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# BIOCHEMICAL AND NUTRITIONAL STUDIES ON INDIGENOUS COTTONSEEDS FOR THE PRODUCTION OF DETOXIFIED COTTONSEED FLOUR

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Free gossypol, total gossypol and available lysine content of certain varieties of indigenous cottonseed meats and meals has been determined with a view to prepare a detoxified cottonseed flour. Free gossypol which is responsible for the toxicity of cottonseed was found to range from 0.9 to 1.45% in meats and from 0.16 to 0.88% in meals. Available lysine ranged between 3.2 to 3.5 g/16g N in meats and 2.1 to 3.3/16 g N in meals which showed damage to protein quality in the latter due to processing.

Experiments were conducted for eliminating free gossypol by solvent extraction and by fixation with certain compounds. It was found that free gossypol could be eliminated more efficiently by ethyl alcohol (which is cheaply available in Pakistan) as compared with other solvent systems. Cottonseed meal containing less than 0.3% free gossypol could be detoxified by means of a mixture of calcium and iron salts but the product was of a dark colour and could possibly be used as animal feed.

Net protein utilisation value showed that edible cottonseed flour prepared by treatment with calcium and iron salts was slightly of better protein quality as compared with alcohol extracted flour.

Cottonseed meal has been highly esteemed as a protein concentrate for animal feeding especially for milch animals. Its use in the ration of monogastric animals such as swine and chicks is limited due to the presence of gossypol, a yellowish green pigment, which has been found to have adverse physiological effects on these animals.[1,2] Gossypol may exist free or in bound form, the latter is the result of Maillard type binding of gossypol with protein moiety. The epsilon amino group of lysine which is free in the processed protein is most sensitive to this type of binding and gets chemically combined with carbonyl group of gossypol or related pigments during cooking or expulsion of oil from cottonseed by hydraulic or expeller process.[3] Although bound gossypol is not considered toxic it reduces the protein value of the meal due to inactivation of a portion of available lysine.[4] Hence, in judging the quality of cottonseed meal, the quantity of both the free and bound gossypol is taken into consideration.

Free gossypol in cottonseed meal is removed by employing solvents or a mixture of solvents amongst which acetone, acetone–hexane–water mixture, and alcohol have been found quite successful.[5,6] Various compounds such as sugar, and enzymes have also been tried to reduce the free gossypol content.[7] The addition of ferrous salts to diets containing cottonseed meal has been shown by various workers to reduce the toxicity of cottonseed meal.[8–10] It is believed that the beneficial effect of added iron are due to the formation of an insoluble iron compound of gossypol and to the oxidation of gossypol thus decreasing its concentration. Bressani *et al.*[7] have shown recently that the addition of iron in the presence of calcium salt with or without cooking reduces the concentration of free gossypol in food mixtures containing cottonseed flour to a very low level.

Pakistan produces about 1,116,000 tons of cottonseed.[11] A good deal of oil is expressed by solvent extraction process. The residue is used as a cattle feed or as fertilizer. Its export is limited because of its gossypol content. Hence the develdevelopment of an economical method for the removal of gossypol is necessary for utilization of this potential source of protein as food.

In the present study gossypol content (free and total) of some indigenous varieties of cottonseed meats and meals obtained from different sources has been determined. Available lysine content was also measured with a view to determine the extent of damage to protein quality due to processing. Attempts were afterwards made to reduce the free gossypol by different treatments, viz. extraction with solvents and fixation of gossypol with certain chmicals. Net protein utilization (NPU) of degossypolised cottonseed flour was determined to ensure that protein quality was not impaired to preclude its use as food or feed.

## Experimental

### *Preparation of the Samples*

Samples of cottonseed were decorticated, the meats finely ground in a pestle and mortar and passed through 60 mesh sieve.

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Samples of hexane-extracted cottonseed meals received from different factories were finely ground and passed through 80 mesh before analysis.

*Treatment of Hexane-Extracted Cottonseed Meals*

(a) *Treatment with Solvents.*—The samples were extracted with (1) ethyl alcohol, (2) acetone, (3) alcohol–acetone (1:1) and (4) water–acetone–hexane (3:53:44) in a soxhlet extractor overnight. The meals were dried under vacuum at 60–65°C to remove the solvent completely.

(b) *Treatment with Chemicals.*—The samples were treated with calcium hydroxide, calcium hydroxide and ferrous sulphate, borax and egg albumin by mixing the compounds in the proportion given in Table 3. A slurry was made by adding small additions of water with constant stirring. It was cooked on a water bath for ca. 15 min with constant stirring and was then dried in an air oven at 60–65°C. The material was finely ground to pass through an 80 mesh sieve.

*Analytical Methods*

Free gossypol was determined according to the method of A.O.C.S.[12] using pure gossypol acetate as standard. Total gossypol was estimated according to the method of Miller.[13]

Available lysine was determined by the method of Conkerton and Frampton.[14]

Net protein utilization at 10% protein level (N.P.U.[10]) was estimated by the method of Miller and Bender[15] using male albino rats weighing 40–45 g. The temperature of the animal house was maintained at 78°F ± 2°F. Food and water were given *ad libitum*. The composition of the various experimental diets and the results of N.P.U. assays are shown in Tables 5 and 6, respectively.

## Results and Discussion

*Free Gossypol, Total Gossypol and Available Lysine Content of Cottonseed Meats and Meals*

Table 1 shows the results of analysis of cottonseed meats and meals. It will be observed that the free gossypol content in meats varied from 0.9 to 1.45% as compared 0.16 to 0.88% in meals. There was also a wide variation in the analytical results of different batches of cottonseed meals from the same source. In meals, free gossypol was less than that found in meats which is expected, as a part of the gossypol is eliminated with the oil and a part of it gets bound with protein. Samples before and after processing were not available and would have given better indication of extent of elimination of gossypol as a result of processing. Available lysine content of the samples showed that some impairment of protein quality had taken place. It will be noted that available lysine content of meats (3.2–3.5 g/16g N) was much higher than those of meals (2.1–3.3 g/16 N).

The available lysine content of indigenous cottonseeds was found to be slightly less than those of United States origin which may be due to varietal differences.

When compared with FAO/WHO/UNICEF standards[16] the available lysine content of indigenous solvent-extracted meals (excepting one sample) was less than specified, i.e. 3.6 g/16 N. Analysis of meats showed that they were already border line with respect to FAO/WHO UNICEF standards. Some damage to lysine is inevitable during processing. At present our mills are geared to get the maximum yield of oil and no attention is paid to the quality of meal. If proper precautions were taken at the expeller stage and at removal of the solvent it may be possible to preclude damage to lysine and produce quality meal fit for human consumption.

*Detoxification of Cottonseed Meal by Solvent Treatment*

Table 2 shows the effect of various solvent treatment on gossypol (free and total) and available lysine content of cottonseed meals. It will be seen that the various solvent systems used were able to reduce the free gossypol content of the meal to the level specified by FAO/WHO/UNICEF (i.e. less than 0.06%). In all these experiments ethyl alcohol appeared to be the most effective. The available lysine content of the solvent-extracted samples was slightly less than that of the original material which showed some damage to protein quality during extraction with solvent and subsequent desolventisation. Although ethyl alcohol has generally not been used as a solvent for degossypolisation, in Pakistan, where alcohol is more cheaply available than acetone, it may be economical to use alcohol for preparation of edible grade cottonseed flour.

*Detoxification of Cottonseed Meal by Treatment with certain Compounds*

As shown in Table 3, 1% calcium hydroxide, 1% calcium hydroxide+0.1% ferrous sulphate, 2.5% gelatin, 5% gelatin, 2% borax, 2.5% egg albumin reduced the free gossypol content but these were not as efficient as organic solvents. It is also clear that calcium hydroxide alone is not as

TABLE 1.—FREE GOSSYPOL, TOTAL GOSSYPOL AND AVAILABLE LYSINE CONTENT OF COTTONSEED MEATS and MEALS.

| | Total gossypol % | Free gossypol % | Bound* gossypol % | Available lysine 6/16g N |
|---|---|---|---|---|
| *Cottonseed meats from Agricultural Research Institute, Tandojam* | | | | |
| Variety M-100 | 1.50 | 1.45 | 0.05 | 3·4 |
| ,, M-4 | 1.38 | 1.32 | 0.60 | 3·5 |
| ,, AC-134 | 1.42 | 1.40 | 0.02 | 3·5 |
| ,, LSS | 0.93 | 0.90 | 0.03 | 3·4 |
| ,, 18829-M | 1.10 | 1.00 | 0.10 | 3·5 |
| ,, TDI | 0.90 | 0.85 | 0.05 | 3·3 |
| ,, 231 R | 1.10 | 1.05 | 0.05 | 3·4 |
| ,, S-57-91 | 1.05 | 0.95 | 0.10 | 3·2 |
| *Pre-pressed, solvent-extracted meals* | | | | |
| Burma Oil Mills, Karachi (1) | 1.70 | 0.60 | 1.10 | 2.9 |
| Burma Oil Mills, Karachi (2) | 0.90 | 0.25 | 0.65 | 3·3 |
| Burma Oil Mills, Karachi (3) | 0.72 | 0.16 | 0.56 | 3.1 |
| Koh-i-Noor Oil Mills, Lahore (1) | 1.30 | 0.30 | 1.00 | 2.1 |
| Koh-i-Noor Oil Mills, Lahore (2) | 1.00 | 0.45 | 0.55 | 2.6 |
| Haji Dossa & Sons, Hyderabad | 1.50 | 0.88 | 0.62 | 2.3 |
| Dawood Industries, Burewala | 0.95 | 0.24 | 0.71 | 3.1 |

*Total gossypol—free gossypol=bound gossypol.

TABLE 2.—EFFECT OF TREATMENT WITH DIFFERENT SOLVENTS ON GOSSYPOL AND AVAILABLE LSYSINE CONTENT OF COTTONSEED MEALS.

| Treatment | Total gossypol % | Free gossypol % | Bound gossypol % | Available lysine g/16g N |
|---|---|---|---|---|
| *Experiment No.* 1 | | | | |
| No treatment (a) | 1.70 | 0.600 | 1.100 | 2.9 |
| Alcohol | 1.08 | 0.024 | 1.056 | 2.6 |
| Acetone | 1.12 | 0.058 | 1.062 | 2.6 |
| Alcohol–acetone (1:1) | 1.11 | 0.032 | 1.078 | 2.6 |
| Water–acetone–hexane (3:53:44) | 1.14 | 0.07 | 1.07 | — |
| *Experiment No.* 2 | | | | |
| No treatment (b) | 0.90 | 0.25 | 0.65 | 3·3 |
| Alcohol | 0.70 | 0.02 | 0.68 | 3.0 |
| Acetone | 0.71 | 0.05 | 0.66 | 2.9 |
| Alcohol–acetone (1:1) | 0.70 | 0.028 | 0.672 | 2.9 |
| Water–acetone–hexane (3:53:44) | 0.71 | 0.058 | 0.652 | — |
| *Experiment No.* 3 | | | | |
| No treatment (c) | 1.30 | 0.300 | 1.000 | 2.1 |
| Alcohol | 1.00 | 0.018 | 0.982 | 1.8 |
| Acetone | 1.03 | 0.045 | 0.985 | 1.7 |
| Alcohol–acetone | 1.02 | 0.028 | 0.992 | 1.7 |

(a) Burma Oil Mills, Karachi, Lot No. 1. (b) Burma Oil Mlls, Karachi, Lot No. 2. (c) Koh-i-Noor Oil Mills, Lahore, Lot No. 1.

Table 3.—Effect of Treatment with Certain Compounds on Gossypol and Available Content of Solvent Extracted Cottonseed Meal having high Gossypol Content.

| Treatment | Total gossypol % | Free gossypol % | Bound gossypol % | Available lysine g/16 g N |
|---|---|---|---|---|
| No treatment | 1.70 | 0.60 | 1.10 | 2.9 |
| % $Ca(OH)_2$ | 1.55 | 0.25 | 1.30 | 2.8 |
| 1% $Ca(OH)_2$ + 0.1% $FeSO_4$ | 1.60 | 0.16 | 1.44 | 2.8 |
| 2.5% Gelatin | 1.58 | 0.24 | 1.34 | 2.9 |
| 5% Gelatin | 1.57 | 0.18 | 1.39 | 2.9 |
| 2% Borax | 1.55 | 0.18 | 1.37 | 2.6 |
| 2.5% Egg albumin | 1.58 | 0.26 | 1.32 | 2.8 |

Table 4.—Effect of Treatment with Various Levels of Calcium Hydroxide and Ferrous Sulphate on Gossypol and Available Lysine Content of Solvent- Extracted Cottonseed Meal having Low Gossypol Content.

| Treatment | Total gossypol % | Free gossypol % | Bound gossypol % | Available lysine g/16g N |
|---|---|---|---|---|
| No treatment | 0.9 | 0.25 | 6.65 | 3.3 |
| 1% $Ca(OH)_2$ | 0.87 | 0.13 | 0.74 | 3.1 |
| 0.5%$Ca(OH)_2$ + 0.05%$FeSO_4$ | 0.87 | 0.12 | 0.75 | 3.1 |
| 1%$Ca(OH)_2$ + 0.1% $FeSO_4$ | 0.88 | 0.08 | 0.80 | 3.1 |
| 1% $Ca(OH)_2$ + 0.15% $FeSO_4$ | 0.87 | 0.05 | 0.82 | 3.1 |
| 1.5%$Ca(OH)_2$ + 0.15%$FeSO_4$ | 0.87 | 0.045 | 0.825 | 3.1 |
| 1.5%$Ca(OH)_2$ + 0.2% $FeSO_4$ | 0.87 | 0.04 | 0.83 | 3.1 |

Table 5.—Percentage Composition of the Experimental Diets.

| | |
|---|---|
| Cottonseed flour* | 20% |
| Maize starch | 30% |
| Potato Starch | 10% |
| Hydrogenated Oil | 15% |
| Glucose | 15% |
| Vitamin mixture17 | 5% |
| Mineral mixture17 | 5% |

* Since treated and untreated cottonseed flour had the same protein content, the above formula was used for the other two diets.

effective as a mixture of calcium hydroxide and ferrous sulphate. Total gossypol and available lysine content practically remained unchanged as compared with control.

In the next experiments, various combinations of calcium hydroxide and ferrous sulphate were tried (Table 4). The best combination was found to be 1.5% calcium hydroxide+0.2% ferrous sulphate, which reduced the free gossypol from 0.175% to 0.04%. But this combination was abandoned in favour of 1.5% calcium hydroxide+ 0.15% ferrous sulphate, as the former produced a very dark coloured flour. Available lysine content practically remained unaltered which indicated that there was no impairment in protein quality as a result of such treatment.

*Biological Value of Proteins of Alcohol-Treated and Calcium Hydroxide and Ferrous Sulphate-Treated Flour*

The result of N.P.U. determination (Table 6) showed no significant difference between the un-

Table 6.—Net Protein Utilization Value (NPU) of the Experimental Diets.

| Experimental diets | | Body N of test group ($B$) mg | Body N of non-protein group ($B_K$) mg | Intake N of non-protein group ($I_K$) | Intake N of the test group ($I$) mg | NPU % | NPU* mean % |
|---|---|---|---|---|---|---|---|
| *A* Cottonseed flour untreated | 1 | 5814 | 3572 | 200 | 5680 | 43 | 42 |
| | 2 | 5492 | ,, | ,, | 5016 | 41 | |
| *B* Cottonseed flour chemically treated (1.5% Ca $(OH)_2$+0.2% $FeSO_4$) | 1 | 4912 | ,, | ,, | 3680 | 42 | 41 |
| | 2 | 4598 | ,, | ,, | 3260 | 40 | |
| *C* Cottonseed flour (alcohol-extracted) | 1 | 4702 | ,, | ,, | 4000 | 33 | 34·5 |
| | 2 | 4474 | ,, | ,, | 3040 | 36 | |

$$*\text{NPU}\,\% = \frac{B-(B_K-I_K)}{I} \times 100$$

treated flour (42%) and that chemically treated (41%) but the alcohol-treated (34.5%) flours gave lower values, indicating that treatment with calcium and iron salts involved less damage to protein than alcoholic extraction. This is also borne out by the available lysine value which is consistantly less in solvent-treated flours as compared with chemically treated flour. This incidentally shows that the determination of available lysine by the method of Conkerton and Frampton[14] can be employed as a quick and reliable method for the evaluation of protein quality and which provides a good measure of the extent of damage to proteins as a result of processing. Similar findings were reported by Bressani *et al.*[7] who found a good correlation between protein efficiency ratio and free amino groups of lysine.

## Conclusion

On the basis of present investigations it may be concluded that it is possible to detoxify cottonseed meal either by reduction of free gossypol to a specified level by extraction with alcohol or by its fixation with a mixture of calcium and iron salts. For cottonseed meals containing more than 0.3% free gossypol, detoxification by means of alcohol is recommended. For lesser amounts of free gossypol, treatment with 1.5% calcium hydroxide 0.15% ferrous sulphate may be employed, but the detoxified flour by this method is of dark brown colour and may be used advantageously as animal feed.

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