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Comparative analysis of nutritional content and antioxidant preservation in barley–wheat products made from various *Hordeum vulgare* genotypes from Sardinia

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ABSTRACT

Since ancient times, barley (*Hordeum vulgare* L.) has been a key cereal in Sardinia, a region recognized as a Longevity Blue Zone characterized by an unusually high prevalence of centenarians. Among the traditional foods historically consumed in this area, whole-grain cereals, including barley, represent a relevant dietary component. Aim: In the present study, the nutritional composition and antioxidant capacity of flour, "carasau" bread, and pasta derived from three Hordeum vulgare varieties and one *Triticum durum* variety were compared. The objective was to evaluate the impact of traditional processing (baking and pasta production) on macronutrients and antioxidant compounds, and to identify the barley genotype that best preserves its compositional characteristics during transformation. A comprehensive chemical analysis was conducted, including macronutrients, fiber, minerals (Fe, Zn, Mg), vitamin E, total polyphenols, and antioxidant capacity assessed by DPPH, ABTS, and ORAC assays. Results: All three *Hordeum vulgare* varieties showed higher dietary fiber, antioxidant levels, and macro- and micro-element concentrations in flour compared to *Triticum durum*. Specific genotypes exhibited superior retention of functional compounds: *H. vulgare* var. Nudum retained higher antioxidant potential in "carasau" bread (average loss 44.8%), while *H. vulgare* var. Nigrum showed less loss in pasta (33.9%). *H. vulgare* var. Nudum also displayed a notably high protein content (18.13 ± 0.92% in flour). Conclusion: The superior compositional profile and processing stability observed in selected barley genotypes highlight their technological and nutritional relevance within cereal-based products. These findings provide compositional evidence supporting the traditional use of barley in regional dietary patterns, including those historically associated with Sardinia. These results also support considering barley as a resilient complementary cereal within diversified production systems.

1. Introduction

Barley (*Hordeum vulgare* L.), an annual herbaceous plant, belongs to the family Poaceae (Gramineae), tribe Triticeae, subtribe Hordeinae, and genus *Hordeum* (Newman & Newman, 2008) and is one of the oldest cultivated crops, domesticated over 10,000 years ago, especially in the Italian region of Sardinia. During the Middle Neolithic period in Sardinia, barley was one of the primary cereals cultivated, alongside other crops such as *Triticum aestivum/durum*. The cultivation of barley played a significant role in the agricultural system of the time, contributing to the diet and subsistence of Neolithic communities (Ucchesu et al., 2017).

Known for thriving in diverse agricultural settings worldwide, even in arid regions with annual rainfall below 250 mm, barley is highly adaptable and resilient to extreme conditions, including heat and drought. (Bustos-Korts et al., 2019)

Barley has been successfully cultivated in Sardinia despite the

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challenging climate, characterized by hot, dry summers and mild, rainy winters, demonstrating high adaptability (Robinson et al., 2007). Over time, barley has progressively acquired traits that facilitate agricultural production, a process initially driven by natural selection in response to environmental factors and later by human artificial selection (von Bothmer & van Hintum, 2003). Sardinia is also recognized as one of the Longevity Blue Zones (LBZ), with one of the highest concentrations of centenarians globally. In addition to studying the potential hereditary traits of this population, researchers found whether modifiable factors such as diet and lifestyle have contributed to the region's longevity (Aliberti et al., 2024; Poulain et al., 2011). Among the foods consumed by this population was bread made from whole grains, such as wheat and barley, often prepared with homemade dough and microbial cultures containing *lactobacilli*. This traditional bread has chemical and physical characteristics distinct from commercially produced bread, which is typically leavened with baker's yeast (Lai et al., 2022). Today, 68% of barley is used as animal feed, 24% for brewing, 2% for biofuels, and 6% for direct human consumption (Tricase et al., 2018).

Barley has a well-balanced nutritional profile, with an average protein content of 10.3%, lipid content of 1.4%, 70% carbohydrates, and 9% fiber, including β-glucans. On average, barley provides 360 kilocalories per 100 grams of edible portion. Additionally, barley contains moderate amounts of vitamins, particularly vitamin E (tocopherols and tocotrienols) and B vitamins (B1, B2, B3). In terms of mineral content, 100 g of barley contains phosphorus (189 mg), potassium (120 mg), and magnesium (79 mg), followed by smaller quantities of iron, calcium, silicon, and zinc (Raj et al., 2023).

Barley has recently gained greater attention also as a food ingredient; for example, Nakov and collaborators explored using hulled barley flour to replace wheat flour in cookies to enhance their nutritional and functional qualities (Nakov et al., 2022). Results showed that adding more hulled barley flour increased dough viscosity, likely due to its high soluble fiber content, with β-glucan being the main factor (Moza & Gujral, 2017; Sapiga et al., 2021). Products containing barley had a lower energy value but were higher in soluble (especially β-glucans) and insoluble fibers.

Given this compositional profile, several studies have investigated the physiological relevance of barley-derived components. Soluble fibers reduce intestinal enzyme activity, lower postprandial glucose levels, and decrease glycemic response (Ahmed et al., 2025). Furthermore, barley-derived functional foods are believed to contribute to improved diabetes management via anti-inflammatory pathways (Ahmed et al., 2025). They also increase short-chain fatty acid production, which is linked to a lower risk of cardiovascular disease (Nirmala Prasadi & Joye, 2020). These effects are largely attributed to the presence of β-glucans, B-complex vitamins, vitamin E (tocopherols and tocotrienols), minerals, and phenolic compounds (Cian et al., 2023; Farag et al., 2022).

Barley is nowadays recognized as a fourth major cereal globally, with significant nutraceutical potential due to its high levels of *β*-glucans, GABA, and various phytochemicals, supporting approved health claims related to glycemic control and cholesterol reduction (Negeyie et al., 2024; Tian et al., 2025).

To date, studies consistently report that one of the main challenges lies in retaining or enhancing these compounds during food preparation: traditional processing, such as dehulling and porridge making, often leads to a significant loss (e.g., TPC losses up to 68.4%) of bioactive components and antioxidant activity compared to the whole grain (Cian et al., 2023; Tian et al., 2025).

Conversely, controlled processing methods, particularly malting (germination), are effectively used to increase functional components such as GABA, free amino acids, and free phenolic acids, thereby boosting the overall antioxidant capacity of derivatives (Cian et al., 2023; Negeyie et al., 2024).

Crucially, recent research highlights that the functional validation of barley remains limited by reliance on *in vitro* data, stressing the urgent need for clinical trials and bioavailability assessments to confirm its therapeutic efficacy in human populations and drive targeted functional food development (Noreen et al., 2025).

The U.S. Food and Drug Administration (FDA) has approved barley for protein enrichment, recognizing its nutritional benefits. The total essential amino acid content (TEEA) in rye and barley was reported to be close to the FAO-recommended dietary requirements for essential amino acids for children aged 6 months to 3 years. Both the FDA and the European Food Safety Authority (EFSA) have approved health claims for barley β-glucan, recognizing barley-based products as "functional foods" (EFSA Panel on Dietetic Products, Nutrition and Allergies NDA, 2011).

The overview presented herein highlights that barley and its derivatives can be a healthy alternative and a promising raw material for improving the nutritional and functional properties of cereal-based products.

To clarify the effects of barley and its derivatives in a health-related dietary context, this study aims to provide valuable insights into the cereal's antioxidant and nutritional properties.

Although Sardinia is recognized as a Longevity Blue Zone, longevity in this population is widely acknowledged to result from the interplay of multiple factors, including genetic background, lifestyle, social structure, and dietary patterns, rather than from the consumption of a single food item (Pes & Poulain, 2016; Poulain et al., 2011, 2014). Studies conducted in Sardinian long-lived populations consistently emphasize the multifactorial nature of exceptional longevity and caution against attributing causal roles to individual dietary components (Pes et al., 2022, 2021).

Within this framework, barley is a traditional and nutritionally relevant cereal in the Sardinian diet, particularly as a component of whole-grain and sourdough-leavened products characteristic of the region's dietary patterns (Lai et al., 2022; Pes et al., 2021). The nutritional interest in barley derives from its high content of β-glucans, phenolic compounds, and micronutrients, whose biological effects are consistent with mechanisms involved in glycemic regulation, lipid metabolism, and modulation of oxidative stress (Nirmala Prasadi & Joye, 2020).

Therefore, the rationale of the present study is to characterize the nutritional and antioxidant properties of barley and its traditionally processed products within a Longevity Blue Zone context, where diet is considered a modifiable contributor to healthy ageing.

Going in this direction, we compared the nutritional values and antioxidant capacity of flour, "carasau" bread (the traditional Sardinian bread), and pasta derived from three *Hordeum vulgare* varieties (*H. vulgare, H. vulgare* var. Nudum, and *H. vulgare* var. Nigrum) with *Triticum durum* var. Biancale, which represents the control sample.

Particular attention has been given to the analysis of nutritional changes during the sample's transformation, especially during cooking.

2. Materials and methods

2.1. Starting material

The samples analyzed in this study consisted of flour, raw pasta, and "carasau" bread produced from three barley varieties (*Hordeum vulgare, H. vulgare* var. Nudum, and *H. vulgare* var. Nigrum) and one durum wheat variety (*Triticum durum* var. Biancale), all cultivated in Sardinia.

The varieties were selected for their historical and geographical relevance to Sardinia and to represent cereal genotypes traditionally used for food and feed, including hulled, naked, and pigmented barley types, as well as a local durum wheat cultivar.

The *Hordeum vulgare* variety (hulled barley), locally known as "Ogliu Sardu," is still widely cultivated across Sardinia, primarily for animal feed. Until the 1960s, however, it was also used for human consumption. In the province of Nuoro, particularly in Oliena, Fonni, Orgosolo, and Nuoro town, this barley flour was used to make a traditional barley bread known as "Ogliathu." Ethnobotanical accounts also describe the use of this barley in herbal decoctions, prepared with wild mallow

(*Malva sylvestris* L.), to promote intestinal motility. This effect is likely attributable to the synergistic action of mallow mucilage and soluble fiber from the barley.

The variety *H. vulgare* var. Nudum (naked barley) is locally called "Tridihogliu, Pede Voe". The term "Tridihogliu" combines Triticum (wheat) and Hogliu (barley), referring to its morphological similarity to durum wheat, for the absence of hulls (glumes), which makes it appear "naked," like wheat. The epithet "Pede Voe" (ox's foot) refers to the shape of the caryopsis, which bears a central depression resembling the imprint of a bovine hoof. This variety was especially prized to produce high-quality "Ogliathu" barley bread, as it required minimal cleaning and milling, yielding refined, palatable flours. It was also used to make barley coffee, which is still consumed locally. Due to its low yield, the variety was gradually abandoned and retained only by landowners with access to larger cultivation areas.

H. vulgare var. Nigrum is a rare-hulled barley with black-colored grains (black barley). Its seeds are extremely difficult to obtain, and this variety is currently grown only in small plots in the Campidano region (southern Sardinia), exclusively for animal feed.

The durum-wheat variety used in this study was *Triticum durum* var. Biancale was formerly widespread across central and northern Sardinia until the 1980s. Today, it is cultivated by only a few traditional farmers, as it has been largely replaced by modern semi-dwarf varieties that are less susceptible to lodging and yield higher yields.

All *Hordeum* genotypes and *Triticum durum* var. Biancale is categorized as a spring or winter cycle, typical of Mediterranean germplasm adaptation. The *Hordeum vulgare* genotypes exhibit high morphological diversity, being found in both 2-row and 6-row spike architectures. The row number metric is not applicable to *Triticum durum*, which possesses a free-threshing spike morphology. Quantitative data for *H. vulgare* var. Nigrum landraces show a TGW mean range of 40.3 mg, while for *T. durum* var. Biancale, TGW data is not specified, but its classification is as high TGW potential.

All grains were milled by local producers using a stone mill to preserve their nutritional and organoleptic properties. While the general stone-grinding process was consistent, minor differences among mills (e.g., stone configuration and rotational speed) could not be fully standardized.

The resulting flours were used to prepare standardized pasta and "carasau" bread samples (recipes for preparation of "carasau" bread and pasta are described in Supplementary material, section "Preparation of "carasau" bread (*pane carasau*) and preparation of Pasta (*maccarones de Ordascia*).

2.2. Sample preparation

2.2.1. "Carasau" bread

The preparation of "carasau" bread was standardised to ensure reproducibility. For each genotype, three independent replicates were produced. In each replicate, 5 kg of flour was mixed with 2 L of water (40% hydration) and a standardised aliquot of traditional sourdough starter ("oghiathu"), previously refreshed under controlled conditions. The dough was kneaded using a semi-professional mixer (Grilletta IM 5S/230, Famag, Italy) for 13 min until homogeneous. It was then allowed to rest for 75 min, covered to prevent moisture loss, at controlled temperatures (40°C in spring and 55°C in winter) to reproduce typical artisanal processing conditions. After resting, the dough was divided into ~200 g portions and rolled into circular sheets (1–2 mm thick). Baking was carried out in a wood-fired oven equipped with a calibrated temperature probe, with chamber air temperature maintained at approximately 500–550°C. Each sheet was baked for ~15 s until inflation occurred. Reported temperatures refer to the oven chamber air temperature; the product's internal temperature is significantly lower due to a short residence time and evaporative cooling. After the first baking, the loaves were manually separated into two layers and subjected to a second toasting step (~500°C for 15 s) to complete dehydration. Samples were cooled at room temperature prior to analysis.

2.2.2. Pasta production

Pasta was prepared following a standardised artisanal protocol. For each genotype, three independent replicates were produced. In each batch, 1 kg of flour was mixed with 400–500 mL of water (40–50% hydration) and 0.1% (w/w) NaCl. The dough was kneaded manually for 10 min until a uniform consistency was achieved. It was then shaped into strips (~1 cm thickness), cut into 4 cm segments, and manually processed to obtain the typical "maccarones de ordascia" shape. Drying was carried out in a temperature-controlled ventilated dryer at 45°C for 8 h, or until constant weight was reached. Samples were stored in airtight containers at room temperature until analysis.

Flours were analysed as such. Pasta and "carasau" bread samples were homogenised using a mortar and subsequently ground with an electric grinder to obtain a uniform powder prior to analysis.

2.3. Physicochemical and macronutrient evaluations

For detailed analytical procedures and validation parameters, see Supplementary Material (Methods section)

2.3.1. Moisture

Moisture content was determined by gravimetric analysis by drying a 5 g sample in a pre-weighed crucible at 103–105°C for 2 h, according to Regulation (EC) No. 152/2009 (Annex III, Part A). Additional procedural details and calculation steps are provided in the Supplementary Material.

2.3.2. Lipid extraction

Lipid extraction was performed by Soxhlet extraction using petroleum ether as solvent, according to Regulation (EC) No. 152/2009 (Annex III, Method H). The extraction was carried out using a Soxhlet apparatus equipped with four independent heating units (B-811, Büchi Labortechnik AG, Flawil, Switzerland).

2.3.3. Protein determination by the Kjeldahl method

Protein content was determined according to ISO 1871:2009 using the Kjeldahl method, comprising digestion, distillation, and titration. Nitrogen content was converted to protein using a factor of 6.25. Analyses were performed using a Kjeldahl digestion and distillation system (K-439 Speed Digester and K-360 Kjelflex, Büchi Labortechnik AG, Flawil, Switzerland).

2.3.4. Carbohydrate analysis by ion chromatography with pulsed amperometric detection

Carbohydrate content was determined by ion chromatography coupled with pulsed amperometric detection (IC-PAD) according to method M.I. 1 Rev. 0. Chromatographic analyses were performed using an ion chromatography system equipped with an anion-exchange column and pulsed amperometric detector. Additional details on sample preparation, chromatographic conditions, and quantification are provided in the Supplementary Material.

2.4. Determination of total dietary fiber content

Total dietary fiber (TDF) content was determined by enzymatic–-gravimetric analysis according to method M.I. 1 Rev. 0, based on sequential enzymatic digestion, ethanol precipitation, and gravimetric quantification. Analyses were performed using standard laboratory equipment for enzymatic digestion, filtration, and gravimetric determination.

2.5. Determination of vitamin E by high-performance liquid chromatography

Vitamin E content was determined by reversed-phase high-performance liquid chromatography with photodiode array detection (HPLC-PDA) according to Rap. ISTISAN 1996/3. Samples were subjected to alkaline saponification followed by the extraction of the unsaponifiable fraction and chromatographic separation. Analyses were performed using an HPLC system equipped with a photodiode array detector. Quantification was carried out using an internal standard (vitamin E acetate). Additional details on sample preparation, chromatographic conditions, and validation parameters are provided in the Supplementary Material.

2.6. Determination of antioxidant capability

2.6.1. Sample extraction procedure

Antioxidant capacity was evaluated by determining total phenolic content (TPC) and radical scavenging activity (DPPH, ABTS), as well as oxygen radical absorbance capacity (ORAC). Antioxidant compounds were extracted using an aqueous ethanol solution (80:20, v/v) under standardized conditions across all matrices to ensure comparability among samples.

2.6.2. Determination of total polyphenols

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method as described by Singleton et al. (1999). Results were expressed as mg of gallic acid equivalents (GAE) per g of sample.

2.6.3. Evaluation of antioxidant activity using DPPH and ABTS

Antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays according to previously described methods (Loizzo et al., 2019; Saltarelli et al., 2019). Results were expressed as a percentage of radical scavenging activity and, where appropriate, as Trolox equivalents.

2.6.4. Oxygen radical absorbance capacity (ORAC) assay

Antioxidant capacity was further evaluated using the oxygen radical absorbance capacity (ORAC) assay, as described by De Bellis et al. (2019). Analyses were performed using a fluorescence microplate reader (Fluostar Optima, BMG Labtech, Offenburg, Germany). Results were expressed as Trolox equivalents per g of sample.

2.7. Elemental analysis

Sample dissolution was performed according to EPA Method 3052 (SW-846 Test Method 3052: Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices, Revision 0). Elemental analysis was performed using ICP-OES in accordance with EPA Method 6010D (Inductively Coupled Plasma–Optical Emission Spectrometry). Quality control included reagent blanks and laboratory control samples (LCS) spiked with known analyte concentrations to assess method accuracy. Precision was evaluated through replicate measurements.

2.8. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9 software. All measurements were carried out in triplicate ($n = 3$) and are reported as mean ± standard deviation (SD). For the nutritional composition of flours, a one-way ANOVA was applied with genotype as the fixed factor, followed by Tukey's HSD post-hoc test ($p < 0.05$). For the processed products (flour, "carasau" bread, and pasta), separate one-way ANOVAs were performed within each genotype, with product as the fixed factor, again followed by Tukey's HSD test ($p < 0.05$). Results of post hoc comparisons are reported using compact letter codes (a, b, c), where different letters indicate statistically significant differences. All statistical evaluations were performed at a significance level of $p < 0.05$.

3. Results and discussion

The comparative analysis of flours and their derivatives, obtained from four different cereal strains (*Hordeum vulgare, H. vulgare* var. Nudum, *H. vulgare* var. Nigrum, and *Triticum durum*), highlighted significant differences in nutritional and antioxidant profiles.

3.1. Moisture and dry matter content

The moisture and dry matter content of each sample was analyzed. Results showed consistent moisture percentages across the four cereal strains (Table 1). Moisture percentages were highly consistent among genotypes within each product category, indicating that varietal differences had a limited impact compared to processing conditions. Flour samples exhibited the highest average moisture content (14%), whereas "carasau" bread and pasta showed lower values, 8.5% and 6.7%, respectively. These differences reflect the processing methods: bread is baked, and pasta is dried, reducing moisture. Minor variations among strains, for example, *T. durum* pasta showing the lowest value at 5.63 ± 0.10%, may be associated with differences in grain composition, such as fiber and hull content influencing water-binding capacity, but remain secondary to the dominant effect of processing.

3.2. Macronutrients and fiber composition

The macronutrient and fiber composition of the four cereal strains analyzed in this study is consistent with values previously reported in scientific literature. According to Sullivan's review, typical wholegrain barley composition includes approximately 10-20% protein, 2-3% lipids, 70% carbohydrates, 11-34% fiber, and 2.5 % minerals, with the remainder attributed to water content (Sullivan et al., 2013). The macronutrients and fiber composition are reported in Table S1 (Supplementary material).

Lipid content was also measured; however, no statistically significant differences were observed among the samples analyzed.

Regarding protein content, the highest values were recorded for *H. vulgare* var. Nudum, with 18.13 ± 0.92% in flour, 18.43 ± 0.53% in bread, and 17.43 ± 0.68% in pasta. In contrast, *H. vulgare* exhibited the lowest protein levels: 10.53 ± 0.54%, 10.60 ± 0.43%, and 10.80 ± 0.24% in flour, bread, and pasta, respectively.

A notable observation emerged concerning *H. vulgare* var. Nudum, whose products consistently displayed lower carbohydrate content than the other strains. This inverse relationship reflected the high protein concentration in this genotype: protein content averaged approximately 18.0% across all products, while carbohydrate content was reduced to an average of about 63.9%.

Regarding sugars, *Triticum durum* flour and pasta display higher sugar levels than Hordeum samples. In some samples, free sugars were not detected, as their concentrations were below 0.1% (Table 2).

Dietary fiber is primarily derived from cereals, legumes, pseudocereals, and other seeds due to their widespread consumption in the human diet. In cereals, fiber is mainly concentrated in the grain's outer

Table 1
Moisture contents in barley and Triticum flour and their derivatives (%).

Product	*Hordeum vulgare*	*H. vulgare* var. Nudum	*H. vulgare* var. Nigrum	*Triticum durum* var. Biancale
Flour	14.27 ±0.70	14.03±0.60	13.74±0.61	14.01±1.10
"Carasau" bread	8.77±0.53	8.53±0.38	8.57±0.18	8.33±0.39
Pasta	6.53±0.27	6.70±0.51	6.16±0.10	5.63±0.10

Table 2
Sugar contents (%) of barley and wheat flours and their derivatives.

Product	*Hordeum vulgare*	*H. vulgare* var. Nudum	*H. vulgare* var. Nigrum	*Triticum durum* var. Biancale
Flour	0.5 ± 0.1	*n.d*	0.3 ± 0.1	1.1 ± 0.1
"Carasau" bread	*n.d*	*n.d*	0.3 ± 0.1	0.3 ± 0.1
Pasta	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	1.3 ± 0.1

n.d. = below 0.1%

layers, such as the pericarp (bran) and hull (seed coat). Several factors influence the total fiber content, including species, genotype, environmental conditions, grain morphology, and processing. In particular, milling processes significantly reduce fiber levels, as most polysaccharides associated with dietary fiber are concentrated in the bran fraction (Serna Saldívar & Hernández, 2020).

The fiber content observed in the barley-derived samples aligned with the literature. Across all product types, barley-derived products consistently showed higher dietary fiber contents than *Triticum durum* var. Biancale. Average fiber values were approximately 7–8% for the three *Hordeum* genotypes, whereas *Triticum durum* exhibited significantly lower levels, averaging about 5%.

In addition, flours generally displayed slightly higher fiber contents than their corresponding processed products, pasta and "carasau" bread. While the higher fiber content of barley-derived products supports their nutritional interest, no conclusions can be drawn from the present data regarding texture or sensory quality, as these aspects were not evaluated.

Evaluation of the main nutritional parameters showed that *Hordeum vulgare* contains 10.53 ± 0.54% protein and 9.40 ± 0.22% fiber. *H. vulgare var.* Nudum exhibited a significantly higher protein content (18.13 ± 0.93%), while *H. vulgare var.* Nigrum showed intermediate values. *Triticum durum* var. Biancale had a protein content of 13.80 ± 0.54% and lower fiber values. Statistical analysis (ANOVA) confirmed significant differences among strains ($p < 0.05$), with significance also validated by Tukey's HSD post-hoc test.

The corresponding compact letter display for flour macronutrients (proteins, fibers, and lipids) is reported in Supplementary Table S2. Fig. 1 provides a graphical representation of mean values (± SD) and highlights statistically significant differences among genotypes.

3.3. Antioxidant activity

Oxidative stress, resulting from an imbalance between antioxidant defenses and reactive oxygen species (ROS), can damage cellular components like lipids, proteins, DNA, and carbohydrates (Baublis et al., 2000; Zhou et al., 2004). This damage contributes to ageing-related diseases such as cancer and cardiovascular disorders (Baublis et al., 2000). Dietary antioxidants help counteract this process, promoting health and reducing disease risk (Baublis et al., 2000).

Phenolic compounds, mainly in the bran and aleurone layers, are key contributors to cereal antioxidant activity (Zieliński & Kozłowska, 2000). Their levels vary across cereal species and are influenced by cultivation and processing (Yu et al., 2002; Yu & Zhou, 2005). Lipophilic antioxidants in cereals include tocopherols and carotenoids (Atanasova-Penichon et al., 2016). Tocopherols (α, β, γ, δ) and tocotrienols are unevenly distributed: tocopherols concentrate in the germ, tocotrienols in the pericarp and endosperm (Falk et al., 2004; Panfili et al., 2003). Barley and wheat are rich in these compounds, which lower LDL cholesterol via antioxidant mechanisms (Baik & Ullrich, 2008; Gutierrez-Gonzalez et al., 2013).

Among the analyzed products, flour and pasta generally showed higher vitamin E content (mg/100 g dry weight) than "carasau" bread, likely due to thermal degradation during baking. Barley-derived bread exhibited higher vitamin E levels than that from *Triticum durum*. Across all products, the three barley strains consistently displayed greater vitamin E content than durum wheat.

Antioxidant activity was evaluated using DPPH, ABTS, and ORAC

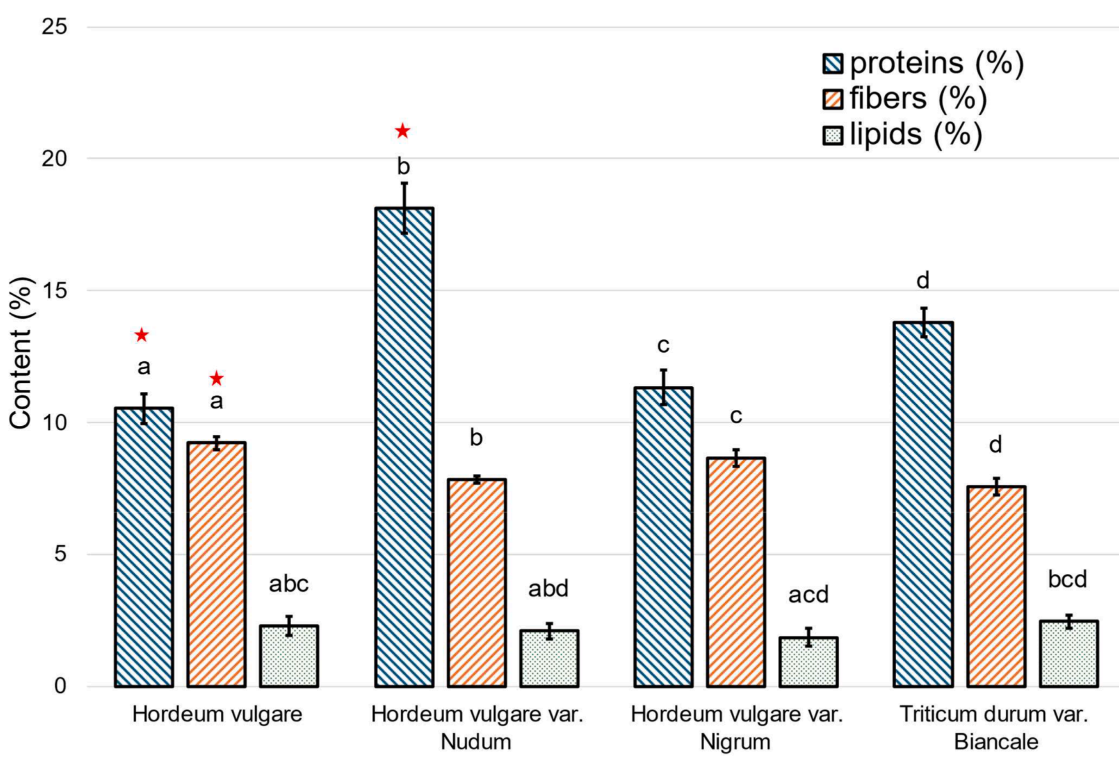

Fig. 1. Nutritional composition of flours from the four strains. The bar chart shows average protein, fiber, and lipid content percentages (% on dry weight basis). Error bars represent standard deviation across three replicates (n=3). Different letters denote significant differences among strains, as determined by the HSD Tukey test ($p < 0.05$). Asterisks indicate significant differences compared with *Triticum durum* var. Biancale ($p < 0.05$).

assays, along with the quantification of total polyphenols and vitamin E. Table S1 (supplementary material) reports the values of these analyses.

Post-hoc comparisons among flour, "carasau" bread, and pasta for all antioxidant parameters are provided in Supplementary Table S3, which reports the statistically significant differences identified by Tukey's HSD within each genotype.

Regarding ABTS radical scavenging activity, flour and pasta samples outperformed "carasau" bread across all cereal types. Moreover, barley-derived samples exhibited slightly higher ABTS activity than those from *T. durum*. Total polyphenol content was significantly higher in flour samples obtained from the three barley strains compared to *T. durum*. This pattern was consistent across all replicates. About DPPH scavenging activity, all barley-derived products (flour, pasta, and "carasau" bread) showed greater radical neutralization capacity than the corresponding samples from durum wheat.

ORAC values, indicating oxygen radical absorbance capacity, were higher in flour and pasta from the barley strains than in *T. durum*. However, this difference was not evident in bread samples, where values were comparable across all cereals.

Hordeum vulgare showed the highest absolute antioxidant values in flour across all parameters, but experienced substantial losses during processing into pasta (–37.4%) and "carasau" bread (–52.4%). Conversely, *H. vulgare var.* Nigrum retained more antioxidant potential in pasta (–33.9%), and *H. vulgare var.* Nudum in "carasau" bread (–44.8%).

Although initially lower in antioxidant content, *Triticum durum* var. Biancale demonstrated comparatively better preservation. Antioxidant profiles of the flours, expressed as normalized relative values, are shown in Fig. 2. Processing-related losses are illustrated in Fig. 3.

Moisture correction was performed using the formula (Eq. 1):

$$\text{Dry basis content} = C_{as\ is} / (1 - U / 100) \quad (1)$$

where $C_{as\ is}$ is the content in the fresh sample, and U is the percentage of moisture.

The percentage loss relative to the flour was calculated using a multivariate approach, based on the mean relative loss of five antioxidant parameters (vitamin E, total polyphenols, DPPH, ABTS, and ORAC), each corrected for moisture. For each strain and processed product (pasta or "carasau" bread), the loss was computed as in formula (Eq. 2):

$$\text{Loss\%} = (1 - C_{product,dry} / C_{flour,dry}) \times 100 \quad (2)$$

where $C_{product,dry}$ and $C_{flour,dry}$ represent the moisture-corrected mean values obtained from three independent replicates. Processing-related losses are illustrated in Fig. 3. The reported value represents the arithmetic mean of the five individual percentage losses calculated for antioxidant-related parameters (vitamin E, total polyphenols, DPPH, ABTS, and ORAC), expressed relative to the corresponding flour on a dry-weight basis. Standard deviations were estimated by propagating the experimental variability of flour and processed products according to standard error-propagation rules applied to the ratio $C_{product,dry} / C_{flour,dry}$.

The reduction in antioxidant activity observed in "carasau" bread can be attributed to several physicochemical mechanisms. Given that the flour blends and their corresponding "carasau" bread samples were prepared under identical conditions, any further differences in antioxidant properties are most likely a consequence of the baking process.

As literature reports, antioxidant compounds naturally present in flours may undergo degradation or transformation during kneading and thermal treatment (Holtekjølen et al., 2008; Sharma et al., 2022). Elevated temperatures encountered during baking can induce degradation, oxidation, and structural alterations of heat-sensitive bioactive molecules such as polyphenols and vitamin E, resulting in diminished radical scavenging activity (Nicoli et al., 1999).

Additionally, polyphenols may interact with proteins or carbohydrates through Maillard-type reactions, forming insoluble or less bioavailable complexes that further reduce antioxidant effectiveness. Lipophilic antioxidants, including tocopherols, are particularly prone to oxidative degradation upon exposure to oxygen, heat, and light during food processing and storage (Holtekjølen & Knutsen, 2011). Compared to baking, pasta processing involves milder thermal conditions but longer exposure times, during which oxidative reactions, leaching phenomena, and interactions with the starch–protein matrix may progressively reduce antioxidant availability.

Collectively, these processes contribute to the significant decrease in antioxidant capacity observed during flour transformation into baked products, as reported in Fig. 3.

In this context, it is important to note that the number of independent genotypes examined ($n = 4$) did not allow for a robust statistical analysis of the correlation between nutritional composition and antioxidant activity. Nonetheless, some mechanistic trends can be identified. Fiber- and β-glucan-rich matrices, as found in barley genotypes, may provide greater protection for phenolic compounds and tocopherols during thermal processing, potentially reducing oxidative degradation.

Similarly, although no formal correlation could be computed, flours with higher vitamin E content generally showed higher ORAC and ABTS values. This tendency weakened in pasta and bread, where vitamin E, being more heat-sensitive than phenolics, experienced comparatively greater processing-related losses.

According to literature data, other traditional cereals such as spelt, emmer, rye, and oats generally show intermediate micronutrient and

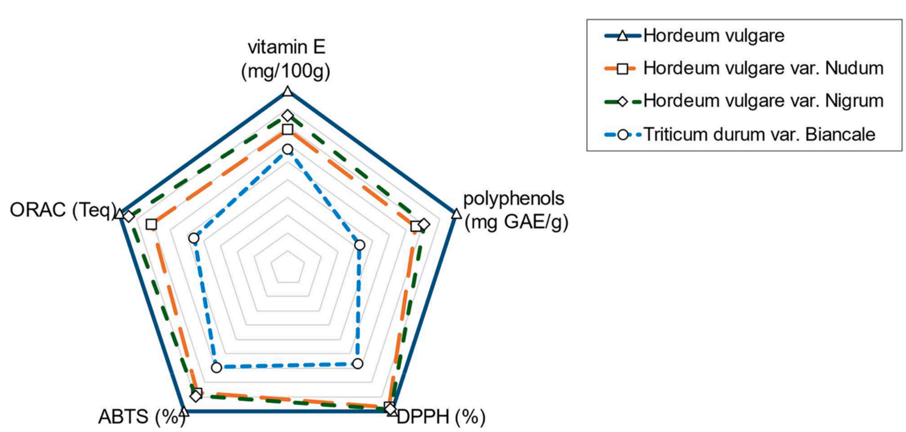

Fig. 2. Relative antioxidant functional profile of flours from four cereal strains. Radar plot based on Z-score standardization of each antioxidant parameter (vitamin E, total polyphenols, DPPH, ABTS, ORAC), followed by min–max rescaling to a 0–1 range. This representation highlights the relative functional positioning of each genotype across antioxidant variables.

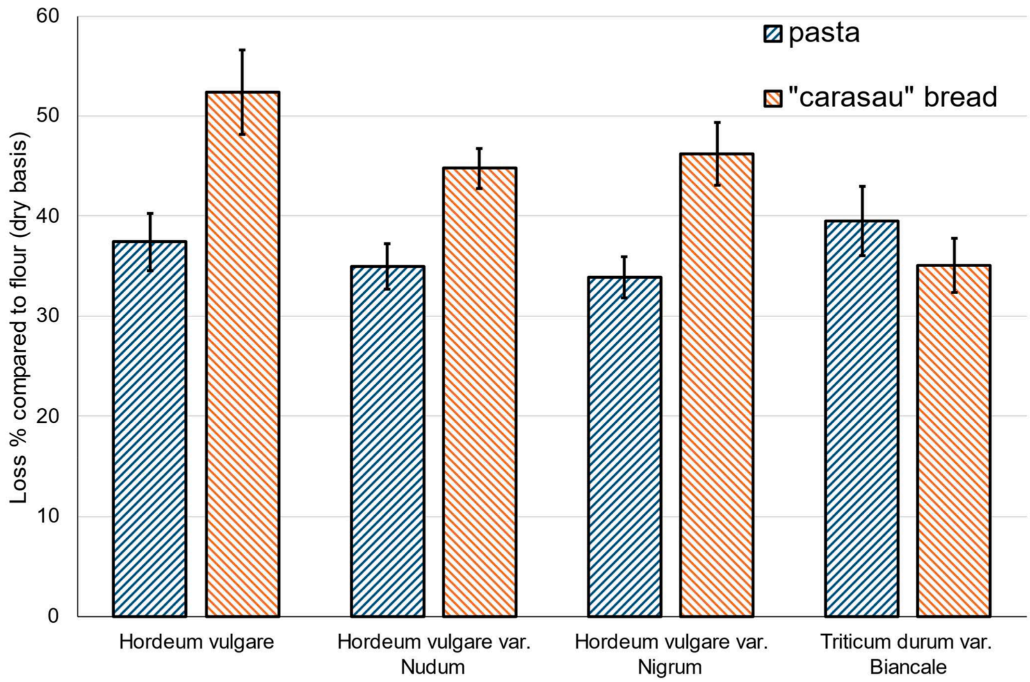

Fig. 3. Mean percentage loss of antioxidant-related parameters after processing into pasta and "carasau" bread, calculated on a dry weight basis. Losses were determined relative to the corresponding flour and averaged across five parameters (vitamin E, total polyphenols, DPPH, ABTS, and ORAC). Bars represent mean percentage loss values for each genotype and product, and error bars indicate propagated standard deviation.

antioxidant retention after processing, placing barley, particularly whole-grain and pigmented genotypes, among the most resilient traditional cereals (Rosa-Sibakov et al., 2015; Van Hung, 2016).

3.4. Integrated evaluation of nutritional and functional potential

A multi-criteria analysis was carried out to integrate the nutritional and functional properties of the different cereal varieties into a single composite index. A nutritional score was calculated for each flour by normalizing the mean values of five parameters on a 0–10 scale: protein, fiber, lipid, total polyphenol, and vitamin E content. Normalization was performed using the formula (Eq. 3):

$$P_i = (X_i - X_{min}) / (X_{max} - X_{min}) \times 10 \quad (3)$$

where X_i is the observed value for a given variety, and X_{min} and X_{max} are the minimum and maximum values recorded for each parameter across all varieties. The overall nutritional score (NS) was then obtained as the arithmetic mean of the five normalized values.

The retention of antioxidant properties during processing into bread and pasta was assessed as the average percentage retention of five antioxidant indicators (vitamin E, total polyphenols, DPPH, ABTS, and ORAC), corrected for moisture and expressed relative to the corresponding flour. For each variety and derivate type, the mean retention values were calculated and normalized on a 0–10 scale, assigning a value of 10 to the variety with the highest retention and 0 to that with the lowest. The antioxidant retention score (ARS) was defined as the average of the normalized values for bread and pasta.

Finally, the final score (FS) was calculated as the arithmetic mean of the nutritional score (NS) and the antioxidant retention score (ARS), weighted equally (50:50), providing a comprehensive index of each cereal variety's nutritional and functional quality.

This balanced weighting follows the standard logic of multi-criteria decision analysis (MCDA), commonly used in nutritional and food-quality assessments, assigning equal importance to parameters with similar physiological relevance when no prior hierarchy exists. The FS index, therefore, represents a novel integrative parameter, introduced in this study to summarize both compositional and functional performance into a single value and to enable direct comparison among varieties. This approach allowed us to compare the overall nutritional–functional performance of different cereal varieties in a single, reproducible metric.

Table 3 reports the nutritional score, ARS, and the final score

Although correlation tests were not statistically feasible with $n = 4$ genotypes, some qualitative associations emerged: varieties richer in vitamin E, fiber, and β-glucans tended to exhibit higher baseline antioxidant capacity, whereas processing altered these relationships due to differential thermal sensitivity of tocopherols and phenolic compounds. The integrated FS index was therefore introduced as a structured comparative tool to jointly consider intrinsic composition and processing stability within the present dataset. Given the limited number of genotypes examined ($n = 4$), the FS should be interpreted as a relative ranking framework rather than an absolute predictive metric. Alternative weighting schemes or normalization strategies could modify the quantitative scores; however, the index provides a transparent and reproducible approach for comparative evaluation.

The multicriteria approach adopted in this study enabled the integration of nutritional attributes and the retention of antioxidant activity in processed products derived from different barley and durum wheat varieties into a single composite index. The results revealed a clear advantage of *Hordeum vulgare* over *Triticum durum*, particularly in protein and fiber content and baseline antioxidant potential (Table S1, Supplementary material).

Among the genotypes evaluated, *H. vulgare* var. Nudum achieved the highest final score (FS: 5.59), reflecting a favorable balance between its nutritional composition (NS: 4.94) and the preservation of antioxidant

Table 3
Integration of nutritional and functional properties.

Variety	NS	ARS	FS
Hordeum vulgare	7.46	1.88	4.67
H. vulgare var. Nudum	4.94	6.23	5.59
H. vulgare var. Nigrum	4.05	6.78	5.42
Triticum durum var. Biancale	2.86	5.00	3.93

compounds during processing (ARS: 6.23). *H. vulgare* var. Nigrum followed with a similar antioxidant retention score (ARS: 6.78) but a slightly lower nutritional score (NS: 4.05), resulting in an FS of 5.42. In contrast, *T. durum* var. Biancale displayed the lowest integrated score (FS: 3.93), driven by a lower nutritional profile (NS: 2.86). More limited antioxidant retention (ARS:5.00). Notably, *H. vulgare* (hulled form) showed the highest nutritional score (NS: 7.46) but suffered the most significant loss of antioxidant activity (ARS: 1.88), which reduced its overall performance (FS: 4.67).

These findings highlight the importance of considering compositional and processing-related stability parameters when assessing the functional quality of cereal-based foods.

A graphical representation of the classification of barley and wheat strains evaluated in this paper is illustrated in Fig. S1 (Supplementary material).

3.5. Micro and macro-essential elements concentration

Comparative analysis of macro and micro-element concentrations across the three different qualities of barley reveals no significant differences among them. However, all considered elements exhibit concentrations significantly higher than those observed in the durum wheat. Consequently, the potential variation in mineral concentrations attributable to processing treatments for baking and pasta production was evaluated using the mean values derived from the three food matrices for comparison. For the mean calculation, the LOD was used when the element concentration fell below it.

The concentration (average of three replicates) of micro and macro-essential elements (Fe, Mn, Cu, Zn; Ca, K, Mg, P, S, Si) for each sample, along with the arithmetical mean and relative standard deviation (SD) for each group of tested samples are reported in Table S4 (Supplementary material).

As illustrated in Fig. 4, mineral concentrations do not change significantly following the processing treatments for baking and pasta manufacturing. Furthermore, the concentrations of all minerals are consistently higher in barley samples than in the wheat control, indicating that the inclusion of barley in a regular diet may contribute to adequate mineral intake, as supported by EFSA guidelines (European Food Safety Authority EFSA, 2017; Meli et al., 2024).

4. Conclusions

This comparative study demonstrated that barley (*Hordeum vulgare*), encompassing hulled, naked, and black-hulled varieties, possesses distinct compositional features, including higher levels of dietary fiber, vitamin E, total polyphenols, and selected essential elements compared to durum wheat (*Triticum durum*). All three *H. vulgare* genotypes exhibited significantly higher concentrations of dietary fiber, vitamin E, total polyphenols, and macro and micro-essential elements in the flour compared to *T. durum*.

A key outcome concerned the retention of functional compounds following traditional food processing. Despite inherent processing-related reductions in antioxidant activity, specific barley varieties showed superior stability: *Hordeum vulgare* var. Nudum retained more antioxidant potential in "carasau" bread, while *Hordeum vulgare* var. Nigrum was the most effective in preserving functional compounds in pasta.

The higher post-processing stability may be associated with the bound phenolic compounds, β-glucans, and thermally generated melanoidins, which can contribute to antioxidant protection during heating (Kaur et al., 2024; Sharma et al., 2021).

The integrated multi-criteria assessment, combining intrinsic nutritional score (NS) and antioxidant retention score (ARS) into a Final Score (FS), confirmed the overall superior performance of the *Hordeum vulgare* varieties compared to *Triticum durum. H. vulgare* var. Nudum achieved the highest overall FS, demonstrating a favorable balance between compositional quality and processing stability.

However, the limited number of independent genotypes examined (n=4) constrained the feasibility of robust statistical analysis of the correlation between compositional parameters and antioxidant activity. The integrated FS index was therefore used to capture both intrinsic composition and processing stability, serving as a synthetic framework supporting the comparative interpretation of barley genotypes within this experimental context.

The integrated NS–ARS framework was developed as a transparent multi-criteria comparative approach to synthesize intrinsic nutritional quality and antioxidant retention after processing. Within the present dataset (n = 4), the FS should be interpreted as a relative ranking tool rather than an absolute predictive model. While alternative weighting schemes or normalization methods may affect quantitative values, the approach provides a reproducible, structured basis for genotype comparison.

This study has some limitations that should be acknowledged. The experimental design focused on a limited number of cereal genotypes grown in a specific geographical area, potentially limiting the generalizability of the findings to other cultivars or production systems. Moreover, the analyses were limited to compositional parameters and antioxidant retention after processing, without directly assessing bioavailability, digestive behavior, or physiological effects. Sensory attributes, consumer acceptability, and technological properties relevant to industrial-scale processing were not evaluated. In addition, the lack of standardization in stone milling may have introduced variability in particle size distribution and in the proportion of outer grain layers in the flour, potentially influencing measured fiber and mineral levels, as well as the extractability of phenolic compounds.

Within these boundaries, the study provides comparative evidence that selected barley genotypes exhibit higher compositional quality and greater antioxidant retention than durum wheat. The integrated NS–ARS approach offers a structured framework for evaluating both intrinsic nutritional characteristics and processing stability, supporting the identification of genotypes with favorable performance profiles.

These findings provide a comparative basis for considering barley as a complementary cereal within diversified cropping and dietary systems, particularly under climate change scenarios (Bustos-Krts et al., 2019). Future research should integrate sensory, digestive, technological, and clinical investigations to further validate and extend the present results.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly and ChaGPT in order to improve language and readability. After using this tool/service, the author reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethical statement

The authors declare that this work did not involve the use of human and animal subjects.

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CRediT authorship contribution statement

Roberta De Bellis: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Francesco Palma:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal

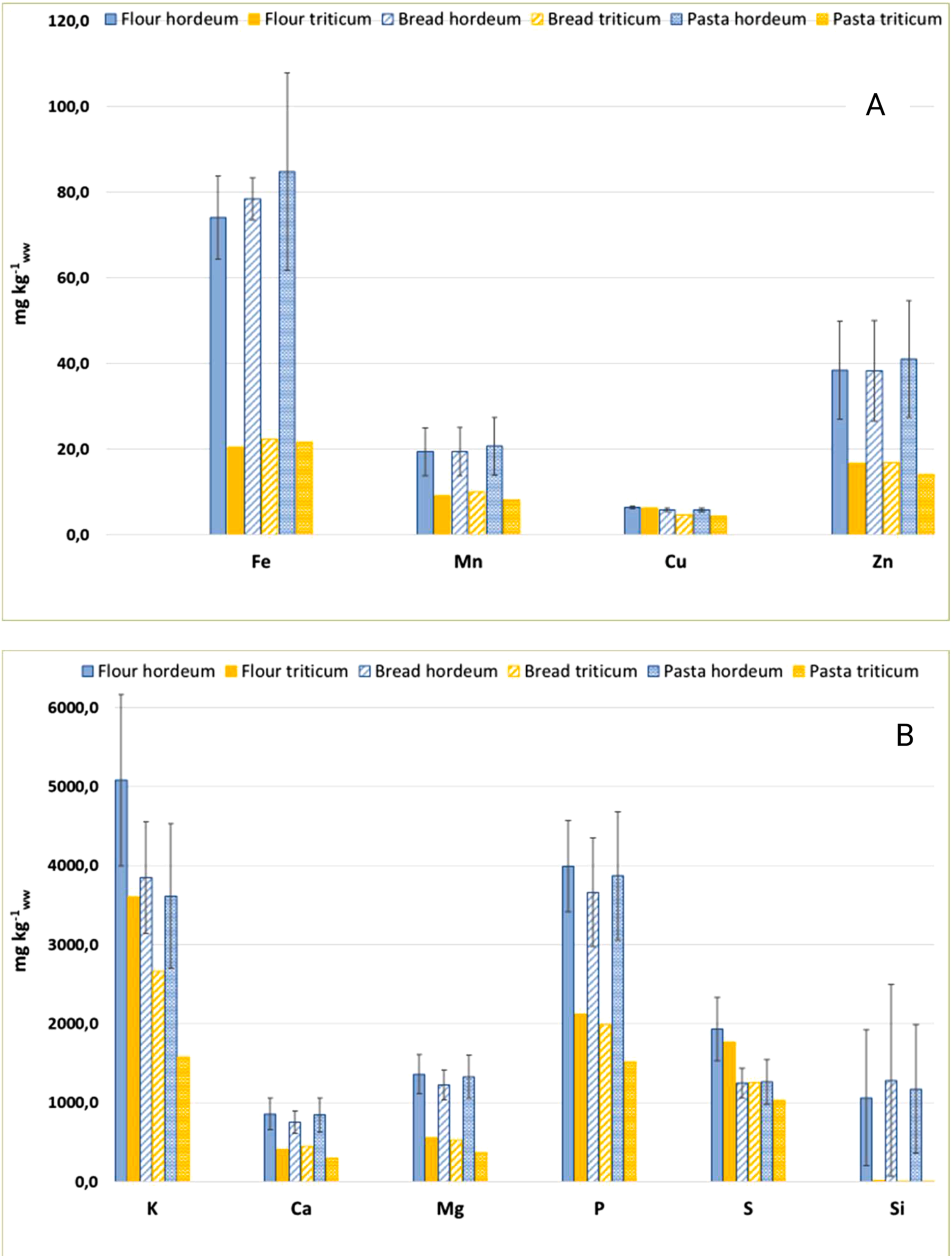

Fig. 4. Comparison between the concentration of micro (A) and macro-essential elements (B) in flour, "carasau" bread, and barley pasta (blue) and the respective durum wheat samples (yellow).

analysis, Data curation, Conceptualization. **Federico Lai:** Resources, Conceptualization. **Angelica Lai:** Resources, Data curation, Investigation. **Laura Brundu:** Methodology, Investigation. **Maria Assunta Meli:** Validation, Data curation. **Carla Roselli:** Formal analysis, Data curation. **Ivan Fagiolino:** Methodology, Investigation. **Antonella Amicucci:** Validation, Funding acquisition. **Laura Chiarantini:** Writing – review & editing, Validation. **Lucia Potenza:** Writing – review & editing, Validation, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper. Two authors, Laura Brundu (Laboratorio Chimico Nuorese, Nuoro, Italy) and Ivan Fagiolino (Gruppo C.S.A. Laboratorio Analisi Ambientali e di Ricerca, Rimini, Italy), are affiliated with private analytical laboratories. These institutions had no role in the design, data collection, analysis, interpretation, or publication decision of this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.102081.

Data availability

Data will be made available upon reasonable request.

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