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Influence of Germination Temperatures on the Chemical Composition of Wheat (*Triticum aestivum* L.) Seeds

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Abstract

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The content of saccharides and lipids in wheat seeds from both conventional and organic agriculture was determined based on the length of germination (0, 2, or 5 days) and germination temperature (10 and 22°C). The content of saccharides was determined by HPLC, and the total lipid content was detected by Soxhlet extraction in the course of germination. While the non-germinated seeds had the highest content of glucose (6.95 mg/g) and fructose (4.37 mg/g), in conventionally grown seeds sucrose (5.03 mg/g) and maltose (3.62 mg/g) were at the highest level. In organically and conventionally produced seeds the contents of fructose, glucose, and maltose increased due to germination. While the content of lipids increased in the seeds from organic production on the second day from 0.46 g/10 g to 0.51 g/10 g (at 10°C) and 0.64 g/10 g (at 22°C). Differences in the content of saccharides and lipids between seeds from organic and conventional agriculture were not unequivocally confirmed.

Keywords: grain; winter wheat; organic farming; content of lipids; content of carbohydrates

Not only the eating habits of the Western world have been changing in the last decades (Jancurová *et al*. 2005), but also the view of nutritional experts on the biological and nutritional value of food has changed. In this regard, the focus also shifted on the biological and nutritional quality of plant germs (Gabrovská *et al*. 2005; Peñas *et al*. 2008). Germination is influenced by the enzyme activity, while the level of activation of individual enzymes depends on the chemical composition of seeds, e.g. in wheat it is mainly the activity of amylase decomposing starches, in sunflower the activity of enzymes decomposing proteins and fat increases. These shifts bring with them changes in the nutritional quality of germinating seeds. According to Katina *et al*. (2007), a change of insoluble components into soluble ones occurs due to their hydration, as well as their chemically complex substances becoming structurally simple.

According to Márton *et al*. (2010) germinating seeds are excellent sources of vitamins, minerals, and proteins. Changes of fatty acid contents take place in the course of germination when, according to Kim *et al*. (2004), the contents of polyunsaturated fatty acids increase, particularly linoleic acid. Saharan and Saroj Bishnoi (2001) further stated that germination improves the bioavailability of mineral substances, particularly Ca, Fe, Zn, Mg, and P. As explained by Sandberg (2002), their increased bioavailability is caused by decreased amounts of anti-nutritional substances, such as phytic acid (Valencia *et al*. 1999) and inhibitors of trypsin and tannins (Sangronis & Machado 2007).

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Further, the enzyme catalysis of proteins into oligopeptides and free amino acids takes place, as well as the contents of sulphure-containing amino acids and nitrates are decreased. Among other changes at the enzymatic level, the content of the enzyme β-galactosidase decreases, while, according to Urbano *et al.* (2005), sucrose, glucose, and fructose increases. Due to the influence of this enzyme, polysaccharides decompose into oligo- and monosaccharides, as well as fatty acids, in the course of germination (Williams *et al.* 1994).

The goal of our study was to monitor these changes in the saccharide and lipid content in germinating wheat seeds in relation to the cultivation system, temperature, and germination time. The growing system was designed because the consumers start to prefer organically grown food as organic farming is an alternative to conventional agriculture. It relies on fertilisers of organic origin such as compost, manure, green manure, and bone meal and it places emphasis on techniques such as crop rotation and companion planting. In general, organic standards are designed to allow the use of naturally occurring substances while prohibiting or strictly limiting synthetic substances. In contrast, intensive (conventional) agriculture is any of the various types of agriculture that involve higher levels of input and output per unit of the agricultural land area. It is characterised by low fallow ratio, higher use of inputs such as capital and labour, and higher crop yields per unit land area.

MATERIAL AND METHODS

As the experimental material, the seeds of wheat (*Triticum aestivum* L.) from conventional and organic production were selected. Seeds of winter wheat cv. Samantha were obtained from the Research Station of the Department of Crop Production (Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague). The weight of the seeds was 20 g and they were germinated in dark at two temperature modes: 10 and 22°C, with air humidity at 72% in a Conviron air-conditioned chamber (Schoeller, Canada). The temperature was constant during the 24-h cycle. The ISTA methodology of testing seed stock was modified. Seeds of experimental plants were germinated in Petri dishes on filter paper. Three samples were taken in the course of germination: on days 0 – 2 – 5. During the individual sampling terms, saccharide and lipid content was identified in the germ.

Saccharides were determined under the following conditions: analytical column Phenomenex Luna 5μ NH_2; precolumn Luna 5μ NH_2 (both Phenomenex, USA); acetonitrile/deionised water mobile phase (75 : 25, v/v), HPLC super gradient acetonitrile (Lach-Ner, Czech Republic), and Milli-Q water, isocratic elution; flow rate 1.25 ml/min; injection 10 μl, column temperature 30°C. The analysis was performed using a Waters High Performance Liquid Chromatograph (WatersThermo Fisher Scientific, USA) with a quaternary pump, refrigerated autosampler, column heater, and RI detector.

Using the Soxhlet extractor (Soxtec system HT6), lipids were extracted using petroleum ether *puriss*, p.a. with a boiling point of 30–60°C. The time of extraction was 4–6 h, at a temperature of 110°C. The content of fats was determined gravimetrically. The results were evaluated by the Statistica 9.0 CZ software using a multifactor analysis of variance of multiple classification (ANOVA) on the level of significance $\alpha = 0.05$, while maintaining 5 replications.

RESULTS AND DISCUSSION

The content of carbohydrates and lipids was monitored in wheat grains during germination. From the results obtained it is evident that the change of fructose content in organic seeds and in conventionally grown seeds displayed an identical trend in relation to both germination temperature and time. At both temperatures (10 and 22°C) the fructose content in organic seeds first decreased on day 2 of germination in comparison with non-germinated seeds. However, by day 5, fructose content conclusively increased at both temperatures (Tables 1 and 2). The content of fructose in germinating seeds grown by conventional methods increased at the temperature of 22°C depending on the germination time (Tables 1 and 2). While in the non-germinated seeds (day 0), the content of fructose was 1.27 mg/g, in seeds germinating at 10°C its content was significantly higher on day 5 reaching 1.80 mg/g and 10.08 mg/g at a temperature of 22°C. From these results it is evident that the fructose content increases due to the germination of wheat seeds. This conclusion is consistent with Zörb *et al.* (2006), who studied the chemical composition of wheat seeds. It also confirmed the conclusions of Bogdan and Zagdańska (2006), who claimed that the increase of fructose concentration in wheat seeds resulted from their rehydration.

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Table 1. Average contents (mean ± standard deviation) of saccharides and lipids in wheat seeds from organic production, depending on germination temperature and time

Temperature (°C)	Day	Fructose	Glucose	Sucrose	Raffinose	Maltose	Lipids (g/10 g)
		(mg/g)					
10	0	4.37 ± 0.07[c]	6.95 ± 0.45[c]	1.60 ± 0.08[b]	1.90 ± 0.22[c]	3.19 ± 0.21[d]	0.46 ± 0.01[bc]
	2	1.24 ± 0.33[d]	2.59 ± 0.28[d]	5.79 ± 0.51[ab]	1.20 ± 0.22[c]	5.76 ± 0.11[c]	0.51 ± 0.13[ab]
	5	5.87 ± 0.09[b]	16.84 ± 0.82[b]	7.82 ± 0.46[ab]	3.27 ± 0.49[b]	27.51 ± 0.58[ab]	0.42 ± 0.04[cd]
22	0	4.37 ± 0.07[c]	6.95 ± 0.44[c]	1.60 ± 0.08[b]	1.90 ± 0.23[c]	3.19 ± 0.21[d]	0.46 ± 0.01[bc]
	2	2.13 ± 0.19	6.08 ± 0.74[c]	12.01 ± 0.40[a]	0.37 ± 0.32[d]	19.36 ± 0.75[b]	0.64 ± 0.04[a]
	5	8.89 ± 0.75[a]	31.76 ± 0.74[a]	12.32 ± 0.38[a]	4.60 ± 0.80[a]	45.23 ± 0.14[a]	0.48 ± 0.06[ab]

The content of the monosaccharide glucose was also monitored in germinating wheat seeds. The seeds germinating at temperatures of 10 and 22°C showed similar changes of glucose content in relation to the germination time and seed source. Organic seeds showed a decrease of glucose content on day 2 of germination compared to non-germinated seeds, while in seeds germinating at temperature of 22°C this difference was statistically insignificant (Tables 1 and 2). A demonstrable decrease of glucose content in wheat seeds was recorded at a temperature of 10°C.

According to Yang *et al*. (2004), the activity of hydrolytic enzymes increases during the germination of wheat. This leads to the decomposition of starch and to an increase of the concentration of monosaccharides, including glucose. A similar trend is evident in barley seeds (Rimsten 2003). The decreased level of glucose on the second day of germination is caused by its consumption during metabolic processes, particularly respiration (Helland *et al*. 2002).

A decisive increase in glucose was recorded among the five-day germinated seeds at both temperatures. These changes in glucose content were also noted in germinating seeds grown by conventional agriculture at a temperature of 10°C. During the effect of higher germination temperatures, glucose content in germinating seeds increased compared to non-germinated seeds. In non-germinated seeds, the glucose content was 2.50 mg/g, while in seeds germinating for five days it reached 31.39 mg/g. Helland *et al*. (2002) also confirmed an increase of glucose content due to gradual germination in their work, monitoring the changes of chemical composition in germinating maize seeds.

Sucrose content also changed, depending on the time and temperature of germination (Tables 1 and 2). Germination at a temperature of 10°C showed the lowest sucrose content in non-germinated seeds from both types of agriculture. Sucrose content increased in the course of germination, thus its highest content was on day 5 of germination. Similarly, the content of sucrose increased in organic seeds at a temperature of 22°C. In conventionally grown seeds, sucrose content conclusively increased between non-germinated seeds and seeds germinating for two days, when its maximum content was recorded. On day 5, sucrose content decreased. According to Bailly *et al*. (2001), the content of sucrose is significant for seed storage and due to germination the sucrose content increases, as is also evident from the results of this research. Mbithi-Mwikya *et al*. (2000) came to similar conclusions in their research.

Table 2. Average contents (mean ± standard deviation) of saccharides and lipids in wheat seeds from conventional production, depending on germination temperature and time

Temperature (°C)	Day	Fructose	Glucose	Sucrose	Raffinose	Maltose	Lipids (g/10 g)
		(mg/g)					
10	0	1.27 ± 0.02[c]	2.50 ± 0.32[c]	5.03 ± 0.04[d]	2.32 ± 0.04[b]	3.61 ± 0.03[d]	0.66 ± 0.18[ab]
	2	1.73 ± 0.97[c]	2.06 ± 0.22[c]	8.02 ± 1.50[c]	1.87 ± 0.12[c]	11.49 ± 0.85[c]	0.38 ± 0.02[c]
	5	1.80 ± 0.14[c]	4.36 ± 0.34[c]	8.00 ± 1.65[c]	0.72 ± 0.07[d]	3.62 ± 0.85[d]	0.81 ± 0.07[a]
22	0	1.27 ± 0.02[c]	2.50 ± 0.33[c]	5.03 ± 0.04[d]	2.32 ± 0.04[b]	3.61 ± 0.03[d]	0.66 ± 0.18[ab]
	2	3.15 ± 0.22[b]	11.80 ± 1.61[b]	14.81 ± 0.33[a]	6.21 ± 0.83[a]	28.92 ± 1.47[bc]	0.39 ± 0.01[bc]
	5	10.08 ± 0.06[a]	31.39 ± 1.32[a]	14.16 ± 0.24[b]	1.53 ± 0.04[c]	44.56 ± 0.86[a]	0.35 ± 0.03[c]

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The content of raffinose in seeds from bio-production and conventional growing showed an identical trend of changes in relation to the time and temperature of germination. Both showed an evident decrease of raffinose content on day 2 of germination. On the contrary, on day 5 of germination, an evident increase of this saccharide was recorded. While in the seeds from bio-production sucrose content increased on day 5, the opposite response was recorded in conventionally grown seeds (Tables 1 and 2). They showed that the highest content of raffinose was found in non-germinated seeds (2.32 mg/g) while the lowest raffinose content was determined on day 5 at both experimental temperatures. On day 5 of germination, the content of raffinose was 0.73 mg/g in seeds germinating at the temperature of 10°C and 1.53 mg/g at 22°C. Sampath *et al.* (2008) also confirmed a decrease of raffinose on day 2 of germination in their work on wheat seeds. These Authors concluded that the decrease of this oligosaccharide at the beginning of germination was caused by increased activity of the enzyme α-galactosidase. The increase of raffinose levels on further days of germination are caused by a gradual transition to autotrophic nurturing. The above conclusion was further confirmed by Corbineau *et al.* (2000).

The last monitored saccharide was maltose (Tables 1 and 2). In this disaccharide, we conclude that its content increases with the length of germination. This trend was recorded at both monitored temperatures. The lowest maltose content was identified in non-germinated seeds from organic production (3.19 mg/g) and conventional production (3.62 mg/g). Its amount on day 5 in seeds germinated at 10°C increased by 24.32 mg/g (organic) and 7.87 mg/g (conventional). Similar results were found in seeds germinating at a temperature of 22°C. In this case, maltose content increased from 3.19 mg/g (organic) and 3.62 mg/g (conventional) to 45.23 mg/g and 44.56 mg/g, respectively.

Mbithi-Mwikya *et al.* (2000) also indicated in their works an increase of maltose content in the course of germination. This increase in maltose due to germination is caused by the activity of hydrolytic enzymes and by the decomposition of starches into soluble sugars (Yang *et al.* 2004; Saman *et al.* 2008). These soluble saccharides are used during respiration.

Aside from saccharides, the content of lipids was also monitored in the germinating seeds, depending on the temperature and time of germination. A different reaction in seeds from organic production and conventionally grown seeds is evident (Tables 1 and 2). The content of lipids increased on day 2 of germination in organically grown seeds at both monitored temperatures (Table 1), when the lipid content in non-germinated seeds was 0.46 g/10 g, while on day 2 lipid content had already reached values 0.51 g/10 g (at 10°C) and 0.64 g/10 g (at 22°C). A decrease of lipid content was recorded on day 5. On the contrary, a 42 and 41% decrease of lipid content was found in conventionally grown seeds at the beginning of germination as opposed to non-germinated seeds. This decrease of lipids was further found on the fifth day in seeds germinating at a temperature of 22°C. Even so, at a temperature of 10°C, the content of lipids evidently increased on day 5 (Tables 1 and 2).

Changes in lipid content depending on germination were also confirmed by Hübke *et al.* (2005). According to Kaukovirta-Norja *et al.* (1998), these changes are caused by the hydrolysis of triacylglycerols and by the activity of lipolytic enzymes. Large amounts of energy and metabolic intermediate products are necessary during germination.

It was confirmed by the results published by Williams *et al.* (1994) and Rose and Pike (2006), who observed that, with the increasing temperature, a reduction of the total lipids occurs due to the increased lipase activity. This was confirmed in both seed types at 22°C. However, at the lower temperature (10°C), this trend was confirmed only in bio-seeds. This difference could be caused by different contents of saturated and unsaturated fatty acids, as exemplified by Hung *et al.* (2011), or by the total lipase activity (Hellyer *et al.* 1999; Paques & Macedo 2006). Ruibal-Mendieta *et al.* (2002) concluded that there were differences in fat content between spelt and wheat bread. This difference is also caused by the method of cultivation. However, this conclusion was confirmed only for wheat bread.

Given the general interest in bio-agricultural products, our study aimed at comparing the chemical composition of non-germinated and sprouted wheat seeds from organic and conventional growing systems. The results did not fully confirm the presumption regarding the difference in the chemical structure between the organically produced germinated wheat seeds and the seeds from intensive agriculture. Nevertheless, it is possible to conclude that non-germinated seeds of organic products had the highest content of glucose and fructose. However, sucrose and maltose were the most abundant in conventionally produced seeds. These changes are caused by the enzymatic

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activity of hydrolytic enzymes whose activity is increased during germination. In both types of grains, the content of fructose, glucose, and maltose increased during germination (5 days). It therefore represents an increase of the amount of nutritionally important oligosaccharides. At a higher temperature (22°C), lipid content in the grains from both cultivation methods decreased during germination whereas at a lower temperature (10°C) lipid content increased in the organic production and decreased in the conventionally produced seeds, which is due to the lipase activity. The results of the comparison of both cultivation methods cannot be clearly generalised because it depends on the plant material source which may originate from different farms or can be purchased at supermarkets. Within one seed source it can be stated that there are no significant differences between the different methods of cultivation.

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