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

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

Department of Biochemistry, Faculty of Agriculture, Shambat, Sudan

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ABSTRACT

*Factors affecting protein extraction from both heated and unheated cottonseed flours were studied. Unheated flour was extracted with NaCl and $CaCl_2$ at five levels of concentration and three levels of solvent to flour ratio. The effect of salt concentration beyond 0·4*M *was more pronounced for $CaCl_2$ than for NaCl. Protein extraction with NaCl was more efficient than with $CaCl_2$ with much of the protein extracted at lower concentrations compared to $CaCl_2$. Heat treatment of the flour resulted in a drop in protein extraction from 74·5 to 55·2 and from 78·7 to 55·0% for $CaCl_2$ and NaCl, respectively. The nature of the salt seemed to have no effect on protein extraction from heated flour. A two step extraction procedure involving successive extraction with water and $CaCl_2$ resulted in improved protein extraction from heated cottonseed flour.*

INTRODUCTION

In recent years attention has been focussed on oilseed crops as largely untapped sources of food proteins. This attention has resulted from grave concern over the lack of adequate protein to provide needed nourishment for a large segment of the world's population in the years ahead. In Sudan about 400 000 tons of cottonseed are produced annually and this output is mainly used for production of edible oil. The resultant cake is used for animal feeding. According to Noyes (1969) cottonseed is only slightly inferior to soybean as a protein source. This is due to a low content of methionine and lysine. Cottonseed meal is being utilized for human consumption in Latin America and, to a limited extent, in other parts of the world (Noyes, 1969). Currently, edible protein is prepared from cottonseed meals by aqueous processing of gland-free meals or from deglanded cottonseed flour. The liquid cyclone process is the method commonly used industrially for preparation of the deglanded cottonseed flour.

Extractions of cottonseed proteins with water, salt and alkaline solutions were carried out by Lawhon *et al.* (1972) and El Tinay *et al.* (1980). Martinez (1964) and Martinez *et al.* (1965) mentioned that cottonseed protein bodies remain intact during oil extraction, grinding and suspension in water. Smith (1971) reported that, unlike those of soybean and peanut, protein bodies of cottonseed do not rupture on suspension in water to produce a water dispersion of storage protein. He mentioned that alkali or salt is needed to rupture the protein body membranes and to solubilize storage proteins of cottonseed. Beradi *et al.* (1969) developed a two-step extraction procedure that isolated water-soluble (non-storage) proteins of low molecular weight which were precipitated out at pH 4·0 and high molecular weight (storage) alkaline soluble proteins which were precipitated out at pH 7·0.

This paper reports on factors influencing aqueous protein extraction from glanded cottonseed flour, namely, optimum extraction time, effect of heat-treatment of cottonseed flour, solvent to flour ratio, type and concentration of salt and single step versus two-step extraction.

MATERIALS AND METHODS

Cottonseed, variety Barac 67B, was obtained from Medani Research Station, Sudan Agricultural Research Corporation. Lints were removed using 50% aqueous sulphuric acid. The delinted seeds were sorted to remove injured or acid-affected seeds. They were then milled using an electric grinder to a mesh size of 80–100. The resultant cottonseed flour was defatted with *n*-hexane at room temperature (25°C) for one portion and in a Soxhlet extractor for the second portion. The oil-free flour was desolventized in open air at room temperature. Cottonseed flours prepared by the two methods were separately kept in tight glass containers and were placed in a refrigerator until used.

Protein extraction

Optimum time was determined keeping other factors constant. Extraction periods tested were 30, 45 and 60 min. One gram of flour was suspended in 10 ml of 1 M $CaCl_2$ and shaken for the specified period. The peptized liquor was separated from insoluble material by centrifugation. Two millilitres of the liquor were taken for nitrogen determination according to the AOAC (1970) micro-Kjeldahl method.

Protein extraction using either $CaCl_2$ or NaCl was carried out with 0·2, 0·4, 0·6, 0·8 and 1 M salt concentrations. The solvent to flour ratios employed were: 10:1, 15:1 and 20:1. Extraction time was 30 min. The peptized liquor was separated from insoluble residue by centrifugation and aliquots were taken for nitrogen determination.

A two-step protein extraction procedure consisted of a water extraction step followed by $CaCl_2$ extraction. One gram of hot defatted cottonseed flour was suspended in 20 ml of distilled water and shaken for 30 min. The peptized liquor was separated by centrifugation and aliquots were taken for nitrogen determination. The insoluble residue was re-extracted with 10 ml of 0·2, 0·4, 0·6, 0·8 or 1 M $CaCl_2$. The peptized liquor was separated by centrifugation and aliquots were taken for nitrogen determination. The protein extracted in the two steps was summed up.

Protein extraction was also conducted using two successive $CaCl_2$ extractions. One gram of the hot defatted cottonseed flour was suspended in 20 ml of 1 M $CaCl_2$ and shaken for 30 min. The peptized liquor was separated by centrifugation and aliquots were taken for nitrogen determination. The insoluble residue was re-extracted using 0·2, 0·4, 0·6, 0·8 or 1 M $CaCl_2$. The protein content of the peptized liquors was determined and the total protein extracted in both steps was summed. All determinations were carried out in triplicate and the mean and standard deviations calculated.

RESULTS AND DISCUSSION

Analytical data on the two cottonseed flours before protein extraction are shown in Table 1. Hot defatted flour differed from the cold extracted flour in total and free gossypol contents. Lower free gossypol content in the hot

TABLE 1
Analytical Data on Cottonseed Flours Used for Protein Extraction

Flour source	*Moisture* (%)	*Oil* (%)	*Protein* (%)	*Crude fibre* (%)	*Gossypol free* (%)	*Total* (%)
Cold defatted	5·30	0·900	47·1	23·4	0·95	1·08
Hot defatted (Soxhlet extracted)	5·35	0·800	48·4	24·9	0·75	0·90

TABLE 2
Effect of Extraction Time in Protein Extraction from Cottonseed Flours

Salt (1 M)	*Flour source*	*Extraction time (min)*	*(%) Protein extraction ± SD*
$CaCl_2$	Hot defatted	30	55·2 ± 0·77
$CaCl_2$	Hot defatted	45	57·0 ± 0·71
$CaCl_2$	Hot defatted	60	56·0 ± 0·25
$CaCl_2$	Cold defatted	30	74·5 ± 0·61
$CaCl_2$	Cold defatted	45	76·2 ± 0·30
$CaCl_2$	Cold defatted	60	77·0 ± 0·69
NaCl	Hot defatted	30	55·0 ± 0·41
NaCl	Cold defatted	30	78·7 ± 0·72

defatted flour may have resulted from binding of gossypol to the protein as a result of heat treatment.

Table 2 shows the effect of extraction time on protein extracted. Increasing extraction time from 30 to 60 min was not accompanied by significant increase in the extractable protein. Beradi *et al.* (1967) and Martinez *et al.* (1967) reported that time above 30 min was not important to

TABLE 3
Effect of $CaCl_2$ Concentration and Solvent to Flour Ratio on Protein Extracted from Hot Defatted Flour

		pH of slurry	*(%) Protein extracted ± SD*
10:1 (Solvent:Flour)			
$CaCl_2$	1 M	4·99	55·2 ± 0·77
	0·8 M	4·93	58·3 ± 0·40
	0·6 M	4·88	59·8 ± 0·80
	0·4 M	4·82	50·0 ± 0·57
	0·2 M	4·80	45·2 ± 0·66
15:1 (Solvent:Flour)			
$CaCl_2$	1 M	5·49	57·7 ± 0·40
	0·8 M	5·30	57·4 ± 0·40
	0·6 M	5·10	60·3 ± 0·83
	0·4 M	4·92	59·7 ± 0·84
	0·2 M	4·78	41·5 ± 0·59
20:1 (Solvent:Flour)			
$CaCl_2$	1 M	5·53	52·1 ± 0·73
	0·8 M	5·36	52·9 ± 0·61
	0·6 M	5·21	53·1 ± 0·32
	0·4 M	5·05	52·9 ± 0·57
	0·2 M	4·91	37·3 ± 0·71

TABLE 4
Effects of NaCl and $CaCl_2$ Concentrations and Solvent to Flour Ratio on Protein Extraction from Cold Defatted Flour

	pH of slurry $(CaCl_2)$	*(%) Protein extracted with* $CaCl_2 \pm SD$	*pH of slurry (NaCl)*	*(%) Protein extraction with* $NaCl_2 \pm SD$
10:1 (Solvent:Flour)				
1M	4·77	74·5 ± 0·61	5·75	78·8 ± 0·72
0·8M	4·76	70·0 ± 0·66	5·73	77·8 ± 0·53
0·6M	4·72	64·5 ± 0·59	5·72	72·6 ± 0·70
0·4M	4·72	55·0 ± 0·70	5·72	65·0 ± 0·11
0·2M	4·71	40·0 ± 0·63	5·69	38·5 ± 0·64
15:1 (Solvent:Flour)				
1M	4·90	70·0 ± 0·31	5·79	78·7 ± 0·30
0·8M	5·00	75·10 ± 0·62	5·47	79·1 ± 0·12
0·6M	5·51	70·0 ± 0·37	5·76	79·5 ± 0·37
20:1 (Solvent:Flour)				
1M	5·71	77·3 ± 0·30	5·85	82·1 ± 0·10
0·8M	5·26	74·0 ± 0·55	5·83	80·5 ± 0·40
0·6M	4·85	74·1 ± 0·46	5·79	81·9 ± 0·33

optimum protein extraction if the extracting solvent contained sufficient ions for maximum nitrogen solubility.

Protein extraction from cold defatted cottonseed flour was significantly higher than that extracted from hot defatted flour (Table 2). Noyes (1969) mentioned that one of the disadvantages of the treatment of cottonseed products with heat to remove gossypol is that it results in protein denaturation.

Table 3 shows the protein extracted from hot defatted cottonseed flour at various salt concentrations and different solvent:flour ratios. The results indicate that protein extractibility increased with increased $CaCl_2$ concentration from 0·2 to 0·6M, beyond which protein extraction became constant or decreased slightly as in the case of the 10:1 solvent to flour ratio. Increasing the solvent to flour ratio from 10:1 to 20:1 did not result in significant increase in protein extraction.

Table 4 shows the effect of varying salt concentration on protein extraction from cold defatted cottonseed flour. Protein extractability improved as the salt concentration was increased. Using varying concentrations of NaCl, the amount of extractable protein increased as the salt concentration was increased from 0·2 to 0·8M. Protein extraction using NaCl was generally higher than that obtained with $CaCl_2$. In general,

TABLE 5
Protein Extracted with Water and $CaCl_2$ in a Two-Step Extraction Procedure (from Hot Defatted Flour)

Step I (20:1 ratio v/wt)	*pH of slurry*	*Step II (10:1 ratio v/wt)*	*pH of slurry*	*(%) Protein extracted in the two steps*	*(%) Protein extracted in Step II ± SD*
Water[a]	6·24	1M $CaCl_2$	6·52	78·9	61·6 ± 1·86
Water	6·24	0·8M $CaCl_2$	6·12	74·0	56·7 ± 1·79
Water	6·24	0·6M $CaCl_2$	5·76	62·7	45·4 ± 2·37
Water	6·24	0·4M $CaCl_2$	5·45	50·8	33·5 ± 0·91
Water	6·24	0·2M $CaCl_2$	5·19	39·6	22·3 ± 1·83
1M $CaCl_2$[b]	5·35	1M $CaCl_2$	7·33	59·7	7·59 ± 0·78
1M $CaCl_2$	5·35	0·8M $CaCl_2$	7·00	57·4	5·28 ± 1·56
1M $CaCl_2$	5·35	0·6M $CaCl_2$	6·82	61·2	9·03 ± 0·88
1M $CaCl_2$	5·35	0·4M $CaCl_2$	6·51	62·6	10·5 ± 1·21
1M $CaCl_2$	5·35	0·2M $CaCl_2$	5·99	60·6	8·48 ± 0·94

[a] Protein extracted in step I was constant (17·3).
[b] Protein extracted in step I was constant (52·1).

protein extraction increased slightly as the solvent to flour ratio increased and this was more pronounced as the salt concentration was decreased from 1 to 0·6M (Table 4).

Table 5 shows protein extraction using a two step procedure involving water extraction followed by salt extraction. The protein extracted in step 1 was constant and amounted to 17·3%. Total protein extraction increased as the salt concentration was increased. Maximum protein extraction (78·9%) was obtained when water extraction was followed by 1M $CaCl_2$ extraction. The improvement in protein extraction caused by successive water and salt extraction may be due to the fact that cottonseed protein is composed of water-soluble and salt-soluble proteins. Mirgani (1974) reported that cottonseed protein is composed of 19·2% albumins, 57·1% globulin and 21·5% glutelins.

Table 5 also shows two step extraction using 1 M $CaCl_2$ in the first step and 1, 0·8, 0·6, 0·4 and 0·2M $CaCl_2$ in the second step. The results indicate that protein extraction was not improved in the two step salt extractions.

CONCLUSIONS

From the results of the present investigation, it can be concluded that for unheated cottonseed flour, NaCl is more efficient than $CaCl_2$ in protein extraction. On the other hand, heating of the flour decreases the percentage of protein extracted in the presence of either NaCl or $CaCl_2$. Improvement of protein extraction in the case of heated flour can be achieved by a 2-step extraction involving water and $CaCl_2$, resulting in extraction of 38·2 g protein from 100 g cottonseed flour.

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