Groups on Physical Properties of Tofu-gel". *Agric. Biol. Chem.* **1971**, *35*, 890–898.
Saio, K.; Terashima, M.; Watanabe, T. "Food Use of Soybean 7S and 11S Proteins. Heat Denaturation of Soybean Proteins at High Temperature." *J. Food Sci.* **1975a**, *40*, 537–540.
Saio, K.; Terashima, M.; Watanabe, T. "Food Use of Soybean 7S and 11S Proteins. Changes in Basic Groups of Soybean Proteins by High Temperature Heating". *J. Food Sci.* **1975b**, *40*, 541–544.
Shimada, K.; Matsushita, S. "Relationship between Thermocoagulation of Proteins and Amino Acid Compositions". *J. Agric. Food Chem.* **1980**, *28*, 413–417.
Simard, C.; Boulet, M. "Distribution des Groupements Sulfhydryles et des Ponts Disulfure dans les Fractions de Protéines de Soja, de Féverole et de Colza". *Can. Inst. Food Sci. Technol. J.* **1978**, *11*, 45–49
Utsumi, S.; Kinsella, J. E. "Structure–Function Relationships in Food Proteins: Subunit Interactions in Heat-Induced Gelation of 7S, 11S, and Soy Isolate Proteins". *J. Agric. Food Chem.* **1985a**, *33*, 297–303.
Utsumi, S.; Kinsella, J. E. "Forces Involved in Soy Protein Gelation: Effects of Various Reagents on the Formation, Hardness and Solubility of Heat-Induced Gels Made from 7S, 11S, and Soy Isolate". *J. Food Sci.* **1985b**, *50*, 1278–1282.
Voutsinas, L. P.; Nakai, S.; Harwalkar, V. R. "Relationships between Protein Hydrophobicity and Thermal Functional Properties of Food Proteins". *Can. Inst. Food Sci. Technol. J.* **1983**, *16*, 185–190.
Watanabe, K.; Klostermeyer, H. "Heat-Induced Changes in Sulphydryl and Disulphide Levels of β-Lactoglobulin A and the Formation of Polymers". *J. Dairy. Res.* **1976**, *43*, 411–418.
Yamagishi, T.; Takaya, M.; Ebina, F.; Yamauchi, F. "Effect of Heating Temperature on Sulfhydryl and Disulfide Contents and State of Aromatic Amino Acid Residues in Soybean Protein". *Agric. Biol. Chem.* **1984**, *48*, 537–539.

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Production Study of a Low-Gossypol Protein Product from Cottonseed Meal[1]

Qiang Zhuge, Elieser S. Posner,* and Charles W. Deyoe[2]

A process was developed to reduce the level of free gossypol in cottonseed meal from either an oil extraction or an expelling process. The level of free gossypol reached was below the maximum allowable in edible meal according to federal regulations (0.045%). The process included extrusion, drying, fine grinding, and air classification. The final coarse product, with particle size larger than 84 μm, and a yield ranging from 67 to 80% by weight of the original raw material, had a protein content of 36.6–45.2%.

Historically, man's interest in cottonseed was as a source of oil. However, problems of malnutrition have been the driving force for research and industry to develop additional protein sources for human consumption. Worldwide, about 5.3×10^7 metric tons of meal is available yearly from glanded cottonseed, i.e. about 1.7×10^7 metric tons of protein that can be potentially used for human consumption (FAO, 1984).

Cottonseed proteins are rarely utilized as a source of edible food by monogastric animals, because of the presence of pigment glands that contain toxic gossypol. Gossypol is a highly reactive yellow polyphenolic binaphtaldehyde. In the metabolically active or free form, it has adverse physiological effects when ingested by monogastric animals (Berardi et al., 1969). Food and Drug Administration (FDA) regulations specifically require that a protein food product made from cottonseed have a content of less than 0.045% free gossypol to be considered edible (*Fed. Reg.*, 1974).

Cottonseed kernels contain about 7% moisture, 30% crude protein, 30% oil, 24% nitrogen-free extract, 4.8 crude fiber, and 4.4% ash (Altschul et al., 1958). Geddes (1951) notes that cottonseed flour is high in vitamins, such as thiamin, riboflavin, and niacin. The protein of cottonseed has been shown to consist of globulin, a pentose-containing protein, and glutelin. Its amino acid composition was compiled by Johns and Jones (1916).

Different approaches were taken in the past in processing the defatted cottonseed meal to a highly concentrated, edible, protein product. Smith (1970) describes the preparation of a glandless cottonseed flour by direct solvent extraction. Air classification can also be used to produce a protein concentrate, as described by Martinez et al. (1967). In this process, the protein bodies are separated from clusters of unruptured cells and cell wall fragments with adhering residual cytoplasm. The process provides two products: one high in nitrogen, for food; and one low in nitrogen but high in cell wall particles, for food or feed.

Kadan et al. (1980) describe a process comprised of five steps by which it is possible to produce from glanded cottonseed a high-protein flour essentially without free gossypol and suitable for human consumption: (a) flaking dehulled glanded cottonseed having moisture contents from 5 to 12%; (b) solvent extracting the cottonseed flakes to reduce fat content; (c) desolvenizing the solvent-extracted flakes; (d) milling the desolvenized flakes into a flour; (e) air classifying the flour to produce a coarse fraction and a fine fraction.

All the above procedures are concerned merely with raw materials produced by oil extraction. The objective of this study was to develop a production procedure of a high-protein product low in free gossypol from a cottonseed meal. The cottonseed meal used could come from an oil-extraction or an oil-expelling process.

MATERIALS AND METHODS

A series of experiments were performed using cottonseed meal that originated from three different oil-removing processes. One batch of cottonseed meal was obtained

Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas 66506.

[1] Contribution 86-244-J.

[2] Research assistant, Associate Professor, Professor and Department Head, respectively.

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Table I. Analytical Results of Three Cottonseed Meals after Air-Classifying Process

	protein,[a] %	ash,[a] %	fat,[a] %	particle size, μm	free gossypol, %	total gossypol	yield, %
expelled raw	36.2	6.1	5.0	68.0	0.072	0.93	
expelled coarse	36.9	5.5	3.4	85.0	0.041	0.74	67
expelled fine	44.6	6.9	5.5	29.0	0.058	1.05	33
Chinese raw	35.2	5.6	8.2	124.0	0.110	0.94	
Chinese coarse	36.6	5.6	5.5	91.0	0.043	0.62	67
Chinese fine	43.0	6.8	9.1	48.0	0.101	1.04	33
extracted raw	44.1	6.3	3.0	68.0	0.278	0.95	
extracted coarse	45.2	6.9	1.9	84.0	0.029	0.78	80
extracted fine	53.0	7.4	3.2	45.0	0.040	0.96	20

[a] Moisture-free basis.

from a solvent-extracting process and the second from an expelling process, both from U.S. manufacturers. The third was a cottonseed meal that originated in Mainland China where it was obtained as a residue from an expelling process. The approximate composition of starting material is indicated in Table I.

The optimal procedure consisted of first using an Extruder Cooker Model X-20 (Wenger Co., Sabetha, KS) for the necessary heat treatment of the raw material. In the case of extracted cottonseed meal a sieving stage was necessary to separate the hulls before extrusion. Temperatures during the extrusion process were 120 °C for the solvent-extracted meal and 150 °C for the expelled meals. The extruded products were cooled and dried, on a batch system. After drying, the products were finely ground on a pin mill, Model 160 Z, operating at 20140 fpm tip speed (Alpine American Corp., Natick, MA).

Different combinations of air classification equipment were tested to produce a product containing a level less than 0.045% free gossypol. To separate the free gossypol from the finely ground product, the Raymond laboratory model air classifier (Combustion Engineering, Abilene, KS) was used.

The Raymond air classifier (two-whizzer type) running at 3600 rpm was set differently for the expelled and extracted meals to achieve optimum results. For the expelled meals, a $7^7/_{16}$-in.-diameter fan plate was used. The upper whizzer had 12 blades, and the lower had 8. For the extracted meal, optimum results were achieved with use of the same fan but 16 and 12 blades on the upper and lower whizzers, respectively. Figure 1 shows the general schematic flow used in the process. Yields are calculated as weight of products divided by the weight of the starting unclassified flours multiplied by 100.

Free-gossypol levels were determined according to Method No. Ba 7-58 of the AOCS (1973). Moisture content was determined according to Method No. 44-40 of the AACC (1983), protein by Kjeldahl Method No. 46-10 of the AACC, fat by Method No. 30-20 of the AACC (1983), and ash by Method No. 08-01 of the AACC (1983). Amino acid analyses of edible products were performed on a Dionex D300 (Sunnyvale, CA) amino acid analyzer after hydrolysis with *p*-toluenesulfonic acid (Liu and Chang, 1971).

The particle size of the different products was determined (Table I) on the Microtrac particle analyzer (Leeds & Northrup Instruments, St. Petersburg, FL).

RESULTS AND DISCUSSION

Table I indicates analytical results of the various products. The cottonseed meal was processed with an extruder, because it is an energy-efficient and convenient method. This can be compared to other suggested processes where the meal is heated at temperatures from 82 to 150 °C for various lengths of time. Extrusion is an instantaneous process that can be incorporated into a continuous line of production.

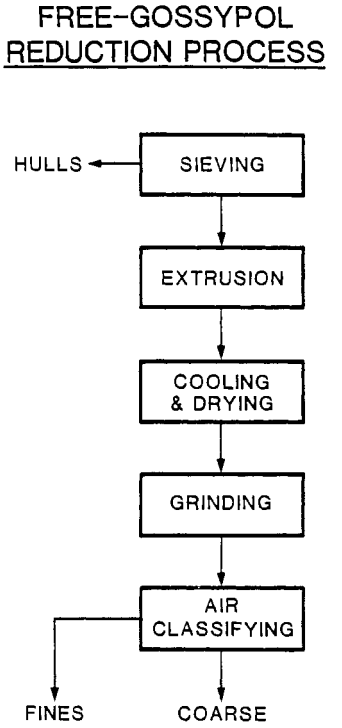

Figure 1. Flow diagram of cottonseed protein extraction process.

Table II. Amino Acid Composition (%) of Low Free-Gossypol Fractions from Different Origins Processed with a Raymond Air Classifier

amino acid	expelled, %	Chinese, %	extr, %
aspartic acid	10.50	10.28	10.28
threonine	3.46	3.66	3.45
serine	5.27	5.39	5.43
glutamic acid	20.56	18.86	19.42
proline	4.05	3.98	3.71
glycine	4.60	4.46	4.48
alanine	4.58	4.46	4.48
half-cystine	1.61	2.43	2.48
valine	3.53	3.70	4.55
methionine	1.84	2.46	2.20
isoleucine	2.36	2.81	2.45
leucine	5.81	5.56	5.40
tyrosine	3.84	4.32	4.05
phenylalanine	5.94	6.48	6.31
histidine	4.95	4.72	5.29
lysine	4.18	4.50	4.20
ammonia	2.14	1.91	1.72
arginine	10.78	10.01	9.78

The products with the low free-gossypol content from the process can be used for human consumption. The fine fraction with higher free-gossypol content and higher protein content (Table I) can be used for rumenant feed.

Percent yield of product from the different raw materials is indicated.

In Table II, grams of amino acids/100 g of protein expressed in percents are shown for the three different low free-gossypol products produced with the Raymond air classifier. Values are corrected to 100% recovery protein basis. No significant difference could be determined among the meals originated from the different sources. However, compared to wheat flour shown by Waggle et al. (1967), the nutritional attribution of low free-gossypol cottonseed meal is much higher.

Because of the higher fat content in the expelled meal, a larger number of coarse particles remained in the finely ground product. In general, the whizzer-type air classifier has a grinding effect on classified stock. In this case, it is possible that this grinding effect eliminated the coarser particles in the meal originating from the expelling process and increased the yield of low free-gossypol product.

On the basis of these findings, it might be suggested that particles below 50 μm contain higher levels of free gossypol. Air classifiers should be adjusted to have the cut point at this particle size.

The whole process can be assembled as a continuous one, starting with sieving out the hulls (if present in the raw meal), extrusion, cooling and drying, fine grinding, and air classification.

A process that can be adapted commercially should have adequate technical-processing tolerances at the various stages to handle variation in raw material. This would ensure final product quality to meet specifications. The results indicate that, even with variability in raw material and its origin, the free gossypol can be reduced efficiently to food standards.

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Registry No. Gossypol, 303-45-7.

LITERATURE CITED

American Association of Cereal Chemists *Approved Methods*; AACC: St. Paul, MN, 1983.

Association of Official Agricultural Chemists *Official Methods of Analysis*; AOCS: Washington, DC, 1973.

Altschul, A. M.; Lyman, C. M; Thurber, F. H. "Cottonseed Meal". In *Processed Plant Protein Foodstuff*; Altschul, A. M., Ed.; Academic: New York, 1958; pp 469–534.

Berardi, L. C.; Goldblatt, L. A. "Gossypol". In *Toxic Constituents of Plant Foodstuffs*; Liener, I. E., Ed; Academic: New York, 1969; pp 211–266.

Food and Agriculture Organization (FAO) of the United Nations *Production Yearbook*, 1984.

Fed. Reg. **1974**, *39* (No. 177), 32735.

Geddes, W. F. "Cereal Grains". In *The Chemistry and Technology of Food* and *Food Products*, 2nd ed.; Jacobs, M. B., Ed.; Interscience: New York, 1951; 3 Vol.

Johns, C. O.; Jones, D. B. *J. Biol. Chem.* **1916**, *28*, 77. Cited by Geddes.

Kadan, R. S.; Freeman, D. W.; Spadaro, J. J.; Ziegler, G. M. "Process for Producing a Low Gossypol Protein Product from Glanded Cottonseed". U.S. Patent No. 4 201 709, 1980.

Liu, T. Y.; Chang, Y. H. "Hydrolysis of Proteins with *p*-Toluenesulfonic Acid". *J. Biol. Chem.* **1971**, *246*(9), 2842.

Martinez, W. H.; Berardi, L. C.; Pfeifer, V. F.; Crovetto, A. J. "The Production of Protein Concentrate by Air Classification of Defatted Cottonseed Flour". *J. Am. Oil. Chem. Soc.* **1967**, *44*, 139A.

Smith, K. J. "Glandless Cottonseed. What Is Its Future in the Cotton Industry's Markets?" *Oil Mill Gazz.* **1970**, *74*(9), 20, 22–23.

Waggle, D. H.; Lambert, M. A.; Miller, G. D.; Farrell, E. P.; Deyoe, C. W. "Extensive Analysis of Flours and Millfeeds Made from Nine Different Wheat Mixes". *Cereal Chem.* **1967**, *44*(1), 48–60.

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Study of Thermal Denaturation of Oat Globulin by Ultraviolet and Fluorescence Spectrophotometry[1]

Ching-Yung Ma* and Ventktesh R. Harwalkar

Thermal denaturation of dilute (<0.05%) oat globulin solutions at high ionic strength was studied by ultraviolet (UV) and fluorescence spectrophotometry. Ultraviolet spectra show a significant red shift of absorption maximum when the protein was heated at 110 °C. Second-derivative and difference-derivative spectra suggest exposure of tryptophan and tyrosine residues in the heated samples. Fluorescence emission spectra show a significant blue shift, indicating protein unfolding. When 1% oat globulin was heat aggregated and fractionated into soluble and insoluble fractions, UV and fluorescence spectra indicate no marked protein unfolding in the soluble fraction but extensive denaturation in the insoluble aggregates. The soluble fraction had significantly higher surface hydrophobicity than the soluble fraction and the unheated protein.

Heat treatments such as drying, roasting, and cooking are routinely used for the processing of oats and other cereal grains. These processes could cause substantial protein denaturation, which is critical to functionality such as gelation, emulsification, and foaming (Kinsella, 1976). Thermal denaturation (or unfolding) of proteins involves conformational changes from the native structure and can be monitored by spectrophotometric techniques such as circular dichroism (CD), ultraviolet (UV), and fluorescence spectroscopy. Spectroscopic studies have been conducted on soy 11S globulin (glycinin) heated in the presence of *N*-ethylmaleimide (NEM), which prevented protein aggregation by blocking sulfhydryl residues and hindering

Food Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

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