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Technological and nutritional optimization of bread using purple, einkorn, and spelt whole grain flours

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Abstract

This study examined how replacing refined bread flour with whole grain flours from purple, einkorn, and spelt wheat at different ratios (0–100%) affects the technological, nutritional, and sensory properties of bread. Einkorn-based bread had the highest protein content (12.44%), while spelt-enriched bread showed the highest total phenolic content (220.8 mg GAE/100g). Regarding dietary fiber fractions, einkorn provided the highest soluble fiber (4.09%), while spelt contributed the most insoluble fiber (5.89%). Distinct amino acid profiles were observed: einkorn showed superior levels of methionine (0.404 g/100g) and phenylalanine (0.590 g/100g); purple and spelt wheats were richer in histidine, arginine, and threonine. Mineral analysis indicated that spelt and einkorn breads contained higher amounts of calcium, copper, iron, potassium, manganese, phosphorus, magnesium, and zinc compared to purple wheat formulations. From a technological perspective, increased substitution levels negatively affected gluten quality, resulting in reduced bread volume and increased crumb firmness. However, at 25% substitution, both einkorn (567.5 mL) and purple wheat (575.0 mL) breads achieved volumes comparable to the control (583.5 mL). Purple wheat bread exhibited the lowest hardness (1890.3 g) and the highest resilience (0.43). Sensory evaluation ranked einkorn highest for crust color, taste, chewability, and overall acceptability. Consequently, the optimal substitution range was identified as 25–50%, balancing nutritional improvement with acceptable sensory quality. Overall, einkorn emerged as the most promising candidate, combining superior nutritional properties with excellent sensory characteristics.

Keywords: ancient wheat, bread, nutrition, purple wheat, einkorn wheat, spelt wheat

1 Introduction

Wheat is one of the most essential food crops globally, providing energy, protein, key minerals, B vitamins, dietary fiber, and phenolic compounds that offer well-known health advantages (Reynolds and Braun, 2022). Globally, wheat supplies approximately 18% of daily caloric needs in developing countries and contributes 20% to the overall protein intake of the world population (Erenstein et al., 2022). Türkiye occupies a unique position in wheat's evolutionary history, serving as both a center of genetic diversity and a primary site of domestication (Özkan et al., 2011). In recent years, consumer interest in ancient wheat varieties has grown markedly, driven by increasing awareness of nutrient-dense grain-based foods and concerns over biodiversity loss in modern agricultural systems (Porto et al., 2025). These ancient wheats—einkorn (*Triticum monococcum* L., diploid), emmer (*Triticum dicoccum* L., tetraploid), and spelt (*Triticum aestivum* ssp. *spelta*, hexaploid)—are characterized as hulled wheats enclosed by tough glumes, which offer superior protection to grains but require dehulling before processing (Jaradat, 2016; Xie et al., 2023). These wheats typically contain higher levels of dietary fiber, protein, trace elements, essential amino acids, and bioactive compounds such as flavonoids and carotenoids (Kulathunga et al., 2020). Notably, studies have identified high levels of bioactive or functional compounds in ancient wheat species, indicating increased potential for health benefits and positive physiological effects (Deyalage et al., 2024). However, ancient wheats pose challenges for baking due to their weaker gluten networks and the presence of hulls, which complicate processing. Several studies have revealed notable differences in gluten protein composition between ancient and common wheats, potentially leading to inferior dough rheology and breadmaking qualities (Porto et al., 2025). Nevertheless, their nutritional advantages continue to draw interest from consumers and researchers alike.

Among ancient wheats, einkorn—locally known as "Siyez" in Türkiye—and spelt are the most recognized. Recent integrated "field-to-consumer" studies have confirmed that einkorn exhibits high protein and gluten content but weak gluten strength, yet its breads feature favorable sensory profiles likely due to aroma, texture, and flavor balance (Correa et al., 2024). Both are now widely available in retail markets. Purple wheat has also attracted interest due to its distinctive purple color, which comes from high anthocyanin content. These pigments provide strong antioxidant activity, and purple wheat also exhibits favorable dough properties, making it suitable for bread applications (Gamel et al., 2023). The growing interest in ancient wheat species stems from their multiple benefits. Farmers appreciate them for crop diversification and their ability to grow in less fertile soils. Bakers value them for creating unique, value-added products. Consumers increasingly prefer them for their superior nutritional content and potential health benefits (Valsalan et al., 2024). Despite this interest, there is still limited comprehensive research on how these flours affect bread quality, sensory properties, and nutritional value—especially when used at different substitution levels (Morey et al., 2025). Current research has explored a range of strategies to enhance ancient grains for baking, including emulsifier treatments and extrusion processing. These techniques have been shown to positively influence dough handling properties and increase bread volume in certain ancient wheat varieties (Valsalan et al., 2025). Studies evaluating whole ancient wheat breads have shown that spelt is significantly preferred to modern whole wheat in flavor and texture characteristics, while consumer knowledge, attitude, and consumption behavior positively correlate with sensory liking (Morey et al., 2025). Furthermore, the incorporation of ancient grain brans has been shown to significantly enhance total dietary fiber (TDF) and polyphenol content while maintaining acceptable sensory and textural properties (Toumi et al., 2025). Beyond processing and formulation, research has also focused on the intrinsic value of the raw material. For instance, bioactive molecules present in the ancient wheat 'Senatore Cappelli' food chain have demonstrated remarkable stability throughout processing, preserving their antioxidant and anti-inflammatory potential (Armeli et al., 2025).

Therefore, the present study aimed to systematically evaluate the effects of partially or completely replacing conventional bread flour with whole grain flours from purple, spelt, and einkorn wheat. Five substitution levels (0%, 25%, 50%, 75%, and 100%) were tested across a comprehensive set of parameters, including flour physicochemical properties, dough rheology, bread texture, nutritional composition, and sensory attributes. The main objective was to determine the substitution levels that optimally balance nutritional enhancement with acceptable technological quality and consumer preference.

2 Materials and Methods

2.1 Raw materials and preparation of flour mixtures

Whole grain flours of purple wheat (PW), spelt (SW), and einkorn (EW) were purchased from a local supplier. Each flour was sieved through a 1 mm screen to remove larger bran particles. For the control sample, Bezostajal wheat was used. The grains were first cleaned using a Brabender Labofix90 device and then milled into flour with

a Chopin Moulin CD1 laboratory mill. Thirteen different flour combinations were prepared. For each ancient wheat type, four substitution levels were used: 25%, 50%, 75%, and 100% ancient wheat flour mixed with control flour. The control flour alone was kept as a reference. Each blend was mixed for 3 minutes using a KitchenAid mixer to ensure homogeneity. The mixtures were then placed in sealed containers and stored at $10 \pm 2^\circ\text{C}$ in a dark environment until analysis.

2.2 Physical and chemical analysis

Flour color parameters (L^* , a^* , b^*) were determined using a Hunterlab MiniScan XE Plus instrument. Moisture content was determined according to AACC Method 44-15.02. Water activity was measured at $25 \pm 1^\circ\text{C}$ using a WaterLab Steroglass meter. Ash content was analyzed following AACC Method 08-01. Protein content was determined using the Dumas combustion method (AACC 46-30). Flour samples were placed in a LECO FP628 nitrogen analyzer to obtain nitrogen content, which was then multiplied by 5.7 to calculate protein percentage. Wet gluten content and gluten index were measured using a Glutomatic 2100 system (Perten Instruments, Sweden) following AACC Method 38-12.02 (AACCI, 2010). Solvent retention capacity was assessed using four different solutions: water, 5% lactic acid, 5% sodium carbonate, and 50% sucrose, following the procedure described by Karaduman (2020). Glutenin swelling index was determined by hydrating flour samples with water, followed by addition of an isopropanol-lactic acid mixture. Samples were vortexed, incubated, centrifuged, and the swollen gel weight was measured relative to the initial flour weight. Amylose content was measured colorimetrically. Flour was first defatted, then starch was extracted using an alkaline solution. Iodine was added to form a blue complex, and absorbance was read at 620 nm. Results were quantified against a standard curve prepared from pure amylose.

2.3 Dough rheology tests

GlutoPeak analysis was performed with a Brabender device. For each measurement, 8.5 g of flour (14% moisture basis) and 9.5 g of 0.5 M CaCl_2 solution were used. The machine operated at 34°C and 1900 rpm for 3 minutes. Four parameters were recorded: maximum torque (BEM), time to reach maximum torque (PMT), torque before the peak (AM), and torque after the peak (PM). Alveograph tests were performed using a Chopin Alveograph instrument according to AACC Method 54-30.02. Water addition was adjusted based on flour moisture, protein content, and SRC values. The test provided values for tenacity (P), extensibility (L), deformation energy (W), and the P/L ratio. Farinograph tests were carried out using a Brabender OHG farinograph following AACC Method 54-21.01. Measurements included water absorption, dough development time, stability, and degree of softening.

2.4 Bread preparation and quality assessment

Bread was produced following a modified AACC Method 10-10A. Each dough consisted of 100 g flour (14% moisture basis), 3% salt, 2% sugar, and 3% fresh yeast. Water addition was based on GlutoPeak absorption values. Mixing was performed for 4 minutes using a KitchenAid mixer. Fermentation was carried out at 30°C with 85% relative humidity. The process included a first proof of 30 minutes, a second proof of 30 minutes, and a final proof of 45 minutes. Baking was done at 230°C for 25 minutes using a Şimşek Laborteknik oven in Turkey. Bread volume was measured using the rapeseed displacement method according to AACC Method 10-05A. Texture profile analysis was performed with a Stable Microsystems TA.XT plus texture analyzer using a 35 mm probe, 40% compression, and a test speed of 2.0 mm/s. Parameters recorded included hardness, springiness, cohesiveness, resilience, gumminess, and chewiness. Crumb microstructure was examined using a JSM-7001F scanning electron microscope (Jeol, Japan) at 500–2000 $\times$ magnification. Samples were lyophilized and coated with gold-palladium before imaging. Sensory evaluation was conducted by 15 trained panelists aged 20–55 years. A 9-point hedonic scale was used, and samples were served in random order at 20°C .

2.5 Nutritional analysis

Total antioxidant activity was measured using the DPPH radical scavenging method. Total phenolic content was determined using the Folin–Ciocalteu method. Both results were expressed as mg gallic acid equivalents (GAE) per 100 g. Mineral content was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer 8000, USA) after microwave digestion with HNO_3 . Certified reference material (SRM 1515 apple leaves) was used for validation. Amino acid composition was determined by high-performance liquid

chromatography (Agilent 1200) after acid hydrolysis and pre-column derivatization with OPA and FMOC. Dietary fiber fractions—soluble, insoluble, and total—were quantified using an enzymatic-gravimetric method. This involved sequential enzymatic digestion with α -amylase, protease, and amyloglucosidase, followed by alcohol precipitation, filtration, and gravimetric determination of residual fiber fractions.

2.6 Statistical analysis

Statistical computations were performed using JMP Pro 13.0.0 (SAS Institute Inc., USA). A full factorial ANOVA model was applied to the flour data. For bread quality parameters, a one-way ANOVA was performed. Whenever the ANOVA indicated significant effects, Tukey's honestly significant difference test was applied for post-hoc mean comparisons, with statistical significance set at $p < 0.05$. The results are presented as mean values accompanied by standard deviations, derived from at least two replicate analyses per sample.

3 Results and Discussion

3.1 Flour properties

Tables 1 and 2 summarize the physicochemical, functional, and rheological characteristics of the flour blends obtained by mixing control flour with purple (PW), spelt (SW), and einkorn (EW) whole grain flours at substitution rates of 25%, 50%, 75%, and 100%.

3.1.1 Physicochemical and functional properties

As the proportion of ancient wheat flour in the blend increased, ash content rose accordingly across all three wheat types (Table 1). When used at 100%, spelt showed the highest ash level (1.870%), followed by einkorn (1.778%) and purple wheat (1.533%); all were considerably higher than the control (0.699%). On average, ash content followed the order spelt (1.278%) > einkorn (1.225%) > purple wheat (1.124%). Increasing the proportion of ancient wheat flour caused a drop in flour brightness (L^*), likely due to greater bran inclusion. Among the three, einkorn gave the highest average L^* (87.62), while purple wheat (87.23) and spelt (87.25) were slightly lower. Einkorn also gave the highest a^* (1.88) and b^* (11.70) values, which can be attributed to its lutein content. In contrast, purple wheat showed lower values (a^* : 1.38; b^* : 9.45), consistent with its anthocyanin-rich pigmentation. Both wheat type and substitution rate had a significant effect on protein content. Spelt had the highest average protein (12.45%, dry basis), with einkorn (12.05%) and purple wheat (11.84%) also surpassing the control (11.23%). Protein levels rose steadily as the share of ancient wheat increased, peaking at 100% substitution. A similar trend was seen for wet gluten: spelt (26.69%) > einkorn (25.26%) > purple wheat (23.83%) > control (23.47%). The SRC profiles highlighted functional distinctions between the three wheat species. Lactic acid SRC values were highest for spelt (92.2%) and einkorn (91.3%), while purple wheat gave a lower value (83.7%). Sodium carbonate SRC ranked SW (92.9%) > EW (87.3%) > PW (82.0%). Water and sucrose SRC followed the same order: SW > EW > PW, with spelt's high sucrose SRC confirming its substantial pentosan content. All SRC values increased with substitution level. All ancient wheats showed substantially lower values in traditional gluten quality tests. The control flour had the highest sedimentation (56.3 mL); among the ancient wheats, purple wheat gave the lowest (34.1 mL), followed by spelt (34.2 mL) and einkorn (37.1 mL). Glutenin swelling index followed similar trends. Amylose content showed minor variation: EW (25.80%), PW (25.52%), SW (24.90%), and control (27.27%).

3.1.2 Rheological properties

Table 2 presents the rheological behavior of the different flour blends. The control flour reached the highest maximum torque (BEM: 47.5 GPU), reflecting its stronger gluten network. For the ancient wheats, BEM values followed the order spelt (44.7 GPU) > purple wheat (42.1 GPU) > einkorn (41.4 GPU), which aligned with their lactic acid SRC rankings. Einkorn gave the highest torque before the peak (AM: 30.3 GPU), indicating a different pattern of early gluten aggregation. Purple wheat took longer to reach peak torque (PMT: 94.0 s) than the control (72.5 s), suggesting that anthocyanins may delay gluten development. SW (60.3 s) and EW (63.7 s) showed faster aggregation. Spelt had the highest torque after the peak (PM: 37.4 GPU), pointing to better stability of its gluten network after maximum development. Alveograph tenacity (P) was greatest for purple wheat (202.9 mm) and

einkorn (200.9 mm), surpassing both the control (193.7 mm) and spelt (188.4 mm). In contrast, extensibility (L) was considerably reduced for all ancient wheats relative to the control (32.3 mm), especially when used at 100% (15.0–17.0 mm). Deformation energy (W) dropped markedly as substitution increased. The control had the highest value (270.3×10^{-4} J), followed by einkorn (192.9×10^{-4} J), purple wheat (180.2×10^{-4} J), and spelt (179.3×10^{-4} J). The lowest values were recorded at 100% substitution, with purple wheat giving 96.5×10^{-4} J. Farinograph water absorption was higher for ancient blends (PW: 61.8%, EW: 60.8%, SW: 60.3%) than control (59.8%). Dough development time was substantially shorter for ancient wheats (SW: 5.4 min, EW: 6.1 min, PW: 7.4 min) compared to control (8.8 min). Dough stability was highest for control (11.8 min), with SW showing relatively better stability (11.0 min) than PW (9.5 min) and EW (9.8 min). Softening degree was markedly higher for ancient blends, particularly PW (123.0 FU at 25% substitution), compared to control (45.5 FU).

3.2 Bread properties

Tables 3 and 4 present the quality characteristics of breads made with 25–100% substitution, covering volume, texture, sensory scores, nutritional composition, and amino acids. Key nutritional indicators are illustrated in Figure 1, while Figure 2 displays SEM images (2000×) of crumb structure.

3.2.1 Crust and crumb color properties

The L, a, and b* color values of bread crust and crumb are presented in Supplementary Figure 1. The control bread exhibited the highest lightness (L*), with crust and crumb values of 57.71 and 71.36, respectively. In the crust, purple wheat bread displayed a higher L* value (54.56) compared to einkorn (52.31) and spelt (50.60). For the crumb, einkorn bread was the lightest (L*: 62.23), followed by spelt (59.57), while purple wheat bread had the darkest crumb (55.94). Among ancient wheat breads, einkorn and spelt formulations maintained relatively higher lightness at 25% substitution in the crumb, with values of 65.72 and 64.36, respectively, whereas 100% purple wheat bread showed the lowest value (45.08). Einkorn breads displayed the highest a* (redness) and b* (yellowness) in both crumb and crust across all substitution levels, consistent with their lutein-rich composition, while purple wheat breads exhibited distinct color profiles attributable to their anthocyanin content. For spelt breads, the crust b* value decreased to 22.19 at 100% substitution, though at other substitution levels, it remained higher than that of einkorn. In the crumb, spelt breads showed b* values higher than purple wheat but lower than einkorn across all substitution levels.

3.2.2 Volume and textural properties

Bread volume was significantly influenced by both the ancient wheat type and the substitution level ($p < 0.05$) (Table 3). The control bread gave the largest volume (583.5 mL), and across all three ancient wheats, increasing the substitution rate led to a steady decline in volume. At 25% substitution, both einkorn (567.5 mL) and purple wheat (575.0 mL) produced volumes close to the control, whereas spelt (542.5 mL) gave a noticeably smaller loaf. When used at 100%, all three wheats gave much smaller loaves, with spelt (377.5 mL) being the smallest, followed by einkorn (387.5 mL) and purple wheat (420.5 mL). Texture analysis showed that hardness rose sharply as more ancient wheat was added. The control was the softest (656.5 g). Among the ancient wheat breads, spelt had the highest average hardness (2519.9 g), followed by einkorn (2097.8 g) and purple wheat (1890.3 g). Cohesiveness followed similar declining trends. Purple wheat and einkorn showed the highest mean resilience (0.43), significantly exceeding spelt (0.41). Springiness was also reduced in ancient wheat breads.

3.2.3 Sensory evaluation

Sensory attributes (crust color, symmetry, pore structure, taste, chewability, overall likeability) were evaluated on a 9-point hedonic scale (Table 3). Scores for all attributes declined with increasing substitution level. Einkorn breads received the highest overall acceptability (mean 6.80), performing best at 25–50% substitution, with high scores for chewability, taste, and crust color. Purple wheat breads also achieved favorable scores (mean 6.09). Spelt breads received the lowest mean scores (5.27), with 100% spelt (SW1) being least preferred, indicating sensory attributes were most adversely affected at high substitution levels.

3.2.4 Nutritional composition

Adding ancient wheat markedly improved the nutritional value of the breads (Table 4, Fig. 1). Average ash content was highest for einkorn (2.681%), followed by spelt (2.491%), purple wheat (2.412%), and the control (1.821%). Ash content also rose with substitution level, reaching 2.838% at 75% and 3.068% at 100%. All breads made with ancient wheat had higher protein than the control (10.43%). Einkorn gave the highest (12.44%), followed by spelt (12.21%) and purple wheat (11.61%). At 100% substitution, both einkorn and spelt surpassed 13.0%. Purple wheat protein increased progressively from 11.01% (25%) to 12.77% (100%), with spelt and einkorn approximately 1% higher at each level (Fig. 1a). Water activity (a_w) values were generally higher in purple wheat breads (mean 0.920), and lower in einkorn and spelt breads (0.882 and 0.897). Total phenolic content (TPC) of all ancient breads exceeded control (130.3 mg GAE/100g). Spelt showed highest TPC (220.8 mg GAE/100g), exceeding 250 mg GAE/100g at $\geq 50\%$ substitution. Einkorn (185.6 mg GAE/100g) reached 230.1 mg GAE/100g at 100%, while purple wheat (170.3 mg GAE/100g) peaked at 200 mg GAE/100g (Fig. 1b). Einkorn contained the highest soluble fiber (4.09%), spelt the highest insoluble fiber (5.89%), with purple wheat intermediate. Total dietary fiber increased with substitution (control: 3.45%), reaching 12.3–13.0% at 100% and 10.4–11.3% at 75% substitution (Fig. 1c). Amino acid profiles revealed significant variations among wheat species. Purple wheat and spelt were richer in histidine, arginine, and threonine. Einkorn excelled in methionine (0.404 g/100g) and phenylalanine (0.590 g/100g). Lysine was highest in purple wheat (0.433 g/100g). Compared to control breads, lysine and arginine contents were higher in purple wheat and spelt breads. Conversely, histidine, arginine, threonine, lysine, leucine, isoleucine, and valine levels were considerably lower in einkorn breads. However, at 100% substitution of einkorn, the contents of histidine, arginine, threonine, valine, leucine, and lysine increased slightly. Mineral composition was substantially enhanced by ancient wheat incorporation. Spelt and einkorn breads showed higher concentrations of zinc, iron, potassium, calcium, copper, magnesium, phosphorus, and manganese than purple wheat. Sulfur was highest in spelt (3215.2 mg kg^{-1}) and lowest in einkorn. Calcium exceeded 500 mg kg^{-1} in spelt and einkorn at $\geq 75\%$ substitution (purple wheat: ~ 400 mg kg^{-1}). Copper reached ≥ 5.0 mg kg^{-1} in spelt and einkorn at 100% substitution. Iron exceeded 70 mg kg^{-1} in einkorn (75–100%) and spelt (100%). Potassium exceeded 5000 mg kg^{-1} in spelt and einkorn at 100% substitution. Magnesium was highest in einkorn (1098.5 mg kg^{-1}). Zinc exceeded 35 mg kg^{-1} in einkorn (100%) and spelt (75–100%). Magnesium, manganese, and phosphorus surpassed 1300 mg kg^{-1} , 30 mg kg^{-1} , and 3700 mg kg^{-1} , respectively, in spelt and einkorn at $\geq 75\%$ substitution. Sulfur remained consistently > 3500 mg kg^{-1} across all spelt breads (Fig. 1 d-l).

3.2.5 Microstructural characteristics

Scanning electron microscopy images (2000 $\times$, Fig. 2) revealed that increasing substitution levels progressively hindered gluten network development across all ancient wheat types. The control bread showed a continuous, well-developed gluten network, with starch granules evenly spread and many small, uniformly arranged gas cells. At 50–100% substitution, high dietary fiber—particularly bran particles—physically disrupted gluten continuity, resulting in compact, dense structures with fragmented matrices and limited porosity. Even at 25% substitution, spelt and einkorn breads showed insufficient gluten development, with irregular gas cell distribution and coalesced voids rather than the fine, uniform pores of the control. This structural deterioration was more pronounced in spelt and einkorn than in purple wheat, consistent with their lower dough stability and weaker gluten strength. The progressive densification of crumb structure with increasing substitution directly correlated with reduced bread volumes and increased hardness (Table 3).

3.3 Discussion

3.3.1 Flour properties

The data in Tables 1 and 2 highlight clear differences in composition and dough behavior between ancient and modern wheats, which in turn shape their baking performance and nutritional value. Ash levels in ancient flours were considerably higher than in the control, rising in a linear fashion as substitution increased. At 100%, spelt (1.870%) and einkorn (1.778%) contained more than double the ash of control (0.699%), reflecting inherent mineral richness (Kohajdová and Karovičová, 2008). The inclusion of bran at higher substitution levels led to a reduction in flour brightness (L^*). Einkorn maintained the lightest color (mean L^* : 87.62) and exhibited the highest a^* (1.88) and b^* (11.70), attributable to its lutein content ($> 90\%$ of total carotenoids in *Triticum*) (Gebruers et al., 2008; Hidalgo and Brandolini, 2014), while purple wheat's lower a^* (1.38) and b^* (9.45) reflected its anthocyanin-dominated pigment profile. Protein levels were notably elevated in the ancient wheats, with spelt (12.45%) and einkorn (12.05%) showing the highest values, followed by purple wheat (11.84%), compared to the control (11.23%). Wet gluten followed the same trend: spelt (26.69%) $>$ einkorn (25.26%) $>$ purple wheat (23.83%) $>$

control (23.47%). Modern wheat's lower protein can be attributed to its larger grain size, starch-dominated endosperm, and the removal of protein-dense bran fractions (Arzani and Ashraf, 2017). However, protein quantity does not equate to baking functionality—a critical distinction. Solvent retention capacity profiles revealed strong glutenin potential in spelt (lactic acid SRC: 92.2%) and einkorn (91.3%), while spelt's elevated sucrose SRC (104.6%) confirmed high pentosan content. Despite these indicators, traditional gluten quality measures revealed lower sedimentation values and glutenin swelling indices for ancient wheats, decreasing progressively with substitution. The control's sedimentation (56.3 mL) was more than triple that of 100% purple wheat (17.0 mL). The coexistence of high protein content with weak gluten functionality is a well-established trait of ancient wheats (Akman and Karaduman, 2021), stemming from variations in gliadin-to-glutenin ratios, bran particle interference, and the lack of specific polymeric protein structures essential for proper viscoelastic properties. Rheological analysis revealed species-specific differences in gluten aggregation, dough strength, and mixing tolerance. Control exhibited the highest maximum torque (BEM), indicating superior gluten development. Spelt showed the greatest gluten strength among ancient wheats, consistent with its higher lactic acid SRC. Purple wheat exhibited prolonged PMT, suggesting slower gluten development due to phenolic interference, while spelt and einkorn aggregated faster. Einkorn showed highest torque before maximum (AM), indicating greater initial resistance; spelt had the highest torque after maximum (PM), reflecting superior post-peak stability. Deformation energy (W) was highest in the control, indicating superior gas retention capacity. For ancient wheats, W values dropped by 25–64% when used at 100%, which corresponded closely with the observed reductions in bread volume. Tenacity (P) was higher in ancient doughs but accompanied by reduced extensibility (L), creating unfavorable P/L ratios—doughs that resist stretching but tear upon expansion, compromising gas retention. Ancient blends showed higher water absorption, reflecting bran and damaged starch. Dough development time was substantially shorter, indicating rapid consistency but inadequate gluten network formation. Dough stability was highest for control, with spelt showing relatively better stability among ancient wheats. The degree of softening was markedly higher for ancient blends, indicating rapid structural breakdown beyond optimal development. Collectively, these profiles describe doughs that develop quickly but lack tolerance to prolonged mixing or fermentation—a significant challenge for high-substitution applications without process modifications.

3.3.2 Bread properties

Bread quality attributes directly reflected flour characteristics, with substitution level and wheat species significantly influencing volume, texture, sensory perception, and nutritional composition. Color parameters (Supplementary Figure 1) revealed species-dependent differences linked to distinct pigment profiles. Einkorn breads gave the highest b^* values (yellowness), with crust and crumb reaching 26.84 and 22.74, respectively, at 100% substitution. This is consistent with the high lutein content of einkorn, which accounts for over 90% of its total carotenoids (Hidalgo and Brandolini, 2014; Gebruers et al., 2008). Purple wheat breads showed distinctly lower L^* and b^* values, with crumb L^* decreasing to 45.08 at 100% substitution, attributable to anthocyanins (Gamel et al., 2023). Spelt breads displayed intermediate color values, consistent with moderate carotenoid content and absence of anthocyanins (Ziegler et al., 2016). Progressive darkening with increasing substitution reflects higher bran incorporation. Bread volume was substantially affected by wheat species and substitution level. Control bread exhibited the highest volume, attributable to its strong, gas-retentive gluten network. A clear inverse relationship between substitution rate and bread volume was observed across all ancient wheats. At 100% substitution, volumes decreased dramatically—spelt showed the greatest reduction, followed by einkorn and purple wheat—directly corresponding to diminished alveograph deformation energy (W) values. At 25% substitution, einkorn and purple wheat maintained volumes statistically comparable to control. Hardness increased markedly with substitution; control bread was softest (656.5 g). Among ancient wheats, purple wheat showed lowest mean hardness (1890.3 g), followed by spelt (2519.9 g) and einkorn (2097.8 g). The substantial dietary fiber content, especially bran particles, contributed to structural firmness and dilution of the gluten network, which negatively impacted gas retention (Gomez et al., 2020). However, at 25% substitution, the base flour's gluten network remained sufficiently intact to uphold gas cell structure. Einkorn breads received the highest overall acceptability scores (mean 6.80), performing best at 25–50% substitution, with high scores for chewability, taste, and crust color. Purple wheat breads achieved favorable scores (mean 6.09), with their distinctive purplish hue adding perceived value. Spelt breads received the lowest scores (5.27), with 100% spelt least preferred due to excessive hardness, reduced resilience, and a potentially stronger, more bitter flavor at high substitution levels—underscoring that nutritional enhancement must be coupled with sensory appeal for consumer adoption. Ancient wheat flours delivered unequivocal nutritional superiority. Ash content mirrored component flours, increasing predictably with substitution: einkorn (2.681%) > spelt (2.491%) > purple wheat (2.412%) > control (1.821%). Protein content of all breads exceeded control (10.43%): einkorn (12.44%) > spelt (12.21%) > purple wheat (11.61%), with values >13.0% in 100% einkorn and spelt breads, and 75% einkorn. Total phenolic content exceeded control (130.3 mg GAE/100g): spelt (220.8 mg GAE/100g), exceeding 250 mg GAE/100g at ≥50%

substitution; einkorn reached 230.1 mg GAE/100g at 100%; purple wheat peaked at 200 mg GAE/100g. Key bioactive compounds in spelt include alkylresorcinols (Grela, 1996), β -sitosterol glycoside (Rozenberg et al., 2003), phenolic acids (Andersson et al., 2010), and tocochromanols (Hussain et al., 2012), with high antioxidant properties (Tóth et al., 2022). Einkorn's bioactive profile includes phytosterols (Nurmi et al., 2008), carotenoids (Brandolini et al., 2008), lutein (Lachman et al., 2013; Hidalgo and Brandolini, 2014), polyphenols (Serpen et al., 2008; Lavelli et al., 2009), and alkylresorcinols (Andersson et al., 2010). Dietary fiber was profoundly enhanced: einkorn showed highest soluble fiber (4.09%), spelt highest insoluble fiber (5.89%). Total dietary fiber increased with substitution, reaching 12.3–13.0% at 100%. Total dietary fiber in einkorn ranges 7.6–9.9% (Løje et al., 2003), while spelt reaches 120 g/kg (Hidalgo and Brandolini, 2014). Spelt grain arabinoxylan averages 5.74% (0.59% water-soluble), with flour values of 1.75% and 0.35% (Escarnot et al., 2015). Amino acid profiles revealed significant variations. Purple wheat and spelt were richer in histidine, arginine, and threonine. Einkorn demonstrated superiority in methionine (0.404 g/100g) and phenylalanine (0.590 g/100g)—essential amino acids often limiting in cereals—making einkorn valuable for improving protein quality. Some einkorn wheats possess higher lysine than common wheat (Løje et al., 2003). Spelt total essential amino acid content differs slightly from common wheat (Dvořáček et al., 2002; Abdel-Aal and Hucl, 2002). Mineral composition was substantially enhanced. Spelt and einkorn exhibited higher concentrations of zinc, iron, potassium, calcium, copper, magnesium, phosphorus, and manganese than purple wheat. Sulfur was highest in spelt (3215.2 mg kg⁻¹) and lowest in einkorn, reflecting spelt's protein composition rich in sulfur-containing amino acids. Key mineral thresholds exceeded: calcium >500 mg kg⁻¹ in spelt and einkorn at ≥75% substitution; copper ≥5.0 mg kg⁻¹ at 100% substitution; iron >70 mg kg⁻¹ in einkorn (75–100%) and spelt (100%); potassium >5000 mg kg⁻¹ at 100% substitution; magnesium highest in einkorn (1098.5 mg kg⁻¹); zinc >35 mg kg⁻¹ in einkorn (100%) and spelt (75–100%); and magnesium, manganese, phosphorus surpassed 1300 mg kg⁻¹, 30 mg kg⁻¹, and 3700 mg kg⁻¹ respectively in spelt and einkorn at ≥75% substitution. Sulfur remained consistently >3500 mg kg⁻¹ across all spelt breads. These substantial increases in iron, zinc, calcium, and magnesium—nutrients of public health concern—position these breads as effective vehicles for improving mineral intake without fortification, corroborating findings that einkorn exceeds commercial wheat in zinc, iron, copper, manganese, calcium, magnesium, potassium, and phosphorus (Erba et al., 2011; Suchowilska et al., 2012), and spelt contains higher protein, lipids, minerals, and antioxidants than modern wheats (Sinkovič et al., 2023).

3.3.3 Heatmap analysis of overall sample characteristics

A heatmap based on standardized Z-scores was constructed to visualize the relationships between all bread samples and measured parameters (Fig. 3). This multivariate approach enabled simultaneous comparison of 13 samples across 45 variables covering flour properties, dough rheology, bread quality, and detailed nutritional composition. The heatmap revealed that substitution level, rather than wheat species, was the primary factor discriminating the samples. Two distinct clusters emerged: the control with low-to-medium substitution levels (25–50%) formed the left cluster, while high substitution levels (75–100%) formed the right cluster. Technological quality parameters (BEM, FSTAB, VOL, COL, TAS, CHE) showed high Z-scores (red) in the control and low-substitution samples (PW4, EW4, SW4, PW3, EW3, SW3), but low Z-scores (blue) in high-substitution samples (PW1, SW1, EW1), where hardness (HARD) was markedly elevated (deep blue). This confirms that substitution rates above 50% progressively compromise bread-making performance due to gluten dilution and bran-induced matrix disruption. Nutritional quality parameters displayed the opposite pattern. Bread protein content (PRT), total phenolic content (TPC), total dietary fiber (TDF), ash content (ASH), dietary fiber fractions (SDF, IDF), essential amino acids (HIS, ARG, THR, MET, VAL, PHE, ILE, LEU, LYS), and all minerals (Zn, Fe, Ca, Cu, K, Mn, Mg, P, S) increased progressively with substitution level, reaching maxima at 100% (PW1, SW1, EW1). This confirms that maximal nutritional benefits come at the expense of technological quality. Among ancient wheats, species-specific profiles emerged clearly: Einkorn (EW) showed the best technological performance, maintaining volume, resilience, and sensory scores comparable to the control at 25–50% substitution, with superior methionine (MET), phenylalanine (PHE), and notable mineral contents (Fe, Mg, P). Spelt (SW) exhibited the highest total phenolic content (TPC), insoluble fiber (IDF), and the highest levels of most minerals (Zn, Fe, Ca, Cu, K, Mn, Mg, P, S), but the poorest technological properties (lowest volume, highest hardness). Purple wheat (PW) showed intermediate technological performance with distinct anthocyanin-related color traits and superior lysine (LYS), while its mineral contents were generally lower than spelt and einkorn. The heatmap clearly demonstrates the opposing patterns of technological and nutritional quality. The optimal balance was identified at 25–50% substitution, with einkorn as the most promising candidate.

3.3.4 Hierarchical cluster analysis

To visualize the overall similarities and differences among the bread samples based on all measured parameters (flour properties, dough rheology, bread quality, and nutritional composition including protein, total phenolic content, dietary fiber, amino acids, and minerals), a hierarchical cluster analysis was performed and presented as a dendrogram (Fig. 4). The analysis separated the four 100% substitution samples (control, PW1, SW1, EW1) into two main clusters. The first cluster grouped the control bread with 100% einkorn (EW1) bread, indicating that einkorn exhibited the closest overall profile to the modern wheat control. This finding is consistent with the superior technological and sensory performance of einkorn observed in this study, particularly in terms of bread volume, crumb hardness, and overall acceptability. The second cluster contained 100% spelt (SW1) and 100% purple wheat (PW1) breads, demonstrating that these two ancient wheats share a more similar characteristic profile, distinct from both the control and einkorn. Within this cluster, spelt and purple wheat were clearly separated as two distinct sub-branches, reflecting their unique compositional and functional differences, such as spelt's higher total phenolic content (TPC), insoluble fiber (IDF), and mineral levels versus purple wheat's distinct anthocyanin-related color traits and superior lysine (LYS). Overall, the dendrogram confirms that einkorn is the most promising ancient wheat for bread production, as it clusters with the modern control. In contrast, spelt and purple wheat require blending with modern flour to achieve optimal bread quality, consistent with their weaker technological performance.

4 Conclusion

The findings demonstrate that incorporating purple, einkorn, or spelt whole grain flours substantially enhances the nutritional profile of bread, increasing protein, dietary fiber, phenolic compounds, and key minerals such as zinc, iron, calcium, and magnesium. However, these nutritional gains involve technological trade-offs, including weaker gluten, reduced dough strength, and smaller loaf volumes at higher substitution levels. The optimal balance between nutritional improvement and acceptable bread quality was achieved at 25–50% substitution. Among the three ancient wheats, einkorn was the most promising, offering strong nutritional benefits alongside the highest sensory ratings for taste, chewability, and overall liking. Purple wheat also performed well, while spelt—despite its nutritional richness—was limited by excessive hardness and lower consumer acceptability. These findings provide a practical framework for developing bread products that combine enhanced nutritional value with desirable sensory characteristics using ancient wheat flours.

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Competing interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: Ethics approval was not required for this study as it involved standard food sensory evaluation with a trained panel, which does not fall under the scope of clinical research or human medical intervention as defined by national regulations.

Informed Consent: Informed consent was obtained from all individual participants involved in the sensory evaluation.

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Authors contributions: Yaşar KARADUMAN (Y.K.) and Ece Dergi (E.D.) conceived the study; Y.K. and E.D. were involved in original draft preparation; Yaşar Karaduman was responsible for conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, visualization, supervision, project administration. Ece Dergi was responsible for methodology, validation, investigation, resources, data curation, writing – review & editing.

Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process: During manuscript preparation, the authors used DeepSeek only for grammar and spelling checking. All AI-suggested changes were reviewed and manually corrected by the authors, who take full responsibility for the final content. No generative AI was used to generate data, figures, or scientific conclusions.

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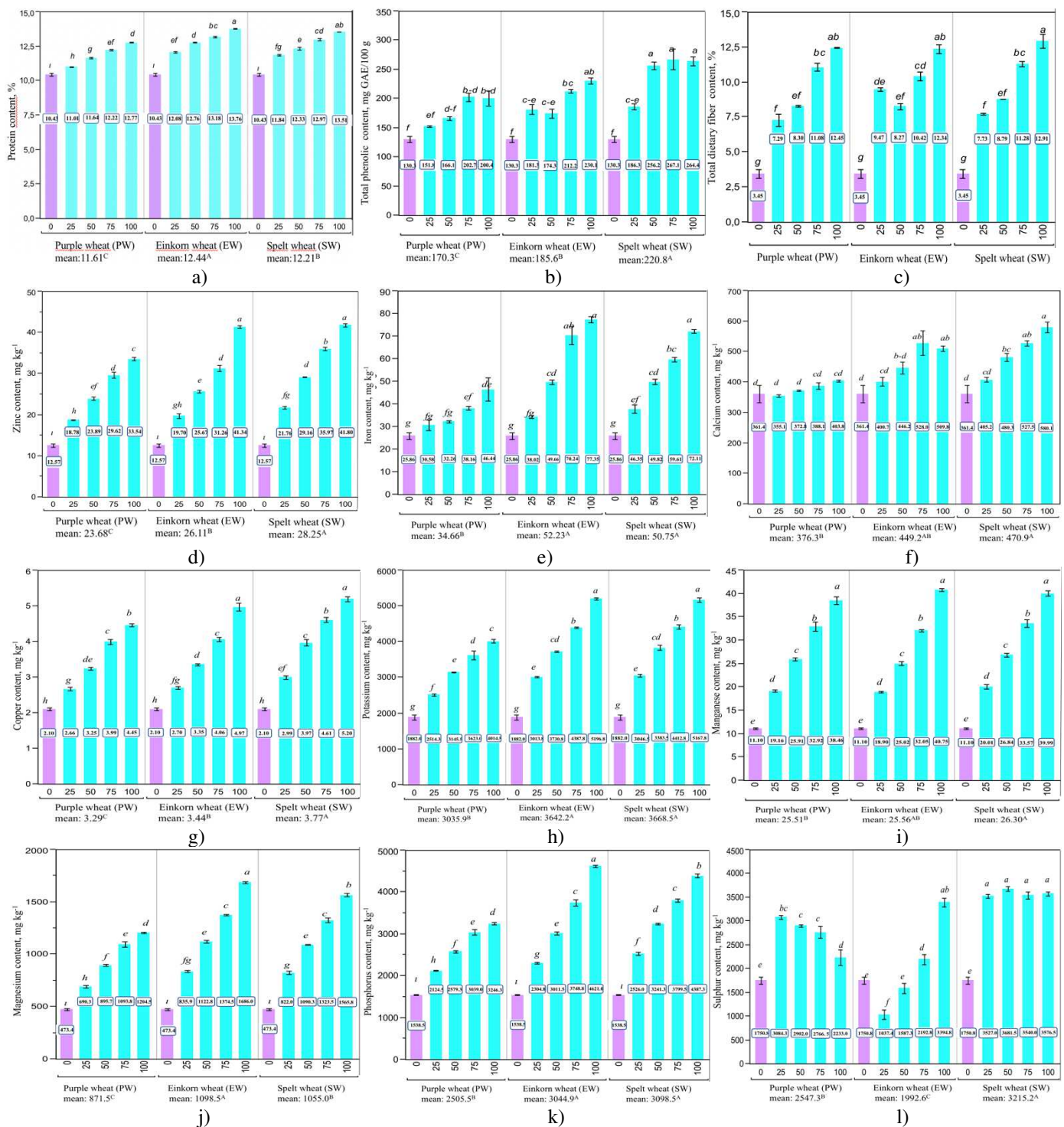
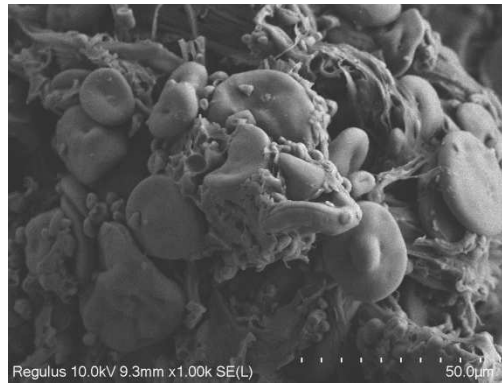
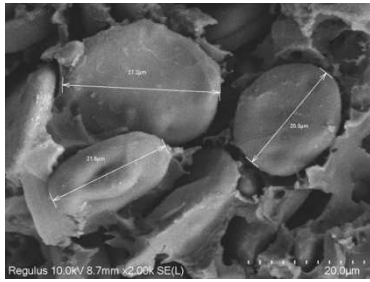


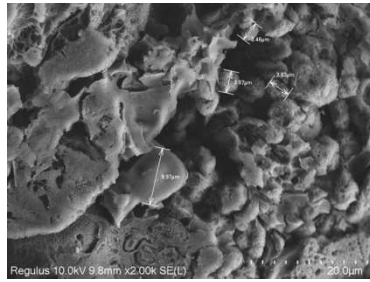
Fig. 1. Nutritional properties of breads formulated with purple, spelt, and einkorn wheat flours at different substitution levels (25-100%): (a) protein content, (b) total phenolic content, (c) total dietary fiber, (d-l) mineral contents (Ca, Cu, Fe, K, Mg, Mn, P, S, Zn)



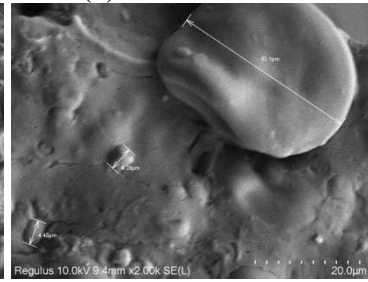
Control bread (a)



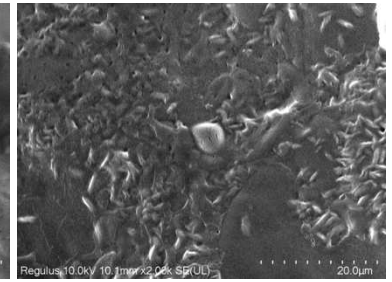
100% purple wheat (b)



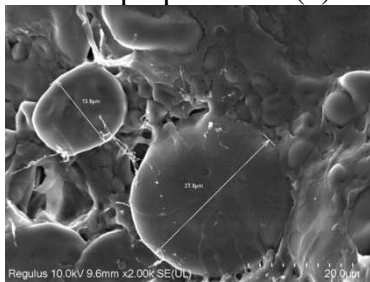
75% purple wheat (c)



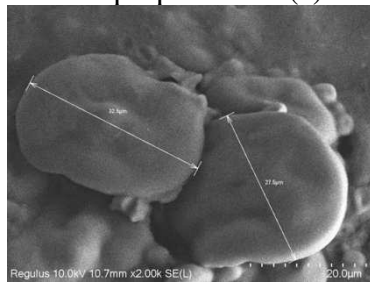
50% purple wheat (d)



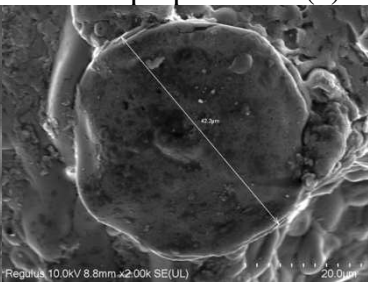
25% purple wheat (e)



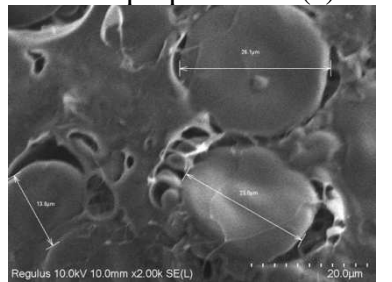
100% spelt wheat (f)



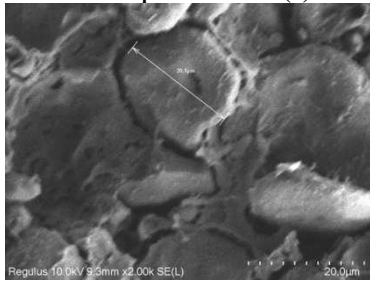
75% spelt wheat (g)



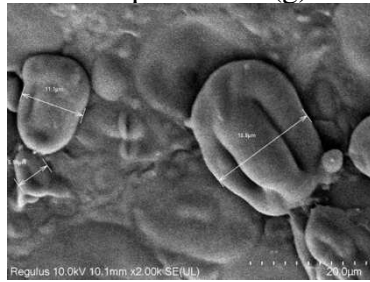
50% spelt wheat (h)



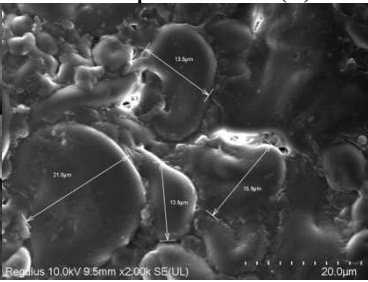
25% spelt wheat (i)



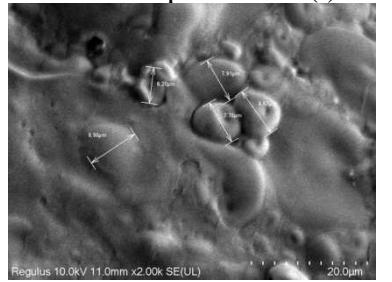
100% einkorn wheat (j)



75% einkorn wheat (k)



50% einkorn wheat (l)



25% einkorn wheat (m)



Fig. 2. SEM images (2000 $\times$) of bread crumb microstructure (a–m) and corresponding bread photographs (1–13). Control: a, 13; Purple wheat: 100% (b, 2), 75% (c, 3), 50% (d, 4), 25% (e, 1); Spelt: 100% (f, 6), 75% (g, 7), 50% (h, 8), 25% (i, 5); Einkorn: 100% (j, 10), 75% (k, 11), 50% (l, 12), 25% (m, 9)

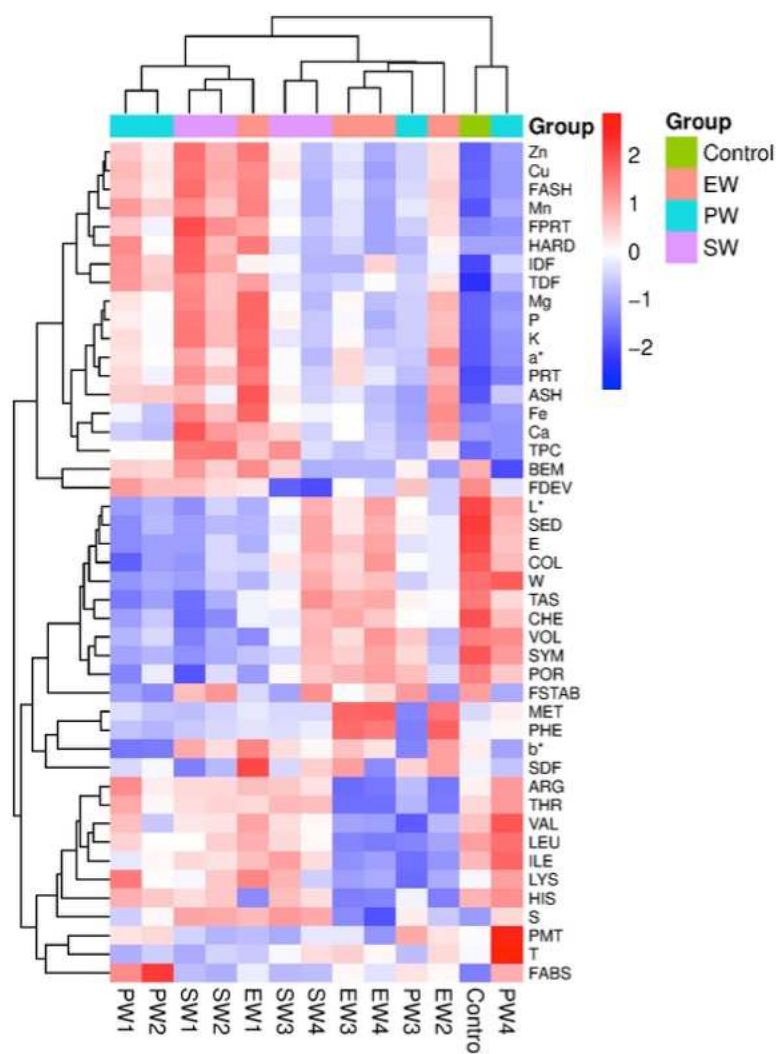


Fig. 3. Heatmap showing hierarchical clustering of 13 bread samples (control; purple wheat, PW1–PW4; spelt, SW1–SW4; einkorn, EW1–EW4) based on 45 flour physicochemical, dough rheological, bread quality, and nutritional parameters. Substitution levels: 1 = 100%, 2 = 75%, 3 = 50%, 4 = 25% ancient wheat flour. Color scale represents standardized Z-scores (red: above mean; blue: below mean). Abbreviations: L*, flour lightness; a*, flour redness; b*, flour yellowness; FASH, flour ash content; FPRT, flour protein content; SED, sedimentation value; BEM, GlutoPeak maximum torque; PMT, GlutoPeak peak maximum time; T, Alveograph tenacity; E, Alveograph extensibility; W, Alveograph deformation energy; FABS, Farinograph water absorption; FSTAB, Farinograph stability; FDEV, Farinograph development time; VOL, bread volume; HARD, bread hardness; COL, crust color; SYM, symmetry; POR, pore structure; TAS, taste; CHE, chewability; ASH, ash content; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; HIS, histidine; ARG, arginine; THR, threonine; MET, methionine; VAL, valine; PHE, phenylalanine; ILE, isoleucine; LEU, leucine; LYS, lysine; PRT, protein content; TPC, total phenolic content; TDF, total dietary fiber; Zn, zinc; Fe, iron; Ca, calcium; Cu, copper; K, potassium; Mn, manganese; Mg, magnesium; P, phosphorus; S, sulphur

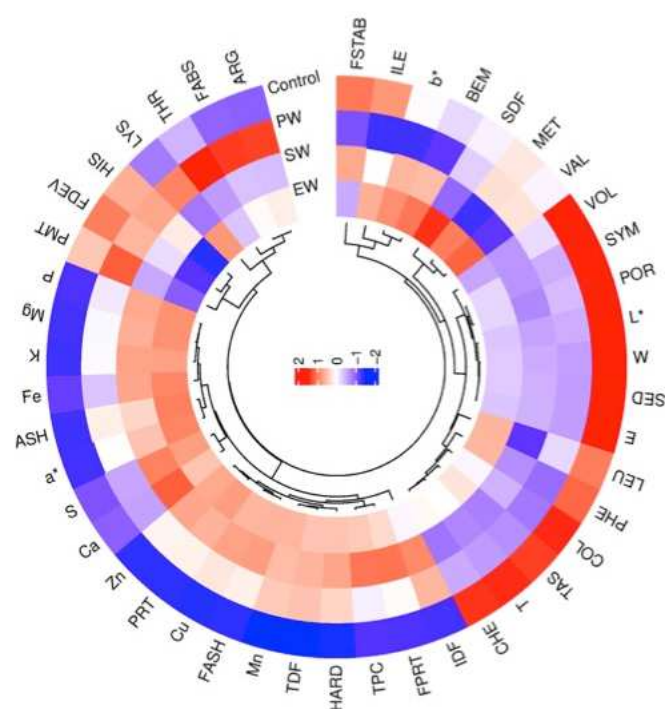


Fig. 4. Dendrogram showing hierarchical clustering of control, purple wheat (PW), spelt (SW), and einkorn (EW) breads based on flour physicochemical properties, dough rheological parameters, bread quality attributes, and nutritional composition. Abbreviations: L*, flour lightness; a*, flour redness; b*, flour yellowness; FASH, flour ash content; FPRT, flour protein content; SED, sedimentation value; BEM, GlutoPeak maximum torque; PMT, GlutoPeak peak maximum time; T, Alveograph tenacity; E, Alveograph extensibility; W, Alveograph deformation energy; FABS, Farinograph water absorption; FSTAB, Farinograph stability; FDEV, Farinograph development time; VOL, bread volume; HARD, bread hardness; COL, crust color; SYM, symmetry; POR, pore structure; TAS, taste; CHE, chewability; ASH, ash content; SDF, soluble dietary fiber; IDF, insoluble dietary fiber; HIS, histidine; ARG, arginine; THR, threonine; MET, methionine; VAL, valine; PHE, phenylalanine; ILE, isoleucine; LEU, leucine; LYS, lysine; PRT, protein content; TPC, total phenolic content; TDF, total dietary fiber; Zn, zinc; Fe, iron; Ca, calcium; Cu, copper; K, potassium; Mn, manganese; Mg, magnesium; P, phosphorus; S, sulphur.

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