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Phytochemical Study of Rice Hull for Phytin Production

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Abstract: Currently, great strides have been made in the field of creating highly effective synthetic medical preparations. Nevertheless, preparations derived from plant raw materials occupy a significant place in medical practice and account for about one third of the total number of medicines. The volumes of annual procurements and processing of plant raw materials amount to tens of thousands of tones. At the same time, for more complete satisfaction of the health needs in medical preparations and expansion of the range of highly effective low-toxic drugs, it is necessary to find new sources of natural bioactive compounds and develop technologies based on them for integrated use of raw materials. A particularly important problem is development of modern technologies for production of biologically active substances from available, cultivated and promising drug raw materials in terms of manufacturing application. It is known that introduction of biometal ions into the molecule of a natural biologically active compound can enhance and expand the range of its therapeutic action. Such coordination compounds, in which molecules of natural compounds act as ligands, and transition metal ions (Cu, Co, Fe, Zn, etc.) as complexing compounds, with relatively low toxicity, have a higher and more diverse biological effect compared to the initial compounds. They also compare favorably from synthetic preparations with much lower toxicity, simpler chemical and technological methods for their production, and, consequently, less complex instrumentation for their production.

Keywords: Rice Hull, Extraction, Inositol Phosphate, Enzyme Preparation, Biopolymers

1. Introduction

One of the new, promising directions in the use of organophosphorus compounds is their use as medical preparations. Currently, more than 100 phosphorus-containing medical preparationsare known.

Organ phosphorus compounds that have a cholinergic action have been used for the first time as phosphorus-containing medical preparations, in particular, in ophthalmology and obstetrics /1/. Recently, phosphorus-containing medical preparations that have anticholinesterase activity and are fundamentally different from the previously used phosphorus-containing medical preparations in terms of chemical structure and mechanism of action have gained recognition in medicine. Along with antiglaucoma and other anticholinesterase agents, phosphorus-containing vitamins and their analogues, preparations for prevention and treatment of diseases of the cardiovascular system, antifungal, antiblastometic neurotropic, antibacterial, anthelmintic, hormonal preparations, metabolic stimulants and other medical preparations areused now.

Among the natural phosphorus-containing compounds, inositol hexaphosphoric acid and its derivatives are of particular importance.

According to the observations of M. A. Tyzhenko /2/, when taking inositol phosphate, in most cases, a sharp improvement in appetite, decrease in mental and physical fatigue and significant increase in working efficiency are observed. In men, after the use of inositol phosphate, an improvement in sexual ability is observed.

The versatile and high biological activity of inositol phosphates is primarily due to the high content of phosphorus and transition metal ions in them. It is known that the main function of phosphorus, which content in the human body reaches 650 g, is associated with the growth and maintenance of the integrity of bone tissue and teeth /216/. Its' another part is located in soft tissues and participates in anabolic and catabolic reactions, which can be seen from the role of phosphate in the formation of high-energy compound (ATP) and phosphorylation of intermediate carbohydrate metabolism products.

Calcium is one of the five (O, C, N, H, Ca) most common elements of a living organism. In addition to building the bone skeleton, calcium ions occupy key positions in many physiological and biochemical processes (for example, in neural excitation transfer, maintaining the integrity of cell membranes, etc.)

Calcium consumption by an adult organism reaches 1.0+1.1 grams/day /4,5,6/. The assumption of a possible high biological activity of metal derivatives has been indeed confirmed on the coordination compounds synthesized later. For example, molybdic salt of meletin /7/ has immunostimulant property, and compounds of inositol hexaphosphoric acid cobalt and iron are recommended for hemolytic anemia treatment /8/.

Recently, inositol phosphate is used in various sectors of the national economy, for example, in the food industry as stabilizing additives to food products in the canning process, it is also used as a fermentation inhibitor, source for inositol production.

It follows from the above that technologies of new coordination compounds based on the inositol hexaphosphoric acidand their dosage form in the form of tablets is relevant. The limited scope of this work has not allowed us to more fully describe all the problems, because each of them can provide a separate review, and we tried only to highlight the key issues here.

2. Research Methods and Materials

2.1. Research Materials

Materials and methods used by us to conduct scientific research comply with the requirements of the General Pharmacopoeia Monograph of the State Pharmacopoeia XI edition, European Pharmacopoeia, the United States Pharmacopoeia, British Pharmacopoeia, corporate standards, All-Russian corporate standards and other normative documents regulating the quality of medical preparations in the Republic of Kazakhstan.

2.2. Research Methods

When processing the obtained research results, the method of variation-statistical analysis has been applied using the Student's validation criterion (P 0.95).

To determine specificity, linear dependence, accuracy and reproducibility of methods for quantitative analysis of active components in medical preparations, the validation method has been used in accordance with the General Pharmacopoeia Monograph "Validation of analytical methods" (Appendix to the State Pharmacopoeia XI. 2002, Russia);

Reviewer Guidance "Validation Chromathographic Methods", Center for Drug Research (CDER), Washington, 1994; Validation of compendial method (USP, 1225), 2002.

3. Results

In the production of rice grits, the grains are subjected to fourfold grinding. Therefore, rice caryopsis waste is a powder of a grayish-straw color and consists of a mixture of different grindingfractions of rice grain and certain amount of finely crushed grains.

In our opinion, it is of some interest to identify the most abundant inositol phosphate grinding fraction. For this, the content of the product in each fraction has been determined [9, 10, 11].

The results of the experiments are shown in Table 1.

Table 1. *Content of calcium and magnesium inositol phosphate in different fractions of rice grinding (in % in air-dry weight of the raw material).*

№	Wastes after the first grinding	Wastes after the second grinding	Wastes after the third grinding	Wastes after the fourth grinding
1	4,98	3,78	1,95	0,89
2	5,12	3,80	2,06	0,75
3	5,08	3,76	2,03	0,78
4	5,01	4,01	2,00	0,98
5	4,96	3,81	1,98	1,02
6	4,97	3,69	1,90	1,04
7	5,02	3,75	1,84	1,02
8	5,06	3,73	1,75	0,88
9	4,98	3,82	1,72	0,96
10	5,04	3,79	1,88	1,06

According to Table 1, it can be seen that the first and second grinding wastes are the richest with inositol phosphate. The amount of wastes of each fraction obtained in the mills is about the same.

In order to improve the quality indicators of the rice production wastes, as a source of obtaining the inositol phosphates, we have developed a scheme for separate collection of wastes.

At the same time, it was possible to obtain raw materials for production of inositol phosphates with a 30–40% higher content of the active substance and consisting only of the first and second grinding fractions.

Dynamics of extraction of minerals and biopolymers from rice hull.

Mineral composition of rice hull may vary depending on soil composition, plant growth conditions and seed maturity, which can affect the mineral composition of inositol phosphates.

Samples of rice hull and calcium and magnesium inositol phosphate obtained under laboratory conditions have been taken as the research objects. Determination of the content of metal cations in the solution has been carried out on atomic absorption spectrophotometer.

The results of the analysis are shown in Table 2.

Table 2. *Mineral composition of rice hull and calcium and magnesium inositol phosphate, %.*

Mineral Sample composition	Calcium	Magnesium	Sodium	Potassium	Phosphorus
Rice hull, sample № 1	0,924	0,074	0,012	0,018	0,914
Rice hull, sample № 2	0,980	0,058	0,018	0,024	0,876
Calcium and magnesium inositol phosphate, sample № 1	21,09	2,75	0,026	0,03	20,30
Calcium and magnesium inositol phosphate, sample № 2	22,14	2,68	0,028	0,025	21,12

From Table 2 it is seen that the rice hull as a source of inositol phosphates contains calcium and magnesium much more than potassium and sodium. This is due to the special role of these cations in plant life. In the samples of inositol phosphate derived from the rice hull, the main content of mineral components is in the cations of calcium and magnesium. The reason for this is that inositol hexaphosphoric acid with alkali earth metals gives neutral salts that are insoluble in water, while their salts dissolve easily in water with alkali metals. Therefore, at the stage of precipitation of calcium and magnesium inositol phosphate in a neutral medium, sodium and potassium salts of inositol hexaphosphoric acid remain in the mother liquor.

With regard to the quantitative ratio of calcium and magnesium in the sample of inositol phosphate, there are significant deviations from the composition known in the literature [12-14], where five magnesium atoms and one calcium atom in the molecule of inositol phosphate are about 14% and 4.9%, respectively.

In the laboratory sample of calcium and magnesium inositol phosphate, magnesium has been found to be less (2.7%), and calcium has been more (22.8%). If at the stage of precipitation of inositol phosphates to introduce additional amount of calcium ions, then even greater increase in calcium (up to 27%) can be achieved, as well as sharp decrease in the content of sodium and potassium due to the removal of water-soluble alkali salts of inositol hexaphosphoric acid.

Consequently, the mineral composition of inositol phosphate depends both on the method of its extraction and on the nature of the initial raw material.

In order to obtain calcium inositol phosphate, where one molecule of inositol hexaphosphoric acid is combined with five calcium atoms and one magnesium atom, conditions of the technological process have been selected with introduction of additional amount of calcium ions. To this end, in the process of obtaining calcium inositol phosphate from the rice hull, the mineral composition of which has already been established, a calculated amount of calcium chloride has been added to the clarified extract. Thus, a technology that allows obtaining samples of calcium inositol phosphatehas been developed. The elemental analysis in%: Ca – 26.8; 26.4; C – 7.6; 7.3; H – 0.65; 0.62; P – 20.4; 20.7; $C_6H_6O_{24}P_6Ca_6$ (see Chapter V).

Along with the minerals, the rice hull contains significant amounts of proteins, starch and other substances. Therefore, in the raw material we have determined the content of crude protein, lipids, ash, crude fiber and starch by the following methods: GOSTs 13979-68, 14176 and 10845-76. The data obtained are shown in Table 3.

Table 3. *Content of the main components in the rice hull, %.*

Content of components	Rice hull			
	sample 1	sample 2	sample 3	sample 4
Moisture	9,40	9,60	9,30	9,70
Calcium and magnesium inositol phosphate	3,86	3,94	3,78	4,02
Crude protein	12,14	10,71	1,64	10,48
Fats	9,84	10,08	9,46	9,18
Crude fiber	4,56	4,45	4,07	4,12
Starch	22,14	21,58	22,17	21,04

When extracting the inositol phosphates and their metal derivatives, along with other substances contained in the raw material, proteins and starch are extracted in large quantities. The content of the latter in the extract increases its viscosity, complicates cleaning from fine particles.

In addition, proteins and starch being in thesolution of inositol phosphates can form complex compounds with inositol hexaphosphoric acid and sharply reduce the yield of the target product. Therefore, we have studied the extractability of inositol phosphates, proteins and minerals from the raw material, depending on the process conditions.

The extractability of minerals from the rice hull as a function of pH of the medium in the range of 4.0–8.0 almost coincides with the extractability of inositol phosphates. This confirms the solubility data for the corresponding salts of inositol hexaphosphoric acid. It can also be assumed here that calcium, magnesium, sodium and potassium salts of inositol phosphates tend to dissolve at low pH values and, conversely, do not dissolve at high pH values.

The above results suggest that it is reasonable to extract the inositol phosphates from the rice hull at pH 2-2.5. In this case, the minimum amount of proteins will be transferred to the extraction. However, it is characteristic of the rice hull that in no case is complete extraction of inositol phosphates achieved. This is explained by the fact that the latter in plants are not only in the form of mineral salts, but also in the form of a complex with carbohydrates, lipids and proteins, and constitute the main part of the biological membrane of the cell. Therefore, directed hydrolysis of biopolymers of plant raw materials could provide an opportunity to increase the extractability of inositol phosphates.

Use of enzyme preparations when preparing the inositol phosphates from the rice hull.

Enzyme technologies have recently been rapidly developing and are widely used in various industries, and in particular in the manufacture of pharmaceuticals. In the production of phytochemicals, enzymes can be used for the targeted hydrolysis of plant materials and clarification of extracts in order to improve filtration and increase the yield of the final product. The basis for increasing the yield is that some enzyme preparations are able to affect the structural elements of plant cells, splitting the biopolymers of the cell membranes, enhancing the diffusion process and simultaneously decreasing the viscosity of the extracts. On the basis of the clarifying effect of enzyme preparations, the splitting of colloidal particles, determiningblushing of the extracts, which are a complex mixture of proteins, polysaccharides, pectin substances, etc., is caused [15].

We have investigated the possibility of using enzyme preparations in obtaining derivatives of inositol phosphates from the rice hull.

It is known that the rice hull contains a large amount of protein and insoluble starch, which complicate the extraction process and separation of the extract from theextraction cake. We have determined the content of the above compounds in the rice hull by known methods. The data are shown in Table 4.

Table 4. *Content of starch and protein in the rice hull.*

Number of the sample of the rice hull	1	2	3	4	5	6	7	8	9
Starch,%	22,4	21,58	21,17	21,04	21,41	22,03	21,27	21,12	22,64
Protein, %	12,14	10,71	11,64	10,48	11,56	11,24	10,96	12,02	10,76

From the results given in the above table it follows that the raw material contains a large amount of proteins and starch, which significantly complicate the extraction stages, and the extract filtration.

We have investigated the enzymatic ability of some enzyme preparations with amylolytic and proteolytic activity, such as amylosubtillin g10x, aminorizinep 10x, glucoamylase, protosubtillin g 10x, amylosubtillin g 3x, amilorizinePx. It has been assumed that the enzymatic splitting of biopolymers of plant raw materials can substantially change a number of stages of production of inositol phosphates from the rice hull.

The optimal conditions for the action of these enzyme preparations are approximately the same, which is convenient for the experiments on the technology of fermentation of plant raw materials.

For the hydrolysis of biopolymers from the rice hull, we have carried out studies on the enzymatic activity of a number of enzyme preparations with amylolytic and proteolytic activity.

Table 5 below shows some characteristics of the enzymes used.

Table 5. *Characteristics of the enzymes used.*

Enzyme preparation	Producer	Activity	Optimal conditions
Amylosubtillin G 10x	Bacillussubtilis	AA 3000 PA 2	pH 6,3
Aminorizine P 10x	Aspergillusorirae	AA 2000 SA 10000 PA 30	pH 4,7 ÷ 5,5 50 ÷ 55°C
Glucoamylase	Aspergillusawomori	GA 2000 AA 50 PA 15	pH 3-5 55 - 60°C
Protosubtillin G 10x	Bacillussubtilis	PA 70 AA 150	pH 4,5 – 6,5 50 – 55°C
Amylosubtillin G 3x	Bacillussubtilis	AA 1000 PA 5	pH 6 -6,3 50 – 55°C

where:
AA – amylolytic activity;
PA – proteolytic activity;
SA – saccharifying activity;
GA– gluco-amylolytic activity.

To determine the optimal conditions for the action of enzymes, we have carried out experiments on the fermentation of plant raw materials under approximately the same conditions, based on the data in the Table. During the hydrolysis of biopolymers from the plant raw materials, the optimal concentration of enzymes is in the range of 0.5 – 2.0% [16]. Based on this, the experiments used 1.0% enzyme solutions.

The fermentation has been carried out as follows:

500 g of the rice hullwere loaded into extractors equipped with a mixer and placed in a water bath and a 1.0% solution of the enzyme was poured, the mass was acidified with a solution of nitric acid to pH corresponding to the optimal activity of the selected enzyme and the mixer was turned on.

The fermentation was carried out for 1 hour. After completion of the fermentation, the suspension was acidified to pH of 2.5, and extraction of inositol phosphates was carried out for 30 minutes.

The extract was separated from the extraction cake and its degree of clarity was determined. The degree of hydrolysis of biopolymers was determined by the amount of glucose formed by the spectrophotometric glucooxidase method [17].

The number of amino acids formed during the hydrolysis of proteins and peptides was determined by the known method [18].

Further, the collected extract was transferred to the stage of obtaining metal complexes of inositol hexaphosphoric acid.

Table 6. *Dependence of the extract's clarity degree, glucose and amino acid yield on the enzyme nature.*

0.1% solutions of the enzymes	The extract's clarity degree, %	Glucose content in the extract, %	Amino acids content in the extract, %	Calcium and magnesium inositol phosphate yield on the raw material mass, %
Amylosubtilling3x	68,7	5,89	1,58	3,74
Amylosubtilling10x	88,2	8,76	5,41	3,96
Aminorizinep10x	94,8	10,84	8,64	4,23
Aminorizineg10x	74,5	5,17	8,12	3,84

From the experimental data shown in the above table, it follows that the best results are obtained when using such enzyme preparations as amylorizin p10x and amylosubtilin g10x.

Considering that amilorizin p 10x has combined effect at pH 4-4.5 it breaks down starch to glucose and at pH 2-2.5 it breaks down protein substances [19]. For further research, we have chosen amilorizin p10x. In addition, production of this enzyme preparation is organized on an industrial scale.

4. Conclusion

Various fractions of the rice grain grinding wastes on the content of inositol phosphate have been studied and rich source of raw materials containing more than 4% of the target product has been proposed.

The solubility of inositol phosphate and kinetics of its extraction from raw materials have been studied, the optimal conditions of the process have been found using Box-Wilson mathematical method of planning of the experiment.

The original method for obtaining inositol phosphate from the rice hull using enzyme preparations has been developed. It has been established that during the fermentation at pH of 4.5, the hydrolysis of carbohydrates and partial extraction of inositol phosphate occurs by increasing the acidity of the medium with pH of 2.5, the hydrolysis of inositol-protein and protein biopolymers has been carried out and more complete extraction of inositol phosphate has been achieved.

The use of enzymatic hydrolysis of biopolymers of the rice hull reduces the viscosity of the extract, improves its filterability and increases the yield of the target product by 30 – 35%.

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