ENZYME ACTIVITY OF DIFFERENT CEREALS GROWN USING ORGANIC AND CONVENTIONAL AGRICULTURAL PRACTICES

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Abstract

Enzymes play an important role in cereal processing not only because in many instances they have an impact on processability, but also they add to final product quality. High activities of different hydrolytic enzymes could cause the losses of grain quality and lead to processing problems and unsatisfactory end-products. However, the information on the impact of various cultural practices and conditions on the variation of enzymes activity levels in cereals is rather limited. The present study is therefore aimed to compare the activity levels of most important hydrolytic enzymes (α-amylase, endoxylanase and protease) in wheat, barely, rye and oats grown by organic and conventional agricultural practices. To address this issue, different registered cultivars and up-and-coming lines of winter wheat (4 varieties), winter rye (3 varieties), spring barley (6 varieties), and oats (3 varieties) grown during 2009 harvest year were involved in the test:The α-amylase activity in organically and conventionally grown cereals varied from 224 till 1335 U (units) g^{-1} and from 814 till 1546 U g^{-1}, endoxylanase activity – from 0.13 till 0.65 U g^{-1} and from 0.06 till 0.15 U g^{-1}, protease activity – from 4.89 till 4.95 U g^{-1} and from 4.87 till 4.95 U g^{-1}, respectively. The data demonstrated that organic wheat, rye, and oats had lower α-amylase activity in compare with conventional counterparts. Also organic rye, barley and oats distinguished much higher endoxylanase activity than conventional ones. Contrary tendency was found during investigation of α-amylase activity in barley and endoxylanase activity in wheat. Comparing protease activity, significant differences have not been found between various agricultural practices. These results warrant further studies investigating links between specific agricultural practices and enzyme activities in important food cereals.

Key words: cereals, enzyme activity, organic farming, conventional farming.

Introduction

Cereals worlwide are extremely important for both human and animal nutrition, hence the great economic importance of their processing into a wide range of products. In the last decade, enzymes have become increasingly important in cereal processing not only because in many instances they have an impact on processability, but also they add to final product quality (Kruger, Lineback, 1987). High activities of different hydrolytic enzymes could cause the losses of grain quality and lead to processing problems and unsatisfactory end-products. These enzymes are mainly responsible for mobilising the insoluble storage reserves in the endosperm: amylases degrade starch into dextrins and glucose, xylanolytic enzymes catalyse the breakdown of non-starch polysaccharides, proteases hydrolyse proteins and produce amino acids.

Factors such as resource availability, soil quality, climate, and insect and animal herbivory pressures are known to affect levels of nutrients in cereals (Brandt, Mølgaard, 2001). However, the information on the impact of various cultural practices and conditions on the variation of enzymes activity levels in cereals is rather limited. Given that increasing evidence indicates a role for hydrolytic enzymes in cereal processing, efforts need to be directed in understanding relationships between cultural practices and activities of different enzymes in cereals.

Conventional, organic, and sustainable agriculture are the primary cultural practices used in the production of foods. The goal of each of these practices differs greatly with respect to crop yield, land and pesticide use, and environmental impact. Conventional agricultural practices utilize high-yield crop cultivars, chemical fertilizers and pesticides, irrigation, and mechanization. Although conventional practices result in reliable high-yield crops, there is concern regarding the negative biological and environmental consequences and long-term sustainability associated with these practices (Robertson et al., 2000). Organic crops cannot be genetically engineered, irradiated, or fertilized with sewage sludge. Additionally, farmland used to grow organic crops is prohibited from being treated with synthetic pesticides and herbicides for at least 3 years prior to harvest.

Most of existing studies have compared the nutritional quality of organically and conventionally grown plants in terms of macronutrients, vitamins, and minerals. *Woese* and others (1997) reported on the quality of foods grown under different production methods and examined between 1926 and 1994. The authors concluded that no major differences in nutrient levels were observed between the different production methods in some cases while in other cases contradictory findings did not permit definitive conclusions about the influence of production methods on nutrient levels. *Worthington* (2001) reviewed a number of studies that compared crops produced with organic fertilizer or by organic farming systems to crops produced using conventional farming systems. It was reported that organic crops contained more vitamin C, iron, magnesium and phosphorus than did conventional crops. *Bourn* and *Prescott* (2002) also compared the effect of inorganic and organic fertilizers on the nutritional value of crops. They concluded that the study results were too variable to provide any definitive conclusions concerning the effect of fertilizer type on mineral and vitamin content of crops. *Davis* and others (2004) compared USDA nutrient content data for 43 garden crops between 1950 and 1999. Statistically reliable declines were noted for 6 nutrients (protein, calcium, potassium, iron, riboflavin, and ascorbic acid), with declines ranging from 6% for protein to 38% for riboflavin. However, they attributed the decreases in nutrient content to changes in the cultivars (plant varieties) used. All these data demonstrate that the results of the nutritional quality of conventionally and organically are difficult to interpret because cultivar selection and growing conditions varied widely and different methods of sampling and analysis were used in the investigations. Additionally, these studies did not address levels of enzyme activities in conventionally and organically grown cereals, although the enzyme activities could be differ between these two practices.

The present study is therefore aimed to compare the activity levels of most important hydrolytic enzymes (α-amylase, endoxylanase and protease) in wheat, barely, rye and oats grown by organic and conventional agricultural practices.

Materials and Methods

The organic and conventional field trials were conducted during 2009 harvest year in Central Lithuania (Dotnuva, 55°24'N, 23°50'E) at the experimental fields of the Institute of Agriculture. The soil of the experimental site is *Endocalcari - Epihypogleyic Cambisol* (CMg-n-w-can) close to neutral acidity, moderately supplied with available phosphorus and potassium, organic matter (humus) in conventional and organic fields respectively 2.6 and 2.4%. Pre-crop – black fallow. The meteorological conditions of cereal growing and harvesting period in 2009 year are shown in Table 1.

Table 1

Meteorological conditions of wheat growing and harvesting during 2009

Parameter	April	May	June	July	August
Average temperature 2009 (ºC)	**8.4**	**12.2**	**14.7**	**18.2**	**16.7**
Average temperature 1924-2006 (ºC)	**5.4**	**11.9**	**15.4**	**16.7**	**16.2**
± deviation over previous period	+3.0	+0.3	-0.7	+1.5	+0.5
Average rainfall 2009 (mm)	**8**	**37**	**96**	**105**	**75**
Average rainfall 1924-2006 (mm)	**42**	**52**	**68**	**79**	**76**
± deviation over previous period	-34	-15	+28	+26	-1
Days with raining ≥ 1 mm	1-3	6-12	6-17	13-18	8-11

Eleven registered cereal cultivars and six up-and-coming lines developed at the Plant Breeding Department of the Institute of Agriculture were involved in the test: winter wheat (*Triticum aestivum* L.) cultivars 'Ada', 'Alma', 'Tauras' and 'Sirvinta 1'; winter rye (*Secale cereale* L.) cultivar 'Joniai' and lines LZI 424, LZI 512; spring barley (*Hordeum vulgare* L.) cultivars 'Simba', 'Luoke', 'Aura DS' and lines 8056-2, 8056-6, 8611; oat (*Avena sativa* L.) cultivars 'Migla', 'Ivory' and line 1551-3. The varieties were grown in 3 replications with a plot size of 5.0 × 1.7 m^2. The crop was sown in a well prepared seedbed with a Hege 80 at a rate of 4.5 million seed ha^{-1}. All fertilities in conventional field trials were applied annually. Winter wheat and rye have got $N_{120}P_{50}K_{50}$, spring barley – $N_{90}P_{60}K_{60}$ and oat – $N_{60}P_{60}K_{60}$ respectively. Phosphorus and potassium fertilisers were applied in autumn, for winter cereals – pre-sowing. Nitrogen was applied in spring – pre-sowing spring crop or after resumption of vegetative growth (BBCH 23-24) of winter crop. Conventional farming field crops as sprayed with herbicides – at the end of tillering - beginning of booting (BBCH 28–30), taking into account prevalent weed species; other pesticides and additional fertilisation were not applied. The organic field was certified for organic agriculture; no agrochemicals and fertilizers were used. Harvest time – fully ripe grain maturity (BBCH 89). The plots were harvested with a Wintersteiger harvester. Combine-harvested grains from each plot were dried and sampled for analyses.

For analytical tests, grains were milled in Laboratory Mill 120 (Perten Instruments AB, Sweden) at a particle size of 0.8 mm and stored by -20 °C. Frozen samples were defrosted and analyzed for α-amylase, endoxylanase and protease activities. For enzyme activity measurements 5 g of milled grains were extracted with 50 ml of particular buffer for 1 hour and centrifuged at 10000×*g* for 20 minutes by 4 °C.

α-amylase activity was determined using an ICC Standard method No. 108 (ICC, 1998). Wheat extract was prepared in the calcium chloride solution (pH 6.0). Soluble starch (1%) was used as substrate. One unit (U) of amylase activity was defined as the amount of amylase which is able to catalyze 1 g soluble starch hydrolysis to dextrins under assay conditions.

Endoxylanase activity was determined by the dinitrosalicylic acid assay (Miller, 1959). Wheat extract was prepared in 10 mM sodium acetate buffer (pH 4.5). One unit (U) of endoxylanase activity was defined as the amount of enzyme required to releases 1 µmol of xylose equivalents per min from the birchwood xylan (5 mg ml^{-1}) under the assay conditions used (pH 4.5; 40 °C). The xylose solution (2.5 mM) was used to prepare xylose standards (0–0.45 µmol ml^{-1}) and construct the calibration curve.

Protease activity was determined by Sigma‘s enzymatic assay of protease using tyrosine as a standard (Sigma Quality Control Test SSCASE01.001, 1999). Wheat extract was prepared in 10 mM sodium acetate buffer (pH 7.5) with 5 mM calcium acetate. Casein solution (0.65%) was used as substrate. One unit (U) of protease activity was defined as the amount of enzyme required to liberate 1 µmol of tyrosine per min per ml under the assay conditions. The tyrosine solution (0.2 mg ml^{-1}) was used to prepare tyrosine standards (0–0.08 mg ml^{-1}) and construct the calibration curve.

All analyses were performed in triplicate. The calculation of the mean values and standard deviations were performed using a Microsoft Excel 2000 program and a statistical program Analyse-it. The means were compared by one-way analysis of variance (ANOVA). The p-values of <0.05 were considered significant.

Results and Discussion

α-amylase, endoxylanase and protease activity levels determined in different winter wheat, winter rye, spring barley and oats cultivars are presented in Table 2.

Table 2

Enzyme activities in conventionally and organically grown cereals

Cereals	Agricultural practice	Enzyme activity (U g^{-1})		
		α-amylase	endoxylanase	protease
Wheat				
Ada	Conventional	1064±103	0.24±0.04	4.95±0.00
Alma		1105±29	0.14±0.03	4.91±0.02
Tauras		1126±29	0.12±0.03	4.92±0.02
Sirvinta1		1546±7	0.11±0.04	4.91±0.01
Average value		***1210±33***	***0.15±0.06***	***4.90±0.01***
Ada	Organic	1026±17	0.10±0.02	4.91±0.00
Alma		1081±39	0.12±0.04	4.89±0.03
Tauras		1038±33	0.17±0.01	4.90±0.01
Sirvinta1		1136±39	0.13±0.01	4.91±0.04
Average value		***1070±49***	***0.13±0.03***	***4.92±0.03***
Rye				
Joniai	Conventional	1053±59	0.11±0.05	4.89±0.01
LZI 424		1131±7	0.12±0.06	4.88±0.00
LZI 512		1012±44	0.15±0.03	4.89±0.00
Average value		***1066±61***	***0.13±0.02***	***4.89±0.01***
Joniai	Organic	999±22	0.80±0.09	4.90±0.01
LZI 424		904±44	0.70±0.10	4.95±0.09
LZI 512		697±74	0.46±0.09	4.90±0.01
Average value		***867±154***	***0.65±0.18***	***4.90±0.03***
Barley				
Simba	Conventional	913±51	0.18±0.01	4.88±0.01
Luoke		986±7	0.03±0.01	4.90±0.00
Aura DS		1100±22	0.14±0.02	4.91±0.02
8056-2		830±7	0.18±0.01	4.93±0.02
8056-6		732±73	0.14±0.02	4.91±0.02
8611		1160±72	0.10±0.01	4.93±0.05
Average value		***954±162***	***0.13±0.06***	***4.91±0.02***
Simba	Organic	1335±55	0.30±0.00	4.88±0.01
Luoke		1057±18	0.16±0.02	4.91±0.03
Aura DS		901±55	0.21±0.04	4.90±0.01
8056-2		1096±12	0.30±0.04	4.93±0.02
8056-6		875±18	0.16±0.01	4.91±0.01
8611		758±12	0.19±0.06	4.88±0.00
Average value		***1004±204***	***0.22±0.07***	***4.89±0.02***
Oats				
Migla	Conventional	834±11	0.06±0.00	4.89±0.00
Ivory		885±50	0.06±0.01	4.87±0.00
1551-3		814±61	0.06±0.05	4.87±0.01
Average value		***844±37***	***0.06±0.01***	***4.88±0.01***
Migla	Organic	810±37	0.10±0.02	4.95±0.04
Ivory		697±61	0.09±0.00	4.89±0.01
1551-3		224±43	0.08±0.01	4.91±0.02
Average value		***577±311***	***0.09±0.01***	***4.91±0.03***

The results showed that organically grown wheat, rye, and oats had lower α-amylase activity in compare with conventionally grown cereals. The average of α-amylase activity in organically grown wheat, rye, oats was found 13, 23 and 46% lower than that measured in conventionally grown samples, respectively. Differences in the levels of α-amylase activity between the organic and conventional barley were not significant. Contrary tendency was found during investigation of endoxylanase activity. In this case, organically grown rye, barley and oats distinguished for higher (5.0, 1.7 and 1.5 times, respectively) average endoxylanase activity than conventionally grown samples. The levels of endoxylanase activity in organic and conventional barley samples had not significant difference. Comparing protease activity, significant differences have not been found between various agricultural practices. The protease activity of organically and conventionally grown cereals was in the range between 4.88 and 4.92 U g^{-1}.

The activities of hydrolytic enzymes, especially amylase, are important factors that may limit the utilization of cereals. α-amylase is unique in modifying starch and its functional properties. In breadmaking, some α-amylase is needed to sustain the production of sugars required for proper fermentation and consequent gas production. However excess α-amylase can have disastrous effects on bread quality (Buchanan, Nicholas, 1980). Endogenous xylanases are involved in re-modeling and expansion of cereal cell walls during normal cell frouwth and development and in more drastic cell wall degradation occuring during seed germination (Dornez et al., 2009). Xylanases hydrolyse the backbone of cereal cell wall arabinoxylans and have a significant impact on bread-making, brewing, animal feed efficiency, pasta production, etc. Proteases hydrolyse the peptide linkage, releasing protein fragments or free amino acids and play important role in biscuit manufacture, where the low-protein soft wheat and plastic properties of dough are required. The data presented showed that the results of the impact of organical and conventional cultural practices on the variation of enzymes activity levels in cereals were difficult to interpret because there were inconsistent differences in the enzyme activities of tested grains. Therefore we can conclude that organic farming system produce cereals of the same quality as conventional ones, but using far fewer external inputs in the form of fertilisers and plant protection agents, thereby safeguarding natural resources. These results are in line with recent studies on organic wheat farming, in which wheat nutritional value (protein content, amino acid composition and mineral and trace element contents) and baking quality were found not to be affected by the farming systems: organically or conventionally (Mader et al., 2007). Thus these findings suggest that organic farming can contribute substantially to solving problems related to high-external-input agriculture and to producers and consumers confidence in organic foods.

Conclusions

1. The results of our study showed the inconsistent variation of enzyme activity levels in tested cereals grown by organic and conventional agricultural practices. The α-amylase activity in organically and conventionally grown cereals varied from 224 till 1335 U g^{-1} and from 814 till 1546 U g^{-1}, endoxylanase activity – from 0.13 till 0.65 U g^{-1} and from 0.06 till 0.15 U g^{-1}, protease activity – from 4.89 till 4.95 U g^{-1} and from 4.87 till 4.95 U g^{-1}, respectively. The data demonstrated that organic wheat, rye, and oats had lower α-amylase activity in compare with conventional counterparts. Also organic rye, barley and oats distinguished much higher endoxylanase activity than conventional ones. Contrary tendency was found during investigation of α-amylase activity in barley and endoxylanase activity in wheat. Comparing protease activity, significant differences have not been found between various agricultural practices. These findings suggest that organic farming system could produce cereals of the same quality as conventional ones, but using far fewer external inputs in the form of fertilisers and plant protection agents.

2. On the other hand, data regarding the levels of enzyme activities in organic food are too limited to allow any conclusion. We feel that these results warrant further studies investigating links between specific agricultural practices and enzyme activities in important food cereals.

References

1. Bourn, D., Prescott, J. (2002) A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Crit Rev Food Sci Nutr*, 42, pp. 1–34.
2. Brandt, K., Mølgaard, J. P. (2001) Organic agriculture: Does it enhance or reduce the nutritional value of plant foods? *J Sci Food Agric*, 81, pp. 924–931.
3. Buchanan, A. M., Nicholas, E. M. (1980) Sprouting, alpha-amylase and bread-making quality. *Cereal Res Commun*, 8, pp. 23–28.
4. Davis, D. R., Epp, M.D., Riordan, H.D. (2004) Changes in USDA food composition data for 43 garden crops, 1950 to 1999. *J Am Coll Nutr*, 23, pp. 669–82.
5. Dornez, E., Gebruers, K., Delcour, J. A., Courtin, C. M. (2009) Grain-associated xylanases: occurence, variability, and implication for cesreal processing. *Trends Food Sci Techn*, 20, pp. 495–510.
6. ICC Standards: Standard Methods of the International Association for Cereal Science and Technology, 7th supplement. (1998) International Association for Cereal Science and Technology (ICC): Vienna, Austria.
7. Kruger, J. E., Lineback, D. R., Stauffer, C. E. *Enzymes and Their Role in Cereal Technology*. (1987) St. Paul, MN: American Association of Cereal Chemists, pp. 403.
8. Mäder, P., Hahn, D., Dubois, D., Gunst, L., Alföldi, T., Bergmann, H., Oehme, M., Amadò, R., Schneider, H., Graf, U., Velimirov, A., Fließbach, A., Niggli, U. (2006) Wheat quality in organic and conventional farming: results of a 21 year field experiment. *J Sci Food Agric*, 87, pp. 1826–1835.
9. Miller, G. L. (1959). Use the dinitrisalicylic acid reagent for determination of reducing sugar. *Anal Chem*, 31, pp. 426–428.
10. Robertson, G. P., Paul, E. A., Harwood, R. R. (2000) Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. *Science*, 289, pp. 1922–1925.
11. Woese, K., Lange, D., Boess, C., Bögl, K. W. (1997) A comparison of organically and conventionally grown foodssresults of a review of the relevant literature. *J Sci Food Agric*, 74, pp. 281–293.
12. Worthington, V. (2001) Nutritional quality of organic versus conventional fruits, vegetables, and grains. *J Altern Complement Med*, 7, pp. 161–73.