The study of the carbohydrate composition of hawthorn fruits

Carbohydrates, in particular monosaccharides, are necessary for the normal functioning of the human body. Therefore, the search for plant sources of available carbohydrates, such as glucose, fructose, and arabinose, is relevant today.

**Aim.** To determine the qualitative composition and the quantitative content of monosaccharides of hawthorn fruits.

**Materials and methods.** Extraction of polysaccharides from the raw material was carried out with purified water in the ratio of the raw material – extractant of 1 : 10 and further treatment of the filtrate with 96 % ethyl alcohol. The preliminary identification of sugars was performed by paper chromatography (PC) after acid hydrolysis. The qualitative composition and the quantitative content of sugars were determined by the gas chromatography-mass spectrometry method on an Agilent 6890N/5973 inert device (Agilent Technologies, USA). The total sugars were isolated by acid hydrolysis using 2 M trifluoroacetic acid. For extraction of free sugars the mixture of methyl alcohol solution and the internal standard (the solution of sorbitol) was added to the powdered raw material.

**Results and discussion.** As a result of chromatography (PC) with the reliable samples glucose and fructose were identified in the raw material. By the chromatography-mass spectrometry study the presence of 5 free sugars was detected, among them glucose (2.02 mg/g), fructose (2.21 mg/g) and sucrose (0.23 mg/g) were determined. After acidic hydrolysis arabinose (1.82 mg/g), xylose (3.88 mg/g), mannose (4.25 mg/g), glucose (5.57 mg/g) and galactose (1.31 mg/g) were identified.

**Conclusions.** The composition of sugars in hawthorn fruit has been found. The highest content has been determined for fructose, glucose, and mannose.

**Key words:** hawthorn; fruits; sugars

N. V. Sydora, A. M. Kovalyova, V. K. Iakovenko

National University of Pharmacy
Monosaccharides are an important part of the daily human diet. These compounds are necessary for the normal functioning of the body [1]. Moreover, glucose is the source of energy for organs and tissues, and it is actively used by the muscles; fructose is involved in metabolic processes; ribose is a component of deoxyribonucleic acid [2]. The search for plants sources of available carbohydrates is relevant today. As we reported earlier, in fruits of North American hawthorn species a significant content of water-soluble polysaccharides was found. Therefore, in our opinion, the profound study of the carbohydrate composition of this raw material is of scientific interest.

The aim of the work is to determine the qualitative composition and the quantitative content of monosaccharides of hawthorn fruits.

Materials and methods

The objects of the study were the hawthorn fruit of the North American group with a sweet, juicy pulp [3, 4]. The raw material was collected in the phase of full ripeness in August 2017 (Botanical Garden of the National University of V. N. Karazin).

Preparation and the study of polysaccharides. Polysaccharides were isolated from the raw material by heating with purified water in the ratio of 1 : 10 and with the subsequent addition of 96 % ethyl alcohol. The precipitate formed was separated by centrifugation for 2 min and dried in a dryer at the temperature of 35 °C [5, 6].

The resulting polysaccharides were hydrolyzed with 10 % sulfate acid while heating. The hydrolyzate was chromatographed in the butanol – acetic acid – water solvent system (4 : 1 : 2) with reliable samples of sugars (D-glucose, L-arabinose, D-fructose, D-xylene) [7]. The chromatograms obtained were treated with aniline phthalate and kept in a drying cabinet for 1 min. After chromatography and processing the formation of brown spots was observed. By color and the value of Rf glucose and fructose were identified in the raw material and compared to the reliable samples [8].

The study of monosaccharides by gas-liquid chromatography-mass spectrometry. The sample preparation of the raw material was based on extraction of the total and free sugars in different conditions.

Isolation of the total sugars. The raw material was powdered, the accurate weight (500 mg) was placed in a round bottom flask, 5 ml of 2 M trifluoroacetic acid was added, and polysaccharides were hydrolyzed for 6 h. Then 2 ml of the hydrolyzate were collected, evaporated, washed with water to remove trifluoroacetic acid. Resuspending was carried out by adding 2 ml of the internal standard (the sorbitol solution) [9].

Isolation of free monosaccharides. Free monosaccharides were extracted from the powdered raw material. For this purpose, the mixture of methanol solution with the internal standard (the sorbitol solution) was added to 500 mg of the raw material (accurate weight). Free polysaccharides were isolated at 80 °C for 4 h using an ultrasound bath.

The qualitative composition and the quantitative content of monosaccharides were determined by the gas chromatography-mass spectrometry method. This method is based on extraction of free sugars and obtaining of acetates of their aldonitrile derivatives.

Experimental conditions: for the chromatographic separation the Agilent 6890N/5973 inert gas chromatography-mass spectrometry system (Agilent Technologies, USA) was used: a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm Agilent Technologies, USA); evaporation temperature – 250 °C, the interface temperature – 280 °C; separation in the programmed temperature, the initial temperature of 160 °C was maintained for 8 min, then raised to 240 °C (with a gradient of 5 °C/min) and maintained at the final temperature of 6 min; 1 μl of the sample were injected in the flow separation mode of 1 : 50. Detection was performed in the SCAN mode (the range of 38-400 m/z). The speed of the gas-carrier was 1.2 ml/min [9, 10].

Results and discussion

Identification of monosaccharides was carried out by comparing the retention time of standard sugars and using the database of the NIST 02 mass spectrometry library. The quantitative analysis was performed by adding the internal standard solution (the sorbitol solution) to the test samples [10].

The mass of monosaccharides (mg) per 1 kg of the raw material was calculated using the formula:

$$X = \frac{S_{\text{st}} \cdot M_{\text{st} \cdot 1000}}{S_{\text{m \cdot st}} \cdot m},$$

where: $S_{\text{st}}$ – is the peak area of a monosaccharide; $M_{\text{st} \cdot 1000}$ – is the mass of the internal standard for the sample; $S_{\text{m \cdot st}}$ – is the peak area of the internal standard; $m$ – is the sample weight.

The chromatogram of free monosaccharides and monosaccharides after hydrolysis are shown in Fig. 1, 2. Monosaccharides identified in the raw material are shown in Table.

As shown in Table, in hawthorn fruit 5 free sugars were detected, of them 3 were identified (glucose, fructose, sucrose); after acid hydrolysis the presence of 6 compounds was detected, among them 5 were identified.
Table

Monosaccharides identified in the raw material

| Compound               | Retention time, min | The quantitative content, mg/g |
|------------------------|---------------------|-------------------------------|
|                        |                     | Free sugars                   |
| Glucose                | 12.77               | 2.02                          |
| Fructose               | 19.02               | 2.21                          |
| Sucrose                | 32.84               | 0.23                          |
|                        |                     | Sugars after acid hydrolysis  |
| Arabinose              | 5.86                | 1.82                          |
| Xylose                 | 6.23                | 3.88                          |
| Mannose                | 10.76               | 4.25                          |
| Glucose                | 12.73               | 5.57                          |
| Galactose              | 13.27               | 1.31                          |
(arabinose, xylose, mannose, glucose, galactose). By the quantitative content in the free form fructose dominated (2.21 mg/g). Among sugars after acid hydrolysis the highest content was determined for glucose (5.57 mg/g) and mannose (4.25 mg/g).

**CONCLUSIONS**

1. In hawthorn fruits the composition of free sugars and sugars after acid hydrolysis has been determined.

2. Dominant sugars are fructose, glucose and mannose.

3. The results obtained indicate the use perspective of hawthorn fruit in medical practice as a source of monosaccharides and water-soluble polysaccharides and create the preconditions for further in-depth study of this raw material.

**Conflict of Interests:** authors have no conflict of interests to declare.

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Information about authors:
Sydora N. V., Candidate of Pharmacy (Ph D), associate professor of the Pharmacognosy Department, National University of Pharmacy. E-mail: sydora2005@gmail.com. ORCID: http://orcid.org/0000-0002-3333-2250
Kovalyova A. M., Doctor of Pharmacy (Dr. habil), professor of the Pharmacognosy Department, National University of Pharmacy. E-mail: allapharm@yahoo.com. ORCID: http://orcid.org/0000-0002-1758-1222
Iakovenko V. K., Doctor of Pharmacy, (Dr. habil), professor of the Industrial Pharmacy and Economy Department. E-mail: v.iakovenko@gmail.com. ORCID: http://orcid.org/0000-0002-9348-7764

Сведения об авторах:
Сидора Н. В., канд. фарм. наук, доцент кафедры фармакогнозии, Національний фармацевтичний університет. E-mail: sydora2005@gmail.com.
ОРЦИД: http://orcid.org/0000-0002-3333-2250
Ковалева А. М., д-р фарм. наук, професор кафедри фармакогнозии, Національний фармацевтичний університет. E-mail: allapharm@yahoo.com.
ОРЦИД: http://orcid.org/0000-0002-1758-1222
Яковенко В. К., д-р фарм. наук, профессор кафедры промышленной фармацевтики и экономики. E-mail: v.iakovenko@gmail.com.
ОРЦИД: http://orcid.org/0000-0002-9348-7764

Information about authors:
Sydora N. V., Candidate of Pharmacy (Ph D), associate professor of the Pharmacognosy Department, National University of Pharmacy. E-mail: sydora2005@gmail.com. ORCID: http://orcid.org/0000-0002-3333-2250
Kovalyova A. M., Doctor of Pharmacy (Dr. habil), professor of the Pharmacognosy Department, National University of Pharmacy. E-mail: allapharm@yahoo.com. ORCID: http://orcid.org/0000-0002-1758-1222
Iakovenko V. K., Doctor of Pharmacy, (Dr. habil), professor of the Industrial Pharmacy and Economy Department. E-mail: v.iakovenko@gmail.com. ORCID: http://orcid.org/0000-0002-9348-7764

Сведения об авторах:
Сидора Н. В., канд. фарм. наук, доцент кафедры фармакогнозии, Національний фармацевтичний університет. E-mail: sydora2005@gmail.com.
ОРЦИД: http://orcid.org/0000-0002-3333-2250
Ковалева А. М., д-р фарм. наук, професор кафедры фармакогнозии, Національний фармацевтичний університет. E-mail: allapharm@yahoo.com.
ОРЦИД: http://orcid.org/0000-0002-1758-1222
Яковенко В. К., д-р фарм. наук, профессор кафедры промышленной фармацевтики и экономики. E-mail: v.iakovenko@gmail.com.
ОРЦИД: http://orcid.org/0000-0002-9348-7764

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