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RESEARCH ARTICLE

Comprehensive study on gluten composition and baking quality of winter wheat

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Abstract

Background and Objectives: Protein and gluten content and composition are important for the baking quality of wheat flours. Our aim was to provide a comprehensive characterization of 82 wheat flours to analyze the influence of protein composition on rheological and baking quality parameters.

Findings: Protein composition, starch gelatinization behavior, as well as rheological (microfarinograph, gluten aggregation, extensibility), and baking parameters were determined. The correlation matrix showed no significant correlations between gluten composition and loaf volume. Parameters of the gluten aggregation test allowed a prediction of gluten, gliadin, and glutenin content with an absolute root mean square error of cross validation of 7.5, 6.0, and 3.2 mg/g, respectively, using partial least squares regression. Starch gelatinization temperature had an effect on gluten aggregation.

Conclusions: The gluten aggregation test was suitable to predict gluten, gliadin, and glutenin content. The lack of correlations between protein composition and loaf volume indicates that baking quality is the result of a complex combination of different parameters.

Significance and Novelty: Our study is the first to comprehensively analyze 82 wheat flours, especially in terms of gluten composition. We show that flour blends can reach excellent baking quality even if quality indicators like crude protein or extensibility are comparatively low.

KEYWORDS

bread, gliadin, glutenin, glutenin macropolymer, starch gelatinization, wheat

1 | INTRODUCTION

Baking quality is one of the most important parameters throughout the value chain of wheat flour. Depending on the perspective, the understanding of baking quality can be quite different. As for consumers, the properties of the baked goods are mainly relevant (e.g., volume, taste and smell, crumb and crust properties). In bakeries, dough handling properties like stickiness or dough stability play an important role as well (Huen et al., 2018). In Germany, wheat cultivars are classified by the Federal Plant Variety Office into four different wheat quality classes, namely C (cookie quality), B (bread quality), A (high quality), and E (elite quality). Parameters like crop

yield, resistances against pathogens and pests, and indicators of dough and baking quality such as falling number, crude protein content, water absorption, and loaf volume are evaluated for overall quality assessment. Commercially available flours are usually mixtures of several wheat cultivars, because millers blend cultivars of similar wheat quality classes to meet customer specifications for each final product.

Gluten proteins are the most important determinant for the baking properties of wheat flour, even if starch is the main component. Gliadins and glutenins build a cohesive protein network during dough preparation, which is responsible for the viscoelastic properties of wheat dough (Amend & Belitz, 1991; Singh & MacRitchie, 2001). An optimal gluten network is necessary for volume increase during proofing, subsequent oven rise, and volume expansion during baking. When a certain temperature is exceeded, protein denaturation and starch gelatinization stabilize the structure of the bread crumb (Falcão-Rodrigues et al., 2005). A high gelatinization temperature of wheat starch is desirable to maximize oven rise (Kusunose et al., 1999).

The best method to evaluate baking quality are baking experiments. Standardized experiments like the rapid mix test were developed for the comprehensive and comparable quality rating (Pelshenke et al., 2007). Microbaking tests were established to reduce the flour amount needed to 10 g per experiment (Kieffer et al., 1993; Thanhaeuser et al., 2014). Because baking experiments are very time- and labor-intensive, other indirect quality parameters were defined to predict baking quality, including protein content (ICC Standard No 167, 2000), wet gluten content (ICC Standard No 137/1, 1994), gluten index (ICC Standard No 155, 1994), or falling number (ICC Standard No 107/1, 1995). Furthermore, the resistance of dough to extension and extensibility from extensograph (ICC Standard No 114/1, 1992) or microscale extension tests can be used as indirect quality parameters (Scherf & Koehler, 2018). In recent studies, the gluten aggregation test was considered as a suitable method to predict loaf volume and rheological parameters like water absorption, dough development, or resistance and extensibility of dough (Bouachra et al., 2017; Geisslitz et al., 2018; Michel et al., 2017).

Loaf volume can be predicted with crude protein content and Zeleny sedimentation volume with acceptable accuracy by means of linear regression models (Laidig et al., 2018). If quality is only rated based on crude protein content, quite different baking quality can be observed for flours with similar protein content, but different gluten composition (Gabriel et al., 2017). Therefore, protein quality should always be considered together with protein quantity. Laidig et al. (2017) observed that the baking quality of winter wheat increased while the crude protein content decreased and ascribed this to increased protein quality. In their study, protein quality was rated based on the Zeleny sedimentation value, but no specific analysis of gluten composition was performed. Therefore, protein quality was rated based on the swelling capability of gluten proteins in lactic acid solution and there is no quantitative data on gluten composition. Thanhaeuser et al. (2014) showed that the content of gliadins, glutenin macropolymer (GMP), glutenins, and gluten is highly correlated to baking quality assessed in the microbaking tests. However, one limitation of this study is that only 13 wheat cultivars were analyzed that had comparatively low crude protein content (8.0%–11.4%).

Studies by Rossmann et al. (2020) and Rekowski et al. (2019) analyzed the effect of late N-fertilization on crude protein content, gluten composition, and baking quality. Weak correlations of loaf volume and crude protein suggested that the changes in gluten composition had a greater influence on the baking quality than the crude protein content. In both studies, only two winter wheat cultivars of wheat quality classes A and B were analyzed in field trials and pot experiments, respectively. Rekowski et al. (2019) also extracted albumins and globulins (ALGL), gliadins, and glutenins and quantified the protein fractions via Bradford assay, but quantitative data for individual gluten protein types was not provided. A comparable result was also described by Xue et al. (2019).

Despite the availability of a number of studies on the effect of wheat protein content and composition on baking quality, only small sample sets were analyzed so far and extensive data on gluten composition is missing in many cases (Rekowski et al., 2019; Rossmann et al., 2020; Thanhaeuser et al., 2014). Furthermore, our project originated with the observation in German bakeries that flours with similar specifications may behave quite differently during manufacturing. Therefore, our main aim was to provide a comprehensive molecular and functional analysis of 82 wheat samples to analyze the influence of protein quantity and quality on rheological and baking quality parameters within a large sample set. Most studies concentrate on the impact of protein fractions on baking quality (Dias et al., 2017; Gabriel et al., 2017). We included pasting properties to analyze the influence of wheat starch and proteins on baking quality. A second aim was to evaluate the suitability of the gluten aggregation test to predict the

content of gluten and its fractions using novel partial least squares (PLS)-regression models.

2 | MATERIALS AND METHODS

2.1 | Wheat samples

Seventy German winter wheat (*Triticum aestivum* var. *aestivum*) grain samples of 24 different cultivars of wheat quality classes B, A, and E from different growing locations and harvest years were obtained from mills and breeders. Samples were tempered with water to a standardized moisture content of 13.5% and milled on a Quadrumat senior mill (Brabender) to yield white flours of type 550 (according to the German flour classification system, i.e., ash content 0.51%–0.63% based on the dry matter). Flour yield was increased using a bran duster (Brabender). Furthermore, 12 commercially available blended flours of type 550 from nine different mills were analyzed. All single cultivars and commercially available flours were without any additives. Single cultivar grains were obtained from the harvest years 2018, 2019, and 2020. The wheat for the commercially available flours was harvested in 2018. In total, 82 flour samples were part of the sample set.

We focused on two main aspects for sample selection. First, we selected the 10 most important German wheat cultivars in terms of production quantity and use. As the cultivation area of spring wheat is only about 2% that of winter wheat in Germany, we only used winter wheat samples. Second, the most important high-molecular-weight glutenin subunit (HMW-GS) alleles (Glu A1: n, 1, 2*; Glu B1: 6 + 8, 7, 7 + 8, 7 + 9, 17 + 18; Glu D1: 2 + 12, 3 + 12, 5 + 10) should be within the sample set. In total, 9 samples were wheat quality class B, 50 were class A, and 11 were class E. Twelve samples were commercially available wheat flours which were a mixture of unknown compositions (Table 1). Wheat quality class C was not considered, because it is not used for bread making. All data presented in the following are grouped according to the wheat quality class and given as the mean and standard deviation of each quality class. In case no significant differences were observed, the minimum and maximum of the total sample set are indicated.

2.2 | Flour characterization

Moisture content was determined with an infrared moisture analyzer MA35M (Sartorius) by heating to 100°C until a constant weight was reached. Crude protein content of wheat flours was determined with the Dumas combustion method. Flour (50 mg) was weighed into a tin foil cup and analyzed with a Leco TruSpec nitrogen analyzer (Leco). A conversion factor of 5.7 was used to calculate crude protein content (ICC Standard No 167, 2000). The enzyme assay K-SDAM (Megazyme) was used to determine the content of damaged starch (ICC Standard No 164, 1996). Falling number was determined according to ICC Standard No 107/1 (1995).

Water absorption and dough development time were determined in a microfarinograph (Brabender). Ten grams of flour (8.6 g dry matter) were kneaded with water in a Z-blade mixer (Brabender) to form a dough with a consistency of 550 BU (Kieffer et al., 1998). The room temperature and humidity for all rheological experiments were constant at 22 ± 2°C and ≥60% relative humidity.

Gluten index was determined following ICC Standard No 158 (1995) with some variations. A dough from 10 g of flour (8.6 g dry matter) and water was prepared as described above. The dough was allowed to rest for 3 min and was then washed in a Glutomatic 2200 (Perten) for 10 min with 0.4 mol/L NaCl solution. After washing and resting for 30 s, the wet gluten was centrifuged in a special sieve for 1 min at 6000 rpm. Gluten index was calculated as the ratio of the part remaining on top of the sieve after centrifugation and the total wet gluten content. Wet gluten was lyophilized to determine dry gluten content.

TABLE 1 Summary of the wheat cultivars within the sample set

Wheat quality class	Number of samples	Cultivars
B	9	Bosporus (1), Dekan (4), Matrix (1), Platin (2), Porthus (1)
A	50	Emerick (1), JB Asano (3), Julius (10), Kashmir (1), Leandrus (2), Linus (4), Meister (8), Opal (2), Patras (4), RGT Reform (11), Spontan (1), Tiger (1), Tobak (2)
E	11	Akteur (3), Axioma (1), Barranco (2), Kerubino (1), Moschus (2), Ponticus (2)
Commercial	12	Unknown

Note: Numbers in parentheses give the number of different samples of each cultivar, either from different locations and/or from different harvest years.

2.3 | Determination of wheat protein composition

Extraction and quantification of ALGL, gliadins, and glutenins were performed according to Wieser et al. (1998). Flour (100 mg) was extracted in three subsequent steps with Na_2HPO_4/KH_2PO_4 buffer (0.67 mol/L, pH = 7.6) containing 0.4 mol/L NaCl, with 60% (v/v) aqueous ethanol and with 0.1 mol/L TRIS/HCl buffer (pH = 7.6)/1-propanol (50/50; v/v) containing 2 mol/L urea and 10 mg/ml dithiothreitol (DTT) to extract ALGL, gliadins and glutenins, respectively. All fractions were analyzed by reversed-phase (RP)-high-performance liquid chromatography (HPLC) on a Jasco XLC HPLC (Jasco Deutschland GmbH) using a Dionex Acclaim 300 C_{18} (3 μm, 2.1 × 150 mm) at 60°C. Water and acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA) were used as eluents with a flow rate of 0.2 ml/min. Linear gradients were used as follows: ALGL: 0 min 20% ACN; 5 min 60% ACN; gliadins and glutenins: 0 min 24% ACN; 15 min 54% ACN. The column was cleaned for 4 min with 90% ACN and for 10 min with 100% water. Proteins were detected by measuring UV absorbance at 210 nm. Injection volumes were 20 μl for ALGL and glutenins and 10 μl for gliadins. For calibration, PWG-gliadin (2.5 mg/ml) was dissolved in 60% aqueous ethanol (v/v), and analyzed to correlate peak areas and protein content. Injection volumes were 5, 10, 15, and 20 μl. The gluten content is the sum of gliadins and glutenins. Gluten protein types are given as percentage in relation to the gluten content to better reflect gluten composition.

2.4 | Determination of GMP

Sodium dodecyl sulfate soluble (SDSS) proteins and GMP were quantified according to Gupta et al. (1993) and Thanhaeuser et al. (2014). Flour (100 mg) was extracted (2 × 1 ml) with 0.05 mol/L Na_2HPO_4 solution (pH = 6.9) containing 1% SDS to extract SDSS. The residue was extracted (2 × 1 ml) with 0.05 mol/L Na_2HPO_4/KH_2PO_4 buffer (pH = 7.5) containing 10 mg/ml DTT. Extracts were analyzed by GP-HPLC using a Jasco Extrema HPLC (Jasco) with a Phenomenex BioSep SEC 3000 column (40 nm, 5 μm, 4.6 × 300 mm) and detection of UV-absorbance at 210 nm. An isocratic flow with water/ACN 50/50 (v/v) with 0.1% (TFA) was used as eluent at a flow rate of 0.3 ml/min. Injection volumes were 7 μl for SDSS and 40 μl for GMP. Chromatograms of GMP showed two peaks, which were not baseline-separated (Mueller et al. 2016). These peaks were quantified separately as HMW-GMP and low-molecular-weight GMP (LMW-GMP). HMW- and LMW-GMP are given as the percentage in relation to the gluten content.

2.5 | Gluten aggregation test

The gluten aggregation test was performed according to Marti et al. (2015) using 8.5 g of flour and 9.5 ml of water. The GlutoPeak (Brabender) was heated to 36°C. All samples and water were preheated. The measurement was carried out for 5 min. The paddle rotated with a speed of 3000 rpm. Data evaluation was performed with the GlutoPeak Software Version 2.2.9 (Brabender) using the "extended" evaluation profile. The curve evaluation includes peak maximum time (PMT), maximum torque (MT), torque 15 s before PMT (ante maximum, AM), and 15 s after PMT (postmaximum, PM). Five areas under the curve are integrated and borders are set depending on the aggregation curve (Supporting Information: Figure S1) as follows: A0 is the beginning of the measurement. A1 is set on the first local maximum when aggregation starts. A2 is a local minimum between A1 and PMT. A3 and A5 are 15 s before and after PMT, respectively. A4 is equal to PMT. All evaluated areas and parameters were considered for correlation analysis.

2.6 | Starch gelatinization and cooling behavior

Analysis of starch gelatinization was performed using the ViscoQuick device (Brabender) and the standard method for starch and flour analysis. Flour (10 g; 8.6 g dry matter) was mixed with 105 g of water. The suspension was heated to 30°C and stirred by a metal paddle with 250 rpm. During the measurement, a temperature profile is used (Supporting Information: Figure S2A). The sample cup is heated to 93°C with 20°C/min. This temperature is held for 3 min. Then, the sample cup is cooled down to 50°C with a cooling rate of −15°C/min. The measurement stops 1 min after 50°C is reached. The software evaluates the following parameters (Supporting Information: Figure S2B): temperature when starch gelatinization starts (a), maximum viscosity hot ($\eta_{max93,}$ sample cup 93°C); (b) and cold ($\eta_{max50,}$ sample cup 50°C; (c) and minimum viscosity η_{min} (d). Breakdown (difference of b and 2) and setback (difference of 2 and 3,) which is the increase of viscosity during the cooling period, are also evaluated. Time and torque are evaluated for points a, b, c, and d. Evaluation points 1, 2, and 3 depend on the temperature profile and, therefore, only torque is evaluated. Only sample curve-dependent parameters (temperature when starch gelatinization starts, η_{max93}, η_{max50}, and η_{min}) as well as breakdown and setback were considered for correlation analysis.

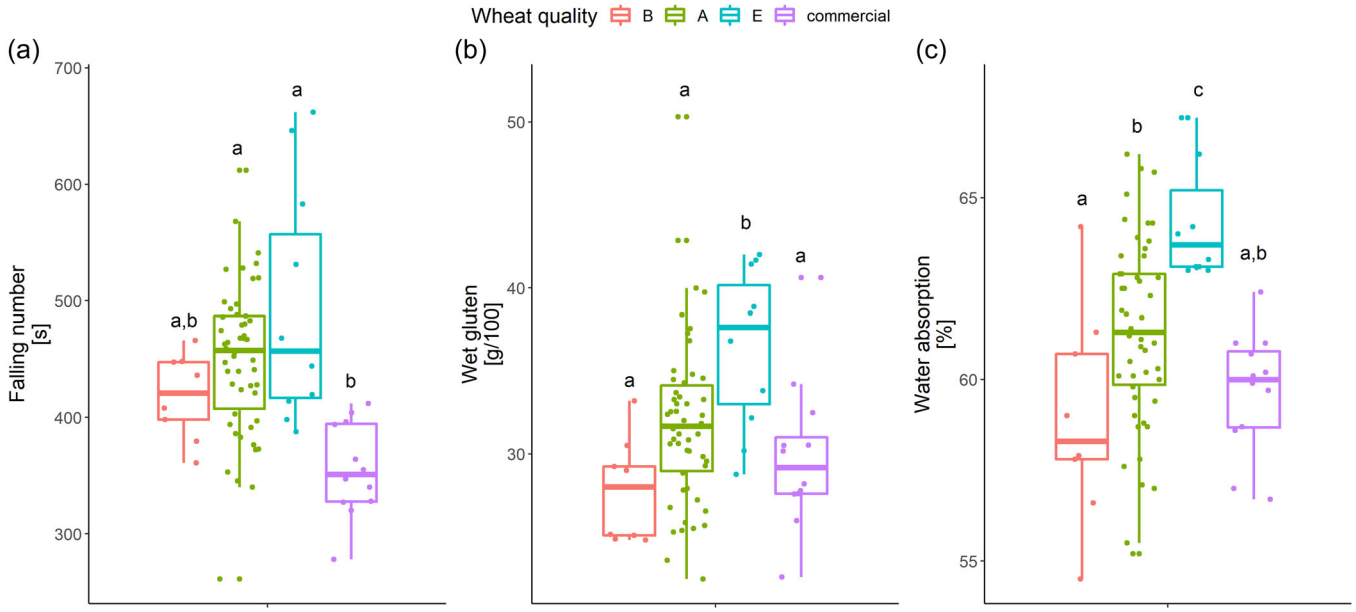

FIGURE 1 Median (line in the box) and ranges of falling number (a), wet gluten content (b), and water absorption (c) of 82 wheat flour samples. Boxes represent the interquartile range and whiskers the minimum and maximum, respectively. Means of groups with different small letters are significantly different (one-way analysis of variance; Tukey's test; $p < .05$). [Color figure can be viewed at wileyonlinelibrary.com]

2.7 | Microscale extension test

Microscale extension tests on dough were performed with a Texture Analyzer TA.XT plus with the SMS/Kieffer Dough and Gluten Extensibility Rig (Stable Micro Systems) according to Köhler and Grosch (1999) with the following modifications. After pressing into a polytetrafluoroethylene mold, the resting time for the dough strands was only 15 min (Scherf & Koehler, 2018). Evaluated parameters are the following: maximal force R_{max}, the distance to R_{max} (E_{Rmax}), the maximal extension when the dough strand breaks (E_{max}), areas under the curve from start to E_{Rmax} (A_{Rmax}), and the area under the whole curve (A_{max}).

2.8 | Microbaking test

The microbaking tests were conducted according to Thanhaeuser et al. (2014). The ingredients were 10 g of wheat flour (8.6 g dry matter), 2% NaCl, 1% shortening (Peter Kölln) and 7% fresh baker's yeast (F.X. Wieninger), and water according to the farinograph. Volume and height/width (H/W) ratio were determined by a VolScan Profiler 600 (Stable Micro Systems). The specific volume was calculated based on the weight of the baked bread rolls after cooling for 2 h.

2.9 | Data analysis

All determinations were performed in triplicate. Means and standard deviations for all quality classes are summarized in Supporting Information: Tables S1–S4. Pearson and Spearman correlation coefficients were calculated in R (Version 4.1.1) using the Hmisc package. Significant correlations were present at a significance level of $p < .05$. Correlations were classified as very weak ($.41 \le r < .54$), weak ($.54 \le r < .67$), medium ($.67 \le r < .78$), and strong ($r \ge .78$). A principal component analysis (PCA) was performed to evaluate multivariate correlations between all parameters using Origin 2020 (OriginLab Corporation). One-way analysis of variance to identify significant differences between samples sorted by quality class was performed in Origin 2020 (OriginLab Corporation). Differences were identified with Tukey's test at a significance level of $p < .05$. The PLS-regressions were calculated with The Unscrambler X (Camo Analytics) using the nonlinear iterative partial least squares (NIPALS) algorithm. All PLS-regression models were cross validated.

3 | RESULTS

3.1 | Flour characterization

Moisture content ranged from 9.6 to 14.0 g/100 g and there were no significant differences between the

means of the four quality classes (Supporting Information: Table S1). The content of damaged starch (3.2%–7.9%) and gluten index (0.2–1.0) also showed no significant differences within the four classes. Falling number (Figure 1a) was significantly lower in commercial wheat flour samples than in samples of classes A and E, but not significantly lower than in samples of class B. The wet gluten content (Figure 1b) of classes A, B, and commercial wheat flours was not significantly different, but that of class E was significantly higher. Class E also had a significantly higher dry gluten content than class A, B, and commercial flours. There was an increasing trend from classes A to E for both wet and dry gluten content.

Water absorption (Figure 1c) rose significantly from class B to E. Commercial flour samples had comparable water absorption as samples of classes A and B. Dough softening of class E was significantly lower than of class A, whereas there were no significant differences between the other quality classes (Supporting Information: Figure S3A). In total, dough softening ranged from 0.0 to 120.0 BU. Dough development time of class E was significantly higher compared to class A. Dough stability was significantly lower for class A compared to class B and commercial flour samples, while class E was not significantly different to the other classes (Supporting Information: Figure S3B and Table S1).

3.2 | Protein content and gluten composition

The crude protein content of flour samples belonging to class E was significantly higher than of classes B and A and the commercial flour samples. An increasing trend was observed from wheat quality classes B to E (Supporting Information: Table S2). The gluten content (Figure 2a) also showed an increasing trend with increasing wheat quality. Commercial flour samples had a gluten content comparable to class B. Significant differences were observed between class E and classes A, B, and commercial flour samples. In total, crude protein and gluten content ranged from 9.7 to 16.4 g/100 g and 5.8 to 12.7 g/100 g, respectively, which is a typical range for German winter wheat flour (Caporaso et al., 2018; Pronin et al., 2020).

No significant differences were observed for the ALGL content (0.6–2.0 g/100 g) (Supporting Information: Figure S4A). Similar to the gluten content, an increasing trend was observed for gliadin, glutenin, and GMP content with increasing wheat quality. The gliadin content of class B and commercial flour samples was significantly lower than that of class E, whereas that of class A was not significantly different compared to the other classes. The glutenin content of classes B, A, and commercial flour samples was significantly lower than that of class E.

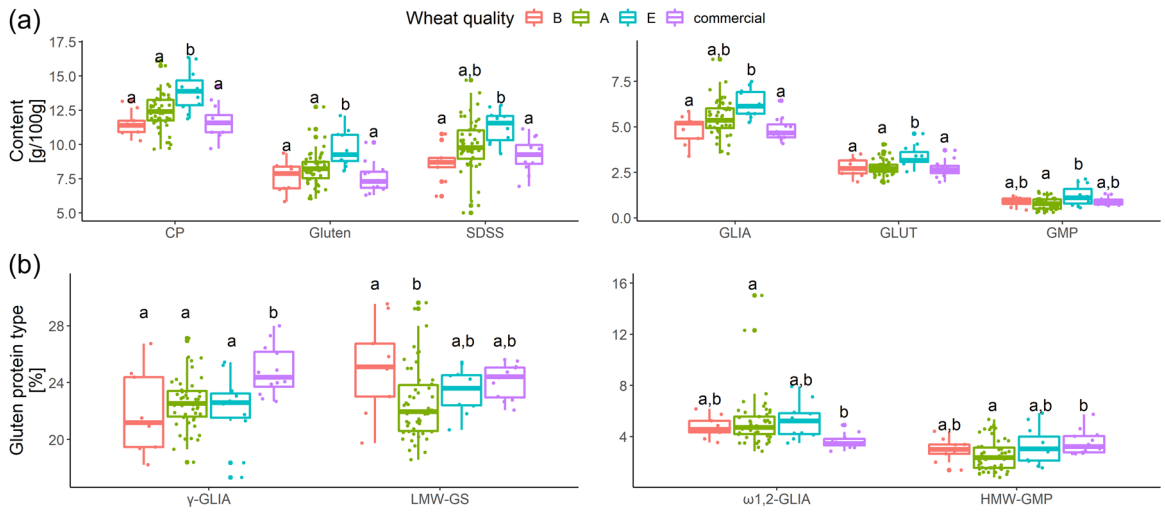

FIGURE 2 Median (line in the box) and ranges of content of crude protein (CP) and wheat protein fractions (a). Gluten protein types and GMP are given as percentage of the total gluten content (b). Boxes represent the interquartile range and whiskers the minimum and maximum, respectively. Means of groups with different small letters are significantly different (one-way ANOVA; Tukey's test; $p < .05$). ANOVA, analysis of variance; GLIA, gliadins, GLUT, glutenins, GMP, glutenin macropolymer, HMW-GS, high-molecular-weight glutenin subunits, LMW-GS, low-molecular-weight glutenin subunits; SDS, sodium dodecyl sulfate; SDSS, SDS-soluble. [Color figure can be viewed at wileyonlinelibrary.com]

GMP content ranged from 0.3 to 2.1 g/100 g and was comparable to earlier reports (Don et al., 2003a; Thanhaeuser et al., 2014). The GMP content of class E was significantly higher than that of class A. SDSS content of class E was significantly higher than of class B and commercial flours.

Proportions of α-gliadins, ω5-gliadins, ωb-gliadins, HMW-GS, and LMW-GMP ranged from 27.9% to 41.2%, 0.8% to 8.0%, 0.5% to 2.3%, 6.3% to 12.3%, and 2.4% to 14.1% of the total gluten content, respectively, and there were no significant differences (Supporting Information: Figure S4B). The percentage of γ-gliadins of the commercial flours was significantly higher compared to the other classes (Figure 2b). The proportion of LMW-GS in flour samples of class A was significantly lower than of class B. For class E and commercial flour samples, there was no significant difference to classes A and B. ω1,2-Gliadins of class A were significantly higher compared to commercial flour samples. HMW-GMP of quality class A was significantly lower compared to commercial wheat flours.

The gliadin/glutenin ratio and SDSS/GMP ratio were not significantly different between wheat quality classes (Supporting Information: Figure S4C). The LMW-GS/HMW-GS ratio was significantly lower for classes A and E compared to class B (2.7 ± 0.5). The LMW-GMP/HMW-GMP ratio of commercial flours was significantly lower than of classes B, A, and E.

3.3 | Gluten aggregation test

The PMT of commercial wheat flour samples was significantly higher than of classes A and E. AM, MT, and PM showed an increasing trend with increasing wheat quality (Figure 3). AM and MT were significantly higher for class A than for commercial flour samples. AM and MT of class E were significantly higher compared to all the other classes. PM of class A was significantly higher than of class B and commercial wheat flours. The mean PM of class E was significantly higher compared to class B, A, and commercial flours.

Areas A01 and A12 did not show significant differences. Area A12 showed a decreasing trend with increasing wheat quality (Supporting Information: Table S3). Area A23 was significantly higher for commercial wheat flours than for class A. Areas A34 and A45 are the areas describing the peak of the aggregation curve. Both areas showed increasing trends with increasing wheat quality. A34 and A45 of class E were significantly higher than of class A. Areas of class B and commercial wheat flours were significantly lower than of classes A and E.

3.4 | Gelatinization behavior

Besides gluten proteins, starch gelatinization is known to influence the baking quality, and therefore, we analyzed the flour gelatinization behavior to exclude this as a potentially confounding factor for loaf volume (Eliasson et al., 1995). Commercial flour samples had significantly higher gelatinization temperatures than the single cultivar flours (Figure 4A and Supporting Information: Table S4). Viscosity parameters η_{max93} and η_{max50} showed no significant differences, but an increasing trend was observed with increasing wheat quality class. Viscosity η_{min} was significantly lower for commercial flours than

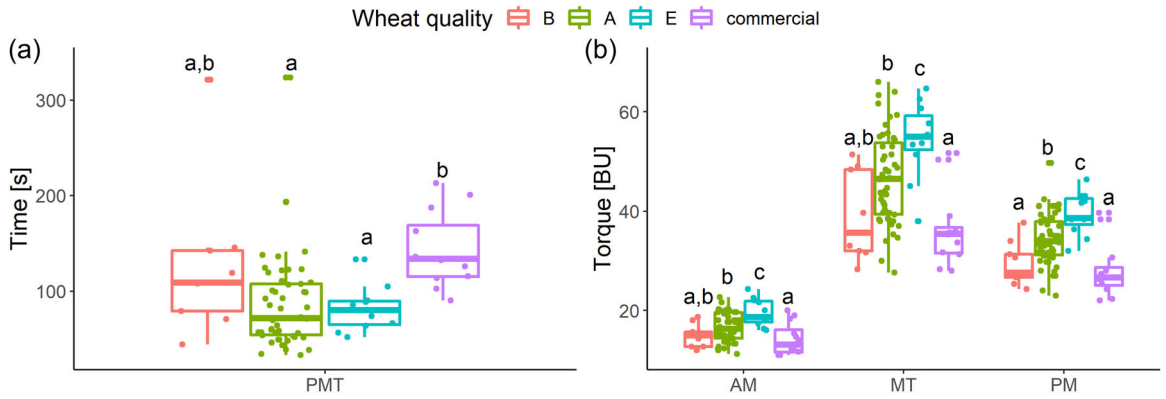

FIGURE 3 Median (line in the box) and ranges of gluten aggregation test parameters of the 82 wheat flour samples, including time (a) and torque at different times (b). Boxes represent the interquartile range and whiskers the minimum and maximum, respectively. Means of groups with different small letters are significantly different (one-way ANOVA; Tukey's test; $p < .05$). AM, ante maximum: torque 15 s before PMT; ANOVA, analysis of variance; BU, Brabender units; MT, maximum torque; PM, postmaximum: torque 15s after PMT; PMT, peak maximum time. [Color figure can be viewed at wileyonlinelibrary.com]

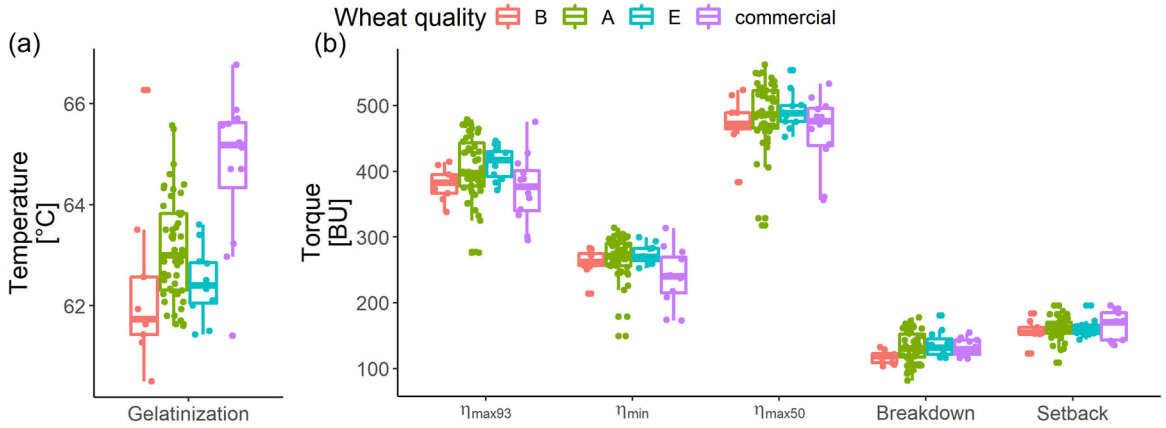

FIGURE 4 Median (line in the box) and ranges of starch gelatinization behavior of the 82 wheat flour samples, including temperature (a) and torque at different times (b). Boxes represent the interquartile range and whiskers the minimum and maximum, respectively. Means of groups with different small letters are significantly different (one-way ANOVA; Tukey's test; $p < .05$). ANOVA, analysis of variance; BU, Brabender units; η_{max93}, maximum viscosity hot; η_{max50}, maximum viscosity cold; η_{min}, minimum viscosity. For visualization of curves showing starch gelatinization behavior, please refer to Supporting Information: Figure S2. [Color figure can be viewed at wileyonlinelibrary.com]

for classes A and E (Figure 4b). Breakdown (82–181 BU) and setback (109–196 BU) were not significantly different.

3.5 | Microscale extension test

Dough extensibility was analyzed with microscale extension tests (Supporting Information: Figure S5). The maximum force needed to extend dough strands was significantly higher for commercial wheat samples than for class A. Extensibility showed an increasing trend for increasing wheat quality (Table S4). Both E_{Rmax} and E_{max} were significantly higher for samples of class A compared to commercial wheat flour samples. E_{Rmax} and E_{max} of wheat quality class E were significantly higher than of class B and commercial flour samples. Similar to the extensibility, the areas under the curve also showed an increasing trend. A_{Rmax} and A_{max} of samples from class E were significantly higher than of class B, A, and commercial flours.

3.6 | Microbaking test

Breads had volumes from 32.0 to 51.7 ml. Loaf volume increased significantly from classes B to E, whereas classes B and A were not significantly different. Commercial flours had loaf volumes between classes A and E and were, therefore, significantly higher than loaf volumes of class B (Figure 5A and Supporting Information: Table S4). The specific volume of baked breads showed comparable results, but significant differences were only observed between classes B and E and commercial flour samples (Figure 5b). Breads with an H/W ratio close to 1 are nearly round, because height and width are almost the same. Breads of class B had the highest H/W ratio and were significantly higher than classes A and E. H/W ratios of commercial wheat flours were not significantly different to the other classes (Figure 5c).

3.7 | Correlation analysis

Pearson correlation coefficients were calculated to analyze the influence of the gluten composition on baking quality and functional properties over all samples (Figure 6) and over samples from each class, respectively (Supporting Information: Figures S6–S9). To include potential nonlinear correlations, Spearman correlation coefficients (data not shown) were also calculated, but essentially agreed with those of Pearson. Gluten aggregation parameters showed several significant correlations to gluten fractions and gluten protein types. PMT was very weakly correlated to the content of damaged starch ($r = -.42$), wet ($r = -0.49$), and dry gluten content ($r = -.49$), gliadin content ($r = -.41$), ratio of LMW-GS/HMW-GS ($r = .52$), and ratio of SDSS/GMP ($r = -0.45$). MT showed strong correlations to wet and dry gluten content ($r = .81$ and $r = .78$, respectively). The total gluten content ($r = .72$) as well as gliadins ($r = .71$), glutenins ($r = .55$), and SDSS ($r = .60$) had medium or weak correlations to MT. All gluten protein types were significantly correlated to MT (r(ω5-gliadins) = .53, r(ω1,2-gliadins) = .44, r(α-gliadins) = .69, r(γ-gliadins) = .56, r(HMW-GS) = .59, r(LMW-GS) = 0.48). AM and PM were also correlated to the

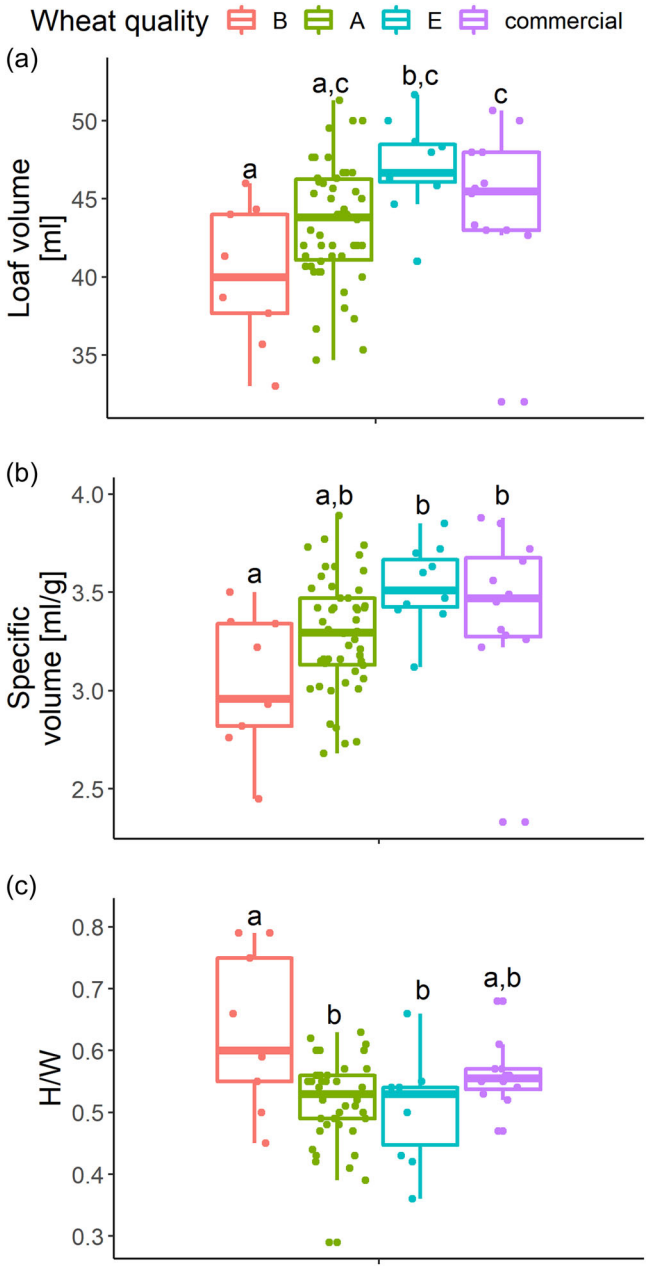

FIGURE 5 Median (line in the box) and ranges of parameters from the microbaking tests, including loaf volume (a) and specific volume calculated from loaf volume divided by bread weight after baking (b) of the 82 wheat flour samples. Height-to-width (H/W) ratio (c) was determined for the middle slice of the bread rolls. Boxes represent the interquartile range and whiskers the minimum and maximum, respectively. Means of groups with different small letters are significantly different (one-way analysis of variance; Tukey's test; $p < .05$). [Color figure can be viewed at wileyonlinelibrary.com]

same parameters as MT. AM was not significantly correlated to γ-gliadin and SDSS content. Correlation coefficients of AM and all protein types were lower than of MT and PM. Areas A01, A12, and A23 did not correlate to any protein fraction or gluten protein type. A01 was only very weakly correlated to the content of damaged starch ($r = .49$). Areas A34 and A45 were significantly correlated to wet ($r = .80$ and $r = .83$) and dry gluten content ($r = .78$ and $r = .81$), crude protein ($r = .68$ and $r = .69$), gluten ($r = .77$ and $r = .76$), gliadin ($r = .73$ and $r = .74$), and glutenin content ($r = .64$ and $r = .61$) as well as all glutenin protein types (r(HMW) = .65; r(LMW) = .60 and r(HMW) = .63; r(LMW) = .55).

Due to significant correlations of gluten aggregation parameters and gluten proteins, the gluten aggregation test was assumed to be suitable to predict gluten composition (Marti et al., 2015). Therefore, PLS-regression models were calculated to predict gluten, gliadin, and glutenin content (Supporting Information: Figure S10). Different combinations of parameters evaluated from the aggregation profiles were assessed to optimize the models for PLS regression. The best results were achieved using all data evaluated as described above (2.5). Gluten, gliadins, and glutenins were predicted with a root mean square error of cross validation (RMSECV) of 7.5, 6.0, and 3.2 mg/g, respectively.

Starch gelatinization temperature was weakly correlated to the content of damaged starch ($r = -.54$). Viscosity parameters η_{min} and η_{max50} were weakly ($r = .54$) and very weakly ($r = .50$) correlated to the falling number.

Correlation analysis of the farinograph parameters revealed that water absorption was correlated to wet gluten ($r = .63$) and dry gluten content ($r = .59$). Furthermore, there were correlations of water absorption and crude protein ($r = .49$), gluten ($r = .59$), gliadin ($r = .57$), and glutenin content ($r = .46$). Dough development time was correlated to GMP ($r = .64$), HMW-GMP ($r = .60$), and LMW-GMP ($r = .64$) as well as SDSS/GMP ratio ($r = -.57$). Very weak correlations were observed for dough stability and GMP ($r = .43$), LMW-GMP ($r = .44$), and SDSS/GMP ratio ($r = -.59$). Dough softening was negatively correlated to GMP ($r = -.54$), HMW-GMP ($r = -.52$), and LMW-GMP ($r = -.54$) as well as SDSS/GMP ratio ($r = .55$).

From the parameters of the microscale extension test, R_{max} was very weakly correlated to the gliadin/glutenin ratio ($r = -.43$), LMW-GMP/HMW-GMP ratio ($r = -.46$), SDSS/GMP ratio ($r = .49$), and HMW-GMP ($r = .45$). E_{max} was correlated to wet gluten ($r = .58$) and dry gluten content ($r = .58$), crude protein ($r = .47$), gluten ($r = .51$), gliadin ($r = .58$), and SDSS content ($r = .50$). Furthermore, ω5-gliadins ($r = .42$) and α-gliadins ($r = .61$) were correlated to E_{max}. E_{max} was also correlated to LMW-GMP/HMW-GMP ratio ($r = .45$). For area A_{max}, correlations to the crude protein content ($r = .48$), gluten content ($r = .47$), as well as wet gluten content ($r = .42$) were observed. Glutenin content ($r = .55$), HMW-GS

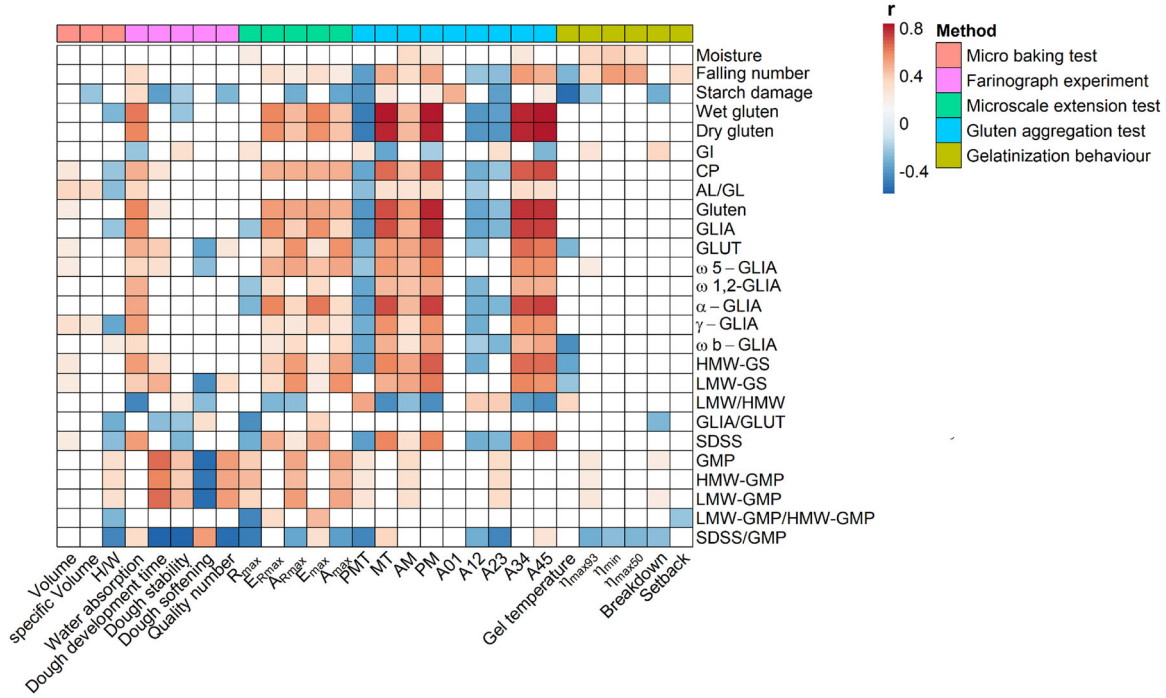

FIGURE 6 Pearson correlation coefficients (r) of gluten composition and functional characteristics of the 82 wheat flour samples determined by microbaking tests, microfarinograph experiments, microscale extension tests, gluten aggregation tests, and gelatinization behavior. The level of significance was set to $p < .05$ and white boxes indicate nonsignificant correlations ($p \geq .05$). For abbreviations, please refer to legends of Figures 1–5 and Supporting Information: Figure S5. [Color figure can be viewed at wileyonlinelibrary.com]

($r = .51$), and LMW-GS ($r = .56$) were correlated to A_{max}. Furthermore, GMP ($r = .50$), HMW-GMP ($r = .50$), and LMW-GMP ($r = .45$) were correlated to the area A_{max}. Similar correlations were observed for E_{Rmax} and A_{Rmax}, respectively. Dry gluten content ($r = .43$) was only correlated to A_{Rmax}. Parameters from microbaking tests did not show significant correlations to any parameter of the gluten composition. The only significant correlation was between H/W ratio and SDSS/GMP ratio ($r = -.46$).

A PCA was calculated considering all parameters of the data set to evaluate multivariate correlations (Supporting Information: Figure S11). Looking at the loadings plot, no correlations of baking quality parameters and functional properties were identified. Wheat quality classes do not cluster in the scores plot.

4 | DISCUSSION

Moisture content, starch damage, falling number, and water absorption of the wheat samples analyzed in our study were in a typical range for wheat samples (Duyvejonck et al., 2011). Falling number, water absorption, and crude protein content are part of the classification scheme of the Federal Plant Variety Office (2021) and, therefore, an increasing trend from quality classes B to E was expected. Extensibility also increased from classes B to E, with E_{max} being strongly inversely correlated to R_{max} ($r = -.79$). The correlations identified for microscale extension test parameters were in line with earlier reports (Wieser & Kieffer, 2001).

Correlations of crude protein, wet and dry gluten as well as gliadin and glutenin content with water absorption are in accordance with literature (Preston et al., 2001; Tipples et al., 1978). The GMP content was not significantly different for all quality classes, whereas the GMP content of class E was slightly higher compared to the others. Dough development time, dough stability, and dough softening are ascribed to the strength of the gluten network (Weegels et al., 1996; Wooding et al., 1999), which is affected by glutenins and GMP (Don et al., 2003b). Flours with higher GMP content were less susceptible for overmixing as dough softening was lower and dough stability was higher.

Inverse trends of PMT and MT were expected and a negative correlation was indeed observed ($r = -.75$). Commercially available flours are usually a mix of different classes to meet customer specifications and mostly have medium-strong gluten properties. Thus, higher PMT and lower MT compared to the other quality classes were expected (Michel et al., 2017). Correlations of gluten, gliadin, glutenin, and SDSS content and MT were already

reported in the literature (Marti et al., 2015), but none between gluten protein types and gluten aggregation parameters. Protein fractions and gluten protein types showed significant correlations to most of the gluten aggregation parameters. As a result, the gluten aggregation test appears to be a suitable method to predict the quantitative gluten composition using PLS-regression models. PLS-regression models were calculated to predict gluten, gliadin, and glutenin content with an RMSECV of 7.48 mg/g (relative error: 5.7%–12.4% for minimum and maximum of the calibrated range), 6.01 mg/g (relative error: 6.9%–17.8%), and 3.23 mg/g (relative error: 6.7%–15.8%), respectively. PLS-regression models for gluten protein types showed relative deviations of up to 100% (data not shown) and were rejected. Gluten aggregation parameters were found to be suitable to predict baking parameters (Geisslitz et al., 2018). However, we found no correlations of gluten aggregation and bread quality parameters in this sample set (data not shown).

There were some correlations of starch properties and parameters of the gluten aggregation test, for example, between PMT and starch damage as well as gelatinization temperature ($r = .56$). Furthermore, the gelatinization temperature was correlated to MT ($r = -.48$) and areas under the curve A23 ($r = .47$), A34 ($r = -.43$), and A45 ($r = -.47$). Flours containing starch with lower gelatinization temperatures have an earlier PMT and a smaller area A23, but larger areas A34 and A45. The energy input of the metal pedal during the measurement of gluten aggregation is very high and the sample slurry temperature increased during measurement. Therefore, starch gelatinization seemed likely to occur as an unintended mechanism influencing the gluten aggregation profiles. In accordance with earlier reports, starch damage was negatively correlated to the gelatinization temperature (Ma et al., 2016) and, therefore, also correlated to PMT.

A high gelatinization temperature is desirable to maximize oven rise (Kusunose et al., 1999). The gelatinization temperature of commercial wheat flours was significantly higher compared to the single cultivar flours and this might be one factor that contributed to their good baking performance despite a comparably low protein content. No differences were observed for all viscosity parameters, which implies that the viscous properties of all analyzed flour samples were very similar. The content of damaged starch was negatively correlated to gelatinization temperature, which is in accordance to literature (León et al., 2006; Liu et al., 2017). The falling number is an indicator of amylase activity in wheat flour with lower starch degradation when the falling number is high. Therefore, viscosity parameters η_{min} and η_{max50} are positively correlated to the falling number (He et al., 2019).

The baking performance of cultivars in the RMT is an important parameter of wheat quality assessment. Therefore, the increasing trend of loaf volume and specific volume from quality classes B to E in the sample set was according to expectations. Commercial wheat flours had comparable baking performance to wheat quality class E. This emphasizes that comparably low crude protein, falling number, and water absorption are sufficient to exceed the baking performance of wheat quality classes B and A.

Several studies identified crude protein, gluten, and GMP content as important quality indicators for the baking quality of wheat flour (Dowell et al., 2008; Sapirstein & Suchy, 1999). In the analyzed sample set, we found no correlation of crude protein and gluten content to loaf volume ($r = .24$ and $r = .22$, respectively) or H/W ratio ($r = -.22$ and no significant correlation, respectively) in the microbaking test. Recent studies discuss that protein quality is more important for baking quality than quantity (Gabriel et al., 2017; Laidig et al., 2017), but we found no significant correlation of protein fractions or gluten protein types and loaf volume or specific volume. Only the ratio of SDSS/GMP was very weakly correlated to the H/W ratio. This parameter is an indicator of the polymerization state of glutenins in flour and gave a hint that breads were less likely to spread laterally with higher GMP content.

Since Payne et al. (1987) established quality scores for HMW-GS alleles, several studies confirmed the result that some alleles are associated with better baking quality than others (Branlard & Dardevet, 1985; Brönneke et al., 2000; Jiang et al., 2019; Jonnala et al., 2010; Luo et al., 2001). Yet other studies report that HMW-GS alleles are not the dominant factor that influences baking quality (Shewry et al., 1992). Scatter plots of gluten content and loaf volume grouped by Glu A1, Glu B1, Glu D1 alleles, and the combined allelic variation (Supporting Information: Figure S12) showed no correlation between different alleles and loaf volume or gluten content in our study. In accordance with Moloi et al. (2017), this implies that other factors are more important than allelic disposition. If baking quality is defined by loaf volume and H/W ratio of breads, our data revealed that baking tests are indeed necessary to determine the baking quality of a large sample set. One limitation of our study is that we only included winter wheat flours from Germany without sufficient information on growing conditions. This is why our data set cannot be directly transferred to international samples or used for the analysis of genetic versus environmental variability regarding protein composition and baking quality.

We are well aware of the fact that the lack of correlation between protein content or individual gluten components and loaf volume in our sample set questions some well-established positive correlations, especially

between HMW-GS or GMP and loaf volume (Don et al., 2006; Thanhaeuser et al., 2014; Wieser & Kieffer, 2001). One aspect to consider is the validity of the microbaking test itself to assess loaf volume. It is based on only 10 g of flour and a comparatively high amount of fresh yeast (7%) and may, therefore, yield different results compared to the RMT or other standardized baking tests. We are currently investigating how well the loaf volumes obtained by the microbaking test relate to other baking tests that are much closer to large-scale industrial baking processes. Another point to consider is that most previous studies only looked at about 10 samples from different quality classes. Due to the inherent differences in protein content and baking quality, this selection may have produced a correlation that is lost in our much larger sample set with 82 samples, of which 50 represent class A. Further, our understanding of wheat glutenin polymers and their interactions remains incomplete (Shewry & Lafiandra, 2022) and requires more fundamental investigations to fully comprehend how gluten proteins determine baking quality. Our study highlights the need to go back to the drawing board and re-think some of the established concepts.

5 | CONCLUSION

Correlation analysis of gluten composition and gluten aggregation test parameters revealed that the gluten aggregation test is suitable as a quick method to predict gluten composition. PLS-regression models with acceptable RMSECV were calculated for gluten, gliadin, and glutenin content. Despite extensive characterization of all flour samples, no factors were identified which had an influence on loaf volume or H/W ratio. The results of microbaking tests were according to the quality classification of wheat samples. Our data indicate that baking quality is the result of a complex combination of different parameters that cannot be approximated by one single parameter. Finding a parameter combining quality traits of starch and protein parameters, should be in focus for further research. Nevertheless, one important novel aspect is that suitable flour blends can reach excellent baking results even with comparably low protein content.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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