METHOD FOR OBTAINING PHYTIN FROM RICE MIDDLINGS

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It has been established that the product of the transformation of phytin (phosphate) is absent from unroasted rice middlings. It is not formed, either, when phytin is heated at 160-200°C for 3 h. It is assumed that the phosphate is possibly formed under the conditions of high temperature and pressure (in the roasting of rice middlings) by the interaction of phytin with other substances present in the rice middlings. It has been found that the use of formalin in the production of phytin is undesirable.

Phytin — the mixed calcium-magnesium salt of inositol hexaphosphate [1] — is widely used in medical practice [2]. We have previously [3, 4] reported a modified method for determining it in rice middlings. The present work was devoted to investigating some chemical questions connected with the isolation of phytin from rice middlings.

Rice middlings contain 45-55% of the rice middlings proper (outer thin skin of the rice grain, rich in phytin) and the remainder of it consists of rice husks and chaff.

It is known that phytin is obtained from roasting rice middlings. Roasting is carried out in order to ensure the natural filtration of the acid extract in the extraction of the phytin. Otherwise, the filtration of the acid extract is very difficult. This phenomenon is due to the fact that on extraction protein and other accompanying substances pass into the acid solution together with the phytin and form a glue-like mass. In addition, the finely dispersed part of the rice middlings may deposit on the filter, which also interferes with filtration.

The process of roasting is accompanied by the coagulation of the protein and other substances, as a consequence of which the rice middlings become free-flowing and the acid solution is easy to filter.

In the roasting of the rice middlings, ~1% of the phytin is lost. The roasting process is carried out at a high temperature and a high pressure in the solid phase, and, possibly, under these conditions a transformation product of phytin — phosphate — is formed which gives a yellow coloration with an aqueous solution of silver nitrate. However, according to the requirements of the State Pharmacopoeia of the USSR, a medicinal preparation of phytin must not contain phosphates [5]. Below we give the results of a determination of the amounts of phytin in roasted and unroasted rice middlings and also of phosphate in the roast middlings from the harvests of previous years:

Harvest	Amount of phytin in the middlings, %		Amount of phosphate in the roasted rice middlings, %
	roasted	unroasted	
1976	3,38	4,42	0,53
1977	3,43	4,25	0,40
1978	3,45	4,38	0,45

Experimental investigations have shown that when rice middlings are extracted with acid in a ratio of 1:3 followed by the washing of the pulp with dilute acid or water and the precipitation of the phytin with ammonia at pH 8.0, this phosphate passes almost completely into the alkaline mother liquor. The technical phytin obtained in this way does not give qualitative reaction for inorganic phosphates.

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On long standing, the alkaline mother liquor sometimes deposits a very small precipitate of a phosphate.

Where the acid extract is saturated with phytin and, accordingly, with phosphate, the phytin precipitated from this solution shows the presence of phosphate, from which it is eliminated by reprecipitation. The alkaline solution on standing also deposits an appreciable amount of a phosphate which, as has been found, has similar properties to phytin: it dissolves readily in acid and is precipitated when the solution is made alkaline, it is sparingly soluble in water and is practically insoluble in ether, acetone, ethanol, and other neutral organic solvents.

We have performed a series of experiments to determine the reasons for the formation of the phytin transformation products. It has been established that unroasted rice middlings, both those obtained freshly and those that have been left for some years, contain no phosphate. Consequently, the formation of phosphate does not depend on the time of storage of the rice middlings.

When pure phytin was heated under various conditions (160-200°C for several hours), no formation of phosphate was observed.

The next question requiring experimental evidence was to investigate the necessity for using formalin in the process of isolating phytin.

In order to prevent a fermentation process in the production of phytin, a certain amount of formalin is added to the acid solution. However, as experiments performed under laboratory conditions at room temperature (summer, 30-35°C) have shown, even when an acid extract is stored for 3-4 days without the addition of formalin, and also when the alkaline solution obtained from this acid extract is stored, no fermentation takes place. Only after several days is the formation of mold observed in the alkaline mother liquor and in the acid extract, but this does not affect the yield of phytin.

The formation of mold is explained by the fact that these solutions are rich in nutrient substances and are an excellent medium for the multiplication of microorganisms.

A process of fermentation with the vigorous evolution of volatile substances with an unpleasant odor is observed when the alkaline solution obtained from the acid extract with addition of formalin is stored for 2-3 days. Under these conditions, the formalin takes part in a condensation reaction with ammonia, and the interaction products possibly under the action of microorganisms decompose with vigorous foam formation and the evolution of volatile low-molecular-weight nitrogen-containing substances.

EXPERIMENTAL

Isolation of Phytin from Unroasted Rice Middlings of the 1976 Harvest. Rice middlings (100 g) were carefully stirred with 300 ml of an acid solution consisting of 30% of ethanol, 1% of nitric acid, and 69% of water for 2-3 min, transferred to a Büchner funnel, and filtered off. The pulp was washed with 500 ml of 30% aqueous ethanol containing no acid. The combined extracts were neutralized with 25% ammonia to pH 8.0. The precipitate of phytin that deposited was filtered off with suction, washed with water, and dried at 100-120°C.

The yield of technical phytin was 4.42 g, which amounts to 4.42% of the air-dry weight of the rice middlings.

The amount of phytin in unroasted rice middlings obtained from the rice harvests of 1977 and 1978 was determined similarly.

Isolation of Phytin from Roasted Rice Middlings of the 1976 Harvest. Rice middlings (100 g) were stirred with 300 ml of 1% nitric acid solution for 2-3 min and were filtered off with suction. The pulp was washed with 500 ml of distilled water. The combined extracts were neutralized with ammonia to pH 8.0 and the precipitated phytin was filtered off with suction and washed with water (alkaline mother solution A).

The yield of technical phytin was 3.38 g, which corresponds to 3.38% of the air-dry weight of the roasted rice middlings. The technical phytin gave no qualitative reaction for inorganic phosphate ($AgNO_3$).

On long standing, mother liquor A deposited crystals of phosphate (30 mg). The amounts of phytin in the roast rice middlings of the 1977 and 1978 harvests were determined similarly.

Isolation of the Product of the Transformation of Phytin from Roasted Rice Middlings. Two 300-g samples of rice middlings were taken. The first was covered with 800 ml of 2% nitric acid and the mixture was carefully stirred and filtered with suction, and the pump was washed with 1000 ml of water.

To the second sample (300 mg) was added the acid extract obtained from the first sample, the mixture was carefully stirred and filtered with suction, and the pulp was washed with the second portion of extract. The mixture was made alkaline with ammonia to pH 8.0, and the phytin was filtered off with suction and washed with water (alkaline mother liquor A).

The yield of technical phytin and phosphate was 19.8 g, which amounts to 3.3% of the weight of the air-dry rice middlings.

On standing, the alkaline mother liquor A deposited small crystals of phosphate. Yield 1.25 g. The phytin transformation product (1.25 g) was dissolved in a small amount of nitric acid, the solution was filtered, the filtrate was made alkaline with ammonia to pH 8.0, and the phosphate that deposited was filtered off with suction. Yield 1.23 g. After two reprecipitations of the technical phytin from the alkaline mother liquor 1.5 g of technical phosphate was obtained.

SUMMARY

1. It has been found that when phytin is heated at 160-200°C for 3 h no phosphate is formed. It is possibly formed at a high temperature and a high pressure by the interaction of phytin with other substances present in the rice middlings.

2. The transformation product of phytin is absent from unroasted rice middlings.

3. It has been established that the fermentation process takes place in the alkaline solution obtained from an acid extract containing formalin; consequently, the use of formalin in the production of phytin is undesirable.

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PREPARATION OF TERT-BUTOXYCARBONYL DERIVATIVES OF AMINO ACIDS USING DI-TERT-BUTYL PYROCARBONATE

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In order to optimize and individualize the process, the influence of conditions of the reactions on the synthesis of Boc derivatives of amino acids using di-tert-butyl pyrocarbonate on the yield of desired product has been studied.

The modern state of peptide synthesis is characterized by the fact that an ever-increasing number of biologically active compounds of peptide nature are becoming products of industrial production. A consequence of this fact is an increase in the demand for the accessibility of auxiliary reagents and, in particular, for the main intermediates of peptide syn-

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