ester, 27174-07-8; *cis*-feruloyl tartaric acid ester, 84518-78-5; *trans*-feruloyl tartaric acid ester, 74282-22-7; isoquercitrin, 482-35-9; catechin, 154-23-4; epicatechin, 490-46-0; caffeoyl tartaric acid ester, 1234-09-9; *p*-coumaroyl tartaric acid ester, 69222-59-9; feruloyl tartaric acid ester, 1044-65-1.

LITERATURE CITED

Albach, R. F.; Kepner, R. E.; Webb, A. D. *J. Food Sci.* **1965**, *30*, 69.
Arnold, R. A.; Noble, A. C.; Singleton, V. L. *J. Agric. Food Chem.* **1980**, *28*, 675.
Baranowski, J. D.; Nagel, C. W. *Am. J. Enol. Vitic.* **1981**, *21*, 5.
Barz, W. *Physiol. Veg.* **1977**, *15*, 261
Barz, W.; Hoessel, W. "Biochemistry of Plant Phenolics"; Plenum Press: New York, 1979; Vol. 12.
De Villiers, J. P. *Am. J. Enol. Vitic.* **1961**, *12*, 25.
Dumazert, G.; Margulis, H.; Montreau, F. R. *Ann. Technol. Agric.* **1973**, *22*, 137.
Duteau, J.; Guilloux M.; Glories, Y.; Seguin, G. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1981**, *292*, 965.
Fleuriet, A. *Physiol. Veg.* **1977**, *15*, 375.
Fleuriet, A.; Macheix, J. J. *J. Chromatogr.* **1972**, *74*, 339.
Genevois, L. *Bull. Soc. Chim. Fr.* **1952**, *19*, 241.
Hartley, R. D.; Buchan, H. *J. Chromatogr.* **1979**, *180*, 139.
Hennig, K.; Burkhardt, R. *Am. J. Enol. Vitic.* **1960**, *11*, 64.
Hrazdina, G.; Franzese, A. J. *Phytochemistry* **1974**, *13*, 225.
Kahnt, G. *Biol. Zentralbl.* **1966**, *85*, 546.
Macheix, J. J. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1971**, *272*, 1097.
Macheix, J. J. *Thèse Doct. Etat Sci. Nat., Paris* **1974a**.
Macheix, J. J. *Physiol. Veg.* **1974b**, *12*, 25.
Nagel, C. W.; Baranowski, J. D.; Wulf, L. W.; Powers, J. R. *Am. J. Enol. Vitic.* **1979**, *30*, 198.
Okamura, S.; Watanabe, M. *Nippon Nogei Kagaku Kaishi* **1979**, *53*, 165.
Okamura, S.; Watanabe, M. *Agric. Biol. Chem.* **1981**, *45*, 2963.
Ong, B. Y.; Nagel, C. W. *J. Chromatogr.* **1978a**, *157*, 345.
Ong, B. Y.; Nagel, C. W. *Am. J. Enol. Vitic.* **1978b**, *29*, 276.
Ribereau-Gayon, J.; Peynaud, E.; Ribereau-Gayon, P.; Sudraud, P. "Traité d'Oenologie—Sciences et Techniques du vin—Analyse et contrôle des vins"; Dunod: Paris, 1972; Vol. 1.
Ribereau-Gayon, P. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1963**, *256*, 4108.
Ribereau-Gayon, P. *Ann. Physiol. Veg.* **1964**, *6*, 119.
Ribereau-Gayon, P. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1965**, *260*, 341.
Ribereau-Gayon, P. *Connaiss. Vigne Vin* **1972**, *6*, 161.
Romeyer, F. *Diplôme d'Etudes Approfondies, Montpellier, Sci. Agron.* **1981**.
Sapis, J. C.; Macheix, J. J.; Cordonnier, R. E. *J. Agric. Food Chem.* **1983a**, preceding paper in this issue.
Sapis, J. C.; Macheix, J. J.; Cordonnier, R. E. *Am. J. Enol. Vitic.* **1983b**, in press.
Scarpati, M. L.; d'Amico, A. *Ric. Sci.* **1960**, *30*, 1746.
Scarpati, M. L.; Oriente, G. *Tetrahedron* **1958**, *4*, 43.
Singleton, V. L. *Am. J. Enol. Vitic.* **1961**, *12*, 1.
Singleton, V. L.; Esau, P. *Adv. Food Res.* **1969**, *17*, 282.
Singleton, V. L.; Noble, A. C. in "Phenolic, sulfur and nitrogen compounds in food flavors"; Charalambors and Katz: Washington, DC, 1976; Vol. 26.
Singleton, V. L.; Timberlake, C. F.; Lea, A. G. H. *J. Sci. Food Agric.* **1978**, *29*, 403.
Sondheimer, E. *Arch. Biochem. Biophys.* **1958**, *74*, 131.
Suzuki, Y.; Shimada, M.; Tadera, K.; Kawai, F.; Mitsuda, H. *Agric. Biol. Chem.* **1970**, *34*, 511.
Tadera, K.; Suzuki, Y.; Kawai, F.; Mitsuda, H. *Agric. Biol. Chem.* **1970**, *34*, 517.
Weurman, C.; de Rooij, C. *Chem. Ind. (London)* **1958**, *16*, 72.
Wulf, L.; Nagel, C. W. *J. Chromatogr.* **1976**, *116*, 271.

Received for review June 9, 1982. Accepted December 8, 1982. This work was performed as a part of a Programmed Thematic Action "Processing, storage and utilization of concentrated grape juices" conducted by the Institut National de la Recherche Agronomique.

Influence of Processing on the Pigmentation of Wild Rice Grain

Steven J. Schwartz, Joachim H. von Elbe, and Robert C. Lindsay*

Chlorophylls, pheophytins, and related derivatives in wild rice grain were analyzed by high-performance liquid chromatography (HPLC) on a reverse-phase column. High-temperature drying (>80 °C) or parching converted chlorophylls to pheophytins, pyropheophytins, and other derivatives which along with melanins contribute to the black color of this grain. HPLC was shown to be a useful technique for monitoring chlorophyll pigment alterations during the processing of wild rice.

Wild rice (*Zizania aquatica*) is an aquatic grass growing in the Upper Great Lakes region of the United States and Canada and is becoming increasingly used as a cereal grain component of many foods (Moore, 1977). This grain is unique with respect to the degree of surface pigmentation of the kernels, and the black appearance of mature seeds is a significant contributor to product identity. Withycombe (1974) studied the pigment systems of wild rice and found evidence of anthocyanin, carotenoid, tannin, and chlorophyll pigments in mature grain. Gutek et al. (1981) studied the chemistry and inheritance of the red pigments in the leaf sheath and staminate florets and found that the production of two anthocyanins was controlled by a dominant gene. However, only polyphenol and chlorophyll pigments systems appear influential in wild rice color (Withycombe, 1974), and Khoo and Wolf (1982) have recently described the location of these pigments in the structure of wild rice kernels.

Processing procedures for wild rice usually include a storage period during which a fermentation occurs, parching or drying from a moisture content of 35–50% to 7–12%, hulling, scarification of kernels, and cleaning (Lindsay et al., 1975). The heat encountered during parching should be sufficient to alter chlorophyll pigments and affect the appearance of kernels. Recently, Schwartz et al. (1981) developed a high-performance liquid chromatography method to monitor chlorophylls and their derivatives in processed foods. In this report, we present results of investigations on the application of this HPLC method to study wild rice chlorophyll pigments and relate this information to the pigment systems that characterize the appearance of wild rice grain.

Department of Food Science, University of Wisconsin—Madison, Madison, Wisconsin 53706.

0021-8561/83/1431-0349$01.50/0

MATERIALS AND METHODS

Green wild rice was obtained from commercial sources.

Processing Conditions. Samples were stored for fermentation at ambient temperature from 0 to 5 weeks. Parching of each sample was accomplished in a rotating perforated drum. Air at 120 °C was passed through the drum for high-temperature drying and at less than 60 °C for low-temperature drying. Samples were hulled and scarified when applicable in small laboratory equipment (Lindsay et al., 1975).

Color Analysis. Wild rice samples which had been scarified were evaluated for color. Color was determined by using a Hunter Color and Color-Difference meter (Model D25). The standard plate for color comparison was the Hunter laboratory standard D25-1301. This is a gray plate with the following Hunter color values: $L = 22.7$, $a = 0.1$, and $b = -0.8$. For measurement of sample color, a 5.75-cm (internal diameter) plastic cup was filled to a depth greater than 1 cm. It had been experimentally determined that at depths greater than 1 cm the color readings were not significantly affected. After determination of the L, a, and b values, the sample was poured from the measuring cup into another cup and then back into the measuring cup again. The color readings were then taken again. This procedure was repeated so that the color values of each sample were measured 6 times.

Preparation of Wild Rice Samples for Pigment Analysis. Twenty-five grams from each sample lot was ground twice in a Wiley mill by using 20 and 40-mesh sieves. Fifty milliliters of acetone was added to 20.0 g of each ground sample, and the mixture was stirred for 10 min and filtered through Whatman No. 1 paper. The residue was washed 3 times with 50 mL of acetone, and the combined extracts were quantitatively transferred to a 250-mL volumetric flask and brought to volume. One hundred milliliters was removed and placed in a 250-mL aluminum foil covered round-bottom flask to prevent exposure to light, and the solvent was removed under reduced pressure. Five milliliters of acetone was added to dissolve the dry residue. The concentrated extract was filtered through a C_{18} Sep-PAK (Waters Associates, Milford, MA) prior to HPLC analysis.

HPLC Analysis. A modified method of Schwartz et al. (1981) was employed to separate and monitor the chlorophyll pigments and derivatives. The gradient elution solvent mixture was eliminated and an isocratic solvent mixture was substituted consisting of ethyl acetate–methanol–water (50:37.5:12.5 v/v/v). Twenty microliters of each pigment extract was injected and separated on a μBondapak C_{18} column (Waters Associates). Eluants were monitored at 654 nm to selectively detect chlorophylls and related pigments.

RESULTS AND DISCUSSION

Figure 1 is a HPLC chromatogram of the chlorophylls and related pigments from a typical fermented and parched wild rice sample. Pheophytins a and b (peaks 5 and 3) are prevalent in these samples as well as some unidentified compounds which absorb light strongly at 654 nm. Pyropheophytin a (peak 6) is also indicated in this sample extract. Fermentation and heat applied during parching would be expected to contribute to the conversion of chlorophylls to pheophytins and pyropheophytins.

Figure 2 is a HPLC chromatogram of the chlorophylls and related pigments from an unfermented ambient air dried sample. The chromatogram indicates the retention of chlorophylls a and b (peaks 2 and 1) and the formation of some pheophytin a (peak 5). Comparison of this sample with other unfermented samples which were hot air dried

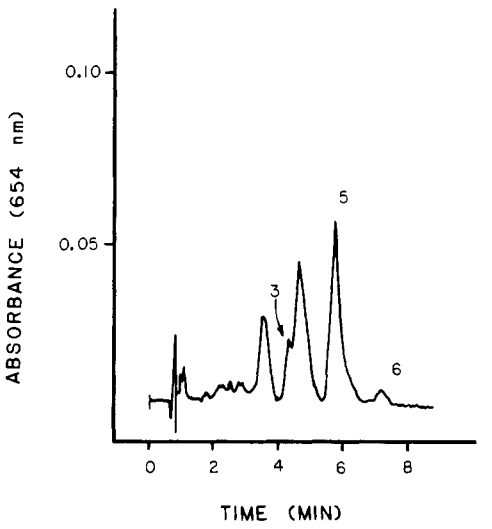

Figure 1. HPLC chromatogram of the chlorophylls and derivatives from a fermented parched wild rice sample. Peak 3 = pheophytin b; peak 5 = pheophytin a; peak 6 = pyropheophytin a.

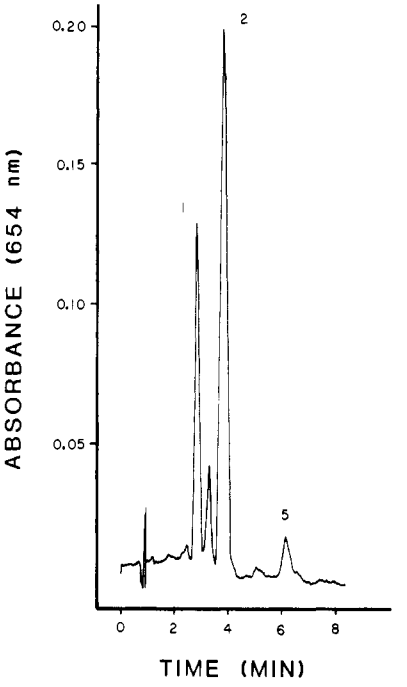

Figure 2. HPLC chromatogram of the chlorophylls and related derivatives from an ambient air dried wild rice sample. Peak 1 = chlorophyll b; peak 2 = chlorophyll a; peak 5 = pheophytin a.

(Figure 3) indicates that destruction of the majority of chlorophylls occurred during the parching treatment. Pheophytins a and b (peaks 5 and 3) were noted in all heat-treated samples. Furthermore, pyropheophytins a and b (peaks 6 and 4) were found in all parched samples, and their contents increased with the severity of heat treatments.

Schwartz et al. (1981) identified pyropheophytins a and b in canned spinach, and formation of the "pyro" derivatives was the result of the high temperatures utilized during the sterilization process. Heat treatments employed during the parching apparently cause similar degradations in wild rice. Pheophytins and pyropheophytins exhibit identical visible light absorption characteristics and therefore would both contribute to the olive-brown pigmentation of wild rice. Other unidentified pigments were also noted in the HPLC chromatograms of parched wild

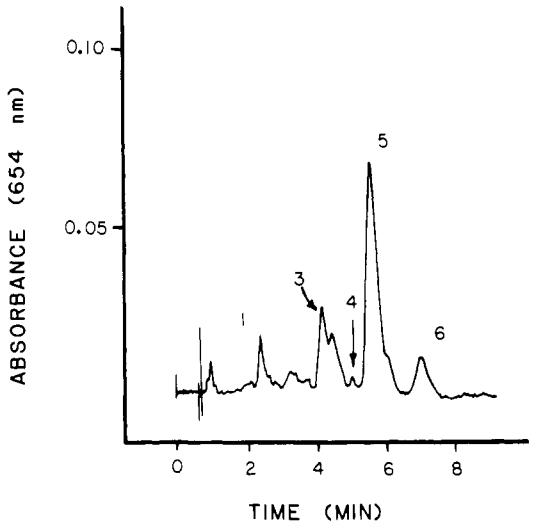

Figure 3. HPLC chromatogram of the chlorophylls and derivatives from an unfermented parched wild rice sample. Peak 3 = pheophytin *b*; peak 4 = pyropheophytin *b*; peak 5 = pheophytin *a*; peak 6 = pyropheophytin *a*.

rice, and the relative areas for these compounds indicate that they also may contribute substantially to overall color.

Most of the chlorophyll-derived pigmentation of wild rice pericarp has been found to reside in a subepidermal layer of cells where both green and brown bodies may occur in the same cell (Khoo and Wolf, 1982). These pigment bodies appear to be derived from chloroplasts. Brown bodies predominate in mature, unparched black kernels. Apparently, these structures are the source of the chlorophyll derivatives observed in this study as well as the pheophytins *a* and *b* which were identified by Withycombe (1974) in acetone extracts of processed wild rice. Khoo and Wolf (1982) also observed other brown pigment bodies in the subepidermis of wild rice kernels which they characterized as phlobaphenes (Singleton, 1972), i.e., oxidation products of tannins. Precursors of these substances apparently are polyphenols that Khoo and Wolf (1982) found localized between the outer and inner seed coat membranes of wild rice. Withycombe (1974) had earlier provided indirect evidence for this pigment system through the demonstration of polyphenoloxidase activity on abraded surfaces of freshly harvested wild rice.

The development and stabilization of the pigments that provide the characterizing visual appearance of wild rice are important to the quality of the finished product. Controlled moisture and temperature parching of wild rice are also important to obtain good-quality products with the absence of undesirable hollow or white centers, limited numbers of broken kernels, and favorable flavor and aroma characteristics (Lund et al., 1975; Anderson et al., 1979a,b). The parching process is usually carried out at temperatures of 80 °C or higher, and as noted above this heat treatment is sufficient to cause alterations in the chlorophyll pigments. The most prevalent degradation pathway of the bright green chlorophyll pigments is the conversion of dull olive pheophytins (Tan and Francis, 1962). This occurs because of the loss of the central Mg atom, and acidic conditions enhance their formation (Schanderl et al., 1962). Other color alterations that occur with chlorophyll pigments result from cleavage of the phytyl ester from chlorophyll or pheophytin forming chlorophyllide or pheophorbide, respectively (Clydesdale et al., 1970), as well as thermal degradations which lead to colorless compounds. It appears preferable to process wild rice grain at a time when maximum chlorophyll–pheophytin concentrations

Table I. Effect of Degree of Scarification upon Hunter Color and Color-Difference *L* Values for Wild Rice

fermentation time, weeks	Hunter *L* values				
	processed only	scarification (no. of passes)			
		2X	4X	6X	8X
0	19.8	20.1	21.1	20.9	21.4
1	20.1	20.6	20.6	22.1	22.6
2	20.3	21.3	21.8	22.2	23.0
3	19.9	20.9	21.8	23.1	23.6
4	20.4	21.0	22.2	22.1	23.3
5	20.6	20.6	21.0	21.7	22.9

are present to minimize losses of chlorophyll contributions through thermal degradation to colorless byproducts. Photooxidation of chlorophylls to colorless degradation products also has been observed when processed wild rice is stored exposed to oxygen and sunlight (Withycombe, 1974). When this occurs processed kernels become somewhat translucent and appear reddish brown in color. These pigments result from sugar–amino or Maillard-type browning and tannins.

Maturation and harvesting of natural-stand wild rice takes place over a period of 2–4 weeks with the ripe kernels being harvested several times throughout the season. Mature freshly harvested wild rice kernels are greenish black in color, and they continue to develop a characteristic deep, shiny black appearance during the early stages of the fermentation process. Manually harvested grain usually provides grain with uniform maturity and pigmentation. However, the advent of mechanized commercial paddy culture of wild rice and the inavailability of uniformly maturing species yield grain which varies considerably in maturity and pigmentation upon harvest.

This rice appears quite green with a high percentage of immature kernels. Very immature kernels are white to light green in color and appear pale yellowish green and translucent when dried. These kernels apparently lack the enzyme system capable of polymerizing the phenolic compounds to produce melanin pigments and contain low levels of chlorophyll. These kernels also represent a loss in yield of high-quality grain and detract from the overall appearance of the finished product. Moderately mature kernels have a greener color than very immature kernels, which is indicative of intermediate levels of chlorophyll deposition, and some tannin pigments. Fully mature fresh kernels are highly pigmented and exhibit a greenish black color. This appearance can be attributed to abundant chlorophyll and tannin or polyphenolic melanin pigments. During fermentation the black color of the more mature kernels intensifies, and this has been attributed to chlorophyll degradations and polyphenolase activity (Lund et al., 1976). Thus, storage of green moderately mature wild rice kernels is desirable to allow maximum enzymatic development of pigments contributing to the black kernel appearance.

Scarification of wild rice is employed to abrade or break the integrity of seed coats to enhance water uptake and thereby reduce cooking time. This process removes portions of the greenish black color in the pericarp and produces lighter colored kernels. Table I lists the Hunter *L* values for samples which were subjected to a fermentation time from 0 to 5 weeks and then parched and given uniform scarification treatments. A trend toward higher *L* values or lighter appearances is noted after successive passes through the scarifier. It is notable that as the fermentation period increased, pigmented seed coats were more easily removed and higher *L* values resulted. Overscarification leads to excessive losses of the blackish

pigmentation, yielding dull grey appearing kernels and a loss of product identity. Thus, adjustments of processing conditions are required to accommodate requirements for both cooking rates and visual characteristics of finished wild rice.

In summary, qualitative effects of usual processing on the chlorophyll components of wild rice grain pigmentation have been studied. The results indicate that HPLC of extracted chlorophylls provides a valuable tool for future research on the effects of processing on wild rice pigmentation.

Registry No. Chlorophyll *a*, 479-61-8; chlorophyll *b*, 519-62-0.

LITERATURE CITED

Anderson, R. A.; Navickis, L. L.; Warner, K. A.; Vojnovich, C.; Bagley, E. B. *Cereal Chem.* **1979a**, *56*, 374.
Anderson, R. A.; Vojnovich, C.; Navickis, L. L.; Bagley, E. B. *Cereal Chem.* **1979b**, *56*, 371.
Clydesdale, F. M.; Fleischman, D. L.; Francis, F. J. *Food Prod. Dev.* **1970**, *4*, 126.
Gutek, L. H.; Woods, D. L.; Clark, K. W. *Crop Sci.* **1981**, *21*, 79.
Khoo, U.; Wolf, M. J., submitted for publication in *Can. Inst. Food Sci. Technol. J.*, 1982.
Lindsay, R. C.; Lund, D. B.; Marth, E. H.; Stuiber, D. A.; Heidemann, R.; Meilinger, J.; Smith, D., Wild Rice Processors Conference, Report of Activities, University of Wisconsin—Madison, Cooperative Extension, 1975.
Lund, D. B.; Heidemann, R.; Lindsay, R. C.; Johnson, C. E.; Marth, E. H.; Stuiber, D. A. *Trans. ASAE* **1976**, *19*, 332.
Lund, D. B.; Lindsay, R. C.; Stuiber, D. A.; Johnson, C. E.; Marth, E. H. *Cereal Foods World* **1975**, *20*, 150.
Moore, K. K. *Food Prod. Dev.* **1977**, *11* (3), 28.
Schanderl, S. H.; Chichester, C. O.; Marsh, G. L. *J. Org. Chem.* **1962**, *27*, 2865.
Schwartz, S. J.; Woo, S. L.; von Elbe, J. H. *J. Agric. Food Chem.* **1981**, *29*, 533.
Singleton, V. L. *Adv. Food Res., Suppl.* **1972**, *3*, 143.
Tan, C. T.; Francis, F. J. *J. Food Sci.* **1962**, *27*, 232.
Withycombe, D. A. Ph.D. Thesis, Department of Food Science, University of Wisconsin—Madison, 1974.

Received for review June 22, 1982. Accepted November 14, 1982. This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin—Madison.

Effect of Acetylation and Succinylation of Cottonseed Flour on Its Functional Properties

E. H. Rahma[1] and M. S. Narasinga Rao*

Cottonseed proteins were acetylated or succinylated to different levels, and the nitrogen solubility in water or 5% NaCl solution, water absorption and oil absorption capacity, emulsification capacity, foam capacity and foam stability, bulk density, and in vitro digestibility of cottonseed flour were determined. Acetylation decreased the nitrogen solubility in water or 5% NaCl whereas succinylation increased it. The water absorption capacity did not change due to succinylation but increased due to acetylation. On the other hand, high levels of acetylation or succinylation decreased the oil absorption capacity. The emulsifying capacity increased initially on acylation and then decreased. Foam capacity increased but foam stability decreased on acylation. Bulk density increased due to modification but in vitro digestibility was not affected.

The presence of gossypol is a limiting factor in the use of cottonseed flour in human foods; gossypol in the free form has been found to be toxic to monogastric animals. In addition it imparts unattractive color and off-flavor to the product (Berardi and Goldblatt, 1969). The liquid cyclone process (LCP) has been used for reducing the gossypol content of cottonseed flour (Riddlehuber and Gardner, 1974). In India this method does not appear to have been commercially used nor has plant breeding for a glandless variety of cottonseed found wide application.

Gossypol reacts with the ϵ-amino groups of the lysine residues of cottonseed proteins (Lyman et al., 1959). A secondary type of interaction has also been reported (Damaty and Hudson, 1974). The interaction of gossypol with proteins, such as bovine serum albumin, has been found to be partly reversible, suggesting noncovalent interaction (Maliwal et al., 1983).

Central Food Technological Research Institute, Mysore-570013, India.
[1]United Nations University Fellow. Present address: Food Sciences and Technology Department, Faculty of Agriculture, University of Monoufeia, Shibin El-kom, Egypt.

In the case of yeast proteins, it has been reported that the chemical modification of the proteins such as succinylation reduces the nucleic acid contamination (Shetty and Kinsella, 1979). It was, therefore, thought desirable to determine if chemical modification of cottonseed proteins would reduce the gossypol content of the flour. Chemical modification has also been reported to alter the functional properties of proteins (Franzen and Kinsella, 1976; Childs and Park, 1976; Beuchat, 1977; Shyama Sundar and Rajagopal Rao, 1978; Choi et al., 1981). In this investigation, the effect of acetylation or succinylation of cottonseed proteins to different levels on the gossypol content and functional properties has been studied.

MATERIALS AND METHODS

Cottonseed Flour. Cottonseed of the variety Varalakshmi was obtained from the Gujarat State Seeds Corp., Ltd., India. It was flaked by using flaking rolls (Model No. 6725, Aktiebolaget, Kvarnmeskiner, Malmo, Sweden) and sieved to remove the husk and remaining fibers, and the fat was extracted with *n*-hexane. The flour was desolventized at room temperature and ground in an Apex communiting mill (Apex Construction, Ltd., London) to pass through a 60-mesh size sieve. The fat content of the flour was 1.22% and protein content 52.6%.

0021-8561/83/1431-0352$01.50/0