



Effect of ascorbic acid on the baking and dough quality of spelt and emmer

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Abstract

Despite the growing interest in ancient wheats such as spelt and emmer, their utilisation is often limited by inferior dough stability and baking properties. This study evaluated the potential of ascorbic acid to improve dough and baking quality. 60 mg/kg of ascorbic acid to a commercial spelt flour led to higher specific bread volume (+7.9%), lower crumb hardness (−22.9%) and higher cell diameter (+17.1%) in the crumb. In emmer, a decrease in dough extensibility (−29.0%) was observed after the addition of 250 mg/kg ascorbic acid. When adding 60 mg/kg ascorbic acid to 24 spelt flours (eight lines from three different locations), we found improvements in specific bread volume (+11.0%), crumb hardness (−20.2%), and maximal dough force (+61.3%). Breads from 25 emmer flours (ten lines from two to three locations) with the addition of 250 mg/kg of ascorbic acid had improved dough strength and cell number (+3.0%) in the crumb, and reductions in chewiness (−10.0%). Further, we found a correlation between the gliadin-to-glutenin ratio and specific bread volume in emmer and identified the trend that HMW-GS Bx14.1 in emmer was associated with higher specific bread volume and a lower crumb hardness. By adding ascorbic acid and extending fermentation to 100 min, we achieved bread quality with only 1% yeast, comparable to that of 3% yeast formulations fermented for 40 min. These findings demonstrate that line selection, ascorbic acid levels, and fermentation times can overcome the lower dough stability of ancient wheats, offering a promising approach to producing high-quality breads.

Keywords Ancient wheat · Dough processing · Long-term fermentation · Microscale extension test · Texture profile analysis · Yeast reduction

Introduction

Spelt (*Triticum aestivum* ssp. *spelta*) and emmer (*Triticum dicoccum*) are ancient wheat species that were once widely cultivated but have since lost importance due to the success of common wheat (*Triticum aestivum* ssp. *aestivum*), which

has higher yields and is easier to produce [1–4]. Common wheat now accounts for 95% of the wheat consumed worldwide [5], but there is a trend toward specialty products connected to tradition and a more sustainable lifestyle [6]. This makes ancient wheats an interesting market opportunity for consumers and bakers [2].

Spelt, and especially emmer, form weaker doughs compared to common wheat, resulting in lower bread volume and quality [7–10]. Traditionally, bread quality is often characterised by a high bread volume, but recent studies also focus on crumb structure and porosity [11–13]. Fine, thin-walled, and uniformly sized cells are known to result in softer, more elastic crumbs, with porous crumbs having a better gas holding capacity and therefore producing a higher bread volume [14].

To improve baking quality, the baking industry commonly uses ascorbic acid, also known as vitamin C [15]. It is authorised as E300 in the European Union and used worldwide as a non-toxic food additive [16]. In dough,

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ascorbate oxidase catalyses the conversion of ascorbic acid to dehydroascorbic acid, which improves weak dough properties [17]. Endogenous glutathione, which can depolymerise the gluten network, is oxidised to the glutathione dimer, and ascorbic acid is regenerated for further oxidation [18]. The mechanisms and applications of ascorbic acid in common wheat have been widely studied [19–22], but are less studied in ancient wheat species. In common wheat flour, differences in baking quality improvement after ascorbic acid addition were observed between flours depending on the composition of high-molecular-weight glutenin subunits (HMW-GS) Dx2 + Dy12 or Dx5 + Dy10, with both studies coming to different results, which indicate that the interaction of HMW-GS and ascorbic acid is responsible for improving the baking quality [20, 23]. Comparable studies are lacking for spelt and emmer.

Studies with spelt commonly used 50–230 mg/kg of ascorbic acid and found an improvement in bread parameters, such as reduced crumb resistance or increased bread volume [24, 25]. Even when there was no improvement in baking quality with a lower amount of 5–10 mg/kg of ascorbic acid, the acceptability improved in sensory tests [26]. For emmer, additions of 30–60 mg/kg of ascorbic acid have been reported [27, 28]. Filipčev et al. found that the magnitude of baking quality improvement after ascorbic acid addition differed between the two analysed spelt cultivars [24]. Furthermore, using three different spelt cultivars, they found a significant impact of the cultivar and the growing year on baking quality [25]. They also analysed HMW-GS variations, crude protein content, and dough parameters, but did not focus on a more detailed characterisation of flour composition, including protein fractions and starch parameters. While these findings indicate a general trend, further research involving a broader range of lines and flour parameters is required to identify specific correlations between flour quality and response to ascorbic acid addition. Similar studies are missing for emmer. Grandal et al. studied one emmer cultivar with addition of 30 and 60 mg/kg of ascorbic acid [28] and focused on dough properties, free and bound thiols and polymeric protein content. They found higher dough strength with ascorbic acid than in a control without addition, but no significant changes in polymeric proteins, accessible sulfhydryl groups, or glutathione in the doughs. However, this study did not include baking quality.

Afzal et al. suggest that emmer baking trials should be carried out with careful, short kneading, less yeast, prolonged dough proofing, and a higher use of ascorbic acid [27]. Similarly, Frakolaki et al. suggest that, due to the weaker structure in spelt, the doughs require modified baking practices, such as shorter mixing time, reduced water addition, and increased ascorbic acid addition, along with a longer dough rest [9]. Long fermentation processes are a

traditional method to make breads. While modern bakeries rely on more efficient bread making, consumers are increasingly looking for better flavour of authentic, artisan-style breads, which can be achieved by long proofing time [29]. Wilson et al. used a dough rest time of 120 min and a further fermentation of 57 min for their 10 g spelt micro baking trials, but did not add ascorbic acid [30]. Wang et al. analysed the addition of 1.5–3% yeast and fermentation times from 60 to 150 min in common wheat bread and found that the specific bread volume of breads with only 1.5% yeast stayed below the breads with 3% yeast, but after a long-term fermentation of 150 min started to reach levels closer to the 3% breads in shorter fermentation time [31]. This indicates that even with a slow fermentation method, a good baking quality can be achieved.

In this study, we analysed the effect of ascorbic acid concentrations of 20–100 mg/kg in spelt and, for the first time, 50–250 mg/kg in emmer on dough and baking properties with commercial flour. We then evaluated the baking quality of eight spelt and ten emmer lines from at least two different locations, using optimised ascorbic acid dosages to correlate improvements with differences in flour composition and identify differences between lines in a larger sample set for the first time. Furthermore, the effects of long-term fermentation with reduced yeast levels and the addition of ascorbic acid were studied on commercial flour. These insights will provide a practical framework for bakers to standardise the production of high-quality breads from spelt and emmer.

Materials and methods

Sample material

Twenty-four spelt samples (eight lines from three locations) and twenty-five emmer samples (ten lines from two to three locations) grown in Southwestern Germany (Hohenheim, Oberer Lindenhof and Ihinger Hof) were used. More information on the lines has been reported earlier in Hempel et al., and can be found in Supplementary Tables 1 and 2 [32, 33]. After dehulling and milling to whole grain flour in a cross-beater mill (Perten Instruments, Hamburg, Germany), the flours were stored dry for at least two weeks at room temperature. The evaluation of different ascorbic acid levels was performed with commercial emmer and spelt flours (SchapfenMühle GmbH & Co. KG, Ulm-Jungingen, Germany).

Reagents

All chemicals and reagents were of analytical or higher grade and purchased from VWR Chemicals (Radnor, PA,

USA), Thermo Fisher Scientific (Waltham, MA, USA) and Carl Roth (Karlsruhe, Germany). Fresh yeast (Wieninger Frische Hefe, REWE, Cologne, Germany) and vegetable fat (“ja! Reines Pflanzenfett”, REWE) were bought in a local supermarket.

Evaluation of optimal ascorbic acid levels in emmer and spelt bread

The recipe and baking method are based on Münzing et al. [34] and Longin et al. [35] and were optimised in previous studies for emmer and spelt [32, 33]. All breads were tested in triplicates. Fifty grams of commercial emmer or spelt flour, 1% sucrose, 1% vegetable fat, 1.5% salt and 3% fresh yeast were mixed for 2 min in a farinograph (Anton Paar TorqueTec GmbH, Duisburg, Germany). Afterwards, water (tempered to 30 °C) was added to achieve a consistency of 350 BU and the dough was mixed further for 4 min at 63 rpm for emmer and at 40 rpm for spelt as in Hempel et al. [32, 33]. The water absorption of each sample without ascorbic acid was measured. For the ascorbic acid addition, a solution of ascorbic acid (0.5% in water (w/v)) was added for spelt to achieve concentrations of 20–100 mg/kg based on flour and of 50–250 mg/kg based on flour for emmer and the volume of the solution was subtracted from the regular water addition to keep hydration levels equal between the doughs with and without ascorbic acid. For emmer, pretests showed that low levels of ascorbic acid (20 and 60 mg/kg) had little to no impact on the specific bread volume (data not shown). For this reason, we decided to test ascorbic acid at higher doses: 0, 50, 100, 150, 200, and 250 mg/kg in emmer and to test 0, 20, 40, 60, 80 and 100 mg/kg ascorbic acid in spelt.

The dough was fermented and baked as described in “Dough processing” and the bread quality was determined as outlined in “Determination of baking quality”. Following the determination of the optimal ascorbic acid concentrations, the 24 spelt and 25 emmer samples, representing eight spelt lines and ten emmer lines from two to three different locations were processed accordingly with addition of 60 mg/kg of ascorbic acid for spelt and 250 mg/kg for emmer.

Microscale extension test

Ten grams of dough, from the development of the optimal ascorbic acid addition with commercial spelt and emmer flour or from the line comparisons with the 24 spelt and 25 emmer samples, were used in the microscale extension test after mixing of the dough. The dough was pressed into greased silicone moulds, and the exposed sides of the dough strands were also greased to prevent drying of the dough

[36, 37]. The pressed dough strands were left to rest at 25 °C for 15 min. Then they were carefully removed and clamped into the SMS/Kieffer dough and gluten extensibility rig in a texture analyser (Stable Micro Systems, Godalming, UK). The test was performed in triplicate dough pieces, and at least three dough strands per dough piece were tested for each replicate.

Dough processing

The doughs were manually rounded and placed in a proofing chamber at 25 °C. The samples were left in the proofer for 20 min, then taken out and carefully folded. Then the dough was proofed once more for 20 min, followed by baking for 10 min at 210 °C in an oven (UNOX Deutschland GmbH, Büren, Germany), with water steaming for 20 s at the beginning. The 20+20 min fermentation time was used for all breads except for breads in Long-term fermentation.

Long-term fermentation

The commercial emmer and spelt flours were used and the yeast amount was reduced from 3% to 1% in the recipe (see “Evaluation of optimal ascorbic acid levels in emmer and spelt bread”). The spelt dough was prepared with 60 mg/kg of ascorbic acid and emmer dough with 250 mg/kg. The dough was prepared as described in “Evaluation of optimal ascorbic acid levels in emmer and spelt bread”. The first fermentation step was extended to 40–60 min. Then the dough was folded and further fermented for 60, 120, or 240 min in the proofer. Breads were baked at 210 °C for 10 min, with steam added for the first 20 s.

Determination of baking quality

The breads were left to cool for 1 h, weighed, and their volumes were measured (VolScan Profiler VSP300, Stable Micro Systems). After cutting the breads into 1.5 cm slices, a slice from the centre was analysed in a TA.XTplus texture analyser (Stable Micro Systems) as described in Stemler and Scherf [38]. The texture analyser was calibrated and a double-compression test with a 20 mm-diameter cylindrical probe was performed. The settings were 40% deformation of the bread slice thickness, a release force of 0.049 N, a waiting time of 10 s between compressions, a pretest speed of 1 mm/s, a test and backtest speed of 0.8 mm/s, and a sampling rate of 200 points per second. The data was evaluated in the software Exponent (version 6.2.6.0, Stable Micro Systems). To analyse crumb characteristics and colour, a second slice was tested in a C-Cell system (Calibre Control, Warrington, UK). Calibration was performed before every measurement

with a calibration card (CC006, Calibre Control) and slices placed on the blue background card.

Statistical analysis

Microsoft Excel was used to calculate means and standard deviations (Microsoft Corporation, Seattle, WA, USA). Analysis of two-sided Student's *t*-tests was performed in OriginPro 2025b (OriginLab Corporation, Northampton, MA, USA). All further analysis was performed in RStudio (2025.9.1.401, Posit Software, PBC, Boston, MA) [39]. Data transformation was performed with package *dplyr*. One-way analysis of variance (ANOVA) with Tukey's post-hoc test ($p \leq 0.05$) was generated with packages *stats*, *emmeans*, *multcomp* and *multcompView*. Parameters of all lines were corrected for location effects using a linear mixed-effects model with location as a random effect using *lme4* package. The residuals from these models were used for principal component analysis (PCA), comparison of lines in one-way ANOVA and grouped Pearson correlations. Grouped Pearson correlations between the flour composition and the change (Δ = value after ascorbic acid – value before ascorbic acid addition) in a baking parameter after ascorbic acid addition were executed with the package *stats*. These correlations were then corrected with the Benjamini-Hochberg correction to control multiple testing errors in the package *stats*. Since a large number of correlations were calculated in one experiment, the correction factor was only applied to biologically relevant correlations of $r \geq 0.40$. PCA was performed with the package *stats* as well. To visualize the statistical results, the package *ggplot2* was used. To identify the effect of HMW-GS variance on selected baking and dough quality parameters, a multivariate analysis of variance (MANOVA) was performed with univariate ANOVAs to check to the individual results in the MANOVA with *stats*.

Results and discussion

Evaluation of optimal ascorbic acid levels in spelt bread

We added 0, 20, 40, 60, 80, and 100 mg/kg of ascorbic acid to a commercial spelt flour to determine the optimal effect on dough and baking properties (Table 1, Supplementary Figs. 1–4). In spelt, we found a slight increase in specific bread volume (+7.9%) with 60 mg/kg, a reduction in hardness (–26.0%) with 80 mg/kg, and a higher cell diameter (+20.2%) with 100 mg/kg of ascorbic acid compared to the control with no addition. Other parameters such as the maximal dough force and the maximal distance till break of the

dough, the elasticity and the cell number of the crumb were not significantly changed. Furthermore, the gumminess of the crumb decreased (–23.1%) with 80 mg/kg and the wall thickness of the cells increased (+5.8%) with 100 mg/kg of ascorbic acid. The results thus show significant effects of ascorbic acid addition on dough and baking properties. Although the highest specific bread volume was observed with 20 mg/kg of ascorbic acid addition, no significant difference in specific bread volume was found between 20 mg/kg and 60 mg/kg addition. In addition, breads with 60 or 80 mg/kg of ascorbic acid addition showed a significantly softer crumb and with 60 mg/kg addition also a higher cell diameter, which indicated an airy bread structure. Therefore, considering the overall bread quality, we recommend the addition of 60–80 mg/kg ascorbic acid to spelt breads.

Filipčev et al. added 20 to 230 mg/kg of ascorbic acid to two spelt lines and found no significant impact on the specific bread volume or the crumb hardness, but a significant reduction of crumb resilience in one cultivar [24]. They concluded that 50 mg/kg of ascorbic acid, 90 mg/kg of citric acid and 60 mg/kg of xylanase were optimal for bread baking with strong cultivars. In weak cultivars, the lower levels of ascorbic acid and citric acid were sufficient, while xylanase levels had to be increased. We observed significant improvements in specific bread volume and crumb hardness, while crumb elasticity remained unaffected. The discrepancy between our findings and the previous studies may stem from their use of a combined experimental design involving citric acid and xylanase, which prevented the effects of ascorbic acid from being isolated and measured independently.

Filipčev et al. analysed the effect of 200 mg/kg of ascorbic acid on three spelt lines and found an increase in specific bread volume and height and a decrease in crumb firmness and resilience, as well as a change in pore structure [25]. Furthermore, they found higher L^* values for two spelt lines after ascorbic acid addition. Except for the increase in L^* value and the decrease in crumb resilience, these results align well with ours. We also show that comparable bread improvements as those reported by Filipčev et al. can be achieved with lower ascorbic acid levels of 60–80 mg/kg.

Evaluation of optimal ascorbic acid levels in emmer bread

When analysing ascorbic acid additions of 0, 50, 100, 150, 200, and 250 mg/kg in emmer breads made with commercial emmer flour, we found a significant decrease in the maximum distance to break for the dough strings (–29.0%) with 250 mg/kg of ascorbic acid (Table 1, Supplementary Figs. 1–4). Otherwise, no significant impact on any other parameter was found. This indicates that the dough strands

Table 1 Spelt and emmer dough and bread characteristics after addition of ascorbic acid (AA in mg/kg) using one commercial flour each

Wheat	AA	Specific bread volume [ml/g]	R_{max} [N]	E_{Rmax}	A_{Rmax}	Hard- ness [N]	Elasticity	Cohesiveness	Gumminess	Chewiness	Springiness	Cell number	Cell diam- eter [mm]	Wall thick- ness [mm]	Colour L*
Spelt	0	2.26 A	0.25 A	19.91 A	1.12 A	36.43 A	2.09 A	7.05 B	0.90 A	0.71 A	5.02 B	7773.33 A	2.64 A	0.44 A	29.10 A
	20	2.45 B	0.28 A	18.88 A	1.12 A	32.64 A	2.02 A	5.64 AB	0.92 A	0.73 A	4.11 AB	7912.33 A	2.92 AB	0.46 B	30.26 A
	40	2.39 AB	0.25 A	20.46 A	1.13 A	33.43 A	1.89 A	5.52 AB	0.92 A	0.72 A	3.99 A	7818.00 A	2.94 AB	0.46 AB	29.01 A
	60	2.43 AB	0.26 A	19.76 A	1.11 A	33.20 A	1.92 A	5.44 A	0.93 A	0.72 A	3.94 A	7746.33 A	3.10 B	0.47 B	29.72 A
	80	2.42 AB	0.26 A	15.84 A	0.88 A	28.43 A	1.60 A	5.22 A	0.95 A	0.74 A	3.86 A	7921.00 A	2.93 AB	0.46 AB	29.53 A
Emmer	100	2.37 AB	0.23 A	18.37 A	0.90 A	32.06 A	1.59 A	5.64 AB	0.92 A	0.71 A	4.02 AB	7138.33 A	3.18 B	0.47 B	29.72 A
	0	2.20 A	0.08 A	16.28 A	0.37 A	31.95 B	0.73 A	8.79 A	0.90 A	0.68 A	6.00 A	7725.33 A	3.05 A	0.45 A	30.19 A
	50	2.21 A	0.10 A	15.11 A	0.38 A	28.36 AB	0.72 A	8.84 A	0.89 A	0.68 A	5.98 A	8312.67 A	2.71 A	0.44 A	31.15 A
	100	2.21 A	0.12 A	15.03 A	0.44 A	26.50 AB	0.78 A	9.28 A	0.93 A	0.69 A	6.38 A	7847.67 A	3.07 A	0.45 A	32.07 A
	150	2.17 A	0.10 A	14.52 A	0.39 A	26.84 AB	0.73 A	8.08 A	0.90 A	0.69 A	5.59 A	7758.67 A	3.10 A	0.46 A	32.87 A
	200	2.19 A	0.12 A	14.68 A	0.44 A	26.02 AB	0.79 A	8.94 A	0.88 A	0.68 A	6.10 A	8066.33 A	2.86 A	0.45 A	32.07 A
	250	2.16 A	0.12 A	13.02 A	0.39 A	22.69 A	0.69 A	8.31 A	0.89 A	0.68 A	5.68 A	7899.00 A	2.99 A	0.45 A	31.73 A

The data are displayed as mean ($n=3$). Different uppercase letters designate significant differences between the parameters between the AA addition levels in one wheat species (one-way analysis of variance, Tukey post-hoc test, $p \leq 0.05$, $n=3$).

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

lost extensibility after the addition of 250 mg/kg ascorbic acid, indicating stronger dough strength. We therefore conclude that the addition only had a small impact on dough properties and no significant impact on baking properties.

Grandal et al. found a significant increase in maximal dough force and a decrease in maximal distance till break of emmer dough with an ascorbic acid addition of 30 and 60 mg/kg [28]. These results match our findings, but the effect was smaller in our experiment, which may be due to the use of different emmer flours. The effects of different levels of ascorbic acid on the baking quality of emmer were not analysed by Grandal et al., thereby precluding comparison.

We found that the addition of 60–80 mg/kg of ascorbic acid for spelt and of 250 mg/kg for emmer was beneficial for the dough or baking quality of bread. One reason we may have observed only a lower effect on baking quality could be the use of commercial flours that are blends of several lines. These flours typically have a higher baking quality compared to flours made of single lines, because line-specific differences are evened out [40]. Therefore, “[Analysis of spelt samples with optimized ascorbic acid addition](#)” and “[Analysis of emmer samples with optimised ascorbic acid addition](#)” focused on breads made from single spelt or emmer lines to determine whether some flours that would otherwise be unsuited for baking could be improved with the addition of ascorbic acid.

Long-term fermentation with reduced yeast content

In long-term fermentation tests with commercial flour, the potential for slower fermentation with only 1% yeast and longer dough fermentation times was studied (Table 2; Fig. 1 Supplementary Figs. 5–7). For both spelt and emmer, the breads with 1% yeast and a fermentation time of 40 min reached significantly lower specific bread volume and produced harder and less elastic crumbs with lower cohesiveness, higher gumminess, higher chewiness and lower springiness than the breads with 3% yeast and the same fermentation time. This is according to expectations and an indicator for underfermentation. These underfermented breads also showed a high crumb cell number, and in emmer, a lower cell diameter, combined with a lower wall thickness of the pores. For spelt, the cell diameter of 1% yeast breads with 40 min fermentation time was not significantly different, but the wall thickness was higher compared to the bread with the same fermentation time but with 3% yeast. Spelt breads with longer fermentation time, but 1% yeast showed specific bread volume, crumb hardness, crumb elasticity, cell number, and cell diameter comparable to those of breads with 3% yeast and 40 min fermentation. After 300 min of fermentation, the cell diameter was significantly larger, and the wall thickness also increased. These

can be seen as the first signs of reduced baking quality due to overfermentation, but even then, the baking quality was comparable after 300 min to that of the 3% yeast bread with 40 min fermentation. Interestingly, the L^* value decreased after 160 min, indicating a darker bread crumb.

The results for emmer breads in long-term fermentation were comparable to those for spelt. After 100 min fermentation time, the 1% yeast breads showed comparable specific bread volume, crumb hardness, elasticity, cohesiveness and springiness to the 3% yeast breads after 40 min fermentation. The difference was a greater variability in the baking quality parameters in the long-term fermentation trials, indicating weaker dough and less baking stability in emmer than in spelt. Especially the breads with a 100 min fermentation time showed a significantly higher elasticity and, therefore, a crumb with higher chewiness. Similar to the spelt experiment, the emmer breads showed a higher cell diameter after 300 min of fermentation and a significantly lower cell number, indicating that the emmer breads begin to lose baking quality after 300 min. They also the same reduction of the L^* value after 160 min of fermentation.

Zaparenko et al. compared one emmer flour with a common wheat flour and found that emmer produced a larger amount of gas and also reached the peak gas formation faster than common wheat [8]. After 60 min of fermentation, gas production slows, then increases again to reach a maximum between 160 and 180 min. This behavior was shared by common wheat and emmer, but with emmer again producing more gas in the second peak. At the same time, they found that a higher amount of gas was released during the fermentation of emmer dough than of common wheat dough and that the dough volume of emmer bread was higher than that of common wheat, with a decrease in volume after a fermentation time of 150–180 min. Troadec et al. analysed french yeast bread with a yeast amount of 0.85% with short-term fermentation for 1 h at room temperature compared to long-term fermentation for 24 h at 6 °C [41]. They found no changes in the protein fractions on SDS-PAGE between the short-term and long-term breads. However, a significantly lower amount of glucose and fructose was detected in the long-term breads compared to the short-term breads, with maltose staying consistent while the amount of ethanol, 3-methylbutanol and phenylethanol increased as a consequence of sugar fermentation. Due to a lower amount of available reducing sugars, lower amounts of furans, ketones and pyrazines were produced during the Maillard reaction in long-term fermented breads during baking. These changes in the composition of breads after long-term fermentation could also happen in our long-term fermentation breads and explain the changes in crumb colour. In Troadec et al., they analysed only the L^* value of the crust and found a higher value in long-term

Table 2 Spelt and emmer bread characteristics after addition of ascorbic acid (60 mg/kg for spelt and 250 mg/kg for emmer) with x min first fermentation and y min final fermentation

Wheat	Fermentation time	Specific bread volume [ml/g]	Hardness [N]	Elasticity	Cohesiveness	Gumminess	Chewiness	Springiness	Cell number	Cell diameter [mm]	Wall thickness [mm]	Colour L*
Spelt	20+20 3%Y	2.43 B	6.66 A	0.93 B	0.72 B	4.75 A	4.39 A	0.33 B	7773.33 AB	2.64 A	0.44 A	29.10 D
	20+20	1.86 A	10.26 B	0.82 A	0.64 A	6.54 C	5.36 B	0.25 A	8679.67 B	3.39 AB	0.46 B	29.05 D
	40+60	2.30 B	6.79 A	0.88 AB	0.70 B	4.76 A	4.22 A	0.32 B	8394.33 AB	3.54 AB	0.47 B	27.15 CD
	40+120	2.35 B	8.33 A	0.92 B	0.70 B	5.83 BC	5.38 B	0.32 B	8286.33 AB	3.30 AB	0.46 AB	24.68 ABC
	40+240	2.37 B	7.30 A	0.92 B	0.72 B	5.24 AB	4.84 AB	0.35 B	7554.00 AB	3.30 AB	0.46 AB	22.87 AB
	60+60	2.42 B	7.31 A	0.88 AB	0.70 B	5.12 AB	4.49 A	0.32 B	7993.67 AB	3.53 AB	0.46 B	26.23 BCD
	60+120	2.35 B	7.35 A	0.88 AB	0.70 B	5.14 AB	4.54 A	0.31 B	8050.67 AB	3.29 AB	0.46 AB	23.87 ABC
	60+240	2.26 B	7.85 A	0.88 AB	0.70 B	5.46 AB	4.82 AB	0.31 B	7220.33 A	3.91 B	0.46 B	21.73 A
	20+20 3%Y	2.16 C	8.79 B	0.90 B	0.68 BC	6.00 AB	5.42 AB	0.32 BC	7899.00 BCD	2.99 A	0.45 AB	30.19 DE
	20+20	1.77 A	11.84 D	0.81 A	0.63 A	7.45 D	6.02 BC	0.25 A	8832.33 D	2.86 A	0.44 A	30.35 E
Emmer	40+60	2.11 BC	9.13 BC	2.34 C	0.72 C	6.54 BC	15.31 D	0.33 BC	8542.00 CD	3.21 AB	0.45 AB	28.32 CDE
	40+120	2.17 C	7.68 A	0.92 B	0.71 BC	5.46 A	5.00 A	0.35 BC	7221.33 B	3.41 AB	0.46 B	27.33 BCD
	40+240	2.04 B	10.00 C	0.92 B	0.70 BC	7.04 CD	6.51 C	0.35 BC	5519.67 A	3.65 AB	0.45 AB	25.29 AB
	60+60	2.10 BC	9.77 BC	0.91 B	0.68 ABC	6.59 BC	5.97 BC	0.31 BC	7670.33 BC	3.14 AB	0.45 AB	28.64 CDE
	60+120	2.02 B	9.87 C	0.89 B	0.66 AB	6.50 BC	5.75 ABC	0.29 AB	7524.67 B	3.57 AB	0.45 AB	25.91 ABC
	60+240	2.06 BC	9.30 BC	0.91 B	0.70 BC	6.54 BC	5.92 ABC	0.35 C	5157.33 A	3.87 B	0.46 AB	24.13 A

The data are displayed as mean ($n = 3$). Different uppercase letters designate significant differences between the parameters after different fermentation times in spelt or emmer doughs (one-way analysis of variance, Tukey post-hoc test, $p \leq 0.05$, $n = 3$).

3%Y, 3% yeast. All other breads were baked with 1% yeast.

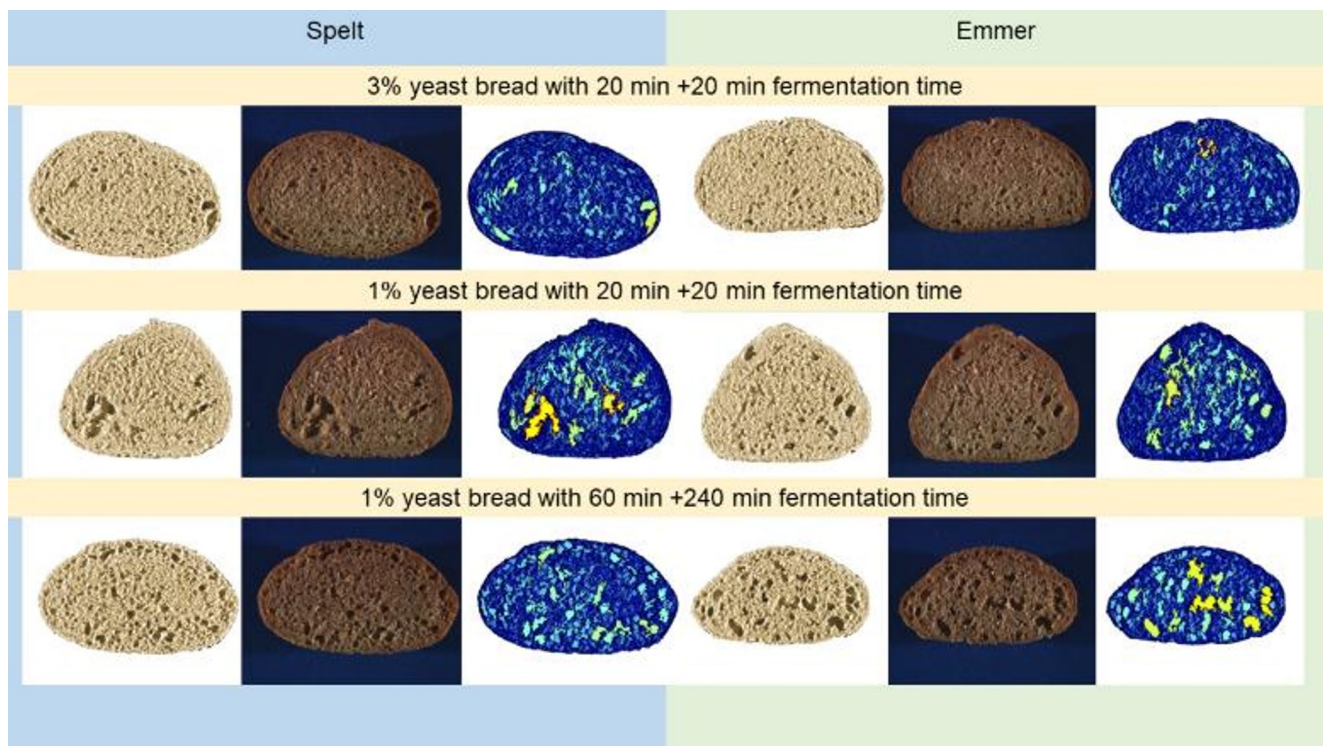


Fig. 1 Comparison of bread slices of spelt (left) and emmer (right) with different amounts of yeast (1 or 3%) and fermentation times using C-cell. Pictures from left to right: crumb view, standard settings, pore view

fermented bread than in short-term breads. Tlapale-Valdivia et al. found that when analysing sweet breads (2.3% yeast, 20% sugar, 12% milk powder, 16% shortening, 20.5% egg) with a comparable mixing time of 6 min, the highest specific bread volume was obtained with 200 min fermentation time [42]. They also detected a significantly lower L^* value after 200 min compared to 0 min fermentation time breads and a higher cell density. They explained the lower L^* value and therefore darker crumb with changes in cell size, and therefore a change in light reflection. In general, the studies mentioned differ significantly in terms of dough processing and bread ingredients and none of these studies used ascorbic acid in their recipe. A direct comparison is therefore not possible, and this study is the first to analyse this specific dough processing method.

Analysis of spelt samples with optimised ascorbic acid addition

We added 60 mg/kg of ascorbic acid to 24 spelt samples and 250 mg/kg of ascorbic acid to 25 emmer samples, representing 8 spelt lines and 10 emmer lines from two to three locations (Fig. 2, Supplementary Figs. 8–10). These samples were then compared to previous results [32, 33], where dough and baking quality of the same samples were measured without the addition of ascorbic acid with the same dough development and recipe. Taking all spelt samples

together, the specific bread volume increased (+ 11.0%), the crumb hardness decreased (−20.2%), and the cell diameter increased (+ 8.7%) with ascorbic acid compared to the control with no addition. The gumminess (− 19.7%) and chewiness (−18.6%) of the crumb decreased, while the cell wall thickness (+ 1.8%) increased. The most noticeable impact of ascorbic acid was observed in dough characteristics, with increased maximal dough force (+ 61.3%) but decreased distance till break (− 49.7%) and area till break (− 23.7%). Other baking parameters were not significantly affected by ascorbic acid. When assessing how different lines responded, we found significant changes (Δ) in dough behaviour, with Badenglanz and Zollernspelz showing an increase in the area till maximum force, and Badenglanz and Zollernspelz also showing significant differences in the area till break compared with the other lines (Table 3). Without ascorbic acid, only 15 of 24 spelt samples could be measured in the microscale extension test because the doughs were too soft to build up sufficient resistance against the hook in the test, whereas with ascorbic acid addition, 22 of 24 spelt samples could be measured. This shows a significant improvement not only in baking quality but also in dough processing, with a stronger dough that is easier for machines and bakers to handle.

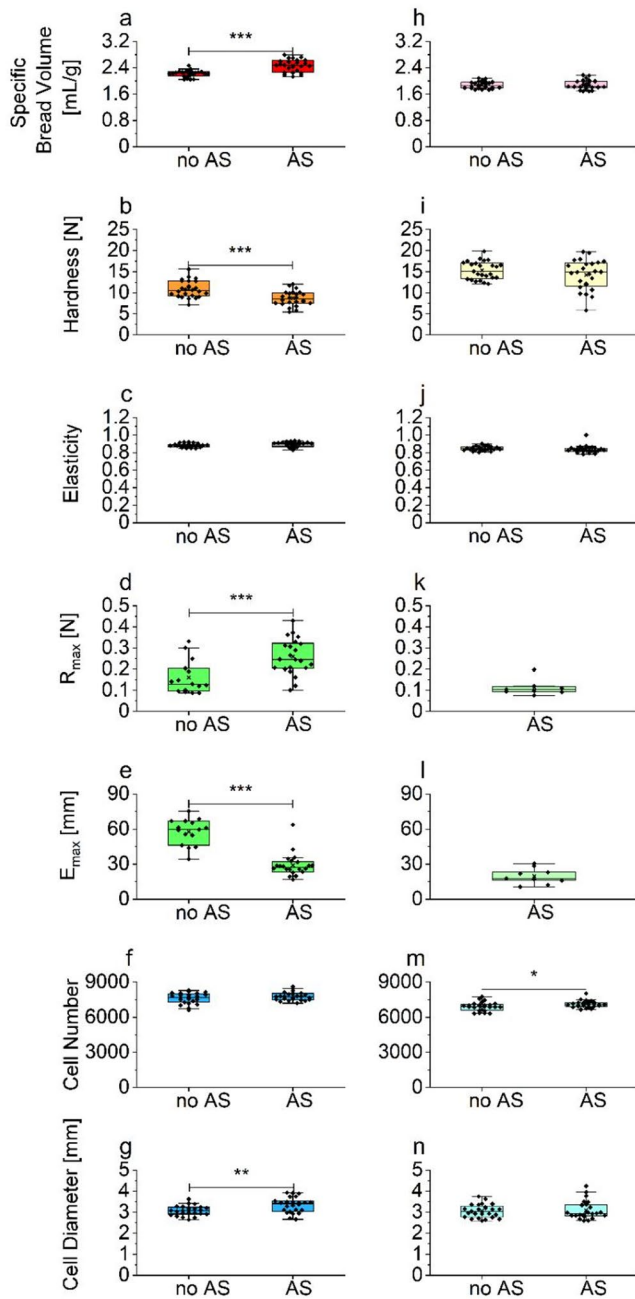


Fig. 2 Dough and bread characteristics of eight spelt lines ($n=24$) (a–g) and ten emmer lines ($n=25$) (h–n) with and without 60 mg/kg of ascorbic acid (spelt) and 250 mg/kg of ascorbic acid (emmer), respectively. (a+h) specific bread volume; (b+i) hardness; (c+j) elasticity; (d+k) maximum force of the dough (R_{max}); (e+l) distance till break (E_{max}); (f+m) cell number and (g+n) cell diameter of the crumb. Asterisks indicate significant differences with $*p \leq 0.05$, $**p \leq 0.01$ and $***p \leq 0.001$ (Student's two-sided t-test, $n=3$). The horizontal line in the box plot shows the median, and the cross shows the mean. The horizontal line in each box plot indicates the median, and the cross indicates the mean. The box represents the interquartile range (IQR), with whiskers extending to $1.5 \times IQR$. Points beyond the whiskers are considered outliers. Results without ascorbic acid are not shown in panels k and l because the dough was too weak for the test to be performed

Analysis of emmer samples with optimised ascorbic acid addition

Considering all emmer samples, only cell number (+3.0%) and colour L^* (+5.8%) increased with ascorbic acid addition, while crumb chewiness (-10.0%) decreased significantly (Fig. 2, Supplementary Figs. 8, 9, 10). All other parameters were not significantly affected. Regarding dough quality, the dough strength of nine emmer samples increased sufficiently with ascorbic acid to be measured in the microscale extension test, whereas without the addition, this test could not be performed on any of the emmer samples. When analysing the different reactions of the specific emmer lines to the addition of ascorbic acid, there was only a significant decrease in specific bread volume for E-160_422 (Table 3). Further, the cell number of bread crumb from Ramses was higher with ascorbic acid addition, while the cell number of E-160_413 and E-160_422 decreased. The wall thickness changed significantly with a reduction for line E-150_415 and an increase for Farro Mutico. Furthermore, there was a decrease in the colour L^* value for E160_422 and an increase for E-150-415, E150_418, and E-150_419 detectable. Considering all results for E-160_422 together, bread made from this line did not improve and even indicated a slight decline in baking quality with ascorbic acid addition.

Correlation analysis between spelt flour composition and baking response to ascorbic acid

To link the composition of the spelt and emmer flours reported earlier in Hempel et al. [32, 33] to changes in dough and bread quality after ascorbic acid addition, we performed a grouped Pearson correlation (Fig. 3, Supplementary Table 3). Across the spelt samples, we found 29 significant ($p \leq 0.05$) correlations after multiple-testing correction of r -values. The strongest correlations ($r = 0.63 - 0.60$) were between the protein composition and changes in the microscale extension test, and therefore the dough properties. A significant correlation with starch content, starch damage, or crude protein content was not detectable. Several important correlations were found between α -gliadins and Δ colour L^* ($r = 0.63$), Δ area till maximal dough force ($r = 0.60$), Δ chewiness ($r = -0.58$), Δ wall thickness ($r = 0.56$), Δ gumminess ($r = -0.56$), Δ hardness ($r = -0.56$), Δ area till break ($r = 0.54$), Δ cell diameter ($r = 0.53$), Δ specific bread volume ($r = 0.52$) and Δ cohesiveness ($r = 0.51$). Besides the significant correlations with the α -gliadins, Δ specific bread volume also correlated with the protein content in the Osborne fractionation ($r = 0.52$), gliadins ($r = 0.51$) and albumins/globulins ($r = 0.45$). The gliadin-to-glutenin ratio correlated with several dough parameters such as Δ area till break ($r = 0.63$), Δ area till maximal force ($r = 0.63$),

Table 3 Differences (Δ =value after ascorbic acid – value before ascorbic acid addition) between the parameters of the 24 spelt and 25 emmer samples

Wheat		Line	Table 2. Parameters and differences between the parameters of the 21 wheat samples														
Bread Volume			Δ Specific	ΔR_{max}	ΔE_{Rmax}	ΔA_{Rmax}	$\Delta Hardness$	$\Delta Elasticity$	$\Delta Cohesiveness$	$\Delta Gumminess$	$\Delta Chewiness$	$\Delta Springiness$	$\Delta Cell$ number	$\Delta Wall$ thickness	$\Delta Cell$ diameter	$\Delta Colour$ L*	
Spelt	Albertino	0.07 A	0.05 A	-7.56 A	-0.12 ABC	-5.11 A	0.04 ABC	-0.44 A	-0.62 A	-0.02 A	-1.42 A	-0.28 A	0.06 A	-27.31 A	0.01 A	0.17 A	-0.16 A
	Badenglanz	-0.02 A	0.07 A	26.47 A	0.91 BC	43.50 A	1.55 BC	-0.26 A	4.31 A	0.20 A	8.33 A	0.22 A	-0.35 A	-126.19 A	0.00 A	-0.12 A	0.17 A
	Frankenkorn	0.01 A	-0.05 A	-9.25 A	-0.67 A	-17.58 A	-1.22 A	-0.34 A	-0.61 A	-0.03 A	-1.42 A	-0.21 A	0.06 A	-244.53 A	0.01 A	0.20 A	-0.20 A
	Rheingold	-0.06 A	-0.01 A	6.80 A	0.34 ABC	13.19 A	0.53 ABC	-1.33 A	-0.64 A	-0.02 A	-1.96 A	-0.83 A	0.04 A	33.36 A	0.00 A	0.12 A	0.33 A
	ZG1814	-0.08 A	0.04 A	-13.75 A	-0.32 AB	-16.01 A	-0.18 ABC	2.60 A	-0.60 A	-0.04 A	0.42 A	1.51 A	0.05 A	42.03 A	-0.01 A	-0.36 A	-0.89 A
	ZG1912	0.06 A	0.00 A	9.28 A	0.17 ABC	18.66 A	0.32 ABC	-1.27 A	-0.62 A	-0.02 A	-2.01 A	-0.80 A	0.06 A	270.03 A	0.00 A	0.17 A	1.36 A
Emmer	Zollernperle	-0.07 A	-0.08 A	-7.47 A	-0.40 AB	-15.65 A	-0.81 AB	1.36 A	-0.60 A	-0.05 A	-0.50 A	0.71 A	0.03 A	-79.75 A	-0.01 A	-0.19 A	-0.53 A
	Zollernspitz	0.08 A	0.02 A	15.40 A	0.80 C	12.40 A	0.98 C	-0.32 A	-0.62 A	-0.03 A	-1.45 A	-0.32 A	0.05 A	132.36 A	0.00 A	0.00 A	-0.08 A
	E-150_415	0.01 BC						1.54 A	-0.01 A	-0.03 A	0.64 A	0.48 A	-0.03 A	55.86 A B	-0.31 A	-0.12 A	0.87 B
	E-150_418	0.05 BC						-0.38 A	-0.02 A	0.01 A	0.09 A	-0.04 A	0.02 A	-135.25 AB	0.13 AB	-0.11 A	0.93 B
	E-150_419	-0.04 AB						0.87 A	-0.01 A	0.00 A	0.67 A	0.53 A	-0.01 A	184.64 AB	-0.02 AB	-0.12 A	1.26 B
	E-150_423	-0.03 ABC						2.17 A	0.01 A	-0.02 A	1.11 A	1.00 A	-0.03 A	-229.69 AB	-0.18 AB	-0.12 A	0.40 AB
	E-160_413	-0.03 ABC						-1.96 A	-0.05 A	0.01 A	-0.96 A	-1.28 A	0.01 A	-468.03 A	0.06 AB	-0.12 A	0.03 AB
	E-160_414	-0.02 ABC						1.22 A	0.02 A	-0.02 A	0.54 A	0.68 A	-0.02 A	115.14 AB	-0.29 AB	-0.13 A	-0.95 AB
	E-160_422	-0.08 A						1.51 A	-0.01 A	-0.03 A	0.56 A	0.35 A	-0.03 A	-369.36 A	-0.03 AB	0.87 A	-2.07 A
	Farro Mutico	0.02 BC						-3.24 A	0.04 A	0.08 A	-1.46 A	-1.01 A	0.09 A	-148.03 AB	0.47 B	-0.11 A	-0.51 AB
Ramses	0.03 BC						-0.26 A	0.01 A	-0.01 A	-0.22 A	-0.07 A	-0.01 A	-0.01 A	838.31 B	-0.15 AB	-0.13 A	0.03 AB

The data are displayed as mean ($n=3$). Different uppercase letters designate significant differences between the spelt and emmer lines (one-way analysis of variance, Tukey post-hoc test, $p \leq 0.05$, $n=3$). Parameters were corrected for location effects using a linear mixed-effects model with location fitted as a random effect. The residuals from these models were used for line comparisons.

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

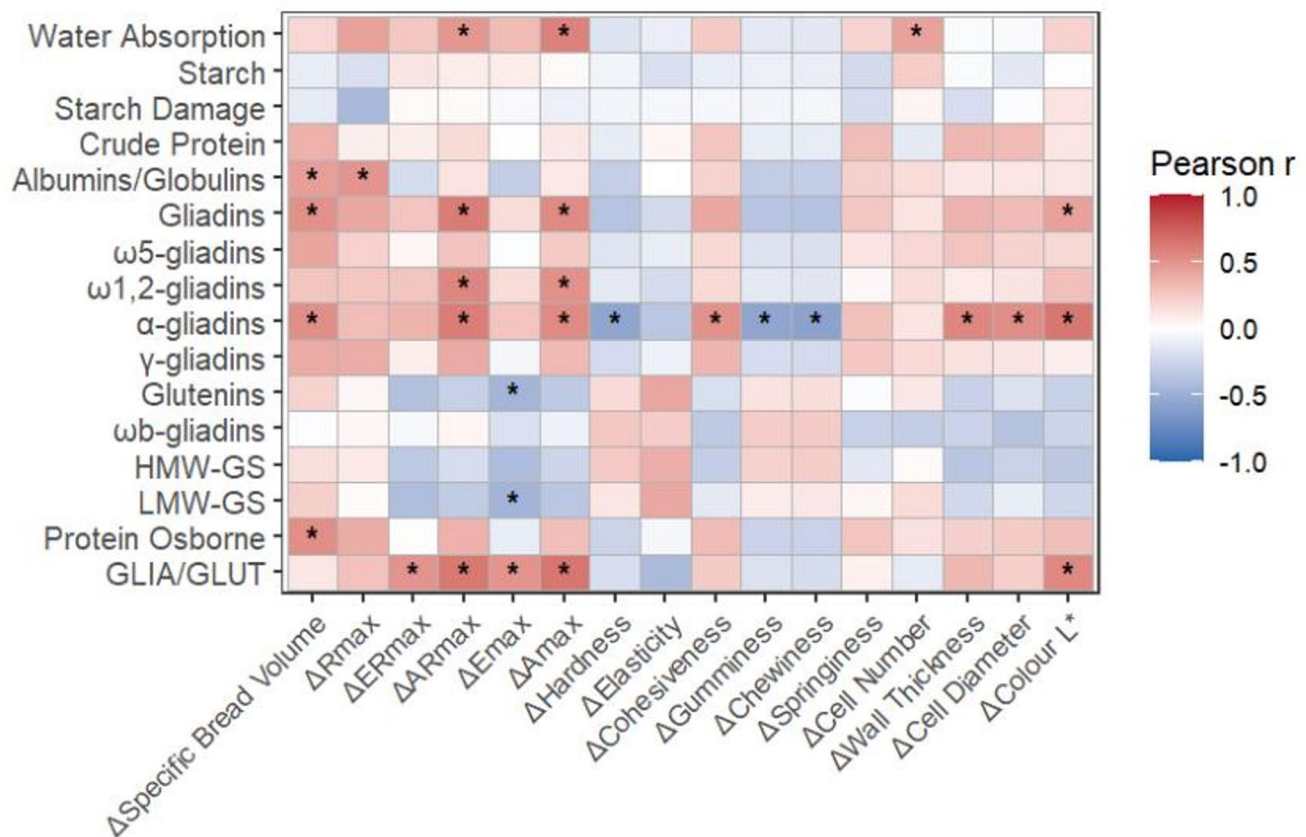


Fig. 3 Grouped Pearson correlation coefficients ($p \leq 0.05$, $n=24$) of analytical parameters with differences in dough or bread quality parameters after ascorbic acid addition of 60 mg/kg of eight spelt lines. Stars on boxes indicate significant results ($p \leq 0.05$). Parameters were corrected for location effects using a linear mixed-effects model with location fitted as a random effect. The residuals from these mod-

els were used for correlation analysis. 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

Δ distance till break ($r = 0.50$) and Δ distance till maximal force ($r = 0.50$). These results indicate that certain changes in dough and baking quality resulting from ascorbic acid addition are correlated with specific flour protein fractions.

Correlation analysis between emmer flour composition and baking response to ascorbic acid

Since the dough strength of emmer doughs before adding ascorbic acid was too low, we included only the baking quality parameters. For emmer, 16 significant correlations were found, with no starch parameters significantly correlating with the baking quality parameters (Fig. 4, Supplementary Table 4). The strongest correlation was found between the water absorption and the Δ gumminess ($r = -0.60$), followed by Δ hardness ($r = -0.55$), Δ chewiness ($r = -0.54$), Δ cohesiveness ($r = 0.46$), Δ springiness ($r = 0.45$) and Δ wall thickness ($r = 0.43$). Δ Specific bread volume correlated significantly only with the gliadin-to-glutenin ratio ($r = -0.52$), and a correlation between the HMW-GS content and Δ cell

number ($r = 0.45$) was also detectable. These results indicate a strong correlation between certain changes in the baking quality of emmer breads after the addition of ascorbic acid and specific protein fractions in the flour.

Every et al. did not find a significant correlation between the volume change of bread after ascorbic acid addition (%), and total protein content, polymeric protein content, or gliadin content in the flours of 19 diverse common wheat cultivars and 19 breeding lines [23]. Only the mixograph dough development time ($r = -0.56$) and the percentual change of maximal dough force ($r = 0.68$) correlated significantly with the percentual volume change. They concluded that a lower dough strength results in a stronger ascorbic acid improvement effect on dough strength and baking quality. Our study also a strong improvement of dough strength with the addition of ascorbic acid, but we also found several protein fractions that correlated significantly with the baking quality of spelt and emmer. Potential reasons why our findings differ from those of Every et al. could be that we analysed spelt and emmer lines instead of common

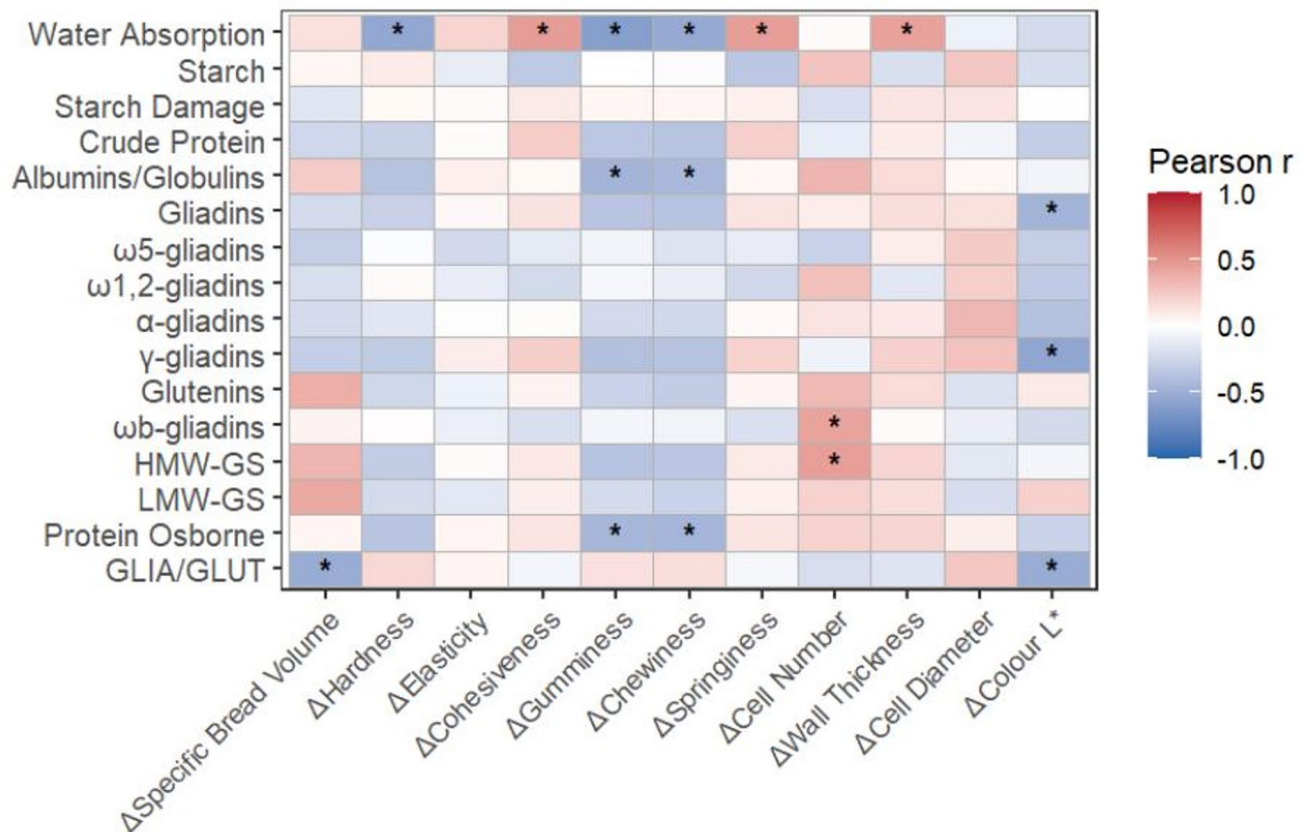


Fig. 4 Grouped Pearson correlation coefficients ($p \leq 0.05$, $n=25$) of analytical parameters with differences in dough or bread quality parameters after ascorbic acid addition of 250 mg/kg of ten emmer lines. Stars on boxes indicate significant results ($p \leq 0.05$). Parameters were corrected for location effects using a linear mixed-effects model

with location fitted as a random effect. The residuals from these models were used for correlation analysis. HMW-GS, high-molecular-weight glutenin subunits; LMW-GS, low-molecular-weight glutenin subunits; GLIA/GLUT, gliadin-to-glutenin ratio

wheat, and that we adjusted the amount of ascorbic acid (spelt: 60 mg/kg, emmer: 250 mg/kg) to the recipe, while Every et al. used 100 mg/kg ascorbic acid for their tests. Aamodt et al. analysed two common wheat lines at two different protein levels, one with HMW-GS Dx5 + Dy10 and the other one with Dx2 + Dy12 [20]. A rise in maximal force of all doughs could be observed, with the strongest rise in the Dx5 + Dy10 line with the lower protein level ($\Delta = 0.166$ N), followed closely by the Dx2 + Dy12 line with the higher protein level ($\Delta = 0.145$ N). Similarly, the bread area improved for all breads after the addition, and the crumb hardness was reduced. The greatest improvements in bread area could be found for the Dx5 + Dy10 line with low protein ($\Delta = +20.14$ cm²) and high protein ($\Delta = +17.83$ cm²) content. Both samples also showed a high reduction of crumb hardness (low protein $\Delta = -2.8$ N, high protein $\Delta = -2.75$ N). The Dx2 + Dy12 line had at lower protein level, 9.86 cm² more bread area, and 2.9 N lower crumb hardness. With the higher protein level, the area increased to 14.26 cm² and the hardness decreased by 1.69 N. These results describe a systematic connection between improvements

in dough and bread properties and quality parameters, but need to be interpreted carefully, since only one line of each HMW-GS variant at two protein levels was analysed, along with other differences in flour composition. We found only a few significant differences in the improvement of dough or bread parameters due to ascorbic acid addition between the spelt and emmer lines, but there were correlations between several dough/baking parameters and protein composition, indicating that the observed improvements are dependent on spelt and emmer flour characteristics, similar to common wheat [20]. A strong negative correlation between Δ specific bread volume and the gliadin-to-glutenin ratio in emmer indicates that flours traditionally considered stronger (i.e., with a lower gliadin-to-glutenin ratio) produce breads with higher specific bread volume changes after ascorbic acid addition and therefore show better baking quality. Such a strong correlation was not found in the spelt breads, where Δ specific bread volume correlated with gliadins, while the lower gliadin-to-glutenin ratio had more of an impact on a higher dough strength. Hrušková and Novotná analysed the effect of ascorbic acid by dividing their flours into groups

with high and low ash content [19]. They found that changes in dough characteristics after ascorbic acid addition allowed a differentiation of these two groups, but this did not translate to differences in specific bread volume. They also described correlations between dough properties and baking volume, and that several dough parameters are influenced by ash content and Zeleny sedimentation value, which is a protein quality parameter. In our study, we did not analyse ash content and focused more on baking parameters than on dough properties; however, these results, together with ours, indicate that flour characteristics and protein parameters are indicators of potential improvements in baking quality with the addition of ascorbic acid. Our results indicate greater potential for improvement with stronger spelt and emmer flours. Therefore, selecting suitable flours is an important step to ensure good quality when adding ascorbic acid.

Analysis of HMW-GS variants and the ascorbic acid improvements in spelt

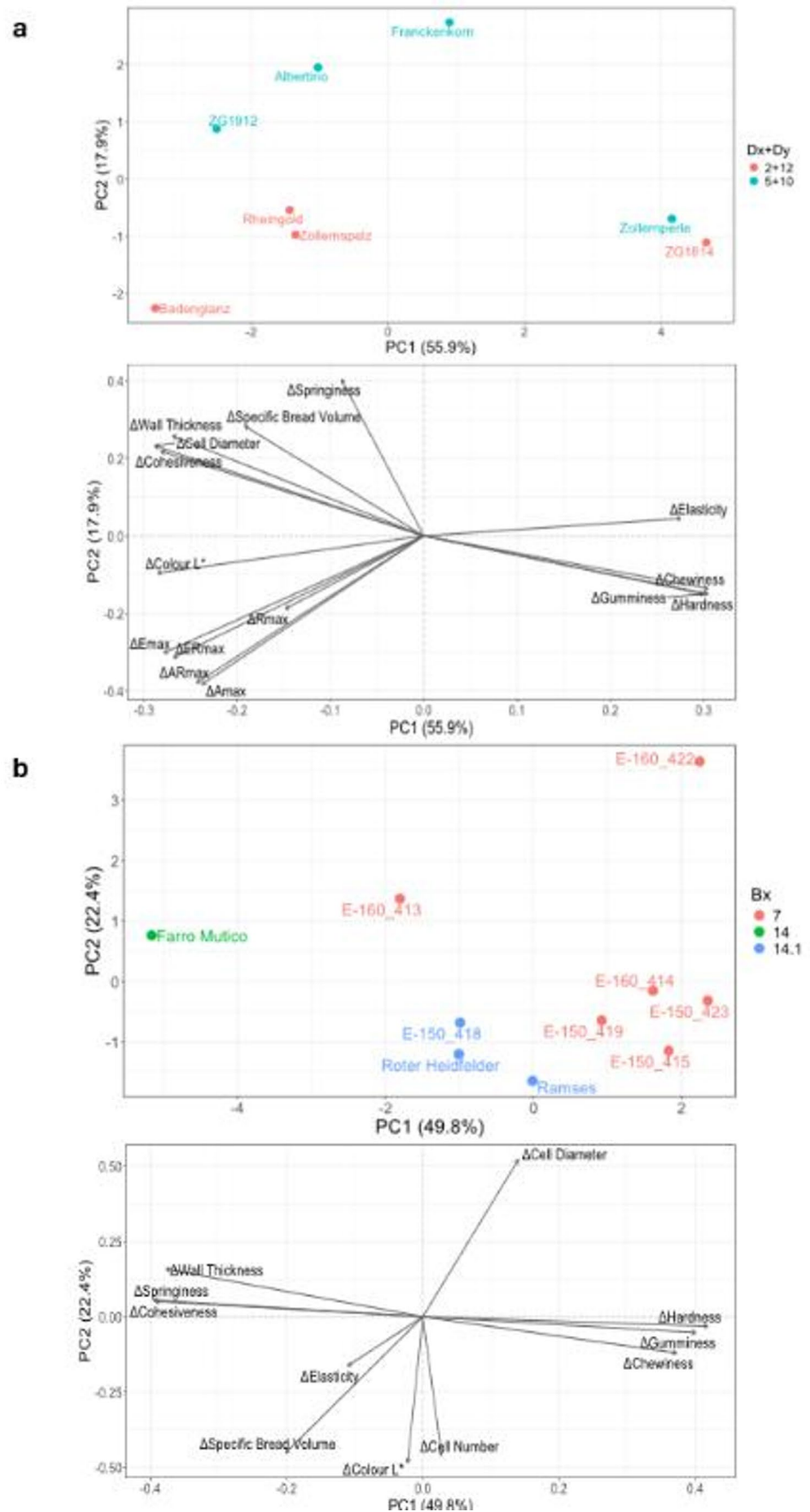
Studies on common wheat suggested that certain HMW-GS variations improve bread quality when combined with ascorbic acid [20, 23]. Of the eight spelt lines, four had the combination Dx5 + Dy10 and four had the combination Dx2 + Dy12. To analyse whether HMW-GS variations affect the improvement in baking quality after ascorbic acid addition, we selected one representative parameter from each quality group (dough parameters, bread volume, crumb texture, and crumb porosity). For spelt, there was no significant impact of the HMW-GS Dx5 + Dy10 and Dx2 + Dy12 on the combined factors Δ maximal dough force, Δ specific bread volume, Δ hardness of the crumb, and Δ cell number detectable (MANOVA, Supplementary Table 5). To further analyse the impact of HMW-GS variance, we also performed a PCA of baking quality for the lines (Fig. 5). There is a slight spatial separation detectable between lines with Dx5 + Dy10 reaching higher levels along the PC2 axis, and lines with Dx2 + Dy12 reaching lower levels. The PC2 axis is mainly driven by positive effects on baking quality, such as Δ specific bread volume ($r = 0.28$), Δ springiness ($r = 0.40$), wall thickness ($r = 0.26$), and Δ cell diameter ($r = 0.23$). This could indicate an improvement in baking quality after the addition of ascorbic acid to spelt breads made from lines with HMW-GS Dx5 + Dy10, which is known as the HMW-GS combination associated with a higher specific bread volume [32]. Nevertheless, the result should only be seen as a first indication, since only eight spelt lines were tested, and Δ specific bread volume, Δ hardness, Δ maximal dough force and Δ cell number did not show significant results in the MANOVA.

Analysis of HMW-GS variants and the ascorbic acid improvements in emmer

For emmer, we analysed 25 samples representing the ten lines, of which six had HMW-GS Bx7, one had Bx14, and three had Bx14.1 [33]. Using MANOVA, we found a significant impact of HMW-GS Bx7, Bx14, or Bx14.1 on the combined factors Δ specific bread volume, Δ crumb hardness, and Δ cell number in emmer in the Pillai's Trace test, but narrowly missed the $p \leq 0.05$ threshold in the Wilks Lambda test ($p = 0.054$) (Supplementary Table 5). The three evaluated parameters were then retested in separate ANOVAs, followed by Tukey's post hoc tests. Lines with the HMW-GS Bx14.1 showed significantly higher improvement of the specific bread volume and reduction of the crumb hardness after the addition of 250 mg/kg ascorbic acid compared to lines with HMW-GS Bx7 (Supplementary Table 6). No significant impact was observed on cell numbers. This trend is also visible in the PCA, with a spatial grouping of the lines with Bx7 in the positive space along the PC1 axis, while Bx14.1 lines clustered in the negative part of the PC1 axis (Fig. 5). The Δ hardness ($r = 0.42$) and Δ gumminess ($r = 0.40$) had the highest impact on the PC1 axis, while the specific bread volume was associated with the negative direction ($r = -0.20$). The PCA therefore also indicates that lines with HMW-GS Bx14.1 could be associated with higher specific bread volume, lower crumb hardness, and therefore better baking quality. These results indicate only that a trend may be visible. Only three lines with the Bx14.1 HMW-GS were analysed, and therefore the results require confirmation with a larger sample size of emmer lines carrying these HMW-GS. Lines with HMW-GS Bx14.1 in emmer were also identified in samples without ascorbic acid, showing higher specific bread volume and better baking quality. This again agrees with the trend found in spelt, that "stronger" flours, characterised by the HMW-GS variance, react positively to the addition of ascorbic acid.

Aamodt et al. analysed the response of five common wheat flours to ascorbic acid addition in correlation with HMW-GS Dx5 + Dy10 and Dx2 + Dy12 and found a longer mixogram peak time in samples with Dx5 + Dy10, except for one line [20]. Using PCA, they found a significantly higher area of bread slices with lines containing HMW-GS Dx5 + Dy10 than with Dx2 + Dy12. One reason why we did not see these effects could be that only one HMW-GS Dy2 + Dy12 line was included in that study, whereas we had four Dx2 + Dy12 lines in the spelt samples to balance the test. Another reason could be that spelt is not comparable to common wheat in this regard, whereas our results for the addition of ascorbic acid to emmer breads indicate a similar trend to that observed in Aamodt et al.. Every et al. describe the opposite effect of HMW-GS variants with the weaker

Fig. 5 Principal component analysis score plots (up) and loading plots (down) with the baking quality parameters of **a** eight spelt lines and **b** ten emmer lines. Parameters of 24 spelt samples and 25 emmer samples were corrected for location effects using a linear mixed-effects model with location fitted as a random effect. The residuals from these models were used for analysis. **a** Lines were grouped by HMW-GS variants Dx2+Dy12 (orange) and Dx5+Dy10 (blue). **b** Lines were grouped by HMW-GS variants Bx7 (orange), Bx14 (green), Bx14.1 (blue). $R_{\max}$, maximal force; $E_{R\max}$, distance to max. force; $A_{R\max}$, area till max. force; $E_{\max}$, distance till break; $A_{\max}$, area till break



Dx2 + Dy12 variation producing a stronger improvement of baking quality after ascorbic acid addition [23]. They explain these effects with a higher percentage of SDS-non-extractable polymeric proteins in lines with Dx5 + Dy10. These proteins result in higher dough strength and, therefore, higher bread volume and baking quality. The Dx2 + Dy12 HMW-GS line showed an increase in the percentage of SDS-non-extractable polymeric proteins after ascorbic acid addition, and Every et al. concluded that this was the reason for the improvement in baking quality.

Therefore, both studies on common wheat do not find the same results regarding the involvement of HMW-GS variants in improving baking quality with ascorbic acid. This shows again that baking quality is a complex characteristic of breads and that even improvements with common baking additives, such as ascorbic acid, are not completely understood and predictable. In our study, we found an indication that the HMW-GS variants could have an impact on the baking quality of emmer breads, but also the gliadin-to-glutenin ratio. A reason why results in this research field differ so much could be the wide variety of lines and differences between wheat species. Our research indicates that the optimal amount of ascorbic acid may be important for improving baking quality. For spelt, we recommend a lower amount of 60 mg/kg for good results, while emmer does not respond strongly to lower amounts of ascorbic acid, which is why we recommend a higher amount of 250 mg/kg. Despite adjusting ascorbic acid levels to improve baking quality, significant variations persist between wheat lines, necessitating careful flour selection. Flours with a lower gliadin-to-glutenin ratio respond more effectively to ascorbic acid in our study design and are therefore preferable especially for emmer breads. Additionally, the changes observed during extended fermentation warrant further investigation into the resulting metabolites and their potential benefits for digestibility and human health. The method development and analysis of breads and dough with the addition of ascorbic acid were prepared with careful thought of appropriate methodology. Nevertheless, further limitations such as the absence of sensory tests should be mentioned. The connection of analytic results and evaluation in a sensory test would strengthen the results for observed traits such as elasticity of breads and crumb colour, but also flavour and acceptance of bread. This would be especially interesting in our long-term fermentation breads, since flavour or consumer acceptability could not be evaluated in our test design. This aspect should therefore be studied further.

Furthermore, significant connections between the HMW-GS variants in emmer and the changes in baking quality after ascorbic acid addition should be further analysed. Analysis of free and bound thiol content in a sample and modifications on cysteine residues on HMW-GS proteins should be

studied to mechanistically connect protein functionality to baking quality and, therefore, further improve the understanding of HMW-GS and baking quality in emmer.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00217-026-05316-0>.

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Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Conflict of interest The authors declare no competing interests.

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

Informed consent Not applicable.

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