INVESTIGATION OF IMMATURE WHEAT GRAIN CHEMICAL COMPOSITION

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Abstract
The present study provides research in content of protein, starch, sugars and concentration of B group vitamins (thiamine, riboflavin, pyridoxine and niacin) in immature wheat (*Triticum aestivum* L.) compared to mature wheat kernels.
The content of protein, starch was determined in kernels using Infratec1241 Grain Analyzer (Sweden), content of sugars was analysed by high- efficiency liquid chromatography HPLC (Shimadzu, Japan). The concentrations of vitamins were determined by high - efficiency liquid chromatography for vitamins B_1, B_2, B_3, B_6. Obtained results suggested that the immature wheat kernels obtained in milk stage showed higher contents of some compounds such as sugars and B group vitamins. The amount of all sugars presented in immature grains is higher and decreased uniformly during maturation. Therefore, immature grains are better sources of the B group vitamin that the mature grains, and therefore more nutritionally efficacious.
Key words: immature wheat grain, protein, starch, sugars, B group vitamins.

Introduction

The nutritional value of food is determined by the content of mainly following substances: fats, carbohydrates, proteins. For a long time it was considered, if human food includes all these nutrients, it fully meets the needs of biological organism. However, the practice does not always confirm the correctness of entrenched ideas about the biological value of food. Practical experience shows that there are diseases, directly related to food. Cereals are basic components of the human diet. Consumption of whole unrefined cereal products is known to contribute significantly to health and chronic disease prevention. Whole cereal grains contain nutritionally significant quantities of dietary fibre, as well as minerals and vitamins that are important for health. Therefore, the interest in cereals as a source of bioactive and functional ingredients has increased (Awika, 2011).

Wheat (*Triticum aestivum* L.) is one of the primary grains consumed by humans. The chemical composition and physical properties of wheat kernels changes during maturing process and depends on the degree of maturity. This is a complex process controlled by several factors. In recent studies it has been proven that wheat during maturation contains many valuable compounds which disappear or lose their unique properties in the mature kernels.

Therefore, the aims of this work are identified and theoretically justify the features of the chemical composition in immature wheat grain. Wheat grains harvested before maturity are a rich source of biologically active substances. Compared to wheat complete ripeness, immature wheat grains contain less starch, more vitamins, fibre and soluble sugars (Yang *et al*., 2012; Iametti *et al*., 2006). Kalnina *et al*. (2015) has informed on contents of vitamins B_1 (thiamine) and B_2 (riboflavin) in mature wheat 'Zentos' (*Triticum aestivum* L.). As reported by other scientists (Yang *et al*., 2012; Merendino *et al*., 2006), the immature grains and immature wholemeal showed higher contents of some important functional compounds such as vitamins C, niacin, dietary fibre, reducing sugars. Wheat kernels harvested during the milk stage suggested its use as an innovative material with interesting functional characteristics to prepare functional foods. Based on the above considerations our research focused in the study of various nutrients including protein, starch, sugars, and vitamins B in immature winter wheat kernels cultivars 'Zentos' compared with mature kernels.

Materials and Methods

Winter wheat (*Triticum aestivum* L.) bread cultivar 'Zentos' from the experimental farm 'Peterlauki' of Latvia University of Agriculture was harvested in immature conditions (milk stage) and mature conditions in 2015. Immature grains sample with initial moisture content 64% was dried in a microwave- vacuum dryer at 45 °C temperatures till moisture content 11.4%.

The content of protein and starch in grains was determined using Infratec1241 Grain Analyzer (Sweden). Content of sugars was analyzed by high-efficiency liquid chromatography. For the analysis of content of individual sugars 5 g of milled samples were extracted with 20 mL deionised water and stirred for 1 h. After one hour 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 – deionised 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).

The analysis of the content of vitamin group B was carried out at the Quality Department JSC 'Grindeks'. In the present study, the concentration of vitamins was determined by high- efficiency liquid chromatography for vitamins B_1, B_2, B_3 and B_6.

HPLC conditions

For simultaneous detection of vitamins, the liquid chromatography method described by Aslam *et al*., (2008), with a few changes was used. The mobile phase used trifluoroacetic acid water solution.

High-performance liquid chromatography (HPLC) analysis was performed with the following parameters: Column – Water Atlantis T3 4.6 × 150 mm, 3.0 µm; Column temperature at 30 °C; Sample temperature 10 °C; Mobile phase – A: 0.05% trifluoroacetic acid water solution (0.5 mL L^{-1}); B: acetonitrile. Flow rate 1.0 mL min^{-1}; Injection volume 10 µL; Injector wash solvent 10% solution of methanol in water for chromatography; Detection – 190 nm – 400 nm (use photodiode array detector) – for identification; 248 and 290 nm for assay; Integration-pyridoxine hydrochloride 248 nm, thiamine hydrochloride 290 nm (Table 1).

Table 1

Gradient cycle

Time, min	A, %*	B, %**	Curve
0.0	100	0	-
4.0	97	3	linear
6.0	90	10	linear
20.0	90	10	linear
30.0	60	40	linear
32.0	60	40	linear
33.0	100	0	linear
40.0	100	0	linear

*A - Mobile phase - A: 0.05% trifluoroacetic acid water solution (0.5 mL L^{-1}).
** - B: acetonitrile.

Statistical Analysis

Statistical analysis was performed with SPSS 23.0 package for Windows 10. Mean arithmetic value and standard deviation were calculated. ANOVA analysis was applied in order to see if there are significant differences between the mature and immature wheat kernels ($p<0.05$).

Results and Discussion

Protein content is one of the most important standards for measuring wheat quality. Proteins are synthesized and accumulated in developing grain at different times and different rates. Immature wheat grains contain 9.8% protein but mature wheat grains – 13.4%. The difference of protein content between immature and mature grains was 3.6%. Similar results have been obtained from other reports (Yang *et al*., 2012; Iametti *et al*., 2006). The protein content of whole wheat increases only very slightly during maturation. The pattern of the changes in total protein during the development of the endosperm resembles the pattern of relative changes in starch (Abou-Guendia & D'Appolonia, 1972.)

Starch content, in fact, increased from 63.7% to 67.6% because during ripening the monosaccharide, disaccharides are converted into storage polysaccharides, in starch. The group of sugars studied in immature and mature grains were the mono- and disaccharides which were extracted with 20 mL deionised water and included glucose, fructose, sucrose and maltose. The changes in individual free sugar content in immature and mature grains are shown in Figure 1. The amount of all sugars presented in immature grains is higher and decreased uniformly during maturation. The concentration of fructose decreased from 1.14 g 100 g^{-1} to 0.27 g 100 g^{-1}. Similar situation we have observed regarding glucose and sucrose. Maltose declined sharply from 2.16 g 100 g^{-1} to 0.59 g 100 g^{-1} in mature grains. The changes in sugars content can be explained that they are involved during maturity in starch and others non-starch oligosaccharides synthesis. Higher sugar content in immature wheat suggested favourable taste with sweetness. The results of present work agree with results of M. Abou-Guendia & B.L. D'Appolonia (1973) and suggest that the sugars in the kernel, which arise by both translocations from plant and by photosynthesis within kernel, are utilized in the rapid process of starch synthesis. The sugar content present in the kernel at any particular stage of maturity theoretically cannot account for the total amount of starch synthesized at the following stage, indicating that translocation and photosynthesis of sugars both are essential for starch accumulation in the kernel.

Vitamins – a group of low-molecular organic compounds are present in small amounts in the diet. Vitamins are essential nutrients that are required in many areas of biochemical metabolism, including deoxyribonucleic acid (DNA) synthesis, energy production and biosynthetic pathways (Ball, 2005).

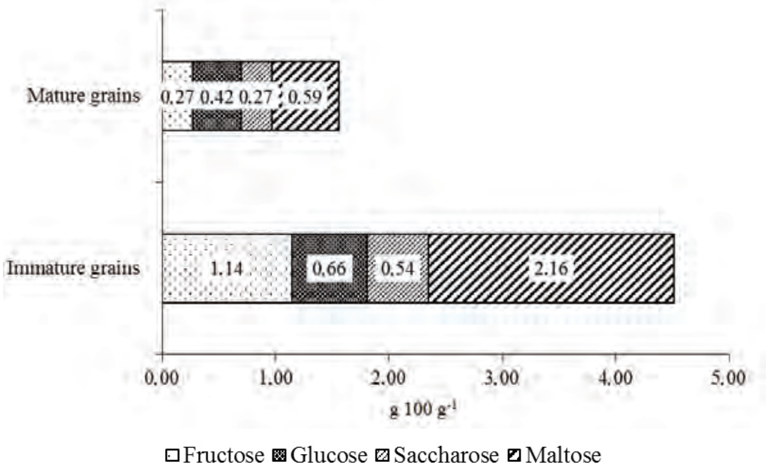

Figure 1. The sugar content in immature and mature wheat grains.

There are several B group vitamins in wheat such as vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (niacin) and B_6 (pyridoxine). In cereal grains, the thiamine is unevenly distributed, being relatively low in the starch endosperm and high in germ. Cereal grains also contain relatively low concentration of flavins, but they are important sources in those parts of the world where cereals constitute the staple diet. In mature cereal grains most of the niacin is concentrated in the aleurone and germ layers. The whole-grain cereals are important source of pyridoxine, where over 90% of the vitamin B_6 is found in the bran and germ. They all, as well proteins and fats (riboflavin, pyridoxine) (Ball, 2005), play an important role in metabolism of carbohydrate (thiamine). B vitamins' concentrations indicate an important variability in wheat cultivars (Batifoulier *et al*., 2006). The contents of B group vitamins into the immature and mature wheat samples were measured and shown in Figure 2.

Niacin (4.45 ± 0.66 mg 100 g^{-1} in immature grains and 3.62 ± 0.15 mg 100 g^{-1} in mature grains) was an abundant component in B vitamins. The level of niacin decreased 20% during grain maturation. The same situation can be observed with content of riboflavin (0.34 ± 0.14) mg 100 g^{-1}) in immature wheat compared to mature (0.08 ± 0.01 mg 100 g^{-1}) wheat samples.

No significant change in thiamine and pyridoxine content was observed during maturation. Similar results were obtained by scientists while evaluating immature grains in two rice cultivars during maturation (Ji *et al*., 2013). The highest content of vitamin $B_{2,}$ $B_{3,}$ and B_6 was found in the mature rice grains. When the variability of B vitamin concentration in mature wheat grain cultivated in France was analysed, the concentration of thiamine, riboflavin, and pyridoxine represented cultivars with higher B vitamins concentration as cultivar 'Zentos' (Batifoulier *et al*.,

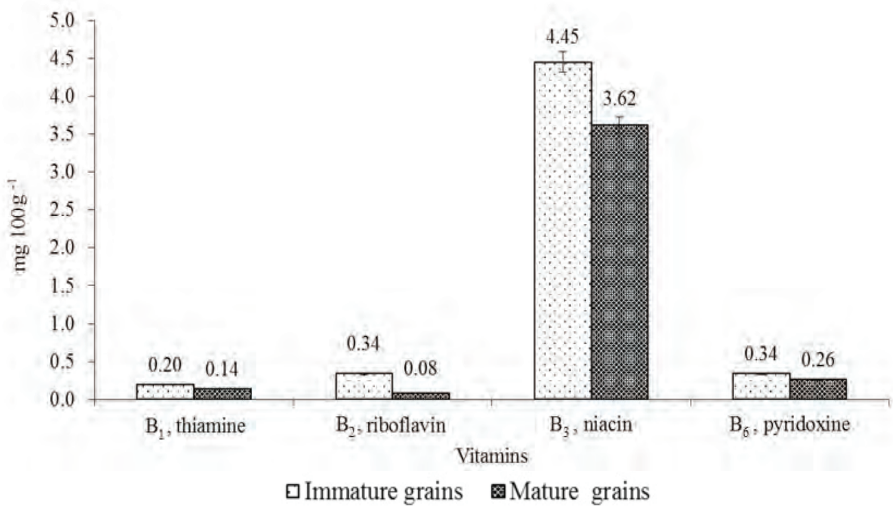

Figure 2. Vitamins in mature and immature wheat, mg 100 g^{-1}.

2006). It means that several factors promote vitamin variability such cultivar, year, growing location and conditions.

The results' interpretation of statistical programs SPSS by a single factor ANOVA showed significant differences between the value of B group vitamins in mature and immature wheat kernels ($p<0.05$).

The values are the means ±standard deviation of four independent experiments. All the differences between mature and immature wheat kernels were significantly different (T-test, $p<0.05$).

The results showed reduction of all group B vitamins during maturation.

Conclusion

In the present research concentrations of B group vitamins (thiamine, riboflavin, pyridoxine and niacin) in immature wheat compared to mature wheat were established. Obtained results suggested that the immature wheat kernels obtained in milk stage showed higher contents of some compounds such as sugars and vitamins B. The amount of investigated sugars presented in immature grains is higher compared with mature grains. Immature grains are better sources of the B group vitamins than the mature grains, and therefore more nutritionally efficacious.

Our results indicate that wheat kernels harvested at milk phase could be representing a valuable ingredient for production of functional foods.

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