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## Comparative Solubility of Protein in Cottonseed Flakes Extracted by Hexane and by Ethanol

By LIONEL K. ARNOLD and BEVERLY JEAN SCHRIVER

### INTRODUCTION

The solvent commonly used in the United States for the extraction of vegetable oils is a petroleum fraction consisting mainly of hexane. Experimental work has been done in this country on the extraction by ethanol of soybean oil by Beckel and associates (1) and of cottonseed and other oils by Rao and associates (4, 5). Ethanol appears promising for use in Asian countries such as India and China largely because it is more readily available than commercial hexane (6). The ethanol extracted cottonseed meal has a lower gossypol content than the hexane extracted meal and for that reason is preferable. However, it is known that ethanol may coagulate some proteins, thus reducing their solubility in water and in aqueous solutions. Because of this relation of solubility and the nutritional value of the protein, determinations of the solubilities of meals produced by the extraction of cottonseed with ethanol and meals produced by the extraction with commercial hexane were made.

The solubility methods used were adapted from the work of Olcott and Fontaine (3) and Lyman, Chang, and Couch (2). Olcott and Fontaine suggest that the solubility of cottonseed meal protein in three per cent sodium chloride solution and in water be used in industry for control of the meal quality. Lyman, Chang, and Couch found a relationship between the solubility of cottonseed meal protein in 0.02 N sodium hydroxide and the nutritional value of the meal for chicks.

### EXPERIMENTAL PROCEDURE

*Oil Removal.* Approximately 15 grams of flaked cottonseed meats were extracted in the rate extraction apparatus, shown in Figure 1, with commercial hexane ("Skellysolve-B") at 144° to 149° F. for two hours. The rate of flow was 10 ml. per minute. Ethanol extractions of flakes from the same batch were made in the same manner except at 168° to 172° F.

*Solubility in 3 per cent NaCl and water* (3). Five gram samples of meal were shaken with 200 ml. of 3 per cent NaCl solution and distilled water respectively in 250 ml. flasks. One ml. of chloroform was added and the mixture was allowed to stand 19 to 24 hours

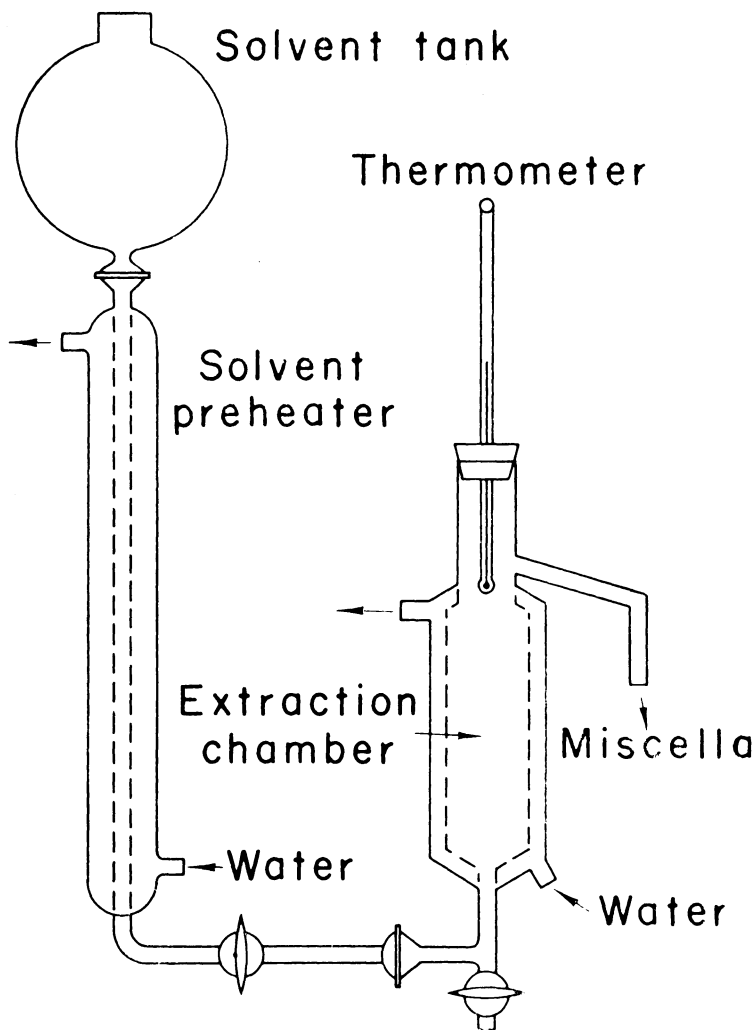


Figure 1. Rate extraction apparatus.

with occasional shaking and then filtered. Protein was determined in 25 ml. aliquots of the filtrate by a semi-micro Kjeldahl method.

*Solubility in dilute sodium hydroxide* (2). One gram samples were shaken with 100 ml. of 0.02 N NaOH and 4 glass beads by a mechanical shaker for one hour at 97° to 101° F. The suspension was then centrifuged for 5 minutes and 25 ml. aliquots analyzed for protein by the semi-micro Kjeldahl method.

*Protein Determination.* The 25 ml. aliquots were digested with sulfuric acid using  $\text{SeOCl}_2$  as a catalyst and perchloric acid as a final oxidizing agent. After cooling the digested samples were trans-

ferred to the distillation flask, shown in Figure 2, and 15 to 17 ml. of 30 per cent NaOH added. The liberated ammonia was steam distilled into 0.01 N HCl and the amount of nitrogen was determined by back titrating with 0.01 N NaOH. All calculations were made on a dry basis with a conversion factor from nitrogen to protein of 6.18.

#### DISCUSSION

The results are summarized in Table 1. They indicate a higher percentage of protein in the meal extracted by ethanol than that extracted by hexane, although both were produced from the same batch of flakes. Apparently the ethanol extracted some non-protein constituents not dissolved by the hexane, thus resulting in a higher percentage of protein.

The relative amount of water soluble protein in the hexane extracted meal was 63.6 per cent more than that in the ethanol product. No determination was made of the amount of water soluble protein in unextracted flakes, but Olcott and Fontaine (3) secured a water solubility of 25 to 30 per cent in cottonseed meals analyzed by them. The relative amount of protein from the hexane extracted meal soluble in the sodium chloride solution was 39.2 per cent greater than that from the ethanol extracted meal. There was less difference in solubilities of the two meals in dilute sodium hydroxide solution, that from hexane being 13.7 more than that from ethanol.

The possible effect of heat on solubility should be considered. The

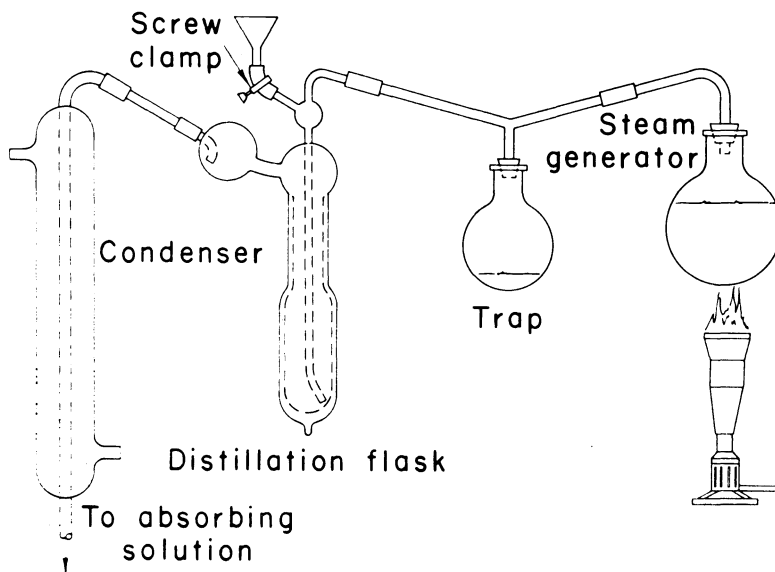


Figure 2. Semi-micro Kjeldahl apparatus.

**Table 1**  
**Proteins in Extracted Cottonseed Flakes**

| Protein Content<br>in per cent         | Extraction Solvent |             |
|--|--------------------|-------------|
|  | Hexane*            | 95% Ethanol |
| Total                                  | 56.4               | 61.5        |
| Soluble in water                       | 11.9               | 7.9         |
| Soluble in NaCl solution               | 40.5               | 31.7        |
| Soluble in NaOH solution               | 44.9               | 43.1        |
| Solubility of Protein<br>in per cent** |                    |             |
| In water                               | 21.1               | 12.9        |
| In NaCl solution                       | 71.8               | 51.5        |
| In NaOH solution                       | 79.6               | 70.1        |

\*"Skellysolve B"

\*\*Numerically equivalent to nitrogen solubility

temperatures used in the experimental extractions were about 23° F. higher with ethanol than with hexane. These temperatures are those practical for industrial operation. Possibly a small part of the lower solubility of the ethanol extracted protein was a temperature effect. Solvent removal from both the experimental meals was at room temperature which is considerably lower than the usual industrial desolventization temperatures. Thus the data apply to the reduction in protein solubility resulting from extraction only and do not include any decrease in solubility which might result from desolventization carried out under usual industrial conditions.

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