PROTEIN AND METABOLITE PROFILES OF WHEAT GRAINS GROWN IN ORGANIC AND CONVENTIONAL AGRICULTURAL SYSTEMS

UDC 633.11 : 577.12

633.11 : 543.4

G. Langenkämper[1], A. Bonte[1], K. Niehaus[2], C. Zörb[1,3], M. G. Lindhauer[1]

[1] *Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Cereals, Schützenberg 12, 32756 Detmold, Germany; e-mail: meinolf.lindhauer@mri.bund.de*

[2] *Bielefeld University, Faculty of Biology, Proteome and Metabolome Research, Bielefeld University, D-33594 Bielefeld, Germany,*

[3] *present address: Christian-Albrechts-Universität Kiel, Institute of Plant Nutrition and Soil Sciences, Hermann-Rodewald-Str. 2, 24118, Kiel, Germany*

ABSTRACT

The market for organic food is increasing steadily during the last years. Since organic food achieves a price premium, there is great economic interest in finding discriminating analytical methods to ensure the authenticity of organic products. Directed at this goal we are employing metabolite- and protein-profiling-techniques on wheat, grown in well controlled organic and a conventional agricultural systems within the DOK field trial, Switzerland.

Using two dimensional gel electrophoresis (2D-GE) and gas chromatography mass spectrometry, expression levels of proteins and metabolite concentrations were determined. Statistical analysis of relative concentrations of metabolites showed that 42 of 50 metabolites were identical in organic and wheat. A group of 8 unrelated metabolites revealed statistically significant changes depending on the farming system. Using 2D-GE, levels of 1049 proteins were recorded in wheat of two harvest years. In both years, levels of 16 proteins were different with a high level of consistency among organic and conventional wheat. Storage proteins, enzymes of carbohydrate metabolism, a peroxidase, and proteins of unknown function were affected by the agricultural regime. These 16 proteins have the potential to serve as biomarkers to prove authenticity of organic wheat. The research is continued using wheat from several cultivars and cropping years.

Key words: authenticity, metabolomics, organic food, profiling, proteomics, wheat

INTRODUCTION

Good indicators for the growing popularity of organic farming and organic products are constant increases in the share of the organically farmed area, particularly in Europe and North America and also increasing global revenues with organic products, which gained approximately 5% to reach 54.9 billion US dollars in 2009 [1] . To regulate and protect organic farming and products, standards and legislation has been developed mainly on a national scale and, in Europe also on level of the European Community. However, there is no international standard for organic agriculture. Where controlling mechanisms are in place, these systems rely heavily on certification, documentation and plausibility, whereas analytical based controls will only be performed in case of suspected fraud [2]. Moreover analytical methods are limited in scope, e. g. testing for residues of plant protection compounds [2]. Against the background of growing global markets, globalisation of trade, the price premium achieved for organic products, and the limited possibilities of control bodies the incentive for fraud with organic products is quite large. Thus, in order to preserve product value and to maintain consumer's trust in organic products, analytical methods capable of authenticating organic products would be highly welcome [2]. Directed at this goal, we are employing metabolite- and protein-profiling-techniques. The main advantage of profiling techniques is that broad spectra of ingredients are analysed, thereby increasing the chance to find potential biomarkers for organic food [3, 4]. In order to test the suitability of the profiling methods, organic and conventional wheat from the thoroughly documented and strictly controlled DOK field trial, established in Switzerland, was used.

MATERIALS AND METHODS

Plant Material. Wheat from the 2003, 2005 (variety Titlis) and 2006 (variety Runal) harvests was obtained from the long-term DOK field trial near Basel, Switzerland, which has been described in detail by Mäder *et al*. [5, 6]. In this work here, wheat grain was used from an organic system, defined as "bio-dynamic" and from a conventional system, defined as an integrated conventional agriculture using mineral fertiliser only. Varieties, crop rotation, tillage, plots size (5 by 20 meters) and number of replicate plots (N = 4) were identical in the different systems. Major differences in the various agricultural systems are the form of plant protection and fertilisation. Long-term mean annual fertilisation was between 34 and 51% lower with respect to N, P and K in the organic systems [5]. The organic systems were fertilised with nitrogen as contained in 10 t ha^{-1} of composted manure and 30 m^3 ha^{-1} of slurry per year. At least three samples of each farming system, originating from the individual replicate field plots were analysed. Whole wheat grains (100 g) were ground in a titan laminated mill using a sieve with 0.5 mm pore size (Retsch, Haan, Germany). The material was further ground with mortar and pistil, using liquid nitrogen. The resulting powder was denoted 'wholemeal'. Storage of wholemeal was at -80 °C until further analyses.

Protein extraction and 2D-GE. Total protein extraction from wholemeal, 2D-GE, image analysis and protein identification were performed as described [3]. For both, organic

and conventional agricultural systems, proteins for 2D-GE were extracted from three field plots. For each field plot three technical 2D-GE replications were done.

Metabolite extraction, GC-MS analysis. Extraction of metabolites from wholemeal using methanol, derivatisation with methoxylamine hydrochloride in pyridine and MSTFA, GC-MS analysis, and data treatment including application of statistics were as detailed in [4].

RESULTS AND DISCUSSION

Metabolite Profiles. GC-MS analysis of wheat grains from the varieties Titlis and Runal, originating from the 2003 and 2006 growing seasons of the DOK-field trial respectively, revealed approximately 250 compound peaks per chromatogram [4, 7]. Altogether 50 metabolites, common to both varieties, were identified in methanol extracts. The identified metabolites could be assigned to five functional groups comprising proteinogenic amino acids (13 compounds), sugar and sugar phosphates (10 compounds), sugar alcohols (4 compounds), organic acids (11 compounds) and a group of other metabolites (11 compounds) [4, 7]. A set of 8 metabolites was found to have significantly different relative concentrations in organic and conventional wheat. Namely, α-alanine, ß-alanine, glycerate, and valine had lower concentrations in the organic Titlis variety and asparagine, lysine, threonine, and tyrosine revealed lower concentrations in the organic Runal variety. A selection of these 8 metabolites is presented in Figure 1.

It is very apparent that significant reductions in metabolite concentrations in organic wheat were mainly occurring in the group of the nitrogen containing free amino acids. Moreover, the only compound with significantly lower concentrations in organic wheat not containing nitrogen, glycerate, is closely linked to the metabolism of a number of the amino acids. This result is consistent with the finding that N supply of the plant is affecting levels of free amino acids and total protein content of wheat [8] – N.B. fertilisation in the organic plots of the field trial was lower.

With respect to the aim of differentiating organic and conventional wheat it is encouraging that GC-MS analysis revealed differences in metabolites of the same functional group in two different varieties and also two growing seasons. At present we are extending the data basis concerning metabolite profiles with two aims. Firstly, the influence of the genetic background is evaluated by examining ten different wheat varieties of one cropping year and secondly, the influence of seasonal effects is evaluated by analysing one variety grown in three cropping years. All wheat samples will be taken from the DOK field trial.

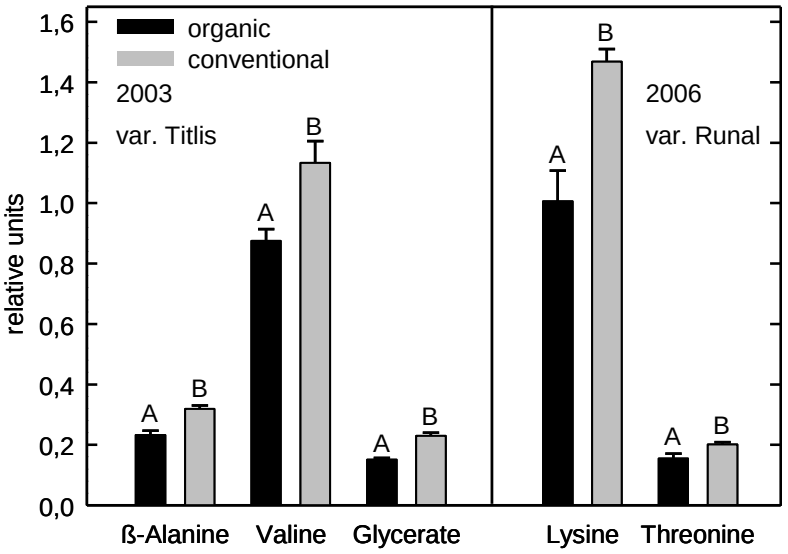

Figure 1. Metabolite concentrations in wheat grain influenced by conditions of cultivation. Wheat varieties Titlis and Runal originated from the 2003 and 2006 growing seasons respectively. Values are means from four replicate field plots and from eight technical replications each. Standard errors of the mean are shown. Statistical significance tests were performed using the Tukey-test algorithm of the Student range. Different letters indicate significant differences of the means (p = 0.05). Reprinted with modifications and permission from J. Agric. Food Chem. (2006) **54**: 8301-8306 and J. Agric. Food Chem. (2009) **57**: 9555-9562. Copyright 2006, 2009 American Chemical Society.

Protein Profiles. A total of 1049 proteins were detected in organic and conventional wheat grain using 2D-GE. A typical 2-DE gel (organic) is presented in Figure 2. Applying image analysis, levels of 25 proteins were found to be different by at least a factor of two between organic and conventional wheat in 2003 and 2005. Of these 25 proteins, 19 were identified (Table 1). Levels of most proteins were higher in conventional wheat, except for two proteins, a peroxidase and an unidentified protein, which were higher in organic wheat (Table 1, No. 49 and 373). The 19 identified proteins (Table 1) could be assigned to two functional groups: *i*) storage proteins comprising four low molecular weight glutenins, two high molecular weight glutenins, one globulin, one serpin, and two triticin precursors and *ii*) enzymes involved in carbohydrate metabolism comprising sucrose synthase, xylanase, glyceraldehyde-3-phosphate dehydrogenase, granule-bound starch synthase precursor, ß-amylase and an aldolase reductase-related protein.

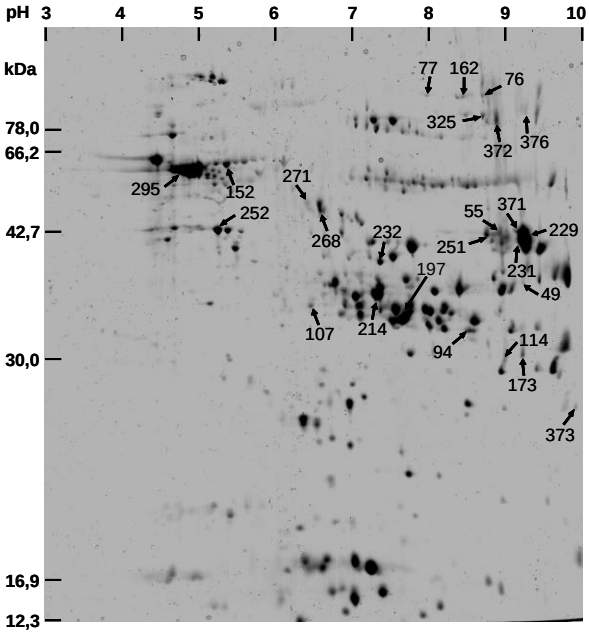

Figure 2. Two-dimensional gel electrophoresis of total protein extracts of organic wheat grain (var. Titlis). Coomassie staining was used. Horizontal: increasing p*I*, ranging from pH 3 to pH 10. Vertical: molecular weight in kDa. Only the 25 proteins which had different levels in wheat grains from organic and conventional agriculture are marked with arrows. Numbers correspond to protein numbers in Table 1, first column. Reprinted with permission from J. Agric. Food Chem (2009) **57**: 2932-2937. Copyright 2009 American Chemical Society.

Protein spot volumes of the 25 proteins (Table 1) were further analysed, taking into account both, consistency of the effect of the agricultural system and seasonal variation in the two cropping years. The calculations performed resulted in a consistency score (Table 1). The development of the score was described in detail [3]. A high consistency score indicates a strong effect of the agricultural system (organic *vs.* conventional) and a weak influence of seasonal parameters. For example, at a consistency score of two the effect of the agricultural system is twice a high as the seasonal variation. If a protein reached of exceeded the consistency score of two, it was considered to be suitable to contribute to a signature of diagnostic proteins, distinguishing organic and conventional wheat. The setting of this threshold is, of course, to some extent arbitrarily. Within the group of 25 proteins showing differential expression levels in organic *vs*. conventional DOK-wheat, 16 proteins pass the consistency score threshold of two (Table 1). In order to extent the present data basis, the protein profiling work will be continued as outlined for the metabolite profiles.

Table 1. Proteins with significantly different levels in organic and conventional wheat grain[a]

Protein No.	Proteins with different levels in organic and conventional wheat grain	Consistency score [b]	Mowse score [c]	Accession No. (gi) [d]
55	low molecular weight glutenin	12.1	73.3	56480772
373	unidentified	12.0		
371	unidentified	6.6		
49	peroxidase 1	4.8	83.7	22001285
162	unidentified	4.7		
107	unidentified	4.3		
114	globulin 1	3.8	72.2	110341795
232	glyceraldehyde 3-phosphate dehydrogenase	3.7	98.5	18978
173	xylanase, family 11	3.5	82.7	51247633
376	unidentified	3.2		
271	triticin precursor	3.0	173.0	7548844
76	high molecular weight glutenin	2.7	97.1	110341796
152	granule-bound starch synthase precursor	2.6	252.0	4588607
229	low molecular weight glutenin	2.6	73.7	17425188
94	xylanase, family 11	2.4	78.9	51247633
252	serpin	2.1	204.0	1885350
268	triticin precursor	2.0	140.0	7548844
197	granule-bound starch synthase precursor	2.0	100.0	4588609
251	low molecular weight glutenin	1.9	94.2	56480772
77	sucrose synthase type 2	1.9	73.2	3393044
295	ß-amylase	1.8	125.0	32400764
214	aldose reductase-related protein	1.8	77.3	167113
372	unidentified	1.7		
325	high molecular weight glutenin	1.7	188.0	22090
231	low molecular weight glutenin	1.7	85.1	47607142

[a]Protein spot volumes were determined with 2-DE in organic and conventional wheat from the cropping years 2003 and 2005. Protein levels were different at least by a factor of two. Proteins were sorted by descending value of the consistency score. [b] A large consistency score indicates a strong influence of the agricultural system and a low influence of seasonal factors on the level of the protein. Protein numbers correspond to numbers in Figure 1. Proteins were identified by MALDI-TOF-MS and database searches (NCBInr). [c] Mowse scores [9] are given. [d] Accession No. from NCBI. Reprinted with permission from J. Agric. Food Chem (2009) **57**: 2932-2937. Copyright 2009 American Chemical Society.

CONCLUSIONS

Metabolite- and protein-profiling-techniques have revealed differences in patterns of a number of metabolites and proteins in organic and conventional wheat, originating from the DOK field trial. Concerning metabolites, decreases of chiefly free amino acids were observed in organic wheat in two different varieties and two cropping years. A signature of 16 proteins with consistent differences in levels in organic and conventional wheat across two cropping seasons was identified. In order to assess, if our finding can be corroborated on a broader basis, we are currently performing further profiling experiments using DOK wheat with two aims. Firstly, the influence of the genetic background is evaluated by examining ten different wheat varieties of one cropping year and secondly, the influence of seasonal effects is evaluated by analysing one variety grown in three cropping years.

ACKNOLEDGEMENT

We thank the Research Institute of Organic Agriculture (Frick, Switzterland) and the Agroscope Reckenholz-Tänikon Research Station ART (Zürich, Switzerland) for making available the wheat grain. The research was financially supported within the "Bundesprogramm Ökologischer Landbau", Project No. 02OE069, of the German Federal Ministry of Food, Agriculture and Consumer Protection.

REFERENCES

1. Willer, H. 2011. The World of Organic Agriculture 2011: Summary. Page 28-34 in: The World of Organic Agriculture - Statistics and Emerging Trends 2011, H. Willer and L. Kilcher (Eds.), Bonn and Frick, Germany and Switzerland.
2. Siderer, Y., Maquet, A., and Anklam, E. 2005. Need for research to support consumer confidence in the growing organic food market. Trends Food Sci. Technol. 16: 332-343.
3. Zörb, C., Betsche, T., and Langenkämper, G. 2009. Search for diagnostic proteins to prove authenticity of organic wheat grains (*Triticum aestivum* L.). J. Agric. Food Chem. 57: 2932-2937.
4. Zörb, C., Langenkämper, G., Betsche, T., Niehaus, K., and Barsch, A. 2006. Metabolite profiling of wheat grains (*Triticum aestivum* L.) from organic and conventional agriculture. J. Agric. Food Chem. 54: 8301-8306.
5. Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P., and Niggli, U. 2002. Soil fertility and biodiversity in organic farming. Science 296: 1694-1697.
6. Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Jossi, W., Widmer, F., Oberson, A., Frossard, E., Oehl, F., Wiemken, A., Gattinger, A., and Niggli, U. 2006. The DOK experiment (Switzerland). Page 41-58 in: Long Term Field Experiments in Organic Farming. ISOFAR Scientific Series No. 1, J. Raupp, C. Pekrun, M. Oltmanns, and U. Köpke (Eds.), Berlin, Germany.
7. Zörb, C., Niehaus, K., Barsch, A., Betsche, T., and Langenkämper, G. 2009. Levels of compounds and metabolites in wheat ears and grains in organic and conventional agriculture. J. Agric. Food Chem. 57: 9555-9562.
8. Barneix, A.J. 2007. Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. J. Plant. Physiol. 164: 581-590.
9. Perkins, D.N., Pappin, D.J.C., Creasy, D.M., and Cottrell, J.S. 1999. Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis 20: 3551-3567.