# Composition and Structure of Phytic Acid Derivatives from Rice Bran

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A novel phosphorus-containing product extracted from rice bran, a rice production by-product from the Far Eastern selection, was isolated. Its composition and structure were determined by chemical methods and IR (Infrared), NMR (Nuclear Magnetic Resonance) ($^1$H, $^{13}$C, $^{31}$P) spectroscopy, and X-ray phase analysis. Physicochemical characteristics such as moisture, bulk density, pH of the aqueous suspension, and water solubility under different conditions of the phosphorus-containing product were obtained.

*Keywords: Rice bran; Phytic acid; Rice production waste*

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## INTRODUCTION

Waste from rice production (straw, husk, and bran) accumulates annually in considerable quantities, and these raw materials can be used to make products in various fields of the chemical industry (Sergienko *et al.* 2004). Studies at the Institute of Chemistry of the Far Eastern Branch of the Russian Academy of Sciences have indicated that wastes of rice production (rice husk, straw, and bran) are useful sources of polysaccharides (Zemnukhova *et al.* 2004), lipids (Zemnukhova *et al.* 2006), amino acids (Zemnukhova *et al.* 2010), and corrosion steel inhibitors (Kharchenko *et al.* 2008). One of the important classes of polyfunctional material extracted from rice bran (RB) is inositol hexaphosphoric or phytic acid and its derivatives (phytin, phosphoinositols). Phosphoinositols exhibit a wide range of properties; namely, they seem to be a candidate as a replacement setting retardant for dicalcium phosphate cements (Meininger *et al.* 2017), they demonstrate antioxidant and antitumor activity in a number of diseases (Kumar *et al.* 2010; Diouf-Lewis *et al.* 2017), and they provide sorption capacity for heavy metal ions (*e.g.*, $Co^{2+}$, $Sr^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Cu^{2+}$, and $Mn^{2+}$) (Makarenko *et al*. 2015). Phytic acid, which is an ester of the cyclic hexahydroxide of myo-inositol and six phosphoric acid residues with the general formula $C_6H_6$ $[OPO(OH)_2]_6$, is a typical plant product. Despite the apparent simplicity of the phytic acid molecule, the composition and structure of its salts are the subject of much debate (Raboy 2003; Sakai *et al.* 2017) insofar as they may vary depending on the type of rice, the place of its growth, and the processing method. Notably, there is no data on the structure of phytic acid derivatives, which are a component of varieties of bran cultivated in Primorsky Krai, available in the literature. Phosphoinositols may not contain all six residues of phosphoric acid, and they may contain from one to five phosphorus-containing groups.

This work is devoted to the study of composition, structure, and physicochemical characteristics of the phosphorus-containing product extracted from the rice bran.

## EXPERIMENTAL

### Obtaining Phosphorus-Containing Product from Rice Bran

All the reagents were of an analytical type and were received from Sigma-Aldrich Co. (Seelze, Germany).

Rice bran of the rice variety Lugovoy harvested in 2016 from Primorsky Krai, Russia was used as a raw material. The rice bran is a product obtained in the process of grinding the kernel after removing the rice husk. The sample of the rice bran was collected at the operating plant of Primorsky Krai for the rice production. According to elemental analysis, the composition of the rice bran is as follows (%): K – 52.86, P – 21.77, Mg – 9.42, Si – 7.43, Ca – 3.49, S – 1.76; Mn – 1.48.

Phytic acid extraction was performed by acid hydrolysis of the raw material (bran) in 1% HCl with stirring for 20 min to 3 h at 20 to 80 °C. The volumetric ratio of solid to liquid was 1:5. The raw materials and filtrate were separated by centrifugation. The filtrate was subject to ultrafiltration through UAM-500 acetate cellulose membranes according to Kolzunova *et al.* (2000). The process was conducted at a pressure drop of 0.1 to 0.4 MPa and $t$ = 20 °C at an installation consisting of a separate cell of UFM-3-2 ($V_1$ = 20, 100, or 1000 mL), a capacity container ($V_2$ = 2000 mL), a magnetic stirrer MM-5, a compressor, a receiver, manometers, and a collection of filtrate. All elements of the separation cell contacting solutions were made of non-corroding, well-sterilized materials. From the purified extract, the phytic acid salt was precipitated with a 10% NaOH solution, at a pH range of 7.0 to 8.0. The salt was then separated from the solution by filtration, washed on a filter with water, and air dried at 25 °C. The yield of the phosphorus-containing product from rice bran was 6%. The yield of the product was calculated relative to the mass of the initial sample of the rice bran.

### Methods of Studying the Phosphorus-Containing Product from Rice Bran

The IR (Infrared) spectra in the range 400 to 4000 $cm^{-1}$ in KBr pellets were recorded by conventional techniques on a Bruker Vertex 70 spectrometer (Karlsruhe, Germany).

The X-ray diffraction patterns were recorded on a D8 Advance diffractometer (Bruker) on Cu $K_\alpha$-radiation. Phase identification was carried out using the EVA program (EVA Software defines a new benchmark for phase analysis) and the PDF-2 powder data bank (The Powder Diffraction File).

The concentration of elements was determined by the semi-quantitative method of the energy dispersive X-ray fluorescence spectroscopy (ED XRFS) on an EDX 800 HS spectrometer (Shimadzu, Kyoto, Japan). A tablet (radiator) with a diameter of 20 mm was pressed from the test sample, and spectra of elements of 100 s were measured in each energy channel. The radiating source was a tube with a Rh - anode. The concentration of elements was calculated by the method of fundamental parameters using software spectrometer without light elements.

The mass fraction of moisture content was determined according to GOST 12597-67 (Russian). “Sorbents. A method for determining the mass fraction of water in active coals and catalysts based on them”.

To determine the bulk density, the test sample was poured into a previously weighed cylinder of 10 cm height, and then the sample cylinder was weighed. The bulk density of the sample was calculated using Eq. 1,

$$\rho_H = \frac{m_1 - m}{V} \tag{1}$$

where $\rho_H$ is the bulk density of the sample (kg m$^{-3}$), $m_1$ is the mass of the measuring cylinder with the sample (kg), $m$ is the mass of the measuring cylinder (kg), and $V$ is the volume of the measuring cylinder (m$^3$).

The solubility of the phosphorus-containing product was determined during 30 min at 25 °C and at the pH values of the medium being 3.4, 5.6, and 8 and in distilled water at 60 and 90 °C. A sample of the phosphorus-containing product weighing 0.15 g and 60 mL of distilled water were added into 100 cm$^3$ beakers. After 30 min, the insoluble residue was filtered through filter paper with a pore size of 2 to 3 µm. The filtrate pH was determined. The residue was dried at 105 °C until it reached a constant weight, and the solubility of the phosphorus-containing product was calculated.

To determine pH of the aqueous extract, 5 g of the sample was placed in a flask with 50 mL of water, and the mixture was boiled with a reflux condenser for 3 min. The flask content was rapidly filtered through a filter paper with a pore size of 2 to 3 µm. After the filtrate was cooled, its acidity was determined on a FiveEasyPlus 20 pH meter (Mettler Toledo, Greifensee, Switzerland).

Measuring the pH of an aqueous suspension of the sample in time was carried out on a pH-meter SevenCompact (Mettler Toledo, Greifensee, Switzerland) using the X-Lab software. For this purpose, 20 mL of distilled water with pH 6.4 to 7.6 was added into a potentiometric cell. Distilled water was pre-boiled for 15 min to remove $CO_2$, and then rapidly cooled to room temperature. The glass electrode was placed into distilled water, and 0.2 g of the sample were added after the stabilization of the potential.

## Demineralization of Phosphorus-Containing Product from Rice Bran

A sample weighing 0.1 g of the phosphorus-containing product that was isolated from rice bran was dissolved in 1 L of distilled water and passed at the temperature 40 °C through a column containing 90 g of strong acid cation-exchange resin KU-2-8hs in the $H^+$-form (analogues - Dowex HCR-S, Marathon C, Monosphere 650 C, Monosphere 500 C). It has been known that copolymers of styrene and divinylbenzene (the basis of sulfocathionites) are characterized of low sorption tendency for low molecular weight anions and neutral compounds. The ion-exchange column after applying the phosphorus-containing substance solution was washed with 0.5 L of distilled water for a long time, which eliminates the loss of non-cationic substances in result of demineralization. The aqueous solutions were combined and evaporated to dryness in vacuum (8 Torr) at 45 °C. In the residue, a colorless amorphous substance was obtained, which is highly soluble in water.

The NMR (Nuclear Magnetic Resonance) ($^1$H, $^{13}$C, $^{31}$P) spectra of the demineralized sample were recorded on Bruker Avance-400 ($^1$H, 400 MHz, $^{13}$C, 100.6 MHz, $^{31}$P, 162 MHz) and Bruker Avance-600 ($^1$H, 500 MHz, $^{13}$C, 150.9 MHz; $^{31}$P, 243 MHz) spectrometers.

## RESULTS AND DISCUSSION

Elemental analyses of the phosphorus-containing product from rice bran indicated that its predominant elements were P (45.1%), Mg (26.8%), K (12.2%), Na (6.3%), Ca (3.9%), Mn (2.3%), and Si (2.1%). Thus, the studied sample was a mixture of phytic acid and its salts, predominantly magnesium and potassium. According to the X-ray diffraction data, the product extracted from the rice bran was amorphous, as described previously by Makarenko *et al*. (2013).

The IR spectrum of the product (Fig. 1) extracted from the rice bran has wide intense absorption bands in the range 1127, 997, and 539 cm$^{-1}$, which according to Nakamoto (1991) could be attributed to valence (symmetric and asymmetric) and deformation vibrations of the P–O bonds in $PO_4^{3-}$ anion. The presence of the OH groups was demonstrated by the availability of the absorption bands in the region of valence (3392 cm$^{-1}$) and deformation (1660 cm$^{-1}$) vibrations. The IR spectrum of the phosphorus-containing sample was similar to the IR spectrum of commercial phytin (Saburov and Kamilov 1989).

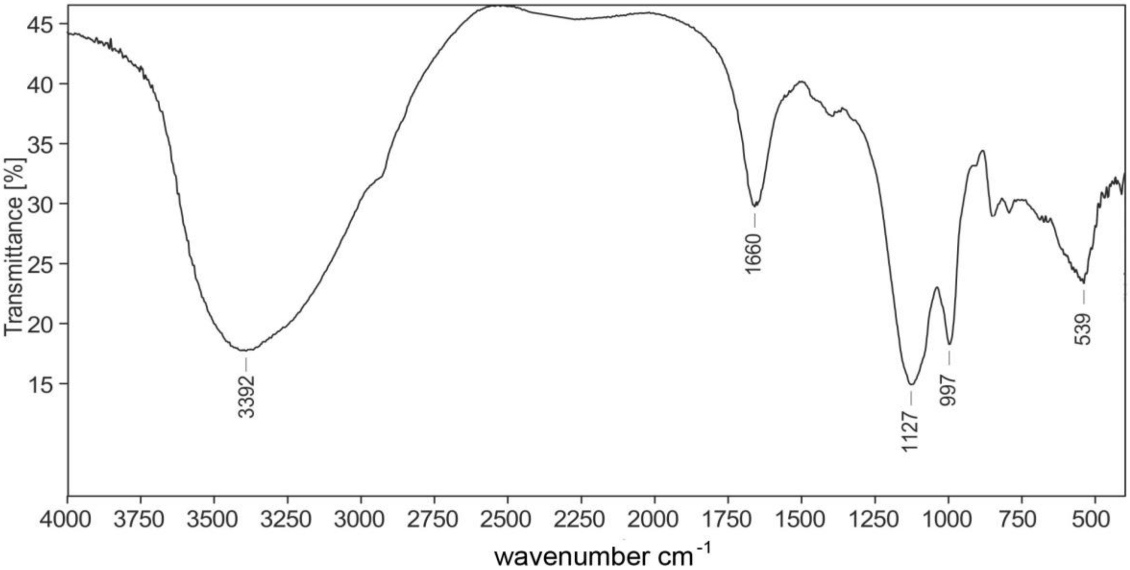

**Fig. 1.** IR spectrum of phosphorus-containing product from rice bran

The resulting product contained 4.5% of sorbed water (moisture) and had a high bulk density (552 kg/m$^3$).

The pH value of the aqueous extract of the phosphorus-containing sample was 6.7. The pH of the aqueous suspension changed over time in the first 270 seconds from 7.5 to 9.4, and then the acidity stabilized (Fig. 2). The high pH value of the medium measured in dynamics compared with the pH of aqueous extract may be due to the hydrolysis of phytic acid salts.

The acidity and temperature of the medium affect the solubility of phytic acid salts (Greiner and Konietzny 2006; Šala *et al*. 2011; see Table 1). With decreased pH, the solubility of the test sample increased. At pH 3, the solubility of the phosphorus-containing product was 0.239 g/100 g, while the mass of the sample residue was 4% of the initial value. In a slightly alkaline medium (pH 8), solubility did not exceed 0.175 g/100 g (70% of the initial mass). With increased temperature, the solubility also increased.

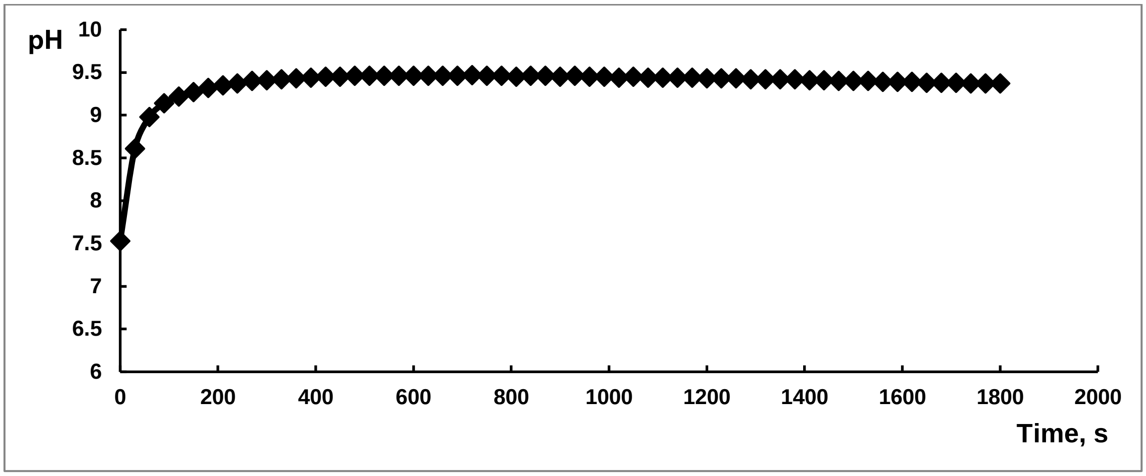

**Fig. 2.** pH change kinetic curve of the aqueous suspension of the rice bran phosphorus-containing sample

**Table 1.** Solubility of the Rice Bran Phosphorus-Containing Sample

| Dissolution Conditions | | Solubility (g/100g of Water) | pH of the Filtrate After Dissolution | Residue Mass (%) |
|---|---|---|---|---|
| pH | t (°C) | | | |
| 3 | 25 | 0.239 | 3.8 | 4.4 |
| 4 | 25 | 0.212 | 4.7 | 15.4 |
| 5 | 25 | 0.198 | 5.7 | 21,0 |
| 6 | 25 | 0.189 | 6.2 | 24.3 |
| 8 | 25 | 0.175 | 8.1 | 70.0 |
| 6 | 60 | 0.182 | 7.6 | 27.2 |
| 6 | 90 | 0.194 | 7.6 | 22.4 |

To determine the structure of the test product extracted from the rice bran, the NMR ($^{1}$H, $^{13}$C, and $^{31}$P) method was used. However, concentrated solutions could not be obtained because the sample had a limited solubility. The colloidal nature in conjunction with the salt nature of the phosphorus-containing fragments led to a strong broadening of the spectral signals and their superposition; the fine structure of the multiplets prevent accurate attribution. For a better resolution of resonances in the NMR spectra, the rice bran phosphorus-containing sample was demineralized using strongly acidic cation exchange resin KU-2-8-hs in the $H^+$ form. The obtained colorless amorphous substance was readily soluble in water. Control over the substance constituents by spectral methods was carried out before and after demineralization. In $^{1}$H NMR spectra of the substance before demineralization, the same signals of protons of inositol hexaphosphate and oligosaccharides are present (in comparison with the demineralized substance), but the signals is a highly broadened, which is explained by exchange processes involving phosphate fragments. The ratio of the integral intensity of the signals in NMR spectra before and after demineralization confirmed the invariance of the composition of the substance. After demineralization, the spectral picture is substantially improved, and it has become possible to determine the values of the spin-spin coupling constants.

In the $^{31}$P NMR spectrum of this demineralized sample (Fig. 3), the three signals $\delta$P –0.5, –1.1, and –1.8 were observed with a ratio of integral intensities of 3:2:1, respectively. Some broadening of the signal at $\delta$P –0.5 along with the reported data (Barrientos *et al.* 1994; Turner *et al.* 2007; Heighton *et al.* 2008) support the structure of the myo-isomer inositol in the inositol esophosphate (Fig. 4). This was also confirmed by $^{1}$H NMR displayed in Fig. 5. In the pH range 0.5 to 10.5, the conformation of phytic acid is stable, and it has one axial and five equatorial phosphate groups. In the $^{31}$P NMR spectrum, it is manifested by four signals in the $\delta$P range from –0.5 to –2.5. In the $^{31}$P NMR spectrum of the sample, the resonances from $C_3$OR and $C_4$OR overlapped, which was reflected in the relative signal intensity at $\delta$P –0.5 (Fig. 3).

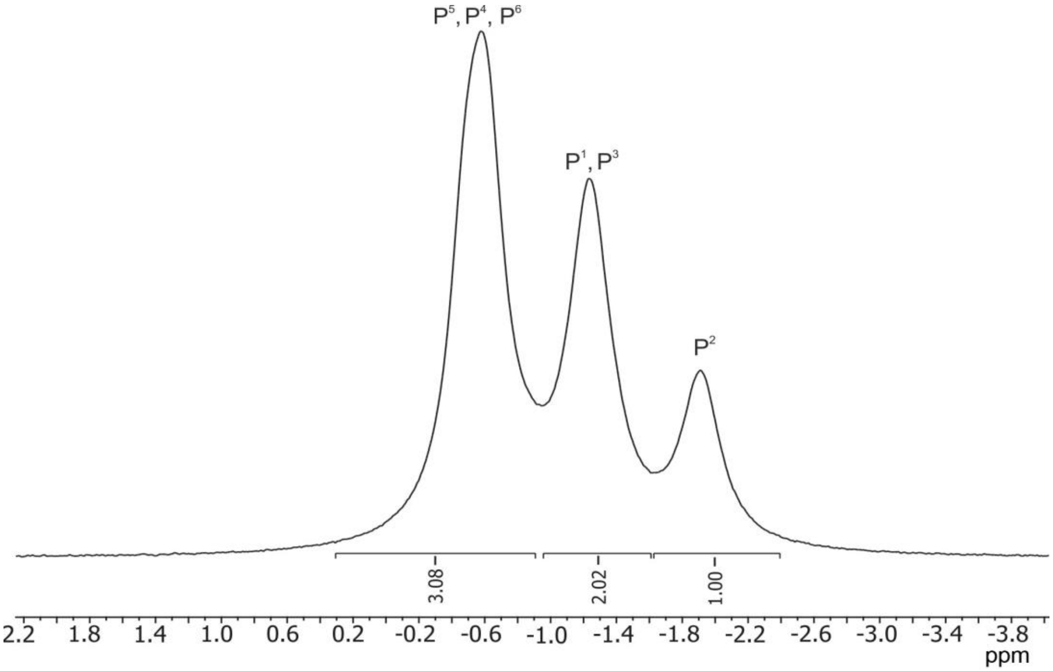

**Fig. 3.** The $^{31}$P NMR spectrum (162 MHz, $D_2O$) of a demineralized phosphorus-containing product from rice bran

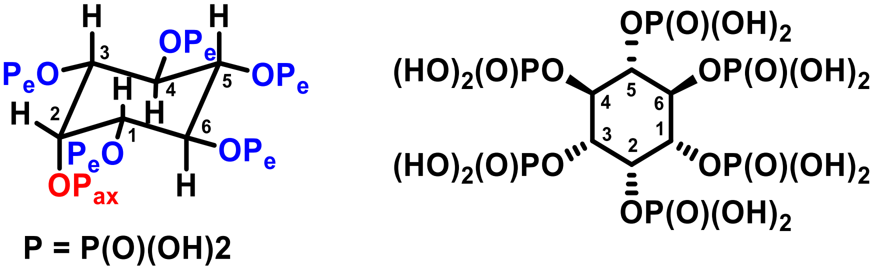

**Fig. 4.** The structure of a demineralized phosphorus-containing product from rice bran

In the $^{1}$H NMR spectrum of the studied substance in the range $\delta$ 3.8 to 4.5, a group of signals from the cyclohexane backbone of phytic acid were observed. At $\delta$ - 4.5 the $H_2$ signal appeared in the form of a broadened doublet with Spin-Spin Coupling Constant from phosphorus ($^{3}J_{POCH}$ 8.6 Hz). The H4 and H6 protons appeared as a broadened pair of doublets ($\delta$ 4.05), whereas the protons $H_1$ and $H_3$ emerged at approximately $\delta$ 3.9 as a pair of doublets (Fig. 5).

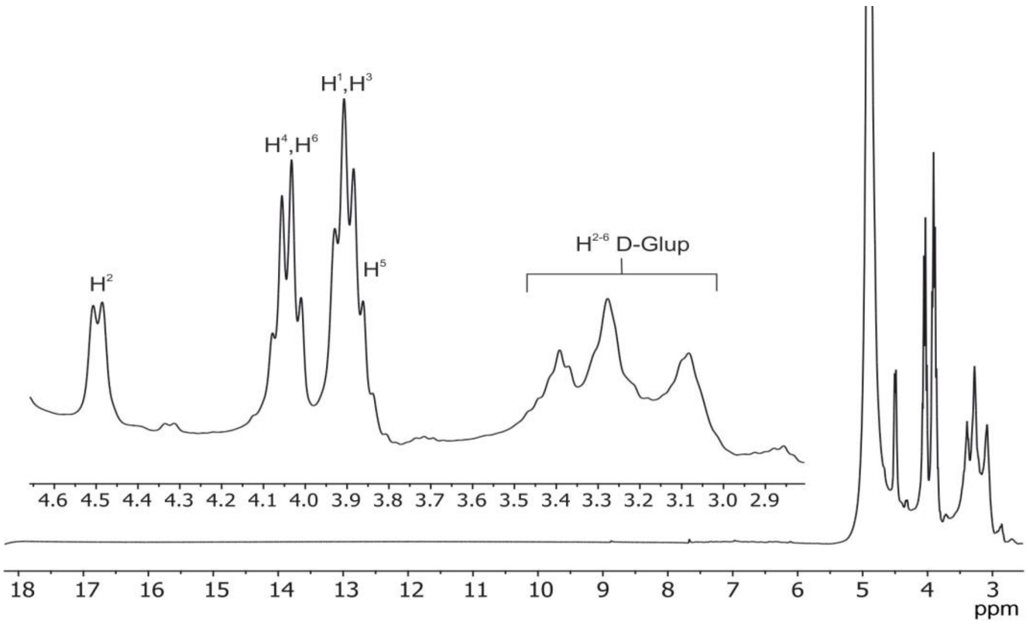

**Fig. 5.** The $^{1}$H NMR (600 MHz, D$_2$O) NMR spectrum of demineralized phosphorus-containing product from rice bran

Along with the signals from phytic acid, the $^{1}$H NMR spectrum contained broadened signals in the range of $\delta$ 3.0 to 3.5. Inspection of the $^{13}$C- and $^{13}$C{$^{1}$H} NMR spectra (Fig. 6) indicated the presence of starch in the sample, which was confirmed by the presence of the broadened signals of D-glucopyranoside fragments at approximately $\delta$C 100 (anomeric carbon) and 60 ppm (C6) and also groups of signals in the range of $\delta$70 to 78 (C2-5). In the $^{13}$C{$^{1}$H} NMR spectrum, availability of four signals at $\delta$72.8, 75.6, 75.9, and 76.7 indicated that the demineralized sample obtained from the rice bean was phytic acid.

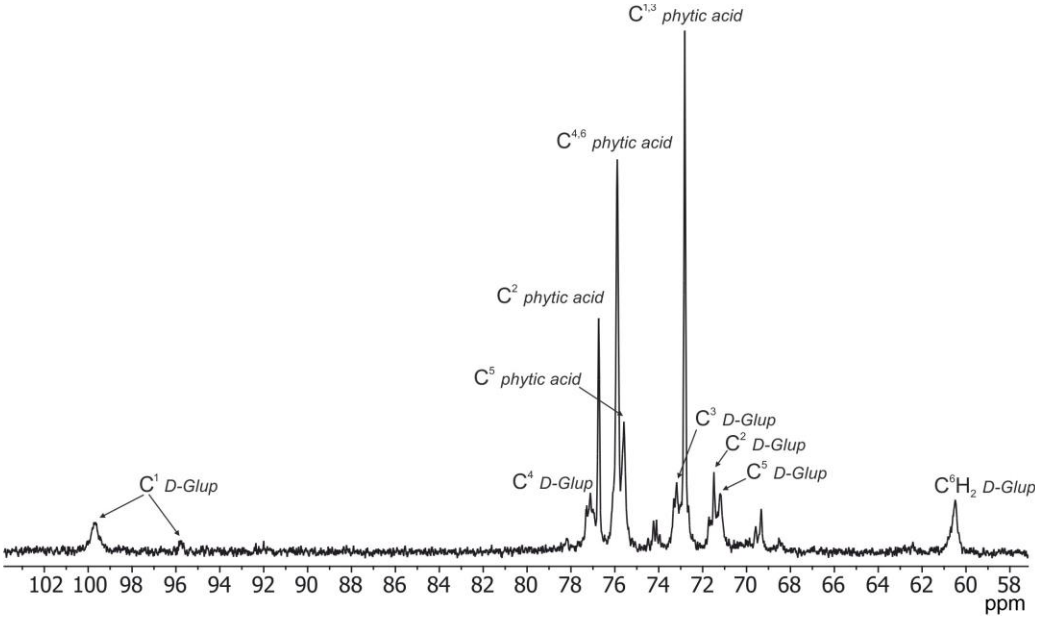

**Fig. 6.** The $^{13}$C- and 13C{1H} NMR spectra (150.9 MHz, D$_2$O) of a demineralized phosphorus-containing product from rice bran

## CONCLUSIONS

1. In this work, a sample of phosphorus-containing product from rice bran that is a by-product from rice production was obtained. By means of energy-dispersive X-ray fluorescence spectroscopy it was found that the sample was a calcium-magnesium salt of phytic acid. The IR spectrum of the phosphorus-containing product is characterized by the intense absorption bands (1127, 997, and 539 cm$^{-1}$) of P–O stretches in the $PO_4^{3-}$ anion, as well as OH groups (3392, 1660 cm$^{-1}$), which emerge in the same ranges as those in the IR spectra of commercial phytin.
2. The physicochemical characteristics of the phosphorus-containing product from the rice bran were studied. Acidities of the aqueous suspension of the phosphorus-containing product vary in time from pH 7.5 to 9.4. Solubility of the phosphorus-containing product in water increases along with the increase of temperature to 90 °C and a decrease of acidity from pH 8 to 3.
3. By NMR ($^1$H, $^{13}$C, $^{31}$P) spectroscopy, this study established the structure of the demineralized phosphorus-containing substance isolated from rice bran, which corresponds to inositol hexaphosphoric acid.

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