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Profiling anti-Nutritional factors and toxic heavy metals in sesame oil: Comparative analysis of four Iranian varieties

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ABSTRACT

Sesame seeds are a globally important food commodity and a high-quality source of cooking oil, particularly in Asia. This study profiled the levels of heavy metals (lead, cadmium, arsenic, mercury) and anti-nutritional factors (oxalate, phytate) in four sesame varieties available in Iran (two domestic: Behbahani, Lari; two imported: Pakistani, Sudanese). Oil was extracted by cold pressing, and analyses were conducted using atomic absorption spectroscopy (AAS) and high-performance liquid chromatography (HPLC). Lead ranged from 1.62-5.45 mg/kg, cadmium 0.45-0.99 mg/kg, phytic acid 0.76-1.50 mg/kg, and oxalic acid 64.01-169.49 mg/kg. The Behbahani variety exhibited the highest levels across all contaminants, while the Sudanese variety showed the lowest. The Pakistani variety had the highest calcium content, and the Sudanese variety had the highest iron, zinc, and magnesium concentrations. These findings highlight the significant varietal differences in sesame oil's chemical composition, which is important for selecting superior genotypes to produce high-quality edible oil.

Keywords: Heavy metals; Anti-nutritional; Sesame oil.

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1. Introduction

Sesame (*Sesamum indicum*), which belongs to the Pedaliaceae family and is cultivated mainly in tropical and subtropical regions, is widely used as an oilseed crop and culinary condiment. Owing to the distinctive aroma of its seeds, sesame is extensively used in the bakery and confectionery industries as a flavoring ingredient. Sesame oil is valued for its mild aroma, pleasant taste, and low freezing point, and is widely regarded as one of the healthiest edible oils. It is commonly used as a cooking oil in many Asian countries; however, compared with other vegetable oils, sesame oil is relatively more expensive (Kheirati-Rounizi et al., 2021; Johnson et al., 1979).

Minerals play essential roles in human physiology, participating in various cellular processes ranging from tissue regeneration to maintaining ionic gradients. The primary source of minerals for humans is the diet. Due to their involvement in crucial physiological functions including skeletal structure development, muscle contraction, and enzymatic metabolic pathways minerals are regarded as essential trace elements for life. Sesame seeds are a rich source of minerals, particularly phosphorus, iron, calcium, and magnesium. Overall, sesame seeds contain approximately 5–7% minerals, with calcium accounting for nearly 1% of total mineral content (Makinde & Akinoso, 2014).

Anti-nutritional factors are natural or synthetic compounds that interfere with the absorption of essential nutrients, potentially adversely affecting health and growth (Wei et al., 2022; Jaffar et al., 2025). Despite the well-documented nutritional value and bioactive compounds of sesame seeds and their hulls, raw sesame also contains anti-nutritional factors such as phytic acid and oxalates, which are mainly concentrated in the seed coat. These compounds reduce mineral bioavailability by forming insoluble complexes with essential elements, including calcium, iron, zinc, and magnesium, thereby impairing intestinal absorption (Bello et al., 2013; Mehari et al. 2025). Phytic acid, the principal storage form of phosphorus in sesame seeds, exhibits strong chelating properties that limit its absorption and negatively affect mineral nutrition. Additionally, heavy metals such as lead, cadmium, arsenic, and mercury can accumulate in edible oils due to soil contamination and agricultural

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practices, posing significant food safety concerns (Rahimi et al. 2020; Mehari et al. 2025; Liu et al. 2024). These toxic elements are not metabolized in the human body, tend to accumulate in tissues, and can disrupt critical cellular functions. Acute exposure is primarily associated with hepatotoxicity, whereas chronic exposure has been linked to cardiovascular diseases, neurological disorders, oxidative stress, and carcinogenesis. Children are particularly vulnerable to these adverse effects (Jacob & Kakulu, 2012). While previous studies examined sesame seeds (Rahimi et al. 2020), no research has compared heavy metal and anti-nutritional accumulation across multiple Iranian sesame oil varieties. This study fills this gap using AAS/HPLC analysis of Behbahani, Lari, Pakistani, and Sudanese oils.

2. Materials and Methods

2.1. Sample collection

Four sesame varieties Behbahani and Lari (domestic), and Pakistani and Sudanese (imported) were obtained in triplicate ($n = 3$) from Qosheh Co., Yazd Province, Iran, in July 2024. Each sample was analyzed individually without pooling. Before the commencement of the experiments, the seeds were cleaned to remove any foreign materials such as dirt, husks, and other impurities.

2.2. Sesame seed dehulling

All four varieties (Behbahani, Lari, Pakistani, Sudanese) underwent identical traditional wet dehulling conditions: seeds soaked in water at 29°C for 4 hours, followed by 2 hours in salt solution to loosen hulls. Hulls settled and were mechanically separated. Seeds were dried at 105°C for 2 hours, ground into fine powder, and stored in glass containers (Amini et al., 2023).

2.3. Raw sesame oil

The oil from each variety of raw, dehulled sesame seeds was extracted using the cold pressing method. After filtration, the oil was stored in glass containers. The cold pressing method was chosen because it yields a light-yellow oil with a pleasant taste and aroma, and due to its high quality, it can be consumed directly without further processing. On the other hand, direct solvent extraction is not suitable due to the high oil content in sesame seeds (Bello et al., 2013).

2.4. Measurement of heavy metals (lead, cadmium, arsenic, and mercury) and mineral salts (calcium, iron, zinc, manganese, and magnesium)

Atomic absorption spectroscopy (AAS) with graphite furnace was used. Samples digested via microwave (Ethos Plus Milestone) with 30% H_2O_2, diluted to 25 mL. Detection limits: Pb (0.01 mg/kg), Cd (0.005 mg/kg), As (0.02 mg/kg), Hg (0.01 mg/kg). Calibration: 5-point curve (0.1-10 mg/L, $R^2>0.999$). Quality control: blanks, spikes, NIST 8414 CRM (recovery 95-102%). Analysis: Spect AA-200 (Italy) with element-specific hollow cathode lamps.

2.5. Measurement of anti-nutritional factors (phytic acid and oxalic acid)

Samples were finely ground, homogenized, and subjected to extraction by mixing at a 1:1 (w/v) ratio with 0.1 M nitric acid. The mixture was stirred for 3 minutes at 300 rpm, followed by centrifugation at 5000 × g for 10 minutes at 4 °C. The resulting supernatant was diluted 1:1 with distilled water containing 0.5% NaCl and centrifuged again at 8000 × g for 5 minutes. The organic phase was subsequently filtered through a 0.45 µm PTFE membrane prior to HPLC analysis .High-performance liquid chromatography (HPLC) was performed using a C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of 0.1% phosphoric acid under isocratic conditions, with a flow rate of 1.0 mL/min. The column temperature was maintained at 30 °C, and detection was achieved using a UV detector set at 210 nm. The injection volume was 20 µL, and the total run time for each sample was 15 minutes . Calibration was conducted using five-point standard curves within the concentration range of 0.1–50 mg/L, yielding correlation coefficients (R^2) greater than 0.999. The limit of detection (LOD) was 0.05 mg/kg for oxalic acid and 0.08 mg/kg for phytic acid, while the limit of quantification (LOQ) was 0.15 mg/kg and 0.24 mg/kg, respectively. Quality control was ensured through the inclusion of blanks and spiked samples, with recovery rates ranging from 92% to 105%.

2.6. Data analysis

All statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). Before analysis of variance (ANOVA), the assumptions of normality and homogeneity of variances were verified. Normality was assessed using the Shapiro-Wilk test ($p \geq 0.05$), and homoscedasticity was confirmed by Levene's test ($p > 0.05$). Mean comparisons were performed using the least significant difference (LSD) test at a significance level of $\alpha = 0.05$. Statistical significance was indicated as follows: ($p \leq 0.05$), ($p \leq 0.01$), while non-significant differences were denoted as ns ($p^* > 0.05$).

3. Results and Discussion

3.1. Heavy metals

3.1.1. Lead

Analysis of variance indicated a significant effect ($p < 0.05$) of variety on lead accumulation (Table 1).

Lead content varied significantly among varieties. The highest concentration was observed in the Behbahanii variety (5.45 mg/kg), whereas the lowest was found in the Sudanese variety (1.62 mg/kg). The Pakistani (2.48 mg/kg) and Sudanese (1.62 mg/kg) varieties were grouped together statistically and significantly differed from the Behbahanii variety (Fig. 1). Variation in lead levels across different varieties is mainly due to genetic differences affecting absorption and accumulation. Some varieties may have more extensive root systems or more active absorption mechanisms for soil elements (Mehari et al. 2025). Environmental and soil conditions, such as contamination near industries or mining areas or heavy fertilizer use, also impact lead accumulation. Additionally, seed lipid and protein composition affect heavy metal uptake.

Table 1. Analysis of variance (ANOVA) results and mean concentrations (mg/kg) of heavy metals, anti-nutritional factors, and minerals in sesame oil from four varieties (Behbahani, Lari, Pakistani, Sudanese).

S.O. V	df	Lead	Cadmium	Arsenic	Mercury	Phytic acid	Oxalic acid	Calcium	Manganese	Iron	Zinc	Magnesium
Variety	3	3.25^{*}	0.0062^{*}	0.059^{**}	0.20^{**}	0.16^{*}	1973.81^{*}	158.74^{*}	0.46^{ns}	17.10^{**}	8.69^{**}	1716.55^{**}
Error	12	0.77	0.0012	0.012	0.01	0.03	22.24	15.65	0.11	0.46	0.25	62.72
C.v. (%)	-	11.33	8.59	10.45	10.02	28.07	19.01	19.70	22.65	4.48	5.90	8.77

Note: ns = non-significant ($p > 0.05$); * = significant at $p < 0.05$; ** = significant at $p < 0.01$. Means followed by the same letter within each row are not significantly different (LSD test, $p < 0.05$). Values represent mean ± standard error (n=3).

Overall, lead content is influenced by genetics, soil, and oil extraction processes. Behbahanii showed the highest lead, possibly due to soil contamination or internal metabolism, while Sudanese and Pakistani had lower levels, indicating potential for producing lower-contaminant oil.

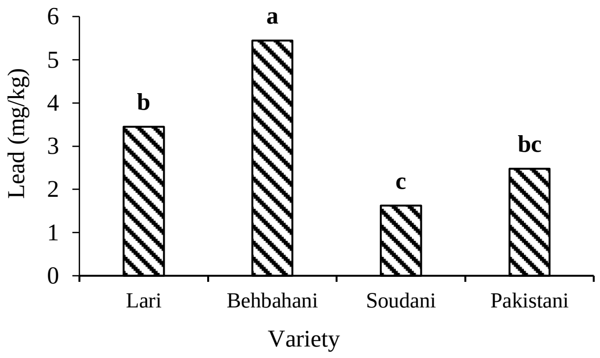

Fig. 1. Mean lead (Pb) concentrations in sesame oil from four varieties. Bars with different letters differ significantly ($p < 0.05$, LSD test). Means with at least one letter in common do not differ significantly from each other at the 5% probability level based on the LSD test.

3.1.2. Cadmium

Analysis of variance indicated a significant effect ($p < 0.05$) of variety on cadmium accumulation (Table 1). Cadmium content varied significantly among varieties. The highest concentration was observed in the Behbahani variety (0.99 mg/kg), whereas the lowest was found in the Sudanese and Lari varieties. The Sudanese and Lari cultivars showed the lowest levels (Fig. 2). Cadmium is a toxic pollutant from anthropogenic sources (Rahimi et al. 2020). The high cadmium content in the Behbahanii variety suggests a greater propensity for uptake. Lari and Sudanese varieties indicate better exclusion. Selecting low-accumulating varieties is an effective strategy to reduce contamination.

3.1.3. Arsenic

Analysis of variance indicated a significant effect ($p < 0.01$) of variety on arsenic accumulation (Table 1). Arsenic content varied significantly among varieties. The highest concentration was observed in the Pakistani variety (2.34 mg/kg), whereas the lowest was found in the Behbahani and Sudanese varieties (Fig. 3). Lari was intermediate, likely due to physiological mechanisms like organic acid secretion or sequestration in roots (Mehari et al. 2025) (Fig. 3).

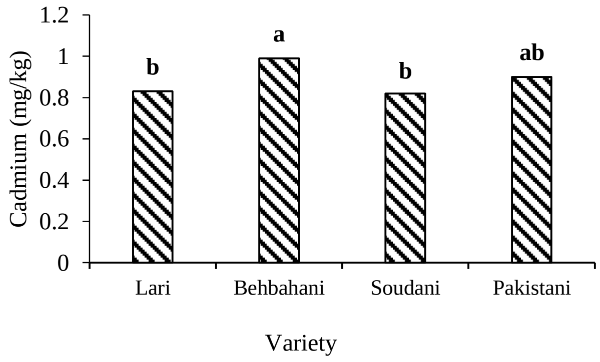

Fig. 2. Mean cadmium (Cd) concentrations in sesame oil from four varieties. Bars with different letters differ significantly ($p < 0.05$, LSD test). Means with at least one letter in common do not differ significantly from each other at the 5% probability level based on the LSD test.

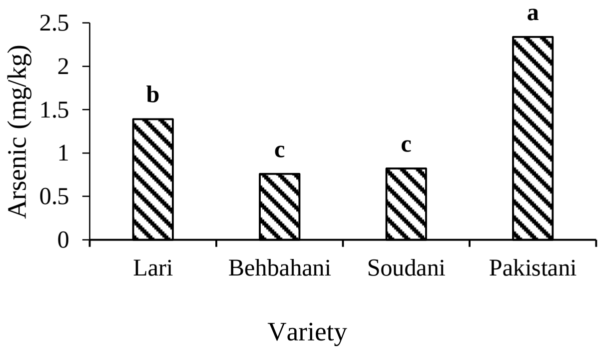

Fig. 3. Mean arsenic (As) concentrations in sesame oil from four varieties. Bars with different letters differ significantly ($p < 0.05$, LSD test). Means with at least one letter in common do not differ significantly from each other at the 5% probability level based on the LSD test.

3.1.4. Mercury

Analysis of variance indicated a significant effect ($p < 0.01$) of variety on mercury accumulation (Table 1). Mercury content varied significantly among varieties. The highest concentration was observed in the Behbahani variety, whereas the lowest was found in the Lari and Sudanese varieties (Fig. 4). Pakistani was intermediate, possibly due to enhanced secretion of metal-chelating compounds and active transport, suggesting effective defense mechanisms like compartmentalization and antioxidant capacity (Liu et al. 2024) (Fig. 4).

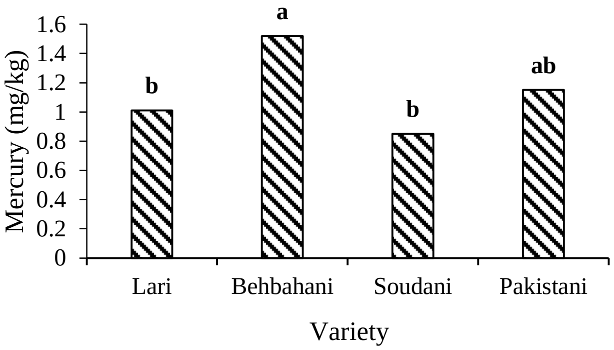

Fig. 4. Mean mercury (Hg) concentrations in sesame oil from four varieties. Bars with different letters differ significantly ($p < 0.05$, LSD test). Means with at least one letter in common do not differ significantly from each other at the 5% probability level based on the LSD test.

3.2. Antinutritional factors

3.2.1. Phytic Acid

Phytic acid content differed significantly among varieties ($p < 0.05$, Table 1). The Behbahani variety exhibited the highest concentration (1.50 mg/kg), while the Sudanese variety showed the lowest (0.76 mg/kg), with significant difference (Fig. 5). Lari and Pakistani were similar. Differences arise from genetics and environmental and nutritional factors (Amini et al., 2023). High phytic acid may impair mineral (Fe, Zn, Ca) absorption but also has antioxidant benefits.

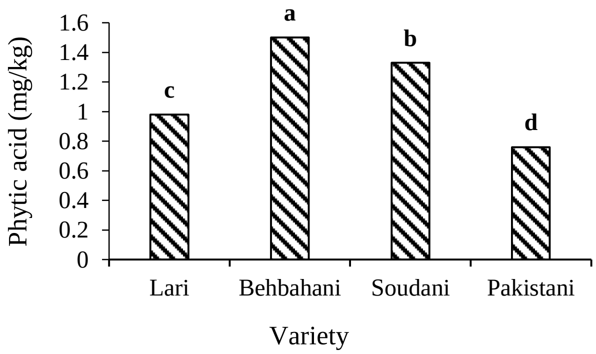

Fig. 5. Mean phytic acid concentrations in sesame oil from four varieties. Bars with different letters differ significantly ($p < 0.05$, LSD test). Means with at least one letter in common do not differ significantly from each other at the 5% probability level based on the LSD test.

3.2.2. Oxalic acid

Oxalic acid content differed significantly among varieties ($p < 0.05$, Table 1). The Behbahani variety exhibited the highest concentration (169.49 mg/kg), followed by Pakistani (130.41 mg/kg) and Lari (103.31 mg/kg), while the Sudanese variety showed the lowest (64.01 mg/kg) (Fig. 6). Genetic and physiological factors influence synthesis (Jacob & Kakulu, 2012). Calcium availability and environmental stresses modulate oxalate formation (Chandrasekaran et al., 2014).

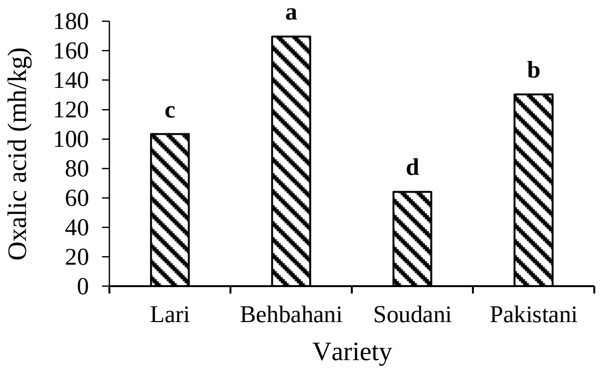

Fig. 6. Mean oxalic acid concentrations in sesame oil from four varieties. Bars with different letters differ significantly ($p < 0.05$, LSD test). Means with at least one letter in common do not differ significantly from each other at the 5% probability level based on the LSD test.

3.3. Mineral content

3.3.1. Calcium

Analysis of variance indicated a significant effect ($p < 0.05$) of variety on calcium content (Table 1). Calcium content varied significantly among varieties. The highest concentration was observed in the Pakistani variety (157.20 mg/kg), with Sudanese (157.20 mg/kg) and Lari (150.25 mg/kg) clustering together, whereas the lowest was found in the Behbahani variety (127.52 mg/kg). Calcium oxalate complexes may form, affecting bioavailability. Variation is due to genetics, environment, and nutrition (Ghavami et al., 2008).

3.3.2. Manganese

No significant differences were observed in manganese content among varieties ($p > 0.05$, Table 1). The Sudanese variety showed the highest concentration, while the Lari variety had the lowest, possibly due to environmental effects.

3.3.3. Iron

Analysis of variance indicated a significant effect ($p < 0.01$) of variety on iron content (Table 1). Iron content varied significantly among varieties. The highest concentrations were observed in the Sudanese (4.82 mg/kg) and Pakistani (4.58 mg/kg) varieties, whereas the lowest was found in the Behbahani variety (2.19 mg/kg).

Table 2. Comparison of heavy metal levels with international safety standards.

Metal	Highest (Variety)	Measured (mg/kg)	Codex ML	EU ML	Stat
Pb	Behbahani	5.45± 0.23	0.1	0.1	54.5x
Cd	Behbahani	0.99± 0.08	0.1	0.1	9.9x
As	Pakistani	2.34± 0.15	0.2	0.1	23.4x
Hg	Behbahani	1.12± 0.09	0.05	0.1	11.2x

Codex STAN 210-1999; EU Reg. 1881/2006. Note: Values represent mean±SD (n=3); ML = Maximum Limit.

Genetic mechanisms, including uptake genes (IRT1, FRO2), play roles (Amini et al., 2023).

3.3.4. Zinc

Analysis of variance indicated a significant effect ($p < 0.05$) of variety on zinc content (Table 1). Zinc content varied significantly among varieties. The highest concentration was observed in the Sudanese variety (11.88 mg/kg), whereas the lowest was found in the Behbahani variety (6.55 mg/kg). Lari and Pakistani varieties showed moderate levels. Efficient uptake is possibly due to root and transporter activity (Amini et al., 2023).

3.3.5. Magnesium

Analysis of variance indicated a significant effect ($p < 0.05$) of variety on magnesium content (Table 1). Magnesium content varied significantly among varieties. The highest concentration was observed in the Sudanese variety (126.47 mg/kg), whereas the lowest was found in the Pakistani variety (66.60 mg/kg). Lari and Behbahani varieties showed moderate levels. Differences are due to genetics and environment.

3.4. Discussion

Our findings demonstrate significant varietal differences in heavy metal and anti-nutritional factor accumulation among four sesame oil varieties from Iran. The Behbahani variety exhibited the highest concentrations of lead, cadmium, mercury, phytic acid, and oxalic acid, whereas the Sudanese variety showed the lowest levels of these contaminants. Genetic factors strongly influence heavy metal and anti-nutrient uptake patterns in sesame, consistent with studies highlighting genotype-specific absorption mechanisms (Mehari et al. 2025; Rahimi et al. 2020). Notably, lead (Pb) and cadmium (Cd) levels were significantly elevated in the Behbahani variety, likely due to root architecture differences, metal transporter expression, and detoxification capacity (Rounizi et al. 2023; Noonan & Savage, 1999). Environmental factors, including soil contamination from industrial/agricultural sources, further contributed to this variability. The Pakistani and Sudanese varieties demonstrated superior heavy metal exclusion, positioning them as promising candidates for safer sesame oil production. Conversely, arsenic (As) was highest in Pakistani oil, while Behbahani and Sudanese varieties showed effective exclusion via organic acid secretion and root sequestration (Mehari et al. 2025; Park et al., 2013). Mercury (Hg) accumulation followed a similar pattern, with Sudanese and Lari varieties exhibiting robust compartmentalization and antioxidant defenses. Heavy metal levels substantially exceeded international safety limits (Table 2). Lead (5.45±0.23 mg/kg, Behbahani) surpassed Codex/EU (0.1 mg/kg) by 54.5-fold. Cadmium (0.99±0.08 mg/kg) exceeded both by 9.9-fold. Arsenic (2.34±0.15 mg/kg, Pakistani) and mercury (1.12±0.09 mg/kg, Behbahani) surpassed Codex (Hg: 0.1 mg/kg) by 11.2-fold and EU (Hg: 0.05 mg/kg) by 22.4-fold (Liu et al. 2024). Sudanese variety approached acceptable limits across all metals, confirming its suitability for safe production.

Sudanese variety levels approached acceptable limits, supporting its selection for safe production. These exceedances highlight urgent food safety concerns for Iranian sesame oil (González-Torres et al., 2023).

Phytic and oxalic acids, key anti-nutritional factors, also varied significantly. Elevated levels in Behbahani oil suggest higher phosphorus storage but reduced mineral bioavailability through chelation (Amini et al., 2023). Sudanese oil's lower levels indicate better nutrient accessibility. Oxalic acid's chelating properties further impair Ca, Mg, and Fe absorption, modulated by genetic and environmental factors (Jacob & Kakulu, 2012).

Essential mineral content (Ca, Fe, Zn, Mg) varied significantly, with Pakistani oil richest in calcium and Sudanese oil in Fe, Zn, and Mg (Amini et al., 2023; Ghavami et al., 2008). These genotypes possess enhanced nutrient uptake/translocation mechanisms beneficial for human nutrition. These results underscore the need for breeding programs balancing high mineral content with low contaminant accumulation. Integrated approaches variety selection and clean soil management are essential for safe, high-quality sesame oil production.

4. Conclusion

This study reveals significant varietal differences in heavy metal and anti-nutritional factor accumulation in sesame oil. Domestic varieties exhibited higher contaminant levels compared to imported ones, highlighting genetic and cultivation influences on food safety profiles. Sudanese and Pakistani varieties are recommended for safer sesame oil production due to superior heavy metal exclusion and enhanced mineral content. Breeding programs should prioritize low-accumulating genotypes alongside soil management strategies to ensure nutritional quality and minimize health risks.

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CRediT authorship contribution statement

Statistical analysis Sajjad Izadi, Ali Sahrae Ardakani, and Amin Ahmadi were responsible for conceptualizing and designing the study, and analyzing and interpreting the data. Amin Ahmadi and Ali Sahrae Ardakani drafted the manuscript and conducted the statistical analysis. The team's collective expertise and diligent efforts ensured the rigorous execution and comprehensive reporting of this research endeavor.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

Data will be made available on request from the corresponding author.

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