EFFECTS OF GERMINATION ON CHEMICAL COMPOSITION OF HULL-LESS SPRING CEREALS

Santa Senhofa[1], Tatjana Ķince[1], Ruta Galoburda[1], Ingmars Cinkmanis[1], Martins Sabovics[1], Ievina Sturite[2]
[1]Latvia University of Agriculture
[2]Norwegian Institute for Agricultural and Environmental Research (Bioforsk)
shenhofa@inbox.lv

Abstract
The objective of the current research was to investigate effects of germination on chemical composition of hull-less barley (*Hordeum vulgare* L. *var. nudum Hook. f.*), hull-less oat (*Avena sativa*), rye (*Secale spp.*), and wheat (*Triticum spp.*) grains for comparison. All the grains were cleaned, washed, steeped and germinated at temperature 35 ± 2 °C and relative humidity 95 ± 2% for 12, 24, 36, and 48 hours. After germination grains were dried till moisture content 14 ± 2%. Main quality parameters such as starch, proteins, b-glucan (in hull-less barley) and individual sugars were determined in cereals during their steeping and germination. Non-germinated grains were used as a control sample. In the present experiments non-significant protein content increase was observed in the analysed hull-less barley, hull-less oat, rye, and wheat during their germination for 48 hours. Starch content in hull-less barley, wheat and rye grains decreased non-significantly during germination for 24 h; opposite results were obtained for hull-less oat grains, where content of starch decreased by 16.7% after steeping and by 26.4% after germination for 24 h. b-glucan content in hull-less barley grains after germination for 48 h decreased by 20.5%. Non-significant changes were obtained in fructose content in analysed cereal grains during germination for 48 h; it was significantly increased after germination for 24 h and in germination for 48 h. Non-significant sucrose content changes were observed in hull-less barley, rye and wheat grains during germination for 12 h and in hull-less oat grains – for 24 h significantly increasing in future germination for 48 h.
Key words: hull-less cereals, germination, sugars, starch, proteins.

Introduction

Cereal grains constitute a major source of energy and nutrients in the world. The benefits of cereals to human health are the subject of extensive research and epidemiological studies, which have linked whole grain intake to the prevention of metabolic syndrome, obesity, and associated chronic diseases such as cardiovascular disease and two types of diabetes. The health benefits of cereals are primarily caused by their phytochemicals including phenolic acids, flavonoids, vitamins, fibre, and minerals, which act together to combat oxidative stress, inflammation, hyperglycaemia, and carcinogenesis (Poutanen, 2012; Wang, Wu, & Shyu, 2013).

Of the various barley (*Hordeum vulgare*) cultivars, hull-less barley has recently been receiving considerable research attention concerning the development of functional food, as it is an excellent source of both soluble and insoluble fibre. Hull-less (or 'naked') barley (*Hordeum vulgare* L. var. *nudum* Hook. f.) is a form of domesticated barley, in which, unlike hulled barleys but similarly to wheat (*Triticum aestivum*), the lemma and palea (hull) are non-adherent to the caryopsis. The total β-glucan content of hull-less barley is higher than that of hulled barley genotypes; whereas the insoluble dietary fibre content is lower (Xue *et al.*, 1997; Blandino *et al.*, 2015). In comparison with other cereals, naked oat (*Avena sativa*) grain is characterised by a larger amount of total protein and crude fat and a smaller one of crude fibre. The characteristic feature of protein is its good amino acid composition with a high nutritive value. A high level of fat is a good source of essential unsaturated fatty acids (Brand & Merwe, 1996; Petkov *et al.*, 2001; Biel, Bobko, & Maciorowski, 2009). The main chemical constituents of the rye (*Secale cereale*) grain are starch, dietary fibre (DF), protein, and mineral matter (ash). The starch content is limited mainly to the endosperm, and contents between 57.1 and 65.6 g 100 g^{-1} of dry matter (DM) are reported in rye. The DF components are found as cell-wall constituents in all parts of the kernel, and the total content of DF reported in rye grain is between 14.7 and 20.9 g 100 g^{-1} of DM. Approximately 25% of the total DF components are water-extractable (Hansen *et al.*, 2004). Wheat is the primary cereal grain produced in the European Union, and bread wheat represents the most important wheat species worldwide to be used as food ingredient in human nutrition (Matsuo, 1994; Rosenfelder, Eklund, & Mosenthin, 2013). Wheat is mainly appreciated as a source of carbohydrates and proteins (albeit poor in some essential amino acids, especially lysine), but contributes also a significant proportion of fibre, minerals, and antioxidant compounds, such as phenolic acids and tocols, to the human diet (Ward *et al.*, 2008; Hidalgo, Scuppa, & Brandolini, 2016).

Germination, a complex process causing physical, chemical and structural changes in grains, has been identified as an inexpensive and effective technology for improving cereal quality. The germination process is characterized by the growth of the embryo of the grain, manifested by the rootlets growth and increase

in length of the shoot (acrospire), with the concomitant modification of the contents of the endosperm (de Pinho Ferreira Guine & dos Reis Correia, 2013). Germination of grain commences with the uptake of water. Once germination is initiated, the predominant endosperm reserves, starch, cell wall, and storage proteins, are mobilized by the action of hydrolytic enzymes, which are synthesized in the aleurone layer and in the scutellum and secreted into the starchy endosperm of germinating grains (Lu, Lim, & Yu, 1998; Shaik *et al*., 2014). During germination, endogenous enzymes of cereal grains are activated, and some major substances such as starch and protein undergo degradation to small molecules. For example, a significant decrease of starch content is found during germination of rice. Furthermore, some functional compounds can be enriched, meanwhile some anti-nutrition factors, such as phytic acid, is degraded during germination. Current studies indicated that germination enriched γ-aminobutyric acid in brown rice (*Oryza sativa* L.), wheat, and foxtail millet (*Setaria italica*). Germinated cereal grains also show higher total phenolic content and antioxidant activity than those of un-germinated rice, wheat and oat. The germination process improves the nutritional quality of cereal. During the process of germination, significant changes in the biochemical, nutritional, and sensory characteristics of cereals occur due to degradation of reserve materials as used for respiration and synthesis of new cell constituents for developing embryo in the seed (Danisova *et al*., 1995; Sharma, Saxena, & Riar, 2016). As compared to un-germinated seed, germinated seeds contain high protein, low unsaturated fatty acids, low carbohydrate, and mineral content (Narsih, 2012; Sharma, Saxena, & Riar, 2016). Alpha-amylase enzyme plays a primary role in native starch granule degradation, and its expression is controlled by both gibberellin and sugar demand/ starvation. Sugar or carbon starvation activates the α-amylase promoter (Lu, Lim, & Yu, 1998; Shaik *et al*., 2014). As a result, during germination amylases are produced and partial breakdown of starch into simple sugars occurs (Chesworth, Stuchbury, & Scaife, 1998). Intense biochemical processes occur during the grain activation (the first stage of germination); as a result, grain biological value increases – the content of vitamins B_2, E and niacin, total sugar, dietary fibre and glucosamine increase; vitamin C is synthesized, and the content of irreplaceable amino acids is increased during the process of protein hydrolysis (Rakcejeva, 2006).

The objective of the current research was to investigate effects of germination on chemical composition of hull-less barley, hull-less oat, and rye, and wheat grains for comparison.

Materials and Methods

The study was realised at the scientific laboratories of the Faculty of Food Technology at Latvia University of Agriculture.

Conventional hull-less barley (′Irbe′), hull-less oat (′Lizete′), rye (′Kaupo′), wheat (′Ellvis′) grains cultivated at State Priekuli Plant Breeding Institute (Latvia) in 2015 were used in the experiments.

All the grains were cleaned, washed and steeped in water in the ratio of 1 : 2 (seeds to water) for 24 ± 1 h at 22 ± 2 °C. After steeping, water was drained, and grains were allowed to germinate in the dark at controlled temperature (35 ± 2 °C) and 95 ± 2% relative humidity (RH) in a climatic chamber ICH110 (Memmert, Germany). Grains were germinated for 12, 24, 36, and 48 hours. After germination grains were dried till moisture content 14 ± 2%. For air drying experiments, a convective dryer 00-800 (Memmert, Germany) was used; drying parameters were as follows: temperature 50 ± 1 °C, air flow velocity 1.2 ± 0.1 m s^{-1}. Grains were placed on a perforated sieve (diameter − 0.185 m), with the diameter of the holes – 0.002 m.

Before testing of parameters grains were ground in laboratory mill 3100 (Perten, Sweden) obtaining fine whole grain flour.

An Infratec™ 1241 Grain Analyzer (Foss, Sweden) was used to analyse starch, protein, and b-glucan concentration in grains according to ISO 12099. The measurements are based on the fact that the main constituents in the grain such as starch and others absorb electromagnetic radiation in the near-infrared region of the spectrum. Sample preparation is not required and the measurements of starch concentration (% of fresh weight basis) are directly displayed after grains are inserted in a pre-calibrated auto-analyser (Singh, Mackill, & Ismai, 2009).

For the analysis of content of individual sugars 5 grams of milled samples were extracted with 20 mL deionized water and stirred for 1 hour. After 1h the extract solution was filtered through the filter paper. The obtained extract was filtered through a high-performance liquid chromatography (HPLC) syringe filter with pore size of 0.45 μm. The content of individual sugars in the grain sample extract filtrate was determined with high-performance liquid chromatography LC 20 Prominence (Shimadzu, Japan). Determination parameters were: detector – refractive index RID-10A; column – Alltech NH2, 4.6 mm × 250.0 mm, 5 μm; temperature 25 °C; isocratic elution regime, mobile phase –A – acetonitrile, B – deionized water (A70:B30); capacity of the injection sample – 10 μL; total time of the analysis – up to 15 min; rate of the flow – 1.0 mL min^{-1}. Acquired data were processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1).

Table 1

Protein content changes in grains during steeping and germination, g 100 g^{-1} DM

Type of cereals	Control	Steeped	Germination time, h			
			12	24	36	48
Hull-less barley	10.5 ± 0.1	11.1 ± 0.1	10.7 ± 0.1	10.6 ± 0.1	10.9 ± 0.1	11.0 ± 0.1
Wheat	9.8 ± 0.1	10.3 ± 0.1	10.2 ± 0.1	10.1 ± 0.1	10.1 ± 0.1	10.0 ± 0.1
Hull-less oats	14.8 ± 0.1	14.9 ± 0.1	14.8 ± 0.1	14.8 ± 0.1	14.9 ± 0.1	15.0 ± 0.1
Rye	7.5 ± 0.1	7.7 ± 0.1	7.6 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	7.8 ± 0.1

The results were processed by mathematical and statistical methods (mean, standard deviation, p-value). Data were subjected to one-way analysis of variance (ANOVA) by Microsoft Office Excel 2007; significance was defined at $p<0.05$. Analyses were completed in triplicate.

Results and Discussion

Non-significant ($p>0.05$) protein content changes were observed in analysed cereals during germination. During germination for 48 h content of proteins in hull-less barley grains increased by 4.5%, in wheat – by 2.0%, in hull-less oats – by 1.3% and in rye – by 3.8%. The increase of protein content (Table 1) in cereals mainly can be explained by the results of M. He *et al.* (2015) for wheat cereals, as during germination, the embryo and endosperm of wheat seeds possibly have a basic pattern of oxygen consumption. They consume plenty of oxygen at the beginning of germination; enter lag period, then oxygen consumption increases sharply when the radicles have broken through the episperm. When germination is completed, oxygen consumption increases continuously in the embryo but decreases in the endosperm. Reserve substances from the endosperm begin to be mobilized when the germination is finished, and more enzymes involved in these processes are synthesized in large quantities. The mobilization also possibly concerns the activation of enzymes and inactivation of inhibitors. Proteins produce amino acids, peptides, and their derivatives, which are used to synthesize new proteins, then transported to the seedling.

Barley has usually been grown for feed uses. However, nowadays, barley is becoming more interesting due to its high content of bioactive compounds. Among these biologically active components, β-glucans play an important role. They are soluble dietary fibres located in the cell wall of the endosperm of barley grains, and their content can reach up to 15%. They contribute to lower cholesterol levels; regulate blood glucose levels, control colon cancer, and increase mineral and vitamin bioavailability (Gómez-Caravaca *et al.*, 2015).

Total content of β-glucan in barley normally ranges from 2 to 8% and depends on both genetic and environmental factors. About 66% of the barley β-glucans are in soluble form (Lee & Bamforth, 2009). In the present research content of b-glucans in analysed hull-less barley was found 4.40 ± 0.08 g 100 g^{-1}. However, significant changes ($p=0.011$) in b-glucans' content were observed in barley during processing as steeping and germination. As results of our experiments demonstrate, content of b-glucans decreased during grain steeping by 6.8%. But after grain germination for 48 h content of glucans decreased by 20.5% comparing with non-germinated hull-less barley grains. More significant ($p=0.024$) b-glucan content decrease was observed in hull-less barley grains after germination for 24 h; as a result, content of analysed parameter decreased by 11.4%, comparing with control grain sample (Fig. 1). O. Marconi *et al.* (2014) indicates that in particular the total β-glucan content decreased during malting due to the action of β-glucanase while β-glucan solubility increased during malting and is positively affected by the germination time.

During the seed germination process, α-amylase is the major enzyme for initial degradation of starch granules and β-amylase is also involved in starch conversion into free simple sugars. A-amylase randomly attacks only the α- (1→4) bonds; the amyloglucosidase (also called glucoamylase) selectively attacks the last bond on the non-reducing terminals, which can act on both the α- (1→4) and the α- (1→6) glucosidic linkages. The patterns of enzyme digestion were to produce large pin holes at starch granule surface in corn and triticale (Li *et al.*, 2011). Figure 2 shows grain starch content changes during germination process.

Non-significant ($p>0.05$) starch content decreases were detected in wheat, hull-less barley, and rye grains after steeping (Fig. 2). However, after hull-less oat steeping content of starch decreased by 16.7% ($p<0.05$) comparing with non-processed cereals. Non-significant ($p>0.05$) starch content decrease was detected in hull-less barley, wheat and

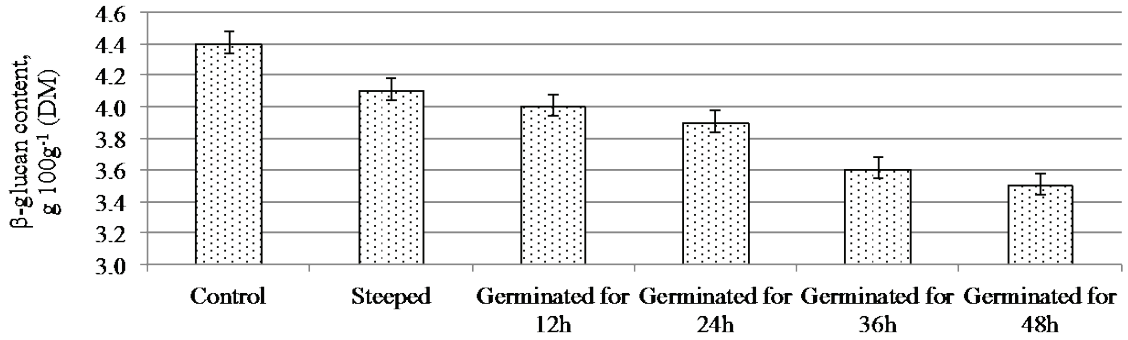

Figure 1. β-glucan content in hull-less barley during its germination.

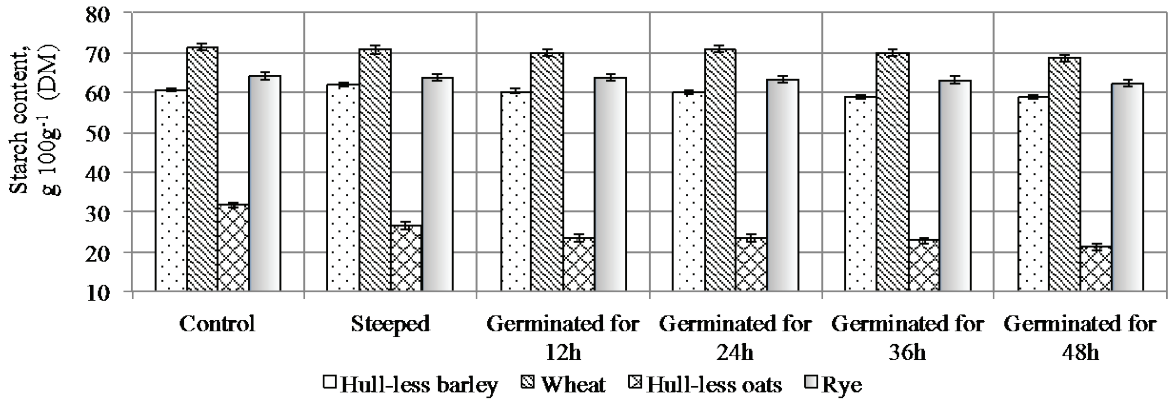

Figure 2. Grain starch content changes during germination.

rye grains after germination for 24 h, the content of starch in presented cereals decreased by 0.8%, 0.7% and 1.3% respectively. Starch content in hull-less oat grains decreased by 26.4% ($p=0.014$) after germination for 24 h, comparing with non-germinated hull-less oat. Non-significant ($p>0.05$) starch content decrease was detected in hull-less barley, wheat, and rye grains after germination for 48 h as follows by 3.5%, 3.9% and 2.7% respectively comparing with non-germinated grains. More pronounced ($p<0.05$) starch content decrease was detected in hull-less oat after germination for 48 h – by 33.6%, comparing with control hull-less oat sample. Similar results were observed in B. Tian *et al.* (2010) research, in oat seeds during germination the contents of protein, starch and phytate decreased significantly, the free amino acids, reducing sugars, free significant correlation among them was found. V. M. E. Andriotis *et al.* (2016) review recent advances in understanding the roles of carbohydrate-active enzymes in starch degradation in cereal grains through complementary chemical and molecular genetics. These approaches have allowed us to start dissecting aspects of starch degradation and the interplay with cell-wall polysaccharide hydrolysis during germination. With a view to improving and diversifying the properties and uses of cereal grains, it is possible that starch degradation may be amenable to manipulation through genetic or chemical intervention at the level of cell wall metabolism, rather than simply in the starch degradation.

During germination the activity of amylases increased progressively, but that of phosphorylase tended to increase during starch degradation (Fig. 3). A new α-amylase isoenzyme band appeared during germination. Glucose was the major product of starch degradations. Sucrose, maltose, maltotriose, raffinose and fructose were also detected. Protease activity reached a maximum on the fifth or sixth day and closely paralleled the increase in amino acids and soluble protein (Basuchaudhuri, 2014). In the present research non-significant changes ($p>0.05$) were obtained in fructose content in wheat, rye, hull-less barley and hull-less oat grains during germination for 48 h. Similar results were reported by D. Charalampopoulos *et al.* (2009), as interestingly the main sugars of the medium (fructose, maltotriose, etc.) and amino nitrogen were consumed in very low quantities during the fermentation – germination process. In the present experiments, non-significant ($p>0.05$) decrease of glucose content was observed

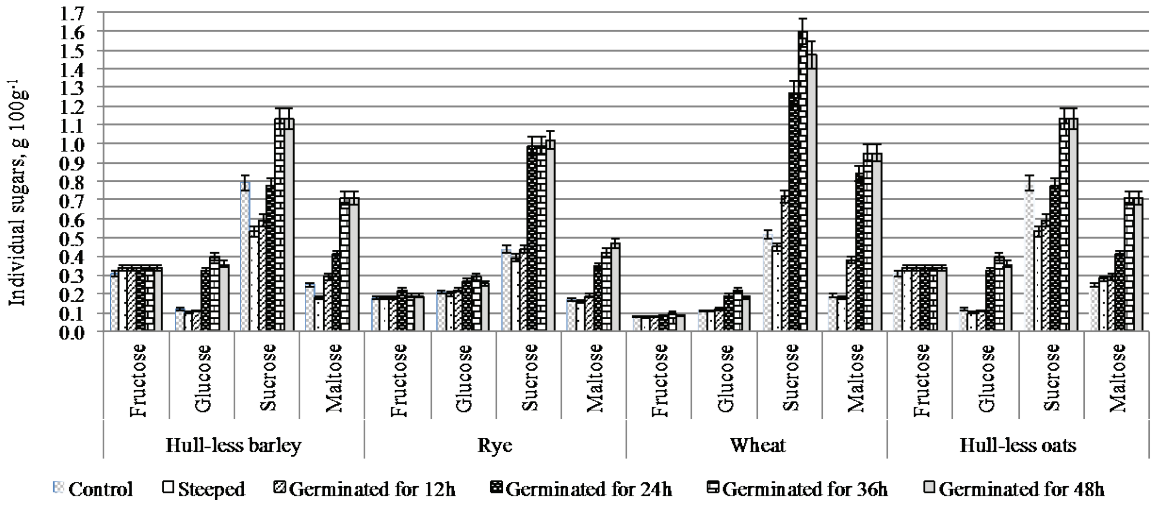

Figure 3. Individual sugars composition in grains.

in analysed cereals during germination for 12 h. However, glucose content significantly increased after germination for 24 h, accordingly, in hull-less barley and hull-less oats – by 62.5%, in rye – by 22.2%, in wheat – by 42.1% comparing with non-germinated cereals. In further germination for 48 h, glucose content changes were not significant ($p>0.05$). The obtained results could be explained with b-glucans breakdown, for example, in hull-less barley, (Fig. 1) into low molecular weight glucans and glucose by the endo-β-glucanases and β-glucosidases (de Pinho Ferreira Guine & dos Reis Correia, 2013). Non-significant ($p>0.05$) sucrose content changes were observed in hull-less barley, rye and wheat grains during germination for 12 h and in hull-less oat grains – for 24 h. On the contrary, sucrose content after 48 h germination in analysed grains increased significantly ($p<0.05$). After germination for 48 h sucrose content in hull-less barley grains increased by 30.1%, in rye – by 56.9%, in wheat – 64.4%, and in hull-less oats – by 46.3%

Significant ($p<0.05$) maltose content changes were detected in the analysed cereals after germination for 24 h; as a result, maltose content in hull-less barley and hull-less oats increased by 39.0%, in rye – by 51.4%, in wheat – by 77.4%. Similarly, the maltose content increased in cereals a further germination for 48 h.

Three enzymes are important for hydrolyzing starch to smaller molecules. They are α-amylase, β-amylase, and glucoamylase. Some of these enzymes (α-amylase and β-amylase) are naturally present in cereal grains and become active during germination. A-amylase displays an endoaction and can hydrolyze the α-1,4 linkage of starch internally and randomly, yielding low molecular weight dextrins. b-amylase is an enzyme having an exoaction. It can hydrolyze starch from the non-reducing chain end. The product removed through β-amylase action is maltose due to the hydrolysis of alternate α-1,4 linkages. β-amylase alone is basically inactive on granular starch but is capable of rapid action when the substrate is solubilized. β-amylase is found in sound, intact cereal grains and the level does not increase much as a result of germination (Liu & Rosentrater, 2011)

Conclusions

In the present research non-significant protein content increase was observed in the analysed hull-less barley, hull-less oat, rye, and wheat during their germination for 48 hours. Germination time has a significant effect on hull-less spring chemical composition resulting change in β -glucan and sugars content especially. Starch content in hull-less barley, wheat and rye grains decreased non-significantly during germination for 24 h. However, opposite results were obtained for hull-less oat grains, where content of starch decreased by 16.7% after steeping and by 26.4% after germination for 24 h. b-glucan content in hull-less barley grains after germination for 48 h decrease by 20.5% resulting starch breakdown. In the present research non-significant changes ($p>0.05$) were obtained in fructose content in analysed cereal grains during germination for 48 h. However, glucose content significantly increased after germination for 24 h, accordingly, in hull-less barley and hull-less oats – by 62.5%, in rye – by 22.2%, in wheat – by 42.1% comparing with non-germinated cereals and in germination for 48 h, glucose content changes were not significant ($p>0.05$). Non-significant ($p>0.05$) sucrose content changes were observed in hull-less barley, rye and wheat grains during germination for 12 h and in

hull-less oat grains – for 24 h significantly increasing in future germination for 48 h. However, maltose content in analysed cereals increase significantly after germination for 24 h – by 39.0% in oat, in rye – by 51.4%, in wheat – by 77.4%.

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References

1. Andriotis, V.M.E., Rejzek, M., Rugen, M.D., Svensson, B., Smith, A.M., & Field, R.A. (2016). Iminosugar inhibitors of carbohydrate-active enzymes that underpin cereal grain germination and endosperm metabolism. *Biochem Soc Trans*., 44(1), 159-165. DOI: 10.1042/BST20150222.
2. Basuchaudhuri, P. (2014). *Cold Tolerance in Rice Cultivation*. CRC Press, pp. 12-13.
3. Biel, W., Bobko, K., & Maciorowski, R. (2009). Chemical composition and nutritive value of husked and naked oats grain. *Journal of Cereal Science*, 49(3), 413-418. DOI: 10.1016/j.jcs.2009.01.009.
4. Blandino, M., Locatelli, M., Gazzola, A., Coïsson, D.-J., Giacosa, S., Travaglia, F., Bordiga, M., Reyneri, A., Rolle, L., & Arlorio, M. (2015). Hull-less barley pearling fractions: Nutritional properties and their effect on the functional and technological quality in bread-making. *Journal of Cereal Science*, 65, 48-56. DOI: 10.1016/j.jcs.2015.06.004.
5. Brand, T.S., Merwe, J.P. (1996). Naked oats (*Avena nuda*) as a substitute for maize in diets for weanling and grower-finisher pigs. *Journal of Animal Feed Science and Technology*, 57(1–2), 139-147. DOI: 10.1016/0377-8401(95)00827-6.
6. Charalampopoulos, D., Pandiella, S.S., & Webb, C. (2009). Uses of Whole Cereals and Cereal Components for the Development of Functional Foods. In *Innovation in Food Engineering*. CRC Press, pp. 638-639.
7. Chesworth, J.M., Stuchbury, T., & Scaife, J.R. (1998). *An Introduction to Agricultural Biochemistry*. Chapman & Hall, pp. 341-342.
8. Danisova, C., Holotnakova, E., Hozova, B., & Buchtova, V. (1995). Effect of germination on a range of nutrients of selected grains and legumes. *Acta Alimentaria*, Vol. 3, pp. 287-298.
9. De Pinho Ferreira Guine, R., & dos Reis Correia, P.M. (2013). Engineering Aspects of Cereal and Cereal-Based Products: *CRC Press*, pp. 53-55.
10. Gómez-Caravaca, A.M., Verardo, V., Candigliota, T., Marconi, E., Segura-Carretero, A., Fernandez-Gutierrez, A., & Fiorenza Caboni, M. (2015). Use of air classification technology as green process to produce functional barley flours naturally enriched of alkylresorcinols, β-glucans and phenolic compounds. *Food Research International*, 73, 88-96. DOI: 10.1016/j.foodres.2015.02.016.
11. Hansen, H.B., Moller, B., Andersen, S.B., Jorgensen, R.J., & Hansen, A.A. (2004). Grain Characteristics, Chemical Composition, and Functional Properties of Rye (*Secale cereale* L.) As Influenced by Genotype and Harvest Year. *Journal of Agricultural and Food Chemistry*, 52(8), 2282-2291.
12. He, M., Zhu, C., Dong, K., Zhang, T., Cheng, Z., Li, J., & Yan, Y. (2015). Comparative proteome analysis of embryo and endosperm reveals central differential expression proteins involved in wheat seed germination. *Journal of Plant Biology*, 15, 97-111. DOI: 10.1186/s12870-015-0471-z.
13. Hidalgo, A., Scuppa, S., & Brandolini, A. (2016). Technological quality and chemical composition of puffed grains from einkorn (*Triticum monococcum* L. subsp. *monococcum*) and bread wheat (*Triticum aestivum* L. subsp. *aestivum*). *LWT - Food Science and Technology*, 68, 541-548. DOI: 10.1016/j.lwt.2015.12.068.
14. Lee, Y.-T., & Bamforth, C.W. (2015). Variations in solubility of barley beta-glucan during malting and impact on levels of beta-glucan in wort and beer. *Journal of the American Society of Brewing Chemists*, 67(2), 67-71. DOI: 10.1094/ASBCJ-2009-0226-01.
15. Li, C.Y., Li, C., Lu, Z.X., Li, W.H., & Cao, L.P. (2012). Morphological changes of starch granules during grain filling and seed germination in wheat. *Starch*, 64, 166-170. DOI: 10.1002/star.201100093166.
16. Liu, K.S., & Rosentrater, K.A. (2016). Distillers Grains: Production, Properties, and Utilization. *CRC Press*, pp. 63-64.
17. Lu, C.A., Lim, E.K., & Yu, S.M. (1998). Sugar Response Sequence in the Promoter of a Rice Alpha-Amylase Gene Serves as a Transcriptional Enhancer. *Journal of Biological Chemistry*, 273(17), 10120-10131. PMID: 9553059.
18. Marconi, O., Tomasi, I., Dionisio, L., Perretti, G., & Fantozzi, P. (2014). Effects of malting on molecular weight distribution and content of water-extractable β-glucans in barley. *Food Research International*, 64, 677-682. DOI: 10.1016/j.foodres.2014.07.035.

19. Matsuo, R.R. (1994). Durum wheat: its unique pasta-making properties. W. Bushuk, V.F. Rasper (Eds.), *Wheat – Production, Properties and Quality, Blackie Academic & Professional.* An imprint of Chapman & Hall. Great Britain, pp. 169-178.
20. Narsih, H.Y. (2012). The study of germination and soaking time to improve nutritional quality of sorghum seed. *International Food Research Journal*, 19(4), 1429-1432.
21. Petkov, K., Biel, W., Kowieska, A., & Jaskowska, I. (2001). The composition and nutritive value of naked oat grain (*Avena sativa* var. *nuda*). *Journal of Animal and Feed Sciences*, 10(2), 303-307.
22. Poutanen, K. (2012). Past and future of cereal grains as food for health. *Trends in Food Science and Technology*, 25(2), 58-62. DOI: 10.1016/j.tifs.2012.02.003.
23. Rakcejeva, T. (2006). Biologically Activated Grain in Wheat Bread Technology. *Resume of the Ph.D. thesis*, Jelgava, Latvia: LLU, PTF. 18 p.
24. Rosenfelder, P., Eklund, M., & Mosenthin, R. (2013). Nutritive value of wheat and wheat by-products in pig nutrition: A review. *Animal Feed Science and Technology*, 185(3–4), 107-125. DOI: 10.1016/j.anifeedsci.2013.07.011.
25. Shaik, S.S., Carciofi, M., Martens, H.J., Hebelstrup, K.H., & Blennow, A. (2014). Starch Bioengineering Affects Cereal Grain Germination and Seedling Establishment. *Journal of Experimental Botany*, 65(9), 2257-2270. DOI: 10.1093/jxb/eru107.
26. Sharma, S., Saxena, D.C., & Riar, C.S. (2016). Analysing the effect of germination on phenolics, dietary fibres, minerals and γ-amino butyric acid contents of barnyard millet (*Echinochloa frumentaceae*). *Food Bioscience*, 13(1), 60-68. DOI: 10.1016/j.fbio.2015.12.007.
27. Singh, S., Mackill, D.J., & Ismai, A.M. (2009). Responses of SUB1 Rice Introgression Lines to Submergence in the Field: Yield and Grain Quality. *Field Crops Research*, 113(1), 12-23. DOI: 10.1016/j.fcr.2009.04.003.
28. Tian, B., Xie, B., Shi, J., Wu, J., Cai, Y., Xu, T., Xue, S., & Deng, Q. (2010). Physicochemical changes of oat seeds during germination. *Food Chemistry*, 119(3), 1195-1200. DOI: 10.1016/j.foodchem.2009.08.035.
29. Wang, C.-Y., Wu, S.-J., & Shyu, Y.-T. (2013). Antioxidant properties of certain cereals as affected by food-grade bacteria fermentation. *Journal of Bioscience and Bioengineering*, 117(4), 449-456. DOI: 10.1016/j.jbiosc.2013.10.002.
30. Ward, J.L., Poutanen, K., Gebruers, K., Piironen, V., Lampi, A.-M., & Nyström, L. (2008). The health grain cereal diversity screen: concept, results, and prospects. *Journal of Agricultural and Food Chemistry*, 56(21), 9699-9709. DOI: 10.1021/jf8009574.
31. Xue, Q., Wang, L., Newman, R.K., Newman, C.W., & Graham, H. (1997). Influence of the hulles, waxy starch and short-awn genes on the composition of barleys. *Journal of Cereal Science*, 26(2), 251-257. DOI: 10.1006/jcrs.1996.0114.