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Does the use of the Tritordeum, an alternative cereal for breadmaking, can be one of the mitigation strategies of acrylamide in bread?

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ABSTRACT

This article presents a comprehensive study comparing the technological quality and baking quality of flours obtained from 11 spring Tritordeum breeding lines with modern cultivars of durum wheat, bread wheat, and naked barley. Tritordeum was characterized by a similar content of protein (14.0 %), ash (0.60 %), and wet gluten (34.3 %) to bread wheat (13.3 %, 0.54 %, and 31.9 %, respectively), but a significantly lower gluten index (51 % vs 90 %). Bread yield was highest in loaves made from durum wheat (142 % on average) and lowest in loaves from bread wheat and barley flour (134 and 133 %, respectively). The bread yield of Tritordeum ranged 133–141 %. The analyzed breeding lines and cultivars were discriminated by k-means clustering. Breeding lines HT438 and HTC1324 were highly similar to bread wheat in terms of all dough rheological properties, milling quality, and baking quality, which indicates that both lines constitute a valuable resource for bread production. Although the Tritordeum was characterized by a similar average asparagine content to common wheat (185 vs 188 mg/kg), the bread obtained from it had a significant lower content of acrylamide (15 vs 21.9 μg/kg). The results can be used to reduce the acrylamide levels in bakery products and risk assessments studies.

1. Introduction

Tritordeum (*Tritordeum martinii* A. Pujadas, nothosp. nov.) is a relatively new allohexaploid crop obtained by crossing diploid wild barley (*Hordeum chilense* Roem. et Schultz) ($H^{ch}H^{ch}$) with tetraploid durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husn.) (AABB) (Martín & Sanchez-Monge Llaguna, 1982). Tritordeum is characterized by a high content of micronutrients, in particular lutein, polyphenols, and macronutrients (Mellado-Ortega & Hornero-Méndez, 2016; Paznocht et al., 2018; Suchowilska et al., 2021, 2023). According to Shewry et al. (2023), Tritordeum grain is a richer source of protein, total phenols, methyl donors, magnesium, and iron than bread wheat and durum wheat grain. It also has higher concentrations of total amino acids (excluding asparagine) and sugars (including raffinose).

According to the cited authors, white flour from bread wheat and Tritordeum has a similar content of arabinoxylan and β-glucan, the main components of dietary fiber, whereas durum wheat flour is much less abundant in these compounds. In addition to the content of compounds with health-promoting properties, the cytogenetic characteristics of Tritordeum have also been analyzed in many studies. According to Delgado et al. (2017), cytogenetic and molecular monitoring of Tritordeum is needed to guarantee the selection of the most stable lines for improvement and sustainable agriculture.

Due to the high value of Tritordeum-based products, this cereal species continues to attract growing interest among breeders, in particular in the Mediterranean Region (Papadopoulos et al., 2024). Tritordeum is drought-tolerant and suitable for cultivation in regions with limited availability of water (Kakabouki et al., 2020), which is a very important consideration in an era of rapid climate change. However, the technological quality of Tritordeum grain and its suitability for the production of bread flour remain insufficiently investigated.

Consumers have a growing demand for novel cereal products characterized by desirable sensory attributes, high nutritional value, and health-promoting properties. Bread continues to be an important part of the human diet as a source of energy, macronutrients (protein and fiber), vitamins, micronutrients, phenolic compounds, and carotenoids. The baking quality of flour is determined by many factors, in particular the quantity and quality of gluten, and the activity of amylolytic and proteolytic enzymes (Carson & Edwards, 2009).

According to Martin et al. (1999), hexaploid Tritordeum resembles

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bread wheat in terms of the baking quality of flour and the rheological properties of dough. The quality of Tritordeum flour is determined by the presence of storage proteins inherited from durum wheat and *Hordeum chilense*. Tritordeum differs from bread wheat in the composition of gluten proteins, and it is characterized by significantly lower levels of gliadins, fewer carbohydrates, and fructans, and a higher content of proteins, dietary fibers, and antioxidants (Vaquero et al., 2018).

Ballesteros et al. (2003) reported that ($1H^{ch}$)1D and (1 A)1D substitution lines did not differ from euploid Tritordeum in terms of agronomic traits, but were characterized by significantly higher gluten strength, alveograph baking value (W), and loaf volume. In turn, Haro et al. (2022) noted that bread made from Tritordeum flour contains fewer peptides associated with gluten proteins, CD-epitopes, and IgE binding sites than bread wheat. Therefore, Tritordeum bread is not suitable for individuals with gluten intolerance, but it offers an alternative for healthy consumers who would like to reduce their gluten consumption without harming their gut health.

Tritordeum is generally well tolerated by individuals with non-celiac wheat sensitivity (NCWS) who do not have to exclude gluten from their diets (Sánchez-León et al., 2021). Russo et al. (2022) found that a diet containing Tritordeum-based foods (bread, bakery products, and pasta) significantly reduced symptoms of diarrhea-predominant IBS in patients. These results were attributed to an overall improvement in the gastrointestinal barrier, as demonstrated by reduced intestinal permeability and a decrease in the markers of intestinal mucosal integrity, mucosal inflammation, and fermentative dysbiosis.

The aim of this study was to determine variability in traits associated with the technological quality of grain and flour and to assess the breadmaking potential of spring Tritordeum lines. The study also explored whether Tritordeum, as an alternative cereal, could serve as a potential strategy for mitigating acrylamide formation in bread, a critical issue in bread production.

2. Materials and methods

2.1. Materials

The study was conducted on 11 Tritordeum breeding lines (*Tritordeum martinii* A. Pujadas, nothosp. nov.), two durum wheat cultivars (*Triticum durum* Desf.), one bread wheat cultivar (*Triticum aestivum* L.), and one naked barley cultivar (*Hordeum vulgare* L.) (Table S1).

2.2. Field experiment

The field experiment was performed in 2021 at the Agricultural Experiment Station in Bałcyny in Poland (53°36′N latitude; 19°5′ E longitude). The experiment was established in randomized block design. Grains were sown at a density of 400 per m^2 in plots with an area of 15 m^2 each. The plots were fertilized with N/P/K at 100/25/80 kg/ha. Chemical plant protection was not applied. Grain were harvested in the over-ripe stage (BBCH 92) (Witzenberger et al., 1989) with a Wintersteiger Classic (Austria) plot harvester. After harvesting, the grain quality parameters listed in the next subsection were determined.

2.3. Analysis of grain quality

Protein content (ISO 20483:2013), gluten content and the gluten index - GI (ISO 21415–2:2015), ash content (ISO 2171:2007), and the falling number - FN (ISO 3093:2009) were evaluated to determine the technological quality of the tested grain samples. Grain vitreousness was analyzed in a Pohl farinator for cutting grains (Bipea method Ref. 204–1104).

2.4. Grain milling

Grain samples were hand-cleaned and tempered to 15 % moisture content. After 24 h, the samples were milled using a Bühler MLU-202 pneumatic laboratory mill (Bühler, Uzwil, Switzerland) to obtain three break flour streams, three reduction flour streams (which were combined to obtain flour samples for analysis), and coarse and fine bran, according to the procedure described by Wiwart et al. (2023). The flour yield and ash content (ISO 2171:2007) of each grain sample were determined.

2.5. Analysis of the baking quality of flour

The flours obtained in the laboratory milling test were analyzed for asparagine content according to the procedure described by Świder et al. (2020) and for starch damage (ISO 17715:2013) in a SDmatic (KPM Analytics, Villeneuve-la-Garenne, France). Particle size distribution was determined by separating flour particles into size fractions using sieves with different mesh sizes (224, 150, 132, 95 μm). The rheological characteristics of dough were measured with a Chopin Mixolab (KPM Analytics, Villeneuve-la-Garenne, France) according to ISO 17718:2013. The Mixolab test involved a standard Chopin + protocol: dough weight – 75 g, water temperature – 30 °C, kneading speed – 80 rpm. The following of the time and temperature settings were applied in the next parts of the analysis: 8 min at 30 °C, heating to 90 °C at 4 °C/min for 15 min, holding at 90 °C for 7 min, cooling down to 50 °C at 4 °C/min for 10 min, and finally at 50 °C for 5 min. The following protein properties of the tested flours were received from the Mixolab curve: dough development time (T1, min), stability time (min), protein weakening (C2, C1-C2, decrease in consistency due to mechanical shear stress in dough, followed by an increase in temperature, based on the calculated difference between points C1 and C2, N × m; slope α, N × m/min). The starch properties were determined based on starch gelatinization (parameters C3, C3-C2, N × m), amylolytic activity (C4, C3-C4, N × m), starch retrogradation (C5, N × m), initial and final temperature of starch gelatinization (D2 and D3, respectively, °C), and starching speed and enzymatic degradation (slopes β and γ, respectively, N × m/min).

2.6. Baking trials

Dough was prepared with the use of the one-step method at 28–30 °C in a standard baking test (Wiwart et al., 2023). Flour (600 g) was combined with water (based on the water absorption capacity determined by Mixolab +3 %), compressed yeast (3 %), and salt (1 %) in a laboratory mixer (KitchenAid, Benton Harbor, MI, USA). The kneading time varied between 3.0 and 6.4 min. The development time (T1) and stability, as determined by the Mixolab, were considered when evaluating the kneading times of the dough. Mixing was divided into two phases. In the first phase, lasting 2 min, all ingredients were slowly incorporated. The "2" speed setting on the mixer, commonly used for mixing and kneading yeast doughs, was used. In the second phase, the "4" speed setting was used to obtain the viscous-elastic structure and properties of a kneaded dough. The decision to stop mixing was made by a single operator for all tested samples. The dough was considered ready when the operator judged that the gluten network had developed sufficient elasticity and extensibility. Dough was fermented for 60 min in a proofing chamber at 30 °C and 75 % relative humidity (RH). After 30 min, it was kneaded by hand for 60 s and divided into three pieces of 250 g each. Each piece was rounded, put in a baking tin, and placed in a proofing cabinet at 30 °C/75 % RH for 34–44 min - the time required for optimal dough development. Loaves were baked in an oven (230 °C, 30 min) (Piccolo Wachtel Winkler, Pulsnitz, Germany). After 24± 1 h each bread sample was evaluated for bread yield, specific volume (in mL per 100 g of bread, with the rapeseed displacement method) and porosity (Różyło et al., 2015), and crumb hardness (Instron 1140, Instron, Norwood, MA, USA) (Wiwart et al., 2023). Flour, crumb and crust color was evaluated in five replicates with a Minolta CR-310 colorimeter (Konica Minolta Sensing Americas, Inc, Ramsey, NJ, USA). The color assessment were carried out in the CIELab system (L^* – lightness, a^* –green-red coordinate, b^* –blue-yellow coordinate). Acrylamide content was determined by GC/MS after bromination (Roszko et al., 2020). The results were validated to confirm that the method performs well at low acrylamide concentrations with LOQ of 10 μg kg^{-1} and recovery and relative standard deviation below 6 %.

2.7. Statistical analysis

The results were analyzed in the Statistica v. 13 program (TIBCO Inc). The normality of distribution was checked with the use of descriptive statistics and the Shapiro-Wilk test. The significance of differences between means was determined by Tukey's range test.

The results were processed by k-means clustering, separately for the quality traits of grain and flour (protein content, ash content, gluten content, gluten index (GI), vitreousness, falling number (FN)) and the rheological properties of dough, and separately for milling and baking quality traits.

3. Results

Grain vitreousness (evaluated before milling) and the technological quality of flour obtained from the grain of the examined cereal species are presented in Table 1. Grain vitreousness was highest in the reference cultivars of durum wheat (93 %) and bread wheat cv. Bombona (72 %), and these values were significantly higher than in the studied Tritordeum lines (50 %) which were characterized by high variability in vitreousness (RSD 44 %). Five Tritordeum lines had desirable, vitreous endosperm. The protein content of Tritordeum grain was similar to that noted in bread wheat cv. Bombona (14.0 % vs 13.3 %), but it was significantly lower relative to durum wheat (15.6 %). No significant differences in gluten content were observed between Tritordeum (34.3 %) and bread wheat cv. Bombona (31.9 %), but this parameter was significantly higher in durum wheat (38.7 %). As expected, the GI was highest in the elite bread wheat cv. Bombona (90 %) and significantly lower in Tritordeum (51 % on average). The high protein content of Tritordeum grain is a trait inherited from durum wheat, but low GI values point to the low quality of gluten proteins. However, the analyzed Tritordeum lines differed considerably in GI values (RSD 79 %), and the GI was the only trait that did not have normal distribution. It should also be noted that the FN of Tritordeum grain was significantly lower relative to both durum wheat and bread wheat (279 s vs 431 s and 335 s, respectively), although only two Tritordeum lines were characterized by FN values below 250 s, and none of these samples contained sprouted grain. Durum wheat and naked barley grain contained significantly more ash than Tritordeum (0.97 % and 0.82 % vs 0.60 %, respectively), but the difference between Tritordeum and bread wheat was not significant.

Bread wheat cv. Bombona was characterized by the highest milling quality expressed by the highest flour extraction rate (69.3 %) (Table 2). The extraction rate of Tritordeum (64.3 % on average) was similar to that noted in durum wheat (63.5 %), but considerable differences were observed between lines (58.5–69.1 %). This parameter exceeded 68 % in three Tritordeum lines (HT438, HTC1324, and HT444), and flours obtained from these lines were relatively low in ash, which suggests that they were characterized by higher milling quality than the remaining lines.

The analyzed cereals differed significantly in the content of damaged starch which is one of the key determinants of the processing suitability of flour in bread production. The content of damaged starch was highest in durum wheat flour (26.1 UCD) and lowest in Tritordeum flour (20.0 UCD on average). The wide range of UCD values for Tritordeum lines (17.0–22.4 UCD) suggests that Tritordeum flour can be used in the production of various types of bread and pastries. The content of asparagine, the main precursor of acrylamide in bread, was similar in flours obtained from Tritordeum and bread wheat cv. Bombona (185 mg/kg and 188 mg/kg, respectively). No significant differences in this parameter were noted relative to durum wheat flour (212 mg/kg), but asparagine content was 5.6 times higher in Tritordeum flour than barley flour (33 mg/kg).

The size of flour particles (granularity) affects water absorption, the physical properties of dough, dough expansion during fermentation, and bread quality (Barak et al., 2014). Fine-grained flours (below 95 μm) are characterized by the most uniform particle size distribution and the highest baking quality. Bread wheat and Tritordeum flours were generally fine-grained with the highest and similar content of fine particles (below 95 μm). Durum wheat flour had coarse granularity with the highest content of particles larger than 224 μm (3.6 %) and particles measuring 150–224 μm (35.1 %). The color lightness L^* of the examined flours ranged from 88.33 for durum wheat flour to 91.33 for barley flour. Negative values of color descriptor a^* (green – red) point to a high contribution of the green component, whereas positive values of descriptor b^* (yellow – blue) point to a high contribution of the yellow component, which was highest in durum wheat flour (19.36).

The parameters describing the rheological properties of dough are presented in Table 3. The water absorption capacity of Tritordeum flours (58.4 %) was similar to that noted in barley and bread wheat flours, but significantly lower than in durum wheat flour (58.4 % vs 68.7 %). A comparison of the five remaining protein quality traits also revealed significant differences between the average values of Tritordeum lines and the reference cultivars, in particular bread wheat cv. Bombona (Table 3). The dough stability time of Tritordeum was significantly shorter in comparison with bread wheat and durum wheat (6.9 min vs 11.5 min and 9.5 min, respectively), but significantly longer than in barley (3.3 min). The analyzed Tritordeum lines were characterized by the highest variability in this parameter (RSD 48 %); its values were not normally distributed and negative kurtosis values indicated a flat distribution. In six Tritordeum lines, dough stability times exceeded 9 min, which points to the desirable properties of dough during mechanical treatment.

Dough consistency measured at point C2 on the Mixolab curve provides important information about changes in the properties of gluten under the influence of mixing and increased temperature. This

Table 1
Basic parameters describing the technological quality of grain of the studied Tritordeum lines and the reference cultivars of durum wheat, bread wheat, and naked barley.

Parameter	Tritordeum (*n* = *11*)						*T. durum* (*n* = 2)	*T. aestivum* cv. Bombona	*H. sativum* cv. Gawrosz
	Mean	Min. ÷ Max.	RSD (%)	Skew.	Kurt.	$p_{(S\text{-}W)}$			
V (%)	50	24 ÷ 82	44	0.22	−1.77	0.150	93***	72**	6***
Pr (% db)	14.0	12.5 ÷ 16.2	9	0.51	−1.16	0.296	15.6**	13.3	10.3***
GL (%)	34.3	25.3 ÷ 41.9	14	−0.05	−0.49	0.514	38.7*	31.9	ND
GI (%)	51	1 ÷ 89	79	−0.48	−2.01	0.002	76	90**	ND
FN (s)	279	186 ÷ 379	19	0.03	0.76	0.840	431***	335**	265
A (% db)	0.60	0.47 ÷ 0.74	15	0.11	−1.13	0.768	0.97***	0.54	0.82***

V – vitreousness; Pr – protein content; GL – gluten content; GI – gluten index; FN – falling number; A – ash content; RSD – relative standard deviation; Skew. – skewness; Kurt. – kurtosis; $p_{(S\text{-}W)}$ – probability in the Shapiro-Wilk test; % db – percentage on a dry basis; *, **, *** - difference relative to Tritordeum lines significant at $p < 0.05$, 0.01, and 0.001, respectively.

Table 2
Parameters of flour obtained from the analyzed grain in the laboratory milling test.

Parameter		Tritordeum (n = 11)						*Triticum durum* (n = 2)	*T. aestivum* cv. Bombona	*H. sativum* cv. Gawrosz
		Mean	Min. ÷ Max.	RSD (%)	Skew.	Kurt.	$p_{(S\text{-}W)}$			
Milling fraction (%)	>224	0.8	0.4 ÷ 1.9	53	1.49	1.93	0.038	3.6***	0.5*	3.5**
	150–224	16.4	11.6 ÷ 19.1	13	−1.15	1.84	0.359	35.1***	12.6***	10.3**
	132–150	12.8	8.9 ÷ 15.9	17	−0.15	−0.59	0.715	18.5***	11.3*	6.3***
	95–132	29.3	24.6 ÷ 34.9	11	0.00	−0.60	0.649	25.7**	35.1***	48.0***
	<95	40.6	36.5 ÷ 47.1	7	1.20	2.45	0.234	17.0***	40.5	31.9***
Flour	FY (%)	64.3	58.5 ÷ 69.1	6	−0.16	−1.31	0.547	63.5	69.3**	30.1***
	SD (UCD)	20.0	17 ÷ 22.4	9	−0.08	−1.27	0.388	26.1***	21.4	23.1*
	Asn (mg/kg)	185	102 ÷ 329	39	1.05	0.23	0.152	212	188	33***
	L^*_{FL}	91.05	90.4 ÷ 92.3	1	0.95	1.18	0.374	88.33***	90.51*	91.33
	a^*_{FL}	−3.01	−4.0 ÷ −1.4	25	0.85	0.99	0.673	−3.70*	−2.52	−1.39*
	b^*_{FL}	12.63	5.1 ÷ 16.2	24	−1.49	3.30	0.083	19.36***	11.59	5.34**

FY - flour yield; SD – starch damage; Asn – free asparagine content; L^*_{FL}, a^*_{FL}, b^*_{FL} –descriptors of flour color (L^* – lightness, a^* –green-red coordinate, b^* –blue-yellow coordinate); RSD – relative standard deviation; Skew. – skewness; Kurt. – kurtosis; $p_{(S\text{-}W)}$ – probability in the Shapiro-Wilk test; *, **, *** -difference relative to Tritordeum lines significant at $p < 0.05$, 0.01, and 0.001, respectively.

Table 3
Rheological properties of dough obtained from the grain of the analyzed cereal species.

Parameter		Tritordeum (n = 11)						*T. durum* (n = 2)	*T. aestivum* cv. Bombona	*H. sativum* cv. Gawrosz
		Mean	Min. ÷ Max.	RSD (%)	Skew.	Kurt.	$p_{(S\text{-}W)}$			
Associated with protein	W_{abs} (%)	58.4	55.4 ÷ 61.7	4	0.17	−1.03	0.831	68.7***	57.7	58.3
	T1 (min)	4.2	2.8 ÷ 6.2	28	0.66	−0.81	0.316	3.5	6.9***	0.9***
	Stability (min)	6.9	2.5 ÷ 10.3	48	−0.30	−2.12	0.010	9.5*	11.5**	3.3**
	C2 (Nm)	0.38	0.20 ÷ 0.53	33	−0.47	−1.47	0.079	0.55***	0.51**	0.39
	C1-C2 (Nm)	0.73	0.61 ÷ 0.92	16	0.54	−1.56	0.057	0.60**	0.60**	0.70*
	α (Nm min^{-1})	−0.09	−0.13 ÷-0.05	32	0.18	−1.24	0.609	−0.11	−0.13**	−0.06**
Associated with starch	C3 (Nm)	1.79	1.65 ÷ 2.04	7	0.65	−0.14	0.403	1.44***	1.92**	1.93**
	C4 (Nm)	1.42	0.84 ÷ 1.98	25	−0.34	−0.55	0.700	1.19	1.70*	1.03**
	C5 (Nm)	2.24	1.29 ÷ 3.08	26	−0.34	−0.32	0.499	1.88	2.33	1.69*
	C3-C2 (Nm)	1.42	1.23 ÷ 1.51	6	−1.06	0.87	0.186	0.89***	1.42	1.54**
	C3-C4 (Nm)	0.37	−0.04 ÷ 0.87	79	0.53	−0.62	0.600	0.25	0.22	0.91**
	D2 (°C)	51.4	50.1 ÷ 53.0	1	0.30	1.07	0.918	49.4***	49.5***	53.5***
	D3 (°C)	73.1	68.5 ÷ 77.0	5	−0.19	−1.79	0.120	69.6**	71.9	74.6
	β (Nm min^{-1})	0.59	0.21 ÷ 0.97	33	0.25	1.81	0.290	0.34**	0.50	0.48
	γ (Nm min^{-1})	−0.05	−0.11 ÷ 0.01	90	−0.48	−1.01	0.323	−0.06	−0.04	−0.15***

W_{abs} – water absorption; T1 – dough development time; C2, C1-C2 – protein weakening; α– indicator of protein weakening; D2, D3 – initial and final temperature of starch gelatinization, respectively; C3, C3-C2 – starch gelatinization; C4, C3-C4 – amylolytic activity; C5 – starch retrogradation; β – starching speed; γ - enzymatic degradation; RSD – relative standard deviation; Skew. – skewness; Kurt. – kurtosis; $p_{(S\text{-}W)}$ – probability in the Shapiro-Wilk test; *, **, *** - difference between the value for cultivar (-s) and the mean for Tritordeum lines significant at $p < 0.05$, 0.01, and 0.001, respectively.

parameter also indirectly describes bread quality (Banu et al., 2011; Dhaka & Khatkar, 2013), and it ranged from 0.20 Nm to 0.55 Nm. Dough consistency was lowest in Tritordeum and barley flours (0.38 Nm and 0.39 Nm on average, respectively) and highest in durum wheat flour (0.55 Nm on average) (Table 3). Low dough consistency at point C2 (below 0.3 Nm) is indicative of considerable dough softening and gluten weakening. Four Tritordeum lines were characterized by low values at point C2 as well as low values of GI (1–5). The higher value of parameter C1-C2 (0.73 Nm) also suggests that Tritordeum flour is less suitable for mechanical processing than bread wheat and durum wheat flour (0.60 Nm).

Starch, which is the largest component in wheat, plays an important role in bread quality, alongside the protein, i.e. setting of the crumb and stalling of bread (Eliasson, 2020). Starch gelatinization (C3) was higher in dough made from bread wheat and barley flours (1.92 Nm and 1.93 Nm, respectively), which indicates that it was more elastic than dough made from Tritordeum flour (1.79 Nm on average). Starch gelatinization was lowest in dough made from durum wheat flour (1.44 Nm on average). Bread wheat flour was also characterized by the highest amylolytic activity (C4, 1.70 Nm), which points to the highest stability of the starch solution during heating, as well as the highest starch retrogradation (C5, 2.33 Nm). These values were lower in barley flour (1.03 Nm and 1.69 Nm, respectively), whereas significant differences in these parameters were observed across Tritordeum lines, which could be attributed to differences in the activity of amylolytic enzymes.

The initial (D2) and final (D3) temperature of starch gelatinization strongly differentiated the compared cereal species and Tritordeum lines. These parameters were highest in barley flour (53.5 °C and 74.6 °C on average, respectively) and lowest in durum wheat flour (49.4 °C and 69.6 °C on average, respectively). All starch parameters were normally distributed, and the highest variability was noted in parameter γ (RSD = 90 %).

The quality of bread obtained in the laboratory baking trial is presented in Table 4, and the cross-sections of bread samples are shown in

Table 4
Quality characteristics of bread obtained from the analyzed cereal species in the laboratory baking test.

Parameter	Tritordeum (n = 11)						*T. durum* (n = 2)	*T. aestivum* cv. Bombona	*H. sativum* cv. Gawrosz
	Mean	Min. ÷ Max.	RSD (%)	Skew.	Kurt.	$p_{(S\text{-}W)}$			
BY (%)	139	133 ÷ 141	2	−1.87	4.72	0.010	142***	134**	133**
V100g (cm^3)	287	223 ÷ 378	17	0.64	−0.71	0.358	415***	420***	181**
H (N)	19.7	12.8 ÷ 26.6	29	0.15	−2.15	0.020	9.1***	12.4**	51.5***
ACR (μg/kg)	15.0	5.0 ÷ 27.4	47	0.37	0.00	0.334	14.7	21.9**	5.0**
L^*_{CRUMB}	70.85	66.14 ÷ 76.84	5	0.20	−1.25	0.602	74.60**	73.65*	66.05**
a^*_{CRUMB}	−1.71	−2.49 ÷ −0.43	34	1.19	1.35	0.169	−2.48**	−1.66***	1.95**
b^*_{CRUMB}	26.50	14.58 ÷ 35.74	25	−0.28	−0.56	0.911	27.37	19.53**	17.18**
L^*_{CRUST}	51.53	46.39 ÷ 56.62	6	0.19	−0.67	0.873	36.31***	44.68***	56.33
a^*_{CRUST}	14.31	12.91 ÷ 15.91	6	0.08	0.49	0.801	13.31**	15.17**	9.01***
b^*_{CRUST}	30.24	24.60 ÷ 34.01	9	−0.44	0.74	0.323	14.95***	24.32***	28.89

BY – bread yield; V100g – loaf volume per 100 g of bread; H – crumb hardness; ACR – acrylamide content; L^*, a^*, b^* - crumb color $_{(CRUMB)}$ and crust color $_{(CRUST)}$ (L^* – lightness, a^* –green-red coordinate, b^* –blue-yellow coordinate); RSD – relative standard deviation; Skew. – skewness; Kurt. – kurtosis; $p_{(S\text{-}W)}$ – probability in the Shapiro-Wilk test; *, **, *** - difference relative to Tritordeum lines significant at p < 0.05, 0.01, and 0.001, respectively.

Fig. S2. Bread yield (quantity of bread obtained per 100 g of flour) was highest for durum wheat (142 % on average) and lowest for bread wheat and barley (134 and 133 %, respectively). The bread yield of the examined Tritordeum lines ranged from 133 % (HTC1324) to 141 % (HTC 2083A, HT157, and HT352) with only minor variability in this parameter (RSD = 2 %). Specific volume per 100 g of bread, a key indicator of bread quality, ranged from 181 cm^3 for barley to 420 cm^3 for bread wheat on average. With the exception of barley flour, all flours met the requirements of Polish Standard PN-A-74105:1992 for specific loaf volume (minimum 200 cm^3) (Wiwart et al., 2023). Loaf volume varied considerably across Tritordeum lines, and accounted for 47 % (line HT352) to 90 % (HTC1324) of that noted in bread wheat.

Bread crumb hardness was a species-specific trait, and it was highest in barley bread (51.5 N) and lowest in durum wheat bread (9.1 N on average). Tritordeum bread was characterized by harder crumb than common wheat bread (19.7 N vs 12.4 N). Acrylamide content varied considerably across the analyzed bread loaves, ranging from 5 μg/kg in bread obtained from barley and Tritordeum lines HT 444 and HTC 2083, to 14.7 μg/kg in durum wheat bread, and 27.4 μg/kg in bread made from the grain of Tritordeum line HT 440.

The analyzed bread loaves also differed considerably in crumb and crust color. Crumb lightness (L^*) was influenced by cereal species, and this parameter was highest in durum wheat bread (L^* = 74.60), and lowest in barley bread (L^* = 66.05). In all bread loaves (excluding bread made from barley flour), crumb color was characterized by negative values of color descriptor a^*, which points to a high contribution of the green component. Crumb color had a high contribution of the red component only in barley bread (a^* = 1.95), whereas crumb color in durum wheat bread had a higher contribution of the green component that bread wheat and Tritordeum bread. All bread loaves were characterized by positive values of color descriptor b^*, which points to a high contribution of the yellow component. Crust color was lighter in Tritordeum bread (L^* = 51.53 on average) than in bread wheat and durum wheat bread (44.68 and 36.31, respectively), while the highest value of this parameter was noted in barley bread (L^* = 56.33). The contribution of the yellow component in crust color was significantly higher in Tritordeum bread (b^* = 30.24) than in bread wheat (24.32) and durum wheat (14.95) bread.

The results were processed by k-means clustering, and all objects were assigned to four clusters. The analysis was performed separately for 22 traits associated with the quality of grain and flour, and the rheological properties of dough, and 20 traits associated with milling and baking quality. The results are presented in Table 5 and Fig. 1. Two durum wheat cultivars (cluster 4), as well as four Tritordeum lines (HT438, HTC1324, HTC 2083, and HTC 2083a) and bread wheat cv. Bombona (cluster 2) were strongly discriminated based on the first group of traits. The objects in clusters 1 and 3 were not strongly discriminated by these traits (Fig. 1). Milling and baking quality traits were strongly discriminating variables, and the four clusters had a different composition than in the previous analysis. Cluster 4 was

Table 5
Classification of all lines and cultivars by k-means clustering, separately for the basic parameters and rheological properties of grain and flour, milling quality and baking quality.

Basic parameters and rheological properties of grain and flour							
Cluster 1		Cluster 2		Cluster 3		Cluster 4	
Line/cultivar	DfC	Line/cultivar	DfC	Line/cultivar	DfC	Line/cultivar	DfC
HT129	4.77	Bombona	6.37	HT440	14.46	Duragold	7.37
HTC2060	18.26	HT438	7.74	JB3	10.46	Floradur	7.37
HT352	13.52	HTC1324	16.58	HT444	8.66		
		HTC2083	7.73	HT157	11.79		
		HTC 2083a	9.52	Gawrosz	22.18		
Traits associated with milling quality and baking quality							
Cluster 1		Cluster 2		Cluster 3		Cluster 4	
Line/cultivar	DfC	Line/cultivar	DfC	Line/cultivar	DfC	Line/cultivar	DfC
Bombona	4.80	HT440	2.64	HT444	2.04	HT438	0.00
HTC1324	4.80	JB3	5.37	HTC2083	5.29		
		HT129	3.18	HTC 2083a	9.96		
		HTC2060	2.57	HT157	2.42		
		HT352	5.01	Floradur	2.42		
		Duragold	2.57				
		Gawrosz	5.01				

DfC – distance from the cluster centroid.

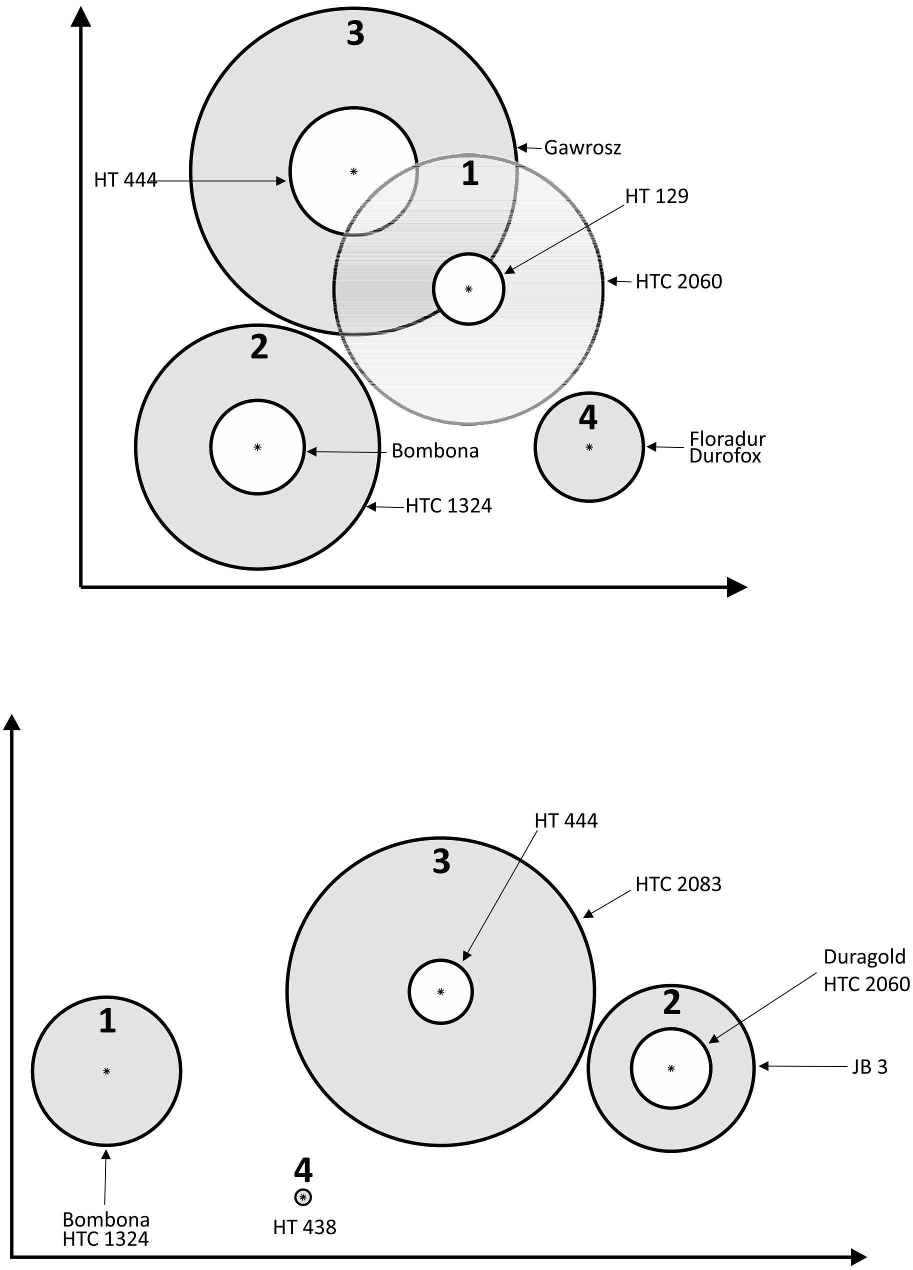

Fig. 1. Graphic interpretation of the results of k-means clustering analysis. The top figure presents the results for 22 traits associated with the quality of grain and flour, and the rheological properties of dough (refer to Table 3), and the bottom figure presents the results for 20 traits associated with the milling and baking quality of the analyzed lines and cultivars (refer to Table 4). The length of cluster radii (1 … 4) and distances between data points and centroids are presented on the same scale in both figures. The figures were generated in CorelDraw® X8 (Corel Corporation).

composed only of Tritordeum line HT 438 (Fig. 1). Cluster 1 contained bread wheat cv. Bombona and Tritordeum line HTC1324, both of which were included in cluster 2 in the previous analysis. Cluster 2 was largest, and it contained five Tritordeum lines, durum wheat cv. Floradur, and barley cv. Gawrosz. Durum wheat cv. IS Duragold was classified in cluster 3.

4. Discussion

This article presents the results of a comprehensive study comparing the technological quality and baking quality of flours obtained from 11 spring Tritordeum breeding lines with flours obtained from modern cultivars of durum wheat, bread wheat, and naked barley. The results of technological quality analyses conducted by other authors indicate that Tritordeum grain is suitable for breadmaking and is characterized by high nutritional value and health-promoting properties (Haro et al., 2022; Sánchez-León et al., 2021). Tritordeum flour is used in commercial bread production (https://www.Tritordeum.com/Tritordeum-bread/; https://www.panetier.es). However, most studies on the technological quality of Tritordeum grain examined a small number of cultivars and traits, and they were conducted mainly in the Mediterranean countries. In the present study, four modern cultivars of three cereal species developed in Central Europe were used as the reference materials. *Triticum durum* is not commonly used for breadmaking, but it is a parental component of Tritordeum, which is why this cereal species was included in the analysis. The second parental component, *H. chilense*, is a species of wild barley that is not used in bread production, which is why the cultivated form of this species (*H. sativum*) was used in the study. In turn, the grain of the elite bread wheat cv. Bombona is characterized by the highest values of traits that determine technological and baking quality.

In the analyzed Tritordeum lines, the values of GI were generally low (51 % on average), whereas gluten content was relatively high (34 %), which is indicative of low gluten strength, mainly due to the low quality of gluten proteins (Martinek et al., 2003). However, Tritordeum lines differed significantly in GI values (RSD = 79 %). This parameter was determined at 1 % in two lines (HT129 and HT440) and at 2 % in line HT352 (weak gluten). In turn, the GI exceeded 80 % (strong gluten) in four lines and reached 89 % in line HTC 2083, which is comparable with the value noted in the elite bread wheat cv. Bombona. Gluten content exceeded 30 % in ten lines, and it was somewhat higher than 25 % in only one line (HTC 2083a). These traits were bound by a strong negative correlation (−0.759) which was significant at $p < 0.01$. Takač et al. (2021) reported similar, but somewhat weaker correlations in bread wheat and spelt. The presence of a strong negative correlation between the analyzed traits (similar gluten content and very high variability in GI values) suggests that Tritordeum lines should be selected for breeding purposes based mainly on GI values.

The ash content of Tritordeum flour ranged from 0.47 % to 0.74 %, and similar values were reported by Nitride et al. (2022). Tritordeum flour contained less ash than durum wheat and barley flours, and it was characterized by higher average values of color lightness L^*. Despite the fact that Tritordeum flour was somewhat more abundant in ash than bread wheat flour (non-significant difference), it was not darker in color.

Cereal grain for breadmaking should be characterized by high flour yield and very low bran content. These parameters have been rarely analyzed in Tritordeum. According to Erlandsson (2010), the flour extraction rate of Tritordeum line HT 1608 was low at 34.5 % and 39.9 %. In the current study, the extraction rate of Tritordeum flour reached 64.3 % and was similar to that noted in the elite bread wheat cv. Bombona. In the work of Martin et al. (1999), this parameter was determined at 67 %, and the cited authors concluded that Tritordeum is characterized by low vitreousness and low hardness of grain, but it has a high flour extraction rate and is therefore more suitable for the production of baked goods than pasta. In three Tritordeum lines (HT438, HTC1324, and HT444), the flour extraction rate exceeded 68 %, and ash content ranged from 0.47 % to 0.57 % (on a dry matter basis), which is indicative of high milling efficiency. Moreover, the differences in flour quality across Tritordeum lines suggest that it is possible to select cultivars with desirable quality attributes.

In the literature, the rheological properties of Tritordeum flour were assessed mostly with the use of an alveograph, and baking strength W was generally lower in Tritordeum than in bread wheat. However, cultivars with desirable rheological characteristics and high baking quality could be selected. Martinek et al. (2003) analyzed the rheological properties of cereals with a Brabender micro-farinograph and found that the water absorption capacity of Tritordeum flour was relatively high and comparable with bread wheat. However, Tritordeum lines were characterized by shorter stability than bread wheat (Martín & Alvarez, 2000; Martinek et al., 2003). Grain samples analyzed in the present study differed in baking quality. Lines HT438, HTC1324, and HT444 were characterized by good baking performance due to high water absorption (59.3 % on average), longer dough development time T1 (5.1 min), longer stability time (9.9 min), and appropriate consistency at point C2 (0.48 Nm). The resulting doughs were cohesive during kneading and were easy to divide and shape into loaves, which suggests that these lines are suitable for automated bakery production.

Tritordeum bread loaves were well-risen and characterized by desirable crust color, relatively even crumb porosity, and desirable, yellowish crumb color that is preferred by consumers. The volume of bread loaves made from Tritordeum flour was smaller than that of bread loaves made from durum wheat and bread wheat flour; however, the volume of loaves made from selected Tritordeum lines was equivalent to 90-72 % of the volume of loaves made from the elite bread wheat cv. Bombona. Similar results were reported by Martín and Alvarez (2000) who found that the volume of Tritordeum bread loaves was equivalent to 92-60 % of the volume of loaves made from hard wheat cv. Yecora. The production technology of Tritordeum bread should be further optimized to increase loaf volume and improve the digestibility of bread while maintaining its high sensory quality. Research has shown that Tritordeum bread could be an alternative for healthy individuals without wheat-related pathologies who want to reduce their gluten consumption without harming their gut health (Haro et al., 2022).

Bread from Tritordeum was characterized by favorable, lower than common wheat bread, acrylamide content, which has been classified as a group 2a carcinogen. However the acrylamide content of all breads did not exceed the levels indicated by the Commission Regulation (EU) 2017/2158 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food (Official Journal of the European Union, L 304, 21. 11. 2017). In the studied flours, the highest content of acrylamide was found in line HT440 (27.4 μg/kg), but it did not exceed 50 μg/kg, the benchmark level for wheat bread, in any of the tested samples. Acrylamide content was significantly correlated with the content of free asparagine ($r = 0.587$, $p < 0.05$). Asparagine and reducing sugars such as glucose, fructose, and maltose are precursors of acrylamide. Raffan and Halford (2019) conducted an extensive review of the literature on acrylamide levels in various food products, the metabolic pathways of acrylamide, and the genetic determinants of free asparagine accumulation in grain. According to Shewry et al. (2023), Tritordeum is more abundant in amino acids, except for asparagine, than other bread cereals. The acrylamide content of wheat and rye bread ranged from below 10 μg/kg to 99 μg/kg in a Polish monitoring study conducted in 2007–2009 (Mojska et al., 2011) and from 3.6 μg/kg to 163 μg/kg in the work of Roszko et al. (2020).

Several factors contribute to the lower acrylamide content in Tritordeum bread compared to common wheat bread. Although both cereals had similar asparagine content, Tritordeum flour had lower starch damage than common wheat flour (20.0 vs. 21.4 UCD). Our research showed that higher acrylamide content in bread was associated with higher asparagine and protein content, weaker gluten network, increased starch damage, and lower final temperature of starch gelatinization. Mulla et al. (2010) found a correlation between starch damaged and acrylamide content in bread. The extent of milling influences reducing sugars (Sadd et al., 2008) and amino acids (Claeys et al., 2005), with Wang et al. (2017) identifying damaged starch content and dough fermentation as major determinants of the acrylamide formation. Increased starch damaged led to higher reducing sugar, which, along with asparagine, are precursors of acrylamide in bread. These findings suggest that acrylamide mitigation in bread can be achieved also by reducing starch damage in wheat flour (Wang et al., 2017).

All of the studied lines and cultivars were discriminated by the k-means clustering analysis. In this approach, data are divided into a predefined number of clusters. The study was conducted on Tritordeum, durum wheat, bread wheat, and barley, and the objects were divided into four clusters. Tritordeum line HTC1324 was most similar to the elite bread wheat cv. Bombona, and these objects were classified in the same cluster based on the quality of grain and flour, and the rheological properties of dough (cluster 2), as well as milling and baking quality (cluster 1). As expected, both durum wheat cultivars were classified into a single, distinct cluster (4) based on the first group of traits, but they differed significantly in terms of milling and baking quality. Durum wheat cv. IS Duragold was highly similar to barley and five Tritordeum lines, whereas durum wheat cv. Floradur was similar to four other Tritordeum lines. Line HT438 was the only object in cluster 4 which was localized in the proximity of cluster 1. This line was highly similar to bread wheat cv. Bombona in terms of the quality of grain and flour and the rheological properties of dough. These observations suggest that Tritordeum lines HTC1324 and HT438 are particularly suitable for breadmaking.

The results of the study clearly indicate that Tritordeum breeding lines can be selected for high technological and baking quality. Considerable variability in the examined lines, as well as many similarities between Tritordeum and bread wheat, including self-pollination, can contribute to breeding success and the emergence of new Tritordeum varieties with high technological quality.

In conclusion, the lower acrylamide content in Tritordeum bread is likely due to its reduced asparagine content, lower starch damage content and denser bread structure. However, other factors, such as sugar composition and Maillard reaction kinetics, may also play a role in this outcome. To fully understand the mechanisms behind the reduced acrylamide formation, further research is needed, including a more detailed analysis of sugar composition and a controlled examination of the Maillard reaction.

CRediT authorship contribution statement

Marian Wiwart: Writing – review & editing, Visualization, Methodology, Investigation. **Anna Szafrańska:** Writing – original draft, Validation, Methodology, Investigation. **Elżbieta Suchowilska:** Writing – original draft, Resources, Investigation, Data curation, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117715.

Data availability

Data will be made available on request.

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