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Gluten protein composition and aggregation properties as predictors for bread volume of common wheat, spelt, durum wheat, emmer and einkorn

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ABSTRACT

The technological properties of the ancient wheat species spelt, emmer and einkorn are considered inferior compared to common wheat and durum wheat, but there are only few comparative studies between ancient and modern wheat species. To help fill this gap, protein content and composition, gluten aggregation, dough and bread properties were determined in a unique set of eight cultivars each of common wheat, spelt, durum wheat, emmer and einkorn grown under standardized conditions at one location in the same year. Spearman correlations and principal component analysis (PCA) revealed that especially the contents of glutenins, high-molecular-weight glutenin subunits and glutenin macropolymer were suitable to predict the volume of breads made from wholemeal flours of the five wheat species using microbaking tests. Based on their proximity to common wheat in the PCA, one cultivar each of spelt, emmer and einkorn was identified that had similar protein analytical, functional and baking properties to common wheat. Furthermore, the characterization of gluten aggregation behavior using the GlutoPeak test (GPT) enabled an estimation of dough properties and bread volume. Therefore, the fast and easy GPT may serve as an alternative to time-consuming and labor-intensive baking tests.

1. Introduction

In the 21st century, the production area of the “ancient” (hulled) wheat species einkorn (*Triticum monococcum* L., diploid), emmer (*T. dicoccum* L., tetraploid) and spelt (*T. spelta* L., hexaploid) is negligibly small compared to that of the “modern” (naked) wheat species common wheat (*T. aestivum* L., hexaploid) and durum wheat (*T. durum* L., tetraploid). Especially in the last decades ancient wheat species were replaced by modern wheat species due to higher grain yields (spelt 37%, emmer 55% and einkorn 62% lower yield compared to common wheat). Ancient species are hulled wheats with a though glume, which has to be separated from the grain in the mill (Longin et al., 2015). Because some consumers associate the consumption of ancient wheats with health benefits, ancient wheat species have been attracting attention in the last 20 years and special products such as bread, pasta and beer have been developed (Longin et al., 2015). Studies on the contents of bioactive components (e.g. dietary fiber components, phenolic acids, folates) in ancient and modern wheats revealed only small differences between modern and ancient wheat species. For example, even though emmer and einkorn contained more of the carotenoid lutein than common wheat, durum wheat had comparable contents of lutein due to its yellow color (Shewry and Hey, 2015). More studies on a wider range of genotypes of ancient and modern wheats grown under standardized conditions are currently needed to assess possible health benefits (Shewry, 2018). Further advantages of ancient wheats include their disease tolerance, adaptation to different climatic conditions, low requirement of fertilizers and potential to increase biodiversity (Longin et al., 2015). In addition, tetraploid and diploid wheat species may contain lower amounts of immunoreactive proteins and peptides compared to hexaploid species. For example, the celiac disease-active 33-mer peptide was not detected in emmer, durum wheat and einkorn samples due to absence of the D-genome, but spelt and common wheat had comparable contents of the 33-mer (Schalk et al., 2017).

The baking quality of wheat flours is mostly determined by gluten quality and quantity. Gluten proteins are storage proteins and divided into gliadins (GLIA) soluble in aqueous alcohol and glutenins (GLUT) soluble in aqueous alcohol only after reduction of disulfide bonds. Contents and composition of GLIA (ω5-, ω1,2-, α- and γ-GLIA) and GLUT (ωb-gliadins, high- (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS)) are typically analyzed by modified Osborne fractionation followed by reversed-phase high-performance liquid chromatography (RP-HPLC) (Wieser et al., 1998). One glutenin

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subfraction with particular importance for baking quality is the polymeric glutenin macropolymer (GMP) that is insoluble in aqueous sodium dodecyl sulfate (SDS) solution. GMP is one of the largest protein-based biopolymers (Don et al., 2003; Weegels et al., 1996) and can be quantitated by gel-permeation (GP-)HPLC (Cinco-Moroyoqui and MacRitchie, 2008). There are only few studies on the content and composition of gluten proteins of the different wheat species. White flours of common wheat had GLIA/GLUT ratios between 1.7–3.1 (Wieser and Kieffer, 2001) and 1.4–2.1 (Thanhaeuser et al., 2014), whereas those of spelt (2.2–9.0) (Koenig et al., 2015), durum wheat (3.1–5.0) (Wieser, 2000; Wieser et al., 2003), emmer (3.5–7.6) (Wieser and Koehler, 2009) and einkorn (4.0–14.0) (Wieser et al., 2009) were higher compared to common wheat. The contents of GLIA ($r = 0.80$), GLUT ($r = 0.76$) and GMP ($r = 0.80$) were suitable to predict the bread volume of common wheat, because of higher correlation coefficients compared to the prediction using crude protein contents ($r = 0.71$) (Thanhaeuser et al., 2014). The GLUT content and especially that of HMW-GS was correlated to the bread volume (Wieser et al., 2009). Regarding GMP, common wheats contained 8–18 mg GMP/g of flour, but there are no studies available for spelt, durum wheat, emmer and einkorn.

Among cereals common wheat is most suitable for bread making because the flour forms a viscoelastic dough when it is mixed with water. In comparison to common wheat the flours of ancient wheat species yield softer doughs with low elasticity and high extensibility because of the poor gluten quality (Sobczyk et al., 2017; Longin et al., 2015; Wieser et al., 2009). The baking quality is usually determined by baking tests which are very time-consuming and labor-intensive. Instead, so-called quality parameters such as crude protein (CP) content (ICC method 167), wet gluten content (ICC method 137/1), micro-scale extension tests (Wieser and Kieffer, 2001) or the Zeleny sedimentation test (ICC method 116/1) are used to predict the baking quality of wheat flours. Another fast and easy method to determine quality-related parameters of wheat flour is the GlutoPeak test (GPT) that registers gluten aggregation properties (torque) during high-speed mixing for a short time (6 min). Parameters such as maximum torque (MT), peak maximum time (PMT) and aggregation time (AGT) are calculated from the respective curve. These parameters were used to predict the gluten and baking quality in white flours of common wheat (Bouachra et al., 2017; Huen et al., 2017; Marti et al., 2015a, 2015b) and durum wheat (Marti et al., 2014). However, no GPT data are available for spelt, emmer and einkorn so far.

Although gluten content and composition of a variety of common wheat, spelt and einkorn cultivars were already characterized, these studies are difficult to compare, because the samples were cultivated in different areas and harvest years, fertilized differently and the grains were milled to white or wholemeal flours. Therefore, the aim of this study was to establish suitable quality parameters to predict the baking quality of wholemeal flours of common wheat, spelt, durum wheat, emmer and einkorn. For this purpose, the results for gluten content and composition and gluten aggregation properties were correlated with the results of baking experiments using eight cultivars of each wheat species grown at the same location (Seligenstadt, Germany) and in the same year (2013).

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical or higher grade and purchased from VWR Merck (Darmstadt, Germany), Serva (Heidelberg, Germany), LECO (Kirchheim, Germany) or Sigma-Aldrich (Steinheim, Germany). Water was deionized by a water purification system Arium 611VF (Sartorius, Goettingen, Germany).

2.2. Wheat samples

Eight cultivars per wheat species of common wheat, spelt, durum wheat, emmer and einkorn were cultivated by the State Plant Breeding Institute (SPBI), University of Hohenheim (Stuttgart, Germany) at Seligenstadt, Germany (Coordinates 49°52′N, 10°8′E) from October 2012 to July 2013. Seligenstadt is located 281 m above sea level and is characterized by 9.4 °C mean annual temperature and 721 mm mean annual precipitation. The fertilization was adjusted to the demands of the wheat species. Common wheat, spelt and durum wheat were fertilized with 95 kg N/ha and emmer and einkorn with 75 kg N/ha. Further information about field trials is available from Longin et al. (2015). An overview of the selected cultivars with sample codes is given in Supplementary Table 1. For common wheat, two cultivars per quality class (E, elite quality; A, high quality; B, bread quality; C, cookie quality) were chosen. For spelt, eight well known cultivars were cultivated. For durum wheat, important winter durum wheat cultivars from Germany, Austria and France were selected. Furthermore, two elite breeding lines of the winter durum wheat breeding program of SPBI were added to the list of durum wheat. As only four approved cultivars of emmer and einkorn each were available, four elite breeding lines of the emmer and einkorn breeding program of SPBI were included. Grains were dehulled in case of spelt, emmer and einkorn and milled into wholemeal flour using a cross-beater mill (Perten Instruments, Hamburg, Germany). The wholemeal flours were stored in closed bottles at room temperature for at least two weeks before they were used for all analyses and techno-functional tests.

2.3. Standard determinations

The nitrogen content of wholemeal flours was determined in triplicate by the Dumas combustion method (ICC standard 167) using a TruSpec Nitrogen Analyzer (Leco, Kirchheim, Germany). Calibration was performed with ethylenediaminetetraacetic acid and the factor 5.7 was used to calculate the CP content. Moisture and ash contents were analyzed according to ICC standards 110 and 104, respectively.

2.4. Analytical Osborne fractionation

Albumin/globulin (ALGL), GLIA and GLUT fractions were extracted stepwise from 100 mg of wholemeal flour and analyzed by RP-HPLC according to Thanhaeuser et al. (2014). Three separate extraction/RP-HPLC experiments were carried out for each flour sample. Injection volumes were optimized for each species and protein fraction. Typical injection volumes were: ALGL of common wheat, 20 µL; ALGL of the other species, 5–10 µL; GLIA of common wheat, 10 µL; GLIA of the other wheat species, 5 µL; GLUT, 20 µL.

2.5. Quantitation of SDS-soluble and GMP fractions

SDSS and GMP fractions were stepwise extracted from 100 mg of wholemeal flour and analyzed by GP-HPLC according to Thanhaeuser et al. (2014). GP-HPLC analysis was performed with slight modifications: elution system, water/acetonitrile/trifluoroacetic acid (TFA) 500/500/1 (v/v/v); isocratic flow rate, 0.3 mL/min. Injection volumes were: SDSS, 10 µL; GMP, 40 µL.

2.6. GlutoPeak test (GPT)

The GPT was performed according to Marti et al. (2015a). The parameters measured automatically by the GPT software were: Time of start of aggregation (lift off time, LOT, in s); maximum torque (MT, in Brabender equivalents, BE); time of maximum torque (peak maximum time, PMT, in s) and aggregation time (AGT, in s, difference between PMT and LOT). Triplicate determinations were made.