# COMPOSITE UTILIZATION OF COTTONSEED KERNELS

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The seeds of the cotton plant occupy the second place after soybeans as a source of vegetable oil. In the 10th Five-Year Plan it is proposed to produce more than 8 million tons of cotton plant from which about 5 million tons of technical seeds can be obtained.

The seed of the cotton plant consists of husk and a kernel that contains oil, proteins, phosphatides, phytin, carbohydrates, tocopherols, carotenoids, sterols, and the toxic pigment gossypol, which is a characteristic component only of cotton seeds and the amount of which varies from 0.8 to 2% of their weight [1].

At the present time, only an edible oil is extracted from cottonseed kernels, and the residue in the form of meal is fed to cattle. In the existing technology of obtaining oil by using high-temperature effects (steaming and pressing), substances still more valuable than the oil – the protein, phytins, and phosphatides – are devalued by interacting with the gossypol and with one another, their amount falls considerably, and the food value of the meal is reduced [2].

Great attention should be devoted to the use of the protein of cottonseed meal as a supplementary food product, particularly in those regions of the terrestrial globe where a considerable deficiency of proteins is experienced [3, 4]. In view of this, questions of the composite treatment of cotton seeds are acquiring great importance – in particular, a method of direct extraction which excludes the action of high tempertures on them. And while the necessity for passing to the direct extraction of the oil from cotton seeds envisaged by the future plan of development of the oils and fats industry is not a matter of doubt at the present time (cotton seeds were processed by this method as early as 1936) [5, 6], the question of the extraction of all the components of the seeds with the aim of their complete utilization has not yet been answered.

The main factor limiting the production of protein and other useful substances from these seeds is that, in the technology for the production of oil used at the present time, the gossypol is distributed between the oil and the meal. It is eliminated from the oil by alkali refining, the consumption of alkali being 1.5-2 times the calculated amount. In the first place, this leads to an increase in the loss of oil and, in the second place, the toxic gossypol remaining in the meal reduces its fodder value. Futhermore, under the influence of high temperatures in the process of roasting and prepressing, the proteins of the kernel are denatured, passing into difficultly assimilable form. The gossypol, by reacting with the ε-amino group of lysine – one of the most important amino acids of cotton seeds – reduces the yield and quality of the protein.

Another serious disadvantage of this technological scheme is its incomplete comprehensiveness: in particular, no use is made of such substances of physiological value as phytin, the proteins, phosphatides, tocopherols, and sterols, and even the same gossypol that today finds independent application.

Two methods have been proposed to get rid of the gossypol. The first is the breeding of gossypol-free varieties of the cotton plant, but this process is laborious and lengthy and moreover, in practice these varieties have proved not to be resistant to any of diseases. The other method is to extract the gossypol into the oil and remove it from the latter by refining and, finally, to use the method of selective extraction for the stagewise isolation of the individual components of the seeds.

In recent years, in order to obtain edible flour the development has begun of a method of separating the gossypol glands from the protein fraction of the seeds. The use of so-called dry fractionation, based on the use of vibrosieves and the aerodynamics and dielectric properties of the gossypol glands has not given the results hoped for [7].

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However, if in the wet fractionation the hexane-defatted flakes are intensively stirred with a mixture of hexane and tetrachloroethylene (sp. gr. 1.378), the glands float and the protein fraction of the kernels sinks. This method is recommended for obtaining gossypol glands with the aim of their further study [8]. In this way it is possible to isolate gossypol glands even from undefatted material, but the oil from this process retains the odor of the tetrachloroethylene.

V. P. Rzhekhin et al. [9] have proposed a mixture of gasoline with carbon tetrachloride in a ratio of 3 : 1 to separate the gossypol glands [9]. The resulting meal contained 0.01% of free gossypol, but the high toxicity of the vapors of the mixed solvent did not permit this method to be recommended for industrial use.

In order to obtain high-quality oils and meal, the method of the "differentiated" precipitation of the gossypol glands has been developed [10] which, in improved form, has found semiindustrial use as the "liquid cyclone" [11]. The method is as follows. The material with a moisture content of 2.5-3% is carefully comminuted to particles of a size of 180-300 mesh, mixed with hexane in a ratio of 1 : 1, and fed into a cyclone where it is separated into two fractions: the first, with a high protein content and almost no free gossypol (0.04-0.07%) and the second enriched with gossypol.

The first fraction can be used as an edible flour. The oil obtained in this way is light in color and easily refined. However, this method does not solve the problem of the use of the fraction enriched with gossypol.

Muller et al. [12] have proposed to treat the second layer with a saturated solution of sodium sulfate which, while dissolving the protein, does not affect the gossypol glands. In this case, to precipitate the dissolved protein the sodium sulfate solution must first be treated with a large amount of water.

One of the most promising methods, permitting the production of high-quality oil, edible protein, phytin, and other substances by the composite treatment of cotton seeds is the stagewise isolation of the components of the seed. V. P. Rzhekhin et al. [13] have recommended the preliminary treatment of the pulp with hydrophilic solvents which would extract the maximum amount of gossypol and free fatty acids and the minimum amount of glycerides. As hydrophilic solvent they selected dilute ethanol.

The oil obtained by pressing or by gasoline extraction from pulp treated in this way and dried in the air was light in color and had an acid number of 0.46-0.8 mg of KOH.

Markman and Rzhekhin [1] consider that other alcohols beside ethanol can be used as hydrophilic solvents.

A. L. Markman et al. have used aqueous acetone for the preliminary treatment of cottonseed pulp in order to extract valuable components from it more completely [14-17]. They have recommended a two-stage extraction of the pulp: the first with 70-75% aqueous acetone, and the second with gasoline after the elimination of the acetone and water from the pulp. In these circumstances, the gossypol and free fatty acids pass into the first extract, and the glycerides into the second; however, the sterols, carotenoids, and phosphatides are distributed between these two fractions.

On varying the water content of the acetone, the authors found that when the acetone concentration was raised from 60 to 80% the amount of gossypol extracted rose slowly, while at a higher concentration the amount of gossypol in the extract fell sharply. The amount of phosphatides in the extract fell, since they are insoluble in acetone.

So far as concerns the fatty acids and neutral fat, with a rise in the concentration of acetone their solubility decreased. Within the range from 0.5 to 1.5 h, the duration of the process has no appreciable influence on the amount of components extracted.

The oil and meal obtained by this scheme contains practically no free gossypol. The acid number of the oil was 0.27 mg of KOH and its color in a 13.5 cm layer was 8 red units.

Pons [18] has also used aqueous acetone for extracting gossypol from cottonseed pulp and has confirmed the results obtained by A. L. Markman et al. In addition, Pons showed one more advantage of the scheme of double extraction, which consists in a reduction of the toxicity of such metabolites as aflatoxins, which are formed as a result of the activity of the microorganisms Aspergillus flavus when the conditions of storing the seeds are unfavorable.

The oil and gossypol have been extracted with 99.9% ethanol [19]. On cooling, the resulting extract separated into two layers: a lower one containing the oil, and an upper one into which the gossypol, phosphatides, and sugars passed.

Ethanol has been used as a vegetable oil extractant in Japan [20], India, Argentina, the USA, and other countries [21, 22]. In the Soviet Union, oil and vitamins are extracted from wheat germs with ethanol [23]. The extraction of vegetable oils has been performed with a mixture of ethanol and isopropanol (8:2) and also with pure isopropanol [24].

Investigations have been carried out in which the oil was extracted with acetone; this does not form azeotropic mixtures with water, which facilitates its regeneration [23].

Acetone is a solvent that is simultaneously lipophilic and hydrophilic. This property of it has been made use of by Vaccarino [25, 26] and also by A. L. Markman et al. [27] for extracting oil and gossypol from cottonseed kernels. According to the results of A. L. Markman et al., the meal obtained after extraction with acetone contained 0.08-0.14% of free gossypol. The oil was refined in the miscella with 5% ammonia solution. The miscella was previously treated with water to give an 80% concentration of acetone. Ammonia gossypolate, soap, and a certain amount of neutral fat passed into the aqueous acetone layer.

Binary mixtures consisting of gasoline (85% by volume) to which 15% by volume of acetone, dichloroethane, or benzene had been added have also been used for the combined extraction of oil and gossypol [27-29].

On comparing various solvents, Markman et al. [28] came to the conclusion that "The best results in relation to the speed and completeness of the extraction of gossypol are obtained by using 70% aqueous acetone as extractant, and the next best after aqueous acetone is dry acetone, followed by gasoline–acetone mixture." They also showed that gasoline is less suitable for this purpose.

Good results have been obtained in the extraction of cottonseed and soybean flakes with binary mixtures consisting of n-hexane and ethanol [30].

Investigations [31, 32] in which the ternary azeotropic mixture of acetone, hexane, and water (42.5:55:2.5) (in some experiments, the hexane was replaced by petroleum ether) was used for the extraction of oil and gossypol are interesting. Although the meal obtained by this method contained insignificant amounts of free gossypol, it possessed an unpleasant smell and, in addition, the separation of the substances extracted by this mixture, and its regeneration, were associated with certain difficulties.

Attempts have been made to extract the oil and gossypol with dichloroethane, carbon tetrachloride, and trichloroethylene, but chlorinated organic solvents are toxic and corrode the apparatus [33-35], and therefore they are unacceptable in the food industry.

There is also information on the use of liquefied gases (butane, propane, and mixtures of them) as solvents for oil [23].

The majority of the investigations mentioned here were directed mainly to obtaining gossypol-free oil and to increasing the fodder value of the meal.

In view of the potential possibility of using the meal as a raw material for obtaining edible protein and phytin, we have proposed in the treatment of cottonseed flake by the method of double extraction, first to extract the oil from it with hexane and then, after drying, to treat it with 70-80% aqueous acetone to extract the gossypol.

The choice of this sequence of solvents, the opposite of that proposed by A. L. Markman et al., is due to an attempt to lower the temperature of distilling off the extractants from the meal, which, in composite treatment after aqueous acetone extraction, is sent for the production of edible protein and phytin.

If, however, the pulp is first treated with aqueous acetone, for subsequent extraction of the oil with a nonpolar solvent such as hexane it must first be carefully dried. The elinination of acetone and water from the pulp requires higher temperatures and the distillation of dry hexane from it, and a rise in temperature is undesirable from considerations of the retention of the protein complex in the meal in its native state.

Since edible protein is obtained with the aid of aqueous solutions, in the proposed sequence of use of solvents the necessity for carefully drying the meal vanishes. Furthermore, in this treatment of the pulp the edible oil does not come into contact with acetone which sometimes contains the toxic methyl ethyl ketone as an impurity.

In our experiments, as a nonpolar extractant we selected hexane, which extracted mainly triglycerides. Hydrophilic solvents were aqueous solutions of alcohols and acetone of various concentrations. For the simultaneous extraction of oil together with some substances accompanying it we used binary mixtures consisting of hexane and acetone (85:15 by volume) and the ternary azeotropic mixture of acetone, hexane, and water (42.5:55:2.5) [36].

Thus, we have studied three variants of the treatment of cottonseed flake: 1) twofold extraction: first hexane and then aqueous acetone; 2) extraction with binary solvents; and 3) extraction with a ternary azeotropic mixture.

With respect to the quality of the oil and meal, the duration of the process, and the consumption of solvents, the best results were obtained by the scheme of twofold extraction. Incidentally, A. L. Markman et al. have also shown that the meal from benzene–acetone extraction is inferior to the meal from acetone extraction in relation to the amount of soluble forms of protein contained in it [29].

In relation to their capacity for extracting free gossypol from petal, dehydrated solvents form the sequence acetone > isopropanol > ethanol [37]. The optimum concentration of the solvents is 70-80%, since a higher concentration leads to a fall in the extractability of the gossypol because of a decrease in the degree of breakdown of the gossypol glands. The optimum extraction temperature is 40°C; at a higher temperature the degree of extraction of the gossypol and of the phosphatides falls because of their interaction with one another with the formation of gossyphosphatides and through the reaction of the gossypol with proteins.

In our experiments, the ratio of material and hexane was 1:6, and the ratio of material and aqueous acetone was 1:8. On extraction with the binary mixture, the liquid: solid ratio was far higher, but the repeated use of a weak miscella for sprinkling the material permitted it to be reduced somewhat.

Since the extraction process is affected not only by the construction of the apparatus but also by the state of the material undergoing extraction we have established the optimum conditions of its preparation for extraction: moisture content of the kernels 10-11%, time of their steeping 2.5 h; thickness of the flake 0.25-0.3 mm, moisture content of the flake 4-5% [37].

The resulting meal contained only 0.02-0.06% of free gossypol and 0.8-0.9% of oil, and the yield of protein from it had risen by 5%, amounting to 20%.

The extraction of the gossypol and the refining of the oil in the miscella was performed by methods developed previously [39]. An aqueous acetone extract was cooled first to +5°C, whereupon the sugars deposited. On cooling to −15°C the precipitate consisted mainly of phosphatides. Then the extract was evaporated at 35°C in vacuum to 50% of its original volume petroleum ether was added, and then, with stirring, a saturated solution of common salt. After settling for one hour, two layers were obtained: a lower layer consisting of water, salt, and a small amount of acetone, and an upper layer containing petroleum ether, acetone, gossypol, and fatty acids.

The acetone was washed out and regenerated, and the residual gossypol being insoluble in petroleum ether, precipitated.

The gossypol was isolated from the hexane–acetone miscella by its treatment with a 3% solution of caustic soda in an amount strictly calculated from the acid number, as has been proposed by A. L. Markman et al. [40]. The solution of alkali was treated with 5 g/liter of sodium hydrosulfite to prevent the oxidation of the gossypolates [41].

After the separation of the lower layer, the miscella was sent to the final refining process, and the lower layer, containing the sodium salts of gossypol and of the fatty acids and a certain amount of neutral oil was separated to isolate the oil and was decomposed with 5% sulfuric acid (pH 5). After settling, the upper layer containing the gossypol and fatty acids was diluted with hexane (1:1), and the fatty acids were separated from the gossypol. The purity of the gossypol isolated from the aqueous acetone extract and from the hexane–acetone mixture amounted to 60-65%.

Thus, with the stagewise extraction of the components from cottonseed kernels a high-quality oil and a meal with a high content of native protein and free from gossypol are obtained. This meal is a good raw material for the production of food and fodder proteins, edible flour, and phytins.

On composite treatment, 1 ton of cotton seeds can give 190 kg of oil, 60 kg of protein, 12 kg of technical phytin, 3.5 kg of technical gossypol, 3.5 kg of sugar, 0.2 kg of sterols, 200 kg of spent pulp, and 500 kg of husks, and also phosphatides and tocopherols.

The rational formulation of the technological scheme for treating cotton seeds excluding the action of high-temperatures will enable the substances mentioned to be obtained in the native state and to be used in the national economy.

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# BISBENZYLISOQUINOLINE ALKALOIDS

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## Distribution in Nature

Bisbenzylisoquinoline alkaloids form a large group of natural bases found in plants of the families of Menispermaceae, Berberidaceae, Ranunculaceae, Lauraceae, Annonaceae, Hernandiaceae, Magnoliaceae, and Nymphaeaceae.

At the present time, more than 150 bisbenzylisoquinoline bases are known the structure of which includes two benzylisoquinoline fragments connected by one, two, or three ether bonds. The number of ether bonds and the positions which they link are used as criteria for classifying these alkaloids. As exceptions, compounds are found with a carbon–carbon bond between the benzyl residues for example, rodiasine, ocotine, and tiliacorine

In recent years, with the development of instrumental methods of determining structure, and also in connection with the discovery of a series of active drugs among bisbenzylisoquinoline alkaloids interest in this class of natural compounds has risen considerably. The list of bimolecular ether alkaloids (see Table 1) is not limited only to bisbenzylisoquinoline bases. It is constantly being supplemented by new types of bases genetically connected with them: benzylisoquinoline-aporphine (see the reviews [120-122]), benzylisoquinoline-proaporphine, bishomobenzylisoquinoline, benzylisoquinoline-benzazpine, aporphine-pavine and others (I-VIII). This shows that the chemistry of these alkaloids is far from being exhausted.

## Chemical Methods of Studying the Structure of the Bisbenzylisoquinoline Alkaloids

The chemical methods of determining the structure of bisbenzylisoquinoline alkaloids consist in the cleavage of their molecules to give simpler fragments of known structure, which provides a possibility of determining the positions of the substituents and of the ether bridges. For this purpose oxidation, reduction, Hofmann degradation, etc., are used. As a rule, phenol-containing alkaloids are previously protected by O-methylation or O-ethylation, and are then cleaved into simpler fragments. Quaternary ammonium salts can, where necessary, be converted into tertiary bases with a yield of 64-84% by heating them with ethanolamine [127] or with sodium thiophenolate [105].

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