et al. (1972) for white rice. Parboiled rice was also found to be easier to dry without checking or cracking of the grain.

Cross-linked rice should have a high potential for commercial incorporation in canned products for the following reasons: less solid loss; sound organoleptic qualities; and stable at pH 5. Hence, it is suitable for tomato-type products, no blanching is required before canning, and the cross-linking operation is very simple with regards to equipment and processing temperature.

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Effect of Processing Conditions on Isolation of Cottonseed Protein by Sodium Hexametaphosphate Extraction Method

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A method for the isolation of cottonseed protein based on extraction with sodium hexametaphosphate (SHMP) and precipitation with acid was investigated. Maximum yields were obtained when extraction was carried out at pH 7.0, with 2% SHMP, at 60° for 30 min, and the curd was precipitated at pH 2–3. The isolate contained up to 94.8% protein, with the main impurity being phosphates. The nutritive value of the isolate was not significantly different from that of degossypolized cottonseed flour.

Present world production of cottonseed is in excess of 20 million tons (Tropical Product Quarterly, 1970), representing a protein potential of some 6 million tons per annum. Although cottonseed was recognized long ago as a possible source of protein for human consumption (Martinez *et al.*, 1970), actual utilization in this direction is negligible. The main reasons for this situation are the presence of gossypol, an antinutritional pigment, and failure to convert cottonseed protein to readily acceptable edible products. The solution to the gossypol problem in processing cottonseed for human food involves at present three different types of approaches: development of gossypol-free (glandless) seed through plant breeding (McMichael, 1959); multisolvent extraction (King *et al.*, 1961; Krishnamoorthi, 1965); and physical separation of the gossypol glands; *e.g.*, by the liquid cyclone process (Gastrock *et al.*, 1969). Glandless or degossypolized cottonseed flours may contain over 60% protein. These flours may be quite acceptable as edible products, provided that the seeds are dehulled completely and excessive heat and moisture are avoided during processing and storage. Further processing of such flours to isolated protein provides a possibility of attaining a higher protein concentration and may be expected to solve some of the acceptability and stability problems associated with the presence of nonprotein constituents.

Several methods for the isolation of cottonseed protein were suggested. They differ mainly in the media used for protein extraction. The media investigated include sodium sulfite (Arthur and Karon, 1948), salt solutions and alkali (Olcott and Fontaine, 1939), water and sodium hydroxide (Berardi *et al.*, 1969; Lawhon and Cater, 1971), and polyphosphates (Chajus and Chajus, 1964).

The purpose of the present work was to determine the effect of various processing conditions on isolation of cottonseed protein, using sodium hexametaphosphate (SHMP) as the extraction medium.

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EXPERIMENTAL SECTION

Preparation of Cottonseed Flour. Fat-free and degossypolized flour was prepared by a two-stage solvent extraction process. First, the decorticated seeds (meats) were gound in aqueous acetone containing 30% water to remove gossypol. This medium is recommended by Pons and Eaves (1967) for the removal of gossypol with minimum extraction of other constituents. Grinding in solvent was done by an Ultra-Turrax colloid blender (Janke & Kunkel A.G., Stauffen, Germany). Subsequently the solvent was filtered off and the meats were reextracted with anhydrous acetone to remove the oil. The extracted flour was dried in open air.

Laboratory Isolation of Proteins. Two-hundred grams of degossypolized fat-free flour was extracted for 1 hr, at 50° and pH 7, in a solution of 40 g of SHMP in 2 l. of water. The extract was separated from the insoluble residue by centrifugation and subsequent filtration. The filtered extract was acidified with diluted HCl to the appropriate pH (usually 2–3). The precipitate (curd) was allowed to settle and the supernatant (whey) was decanted off. The curd was washed with water by resuspension and centrifugation. Finally the washed curd was dried in a vacuum oven at 50°.

Pilot Plant Isolation of Protein. Batches of 1 kg of flour were extracted with 10 l. of water and 200 g of SHMP. The procedure for extraction and precipitation was the same as described above, but drying of the curd was different. A suspension of curd in water was spray-dried, using a Niro-Production Minor apparatus (Niro Atomizer A/S, Copenhagen, Denmark). Air temperature was 190° at the inlet and 90–100° at the outlet.

Analytical. Moisture, fat, total nitrogen, free and total gossypol were determined following methods described by the American Oil Chemists' Society (1959). Phosphorus was determined according to the colorimetric molybdovanadate method described in AOAC (1970).

Nutritive Value. Net protein utilization (NPU) was determined according to Miller and Bender (1955) on individual animals. Weanling albino rats of the Charles River C.D. strain, age 21 days, were used. Three males and three females were used for each test material. The feeding period was 10 days.

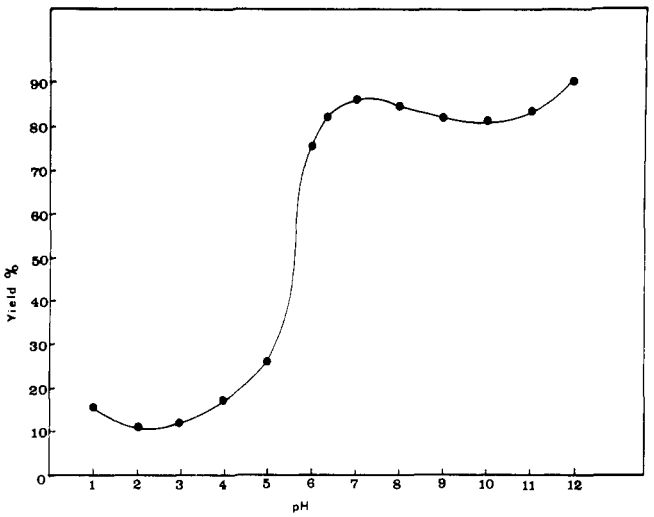

Figure 1. Effect of pH on extraction yield.

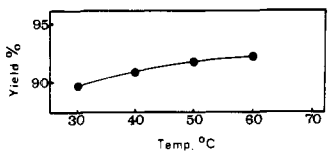

Figure 2. Effect of temperature on extraction yield.

Replications. Chemical determinations were carried out in triplicate and pilot plant runs were in duplicate. The results reported are average values. Animal feeding tests were done only once, using the number of animals specified above.

RESULTS AND DISCUSSION

Extraction Yields. Extraction yield is defined as that portion of the total nitrogen of the meal which is extracted.

The effect of pH on extraction yield is shown in Figure 1. Maximum extraction yield (86%) occurs at pH 7.0. There is a further slow increase at pH values above 11. Olcott and Fontaine (1939) and Mizrahi *et al.* (1968) reported maximum yield (90%) at pH 10, but found that extraction at high pH caused side effects such as darker color and sulfite-like odors.

Temperature affects extraction yields (Figure 2). There is a slight increase in yield as the temperature of extraction is raised from 30 to 60°. Liener (1958) gives reasons why the temperature should not be raised above 55° (damage to nutritive value resulting from a decrease in availability of lysine). Therefore the temperature of 50° was used for extraction in all further experiments.

Figure 3 shows the effect of extraction time at 50° and pH 7 on yield. 87% extraction is achieved already after 30 min. There is a further slow increase in yield thereafter. Chajus and Chajus (1964) report a yield of 70% after 2 hr, working at 25°. The higher temperature or differences in the nitrogen solubility of the raw material may be the reason for the difference in extraction rates.

The effect of SHMP concentration on extraction yield is shown in Figure 4. Maximum extraction is attained at 2% SHMP. Extraction time in this experiment was 30 min.

The effect of replacing SHMP by sodium chloride totally or partially is shown in Figure 5. Extraction yield in 2% NaCl with no SHMP is only 88%, while the yield in 2% SHMP with no NaCl is nearly 93%. For binary mixtures extraction yields assume intermediate values. Extraction time in this experiment was 60 min.

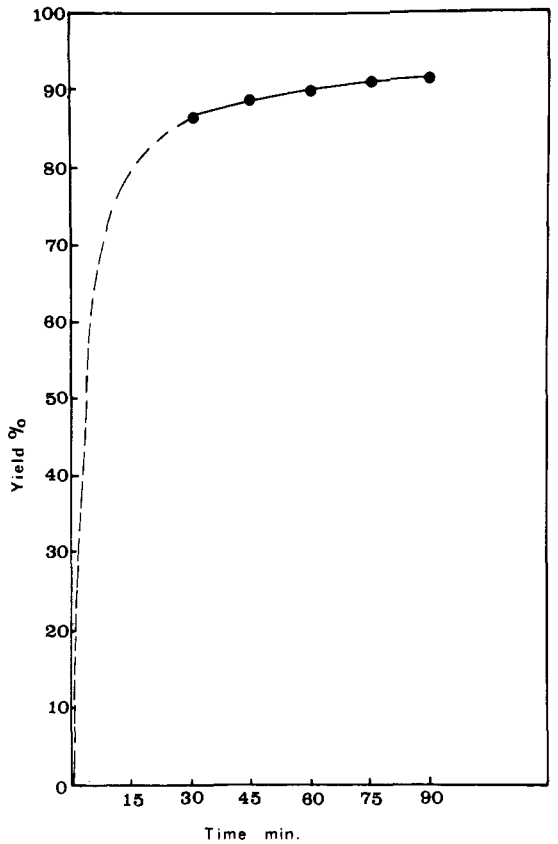

Figure 3. Effect of extraction time on extraction yield.

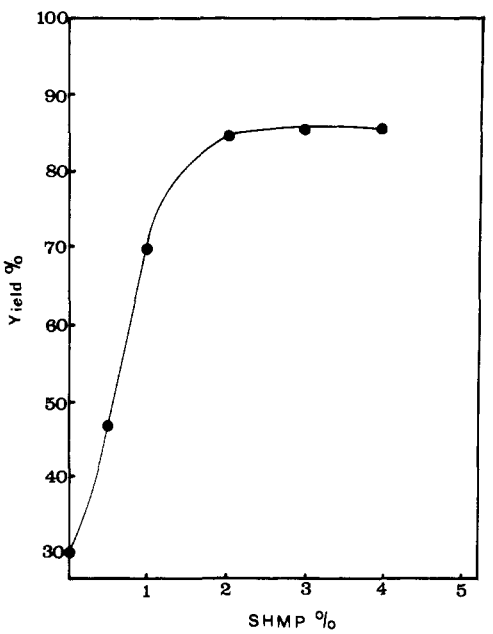

Figure 4. Effect of SHMP concentration on extraction yield.

Precipitation Yield. Precipitation yield is defined as that portion of total nitrogen of the extract which is recovered in the precipitate (curd).

The extract at pH 7 was acidified to various values of pH and the corresponding yield of precipitation (*i.e.*, percentage of the solubilized protein recovered as curd) was determined. Maximum recovery (above 88%) is achieved at pH 2–3 (Figure 6). Former reports (Mizrahi *et al.*, 1968; Olcott and Fontaine, 1939) indicate maximum precipitation at pH 4 when the extractant was NaOH or NaCl.

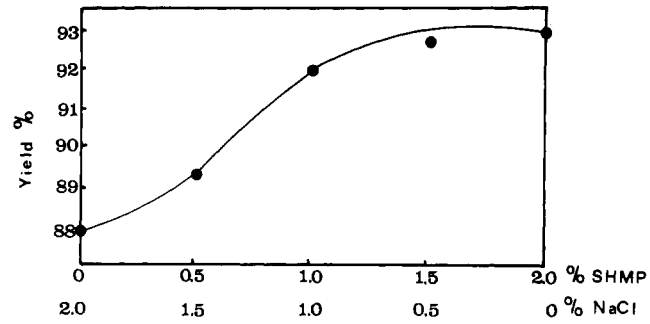

Figure 5. Effect of NaCl on extraction with SHMP.

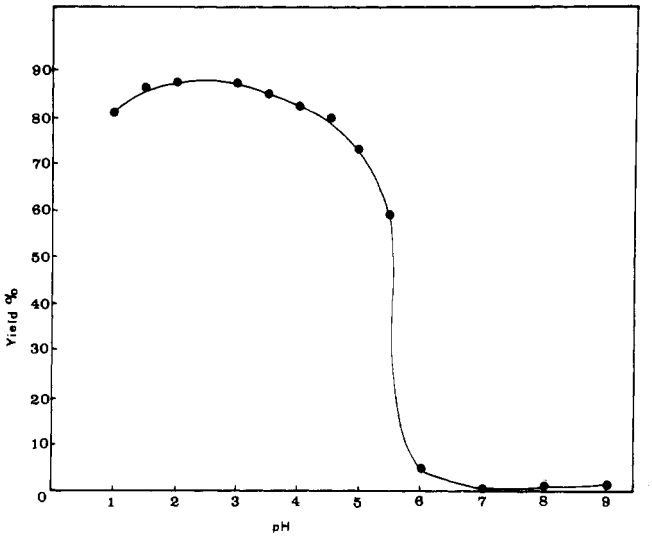

Figure 6. Effect of pH on precipitation yield.

The isolated protein was dissolved in a solution of NaOH (pH 10) and reprecipitated by acidification. Maximum recovery occurred at pH 4, as reported by the workers mentioned above. Thus, the shift in isoelectric point seems to be due to changes in acid-base equilibrium caused by the presence of polyphosphate anion.

Sensory Characteristics and Purity of the Isolated Protein. The spray-dried isolated protein was free of odor and essentially bland except for a very slight salty-metallic aftertaste, probably due to the phosphate. Its color was light cream.

Table I shows the effect of precipitation pH on the purity of the isolated protein. Purity is expressed as protein (N × 6.25) content of the curd. The highest purity corresponds to the highest precipitation pH of 4.5. As the pH is lowered, purity passes through a minimum (75.5%) at pH 3.5. At pH 2.5, purity is somewhat higher (85.2%) but not as good as at pH 4.5. Thus, the increase in precipitation yield is accompanied with some loss in isolate purity.

One of the impurity factors seems to be residual phosphate, carried along from the extraction medium. The level of phosphorus content of the isolate is affected by the pH of precipitation. It is lowest at pH 4.5 and increases as the pH of precipitation is lowered. The phosphorus content of isolated protein from NaCl extract was found to be 0.98%. Thus the higher phosphorus content of the SHMP isolate must be due to entrainment from the extraction medium. However, this impurity seems to be strongly attached to the protein as can be seen from Table II. The effect of six consecutive washings was the reduction of phosphorus content from 4.55 to 3.75%, on a dry basis.

Table I. Effect of Precipitation pH on the Protein Purity and Solubility

Precipitation pH	Percent protein (N × 6.25) in curd, dry basis	Phosphorus %, dry basis
2.5	85.22	3.95
3.0	81.31	4.00
3.5	75.50	3.75
4.0	88.96	3.20
4.5	94.82	2.45

Table II. Nutritive Value of Isolated Cottonseed Protein

Material	pH of precipitation	NPU
Defatted, gossypol free flour		60.0
Isolated protein	2.5	54.0
Isolated protein	3.0	54.2
Isolated protein	3.5	51.4
Isolated protein	4.0	51.0
Isolated protein	4.5	53.2
Casein		72.3

Nutritive Value. The nutritive value of the isolated protein (expressed as NPU) is slightly inferior to that of the flour from which it was made (Table II). The difference was significant at the 95% confidence level. Thus, unlike in soybeans (Cogan *et al.*, 1968), isolation does not impair considerably the nutritive value of cottonseed protein. The effect of pH of precipitation on the NPU of the isolate was not significant at the 95% confidence level.

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