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Effects of iodine, selenium, and zinc biofortification on wheat flour and dough properties for bread making

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ABSTRACT

Micronutrient deficiencies, or hidden hunger, remain a critical global nutritional challenge. Agronomic biofortification of staple crops like wheat with iodine (I), selenium (Se), and zinc (Zn) represents a promising solution to address this issue. This study assessed the impact of foliar-applied I, Se, and Zn, individually and in combination, on flour and bread quality in two wheat cultivars. In Bezostaja-1, biofortified whole wheat flour reached 619 µg/kg I, 623 µg/kg Se, and 75 mg/kg Zn, with white flour retaining 32 %, 74 %, and 23 %, respectively. Co-application of nutrients reduced protein content by ~10 % and slightly decreased dough gas retention (77 %–75 %) yet had minimal effect on bread volume. Individually, Zn biofortification reduced loaf volume, whereas Se and I treatments maintained it. Moreover, pan loaf bread preserved more micronutrient concentrations than flatbread. Despite variations in micronutrient retention among bread types, biofortified wheat presents a sustainable approach to enhancing dietary micronutrient intake without compromising bread-making quality.

1. Introduction

Micronutrient deficiencies, often referred to as “hidden hunger,” affect over 2 billion people worldwide, leading to serious health implications and economic burdens (Gödecke et al., 2018). Though generally associated with undernutrition in developing regions, inadequate intake of essential elements such as iodine (I), selenium (Se), and zinc (Zn) is also becoming more common in high-income countries, driven in part by shifting dietary patterns and reduced salt consumption (Assunção et al., 2022). Moreover, micronutrient availability is shaped not only by diet, but also by soil factors that influence plant uptake. Limited phytoavailability of I, Se, and Zn in cultivated soils can constrain their accumulation in edible plant tissues and, in turn, reduce dietary intake. Research indicates that soil characteristics such as pH, organic matter content, and soil moisture affect the plant-available pool of micronutrients. For example, the bioavailability of iodine is higher in acidic conditions where iodide is the main form whereas the bioavailability of Se increases with increasing of soil pH; in contrary the bioavailability of Zn decrease in this range. Furthermore, higher soil organic matter in many cases decreases the bioavailability of I, Se and Zn due to binding effect thereby reducing their mobility (Cakmak, 2008; Cakmak et al., 2017; Kihara et al., 2020).

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Given these limitations, agronomic strategies such as biofortification have been developed to enhance the micronutrient content of staple crops. Among these, foliar application has proven particularly effective for increasing grain concentrations of Zn, I, and Se across diverse crops and environments (Budke et al., 2021; Cakmak & Kutman, 2018; Lyons, 2018; Prom-u-thai et al., 2020; Ram et al., 2024; Zou et al., 2019). Most studies, however, have focused on single-nutrient applications. The co-application of multiple micronutrients ($\geq$3), although potentially more beneficial, remains largely unexplored. Furthermore, few studies have investigated the stability of these nutrients during post-harvest processing or their effects on the physicochemical and rheological properties of wheat flour and dough.

These functional properties are critical to the performance of flour in bread-making applications. Biofortification of wheat with Zn and Se has been reported to influence protein content, gluten network formation, and bread volume (Khan et al., 2023; Kong et al., 2024). Selenium, for example, can enhance dough elasticity and fermentation kinetics by disrupting disulfide bonds within gluten proteins, improving both antioxidant capacity and shelf life (Du et al., 2023). Zinc enrichment, on the other hand, has been associated with increased dough stiffness and reduced extensibility, which may negatively affect bread texture (Karaduman et al., 2023). However, the effects of iodine biofortification, whether applied alone or in combination with Zn and Se, on flour functionality and dough rheology remain poorly understood. This knowledge is important for developing nutritionally enhanced flours that retain the processing qualities required for industrial-scale production of wheat-based products.

In addition to functional properties, the effectiveness of biofortification depends on the retention of micronutrients throughout post-harvest processing and food preparation. Studies indicate that Zn retention in biofortified whole wheat flour is relatively high, with milling losses mainly affecting the bran and germ fractions (Velu et al., 2014). Baking and fermentation processes cause minor Zn losses, preserving a substantial proportion of its bioavailability in the final baked product (Rosado et al., 2009). Likewise, Se remains largely stable during milling and baking, with minimal volatilization losses occurring at high temperatures (Poblaciones et al., 2021). Its chemical form plays a critical role in retention, as organic Se species are more stable than inorganic forms (Lyons, 2018). In contrast, iodine retention is highly variable due to its volatility and susceptibility to thermal sensitivity. Losses can occur during baking; however, strategies such as iodine-binding carriers and controlled fermentation may help mitigate these effects (Zimmermann & Andersson, 2021). Overall, the retention of biofortified minerals in wheat-based products is influenced by processing methods, mineral speciation, and interactions with flour components. Further research is needed to optimize processing conditions and improve micronutrient stability in bread-making applications.

Therefore, this study aims to: i) investigate variations in I, Se, and Zn concentrations in wheat grains following foliar biofortification, applied individually or in combination under field conditions; ii) evaluate the impact of biofortification treatments on the accumulation of other elements in the grains; iii) examine the distribution of these micronutrients across different milling fractions; iv) assess the effects of biofortification on flour and dough physicochemical characteristics; v) analyse dough development and gas release dynamics; and vi) determine the retention of I, Se and Zn in bread produced from biofortified wheat using different baking processes.

2. Materials and methods

2.1. Biofortification field trials

Field experiments were conducted in 2021 and 2022 using two bread wheat (*Triticum aestivum L.*) cultivars, Bezostaja-1 and Nacibey, at the Transitional Zone Agricultural Research Institute, Eskisehir, Turkey. A split-plot randomized complete block design with four replicates was employed. Plots (30 m^2 each) received uniform basal fertilization and were subjected to foliar applications of I (0.04 % w/v KIO_3), Se (0.001 % w/v Na_2SeO_4), Zn (0.50 % w/v $ZnSO_4 \cdot 5H_2O$), either individually or in combination, at stem elongation, heading, and early milk stages. Those selected doses and application timings were based on optimized application protocols established by the HarvestPlus and HarvestZinc global agronomic biofortification programs. These concentrations have been used in previous multi-country studies and are recognized for effectively achieving nutritionally desirable levels of these micronutrients in wheat grain (Zou et al., 2019). Control plots received no foliar treatment. Soil characteristics and field management are provided in Annex B.1.

2.2. Determination of grain yield and mineral content

At maturity, yield was recorded, and grains were prepared for mineral analysis. Following an acid digestion, I and Se total concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS; NexION 350D, PerkinElmer) and Zn, iron (Fe), and sulfur (S) were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES; iCAP 7200, Thermo Fisher Scientific). Quality control procedures included the use of internal standards, blanks, and certified reference materials, as detailed in Annex B.2. The same analytical protocols were applied to the analysis of flour fractions and bread.

2.3. Milling and experimental design

Harvested grains were milled into whole wheat flour (WWF) or white flour (WF), with bran and shorts as by-products of WF. Whole wheat flour was obtained using a FOSS Analytical mill (Hilleröd, Denmark) and retained all components of the grain (97 % extraction rate). For the production of WF, the wheat grains were first tempered for 24 h to achieve a target moisture content of 15.5 % and then milled using a Bühler MLU-202 laboratory roller mill (Bühler Group, Switzerland). WF extraction rate was 72 %. WWF was used in both flatbread and pan loaf production, whereas WF was used exclusively for pan loaf. All flour fractions were stored at −18 °C until analysis.

2.4. Flour and dough characterization

Flour quality was assessed using standard ICC and ISO methods (Annex B.3.). Moisture, ash, and protein contents were determined, and gluten index was calculated to assess gluten strength. Dough functionality was evaluated using farinograph and falling number measurements to determine water absorption, development time, and alpha-amylase activity. Gas production and retention during proofing were analyzed with a rheofermentometer, and retention coefficient was calculated using Eq. [1].

$$\textit{Retention coefficient}\ (\%) = \left(\frac{\textit{The volume of gas retained in the dough}}{\textit{Total volume of produced gas}}\right) x100 \qquad \text{Eq.}[1]$$

2.5. Baking performance

For flatbread preparation, 300 g of WWF was mixed with 500 ml of boiling water (85–90 °C). The dough was manually kneaded for 3 min, then covered and rested for 15 min at room temperature (22–25 °C). It was then divided into eight equal portions, each rolled to a thickness of 0.5 mm, shaped into spheres, and cut into 9 cm circles. Baking was carried out on an electric hot plate for 1.5 min on each side.

Pan loaf preparation included 300 g of flour (either WWF or WF), 4.5 g iodized salt, 3 g instant dry yeast, 0.0075 g vitamin C, malt, and water. Water and malt quantities were adjusted based on farinograph and falling number test results, respectively. Dough mixing was performed using a spiral dough mixer (Diosna SP12, Germany). Proofing was carried out in three stages in a proofer (Panem FPC 5 HR, France) maintained at 30 °C and 85 % relative humidity: pre-proofing for 10 min, intermediate proofing for 30 min with shaping using calibration slats, and final proofing for 65 min in greased tins.

Baking was conducted in an FP 12 oven (Miwe aero, Germany) in three phases: 2 min at 230 °C with 200 ml steam injection, followed by 13 min at 230 °C with the steam valve closed, and a final 5 min at 200 °C with the valve open. After cooling, bread height and loaf volume were measured using a Volscan Profiler 600 (Stable Micro Systems, United Kingdom) and precision calipers (accuracy 0.01 mm). Loaf volume per kilogram of flour (VOLfc) was calculated using Eqs. [2] and [3] as described by Hellemans (2020). Micronutrient content of baked bread was analyzed as described above in section 2.2.

$$flour : dough - ratio = \frac{[flour\ weight]}{[flour + water + yeast + malt + salt + ascorbic\ acid]} \qquad \text{Eq.[2]}$$

$$VOLfc = \left(\frac{\left[\frac{\text{Volume}}{100}\right]}{flour : dough - ratio}\right) x1000 \qquad \text{Eq.[3]}$$

2.6. Statistical analysis

All measurements were conducted in triplicate, except for gluten, which was analyzed in duplicate due to sample limitations, and water absorption, which was measured once as to define the formulation parameters for the baking trials. Data were statistically evaluated using SPSS Statistics (v29; IBM Corp., Armonk, NY), and RStudio (v2024.04.2). ANOVA with Tukey's HSD ($p < 0.05$) was used for significance. Pearson's correlation models were used to assess elemental uptake and nutrient enrichment effects.

3. Results

3.1. Impact of biofortification on grain mineral concentration

Foliar biofortification significantly increased I, Se, and Zn concentrations in both wheat cultivars, Bezostaja-1 and Nacibey ($p < 0.05$, Fig. 1). In 2021, grain iodine levels rose from 25 to 557 μg/kg in Bezostaja-1 and from 8 to 254 μg/kg in Nacibey following biofortification treatment. In 2022, however, iodine concentrations declined by 83 % in Bezostaja-1 and 61 % in Nacibey grains. The cause of this reduction remains unclear (see Discussion). Zinc concentrations also increased in 2021, from 15 mg/kg (unsprayed) to 58 mg/kg in Bezostaja-1, and up to 40 mg/kg in Nacibey. Selenium levels rose from 34 to 436 μg/kg in Bezostaja-1 and reached 279 μg/kg in Nacibey. The highest Se concentration was recorded in 2022 in Nacibey, at 617 μg/kg.

The co-application of nutrients resulted in lower grain iodine concentrations compared to its individual treatment in both cultivars. For Se and Zn, this reduction was observed only in Bezostaja-1, which may be attributed to interactive effects during nutrient uptake and translocation (Cakmak et al., 2020). Nevertheless, in the Nacibey cultivar, combined biofortification led to higher concentrations of Zn and Se compared to individual applications. In addition to these results, Zn treatments contributed to an increase in Fe content in the grain, with Fe levels rising by 11 %–49 % relative to the control.

3.2. Yield and other nutrients accumulation

Overall, foliar biofortification did not influence grain yield across wheat cultivars or treatments. The mean grain yield was 4.19 t/ha for cultivar Bezostaja-1 and 3.29 t/ha for cultivar Nacibey. Notably, control grains from Nacibey exhibited yields below the annual average of the treatments, possibly due to variations in soil conditions or farming practices (Table A.1). However, in 2022, these cultivars demonstrated their highest yields, averaging 7.25 t/ha across all biofortification

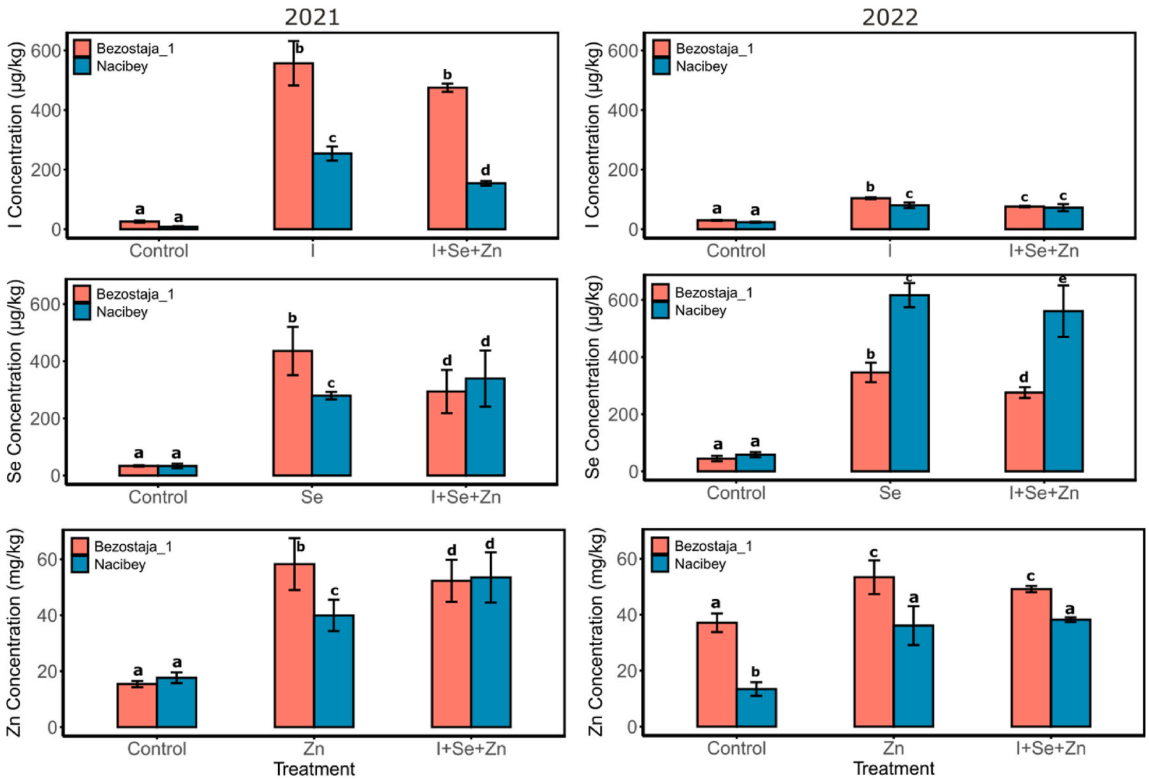

Fig. 1. Iodine, Se, and Zn concentrations in the wheat grains following the biofortification treatments, for two wheat cultivars (Bezostaja-1 and Nacibey) in two harvest years (2021 and 2022). Letters above the bars indicate significant differences between treatments and wheat cultivars within one cropping season ($p \leq 0.05$).

treatments Furthermore, moderate correlations were observed between Zn and Fe, as well as Zn and S ($p < 0.05$). A strong positive correlation was also found between S and Fe ($r = 0.82$, $p < 0.01$), irrespective of treatment, indicating potential co-accumulation or shared physiological pathways (Fig. 2).

3.3. *Nutritional content and functional properties of flour*

3.3.1. *Mineral content*

The milling process of biofortified wheat grains produced flours with differing levels of enhanced mineral concentrations. Whole wheat flour, which includes the bran and germ, retained I, Se, and Zn levels comparable to their corresponds grains of both Bezostaja-1 and Nacibey cultivars (Table 1). White flour, primarily composed of endosperm, had lower mineral concentrations than WWF but higher concentrations than untreated WF. In Nacibey, biofortified WF contained 65 μg/kg I, 288 μg/kg Se, and 14 mg/kg Zn, compared to 45 μg/kg I, 33 μg/kg Se, and 4 mg/kg Zn in untreated the flour. Similarly, in Bezostaja-1, biofortified WF contained 203 μg/kg I, 295 μg/kg Se, and 11 mg/kg Zn, whereas the control had only 1 μg/kg I, 31 μg/kg Se, and 4 mg/kg Zn.

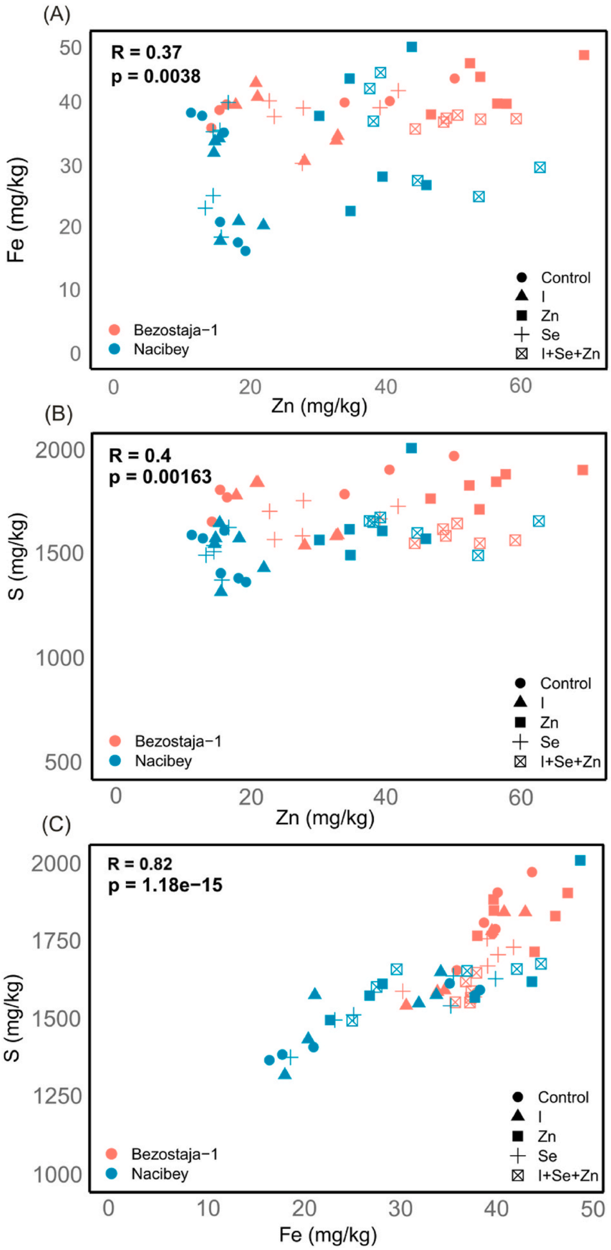

Fig. 2. Correlations between grain mineral concentrations in two wheat cultivars (Bezostaja-1 and Nacibey) grown under five treatment conditions (Control, I, Se, Zn, and I + Se + Zn). Panel (A) shows the relationship between Fe and Zn; (B) between sulfur (S) and Zn; and (C) between S and Fe. Cultivars are differentiated by color, and treatments are represented by distinct point shapes. Pearson's correlation coefficient (R) and p-values are included to reflect the strength and significance of each relationship.

During WF production, a substantial proportion of the minerals remained in the bran and germ. As a result, the retention rates of minerals in the WF were 68 % for I, 74 % for Zn, and 25.5 % for Se. The remaining micronutrient-rich by-products could be repurposed as functional ingredients to enhance nutritional value and promote sustainability.

3.3.2. *Functional properties*

3.3.2.1. Protein, gluten and ash content. Foliar biofortification had a reducing effect on protein content, with responses varying by cultivar, micronutrient treatment, and experimental year (Table A.2). In Bezostaja-1, the co-application of I, Zn, and Se resulted in a reduction of protein content by 16.81 % in 2022 and 9.26 % in 2021. Individual micronutrient treatments showed diverse responses: Se application consistently reduced protein content by 9.86 % in 2022 and 6.87 % in 2021, while Zn led to a 16.09 % decrease in 2022 but an increase of 3.37 % in 2021. Iodine had a minimal effect, reducing protein content by 1.40 % in 2022. In Nacibey (2022), combined treatment resulted in a moderate protein content reduction of 3.69 %. The highest decrease was observed with Se application (4.55 %), followed by Zn (3.69 %), while iodine had the least impact (2.09 %). Despite these reductions, both biofortified cultivars-maintained protein levels between 10 % and 15 %, which positively contributes to flour strength.

The quality of flour, dough, and bread is predominantly determined by gluten proteins, which constitute approximately 80–85 % of the total protein content. These proteins are primarily composed of gliadin and glutenin (Guo et al., 2021). In our study, WWF contains 37 %–50 % wet gluten for Bezostaja-1 and 29 % to 38 % for Nacibey (Table 2). In comparison to their gluten indices, WWF showed lower gluten indices compared to the white flour (62–81 %). Moreover, the ash content remained relatively constant across the different treatments for both WF and WWF, remaining within the range of referenced commercial flours from 1.5 % to 2 % for WWF and 0.5 %–0.7 % for WF. However, an exception was observed in the Bezostaja-1 WWF in 2022, where significant variation between the biofortified treatments and the control was noted. The maximum ash content was recorded in the iodine treatment (2.25 %).

3.3.2.2. Mixing and dough properties. The falling number analysis revealed distinct sensitivities of Bezostaja-1 flour compared to Nacibey across treatments (Table 2). In WWF, the control group showed the highest falling number 418 s, which significantly decreased with biofortification treatment ($p < 0.05$). Zinc treatment had the most substantial reduction 171 s, followed by iodine 232 s and Se 264 s. The combined treatment showed a partial mitigating effect, resulting in a falling number of 289 s, higher than individual treatments but significantly lower than the control. A similar trend was observed in WF, where the control recorded the highest value 331 s. Zinc treatment again caused the larger reduction 187 s, followed by iodine 210 s and Se 240 s, while the combined treatment yielded an intermediate value 250 s. In contrast, Nacibey exhibited less sensitivity to treatments. In whole wheat flour, the control maintained a falling number of 488 s, with no significant change from Se treatment 482 s. However, individual biofortification of I, Zn, and their co-application with Se resulted in moderate reductions to 478 s, 472 s, and 448 s, respectively. In WF, falling number values were stable across all conditions, with the control 447 s

Table 1
Iodine, Se and Zn concentrations in wheat grain fractions after milling per treatment, harvesting year and wheat cultivar.

Flour type	Treatment	I (μg/kg)		Treatment	Se (μg/kg)		Treatment	Zn (mg/kg)	
		Bezostaja-1	Nacibey		Bezostaja-1	Nacibey		Bezostaja-1	Nacibey
2022									
Whole wheat flour	*Control*	30 ± 2.5	32 ± 0.1	*Control*	47 ± 4.6	60 ± 6.8	*Control*	43 ± 0.8	13 ± 0.5
	I	103 ± 3.4^{*a}	92 ± 6.3*	*Se*	309 ± 23^{*a}	623 ± 60^{*a}	*Zn*	56 ± 1.3^{*a}	30 ± 2.4^{*a}
	I + Se + Zn	136 ± 5.2^{*b}	92 ± 4.8*	*I + Se + Zn*	261 ± 9.2^{*b}	500 ± 9.3^{*b}	*I + Se + Zn*	49 ± 2.1^{*b}	37 ± 2.4^{*b}
2021									
Whole wheat flour	*Control*	11 ± 2.5	8 ± 0.8	*Control*	35 ± 2.8	42 ± 1.7	*Control*	20 ± 5.1	19 ± 0.1
	I	619 ± 9.1^{*a}	210 ± 9.2^{*a}	*Se*	384 ± 1.6^{*a}	401 ± 21^{*a}	*Zn*	54 ± 2.4^{*a}	45 ± 2.0^{*a}
	I + Se + Zn	474 ± 14^{*b}	167 ± 8.3^{*b}	*I + Se + Zn*	251 ± 5.1^{*b}	333 ± 17^{*b}	*I + Se + Zn*	50 ± 0.7^{*b}	50 ± 1.8^{*b}
White flour	*Control*	1 ± 0.3	45 ± 7.1	*Control*	31 ± 2.5	33 ± 0.3	*Control*	4 ± 0.3	4 ± 3.1
	I	203 ± 1.6^{*a}	65 ± 1.6*	*Se*	295 ± 0.4^{*a}	288 ± 13^{*a}	*Zn*	11 ± 0.7^{*a}	14 ± 0.2*
	I + Se + Zn	147 ± 2.4^{*b}	54 ± 7.6	*I + Se + Zn*	205 ± 3.1^{*b}	216 ± 4.8^{*b}	*I + Se + Zn*	10 ± 0.7^{*b}	15 ± 0.5*
Shorts	*Control*	12 ± 3.1	183 ± 32	*Control*	38 ± 2.6	147 ± 16	*Control*	35 ± 1.6	51 ± 3.4
	I	917 ± 4.2^{*a}	191 ± 31^{*a}	*Se*	420 ± 46^{*a}	338 ± 20^{*a}	*Zn*	111 ± 3.2^{*a}	103 ± 7.2^{*a}
	I + Se + Zn	590 ± 70^{*b}	314 ± 10^{*b}	*I + Se + Zn*	286 ± 17^{*b}	192 ± 2.5^{*b}	*I + Se + Zn*	82 ± 3.7^{*b}	78 ± 3.5^{*b}
Bran	*Control*	103 ± 7.5	71 ± 4	*Control*	87 ± 6.6	51 ± 2.9	*Control*	53 ± 3.1	54 ± 1.80
	I	1631 ± 144^{*a}	678 ± 54^{*a}	*Se*	693 ± 57^{*a}	486 ± 15^{*a}	*Zn*	184 ± 15^{*a}	124 ± 2.7^{*a}
	I + Se + Zn	1461 ± 92^{*b}	767 ± 91^{*b}	*I + Se + Zn*	497 ± 29^{*b}	420 ± 16^{*b}	*I + Se + Zn*	150 ± 11^{*b}	148 ± 1.9^{*b}
Sig.	*Cultivar (C)*	***		*Cultivar (C)*	***		*Cultivar (C)*	***	
	Year (Y)	***		*Year (Y)*	***		*Year (Y)*	ns	
	Flour type (F)	***		*Flour type (F)*	***		*Flour type (F)*	***	
	Treatment (T)	***		*Treatment (T)*	***		*Treatment (T)*	***	
	$C \times Y$	***		$C \times Y$	***		$C \times Y$	***	
	$C \times F$	***		$C \times F$	***		$C \times T$	*	
	$C \times T$	***		$C \times T$	ns		$Y \times T$	***	
	$Y \times T$	***		$Y \times T$	***		$F \times T$	***	
	$F \times T$	***		$F \times T$	***		$C \times Y \times T$	*	
	$C \times Y \times T$	***		$C \times Y \times T$	***				
	$C \times F \times T$	***		$C \times F \times T$	***				

Between control and biofortification treatments (*) indicate significant differences ($p \leq 0{,}05$). Values within the same flour type, year and wheat cultivar with different letters are significantly different from each other ($p \leq 0{,}05$). ns: not significant, * and *** indicate significance (Sig.) at ($p < 0.05$, $p < 0.001$).

showing minimal differences from iodine 454 s, Se 442 s, Zn 447 s, and the combined treatment 444 s. Interestingly, these patterns were not replicated in the second harvest year (2022), where no significant differences were observed among treatments for either Bezostaja-1 or Nacibey. except for a minor reduction observed in the cocktail treatments of Nacibey WWF in 2022. The falling number consistently exceeded the quality threshold of 250 s, indicating a reduced α-amylase activity (Knapowski et al., 2009).

Furthermore, higher water absorption rates were observed in whole wheat flour, due to its high fibre content, approximately 66 % in Bezostaja-1 and 62 % in Nacibey, compared to 58 % in white flour (Table A.3). Dough development times varied between cultivars, with Bezostaja-1 consistently displaying shorter development times than Nacibey in both WWF and WF. Additionally, development times were longer in WF than in WWF. The biofortification treatments were associated with variations in dough stability and softening values across cultivars and flour types. In Bezostaja-1, WWF showed a softening value of 83 FU, while white flour reached 87 FU. In Nacibey, softening values were 89 FU for WWF and 57 FU for WF under their respective treatments. Although these results demonstrate differences in dough properties across cultivars and treatments, the analyses were conducted with a single replication due to material limitations. Therefore, further investigations with additional replications are essential to confirm these findings and gain a better understanding of the underlying mechanisms.

3.3.2.3. Dough development and gas release. The assessment of biofortification on dough development and gas release showed that dough properties, including maximum dough height, final dough height, and weakening capacity, remained stable across treatments (Table 3). However, Zn-treated dough demonstrates the highest weakening capacity (47 %) compared to the control (34 %), though the difference was not statistically significant. The onset of CO_2 release was consistent across treatments (49–55 min), while total gas volume was higher in all biofortified samples than in the control. Zinc treated dough had the highest gas production, followed by Se and I. Retention efficiency declined in all biofortified treatments by the end of the experiment, with the most pronounced decrease in Zn-treated dough (72 %) compared to the control (77 %). The co-application of I, Se, and Zn resulted in an intermediate retention coefficient (75 %), partially compensating for the negative impact on retention observed in treated samples.

3.4. Baking performance

Despite the influence of biofortification on dough expansion and gas retention capacity, fermentation height and bread volume were observed to be slightly higher in the I, Se, and control treatments compared to the combined application in Bezostaja-1, while the Zn treatment resulted in the lowest values (Fig. 3). In Nacibey, the co-application treatment had the lowest fermentation height and bread volume.

Moreover, due to the adverse effects of bran and fibre on gluten network development and gas retention the calculated potential loaf volume in the whole wheat pan loaf from both Bezostaja-1 and Nacibey cultivars remained below 4000 mL/kg flour. In contrast, pan loaf made from WF consistently achieved the highest volumes, exceeding 6000 mL/kg flour under the control, iodine, and co-application (I, Se, Zn) treatments (Fig. 4). Zinc treatment resulted in both flour types to the lowest loaf volumes. In addition, significant interaction between flour type and biofortification treatment ($p < 0.05$) was observed. For instance, Se treatment reduced loaf volume in WF formulations compared to the control, whereas its effect on whole wheat pan loaf was negligible.

Cultivar effects are significant but secondary to flour type, with Nacibey generally achieving higher loaf volumes, particularly under control and iodine treatments ($p < 0.001$). The cultivar and treatment interaction shows that while Nacibey maintains relatively stable loaf volumes under Se and Zn, Bezostaja-1 experiences a sharper decline, particularly at lower fermentation heights ($p = 0.001$). These indicate the importance of cultivar selection and flour type in optimizing baking performance under biofortification strategies.

3.5. Relationship between biofortification treatments, dough and bread properties

To further evaluate the effects of biofortification on quality-related parameters in WWF, Pearson correlation analysis was conducted (Table 4). Significant correlations ($p < 0.05$) with coefficients exceeding ± 0.8 highlighted the influence of I, Zn, and Se treatments on dough and bread-making characteristics. A strong positive correlation was observed between protein content and wet gluten ($r = 0.94$, $p < 0.05$), indicating that higher protein levels are closely associated with increased wet gluten formation, which is critical for dough elasticity and gas retention during baking. Conversely, a negative correlation between protein content and the gluten index ($r = -0.91$, $p < 0.05$) points to a decline in gluten quality with increasing protein content.

Selenium treatment correlated negatively with protein content ($r = -0.89$, $p < 0.05$), reflecting a reduction in protein levels in response to elevated Se concentrations. Similarly, water absorption was negatively correlated with Se levels ($r = -0.94$, $p < 0.05$), implying lower water-binding capacity in dough containing higher Se content.

A perfect positive correlation between gluten index and loaf volume per kilogram of flour ($r = 1.00$, $p < 0.001$) emphasizes the central role of gluten strength in determining loaf expansion potential. Furthermore, falling number displayed strong negative correlations with both loaf volume ($r = -0.86$, $p < 0.05$) and bread volume ($r = -0.84$, $p < 0.05$), indicating that increased enzymatic activity, inferred from lower falling number values, is associated with reduced loaf and bread volume.

Moreover, although some correlations were relatively strong (e.g., $r = -0.81$ between ash and protein), the corresponding p-values (e.g., $p = 0.0968$) did not reach statistical significance, primarily due to the limited sample size, as the analysis was based on treatment means averaged across three replicated plots.

While averaging improves the precision of treatment effect estimates by reducing within-group variability, it also reduces the number of independent observations, thereby limiting statistical power and potentially obscuring statistically significant relationships.

3.6. Mineral enriched bread

Biofortification effectively increased the content of all target minerals in the final bread products. Specifically, the use of I-biofortified flour led to a significant rise in iodine concentration (Fig. 5). In whole wheat flatbreads, control bread contained 22–25 μg/kg of I, which increased substantially under both individual and combined biofortification treatments. For the Bezostaja-1 cultivar, concentrations reached 340 μg/kg and 284 μg/kg, respectively, while Nacibey showed similar increases, reaching 143 μg/kg and 121 μg/kg. The highest iodine enrichment was observed in whole wheat pan loaf made with both biofortified flour and iodized salt, peaking at 650 μg/kg for Bezostaja-1 and 405 μg/kg for Nacibey. A comparable trend across bread types and treatments was noted in products made from second-year grains (results not presented).

Similarly, whole wheat pan loaf consistently showed higher Se concentrations than flatbread. In Nacibey, Se reached 309 μg/kg in pan loaf compared to 222 μg/kg in flatbread. In Bezostaja-1, concentrations were 293 μg/kg and 196 μg/kg, respectively. Zinc showed a similar pattern, with higher concentrations in pan loaf. For whole wheat flatbread made from Bezostaja-1, Zn concentration was 45 mg/kg with individual treatment and 31 mg/kg with the combined treatment. In pan loaf, the corresponding Zn concentrations were 43 mg/kg and 39 mg/kg, respectively. The same trend was observed in Nacibey across the bread

Table 2

Moisture, gluten properties, falling number, and ash content are expressed on a dry matter basis for biofortified grain flour, categorized by treatment and wheat cultivar.

Flour type	Treatment	Moisture (%)		Ash (%)		Wet gluten (%)		Gluten index (%)		Water binding capacity (%)		Falling number (s)	
		Bezostaja-1	Nacibey	Bezostaja-1	Nacibey	Bezostaja-1	Nacibey	Bezostaja-1	Nacibey	Bezostaja-1	Nacibey	Bezostaja-1	Nacibey
2022													
Whole wheat flour	*Control*	11 ± 0.13	11 ± 0.02	2.14 ± 0.01	1.76 ± 0.01	50 ± 0.82	38 ± 1.17	17 ± 14.3	56 ± 7.15	33 ± 0.77	26 ± 0.92	356 ± 17	444 ± 2
	I	12 ± 0.38	11 ± 0.09	2.25 ± 0.00*^{a}	1.74 ± 0.01	42 ± 0.42	34 ± 0.77	36 ± 1.57	62 ± 2.11	28 ± 0.25	23 ± 0.49	353 ± 7	455 ± 7
	Se	11 ± 0.18	11 ± 0.13	2.08 ± 0.01*^{b}	1.68 ± 0.02*	39 ± 0.62	34 ± 0.09	38 ± 3.33	72 ± 5.68	26 ± 0.71	23 ± 0.04	370 ± 6	463 ± 11
	Zn	11 ± 0.30	12 ± 0.14	2.03 ± 0.01*^{c}	1.67 ± 0.02*	50 ± 2.01	34 ± 0.76	36 ± 8.74	74 ± 4.78	33 ± 2.11	23 ± 0.53	386 ± 4	461 ± 10
	I + Se + Zn	12 ± 0.20	11 ± 0.19	2.08 ± 0.01*^{b}	1.72 ± 0.01	37 ± 1.43	32 ± 0.66	25 ± 18.9	80 ± 0.11	25 ± 1.35	21 ± 0.50	375 ± 27	477 ± 13*
2021													
Whole wheat flour	*Control*	10 ± 0.05	14 ± 0.14	1.56 ± 0.01	1.64 ± 0.03	32 ± 0.71	26 ± 0.33	33 ± 0.73	73 ± 19.1	21 ± 0.71	18 ± 0.93	418 ± 1	488 ± 19
	I	10 ± 0.03	13 ± 0.37	1.56 ± 0.00	1.65 ± 0.04	34 ± 1.70	29 ± 0.00	23 ± 0.54	52 ± 5.06	22 ± 1.13	20 ± 0.09	232 ± 2*	478 ± 6*
	Se	10 ± 0.08	12 ± 0.08	1.56 ± 0.01	1.60 ± 0.01	30 ± 0.42	30 ± 1.71	38 ± 1.24	53 ± 0.76	20 ± 0.07	21 ± 1.32	264 ± 2*	482 ± 11
	Zn	10 ± 0.03	12 ± 0.63	1.55 ± 0.01	1.71 ± 0.01*	35 ± 1.70	32 ± 1.02	26 ± 1.15	49 ± 4.38	23 ± 1.06	22 ± 1.16	171 ± 2*	472 ± 15*
	I + Se + Zn	10 ± 0.04	13 ± 0.15	1.59 ± 0.04	1.71 ± 0.02*	30 ± 0.14	32 ± 2.63	45 ± 3.92	25 ± 13.5	21 ± 0.07	22 ± 1.76	289 ± 1*	448 ± 5*
White flour	*Control*	13 ± 0.01	14 ± 0.34	0.47 ± 0.00	0.68 ± 0.00*	37 ± 0.28	30 ± 0.14	67 ± 0.39	75 ± 3.01	24 ± 0.02	20 ± 0.16	331 ± 5	447 ± 22
	Iodine	13 ± 0.01	14 ± 0.01	0.48 ± 0.01	0.63 ± 0.00*	41 ± 0.05	31 ± 0.28	62 ± 0.22	81 ± 0.23	26 ± 0.23	21 ± 0.14	210 ± 2*	454 ± 27
	Se	13 ± 0.04	14 ± 0.01	0.46 ± 0.01	0.63 ± 0.01*	33 ± 0.53	31 ± 0.67	73 ± 0.10	80 ± 1.81	21 ± 0.20	21 ± 0.18	240 ± 6*	442 ± 20
	Zn	13 ± 0.03	14 ± 0.24	0.50 ± 0.00*	0.65 ± 0.00*	37 ± 0.07	36 ± 0.35	71 ± 0.47	70 ± 1.14	24 ± 0.14	24 ± 0.28	187 ± 8*	447 ± 19
	I + Se + Zn	13 ± 0.06	14 ± 0.00	0.46 ± 0.01	0.62 ± 0.01*	42 ± 7.50	33 ± 0.71	64 ± 0.29	74 ± 3.72	25 ± 2.91	23 ± 0.67	250 ± 6*	444 ± 17
Sig.	*Cultivar (C)*	***		***		***		***		***		***	
	Treatment (T)	***		***		***		***		***		***	
	Year (Y)	***		***		***		*ns*		***		***	
	C × T	***		***		***		*		***		***	
	Y × T	***		***		***		***		***		***	

Between control and biofortification treatments (*) indicate significant differences ($p \leq 0,05$). Values within the same flour type, year and wheat cultivar with different letters are significantly different from each other ($p \leq 0,05$). ns: not significant, * and *** indicate significance (Sig.) at ($p < 0.05$, $p < 0.001$).

Table 3

Effect of biofortification on dough development and gas release characteristics of whole wheat flour of Bezostaja-1.

Treatment	Hm (mm)	h (mm)	DWC (%)	Tx (min)	Vt (ml)	CR (%)
Control	32.9 ± 0.2^{a}	21.6 ± 0.7^{a}	34.3 ± 2.2^{a}	54.1 ± 4.7^{a}	1593 ± 11^{a}	77.1 ± 0.1^{a}
I	33.4 ± 0.9^{a}	21.1 ± 3.2^{a}	37.2 ± 8.2^{a}	49.0 ± 7.1^{a}	1734 ± 60^{b}	73.5 ± 0.5^{b}
Se	32.1 ± 1.6^{a}	20.1 ± 2.0^{a}	37.5 ± 3.8^{a}	49.0 ± 4.6^{a}	1755 ± 30^{b}	73.6 ± 1.5^{b}
Zn	32.6 ± 0.7^{a}	17.3 ± 3.1^{a}	47.0 ± 8.9^{a}	55.0 ± 3.8^{a}	1781 ± 36^{b}	72.3 ± 0.8^{b}
I + Se + Zn	32.6 ± 1.2^{a}	21.0 ± 1.3^{a}	35.7 ± 4.4^{a}	53.0 ± 3.1^{a}	1726 ± 33^{b}	74.9 ± 1.7^{ab}

Values within the same parameter with different letters are significantly different from each other ($p \leq 0.05$). Hm: Maximum height during the dough development. h: The dough development height at the end of the test. DWC: Describes the drop in development height from its maximum (Hm) to the height after 3 h (h) in percentage. Tx: The time it takes until the dough starts releasing CO2 (dough porosity time). Vt: total volume of produced gas and CR: the retention coefficient.

types and treatments.

When examining the proportion of each nutrient carried through the baking process, the results showed that mineral stability varied significantly ($p < 0.001$). This variation appeared to depend on the chemical properties of each element, as well as the baking method and flour type. Iodine and Zn consistently showed higher retention in whole wheat pan loaf compared to flatbread. Still, the difference between the two elements was pronounced: mean retention in whole wheat pan loaf was approximately 98 % for I and 32 % for Zn. Selenium showed the lowest stability, with average retention 27 % in both bread types. Moreover, substituting whole wheat flour with refined flour led to a significant additional decrease in mineral retention ($p < 0.01$), resulting in final retention values of 52 % for I, 25 % for Zn, and 20 % for Se in the white pan loaf.

4. Discussion

4.1. Grain yield and grain concentrations of micronutrients

Foliar biofortification treatments applied in this study had no adverse effects on grain yield across cultivars and harvest seasons (Table A.1), which is consistent with previous findings (Cakmak, McLaughlin, & White, 2017; Stroud et al., 2010; Zou et al., 2012, 2019). In fact, a positive yield trend was observed following Se individual application. This aligns with Schiavon et al. (2020), who reported that Se can promote plant growth and improve yield performance, particularly under stress conditions, due to its role in enhancing antioxidant defences and reducing oxidative damage caused by abiotic stressors. In parallel, foliar application of I, Se, and Zn led to significant increases in their respective concentrations in wheat grain ($p < 0.05$), demonstrating the efficacy of this approach for improving the nutritional quality of the crop. Similar improvements have been reported in earlier field-based studies (Cakmak et al., 2020; Zou et al., 2019), reinforcing the potential of foliar biofortification to address micronutrient deficiencies through staple cereals. Among the applied micronutrients, Se showed the highest translocation efficiency from leaves to grain, likely due to its mobility through both xylem and phloem pathways (Lan et al., 2021; Zhou et al., 2020). In contrast, Zn and particularly iodine have limited phloem mobility, which restricts their transport to developing grains (Cakmak et al., 2010; Hurtevent et al., 2013; Medrano-Macías et al., 2016; Prom-u-thai et al., 2020). Nevertheless, substantial increases in grain content were achieved through foliar application of both elements. As previously discussed by Cakmak, McLaughlin, and White (2017) and Prom-u-thai et al. (2020), this is likely due to direct deposition onto reproductive tissues during spraying, particularly when applied during early grain development. The lower iodine uptake observed in 2022, compared to 2021, may have been caused by limited contact with florets during the spraying treatment (Fig. 1).

Moreover, the observed differential accumulation of I, Se, and Zn across treatments and cultivars points to distinct physiological mechanisms governing micronutrient partitioning under multi-element fertilization. The consistent reduction in grain iodine concentrations when co-applied with Se and Zn, irrespective of the cultivar, significantly suggests a competitive antagonism in this system ($p < 0.05$). This is due to saturation or mutual interference within the phloem loading and translocation pathways responsible for transporting these micronutrients from the flag leaf to the developing grain (Cakmak et al., 2020; Zou et al., 2019). In contrast, Se and Zn treatments showed a significant genotype-by-treatment interaction ($p < 0.05$). In Bezostaja-1, the simultaneous application of all three elements reinforced the inhibitory trend, resulting in lower Se and Zn accumulation compared to their individual applications. This reflects the cultivar limited capacity to manage the micronutrient cocktail's competitive dynamics, specifically due to constraints in utilizing key mechanisms like high-affinity

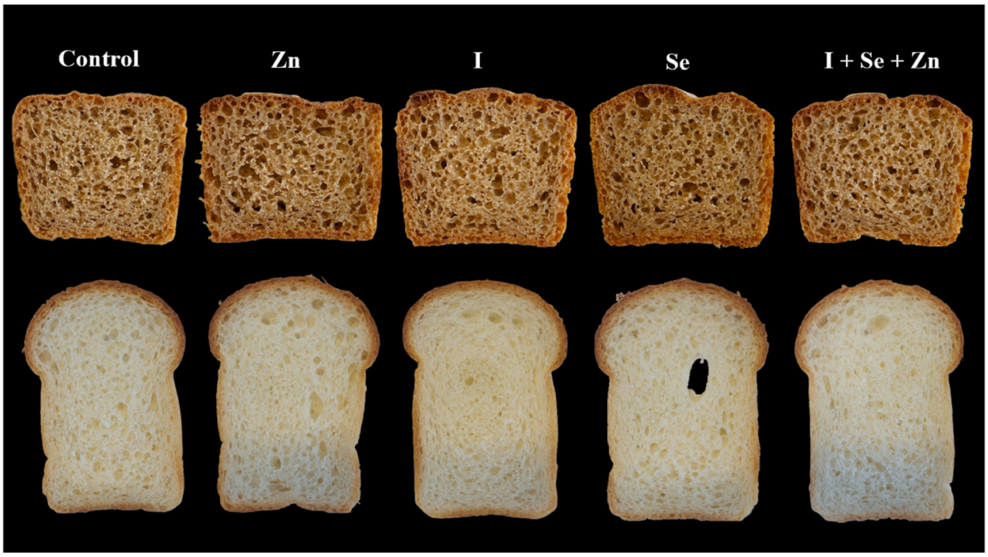

Fig. 3. Cross-sections of pan loaf prepared from different biofortified flour treatments derived from Bezostaja-1 (2021) grains.

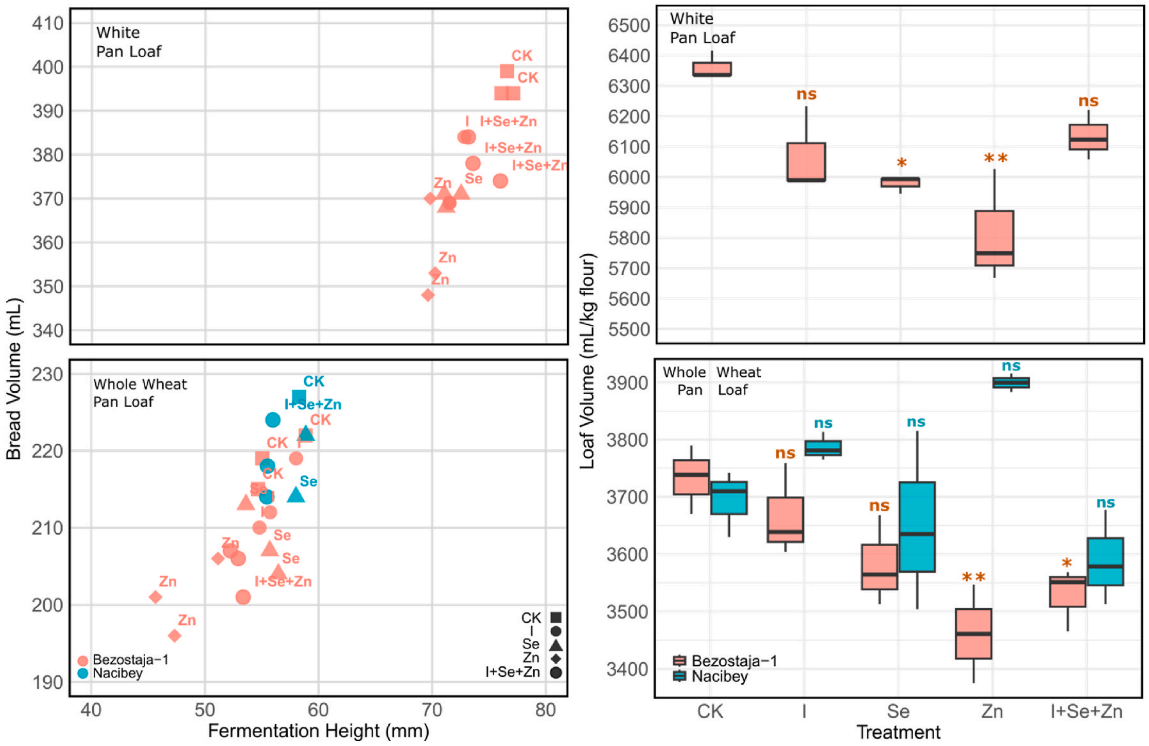

Fig. 4. Impact of biofortification treatments on bread volume, fermentation height, and loaf volume across cultivars and bread types made from 2021 harvested grains.

(Left) Effect of biofortification treatments on pan loaf volume and fermentation height for different bread types and wheat cultivars. (Right) Comparison of loaf volume under different biofortification treatments, categorized by bread type. Statistical significance indicates differences in loaf volume compared to each bread type control (CK) within the same cultivar. ns: not significant, * ($p < 0.05$), ** ($p < 0.01$).

transporters or efficient chelation systems (Chaukhe et al., 2025). Studies using combined foliar applications of Zn and Se in wheat have reported similar instances of antagonism or limited benefit from co-application, emphasizing the variability of these interactions (García-Latorre et al., 2025; Kong et al., 2024). The Nacibey cultivar, however, exhibited a distinct synergism for Se and Zn accumulation under the combined treatment. This synergistic response implies that Nacibey possesses genotypic traits that are either induced or maximally utilized when multiple micronutrients are simultaneously supplied. Plausible mechanisms include the upregulation of Zn and Se-specific transporters or an enhanced ability to synthesize and utilize phytosiderophores or other chelating compounds that facilitate the efficient co-transport and partitioning of these elements into the grain (García-Latorre et al., 2025; Kong et al., 2024).

Furthermore, Zn and Fe in the grain showed a positive relationship (Fig. 2), which may be attributed to Zn spray-induced production of Zn-binding compounds in the grain that also exhibit a high affinity for Fe. (Cakmak et al., 2010; Kutman et al., 2011; Ram, Naeem, Rashid, Kaur, Ashraf, Singh Malik et al., 2024). Interestingly, a strong relationship was also observed among S, Zn, and Fe concentrations in the grain. This may reflect the role of S-rich compounds, such as cysteine-rich proteins, which possess high metal-binding capacity and could facilitate Zn and Fe accumulation in grain tissues (Kawakami & Bhullar, 2020; Persson et al., 2016). These findings align with earlier reports of positive correlations among S, Zn, and Fe concentrations in wheat, including a study of 66 cultivars grown under field conditions (Morgounov et al., 2007). In addition, the seasonal variations in grain concentrations have likely resulted from environmental factors such as temperature and precipitation, which influence nutrient uptake and translocation (Ebrahimi et al., 2019; Rahman et al., 2020; Ram et al., 2024).

4.2. Nutritional composition and milling effects

The milling process significantly affected the retention of biofortified minerals, with WWF retaining the highest concentrations of I, Se, and Zn (Gómez et al., 2020). Although WF had lower nutrient levels as a result of refining, its biofortified version still provided a nutritional advantage over the non-biofortified flour. This highlights the value of biofortification not only in enhancing whole grains but also in improving the micronutrient profile of refined products, which dominate wheat consumption in many regions. The remaining mineral-enriched milling fractions, particularly bran and shorts, which retain 68 %, 74 %, and 26 % of the enriched I, Zn, and Se, respectively, are not commonly used in conventional baking processes but could be reintroduced as functional ingredients in food formulations thereby enhancing mineral intake and supporting dietary recommendations for increased fibre consumption.

4.3. Protein and gluten properties

While biofortification effectively improved grain micronutrient concentrations, it was associated with a reduction in wheat protein content, particularly under treatments involving Se, Zn, and their combined application with iodine (Table A.2). The consistent decrease in protein content observed with Se application may be partially attributed to a dilution effect associated with increased grain yield (Table A.1). Similar outcomes have been reported in previous studies, where Se and Zn negatively affected protein accumulation through various mechanisms (Hawrylak-Nowak et al., 2015; Wang et al., 2020). For example, Se can substitute for S in S-containing amino acids, such as methionine and cysteine, potentially disrupting disulfide bond formation and altering protein conformation, stability, and functionality (Haug et al., 2007). Regarding baking quality, WWF demonstrated lower gluten quality compared to WF, a result of bran components disrupting

Table 4
Correlation among the dough and bread proprieties.

	Protein	Ash	Wet gluten	Gluten index	Water binding capacity	Falling number	Water absorption	Dough development	Gas retention coefficient	Loaf volume	Bread volume	I	Se	Zn
Protein	1	−0.81	0.94*	−0.91*	0.78	−0.22	−0.17	−0.57	−0.09	0.01	0.03	−0.23	−0.89*	0.00
Ash		1	−0.68	0.79	−0.40	0.26	−0.03	0.15	0.23	−0.28	−0.29	0.54	0.47	0.23
Wet gluten			1	−0.92*	0.92*	−0.48	0.10	−0.80	−0.35	−0.32	−0.31	0.02	−0.87	0.21
Gluten index				1	−0.71	0.39	−0.14	0.67	0.44	0.26	0.23	−0.04	0.78	0.15
Water binding capacity					1	−0.57	0.16	−0.89*	−0.31	−0.47	−0.47	0.12	−0.80	0.54
Falling number						1	−0.87	0.59	0.86	0.64	0.67	−0.17	0.03	−0.58
Water absorption							1	−0.34	−0.94*	−0.67	−0.69	0.35	0.34	0.31
Dough development								1	0.51	0.79	0.78	−0.56	0.71	−0.40
Gas retention coefficient									1	0.72	0.73	−0.42	−0.07	−0.14
Loaf volume										1	1***	−0.86	0.17	−0.35
Bread volume											1	−0.84	0.14	−0.40
I												1	−0.09	−0.01
Se													1	−0.03
Zn														1

The Pearson correlation analysis shows the relationships among various quality parameters of Bezostaja-1 (2021) whole wheat flour, focusing on the effects of treatments with Selenium (Se), Zinc (Zn), and Iodine (I) on dough-related properties. The statistical significance of these correlations is indicated by * ($p \leq 0.05$), and *** ($p \leq 0.001$).

gluten matrix development during dough formation (Wang et al., 2002). The impact of biofortification on gluten content and gluten index was generally limited; however, minor reductions were detected under certain treatments, particularly in whole wheat formulations (Table 2). In addition, the ash content remained relatively constant across the different treatments for both white and whole wheat flours, remaining within the range of commercial flours from 1.5 % to 2 % for WWF and 0.5 %–0.7 % for WF (Andersson et al., 1993; Bodor et al., 2024).

4.4. *Dough and fermentation performance*

The enzymatic activity and starch characteristics of Bezostaja-1 and Nacibey wheat flours were affected by I, Se, and Zn biofortification (Table 3). These changes may influence flour behaviour during processing and baking. The untreated flour consistently exhibited higher resistance to starch hydrolysis in both WWF and WF. Meanwhile, the biofortified flour shows significantly lower falling numbers, with the strongest reduction under Zn treatments, followed by I and Se. This reduction could be interpreted as an increase of α-amylase activity; however, Zn is not known to directly activate this enzyme in wheat-based systems. While Zn is a cofactor for many enzymes, current evidence does not support its role in stabilizing or enhancing α-amylase activity in cereals. Instead, Calcium (Ca^{2+}) is a well-established cofactor essential for stabilizing and activating α-amylase in cereals. Therefore, the observed decrease in falling number following Zn biofortification is more likely due to indirect effects, such as altered expression of endogenous enzymes during grain development or increased starch vulnerability to hydrolysis activation (Cakmak, 2008; Velu et al., 2014). The partial attenuation of this effect under combined treatments suggest mineral interactions may buffer the individual impact of Zn. In addition, it was observed that Zn biofortification enhanced yeast fermentation, likely by acting as a cofactor in glycolytic and fermentative pathways. However, this improvement was accompanied by a weakening of the gluten network, resulting in reduced gas retention and dough stability. This structural weakening aligns with findings that Zn can interfere with gluten protein cross-linking, particularly by interacting with sulfhydryl groups and disrupting disulfide bond formation, as observed by Karaduman et al. (2023). In contrast, Se biofortification enhanced dough elasticity and gas retention by preventing oxidative degradation of gluten proteins through its antioxidant activity and by contributing to yeast metabolism (He et al., 2023). The combined treatment resulted in a more balanced effect, counteracting Zn-induced gluten weakening while preserving fermentation efficiency. This aligns with Verheyen et al. (2015) indicating that excessive gas production can exceed the retention capacity of weakened dough matrices, causing early gas loss and reduced loaf volume.

4.5. *Baking performance*

Pan loaf characteristics were largely influenced by flour type and cultivar. Whole wheat formulations consistently yielded lower loaf volumes than those made from WF (Fig. 4), reflecting the negative effect of bran on gluten development and gas retention capacity (Gan et al., 1995). Although biofortification with I, Se and Zn did not significantly influence fermentation height or final loaf volume, Zn treatments consistently resulted in the smallest loaf across both flour types. This supports the findings of Karaduman et al. (2023), who reported that Zn can impair gluten network formation by interacting with sulfhydryl groups, thereby disrupting disulfide bond formation, and consequently reducing dough strength and gas retention. Additionally, cultivar-specific responses were evident, particularly for I and Se treatments, emphasizing the influence of genetic background in shaping dough resilience and baking performance under biofortification.

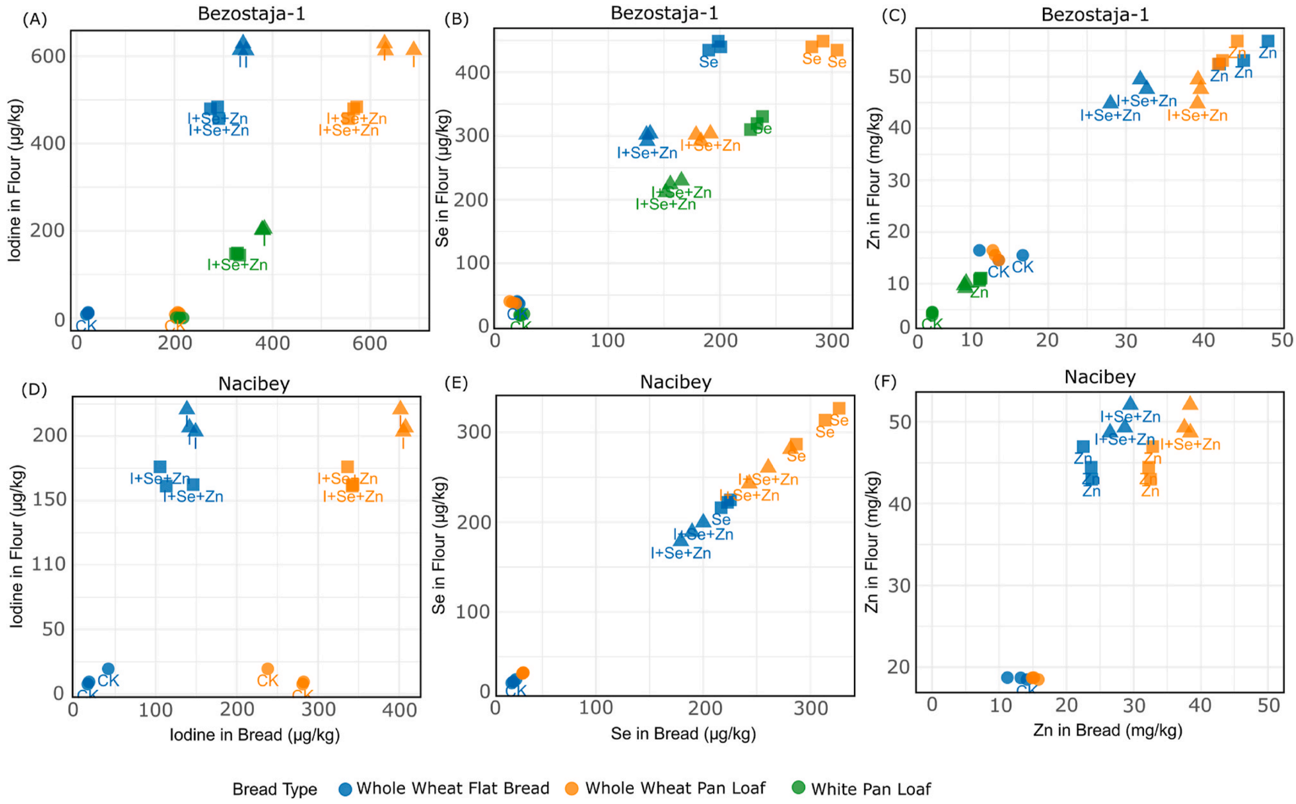

Fig. 5. Retention of iodine, Se, and Zn in bread from biofortified flour to bread across wheat cultivars. Treatments include Control (CK), I, Se, Zn, and I + Se + Zn. Panels A to C represent *Bezostaja-1*; panels D to F represent *Nacibey*.

4.6. Bread mineral retention

The differential retention observed among the I, Zn, and Se in bread is primarily attributable to their distinct chemical interactions within the bread matrix and their stability under thermal processing. The high retention observed for both I and Zn (approximately 98 % in whole wheat pan loaf) indicates that, whether biofortified or added through ingredients, both remain in a thermostable, non-volatile forms when embedded in a fiber- and protein-rich matrix. However, retention drops significantly in white pan loaf, with iodine decreases to 52 % and Zn to 25 %, reflecting the lower retention in WF. Moreover, the high retention observed in pan loaf made with biofortified flour is consistent with previous studies showing that both biofortified flour and iodized salt can deliver thermally stable iodine during baking, whether used individually or in combination (Belarbi et al., 2026; Longvah & Upadhyay, 2015).

However, when formulated as whole wheat flatbread, iodine retention decreased further to 32 %, compared to their corresponding values in pan loaf. The high-temperature and short-duration baking conditions used for flatbreads may promote higher volatilization or thermal degradation than the baking conditions used for pan (Dulova et al., 2020; Haldimann et al., 2005; Rana & Raghuvanshi, 2013). For Zn, the difference in retention relates directly to phytase activity and fermentation time. In flatbreads, the lack of fermentation and high baking temperatures inhibit phytase, leaving Zn bound in insoluble phytate complexes. This results in higher total retention (32 % compared to 25 %) but lower bioavailability. In contrast, the longer fermentation time in pan loaf allows phytase to break down phytate, reducing Zn binding and improving its bioavailability (Z. Wang & Wang, 2024).

For Se, the consistently low retention across both bread types (27 %) points toward a different mechanism: thermal degradation of organic Se compounds (e.g., selenomethionine) or the formation of volatile Se species that escape during high-heat exposure (Pérez et al., 2018; Xie et al., 2023). High temperatures used in roasting and baking are known to significantly increase Se loss, a process that is less reliant on the whole wheat matrix than I or Zn stability (Pérez et al., 2018).

According to the Recommended Dietary Allowances (RDA) established by the Institute of Medicine (IOM), the daily requirements are 150 µg for I, 55 µg for Se, and 11 mg for Zn (IOM, 2000, 2001). A 100 g serving of biofortified bread can provide up to 43 % of I, 56 % of the Se, and 41 % of the Zn RDA (Table 5). Considering that daily bread consumption often exceeds 100 g in many populations, the nutritional contribution of biofortified bread can be higher. For instance, at 400 g/day the estimated intake would increase to approximately 260 µg of I (173 % RDA), 124 µg of Se (225 % RDA), and 18 mg of Zn (164 % RDA). These values remain well below the established tolerable upper intake levels of 1100 µg for I, 400 µg for Se, and 40 mg for Zn (IOM, 2000, 2001). These findings demonstrate the potential of biofortified flour for use in a variety of bread formulations, especially for improving micronutrient intake in vulnerable populations such as pregnant women, children, and individuals with restricted diets or malabsorption disorders.

4.7. Limitations

The present study was limited to two wheat cultivars cultivated on a single site. Nutritional evaluation was based on compositional data, without assessing the bioavailability of the micronutrients. Additionally, no sensory or consumer-level assessments were conducted. Further research is needed to investigate nutrient speciation, plant physiological responses, and bioaccessibility, in order to better understand the nutritional implications of multi-nutrient biofortification. Advancing these areas of research will support the integration of biofortified wheat into agricultural and nutritional interventions aimed at alleviating micronutrient deficiencies.

Table 5

Mineral content per 100 g of different bread types made from individual biofortification and their contribution to the Recommended Dietary Allowances (RDA).

Wheat Cultivar	Flour Type	Bread Type	I (μg/100g)	Se (μg/100g)	Zn (mg/100g)	I % RDA	Se % RDA	Zn % RDA
Bezostaja-1	Whole Wheat Flour	Flat Bread	34	20	4.5	23 %	36 %	41 %
		Pan Loaf	65	29	4.3	43 %	53 %	39 %
Nacibey		Flat Bread	14	22	3.1	9.5 %	40 %	28 %
		Pan Loaf	40.5	31	3.9	27 %	56 %	35 %
Bezostaja-1	White Flour	Pan Loaf	38	23	1.1	25 %	42 %	10 %

5. Conclusion

Micronutrient deficiencies remain a major global health challenge, particularly in populations with limited dietary diversity and insufficient intake of micronutrients. This research integrates multi-year field trials with two wheat cultivars, combined foliar treatments of I, Se, and Zn, and a full evaluation of micronutrient dynamics from grain through milling to different bread types. The findings demonstrate that foliar biofortification is an effective, scalable approach for increasing the concentrations of I, Se, and Zn in wheat grain and its processed products. While individual applications enhance specific nutrient uptake, combined treatments result in a more balanced micronutrient profile, although potential antagonistic interactions during foliar absorption and translocation must be considered. Post-harvest processing steps, particularly milling and baking, substantially influence micronutrient retention and, consequently, the nutritional composition of the final products. Moreover, whereas Zn application alone was associated with a slight reduction in loaf volume, Se and I biofortification maintained overall baking performance and dough functionality. Aligning biofortification strategies with industrial processing workflows provides a promising approach to developing micronutrient-enriched wheat-based foods, thereby contributing to improved nutritional quality of staple products and supporting efforts to mitigate hidden hunger.

CRediT authorship contribution statement

Hind Belarbi: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fassil Kebede:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization. **Ingrid De Leyn:** Writing – review & editing, Resources, Methodology, Data curation. **Filip Van Bockstaele:** Writing – review & editing, Methodology. **Pieter Vermeir:** Methodology, Investigation, Formal analysis. **Erdinç Savaşlı:** Methodology, Investigation. **Ismail Cakmak:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Gijs Du Laing:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

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

During the preparation of this study, the author(s) used ChatGPT to improve the readability and language of the manuscript. After utilizing this tool, the author(s) reviewed and edited the content as needed and take full responsibility for the final published article.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mohammed VI Polytechnic University reports financial support was provided by OCP Group. If there are other authors, they 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.2026.119099.

Data availability

Data will be made available on request.

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