Journal of Cereal Science 126 (2025) 104303

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Evaluation of einkorn, emmer and spelt genotypes through an integrated approach: agronomic performance, chemical composition, aromatic profile, bread characteristics and consumers' perception

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ARTICLE INFO

Keywords:
Farro species
Yield
Quality
Polyphenols
VOCs
Mediterranean region
Durum wheat

ABSTRACT

This study evaluated 16 genotypes of ancient hulled wheats—einkorn (*Triticum monococcum*), emmer (*T. dicoccum*), and spelt (*T. spelta*)—grown in two contrasting soils under semi-arid Mediterranean conditions, where data on their adaptability remain limited. Three durum wheat (*T. durum*) genotypes served as controls. A comprehensive "field-to-consumer" approach assessed agronomic performance, grain quality, total phenolic content (TPC), volatile organic compounds (VOCs), bread characteristics, and sensory traits. Einkorn showed low yield potential—less than half that of other species under optimal conditions—though this gap narrowed under less favorable conditions; it had high protein and gluten content, weak gluten strength, and low TPC; its breads featured favorable sensory profiles likely due to aroma, texture, and flavor balance. All these attributes suggest einkorn's suitability for low-input or organic systems in marginal Mediterranean areas, where quality and market value may offset lower yields. Emmer exhibited substantial intraspecific variability, but no single genotype consistently excelled across all the traits examined, thus indicating opportunities for targeted breeding. Spelt displayed limited agronomic and grain quality intraspecific variability but notable genotypic differences in VOCs and sensory attributes. Correlations revealed complex relationships between VOCs and TPC; some volatiles increased with TPC, which negatively correlated with bread sensory scores, highlighting trade-offs between nutraceutical content and sensory quality. Although VOC profiles in wholemeal flour only weakly predicted bread sensory attributes, specific compounds (e.g., some alkanes and esters) correlated positively with bread odor. These findings highlight hulled wheats' potential to diversify Mediterranean cropping systems, meet evolving consumer demands, and support climate-resilient agri-food chains. The comprehensive dataset generated provides a valuable resource for future genetic improvement programs.

1. Introduction

The hulled wheat species einkorn (*Triticum monococcum*), emmer (*T. dicoccum*), and spelt (*T. spelta*), collectively referred to as "farro" (van Slageren and Payne, 2013), are among the earliest domesticated cereal crops, with a history of cultivation that spans approximately 10,000 years (Nesbitt and Samuel, 1996; Salamini et al., 2002). Originating in the Fertile Crescent, and subsequently grown across Europe, Asia, and Africa for millennia, these ancient wheats played a pivotal role in the development of early agricultural societies. Despite their historical and agronomic importance, hulled wheats have gradually fallen out of favor over the past centuries, largely supplanted by free-threshing species such as common wheat (*T. aestivum*) and durum wheat (*T. durum*). This transition has primarily been driven by the higher productivity, improved post-harvest handling, and technological properties of modern wheat varieties, which better align with the demands of industrialized food systems (Longin et al., 2016; Shewry, 2018).

In recent years, farro species have attracted renewed interest, largely

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https://doi.org/10.1016/j.jcs.2025.104303
Received 18 July 2025; Received in revised form 1 October 2025; Accepted 21 October 2025

in response to growing consumer demand for traditional, locally sourced, and nutrient-rich food products (Shewry, 2018; Costanzo et al., 2019; Karakas et al., 2021). These ancient wheats are gaining recognition not only for their nutraceutical properties but also for their distinctive sensory attributes in breadmaking and other food applications (Brandolini et al., 2008). Moreover, farro species are increasingly capturing the attention of farmers in developed countries, due to their potentially greater adaptability to low-input and organic farming systems, compared to modern wheat varieties (Konvalina et al., 2014; Longin et al., 2016; Bencze et al., 2020). This trend aligns with broader efforts to promote agrobiodiversity, support sustainable agricultural practices, and enhance the value of underutilized crops.

The Mediterranean region is generally characterized by low crop productivity, primarily due to a combination of edaphic and climatic constraints, including limited annual precipitation and highly variable rainfall patterns. Moreover, current climate change projections suggest increasingly adverse conditions, marked by significant temperature increases and concurrent declines in rainfall, which may have particularly severe impacts on rainfed agricultural systems (Abd-Elmabod et al., 2020). In this context, identifying crop species and genotypes with high adaptability and rusticity is crucial to ensuring the future economic viability of agriculture while promoting sustainability and resilience.

In this study, conducted in a typical semi-arid Mediterranean environment, we evaluated sixteen recently developed varieties of einkorn, emmer, and spelt through an integrated approach that included a comparison of their agronomic, qualitative, and nutraceutical traits with those of both old and modern durum wheat genotypes commonly cultivated in the same environment; and an assessment of their technological performance in breadmaking, along with the sensory profile of the resulting breads. The objectives are: i) to evaluate the three hulled-wheat species under rainfed systems in the semi-arid Mediterranean region—an environment in which data on their adaptability remain scarce—, and to identify promising genotypes that combine agronomic and nutritional value and sensory quality, or which may be valuable for use in breeding programs; ii) to define appropriate quality and aromatic parameters to predict the baking performance of wholemeal flours from einkorn, emmer, and spelt. Thus, this research aims to provide valuable insights into the valorization and reintroduction of hulled wheats within Mediterranean agro-ecosystems, an objective that is particularly relevant in the context of ongoing climatic challenges and the growing need to diversify cropping systems, especially in marginal and rainfed areas.

2. Materials and methods

2.1. Experimental sites

The experiment was carried out during the 2021–2022 growing season at two experimental sites (A and B), situated at the Pietranera farm (A. & S. Lima Mancuso Foundation) in inland Sicily, Italy (approximately 30 km north of Agrigento; 37°30′N, 13°31′E; 178 m a.s.l.). The two sites, situated about 300 m apart, differ markedly in their soil characteristics (Table 1). The farm extends over approximately 700 ha and encompasses a wide range of soil types, distinguished by their physical and chemical properties.

According to the Soil Survey Staff (2006), the soil at site A is classified as Typic Xerorthent (Entisol) (Table 1). It is light brown in color, has a sub-alkaline pH, an unstable structure, low organic matter content, and a medium–low yield potential, while site B is characterized by a Vertic Xerochrept (Inceptisol) soil, which is well structured, clayey in texture, sub-alkaline, and richer in organic matter and fertility elements (N, P, K, etc.), resulting in a medium–high yield potential.

The farm is set within a semi-arid Mediterranean climate zone, with an average annual rainfall of 581 mm. The dry season extends from May to September. The annual mean minimum and maximum temperatures are 10.0 °C and 23.3 °C, respectively. Climatic data, including air temperature and precipitation, were collected from a weather station located within 200 m of both experimental sites.

Table 1
Physical and chemical characteristics of the top layer (0–40 cm) of the two soil types where the experiment was conducted.

Soil characteristic	Unit	Site A	Site B
		Typic Xerorthent (Entisol)	Vertic Xerochrept (Inceptisol)
Particle size analysis			
Clay	g kg^{-1}	209	525
Silt	g kg^{-1}	461	227
Sand	g kg^{-1}	330	248
pH (1:2.5 H_2O)		7.84	8.20
Total C (Walkley Black)	g kg^{-1}	9.3	16.8
Total N (Kjeldahl)	g kg^{-1}	1.20	1.78
Available P (Olsen)	mg kg^{-1}	16.3	40.1
Cation exchange capacity	cmol + kg^{-1}	18.4	35.0
Water content at			
field capacity	cm^3 cm^{-3}	0.288	0.420
permanent wilting point	cm^3 cm^{-3}	0.137	0.223

2.2. Experimental design and management

A total of sixteen genotypes representing the three hulled wheat species (einkorn, emmer, and spelt) were evaluated, alongside three durum wheat genotypes used as controls (Table 2). A randomized block design with four replicates was adopted at both sites. Each elementary plot measured 10 m^2 and consisted of eight rows, spaced 0.18 m apart and 7 m in length.

The preceding crop at both sites was berseem clover (*Trifolium alexandrinum*). Soil tillage included plowing to a depth of 25–30 cm at the end of summer, followed by harrowing immediately before sowing to eliminate emerged weeds and prepare a suitable seedbed. Sowing was performed in December 2021 using a plot seeder, at a seeding rate of 350 viable seeds m^{-2} across all genotypes and both sites. Nitrogen fertilization was applied at both sites at a rate of 46 kg ha^{-1} as urea, at the end of February 2022 (late tillering stage). No phosphorus or potassium fertilizers were applied. Weed control was carried out in March 2022 using a post-emergence systemic herbicide (Zypar®, Corteva Agriscience Italia srl), consisting of Halauxifen-methyl, Florasulam, and Cloquintocet-mexyl, applied at a rate of 0.75 L ha^{-1} for the control of

Table 2
Studied species and genotypes.

Species	Name of variety (V) or landrace (L)	Acronym	Year of release
Einkorn	Monlis (V)	Monl	2006
(*T. monococcum*)	Norberto (V)	Norb	2017
	Unknown (L)	UMon	n.a.[a]
Emmer	Davide (V)	Davi	2009
(*T. dicoccum*)	Giovanni Paolo (V)	GiPa	2008
	Padre Pio (V)	PaPi	2016
	Rosso Rubino (V)	RoRu	2009
	Unknown (L)	UDic	n.a.[a]
	Yakub (V)	Yaku	2009
	Zefiro (V)	Zefi	2006
Spelt	Benedetto (V)	Bene	2010
(*T. spelta*)	Giuseppe (V)	Gius	2008
	Maddalena (V)	Madd	2017
	Pietro (V)	Piet	2010
	Rita (V)	Rita	2016
	Rossella (V)	Ross	2016
Durum wheat	Antalis (V)	Anta	2013
(*T. durum*)	Aureo (V)	Aure	2009
	Perciasacchi (L)	Perc	<1915

[a] Not available.

broadleaf weeds. Additionally, no fungicide or insecticide treatments were applied during the entire trial period.

2.3. Agronomic parameters and quality of grains

At both experimental sites, the following agronomic traits were recorded for each plot: heading and maturity dates; plant height (based on 10 measurements per plot at maturity); lodging area and intensity (expressed as a percentage); number of spikes m^{-2} (calculated from the number of spikes in a 6 m row); number of kernels per spike; grain yield; 1,000-kernel weight. For each hulled wheat genotype, a 30 g sample of hulled kernels was manually dehulled to assess husk incidence and calculate the net grain yield.

In addition, grain samples were analyzed for protein and moisture contents (%) using the Infratec 1241 NIR grain analyzer (Foss, Hillerød, Denmark). Wholemeal flour was obtained by grinding the grains to 0.75 mm using a Retsch ZM 200 laboratory mill (Retsch GmbH, Haan, Germany). The following flour parameters were then determined: ash content (AACC Method 08–03.01), gluten content and gluten index using the Glutomatic System [Glutomatic 2200, Centrifuge 2015; Glutork 2020 (Perten Instruments AB, Huddinge, Sweden)] according to AACC Method 38-12.

2.4. Determination of total phenolic content (TPC)

The total phenolic content (TPC) of wholemeal flour from all genotypes was determined following the method described by Yu et al. (2002) and Lu et al. (2015), with slight modifications. Phenolic compounds were extracted by mixing 1 g of wholemeal flour with 10 mL of cold 80 % ethanol. The mixture was centrifuged at 10,000 rpm for 10 min. Subsequently, 5 mL of 6 M sodium hydroxide were added at room temperature, and the solution was stirred for 1 h. After stirring, the mixture was acidified to pH 2 using 6 M hydrochloric acid. The supernatant was then extracted with ethyl acetate and centrifuged at 10,000 rpm for 10 min. This extraction step was repeated three times. The ethyl acetate phase was evaporated at 45 °C until a dry residue was obtained, which was then reconstituted in 3 mL of methanol. The resulting extracts were stored at −20 °C until analysis.

TPC was quantified using the method of Singleton et al. (1999) and Tian and Li (2018), with some modifications. Briefly, 0.1 mL of extract was mixed with 7.9 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent. After 5 min, 1.5 mL of 20 % sodium carbonate solution were added. The reaction mixture was incubated at room temperature in the dark for 2 h to allow for color development. Absorbance was measured at 765 nm using a Shimadzu UV Mini 1240 spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used as the calibration standard, and TPC was expressed as micromoles of gallic acid equivalents per gram of flour (μM GAE g^{-1}).

2.5. Dough preparation, baking process, and sensory evaluation of breads

Baking tests were conducted using wholemeal flour obtained from grains harvested at site B. Due to budget limitations, the number of experimental samples was reduced. Grains from site B were selected for this analysis, as they exhibited greater variability in both agronomic and quality traits compared to those from site A.

For each genotype, 300 g of dough was prepared using wholemeal flour. Following the method described by Alfonzo et al. (2016), 3 g of fresh baker's yeast (La Parisienne, AB Mauri Italy SpA, Casteggio, Italy), corresponding to 1 % (w/w) of the dough weight, were added to initiate fermentation. The yeast, composed of *Saccharomyces cerevisiae* at a cell density exceeding 7 Log CFU/g, was suspended in sterile tap water prior to mixing with the flour. All doughs were manually mixed in sterile 1-liter glass beakers under a laminar flow hood.

The doughs were then transferred to rectangular stainless steel pans in accordance with AACC Method 10-10B and incubated at 25 °C for 2 h. Each baking test was performed in duplicate and repeated after two weeks, representing independent replicates.

Dough fermentation was monitored by measuring pH, total titratable acidity (TTA), and yeast population growth at the beginning and after 2 h of fermentation, as described by Ruisi et al. (2021).

After fermentation, the doughs were baked in an industrial oven (Electrolux, Pordenone, Italy) using a two-phase baking program: an initial steam phase for 5 min at 200 °C, followed by a combined air and steam phase for 15 min at the same temperature. Upon baking, breads were cooled at room temperature for 30 min before further evaluation. Breads were assessed based on the following parameters: weight loss, height, specific volume, crumb firmness (as an indicator of crumb density), and crust and crumb color. In addition, image analysis was performed. Weight loss was calculated as the difference in bread weight before and after baking. Bread height was measured using a digital caliper (841–2518, RS Components srl, Sesto San Giovanni, Italy). Bread volume was determined using a bakery product volumeter (ErreCi s.r.l., Merate, Italy), applying the rapeseed displacement method (AACC Method 55–50.01). Crumb firmness was measured using an Instron 5564 texture analyzer (Instron Corp., Canton, MA), according to the procedure described by Corsetti et al. (2000). Specifically, 25 mm-thick slices were compressed to 40 % of their original height using cylindrical probes with a diameter of 38.1 mm. Crust and crumb color were measured with a Chroma Meter CR-400C (Minolta, Osaka, Japan), following the protocol of Settanni et al. (2013). Image analysis of the crumb structure included the calculation of void fraction, cell density (number of alveoli per unit area), and mean cell area, as described by Settanni et al. (2013).

Sensory analysis of the laboratory-produced breads was performed by a panel of 13 trained judges (male and female), aged between 20 and 60 years. The evaluation followed the protocol described by Alfonzo et al. (2016) and was conducted in accordance with ISO 13299 (2003) guidelines. Panelists assessed the breads using 23 sensory descriptors: crust color, crust thickness, crumb color, porosity, alveolation, uniformity of alveolation, aroma intensity, bread aroma, yeast aroma, sourdough aroma, off-odors, flavor intensity, bread flavor, yeast flavor, sourdough flavor, off-flavors, saltiness, acidity, bitterness, flavor persistence, mouthfeel, crispness, and overall evaluation. All attributes were scored on a numerical scale from 0 to 10, where 0 indicated the absence of the characteristic and 10 represented the highest intensity perceived.

2.6. Determination of volatile organic compounds (VOCs)

The volatile organic compounds (VOCs) from the 20 wholemeal flour samples were extracted using headspace solid phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (Agilent 6890 GC system, DB5-MS column and MS5973 quadruple MS). The gas chromatograph injector was set to 280 °C and operated in splitless mode, using continuous helium gas flow as the carrier. The column was initially held at 40 °C for 5 min, followed by a gradient of 10 °C/min up to 250 °C, held for 20 min. Mass spectra were then recorded between 40 and 550 atomic mass units using an electron impact ionization energy of 70 eV. Commercial Carbowax-divinylbenzene fiber (CW-DVB, 65 μm, Supelco, Bellefonte, PA, USA) was used as the stationary phase, with injections performed using a manual SPME holder from the same manufacturer. Prior to use, new fibers were conditioned according to the manufacturer's instructions by heating in the injector (200 °C, split mode) for 30 min. The fiber was then immersed in the vial containing the sample. Specifically, headspace samples were prepared by placing 4 g of wholemeal flour inside a 22 ml amber vial, sealed with a screw cap with a polytetrafluoroethylene/silicone septum (Supelco, Bellefonte, PA, USA). VOCs were collected from the headspace of the glass vial by inserting the SPME needle through the septum and exposing the fiber for 1 h. The fiber was then thermally desorbed into the GC inlet for 1 min and the analytes were transferred to the GC column. Compounds were identified by comparing retention indices and mass spectra with those reported in the databases (www.pherobase.com; NIST library, 2010). Linear retention indices were calculated using a standard mixture of n-alkanes (C7–C30) injected immediately before the samples. Where available, compound identities were further confirmed using authentic standards (Sigma-Aldrich, Milan, Italy). Peak areas were quantified using MSD ChemStation software. Possible contaminations were eliminated from the analysis by subtracting background signals arising from the empty headspace of the vials used for volatile extraction.

2.7. *Statistical analysis*

Data were analyzed using R software (R Core Team, 2024) to assess differences both among accessions and species, as well as their interactions with the experimental site (i.e., site × genotype and site × species interactions). A mixed-effect model was fitted using the “aov()” function, specifying “block” as random factor nested within “site” as fixed factor to account for the experimental design. Model residuals were checked for heteroscedasticity and a normal distribution. When parametric assumption where violated data were transformed accordingly.

Data from the baking tests (technological and sensory parameters) and VOC analysis, which were conducted exclusively at site B, were analyzed including “block” as the sole random factor. Following ANOVA, pairwise comparisons were performed using the Least Significant Difference (LSD) test with Bonferroni adjustment for multiple comparisons, using the “LSD.test()” function from the “agricolae” package (de Mendiburu, 2023), with significance level set at 0.05.

Linear correlation coefficients were calculated across all measured variables to identify significant relationships between grain quality and bread technological traits, while Spearman’s rank-order correlation was used between VOCs and bread sensory traits. Finally, Principal Component Analysis (PCA) was conducted to assess variation among treatments and to determine which variables contributed most to group separation. PCA was performed using the “FactoMineR” package (Le et al., 2008) and visualized with the “factoextra” package (Kassambara and Mundt, 2020).

3. Results and discussion

3.1. *Weather conditions*

During the growing season, total rainfall amounted to 845 mm, 45 % more than the long-term average. Rainfall occurred mainly between October and December (611 mm) and during May (110 mm) (Fig. S1). Mean winter temperatures were comparable to the historical average, while autumn and spring temperatures were slightly higher (+0.6 °C). Overall, climatic conditions were favorable for crop development. In particular, the abundant rainfall and its well-distributed pattern prevented waterlogging and ensured sufficient water availability throughout the season.

3.2. *Agronomic and qualitative traits*

Site did not affect the heading dates of the genotypes (interactions site × genotype and site × species not significant; Table 3). On average, species differed markedly: durum wheat was consistently the earliest, while einkorn showed the latest heading. Overall variability ranged from 123 days after sowing (DAS) for the emmer variety Davide to 150 DAS for the einkorn variety Monlis (Fig. S2).

Table 3
Analysis of variance (ANOVA) of agronomic and qualitative traits: P values of site, genotype, and species and their interactions.

Trait	Site	Genotype	Site × Genotype	Species	Site × Species
Heading date (days after sowing)	0.008	<0.001	0.081	<0.001	0.678
Plant height (cm)	<0.001	<0.001	0.010	<0.001	0.684
Grain yield (t ha^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001
Spikes m^{-2}	0.002	<0.001	<0.001	<0.001	0.178
Kernels spike^{-1}	0.095	<0.001	<0.001	0.002	0.015
1000-kernel weight (g)	0.114	<0.001	<0.001	<0.001	0.002
Protein content (%)	<0.001	<0.001	<0.001	<0.001	<0.001
Gluten content (%)	<0.001	<0.001	<0.001	<0.001	0.179
Gluten index (%)	0.081	<0.001	<0.001	<0.001	0.122
Total phenolic content (μM g^{-1})	0.492	<0.001	<0.001	<0.001	<0.001

Plant height varied significantly between sites and among genotypes (Fig. S3). At Site A, the average height was 96 cm, whereas at the more fertile Site B, it reached 113 cm. Despite the statistical significance of the site × genotype interaction, the overall variation in genotype performance across the sites was relatively modest. On average, the shortest genotypes were Giovanni Paolo, Pietro, and the two modern durum varieties, Antalis and Aureo, all of which exhibited heights below 90 cm even under the most favorable conditions (i.e., at Site B). In contrast, the tallest plants included the durum landrace Perciasacchi (average 131 cm) and three einkorn genotypes (Monlis, UMon, and Norberto), with average heights ranging from 116 to 130 cm.

At Site A, where plant height was more limited, no lodging was observed in any genotype (data not shown). In contrast, at Site B, severe lodging was recorded in the emmer variety Zefiro, while moderate lodging was observed in Perciasacchi, Yakub, and UDic. Notably, the three einkorn genotypes showed no lodging despite their height, likely due to a combination of factors, including high culm flexibility (Jaradat, 2019), smaller spike size (and thus lower weight), and the absence of heavy post-anthesis rainfall, as flowering occurred very late in this species.

At Site A, the average number of spikes m^{-2} was 211 (Table S1). At Site B, the higher soil fertility promoted tillering, resulting in a 50 % increase (313 spikes m^{-2}). Significant differences were observed among species (einkorn > emmer = spelt > durum wheat), thereby confirming previous findings (Giraldo et al., 2016; Jaradat, 2019). Under the less favorable conditions (i.e., Site A), variability in this trait was generally low (excluding einkorn). As soil fertility increased (i.e., Site B), responses were more heterogeneous: some genotypes (Padre Pio, Maddalena, Giuseppe) showed a marked increase in spike number per unit area (>80 %), while others (Zefiro, Perciasacchi, UMon, Norberto) showed minimal changes (<20 %). Further investigation is needed to clarify the underlying causes of these contrasting responses.

In the more favorable site (Site B), average net (i.e. dehulled) grain yield reached 3.51 t ha^{-1}, while at Site A it was nearly halved (1.81 t ha^{-1}; Fig. 1). This outcome was expected, given the marked differences in soil physical and chemical fertility between the two sites. At Site A, yield differences among genotypes were generally modest, ranging from 1.42 to 2.34 t ha^{-1} for the emmer varieties Giovanni Paolo and Zefiro, respectively. Among species, einkorn showed substantially lower average grain yield compared to the others, corroborating previous findings (Troccoli and Codianni, 2005; Longin et al., 2016). In contrast, at Site B, genotype differences were more pronounced, with yields ranging from 1.70 t ha^{-1} (emmer variety Rosso Rubino) to 5.25 t ha^{-1} (durum variety Antalis).

Genotypes differed in their ability to exploit improved growing conditions. For example, Antalis showed a more than threefold increase in grain yield at Site B compared to Site A, while the three einkorn genotypes and some emmer genotypes (UDic, Yakub, and Zefiro) displayed modest increases (around 50 %). In contrast, other emmer genotypes

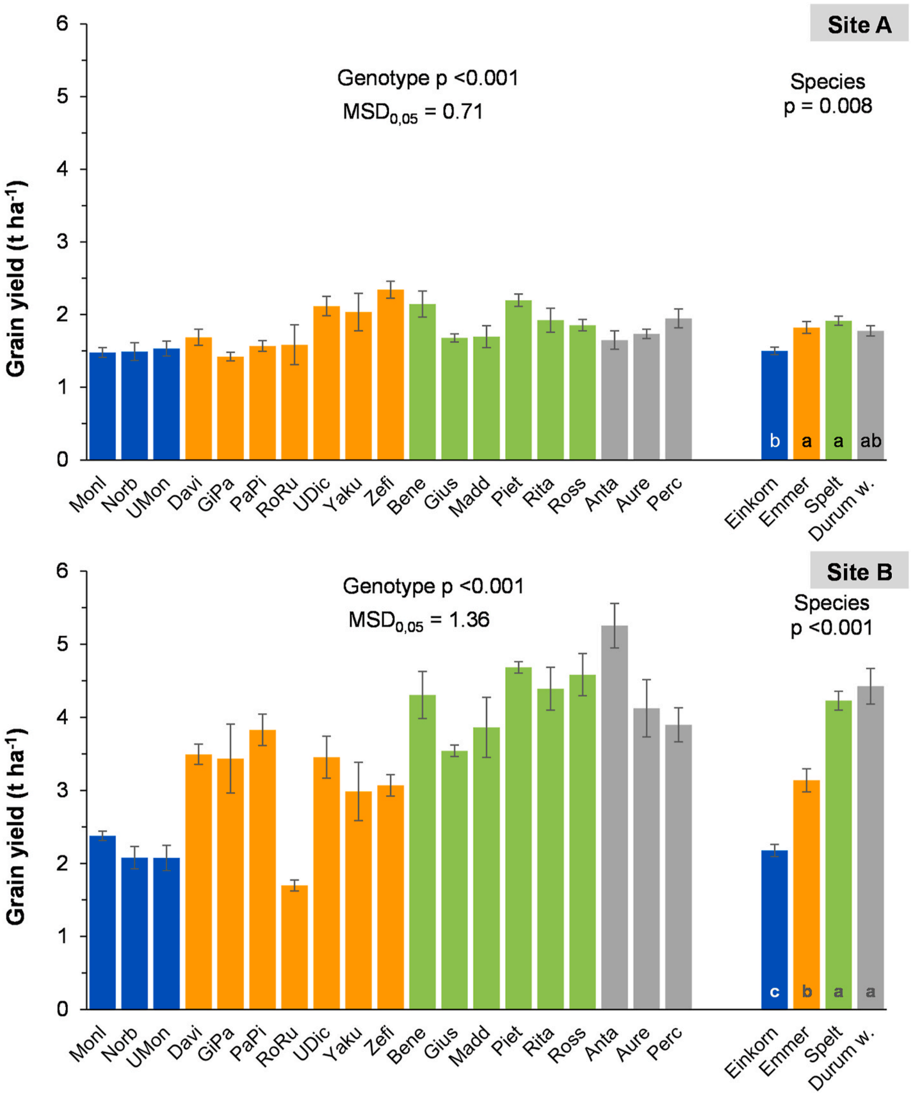

Fig. 1. Grain yield (t ha^{-1}) of the genotypes (left) and species (right) at Site A (top) and Site B (bottom). The histograms on the left represent the mean ± standard error (n = 4); the P value and the minimum significant difference (MSD) for P = 0.05 are also reported. Different letters denote significant differences among species at P ≤ 0.05.

(Padre Pio, Davide, and Giovanni Paolo) responded more efficiently to improved soil fertility, with yield increases of approximately 120 %, a trend also observed in all spelt accessions.

These findings have practical implications. In general, under favorable conditions—specifically those characterized by higher soil fertility as defined in this study—, the adoption of modern durum wheat varieties (e.g., Antalis) appears advantageous due to their more efficient utilization of increased resource availability. Conversely, under less favorable growing conditions, differences among genotypes tend to diminish, broadening the range of viable options to include other species, particularly einkorn, which benefits from a higher market value (Bencze et al., 2020). This may be especially relevant in organic farming systems, where the economic benefits of einkorn are complemented by agronomic advantages linked to its greater plant height. Indeed, several studies have demonstrated a strong positive relationship between plant height and competitive ability against weeds (Gonzalez Ponce and Santin, 2001; Ruisi et al., 2015), the management of which remains a major challenge in organic farming systems (Bàrberi, 2002).

Substantial differences in 1,000-kernel weight were observed among genotypes and species, with site-dependent variation (Table S1). The lowest values were recorded in the einkorn genotypes (average: 17 g), while the highest were observed in durum wheat (average: 38 g). Within this species, the highest 1,000-kernel weight was measured at both sites in Perciasacchi (43 g on average), and among emmer genotypes, in Davide (40 g on average). Soil fertility differences between the two sites had a marked and variable impact on this trait across genotypes. At the lower fertility site (Site A), all einkorn genotypes showed reduced 1,000-kernel weight (−11 %), whereas all spelt genotypes exhibited an increase (+25 %) under improved fertility conditions. Emmer genotypes displayed heterogeneous responses, with changes ranging from −9 % to +16 %, confirming trends previously observed for grain yield.

At Site A, the average number of grains per spike was 34, ranging from 22.6 (einkorn UMon) to 45.6 (spelt Benedetto; Table S1). Improved soil fertility had highly variable effects on spike fertility across genotypes. Notable increases were observed in the modern durum wheat varieties (Antalis and Aureo), whereas modest increases, or even reductions, were recorded in several genotypes from other species (e.g., Rosso Rubino, Rossella, and Giuseppe).

Overall, based on the yield component analysis, the increase in grain yield observed under higher soil fertility conditions appeared to be primarily driven by an increased number of spikes per unit area, with more limited contributions from the number of grains per spike and 1,000-kernel weight.

Significant differences in grain protein content were observed between the two sites. At Site A, the average was 13.2 %, while at Site B it exceeded 16 % (Fig. 2). Wide variation was also recorded among species and genotypes, with responses differing markedly by site (Table 3). At Site A, no significant differences were found among species, but substantial variation was observed within each species. In emmer, for example, high protein content was recorded in Davide and Giovanni Paolo (15.7 %), whereas Rosso Rubino and Zefiro showed much lower values (11.2 % and 11.3 %, respectively). Among durum wheats, Antalis averaged 12.6 % and Perciasacchi 13.3 %, while Aureo exhibited a particularly high value (14.5 %). This result was expected, as Aureo has been selected for high grain quality, especially protein content, and is favored by the pasta industry. In contrast, the protein content observed in Perciasacchi was unexpected as previous studies consistently reported much higher values for this landrace compared to modern durum varieties (Ficco et al., 2019; Ruisi et al., 2021). A possible explanation for this may be that, in the current study, grain yields were low at site A, with no significant differences observed among the durum wheat genotypes, while Perciasacchi usually yields significantly less than modern varieties. Thus, it is reasonable that, under such conditions, the “dilution effect” typically associated with increased grain yield likely did not occur. In spelt, the average protein content was 12.9 %, with minimal variation among genotypes, except for Giuseppe, which reached 14.3 %.

At Site B, substantial differences in grain protein content were observed among species and genotypes. The highest protein content values (>18 %) were recorded in the three einkorn accessions, confirming the findings reported by Gazza et al. (2023), whereas the lowest protein content was observed in Antalis (approximately 13 %). In contrast to Site A, a clear negative linear relationship between grain yield and protein content was evident ($R^2 = 0.84$), consistent with previous studies (Ruisi et al., 2015; Laidig et al., 2017).

Interestingly, genotypes responded differently to changes in growing conditions. Some genotypes, including the three einkorn accessions and the emmer genotypes Rosso Rubino, UDic, Yakub, and Zefiro, showed only modest yield increases under improved fertility, but a substantial rise in protein content. In contrast, the modern durum variety Antalis markedly increased its grain yield without a significant change in protein content. Finally, other genotypes, including all spelt accessions, the durum varieties Aureo and Perciasacchi, and the emmer genotypes Davide, Giovanni Paolo, and Padre Pio, responded to enhanced soil fertility by increasing both grain yield and protein content in a more balanced manner. These patterns likely reflect differences in nitrogen use efficiency among genotypes. This suggests that optimizing genotype performance and meeting specific production goals will require tailored agronomic management strategies.

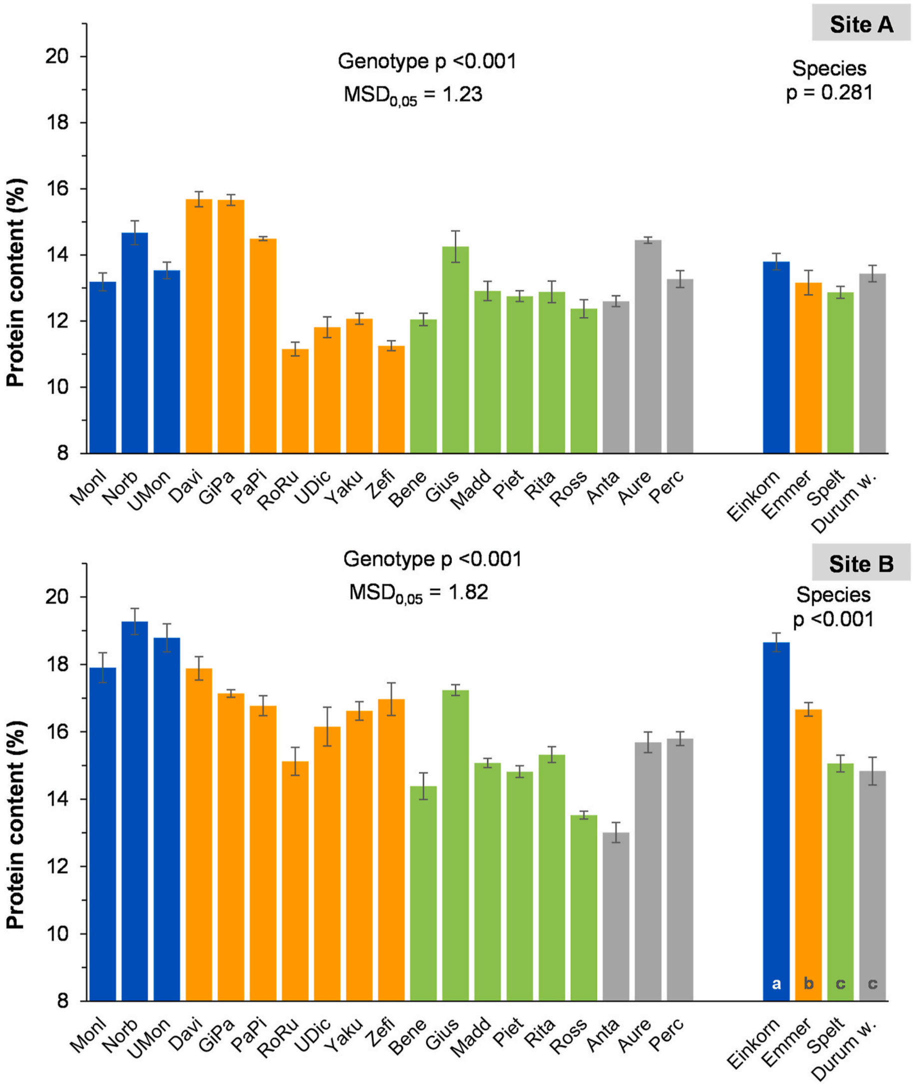

Fig. 2. Protein content (%) of grains of the evaluated genotypes (left) and species (right) at Site A (top) and Site B (bottom). The histograms on the left represent the mean ± standard error (n = 4); the P value and the minimum significant difference (MSD) for P = 0.05 are also reported. Different letters denote significant differences among species at $P \leq 0.05$.

Gluten content varied according to site, species, and genotype, following trends similar to total protein (Fig. S4). However, the proportion of gluten relative to total protein was, on average, lower at the more fertile site (68.6 % at Site B vs. 75.4 % at Site A). Interestingly, the increase in gluten content did not consistently mirror the increase in protein content, suggesting a shift in protein composition. Specifically, higher soil fertility may promote the synthesis of non-gluten proteins (e.g., enzymatic proteins) over storage proteins, thereby altering the gluten-to-protein ratio. This finding contrasts with reports in wheat by Rekowski et al. (2019) and Horvat et al. (2021). However, Horvat et al. (2021) pointed out that the proportion of the non-gluten protein fraction relative to the total grain protein content varies depending on the genotype, which may partially explain the discrepancies observed in the present study.

Regarding gluten quality, modest but significant differences between the two sites were observed for gluten index (GI), while genotype and species differences were more pronounced (Fig. 3). Each genotype and species exhibited relatively stable behavior across sites. Some studies have indicated that genotype × environment interactions play a major role in determining gluten quantity, but a minor role in gluten quality (Ames et al., 1999; Vida et al., 2014). In both sites, einkorn accessions showed the lowest GI values, averaging 35.9. For Monlis, GI could not be determined because the gluten was completely washed out during the test, a phenomenon also reported in other einkorn genotypes (Hlisnikovský et al., 2019). This result is consistent with the known protein profile of einkorn, which differs from other *Triticum* species in several respects: it has a higher gliadin-to-glutenin ratio and contains lower levels of both high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits, which are in general positively correlated with gluten strength and, consequently, with baking performance (Wieser et al., 2009; Geisslitz et al., 2019; Mefleh et al., 2022). Particularly high GI values were observed in the modern durum wheat varieties Antalis and Aureo, but not in the landrace population Perciasacchi, reflecting the role of gluten quality as a key selection target in modern breeding. Among spelts, all genotypes, except Benedetto, exhibited particularly high GI values. Emmer accessions, by contrast, showed generally low GI values, ranging from 25.4 in Yakub to 65.1 in Padre Pio.

3.3. Total phenolic content (TPC)

The analysis of TPC data revealed significant differences among the evaluated genotypes and species, as well as significant interactions between site × genotype and site × species (Table 3). At Site A, the highest

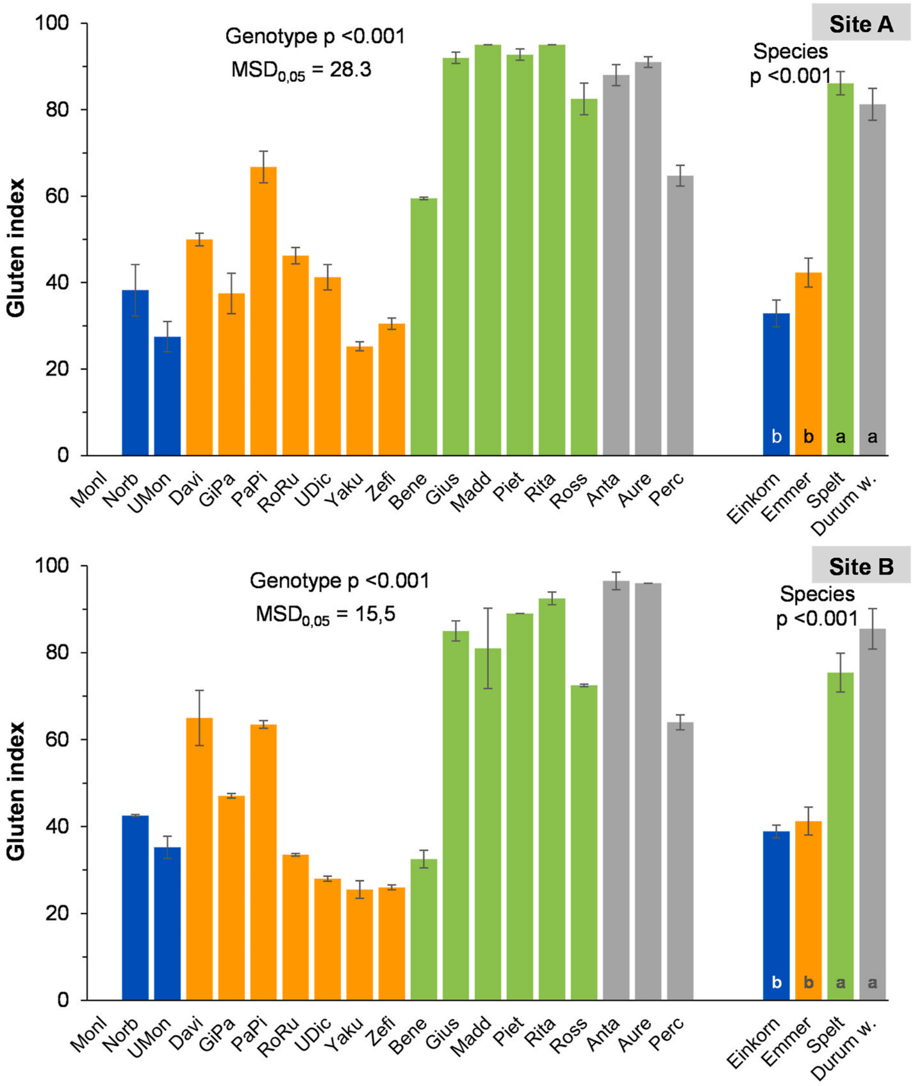

Fig. 3. Gluten index of wholemeal flours of the evaluated genotypes (left) and species (right) at Site A (top) and Site B (bottom). The histograms on the left represent the mean ± standard error (n = 4); the P value and the minimum significant difference (MSD) for P = 0.05 are also reported. Different letters denote significant differences among species at $P \leq 0.05$.

TPC values were recorded in the durum wheat accessions (average: 6.18 μM g^{-1}, with modest variation among genotypes; Fig. 4) and in spelt (average: 6.17 μM g^{-1}, range: 4.95–6.85 μM g^{-1}). In contrast, the lowest values were observed in the einkorn genotypes (average: 4.02 μM g^{-1}, range: 3.40–5.10 μM g^{-1}). Emmer showed an intermediate average TPC of 5.33 μM g^{-1}, with relatively limited variation among genotypes (range: 4.25–5.85 μM g^{-1}).

At Site B, the mean TPC values were comparable to those recorded at Site A (5.79 vs. 5.46 μM g^{-1}, respectively; Fig. 4), although considerable differences among species were detected. Notably, einkorn exhibited a significant average increase in TPC (+48 %), whereas no substantial changes were observed in the other three species. The response of genotypes appeared highly variable; in some genotypes (UMon, Monlis, and Rosso Rubino), improved soil fertility conditions led to pronounced increases in TPC values (>50 %), while in others (such as Rita and Antalis), significant decreases were recorded (approximately −20 %).

Overall, the analysis of TPC data indicates that species and genotypes respond differently depending on the growing conditions. The existing literature on TPC in *Triticum* species presents highly inconsistent findings. Brandolini et al. (2013) reported similar TPC values in einkorn, emmer, and spelt, with significantly higher levels in durum and common wheat. Conversely, Serpen et al. (2008) observed higher TPC in emmer compared to einkorn and common wheat. Abdel-Aal and Rabalski (2008) found lower TPC values in durum and common wheat than in einkorn, emmer, and spelt. Such discrepancies may be attributed to various factors, including the pedoclimatic conditions of the experimental sites, agronomic practices, and the genetic materials under investigation (Dapčević-Hadnađev et al., 2022).

It is worth noting that, in the present study, on average, the more favorable cultivation conditions at Site B did not lead to significant changes in TPC. This finding contrasts with previous literature, which generally indicates that many antioxidant compounds, including polyphenols, are synthesized by plants as a response to abiotic stress, typically associated with water availability, temperature, and nutrient limitations (Stracke et al., 2009; Lachman et al., 2012; Lu et al., 2015; Zrcková et al., 2019). It is plausible that such stress conditions were more prevalent at Site A, which presented less favorable growing conditions. In this experiment, the variation in TPC in response to site conditions proved to be highly variable, confirming the unpredictable nature of these responses. The underlying mechanisms are undoubtedly complex, involving climatic, soil-related, agronomic, and genetic factors, which warrant further detailed investigation.

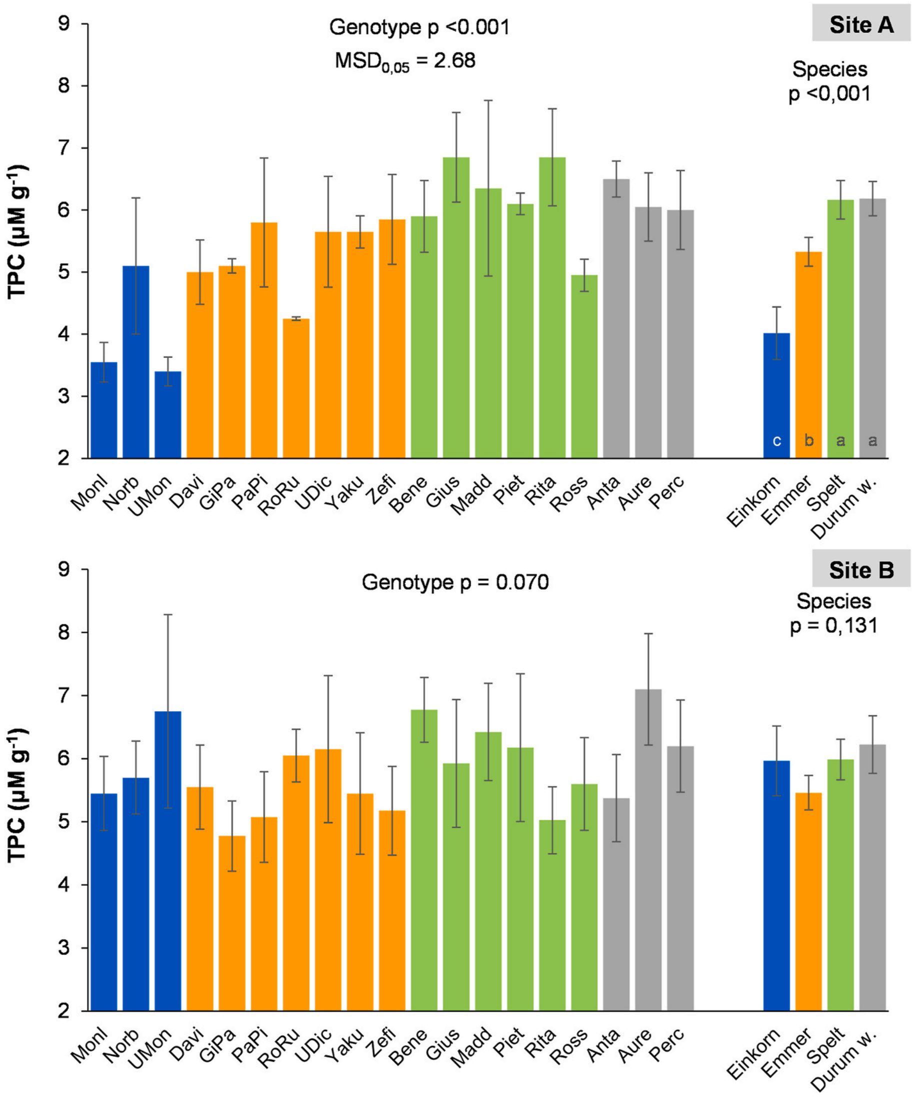

Fig. 4. Total phenolic content (TPC) of the wholemeal flours of the evaluated genotypes (left) and species (right) at Site A (top) and Site B (bottom). The histograms on the left represent the mean ± standard error (n = 4); the P value and the minimum significant difference (MSD) for P = 0.05 are also reported. Different letters denote significant differences among species at P ≤ 0.05.

3.4. Principal Component Analysis (PCA)

A multivariate analysis (Principal Component Analysis, PCA) was conducted using the collected and derived data to explore similarities and differences among the evaluated genotypes and to identify the traits that most significantly influenced these patterns. The first two principal components accounted for approximately 62 % of the total variance, which is considered sufficient for a two-dimensional representation. Three einkorn genotypes were clearly separated from all others (Fig. 5), although they exhibited a high degree of similarity among themselves. Notably, the einkorn accessions displayed highly distinctive characteristics, including late heading, low productivity, reduced kernel weight, and high protein content. The other species showed substantial intraspecific variability, leading to considerable overlap within their respective groupings. This result was somewhat expected; for example, the durum wheat material included two modern varieties and one old population with markedly different phenological, morphological, productivity, and grain quality traits. The broad variability observed in both emmer and spelt, in terms of agronomic and quality characteristics, is particularly noteworthy and suggests the potential to identify genotypes well-suited to diverse agricultural and environmental conditions within the Mediterranean region.

3.5. Volatile organic compounds (VOCs)

A total of 35 VOCs were identified in the wholemeal flours of the tested genotypes (Table S2). The most represented chemical groups were aldehydes (6 compounds), alkanes (6), terpenes (3), esters (3), and alcohols (3). Nine compounds were detected but not identified. Among the most abundant were (E)-2-octenal (aldehydes), 4,6-dimethyldodecane (alkanes), estragole (terpenes), and 2-methylbutyl 3-methylbutanoate (esters). Despite significant differences among genotypes, nine compounds were consistently detected across all samples. Conversely, three compounds (hexan-1-ol, hexanal, and (E)-2-decenal) were present in 40 % or fewer of the samples. Nearly all detected compounds showed significant differences among genotypes.

The heatmap (Fig. 6) highlights a group of 12 genotypes with generally similar aromatic profiles, including all emmer accessions, two einkorns (Monlis and UMon), and two spelts (Rita and Giuseppe). The aromatic profiles within this group were generally subdued. Notably, some accessions, such as the einkorns Monlis and UMon and the emmer Zefiro, exhibited a particularly low number of detected compounds (<20). The modern durum wheat varieties Antalis and Aureo displayed similar aromatic profiles of medium to high intensity, while the old durum wheat population Perciasacchi showed a distinctly intense aromatic profile, clearly standing out from all other accessions. This result is consistent with previous reports describing significant differences in aromatic profiles between modern durum wheat varieties and local populations (Vita et al., 2016). The other genotypes exhibited highly specific aromatic profiles, distinct both from each other and from the previously mentioned groups, as confirmed by PCA (Fig. S5). Overall, the results indicate some degree of intraspecific variability in all species except emmer, despite the limited number of genotypes evaluated per species (3–7). This variability hinders the identification of species-specific aromatic profiles, an outcome that was somewhat unexpected and holds significant methodological implications for the design of future comparative studies across *Triticum* species. Many existing studies have relied on a limited number of genotypes per species (Makhoul et al., 2015; De Flaviis et al., 2021, 2023; Frankin et al., 2023). However, based on our results, future research should include a broader range of genotypes to more effectively capture both intra- and interspecific variability.

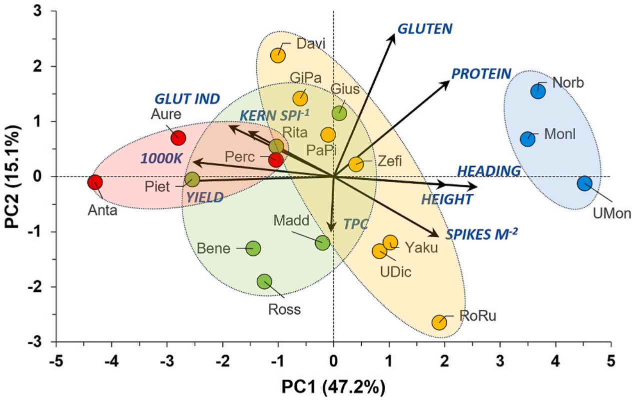

Fig. 5. Biplot of the principal component analysis (PCA) based on the agronomic and quality traits assessed on the 19 genotypes evaluated at site B. The percentage of total variance explained by the first two principal components (PC1 and PC2) is shown in parentheses. The 10 arrows originating from the origin (coordinates 0,0) represent the original variables: date of heading (HEADING); maximum plant height (HEIGHT); number of spikes per m^2 (SPIKES M^{-2}); grain yield (YIELD); number of kernels per spike; (KERN SPI^{-1}) weight of 1000 kernels (1000K); protein content in the grain (PROTEIN); gluten content in the grain (GLUTEN); gluten index (GLUT IND); total phenolic content in the grain (TPC). The length of each arrow is proportional to its contribution to the first two principal components. Each ellipse encompasses the evaluated genotypes for each species (blue: einkorn; yellow: emmer; green: spelt; red: durum wheat).

3.6. Fermentation process

Initial pH values of doughs made from wholemeal flours of the tested genotypes ranged from 6.06 (durum wheat Aureo) to 6.37 (emmer Yakub), with statistically significant differences among genotypes (Table S3). After fermentation, pH values decreased slightly in all genotypes, with the largest drop recorded in spelt Maddalena and emmer Yakub (−0.77 pH) and the smallest in emmer Giovanni Paolo (−0.32 pH); these differences were also statistically significant. Total titratable acidity (TTA) at the start of fermentation averaged 3.02 mL NaOH 0.1 N, with minor variations among genotypes (range: 2.83–3.20 mL NaOH 0.1 N). By the end of fermentation, TTA increased significantly, showing notable differences both among genotypes and species. The highest TTA (5.00 mL NaOH 0.1 N) was found in spelt Rita, and the lowest (4.30 mL NaOH 0.1 N) in modern durum wheat Aureo. Among species, einkorn had the lowest average TTA after 2 h (4.38 mL NaOH 0.1 N), while spelt had the highest (4.76 mL NaOH 0.1 N). Although pH and TTA were inversely correlated, TTA increases were not always proportional to pH decreases across genotypes.

Notably, TTA increases were related to the ploidy level of the species: highest in hexaploid spelt (+1.70 mL NaOH 0.1 N on average), lowest in diploid einkorn (+1.33 mL NaOH 0.1 N), and intermediate in tetraploid durum wheat and emmer (+1.56 mL NaOH 0.1 N). This pattern may reflect differences in flour characteristics; spelt flour typically has a finer particle size than flours from other species (Stoddard, 1999; Gargano et al., 2024). Larger particle sizes reduce surface area available to bacteria and yeasts, decreasing fermentable carbohydrate utilization and resulting in smaller TTA increases after fermentation (Ruisi et al., 2021). Another explanation, suggested by Gaglio et al. (2019), involves differences in flour buffering capacities due to varying protein contents, which are higher in einkorn and lower in hexaploid species like spelt.

Initial yeast inoculum levels ranged from 6.55 (Zefiro) to 7.47 (UMon) Log CFU/g. After 2 h of fermentation, yeast densities increased to between 7.17 (Zefiro) and 8.09 (Monlis) Log CFU/g. Yeast levels at both fermentation stages align with values reported for *Saccharomyces cerevisiae* in similar studies (Alfonzo et al., 2021; Ruisi et al., 2021).

3.7. Characteristics of breads

Table 4 reports the results of the analysis of breads made from

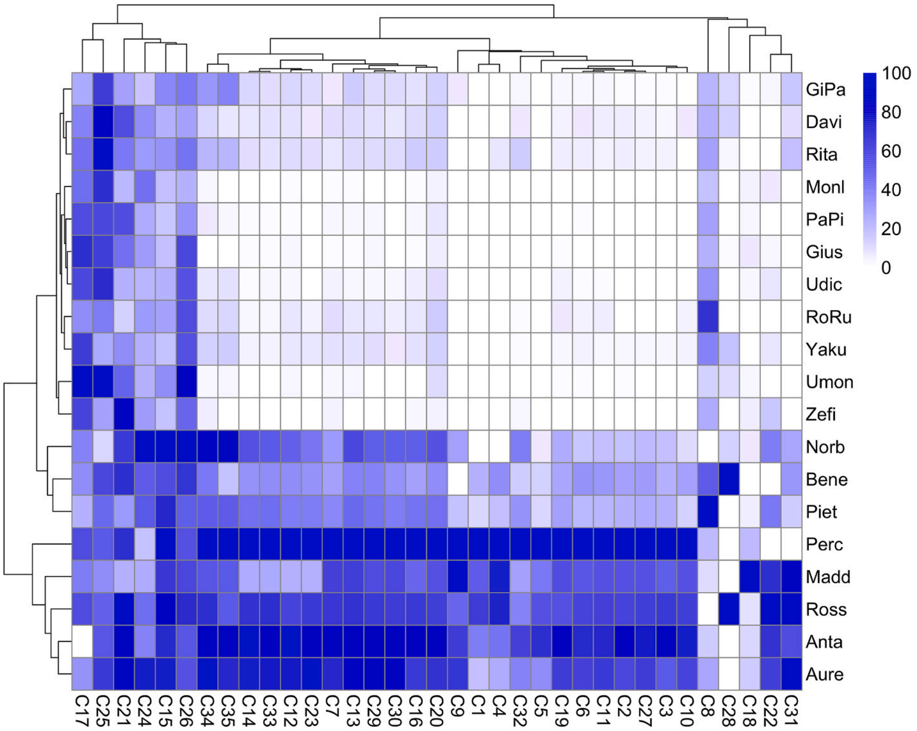

Fig. 6. Heatmap and hierarchical cluster analysis conducted on volatile organic compounds (VOCs; C1–C35, see Table 5) detected in the wholemeal flours of the 19 genotypes evaluated at site B. The color intensity in each cell represents the level of each compound in each genotype (100 = maximum value). The dendrograms, based on the VOC dataset, indicate the levels of similarity among the evaluated genotypes and among the detected VOCs.

Table 4
Characteristics of the breads obtained from the wholemeal flours of the genotypes and species under evaluation at site B. P values for Genotype and Species are also reported.

Species	Genotype	Weight loss (%)	Height (mm)	Specific volume (cm^3 g^{-1})	Firmness (N mm^{-2})	Void fraction (%)	Cell density (n cm^{-2})	Mean cell area (mm^2)
Einkorn	Monl	10.6	24.5	1.69	0.076	30.9	117.2	0.266
	Norb	16.9	52.5	3.89	0.033	35.4	33.3	1.222
	UMon	10.9	25.5	1.76	0.063	33.5	108.4	0.313
Emmer	Davi	13.7	61.5	4.38	0.019	34.6	51.6	0.670
	GiPa	14.4	41.0	2.94	0.034	32.0	56.9	0.571
	PaPi	10.7	36.5	2.51	0.026	39.7	61.8	0.641
	RoRu	13.5	29.0	2.06	0.067	38.5	61.3	0.638
	UDic	13.3	45.8	3.25	0.020	40.4	51.9	0.791
	Yaku	14.5	50.0	3.60	0.010	36.6	45.3	0.810
	Zefi	12.5	40.0	2.81	0.023	34.1	42.7	0.838
Spelt	Bene	12.7	37.0	2.60	0.051	33.7	50.8	0.669
	Gius	9.8	41.5	2.83	0.055	27.9	84.1	0.332
	Madd	12.6	30.5	2.15	0.054	35.8	67.3	0.551
	Piet	13.8	35.5	2.53	0.034	34.5	76.1	0.474
	Rita	13.6	37.0	2.64	0.094	31.5	88.9	0.355
	Ross	14.6	48.0	3.45	0.050	35.0	41.3	0.854
Durum wheat	Anta	12.7	28.0	1.97	0.040	34.3	60.6	0.566
	Aure	16.5	28.0	2.06	0.064	32.0	59.1	0.537
	Perc	13.2	24.5	1.74	0.058	33.9	67.7	0.501
Mean		13.2	37.7	2.68	0.046	34.4	64.5	0.610
P value		<0.001	<0.001	<0.001	<0.001	0.020	<0.001	<0.001
MSD$_{0.05}$		1.55	2.38	0.172	0.018	5.82	15.15	0.285
Species mean								
Einkorn		12.8	34.2 b	2.44 bc	0.058 a	33.3 b	86.3 a	0.600
Emmer		13.2	43.4 a	3.08 a	0.029 b	36.5 a	53.1 c	0.708
Spelt		12.8	38.3 b	2.70 b	0.056 a	33.1 b	68.1 b	0.539
Durum wheat		14.1	26.8 c	1.92 c	0.054 a	33.4 ab	62.5 bc	0.535

wholemeal flours of the evaluated genotypes. The average weight loss was 13.2 %, with statistically significant differences among genotypes, but not among species. The lowest weight loss was recorded in spelt Giuseppe (−9.8 %), while the highest was in einkorn Norberto (−16.9 %). These results are consistent with, or slightly lower than, those reported in previous experiments on wholemeal breads made from both durum and common wheat grown in the same environment (Ruisi et al., 2021; Cirlincione et al., 2022).

Bread height varied significantly among genotypes, ranging from 24.5 mm (einkorn Monlis) to 61.5 mm (emmer Davide). Significant differences were also observed among species (from 26.8 mm in durum wheat to 43.0 mm in emmer), with considerable variability within each species. A similar trend was observed for specific volume, which ranged from 1.69 cm^3 g^{-1} (einkorn Monlis) to 4.38 cm^3 g^{-1} (emmer Davide), closely mirroring bread height due to the strong correlation between these two traits. Both bread height and specific volume are influenced by the amount of CO_2 produced during fermentation and by the dough's ability to retain gas (Škrobot et al., 2022), which depends on gluten content and properties, which together determine the formation of a more or less robust gluten network. Therefore, in einkorn, the reduced bread height and volume may be associated with its high protein and gluten content, whereas in durum wheat, the limited rise is likely attributable to the high dough tenacity, a characteristic typical of modern varieties of this species (Peña et al., 2002). The variability observed within species suggests that other factors may also influence bread volume and structure, which should be further investigated.

The average firmness of the breads was 0.046 N mm^{-2}, with significant differences among both genotypes and species. The highest firmness was recorded in spelt Rita (0.094 N mm^{-2}), while the lowest was in emmer Yakub (0.010 N mm^{-2}). Among species, breads made from emmer wholemeal flours had the lowest average firmness (0.029 N mm^{-2}), while those from einkorn and spelt showed the highest values (on average, 0.058 and 0.056 N mm^{-2}, respectively), though variability within species was substantial. Firmness was negatively correlated with bread height (data not shown), in line with previous studies (Chin et al., 2009). Furthermore, similar to what was observed for bread height and volume, firmness appeared to be related to the rheological properties of the dough; in particular, bread firmness was directly proportional to dough tenacity, as indicated by the GI ($r = 0.430$; Fig. S6), consistent with observations by Ruisi et al. (2021). However, the data indicate that GI alone is not a reliable predictor of bread-making performance. While the GI value may offer some insights, it should be used to assess wheat quality only in combination with other parameters, such as protein and gluten content.

Image analysis revealed significant differences among the genotypes for all three parameters considered: void fraction, cell density, and mean cell area (Table 4). The UDic genotype exhibited the highest void fraction (40.4 %), while spelt Giuseppe had the lowest (27.9 %). Among species, void fraction ranged from 33.1 % in spelt to 36.5 % in emmer. Overall, this trait was negatively correlated with both gluten content and GI ($r = -0.455$ and -0.449, respectively; Fig. S6). Significant differences in cell density were also observed among genotypes. Monlis and UMon showed the highest alveoli counts per cm^2 (117.2 and 108.4, respectively), while Norberto had the lowest (33.3 per cm^2). Among species, einkorn showed the highest average cell density (86.3 per cm^2) and emmer the lowest (53.1 per cm^2), although considerable variability was noted within each species. The mean cell area ranged from 0.266 mm^2 in Monlis to 1.222 mm^2 in Norberto, with significant differences among genotypes but not among species. A strong inverse correlation ($r = -0.885$; Fig. S6) was found between the number of alveoli per unit area and their average size. It is worth noting that bread height and volume were negatively correlated with cell density ($r = -0.614$ and -0.638, respectively; Fig. S6) and positively correlated with mean cell area ($r = 0.636$ and 0.668, respectively; Fig. S6). This indicates that breads with greater rise had fewer but larger alveoli per unit area, whereas breads with lower height and volume had a denser crumb structure, characterized by a higher number of smaller alveoli.

Significant differences were observed among genotypes for all three colorimetric parameters (L*, a*, and b*) in both the crust and crumb (Table S4). The yellow index (b*) was particularly high in all einkorn genotypes, averaging 37.87 in the crust and 27.80 in the crumb. In contrast, spelt breads showed particularly low b* values, averaging 26.61 in the crust and 17.51 in the crumb, with modest differences among genotypes. High b* values were also observed in both the crust and crumb of bread made from the wholemeal flour of the modern durum wheat variety Aureo. This was somewhat expected, as increasing yellowness in grain and flour, and consequently in processed products, has been one of the main goals in durum wheat breeding programs (Clarke et al., 1998), as this trait is highly appreciated by consumers (Boukid et al., 2020). Therefore, the high b* values recorded in breads made from einkorn genotypes and the modern durum wheat variety Aureo can be attributed to the natural color of the wholemeal flours. Within emmer, unlike einkorn and spelt, there was considerable variability in b* values among genotypes, both in the crust and crumb. Some genotypes (e.g., Giovanni Paolo, Padre Pio, UDic) had b* values only slightly lower than those recorded in einkorn, while others (e.g., Rosso Rubino, Yakub, Zefiro) had values more similar to those observed in spelt.

3.8. Sensory analysis

The sensory analysis revealed that the emmer genotypes Rosso Rubino and Zefiro obtained the highest scores for crust color (6.52 and 6.12, respectively) and crumb color (6.90 and 6.50, respectively), indicating a more golden crust and an appealing crumb (Fig. 7). In contrast, breads made from spelt, particularly the Maddalena and Giuseppe genotypes, exhibited paler colors and received lower scores (>3.00). Emmer genotypes Davide and Yakub were characterized by thicker crusts, with scores of 5.31 and 5.26, respectively, while breads from spelt, especially Giuseppe, had thinner crusts (2.69), which negatively affected their crunchiness.

The highest porosity was observed in breads made from the durum wheat variety Aureo and the emmer genotype Giovanni Paolo (7.18 and 6.91, respectively), indicating a lighter, more aerated structure. Spelt Rossella also showed good porosity (6.11), whereas emmer Padre Pio and einkorn Monlis had more compact crumb structures (2.11 and 2.61, respectively). A wide variability in alveolation was observed among the genotypes. Emmer Giovanni Paolo and einkorn Norberto exhibited good alveoli formation (5.76 and 6.25, respectively), while emmer Padre Pio and UDic had lower scores (2.36 and 2.70, respectively). The most uniform alveoli were recorded in emmer Zefiro and Giovanni Paolo (5.90 and 5.42, respectively), while einkorn Monlis displayed the least uniformity (3.32).

Significant differences were also observed in the intensity and type of odors and aromas perceived. Breads made from Aureo (durum wheat) and Norberto (einkorn) were particularly appreciated for their intense odor and typical "bread-like" aroma, which are key attractive sensory traits. Conversely, breads made from Giuseppe (spelt) and Padre Pio (emmer) received lower scores for odor and aroma intensity. The bread from Aureo had a strong yeast odor (6.16), although this was not fully matched by a corresponding yeast aroma. Einkorn breads achieved high aroma intensity scores (average 5.82), while spelt breads, particularly from the Benedetto genotype, received lower scores (3.56). The intensity of the "bread aroma" varied considerably among genotypes, with Giovanni Paolo and Giuseppe at opposite extremes (3.62 and 5.25, respectively). Off-flavors were detected in einkorn UMon and emmer Davide breads. Breads from einkorn genotypes Monlis and UMon were perceived as slightly sweeter, whereas those made from Giuseppe and Aureo were less sweet. These differences may reflect variations in the sugar composition of the raw materials, which influence fermentation dynamics during dough leavening and consequently affect the chemical characteristics of the final product (Struyf et al., 2017; Dong and

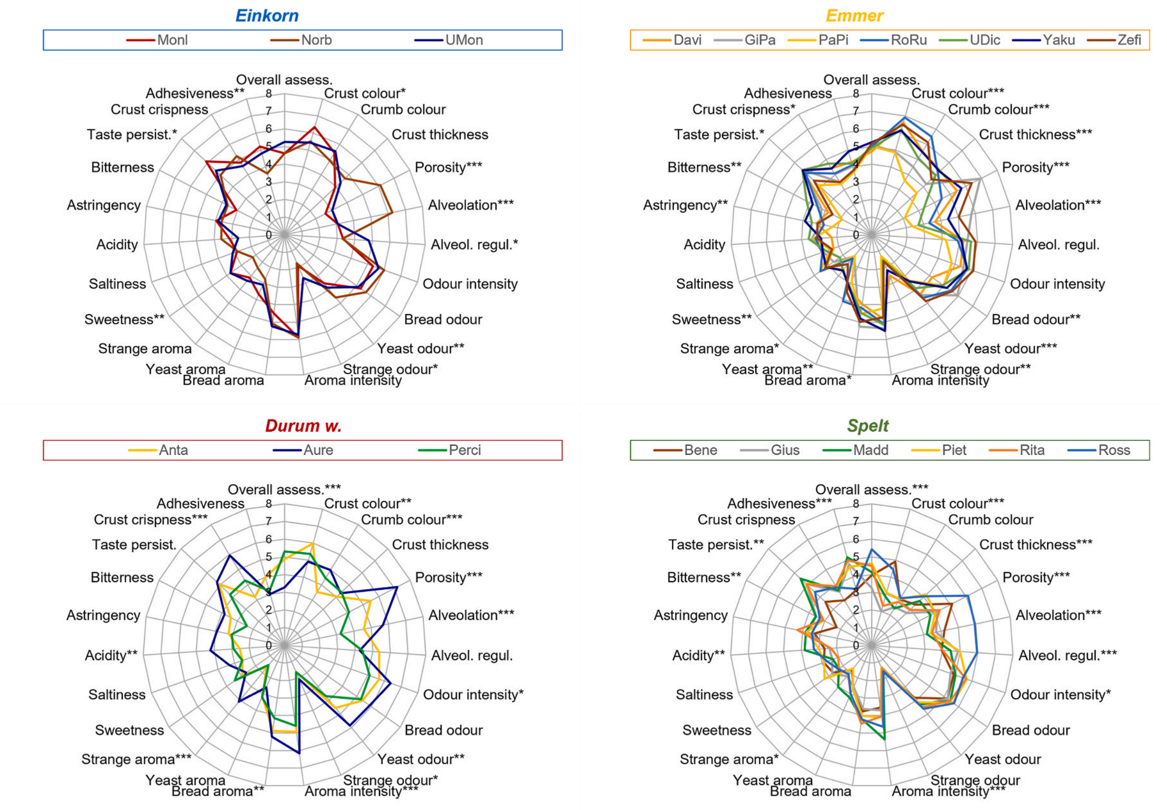

Fig. 7. Differences among genotypes, separately for each species, for the sensory traits measured on the breads made from the corresponding wholemeal flours (the analysis was conducted on material from evaluation at site B). Asterisks indicate the level of significance of the differences among genotypes within each species (*, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001).

Karboune, 2021).

The highest saltiness scores were recorded in breads made from durum wheat Aureo and einkorn UMon, while the lowest were observed in those from spelt Rita and emmer Davide. Einkorn genotypes also exhibited high scores for taste persistence. Overall, the panelists expressed a preference for breads made from the wholemeal flours of emmer and einkorn, which generally achieved higher average scores for aroma, texture, and flavor compared to those from other species (Fig. S7). Multivariate analysis (PCA) confirmed a significant diversity among genotypes (Fig. 8), with Aureo distinctly separated from the others and spelt genotypes forming a separate cluster from the remaining species.

4. Concluding remarks

This study, conducted across two distinct sites, comprehensively investigated the agronomic performance, grain quality, nutraceutical properties, and volatile organic compound (VOC) profiles of various genotypes from different *Triticum* species. Additionally, the technological and sensory characteristics of the derived bread products were thoroughly assessed. The analysis revealed that einkorn, without significant differences among genotypes, exhibited a low yield potential, producing grain quantities less than half those of the other species under optimal conditions. However, this yield gap was substantially reduced under less favorable growing conditions. Beyond its agronomic performance, einkorn displayed distinctive qualitative and technological attributes: it was characterized by high protein and gluten content, low TPC, and weak gluten strength. Breads produced from einkorn wholemeal flour showed more intense crust and crumb coloration (likely attributable to elevated carotenoid levels), reduced loaf height, and a denser crumb structure. Sensory evaluation indicated that einkorn

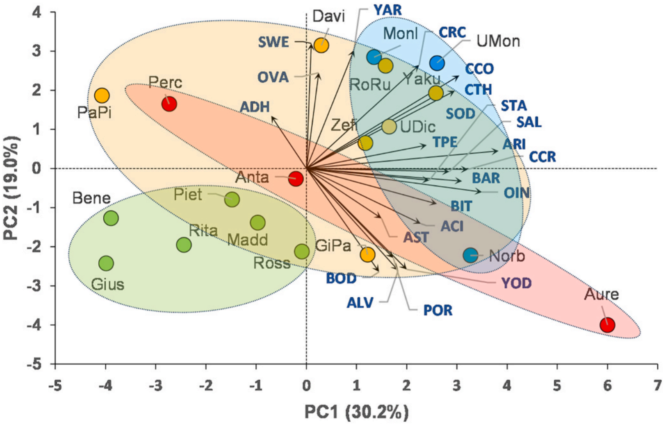

Fig. 8. Biplot of the principal component analysis (PCA) based on the sensory properties of breads made from the wholemeal flours of 19 genotypes evaluated at site B. The percentage of total variance explained by the first two principal components (PC1 and PC2) is shown in parentheses. The 23 arrows originating from the origin (coordinates 0,0) represent the original variables: acidity (ACI); adhesiveness (ADH); alveolation (ALV); alveolation regularity (ALR); aroma intensity (ARI); astringency (AST); bread aroma (BAR); bitterness (BIT); bread odor (BOD); crumb color (CCO); crust crispness (CCR); crust color (CRC); crust thickness (CTH); odor intensity (OIN); overall assessment (OVA); porosity (POR); saltiness (SAL); strange odor (SOD); strange aroma (STA); sweetness (SWE); taste persistency (TPE); yeast aroma (YAR); yeast odor (YOD). The length of each arrow is proportional to its contribution to the first two principal components. Each ellipse encompasses the evaluated genotypes for each species (blue: einkorn; yellow: emmer; green: spelt; red: durum wheat).

breads were particularly appreciated, presumably due to a favorable balance of aroma, texture, and flavor. Taken together, these findings suggest that einkorn could represent a valuable crop option for low-input and/or organic systems in marginal Mediterranean environments, where its lower yield could be compensated by other agronomic advantages and by the superior quality and potential market value of its derived products. Among the emmer genotypes, substantial intraspecific variability was observed across most of the parameters examined. Despite this variability, no single genotype demonstrated superior performance across agronomic, qualitative, technological, and sensory dimensions. Nevertheless, the wide variability within this species, combined with the generally positive sensory evaluation of emmer breads, suggests that targeted selection of genotypes suited to specific Mediterranean environments and cropping systems is a promising avenue. Spelt exhibited limited variability in agronomic performance and grain quality but showed considerable differences in VOC profiles, bread characteristics, and sensory attributes.

In this study, correlating VOC data with TPC revealed that the emission of certain volatile compounds (three alkanes and one terpene; r values > 0.44) significantly increased with higher TPC levels (Fig. S8). To date, these relationships have been scarcely investigated in cereals, despite their potential relevance considering both the health benefits generally associated with phenolic compounds and the impact of the aromatic profile of cereal raw materials on the sensory properties of processed products.Furthermore, a significant negative correlation ($r = -0.454$; Fig. S8) was found between the overall sensory score and the TPC in the wholemeal flours of the tested genotypes. Polyphenols are known to interfere with the Maillard reaction, potentially affecting the flavor and aroma of processed products. Previous studies have shown that phenolic compounds can impart undesirable flavors to plant-based foods (Drewnowski and Gomez-Carneros, 2000). Specifically, in cereal-based products, phenolic compounds have been associated with bitterness (Heiniö et al., 2008; Challacombe et al., 2012) and astringency (Kobue-Lekalake et al., 2007). In this study, TPC levels were positively correlated with the perception of strange aroma ($r = 0.488$) and salty taste ($r = 0.490$). These relationships underscores the complex trade-offs between nutraceutical content and sensory quality in wholemeal cereal products.

Overall, no strong relationships were identified between the VOCs present in the wholemeal flours and the sensory evaluations of the breads. This was expected, as many VOCs can be transformed or lost during fermentation and baking (Şerban et al., 2023; Păucean et al., 2024). Nevertheless, significant correlations were found between bread and yeast odors and certain alkanes present in the wholemeal flours (Fig. S8), particularly with tetradecane. Additionally, bread odor was positively correlated with the presence of esters and negatively correlated with aldehydes (Fig. S8). These results are partially consistent with previous studies, which suggest that cereal-based products with higher levels of aromatic compounds such as alcohols, ketones, and esters, and lower levels of acids and aldehydes, tend to be preferred by consumers (Plessas et al., 2005; Birch et al., 2013; Heitmann et al., 2017).

Taken together, these findings underscore the potential of hulled wheats as valuable resources for diversifying Mediterranean arable systems—particularly under low-input and organic conditions—enhancing their resilience and providing high-quality, nutritionally distinctive grain products. Finally, the comprehensive dataset acquired for the diverse genotypes evaluated can serve as a valuable resource for their use in future genetic improvement programs.

CRediT authorship contribution statement

Antonella Lo Porto: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Rosolino Ingraffia:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Gaetano Amato:** Writing – review & editing, Visualization, Funding acquisition, Data curation, Conceptualization. **Alfonso Salvatore Frenda:** Resources, Investigation. **Giacomo Gargano:** Writing – review & editing, Visualization, Investigation, Data curation. **Salvatore Guarino:** Writing – review & editing, Resources, Investigation, Data curation. **Paolo Ruisi:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Enrico Viola:** Writing – review & editing, Investigation, Data curation. **Luca Settanni:** Writing – review & editing, Resources, Investigation, Data curation. **Dario Giambalvo:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Conceptualization.

Ethical statement

This work did not involve the use of human and animal subjects.

Funding

This research was carried out within the framework of the projects: 1) "Costruzione di una filiera basata sui cereali minori per la valorizzazione di contesti marginali in ambiente mediterraneo" (Ministero dell'Università e della Ricerca, PON Ricerca e Innovazione, 2014–2020 – Azione IV.6 "Contratti di ricerca su tematiche Green"); 2) "Valorizzazione delle varietà antiche di frumento e sviluppo di un sistema di tracciabilità genetica – ANCIENT" (PSR Sicilia, 2014–2020, Misura 16 – Sottomisura 16.1).

Declaration of competing interest

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

Acknowledgements

The authors gratefully acknowledge Prometeo S.r.l. (Urbino, Italy), Agroservice S.p.a. (San Severino Marche, Italy), and Research Center for Cereal and Industrial Crops – CREA-CI (Foggia, Italy) for providing grains of the farro genotypes used in the present study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2025.104303.

Data availability

Data will be made available on request.

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