ISOLATION OF PROTEINS FROM COTTONSEED MEAL

I. THE AMMONIA METHOD OF ISOLATING PROTEIN

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Cottonseed meal contains about 50% of protein. There are a number of methods for isolating protein [1-3]. We have used the alkaline method of extraction, which enables the protein to be obtained with the maximum yield (32-34%). We have used various solutions for extraction. The total yields of extracted protein and the percentage nitrogen contents are given in Table 1.

The best results with respect to the total yield of protein and to the percentage nitrogen content were achieved when the meal was extracted with a 0.27% solution of ammonia. The optimum conditions for extraction were selected: the yield of extracted protein and the nitrogen content were determined as functions of the time and temperature of heating and of the pH of the extracted solution. It was found that the optimum conditions are pH 11, temperature of heating 90°C, and time of heating 45 min.

To improve the quality of the protein obtained, the extracts were treated with calcium salts [4, 5] in various concentrations (0.0065-0.5 M), with their addition both to the extracted mass before heating and to the extract isolated after the centrifuging of the residual meal. In both cases a considerable decrease in the yield of protein was observed, while the percentage nitrogen content increased slightly (see Table 1). In addition, we studied the action of hydrogen peroxide on the yield and quality of the protein. The yield of protein fell by 5%, and the percentage nitrogen content rose from 12.6 to 13.2% on extraction with NaOH and from 13.5 to 15.5% on extraction with NH_3. The color of the protein was lighter and, in addition to this, the amount of bound gossypol in it had fallen sharply (Table 2).

Table 2 gives the results of chemical analyses on the basis of which it is possible to estimate the food value of the proteins obtained by the alkaline method.

The amino-acid composition of the protein on extraction with ammonia was as follows (%): lysine 2.5; histidine 1.45; arginine 10.65, aspartic acid 7.37; threonine 2.15; serine 3.76; glutamic acid 14.55; glycine 3.62; alanine 3.6; valine 5.44; isoleucine 2.8; leucine 5.51; tyrosine 2.35; phenylalanine 5.2; and proline 6.0. Essential amino acid index (EAAI) taking into account the amounts of all ten essential amino acids, 67.7%. Moisture content of the protein 6.2%, ash content 1.55%.

A spectroscopic analysis of the ash showed the presence in the protein of 0.034% of Si, 0.34% of Ca, 0.34% of Mg, 0.19% of P, and 0.02% of Cr, other elements being present only in trace amounts of 10^{-3}-10^{-4}%.

EXPERIMENTAL

Protein. The amount of protein in the solution was determined by the biuretic reaction and by the Warburg–Christiani method [6].

Nitrogen. The percentage nitrogen content of the protein was determined by Conway's microdiffusion method and by the Dumas combustion method.

Lysine. The amount of assimilable lysine was determined by Carpenters' method [7] modified for protein from plant sources. The amount of DNP lysine was determined from a calibration curve plotted for pure E-DNP lysine · HCl. For converting E-DNP-lysine into lysine we used a factor of 0.419 [8].

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TABLE 1

Alkaline solution	Yield of protein in the precip.	Total extractable protein, %	Nitrogen content
NaOH	25	31	12,5–12,8
Na_2CO_3	14	20–23	12,6
NH_3	32–34	39–40	13,5–14
NaOH*	15–18	20–22	13,5
NH_3*	20–22	25–28	14,3–14,5

* Treated with calcium salts.

TABLE 2

Extracting solution	Yield of prot. in the precip.	Protein content	Assimilable lysine, g/16 g of N	Solubility in 0.02 NaOH	Gossypol content, %	
	%				total	free
NaOH	27–30	81,3	3,1	100	1,45	0,02
NH_3	32–34	87,5	3	100	1,45	0,015–0,025
$NH_3+H_2O_2$	28	91	3,4–3,5	100	0,38–0,45	0,015–0,022

Gossypol. The amount of total gossypol was determined by Smith's method [9].

Extraction of the Protein from the Meal. The meal ground in a ball mill (5 g) was extracted with 45 ml of 0.27% NH_3 at pH 10.5-11, and the extract was heated at 90°C for 45 min and was centrifuged at 6000 rpm for 15 min. The precipitate was washed twice in ammoniacal water at 70°C with stirring.

The supernatant liquids were combined with the main extract, and the protein was precipitated with 1 N HCl, the pH being brought to 3.8–4, and was then centrifuged at 6000 rpm for 15 min. The protein was washed with acidified water at pH 4.5-5 and was dried either with acetone or by lyophization (II).

Meal was extracted similarly with a 0.25% solution of NaOH and with a 0.2% solution of Na_2CO_3.

Determination of the Yield of Protein and of the Percentage Nitrogen Content. As a Function of the Time of Heating. The meal was extracted by the given method for 15-19 min with an interval of 15 min and the extract was worked up as described above.

As a Function of the Temperature of Heating. The meal was extracted under the same conditions in the temperature range from 60-90°C (every 5°C). The protein was isolated as described above.

As a Function of the pH of the Extracted Solution. The meal was extracted with ammonia solution at pH 7.5-11.5 at intervals of 0.5 pH unit.

Acid Hydrolysis of the Protein. The protein (3-5 mg) was hydrolyzed with 8 ml of 6 N HCl under vacuum at 110°C for 24 h. The hydrolyzate was dried in a rotary evaporator. The amount of amino acids was determined on a type AAA-881 amino-acid analyzer.

Preliminary Washing of the Meal with Water. The meal was suspended in water acidified with 1 N HCl, or 1 N H_2SO_4 to pH 5.5 in a ratio of 1:5, and the suspension was stirred at 65-70°C for 15 min and was then centrifuged. The solid matter was used for further extraction by the method described above, and the percentage of protein in the supernatant liquid was determined. The losses of protein amounted to 5-6% and of nitrogen to 13.2-13.6%.

Treatment of the Extracted Protein with Calcium Salts. The alkaline extract was treated with 0.0065 N $CaCl_2$ (0.3-6 ml), left for 12 h, acidified with 1 HCl, and centrifuged. The percentage nitrogen contents of all the samples were determined. On the addition of 6 ml of $CaCl_2$ the yield of protein in the precipitate decreased by a factor of 2, and the percentage of nitrogen by a factor of 13; when similar amounts of $CaCl_2$ were used, no appreciable increase in the amount of nitrogen was observed and the yield of protein fell by a factor of 1.2-1.3. To the alkaline extract obtained from 15 g of meal was added 0.3 g of $Ca(OH)_2$ + 0.55 ml of 50% NaOH + 0.42 g of Na_2CO_3, and the mixture was left for 12 h. Then it was centrifuged and after 2 h it was acidified with 1 N HCl. The yield of protein was 6-10% and the percentage of nitrogen 13.5-14.5.

Treatment of the Extracted Protein with Hydrogen Peroxide. To an alkaline extract or to a suspension of the meal in an alkaline solution was added 30% H_2O_2 (0.04-0.3 mole per gram of dry meal). The extract

was additionally heated at 70°C for 30 min. The subsequent working up was performed by the method described above.

CONCLUSIONS

The optimum conditions for the extraction of protein from cottonseed meal with a solution of ammonia has been selected, giving a yield of protein of 34% with a nitrogen content of 13.5-14%. The chemical characteristics of the protein are given.

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