, probably phospholipids which normally are removed in miscella refining of glanded seed oil, must be considered in refining glandless cottonseed oil (61).

The 1977 conference on "Glandless Cotton: Its Significance, Status, and Prospects" showed that oil mill operators had considerably more knowledge about processing glandless cottonseed than was available in the public literature. Estimated advantages of processing glandless cottonseed over glanded seed included: (a) 50% reduction in electrical energy used for flaking; (b) exchange of expensive prepress operations for larger solvent extractors with lower maintenance costs, and 50% reduction in processing energy required after flaking; (c) ability to hold crude cottonseed oil for 3 mo or longer without setting of color (as typically occurs with glanded seed oils), thus eliminating needs for miscella or on-site conventional refineries; (d) 3% less refining loss of glandless seed oil, with reduced requirements for alkali; (e) reduction of bleaching earth requirements by 50% (to a level of 0.5%

TABLE 1

Comparative Analysis Means, Eight Varieties Each of Glanded and Glandless Cottonseed and Their Products (58)[a]

Product and assay	Glanded cottonseed	Glandless cottonseed
Whole cottonseed		
Oil (%)	21.0	21.1
Iodine no.	108.9	109.9
Protein (N × 6.25; %)	23.1	22.5
Wt. 100 fumed kernels (g)	10.0	10.6
No. fumed seed/100 ml	542	510
% Kernels in lint-free seed	61.7	59.6
Cottonseed kernels		
Oil (%)	37.8	39.7
Protein (N × 6.25; %)	39.3	38.9
Crude fiber (%)	1.6	1.7
Total phosphorous (%)	0.8	0.9
Total sugars (%)	7.4	6.8
Total gossypol (%)	1.2	0.02
Wt. 100 kernels (g)	6.5	7.0
No. kernels/100 ml	912	844
Hexane-extracted flour (meal)		
Oil (%)	0.8	0.8
Protein (N × 6.25; %)	63.2	62.6
Crude fiber (%)	2.7	2.8
Ash (%)	8.0	7.8
Total phosphorous (%)	1.3	1.4
Total sugars (%)	13.4	13.7
Total gossypol (%)	1.6	0.02
Color, Hunter "L" values		
Dry	84.3	89.8
Wet (5 water:1 flour)	48.1	71.3
Crude oil		
Cyclopropenoid fatty acids (%)	0.23	0.23
Fatty acids (%)		
Myristic	0.9	0.7
Palmitic	23.0	22.6
Stearic	2.2	2.1
Oleic	17.7	17.7
Linoleic	55.8	56.5
Unknown	0.4	0.4
Refined oil		
Refined oil color, red	6.9	3.7
Bleached oil color, red	2.9	2.2

[a]Dry weight basis.

earth used); and (f) marketing of a light-colored oil, which is more competitive and does not need the light hydrogenation required for soy oil (costing approximately $0.006/lb in 1977) (34).

The advent of glandless cottonseed as a new crop also led to new perspectives in research. Velasco (62) reported that, in the absence of gossypol, analysis of free fatty acids by conductivity was successful in both petroleum ether extracts and crude oil of glandless cottonseed. Cross et al. (63) reported that extraction rates of oil from glandless cottonseed flakes are about the same as from glanded flakes, using commercial hexane, nearly normal hexane, or a mixed solvent of acetone, hexane and water (39:60:1).

An exhaustive review of lecithin chemistry and uses (64) concluded that glandless cottonseed might be a source of a new type of lecithin, previously unavailable because of its binding with gossypol during cooking and pressing of glanded seed and the refining of its crude oil. Among the common oilseeds, cottonseed has the highest content of phospholipids after soybeans, present at approximately 2.2% in the oil. Cottonseed phospholipids contain less phosphatidylcholine, but more phosphatidylethanolamine and phosphatidylinositiol (33, 45%; 22, 15%; and 37, 25%, respectively) than do soybean phospholipids, the major commercial source of lecithin. Several potential uses of cottonseed lecithin have been suggested; it contains no fatty acid with more than two double bonds and is expected to be more stable to oxidation in food and industrial uses than is soybean lecithin.

FEED USES OF MEAL

Glandless cottonseed meal (GCSM) is attractive to the feed industry for feeding monogastric animals, including poultry, swine and horses, and also as a milk replacer for calves whose rumen is still in the nonfunctioning state. A free gossypol level of 0.04% in cottonseed meal, fed at levels of 10% to 20% depending upon the use, has been established as generally safe. Further, gossypol can be essentially deactivated by addition of selected forms of iron to the diet, thus permitting use of cottonseed meals at even higher levels. Given a price of $187/ton for prepress solvent-extracted glanded meal, glandless meal has been estimated to be worth $9 more per ton when used in a 16% protein formula for growing swine (65).

Research has shown that, at equivalent fiber levels, the metabolizable energy of solvent extracted GCSM is approximately 20% higher than that of glanded prepress solvent extracted meal (65). Harper (66) reported that GCSM contained approximately 17% more lysine than glanded meals produced in the same oil mill. Johnston and Watts (67,68) have shown that GCSM have a greater protein efficiency in feeding broilers than glanded meals prepared by similar processes.

Commercial GCSM intended for poultry feeding receive considerable heat during processing to intentionally bind the gossypol. Thus, it would be expected that low heat treatment would be desirable in GCSM production. However, Johnston and Watts (69) found that heating glandless cottonseed before hexane extraction substantially improved protein efficiency in growing broilers. Heating glandless cottonseed with 12% added water for 10 min at 82 C, followed by 105 C for 20 min before hexane extraction, resulted in increased broiler gains. The same heat and moisture treatment had no effect when applied to GCSM extracted with hexane without prior heat treatment. Heating before extraction would be part of the normal conditioning process before flaking in extraction of glandless cottonseed. In a series of studies, Johnston and Watts (68–70) concluded that GCSM was equal to soybean meal in supporting chick growth. Waldroup et al. (71) found that GCSM could replace all or part of the soybean meal in a practical broiler ration. Lysine supplementation was necessary for optimum performance only when more than 75% of the soybean meal was replaced by GCSM. Anderson and Warnick (72) reported that lysine and methionine were about equally limiting in GCSM. Fisher and Quisenberry (73) observed a 27% increase in net protein utilization of GCSM over glanded meal when both were similarly supplemented with five essential amino acids, and concluded that supplemented GCSM is equivalent to methionine-supplemented soybean meal.

Heywang and Vavich (74) and Heywang et al. (75) reported discoloration of stored eggs from hens fed GCSM prepared by pilot plant hexane extraction. Discoloration did not occur when the GCSM was prepared in a pilot plant screwpress.

Roberson (76) reported that replacing part or all of the soybean meal in a laying hen diet with GCSM did not affect numbers, weight or shell thickness of eggs produced, or feed conversion ratio, mortality or body weight gain of hens. Discoloration of yolks and incidence of pink whites were higher than expected for GCSM, possibly because of the higher fat content of the pilot plant-prepared GCSM compared to commercially extracted glanded cottonseed meal. It was believed that cyclopropenoid fatty acids in the residual oil of the cottonseed meal may have increased the incidence of yolk discoloration. Interior quality of eggs from birds receiving GCSM deteriorated more rapidly (by discoloration of yolks and whites, and thinning of whites) than from hens receiving only soybean meal. It was concluded that GCSM protein was of excellent quality and about equal to soybean meal in sustaining the performance of laying hens.

In 1983, a commercial oil mill run of an Acala-type glandless cottonseed produced a lowfat meal that was used in a series of feeding studies. Reid et al. (77) fed this meal at the 5, 10 and 15% levels to laying hens for 336 days and found no significant difference in egg output or egg weight compared with those obtained using a soybased diet. Egg production appeared to trend downward with increasing levels of GCSM, while egg weight showed a slight increase. No changes were found in yolk fatty acids, and discolorations were not present after 56 days' storage.

Growth trials with 28-day-old pigs (7.5 kg) and growing finishing pigs (19–97 kg) were conducted by LaRue et al. (78). In the trials, GCSM was substituted for corn-soybean meal-based diets at 20% increments from 0 to 100%. Lysine was added to the diets to make them equal to control diets. Pigs fed up to 40% GCSM proteins performed equal to those fed the basal diet. Above this level (60 to 100%), a linear reduction in daily gain occurred. This study also showed that GCSM had digestibilities equal to or better than soybean meal for nitrogen and all essential amino acids, as measured at the end of the small intestine.

A series of catfish studies (79) was concluded using an extended pond feeding study. Fish fed GCSM for 176 days had feed conversions and dress out percentages equal to those of fish fed a commercially prepared extruded soybean feed used as a control. However, fish on the GCSM diet were higher in fat and lower in moisture, protein, ash and edible tissue than fish on the soybean control diet. Although measured lysine levels apparently were deficient in the GCSM feed, growth and feed conversions were unaffected.

Lawrence (80) compared shrimp growth using a commercial feed, a proven experimental feed containing squid meal, and an experimental feed without squid meal. Each of these feeds was supplemented with 4.5, 9 and 13.5% GCSM prepared in a pilot plant. Two sizes of shrimp (*Penaeus setiferus*) were used (0.18–0.19 g and 1.7–1.9 g). The survival of both sizes was excellent, with the smaller shrimp showing greater percent growth, and the larger shrimp showing slightly larger weight gain. There was no performance difference between the two experimental diets. Animals receiving GCSM had greater weight gain and slightly better survival than those fed unsupplemented diets. Similar nutritional responses to the diets with and without GCSM indicate that GCSM can be used in shrimp feeding.

PROTEIN FOODS PROCESSING

General. The processing of edible protein products has

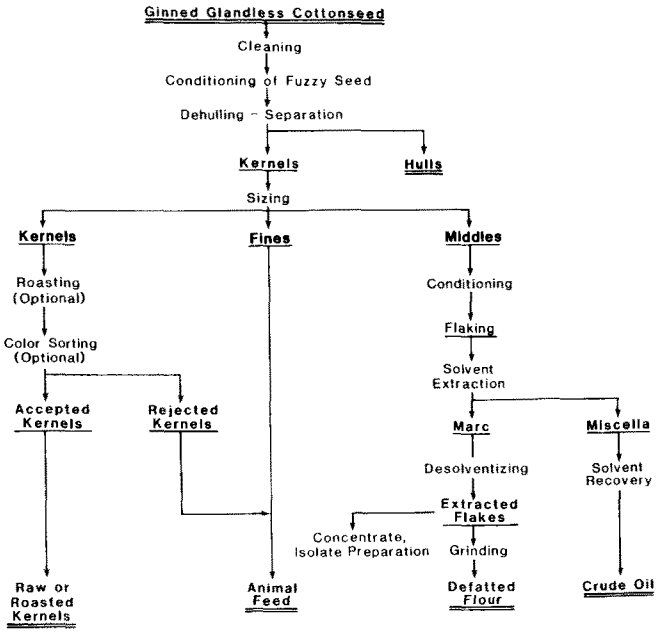

FIG. 2. General flow chart for production of glandless cottonseed food ingredients.

been summarized by Lusas et al. (81) (Fig. 2). Ginned cottonseed is first cleaned, conditioned, dehulled and separated to produce kernels. The kernels may be size-sorted and the larger particles roasted and color sorted. The "middles" (small or broken kernels) may then be further conditioned, flaked and solvent-extracted. Upon grinding, extracted flakes become flour. The defatted flakes can be reextracted with acidified water or ethanol to remove the soluble sugars and flavor compounds, and dried to produce protein concentrate. If a spray dryer is used in preparing concentrates, the defatted flakes are preferably ground into flour before extraction. The flour also can be used to make either a single ("classical") isolate by extracting the protein with alkali followed by precipitation at one pH level, or two protein isolate fractions (storage protein and nonstorage protein) by extracting and reprecipitating by two major routes. Additional techniques, such as extraction of oil by aqueous processing (AP), and use of ultrafiltration (UF) membranes to recover protein, followed by reverse osmosis (RO) treatment of the UF permeate to recover and recycle part of the processing water, also have been evaluated.

Kernels. Glandless cottonseed kernels ("meats") are prepared either for direct use as whole nut substitutes and food ingredients, or as hull-free intermediates in the production of flours and food protein concentrates or isolates. Thirty percent or more breakage is often experienced in hulling unconditioned seed, and it is desirable to maximize production of the more valuable whole kernels and divert the "middles" fraction to the manufacture of food proteins or oil milling. Where the main objective is to produce food proteins, less care needs to be taken to minimize breakage during dehulling.

At the Food Protein Research and Development Center, seed is cleaned by a Bauer two-tray cleaner, a piece of equipment commonly used in the cottonseed processing industry. Seed produced on the Texas High Plains often is received at about 6% moisture, and breakage during hulling can be decreased by preconditioning (82). The most effective treatment found thus far consists of steaming delinted seed containing 6% moisture kernels at atmospheric pressure for 4 min, then drying with indirect heat for 2 min, followed by dehulling while hot (83). Hulling is enhanced without adding appreciable moisture to the kernels or inducing noticeable lipase activity. By this steaming/drying/hot hulling process, the yield of kernels retained on a 3.2 × 6.4 mm screen was increased from 47.7% to 84.6%. Higher yields of acceptable kernels were achieved by this method than by equilibrating seed to 8% kernel moisture before hulling.

Separation after dehulling is enhanced if the seed is not delinted. Optimum linters content is about 8–10%, and equipment throughput decreases and kernel losses increase if linters content exceeds 10% (82–84). Good hulling results have been obtained with Chandler and Murray-Carver bar-type dehullers, with the gap between the knives increased to induce impact hulling, rather than slicing. Kernels also have been produced with a Bauer Laboratory disc huller. After dehulling, the kernels and hulls are separated on a Bauer or Murray-Carver shaker table, clothed with a 4.8 mm round hole screen followed by a 6.4 mm round hole screen, and using aspiration. An additional purifier is then used to further remove hulls and size grade the seed into three fractions: "kernels," retained on a 4.0 mm (round hole) screen); "middles," passing the 4.0 mm screen but retained on a 2.4 mm screen; and "fines," passing the 2.4 mm screen. The Bauer and Murray-Carver separated systems are capable of separating undehulled seed so it can be recycled through the dehuller.

One ton (907 kg) of cleaned fuzzy glandless cottonseed yields approximately 249 kg kernels, 179 kg middles, 57 kg fines ("peppers"), and 431 kg fuzzy hulls and entangled seed. The ratio of kernels to middles can be

altered by changing the purifier screen sizes. The middles may be roasted for direct food use, but are mainly considered a starting material to produce defatted flour and protein concentrates and isolates. A means to effectively separate broken kernels from hull pieces in the fines fraction has not been developed, and current recommendations are that this stream be sent to a solvent extraction line. Because of the presence of meats and small undecorticated seeds entrapped in the fuzzy hulls, it may be desirable to send this fraction through a closely set dehuller and then to an oil extraction line also.

Some cottonseed oil mill operators have been concerned that development of glandless cottonseed for food uses may divert seed from the oil milling industry. Actually, a segregated dehulling line to produce edible kernels from glandless cottonseed could be most profitable for an established oil mill that can extract the by-product streams. Under this arrangement, processing of glandless cottonseed kernels would become a high return operation to the oil mill, rather than a separate industry competing for the same seed source.

In 1968, the Food Protein Research and Development Center demonstrated the processing of glandless cottonseed kernels into nut-like products called "Tamunuts®." Various roasting methods were tried by Lawhon et al. (85,86), including dry roasting at several temperatures, roasting under vacuum followed by steam injection, pressure steaming followed by oven roasting, and deep fat frying in various oils (including corn, cottonseed, peanut, safflower, soybean and sunflower oils). The product most preferred by taste panel was made by dry roasting kernels to an end temperature of 141 C. If a salted product was desired, 2.5% of a hot 1:1 mixture of peanut and coconut oils was added to improve adhesion of salt. Later, a non-pressurized roaster design was developed. This roaster initially retains the steam atmosphere resulting from heating the kernels and produces a less hard product (87).

In the currently preferred process, the above described roaster is preheated to a wall-temperature of 204 C, then charged with 11 kg of raw kernels. The batch is then heated with stirring to 110–113 C (requiring about 40 min after roasting begins), and the vents are opened to allow the steam to escape and develop the texture desired in the finished product. Roasting is continued to a batch temperature of 130 C (requiring another 35 to 50 min) and the Tamunuts® are discharged. The product is then color sorted with a Geosource Electronic System Division gravity sorter, model GB-103, set for monochromatic sorting. Removal of necrotic, damaged and gossypol-containing seed is enhanced by their darkening during roasting, but the kernels can be sorted before roasting if the processor is satisfied with the resulting separation efficiency. The kernels can then be returned to the roaster and hot oil and salt added if a salted product is desired. Unsalted Tamunuts® prepared in the above fashion contain approximately 39% protein, 32% fat, 17% carbohydrate, 5% ash, 4% crude fiber and 2% moisture. The warm "nuts" are packed into metal cans with pull-top lids and vacuum/nitrogen packed using a Rooney Semi-Automatic closing machine. A vacuum of 381–457 mm is drawn and released with nitrogen for two cycles; on the third drawing of vacuum, the can is sealed. A polyethylene overcap is provided with each can for reclosing to extend freshness after opening. Dry roasted, unsalted Tamunuts® have kept their freshness for three years at room temperatures (21–27 C) when sealed in cans in this manner.

Development of food uses for whole kernels led to the question of how best to handle glandless cottonseed for an export market. Grey (fuzzy) cottonseed weighs about 400 kg/m^3, completely delintered seed weighs about 641 kg/m^3, and dehulled seed (kernels) weighs about 625 kg/m^3. Because of dehulling yields and differences in density, reductions of approximately 50% in shipping weight, and 82% in storage space (volume) might be realized if techniques could be developed to ship and store dehulled seed rather than grey seed. Another potential advantage of marketing dehulled seed might be its comparability with conventional grain systems which cannot convey grey cottonseed. Also, space, equipment, capital, energy and labor costs for dehulling would be avoided by the kernel purchaser, and the cottonseed shipper would sell a value-added product.

During a series of studies to determine conditions for optimum handling of dehulled glandless cottonseed, Johnson (88,89) found that enzyme-catalyzed lipolysis is the primary spoilage mechanism in raw kernels. Deterioration was proportional to the extent of mechanical damage caused by dehulling, with kernels being the most stable fraction and the fines being the least stable. Rates of lipolysis in dehulled kernels were greatly affected by moisture content, but not appreciably by temperature (Fig. 3). It was found that 2.25% free fatty acid content is the maximum level acceptable to an untrained taste panel. Raw seed purchased for processing should contain considerably less free fatty acid.

Autoxidation is the primary spoilage mechanism in roasted seed; however, cottonseed is unusually stable to lipid oxidation, indicating the presence of high levels of natural antioxidants. A peroxide value of 54 meq/kg oil was found to be a critical level for consumer acceptance. When roasted product exceeds these levels, the sample should be considered spoiled and removed from market channels. Noticeable lipid oxidation did not occur during dehulling or storage of raw kernels. No evidence of lipoxygenase-catalyzed oxidation was found in either glanded or glandless cottonseed (88,89).

It also was found that when raw kernels with a moisture content of 9.8% or more were roasted, they become soft-textured and also dark colored on the surface. Both attributes were offensive to panelists. These observations explained a problem noticed in this laboratory over several years—glandless cottonseed that had become wet during storage in modules or wagons awaiting ginning often turned brown upon dehulling and roasting. A dehulling-roasting test for seed at the receiving dock, as is sometimes used in accepting confectionery sunflower seed, might be a simple means to predict whether specific shipments of glandless cottonseed should be used to make kernel-type products.

Recommendations developed by this study were: (a) keep the whole seed intact as long as possible, as cool as possible, and under 9% moisture (whole seed at 9% moisture will have kernels at 8% moisture or less, and is more stable than dehulled kernels); (b) do not use raw

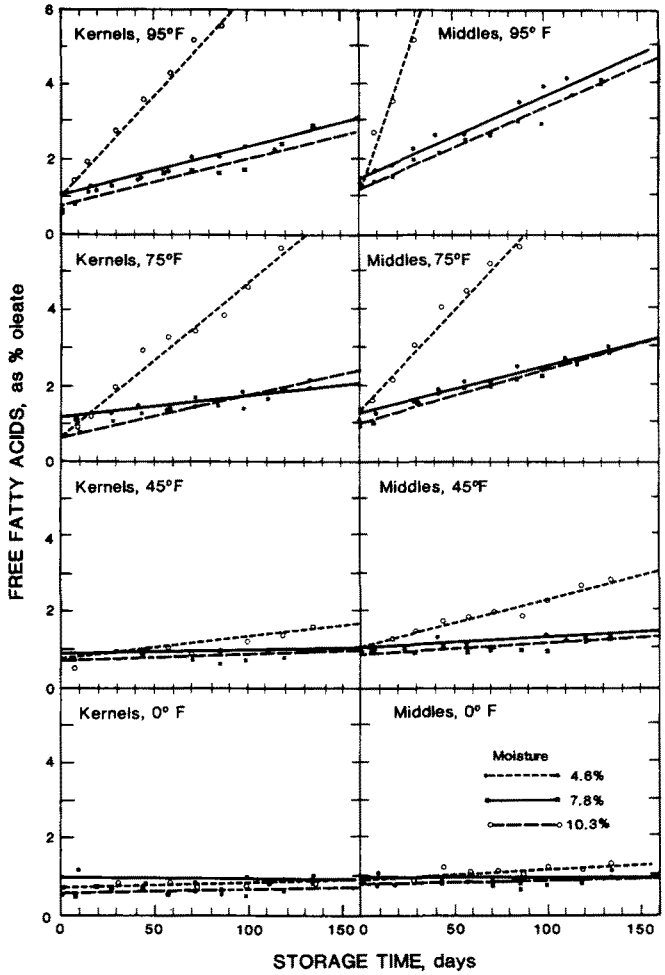

FIG. 3. Free fatty acid development during storage of dehulled glandless cottonseed kernels and middles at selected storage temperatures and moisture contents (89).

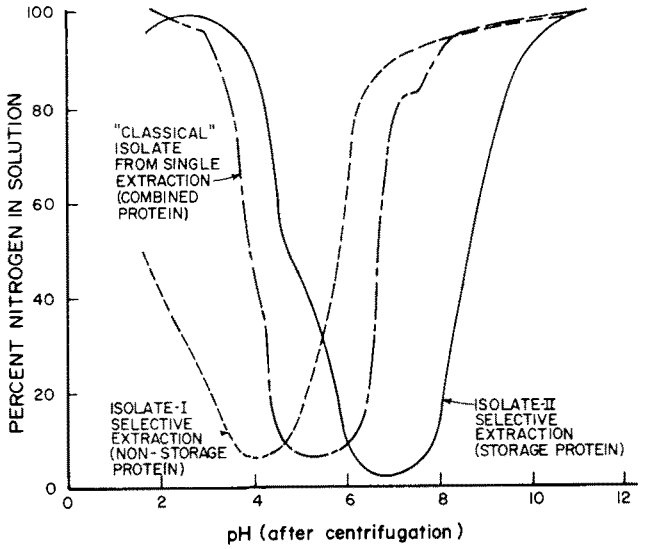

FIG. 4. Nitrogen solubility curves of 1% solutions of cottonseed protein isolates from the single-step and selective extraction processes (92).

kernels with greater than 1.0% free fatty acid and 8% moisture (roasted seed becomes organoleptically unacceptable at 2.25% free fatty acid content, but should be removed from the marketplace at much lower levels); (c) store all dehulled raw kernels as dry and cool as possible (kernels with 0.5% initial free fatty acid and 8% moisture or less can be safely stored at 5 C for approximately one year); (d) do not dry dehulled kernels to less than 8% moisture (drying of dehulled, mechanically damaged kernels to lower than 8% moisture increases their free fatty acid content and does not significantly reduce hydrolysis rates); (e) package roasted kernels under vacuum or nitrogen in relatively oxygen- and moisture-proof containers or flexible packaging (packing under CO_2 does not increase product protection [88,89]).

Food protein flour. Glandless cottonseed flour is prepared by extracting dehulled glandless seed flakes with hexane, desolventizing and grinding the resulting meal. Sound, clean seed, with a free acid content of less than 1.5%, should be used. Glandless seed should be processed, segregated from glanded, in facilities suitable for preparing food-grade products. A typical process would be to rapidly temper approximately 11.5%, heat to a temperature of 80 C, flake to 0.35–0.30 mm thickness, extract with hexane, desolventize, and grind to pass a 100-mesh screen. FDA requires that residual oil content be less than 1.0% and residual solvent less than 60 ppm (44). Economics of making glandless cottonseed flour for three sizes of mills, processing 100, 200 and 400 tons of seed per day (300 days/yr) have been estimated by Clark et al. (90). Current input costs for seed, energy, labor and other materials would be required to update the estimates. Wan et al. (91) reported that wetted flours always are darker than dry flours, and that the factors most responsible are gossypol, intact gossypol-containing glands, and hull particles. Also, the natural flavonoids in cottonseed may cause yellow colors if the flour is wetted under alkaline conditions (higher than pH 8.2). The protein content of defatted cottonseed flour usually is higher than 55%.

Concentrates from defatted flour. Glandless cottonseed concentrates are essentially defatted flours from which the sugars and other soluble compounds have been leached, followed by drying. Through this processing, lower flavor content products are produced, containing 70% or more protein on a dry weight basis.

Glandless cottonseed concentrates and isolates have been made in experimental quantities only. The processing of cottonseed concentrates and isolates differs considerably from preparing similar products from soybeans. The primary difference is the presence of two protein fractions in cottonseed whose solubilities differ appreciably with pH (Fig. 4). Storage protein (SP) is soluble at high and low pHs, while maximum solubility of nonstorage protein (NSP) is near neutrality. Storage protein is the major protein in cottonseed, present in 2.5 times the quantity of nonstorage protein. Storage protein is believed to originate from discrete bodies deposited within the seed cell, and the nonstorage protein to be the "cement" which holds the different structures in the cell together. Thus, extraction conditions must be chosen carefully to obtain the type of protein desired, and especially to minimize loss

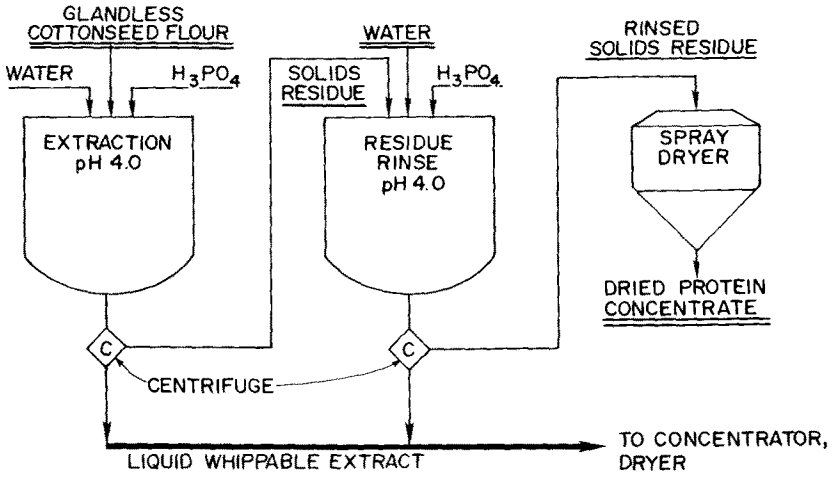

FIG. 5. Simplified flow chart for protein concentrate production by the acidic water extraction process (81).

of soluble protein in preparation of concentrates.

Concentrates have been prepared from flour by a dry air classification method (93), and by acidic water extraction (94) (Fig. 5). In the latter method, glandless cottonseed flour is extracted with phosphoric acid at pH 4.0; the liquid fraction is separated by decanter, and the solids are washed again with phosphoric acid rinse and then spray- or drum-dried. If desired, a neutralized form of concentrate can be produced by adjusting the pH to approximately 7.0 before drying. Protein concentrates are less expensive than isolates, and are equally suitable for certain applications.

Isolates from defatted flour. As summarized by Martinez and Hopkins (95), three major techniques exist for making glandless cottonseed isolates. In the "classical" procedure (Fig. 6), ground flour is extracted with dilute alkali (at pH 10) and the insoluble residue removed by continuous centrifuging or decanting. The clarified liquor is then precipitated at one pH (5.0), and the resulting solids are concentrated by centrifugation and then dried to produce a mixture of SP and NSP. The solubles, as would be extracted in making concentrates, remain in the whey.

The "selective extraction" procedure developed by Berardi et al. (92) (Fig. 7) consists of leaching the proteins soluble at neutrality with water, followed by centrifugation. The resulting liquor is acidified to pH 4 to precipitate a protein curd. After centrifuging, the curd is dried to produce an NSP isolate and the whey contains the insolubles normally removed by acidic water preparation of glandless cottonseed concentrate. The residue from the original water leaching is then solubilized in alkali (pH 10) and centrifuged to remove insolubles, and the clarified liquor is precipitated at pH 7. The resulting curd is concentrated by centrifugation, then dried to produce an SP isolate (95).

In the "selective precipitation" procedure (92,96,97) (Fig. 8), the protein is extracted from the flour by alkali (pH 10) and the residue is removed by centrifugation. The liquor is first precipitated at pH 7, and the storage protein curd removed by centrifugation and then dried. The liquor is further acidified to pH 4 to precipitate the nonstorage protein, which is removed by centrifugation

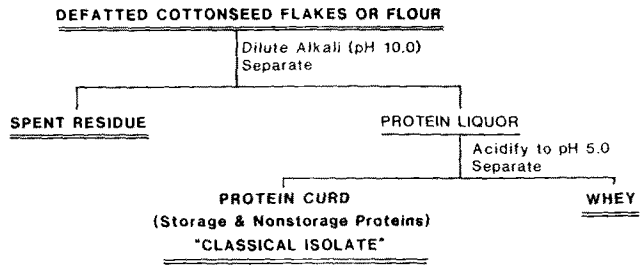

FIG. 6. Flow chart of classical procedure for preparing glandless cottonseed protein isolate (95).

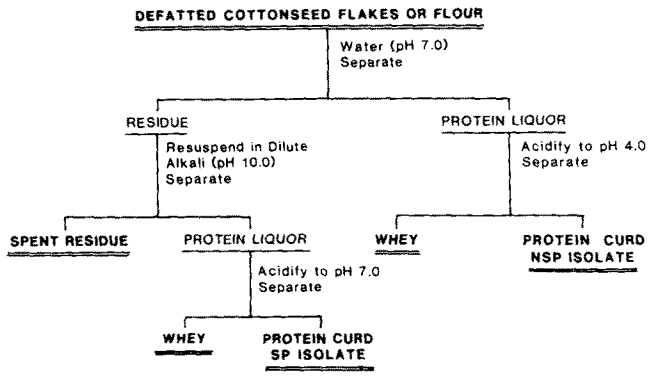

FIG. 7. Flow chart of the selective extraction procedure for preparing nonstorage protein (NSP) and storage protein (SP) (95).

and dried. The solubles remain in the whey. By either the selective extraction or selective precipitation method, a relatively pure storage protein fraction, containing over 90% protein (dry weight basis), is prepared. However, the nonstorage protein, usually slightly less than 90% protein content and technically a "concentrate," analyzes lower for protein content when made by the selective extraction method. In either case, the separated nonstorage protein curd can be adjusted to pH 7.0 before drying to

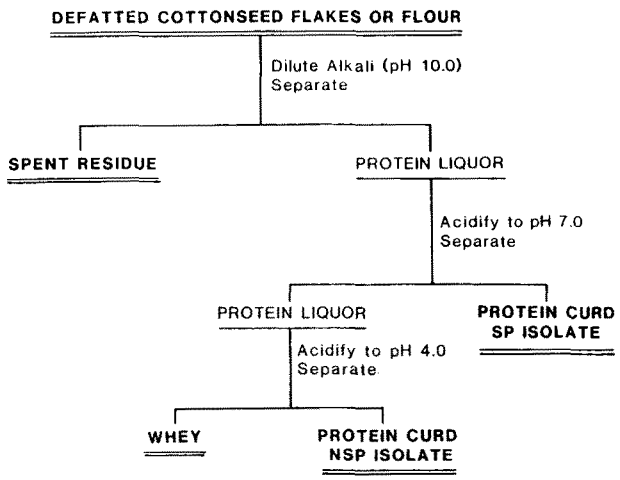

FIG. 8. Flow chart of the selective precipitation procedure for preparing storage protein (SP) and nonstorage protein (NSP) (95).

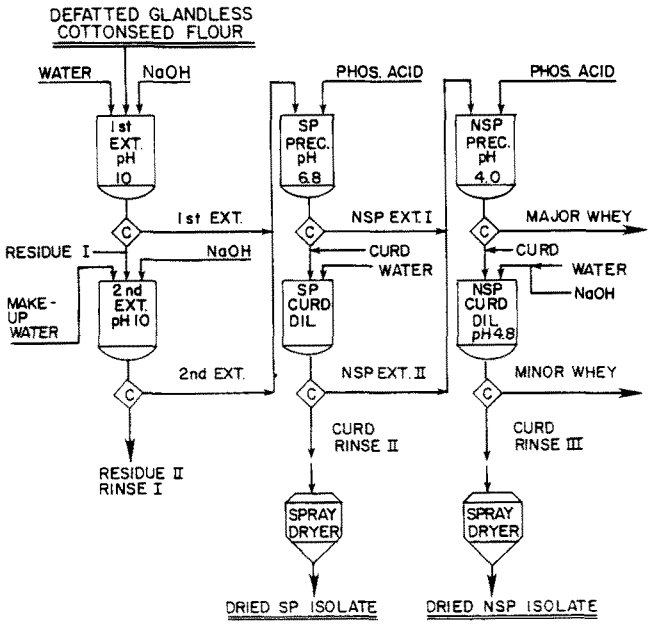

FIG. 9. Flow chart of two-step extraction process for preparing glandless cottonseed protein isolates (81).

produce a sodium proteinate form of nonstorage protein.

Actual preparation of protein isolates is more complex than is indicated in Figures 6-8. For example, the procedure used at the Food Protein Research and Development Center (81) for making storage and nonstorage protein by a modified selective precipitation procedure (Fig. 9) includes secondary re-extraction of the flour and rinsing of the storage protein curd to increase the total proteins recovered.

Aqueous extraction processing. Aqueous extraction processing (AEP) uses the ancient principle of mixing ground dehulled oilseeds in vats of hot water, and skimming off the oil rising to the surface. In modern AEP, gravity separation is replaced with mechanical centrifuges, and the emphasis is on operating conditions which cause the least damage to the nutritional value of the food proteins (98). Generally, residual oil contents of AEP food proteins are positively related to the phospholipid contents of the respective oilseeds and are substantially higher than for products processed from solvent extracted flakes or flours. Although they contain substantially more oil, AEP food ingredients are remarkably stable. A flour-like product results if the entire water dispersion solution is dried after removal of oil by centrifugation. However, the dispersion can be further treated to produce AEP equivalents of food protein concentrates or isolates. Preparation of glandless cottonseed protein concentrate is depicted in Figure 10 (99). In this process, dehulled glandless cottonseed is ground dry, leached with water acidified to pH 4.0 with phosphoric or hydrochloric acid, and separated by centrifuge (6,000 × gravity). The liquid fraction is further separated by a three-phase centrifuge (6,000 × gravity) into an oil emulsion which is later broken to obtain crude oil, a whey fraction, and residual solids which then may be returned to the leached solids for additional washing and drying into protein concentrate.

The flow chart for preparing AEP glandless cottonseed protein isolate by the process of Rhee et al. (100) is shown in Figure 11. Hull-free cottonseed kernels are ground dry, mixed with water, and adjusted to pH 10 with NaOH to solubilize the protein and sugars. The undissolved solids, consisting mainly of fibrous materials, are rinsed and dried. The protein is separated by a continuous centrifuge into oil emulsion, liquid extract and sludge fraction. The liquid extract is acidified to pH 4.5 to precipitate the proteins as a "classical-type" isolate, which subsequently is washed and dried. The whey fraction, containing sugars and some soluble proteins, can then be concentrated and dried or processed further. The oil emulsion is broken by various means to recover the oil, and several procedures have been optimized in laboratory and pilot plant-scale trials.

Process engineering research has shown that aqueous extraction of glandless cottonseed is technologically feasible, producing high quality oil and protein. Also, nongossypol pigments can be removed. Potential advantages of aqueous extraction processing over solvent extraction include: (a) lower capital costs by building smaller installations, or retrofitting the process into milk drying plants for operation during periods of low milk production; (b) safer operations; (c) production of a broader variety of tailored or modified products; and (d) opportunity to use selected chemicals to inactivate undesirable substances, including aflatoxins (101). However, because of the higher residual oil content in the meal and greater energy requirements to evaporate water rather than hexane, AEP probably can be rationalized only for preparation of food proteins, not feed meals.

Industrial membrane processing. The principle in industrial membrane processing (IMP) is to use membranes of selected pore ("cut off") sizes to separate (or "sieve") compounds according to molecular weight (MW) and configuration. Although IMP separations can be made from mixtures containing oil, it usually is preferable to use solvent-extracted flours, or the liquid extract from an AEP after separation of the oil emulsion. Ultrafiltration (UF) membranes (10,000-18,000 MW) are used to

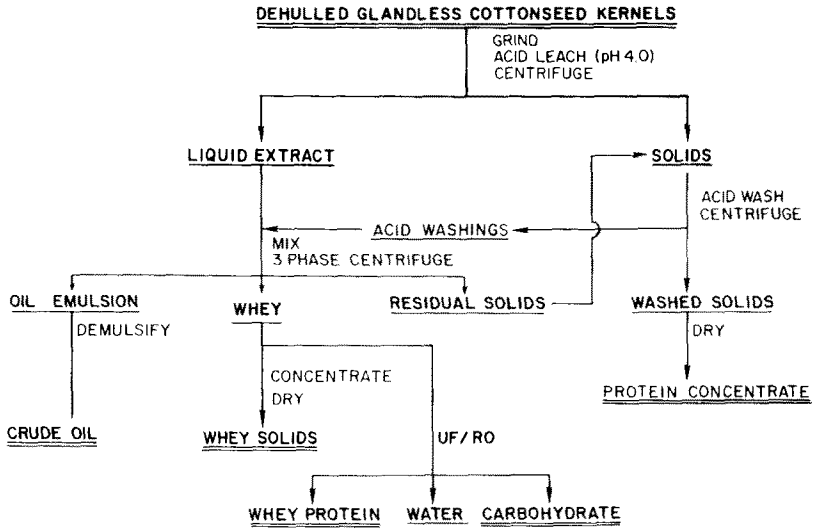

FIG. 10. Flow chart for continuous preparation of protein concentrates by aqueous extraction processing of undefatted glandless cottonseed kernels (99).

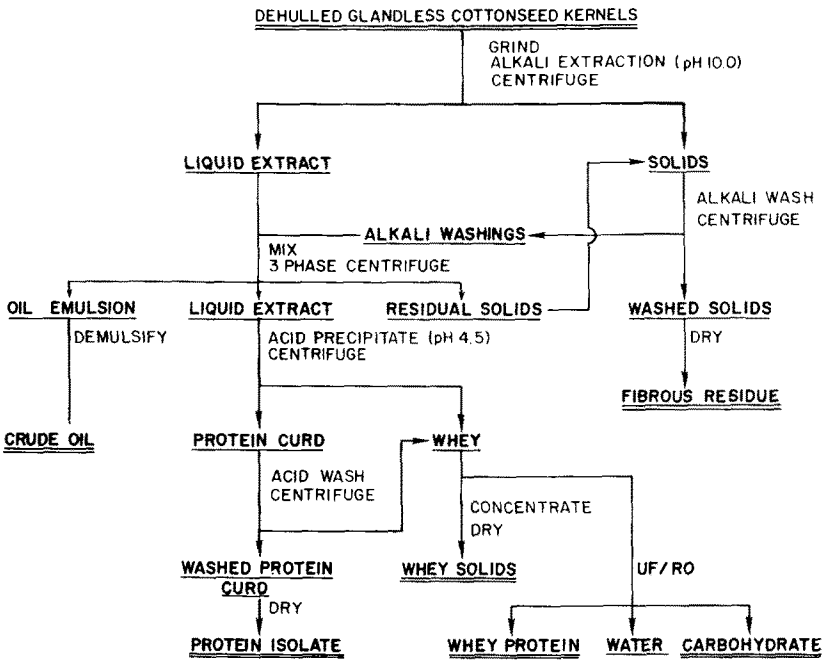

FIG. 11. Flow chart for continuous preparation of glandless cottonseed protein "classical" isolate by aqueous extraction processing (81).

separate proteins (as "retentate") from smaller molecules, including water and soluble compounds, which pass through the membrane as "permeate." The permeate may then be treated with smaller cut-off reverse osmosis (RO) membranes (200–500 MW) to concentrate the solution. Here, the soluble compounds are held back as retentate, and the permeate (water) may be reused for processing. Microbial growth in IMP processes can be avoided by operating at temperatures in excess of 65 C. The practical maximum achievable solids concentration for RO is approximately 25%. Whereas not all excess water can be separated by RO, that which can be removed requires approximately 10% of the energy required for concentration by evaporation. Concentration by RO is economically attractive in wet processes, even if used in conjunction with traditional concentration processes.

Approximately 20–30% of original flour nitrogen is lost in the wheys of cottonseed isolates prepared by the classical process (102) and can be recovered by UP and RO membranes (103–105). When food protein isolates are prepared directly by UP and RO, generation of by-product wheys is greatly reduced (103,106–108). Also, yields of sellable products are increased (107), ingredients with unique properties are produced (109) and the nutritional

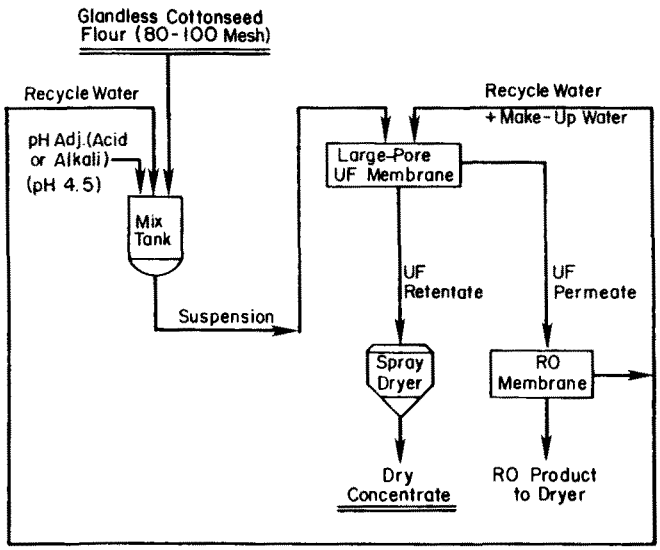

FIG. 12. Simplified flow diagram for production of glandless cottonseed protein concentrate with UF and RO membranes (110).

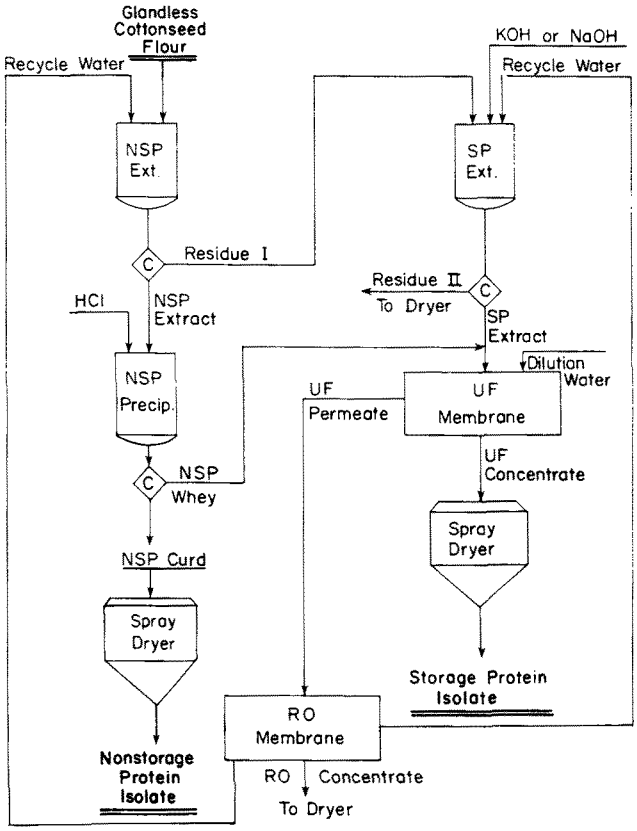

FIG. 13. Simplified flow diagram for preparation of glandless cottonseed protein isolates using industrial membranes (113).

quality (Protein Efficiency Ratio—"PER") of the resulting protein enhanced.

A patented process for preparation of glandless cottonseed protein concentrate from solvent-extracted flour using industrial membranes is depicted in Figure 12 (110).

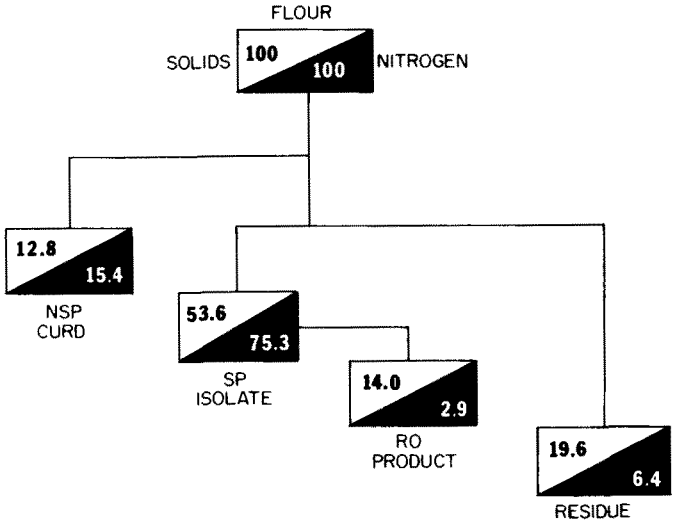

FIG. 14. Distribution of flour solids and nitrogen in improved cottonseed protein membrane isolation process (113).

Defatted flour is sieved through an 80-mesh screen to break up any agglomerates and remove hull particles, and suspended in acidified water at pH 4.0–4.5. This solution is passed through an UP membrane (100,000 MW cutoff) and the retentate either dried directly or neutralized before drying. The permeate may then be passed through an RO membrane to concentrate the soluble solids and to recover water which may be reused in the process (111).

Combinations of various techniques can be used to prepare SP and NSP cottonseed isolates employing IMP. In the Lawhon and Manak patent (112) (Fig. 13), protein is extracted from defatted flour with water at pH 6.7, and pH is adjusted to approximately 4.0 to precipitate a nonstorage protein curd. After drying, the resulting product contains approximately 80% protein. The residue from the original extraction step is next extracted with sodium hydroxide (pH 9.5–10.0) to solubilize the storage protein. After the insoluble fraction has been removed by centrifugation, the extract is combined with the previously made NSP whey, adjusted to approximately pH 7.0, passed through a UP membrane to concentrate the protein solution, and then spray-dried to produce an SP isolate containing approximately 92% protein on a dry weight basis. The distribution of solids from the process (113) is shown in Figure 14. In accompanying research, it was found that NaOH and KOH were nearly as effective in extracting storage protein, but $Ca(OH)_2$ solubilized only about one-fifth as much protein as the other two hydroxides.

A more recent process and product patent by Lawhon (111) produces light-colored, bland glandless cottonseed isolates and concentrates. The principle of this process is to pass the alkali-extracted protein solution, after centrifuging, through a large pore (100,000 MW cutoff) membrane. The soluble components, including flavor- and color-causing compounds, permeate the membrane, alone or in combination with the smaller molecular weight protein molecules (part of the 2S fraction). The retentate, consisting primarily of 7S and 11S-type proteins, is then spray-dried to produce the protein isolate(s).

Techniques have been developed to increase the yields

of sellable products, improve product purity and increase membrane throughput. Economic analyses (104) show that membrane processing is economically feasible under certain conditions.

Next month: Part II—A Look at "Characteristics and Uses of Glandless Cottonseed for Protein Ingredients."

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