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Classification of spelt cultivars based on differences in storage protein compositions from wheat

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ABSTRACT

Wholemeal flours from 62 spelt and 13 wheat cultivars were studied. The quantitative protein compositions of the Osborne fractions determined by reversed-phase high-performance liquid chromatography, showed that the chromatograms of the reduced gliadin fractions were most suitable for the distinction of spelt from wheat and for the classification of spelt. The patterns of the reduced spelt gliadins showed one to three markers that were not present in wheat. Based on these markers, spelt cultivars were classified into three groups ranging from 'typical spelt' to 'similar to common wheat'. Marker 1 was identified as ω1,2-gliadin and markers 2, 3a and 3b were identified as γ-gliadins by means of N-terminal sequence analysis and determination of the relative molecular mass by mass spectrometry. As glutenin-bound ω-gliadins were present in wheat and absent in spelt, this protein type may be used to detect and quantitate small amounts of wheat in spelt products.

1. Introduction

Spelt (*Triticum aestivum* ssp. *spelta* (L.) Thell.) was the most important cereal in southern Germany, Austria, and Switzerland one hundred years ago due to its robustness, modesty with regard to climate and soil, nitrogen utilisation efficiency, and resistance to disease. During the 20th century, common (bread) wheat (*T. aestivum* ssp. *aestivum* L.) almost completely replaced spelt because of higher yields, lower processing costs and better baking performance. Since more than 10 years, however, spelt products have gained increasing popularity. Pleasant taste and aroma, good digestibility and a better tolerance (in the case of wheat sensitivity) have been suggested as the major reasons (Kling, 1988). Moreover, as spelt has fewer requirements regarding fertilisation and use of pesticides or herbicides compared to wheat, it is now being increasingly cultivated in water-protected areas and in organic farming (Kohajdova & Karovicova, 2008). To compensate for the detrimental properties of spelt in agriculture and processing, but to preserve its desirable properties, systematic crossbreeding of spelt and wheat was started at the beginning of the 20th century. Since that time, in addition to pure spelt, spelt/wheat crossbreeds have been cultivated and processed into food products. The assignment of crossbreeds to the subspecies spelt is exclusively done on the basis of morphological properties such as shape of the ears and the fact that the husks remain attached to the kernels after threshing. Typical constituents of the kernels that determine the quality and characteristics of spelt are not accounted for at all and information on the degree of wheat crossing has not been available to both producers and consumers of spelt products up until now. The classification of spelt cultivars, according to crossbreeding with common wheat, may be of special interest for persons sensitive to wheat and tolerant towards spelt (Catassi et al., 2013; Ludvigsson et al., 2013).

Previous studies indicated that differences in the protein patterns allow a reliable distinction of spelt and wheat (Schober & Kuhn, 2003; Wieser, 2006), although spelt and wheat kernels contain similar endosperm proteins due to their close botanical relationship. The proteins can be grouped into the so-called Osborne fractions (albumins/globulins (ALGL), gliadins (GLIA), glutenins (GLUT)) and subgrouped into the different gluten protein types (ω5-, ω1,2-, α-, γ-gliadins; high- and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS)) (Wieser, 2000). GLIA are the major protein fractions of both species and their patterns are characterised by numerous components. Variations in their patterns have been effectively used for the identification and differentiation of cultivars since decades. Acid gel and SDS gel electrophoresis, gel isoelectric focusing, and reversed-phase high-performance liquid chromatography (RP-HPLC) have been applied in particular for the differentiation of wheat cultivars worldwide (Cornell &

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Hoveling, 1998; Radic, Günther, Kling, & Hesemann, 1997; Radic-Miehle, Saam, Hüls, Kling, & Hesemann, 1998; Wrigley & Bietz, 1988).

Capillary zone electrophoresis (CE) of GLIA from 27 European spelt samples showed that differences, even of closely related cultivars and wheat elements, were detected in the patterns of some wheat/spelt crossbreeds (Schober & Kuhn, 2003). Likewise, RP-HPLC of GLIA allowed the differentiation of spelt and wheat cultivars and the determination of the degree of wheat crossing in spelt (Wieser, 2006). 23 spelt cultivars were classified into five groups ranging from pure spelts to spelts similar to wheat. Crossbreeds with identical parents (spelt and wheat) showed different GLIA patterns and a different degree of wheat crossing. Thus, the pedigree did not allow any conclusion on the real content of wheat elements. Wheat contaminations in spelt products could not be determined for quality and authenticity control of cereal seeds and pure cultivars until recently. Mayer et al. (2012) demonstrated that a simple and fast detection of wheat in spelt flours can be achieved by polymerase chain reaction–restriction fragment length (PCR–RFLP) analysis in combination with lab-on-a-chip capillary gel electrophoresis.

The aim of the work presented here was, therefore, to expand and optimise the previous studies (Wieser, 2006) on classifying different spelt cultivars according to the degree of wheat crossing based on protein markers typical of pure spelt. Additionally, the effect of wheat crossing on the quantitative protein composition was studied.

2. Materials and methods

2.1. Flours

Dehulled kernels of 62 spelt cultivars (Table 1) and 13 wheat cultivars (cv. Akteur, Bussard, Cubus, Dekan, Herrmann, Impression, Manager, Maris Hustler, Mulan, Potenzial, Tommi, Virtus, Winnetou) were obtained from different breeders. They had been grown at different locations and harvested in the years 2007–2009. Dehulled kernels (approximately 10 g) were milled into wholemeal flours by means of an ultracentrifugal mill (ZM 200, Retsch, Haan, Germany) using a 0.08 mm sieve.

2.2. Crude protein content

The nitrogen content of flours was measured by the Dumas combustion method by means of a FP-328 instrument (Leco, Moenchengladbach, Germany). The nitrogen content was multiplied with a factor of 5.7 to calculate the crude protein content.

2.3. Protein extraction

Flours (100 mg) were extracted sequentially with a buffered salt solution (2 × 1.0 ml; 0.4 mol/l NaCl/0.067 mol/l Na_2HPO_4/KH_2PO_4, pH 7.6) for 10 min at room temperature (RT; approximately 20 °C) (ALGL), with 60% (v/v) ethanol (3 × 0.5 ml) for 10 min at RT (GLIA), and with glutenin extraction solution (2 × 1.0 ml; 50% (v/v) 1-propanol/0.05 mol/l Tris–HCl, pH 7.5/1% (w/v) dithioerythritol (DTE)) for 30 min at 60 °C under nitrogen (GLUT) (Wieser, Antes, & Seilmeier, 1998). Each extraction step was initiated by vortexing for 2 min at RT and continued with magnetic stirring. The suspensions were then centrifuged for 20 min at 3550g and 23 °C. The corresponding supernatants were combined and diluted to 2.0 ml with the respective extraction solvent. Three separate extraction experiments were carried out for each flour sample. For the reduction of disulphide bonds, aliquots of the GLIA extracts (1 ml) were incubated with DTE (10 mg/ml) at 60 °C for 30 min.

2.4. RP-HPLC

Protein extracts were filtered through a 0.45 μm membrane and used for RP-HPLC analysis under the following conditions (Wieser et al., 1998): instrument, Spectra System with a ChromQuest software (Thermo Finnigan, Schwerte, Germany); column, Nucleosil 300-3-C_{18}, 2.1 × 150 mm (Dionex, Germering, Germany); temperature, 60 °C; injection, 20 μl (ALGL, GLUT), 10 μl (GLIA, reduced GLIA); elution system, trifluoroacetic acid (TFA) (0.1%, v/v) in water (A) and TFA (0.1%, v/v) in acetonitrile (B); linear gradients, 0 min 0% B, 1 min 20% B, 15 min 60% B (ALGL), 0 min 0% B, 1 min 31% B, 25 min 56% B (GLIA, reduced GLIA, GLUT); flow rate, 0.3 ml/min; detection, UV absorbance at 210 nm; calibration reference: PWG-gliadin (Van Eckert et al., 2006); integration, ChromQuest software. For the isolation of protein markers, corresponding RP-HPLC eluates were collected manually from numerous runs and freeze-dried.

2.5. Determination of the relative molecular mass (M_r)

The M_r of isolated protein markers was determined by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) using a MALDI Ultraflex instrument (Bruker, Bremen, Germany). Protein solution (1 μl; 3 mg/ml) in acetonitrile/water (30/70, v/v) containing TFA (0.1%, v/v) was applied onto a multi target plate (MTP; 384 targets; ground steel) by the dried-droplet-method using sinapic acid solution (1 μl; 10 mg/ml) in 30% (v/v) aqueous acetonitrile containing TFA (0.1%, v/v). The laser energy (smartbeam2, modified Nd:YAG-laser), laser frequency: 1000 Hz) was set to 50–60%. A mass range from m/z 20,000 to m/z 100,000 was used (1000–10,000 shots per target). The equipment was externally calibrated with a Protein II standard of Bruker Daltonics (trypsinogen $[M+H]^+$ m/z 23,982, protein A $[M+H]^+$ m/z 44,613, bovine serum albumin (BSA) $[M+H]^+$ m/z 66,463).

2.6. N-terminal sequence analysis

N-terminal sequence analysis was carried out by automated Edman degradation on a protein sequencer Procise 492 (Applied Biosystems, Carlsbad, California), running in the pulsed-liquid mode. Isolated protein marker fractions were dissolved in acetonitrile/water (30/70, v/v) containing TFA (0.1%, v/v); the amount of protein applied onto the sequencer was between 50 and 100 pmol.

2.7. Statistics

Statistical significances of the differences between the spelt and wheat groups were determined by one-way analysis of variance (ANOVA) with Holm-Sidak's test as post hoc procedure using the software SigmaPlot 11.0 (Systat Software, San José, CA, USA). Differences were judged to be significant at $p < 0.05$. Principal component analysis (PCA) was carried out to determine whether the observed quantitative differences of the GLUT fraction, HMW-GS, LMW-GS and glutenin-bound ω-gliadins (ωb-gliadins) could be used to differentiate between spelt groups and wheat. The software XLStat 2014 (Addinsoft, New York, NY, USA) was used for PCA.

3. Results and discussion

3.1. RP-HPLC patterns of Osborne fractions

Kernels of 62 spelt and 13 wheat cultivars were milled into wholemeal flours and proteins were stepwise extracted according to a modified Osborne fractionation. The obtained ALGL, GLIA, and