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The effects of milling and processing on bioactive compounds in bread wheat and tritordeum

Claudia Sardella [a], Barbora Burešová [b], Zora Kotíková [b], Luboš Paznocht [b], Francesca Vanara [a], Matyáš Orsák [b], Massimo Blandino [a,*]

[a] Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, Turin, Grugliasco 10095, Italy
[b] Department of Chemistry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129 Suchdol, Prague 165 00, Czech Republic

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ABSTRACT

Tritordeum, a novel cereal species, is gaining attention for its health-promoting phytochemicals, yet the effects of milling and food processing on its bioactive compounds has not been fully explored. This study compared the contents of β-glucans, carotenoids, tocols, and phenolic acids in tritordeum and bread wheat, analyzing roller and stone milled flours and four products: fresh pasta, breadsticks, bread, and pizza. Stone milling preserved more β-glucans, tocols, and phenolic acids, whereas roller milling favored carotenoid retention, particularly in tritordeum flours. Although processing caused substantial losses, tritordeum products retained more carotenoids (with both milling methods) and greater amounts of tocols and soluble phenolic acids (with stone milling) than the wheat counterparts. These findings highlight how the combination of milling and processing conditions can optimize the health-related quality of cereal-based foods. This study provides the first integrated assessment of tritordeum phytochemicals across multiple processing steps, underscoring its potential as a functional ingredient in staple products. Future studies should investigate the phytochemical bioavailability and sensory properties of tritordeum-based products.

1. Introduction

Consumer demand for foods with added health value has promoted research on cereals enriched in bioactive compounds (Bangar and Kaushik, 2022). Beyond their role as staple sources of digestible starch and proteins, cereals, especially whole grain, are important providers of non-nutrient compounds with biological activity, such as indigestible carbohydrates and phytochemicals (Liu et al., 2007). The inclusion of whole grains in the human diet has been associated with a risk of lifestyle diseases, such as cardiovascular disorders, type 2 diabetes, obesity, and certain cancers (Capurso, 2021). These protective effects are attributed to the synergistic contribution of dietary fiber and a wide range of phytochemicals with antioxidant activity, including phenolic acids, carotenoids, and tocols (Zhu and Sang, 2017).

Recent breeding strategies have aimed to enhance the phytochemical composition of cereals through either the selection of improved varieties (Padhy et al., 2022) or the development of new species (Ávila et al., 2021), to promote their use in functional food production.

Breeding efforts in the 1970s led to the development of tritordeum (× *Tritordeum martinii* A. Pujadas, nothosp. nov.), an intergeneric amphiploid derived from *Triticum durum* Desf. and *Hordeum chilense* Roem. et Schultz (Papadopoulos et al., 2023). Currently, tritordeum is available on the market as a minor cereal and represents a promising alternative to bread wheat, as its rheological and technological properties are suitable for bread making (Martín et al., 1999; Vaquero et al., 2018). However, the intense yellow color of its grains and derived products confers a clear differentiation from standard bread wheat and high functional food potential. This trait results from an exceptionally high concentration of carotenoids, mainly lutein, in the endosperm, which can be up to five times higher in tritordeum than in durum wheat (Atienza et al., 2007).

Tritordeum is also rich in proteins, minerals, methyl donors (Phuong et al., 2017; Shewry et al., 2023), flavonoids (Suchowilska et al., 2021), phenolic acids (Giordano et al., 2019), and tocols (Lachman et al., 2018). However, most previous studies have focused on grain composition or flour properties (Wiwart et al., 2025), while little attention has

* Corresponding author.
E-mail addresses: claudia.sardella@unito.it (C. Sardella), buresovab@af.czu.cz (B. Burešová), kotikova@af.czu.cz (Z. Kotíková), paznocht@af.czu.cz (L. Paznocht), francesca.vanara@unito.it (F. Vanara), orsak@af.czu.cz (M. Orsák), massimo.blandino@unito.it (M. Blandino).

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been given to how milling and subsequent food processing influence its bioactive composition.

Milling is a critical step in cereal processing that determines the distribution of phytochemicals among flour fractions (Giordano et al., 2019). Stone milling preserves most of the bran and germ, thereby producing whole or semi-whole flours with enhanced fiber, tocol, and phenolic acid contents (Fărcaș et al., 2022). By contrast, roller milling produces refined flour and may result in lower concentrations of certain compounds while favoring the retention of others, such as carotenoids, which are mainly present in the endosperm (Giordano et al., 2019).

Subsequent mechanical and thermal processing steps, such as kneading, dough fermentation, baking, and boiling, strongly impact phytochemical content and composition, potentially influencing their bioavailability, bioactivity, and associated health benefits (Cilla et al., 2018). Carotenoids and tocols are highly sensitive to oxidation during mixing and heating (Paznocht et al., 2019), yet thermal treatments can also improve their extractability through matrix disruption (Elvira-Torales et al., 2019). Thermal processing may also decrease the levels of phenolic acids in the soluble form (Yu and Beta, 2015), but it may also release those bound to the grain cell wall structures (Tian et al., 2021). Since soluble and bound phenolic acids differ in absorption pathways and physiological effects (Roasa et al., 2021), and may also interact within the gastrointestinal tract (Çelik and Gökmen, 2022), it is crucial to evaluate how technological processes alter their relative profiles.

To date, the literature has primarily characterized the phytochemical profile of tritordeum at the grain or flour level (Suchowilska et al., 2021; Shewry et al., 2023), with limited information on the retention of these compounds in final food products. To the best of the authors' knowledge, no work has systematically examined the combined effects of different milling strategies (stone vs roller milling) and subsequent processing on the bioactive composition of tritordeum. Addressing this gap is essential to evaluate its actual nutritional potential in commonly consumed foods, and its role in the development of new products with enhanced phytochemical profiles.

Although previous studies have explored the effects of different processing steps on cereal phytochemicals (Abdel-Aal and Rabalski, 2013; Burešová et al., 2021; Paznocht et al., 2019; Yu and Beta, 2015), comparisons among final products remain limited due to differences in cereal type, genotype, processing protocols, and extraction methods. In this study, four food prototypes were selected: fresh pasta, breadsticks, bread, and pizza bases. These products represent typical items of the Mediterranean diet (Capurso, 2021), prepared with similar ingredients, but differing in processing conditions, such as boiling, baking times, and leavening length, which can differentially affect phytochemical stability.

Therefore, the objectives of this present study were (1) to investigate the effects of roller and stone milling on β-glucan, carotenoid, tocol, and phenolic acid contents in tritordeum compared with bread wheat, and (2) to determine the retention levels of these compounds in four standard food prototypes.

2. Materials and methods

2.1. Plant material and milling processes

One variety of tritordeum (Bulel) and one of bread wheat (Graindor), used as a control, were cultivated in the 2020–21 growing season in north-west Italy. An amount of 100 t of tritordeum and wheat grains were ground by either roller (GBS S.n.c., Leinì, Italy) or stone milling (Partisani S.r.l., Forlì, Italy), in an industrial plant (Molini Bongiovanni S.p.A., Cambiano, Italy) to obtain refined and semi-whole flours, respectively. The milling workflow is detailed in Figure S1. Samples were collected from the opening slits of the milling plant, according to the sampling method reported in European Commission Regulation (EC) No. 401/2006 (European Commission, 2006). An aggregate representative sample was obtained for each species and milling process. After stone milling, a further sifting process was performed using a single channel square purifier (GBS S.n.c., Leinì, Italy) to separate a 10 % of bran and brown flour. The milling yield was calculated and reported in Figure S1. The particle size of the refined and semi-whole flour was measured using a set of 6 sieves (Retsch, Haan, Germany) with progressively smaller mesh sizes (μm) and reported in Table S1.

2.2. Proximal composition analyses of the flour

Aggregate samples of the 4 flours used in this study were collected from the milling plant and stored at + 4 °C until being processed. The proximal composition is reported in Table S2. Data are reported as a single representative determination for each sample. The protein content was determined according to AACC method 39–10.01 (AACC International, 2008) by means of an NIR System Model 6500 (FOSS Italia S.r.l., Padua, Italy). The determination was based on a calibration of 900 samples that were analyzed using standard methods. The calibration range values were 7.3–18 %. The standard error of cross-validation (SEC) was 0.28 %, and the coefficient of determination (R^2) was 0.98 %. The starch content was measured according to Commission Regulation (EU) No 118/2010 of 9 February 2010, Annex I (European Commission, 2010). The fat, total soluble and insoluble dietary fiber, and ash contents were measured according to Soxhlet (AOAC 2003.05), enzymatic-gravimetric (AOAC 991.43), and muffle furnace (AOAC 923.03) methods, respectively (AOAC International, 2005).

2.3. Technological processes

The refined and semi-whole flours of both tritordeum and wheat were then used to make 4 different types of products (fresh pasta, breadsticks, bread, and pizza). Three trials were performed for each product formulation, and three representative samples were collected for each treatment, which was defined as a combination of species, milling degree, and processing type. The grains, the refined and semi-whole flours, and the developed products are presented in Figure S2. The experimental workflow is detailed in Figure S3. The products were selected because they have similar formulations but different processing procedures, i.e. specific technological parameters in the kneading, leavening, and cooking/baking stages, as detailed below and summarized in Figure S3.

2.3.1. Preparation of fresh pasta samples

For the preparation of fresh pasta, an aliquot of 2 kg of flour was mixed with water (40 °C) at different ratios, depending on the type of flour and on the basis of their farinograph water absorption (roller milled tritordeum 46.5 % and wheat 40.0 %, stone milled tritordeum 50.0 % and wheat 44.1 %), and the obtained dough was then processed in a planetary kneading machine (Kenwood, De' Longhi Appliances S.r.l., Treviso, Italy). The dough was left to rest under vacuum and then rolled into 1 mm thick pasta sheets using a continuous press (Imperia & Monferrina S.p.a., Moncalieri, Italy). The sheets were shaped into 10 × 10 cm squares and stored at 4 °C. The obtained pasta was cooked in boiling distilled water (pasta:water ratio = 1:10) for the optimum time, which had been determined by ten individuals through a sensory evaluation of the products, cooked for different time periods.

2.3.2. Preparation of breadstick samples

Breadsticks were produced in a local factory by means of an industrial process (Giovanni Cane S.r.l., Alessandria, Italy) in which 15 kg of flour was mixed with corn oil (4 %), fresh yeast (3 %), salt (1 %), and water in different ratios, depending on the type of flour (roller milled tritordeum 27.9 % and wheat 26.7 %, stone milled tritordeum 29.6 % and wheat 27.5 %). The dough was left to rest and then sheeted and folded for about 10 min. After leavening, the dough was shaped using stainless steel molds (Torneria Automatica Tealdi S.n.c., Clavesana,

Italy) at 27 °C and 45 % relative humidity. The breadsticks were baked in a continuous tunnel oven (Tibiletti Forni S.r.l., Novara, Italy).

2.3.3. Preparation of bread samples

For the preparation of bread, an aliquot of 3 kg of flour was mixed with fresh yeast (3 %), salt (2.5 %), and water in different ratios, depending on the type of flour (roller milled tritordeum 60.3 % and wheat 53.6 %, stone milled tritordeum 66.6 % and wheat 58.4 %) and processed in a spiral kneading machine (Esmach Ali Group S.r.l., Grisignano di Zocco, Italy). The dough was left to rest at 70 % relative humidity in a proofing chamber (Esmach Ali Group S.r.l) and then manually divided into 750 g loaves, which, after leavening, were baked in a bakery oven (Moretti Forni S.p.A., Mondolfo, Italy).

2.3.4. Preparation of pasta samples

For the preparation of pizza, an aliquot of 3 kg of flour was mixed with fresh yeast (1 %), salt (2.5 %), and water in different ratios, depending on the type of flour (roller milled tritordeum 60.3 % and wheat 53.6 %, stone milled tritordeum 66.6 % and wheat 58.4 %) and processed in a spiral kneading machine (Esmach Ali Group S.r.l.). The dough was left to rest and then manually divided into 300 g loaves. After leavening for 17 h, the loaves were kneaded by hand and left to rest for another 2 h. The loaves were then shaped and baked in a bakery oven (Moretti Forni S.p.A.) without the addition of any ingredients as toppings.

2.4. Analytical determinations

2.4.1. Sample preparation and moisture analysis

Three representative samples of flours and cooked/baked products for each treatment were separately collected and immediately stored at –25 °C. Each sample underwent separate analytical determinations. The products were freeze-dried using a Lyovapor L-200 Freeze Dryer (Buchi, Flawil, Switzerland), ground to a fine powder (particle size <500 μm) with a Cyclotec 1093 sample mill (Foss, Padua, Italy), and stored at –25 °C until being analyzed. The moisture content of the flours and lyophilized products was determined by oven-drying (Falc Instrument S.r.l., Treviglio, Italy) the samples at 105 °C to a constant weight, and the results of the chemical analyses were expressed on a dry weight (DW) basis.

2.4.2. Chemicals

Ethanol (CHROMASOLV®, 99.8 %), ethyl acetate (CHROMASOLV®, 99.8 %), hydrochloric acid (37 %), methanol (CHROMASOLV®, 99.9 %), sodium hydroxide (≥98 %), butylated hydroxytoluene (BHT, ≥99 %), *tert*-butyl methyl ether (HPLC grade), and phenolic acid standards (caffeic acid ≥98 %, *p*-coumaric acid ≥98 %, ferulic acid ≥99 %, gallic acid ≥99 %, protocatechuic acid ≥99 %, *p*-hydroxybenzoic acid ≥99 %, sinapic acid ≥98 %, syringic acid ≥95 %, and vanillic acid ≥97 %) were purchased from Merck KGaA (Darmstadt, Germany). Lutein (≥95 %) and zeaxanthin (≥98 %) standards were obtained from Extrasynthese (Genay, France). A tocotrienol and tocopherol mixed standard solution was obtained from ChromaDex (Irvine, CA, USA). Acetone, hexane, sodium chloride, potassium hydroxide, and pyrogallol (all GR grade) were obtained from Lachner (Neratovice, The Czech Republic). Water (HPLC grade) was prepared using an ELGA PURELAB Ultra system (M-medical, Cornaredo, Milan, Italy).

2.4.3. β-Glucan content

The β-glucan content (AACC International, 2008) was determined using a Megazyme mixed-linkage β-glucan assay kit, according to the producer's instructions (Megazyme International Ireland Ltd, Wicklow, Ireland). The quantification followed Megazyme protocol and total β-glucan was expressed in g 100 g^{-1} DW.

2.4.4. Sample extraction and chromatographic analysis of carotenoids

The carotenoids were extracted and analyzed by means of HPLC–DAD, according to the method described by Paznocht et al. (2019). Briefly, 0.5 g of homogenized sample were extracted with 4 mL ethanol/acetone/hexane mixture (1:1:2, *v/v/v*), vortexed and left to stand for 24 h in a refrigerator (4 °C). The sample was vortexed for 1 min (Basic 3, IKA Werke, Staufen, Germany), sonicated for 10 min in an ultrasonic bath (PS 04, Powersonic-Notus, Ltd., Vráble, Slovakia) and centrifuged at 8228 rcf for 5 min. Then, 3 mL of the supernatant were transferred into another glass tube and the sediment was re-extracted with 4 mL of the extraction mixture. Both supernatants were combined and evaporated under nitrogen steam (40 °C; Rotavapor R-200; Büchi Labortechnik, AG, Flawil, Switzerland). The dry residue was reconstituted with 0.2 mL ethanol/acetone (3:2, *v/v*) solution containing 0.2 % BHT and filtered through a syringe filter (PVDF, 0.45 μm) into an amber HPLC vial. Analyses were carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA) with a quaternary pump, autosampler, column heater, and diode array detector. The analytes were separated by gradient elution on an YMC C30 Carotenoid Column (150 mm × 3.0 mm, S-3 μm; YMC Co., Kyoto, Japan). The operating conditions are detailed in Paznocht et al. (2019). Carotenoids were identified by comparing retention times and absorption spectra with those of analytical standards. The total carotenoid content (TCC) was expressed as the sum of all the determined lutein isomers, zeaxanthin, and xanthophyll esters (mg kg^{-1} DW). Limits of detection (LOD) for lutein and zeaxanthin (0.006 and 0.012 mg kg^{-1} DW, respectively) were calculated using the formula 3.3 × (σ/S) (Q2B CH, 1996). The presence of xanthophyll esters was confirmed by alkaline hydrolysis of the sample extract. Since esterification with fatty acids does not affect chromophore properties and most of the esters in wheat are lutein esters with only a minor proportion of antheraxanthin and zeaxanthin esters (Paznocht et al., 2019), xanthophyll esters were quantified using the all-*E*-lutein calibration curve. The *Z*-isomers of lutein were confirmed by photoisomerization of lutein by iodine and their quantification was based on the calibration curve of all-*E*-lutein. Figure S4 shows detection wavelengths and illustrative chromatograms of the standard matrix (A) and of the semi-whole tritordeum samples (B–F), displaying the differences in processing from flour (B) to fresh pasta (C), breadsticks (D), bread (E), and pizza (F).

2.4.5. Sample extraction and chromatographic analysis of tocols

The extraction, chromatographic separation, identification, and quantification of the tocols, by means of HPLC–FLD, were described in detail in a previous study by Burešová et al. (2021). In brief, 0.5 g of homogenized sample was combined with 0.5 mL of 10 M KOH, 0.5 mL of 95 % ethanol, 0.5 mL of 0.15 M NaCl, and 1.25 mL of 0.48 mol/L ethanolic pyrogallol in a 50-mL plastic screw-capped Falcon tube and incubated at 80 °C for 30 min in a shaking water bath (VWR International, Leuven, Belgium), vortexing every 10 min (Basic 3, IKA Werke, Staufen, Germany). After cooling the sample to an ambient temperature in an ice bath, 0.75 mL of 0.15 M NaCl was added, and the resulting mixture was extracted twice with 6.5 mL of hexane/ethyl acetate (9:1, *v/v*), while supported by vortexing and shaking for 15 min (GFL 3006, Burgwedel, Germany). The separation of immiscible phases was facilitated by 5 min of centrifugation (3186 rcf, Eppendorf, Hamburg, Germany). Subsequently, the organic phase was transferred into a 20-mL glass test tube and evaporated to dryness under a nitrogen stream (40 °C). The dry residue was reconstituted with 0.5 mL of methanol and filtered through a syringe filter (PVDF, 0.45 μm) into a HPLC vial. The analyses were performed using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) with a quaternary pump, column heater, autosampler, and fluorescence detector. The analytes were separated by isocratic elution on an C30 analytical column Develosil 5 μm (250 × 4.6 mm; Phenomenex, Torrance, CA, USA) with a C18 (12.5 × 4.6 mm) guard column. The mobile phase used was H_2O/methanol (3:97, *v/v*). The operating conditions are detailed in Burešová et al. (2021). Tocols

were identified by comparing retention times with those of analytical standards. The total tocol content (TTC) was expressed as the sum of all the determined tocopherols (T) and tocotrienols (T3) (mg kg^{-1} DW). LODs (signal/noise = 3) expressed in mg kg^{-1} for individual tocols were as follows: δ-tocotrienol (δ-T3) 0.015; γ-tocotrienol (γ-T3) 0.020; β-tocotrienol (β-T3) 0.020; α-tocotrienol (α-T3) 0.050; δ-tocopherol (δ-T) 0.030; γ-tocopherol (γ-T) 0.040; β-tocopherol (β-T) 0.040; and α-tocopherol (α-T) 0.090. Figure S5 shows detection wavelengths and illustrative chromatograms of the standard matrix (A) and of the semi-whole tritordeum samples (B–F), displaying the differences in processing from flour (B) to fresh pasta (C), breadsticks (D), bread (E), and pizza (F).

2.4.6. Sample extraction and chromatographic analysis of phenolic acids

The soluble (SPAs) and cell wall-bound phenolic acids (CWBPAs) were individually extracted through a two-step method, analyzed by means of HPLC–DAD, and expressed in mg kg^{-1} DW as the sum of all the determined compounds. Details of the sample preparation procedure as well as of the chromatographic conditions were given in Giordano et al. (2019). Briefly, the SPAs were extracted from a 0.1250 g sample twice with 1 mL of an 80:20 (v/v) ethanol:water solution in an ultrasound bath (Bandelin Electronic, Berlin, Germany) at 4 °C for 10 min. The supernatants were collected and then evaporated to dryness under a nitrogen stream. The samples were hydrolyzed with 2 M NaOH (400 μL) under continuous stirring at 4 °C for 2 h. After acidification to pH 2 with HCl 6 N (160 μL), the SPAs were extracted three times with 500 μL of ethyl acetate. The combined supernatants were evaporated to dryness under a nitrogen stream and then reconstituted in 100 μL of a 80:20 (*v/v*) methanol:water solution. For the CWBPAs, the SPAs were first separated following the previously described extraction. Then, hydrolysis was performed by adding 400 μL of 2 M NaOH to the remaining pellet. The mixture was stirred continuously at 4 °C for 4 h. After acidification to pH 2 with HCl 12 N (120 μL), the CWBPAs were extracted with 800 μL of ethyl acetate and then centrifuged at 10,600 ×*g* for 2 min at 4 °C. The extraction was repeated once more. The combined supernatants were evaporated to dryness under a nitrogen stream, and then reconstituted in 200 μL of a 80:20 (*v/v*) methanol:water solution. Both the SPA and CWBPA extracts were filtered through a 0.2 μm filter and then analyzed using a high-performance liquid chromatography Agilent 1200 Series (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 1200 Series diode array detector. Separations were carried out using a 150 × 4.6 mm, 5 μm, Gemini RP18 column (Phenomenex, Torrance, CA, USA) protected by a guard column at 35 °C. The mobile phase consisted of 0.1 % acetic acid in water (solvent A) and 0.1 % acetic acid in methanol (solvent B), and the separation was performed using the gradient previously described in Giordano et al. (2019). Phenolic acids were identified using the retention times and the UV/Vis spectra of their respective standards. Quantifications of individual compounds were obtained through the corresponding calibration curves, as reported in Giordano et al. (2019). LODs, calculated using the formula 3.3 × (σ/S) (Q2B CH, 1996), in mg kg^{-1} for individual phenolic acids were as follows: gallic acid 0.100; protocatechuic acid 0.100; hydroxybenzoic acid 0.193; vanillic acid 0.150; caffeic acid 0.036; syringic acid 0.317; *p*-coumaric acids 0.150; ferulic acid 0.367; sinapic acid 0.575. Figure S6 shows detection wavelengths and illustrative chromatograms of the standard matrix (A) and the semi-whole tritordeum samples for SPAs and CWBPAs, displaying the differences in processing from flour (B–C) to fresh pasta (D–E), breadsticks (F–G), bread (H–I) and pizza (J–K).

2.5. Statistical analyses

The data are reported as the mean and standard deviation from three different analytical determinations on separate and representative samples for each treatment, which was defined as a combination of species, milling degree, and processing type. The data are expressed on a DW basis. The obtained data were compared by means of an analysis of variance (ANOVA) to evaluate the effect of the species (S; $n = 2$), the milling degree (M; $n = 2$), the type of processing (P; $n = 4$), and their interactions on the bioactive compound content of the flours ($n = 12$) and of the final products ($n = 48$). Means were identified as significantly different ($p < 0.05$) on the basis of the Ryan–Einot–Gabriel–Welsch F (REGW–F) statistical test. Prior to the analysis, a graphical method was used to verify the basic assumptions (Onofri et al., 2016). The analyses were carried out by means of the SPSS for Windows statistical package, version 28.0 (SPSS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Determination of β-glucans

Three-way ANOVA showed significant effects ($p < 0.001$) of all the three investigated factors (species, milling degree, and processing type) on the β-glucan contents of the analyzed samples (Table 1). The milling degree accounted for most of the observed variation (71.4 %), and this was followed by the species (14.7 %) and the processing type (10.6 %). The S × P and M × P interactions were significant ($p < 0.001$), although they only accounted for a small share of the total variation.

The wheat samples showed significantly higher β-glucan levels than the samples made of tritordeum (+18 %; average value of 0.20 vs 0.17 g 100 g^{-1}). The highest β-glucan value (Fig. 1A) was observed in stone milled wheat flour (0.31 g 100 g^{-1}), which was 41 % higher than its tritordeum counterpart (0.22 g 100 g^{-1}). Roller milling resulted in lower values for both species (0.24 and 0.15 g 100 g^{-1} for wheat and tritordeum, respectively). Consistently with other literature data comparing tritordeum with wheat, barley (Giordano et al., 2019) and triticale (Rakha et al., 2012), the β-glucan content of tritordeum was observed to be lower than that of bread wheat, closer to that of durum wheat, and not as high as that of barley varieties. However, the aforementioned studies focused solely on the grain content, and they reported a β-glucan concentration for tritordeum grains that was 3 times higher than that observed in the stone milled tritordeum flour analyzed in the present study. This difference likely reflects the sieving step applied after stone milling in the present work. This step removed some of the intermediate kernel layers that were previously identified as rich in β-glucans in tritordeum (Giordano et al., 2019). The stone milled samples were characterized by a higher average β-glucan content than roller milled samples (0.23 vs 0.14 g 100 g^{-1}; +64 %). The derived products also exhibited smaller percentage losses compared with roller-milled counterparts (Fig. 1A). Furthermore, the initial β-glucan contents observed in the flours decreased significantly, at different rates, according to the species and the type of process. The higher levels observed in wheat than in tritordeum flour were maintained throughout production, although wheat showed higher percentage losses than

Table 1
Level of significance of the three-way ANOVA analysis performed to evaluate the contribution of the species, degree of milling, type of processing, and their interaction to the biologically relevant compounds of the analyzed samples. The results are expressed as percentages of the total mean square.

Factors	β-glucans	TCC	TTC	SPAs	CWBPAs
Species (S)	14.7 ***	43.8 ***	0.3 ***	9.2 ***	0.1 *
Milling degree (M)	71.4 ***	0.4 ***	8.7 ***	72.7 ***	98.2 ***
Processing type (P)	10.6 ***	44.2 ***	85.2 ***	6.5 ***	1.2 ***
S × M	0.2	0.5 ***	0.4 ***	7.7 ***	0.2 *
S × P	2.3 ***	10.7 ***	0.01	1.5 ***	0.02
M × P	0.7 ***	0.2 ***	5.1 ***	1.8 ***	0.2 ***
S × M × P	< 0.01	0.2 ***	0.3 ***	0.5**	0.04
Error	0.1	0.01	0.01	0.1	0.02

TCC, total carotenoid content; TTC, total tocol content; SPAs, soluble phenolic acids; CWBPAs, cell wall-bound phenolic acids. The indicated factors are statistically significant, according to the REGW-F test [(*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$].

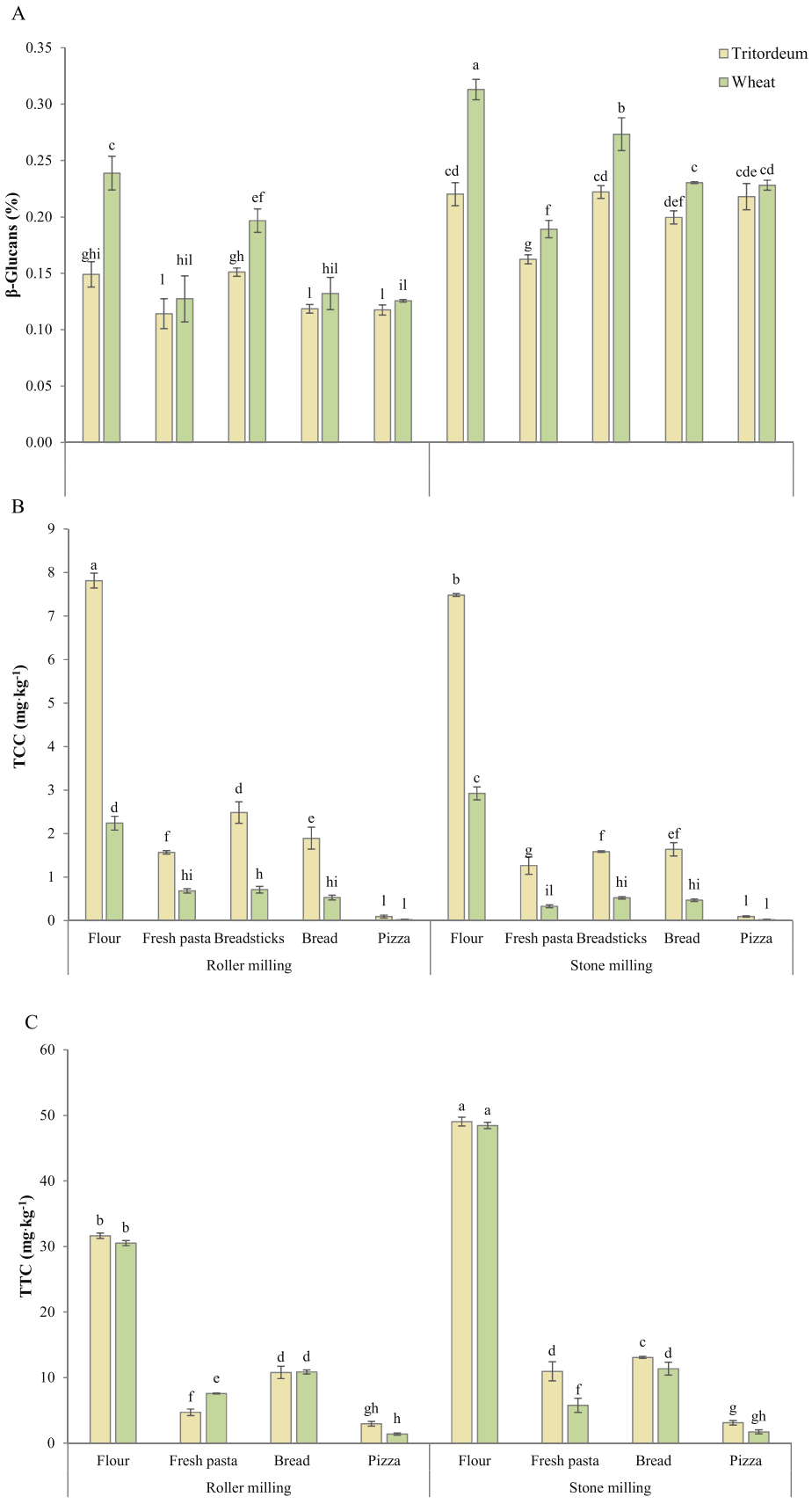

Fig. 1. The β-glucan (A), carotenoid (TCC; B), and tocol (TTC; C) contents of the tritordeum and wheat samples of flour, fresh pasta, breadsticks, bread, and pizza, obtained from roller and stone milling. Each column represents the mean from three different analytical determinations on separate and representative samples for each treatment. Error bars represent standard deviation. The results are expressed on a DW basis. Different letters above the columns indicate significant differences for $p < 0.05$, according to the REGW-F test.

tritordeum, especially when roller milled, and this led to less marked differences between the wheat and tritordeum derived products (Fig. 1A), with statistically similar values for all products except breadsticks. Breadstick production preserved β-glucans most effectively among all product types, retaining 83 % of the initial value (i.v.) in wheat flour and statistically similar values to the i.v. in tritordeum. Pasta showed the lowest retention (65 % i.v.), though significant differences among pasta, bread, and pizza were only observed for stone milled flours.

Cereal β-glucans are linear long-chain homopolymers of high molecular weight, formed by D-glucose residues linked by β-(1,4) bonds and interspersed with β-(1,3) linkages (Lante et al., 2023). Processing and cooking conditions can alter the polymer structure, that is, they change the molecular weight and extractability, and therefore viscosity, which is associated with the physiological functions of cereal β-glucans (Henrion et al., 2019). De Paula et al. (2017) found the highest viscosity in cooked pasta with the lowest β-glucan concentration among samples with different barley flour substitutions, highlighting that the solubility and molecular weight of β-glucans play a more significant role in determining viscosity than the total content (Regand et al., 2009). Future work should therefore evaluate tritordeum β-glucans in terms of solubility, molecular weight distribution, and viscosity (Ames et al., 2019) to better assess their nutritional potential and validate functional efficacy.

3.2. Determination of carotenoids

The three investigated factors all had significant effects on the TCC, as did their interactions ($p < 0.001$; Table 1). Most of the observed variation was explained by the species (43.8 %) and the processing type (44.2 %). As expected, tritordeum exhibited 2–4 times higher TCC than wheat (Fig. 1B). The TCC was significantly higher in roller milled samples (+10 %; 1.80 vs 1.63 mg kg^{-1}), and technological processing reduced carotenoid levels to varying extents depending on the process and cereal type (S × P = 10.7 %).

The high initial TCC of tritordeum flour (7.65 mg kg^{-1} as the average of roller and stone milling, Table 2) is in agreement with the observations of previous authors (Giordano et al., 2019; Rodríguez-Suárez et al., 2024) and is comparable to that of einkorn (*T. monococcum* L. subsp. *monococcum*), which has the highest carotenoid content of all cultivated wheats (Hidalgo et al., 2010). Significantly higher values of TCC were found in the whole-grain flour obtained from the tritordeum breeding line HT-439 by Paznocht et al. (2018) (12.16 mg kg^{-1} DW) or, and even more so, in that obtained by Suchowilska et al. (2021), who reported an average of 17.09 mg kg^{-1} DW for eleven spring tritordeum lines. The variability in TCC across studies likely reflects differences in genotype, environmental conditions, milling degree, and analytical methods (Lachman et al., 2017). Overall, the TCC of tritordeum flour was about 3 times higher than that of the control bread wheat variety, being in line with previous data (Giordano et al., 2019; Paznocht et al., 2018). This enrichment in carotenoids may be related to the *Hordeum chilense* genome contribution, which provides enhanced carotenoid biosynthetic activity compared with durum or bread wheat (Paznocht et al., 2018).

The carotenoid profiles revealed that all-*E*-lutein was the most abundant carotenoid in free form in both tritordeum and wheat flour, accounting for 68 and 61 % of the TCC, respectively (Figure S7), with average contents of 5.17 and 1.58 mg kg^{-1} (Table 2), accompanied by its *Z*-isomers (10 vs 7 %; 0.73 vs 0.18 mg kg^{-1}). The results of the present study confirm the relatively larger amounts of xanthophylls esterified with fatty acids in tritordeum than in wheat (1.50 vs 0.70 mg kg^{-1}), which were dominated by lutein monoesters, as reported in previous works (Atienza et al., 2007; Mellado-Ortega and Hornero-Méndez, 2018; Paznocht et al., 2018).

The significantly higher TCC found in refined tritordeum flour than in semi-whole flour (7.81 vs 7.48 mg kg^{-1}; Fig. 1B) is consistent with the known lutein gradient across kernel layers, increasing from outer to inner fractions (Giordano et al., 2019). This larger amount of TCC was preserved after the roller milling procedure applied in the present study. On the other hand, the lutein content of the wheat flour was very low (Table 2). In wheat, zeaxanthin is mainly concentrated in the intermediate layers and germ fractions (Mellado-Ortega and Hornero-Méndez, 2018), which are retained during stone milling but discarded during roller milling. As a result, stone milled wheat flour contained significantly higher amounts of carotenoids than its roller milled counterpart (Fig. 1B).

Overall, the TCC retention rates were higher for the roller milled products than for the stone milled ones (20 vs 14 % i.v.). Different shares of preserved carotenoids were found for the final products from the i.v. of the flour (Fig. 1B): breadsticks > bread > fresh pasta > pizza (26 > 22 > 19 > 1 %). The reduction percentage was similar for both tritordeum and wheat, but tritordeum supplied considerably more carotenoids than wheat to the end products, due to the significantly higher initial content. Notably, tritordeum breadsticks preserved the greatest TCC (2.48 mg kg^{-1}), with values statistically similar to the i.v. of the wheat flour (2.24 mg kg^{-1}). Significant carotenoid losses were reported during the manufacturing of different end products in previous publications. A lower TCC decrease was found by Hidalgo et al. (2010) in the breadcrumbs (up to 74 %) and in the bread crust (up to 45 %) of wheat flour, while a comparable retention was observed for raw dry pasta (up to 22 %). The different outcomes observed for products characterized by different time-temperature processing conditions (Table S3) confirm the important role of heat and of the cooking stage on the stability of these bioactives. The authors of two bread-making experiments observed a similar TCC retention for unleavened flatbreads (26 %; Burešová et al.,

Table 2
The carotenoids (an average ± standard deviation; mg kg^{-1}) detected in the tritordeum and wheat samples of flour, fresh pasta, breadsticks, bread and pizza, expressed as the average of roller and stone milling.

Species	Processing type	Lutein	Lutein *Z*-isomers	Xanthophyll esters	Zeaxanthin	TCC[a]
Tritordeum	Flour	5.17 ± 0.19 a	0.73 ± 0.03 a	1.50 ± 0.10 a	0.25 ± 0.05 a	7.65 ± 0.21 a
	Fresh pasta	0.86 ± 0.13 d	0.13 ± 0.01 d	0.39 ± 0.10 c	0.03 ± 0.01 cde	1.42 ± 0.21 c
	Breadsticks	1.35 ± 0.41 bc	0.30 ± 0.08 b	0.31 ± 0.05 cd	0.07 ± 0.01 c	2.03 ± 0.52 b
	Bread	1.10 ± 0.16 cd	0.22 ± 0.04 c	0.39 ± 0.05 c	0.05 ± 0.01 c	1.77 ± 0.23 bc
	Pizza	0.09 ± 0.02 f	< LOD	< LOD	< LOD	0.09 ± 0.02 e
Wheat	Flour	1.58 ± 0.21 b	0.18 ± 0.02 c	0.70 ± 0.12 b	0.12 ± 0.06 b	2.58 ± 0.40 b
	Fresh pasta	0.29 ± 0.13 ef	0.01 ± 0.01 e	0.21 ± 0.06 d	< LOD	0.51 ± 0.20 d
	Breadsticks	0.48 ± 0.10 e	0.09 ± 0.02 d	< LOD	0.04 ± 0.01 cd	0.62 ± 0.11 d
	Bread	0.25 ± 0.04 ef	0.03 ± 0.01 e	0.22 ± 0.02 d	< LOD	0.50 ± 0.05 d
	Pizza	0.02 ± 0.003 f	< LOD	< LOD	< LOD	0.02 ± 0.003 e
	p-value	***	***	***	***	***

LOD, limit of detection; TCC, total carotenoid content. The data are reported as the mean ± standard deviation from three different analytical determinations on separate and representative samples for each treatment. The results are expressed on a DW basis. Means followed by different letters are significantly different, according to the REGW-F test [(*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$, and ns, non-significant].

[a] The sum of all the determined carotenoids.

2021) and leavened buns (27 %; Paznocht et al., 2019) of colored-grain wheat. In the present study, the breadsticks benefited from milder baking conditions than the bread and pizza. The particularly low retention in pizza is likely due to the high baking temperatures, which the thin product reached much faster than the other products. Kneading also influenced carotenoid stability, as longer mixing promoted lipoxygenase activity, accelerating carotenoid oxidation (Burešová et al., 2021; Fratianni et al., 2012; Hidalgo et al., 2010). Consistently, products with shorter kneading and fermentation (breadsticks; Figure S3) retained more carotenoids than those requiring longer processing (bread and pizza), a result that is consistent with previous studies (Leenhardt et al., 2006; Paznocht et al., 2019). As far as pasta is concerned, previous publications reported a greater role of kneading than extrusion and drying, with the total carotenoids showing a significant loss, that is, from 22 % to 35 %, only during this step (Chiremba et al., 2015; Fratianni et al., 2012). In the present study, the pasta procedure was characterized by a higher carotenoid degradation than the breadsticks, and the losses should therefore mainly be ascribed to the cooking phase, thereby highlighting the importance of considering the impact of the cooking phase on the retention of phytochemicals at the moment of consumption.

Boiling may also induce carotenoid (*E*- to *Z*-) isomerization (Honda et al., 2018). The lutein *Z*-isomers have shown higher antioxidant activity and solubility, which in turn results in improved bioaccessibility, than all-*E* counterparts (Yang et al., 2020). Burešová et al. (2023) observed losses of 13-*Z*-lutein and 13'-*Z*-lutein after boiling colored-wheat grains, but increased contents of 9-*Z*-lutein and 9-*Z*'-lutein, thus indicating a pronounced stability and/or isomerization of the two *Z*-forms. However, Oduro-Obeng et al. (2021) observed a decrease in 9-*Z* and 13'-*Z*-lutein after boiling durum wheat pasta. They attributed this reduction to the degradation of carotenoids into apocarotenoids, and they only noted an increase in (*E*- to *Z*-) isomerization after prolonged boiling. No carotenoid isomerization was detected in any of the products in the current study, a result that is consistent with the findings of other studies (Kean et al., 2011; Kotíková et al., 2016) on sorghum porridge and cooked potatoes.

Xanthophyll esterification is a common mechanism in plant to increase liposolubility and sequester carotenoids, and it has been proposed as an effective strategy to enhance their stability and bioaccessibility in foods (Yang et al., 2020). Esterified xanthophylls degraded to a different extent depending on the species (from 21 % to 26 % i.v. for tritordeum products and from 0 % to 32 % for wheat products) and the process. However, the extent of degradation was similar to that of their free form and to what Paznocht et al. (2019) and Burešová et al. (2021) observed in other baking experiments (up to 21 and 28 % i.v., respectively). A higher retention was observed in the wheat grains after boiling (up to 58 % i.v.) by Burešová et al. (2023). In the present study, as in the cited works, xanthophyll esters were not found to be more resistant to technological processing, therefore the hypothesis of higher stability of xanthophyll esters presented by other authors (Mattera et al., 2017; Mellado-Ortega and Hornero-Méndez, 2012) was not confirmed.

3.3. Determination of tocols

The processing type significantly ($p < 0.001$) contributed to the variation in the levels of TTC, and this was followed by the milling degree (85.2 and 8.7 %; Table 1). The species and the S × M, M × P, and S × M × P interactions all showed significant effects ($p < 0.001$), but only explained a small share of the variation.

As tocols are more abundant in the germ and less in the endosperm, the TTC of the stone milled flour was on average higher than that of the roller milled flour (48.75 vs 31.07 mg kg^{-1}). A slightly higher, though not significant, TTC was found in tritordeum flours compared with wheat (Fig. 1C). To the best of the authors' knowledge, very little information is available on the concentration and composition of tocols in tritordeum. The concentration in the tritordeum stone milled flour of the present study (49.05 mg kg^{-1}) was higher than that observed by Lachman et al. (2018) in spring tritordeum (30.26 mg kg^{-1}). The variations in the TTC may have been caused by environmental conditions or genetic factors (Lachman et al., 2018; Lampi et al., 2010). Similarly to the work of Lachman et al. (2018), four of the eight forms of tocols were detected in tritordeum (Table 3 and Figure S7): β-T3 (25.35 mg kg^{-1}; 63 % of the TTC) > α-T (6.39 mg kg^{-1}; 16 %) > α-T3 (5.57 mg kg^{-1}; 14 %) > β-T (2.94 mg kg^{-1}; 7 %). δ-T3 was also identified, but at much lower levels (0.09 mg kg^{-1}). Greater differences between wheat and tritordeum flour were observed for the β-T and α-T3 proportions (12 vs 7 %, and 6 vs 14 %, respectively). This resulted in a higher overall proportion of tocotrienols (77 %) in the TTC of tritordeum flour than in wheat flour (71 %), both of which were dominated by β-T3.

Processing caused a significant degradation of TCC, with retention varying by product. Because of the specific formulation of breadsticks, which, in this case, involved the addition of 4 % corn oil to the dough, these products were not included in the statistical comparison, as the addition of oil led to the detection of some compounds (γ-T3, δ-T, and γ-T; data not shown) that are typically detected in corn oil (Moreau and Hicks, 2005; Wen et al., 2020), and which were not present in the initial flour (Figure S7). Bread retained the largest amount (11.52 mg kg^{-1}; Table 3), followed by pasta and pizza (7.25 and 2.29 mg kg^{-1}, respectively). The milling degree influenced the share of preserved TTC (Fig. 1C), with the largest proportion being retained in the products prepared with roller milled flour (21 % i.v.) and the smallest in the products prepared with stone milled flour (16 % i.v.). Within the stone milled products, tritordeum samples showed higher retention levels (18 % i.v.) than the wheat counterparts (13 % i.v.). The degradation of tocols and carotenoids seems to have been caused by the same factors, i.e. direct oxygenation and enzyme-controlled oxidation during kneading and leavening as well as heat destruction during baking/cooking (Burešová et al., 2021). Lower TTC losses in roller milled products could be ascribed to reduced lipoxygenase activity and lower polyunsaturated fatty acid content (Leenhardt et al., 2006) than the stone milled flour containing the germ, as well as to a higher concentration of other protective lipophilic antioxidants, such as carotenoids, as observed in tritordeum. These conditions may promote antioxidant regeneration phenomena during processing (Çelik and Gökmen, 2022). The limited retention in the pizza could be ascribed to the fermentation step, during which the oxidative enzymes may be active for longer, and by the more intensive heat treatment. The retention of tocols in cooked fresh pasta reached an intermediate level for bread and pizza. Cooking the pasta in large amount of water did not increase the tocol level, which can occur as a result of the leaching of soluble dry matter and the release of bound tocols, as has been reported after the conventional boiling of rice and vegetables (Finocchiaro et al., 2007; Fratianni et al., 2021). However, it did result in a loss of TTC, which could be ascribed to oxidative destruction because of heating, as reported in previous works that focused on the cooking of brown rice (Pascual et al., 2013). In all the processes, and especially after pizza baking, the tocotrienol-to-tocopherol ratio increased, indicating greater thermal stability of tocotrienols. Cereal grains are the second most important source of tocols in diets, after vegetable oils, and are considered a unique source due to the predominance of tocotrienols, which have a variety of beneficial functions, over tocopherols (Tiwari and Cummins, 2009). The degradation of tocols in all the considered processes was found to be lower than that of carotenoids, thereby supporting the higher thermostability suggested by some other authors (Burešová et al., 2021; Fratianni et al., 2012; Fratianni et al., 2021; Hidalgo and Brandolini, 2010; Leenhardt et al., 2006; Table S3).

3.4. Determination of phenolic acids

The three investigated factors and their interactions significantly affected the SPAs ($p < 0.01$; Table 1), which were extracted and analyzed as a combination of the soluble-free and soluble-conjugated

Table 3
The tocols (an average ± standard deviation; mg kg^{-1}) detected in the tritordeum and wheat samples of the flour, fresh pasta, bread, and pizza, expressed as the average of roller and stone milling.

Species	Processing type	Tocotrienols				Tocopherols				TTC[a]
		δ-T3	γ-T3	β-T3	α-T3	δ-T	γ-T	β-T	α-T	
Tritordeum	Flour	0.09 ± 0.03 a	< LOD	25.35 ± 3.98 a	5.57 ± 1.57 a	< LOD	< LOD	2.94 ± 1.15 b	6.39 ± 2.84 a	40.34 ± 9.55 a
	Fresh pasta	< LOD	< LOD	6.25 ± 2.23 bc	0.40 ± 0.44 c	< LOD	< LOD	0.72 ± 0.40 c	0.46 ± 0.50 b	7.83 ± 3.57 bcd
	Bread	0.02 ± 0.03 c	< LOD	9.16 ± 0.78 b	0.91 ± 0.12 c	< LOD	< LOD	0.98 ± 0.31 c	0.85 ± 0.28 b	11.93 ± 1.40 b
	Pizza	< LOD	< LOD	2.58 ± 0.27 d	0.26 ± 0.05 c	< LOD	< LOD	0.18 ± 0.05 c	< LOD	3.02 ± 0.33 cd
Wheat	Flour	0.06 ± 0.01 b	< LOD	25.65 ± 3.44 a	2.41 ± 0.80 b	< LOD	< LOD	4.59 ± 1.89 a	6.77 ± 3.71 a	39.48 ± 9.83 a
	Fresh pasta	< LOD	< LOD	5.74 ± 1.18 c	< LOD	< LOD	< LOD	0.93 ± 0.18 c	< LOD	6.67 ± 1.20 bcd
	Bread	< LOD	< LOD	8.89 ± 0.68 bc	0.17 ± 0.19 c	< LOD	< LOD	1.39 ± 0.32 c	0.66 ± 0.35 b	11.11 ± 0.71 bc
	Pizza	< LOD	< LOD	1.44 ± 0.20 d	< LOD	< LOD	< LOD	0.11 ± 0.13 c	< LOD	1.55 ± 0.30 d
	p-value	***	ns	***	***	ns	ns	***	***	***

δ-T3, δ-tocotrienol; γ-T3, γ-tocotrienol; β-T3, β-tocotrienol; α-T3, α-tocotrienol; δ-T, δ-tocopherol; γ-T, γ-tocopherol; β-T, β-tocopherol; α-T, α-tocopherol; LOD, limit of detection; TTC, total tocol content. The data are reported as the mean ± standard deviation from three different analytical determinations on separate and representative samples for each treatment. The results are expressed on a DW basis. Means followed by different letters are significantly different, according to the REGW-F test [(*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$, and ns, non-significant].

[a] The sum of all the determined tocols.

fractions. Regarding the bound fraction (CWBPA), the three factors and the S × M and M × P interactions showed significant effects ($p < 0.05$), although the milling degree explained most of the observed variation (98.2 %).

The highest concentration of SPAs was found in tritordeum samples (+33 % compared to the wheat samples, 41.16 vs 31.78 mg kg^{-1}), and in particular in stone milled flour (+85 %, 91.90 vs 49.79 mg kg^{-1} in wheat; Fig. 2A). Differences in the CWBPA content were significant, although far less pronounced (+3 % in the tritordeum samples, 151.49 vs 146.96 mg kg^{-1}; Fig. 2B), and only accounted for a small share of the variation (0.1 %; Table 1). The SPAs in tritordeum flour represented a higher proportion of the total phenolic acids than in wheat (29 vs 19 %). Sinapic acid was the major SPA in both species, accounting for 53 % and 39 % of total SPAs in tritordeum and wheat flours (Figure S7), respectively (23.99 and 18.30 mg kg^{-1}; Table 4). Ferulic acid dominated the bound fraction (127.03 and 119.00 mg kg^{-1}; 84 and 82 % of total CWBPAs). Interestingly, gallic acid was the second most abundant SPA in tritordeum flour (16.78 mg kg^{-1}; 28 % of the total SPAs), whereas it was negligible in wheat (0.52 mg kg^{-1}; 2 %). Vanillic acid represented 6 and 7 % of the total SPAs in tritordeum and wheat flours, with no significant differences between species (3.19 mg kg^{-1} on average). The soluble protocatechuic, hydroxybenzoic, caffeic, syringic, and *p*-coumaric acid contents were each < 3.00 mg kg^{-1}, and they accounted for less than 4 % in all the flours. The CWBPA composition was less heterogeneous (Figure S7). After ferulic acid, sinapic acid accounted for 10–12 % of the total CWBPA content, with no significant differences between tritordeum and wheat flour (15.90 mg kg^{-1} on average; Table 4), followed by *p*-coumaric acid (4.87 mg kg^{-1}; 3 %). Only a few works have investigated the phenolic acid profile of tritordeum whole grains and compared it with that of durum wheat (Giordano et al., 2019; Montesano et al., 2021; Suchowilska et al., 2021) or with that of bread wheat or barley (Giordano et al., 2019). Giordano et al. (2019) observed SPA and CWBPA concentrations in tritordeum of 58 and 872 mg kg^{-1}, which were 33 % higher and 12 % lower than in bread wheat. The phenolic acid concentration ratio in tritordeum determined by these authors was similar to that obtained in our study, although they did not identify gallic acid. In contrast to our findings, Montesano et al. (2021) reported a markedly different profile of bound phenolic acids, with cinnamic and gentisic acids being the most abundant in tritordeum lines. Suchowilska et al. (2021) also found an abundance of cinnamic acid, the second most abundant after ferulic acid. It is worth noting that the phenolic acid content observed in the present study (Table 4) was overall lower than in the cited experiments, in which whole grains were analyzed.

The milling degree was responsible for most of the observed variation (72.7 % for SPAs and 98.2 % for CWBPAs; Table 1) for both fractions. Giordano et al. (2019) reported a decreasing concentration of tritordeum SPAs and CWBPAs from the outermost to the innermost kernel layers, with a similar pattern to that of durum and bread wheat, thereby supporting the significantly higher content of both forms observed in the stone milled samples of the present study (+130 % compared to the refined samples for SPAs, 51.54 vs 22.41 mg kg^{-1}; +160 % for CWBPAs, 215.61 vs 82.84 mg kg^{-1}). The cited authors also observed a 36 % higher concentration of CWBPAs in the residual pearled grain of tritordeum than bread wheat, thus suggesting the use of both whole and refined tritordeum flour for enhanced health-promoting properties.

Furthermore, processing had significant effects ($p < 0.001$) on the total SPA and CWBPA contents, even though it only accounted for a small share of the variation (6.5 and 1.2 %; Table 1). To the best of the authors' knowledge, this is the first study to have investigated the fate of tritordeum phenolic acids during different types of processing. In roller milled samples, total SPA and CWBPA contents were largely unchanged, except for a significant SPA reduction during tritordeum pasta-making (to 35 % of i.v.). Only in the case of breadsticks did a marked increase in CWBPAs occur (up to 119 and 128 % for tritordeum and wheat, respectively) during production, although the level was only significantly higher than the i.v. of the flour for wheat. In stone milled samples, losses were greater because of the higher initial concentrations. In terms of SPA content, tritordeum retained the largest proportion in breadsticks and pizza (69 % i.v. on average) and the lowest in bread and pasta (49 % i.v. on average). In wheat, significant losses occurred only after pasta making (47 % i.v.). For CWBPAs, bread and pasta samples achieved similar retention rates, with a limited but significant drop to 89 % i.v., and statistically comparable final levels (194 mg kg^{-1} on average; Fig. 2B). No significant variation in the total content was detected in the pizza samples (224 mg kg^{-1} on average), compared to the flours, or between tritordeum and wheat. Breadsticks again exhibited significant increases of up to 117 % (tritordeum) and 112 % (wheat) resulting in final concentrations of 259 and 240 mg kg^{-1}, respectively. Changes in individual phenolic acids during processing have already been reported in the literature (Table S3), albeit with conflicting results, as the effect of baking or cooking on free or bound phenolic acids seems to be dependent on the species or variety as well as on the processing conditions (Abdel-Aal and Rabalski, 2013; Tian et al., 2021). Among the products obtained from tritordeum, free sinapic acid was found to be preserved the most in the case of breadsticks, while a decrease, although not significant, occurred in all the other cases (Table 4). The gallic acid content decreased significantly in tritordeum products, except for pizza, by as much as 8 times (bread). On the other hand, the gallic acid in wheat products increased to various extents, albeit not significantly, with the highest increase in pizza (19 times higher than flour). Regarding the

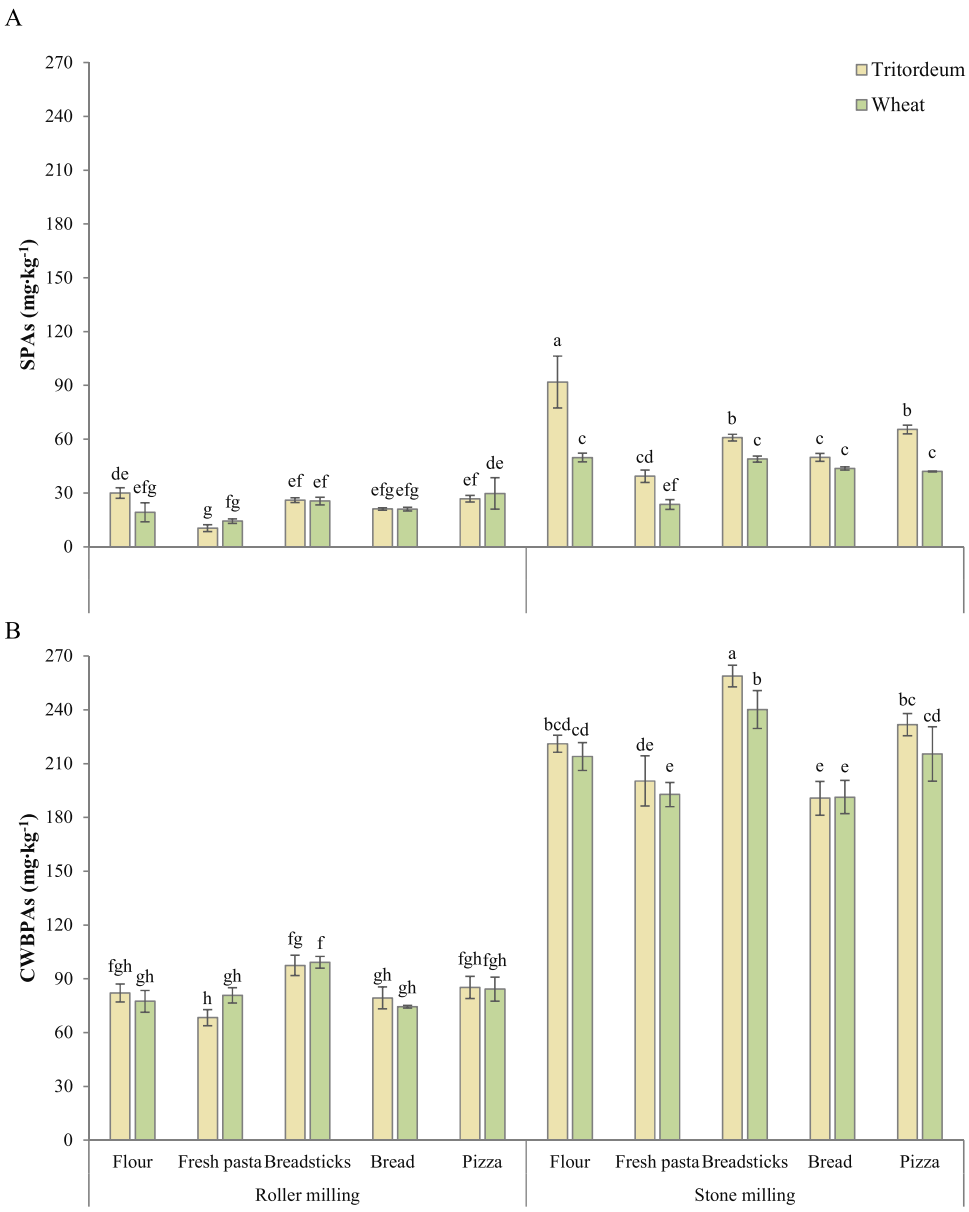

Fig. 2. The soluble (SPA; A) and cell wall-bound (CWBPA; B) phenolic acid contents of the tritordeum and wheat samples of flour, fresh pasta, breadsticks, bread, and pizza, obtained from roller and stone milling. Each column represents the mean from three different analytical determinations on separate and representative samples for each treatment. Error bars represent standard deviation. The results are expressed on a DW basis. Different letters above the columns indicate significant differences for $p < 0.05$, according to the REGW-F test.

CWBPAs, the predominant ferulic and sinapic acids were generally not influenced by processing, except for the increase of ferulic acid in breadsticks (Table 4). Different processing steps may have competing effects on phenolic acid dynamics. The metabolic activity of yeast may partially degrade the phenolic-carbohydrate complex during the fermentation process, releasing soluble phenolic acids or converting them into other derivatives. This process was found to be highly dependent on the wheat variety (Tian et al., 2021). The same authors found a positive correlation between the decrease of soluble ferulic acid and its polymerization and incorporation in Maillard reaction products (MRPs) during thermal processing, which they suggested led to reduced extractability but increased antioxidant activity. High temperatures during the baking process can lead to decomposition of the free fractions, as observed in dry-extruded corn snacks (Bresciani et al., 2021b), as well as to the release of some phenolic acids from the phenolic-carbohydrate complex (Zeng et al., 2016). Abdel-Aal and Rabalski (2013) reported an increase in free ferulic acid and a concomitant decrease of its bound form during the baking of an unleavened one-layer flat bread. On the other hand, no changes in insoluble phenolic acid levels (Mattila et al., 2005) or even a slight increase in the ferulic acid and total phenolic acids (Lu et al., 2014) were observed during bread-making processes. In the present study, the variations in the level of the individual compounds were different throughout processing, and appeared to depend on both the species and the milling degree of the flour. Overall, the phenolic acids were better preserved after the baking processes, which, as in the case of breadsticks, proved to be beneficial for the bound fraction. As for the only boiled product, i.e. fresh pasta, the process generally reduced the total phenolic acid content, with overall losses of all the soluble compounds and of the bound ferulic acid. As no extrusion or drying process was involved, the losses may be ascribed to degradation, due to the heat treatment, and to leaching into the cooking water. Moreover, hydrolysis of the methoxy group in bound ferulic acid may have occurred in the hot, aqueous environment, and this could have led to the formation of caffeic acid.

Table 4
The soluble (SPAs) and cell wall-bound (CWBPAs) phenolic acids (average ± standard deviation; mg kg^{-1}) detected in the tritordeum and wheat samples of flour, fresh pasta, breadsticks, bread, and pizza, expressed as the average of roller and stone milling.

Phenolic acids	Species	Processing type	GA	PRO	HYD	VA	CAF	SYR	COU	FER	SIN	Total content[a]
SPAs	Tritordeum	Flour	16.78 ± 17.97 a	2.22 ± 3.46 a	1.74 ± 0.83 a	3.79 ± 2.09 a	0.58 ± 0.16 ab	1.35 ± 0.74 a	1.10 ± 0.57 a	9.36 ± 4.73 a	23.99 ± 13.27 a	60.91 ± 35.20 a
		Fresh pasta	2.21 ± 2.35 b	0.28 ± 0.68 a	1.48 ± 0.63 a	3.24 ± 2.03 a	0.16 ± 0.17c	0.99 ± 0.64 a	0.62 ± 0.29 a	8.46 ± 6.60 a	7.45 ± 6.85 ab	24.89 ± 16.02 ab
		Breadsticks	4.08 ± 1.89 b	0.96 ± 0.39 a	1.99 ± 0.68 a	3.61 ± 1.84 a	0.49 ± 0.18 ab	1.43 ± 0.69 a	1.09 ± 0.43 a	9.16 ± 4.23 a	20.64 ± 10.56 a	43.45 ± 19.15 ab
		Bread	2.01 ± 0.83 b	< LOD	1.72 ± 0.57 a	3.72 ± 1.68 a	0.74 ± 0.27 a	1.09 ± 0.46 a	1.00 ± 0.44 a	8.50 ± 3.70 a	16.68 ± 8.07 ab	35.47 ± 15.84 ab
		Pizza	7.78 ± 2.34 ab	1.91 ± 2.31 a	1.96 ± 0.85 a	3.82 ± 2.14 a	0.39 ± 0.30 abc	1.23 ± 0.62 a	0.97 ± 0.48 a	10.38 ± 5.67 a	17.66 ± 10.72 ab	46.11 ± 21.25 ab
	Wheat	Flour	0.52 ± 0.85 b	0.61 ± 0.81 a	1.28 ± 0.59 a	2.58 ± 1.32 a	0.47 ± 0.22 abc	1.13 ± 0.53 a	0.94 ± 0.44 a	8.66 ± 4.04 a	18.30 ± 9.53 ab	34.50 ± 17.14 ab
		Fresh pasta	1.05 ± 0.70 b	0.47 ± 0.60 a	1.16 ± 0.27 a	2.44 ± 1.03 a	0.17 ± 0.18 bc	1.02 ± 0.25 a	0.53 ± 0.13 a	8.19 ± 2.63 a	3.93 ± 1.13 b	18.97 ± 5.40 b
		Breadsticks	8.10 ± 1.25 ab	0.81 ± 0.16 a	1.60 ± 0.64 a	2.19 ± 1.03 a	0.52 ± 0.30 ab	1.28 ± 0.50 a	0.92 ± 0.29 a	7.32 ± 3.09 a	14.46 ± 6.68 ab	37.21 ± 12.94 ab
		Bread	4.54 ± 1.88 ab	0.43 ± 0.30 a	1.32 ± 0.52 a	2.54 ± 1.32 a	0.72 ± 0.21 a	0.99 ± 0.48 a	0.87 ± 0.35 a	7.83 ± 3.71 a	13.11 ± 7.23 ab	32.34 ± 12.44 ab
		Pizza	10.06 ± 8.45 ab	0.50 ± 0.70 a	1.26 ± 0.53 a	2.40 ± 1.31 a	0.41 ± 0.16 abc	0.98 ± 0.48 a	0.75 ± 0.27 a	8.57 ± 3.79 a	10.95 ± 6.17 ab	35.88 ± 8.73 ab
		p-value	**	ns	ns	ns	***	ns	ns	ns	**	*
CWBPAs	Tritordeum	Flour	1.84 ± 1.36 d	< LOD	0.68 ± 0.23 a	1.11 ± 0.55 a	0.69 ± 0.23 abc	0.84 ± 0.26 a	4.74 ± 3.27 a	127.03 ± 65.28 a	14.63 ± 7.38 a	151.57 ± 76.27 a
		Fresh pasta	6.93 ± 2.59 c	< LOD	0.69 ± 0.23 a	1.06 ± 0.44 a	0.64 ± 0.26 abc	0.75 ± 0.30 a	4.41 ± 2.97 a	106.01 ± 58.93 a	13.82 ± 8.28 a	134.32 ± 72.88 a
		Breadsticks	11.19 ± 2.55 ab	< LOD	0.86 ± 0.27 a	1.44 ± 0.78 a	< LOD	0.98 ± 0.45 a	5.35 ± 3.29 a	143.02 ± 73.19 a	15.28 ± 8.39 a	178.12 ± 88.52 a
		Bread	2.16 ± 0.44 d	0.06 ± 0.15 b	0.57 ± 0.19 a	0.91 ± 0.38 a	0.37 ± 0.23 abcd	0.46 ± 0.19 a	3.72 ± 2.16 a	112.17 ± 51.57 a	14.58 ± 6.43 a	134.99 ± 61.40 a
		Pizza	11.09 ± 3.03 b	< LOD	0.81 ± 0.32 a	1.45 ± 0.67 a	0.25 ± 0.28 cd	0.97 ± 0.46 a	4.88 ± 3.37 a	123.46 ± 66.21 a	15.54 ± 6.42 a	158.45 ± 80.48 a
	Bread wheat	Flour	1.09 ± 0.49 d	< LOD	0.66 ± 0.27 a	1.30 ± 0.53 a	0.78 ± 0.22 a	0.67 ± 0.25 a	5.00 ± 2.83 a	119.00 ± 62.80 a	17.17 ± 8.19 a	145.67 ± 75.05 a
		Fresh pasta	14.38 ± 2.42 a	0.70 ± 0.41 ab	0.82 ± 0.28 a	1.06 ± 0.56 a	0.70 ± 0.27 ab	0.84 ± 0.40 a	4.77 ± 2.60 a	97.85 ± 50.64 a	15.64 ± 7.46 a	136.76 ± 61.51 a
		Breadsticks	13.43 ± 1.36 ab	1.32 ± 1.83 a	0.84 ± 0.36 a	1.43 ± 0.68 a	0.43 ± 0.47 abcd	1.04 ± 0.40 a	5.74 ± 3.09 a	127.97 ± 63.61 a	17.47 ± 9.57 a	169.68 ± 77.52 a
		Bread	2.89 ± 0.48 d	< LOD	0.63 ± 0.29 a	0.99 ± 0.42 a	0.33 ± 0.36 bcd	0.58 ± 0.12 a	4.28 ± 2.30 a	106.66 ± 54.09 a	16.53 ± 7.10 a	132.88 ± 64.30 a
		Pizza	14.26 ± 3.13 a	< LOD	0.74 ± 0.21 a	1.33 ± 0.62 a	0.34 ± 0.17 bcd	0.86 ± 0.40 a	4.87 ± 2.75 a	108.05 ± 59.01 a	19.35 ± 8.81 a	149.80 ± 72.60 a
		p-value	***	**	ns	ns	***	ns	ns	ns	ns	ns

LOD, limit of detection; SPAs, soluble phenolic acids; CWBPAs, cell wall-bound phenolic acids. The data are reported as the mean ± standard deviation from three different analytical determinations on separate and representative samples for each treatment. The results are expressed on a DW basis. Means followed by different letters are significantly different, according to the REGW-F test [(*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$, and ns, non-significant].

[a] The sum of all the determined SPAs and CWBPAs: gallic acid (GA), protocatechuic acid (PRO), hydroxybenzoic acid (HYD), vanillic acid (VAN), caffeic acid (CAF), syringic acid (SYR), *p*-coumaric acid (COU), ferulic acid (FER), sinapic acid (SIN).

The only significant increase observed in the bound fraction during both baking and boiling was in gallic acid, which could have been due to the enhanced availability and extractability of hydrolysable tannins (gallotannins), as a result of the disruption of cell membranes caused by thermal stress. Rocchetti et al. (2017) reported significant losses of both soluble and bound phenolic acids after the cooking of different types of gluten-free pasta, although not for black rice pasta, which showed a significant increase in free phenolics. A diminished amount of phenolic compounds was also reported for both buckwheat pasta (Verardo et al., 2011) and brown and polished rice (Ti et al., 2015) after boiling. Conversely, Bresciani et al. (2021a) observed an increase in free phenolic acids and either a reduction or no changes in the bound fraction after the cooking of high-amylose gluten-free pasta. Overall, baking processes tended to preserve phenolic acids more effectively than boiling, particularly in the bound fraction, confirming the stabilizing effect of dry heat. However, in products such as bread, where longer baking times are required, a partial degradation of bound phenolic acids may still occur due to extended exposure to heat and oxygen. Since the structure of phenolic acids may ultimately affect their bioavailability for absorption and subsequent physiological effects (Çelik and Gökmen, 2022), understanding the simultaneous incorporation and release of these compounds during processing may be particularly important to corroborate potential health effects of end products.

4. Conclusions

This study revealed that tritordeum flour contained significantly higher concentrations of key phytochemicals, especially carotenoids and soluble phenolic acids, than conventional bread wheat flour. However, the applied milling and processing methods significantly influenced the distribution of these compounds in the derived flours and their retention in the final food products.

Stone milling helped retain the majority of the analyzed compounds: semi-whole flours of both tritordeum and wheat showed higher concentrations of tocols, soluble and bound phenolic acids, and β-glucans than their refined counterparts. However, roller milling was advantageous for maximizing the carotenoid concentration, albeit this was observed only for tritordeum, due to the higher contribution of lutein to its carotenoid profile and a more homogenous distribution within the kernel layers.

Processing led to substantial reductions in most of the monitored phytochemicals, although the extent of losses varied with the cereal type, milling degree, and processing treatment. Lipophilic antioxidants, such as carotenoids and tocols, benefited from shorter fermentation and gentler heat transfer (as adopted for the breadsticks and bread production), while they were almost completely degraded during short but intense thermal treatments (as for the pizza). Phenolic acids were generally not compromised by the processes when refined flour was used, whereas more pronounced changes occurred in semi-whole products: bound phenolic acids seemed to be more susceptible to degradation during prolonged baking (e.g., for the bread), while soluble fractions were prone to leaching during cooking (e.g., for the fresh pasta). Notably, in stone milled tritordeum flour, the bound fraction of phenolic acids was not significantly reduced after pizza baking and even increased during breadstick production, suggesting cereal-specific matrix interactions that may enhance stability. The use of tritordeum was found to be advantageous to retain higher carotenoid levels than wheat in both roller and stone milled products. The highest carotenoid content was found in roller milled tritordeum breadsticks, which was nearly 3.5 times more than the wheat counterpart. In addition, despite statistically similar initial values in flour, stone milled tritordeum products retained higher tocol levels than their wheat counterparts, suggesting possible regeneration phenomena involving other antioxidants, such as carotenoids.

Overall, our study shows that the combination of milling and processing conditions can either enhance or diminish the potential health benefits of cereal-based products. These findings provide manufactures with practical guidance for selecting milling methods and technological parameters that preserve the phytochemical content and antioxidant potential of bakery and pasta products. For consumers, the use of tritordeum could support the development of foods with potential health benefits linked to higher antioxidant intake and diverse profile.

Further research should focus on linking these compositional advantages to health-relevant outcomes through bioavailability studies. Additionally, research should integrate consumer acceptance and sustainability potential to assess the feasibility of large-scale applications of tritordeum-based products.

Abbreviations

ANOVA, analysis of variance; CWBPAs, cell wall-bound phenolic acids; DAD, diode array detection; DW, dry weight; FLD, fluorescence detector; FW, fresh weight; i.v, initial value of the flour; HPLC, high-performance liquid chromatography; LOD, limit of detection; REGW–F test, Ryan–Einot–Gabriel–Welsch F test; SPAs, soluble phenolic acids; TCC, total carotenoid content; TTC, total tocol content.

CRediT authorship contribution statement

Sardella Claudia: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Burešová Barbora:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Kotíková Zora:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Orsák Matyáš:** Writing – review & editing, Validation, Supervision, Resources, Methodology. **Blandino Massimo:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Paznocht Luboš:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis. **Vanara Francesca:** Writing – review & editing, Visualization, Investigation, Formal analysis.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.108591.

Data availability

Data will be made available on request.

References

AACC International, 2008. AACC Approved Methods of Analysis, eleventh Ed. Cereals & Grains Association.

Abdel-Aal, E.-S.M., Rabalski, I., 2013. Effect of baking on free and bound phenolic acids in wholegrain bakery products. J. Cereal Sci. 57, 312–318. https://doi.org/10.1016/j.jcs.2012.12.001.

Ames, N., Storsley, J., Thandapilly, S., J., 2019. Functionality of Beta-glucan from Oat and Barley and Its Relation with Human Health. In: Beta, In.T., Camire, M.E. (Eds.), Cereal Grain-Based Functional Foods: Carbohydrate and Phytochemical Components. Royal Society of Chemistry, pp. 147–166.

AOAC International, 2005. Official Methods of Analysis of AOAC International, eighth Ed. Association of Official Analytical Chemists.

Atienza, S.G., Ballesteros, J., Martín, A., Hornero-Méndez, D., 2007. Genetic Variability Of carotenoid concentration and degree of esterification among *Tritordeum* (× *Tritordeum* Ascherson et Graebner) And Durum Wheat Accessions. J. Agric. Food Chem. 55, 4244–4251. https://doi.org/10.1021/jf070342p.

Ávila, C.M., Rodríguez-Suárez, C., Atienza, S.G., 2021. Tritordeum: creating a new crop species—the successful use of plant genetic resources. Plants 10, 1029. https://doi.org/10.3390/plants10051029.

Bangar, S.P., Kaushik, N., 2022. Functional Cereals: functional components and benefits. In: Punia Bangar, S., Kumar Siroha, A. (Eds.), Functional Cereals and Cereal Foods. Springer, Cham, pp. 3–25. https://doi.org/10.1007/978-3-031-05611-6_1.

Bresciani, A., Giordano, D., Vanara, F., Blandino, M., Marti, A., 2021a. High-amylose corn in gluten-free pasta: strategies to deliver nutritional benefits ensuring the overall quality. Food Chem. 353, 129489. https://doi.org/10.1016/j.foodchem.2021.129489.

Bresciani, A., Giordano, D., Vanara, F., Blandino, M., Marti, A., 2021b. The effect of the amylose content and milling fractions on the physico-chemical features of co-extruded snacks from corn. Food Chem. 343, 128503. https://doi.org/10.1016/j.foodchem.2020.128503.

Burešová, B., Paznocht, L., Jarošová, V., Doskočil, I., Martinek, P., 2023. The effect of boiling and *in Vitro* digestion on the carotenoid content of colored-grain wheat. J. Food Compos. Anal. 115, 105002. https://doi.org/10.1016/j.jfca.2022.105002.

Burešová, B., Paznocht, L., Kotíková, Z., Giampaglia, B., Martinek, P., Lachman, J., 2021. Changes in carotenoids and tocols of colored-grain wheat during unleavened bread preparation. J. Food Compos. Anal. 103, 104108. https://doi.org/10.1016/j.jfca.2021.104108.

Capurso, C., 2021. Whole-grain intake in the mediterranean diet and a low protein to carbohydrates ratio can help to reduce mortality from Cardiovascular disease, slow down the progression of aging, and to improve lifespan: a review. Nutrients 13, 2540. https://doi.org/10.3390/nu13082540.

Çelik, E.E., Gökmen, V., 2022. Interactions between free and bound antioxidants under different conditions in food systems. Crit. Rev. Food Sci. Nutr. 62, 5766–5782. https://doi.org/10.1080/10408398.2021.1892584.

Chiremba, C., Pozniak, C.J., Fu, B.X., 2015. Changes in semolina yellow pigment content and carotenoid composition during pasta processing. Cereal Chem. 92, 551–556. https://doi.org/10.1094/CCHEM-04-15-0065-R.

Cilla, A., Bosch, L., Barberá, R., Alegría, A., 2018. Effect of processing on the bioaccessibility of bioactive compounds – a review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols. J. Food Compos. Anal. 68, 3–15. https://doi.org/10.1016/j.jfca.2017.01.009.

De Paula, R., Abdel-Aal, E.-S.M., Messia, M.C., Rabalski, I., Marconi, E., 2017. Effect of processing on the beta-glucan physicochemical properties in barley and semolina pasta. J. Cereal Sci. 75, 124–131. https://doi.org/10.1016/j.jcs.2017.03.030.

Elvira-Torales, L.I., García-Alonso, J., Periago-Castón, M.J., 2019. Nutritional importance of carotenoids and their effect on liver health: a review. Antioxidants 8, 229. https://doi.org/10.3390/antiox8070229.

European Commission (2006). Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. OJ of the European Union, L 70, 12–34.

European Commission (2010). Commission Regulation (EU) No 118/2010 of 9 February 2010 amending Regulation (EC) No 900/2008 laying down the methods of analysis and other technical provisions necessary for the application of the arrangements for imports of certain goods resulting from the processing of agricultural products. OJ of the European Union, L 37, 21–25.

Fărcaş, A.C., Socaci, S.A., Nemeş, S.A., Pop, O.L., Coldea, T.E., Fogarasi, M., Biriş-Dorhoi, E.S., 2022. An update regarding the bioactive compound of cereal By-products: HEalth Benefits and Potential Applications. Nutrients 14, 3470. https://doi.org/10.3390/nu14173470.

Finocchiaro, F., Ferrari, B., Gianinetti, A., Dall'Asta, C., Galaverna, G., Scazzina, F., Pellegrini, N., 2007. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. Mol. Nutr. Food Res. 51, 1006–1019. https://doi.org/10.1002/mnfr.200700011.

Fratianni, A., Criscio, T.D., Mignogna, R., Panfili, G., 2012. Carotenoids, tocols and retinols evolution during egg pasta – making processes. Food Chem. 131, 590–595. https://doi.org/10.1016/j.foodchem.2011.09.034.

Fratianni, A., D'Agostino, A., Niro, S., Bufano, A., Paura, B., Panfili, G., 2021. Loss or Gain of Lipophilic Bioactive Compounds in Vegetables after Domestic Cooking? Effect of Steaming and Boiling. Foods 10, 960. https://doi.org/10.3390/foods10050960.

Giordano, D., Reyneri, A., Locatelli, M., Coïsson, J.D., Blandino, M., 2019. Distribution of bioactive compounds in pearled fractions of tritordeum. Food Chem. 301, 125228. https://doi.org/10.1016/j.foodchem.2019.125228.

Henrion, M., Francey, C., Lê, K.-A., Lamothe, L., 2019. Cereal B-Glucans: the impact of processing and how it affects physiological responses. Nutrients 11, 1729. https://doi.org/10.3390/nu11081729.

Hidalgo, A., Brandolini, A., Pompei, C., 2010. Carotenoids evolution during pasta, bread and water biscuit preparation from wheat flours. Food Chem. 121, 746–751. https://doi.org/10.1016/j.foodchem.2010.01.034.

Hidalgo, A., Brandolini, A., 2010. Tocols stability during bread, water biscuit and pasta processing from wheat flours. J. Cereal Sci. 52, 254–259. https://doi.org/10.1016/j.jcs.2010.06.002.

Honda, M., Maeda, H., Fukaya, T., Goto, M., 2018. In: Zepka, L.Q., Jacob-Lopes, E., De Rosso, V.V. (Eds.), Progress in carotenoid research. BoD–Books on Demand, pp. 139–159.

Kean, E.G., Bordenave, N., Ejeta, G., Hamaker, B.R., Ferruzzi, M.G., 2011. Carotenoid bioaccessibility from whole grain and decorticated yellow endosperm sorghum porridge. J. Cereal Sci. 54, 450–459. https://doi.org/10.1016/j.jcs.2011.08.010.

Kotíková, Z., Šulc, M., Lachman, J., Pivec, V., Orsák, M., Hamouz, K., 2016. Carotenoid profile and retention in yellow-, purple- and red-fleshed potatoes after thermal processing. Food Chem. 197, 992–1001. https://doi.org/10.1016/j.foodchem.2015.11.072.

Lachman, J., Hejtmánková, A., Orsák, M., Popov, M., Martinek, P., 2018. Tocotrienols and tocopherols in colored-grain wheat, tritordeum and barley. Food Chem. 240, 725–735. https://doi.org/10.1016/j.foodchem.2017.07.123.

Lachman, J., Martinek, P., Kotíková, Z., Orsák, M., Šulc, M., 2017. Genetics and chemistry of pigments in wheat grain – a review. J. Cereal Sci. 74, 145–154. https://doi.org/10.1016/j.jcs.2017.02.007.

Lampi, A.-M., Nurmi, T., Piironen, V., 2010. Effects of the environment and genotype on tocopherols and tocotrienols in wheat in the HEALTHGRAIN diversity screen. J. Agric. Food Chem. 58, 9306–9313. https://doi.org/10.1021/jf100253u.

Lante, A., Canazza, E., Tessari, P., 2023. Beta-glucans of cereals: functional and technological properties. Nutrients 15, 2124. https://doi.org/10.3390/nu15092124.

Leenhardt, F., Lyan, B., Rock, E., Boussard, A., Potus, J., Chanliaud, E., Remesy, C., 2006. Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. J. Agric. Food Chem. 54, 1710–1715. https://doi.org/10.1021/jf052243m.

Liu, R.H., 2007. Whole grain phytochemicals and health. J. Cereal Sci. 46, 207–219. https://doi.org/10.1016/j.jcs.2007.06.010.

Lu, Y., Luthria, D., Fuerst, E.P., Kiszonas, A.M., Yu, L., Morris, C.F., 2014. Effect of Processing on phenolic composition of dough and bread fractions made from refined and whole wheat flour of three wheat varieties. J. Agric. Food Chem. 62, 10431–10436. https://doi.org/10.1021/jf501941r.

Martín, A., Alvarez, J.B., Martín, L.M., Barro, F., Ballesteros, J., 1999. The development Of Tritordeum: a novel cereal for food processing. J. Cereal Sci. 30, 85–95. https://doi.org/10.1006/jcrs.1998.0235.

Mattera, M.G., Hornero-Méndez, D., Atienza, S.G., 2017. Lutein ester profile in wheat and tritordeum can be modulated by temperature: evidences for regioselectivity and fatty acid preferential of enzymes encoded by genes on chromosomes 7D and $7H^{ch}$. Food Chem. 219, 199–206. https://doi.org/10.1016/j.foodchem.2016.09.133.

Mattila, P., Pihlava, J., Hellström, J., 2005. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. J. Agric. Food Chem. 53, 8290–8295. https://doi.org/10.1021/jf051437z.

Mellado-Ortega, E., Hornero-Méndez, D., 2012. Isolation and identification of lutein esters, including their regioisomers, in tritordeum (×*Tritordeum* Ascherson et Graebner) grains: Evidence for a preferential xanthophyll acyltransferase activity. Food Chem. 135, 1344–1352. https://doi.org/10.1016/j.foodchem.2012.05.046.

Mellado-Ortega, E., Hornero-Méndez, D., 2018. Effect of lutein esterification on the differential distribution of carotenoids in germ and endosperm fractions from tritordeum grains. J. Cereal Sci. 79, 462–468. https://doi.org/10.1016/j.jcs.2017.12.006.

Montesano, V., Negro, D., De Lisi, A., Urbano, M., Sarli, G., Laghetti, G., 2021. Agronomic performance and phenolic profile of Tritordeum (x *Tritordeum martinii* A. Pujadas) lines. Cereal Chem. 98, 382–391. https://doi.org/10.1002/cche.10378.

Moreau, R.A., Hicks, K.B., 2005. The composition of corn oil obtained by the alcohol extraction of ground corn. J. Am. Oil Chem. Soc. 82, 809–815. https://doi.org/10.1007/s11746-005-1148-4.

Oduro-Obeng, H., Fu, B.X., Beta, T., 2021. Influence of cooking duration on carotenoids, physical properties and in vitro antioxidant capacity of pasta prepared from three Canadian durum wheat cultivars. Food Chem. 363, 130016. https://doi.org/10.1016/j.foodchem.2021.130016.

Onofri, A., Seddaiu, G., Piepho, H.-P., 2016. Long-Term Experiments with cropping systems: Case studies on data analysis. Eur. J. Agron. 77, 223–235. https://doi.org/10.1016/j.eja.2016.02.005.

Padhy, A.K., Sharma, A., Sharma, H., Rajput, R., Pandey, A., Srivastava, P., Bains, N.S., 2022. Bread wheat with enhanced grain carotenoid content: a novel option for wheat biofortification. Mol. Breed. 42, 67. https://doi.org/10.1007/s11032-022-01338-0.

Papadopoulos, G., Mavroeidis, A., Stavropoulos, P., Anastasopoulos, V., Beslemes, D., Tigka, E., Kakabouki, I., 2023. Tritordeum: a versatile and resilient cereal for Mediterranean agriculture and sustainable food production. Cereal Res. Commun. 52, 323–331. https://doi.org/10.1007/s42976-023-00401-6.

Pascual, C.D.S.C.I., Massaretto, I.L., Kawassaki, F., Barros, R.M.C., Noldin, J.A., Marquez, U.M.L., 2013. Effects of parboiling, storage and cooking on the levels of tocopherols, tocotrienols and γ-oryzanol in brown rice (*Oryza sativa* L.). Food Res. Int. 50, 676–681. https://doi.org/10.1016/j.foodres.2011.07.013.

Paznocht, L., Kotíková, Z., Orsák, M., Lachman, J., Martinek, P., 2019. Carotenoid changes of colored-grain wheat flours during bun-making. Food Chem. 277, 725–734. https://doi.org/10.1016/j.foodchem.2018.11.019.

Paznocht, L., Kotíková, Z., Šulc, M., Lachman, J., Orsák, M., Eliášová, M., Martinek, P., 2018. Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains. Food Chem. 240, 670–678. https://doi.org/10.1016/j.foodchem.2017.07.151.

Phuong, L.M., Lachman, J., Kotíková, Z., Orsák, M., Michlová, T., Martinek, P., 2017. Selenium in colour-grained winter wheat and spring tritordeum. Plant Soil Environ. 63, 315–321. https://doi.org/10.17221/259/2017-PSE.

Q2B CH, 1996. Validation of analytical procedures: Methodology. International Conference on Harmonization, Geneva, Switzerland.
Rakha, A., Saulnier, L., Åman, P., Andersson, R., 2012. Enzymatic fingerprinting of arabinoxylan and β-glucan in triticale, barley and tritordeum grains. Carbohydr. Polym. 90, 1226–1234. https://doi.org/10.1016/j.carbpol.2012.06.054.
Regand, A., Tosh, S.M., Wolever, T.M.S., Wood, P.J., 2009. Physicochemical Properties of β-Glucan in Differently Processed Oat Foods Influence Glycemic Response. J. Agric. Food Chem. 57, 8831–8838. https://doi.org/10.1021/jf901271v.
Roasa, J., De Villa, R., Mine, Y., Tsao, R., 2021. Phenolics of cereal, pulse and oilseed processing by-products and potential effects of solid-state fermentation on their bioaccessibility, bioavailability and health benefits: a review. Trends Food Sci. Technol. 116, 954–974. https://doi.org/10.1016/j.tifs.2021.08.027.
Rocchetti, G., Lucini, L., Chiodelli, G., Giuberti, G., Montesano, D., Masoero, F., Trevisan, M., 2017. Impact of boiling on free and bound phenolic profile and antioxidant activity of commercial gluten-free pasta. Food Res. Int. 100, 69–77. https://doi.org/10.1016/j.foodres.2017.08.031.
Rodríguez-Suárez, C., Requena-Ramírez, M.D., Mazzucotelli, E., Mastrangelo, A.M., Marcotuli, I., Gadaleta, A., Martín, A., Hornero-Méndez, D., Atienza, S.G., 2024. Prospects for tritordeum (×*Tritordeum martinii* A. Pujadas, Nothosp. Nov.) cereal breeding: Key points for future challenges. Plant Breed. 143, 763–772. https://doi.org/10.1111/pbr.13207.
Shewry, P.R., Brouns, F., Dunn, J., Hood, J., Burridge, A.J., America, A.H.P., Gilissen, L., Proos-Huijsmans, Z.A.M., Van Straaten, J.P., Jonkers, D., Lazzeri, P.A., Ward, J.L., Lovegrove, A., 2023. Comparative compositions of grain of tritordeum, durum wheat and bread wheat grown in multi-environment trials. Food Chem. 423, 136312. https://doi.org/10.1016/j.foodchem.2023.136312.
Suchowilska, E., Wiwart, M., Przybylska-Balcerek, A., Stuper-Szablewska, K., 2021. The profile of bioactive compounds in the grain of various x *Tritordeum* genotypes. J. Cereal Sci. 102, 103352. https://doi.org/10.1016/j.jcs.2021.103352.
Ti, H., Zhang, R., Li, Q., Wei, Z., Zhang, M., 2015. Effects of cooking and in vitro digestion of rice on phenolic profiles and antioxidant activity. Food Res. Int. 76, 813–820. https://doi.org/10.1016/j.foodres.2015.07.032.
Tian, W., Chen, G., Tilley, M., Li, Y., 2021. Changes in phenolic profiles and antioxidant activities during the whole wheat bread-making process. Food Chem. 345, 128851. https://doi.org/10.1016/j.foodchem.2020.128851.
Tiwari, U., Cummins, E., 2009. Nutritional importance and effect of processing on tocols in cereals. Trends Food Sci. Technol. 20, 511–520. https://doi.org/10.1016/j.tifs.2009.06.001.
Vaquero, L., Comino, I., Vivas, S., Rodríguez-Martín, L., Giménez, M.J., Pastor, J., Sousa, C., Barro, F., 2018. Tritordeum: a novel cereal for food processing with good acceptability and significant reduction in gluten immunogenic peptides in comparison with wheat. J. Sci. Food Agric. 98, 2201–2209. https://doi.org/10.1002/jsfa.8705.
Verardo, V., Arráez-Román, D., Segura-Carretero, A., Marconi, E., Fernández-Gutiérrez, A., Caboni, M.F., 2011. Determination of free and bound phenolic compounds in buckwheat spaghetti by RP-HPLC-ESI-TOF-MS: effect of thermal processing from farm to fork. J. Agric. Food Chem. 59, 7700–7707. https://doi.org/10.1021/jf201069k.
Wen, Y., Xu, L., Xue, C., Jiang, X., Wei, Z., 2020. Assessing the impact of oil types and grades on tocopherol and tocotrienol contents in vegetable oils with chemometric methods. Molecules 25, 5076. https://doi.org/10.3390/molecules25215076.
Wiwart, M., Szafrańska, A., Suchowilska, E., 2025. Does the use of the Tritordeum, an alternative cereal for breadmaking, can be one of the mitigation strategies of acrylamide in bread? LWTFood Sci. Technol. 223, 117715. https://doi.org/10.1016/j.lwt.2025.117715.
Yang, C., Zhang, L., Tsao, R., 2020. Chemistry and biochemistry of dietary carotenoids: bioaccessibility, bioavailability and bioactivities. J. Food Bioact. 10, 32–46. https://doi.org/10.31665/JFB.2020.10229.
Yu, L., Beta, T., 2015. Identification and Antioxidant Properties of Phenolic Compounds during Production of Bread from Purple Wheat Grains. Molecules 20, 15525–15549. https://doi.org/10.3390/molecules200915525.
Zeng, Z., Liu, C., Luo, S., Chen, J., Gong, E., 2016. The profile and bioaccessibility of phenolic compounds in cereals influenced by improved extrusion cooking treatment. PLoS One 11, e0161086. https://doi.org/10.1371/journal.pone.0161086.
Zhu, Y., Sang, S., 2017. Phytochemicals in whole grain wheat and their health-promoting effects. Mol. Nutr. Food Res. 61, 1600852. https://doi.org/10.1002/mnfr.201600852.