hybridization attempts between the soybean and the species in the subgenus *Glycine* as a means of producing a low linolenic acid soybean. However, the wild species germplasm might have other chemical or agronomic characteristics of use to the soybean breeder. An understanding of the variability that does exist between and among the wild *Glycine* species is required before they can be utilized in any breeding projects.

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♣ Effect of Ultrasonic Comminution on Liquid Classification of Cottonseed Protein and Gossypol Pigment Glands[1]

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ABSTRACT

Slurries of pin-milled full-fat and flaked, extracted cottonseed were ultrasonically comminuted in hexane and liquid-classified using laboratory differential settling techniques. Sonication of full fat cottonseed slurries increased the liquid classified protein fraction recovery from 25.9% (nonsonicated control) to over 60%, while the protein content of the fraction remained basically constant at 67%, and free gossypol increased slightly from 0.027 to 0.032%. Sonication of flaked, solvent extracted (fat free), slurried cottonseed yielded a 28% classified fraction containing 72% protein and 0.032% free gossypol. Although it was demonstrated on laboratory scale only, ultrasonic comminution may make the price of edible cottonseed protein concentrates produced from glanded seed (via a process such as the liquid cyclone process) competitive with other edible protein products.

INTRODUCTION

Wet disintegration of cottonseed, in conjunction with liquid classification into whole, toxic, gossypol-containing pigment gland and protein meal fractions, was intially investigated by Boatner and Hall in 1946 (1). They disintegrated cottonseed flakes in a specific gravity-adjusted mixture of chlorinated solvents and hexane in a Waring blender, and then removed the floating pigment glands. Vix et al. (2) also disintegrated flakes in a blender but restricted their investigations to a hexane medium in which pigment glands and coarse meal settled to the bottom, and a slow-settling, protein rich, gland free fraction was siphoned from the top. Gastrock et al. (3) capitalized on these distinctive settling characteristics and developed the first continuous wet classification process, the liquid cyclone process (LCP). Gardner et al. (4) and Gastrock (5) improved the LCP, but it has never been fully commercialized.

All past cottonseed classification research used conventional mechanical disintegration methods with blenders and pin and stone mills. Many researchers have used another mechanical disintegration method, sonication, as an aid in extracting vegetable oils (6-8) and protein (9-11), but information about effects of sonication on pigment gland rupture and protein classification is lacking. Ultrasonic disintegration of a slurry is achieved by applying electrical energy to a crystal, which changes shape in tune with the applied electrical field, creating pulsations or ultrasonic waves. The waves, consisting of alternate compressions and rarefactions (a phenomonon known as cavitation), cause formation and collapse of microscopic bubbles that can produce local pressure changes as high as 20,000 atm. These extremely high shearing pressures and shock waves produce the disintegration effect.

EXPERIMENTAL PROCEDURES

Materials

The 1977 crop, ginned, glanded cottonseed was obtained from oil mills located in 4 different, major cottonseed growing areas. The solvent used was Skellysolve B, a commercially available hexane high in *n*-hexane.

Methods

Hulling. All seed samples were hulled in pilot plant Carver equipment to yield whole and cracked meat fractions containing less than 3% hulls.

Flaking. Flakes ca. 0.30 mm (0.012 in.) thickness, were obtained by processing the hulled meat fractions without prior conditioning through Allis Chalmers flaking rolls. In preparing defatted flakes, both raw and dried flakes were batch-extracted 9 times with hexane. Each extraction consisted of portions of hexane equivalent to the flaked sample weight, and lasted 20 min.

[1] Presented at the AOCS Annual Meeting, 1981, New Orleans.

Drying. Whole and cracked meats were dried on a pilot-plant continuous-belt dryer using air at 82 C (180 F). Flaked cottonseed meats were dried in batches on trays in a forced-draft oven, also at 82 C.

Grinding. Dried meat samples were comminuted using a commercial 250 CW Alpine American Contraplex pin mill for differential settling tests (DST) and LCP runs. The mill side pin disc was operated at 7,300 rpm, and the door disc countercurrently at 2,575 rpm. For lower Rio Grande Valley seed, the speed on the mill side disc was reduced to 5,800 rpm to minimize gland damage in this high-gossypol-content seed.

Sonication. Hexane was added to 100.0 g of pin milled, full-fat meats to bring the total vol to 250 mL in a 500-mL glass beaker. The probe of a Braunsonic 1510 sonicator (B. Braun Instruments, San Francisco, CA) was inserted ca. 2.5 cm (1.0 in.) into the mixture and operated at maximal output power (400 W) for .5, 1, or 3 min. For extracted flake samples, hexane was added to 275 mL of solvent-damp flakes to bring the total vol to 400 mL in a 600-mL glass beaker. The nominal 275 mL volume of solvent-damp flakes was chosen because it approximates the solids contained in 100 g of pin-milled full-fat meats. The sonic probe was again inserted ca. 2.5 cm into the mixture and operated at maximal output power for 1, 3, or 5 min.

Differential settling test. The technique used for the DST was essentially as previously described by Hron (12), using 100 g of pin-milled meats directly, or sonicated slurry samples.

Liquid cyclone process. The LCP pilot plant data were obtained by processing 36-kg (80-lb) batches of pin-milled meats as previously described by Gardner et al. (4).

Analytical measurements. Moisture, nitrogen, and free and total gossypol were determined according to standard AOCS methods (13). All DST and LCP reported data were run in duplicate.

RESULTS AND DISCUSSION

DST data in Table I show that a .5-min sonication of pin milled, full-fat Texas High Plains cottonseed hexane slurries increased recovery of the concentrated protein fraction from 25.9% (nonsonicated control) to 61.8%, (a 140% increase), with only slight changes in protein content. The change in free gossypol from 0.027 to 0.032% is within the accuracy of ± 0.005% claimed by Pons for the method (14). When compared to the LCP, the .5-min sonication, using identical pin-milled meats, increased protein concentrate recovery 27%, with little difference in free gossypol and protein contents. Similar results were found with Mississippi Delta seed. Extending sonication to 3 min for the Mississippi Delta seed was impractical because it increased recovery only 2.5% over the 1-min treatment while increasing free gossypol content to 0.069%.

Data for lower Rio Grande Valley seed are reported in Table I to show the effect of sonication on a seed high in free gossypol. (In a recent unpublished survey by the authors, seed samples obtained from oil mills located in the 7 major U.S. cottonseed-growing areas were found to have free gossypol contents ranging from 0.768 to 1.32%; the highest amount was from Lower Rio Grande Valley seed.) Although the free gossypol content of the DST and LCP samples obtained from this seed is above the FDA permitted level of 0.045% (15), sonication of a milled-meat slurry sample resulted in doubling the recovery rate with only a small increase in free gossypol content.

Because direct sonication (bypassing the pin-milling step) of these same meats resulted in recoveries of only 5%, it was discontinued. Further investigations of direct sonication, using dried, solvent-extracted, Lower Rio Grande Valley flakes, produced DST recoveries of 12.4 and 26.9%, respectively, edible free gossypol levels were 0.024% for both (Table II). Similar results were obtained with Texas High Plains and Mississippi Delta flakes. Although identical sonication treatments of all untreated (not dried), extracted flake samples yielded higher DST recoveries than dried flake samples, excessive gland rupture occurred and free gossypol was found to be above levels permitted by FDA. Apparently, as was previously reported (4,11), seed moisture and absolute gossypol content remain key factors in obtaining edible protein concentrates from glanded cottonseed. Protein contents reported in Table II are generally

TABLE I

Effects of Sonicating Pin-Milled, Full-Fat-Meat/Hexane Slurries

Meats source and treatment[a]	Concentrate recovery (%)[b]	Free gossypol (%)[c]	Total gossypol (%)[c]	Protein (%)[d]
Texas High Plains:				
Control	25.9	0.027	0.078	67.4
Sonicated .5 min	61.8	0.032	—	66.8
Sonicated 1 min	63.5	0.033	0.137	67.9
Pilot-plant LCP	48.7[e]	0.027	0.081	64.7
Mississippi Delta:				
Control	21.2	0.035	0.134	72.0
Sonicated .5 min	52.5	0.043	0.137	70.4
Sonicated 1 min	54.3	0.041	0.134	73.5
Sonicated 3 min	56.8	0.069	0.240	68.7
Pilot-plant LCP	42.8[e]	0.043	0.184	68.9
Lower Rio Grande Valley:				
Control	13.0	0.050	0.110	64.3
Sonicated .5 min	26.4	0.057	0.157	67.9
Pilot-plant LCP	39.2[e]	0.081	0.226	64.1

[a]Meats contain <3% hulls and <2% moisture.

[b]"Overs" weight-percent fraction from differentially settled slurry samples.

[c]As-is basis.

[d]Moisture-/and oil-free basis, N X 6.25.

[e]Represents "overs" fraction from pilot-plant LCP.

TABLE II

Effects of Sonicating Solvent-Extracted-Flake/Hexane Slurries

Seed source and treatment	Sonication time (min)	Concentrate recovery (%)[a]	Free gossypol (%)[b]	Total gossypol (%)[b]	Protein (%)[c]
Texas High Plains:					
Untreated, extracted flakes[d]	1	25.7	0.061	0.164	72.7
	3	29.0	0.066	0.200	67.6
Dried, extracted flakes[e]	1	17.6	0.025	0.056	73.1
	3	28.0	0.032	0.076	72.1
Mississippi Delta:					
Untreated, extracted flakes[d]	5	29.0	0.081	0.321	72.1
Dried, extracted flakes[e]	5	21.5	0.036	0.127	70.9
Lower Rio Grande Valley:					
Untreated, extracted flakes[d]	1	17.1	0.076	0.188	70.7
Dried, extracted flakes[e]	1	12.4	0.024	0.061	69.6
	5	26.9	0.024	0.076	69.5

[a]"Overs" weight-percent fraction from differentially settled slurry samples.
[b]As-is basis.
[c]Moisture-/and oil-free basis, N × 6.25.
[d]Solvent-extracted flakes obtained from meats containing <3% hulls and >7% moisture.
[e]Flakes dried to <2% moisture prior to extraction.

equal to those in Table I for sonicated slurry samples, and are equal to or slightly higher than those reported for both the nonsonicated LCP and control samples, indicating that sonication can assist in obtaining higher recoveries and also in protein classification.

Data in Tables I and II were for meats containing 3% hulls. In order to simulate oil-mill processing, 1.6 kg (3.6 lb) of hulls were added back to 6.4 kg (15 lb) of full-fat meats prior to flaking to produce the equivalent of a 43%-protein extracted meal. Table III compares effects of sonicating extracted flake slurries with and without addition of hulls. In all cases, both the untreated and the 43%-protein-equivalent extracted flakes produced lower recoveries and higher free gossypol levels. The exceptionally high free gossypol content of 0.060% for the untreated, 43%-protein flakes sonicated 5 min was probably due to both moisture and the excess hulls present. Gardner et al. (16) also reported higher free gossypol when they processed pin-milled, full-fat meats containing over 3% hulls through the LCP. LCP and DST data for pin-milled, full-fat meats from Central East Texas are included in Table III to show that sonication can increase the protein content of a bench-top liquid-classified sample to that of a true protein concentrate (+70%).

The ability to produce an edible protein concentrate by sonicating and liquid classifying an oil-mill mare suggests a potential process in which ginned seed is delintered, hulled and flaked as is normally done; contrary to normal operations, conditioning by drying the flakes to ca. 2% moisture would be done after flaking (Fig. 1). Such a change is necessitated by the extreme sensitivity pigment glands have shown to moisture (17). Yatsu et al. (18) found that moisture ruptures glands and, more importantly, results in dispersion of minute pigment spherules contained within the

TABLE III

Effect of Moisture and Hull Content on Sonication of Extracted-Flake/Hexane Slurries

Sample description[a]	Sonication time (min)	Concentrate recovery (%)[b]	Free gossypol (%)[c]	Protein (%)[d]
Untreated, extracted flakes[e]	1	14.6	0.031	72.1
	5	32.2	0.032	71.3
Dried, extracted flakes[f]	1	8.9	0.013	72.7
	5	23.8	0.014	72.1
Untreated, 43% protein, extracted flakes[g]	1	9.9	0.038	73.7
	5	25.5	0.060	71.2
Dried, 43% protein, extracted flakes[h]	1	5.8	0.017	70.6
	5	13.3	0.018	69.7
Full-fat pin-milled	–	23.1	0.033	66.3
LCP	–	45.1[i]	0.030	66.1

[a]Central east Texas seed.
[b]"Overs" weight-percent fraction from differential settling test.
[c]As-is basis.
[d]Moisture-/and oil-free basis, N × 6.25.
[e]Solvent-extracted flakes obtained from meats containing 3% hulls and 8.2% moisture.
[f]Flakes dried to 1.3% moisture prior to extraction.
[g]Same as e, except hulls added prior to flaking to reduce protein content to ca. 43%.
[h]Same as f, except hulls added prior to flaking to reduce protein content to ca. 43%.
[i]Represents "overs" fraction from pilot plant LCP.

glands. These spherules are only 0.1-1.5 μ in diameter, and, once released from their water-soluble matrix, cannot be removed by normal liquid classification techniques.

Oil mills purposely capitalize on the sensitivity of glands to water. Routinely, heat and moisture are used to maximize oil extraction and to fix or bind the released pigments by reacting them with the protein fraction. This binding prevents the pigments from entering the oil fraction during extraction and causing color problems during refining. In our proposed process, the conditioned (dried) flakes are extracted by conventional solvent extraction equipment. The refined oil produced is expected to be of prime quality as a result of the previous drying step and minimal gland rupture. The drained, extracted flakes (marc) are reslurried with hexane and screened to separate proteinaceous material from excess hulls initially needed for efficient extraction. The slurry is then milled with an inline ultrasonic mixer (such as that made by Lewis Corp., Oxford, CT), and fed to a liquid cyclone for classification into overflow and underflow fractions. The overflow slurry fraction contains the protein concentrate, and can be concentrated either before being filtered on a rotary vacuum drum dryer or directly drum dried, desolventized, stripped, milled and packaged. The opportunity to drum-dry the slurry concentrate is significant because it could eliminate the technical problems in filtration and desolventization associated with the original LCP. The underflow slurry could be added to the screened hull fraction, desolventized, packaged and used for animal feed. It is notable that the amount of marc processed through the sonicator and liquid cyclone steps can be treated as a sidestream, and completely controlled to yield the amount of protein concentrate desired.

Although demonstrated on a laboratory scale, ultrasonic comminution has the potential to dramatically improve the economics of the existing LCP by increasing recoveries to over 60%, thus making the process more appealing to industry. Alternately, it could be incorporated into standard oil-mill processing operations by modification of the conditioning step to produce protein concentrate from glanded cottonseed economically and at any desired rate or quantity.

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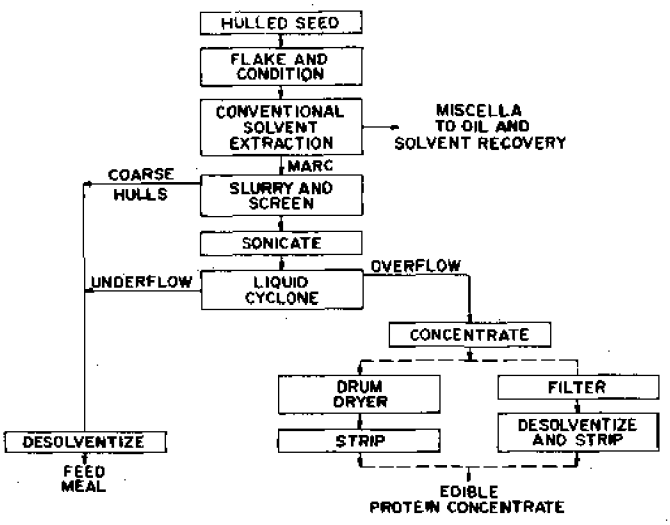

FIG. 1. Flowsheet for production of cottonseed protein concentrate from oil mill marc.

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