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β-GLUCANS CONTENT AS A PERSPECTIVE TRAIT IN THE BARLEY BREEDING FOR FOODSTUFF USE
(review)

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S u m m a r y

On the ground of data of foreign literature the authors consider the target utilization of barley in connection with β-glucans in grain. The authors analyzed the influence of a genotype, climatic conditions and agronomic methods of plant growing on β-glucans content in barley corn. The information was presented about physiologo-biochemical parameters β-glucans, which may be helpful for a development of indirect estimation of breeding material. Thus, it was fixed the negative correlation between β-glucans content in barley grain on the one hand and value of 1000seeds mass, amylose and starch content, ash percent, corn yield — on the other hand. The strong positive correlation was found between the content in corn of β-glucans, lipids, protein and the grain-unit and its hardness. The existence of such correlations makes possible the development of indirect estimation during barley breeding on heightened/reduced β-glucans content in grain. It was shown, that glumaceous amylose-free varieties are more advanced agronomically (in arid climate, especially), as they are able to realize a substantial potential of yield with high content of β-glucans. The authors made a conclusion about an importance of genetic variability in content of β-glucans in barley grain for successful breeding on this determinant.

Keywords: β-glucans, barley, grain, starch, fibre, dietary nutrition, hardness, cell wall, endosperm, evaluation.

Barley is one of major cereal crops in the world; its gross grain yield is estimated as the fifth after wheat, maize, rice, and sorghum.

Healthy functional nutrition has been in focus of increasing interest in recent years, especially soluble dietary fiber from cereals as dietary supplements. The recently adopted regulations of the US Food and Drug Administration permit the use of barley food products in order to reduce the risk of cardiovascular disease and allow official registration of such products as dietary supplements reducing blood cholesterol level; previously the same notice was announced for products derived from oats (Anonymous, 2005; 1997; cited from 1). Cell walls of the endosperm of barley, oats, and other cereal crops were found to contain specific polysaccharides so-called (1,3;1,4)-β-D-glucans having the property to decrease blood cholesterol and sugar levels, reduce the risk of cardiovascular disease and diabetes, and efficient prevention and treatment of some serious human diseases including colon cancer; these substances may help to reduce body weight due to a lasting sense of satiety, they strengthen the immune system and show antimicrobial activity (2-5). These facts explain the current regain of interest to barley as a food product in some European countries (6).

β-Glucans and intended use of barley. The mechanisms by which soluble dietary fibers such as β-glucans promote the reduce in blood cholesterol and glucose levels are yet insufficiently clear and widely discussed in the literature. Most hypotheses assume that high viscosity of contents in the gastrointestinal tract may suppress the absorption of cholesterol, bile acids, and their metabolites (7-10). Furthermore, the said polysaccharides inhibit the absorption of nutrients, primarily carbohydrates, reduce hyperglycemia and insulin secretion. The latter positively affects the state of health in cases of diabetes II type, and also contributes to a certain indirect effect of the decline in blood cholesterol level (7, 8, 11).

Clinical observation of more than 400 patients has revealed positive effects of diets supplemented with β-glucans and wholegrain barley foods: the decrease in blood levels of glucose (3, 12), total cholesterol (13), low density lipoprotein, and triglycerides (2, 14). In 90 volunteers (men and women) receiving the diet with barley β-glucans for 6 weeks resulted in a notable reduce in body weight (15).

The variety in food enriched with dietary fibers is an important target in development of innovative approaches to grain food technology, such as adding β-glucans in yogurt (16) or in wheat flour for bakery (3, 17, 1). Along with it, healthy nutrition considers the value of products prepared from other cereal crops, particularly rye and oats. In rodent experiments, β-glucans derived from both barley and oat grain provided almost equal activity of reducing blood cholesterol level (19).

However, it should be noted that some studies didn't reveal any significant physiological effects of diets supplemented with barley β-glucans. For example, clinical research on men with high cholesterol level haven't proved the reduce of cardiovascular disease risk (20), and some cases didn't show an obvious correlation between the viscosity of β-glucans and the decrease in blood cholesterol level (21). Possibly, this occurred due to diets, doses, or properties of β-glucans used in these experiments.

Along with the abovementioned positive effects of polysaccharides from cell walls of the cereal endosperm in a human diet, β-glucans often act as a negative factor in assimilation of dietary nutrients by non-ruminant animals. High viscosity of mucus-forming polysaccharides may obstruct the digestion and assimilation of nutrients from the gastrointestinal tract. Domestic animals show the accompanying symptoms – low body weight gain and unwholesome appearance (4, 22).

In brewing industry, high content of β-glucans in cell walls of the cereal endosperm is an important factor affecting quality of malt. It is known that slow degradation of cell walls in germinating barley grains can decrease the yield of starch, protein, and other components of the endosperm and, therefore, reduce the volume of resulting extract (malt). Arabinoxylans and β-glucans in the cell walls are the barrier for hydrolytic enzymes providing a chemical attack of starch and protein molecules, and, therefore, these polysaccharides determine viscosity of the obtained extract and filtration rate of beer (23). That's why it is so important that barley varieties for brewery must keep low content of key polysaccharides of the cell wall and/or rapid synthesis (activation) of enzymes capable of hydrolysis of such polysaccharides (4, 24).

M o l e c u l a r   s t r u c t u r e   o f   β - g l u c a n s. The main component of soluble dietary fibers of cereals are (1,3; 1,4)-β-D-glucans. This collective term describes high molecular weight glucose polymers with glycosidic bonds β (1-3) and β (1-4).

Molecules of β-glucans are linear homopolisaccharides consisting of D-glucopyranose residues bound with β-1-4 bonds and arranged in blocks of units separated by β-1-3-bonds (25). Most of segments within these blocks are tri- and tetramers, while polymeric chains usually contain longer blocks (26). Differences in chemical structure and composition of β-glucans are associated with the ratio between trimers and tetramers, β(1-4)/β(1-3) bonds, and the content of long cellulose oligomers (27, 28).

β-Glucans derived from barley grain have the relative content of saccharides 52-69 %, tetrasaccharides – 25-33 %, the ratio of tri-/tetrasaccharides – 1.8-3.5. This ratio depends on the chemical structure of cereal β-glucans. Each cereal species has its own unique ratio comparable with individual fingerprints (29-34).

The ratio between tri- and tetrasaccharides in β-glucans is higher, firstly, in barley varieties with low amylose content (so-called Waxi-forms) than in varieties with normal content of this component of starch, and, secondly, in cell walls of the aleurone layer as compared with the endosperm (26, 35). Differences in this ratio depend on the genotype and external factors that prevailed during formation and maturation of grain in cereals (36-38). Furthermore, the ratio between tri- and tetrasaccharides in β-glucans depends on conditions of isolation, in particular, increasing the temperature from 40 to 65 ºC during water extraction of β-glucans yields in higher proportion of trisaccharides (27, 39).

Molecular weight of barley β-glucans is the six- or seven-digit number (31½103-2700½103 Da). This important characteristic varies due to several factors: variety, environmental conditions of growth, as well as techniques of isolation, purification, and measurement (1, 27). The molecule of β-glucan has a shape of a curved chain whose model looks like a cylinder with length of 3.5-3.8 nm and a diameter of about 0.45 nm (40).

Biosynthetic pathways of many polysaccharides (starch, cellulose, glycogen) are studied quite well, but reactions leading to formation of β-glucans and processes of their accumulation in cell walls of the endosperm are yet insufficiently understood. Some findings show that biosynthesis of cell walls during grain formation is associated with morphological changes at ripening (4).

Studies show that molecular structure and structural organization of β-glucans and arabinoxylans from oat and barley are important determinants of their physical properties, such as water solubility, viscosity (gelling), and digestibility. These properties determine functionality of these polysaccharides and their physiological role in the gastrointestinal tract (1, 32, 33, 41, 42). It was experimentally recorded the difference in viscosity of β-glucan fractions extracted with water at temperatures of 45 and 90 ºC. This was also confirmed by variations in molecular weight of isolated polysaccharides derived from different barley varieties (37).

Content of β-glucans in barley grain. β-Glucans are polysaccharides present solely in the family *Poaceae* (*Gramineae*) as components of cell walls of barley, oats, wheat, rye, corn, rice, and sorghum (43, 44). Cell walls of cereals also contain high content of heteroxylanes and a small amount or total absence of cellulose and pectin. In the aleurone layer and endosperm cell walls consist mainly of (1,3; 1,4)-β-D-glucans and arabinoxylans. For example, cells of the starchy endosperm in mature barley grain are surrounded by the thin walls containing approximately 70 % β-glucans, 25 % arabinoxylans, 2% cellulose, and 2% glucomannans (45, 46). Polysaccharides β-glucans form the inner lining of cell walls in the endosperm of barley (47, 48).

Hulling off the outer layers including glumes from cereal kernels reduces proportion of insoluble dietary fibers, protein, free lipids, and ash, while this increases the proportion of starch and β-glucans (49, 50). Hulled grains of covered barley or grains of hullless barley contain 11-20 % of dietary fibers and 3-10 % of soluble dietary fibers relative the total amount (51).

Barley and oats are considered as champions among cereal crops by the content of β-glucans (52, 53). This characteristic of quality in wheat, barley, oats, and rye grain amounts to, respectively, 0.6; 4.2 ; 3.9 ; 2.5%, and in the separated endosperm – 0.3 ; 4.1 ; 1.8 ; 1.7 % of dry biomass (54).

Whole grain of barley keeps an average content of β-glucans 3-9% (55-59). However, there are some notable mutants of barley with low accumulation of starch and increased – β-glucans that may partially or completely replenish the loss of starch. So, high-lysine mutant barley Riso 13 and 29 develops the content of starch reduced by 30 %, β-glucans – increased by 20% (60). The professor R.F. Eslick from the University of Montana (USA) has established the hullless barley isoline with very low content of amylose Prowashonupana (high protein, waxy, short awn nude "Compana") and extremely rich in β-glucans – up to 15-18 % (57, 61).

Foods produced from cereals with low starch and high β-glucan content may be valuable in a low-calorie human diet (62). The six recently established barley lines are assumed to be possible components of functional foods due to high content of soluble and insoluble dietary fiber and low energy value (60).

Grain hardness and content of β-glucans. It was experimentally found that hardness of cereal grains is largely associated with the degree of adhesion between starch granules and protein matrix in the endosperm (63, 64). As a rule, soft varieties are used for malt, and non-brewing varieties are hard. In brewing technology, the quality of malt is predicted from the varietal indicator associated with measured physical hardness of grains (65). Usually, hardness of barley grain is determined with the instrument for measuring hardness of individual grains – Single Kernel Characterization System (SKCS 4100, "Perten Instruments, Inc.", IL USA) (66). Another method of screening grain hardness is based on light reflection in near infrared spectral region (67, 68).

Commonly, hardness of barley grains evaluated as the energy expenditure for grinding significantly affects the quality of malt and it is negatively correlated with volume of the aqueous extract of barley and endosperm modification (69, 70). Hardness of barley kernels depends on components of cell walls in the endosperm – β-glucans and arabinoxylans, as well as protein content in grains. Contents of these substances in grain are in strong positive correlation with grain hardness index (71, 72). The observed effect may occur due to the difference in thickness of cell walls in the endosperm of barley lines with unequal content of β-glucans (61).

According G.P. Fox et al. (68), grain hardness of barley is mainly associated with two factors – environmental conditions during formation and maturation of the grain, and its genotype (variety). Experimental data confirm the influence of growth conditions on protein content, which, in turn, affects grain hardness. These authors believe that the observed dependence between grain hardness and the genotype allows breeding barley varieties with very hard or very soft kernels.

A large number of barley varieties and breeding lines grown in different condition were tested for suitability for brewing using the criteria of measured grain hardness and recorded size of particles after grinding, as well as resistance of grains to grinding (68).

A. Lazaridou et al. (66) studied the process of extraction of highly purified polysaccharides of cell walls from the endosperm of barley grown under different conditions (three variants of experiments). The obtained samples had different grain hardness, contents of protein and β-glucans. Using several methods (extraction with water, sodium hydroxide, and barium hydroxide solution; treating with malt enzymes) there were demonstrated substantial modifications and capacity of being attacked by enzymes in different polysaccharides from cell walls of the endosperm, which, apparently, depended on chemical composition and properties of these substances formed under the influence of growing conditions.

Components of cell walls in the endosperm (β-glucans and arabinoxylans) determine its structure and physical properties, and play a significant role in swelling during germination. Thus, examination of ten barley varieties grown for 2 years in different conditions (nine variants) showed that relative absorption of water by kernels is negatively correlated with β-glucan content in the endosperm, as well as with grain hardness index (72).

Probably, hardness of grains is not related with their absolute weight. According to the literature, barley grains of various weight have a very small difference in relative water absorption in the first 10 hours of swelling (73). The rate of water absorption by kernels grows with increase in temperature, as was found in model experiments (74).

The growing interest to foods with barley not requiring special processing technologies (more intense physical treatment) necessitates production of grain with a specific hardness index. In the view of B.B. Baik and S.E. Ulrich (75), it is important to find out how the structure of cell walls in the endosperm is correlated with grain hardness, and which component (starch, protein, β-glucans) is the most significant (quantitatively and qualitatively) for variation of hardness, and finally, which contributions are provided by the genotype and environment to the grain hardness index of barley.

Effects of genotype and environment on β-glucan content in grain. β-Glucan content in grain is largely determined by the genotype and climatic conditions of plant growth (55, 76-79). Some researchers show that genetic factor has the largest contribution in variation of β-glucan content (53, 80-84), while others assume the crucial importance of environmental conditions (77, 85, 86).

In experiments on 33 barley varieties and lines grown for 2 years in two dry regions of the USA it was found that variation of β-glucan content in grain depended on the genotype by 66% (83) or 51% (82). In the latter case, protein content in grain was affected by environmental conditions by 69%, grain yield and natural weight – by 83 and 70%, respectively. Along with it, C.E. Fastnaught et al. (77) found that conditions of growing notably affect contents of starch, β-glucans, and protein in barley grain, so they concluded the need for quality standards and tests for food barley.

The study of 9 varieties of barley and 10 varieties of oats has revealed intervarietal differences in content of β-glucans persistent by years of the experiment (53). Genotypic analysis of 33 barley genotypes grown for 2 years in nine different geographical regions showed that genotypic variation in content of β-glucans is quite important for success in breeding for this quality trait (87).

A.A. Chernyshova et al. (88) evaluated genetic component of variation in β-glucan content in oat grain as well as in progeny of crosses between the samples with a high content of β-glucans and samples with elite agronomic properties. The research involved 24 varieties and lines; its findings revealed significant variability in content of β-glucans in grain as a promising basis for breeding of this economically useful trait.

Along with genetic factors and environmental conditions, β-glucan content depends on developmental phase of plants: it gradually increases during formation of grain up to complete ripening when it reaches a plateau or may be somewhat reduced (44).

Postharvest storage of grain also influences accumulation of the said polysaccharides. The content of soluble β-glucans in both oats and barley reduces during storage in a cornloft, especially at 25 ºC as compared with storage in a refrigerator (about 8 ºC) (52). In conditions close to optimum, β-glucan content in grain remains unchanged through a 6-month storage (44).

Along with the abovementioned factors, the content of β-glucans in grain is determined by weather conditions of growing season (53, 87), primarily, by air temperature and moisture. Growth of air temperature positively affects accumulation of these polysaccharides, while increase in moisture has a negative effect. Cultivation of 8 two-rowed barley varieties up to maturation in climatic chambers with five temperature regimes has shown that growth in air temperature stimulates accumulation of water-soluble β-glucans (89) and increase of their molecular weight. Low temperature of growing season reduces the content of β-glucans (90). In experiments with artificial irrigation, increased rates of watering inhibited accumulation of these polysaccharides in barley grain (91).

Agronomic factors (fertilizers, selected predecessor crops, etc.) also affect β-glucan content in grain. High doses of nitrogen fertilizer increase the content of β-glucans in barley grain (91). Oats grown after another cereal crop manifested higher accumulation of β-glucans than in the variant of a leguminous predecessor (92).

I n d i r e c t i n d i c a t o r s o f β - g l u c a n c o n t e n t i n g r a i n. Total content of β-glucans in grain of cereals is commonly measured by the biochemical method developed by B.V. McCleary and R. Codd (93). As a rule, β-glucan content in samples is determined using a special reagent kit by "Megazyme International Ireland Ltd." (Bray Business Park, Bray, Co. Wickow, Ireland) (53, 66). In grinded barley grain is tested by the biophysical method with reflection in the near infrared spectral region (94, 95).

In practical breeding it is quite important to have simple and rapid methods allowing evaluation of quality characters of agricultural plants, in particular, in breeding cereal crops for β-glucan content in grain. Solving this problem has been the target of numerous studies aimed at finding natural correlations between the content of these polysaccharides in grain and various morphological, physical, chemical, and physiological parameters of grains and substances isolated from them. Along with it, molecular markers are used in breeding barley for high or low β-glucan content in grain (96).

Thus, it was found the unstable obviously negative correlation between the accumulation of β-glucans in barley grain, on the one side, and 1000-grain weight (78, 91), contents of amylose (77, 82, 83, 97), starch (60), percentage of ash (79), grain yield size (82, 88) – on the other. In oat it was observed a strong positive correlation between β-glucan content in grain, on the one hand, and the protein content and natural weight – on the other, as well as the negative correlation between accumulation of β-glucans and duration of the period germination-seedlings (87). Other researchers haven't found any correlations between β-glucan content in barley grain and accumulation of protein (72, 79) or 1000-grain weight (79). In barley it was recorded a strong positive correlation between β-glucan content and lipid fractions in grain (79), protein content (82, 91), as well as natural weight of grain (79).

Six-rowed barley and two-rowed barley were found to have no significant differences in β-glucan content in grain (77). Grain size (the trait largely determined by the genotype than by growth conditions) (98) is positively correlated with the content of β-glucans and negatively – with the ratio pentosans/β-glucans (56). In spring barley forms, the content of protein and soluble fiber is higher compared with winter forms (A. Batal, N. Dale, 2009; cited on 79).

Comparison of hulless and covered barley was performed by Norwegian scientists S.H. Knutsen and A.K. Holtekjolen (99) upon 16 varieties of hulless and covered barley, as well as by American researchers (79) on 14 samples of covered and 37 – hulless barley; according to their findings, these two forms have no significant differences in contents of β-glucans and water-soluble arabinoxylans in grain. Other authors reported that hulless barley exceeds covered barley in content of β-glucans (77, 100). The highest content of β-glucans was recorded in grain of Tibetan hulless barley (55). As a rule, hulless varieties develop lower average yield than covered cereals. That's why the most promising agronomic varieties (especially in dry climate) are covered amylose-free cultivars capable to realize a potential significant yield with high β-glucan content (83).

Accumulation of β-glucans in oat grain was found to be the factor reducing the rate of seed germination. The experiment was conducted using Monte Carlo method with simulated conditions affecting the content of β-glucans in covered and hulless oats during vegetation. The analysis revealed a positive correlation between the delay of sprouting and β-glucan content in grain (correlation coefficients 0.32 and 0.25, respectively) (84). Such differences were caused by both genotype and environmental factors, and the latter may be stronger assuming their role in absorption of water by swelling seeds, in reports of some authors (86).

It was experimentally proved that the content of water-soluble arabinoxylans in rye grains rather depends on viscosity of an aqueous extract of flour measured on a viscometer (101). This physicochemical parameter can be used in selection, and crosses between the selected lines may lead to hybrids whose viscosity index of an aqueous extract of grain will be significantly higher or lower than that of a standard variety. The first ones may be valuable for food purposes, the second ones – for animal forage.

There is the data (60) showing that the barley grain with high content of β-glucans and reduced amount of starch contains an increased proportion of dry matter (and, respectively, less water). The authors suppose that this fact occurred due to reduced water binding capacity in cell walls of the endosperm than in crystalline structures of starch in amyloplasts.

It should be emphasized that correlation between β-glucan content in barley grain and some agronomic properties is generally insignificant (87).

Modern spectroscopy in the near infrared spectral region provides an automated approach to segregation of barley and wheat grains into three groups of food (forage) quality. This target is implemented in the specially designed Grader BoMill TriQ single seed pilot NIR sorter ("BoMill AB"-Lund", Sweden) (102). Its optical fibers record the reflectance spectrum of single grains, which signal then passes through computer processing and data analysis, and, as a result, pneumatic system separates the grains into three fractions: low, medium, and high quality. The first class grain is intended for animal forage, the third – for production of high-quality bread. The grader has working capacity of up to 10 t/h and suitable for use in industrial conditions as well as in breeding purposes.

Thus, barley grain has high content of the soluble dietary fiber β-glucans in cell walls of the endosperm, for which it is assumed as a promising food capable of reducing cardiovascular disease risk. In barley grain with significantly increased content of β-glucans, two other important quality parameters are suppressed – forage value and suitability for brewery use. β-Glucans are high molecular weight polymers of glucose bound with β(1-3) and β(1-4) glycosidic bonds; a whole barley grain contains an average of 3-9 % of these substances. Some mutant barley forms have the grain with low accumulation of starch and high content of β-glucans that fully or partially compensate the reduce in starch. The content of β-glucans in barley is largely determined by the genotype and climatic conditions of growing season; this parameter is positively correlated with air temperature and the dose of nitrogen fertilizer, and negatively – with moisture supply. β-Glucan content in barley grain is negatively correlated with and 1000-grain weight, amylose content, accumulation of starch, ash fraction, and grain yield. It was found a strong positive relationship between the amount of β-glucans and lipids in grain and protein content, as well as grain unit and hardness. These correlations allow indirect evaluation of barley in breeding work aimed at creation of forms with increased/decreased content of β-glucans in grain. Genotypic variation in accumulation of β-glucans is essential basis for progress in breeding of this economically important property. Covered amylose-free barley varieties are considered as the most promising for cultivation, especially in arid areas, due to capability of realizing a significant potential yield with a high content of β-glucans.

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