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Search for Diagnostic Proteins To Prove Authenticity of Organic Wheat Grains (*Triticum aestivum* L.)

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Research comparing the biochemical composition of wheat grains from organic or conventional agriculture has used the targeted analytical approach. To obtain a more comprehensive record of the food's composition, we employed protein profiling techniques. Levels of 1049 proteins were recorded in wheat grains (*Triticum aestivum* L., cv. Titlis) of two growing seasons from a rigorously controlled field trial in Switzerland, containing organic and conventional plots. Levels of 25 proteins were different between organic and conventional wheat in both years. Storage proteins, enzymes of carbohydrate metabolism, a peroxidase, and proteins of unknown function were affected by the agricultural regime. Total protein content was lower in organic wheat. We consider these differences negligible with regard to nutrition in an average diet and propose that food quality of conventional and organic wheat grown in the field trial was equal. Applying various filters and calculations, one of which takes seasonal influences into account, 16 of the 25 proteins with different levels in organic and conventional wheat were retained. These 16 "diagnostic" proteins have the potential to afford a signature to prove authenticity of organic wheat.

KEYWORDS: Authenticity; conventional; organic; *Triticum aestivum*; two-dimensional gel electrophoresis; wheat grain

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop and food commodity worldwide. According to the Food and Agriculture Organization of the United Nations, the worldwide production of wheat was an estimated 603 million metric tons in 2007 (http://www.fao.org/giews) (*1*). Organic agriculture is practiced in more than 120 countries of the world, and the share of organically farmed area has been increasing during the last years, particularly in North America and Europe (*2*). Today, even "conventional" supermarkets offer food from organic agricultural production. This rising interest in organic food can partially be explained by a growing anxiety about food safety resulting in a change of consumer behavior. Surveys have shown that many consumers expect health benefits from organic food assuming that nutrient content and composition of organic food is favorable (*3*). Despite the many publications, no final answer concerning the nutritional quality of conventional vs organic plant food can be given to date because sound comparative data is scarce (*4*). Main drawbacks in most investigations are that (i) only a very small portion of the huge number of nutrients was analyzed (denoted here targeted approach), and that (ii) food from the marketplace was analyzed in most cases. This implies that the origin and history of the food could not be documented with sufficient certainty (cultivar, production sites, harvest, and storage conditions). In order to overcome these shortcomings, first we resorted in our work to wheat (cv. Titlis), produced under thoroughly documented and strictly controlled experimental conditions of the DOK [biodynamic, bioorganic and "konventionell" (conventional) farming] field trial (*5*). This long-term experiment was started in 1978 by the Research Institute of Organic Agriculture (FiBL, Frick, CH) and the Agroscope Reckenholz-Tänikon Research Station (ART, Zürich, CH), and has been continued ever since (*5, 6*). Second, we used modern and generally accepted broadband profiling techniques. Main advantages of profiling techniques are that broad spectra of ingredients are analyzed, whereas the targeted approach implies restriction to a choice of substances. Profiling techniques are expected to be excellent tools (i) to assess the quality of the wheat grain for human and animal nutrition and (ii) to provide evidence for proof of authenticity, i.e. a signature for organically produced wheat. From a plant biologist's point of view, the general question arises of the likelihood that organically produced wheat differs from conventional plants. There is convincing proof that plant nutrition and soil quality are distinctly different in both agricultural regimes (*5*). It is therefore plausible and probable that the agricultural regime would affect the plant's transcriptome, metabolome and proteome in total. In fact, a previous report has shown that the metabolome is modulated, though moder-

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ately, by the agricultural regime (*7*). Similar attempts of differentiating wheat from high and low input agricultural regimes were made by identifying "diagnostic genes" using wheat microarrays (*8*). Further, effects of the agricultural production system on protein profiles of potatoes were described very recently (*9*). In this work here effects of the agricultural regime on the proteome of wheat grains are reported. Results are viewed with regard to nutritive quality, and results are integrated with view on the search for diagnostic proteins to prove authenticity of organic wheat grains.

MATERIALS AND METHODS

Plant Material: DOK Field Trial. Wheat grains (*T. aestivum* L., cv. Titlis) from the 2003 and 2005 crops were obtained from the long-term DOK field trial in Therwil near Basel, Switzerland (7° 33′ E, 47° 30′ N). In 2004, wheat was not grown in the DOK field trial. Refer to Mäder et al. (*5*, *6*) for a detailed description of the field trial. In our study, wheat grain was used from an organic regime, defined as "biodynamic", and from a conventional regime, defined as an integrated conventional agricultural regime using mineral fertilizer only. Long-term mean annual nutrient supply with respect to N, P and K was between 34 and 51% lower in the organic regimes (*5*). The organic wheat plots were fertilized with very low doses of mineral nitrogen fertilizers as contained in 10 t ha^{-1} of composted manure and 30 m^3 ha^{-1} of slurry each year. The conventional plots received 140 and 130 kg mineral N ha^{-1} in 2003 and 2005, respectively. Crop rotation and varieties were identical in all systems. Crop rotation in the period 1999 to 2005 comprised potatoes, soy bean, maize, a grass−clover mixture and winter wheat. In both years, maize was the preceding crop. For each of the two wheat agricultural regimes, samples of at least three individual field plots were analyzed. The size of each field plot was 5 m by 20 m.

Protein Extraction. After harvest, wheat grains were initially kept at ambient temperature (18 to 20 °C). Within four weeks after harvest, wheat grains (100 g) were ground in a titan laminated mill using a 500 μm sieve (Retsch, Haan, Germany). The material was further ground in a mortar with liquid nitrogen to yield a fine homogeneous powder denoted meal. The meal was stored at −80 °C until analysis. Proteins were extracted from 200 mg of meal with a modified precipitation method using DTT, TCA and acetone (all from Roth, Karlsruhe, Germany) (*10*, *11*). This method removes material such as organic acids, phenolic compounds, pigments, terpenes and inhibitory ions that can interfere with two-dimensional gel electrophoresis (2-DE) (*10*−*12*). To inhibit proteases, the strong denaturants urea and TCA as well as a protease inhibitor cocktail containing 1,10-phenanthroline, pepstatin A, leupeptin, bestatin, and (L-3-*trans*-carboxyoxiran-2-carbonyl)-L-leucyl-agmatin (Sigma-Aldrich, Munich, Germany) were present in the extraction medium. An aliquot of 1.8 mL of 10% TCA in acetone was added to 200 mg of wheat meal. After vortexing, the suspension was incubated for 10 min in an ice cold ultrasonic bath and incubated at −20 °C for approximately 24 h before centrifugation (20000*g*, 15 min, 4 °C). The residue containing the proteins was resuspended in 1 mL of ice-cold solution, containing 50 mmol/L DTT and 2 mmol/L EDTA (Roth) in acetone. The suspension was sonicated for 5 min in the cold. After centrifugation as above, the procedure was repeated and pellets were dried in N_2 atmosphere. For each sample, four aliquots of meal were treated as described above and the resulting pellets were combined and resuspended in 1 mL of protein sample buffer containing 8 mol/L urea (VWR International, Darmstadt, Germany); 2 mol/L thiourea (GE Healthcare, Freiburg, Germany); 2% immobilized pH gradient (IPG) buffer (v/v, pH 3−10, GE Healthcare); 4% CHAPS (Roth); 30 mmol/L DTT; 20 mmol/L Tris-base, pH 8.8 and 5 mmol/L protease inhibitor cocktail (Sigma-Aldrich). To resolve the proteins, suspensions were incubated for 2 h at 33 °C followed by 10 min in an ice-cold ultrasonic bath. After vortexing and centrifugation at 18000*g* for 30 min, the supernatant was retained. A 2-D Clean-Up Kit (GE Healthcare) was used on the protein solution to improve the quality of resolution of subsequent separation by isoelectric focusing (IEF). The protein concentration of the solution was determined using a 2-D Quant protein determination kit (GE Healthcare) with bovine serum albumin as a standard. Total protein in grains was determined using the procedure of Kjeldahl (*13*). The factor 5.7 was used to calculate protein from N-concentration.

IEF and SDS−PAGE. 2-DE was performed, with some modifications, using published protocols (*14*, *15*). IPG gel strips with a linear gradient over pH 3−10 (18 cm; GE Healthcare) were rehydrated for 18 h with 500 μg protein dissolved in 340 μL protein sample buffer (composition see Protein Extraction). IEF was carried out with a Multiphor II apparatus (GE Healthcare). An electrode paper strip (Whatman, Dassel, Germany) soaked in DTT solution (1 mol/L) was placed at the alkaline end of the strip (cathode) to prevent renaturation of basic proteins. The strips were covered with paraffin oil to prevent evaporation. IEF was carried out at 20 °C with 500 V for 0.5 h; 1000 V for 0.5 h; 1500 V for 0.5 h; 2000 V for 0.5 h; 2500 for 0.5 h and 3000 V for 19 h. A 20 × 20 cm vertical gel electrophoresis chamber (Protean II xi 2-D Cell, BioRad, Munich, Germany) was used for second dimension SDS−PAGE. The strips were placed in equilibration buffer (50 mmol/L Tris-base, pH 8.8; 6 mol/L urea; 30% glycerol; 2% (w/v) SDS; bromophenol blue, 0.001% (w/v) containing 1% DTT (w/v) and gently agitated for 10 min. The strips were then incubated under gentle agitation in equilibration buffer with 4% (w/v) iodoacetamide (omitting DTT) for another 10 min and stored at −20 °C. For electrophoresis, the strips were rinsed three times in a tray for 3 min with SDS−PAGE running buffer (25 mmol/L Tris-base; 192 mmol/L glycine; 0.1% (w/v) SDS). Gels had 12.5% (v/v) acrylamide. Molecular weight standards in the range from 12.3 to 78.0 kDa (Merck, Darmstadt, Germany) were placed at the acidic end of the gel which was subsequently sealed with 1% (w/v) agarose containing 0.001% (w/v) bromophenol blue. Electrophoresis was carried out at constant current (45 mA per Gel) and 10 °C. The run was stopped when the bromophenol blue reached the gel margin. Proteins in the gel were fixed with 50% ethanol and 12% acetic acid. Coomassie staining was done according to a hot-staining protocol with Coomassie R350 tablets (GE Healthcare) (*15*). The gels could be stored in 5% glycerol at 4 °C for several weeks without any signs of deterioration.

Image Analyses. Gels were scanned in the transillumination mode (HP Scan-Jet 4890, biostep, Jahnsdorf, Germany) at 300 dpi and 16 bits per pixel. Computer-assisted 2-DE gel analysis was performed with the software Delta2D 3.4 (Decodon, Rostock, Germany), following published guidelines (*16*). Briefly, 2-DE gel images were warped using a group warping strategy to obtain a so-called average fusion gel for each agricultural regime. A virtual master fusion gel was created by the software using every matched protein spot from all gels of all agricultural regimes. This master fusion gel was used to delete artifacts, specks and freckles on individual gels before further processing. Subsequently, normalized protein spot volumes were determined on all individual gels.

Data, Replication, Statistics. For two crops (2003 and 2005), the reliability of the results was ensured by the following measures: (i) three technical 2-DE gel replications from grain of each field plot were done; (ii) for both organic and conventional agricultural regimes, three field plots each were analyzed. For the three field plots, virtual average 2-DE gels including standard error of the mean were calculated and (iii) Student's *t* tests were performed to evaluate the reproducibility of the protein spot volume. Protein spots with a *t* test value below 90% ($p \geq 0.1$) were disregarded; (iv) differences in protein spot volumes from organic and conventional wheat were disregarded, unless different by at least a factor two and $p \leq 0.05$ (Student *t* test).

Calculations To Distinguish Effects of Agricultural Regime and Cropping Year. In order to determinate if the differences in protein spot volumes between organic and conventional agriculture were consistent in two cropping years, a number of calculations was performed. In this section we are giving the equations; the rationale for the calculations is explained in the Results and Discussion. For individual proteins the ratio of protein spot volumes for organic and conventional wheat was denoted agricultural effect (a, a', b, b'), which was calculated separately for the two cropping years according to the equations

$$a = 100 - (\beta/\alpha \times 100)\ [\%] \quad (1)$$

or

$$a' = 100 - (\alpha/\beta \times 100)\ [\%] \quad (1')$$

and

$$b = 100 - (\delta/\gamma \times 100)\ [\%] \quad (2)$$

or

$$b' = 100 - (\gamma/\delta \times 100)\ [\%] \quad (2')$$

where α = protein spot volume in conventional wheat in 2003, β = protein spot volume in organic wheat in 2003, γ = protein spot volume in conventional wheat in 2005 and δ = protein spot volume in organic wheat in 2005. If $\alpha > \beta$, eq 1 was used, and if $\beta > \alpha$, calculations were done according to eq 1′. If $\gamma > \delta$, eq 2 was used, and if $\delta > \gamma$, calculations were done according to eq 2′. The difference between the agricultural effect in 2003 and 2005 was denoted seasonal influence. Seasonal influence and the mean agricultural effect of both growing seasons were used to calculate a consistency score (c or c') according to the equation

$$c = (a + b)/|(a - b)| \times 2 \quad (3)$$

or

$$c' = (a' + b')/|(a' - b')| \times 2 \quad (3')$$

where $a \neq b$ and $a' \neq b'$. The higher the consistency score, the stronger was the mean agricultural effect of the two growing seasons and the weaker was the seasonal influence affecting protein expression.

Protein Identification. Mass spectrometry and protein identification were performed at the Center for Molecular Medicine, University of Cologne, Germany. For automatic InGel digestion, selected protein spots were excised with a Proteineer sp robot (Bruker Daltonics, Bremen, Germany) to yield 1.5 mm (diameter) gel plugs. The plugs were transferred onto 96 well plates. Automatic in gel digestion and preparation of the MALDI-MS targets was carried out with a PROTEINEER digestion robot (Bruker Daltonics). Predefined protocols recommended by the manufacturer were used with minor modifications. The excised gel plugs were washed three times by incubation in a mixture of 10 mmol/L ammonium bicarbonate in acetonitrile and 10 mmol/L ammonium bicarbonate (v/v) at room temperature. After adding acetonitrile the gel plugs were shrunk by drying in a flow of N_2, cooled to 8 °C, and soaked with a solution of 10 ng/μL trypsin (Promega, Mannheim, Germany) in 10 mmol/L ammonium bicarbonate. After 30 min, excess solution was removed and replaced by 4 μL of 10 mmol/L ammonium bicarbonate. Digestion was allowed to proceed for 4 h at 30 °C. Peptides were extracted by incubation with 10 μL of 0.5% trifluoroacetic acid in 50% acetonitrile/water (v/v) for 30 min. An 1 μL aliquot of this extract was combined with 1 μL of 2,5-dihydroxybenzoic acid (2.5 mg/mL in 0.1% trifluoroacetic acid/acetonitrile, 2:1, v/v) on the MALDI-MS target (600 μm AnchorChip, Bruker Daltonics). In peptide mass fingerprint analysis, automatic acquisition of mass spectra was performed on a Bruker Reflex IV MALDI-TOF-MS controlled by FlexControl 1.3 software (Bruker Daltonics). Methods for automatic data acquisition were defined in the auto execute module integrated in the FlexControl. All spectra were acquired in the positive ion mode in 20 acquisition cycles using external calibration. In each cycle, 5 shots were stored in the temporal acquisition buffer for evaluation by the software. Spectra with a minimum signal-to-noise ratio of 10 and a minimum resolution of 4500 for the most intense peak in the mass range from m/z 1200 to m/z 3000 were added to the sum. The raw spectra were processed by Flexanalysis 2.4 and the generated peak lists were transferred to Proteinscape 1.3 which triggered database searches in NCBInr release 20070824 using MASCOT 1.9. The searches were restricted to green plants and trypsin specificity with one missed cleavage allowed. The maximum mass error was 100 ppm for externally calibrated spectra. The molecular weight of the proteins was calculated using standards ranging from 12.3 to 78.0 kDa (Merck) applied to the gels. The isoelectric point (pI) of the protein spots was calculated from their position on the IEF-strips as indicated by the manufacturer's specification (GE Healthcare). To ease reading, the term "protein" is used when referring to a spot on 2-DE gels in the following sections, assuming that each spot in 2-DE gels to corresponds to one single protein or polypeptide with or without a posttranscriptional modification.

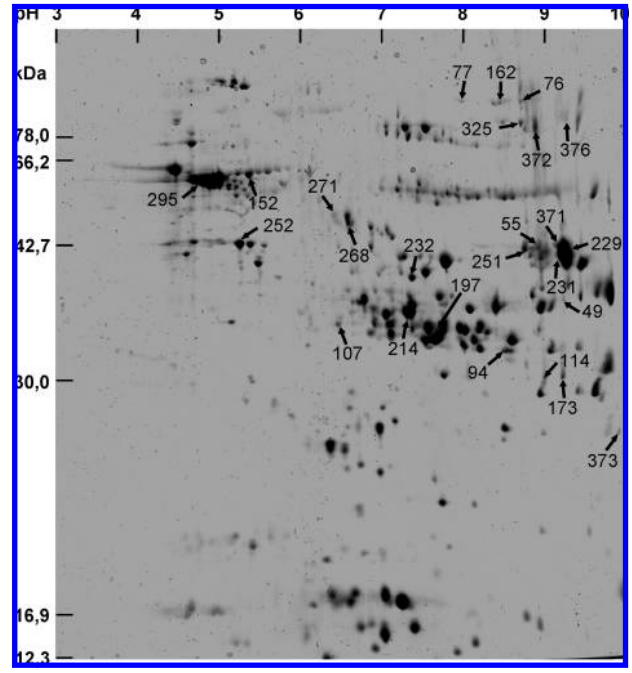

Figure 1. Two-dimensional gel electrophoresis of total protein extracts of organic wheat grain (cv. Titlis). Coomassie staining was used. Horizontal: increasing pI from range pH 3 to pH 10. Vertical: molecular weight in kDa. To obtain a good resolution of the large majority of the proteins between 16 and 80 kDa, electrophoresis was stopped when the smallest standard protein (12.3 kDa) had reached the end of the gel. Only the 25 proteins which had different levels in wheat grains from organic and conventional agriculture are marked with arrows. Results of the protein quantification are presented in **Figure 2** and in Supporting Information. Numbers correspond to protein numbers in **Table 1**, first column. Proteins were analyzed by MALDI-TOF-MS and identified from protein-sequence database interrogation (NCBInr).

RESULTS AND DISCUSSION

Agricultural Regime, Wheat Grain Protein Content and Protein Profile. Grain total protein content is a widely accepted criterion to estimate the baking quality of wheat flour. We determined protein levels of 15.6 ± 0.20% and 13.4 ± 0.11% for conventional and 13.2 ± 0.47% and 11.4 ± 0.09% for organic wheat grain of the 2003 and 2005 crops, respectively. These results are in line with those of others reporting that organic agriculture yields wheat with a reduced protein content (*3*, *17*).

Analysis of the proteome using 2-DE revealed a total of 1049 proteins in both organic and conventional wheat grains. A specimen for a wheat grain 2-DE gel (organic) is depicted in **Figure 1**. As far as the resolution of our analytical setup is concerned, the detection of 1049 proteins, 132 of which were identified by MALDI-TOF-MS analysis and database search, is in the same range as that obtained by Skylas et al. (*18*). These workers reported 1300 polypeptides and identified 177 proteins.

A set of 25 proteins differed markedly at a high level of statistical significance ($p \leq 0.05$) between organic and conventional in 2003 and in 2005 (**Table 1**, **Figure 2** and Supporting Information). To ensure high reliability, only proteins with a difference in levels of at least a factor of 2 between organic and conventional were taken into further account. Of the 25

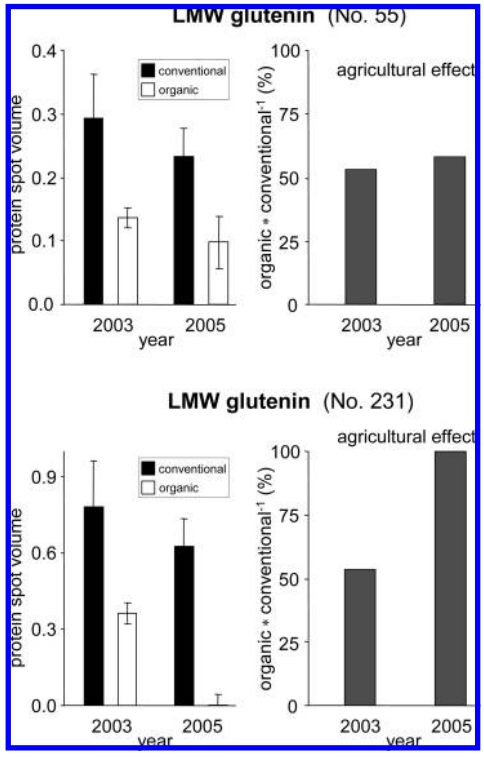

Figure 2. Levels of two low molecular weight glutenins vary in wheat from organic and conventional agriculture. Bar diagrams on the left show protein spot volume, determined with 2-DE, in wheat from conventional (filled bars) and organic (open bars) agriculture from the cropping years 2003 and 2005. Results represent averages of three DOK field trial replicates and three 2-DE gel replicates each. Error bars indicate SEM. Protein levels were different by at least a factor of 2 (Student *t* test values $p \leq 0.05$). Bar diagrams on the right show the ratios of protein spot volume for organic and conventional farming. Consistency scores were 12.1 for protein 55 and 1.7 for protein 231 (see **Table 1**).

proteins fulfilling this criterion, 19 were identified (**Table 1**). For the majority of the proteins, the level was higher in conventionally produced wheat and solely two proteins, a peroxidase and an unidentified protein, were lower in conventional wheat (**Table 1**, No. 49 and 373, and Supporting Information). With regard to plant metabolism, we identified two functional protein groups that were responsive to the agricultural regime. One group comprised storage proteins, namely four low molecular weight (LMW) glutenins, two high molecular weight (HMW) glutenins, one globulin, one serpin, and two triticin precursors. The second group comprised enzymes involved in carbohydrate metabolism, namely sucrose synthase, xylanase family 11, glyceraldehyde-3-phosphate dehydrogenase, granule-bound starch synthase precursor, β-amylase and an aldolase reductase-related protein. It is emphasized that these effects of the agricultural regimes were observed in two crops, 2005 and 2003. No data are available for 2004, since in this year there was no wheat crop in the DOK field trial. With respect to wheat food quality, we do not consider the observed changes in total protein content and in the levels of the proteins analyzed by 2-DE and mass-spectrometry as nutritionally relevant in an average diet. The same conclusion was drawn previously on the basis of metabolite profiling and other data using DOK-wheat (*7*, *17*, *19*). On the basis of our data, it appears to be justified to state that the nutritional food quality of the DOK-wheat from organic and conventional agriculture is equal.

Differentiation between Effects of Agricultural Regimes and Cropping Year. Different weather conditions and other factors, like presence of pathogens, during the two cropping years may have interfered with specific effects of the agricultural regimes, hampering proof of authenticity via protein profiling analysis. To take account of this complex situation, spot volumes for the set of 25 proteins (**Table 1**) were further examined. For these 25 proteins the ratio of protein spot volumes in organic and conventional wheat was calculated for the crops of 2003 and 2005. This ratio of protein spot volumes was denoted agricultural effect. The agricultural effect on individual proteins varied in crops of 2003 and 2005 (**Figure 2** and Supporting Information, right panel). **Figure 2** illustrates the situation for two selected proteins. For protein 55, the agricultural effect was quite similar in both years, i.e. 54% in 2003 and 58% in 2005. In contrast, the agricultural effect for protein 231 was highly different in both years, with 54% in 2003 and near 100% in 2005, indicating that levels of protein 231 were affected by seasonal parameters. The modulation of the agricultural effect in the two cropping years is denoted seasonal influence from here on. The finding that seasonal influence is highly variable for the proteins responding to the agricultural regime should be taken into account when attempting to identify a protein signature for organically produced wheat.

Suitability of Proteins To Prove Authenticity. Our paradigm is that the stronger the mean agricultural effect and the weaker the uncontrollable seasonal influence, the better a protein is suitable to serve as a diagnostic protein in a signature for organic wheat production. **Figure 2** and Supporting Information show that, among the 25 proteins, the magnitudes of both agricultural effect and seasonal influence varied to a large extent. To estimate the "degree of diagnostic suitability" of the 25 proteins, a simple calculation was made based on the mean agricultural effect in the two cropping seasons and seasonal influence. The result of the calculation is a numerical "consistency score" (**Table 1**; for calculations see Materials and Methods). The higher the consistency score, the stronger was the mean agricultural effect and the weaker was the seasonal influence. For example, if a consistency score of 2 is calculated, the mean agricultural effect is twice as large as the seasonal influence. At this stage of our work, we have, arbitrarily to some extent, set the consistency score to a value of 2 as a minimum margin of safety. This means that any protein that exceeds the threshold value of 2 is considered suitable for contributing to the proposed signature. Of the 25 proteins with different expression in organic and conventional DOK-wheat, 16 proteins pass this threshold. The maximum consistency score of about 12 was calculated for a LMW glutenin and for an unknown protein. The results taken together demonstrate that, although there are marked seasonal influences, a considerable number of "diagnostic proteins" of diverse functions could be identified which are deemed suitable to constitute a signature identifying organically produced DOK-wheat.

When analyzing commercial wheat for its authenticity, organic or conventional, it is probable that more interfering factors come into play by comparison with wheat from the DOK field trial. Main additional factors probably introducing more bias are differences in wheat variety and soil type. Nevertheless, applying the presented approach to determine "suitability" of a protein for a signature using the profiling technique 2-DE, it should be possible to find out whether wheat of diverse sources can be identified as organic and conventional. Moreover, the proposed protein signature,

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	Mowse score	p*I*[b]	MW [kDa][b]	Accession No. (gi)[c]
55	low molecular weight glutenin	12.1	73.3	9.08	46.7	56480772
373	unidentified	12.0		9.95	27.6	
371	unidentified	6.6		9.04	48.6	
49	peroxidase 1	4.8	83.7	9.52	42.3	22001285
162	unidentified	4.7		8.57	83.4	
107	unidentified	4.3		6.56	35.7	
114	globulin 1	3.8	72.2	9.03	32.4	110341795
232	glyceraldehyde 3-phosphate dehydrogenase	3.7	98.5	7.44	43.3	18978
173	xylanase, family 11	3.5	82.7	9.37	31.9	51247633
376	unidentified	3.2		9.00	78.0	
271	triticin precursor	3.0	173	6.66	52.8	7548844
76	high molecular weight glutenin	2.7	97.1	8.87	83.4	110341796
152	granule-bound starch synthase precursor	2.6	252	5.46	59.1	4588607
229	low molecular weight glutenin	2.6	73.7	9.14	48.7	17425188
94	xylanase, family 11	2.4	78.9	9.52	33.9	51247633
252	serpin	2.1	204	5.28	50.2	1885350
268	triticin precursor	2.0	140	6.78	51.5	7548844
197	granule-bound starch synthase precursor	2.0	100	7.64	36.0	4588609
251	low molecular weight glutenin	1.9	94.2	8.79	49.4	56480772
77	sucrose synthase type 2	1.9	73.2	8.01	83.0	3393044
295	β-amylase	1.8	125	4.73	57.3	32400764
214	aldose reductase-related protein	1.8	77.3	7.38	35.9	167113
372	unidentified	1.7		9.01	74.6	
325	high molecular weight glutenin	1.7	188	8.85	75.3	22090
231	low molecular weight glutenin	1.7	85.1	9.09	46.6	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 2 (see **Figure 2** and Supporting Information). Proteins were sorted by descending value of the consistency score (for the calculations see Materials and Methods). A large consistency score indicates a strong influence of the agricultural regime 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). [b] p*I* and MW were determined from 2-DE gels. [c] Accession No. from NCBI.

which may comprise a relatively small number of proteins when commercial wheat is under investigation, can be complemented by suitable "diagnostic" metabolites and "diagnostic" genes. For example, our previous metabolite profiling work has identified metabolites whose concentrations were changed by the farming system (*7*). Similarly, the transcriptional profiling work of Lu et al. (*8*) has revealed gene expression changes correlating with differing agricultural regimes. By finally combining the results of different profiling techniques, we consider it an achievable goal to identify a profiling based signature that can discriminate organic wheat against conventional wheat from the marketplace.

ABBREVIATIONS USED

2-DE, two-dimensional gel electrophoresis; CHAPS, 3-[3-(cholamidopropyl) dimethylammonio]-1-propane sulfonate; DOK, biodynamic, bioorganic and "Konventionell" (conventional); DTT, dithiothreitol; HMW, high molecular weight; IEF, isoelectric focusing; IPG, immobilized pH gradient; LMW, low molecular weight, MALDI-TOF-MS, matrix assisted laser desorption/ionization-time-of-flight-mass spectrometer; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TCA, trichloroacetic acid.

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Supporting Information Available: Figure 1 depicting levels of 23 proteins in wheat from organic and conventional agriculture. This material is available free of charge via the Internet at http://pubs.acs.org.

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