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Comprehensive analysis of spelt flour composition and breadmaking quality

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ABSTRACT

Spelt is an ancient wheat variety with less-developed and less-studied baking properties. We developed an optimised baking procedure for spelt and analysed 30 spelt lines from three locations each for dough and baking quality as well as protein and starch composition. Spelt dough with 3% yeast, a mixing rate of 40 rpm, and a dough consistency of 350 BU produced the highest specific bread volume in our study design. Overall, the environment had a greater impact on most parameters than genetics. Correlation analysis revealed positive correlations between crude protein, gliadins, α-gliadins, and γ-gliadins and the maximum torque in the Gluto-Peak test, respectively, as well as water absorption. Regarding baking quality, we did not find a significant and high correlation between the specific bread volume and any of the analysed analytical parameters, except for the composition of high-molecular-weight glutenin subunits (HMW-GS). Lines with HMW-GS Dx5 +Dy10 produced breads with a higher specific bread volume than those with the combination Dx2 +Dy12. Our study shows that gluten quality parameters, such as HMW-GS composition, better indicate baking quality in spelt than does the quantitative protein composition.

1. Introduction

Spelt (*Triticum aestivum* ssp. *spelta*) is an ancient wheat species closely related to modern common wheat (*Triticum aestivum* ssp. *aestivum*) (Haas et al., 2019). Common wheat is used for baking, and with 95% of the global wheat production, it is the most important wheat species (Sousa et al., 2021). Spelt was widely cultivated in Europe during the early and middle Bronze Age, until its importance decreased because of a lower yield compared to common wheat and the tough glumes making production more labor-intensive than common wheat (Longin et al., 2016; Lechterbeck and Kerig, 2024). In the last few years, a renewed interest in spelt can be observed among consumers, bakers and farmers (Rapp et al., 2017). One of the reasons is that spelt is perceived as healthier than common wheat (Zimmermann et al., 2022). Consumers increasingly value high-quality products connected to pleasure, culture and tradition, and they care about a wholesome diet with a more sustainable lifestyle (Ranhotra et al., 1995). An essential factor in making spelt products more attractive to consumers is to improve the baking quality, which is lower compared to common wheat. Dough made of spelt flour is weaker, less elastic and produces breads with a lower bread volume (Frakolaki et al., 2018; Huertas-García et al., 2023). Predicting the baking quality of spelt and improving the baking process are therefore important steps to help increase its market share and consumer interest.

Bread is a complex system in which starch, proteins, enzymes, non-starch polysaccharides and other ingredients interact with each other (van Rooyen et al., 2023). When gluten proteins are hydrated and mixed, they build a network stabilised mainly by disulfide bonds. During dough fermentation, the gluten network restrains gas release from the dough and is crucial for the development of bread structure and its quality (Magalhães et al., 2025). The content of damaged starch is primarily determined by the milling procedure and wheat kernel hardness.

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It affects dough water absorption capacity and yeast gas production, because it swells and hydrates more easily, is rapidly hydrolysed into maltose and maltooligosaccharides by amylases, and these reducing sugars drive yeast fermentation and Maillard reactions, affecting end-product quality (Struyf et al., 2017; van Rooyen et al., 2023). Tóth et al. (2022) and Wilson et al. (2008) already measured starch content and starch damage in spelt cultivars, but did not correlate these measurements with baking trials. Nevertheless, most studies on baking quality focus on the protein fraction and do not include total starch content and starch damage (Wilson et al., 2008).

The quality of common wheat is mainly predicted based on its positive association with the grain protein content, of which about 70–80% is the storage protein, called gluten. Protein content is most commonly measured by near-infrared spectroscopy (Du et al., 2022; Wieser et al., 2023). Gluten proteins are separated into the monomeric gliadins (ω1, 2-, ω5-, α- and γ-gliadins) and the polymeric glutenins. After reduction of the disulfide bonds, glutenins can be separated into low-molecular-weight glutenin subunits (LMW-GS) and high-molecular-weight glutenin subunits (HMW-GS) (Wieser et al., 1998). A high content of gluten proteins, especially glutenins, is associated with a high bread volume and a good baking quality (Thanhaeuser et al., 2014). In most studies, good baking quality is determined by a high bread volume (Schuster et al., 2023; Thanhaeuser et al., 2014; Geisslitz et al., 2018). This parameter is very important, but other quality parameters, such as bread texture and porosity, are also important for baking quality and are rarely studied. Geisslitz et al. (2018) found correlations between a high bread volume and high glutenin content when analysing eight spelt cultivars. In contrast, Schuster et al. (2023), who used a larger sample set of 82 common wheat samples, did not find a correlation between bread volume and protein composition. Studies on common wheat also show a correlation between certain HMW-GS and bread volume. As a hexaploid wheat species, spelt can produce five different HMW-GS: the x-types (Ax, Bx, and Dx) and the y-types (By, Dy) (Geisslitz et al., 2020). The HMW-GS combination Dx5 +Dy10 produced a superior baking quality to the HMW-GS combination Dx2 +Dy12 (Shewry et al., 1992). It is known that spelt has HMW-GS Dx and Dy similar to those of common wheat; however, limited information exists on how the HMW-GS combination of spelt affects its baking quality (Geisslitz et al., 2020). To determine gluten quality, the GlutoPeak test, an aggregation test of the gluten proteins in the flour samples, is commonly used (Marti et al., 2015). Geisslitz et al. (2018) found correlations between bread volume and glutenin content when analysing the different species common wheat, spelt, durum wheat, emmer and einkorn. Again, only studies with comparatively small sample sets are available.

Another way to predict the baking quality of bread is to use a microscale extension test, often referred to as Kieffer rig test (Kieffer et al., 1998). Sobczyk et al. (2017) analysed the extensibility of spelt dough using the Extensograph. They focused on differences in dough and baking properties between old spelt cultivars and newer lines, and also detected lower dough resistance to extension in spelt compared to common wheat. They also found large differences between the lines, indicating that spelt exhibits wide variations in rheological properties. This is why a large sample set needs to be studied. In Tóth et al. (2022), a sample set of 90 spelt cultivars was analysed for physical traits (thousand kernel weight), compositional traits (crude protein, wet gluten content, starch content, starch properties, β-glucan) and dough properties (water absorption, dough development time, dough stability, dough softening, gluten index and Zeleny sedimentation). They found higher protein and gluten content in spelt than in common wheat, but weaker dough strength and stability. Additionally, the baking quality and protein composition of the spelt cultivars were not directly analysed.

Takač et al. (2022) indicated that spelt composition, including starch and protein, not only depends on genetic factors but also on the environment. These factors need to be considered when analyzing spelt composition. Furthermore, Korczyk-Szabó and Lacko-Bartošová (2012) found an impact of growing year and genetics on the specific bread volume of spelt, but only for a set of four spelt cultivars.

Another way to improve the baking quality of spelt is to adjust the baking recipe. Standard baking procedures are intended for common wheat (International Association for, 1980). Studies found that weaker doughs, like spelt doughs, tend to react faster to strong mechanical forces and have a higher water absorbance compared to common wheat (Rodríguez-Quijano et al., 2019). Sobczyk et al. (2017) also suggested a longer kneading time at a lower speed of mixing for spelt doughs. Studies that optimise the dough preparation of spelt and focus on the speed of mixing and water addition are, therefore, necessary but have not been performed to our knowledge.

To summarise, the market for ancient wheat products, such as spelt, is rising; however, to support this growth, the baking quality of spelt in relation to its protein composition needs to be studied and improved. Existing studies on spelt baking quality are often limited by small sample sets and focus mainly on bread volume. Since spelt has a weaker gluten network compared to common wheat, studies suggest that the baking protocol for common wheat is not suitable for spelt, and an optimised processing protocol is required.

Therefore, this study aimed to comprehensively analyse flours of 30 spelt lines from three locations for the first time to determine the environmental and genetic impact on each in terms of starch content, starch damage, protein content, protein composition, and different functional tests, and relate these parameters to the baking quality assessed using a newly developed, optimised baking procedure for spelt. Our approach also defined the baking quality not only by specific bread volume but also by crumb texture and pore profile to provide further new insights into relationships between flour composition and functionality.

2. Materials and methods

2.1. Spelt samples

Ninety dehulled spelt grain samples, containing 30 spelt lines harvested in 2021 from the three locations Hohenheim (HOH), Oberer Lindenhof (OLI), and Ihinger Hof (IHH), were milled in a cross-beater mill (Perten Instruments, Hamburg, Germany) to whole wheat flours. The field trials were conducted as winter cropping, i.e., sowing in October 2020 and harvest in July 2021. Field plot size ranged between 5 and 9 m^2, sowing density from 280 to 350 dehulled grains per m^2, and number of rows from 6 to 7 with row spacing of 0.18 m depending on the location. All plots were machine-planted and combine-harvested and were grown under comparable conditions according to common practice in conventional farming using twice herbicides and fungicides. For HOH, the amount of nitrogen fertilizer applied was 150 kg/ha, for OLI 147 kg/ha and for IHH, 159 kg/ha. Further information on the cultivation locations and the spelt lines is given in Supplementary Tables S1 and S2. After milling, all flours were stored dry and cool for two weeks before starting the experiments. For method development, one commercial spelt flour was provided by SchapfenMühle GmbH & Co. KG (Ulm-Jungingen, Germany).

2.2. Reagents

Chemicals were of analytical or higher grade and purchased from Carl Roth (Karlsruhe, Germany), VWR Chemicals (Radnor, PA, USA) and Thermo Fisher Scientific (Waltham, MA, USA). Total Starch Assay Kit (AA/AMG) and Starch Damage Assay Kit were obtained from Megazyme (Wicklow, Ireland). Baking ingredients (fresh yeast and vegetable fat) were bought at a local supermarket.

2.3. Hulled grain yield

The hulled grain yield was determined as in Longin et al. (2016). Therefore, the plot was harvested and the samples dried for three days

before weighing to determine the hulled grain yield in decitons/hectare. Afterwards, the spelt samples were dehulled with a stone mill with a hard rubber application and cleaned with a Mini-Petkus seed cleaner (Röber, Bad Oeynhausen, Germany). The resulting grains were then used for quality analyses.

2.4. Starch content and starch damage

Total starch was determined according to ICC Standard 76–13.01, and starch damage was determined according to ICC Standard 164. All ICC Standards referred to in 2 have undergone full method validation.

2.5. Crude protein content

The determination of the crude protein content was carried out using 200 mg of flour according to ICC Standard 167 using a Dumatherm nitrogen analyser (Gerhard Instruments, Königswinter, Germany). The protein content was calculated using the factor 5.71.

2.6. Protein composition

Protein fractions were extracted in triplicate from 100 mg of flour each according to Wieser et al. (1998). The albumins/globulins were first extracted twice with 1 mL of extraction solution A (0.4 mol/L NaCl, 0.067 mol/L Na_2HPO_4/KH_2PO_4 (pH 7.6)) for 10 min at 22 °C. Gliadins were extracted from the residue three times with 0.5 mL of extraction solution B (60% (v/v) aqueous ethanol) for 10 min at 22 °C. Glutenins were extracted twice with 1 mL of extraction solution C (50% 1-propanol, 2 mol/L urea, 0.05 mol/L Tris-HCl (pH 7.5) + 1% (w/v) dithiothreitol) at 60 °C for 30 min. After adding the extraction solution, all samples were vortexed for 2 min and stirred. After the extraction, the samples were centrifuged for 30 min at 25 °C with 3550 rcf. Supernatants of each fraction were collected and diluted to 2 mL with the respective extraction solution. Before HPLC analysis, all samples were filtered through 0.45 μm syringe filters with a regenerated cellulose membrane (WICOM, Heppenheim, Germany).

The extracts were measured on a Thermo Scientific Vanquish UHPLC using an Acclaim 300 C18, 3 μm (30 nm, 2.1 × 150 mm) column with a diode array detector at 210 nm. Quantification was done with the Prolamin Working Group (PWG)-gliadin standard (distributed by Arbeitsgemeinschaft Getreideforschung e.V., Detmold, Germany) at a concentration of 2.5 mg/mL by injecting 2.5–20 μL. The resulting calibration was used to calculate the content of each protein fraction based on the corresponding peak area. The injection volume for albumins/globulins was 20 μL, for gliadins 10 μL, and for glutenins 25 μL.

2.7. Composition of HMW-GS

The experiment was carried out according to Geisslitz et al. (2020). Flour (50 mg) was mixed twice with 0.5 mL of 50% aqueous 1-propanol (v/v) and 1% dithiothreitol and heated at 60 °C for 30 min. The samples were centrifuged (25 min at 3550 rcf) and the collected supernatants were diluted to 1 mL. 1-propanol (0.25 mL) was added to the supernatant, and the mixture was stirred for 30 min. The solution was centrifuged (25 min at 3550 rcf), and the residue was mixed with 200 μL of NuPAGE LDS Sample Buffer (1x) (Thermo Fisher Scientific). This mixture was incubated overnight after which dithiothreitol was added, and the solution was shaken for 10 min at 60 °C. After cooling, 12 μL of the solution was applied to the chambers of a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel (Novex 6% Tris-Glycine Plus WedgeWell Gel (Thermo Fisher Scientific)). A protein ladder (5 μL) (PageRuler Unstained Protein Ladder covering 10 kDa to 200 kDa with 14 proteins, Thermo Fisher Scientific) was applied to estimate the molecular weights of the proteins. The gels were run in a gel chamber (Bio RAD PowerPac HV, Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 225 V and 125 mA with running buffer (25 mmol/L Tris, 192 mmol/L glycine, pH 8.3). After fixing, staining, and destaining, the gels were scanned with a Gel Doc EZ Imager (BioRad).

2.8. Gluten Aggregation Test

The GlutoPeak test was performed according to the manufacturer's instructions (Brabender, Duisburg, Germany) (Güçbilmez et al., 2019). Flour (7.74 g, calculated as dry weight) was weighed, along with 9 mL of water (tempered to 36 °C), and placed into the GlutoPeak (Model 803400). The suspension was mixed at 2750 rpm and 36 °C using the preinstalled method "rapid flour check" and the "extended" evaluation method with the GlutoPeak Software Version 2.2.6. The GlutoPeak software generates a torque against time curve. The time the aggregation started (lift-off time, LOT in [s]) was manually evaluated, and the time when maximum torque was reached (peak maximum time, PMT in [s]) was automatically calculated by the software. Subtracting LOT from PMT results in the aggregation time (AGT, in [s]). Furthermore, the maximum torque (MT, in Brabender units (BU)) was calculated automatically by the software.

2.9. Development of an optimised microbaking test for spelt

To reduce the amount of flour needed for the development of a baking method for spelt breads, we used the Box-Behnken-Design, a three-level incomplete factorial design (Ferreira et al., 2007). We chose to optimise the amount of yeast (1, 2, and 3%), the speed of mixing (40, 51.5, and 63 rpm), and the dough consistency (350, 450, and 550 BU) by adding different amounts of water (Supplementary Table S3). For the three equidistant levels used in the design, we referred to ICC Standard No. 115/1, which specifies 63 rpm and 500 BU for water absorption testing using the farinograph (International Association for Cereal Science and Technology, 1992).

The baking procedure was adapted from Münzing et al. (2009) and Longin et al. (2014). Flour (50 g) was mixed with 1% sucrose, 1% vegetable fat ("ja! Reines Pflanzenfett", REWE, Cologne, Germany), 1.5% salt, and 1%, 2% or 3% fresh yeast for 2 min in a farinograph. Subsequently, water tempered to 30 °C was added to reach a consistency of 350 BU, 450 BU or 550 BU. The dough was then kneaded for 4 min with mixing speeds ranging from 40 rpm, 51.5 rpm or 63 rpm. The dough was manually shaped round and placed in a proofing chamber to rest for 20 min at 25 °C. After resting, the dough was sheeted and folded again and proofed for another 20 min in the proofing chamber (25 °C). The dough was baked in an oven (UNOX Deutschland GmbH, Büren, Germany) at 210 °C for 10 min, with steam applied during the first 20 s. After a cooling period of 1 h, the breads were weighed, and the bread volume was measured using a laser scanner (VolScan Profiler VSP300, Stable Micro Systems, Godalming, UK). The bread was sliced into 1.5 cm thick slices. One slice from the centre of the loaf was analysed in the TA. XTplus texture analyser (Stable Micro Systems, Godalming, UK) as reported in Stemler and Scherf (2022). The system was regularly calibrated with a test weight. A 20 mm diameter cylindrical probe was used in a double compression test. The sample was deformed to 40% of its height with a release force of 0.049 N and a 10 s waiting time between measurements. Pretest speed was set to 1 mm/s, while test and backtest speeds were set to 0.8 mm/s with a measurement data rate of 200 measuring points per second. The texture data was evaluated in the software Exponent (Version 6.2.6.0) by Stable Micro Systems. The software presets the texture parameters and includes hardness (maximum of the first compression peak), elasticity (ratio of the first decompression to the first cycle compression), cohesiveness (ratio of positive force area during second compression to during the first compression), gumminess (hardness x cohesiveness), chewiness (hardness x cohesiveness x springiness) and springiness (ratio of time difference during second compression to during first compression). Another slice was examined using the C-Cell system (Calibre Control, Warrington, UK) for crumb structure and colour. The system was calibrated with

a calibration card (CC006, Calibre Control), and samples were placed on a blue background.

2.10. Application of the optimised microbaking test

All 90 spelt samples were baked with the optimised parameters: 3% yeast, 40 rpm for speed of mixing and water added until a consistency of 350 BU was reached. The breads were analysed as described above.

2.11. Microscale extension test

Approximately 10 g of dough was taken after kneading in the farinograph for the microscale extension test. According to Hoeller and Scherf (2024) and Scherf and Koehler (2018), this dough was pressed into a slightly greased silicone mould. The exposed areas of the mould were also greased to prevent the dough from drying out. The pressed dough was allowed to rest in the mould for 15 min at 25 °C. After resting, the strands were carefully removed from the mould and placed onto an SMS/Kieffer dough and gluten extensibility rig in a texture analyser (Stable Micro Systems). The test was carried out in triplicate, with at least three strands analysed per replicate.

2.12. Statistical analysis

Mean values ± standard deviations of triplicates were calculated using Microsoft Office Excel (Microsoft Corporation, Seattle, WA, USA). The Box-Behnken-Design was calculated and evaluated in OriginPro 2023 and 2025 (OriginLab, Northampton, MA, USA) using the Add-on "Design of Experiments". One- and two-way ANOVAs (analysis of variance) (factors: line and location) with Tukey's post-hoc test ($p \leq 0.05$) were performed in OriginPro. RStudio (2024.12.01 and 2025.09.01, Posit PBC, Boston, MA, US) was used to conduct advanced statistical analysis and data visualisation. The correlation plots were created using the corrplot package, and the heatmap comparing the influence of line and location was generated with reshape2 and pheatmap packages. For ridge regression on the normalized numerical data, the glmnet package was used.

3. Results and discussion

Fig. 1 provides an overview of all the analytical parameters discussed in the following sections. Fig. 2 and Supplementary Table S4 show the impact of the factors line and location on the analysed parameters.

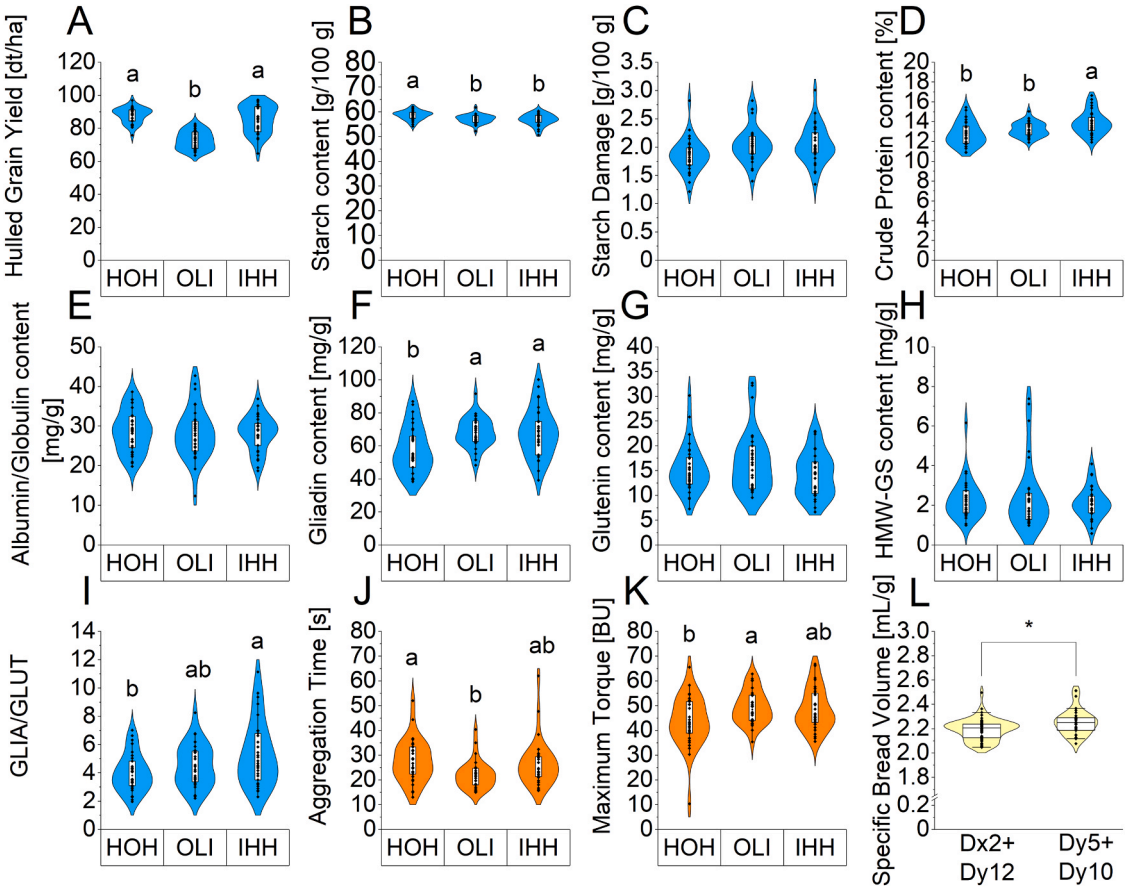

Fig. 1. Comparison of 30 spelt lines grown at three growing locations, respectively: (A) hulled grain yield (B) starch content; (C) starch damage; (D) crude protein content; (E) albumin/globulin content; (F) gliadin content; (G) glutenin content; (H) content of high-molecular-weight glutenin subunits (HMW-GS); (I) gliadin-to-glutenin ratio (GLIA/GLUT); (J) aggregation time; (K) maximum torque. The width of the violin plot indicates data density. Violins with different letters differ significantly (one-way analysis of variance, Tukey-post-hoc test, $p \leq 0.05$, three locations with 30 lines each, $n = 2$ for hulled grain yield, crude protein, starch and starch damage, $n = 3$ for all others). (L) Comparison of specific bread volume of lines with HMW-GS combination Dx2 +Dy12 and Dx5 +Dy10. * Significant difference (two-sample-*t*-test, $p \leq 0.05$). The horizontal line in the box plot shows the median, and the hollow point shows the mean. Box indicates interquartile range (IQR) with whiskers at 1.5 x IQR. Points outside the whiskers indicate outliers. Abbreviations: HOH, Hohenheim; OLI, Oberer Lindenhof; IHH, Ihinger Hof. Gliadin and glutenin protein types are displayed in Supplementary Fig. S1.

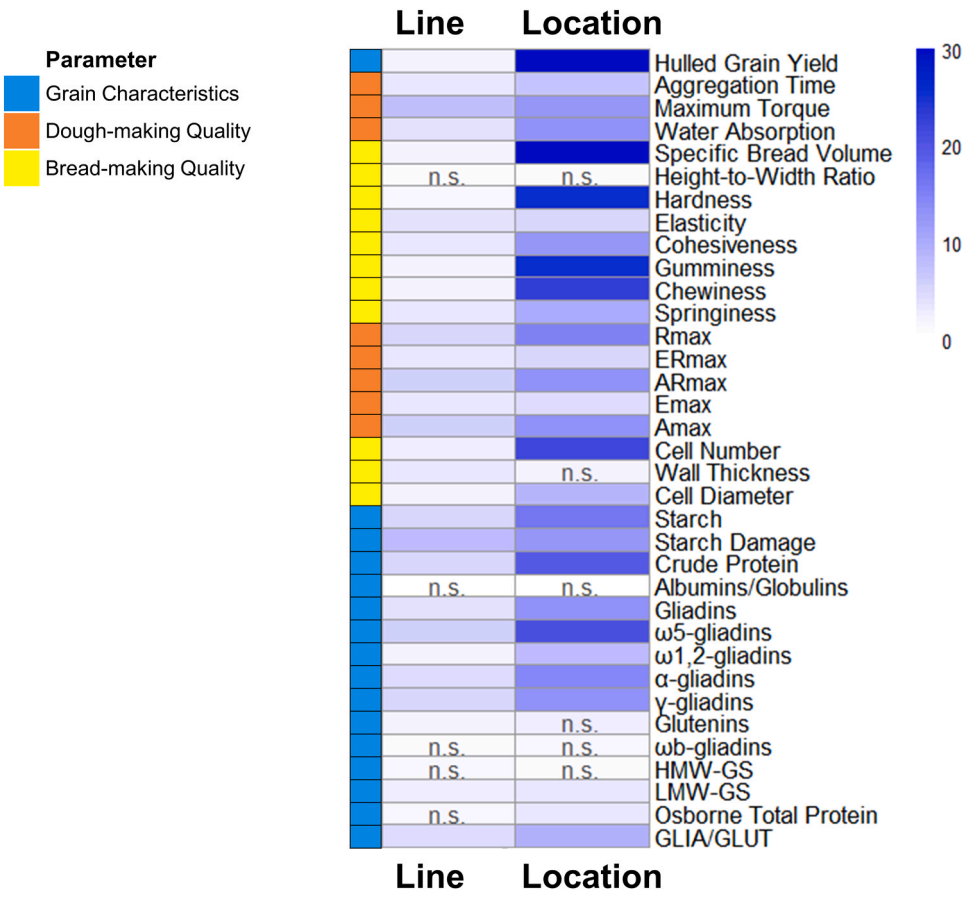

Fig. 2. Impact of the factors line and location on 35 different parameters assessed for the spelt samples. F-values of two-way ANOVAs (factors: line and location, $p \leq 0.05$, $n = 2$ for crude protein, starch and starch damage, $n = 3$ for all others) for the analytical and baking quality parameters of 90 spelt samples. Non-significant F-values are labelled with n.s (non-significant). Abbreviations: R_{max}, maximal force; E_{Rmax}, distance to max. force; A_{Rmax}, area till max. force; E_{max}, distance till break; A_{max}, area till break; HMW-GS, high-molecular-weight glutenin subunits; LMW-GS, low-molecular-weight glutenin subunits; GLIA/GLUT, gliadin-to-glutenin-ratio. For more details, please refer to Supplementary Table S4.

3.1. Hulled grain yield

The hulled grain yield of 90 spelt samples was between 63.6 and 97.2 dt/ha (Fig. 1A) and showed a very high and significant F-Value of 69.4 for the location and an F-Value of 2.40 for the line effect. When comparing the three locations, OLI produced a significantly lower hulled grain yield than the two other locations. In Dorval et al. (2015), four spelt cultivars from three different locations produced hulled grain yields between 27.3–44.2 dt/ha in eastern Canada. In Pospišil et al. (2011), two spelt cultivars had a hulled grain yield between 58.0 and 76.8 dt/ha, which is also comparable to our results.

3.2. Starch content and starch damage

The starch content of the 90 spelt samples was between 50.6% and 62.0% and starch damage percentages were between 1.2% and 3.0% (Fig. 1B-C). The location had a significantly higher impact than the genetics (starch: location F=14.4; line F=4.8; starch damage: location F=13.4; line F=10.6). The samples from HOH had a significantly higher starch content compared to those from OLI and IHH. There was no significant difference between the three locations concerning starch damage. Since all samples were milled in the same mill, with the same procedure, which determines the degree of starch damage, no significant difference was expected. Wilson et al. (2008) reported the starch content of five spelt cultivars from three different years of 72.4–82.0% and starch damage of 2.1–3.2%. The values for starch damage matched our results, but the starch content was higher in their study compared to ours. Wilson et al. (2008) isolated starch by sedimentation out of the flour, while we chose to use an enzymatic assay, which may explain the different results. Tóth et al. (2022) reported a starch content of 49.9–57.8% determined by NIR in 90 spelt lines grown over three years. These results are comparable to our results.

3.3. Crude protein content

The crude protein content ranged from 10.9 to 16.6 g/100 g (Fig. 1D). The line (F=5.04) and the location (F=19.99) had a significant influence on the crude protein content, with samples from IHH having a significantly higher content compared to those from HOH and OLI. Geisslitz et al. (2018) detected crude protein contents in eight spelt lines ranging from 10.8 to 16.1 g/100 g under similar conditions. The 90 spelt cultivars from three years analysed by Tóth et al. (2022) had crude protein contents ranging from 12.2 to 22.2 g/100 g. Therefore, our results align well with the literature.

3.4. Protein composition

The albumin/globulin content ranged from 12.3 to 42.7 mg/g (Fig. 1E). The gliadin content ranged from 38.3 to 100.1 mg/g (Fig. 1F) and that of ω5-gliadins from 1.6 to 9.6 mg/g, ω1,2-gliadins from 3.1 to 20.1 mg/g, α-gliadins from 18.1 to 47.5 mg/g and γ-gliadins from 12.1 to 41.0 mg/g (Supplementary Figure S1A-D). The glutenin content ranged from 3.4 to 32.7 mg/g (Fig. 1G), comprising 0.01–2.3 mg/g of ωb-gliadins, 0.3–9.5 mg/g of HMW-GS and 3.1–24.6 mg/g of LMW-GS

(Supplementary Figure S1E-G; HMW-GS in Fig. 1H). The gliadin-to-glutenin ratio is an important factor for a better comparison of the protein composition. This ratio ranged from 2.0 to 11.1 (Fig. 1I). We found no significant differences in albumin/globulin or glutenin content or in the corresponding glutenin types across the three locations. The gliadin content of the spelt samples grown at HOH was significantly lower than that of samples from OLI and IHH. The same was true for the gliadin types, while the gliadin-to-glutenin ratio was significantly lower in HOH.

There was a significantly higher impact of the environment (F=9.1–22.8) than the line (F=2.4–5.4) on the gliadin content. A similar result was found for the gliadin types ω5-, ω1,2-, α- and γ-gliadins. There were no significant results or only results with a small F-value for the environmental and genetic impact on glutenins and glutenin types.

When calculating the percentages of gliadins and glutenins relative to the total amount of proteins determined by HPLC, the spelt samples contained 48.1–74.6% of gliadins and 3.0–24.6% of glutenins. Geisslitz et al. (2018) found percentages of 57.1–65.9% for gliadins and 14.7–21.7% for glutenins as well as gliadin-to-glutenin ratios of 2.8–4.0. Therefore, the protein composition in our study is comparable to that of Geisslitz et al. (2018). Koenig et al. (2015) analysed 62 spelt cultivars and their protein content and composition, resulting in 52.6–71.8% of gliadins, 7.7–25.0% of glutenins and gliadin-to-glutenin ratios of 2.2–9.0. These results align very well with our findings. The large number of samples can explain the large variation of contents in our sample set compared to the smaller variation in Koenig et al. (2015).

3.5. Gluten aggregation test

In the gluten aggregation test, we focused on the two parameters AGT and MT. The parameters were chosen because studies, such as Geisslitz et al. (2018), found correlations between the bread volume of eight cultivars from five wheat species and the PMT, AGT, and MT. Since the AGT is calculated from the PMT, we decided to focus only on the AGT and MT. The MT ranged from 10.3 to 66.8 BU and the AGT from 13.0 to 62.0 s (Fig. 1J-K). The location showed a higher impact on the MT (F=13.1) and the AGT (F=7.1) than the genetic factor (MT: F=7.97; AGT: F=3.32). In lines from HOH, the AGT was significantly higher and the MT was lower than in those from OLI.

Hadnađev et al. (2022) used a similar experimental setup and found a MT of 44.5 BU and a PMT of 120.5 s in a gluten aggregation test with one spelt cultivar. This MT result fits very well with our results, but the PMT value was higher. Geisslitz et al. (2018) reported a MT of 28.3–45.3 BU and an AGT of 34.7–65.0 s for eight spelt cultivars. Both ranges agree well with our results.

3.6. Development of an optimised microbaking test for spelt

Since the specific bread volume is one of the most important factors concerning baking quality, the baking method was developed to produce an optimized high specific bread volume. We tested the three parameters, speed of dough mixing (40, 51.5 and 63 rpm), dough consistency after water addition (350, 450 and 550 BU) and amount of yeast (1, 2 and 3%) for their impact on the baking quality. Only the amount of yeast had a significant impact on the specific bread volume (standardised effect value: 13.1) (Fig. 3A). The main effect plot shows that the addition of 3% yeast to the dough produced breads with a higher specific bread volume compared to those with 2% and 1% yeast (Fig. 3B). We then used a Box-Behnken experimental design to maximise the parameters to a high specific bread volume with a lower limit of 2.2 mL/g and an upper limit of 3.0 mL/g. With a combination of 3% yeast, 350 BU consistency, and 40 rpm, the software predicted a specific bread volume of 2.4 mL/g (95% confidence interval: 2.3–2.5 mL/g). This fit was verified in a baking test (Supplementary Figure S2), and the breads reached a specific bread volume of 2.3 mL/g. The fit had an R^2 value of 0.88, which is considered a good fit for a baking trial (Supplementary Figure S3)

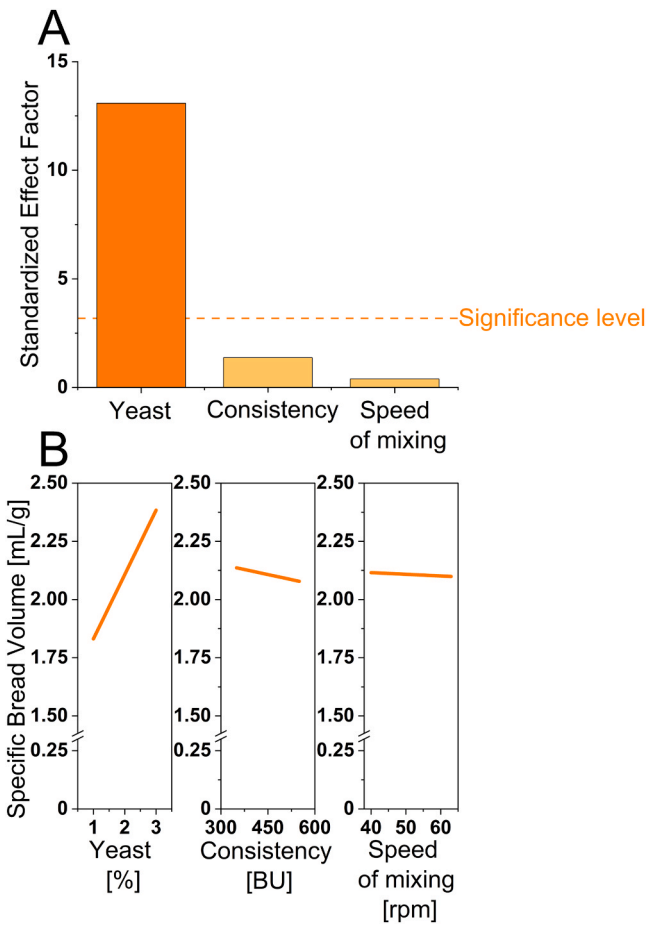

Fig. 3. Development of an optimised microbaking test for spelt using design of experiments. (A) Standardized effect factor: t-Values for yeast addition, consistency of the dough and speed of mixing in the spelt baking method development. H0 = parameter does not affect the specific bread volume. The significance level is set to 0.05; n = 3. (B) Main-Effect-Plot of the amount of yeast, the consistency of the dough and the speed of mixing of the dough on the specific bread volume (n = 3).

(Maruyama et al., 2013). Therefore, further baking trials used the optimised combination of parameters suggested by the software design.

Rodríguez-Quijano et al. (2019) showed that spelt has rheological differences from common wheat, with lower water absorption capacity, strength, tenacity, a shorter dough development time and a quicker dough weakening. They concluded that these parameters match a dough with a weak gluten network; however, they did not perform baking tests. In our optimisation, we found that higher water absorption and lower speed of mixing produced a higher specific bread volume. These effects had no significant ($p > 0.05$) impact in our trials, but showed a similar trend to the observations by Rodríguez-Quijano et al. (2019).

3.7. Application of the microbaking test

The optimised microbaking test was applied to the 90 spelt samples and the baked breads were characterised in terms of specific bread volume, height-to-width ratio, hardness, elasticity, cohesiveness, gumminess, chewiness, springiness, number of cells, wall thickness, and cell diameter. All parameters were analysed, but only essential parameters from each experiment were selected for discussion and are shown in Fig. 4. For further information on the results, see Supplementary Figures S4–S6.

The water absorption needed to reach a consistency of 350 BU ranged from 62.5% to 75.5% (Fig. 4A). The resulting specific bread

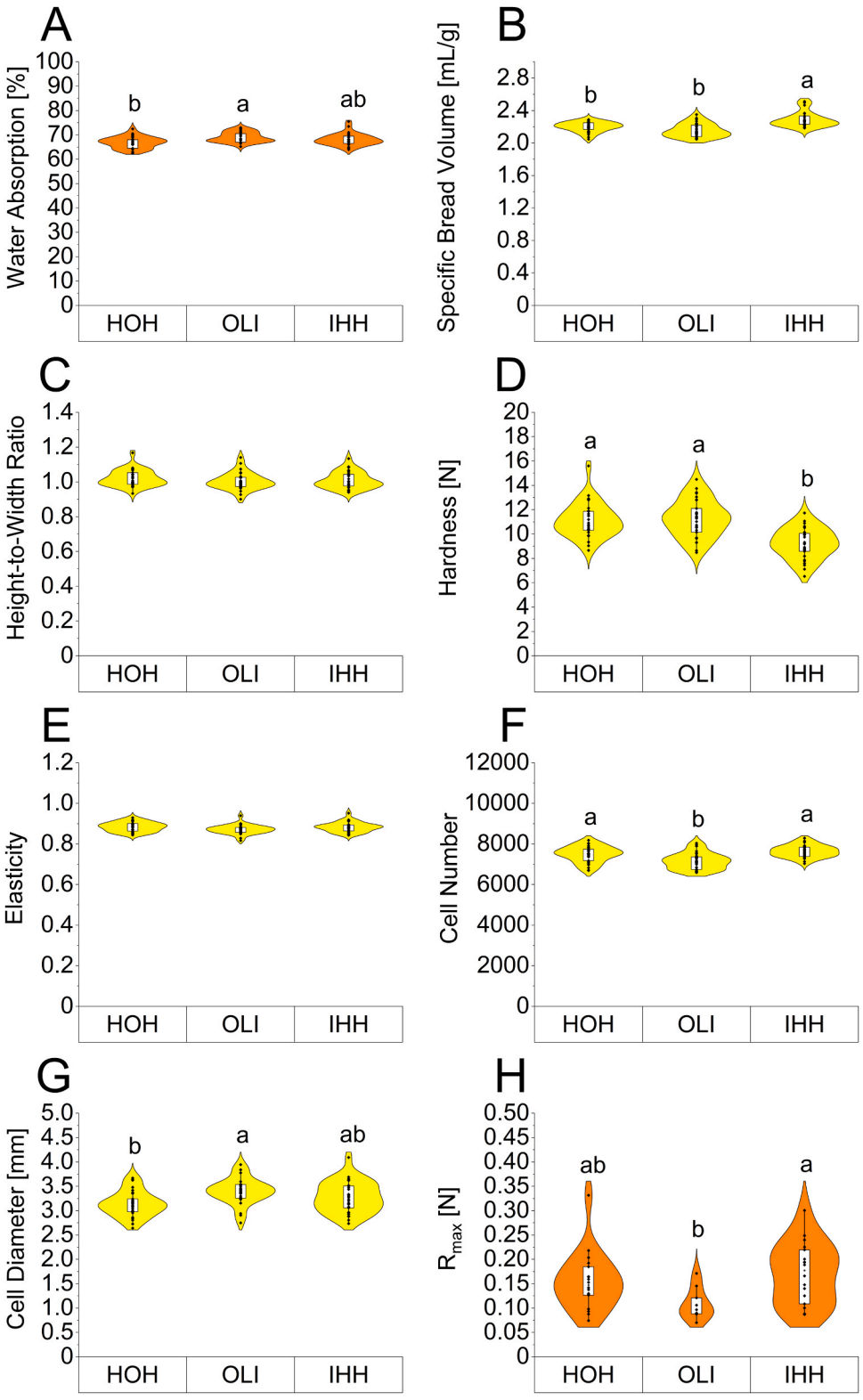

Fig. 4. Results for (A) water absorption; (B) specific bread volume; (C) height-to-width ratio; (D) hardness; (E) elasticity; (F) cell number; (G) cell diameter and (H) maximum force of the dough (R_{max}). The width of the violin plot indicates data density. Violins with different letters differ significantly (one-way analysis of variance, Tukey-post-hoc test, $p \leq 0.05$, three locations with 30 lines each, $n = 3$). Abbreviations: HOH, Hohenheim; OLI, Oberer Lindenhof; IHH, Ihinger Hof. Box indicates interquartile range (IQR) with whiskers at 1.5 x IQR. Points outside the whiskers indicate outliers. For further determination of the parameters of dough and bread, refer to Supplementary Figs. S4-S6.

volume ranged from 2.05 to 2.51 mL/g (Fig. 4B), with samples from IHH producing significantly higher specific bread volume than those from HOH or OLI. The specific bread volume was affected more by the environment (F=28.7) than by the genetic background. The measured specific bread volume was higher than in comparable studies, like Geisslitz et al. (2018). They found bread volumes between 1.7 and 2.3 mL/g in eight spelt cultivars. This indicates that the optimised microbaking test could be responsible for this effect. The height-to-width ratio ranged from 0.90 to 1.17 and was similar for all breads (Fig. 4C). This could be why we did not find a significant impact of the environment or genetics on these results.

When analysing the texture of the bread, we focused on the hardness (Fig. 4D) and elasticity of the crumb (Fig. 4E). The hardness ranged from 6.5 N to 15.6 N, and the elasticity ranged from 0.82 to 0.95, revealing a high variance in crumb hardness but a lower difference in elasticity. The impact of the location (F=24.1) was higher than that of the genetic background (F=2.0), and the crumb hardness of breads made with lines from IHH was significantly lower than that of samples from HOH and OLI. The impact of the location (F=5.4) and the genetics (F=3.9) were lower on the elasticity of the crumb, and no significant location-dependent differences between the elasticity of the breads were observed. Frakolaki et al. (2018) compared a spelt bread to a common wheat bread, and the crumb hardness of 13.6 N fits our results.

Comparing the pore structures of the breads, we measured 6593–8289 cells and cell diameters between 2.64 and 4.09 mm (Fig. 4F-G). The environmental factor was higher for the cell number (F=17.3) and the cell diameter (F=8.8) than the genetics (cell number F=2.5; cell diameter F=2.0). The breads made from lines grown at OLI showed significantly lower cell numbers and larger cell diameters than those from lines of HOH and OLI. This indicates a less fine crumb structure in these breads. In Ktenioudaki et al. (2010) eight wheat cultivars were tested in a similar C-Cell test. The breads with the best baking results had a fine crumb structure (cell diameter: 2 mm), while even the breads with a coarse appearance (cell diameter: 2.3 and 2.6 mm) showed a lower cell diameter than our breads. Wide differences in the hardness and structure of the crumb indicate that good baking performance can be achieved with spelt when high-quality flour is selected.

3.8. Microscale extension test

Only 45 of the 90 spelt samples could be measured in this test. The other samples showed too little resistance against the hook (5 kg measurement cell). The evaluated factors are the maximal force, the area till max. force, the distance to max. force, the distance till break and the area till break. We focused on the maximal force since further factors did not provide further insights. The 45 samples reached maximal forces of 0.07–0.33 N (Fig. 4H). The genetic (F=5.1) and the environmental impact (F=15.2) were significant, with the environmental factor again being the larger impact factor. The maximal force of the doughs made of samples from OLI was significantly lower than that of the doughs made from samples of IHH.

In Kieffer et al. (1998), twenty-five common wheat flours were mixed into doughs and analysed. They found maximal forces between 0.08 and 0.43 N. This shows that, comparable to our experiments, there was a large variability in maximal resistance depending on the sample.

3.9. Composition of HMW-GS

In the 30 spelt lines, we had 20 lines expressing the Ax HMW-GS and 11 that did not. Except for the lines Badenkrone with Bx7 and missing By protein and ZG1814 with Bx7 +By9 HMW-GS, we found the HMW-GS Bx6.1 +By22 in all other lines. Eleven lines with the HMW-GS variants Dx5 +Dy10 and 20 lines with the HMW-GS Dx2 +Dy12 were detected (Supplementary Figure S7). Since the HMW-GS composition is defined on a genetic level, only one location per line was analysed. Chňapek et al. (2014) found that 82% of 41 spelt cultivars showed the Dx2 +Dy12 combination. We also observed that 65% of the 30 spelt lines had this HMW-GS combination.

The results of the HMW-GS composition cannot be directly correlated with the specific bread volume, as the composition is qualitative rather than numerical. Using a Student's two-sided *t*-test, we found that lines with the HMW-GS combination Dx5 +Dy10 produced breads with a higher specific bread volume than lines with the combination Dx2 +Dy12 (Fig. 1L). To our knowledge, there are no studies that directly correlate the HMW-GS composition of spelt with its baking quality. But since common wheat and spelt are closely related, the same Dx and Dy HMW-GS can be found in common wheat and spelt. Brönneke et al. (2000) found a correlation between higher bread volume and the HMW-GS combination Dx5 +Dy10 in common wheat, using a sample set of 153. Jiang et al. (2019) found a superior bread crumb structure with the HMW-GS Dx5 +Dy10. In contrast, Schuster et al. (2023) found no correlation between the HMW-GS composition and the bread volume while analysing 82 common wheat samples. An explanation for the improved baking quality can be found in the structure of Dx5, which features an additional cysteine residue at the N-terminal end of the repetitive region (Shewry et al., 1997). Brönneke et al. (2000) conclude that this leads to more inter- and intramolecular disulfide bonds and to a more linked gluten network.

3.10. Pearson correlations between dough and baking quality and analytical parameters

A grouped Pearson correlation between analytical parameters and dough and baking quality parameters is shown in Fig. 5. For further information and an ungrouped correlation, please refer to Supplementary Table S5 and Figure S8. The highest correlation coefficients were found between the results of the GlutoPeak test and certain parameters in terms of protein content and composition. A high content of crude protein (r = 0.70), gliadins (r = 0.69), α-gliadins (r = 0.61) and γ-gliadins (r = 0.60) was positively correlated with the MT reached in the gluten aggregation test and negatively with the AGT (gliadins: r = -0.52; α-gliadins; r = -0.51; γ-gliadins: r = -0.45). A high content of crude protein (r = 0.58), gliadins (r = 0.58), α-gliadins (r = 0.50) and γ-gliadins (r = 0.51) was also positively correlated with the water absorption. A weak correlation was observed between the maximum force of the dough in the microscale extension test and the content of α-gliadins (r = -0.44). Other significant correlations were found between the hulled grain yield and the aggregation time (r = 0.41), the maximum torque (r = -0.44) and the water absorption (r = -0.53). Concerning the baking quality, there were no significant correlations between the specific bread volume, the height-to-width ratio of the breads or the crumb hardness. Only a weak correlation was found between the specific bread volume and the hulled grain yield (r = 0.33) and the AGT (r = 0.37), respectively. Furthermore, the crumb elasticity (r = 0.40) and the cohesiveness (r = 0.45) correlated weakly with the crude protein content.

3.11. Ridge regression between specific bread volume, water absorption, gluten aggregation behaviour and analytical parameters

To further analyse the relationship between specific bread volume, water absorption, gluten aggregation behaviour and flour composition we used ridge regression (Table S6). The most influential effects on higher specific bread volume in the model were lower starch damage (β=-0.02), lower ω-gliadins (β=-0.02), and higher protein content (β=0.01). For dough quality, we examined gluten aggregation behaviour and found that a long gluten aggregation time was associated with lower starch damage (β=-2.37), higher ωb-gliadin (β=0.24) and ω5-gliadin (β=0.19) content, and lower crude protein (β=-0.23) and higher HMW-GS (β=0.17) content. For a high maximum torque, the model indicated that high starch damage (β=3.36) and crude protein content (β=1.81) were the most influential. The two parameters with the largest

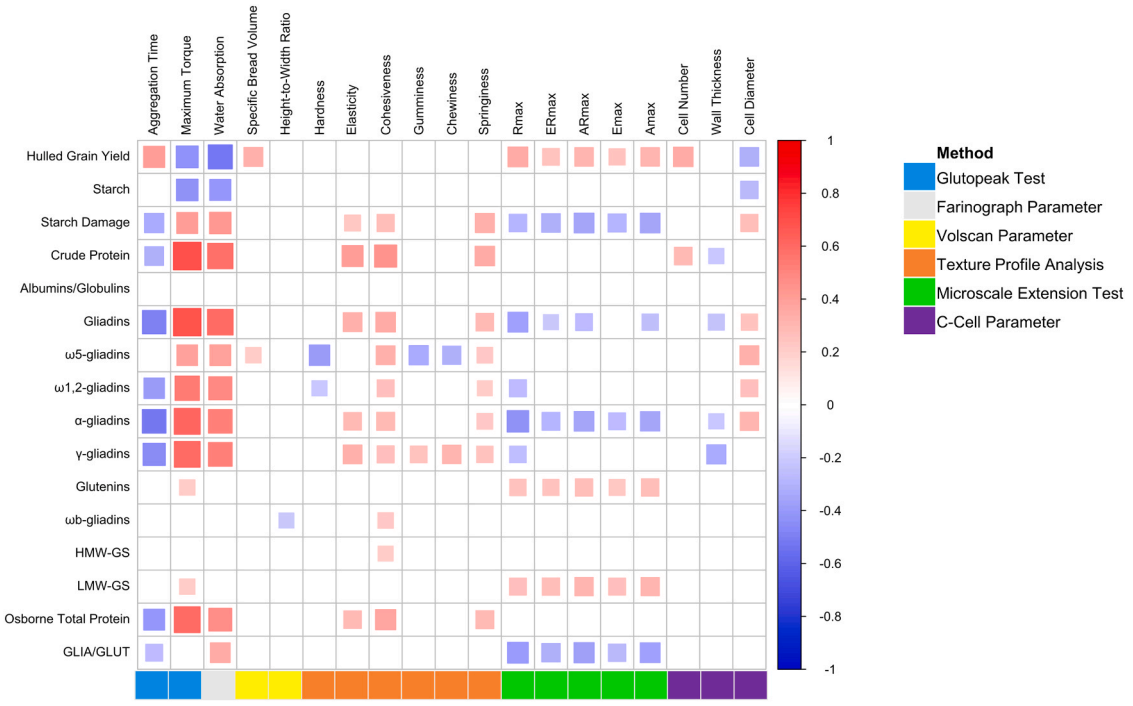

Fig. 5. Grouped Pearson correlation coefficients ($p \leq 0.05$, $n = 2$ for crude protein, starch and starch damage, $n = 3$ for all others) of analytical parameters with dough or bread quality parameters of the 90 spelt samples. Empty boxes indicate non-significant results ($p > 0.05$). Abbreviations: R_{max}, maximal force; E_{Rmax}, distance to max. force; A_{Rmax}, area till max. force; E_{max}, distance till break; A_{max}, area till break; HMW-GS, high-molecular-weight glutenin subunits; LMW-GS, low-molecular-weight glutenin subunits; GLIA/GLUT, gliadin-to-glutenin ratio. For more details, please refer to Supplementary Fig. S8 and Table S5.

influence on water absorption were starch damage (β=1.03) and protein content (β=0.31). This shows that starch damage and, therefore, the milling and kernel selection are important for baking and dough quality, even if there were no significant differences between the locations (Fig. 1). We also found an impact of HMW-GS on dough quality, which is not directly reflected in baking quality. Since the different HMW-GS combinations are not numerical data, these results cannot be included in the ridge regression.

3.12. Limitations

The study was designed and executed to produce reliable, conclusive results, but we encountered certain limitations. Due to the limited amount of flour, we were only able to make breads at a scale of 50 g of flour and standard tests like Extensograph could not be performed. Rheological tests, such as the microscale extension test, were only applicable to 45 of 90 spelt samples because the resistance of the remaining doughs was too low during the test. This excluded the weaker samples from the statistical evaluation, resulting in potential bias. Our study focused on protein and starch, but other minor flour components, such as lipids and enzymes, can also affect baking performance. This would need to be studied further. Furthermore, we used a common amount of yeast in our baking recipe (Wang et al., 2024). Studies with lower yeast levels could find a more subtle impact of the gluten network on baking quality. This could also be studied in long-term experiments to avoid underfermentation of breads with lower yeast amounts.

3.13. Future directions

Geisslitz et al. (2018) found high correlations between the AGT and bread volume in eight spelt cultivars. In our study, we only found a weak correlation to the AGT. This could be linked to different baking recipes, a larger sample size, and newly developed lines. In Nagel-Held et al. (2024), 1112 common wheat samples from four locations and two years were analysed for their baking quality. In this study, they found moderate correlations between the bread volume and grain protein content, SDS sedimentation volume, extensibility, water absorption and Glutopeak results. Schuster et al. (2023) also found correlations between certain gluten fractions and the water absorption and MT in the Gluto-Peak test, but no correlations with the specific bread volume based on a set of 70 wheat samples (24 common wheat cultivars, from different locations and harvest years). Our study used a sample set of similar size and showed quite comparable results. This indicates that the prediction of baking quality in wheat bread cannot be easily done by identifying the protein composition of the flour. Studies like Ziegler et al. (2025) found that the prediction of baking quality can be better achieved by combining different data types, like farinograph data with spectroscopy data. Selga et al. (2024) predicted bread volume with models based on rheological tests combined with protein and damaged starch analysis and also found a complex correlation of the analysed parameters to the bread volume (Selga et al., 2024). This indicates, together with our results, that more multifactorial studies are needed to deepen our understanding of wheat and spelt baking. Our results also show that gluten quality, as analysed in the SDS-PAGE, can be a better indicator of baking quality. Galimova et al. (2023) found that 80–92% of the analysed common winter wheat samples carried the Dx5 +Dy10 combination, indicating that this beneficial HMW-GS combination is more prominent in common wheat than in spelt, where 65% had the unfavourable Dx2 +Dy12 combination in our set. This is why breeding programs should not only focus on high protein content but also favourable protein quality (Werrie et al., 2025). This link should be studied more in the future to guide the selection of suitable spelt flours to make high-quality breads. Since the HMW-GS types differ in the number of cysteine residues, these should be studied further in combination with quantities of low-molecular weight thiols to gain insights into their functionality in dough and bread.

Abbreviations

AGT aggregation time

ANOVA	analysis of variance
BU	Brabender units
HMW-GS	high-molecular-weight glutenin subunits
HOH	Hohenheim
IHH	Ihinger Hof
LMW-GS	low-molecular-weight glutenin subunits
LOT	lift off time
MT	maximum torque
OLI	Oberer Lindenhof
PMT	peak maximum time
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

CRediT authorship contribution statement

Christina Hempel: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Katharina Anne Scherf:** Writing – review & editing, Supervision, Resources. **C. Friedrich H. Longin:** Writing – review & editing, Resources, Conceptualization. **Sabrina Geisslitz:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Informed consent

Not applicable.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

The first author used ChatGPT (OpenAI, 2025) for language refinement and grammatical corrections, and for support in R coding used in this manuscript. The content was reviewed and edited after application, and the authors take full responsibility for the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2026.109007.

Data availability

Data will be made available on request.

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