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.

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

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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).

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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%.

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Reagents. Pepsin, pancreatin, and 1-fluoro-2,4-dinitrobenzene were from E. Merck, West Germany.

Acetylation. A 25% (w/v) suspension of cottonseed flour was prepared in distilled water at room temperature (~28°C), and the pH adjusted to 7.5 by the addition of 2 N NaOH. Acetic anhydride was added to the suspension over 30–90 min at the levels of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 g/g of protein; the weight of acetic anhydride was determined by its specific gravity. The pH was maintained in the range 7.5–8.5 at room temperature by the addition of 2 N NaOH. The final pH was adjusted to 7.5, and the slurry was left for 2 h at room temperature and dialyzed against distilled water for 12–16 h. It was then freeze-dried and ground to pass through a 60-mesh size sieve.

Succinylation. The same procedure as for acetylation was used for succinylation with succinic anhydride. The levels used were 0.2, 0.4, 0.6, 0.8, and 1.0 g/g of protein.

Estimation of Chemical Modification. The extent of chemical modification was estimated by determining the available lysine content of the proteins. This was determined by the procedure of Carpenter (1960) using 1-fluoro-2,4-dinitrobenzene reagent. Modification is expressed as percent reduction in available lysine residues.

Total, Free, and Bound Gossypol. Total and free gossypol was determined by the method of Walter et al. (1950, 1949). Bound gossypol was calculated by difference.

Nitrogen Solubility. Nitrogen solubility in water and 5% NaCl solution was determined by the method described by Rahma and Narasinga Rao (1979).

Water Absorption Capacity. To 0.5 g of the flour 15 mL of water was added in a weighed 20-mL, glass centrifuge tube. It was agitated on a Vortex mixer for 2 min and then centrifuged for 20 min at 3000 rpm. The clear supernatant was decanted and discarded. The adhering drops of water were removed and the tube was weighed. The weight of water bound per 100 g of flour was calculated and expressed as water absorption capacity.

Oil Absorption Capacity. This was determined in the same way as water absorption capacity except that 10 mL of refined groundnut oil was added to 0.5 g of cottonseed flour. The oil absorption capacity is expressed as milliliters of oil bound per 100 g of cottonseed flour.

Emulsifying Capacity. This was determined by the method described by Webb et al. (1970). It is expressed as mililiters of oil emulsified per gram of cottonseed flour.

Foam Capacity and Foaming Stability. The procedure of Huffman et al. (1975) was used. One gram of cottonseed flour was added to 50 mL of distilled water and blended in a Waring Blendor at 1600 rpm for 5 min. The mixture was poured into a 100-mL graduated measuring cylinder and the total volume recorded after 30 s as foam capacity. After the mixture was allowed to stand for 30 min at room temperature, the foam volume was recorded as foam stability.

Bulk Density. A weighed 15-mL gradulated glass centrifuge tube was filled to 5 mL with the sample by constant tapping until there was no further channge in the volume. Then its weight was recorded. From the difference in weight, density was calculated.

In Vitro Digestibility Index. This was determined by the method of Akeson and Stahmann (1964) using the pepsin–pancreatin enzyme system. The results are expressed as percent protein digested.

All the measurements were made in triplicate and the values reported are the average of three measurements.

RESULTS AND DISCUSSION

The extent of lysine modification increased as the ratio of acetic anhydride or succinic anhydride to the protein increased (Figure 1). The rate of modification with acetic anhydride was greater than with succinic anhdyride. At a ratio of 0.4 g of acetic anhydride/g of protein nearly 86% of the lysine residues had been modified whereas at the same ratio of succinic anhydride only about 55% modification had occurred. At a ratio of 1 g of anhydride/g of protein, acetic anhydride modified about 90% residues and succinic anhydride 73%. Thus, acetic anhydride appeared to be a better acylating agent for cottonseed proteins than succinic anhydride. Shyama Sundar (1980) has reported that acetic anhydride and succinic anhydride are equally effective in acylating arachin.

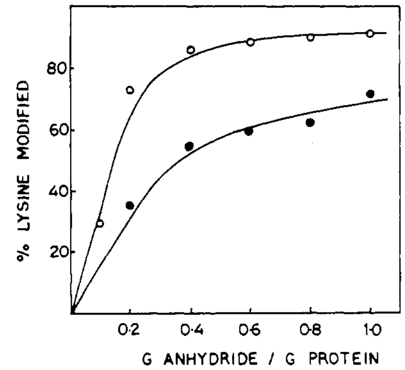

Figure 1. Percent lysine modification as a function of anhydride concentration: (O) acetic anhydride; (●) succinic anhydride.

Table I. Effect of Acetylation or Succinylation on the Total and Free Gossypol Content of Cottonseed Flour

acetylation			succinylation		
% modification[a]	total gossypol, %	free gossypol, %	% modification[a]	total gossypol, %	free gossypol, %
0	1.72	1.52	0	1.72	1.52
73	1.62	0.75	35	1.57	0.46
86	1.64	0.64	55	1.60	0.45
88	1.56	0.61	60	1.52	0.55
90	1.55	0.96	63	1.50	0.58
91	1.51	0.99	73	1.46	0.68

[a] Modification is expressed as percent reduction in available lysine.

Acetylation or succinylation of cottonseed flour did not significantly decrease the total gossypol content (Table I). It decreased only by about 12–15%. The free gossypol content, however, decreased almost to 50% level at 88% acetylation and 33% at 55% succinylation. Higher levels of acetylation or succinylation were less effective. Even at the lowest level of gossypol the free gossypol content was much higher than the content accepted for edible cottonseed flour (Protein Advisory Group, 1970). Thus, acylation of cottonseed proteins did not appear to be an effective method for reducing gossypol content to prepare edible cottonseed flour.

The nitrogen solubility of acetylated and succinylated cottonseed flour in water and 5% NaCl solution is shown in Figure 2. The nitrogen solubility of acetylated protein in water showed a marginal increase up to 73% acetylation and then decreased. On the other hand succinylation lead to increased solubility in water (Figure 2A). In 5% NaCl solution the nitrogen solubility of acetylated proteins decreased continuously; succinylated proteins showed a decrease and then attained the value of the untreated flour. Interestingly, the solubility of acetylated protein in water or 5% NaCl solution was the same at higher levels of modification.

Choi et al. (1981) have reported that the solubility of protein isolate prepared from succinylated, glandless

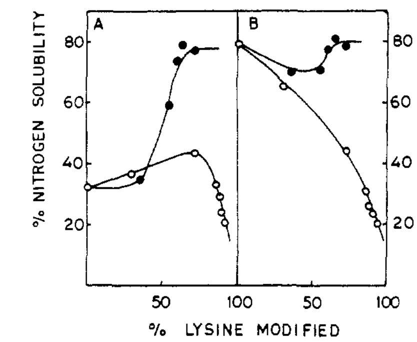

Figure 2. (A) Nitrogen solubility in water of cottonseed flour as a function of percent lysine modification: (O) acetylated; (●) succinylated. (B) Nitrogen solubility in 5% NaCl solution of cottonseed flour as a function of percent lysine modification: (O) acetylated; (●) succinylated.

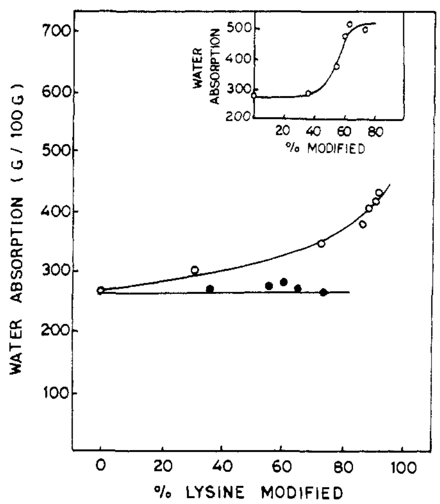

Figure 3. Water absorption capacity of cottonseed flour as a function of percent lysine modification: (O) acetylated; (●) succinylated. (Inset) Water absorption of succinylated cottonseed flour corrected for solubilized protein, as a function of percent lysine modification.

cottonseed flour was higher in water and 4% NaCl than that of the isolate prepared from the untreated flour; further, the higher the level of modification the greater was the solubility. Childs and Park (1976), who acetylated glandless cottonseed flour, have not reported its nitrogen solubility. Franzen and Kinsella (1976) have reported that acetylation and succinylation of soy protein isolate increased the nitrogen solubility in water (pH 7.0).

During acetylation of the proteins a hydrophobic group is introduced into the protein molecule whereas during succinylation a hydrophilic group is introduced. Introduction of hydrophobic residues would tend to reduce solubility in aqueous media. Also, the decrease would depend upon the number of groups acetylated.

The water absorption capacity of acetylated cottonseed flour was higher than that of the untreated flour; it increased with an increase in the degree of modification (Figure 3). Our observation with acetylated flour agrees with the observation of Childs and Park (1976). Succinylation did not change the water absorption capacity. Childs and Park (1976) and Choi et al. (1981) have reported that succinylation of glandless cottonseed flour increased the water absorption capacity. Further, the increase was dependent on the level of succinylation (Choi et al., 1981). The measurements of Childs and Park (1976) were made in 0.02 M citrate buffer of pH 3.5 and those of Choi et al. (1981) were made with hot-water-insoluble protein. Our measurements were made in water at 28 °C.

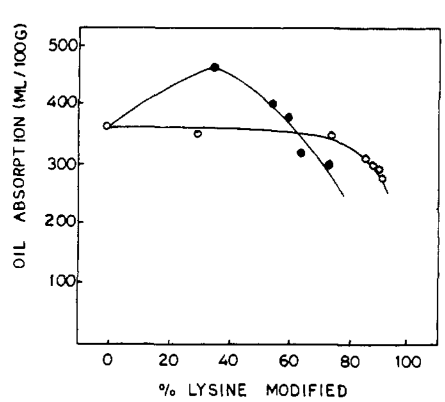

Figure 4. Oil absorption capacity of cottonseed flour as a function of percent lysine modification: (O) acetylated; (●) succinylated.

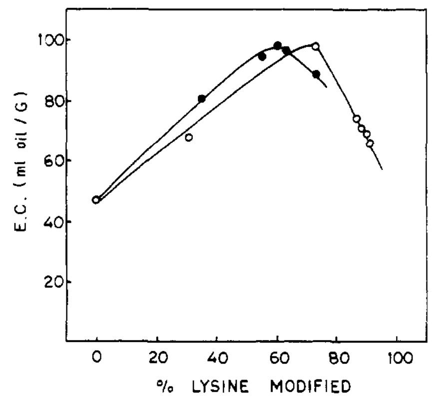

Figure 5. Emulsifying capacity of cottonseed flour as a function of percent lysine modification: (O) acetylated; (●) succinylated.

Reference to Figure 2 shows that succinylated proteins are readily soluble in water. Therefore, a considerable portion of the protein is leached out during the experiment. If correction for the leached out protein was made, the water absorption capacity of succinylated protein was indeed found to be higher than that of the untreated flour (Figure 3, inset). However, such a correction ignores the contribution of other constituents of cottonseed flour to water absorption capacity.

The oil absorption capacity of acetylated protein was unaffected up to 73% lysine modification and then showed a decrease (Figure 4). The succinylated flour showed an initial increase up to 35% modification and then showed a decrease. Childs and Park (1976) and Choi et al. (1981) have reported that both acetylation and succinylation of glandless cottonseed flour increased oil absorption capacity. Beuchat (1977) has reported that the oil absorption capacity of peanut flour was not markedly affected by succinylation to various levels. Thus, the effect appears to be dependent on the type of protein.

The emulsifying capacity (EC) of cottonseed flour increased with acetylation or succinylation up to about 65% modification and then decreased (Figure 5). Even at the highest level of acetylation or succinylation, EC was higher than that of the untreated flour. Childs and Park (1976) have reported that acetylation of glandless cottonseed flour did not significantly affect EC. However, succinylation increased it significantly. The protein isolate from succinylated glandless cottonseed flour also had a higher EC (Choi et al., 1981). At 40% and 54% levels, the increases were about 50% and 100%, respectively. We observe that at 40% and 60% levels, the increases are about 90% and 110% (Figure 5). Franzen and Kinsella (1976) have reported that succinylated soy protein isolate had a higher EC than the untreated isolate.

Table II. Effect of Acetylation or Succinylation on Foam Capacity and Foaming Stability of Cottonseed Flour

acetylation			succinylation		
% modification	foam capacity, mL	foam stability, mL	% modification	foam capacity, mL	foam capacity, mL
0	74.0	30.0	0	74.0	30.0
73	81.0	28.0	35	75.0	25.0
86	85.0	24.0	55	76.0	24.0
88	86.0	39.0	60	82.0	24.0
90	87.0	40.0	63	83.0	21.0
91	88.0	34.0	73	86.0	22.0

Table III. Effect of Acetylation or Succinylation on Protein Digestibility of Cottonseed Flour

acetylation		succinylation	
% modification	digested protein, %	% modification	digested protein, %
0	85	0	85
73	85	35	82
86	87	55	76
88	84	60	76
90	81	63	77
91	75	73	82

Table IV. Effect of Acetylation or Succinylation on the Bulk Density of Cottonseed Flour

acetylation		succinylation	
% modification	bulk density, g/mL	% modification	bulk density, g/mL
0	0.29	0	0.29
73	0.45	35	0.34
86	0.51	55	0.43
88	0.54	60	0.46
90	0.56	63	0.50
91	0.59	73	0.52

The foam capacity of cottonseed flours increased slightly due to acetylation or succinylation (Table II). At maximum modification of lysine groups, by acetylation or succinylation, the increase in foam capacity was about 17%. However, foam stability decreased due to acylation. Childs and Park (1976) have also reported similar results with acetylated and succinylated glandless cottonseed flour.

The protein digestibility of acetylated or succinylated flour was not significantly altered (Table III). They had the same value as the untreated flour.

The bulk density of the cottonseed flour increased due to acetylation or succinylation (Table IV). At the highest level of modification, the increase was almost 2-fold. Choi et al. (1981) have reported that the protein isolates prepared from succinylated glandless cottonseed flour had a lower bulk density than the isolate from the untreated flour.

Registry No. Gossypol, 303-45-7; L-lysine, 56-87-1.

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