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Weight method for determination of soluble β-glucans in barley grain

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Background. Barley (*Hordeum vulgare* L.) is an important source of nutrients, such as starch, protein, and various dietary fibers. β-Glucans are soluble fibers found in high amounts in oat and barley grain, so they are becoming increasingly interesting due to their numerous functional and bioactive properties. The increased interest in β-glucans as a dietary supplement and a functional component of food calls for a convenient, inexpensive and affordable method for quantitative determination of these compounds.
Materials and methods. An overview is given on the existing techniques for determining and isolating β-glucans in cereals: IR spectroscopy, enzymatic, colorimetric, and alkaline-enzymatic methods. Their advantages and disadvantages are shown. The disadvantages of the methods considered include high costs of reagents and equipment, duration of performance, and labor intensity.
Results. This study promotes the weight method for isolation and quantitative determination of β-glucans in the grain of covered and naked barley. It is based on the modified alkaline method adapted to barley; we developed it earlier for oat grain. The amount of β-glucans in the grain of the studied barley accessions ranged from 4.12±0.23% to 5.34 ± 0.31% for naked cultivars, and from 3.57 ± 0.18% to 4.29 ± 0.32% for covered ones.
Conclusion. Based on the conducted research, optimal conditions for the isolation and quantitative determination of β-glucans from barley grain were selected: centrifugation modes, temperature and extraction ratio, concentration of compounds, precipitation and drying procedures. The main advantage of the described method is its accessibility and practical applicability when conducting mass analysis, including studying a collection of cereal crops.

Keywords: β-glucans, *Hordeum vulgare* L., nonstarch polysaccharides, naked barley, covered barley, alkaline method

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ИЗУЧЕНИЕ И ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ РАСТЕНИЙ

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Весовой метод определения растворимых β-глюканов в зерне ячменя

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Актуальность. Ячмень (*Hordeum vulgare* L.) является важным источником питательных веществ, таких как крахмал, белок и различные пищевые волокна. β-Глюканы представляют собой растворимые волокна, в большом количестве присутствующие в зерновках овса и ячменя; они вызывают все больший интерес благодаря своим функциональным и биологически активным свойствам. В связи с повышенным интересом к β-глюканам как к биологически активной добавке и функциональному компоненту пищи существует потребность в удобном, недорогом и доступном методе количественного определения данных веществ.
Материалы и методы. В статье приводится обзор существующих методов определения и способов выделения β-глюканов из зерновых культур: ИК-спектроскопия, ферментативный, колориметрический, щелочно-ферментативный. Показаны их достоинства и недостатки. Среди недостатков рассмотренных методов необходимо отметить высокую стоимость реактивов и оборудования, длительность выполнения, трудоемкость.
Результаты. В данном исследовании приводится разработка весового метода для выделения и количественного определения β-глюканов в зерне пленчатого и голозерного ячменя. За основу взят модифицированный и адаптированный для ячменя щелочной метод, разработанный нами ранее для овсяного зерна. Количество β-глюканов в зерне исследованных образцов ячменя составило от 4,12 ± 0,23% до 5,34 ± 0,31% для голозерных сортов и от 3,57 ± 0,18% до 4,29 ± 0,32 % для пленчатых.
Заключение. На основе проведенного исследования подобраны оптимальные условия выделения и количественного определения β-глюканов в зерне ячменя: установлены режимы центрифугирования, температура и соотношение экстрагирующих веществ, их концентрация, порядок осаждения и сушки. Основным преимуществом описанного метода является его доступность и практичность при проведении массового анализа, в том числе при изучении коллекции зерновых культур.

Ключевые слова: β-глюканы, *Hordeum vulgare* L., некрахмальные полисахариды, голозерный ячмень, пленчатый ячмень, щелочной метод

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Introduction

Barley (*Hordeum vulgare* L.) is considered a valuable food, fodder, and industrial crop. In recent years, this cereal has been recognized as a rich source of various functional ingredients due to its high content of fiber, minerals, and antioxidants, and its ability to have a beneficial effect on consumer health and reduce the risk of various diseases (Shewry et al., 2009).

There are opportunities to expand the application range of barley grain through the development of cultivars with new grain characteristics for the processing industry. It is possible due to the donors and genetic sources, indirect and direct methods for evaluating grain material for the breeding of barley cultivars with the following properties: high protein content (Kovaleva et al., 2017); low gluten content (the first highly productive cultivars have been obtained, which would diversify the diet of patients with celiac disease); high activity of phytinases or low phytin content; a high level of anthocyanidins (for example, with purple grain color); decreased or increased content of *β*-glucans (Konarev et al., 2019).

Beta-glucans ([1,3;1,4]-*β*-D-glucans) belong to the class of indigestible polysaccharides contained in cereals, yeast, bacteria, algae and fungi (Wood, Beer, 1998). The beneficial role of *β*-glucans in insulin resistance, hypertension, obesity, and dyslipidemia has been proven (Wood, 2010; Regand et al, 2011). The ability of *β*-glucans to form highly viscous solutions in the intestine is the basis of their health benefits. These compounds contribute to the nutrition and growth of intestinal bacteria that synthesize vitamins, enzymes, and other compounds. The physiological effects of *β*-glucans are mainly explained by their physicochemical and structural characteristics (Bechtel et al., 2009; Regand et al., 2011; Chang et al., 2013; Harland, 2014; Krasilnikov et al., 2014).

Along with other insoluble dietary fibers (lignin, fiber, hemicelluloses, etc.), insoluble *β*-glucans are not exposed to digestive enzymes, and therefore they are not broken down and are not absorbed by the body (Dikeman, Fahey, 2006). A research study (Gajdošová et al., 2007) showed that the content of soluble (the most valuable) glucans decreased in the following order: barley (3.75–7.96% DW) > naked oats (3.91–7.47%) > covered oats (1.97–4.09%), while the content of insoluble glucans in covered oat forms (33.73 – 13.79%) > barley (10.89 – 21.70%) > naked oats (5.15 – 10.80%).

Cereals are a valuable source of glucans and thus they can satisfy the first and decisive condition of their use – nutritional supplements and ingredients to functional foods. In addition, no side effects have been reported for humans after consuming a diet rich in *β*-glucans from oatmeal, or barley flour, or their extracts. At the same time, there are antinutritional properties in feed grains, and it needs to be heeded by feed producers (Faure et al., 2015; Loskutov, Polonskiy, 2017). In addition, for brewing technology, it is necessary that malt (wort) contained as few *β*-glucans as possible, since they contribute to an increase in the viscosity of wort and beer, reducing output and filterability.

Such facts indicate the importance of the quantitative determination of *β*-glucans in various grain raw materials. However, separation and determination of pure *β*-glucan is a complex and expensive process since in cereals they are present in the bound form and concentrated mainly in the aleurone and subaleurone layers, which also contain starch, proteins and lipids that interfere with their determination (Bechtel et al., 2009).

The objective of the study was to develop an affordable and inexpensive method for quantitative assessment of *β*-glucans in the grain of naked and covered barleys for mass analysis.

Materials and methods

A number of methods for the quantitative determination of glucans are discussed in publications. The most widely used is the biophysical enzymatic method using lichenase and *β*-glucosidase (McCleary, Codd, 1991; Lee et al., 1997). However, this method is considered time-consuming and requires many operations. In addition, the cost of a set of reagents supplied by Megazyme can be quite significant if you need to analyze a large number of samples, which is not always convenient for everyday and frequent analyses. At the moment, the availability of imported reagents supplied by foreign companies is also an important factor.

There is a method for photometric determination of *β*-glucans using the fluorescent brightening agent calcofluor white. The method is based on the formation of a complex of *β*-glucans with a dye, subsequent photometric measurement of the optical density of the modified liquid phase, and subsequent determination of the concentration of *β*-glucans (Tikanoja et al., 2014).

Currently, the potential of the method in the near-infrared region for the determination of *β*-glucans in various cereals is being studied; it is considered as fast, accurate, and cost-effective. However, this technique requires device tuning, and preliminary construction of calibration curves obtained on a large volume of material using classical analysis methods (Blakeney, Flinn, 2005).

Another interesting method is the extraction of *β*-glucans from grain by alkaline and two-stage alkaline-enzymatic methods. In published sources (Salomatov, 2015; Gematdinova et al., 2017), the main stages of the alkaline extraction method were studied. However, the proposed methods have a number of disadvantages associated with the duration of the procedure and the lack of a possibility to accurately determine *β*-glucans due to the presence of accompanying compounds. These methods are mainly intended for the isolation, purification and concentration of glucans, which can then be used as a dietary supplement.

In our study, we used a modified alkaline method for oat grain based on the previously developed technique (Popov et al., 2021), since each crop has its own characteristics and requires appropriate adaptation and specific extraction modes. The time of the experiment and the number of operations are reduced, while the yield and purity of the final product are increased, which makes it possible to quantify the glucans in barley grain.

The results were compared with the data obtained on the same barley accessions by the arbitration enzymatic method AOAC 995.16 and ICC Standard Method No. 168 for *β*-glucans (Megazyme) (Polonskiy et al., 2021). The developed method was tested on naked and covered barley cultivars (Table 1).

Devices and materials used for the determination of *β*-glucans:

– a centrifuge for large volumes with cooling SL16R, Thermo FS or similar;

– a pH meter, ANION-4100 or similar;

– a laboratory mill, LMT-1 or similar;

– reagents: hydrochloric acid (chemically pure CP); sodium hydroxide (CP); phosphoric acid (CP); ethyl alcohol (CP).

Flour was obtained by direct grinding of whole barley grain (naked) and with husks (for covered cultivars).

Table 1. **Description of the accessions used in the study**

Таблица 1. **Характеристика образцов используемых в исследовании**

Accession name (*Hordeum vulgare* L.)	VIR catalogue No.	Type of grain	Variety	Origin
Korona Laschego	27471	Naked	*celeste*	Poland
Nudum 7566	29453		*nudum*	Kyrgyzstan
Omsky Golozerny 1	30919		*nudum*	Omsk Province
Nudum 95	31125		*nudum*	Chelyabinsk Province
Mayak	29622	Covered	*nutans*	Krasnoyarsk Territory
Acha	30243		*nutans*	Novosibirsk Province
Zolotnik	30845		*medicum*	Altai Territory
Biom	30984		*nutans*	Novosibirsk Province

Results and discussion

The weight method for determining *β*-glucans in barley grain includes sequential step-by-step separation of related compounds from flour samples (protein, starch, sugars, and nonstarch polysaccharides), isolation of *β*-glucans with alcohol, drying them to a constant mass followed by weighing and determination of the percentage content in flour with further conversion to dry matter.

Stages of quantitative determination of β-glucans in barley:

1. Preground barley (whole flour) was weighed in plastic centrifuge tubes, treated with 50% ethyl alcohol in a ratio of 1:10 (m/v) to extract free sugars, part of proteins, lipids, and other substances. Extraction was carried out at a temperature of 60°C for 20 minutes, which also contributes to the inactivation of *β*-glucanase, which destroys glucans (Skendi et al., 2003). Then the suspension was centrifuged at a rotation speed of 5 thousand rpm for 10 minutes and a temperature of +20°C. Thereafter, the ethyl alcohol was drained and the resulting precipitate was used for further research.

2. The release of *β*-glucans from the flour endosperm and aleurone layer was carried out in an alkaline solution by holding the precipitate in the same test tubes in a 5% sodium hydroxide solution at a ratio of 1:20 (m/v) and a temperature of 45°C for 60 minutes. For better mixing and lump formation prevention the precipitate must first be dissolved in a small amount of an alkali solution, carefully kneading the lumps formed with a glass stick. The suspension was stirred every 10 minutes.

3. Then the alkaline extract was neutralized with concentrated hydrochloric acid in centrifuge tubes to pH 2.0...3.0 controlling the acidity using a pH meter or litmus test. The mixture was thoroughly mixed until a homogeneous milky white color. The acidic environment further contributes to the formation of a dense precipitate consisting of protein substances and polysaccharides, and a better separation and transition of *β*-glucans into the supernatant.

4. Phosphotungstic acid (10%) was added to the resulting solution in a ratio of 1:1.5 (m/v), mixed, and centrifuged with cooling in the same tubes at a rotation speed of 10,000 rpm for 20 minutes and a temperature of +10°C. Phosphotungstic acid contributes to the formation of a dense precipitate, more complete precipitation of protein and polysaccharides, while simple sugars, amino acids and *β*-glucans remain in the supernatant (Fig. 1).

5. *Beta*-glucans were isolated from the pellet by adding a 2-fold volume of 96% ethyl alcohol and holding the mixture for 30 minutes at a temperature of +20°C (Fig. 2). For better mixing, the pellet from centrifuge tubes was poured into ethyl alcohol measured in measuring cylinders by 50 or 100 cm^3.

6. After the *β*-glucan fibers surfaced, they were extracted and placed in predried and weighed porcelain cups and dried

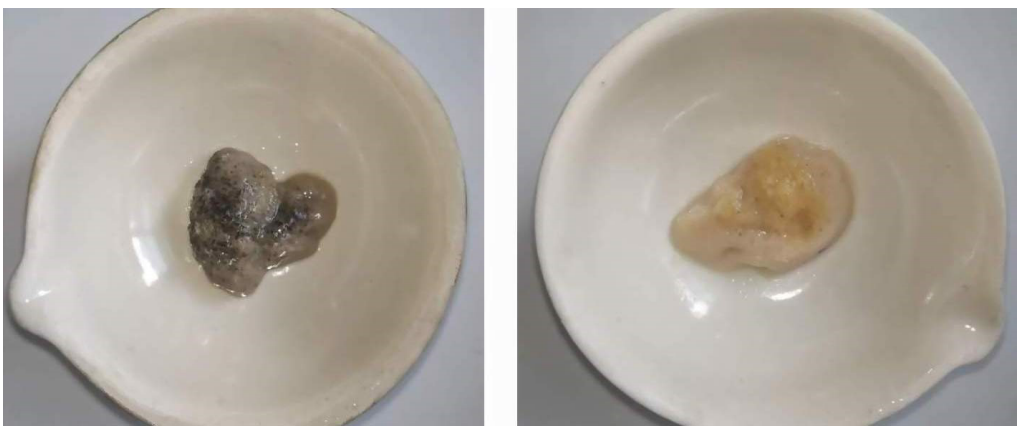

Fig. 1. **A precipitate consisting of insoluble fiber, protein, and starch**

Рис. 1. **Осадок, состоящий из нерастворимых волокон, белка и крахмала**

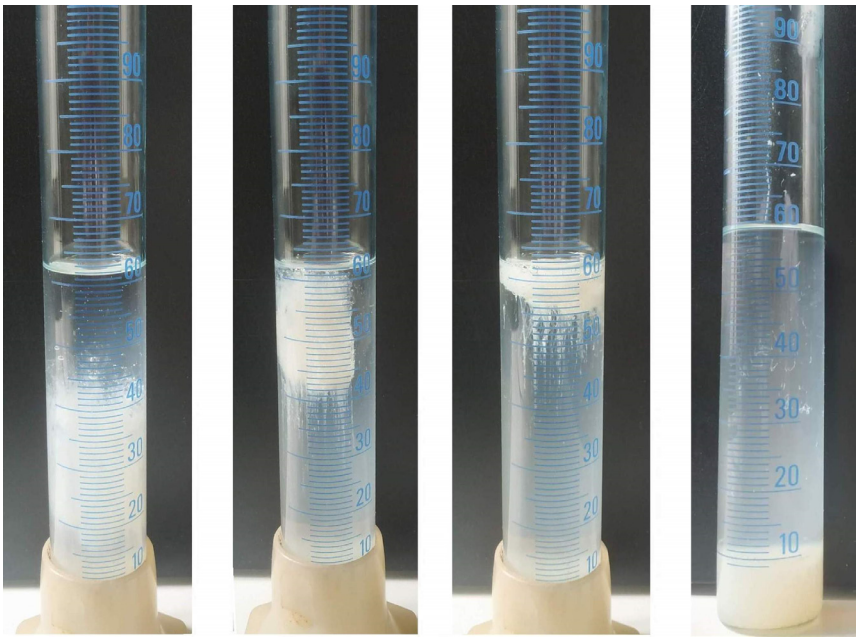

Fig. 2. The stages of the surfacing of *β*-glucans fibers in ethanol
Рис. 2. Этапы всплытия волокон β-глюканов в этаноле

Fig. 3. *Beta*-glucan fibers before (left) and after (right) drying
Рис. 3. Волокна β-глюканов до (слева) и после (справа) высушивания

at a temperature of 75°C to a constant mass (Fig. 3). The precipitate containing sugars, oligosaccharides and other fractions was not taken into account.

7. The quantitative content of *β*-glucans in terms of dry flour weight (x, %) was calculated by the formula:

$$x = \frac{(m_1 - m_2) \cdot 100 \cdot 100}{m \cdot c},$$

where
m_1 is the mass of the porcelain cup with dried *β*-glucans, g;
m_2 is the mass of the porcelain cup, g;
m is the mass of the flour sample, g;
c is the dry matter content in the flour sample, %.

The dry matter content of flour was determined by a method based on weighing a part of the grinded medium sample before and after drying to a constant mass at a temperature of 100–102°C (Ermakov, 1987).

The obtained *β*-glucans were tested for the presence of impurities and compared with barley accessions with a known content of soluble glucans determined by the standard enzymatic method (Table 2). A qualitative reaction to starch with Lugol's reagent did not reveal a characteristic blue color, nitrogenous substances investigated by the Kjeldahl method were also not detected.

As seen in Table 2, the content of glucans according to the developed gravimetric method ranged from 4.12 ± 0.23% to 5.34 ± 0.31% for naked barley cultivars and from 3.57 ± 0.18% to 4.29 ± 0.32% for covered barley cultivars, which is comparable to the data obtained by the standard enzymatic method.

Thus, the developed weight method for determining *β*-glucans in barley grain provides direct isolation of *β*-glucans from samples, purification from various accompanying substances, and calculation of the percentage content of the sample's weight.

Table 2. ***Beta*-glucans in barley accessions**

Таблица 2. Бета-глюканы в образцах ячменя

Type of grain	VIR catalogue No.	*β*-glucans (DW), %			Difference	
		Weight method	Standard method. Average data for 3 years*		Abs.	%
			$\bar{X}$	Cv		
Naked	к-27471	4.12±0.23	3.81	8.7	+0.31	8.1
	к-29453	4.44±0.19	4.27	14.4	+0.17	4.0
	к-30919	4.55±0.12	4.30	10.1	+0.25	5.8
	к-31125	5.34±0.31	5.21	4.9	+0.13	2.5
Covered	к-29622	3.57±0.18	3.43	7.0	+0.14	4.1
	к-30243	4.17±0.08	4.51	10.4	-0.34	8.2
	к-30845	4.29±0.32	4.38	14.7	-0.09	2.1
	к-30984	3.70±0.24	3.89	14.0	-0.19	5.1

Note: * – standard method for *β*-glucans (Polonskiy, 2021)

Примечание: * – стандартный метод для β-глюканов (Polonskiy, 2021)

Conclusion

Published data on the quantity of *β*-glucans in the grains of different crops vary greatly and exhibit significant intervarietal differences. The quantitative content of *β*-glucans in barley ranges from 3 to 11%, in oats from 1.8 to 7.5%, in rye from 1 to 2%, and in wheat < 1%, while trace amounts are found in other types of grains (Loskutov, Polonskiy, 2017). Considering such a significant varietal range of variability in the content of glucans in different crops, it is necessary to study the genetic diversity of cereals and cereal crops in order to isolate accessions (sources) for different directions in breeding, particularly for use in feed production, food, and brewing industries.

The proposed method for determining *β*-glucans from barley grain can be successfully used to analyze naked and covered cultivars during screening. The advantages of the method include the availability of the reagents and equipment, as well as insignificant time costs for preparation and performance of the analysis. It should be noted separately that the method provides an opportunity of quantitative determination of the product obtained, thanks to the virtually complete absence of accompanying impurities.

When studying a cereal gene pool, particularly barley as a crop characterized by high quantitative content of glucans, this method can be useful for constructing calibration curves using near infrared spectroscopy, which would help to preserve valuable breeding material and possibly lead to the discovery of new, highly specialized and versatile uses of these crops in food industry. This will contribute to the development of commercial barley cultivars for feed and food production (Zhu et al., 2016).

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