Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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V. Lakshman[a]; D. N. Bhowmick[a]
[a] DEPARTMENT OF CHEMICAL TECHNOLOGY, UNIVERSITY OF BOMBAY MATUNGA, BOMBAY, INDIA

To cite this Article Lakshman, V. and Bhowmick, D. N.(1994) 'Effect of Processing Parameters on Gossypol Levels in Protein Isolates from Cottonseed by Alkali Extraction/Ultrafiltration/Diafiltration', Separation Science and Technology, 29: 15, 2035 — 2046
To link to this Article: DOI: 10.1080/01496399408002188
URL: http://dx.doi.org/10.1080/01496399408002188

SEPARATION SCIENCE AND TECHNOLOGY, 29(15), pp. 2035–2046, 1994

Effect of Processing Parameters on Gossypol Levels in Protein Isolates from Cottonseed by Alkali Extraction/Ultrafiltration/Diafiltration

V. LAKSHMAN and D. N. BHOWMICK*
DEPARTMENT OF CHEMICAL TECHNOLOGY
UNIVERSITY OF BOMBAY
MATUNGA, BOMBAY 400019, INDIA

ABSTRACT

Three different varieties of cottonseed (S1, S2, and S3) were dehulled, separated, and defatted by hexane extraction under controlled conditions (moisture < 3%, temperature < 50°C). The defatted flours, designated as CS1 and CS2 (glandless) and CS3 (glanded), which had free-gossypol levels of 0.15, 0.28, and 0.68%, respectively, and protein levels of 61.4, 61.7, and 58.5%, respectively, were taken for aqueous extraction using NaOH as the alkali in the presence of $Na_2S_2O_4$, a reducing agent. The extract, after centrifugation, was immediately taken for ultrafiltration (UF) using polysulfone membranes, followed by diafiltration (DF). Experiments at 40 and 60°C, to examine the UF performance and gossypol binding effect, were carried out with strict control of the feed pH. The intensely yellow-colored permeates, probably due to alkali-soluble sodium gossypolate and gossypol-like pigments, were checked for color intensity as a qualitative measure in ultrafiltration concentrate. The intensity was found to be on the decline, less during UF and more during diafiltration. The final UF/DF dried products were analyzed for free gossypol (FG), bound gossypol (BG), total gossypol (TG), and protein. Protein isolates (PI) from Samples CS1 and CS2 were found to have very low FG, with little effect of the processing conditions on binding of gossypol with the protein. PI from Sample CS3 was found to have slightly high FG with relatively high BG. The effect of temperature was found to result in high permeation rates without much effect on the rejection of the components and the binding of gossypol. The gossypol and protein contents of three UF/DF dried proteins were 0.006, 0.012, and 0.041% FG, and 89.4, 90.1, and 86.4% protein. The colors of PI from

* To whom correspondence should be addressed.

Samples CS1 and CS2 were lighter while PI from Sample CS3 was relatively darker.

Key Words. Ultrafiltration; Diafiltration; Gossypol; Defatted flour and cottonseed

INTRODUCTION

India is one of the major oilseed producing countries in the world, and ranks first in Asia and third in the world in cottonseed production, which is presently estimated to be around 32.5 million metric tons (1). Despite the surplus availability, there has been little effort directed toward the ultilization of cottonseed defatted flour. Cottonseed meal is an excellent source of protein and its potential value in the human diet has been well documented (2, 3). The use of cottonseed meal is restricted to animal feed or fertilizer due to the presence of a toxic substance, called "gossypol," present in a cellular structured gland. This yellow pigment constitutes 95% of the overall pigmentation present in the glands, which in turn constitute 2–4% of the seed kernel of the glanded cottonseed variety (4).

A part of the gossypol (1,1′,6,6′,7,7′-hexahydroxy-5-5′-diisopropyl-3-3′-dimethyl-[2,2′-binaphthalene]-8,8′-dicarboxaldehyde) becomes associated with the native protein under certain processing conditions, especially at high levels of moisture and temperature. This form of gossypol, known as "bound gossypol" (BG), has been reported to be physiologically ineffective (5). A further portion of gossypol which remains in the free form, known as "free gossypol" (FG), has been mainly responsible for toxicological effects in monogastric animals including human (5).

The gossypol levels in the specifications of proteins, meant for human consumption and laid down by WHO/FAO/UNICEF/Protein Advisory Group, are FG $< 0.045\%$ and total gossypol (TG) $< 1.0\%$. Therefore, the objectives of many processes have been the protein content, nutritive value, and gossypol levels.

Various methods that have been used/proposed to detoxify or reduce gossypol in cottonseed proteins, and the reasons for their success and/or failure are highlighted elsewhere (6). Alternative methods for detoxification presented in Reference 6 are reviewed in the present article, with additional comments to highlight the relevance of our work.

1. Controlled heating in the presence of moisture causes gossypol to interact with other components. This results in reduced digestibility owing to both denaturation and chemical modification of the protein. Also, there is a loss of biological value due to the interaction of the essential amino acid, lysine (7).

2. Complexing of gossypol with metal ions or chemical combination with aniline, ammonia, boric acid, etc. Removal of these complexes has been reported to be a problem (8). This is discussed later.

3. Air classification or liquid medium separative technique, such as the liquid cyclone process. This has been reported to be the only effective method, but it creates a byproduct problem. It is also uneconomical on a large scale (9).

4. Solvent extraction of cottonseed meats (oil-bearing), either to extract gossypol along with oil or as a secondary extraction of cottonseed meals (oil-free) to extract only gossypol by using various pure organic solvents and their combinations, including aqueous–organic azeotropic mixtures (10–13).

5. Cultivation of gossypol-free seeds (glandless) from selective breeding programs. The reasons for the small success of this on an overall basis has been attributed to poor lint quality, late maturity, and, most importantly, reduced resistance to insect infestation (7).

The last two approaches listed above seem to be the best alternatives for the preparation of cottonseed proteins free of gossypol as potential sources of proteins for food use.

Unfortunately, success in the evolution of glandless cottonseed in India is meagre owing to climatic and other factors related to cultivation, and many varieties have up to 0.27% free gossypol. Thus, the glanded cottonseed variety is still the predominantly grown variety, and it has free gossypol levels up to 2% (14, 15).

Not all extractions using different solvent systems resulted in the reduction of FG to the safer limits (<0.045%) meant for human consumption, and if they did, it was at the expense of either large meal-to-solvent ratios or more extraction steps involving two or more solvents (16).

Ultrafiltration was used in the present work, keeping in view the solution characteristics (aqueous extract from defatted flour) as well as the concentration/fractionation and purification capacity of this simple but effective separation technique.

The success of membrane ultrafiltration is now widely known in the dairy industry as a commercial process (17). Among numerous other applications, it has gained wide acceptance in oilseed flour extract processing for the production of protein isolates from soybean (18, 19) and rapeseed (20, 21). Work to date has been reported only on glandless cottonseed (with very low or no free gossypol) in the preparation of protein isolates by ultrafiltration (17, 19, 22, 23).

The first objective of the present work is to see the effects of direct aqueous alkali extraction/ultrafiltration/diafiltration on the quality of protein isolates prepared from defatted cottonseed flours having varying ini-

tial free gossypol levels. For this purpose, two glandless cottonseed meals (S1 and S2) were hexane extracted and gave defatted flours having free gossypol levels of 0.15 and 0.28%, respectively.

Second, to determine the extent of free gossypol levels in the starting defatted flours that are considered for the proposed treatment. For this, a glanded cottonseed variety with a relatively high free gossypol level (0.68%), in addition to the two glandless cottonseed varieties with low gossypol levels, was selected.

MATERIALS AND METHODS

Extract Preparation

Two glandless and one glanded cottonseed varieties from different breeding stations were collected through Cotton Technological Research Laboratory, Bombay, India. The seeds were dehulled, separated, and ground (at <3% moisture) in a disc mill in discrete modes so as not to allow the grinding temperature, due to friction, to exceed 50°C. The ground meal was then hexane extracted, followed by desolventization at mild temperatures. The defatted flour was then extracted (solids/water ratio = 1:20, pH 10, extraction time = 30 minutes) in an aqueous medium using NaOH as the alkali. To this, 1% (w/v) sodium dithionite was added. The insoluble material was removed by centrifugation in a Sharples super centrifuge (22,000*g*), and the extracts collected were immediately taken for ultrafiltration.

Ultrafiltration

Ultrafiltration was performed in a Sartocon II crossflow system (Sartorius GmbH, Germany) using a polysulfone mini-module of 0.2 m^2 area. The feed was pumped from a solution container and circulated through the whole system, i.e., the retentate was fed back to the feed tank. Permeate at different stages of UF and DF, along with the corresponding retentate samples, were collected for analysis.

A discontinuous mode of DF (i.e., dilution and reconcentration of UF concentrate) was performed. The feed pH (9.5) was strictly controlled throughout the UF/DF operation. The feed solution flow rate was 12 L/min, and the operating pressure was 200 kPa. The temperature of the solution was regulated by a coil connected to the temperature control bath and inserted in the sample container.

Experiments with Sample CS2 were made at 40 and 60°C, and the rest of the experiments were carried out at 60°C.

The steps involved in the preparation of protein isolates from cottonseed are seed processing, extraction, ultrafiltration, diafiltration, and drying, as shown in Fig. 1.

Analysis

Moisture, free gossypol, and total gossypol were determined according to the standard AOCS methods (25). Nitrogen content was determined by the micro-Kjeldahl method (26).

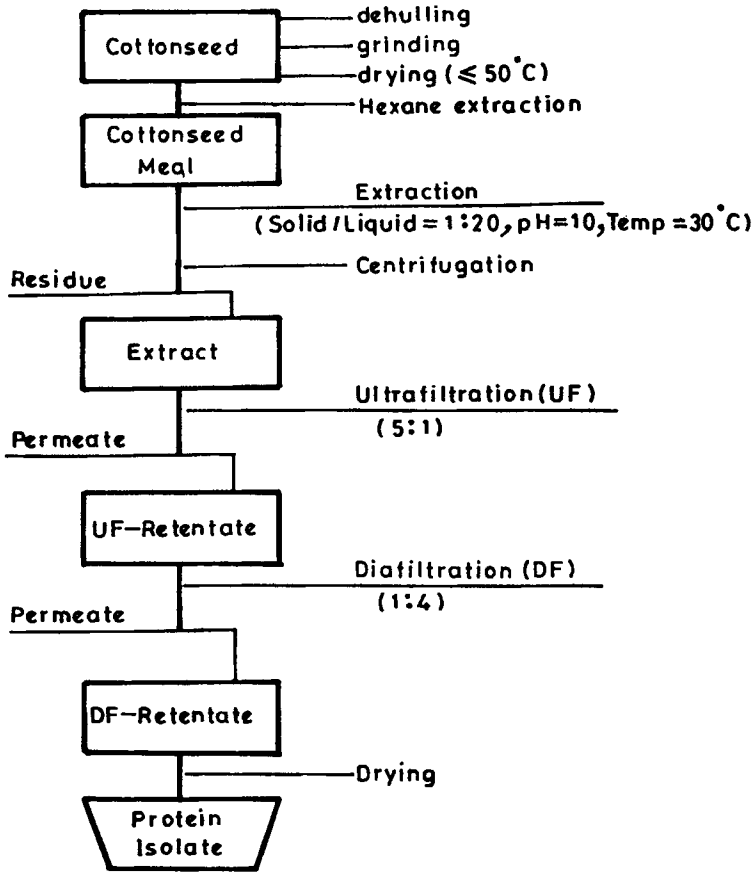

FIG. 1 Flow chart for the preparation of cottonseed protein isolate by extraction/ultrafiltration/diafiltration.

RESULTS AND DISCUSSION

The optimum conditions for seed processing are reported elsewhere (6). In the present case, dehulling was done at a moisture level of 6–7%, and the kernels thus obtained were further dried to a moisture content of 3% at a temperature not exceeding 50°C before hexane extraction. The pigment glands of the kernels remain intact under these conditions due to low moisture (glands rupture in contact with water) and moderate temperatures. As a result, the gossypol is not released and does not combine with either the protein or any other constituent (27). This is evident from the composition of the meals and defatted flours as shown in Table 1. However, some binding of gossypol seems to be inevitable. The purpose of processing the seed under controlled conditions in the present work is to obtain defatted flours having the lowest possible BG. The optimum extraction conditions are reported in Table 2. NaOH was selected as the alkali because it extracts more solids and protein from defatted flour compared to other hydroxides like $Ca(OH)_2$ and KOH.

When gossypol is extracted into an aqueous alkaline solution along with protein and other water-soluble components, it is expected to form a complex, disodium salt of gossypol (23). This has been reported to be unstable and may even result in oxidative products at very high pH values (>11).

TABLE 1
Composition of Undefatted Meals and Defatted Flours

Sample description[a]	Sample type	Moisture (%)	Gossypol (%)[b]			Protein (%)
			FG	BG	TG	
S1	Glandless (low gossypol)	5.90	0.12	0.03	0.15	41.5
CS1	—	2.10	0.15	0.055	0.21	61.4
S2	Glandless (low gossypol)	6.20	0.21	0.08	0.29	40.2
CS2	—	1.80	0.28	0.13	0.41	61.7
S3	Glanded (high gossypol)	6.40	0.51	0.25	0.76	38.6
CS3	—	1.85	0.68	0.36	1.10	58.9

[a] CS1, CS2, and CS3 are defatted flours from the corresponding meats S1, S2, and S3.
[b] FG, BG, and TG are free gossypol, bound gossypol, and total gossypol, respectively.

TABLE 2
Extraction Parameters

Solid/water ratio	1:20
Extraction pH	10.0
Extraction temperature	30°C
Extraction time	30 minutes

However, by the addition of $Na_2S_2O_4$, a reducing agent, at 1% by weight in NaOH solution, the oxidative decomposition of gossypol was reported to have been reduced (24). Also, the operating pH in the present work is 9.5, which is away from the critical value. Gossypol and gossypol-like pigments, either as sodium salts or in association with other components (other than protein), should be able to permeate during UF and DF (a solvent washing step).

During UF, the most critical parameter in our study was the pH of the feed solution. The solution was found to become turbid with a drift toward acidic pH, indicating the precipitation of protein. This was also observed by a decline in the permeation rate caused by the onset of concentration polarization (CP) and possible fouling. CP is a detrimental phenomenon occurring due to the accumulation of solutes on the membrane surface, and fouling is due to the interaction of the solute–membrane system (28).

There is a possibility that gossypol and gossypol-like pigments may also precipitate and adsorb on the protein due to its insoluble nature in water and in acidic medium. Therefore, experiments were carried out to minimize CP at the most appropriate conditions, i.e., low pressures and high flow rates across the membrane with a strict control of feed pH at 9.5.

The permeate from UF was yellow, as is sodium gossypolate. During UF, the permeate color did not change significantly with a change in concentration, but in the DF stages it did. This change in the permeate solution was used as a means of measuring the intensity of the color of the permeate, and hence its concentration, during UF/DF. To the same volumes of permeate (at the same pH) collected at different stages of UF/DF, the amount of acid (HCl) needed to make the solution colorless was found to be on the decline, thus strengthening our physical observation and probably indicating some kind of correlation. These data are not reported in the present work because we have only used it as a physical means of observation to see the effect of operating parameters on the final protein isolates.

Compositional data on ultrafiltered/diafiltered products (dried), undefatted meals, and defatted flours are shown in Table 3.

The operating temperature was found to have no significant effect on UF/DF products in terms of gossypol levels except that there was slight

TABLE 3
Composition Comparison of Undefatted Meals, Defatted Flours, and UF/DF Protein Isolates

Sample description	Sample type	Moisture (%)	Gossypol (%)			Protein (%)
			FG	BG	TG	
S1	Glandless (low gossypol)	5.90	0.120	0.030	0.150	41.5
CS1	—	2.10	0.150	0.055	0.210	61.4
(UF/DF)CS1	—		0.006	0.070	0.076	89.4
S2	Glandless (low gossypol)	6.20	0.210	0.080	0.290	40.2
CS2	—	1.80	0.280	0.130	0.410	61.7
(UF/DF)CS2	—		0.012	0.140	0.152	90.1
S3	Glanded (high gossypol)	6.40	0.510	0.250	0.760	38.6
CS3	—	1.85	0.680	0.360	1.100	58.5
(UF/DF)CS3	—		0.041	0.470	0.510	86.4

increase in the protein content, possibly owing to high permeation rates of unrejected solutes (Table 4). Our experiments were therefore carried out at 60°C to maintain high permeation rates and avoid any microbial growth.

The color of the PI (dried) prepared from Sample CS2 (at a UF operating temperature of 40°C) was found to be slightly darker at a protein content of 89.1%. Protein isolates prepared from Samples CS1 and CS2 at a UF operating temperature of 60°C were lighter and had protein contents of 90.2 and 88.7% (dry basis), respectively. Although the free gossypol content in Sample CS3 was reasonably reduced, the result was a slight in-

TABLE 4
Effect of UF/DF Operating Temperature on Binding of Gossypol (protein isolate from Sample CS2)

Sample description	Sample type	Moisture (%)	UF feed temperature (°C)	Gossypol (%)		
				FG	BG	TG
CS2	Glandless	1.8	—	0.280	0.130	0.410
(UF/DF)CS2	—	—	60	0.012	0.110	0.126
(UF/DF)CS2	—	—	40	0.010	0.070	0.080

crease in bound gossypol with a protein content of 86.4% (dry basis), and it was comparatively darker than the rest of the samples. Experiments with samples having free gossypol levels in the intermediate range of Samples CS2 and CS3 could not be carried out because of their nonavailability.

The overall effect of processing conditions on gossypol levels (free gossypol, bound gossypol, and total gossypol) is shown in Fig. 2. The x-axis indicates the various processing stages (1 = undefatted meal, 2 = defatted flour, and 3 = UF/DF product), and the y-axis denotes % gossypol.

The increase of both free and bound gossypol between Stages 1 and 2 is obviously due to oil removal. The trends in bound gossypol levels between

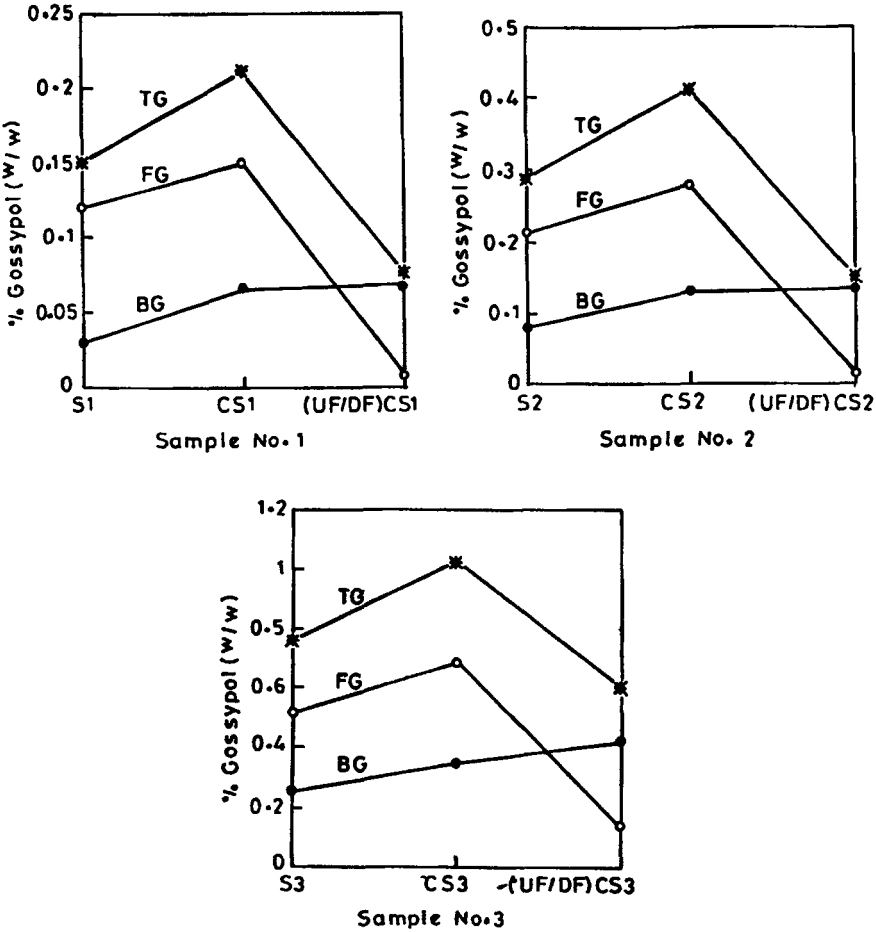

FIG. 2 Effect of processing conditions on gossypol binding.

Stages 2 and 3 are more or less constant with respect to relatively low gossypol samples (S_1 and S_2), indicating minimum binding, whereas in Sample S3 it appears that binding does take place owing to the high initial amount of free gossypol. However, the corresponding free gossypol levels and hence the total gossypol levels are on the decline in all samples.

Therefore, it appears that samples with initial free gossypol above the safe limit for consumption, i.e., above 0.045% and up to about 0.3%, can be directly treated by the present system after the seed is processed under controlled conditions.

The reduction of gossypol levels in the final protein isolates by the present method raises two questions:

1. Is it because of the interaction or combination with other components?
2. Is it because of the permeation of gossypol and/or gossypol-like pigments?

The first point seems to be unlikely as there were no significant changes in the bound gossypol levels of defatted meals and UF/DF (dried) proteins.

Hence, to strengthen the second possibility, more work with respect to the chemical stability of the color imparting gossypol or gossypol-like pigments needs to be done. Also, the proteins produced in the present work need to be analyzed for such functional properties as nitrogen solubility and nutritive value in terms of available lysine.

It was reported that there is hardly any correlation between levels of FG and the available lysine (29). This is one reason for omitting that aspect in the present work. However, that may be the only way to judge the validity. This will be the subject of a future communication.

CONCLUSIONS

The presence of gossypol in cottonseed has restricted its use in the food of nonruminants and humans.

Various methods used in the detoxification of gossypol suffer from some disadvantages. The liquid cyclone process still seems to be the best alternative for glanded cottonseed, but at the expense of high energy costs. Solvent extraction methods appear to be the second best alternative to obtain defatted flours and hence protein concentrates/isolates having very low free and bound gossypol levels, although this method involves multiple extractions in different solvent systems.

Success in developing glandless cottonseed has been meagre, and a major portion of the varieties now available still contain objectionable FG levels for human consumption.

There is a possibility of obtaining defatted flours with reduced meal-to-solvent ratios or by a limited number of extraction steps, but their gossypol levels may still be unacceptable.

Under the present circumstances, the process described here might suffice.

Finally, the present work gives scope for the exploration of new or better reagents with the ability to form a complex with gossypol in the extraction step in order to exploit the finer points of the ultrafiltration separation technique.

ACKNOWLEDGMENT

Financial support was provided by CSIR, New Delhi, India.

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Received by editor October 4, 1993
Revised February 7, 1994