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# ISOLATION OF RAFFINOSE FROM COTTONSEED MEAL

I. A. Ivanova, M. T. Turakhozhaev,
and T. T. Shakirov

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The solubility of raffinose in various solvents has been studied. The influence of several factors on the process of extracting raffinose from cottonseed meal has been investigated. The optimum conditions of the extraction process have been established.

Raffinose is a nonreducing trisaccharide consisting of D-galactose, D-glucose, and D-fructose residues [1]. It is used in agriculture for the development of pedigree stock breeding and as a reagent for scientific-research purposes.

Raffinose is present in sugar beet at a level of 0.01-0.02% [2, 3] and also in the manna of several Australian eucalyptuses [4], and in rice, wheat, millet, soybean, pea, and jute seeds [5, 6]. The level of raffinose in jute amounts to 2%, which has permitted the seeds of this technical crop to be used as a raw material on the industrial scale [6]. The most accessible source of raffinose consists of cotton seeds and cottonseed meal.

There is contradictory information in the literature concerning the amount of raffinose in cotton seeds: It ranges from 1.5 to 4.0% by weight or, according to some reports, to 9%, which is apparently due to a dependence on the amount of carbohydrate synthesized on the variety of cotton plant and the soil and climatic conditions [7-9].

We have adopted a method of obtaining raffinose from cottonseed meal [10]. The method is based on the extraction of the raw material with aqueous ethanol with elimination of resinous substances from the concentrated extract, and the subsequent crystallization of the raffinose.

In order to select solvents for the extraction and further purification of the raffinose, we studied its solubility in organic solvents and aqueous organic mixtures (Table 1). For the experiment we used raffinose corresponding to TU [Technical Specification] 6-09-50-2374-81.

As can be seen from Table 1 the best solvents for extracting raffinose from the meal are aqueous ethanols, and for freeing it from ballast substances those of hydrophilic nature, such as a mixture of acetone, ethanol, and water. In order to determine the optimum concentration of ethanol for extracting raffinose, we performed a series of experiments in which 0.5-kg portions of ground cottonseed meal were extracted with ethanol of various concentrations at room temperature. Meal from cotton seeds of the 1981 harvest produced by the Kokand oils and fats combined was used.

Extraction was performed by the steeping method, and a total of these extractions, each of 3 h, was carried out. The combined extract was concentrated to 1/10 of its initial volume and was treated with gasoline to eliminate resinous substances, after which it was diluted with a twofold amount of acetone and was left for crystallization. The crystals that deposited were filtered off with suction and recrystallized from aqueous ethanol (Table 2).

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TABLE 1.

| Solvent | Solubility, g/100 ml | |
|---|---|---|
| | at 20℃ | at the boiling point |
| Ethanol, % | | |
| 95 | 0,050 | 2,25 |
| 80 | 0,192 | 12,65 |
| 70 | 0,357 | 82,62 |
| 60 | 0.812 | 96,14 |
| 50 | 1,256 | 124.48 |
| Acetone, % | | |
| 100 | 0,018 | 0,81 |
| 80 | 0,030 | 1,11 |
| 60 | 0,418 | 66,75 |
| 40 | 1,839 | 142.8 |
| 20 | 4,489 | 201,4 |
| Water | | |
| | 9.430 | 335,4 |
| Ethanol—acetone—water | | |
| 1:1:1 | 0,251 | 50,76 |
| 2:2:1 | 0,059 | 10.67 |
| 1:2:1 | 0,055 | 6,88 |
| 1:2:0.5 | 0,022 | 2.68 |

It can be seen from Table 5 that the best yield of raffinose was obtained on extraction with 85% ethanol. With an increase in the strength of the ethanol, the solubility of raffinose falls appreciably, which apparently explains the low yield of the desired product from jute seeds [6] when 95% ethanol was used as extractant. At concentrations of ethanol below 85%, the total amount of extractive substances increased, which led to a high yield of technical raffinose. However, the increase in the yield of technical product took place through a rise in the proportion of ballast substances of hydrophilic nature, which, in the final account, led to a loss of the main product on purification by recrystallization.

We also considered the influence of the temperature on the extraction process, for which purpose 0.5-kg portions of ground meal were extracted with 85% ethanol at various temperatures (Table 3).

The best yield of raffinose was obtained at room temperature. It is known that with a rise in temperature the solubility of raffinose increases and the extraction process intensifies. However, in this case the presence of a large amount of ballast substances passing into the extract with a rise in the temperature did not permit a favorable effect to be achieved.

Concentration of the extract obtained at 50-70°C led to the deposition of a resinous precipitate which entrained a considerable part of the main product.

Appreciable losses also took place in the process of eliminating ballast substances of hydrophilic nature from the concentrated extract by treatment with gasoline which, in the final account, lowered the yield of raffinose at the stage of obtaining the technical product.

One of the main factors affecting the raffinose extraction process is the time of steeping. To determine the optimum time between extractions, 0.5-kg portions of ground meal were extracted under indentical conditions with 85% ethanol, only the time between extractions being varied. A total of three extractions was performed (Table 4).

As can be seen from Table 4, for the exhaustive extraction of raffinose from cottonseed meal three extractions must be performed at 3-h intervals, a further increase in the time of steeping giving no appreciable positive effect.

## EXPERIMENTAL

Determination of the Solubility of Raffinose. Raffinose obtained from cottonseed meal and corresponding to the requirements of TU 6-09-50-2374-81, of ch. ["pure"] grade was used. The solubility was determined in accordance with Xth edition of State Pharmacopoeia.

TABLE 2.

| Concentration of ethanol, % | Yield of raffinose | | |
|---|---|---|---|
| | g | | % on the weight of the raw material |
| | technical | purified | |
| 80 | 25.10 | 13,25 | 2.65 |
| 85 | 19,25 | 14.50 | 2,90 |
| 90 | 13,70 | 10.95 | 2,19 |
| 95 | 1,40 | 1.25 | 0,50 |

TABLE 3.

| Extraction temperature, ℃ | Yield of raffinose | | |
|---|---|---|---|
| | g | | % on the weight of the raw material |
| | technical | purified | |
| 20 | 29,25 | 14,35 | 2.87 |
| 30 | 29,00 | 14,00 | 2.80 |
| 40 | 17,80 | 10.90 | 2.18 |
| 50 | 10,42 | 6.80 | 1.36 |
| 60 | 9,93 | 6,60 | 1.32 |
| 70 | 8,91 | 6,40 | 1,28 |

TABLE 4.

| Interval between extractions, h | Yield of raffinose | | |
|---|---|---|---|
| | g | | % on the weight of the raw material |
| | technical | purified | |
| 0.5 | 12,2 | 9,0 | 1,80 |
| 1 | 13.2 | 10,2 | 2.10 |
| 2 | 17,5 | 12,3 | 2,40 |
| 3 | 19.2 | 14,5 | 2,90 |
| 4 | 19,5 | 14.6 | 2,95 |

Extraction of Raffinose. The ground meal (0.5 kg) was charged into a glass diffuser, the extractant was added until a free surface had been formed (0.75 liter), and after some time it was poured off. The extracts were combined and concentrated in a vacuum evaporating apparatus to 1/10 of their initial volume (the temperature of distillation did not exceed 50°C; vacuum of 500-600 mm Hg).

Elimination of Resinous Substances. The concentrated extract was treated in a separatory funnel with an equal volume of gasoline. The mixture was shaken for 3 min and, after settling, the lower layer was taken off. This operation was repeated twice.

Production of Technical Raffinose. A double amount of acetone was added to the concentrated extract and it was left for crystallization. The crystals that deposited were separated off after 2 days and were then washed with a small amount of acetone and dried in the air.

Recrystallization of Raffinose. The technical raffinose was dissolved in a tenfold amount of 70% ethanol at 70-80°C, and the solution was filtered through a hot filter and left for crystallization. After 2 days the crystals of raffinose that had deposited were separated off and washed with a small amount of 70% ethanol. The yield of air-dried crystals was 14.5 g, or 2.9% of the weight of the initial meal.

## SUMMARY

1. The solubility of raffinose in various solvents has been investigated.

2. The influence of several factors on the process of extracting raffinose from cottonseed meal has been studied and the optimum conditions of extraction have been determined.

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# QUANTITATIVE DETERMINATION OF RAFFINOSE IN COTTONSEED MEAL

R. A. Mirkina and T. T. Shakirov

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A chromatographic method is proposed for the quantitative determination of raffinose in cottonseed meal. The relative error of a single determination is ±4.0%.

We have previously [1] reported the isolation of raffinose from cottonseed meal. Raffinose pentahydrate has been proposed for use in animal husbandry for the breeding of cattle and in bacteriology for the preparation of media.

We have developed a procedure for the quantitative determination of raffinose in cottonseed meal. It consists in the extraction of the total saccharides from raw material with 80% ethanol, chromatographic separation in a thin layer of silica gel, and the photoelectric determination of the raffinose by means of its color reaction with anthrone [2]. In studying the stage of chromatographic separation of the total saccharides, we have carried out experiments with different solvents and solvent systems. The most suitable proved to be the methyl ethyl ketone—acetic acid—water (5:4:1) system.

To confirm the completeness of desorption of the raffinose from the silica gel, a standard solution of raffinose was chromatographed with the subsequent quantitative determination of the raffinose in the eluate:

| Deposited, mg | Found, mg |
|---|---|
| 0,067 | 97.5 |
| 0.075 | 98,0 |
| 0,080 | 97,8 |

Using the method developed, we determined the amount of raffinose in the meals from cottonseeds of the 1980-1982 harvests. The metrological characteristics of the results of the analysis of the means are given below:

| $f$ | $\overline{X}$ | $S$ | $P$, % | $t(p,f)$ | $\Delta\overline{X}$ | $E$,% |
|---|---|---|---|---|---|---|
| 5 | 4.44 | ±0,1245 | 95 | 2.571 | ±0,154 | ±3.47 |
| 5 | 3,91 | ±0.1345 | 95 | 2,571 | ±0,154 | ±3.94 |
| 5 | 4.17 | ±0.1020 | 95 | 2.571 | ±0.116 | ±2,78 |

The absence of a systematic error of the method was shown by experiments with the addition of a definite amount of a standard solution of raffinose to an extract with a known concentration of raffinose:

| Found in extract, g | Added, g | Found, g | Relative error, % |
|---|---|---|---|
| 0.0391 | 0,0112 | 0.0485 | −3,5 |
| 0,0391 | 0,0093 | 0,0498 | +2,7 |
| 0.0391 | 0,0045 | 0,0422 | −3.2 |

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