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Levels of Compounds and Metabolites in Wheat Ears and Grains in Organic and Conventional Agriculture

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In this work, wheat from two farming systems, organic and conventional, was analyzed. Organic agriculture is one of the fastest growing sectors in the food industry of Europe and the United States. It is an open question, whether organic or conventional agricultural management influences variables such as metabolism, nutrient supply, seed loading and metabolite composition of wheat. Our aim was to detect if organic or conventional farming systems would affect concentrations of metabolites and substances in developing ears and in corresponding matured grain. Therefore, broadband metabolite profiles together with lipids, cations, starch and protein concentrations of wheat ears in the last phase of grain development and of matured grain from organic and conventional agriculture of a rigorously controlled field trial with two organic and two conventional systems were examined. It appears that seed metabolism and supply of developing ears differ in organic and conventional agriculture. However, the differences in 62 metabolite concentrations become marginal or disappear in the matured grains, indicating an adjustment of nutrients in the matured grain from organic agriculture. This result suggests a high degree of homeostasis in the final seed set independent of the growing regime.

KEYWORDS: Wheat; organic agriculture; conventional agriculture; metabolite profiling; lipids; cations

INTRODUCTION

Organic agriculture is a fast growing sector in the food industry of Europe and the United States (*1*). Strict rules are applied to grow organic crops such as wheat, including a ban on the use of synthetic fertilizers. For instance nitrogen inputs must come from animal manure or slurries, green manures, crop residues, legumes and residual soil organic matter (*2*). Despite the increasing interest in organic wheat, little is known about potential differences in ear development in terms of supply and transfer of metabolites and substances. Differences in metabolite concentrations between ears grown solely with organic fertilizer versus conventional conditions might account for possible differences in composition of matured grain. Moreover, the evaluation of a series of compounds will contribute to the physiological knowledge on seed development.

Environmental factors such as the different agricultural management, fertilization levels, or soil conditions are expected to affect gene expression and induce different concentrations of compounds in developing ears and in matured grains. Supply of plant nutrients under organic and conventional agriculture primarily depends on the concentration and the form of ions available in the soil and possibly additional factors such as the pH of the soil solution. Organic fertilizers can contain a wide range of organic-nitrogen compounds such as amino acids, amino sugars and heterocyclic nitrogen compounds (*3*). A major difference in conventional agriculture, besides the different agricultural management influencing the soil pH (*4*), is the extra fertilization with nitrogen at the beginning of the development of ears, which primarily increases the protein content of the grain. Individual wheat storage proteins, which represent a high proportion of total protein, are affected by this late nitrogen fertilization (*5*). Grain nitrogen levels depend at least on two factors: continued nitrate uptake by roots and the remobilization of nitrogenous compounds from leaves and stems during grain filling (*6*). But also non-protein compounds are expected to change under different growth conditions. Photoassimilates, namely, carbohydrates, are transported from sites of production to sites of growth and storage (*7*). Environmental variables affect the development of leaf size and hence photosynthetic machinery to generate photoassimilates for seed loading. On the other hand increase of yield from greater seed biomass gained at the expense of vegetative organs competes for the available nutrient pool. Final seed biomass is a function of both filling rate and filling duration. Filling rate is a function of seed cell number and activities of seed physiological processes (*8*). The potential size that a seed may

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achieve at maturity is positively related to cell number and often inversely related to the number of seeds developing on the plant. Both seed and cell numbers are set during the prestorage phase of seed development (*8*).

A differing agricultural management with its direct acting variables such as nutrient availability and supply as well as the indirect variables such as different soil and microclimate conditions might have a distinct influence on the composition of metabolites and substances in ears and the quality of matured grains.

Here, we analyzed wheat originating from different growing regimes of a rigorously controlled field trial with two types of organic and two types of conventional field plots. A broadband metabolite profile, ions, and lipids were examined in wheat ears during the last phase of ear development and in the corresponding matured grains originating from organic and conventional agriculture. Our aim was to detect if different farming systems affect concentrations of metabolites and other substances in developing ears and in the corresponding matured grains.

MATERIALS AND METHODS

Plant Material. Wheat grains were harvested in 2006 from the "DOK" field trial near Basel, Switzerland. Material was obtained from two organic farming systems, namely, biodynamic (D) and bioorganic (O), and from two conventional systems, one using mineral fertilizer (M) and the latter using mineral fertilizer plus farmyard manure (K). Crop rotation, varieties, and tillage were identical in all systems (*4*). Plants in the crop rotation were (1) potatoes; (2a) winter wheat, (b) green manure, clover-grass; (3a) soy bean, (b) green manure, clover-grass; (4) maize; (5) winter wheat (cv. Runal, for the year 2006). The conventional systems received ca. 130 kg of water-soluble nitrogen ha^{-1}, both organic systems ca. 20 kg of soluble nitrogen ha^{-1}. Four samples of each farming system, originating from the four field replicate plots from the DOK trial, were taken. Wheat grains (100 g) were crushed in a Titan laminated mill using a 0.5 mm sieve (Retsch, Haan, Germany). The material was then ground using a mortar to a fine homogeneous powder denoted wholemeal. Wheat ears from each field plot were collected 4 weeks before harvest. Directly at the field, the ears were shock frosted in liquid nitrogen and afterward stored at −80 °C. The material was than crushed in a mortar under liquid nitrogen, and the fine powder was stored at −80 °C until extraction.

Analytical Procedures of Sugar Concentration Measurements. Soluble sugars from 4 g of wholemeal from grains and ears were extracted with 50 mL of 80% ethanol at 60 °C for 30 min. The extraction was repeated once (*9*). The two extracts were combined and centrifuged for 10 min at 1500*g* at room temperature. The supernatant was concentrated by rotary evaporation from 100 mbar to 30 mbar, and the dry residue was resuspended in 8 mL of water (double distilled) and sonicated for 3 min in an ultrasonic bath. The suspension was centrifuged for 10 min at 20 °C and 1500*g*. The supernatant was filtrated through a Strata X 60 mg cartridge (Phenomenex, Germany) which was previously equilibrated with 2 mL of methanol and 2 mL of water, subsequently. To 4 mL of the filtrate was added 6 mL of acetonitrile. After centrifugation (see above), 50 μL of the supernatant was applied to the high performance liquid chromatography system (HPLC) for sugar separation. Analysis was done by HPLC according to Zörb et al. (*10*). The chromatographic equipment consisted of a model Kontron HPLC system, a Geminyx data module (Spectro BioNova, Germany), and an RI Detector model 7515A (ERC, Germany). A stainless-steel analytical column CC 250/4.6 Nucleosil 100-5 NH_2 (Macherey & Nagel, Germany) was used together with a precolumn CC 8/4 Nucleosil 100-5 NH_2 (Macherey & Nagel, Germany). The mobile phase was degassed (acetonitrile−water 6:4 w/w), and the flow rate was set to 0.5 mL/min. The column temperature was set at 30 °C. External standards were used for calibration. A linear response was obtained in the range of 0.0−4.0 mg mL^{-1} with a correlation coefficient of 0.99.

Metabolite Extraction. The metabolites were extracted from 10 to 20 mg of wholemeal with 1 mL of 80% methanol, containing 10 μM ribitol as internal standard in a FastPrep Instrument (Qbiogene, Heidelberg, Germany), using 1 mm zirconia beads (Roth, Karlsruhe, Germany). Extracts were treated three times at 6.5 m/s for 45 s. Metabolite extraction was enhanced by incubation at 70 °C for 15 min with 1,400 rpm in a Thermomixer (Eppendorf, Hamburg, Germany). After 10 min centrifugation at 15000*g* at room temperature, the clear supernatant was transferred to 1 mL glass vials (Supelco, Bellfonte, California) and evaporated in a nitrogen stream. Metabolites were derivatized with 75 μL of methoxylamine hydrochloride in pyridine (20 mg/mL; g/v) for 90 min at 37 °C and 75 μL MSTFA for 30 min at 37 °C (*11*). All chemicals and standard compounds were purchased from Sigma-Aldrich-Fluka (Taufkirchen, Germany), Merck (Darmstadt, Germany) or Macherey-Nagel (Düren, Germany).

GC−MS Analysis. Sample volumes of 1 μL were analyzed with a TraceGC gas chromatograph coupled to a PolarisQ ion trap mass spectrometer equipped with an AS2000 auto sampler (Thermo Electron, Dreieich, Germany). Derivatized metabolites were evaporated at 250 °C in splitless mode and separated on a 30 m × 0.25 mm VF-5MS capillary column with 0.25 μm coating equipped with an integrated 10 m guard column (Varian, Darmstadt, Germany). Helium carrier gas flow was adjusted to 1 mL/min. The interface and ion source temperatures were set to 250 °C. Oven temperature was kept constant for 3 min at 80 °C and subsequently raised to 325 at 5 °C/min. The system was equilibrated for 5 min at 80 °C after each analysis. Mass spectra were recorded at 2 scans/s with a scanning range of 50−550 m/z. Metabolites were identified by comparison to purified standards and the NIST 2005 database (NIST, Gaithersburg, MD). In addition, the freely available Golm Metabolome Database (*12*) was of particular help to identify several metabolites. Relative levels of selected metabolites were determined automatically by integrating the peak areas of selective ions (*13*) using the processing setup implemented in the Xcalibur 1.4 software (Thermo Electron, Dreieich, Germany). Relative response ratios were calculated by normalizing the respective peak areas to the peak area of the internal standard and dividing the value by the weight of the extracted sample.

Other Measurements. Nitrogen levels were determined in ears and grains using the procedure of Dumas (ICC 167).

Thousand seed weight was determined by recording three times the mass of 100 grains.

Lipids were extracted according to published methods (*14*, *15*) and separated using thin layer chromatography technique (TLC). TLC plates were digitalized and relatively quantified by densitometry analysis of the thin layer chromatography.

The antioxidative capacity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) as described (*16*).

Cations concentrations were analyzed according to Langenkämper et al. (*17*) using an atomic absorption photometer.

Data, Replication, Statistics. For the determination of thousand seed weight, antioxidative capacity, cation and protein concentration, lipids and fatty acids four biological replicates from each condition (D, O, K, M) originating from the four field replicate plots were analyzed. Each sample was extracted and analyzed at least two times for each procedure. Statistical treatment was checked on the basis of the 5% level using the Tukey-test algorithm of Student range using SAS software (Institute Inc., Cary, NC). Concerning reproducibility six aliquots of one sample were extracted in parallel and taken separately through the sample preparation and GC−MS analysis procedure for all individual metabolites. Of these six analyses standard deviations were calculated. Relative standard deviations for levels of 74 metabolites were below 10%. In the subsequent analyses of all wheat grain samples only levels of the 74 metabolites were determined. In order to analyze the accuracy of the system seven internal technical replications were made; values were only accepted when the standard error was below 5%. In addition, calibration curves for 30 commercially available metabolites were generated. These measurements revealed linearity of detection for most metabolites in the range of 100 fmol to 1 nmol. Samples were randomized prior to GC−MS injection in order to prevent bias due to instrument performance.

The reproducibility of the extraction of soluble sugars using HPLC was calculated from three independent extractions, and the relative standard error of the means ranged between 0.1% and 5.6%. The recovery of sugars tested was 98% for kestotetraose, 95% for glucose, 90% for sucrose, 86% for fructose, 85% for raffinose, 84% for maltose, 81% for 1-kestose, and 78% for stachyose.

RESULTS

Thousand Seed Weight, Nitrogen, Antioxidative Potential, and Starch in Wheat Ears and Grains. The thousand seed weight

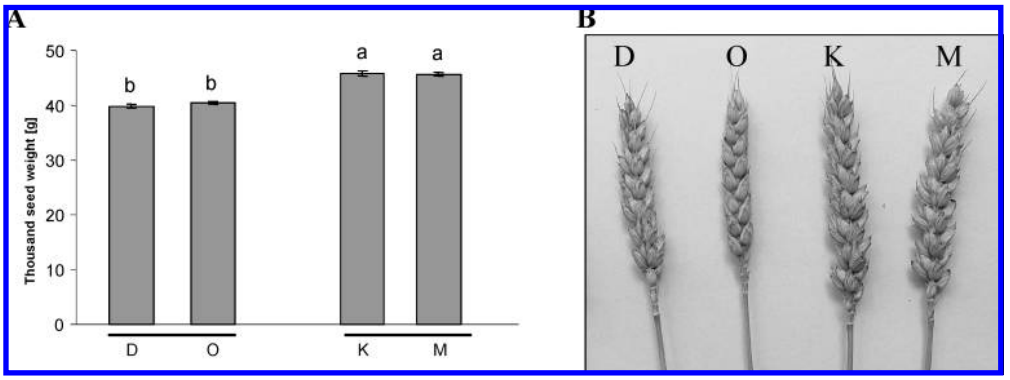

Figure 1. (**A**) Thousand seeds weight of matured wheat grains (*Triticum aestivum* L. cv. Runal) from the DOK field trial (see Materials and Methods). Standard errors of the mean (SE) were calculated from all 4 field plot replicates. Statistical significance tests ($p = 0.05$) of all four treatments were performed using the Tukey-test algorithm of Student range. Different letters indicate significant differences of the means. (**B**) Sample picture of single ears from the DOK field trial, Switzerland. The ears from the field plots were collected 4 weeks before harvest. Two organic farming systems: D, biodynamic; O, bioorganic. Two conventional farming systems: K, organic-mineral; M, mineral.

Table 1. Protein Concentrations (In Percent of Dry Matter) from Wheat Ears and Grains[a]

treatment	ear (%)	grain (%)
D	14.3 ± 0.1 b	10.9 ± 0.5 bc
O	14.2 ± 0.1 b	10.7 ± 0.1 c
K	14.5 ± 0.1 ab	14.5 ± 0.2 ab
M	15.6 ± 0.2 a	14.1 ± 0.2 a

[a] The nitrogen concentrations were determined using the method of Dumas, and the factor of 5.7 was applied to calculate protein concentrations. Standard errors of the mean (SE) were calculated from all 4 field plot replicates. Statistical significance was tested separately for ears and grains using the Tukey-test algorithm of Student range ($p \leq 0.05$). (O, bioorganic; D, biodynamic; K, conventional; M, mineralic fertilizer). Different letters indicate significant differences ($p \leq 0.05$) of the means.

(**Figure 1A**) of matured grains was significantly higher in conventional wheat grains (K and M) compared to organic wheat grains (D and O). The differences in biomass formation from organic (D and O) and conventional (K and M) agriculture are illustrated by a photograph of the ears which was taken four weeks before harvest (**Figure 1B**). The ears at this developmental stage were used for the analyses, whereas matured grains and ripe ears are not shown. Moreover, the concentration of protein in ears and grains (**Table 1**) was higher in conventional wheat compared to wheat grains from organic agriculture.

The total antioxidative capacity (**Figure 2**) did not show significant differences between organic or conventional wheat ears or grains, respectively.

K^+, Ca^{2+}, Mg^{2+}, and Na^+ Concentrations in Ears and Grains. The concentrations of cations measured in grains were in the range formerly published harvests of the DOK field trial (*18*). In matured grains the concentrations of K^+ and Mg^{2+} were significantly lower in conventional agriculture, whereas Ca^{2+} and Na^+ concentrations were in the same range (**Table 2**). The concentrations of K^+, Ca^{2+}, Mg^{2+}, and Na^+ in ears did not differ significantly by comparing the different farming regimes (**Table 2**). Comparing ion levels of ears and grains K^+ and Na^+ were in the same range, but the Mg^{2+} concentrations in ears were about 20% lower than in grains. Adversely, the Ca^{2+} concentrations in ears of each treatment were up to 60% higher than in grains.

Sugar Concentrations in Ears and Grains. The supply with assimilates from the sink organs of the plant at the different farming regimes was examined by measuring the sugar concentrations in ears using HPLC. Sugar concentrations in ears decreased in the following order: sucrose ≫ 1-kestose ≫ fructose

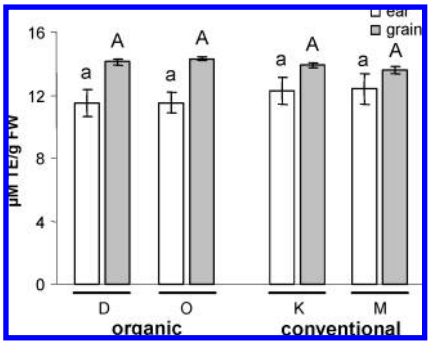

Figure 2. Antioxidative capacity expressed as μmol of Trolox equivalents (TE) per g of ears and grains. Standard errors of the mean (SE) were calculated from all 4 field plot replicates. Statistical significance tests ($p = 0.05$) of all four treatments were performed separately for ears and grains using the Tukey-test algorithm of Student range. Identical letters indicate no significant differences of the means. D, biodynamic; O, bioorganic; K, organic-mineral; M, mineral.

Table 2. Compilation of Mineral Nutrient Concentrations in Wheat Ears and Grains from the DOK Field Trial (μg g DW^{-1})[a]

	μg g^{-1} dry weight			
	K^+	Ca^{2+}	Mg^{2+}	Na^+
		Ears		
D	4310 ± 85 a	387 ± 37 a	1026 ± 48 a	61 ± 5 a
O	4571 ± 208 a	390 ± 42 a	1003 ± 44 a	66 ± 9 a
K	4466 ± 120 a	471 ± 27 a	1132 ± 84 a	76 ± 15 a
M	4272 ± 164 a	454 ± 93 a	960 ± 64 a	61 ± 4 a
		Grains		
D	4429 ± 61 a	321 ± 52 a	1254 ± 25 a	116 ± 31 a
O	4557 ± 69 a	270 ± 2 a	1215 ± 11 ab	62 ± 5 a
K	3805 ± 68 b	294 ± 5 a	1169 ± 12 b	97 ± 19 a
M	3972 ± 64 b	295 ± 12 a	1200 ± 16 b	61 ± 8 a

[a] Standard errors of the mean (SE) were calculated from all 4 field plot replicates. Statistical significance tests ($p = 0.05$) were performed separately for ears and grains using the Tukey-test algorithm of Student range. Different letters indicate significant differences ($p \leq 0.05$) of the means. D, biodynamic; O, bioorganic; K, organic-mineral; M, mineral.

> glucose = kestotetraose = maltose > raffinose > stachyose > kestopentaose (**Table 3**). Verbascose and xylose were below the detection limit. In ears, no significant differences between the farming systems with regard to kestotetraose, kestopentaose,

Table 3. Concentrations of Carbohydrates in Wheat Ears and Grains from the DOK Field Trial (mg g DW^{-1})[a]

	mg g^{-1} dry weight								
	sucrose	1-kestose	fructose	glucose	K-tetraose	maltose	raffinose	stachyose	K-pentaose
					Ears				
D	7.69 ± 0.43 ab	3.03 ± 0.09 a	1.17 ± 0.12 a	0.86 ± 0.12 ab	0.86 ± 0.02 a	0.75 ± 0.03 a	0.31 ± 0.03 a	0.17 ± 0.01 a	0.01 ± 0.01 a
O	6.79 ± 0.33 b	3.19 ± 0.14 a	0.96 ± 0.1 a	0.64 ± 0.07 b	0.8 ± 0.04 a	0.82 ± 0.05 a	0.39 ± 0.04 a	0.13 ± 0.01 a	0.03 ± 0.01 a
K	8.58 ± 0.45 a	2.84 ± 0.1 a	1.34 ± 0.1 a	1.09 ± 0.09 a	0.80 ± 0.04 a	0.70 ± 0.02 a	0.40 ± 0.02 a	0.21 ± 0.02 a	0.02 ± 0.01 a
M	8.51 ± 0.48 ab	2.98 ± 0.11 a	1.22 ± 0.09 a	0.97 ± 0.08 ab	0.85 ± 0.02 a	0.79 ± 0.04 a	0.41 ± 0.04 a	0.18 ± 0.02 a	0.02 ± 0.01 a
					Grains				
D	5.68 ± 0.08 a	2.86 ± 0.08 a	0.30 ± 0.01 bc	0.16 ± 0.01 ab	0.67 ± 0.02 a	1.53 ± 0.05 a	2.80 ± 0.02 a	0.05 ± 0.01 a	0.22 ± 0.07 ab
O	5.83 ± 0.04 a	2.92 ± 0.04 a	0.27 ± 0.01 c	0.15 ± 0.01 b	0.66 ± 0.01 ab	1.56 ± 0.03 a	2.76 ± 0.02 a	0.04 ± 0.001 a	0.07 ± 0.04 b
K	4.95 ± 0.15 b	2.48 ± 0.10 b	0.31 ± 0.01 bc	0.17 ± 0.01 ab	0.60 ± 0.02 b	1.09 ± 0.05 c	2.55 ± 0.09 b	0.02 ± 0.001 b	0.33 ± 0.05 a
M	5.40 ± 0.10 a	2.82 ± 0.08 a	0.36 ± 0.01 a	0.19 ± 0.01 a	0.68 ± 0.01 a	1.26 ± 0.01 b	2.84 ± 0.05 a	0.02 ± 0.001 b	0.38 ± 0.02 a

[a] Standard errors of the mean (SE) were calculated from all 4 field plot replicates. Statistical significance tests ($p = 0.05$) were performed separately for ears and grains using the Tukey-test algorithm of Student range. Different letters indicate significant differences ($p \leq 0.05$) of the means. D, biodynamic; O, bioorganic; K, organic-mineral; M, mineral.

Table 4. Compilation of the Metabolites Identified and Quantified by Gas Chromatography−Mass Spectrometry in Methanol Extracts of *Triticum aestivum* L. from Organic and Nonorganic Ears and Grains[a]

amino acids	sugars	sugar alcohols	organic acids	others
α-alanine (116)	**fructose-6-P (315)**	**glycerate (189, 192)**	**citrate (257)**	*O*-acetyl homoserine (128)
***β*-alanine# (248)**	**gluconate (333)**	**myo-inositol (305)**	DHAP (400)	***O*-acetyl serine (174)**
arginine# (256)	**gluconate 6-P* (387)**	**myo-inositol-P (318)**	**fumarate (245)**	**adenine (264)**
asparagine# (188)	**glucose-6-P (387)**	**pinitol (260)**	**2-hydroxyglutarate (203, 247)**	**adenosine (236)**
aspartate (232)	**ribose (217)**	**(ribitol, IS)**	isocitrate (245, 319)	agmantin# (174)
cysteine (220)	**ribose-5-P (217)**		2-isopropylmalmate (275)	**2-aminoadipate# (260)**
glutamate (154)	ribulose-5-P (357)		α-ketothio-aminobutyr. (218)	**4-aminobutyrate (174)**
glutamine (155)	trehalose (361)		**α-ketoglutarate (198)**	**citrulline (142)**
glycine (174)			**α-ketocaproate (110)**	cystathionine* (218)
histidine# (154)	**fructose (307)**		lactate (191)	**glycerat-2-P (315)**
isoleucine# (158)	**glucose (319)**		*maleic acid (245)*	glycerat-3-P (299)
leucine# (158)	**maltose (361)**		**malate (245, 307)**	**α-glycerophosphate (357)**
lysine# (204)	**raffinose* (361)**		2-methylcitrate (287)	**homocysteine (234)**
methionine# (176)	**sucrose (361)**		2-methylisocitrate (259)	**homoserine# (218)**
phenylalanine# (192)			**2-phosphoglycerate (277, 299, 459)**	5-methyl-thioadenosine (236)
proline# (142)			**pyruvate (174)**	**pantothenic acid (201)**
serine# (204)			**succinate (247, 409)**	**shikimate (204)**
threonine# (101)				spermin (144)
thymine (255)				**spermidine (144)**
tryptophan (202)				**thymine (255)**
tyrosine# (218)				**uracil (255, 241)**
valine# (144)				**urea (189)**

[a] Abbreviations: P, phosphate; IS, internal standard. *m/z* values in brackets are the selective ions used for quantification. *Metabolites identified using the NIST 2005 database, others through purified standards. Metabolites detected in ears and grains, bold; metabolites in grains, normal print. #Metabolites with significant differences between organic and conventional agriculture; for details see histograms in **Figure 4**.

stachyose, maltose, 1-kestose, raffinose, and fructose were detected. Only a difference for sucrose and glucose was found with higher concentrations in ears from conventional agriculture.

The sugar concentrations in grains decreased in the following order: sucrose ≫ 1-kestose = raffinose > maltose ≫ kestotetraose ≫ fructose = kestopentaose > glucose ≫ stachyose (**Table 3**). Verbascose and xylose were below the detection limit.

Additionally, five of the sugars quantified by HPLC were detected using GC−MS in the metabolite profile, likewise (**Table 4**). Parallel results of these sugars from the metabolite profile were therefore not shown in detail.

Metabolite Profile. The GC−MS chromatograms from wheat contained about 250 compounds (as different peaks) (**Figure 3**). An overlay of the chromatograms from equal amounts of ears and grains showed higher peaks mostly in ears. In total 74 and 63 metabolites were identified from methanol extracts of ears and grains, respectively (**Table 4**). The metabolites were classified in five groups containing amino acids (*22*), sugars and sugar derivates (*13*), sugar alcohols (*5*), organic acids (*16*), and other metabolites (*22*) (**Table 4**). Of these, 17 metabolites showed differences comparing organic and conventional ears or grains (**Table 4**, **Figure 4**). Most of the compounds, with higher concentrations in conventional wheat, were amino acids. In particular 14 metabolites from ears (arginine, asparagine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, tyrosine, valine, agmantin, 2-aminoadipate, homoserine) and 11 from grains (ss-alanine, arginine, asparagine, isoleucine, leucine, lysine, phenylalanine, proline, threonine, thyrosin, L-homoserine) showed significant differences between organic and conventional wheat (**Figure 4**; **Table 4**). The metabolite concentrations in ears were except of asparagines, generally higher as compared to grains (**Figure 4**). Amino acids such as lysine and histidine containing additional nitrogen in the side chain were significantly higher especially in ears grown under conventional inorganic conditions. Agmantin was only detected in ears.

Lipid Compounds. Concentrations of free fatty acids were up 5- to 10-fold higher in comparison to a number of diverse lipid compounds measured (**Table 5**). There was no difference between organic and conventional wheat with regard to free fatty acids and also there were no significant differences of 1,2-diacylglyceride.

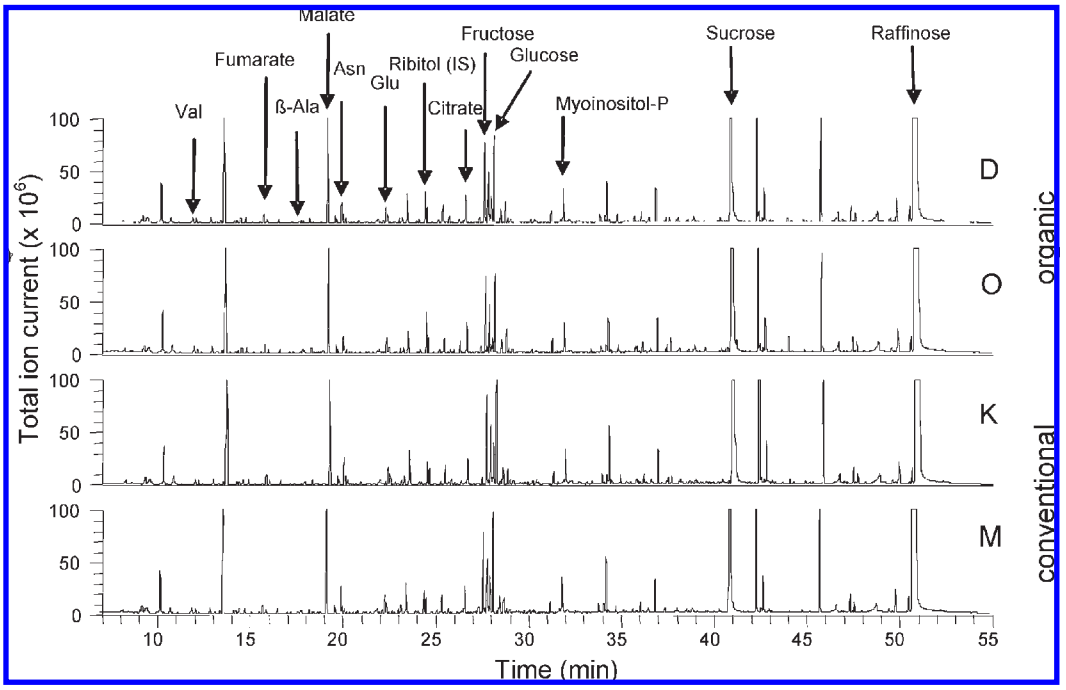

Figure 3. Gas chromatography−mass spectrometry total ion chromatograms of methanolic metabolite extracts from ears of wheat from two organic agricultures (D, biodynamic; O, bioorganic) and two conventional agricultures (K, organic-mineral; M, mineral), respectively (see Material and Methods). Val, valine; ss-Ala, ss-alanine; Glu, glutamate; Asn, aspartate; Fruc, fructose; Gluc, glucose; Suc, sucrose; Raf, rafinose; ribitol (IS), internal standard.

A 2.5-fold higher concentration of 1,2-diacylglyceride in grains as compared to ears indicates a sufficient source for biosynthesis of complex lipids. The content of triacylglycerides shows a tendency of lower levels in organic ears but no clear difference (**Table 5**). The concentrations of sterol lipids in grains were low, without differences between organic and conventional agriculture (**Table 5**). Accordingly, the concentrations of sterol lipids were in the range as reported in the literature (*19*, *20*). Several of the detected phosphoglycerides are ubiquitous in plant membranes. The content of phosphatidylethanolamine and phosphatidylcholine in grains is 6- to 10-fold higher compared to ears, indicating the process of membranes biogenesis in the last phase of grain development. No significant difference in the content of phosphatidylinositol and lysophosphatidylinositol was detected concerning the comparison of ears and grains and the different regimes, respectively (**Table 5**). Phosphatidic acid was at the detection limit in grains, as reported formerly (*21*) and not detectable in ears.

DISCUSSION

Our results indicate that seed metabolism and supply of developing ears differ in organic and conventional agriculture. However, the differences in 62 metabolite concentrations become marginal or disappear in the matured grains, indicating an adjustment of nutrients in the matured grain from organic agriculture. This result suggests a high degree of homeostasis in the final seed set independent of the growing regime.

It has been reported that antioxidants can lower the risk of developing cardiovascular disease, certain types of cancer and age-related degenerative processes due to their radical scavenging capacity (*22*). The DPPH radical scavenging assay is one of the most common applied methods to evaluate the total antioxidant activity. However, similar antioxidative equivalents (**Figure 2**) did not provide evidence of increased radical scavenging capacity of one agricultural system. These results agree well with those obtained with another wheat cultivar (*17*). Differences in yield height and protein concentration under organic agriculture (*4*, *6*, *23*, *24*) demonstrate that plants can adjust the seed weight or the number of seeds and the seed protein quantity under different growth conditions. The results reported in this work showed that thousand seed weight and crude protein content were reduced in matured organic wheat according to a lower N supply in both organic systems.

The slightly lower concentrations of K and Mg in conventional grains (**Table 2**), without being in deficit for seed development, may depend on higher seed volumes. Thus, the similarity of concentrations in grains implies a sufficient supply and translocation of the macronutrients K and Mg (and the beneficial element Na) into the developing grain. In both farming systems Ca concentrations did not show significant differences (**Table 2**). Particularly, compared to grain, the concentration of Ca was 17−37% higher in ears, respectively. Especially the Ca consumption of the seed is high during the late seed development phase, when cell division, cell wall expansion, and storage overlap and Ca has to be transported to the developing grain. However, the Ca supply of both systems was sufficient, when compared to concentrations of Ca in wheat published previously (*21*).

To the best of our knowledge we show the first report on metabolite profiles of wheat ears (plus corresponding grains). We identified 74 metabolites by gas chromatography−mass spectrometry in methanolic extracts from wheat ears (**Table 4**). By comparing metabolite concentrations of ears and grains it was clearly shown that the majority of metabolites were higher concentrated in ears (**Figure 4**; **Table 4**). Especially amounts of free amino acids were higher in ears, revealing the capacity to build up storage proteins such as glutens in matured grains. Amino acids released in this way are exported to the ear via the phloem, where they are used to synthesize the grain proteins. Storage proteins account for about 50% of the total protein in matured cereal grains, and specific proteins have important impact on their nutritional quality (*25*). Amino acid derivates such as agmanitine, 2-aminoadipate, and L-homoserine were higher under conventional agriculture (**Figure 4**). The results

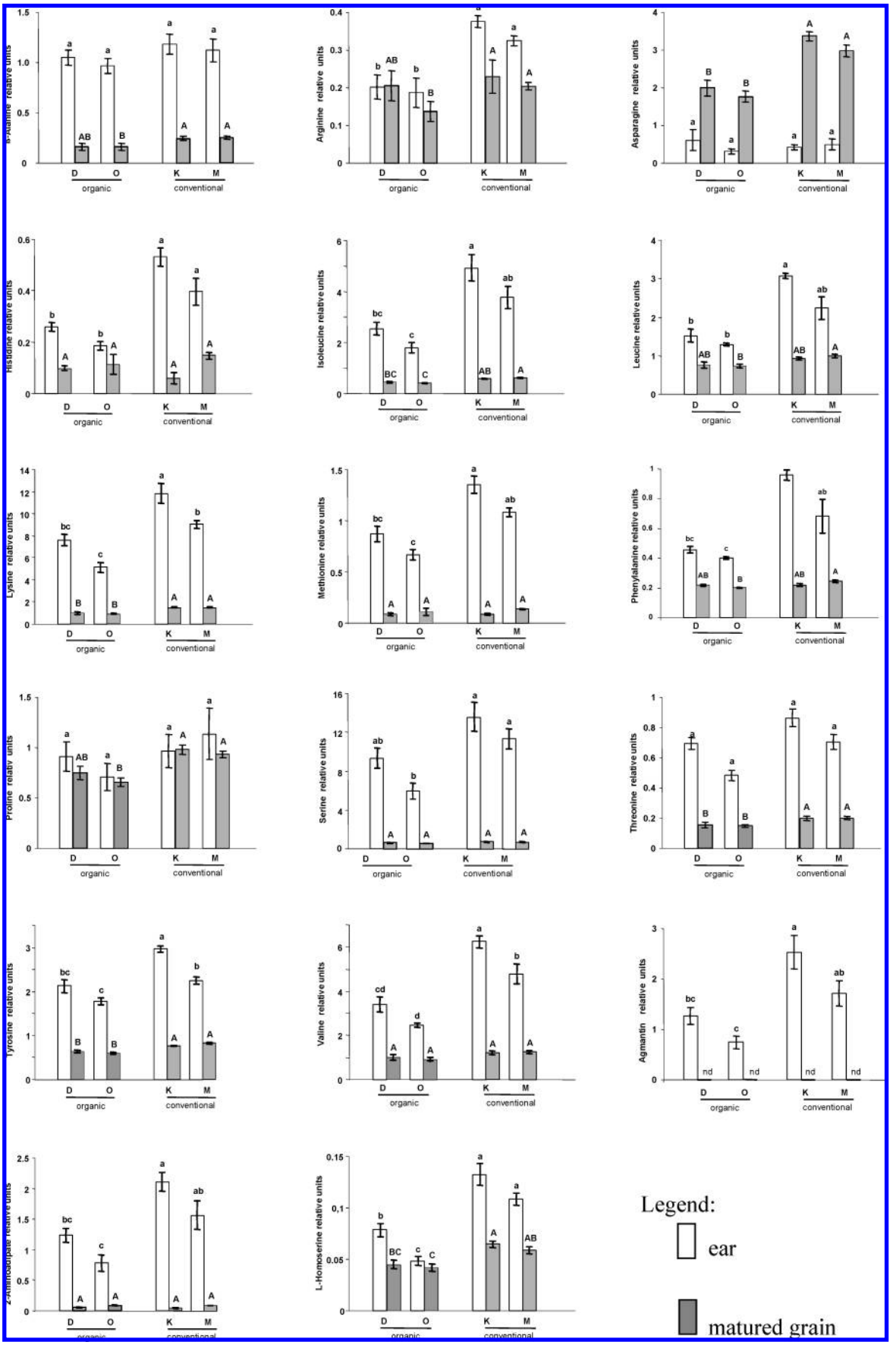

Figure 4. Metabolite concentrations of wheat ears (white bars) and grains (gray bars) influenced by the conditions of cultivation. Values are means from four replicate field plots and from eight technical replications each ± standard error of the mean. Statistical significance tests ($p = 0.05$) of all four treatments were performed separately for ears and grains using the Tukey-test algorithm of Student range. Different letters indicate significant differences of the means ($p \leq 0.05$); uppercase letters correspond to matured grain and lowercase letters to ears. D, biodynamic; O, bioorganic; K, organic-mineral; M, mineral.

Table 5. Compilation of Free Fatty Acids and Lipids Identified and Quantified by TLC of *Triticum aestivum* L. from Organic and Nonorganic Ears and Grains[a]

	rel units							
	ears				grains			
	organic		conv		organic		conv	
lipid fractions	D	O	K	M	D	O	K	M
free fatty acids	499 a	474 a	460 a	494 a	1627 a	1605 a	1521 a	1583 a
triacylglycerides	78 ab	32 b	111 a	108 a	171 a	163 a	173 a	212 a
1,2-diacylglycerides	40 a	45 a	39 a	38 a	125 a	110 a	103 a	108 a
1,3-diacylglycerides	33 a	10 b	48 a	29 ab	20 a	19 a	19 a	16 a
phosphatidylinositol	33 ab	20 b	35 ab	37 a	28 a	25 a	28 a	31 a
sterol lipids	23 a	31 a	25 a	31 a	186 a	174 a	166 a	181 a
cerebrosides	22 a	23 a	25 a	26 a	121 a	122 a	104 a	126 a
phosphatidylethanolamine	17 a	14 a	21 a	21 a	121 a	122 a	104 a	126 a
gangliosides	8 a	10 a	8 a	9 a	59 a	56 a	48 a	54 a
arachidolylphosphatidylcholine	2 a	2 a	2 a	3 a	20 a	17 ab	12 ab	10 b
lysophosphatidylinositol	nd	nd	nd	nd	75 a	72 a	48 a	52 a
phosphatidic acid	nd	nd	nd	nd	7 a	9 a	8 a	8 a

[a] Standard errors of the mean (SE) were calculated from all 4 field plot replicates. Statistical significance tests (p = 0.05) were performed separately for ears and grains using the Tukey-test algorithm of Student range. Different letters indicate significant differences of the means. nd, below detection limit. D, biodynamic; O, bioorganic; K, organic-mineral; M, mineral.

show that concentrations of metabolites containing nitrogen are influenced by organic and conventional agriculture. Regulation of the expression of genes responsible for the synthesis of storage proteins was reported to be closely linked with environmental variables (*26*). Contrasting to this finding, Mäder et al. (*18*) did not report significant differences in amino acid levels in wheat of the DOK trial from different growing conditions in the 1993 harvest. These dissenting results may be ascribed to different weather conditions, different wheat varieties or the nitrogen fertilization. Besides N-metabolites, we (*27*) detected significant though small differences for glycerate, hydroxyglutarate and myoinositol with lower amounts in organic agriculture, respectively. The 6- to 10-fold higher content of phosphatidylethanolamine and phosphatidylcholine in grains compared to ears is probably associated with the process of membrane biogenesis in the last phase of grain development. Free sugars primarily serve as precursors for the conversion into starch. Sucrose is the principally imported sugar in wheat cells (*7*). The concentration of free sugars such as sucrose, 1-kestose, fructose and glucose decreased during the development of matured grains (**Table 3**). Absolute changes in concentration were relatively minor (10 to 30%), except for stachyose and K-pentaose, where absolute differences of 60% and 82% were detected. Because of the lack of clear differences between the major free sugars and starch in wheat grains from organic and conventional agriculture, it can be assumed that the photosynthetic rate and remobilization of assimilates from stems and inflorescences did not limit the grain development under organic agriculture. Additionally, the absence of significant differences in most sugars, sugar alcohols, and organic acids indicates that the ears were well supplied with sufficient assimilate source.

Wheat plants react to a decreased and different nutrient supply in organic agriculture by reduction of tillers at an early developmental phase and a reduced seed weight, often accompanied by a plant-compatible reduction of storage proteins (*23*, *24*, *28*). Additionally, different N supply exerts a significant effect on grain composition. Overall, our data show that differences in concentrations of metabolites are pronounced in developing wheat ears of organic and conventional agriculture. However, these differences in metabolite concentrations were greatly reduced in matured grains of both farming regimes.

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LITERATURE CITED

(1) Greene, C.; Dobbs, T. Organic wheat production in the United States: expanding markets and supplies. In *Wheat situation and outlook yearbook*; WHS-2001; Economic Research Service, USDA: Washington, DC, 2001; pp 31−37.

(2) Lu, C.; Hawkesford, M. J.; Barraclough, P. B.; Poulton, P. R.; Wilson, I. D.; Barker, G. L.; Edwards, K. J. Markedly different gene expression in wheat grown with organic or inorganic fertilizer. *Proc. R. Soc.* **2007**, *272*, 1901–1908.

(3) Shannon, D.; Sen A. M.; Johnson, D. B. A comparative study of the microbiology of soils managed under organic and conventional regimes. *Soil Use Manage.* **2002**, *18* (Suppl. S1), 274−283.

(4) Mäder, P.; Fliessbach, A.; Dubois, D.; Gunst, L.; Fried, P.; Niggli, U. Soil fertility and biodiversity in organic farming. *Science* **2002**, *296*, 1694–1697.

(5) Wieser, H.; Seilmeier, W. The influence of nitrogen fertilization on quantities and proportions of different protein types in wheat flour. *J. Sci. Food Agric.* **1998**, *76*, 49–55.

(6) Masclaux, C.; Quillere, I.; Gallais, A.; Hirel, B. The challenge of remobilisation in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. *Ann. Appl. Biol.* **2001**, *138*, 69–81.

(7) Van Bel, A. J. E.; Offler, C. E.; Patrick, J. W. Sources and Sinks. In *Photosynthesis and Partitioning*; Elsevier Ltd: 2003; pp 724−734.

(8) Patrick, J. W.; Offler, C. E.; van Bel, A. J. E. *Nutrient Loading of Seeds*; Elsevier Ltd: 2003; pp 1240−1249.

(9) Munzquiz, M.; Rey, C.; Cuadrago, C.; Fenwick, G. R. Effect of germination on the oligosaccharide content of lupine species. *J. Chromatogr.* **1992**, *607*, 349–352.

(10) Zörb, C.; Betsche, T.; Langenkämper, G.; Zapp, J.; Seifert, M. Free sugars in spelt wholemeal and flour. *J. Appl. Bot. Food Qual.* **2007**, *81*, 172–174.

(11) Barsch, A.; Patschkowski, T.; Niehaus, K. Comprehensive metabolite profiling of *Sinorhizobium melilotii* using gas chromatography-mass spectrometry. *Funct. Integr. Genomics* **2004**, *4*, 219–230.

(12) Kopka, J.; Schauer, N.; Krueger, S.; Birkmeyer, C.; Usadel, B.; Bergmüller, E.; Dormann, P.; Weckwerth, W.; Gibon, Y.; Stitt, M.; Willmitzer, L.; Fernie, A.; Steinhauser, D. 2005 GMD@CSB.DB: The Golm Metabolome Database. *Bioinformatics* **2005**, *21*, 1635-1638.

(13) Fiehn, O.; Kopka, J.; Dormann, P.; Altmann, T.; Trethewey, R. N.; Willmitzer, L. Metabolite profiling for plant functional genomics. *Nat. Biotechnol.* **2000**, *18*, 1157–1161.

(14) Vuorela, P.; Vuorela, H.; Suppula, H.; Hiltunen, R. Development of a quantitative TLC assay for phospholipid products. *J. Planar Chromatogr.—Mod. TLC* **1995**, *9*, 254–259.

(15) Helmrich, G.; Köhler, P. Einfache dünnschichtchromatographische Quantifizierung von Phospholipiden. *Getreide, Mehl Brot* **2002**, *56*, 195–197.

(16) Miller, H. E.; Tiegelhof, F.; Marquart, L.; Prakash, A.; Kanter, M. Whole-grain products and antioxidants. *Cereal Foods World* **2000**, *45*, 59–63.

(17) Langenkämper, G.; Zörb, C.; Seifert, M.; Mäder, P.; Fretzdorff, B.; Betsche, T. Nutritional quality of organic and conventional wheat. *J. Appl. Bot. Food Qual.* **2006**, *80*, 150–154.

(18) 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. Wheat quality in organic and conventional farming: results of a 21 year field experiment. *J. Sci. Food Agric.* **2007**, *87*, 1826–1835.

(19) McDonnell, E. M.; Pulford, F. G.; Mirbahar, R. B.; Tomos, A. D.; Laidman, D. L. Membrane lipids and phosphatidylcholine turnover in embryos from germinaging low and high vigor wheat (*Triticum aeastivum*). *J. Exp. Bot.* **1982**, *33*, 631–642.

(20) Singh, S. P.; Paleg, L. G. Low temperature-induced GA_3-sensitivity of wheat. V. Sterol conversions in the wheat aleurone tissue during imbibition. *Austr. J. Plant Physiol.* **1985**, *12*, 549–555.

(21) Pomeranz, Y. Chemical composition of kernel structures. In *Wheat Chemistry and Technology*, 3rd ed.; Pomeranz, Y., Ed.; American Association of Cereal Chemists, Inc: St. Paul, MN, 1988; pp 97−158.

(22) Stanner, S. A.; Huges, J.; Kelly, C. N. M.; Buttriss, J. A review of the epidemiological evidence for the antioxidant hypothesis. *Public Health Nutr.* **2004**, *7*, 407–422.

(23) Steineck, O.; Liebhard, P. Qualität und Ertrag von Brotgetreide aus naturwissenschaftlich-biologischer und alternativ-biologischer Pflanzenproduktion. *Allg. Mühlen-Markt* **1984**, *85*, 35–40.

(24) Eltun, R. The Apelsvoll cropping system experiment III. Yield and grain quality of cereals. *Norw. J. Agric. Sci.* **1996**, *10*, 7–12.

(25) Shewry, P. R.; Halford, N. G. Cereal seed storage proteins: structures, properties and role in grain utilization. *J. Exp. Bot.* **2002**, *53*, 947–58.

(26) Barneix, A. J. Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. *J. Plant Physiol.* **2007**, *164*, 581–90.

(27) Zörb, C.; Langenkämper, G.; Betsche, T.; Niehaus, K.; Barsch, A. Metabolite profiling of wheat grains (*Triticum aestivum* L.) from organic and conventional agriculure. *J. Agric. Food Chem.* **2006**, *54*, 8301–8306.

(28) Bolling, H.; Gerstenkorn, P.; Weipert, D. Vergleichende Untersuchungen zur Verarbeitungsqualität von alternative und konventionell angebautem Brotgetreide. *Getreide, Mehl Brot* **1986**, *40*, 46–51.

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