*Food Chemistry* **30** (1988) 19–27

# Aqueous Protein and Gossypol Extraction from Glanded Cottonseed Flour: Factors Affecting Protein Coagulation and Gossypol Content

A. H. El Tinay, A. M. Nour, S. H. Abdel-Karim & S. O. Mahgoub

Department of Biochemistry, Faculty of Agriculture, Shambat, Sudan

(Received 15 September 1987; revised version received and accepted 30 November 1987)

*ABSTRACT*

*Protein extracted from cold- and hot-defatted cottonseed flour was recovered by isoelectric coagulation, dialysis, isoelectric coagulation after dialysis and isoelectric coagulation followed by dialysis of the whey. Isoelectric precipitation resulted in poor protein recovery, but dialysis caused considerable improvement in protein recovery. Isoelectric precipitation followed by coagulation of the remaining protein in the whey by dialysis resulted in the highest protein recovery. Isoelectric coagulation, after dialysis of the extract, gave a low protein recovery compared to that obtained by dialysis, but higher than that obtained by isoelectric coagulation alone. The protein and gossypol contents of protein isolates obtained from hot-defatted cottonseed flour were determined. The protein content of isolates obtained by dialysis was markedly high (above 90%) and their free gossypol content was low compared to isolates obtained by isoelectric coagulation. Increase of solvent to flour ratio decreased both total and free gossypol contents of protein isolates obtained by both methods.*

## INTRODUCTION

The biggest problem associated with cottonseed is the toxic pigment gossypol which must be removed before being eaten by monogastric animals (Noyes, 1969). Eckey (1954) reported that *Gossypium hirsutum* contains



*Food Chemistry* 0308-8146/88/$03·50 

1·4% to 2·1% gossypol and *Gossypium barbadenese* contains 1·9% to 3·4%. Total gossypol comprises free and bound gossypol. Only the free gossypol is of concern with regard to toxicity; bound gossypol is not toxic. Edible grade cottonseed flour should not contain more than 0·06% free gossypol (Noyes, 1969).

The problems associated with protein isolation processes which produce liquid by-products are how to recover or properly precipitate these proteins. Fox *et al.* (1957) reported that, at the isoelectric point, the solubility of proteins is minimal, because proteins are more soluble when combined with bases or acids than in neutral states. They also mentioned that addition of salts increases water solubility of most proteins at the isoelectric point. Altschull (1958) showed that proteins can be precipitated by heat, dialysis, electrodialysis, salts, acids, bases or organic chemicals. Mattil (1971) reported that lowest nitrogen solubility of cottonseed protein is at pH 4·0. Beradi *et al.* (1969) reported that the isoelectric point for water-soluble proteins is at pH 4·0 and that of storage proteins is at pH 7·0 and that a combination of the two groups in the proportion found in the flour has minimum solubility at pH 5·0. El Tinay *et al.* (1980) reported that a protein coagulation of 96% could be obtained at pH 4·5. Pant & Tulsiani (1969) reported that treatment of protein fractions with strong acids and bases decreased their nutritive value; thus the best method for isolating protein from dry seeds would be to extract them with NaCl followed by precipitation by dialysis of the extract. Such protein precipitates seem to be of high purity since they contain less non-protein material compared with those isolated by other methods.

This paper reports on factors affecting protein and gossypol recovery as well as the protein content of the isolates obtained from glanded cottonseed flour salt extracts.

## MATERIALS AND METHODS

Materials and protein extraction and determination were as described in 'Aqueous protein and gossypol extraction from glanded cottonseed flour: factors affecting protein extraction (*Food Chemistry*, **29**, 1988, 57–63)'.

### Protein coagulation

Protein coagulation at the isoelectric point was carried out by taking 40 ml from each salt extract and the pH was adjusted to 4·5 using 1N HCl. The protein precipitated was separated by centrifugation. The protein content of

the whey was determined and the protein precipitate was dried at 50°C in a vacuum oven for 10 h.

Protein coagulation by dialysis was carried out in a 3·5 cm diameter dialysis bag of 10 cm length. The dialysis bags were boiled in distilled water before use and were put in containers containing distilled water and kept in a refrigerator. Ten millilitres of the protein extract were placed in each dialysis bag and were sealed. The bags with contents were placed in large containers and were dialyzed against distilled water. The water was changed until it was free from salt. The contents of each dialysis bag were transferred to a 50 ml volumetric flask. Volumes were made to 50 ml with distilled water. The precipitate was removed by centrifugation. The nitrogen in the whey was determined by the micro-Kjeldahl method. The precipitate was dried at 50°C for 10 h in a vacuum oven and its nitrogen content determined.

Protein was also coagulated at the isoelectric point followed by coagulation of the whey protein by dialysis as described above. Also, the protein was coagulated by dialysis and this was followed by isolelectric precipitation of the protein in the whey.

### Gossypol determination

Free gossypol was determined according to the AOCS (1969) method, Ba 7-58. Total gossypol was determined by the same method after acid hydrolysis using 0·1% oxalic acid.

All determinations were carried out in triplicate and the mean and standard deviation calculated.

## RESULTS AND DISCUSSION

Protein precipitation at pH 4·5 in extracts using $CaCl_2$ at 10:1 solvent to flour ratio ranged from 20·1 to 65·0% (Table 1). These results are similar to those obtained by El Tinay *et al.* (1980). Protein coagulation at pH 4·5, using NaCl at 10:1 solvent to flour ratio, ranged from 42·0% to 76·1%. Protein coagulation increased markedly as $CaCl_2$ concentration increased. Protein coagulation from NaCl extracts increased markedly from 0·2 to 0·4M. Table 1 also shows the effect of solvent to flour ratio on protein coagulation at the isoelectric point. Protein coagulation decreased with increasing solvent to flour ratio.

Protein coagulation from hot-defatted cottonseed flour precipitated at the isoelectric point is shown in Table 2. Protein coagulation using $CaCl_2$ extracts at 10:1 solvent to flour ratio ranged from 31% to 53%; at 15:1 solvent to flour ratio it ranged from 34·00% to 55·95%; and at 20:1 solvent

**TABLE 1**
Isoelectric Protein Precipitation from Cold-Defatted Flour Extracts using $CaCl_2$ and NaCl

| | *Cold-defatted flour* | | | |
|---|---|---|---|---|
| *Extraction method* *Solvent:Flour* | *Protein precipitated from $CaCl_2$ extracts at isoelectric pH ± SD (%)* | *Protein isolate yield (g per 100 g flour)* | *Protein precipitated from NaCl extracts at isoelectric pH ± SD (%)* | *Protein isolate yield (g per 100 g flour)* |
| 10:1 | | | | |
| 1M Salt | 65·01 ± 1·91 | 22·80 | 75·00 ± 2·16 | 27·81 |
| 0·8 | 57·85 ± 2·36 | 19·07 | 76·10 ± 1·73 | 27·87 |
| 0·6 | 50·62 ± 2·53 | 15·38 | 70·72 ± 1·68 | 24·18 |
| 0·4 | 42·30 ± 1·76 | 10·95 | 67·01 ± 1·84 | 20·50 |
| 0·2 | 20·10 ± 2·11 | 3·97 | 42·11 ± 0·92 | 7·61 |
| 15:1 | | | | |
| 1M | 60·11 ± 0·88 | 21·75 | 70·20 ± 0·88 | 25·99 |
| 0·8 | 50·10 ± 0·92 | 17·71 | 71·81 ± 0·67 | 26·75 |
| 0·6 | 45·90 ± 0·80 | 15·13 | 69·00 ± 0·77 | 25·84 |
| 0·4 | | | | |
| 0·2 | | | | |
| 20:1 | | | | |
| 1M | 54·92 ± 1·31 | 19·99 | 62·13 ± 1·14 | 24·01 |
| 0·8 | 49·13 ± 1·66 | 17·12 | 60·28 ± 0·83 | 22·86 |
| 0·6 | 40·01 ± 1·01 | 13·94 | 60·33 ± 0·92 | 23·14 |
| 0·4 | | | | |
| 0·2 | | | | |

to flour ratio it ranged from 18·51% to 44·02%. The results show that the increase of solvent to flour ratio did not improve protein coagulation at pH 4·5. Protein coagulation was generally poor even at 1M $CaCl_2$ concentration.

Results of protein coagulation at the isoelectric pH after dialysis of the extracts are shown in Table 2. The results indicate that exposure of protein extracts to dialysis affected protein precipitation at the isoelectric point. This may be due to the fact that the presence of salts increases the solubility of proteins at the isoelectric pH. Protein coagulation in extracts of $CaCl_2$ at 10:1, 15:1 and 20:1 solvent to flour ratios, ranged from 45·0% to 67·5%, 40·0% to 60·0% and from 40·0% to 60·1%, respectively. Protein coagulation decreased as the solvent to flour ratio increased. Also, 0·2 and 0·4M $CaCl_2$ concentrations gave low protein precipitation.

Table 2 shows results for protein coagulation by dialysis. It ranged from 64·0% to 82·0%, 63·3% to 80·0% and from 65·1% to 75·0% using 10:1, 15:1

**TABLE 2**

Protein Coagulation by Isoelectric Precipitation, Isoelectric Coagulation after Dialysis of Extracts, Coagulation by Dialysis alone and Isoelectric Precipitation and Dialysis of the Whey from Hot-Defatted Flour Extracts

| | *Hot-defatted flour* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Extraction method* *Solvent:Flour* | *Protein precipitated at isoelectric pH ± SD (%)* | *Protein isolate yield (g per 100 g flour)* | *Protein precipitated at isoelectric pH after dialysis of protein extract ± SD (%)* | *Protein isolate yield (g per 100 g flour)* | *Protein coagulated by dialysis ± SD (%)* | *Protein isolate yield (g per 100 g flour)* | *Total protein precipitated by isoelectric coagulation and dialysis of the whey ± SD (%)* | *Protein yield (g per 100 g flour)* |
| 10:1 | | | | | | | | |
| 1M Salt | 50·12 ± 1·37 | 13·36 | 62·51 ± 1·12 | 16·70 | 80·60 ± 0·34 | 21·15 | 83·31 ± 0·69 | 22·22 |
| 0·8 | 52·98 ± 1·54 | 14·95 | 65·89 ± 0·97 | 18·61 | 81·21 ± 0·17 | 22·84 | 89·90 ± 1·23 | 25·35 |
| 0·6 | 39·50 ± 1·11 | 10·86 | 67·51 ± 0·79 | 19·54 | 81·96 ± 0·11 | 23·74 | 80·11 ± 1·23 | 23·16 |
| 0·4 | 33·19 ± 0·66 | 8·03 | 50·11 ± 1·00 | 12·10 | 75·13 ± 0·56 | 18·15 | 87·21 ± 0·68 | 21·05 |
| 0·2 | 31·00 ± 1·20 | 6·78 | 45·12 ± 1·23 | 9·84 | 64·22 ± 0·43 | 13·00 | 85·22 ± 1·00 | 18·63 |
| 15:1 | | | | | | | | |
| 1M | 45·02 ± 0·82 | 12·57 | 59·50 ± 1·53 | 16·61 | 77·88 ± 0·28 | 21·78 | 84·50 ± 1·54 | 23·59 |
| 0·8 | 35·10 ± 0·83 | 9·75 | 37·52 ± 1·22 | 15·97 | 80·01 ± 0·36 | 22·23 | 91·49 ± 1·13 | 25·42 |
| 0·6 | 55·95 ± 1·36 | 16·33 | 60·11 ± 1·13 | 17·51 | 78·20 ± 0·17 | 22·82 | 86·52 ± 0·71 | 25·50 |
| 0·4 | 34·22 ± 0·59 | 9·83 | 45·00 ± 0·46 | 13·00 | 74·00 ± 0·40 | 21·39 | 86·53 ± 0·22 | 25·00 |
| 0·2 | 37·51 ± 1·33 | 7·54 | 40·13 ± 0·98 | 8·04 | 63·34 ± 0·38 | 12·73 | 82·11 ± 0·46 | 16·69 |
| 20:1 | | | | | | | | |
| 1M | 44·02 ± 1·36 | 11·10 | 60·10 ± 1·33 | 15·16 | 75·14 ± 0·63 | 18·92 | 77·78 ± 0·68 | 20·13 |
| 0·8 | 39·00 ± 0·44 | 9·98 | 51·00 ± 0·87 | 13·05 | 68·70 ± 0·45 | 17·58 | 78·99 ± 0·44 | 20·22 |
| 0·6 | 36·21 ± 0·75 | 9·25 | 55·14 ± 1·21 | 14·13 | 72·99 ± 0·45 | 18·75 | 80·13 ± 1·02 | 20·55 |
| 0·4 | 24·50 ± 1·11 | 6·27 | 40·21 ± 0·66 | 10·29 | 74·10 ± 0·22 | 18·94 | 82·50 ± 0·66 | 21·11 |
| 0·2 | 18·51 ± 0·56 | 3·34 | 39·97 ± 1·35 | 7·22 | 65·09 ± 1·00 | 11·73 | 77·93 ± 0·36 | 14·09 |

**TABLE 3**

Protein, Free and Total Gossypol Content of Protein Isolates obtained by Isoelectric Coagulation and by Dialysis

| *Extraction method* *Solvent: Flour* | *Protein content of isoelectric isolates* (%) | *Protein content of dialysis isolated* ± *SD* (%) | *Free gossypol content of isoelectric isolates* ± *SD* (%) | *Free gossypol content of dialysis isolates* ± *SD* (%) | *Total gossypol content of isoelectric isolates* ± *SD* (%) | *Total gossypol content of dialysis isolates* ± *SD* (%) |
|---|---|---|---|---|---|---|
| 10:1 | | | | | | |
| 1M $CaCl_2$ | 55·0 ± 1·34 | 91·4 ± 0·32 | 0·04 ± 0·0059 | 0·02 ± 0·0017 | 0·54 ± 0·0236 | 0·50 ± 0·0473 |
| 0·8 | 60·3 ± 0·93 | 96·3 ± 0·17 | 0·05 ± 0·0043 | 0·01 ± 0·0030 | 0·53 ± 0·0550 | 0·53 ± 0·0233 |
| 0·6 | 67·1 ± 1·11 | 94·0 ± 1·55 | 0·05 ± 0·0025 | 0·02 ± 0·0034 | 0·60 ± 0·0291 | 0·51 ± 0·0187 |
| 0·4 | 65·0 ± 1·00 | 93·0 ± 0·41 | 0·06 ± 0·0031 | 0·02 ± 0·0022 | 0·30 ± 0·0473 | 0·40 ± 0·0231 |
| 0·2 | 70·3 ± 1·31 | 95·3 ± 0·97 | 0·09 ± 0·0022 | 0·04 ± 0·0057 | 0·35 ± 0·0280 | 0·30 ± 0·0426 |
| 15:1 | | | | | | |
| 1M | 65·0 ± 0·71 | 94·3 ± 0·17 | 0·03 ± 0·0045 | 0·01 ± 0·003 | 0·54 ± 0·0412 | 0·50 ± 0·0147 |
| 0·8 | 65·8 ± 0·56 | 96·3 ± 0·84 | 0·03 ± 0·0052 | 0·01 ± 0·0036 | 0·50 ± 0·0364 | 0·50 ± 0·0163 |
| 0·6 | 70·3 ± 1·11 | 96·0 ± 0·55 | 0·04 ± 0·0082 | 0·01 ± 0·0015 | 0·39 ± 0·0139 | 0·48 ± 0·0350 |
| 0·4 | 72·2 ± 1·67 | 98·9 ± 0·21 | 0·06 ± 0·00069 | 0·02 ± 0·0024 | 0·32 ± 0·0455 | 0·40 ± 0·0226 |
| 0·2 | 70·0 ± 0·85 | 98·4 ± 0·13 | 0·10 ± 0·0244 | 0·02 ± 0·0039 | 0·32 ± 0·0236 | 0·35 ± 0·0308 |
| 20:1 | | | | | | |
| 1M | 66·1 ± 0·67 | 99·2 ± 0·77 | 0·03 ± 0·0066 | 0·01 ± 0·0027 | 0·32 ± 0·0251 | 0·46 ± 0·0183 |
| 0·8 | 67·8 ± 0·46 | 98·5 ± 0·31 | 0·02 ± 0·0071 | 0·01 ± 0·0019 | 0·30 ± 0·0393 | 0·47 ± 0·0445 |
| 0·6 | 75·0 ± 1·37 | 98·4 ± 0·13 | 0·04 ± 0·0034 | 0·01 ± 0·0011 | 0·35 ± 0·0126 | 0·40 ± 0·0177 |
| 0·4 | 76·2 ± 2·37 | 97·6 ± 0·42 | 0·05 ± 0·0016 | 0·01 ± 0·0008 | 0·30 ± 0·0277 | 0·27 ± 0·0251 |
| 0·2 | 74·9 ± 1·56 | 97·3 ± 0·30 | 0·10 ± 0·0236 | 0·01 ± 0·0020 | 0·28 ± 0·0117 | 0·30 ± 0·0117 |

and 20:1 solvent to flour ratios, respectively. Dialysis resulted in higher protein coagulation compared to isoelectric precipitation and isoelectric coagulation after dialysis. This may be due to the fact that most of the cottonseed proteins are globulins which are salt-soluble proteins. Data on protein coagulation by dialysis is not available. However, Murray *et al.* (1981) obtained protein isolates of high purity by subsequent dilution of the salt extract.

Table 2 shows results obtained from isoelectric precipitation of the protein followed by protein coagulation in the whey by dialysis. Total protein precipitated ranged from 80·0% to 89·9%, 83·0% to 91·5% and from 79% to 82·5% using 10:1, 15:1 and 20:1 solvent to flour ratios, respectively (Table 2). Precipitation of proteins from salt extracts at pH 4·5 followed by coagulation of the protein remaining in solution by dialysis resulted in higher protein recovery compared to that induced by dialysis or isoelectric precipitation separately.

The protein content of the isolates prepared by isoelectric coagulation is shown in Table 3. Using 10:1 solvent to flour ratio, it ranged from 55·0% to 70·3%, with 15:1 it ranged from 65·0% to 72·2% and with 20:1 it ranged from 66·1% to 76·2%. The protein content of the isolates increased with increasing solvent to flour ratio and decreased with increasing $CaCl_2$ concentration.

The protein content of isolate obtained by dialysis is shown in Table 3. It ranged from 91·4% to 96·3%, 94·3% to 98·9% and from 97·3% to 99·2% at 10:1, 15:1 and 20:1 solvent to flour ratio, respectively. These values are markedly high compared to the protein contents of isolates obtained by isoelectric precipitation. Murray et al. (1981) used subsequent dialysis of NaCl extracts of faba bean proteins and obtained protein contents which amounted to 95·6%.

Results in Table 3 show the free gossypol content of protein isolates obtained by isoelectric coagulation. Using 10:1, 15:1 and 20:1 solvent to flour ratios, the free gossypol ranged from 0·040% to 0·090%, 0·030% to 0·10% and from 0·020% to 0·10%, respectively. The free gossypol content of protein isolates increased with decreasing salt concentration and decreasing solvent to flour ratio. The total gossypol content of isolates obtained by isoelectric precipitation is shown in Table 3. Using 10:1, 15:1 and 20:1 solvent to flour ratios, the total gossypol contents ranged from 0·30% to 0·60%, 0·32% to 0·54% and from 0·28% to 0·35%, respectively. Total gossypol content was low at lower salt concentration and at 20:1 solvent to flour ratio.

The free gossypol content of isolates coagulated by dialysis is shown in Table 3. Using 10:1, 15:1 and 20:1 solvent to flour ratios, the free gossypol ranged from 0·010% to 0·040%, 0·010% to 0·020% and was 0·010%,

respectively. Variations in free gossypol content between isolates obtained by dialysis at different $CaCl_2$ concentrations and different solvent to flour ratios were very low. This was more pronounced with higher solvent to flour ratios. The total gossypol content of isolates coagulated by dialysis is shown in Table 3. Using 10:1, 15:1 and 20:1 solvent to flour ratios, the total gossypol ranged from 0·30% to 0·53%, 0·35% to 0·50% and from 0·27% to 0·47%, respectively.

Isolates obtained by isoelectric precipitation and those obtained by dialysis differed in their free gossypol content, but were similar in their total gossypol content. The free gossypol content of isolates coagulated by dialysis was low compared to that obtained by isoelectric precipitation.

## CONCLUSIONS

The best method for protein recovery from hot defatted cottonseed flour is coagulation by dialysis. This method resulted in a protein recovery of 81·2% and a protein yield of 23·9 g per 100 g of flour having 0·010% free gossypol and 0·53% total gossypol. This level of free gossypol in the resultant protein isolate is far below the toxic limit (0·06%) and the isolate can be considered an edible grade protein.

## REFERENCES

Altschull, A. M. (1958). *Processed plant protein foodstuffs*, Academic Press, New York.

AOCS (1969). *Official and tentative methods of the American Oil Chemists Society*, Champaign, III. Method Ba 7–58.

Beradi, L. C., Martinez, W. H. & Fernandez, C. J. (1969). Cottonseed protein isolates: Two step extraction procedure. *Journal of Food Technology*, **23** (10), 75–82.

Eckey, E. W. (1954). *Vegetable fats and oils*, Reinhold Pub. Corp., New York.

El Tinay, A., Chandrasehkar, H. & Ramantham, G. (1980). Protein and gossypol extractability from cottonseed flour. *Journal of the Science of Food and Agriculture*, **31**, 38–42.

Fox, W., Foster, E. R. & Joseph, F. (1957). *Introduction to protein chemistry*, John Wiley and Sons, London.

Mattil, K. F. (1971). The functional requirements of proteins for food. *Journal of the American Oil Chemists Society*, **48**, 477–80.

Murray, E. D., Myers, C. D., Barker, L. D. & Maurice, T. J. (1981). Functional attributes of proteins. A non-covalent approach to processing and utilizing plant proteins. (Stanley, D. W., Murray, E. D. & Less, D. H. (Eds)), Food and Nutrition Inc., Westport, USA.

Noyes, R. (1969). *Protein Food Supplements. Food Processing Review No. 3*, Noyes Development Corporation, Park Ridge, New Jersey, USA, 232–45.

Pant, R. & Tulsiani, D. R. P. (1969). Solubility, amino acid composition and biological evaluation of protein isolation from leguminous seed. *Journal of Agriculture and Food Chemistry*, **17**, 361–6.