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The Effect of Soaking Duration on Zinc Leaching and Apparent Zinc Retention in Wheat (*Triticum aestivum* L.)

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ABSTRACT

Zinc is an essential micronutrient whose concentration in cereal foods can be altered by production practices and post-harvest processing. This study evaluated the effect of soaking duration on zinc release into soaking water and apparent zinc retention in wheat (Triticum aestivum L.). Wheat grains obtained from Hausa Quarters, Ekpoma, Edo State, Nigeria, were cleaned and divided into 100-g portions. Each portion was soaked in 400 mL of distilled water at room temperature for 2, 4, 8, 16 or 24 h. Soaking filtrates were separated, filtered, acid-digested and analyzed for zinc by atomic absorption spectrometry at 213.9 nm. The recorded initial zinc content was 2.70 mg/100 g. Under the stated analytical scheme 1.0 mL filtrate aliquot diluted to a final digest volume of 100 mL. Zinc recovery increased from 0.01092 mg at 2 h to 0.16881 mg at 24 h, corresponding to calculated zinc losses of 0.40% and 6.25%, respectively, and apparent retentions of 99.60% and 93.75%. Descriptive regression gave $R^2 = 0.9374$ for zinc recovery and $R^2 = 0.9372$ for apparent retention. The findings indicate progressively greater reported zinc release with prolonged soaking.

Keywords: wheat; zinc; soaking duration; zinc leaching; apparent mineral retention; atomic absorption spectrometry; post-harvest processing

INTRODUCTION

Zinc is an essential trace element required for normal cellular metabolism, enzyme function, protein synthesis, nucleic-acid metabolism, immune function and growth. Wheat is an important cereal in human diets, and its zinc concentration can contribute materially to dietary zinc intake. The nutritional contribution of wheat, however, depends not only on the amount of zinc accumulated before harvest but also on how much of the mineral remains after cleaning, soaking, milling, cooking and other post-harvest operations.

Grain zinc concentration is influenced by genotype, soil properties, environmental conditions and agronomic management. Agronomic biofortification has consequently received considerable attention as a strategy for increasing zinc concentration in wheat. A global meta-analysis found that soil, foliar and combined soil-plus-foliar zinc applications can increase wheat grain zinc concentration, with response influenced by application rate, initial grain zinc status and timing of application (Hui et al., 2025). Zinc fertilization has also been associated with improved wheat growth, zinc uptake and grain nutritional characteristics (Makhdum et al., 2024; Zulfiqar et al., 2020). Zinc-solubilizing bacteria provide another approach for improving zinc availability and wheat zinc accumulation (Ali et al., 2023).

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Production-stage zinc enrichment does not guarantee complete mineral retention during food processing. Post-harvest handling can substantially influence micronutrient retention. A systematic review of biofortified crops found considerable variability in iron and zinc retention according to processing method and crop, and highlighted the importance of processing practices in determining micronutrient levels at consumption (Huey et al., 2023). Thus, production-stage biofortification and post-harvest retention should be considered as complementary components of the food-to-nutrition pathway.

Soaking is a common preparatory operation for cereals and legumes. Water contact hydrates grain tissues and can facilitate movement of soluble or weakly associated mineral fractions into the surrounding medium. Lestienne et al. (2005) reported that 24-h soaking caused leaching of iron and, to a lesser extent, zinc from several whole cereals. Their findings support the hypothesis that prolonged soaking can contribute to mineral losses, although the magnitude of loss is crop- and process-dependent.

The nutritional meaning of mineral loss is more complex than total zinc concentration alone. Phytate is an important mineral-binding compound in cereals and can reduce zinc bioavailability. Soaking can therefore have two simultaneous effects: some zinc may move into the soaking medium, while phytate and phytase activity may also change. Luo and Xie (2013) reported reductions in zinc content after soaking faba beans together with reductions in phytate and improved in-vitro mineral availability. Consequently, a reduction in total zinc should not automatically be interpreted as an equivalent reduction in nutritionally available zinc.

Recent wheat research has predominantly emphasized agronomic biofortification, zinc fertilization and improved zinc availability at the production stage (Ali et al., 2023; Hui et al., 2025; Makhdum et al., 2024; Zulfiqar et al., 2020). Comparatively less attention has been given to the post-harvest fate of endogenous zinc during prolonged water soaking of harvested wheat. The present study addresses this gap by examining zinc released into soaking water over 2–24 h.

The objectives were to determine the initial zinc concentration of wheat; quantify zinc in soaking filtrates after 2, 4, 8, 16 and 24 h; estimate reported zinc loss and apparent retention; describe the relationship between soaking duration and zinc recovery/retention; and interpret the findings in relation to post-harvest mineral retention and nutritional quality.

MATERIALS AND METHODS

Study design

The study was designed as a laboratory soaking experiment in which soaking duration was the principal experimental factor. Harvested wheat grain was exposed to distilled water for 2, 4, 8, 16 and 24 h at a fixed grain-to-water ratio of 100 g to 400 mL (1:4, w/v). Zinc in the soaking liquid was subsequently determined using atomic absorption spectrometry (AAS).

Sample collection and preparation

Wheat grains (Triticum aestivum L.) were purchased from Hausa Quarters, Ekpoma, Esan West Local Government Area, Edo State, Nigeria. The grains were manually sorted to remove broken kernels, stones, dust, and other visible foreign materials, and then stored in airtight plastic containers at room temperature. A separate portion of untreated grain was powdered for determination of the initial zinc concentration.

The wheat sample used in this study was obtained from a local market in Ekpoma, Edo State, Nigeria, and the specific variety and agronomic history were not available. Wheat grain zinc concentration can vary considerably depending on genotype, soil zinc availability, zinc fertilization practices, and environmental conditions. The initial zinc concentration of 2.70 mg/100 g is within the range reported for wheat grown in Nigeria and other regions, although it is lower than the concentrations achieved through agronomic biofortification (Hui et al., 2025; Zulfiqar et al., 2020). The findings of this study should be interpreted as representative of wheat with this initial zinc concentration; the magnitude of zinc leaching may differ for wheat with higher or lower zinc concentrations. Future studies should include samples with well-documented

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agronomic history to enable comparisons across different production systems and to assess the generalisability of the findings.

Chemicals and equipment

Analytical-reagent-grade chemicals and distilled water were used. Laboratory equipment included an analytical balance, sieve, Whatman No. 1 filter paper, Kjeldahl digestion flasks, measuring cylinders, pipettes, glass funnels, retort stand and clamp, heating source and an atomic absorption spectrophotometer.

Soaking procedure

Five 100-g portions of cleaned wheat grain were placed in separate 600-mL plastic containers. Each portion was combined with 400 mL distilled water and soaked at room temperature for 2, 4, 8, 16 or 24 h. At the end of each period, the liquid was decanted and the grains separated using a laboratory sieve. The soaking liquid was filtered through Whatman No. 1 filter paper and collected in labelled containers. Recorded filtrate volumes were 364, 349, 339, 332 and 331 mL for the 2-, 4-, 8-, 16- and 24-h treatments, respectively.

Acid digestion

A 1.0-mL aliquot of each filtrate was transferred into a Kjeldahl digestion flask with the recorded digestion reagents and heated until a clear digest was produced. The digest was quantitatively transferred into a 100-mL volumetric flask and diluted with distilled water. The same procedure was used for the powdered wheat sample. A reagent blank containing all reagents but no sample was carried through the procedure.

Zinc determination by AAS

Zinc was determined by atomic absorption spectrometry at 213.9 nm. Zinc standards prepared from certified stock solution were measured with reagent blanks and sample digests. The recorded calibration range was 0.000–0.025 mg/L.

Calculation of Zinc Recovery, Zinc Loss and Apparent Retention

The AAS concentrations reported in Table 2 are treated as concentrations in the final 100-mL digest prepared from a 1.0-mL aliquot of the corresponding soaking filtrate. Under this explicitly stated dilution scheme, the calculation is internally consistent. The mass of zinc represented by the analyzed aliquot was calculated as:

Zn in 1-mL aliquot (mg) = C_{AAS} (mg/L) × 0.100 L

Because the analyzed aliquot was 1.0 mL, the total zinc recovered in the complete filtrate was calculated by scaling the aliquot mass by the recorded filtrate volume:

Zn recovered (mg) = [$\mathbf{C_{AAS}}$ × 0.100] × $V_{filtrate}$

where $\mathbf{C_{AAS}}$ is the zinc concentration measured in the final digest (mg/L) and $V_{filtrate}$ is the complete soaking-filtrate volume expressed in mL.

The factor 0.100 L accounts for the final digest volume, while the numerical value of $V_{filtrate}$ scales the 1-mL aliquot to the total filtrate.

For example, at 2 h: Zn in aliquot = 0.0003 mg/L × 0.100 L = 0.000030 mg; total Zn recovered = 0.000030 mg × 364 = 0.01092 mg.

Percentage zinc loss was calculated relative to the recorded initial zinc content of the 100-g wheat sample:

Zn loss (%) = [Zn recovered / Zn initial] × 100

Apparent zinc retention (%) = 100 − Zn loss (%) The recorded initial zinc content was 2.70 mg/100 g.

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Statistical analysis and quality control

Descriptive analysis was performed using the recorded treatment-level zinc recovery, percentage zinc loss and apparent zinc retention values. Simple linear regression was used to describe the relationship between soaking duration and zinc recovery. The coefficient of determination (R^2) was calculated from the five treatment-level observations. The regression was used only to describe the observed trend and was not interpreted as a replacement for ANOVA or as evidence of significant differences among treatment means. Based on the available values, zinc recovery increased with soaking duration, with a descriptive regression coefficient of determination of approximately **$R^2 = 0.9374$**. Apparent zinc retention showed the corresponding decreasing trend.

Quality-control procedures included the use of reagent blanks and zinc calibration standards. The calibration data showed a highly linear response over the recorded concentration range.

Table 1. Recorded zinc calibration standards and absorbance values.

Zn concentration (mg/L)	Absorbance
0.000	0.000
0.005	0.233
0.010	0.466
0.015	0.700
0.020	0.933
0.025	1.167

Linear regression of the recorded calibration points gave $y = 46.680x - 0.0003$ with $R^2 = 0.999999$.

RESULTS

Zinc calibration performance

The six recorded standards produced a highly linear response across 0.000–0.025 mg/L. The calibration equation was $y = 46.680x - 0.0003$ with $R^2 = 0.999999$. The near-zero intercept and high R^2 indicate excellent linearity of the supplied calibration points as shown in Figure 1; however, linearity alone does not establish analytical accuracy, precision or recovery.

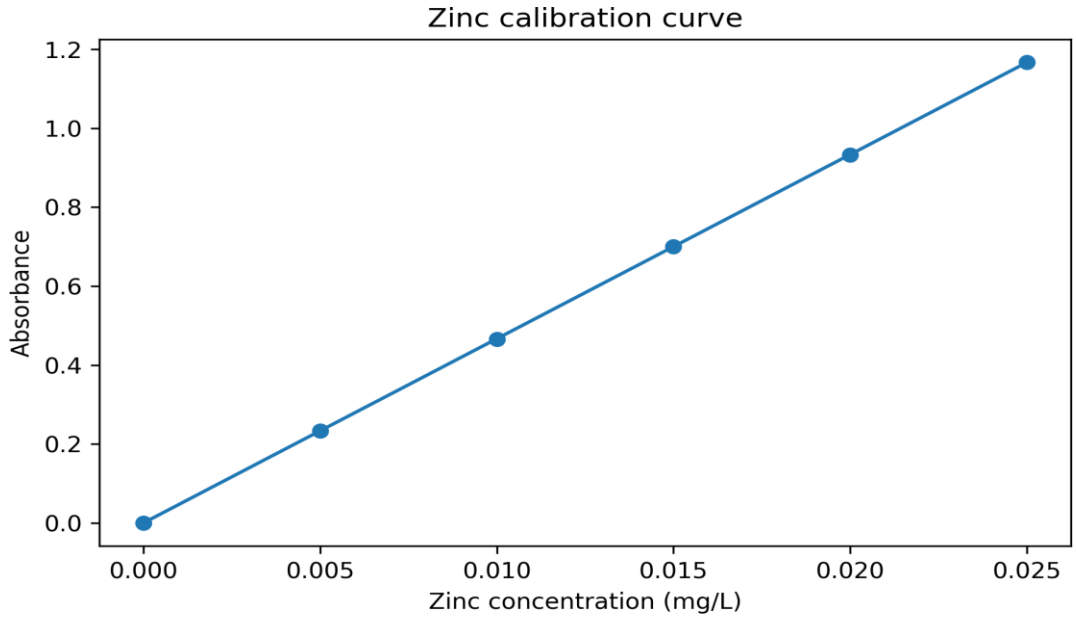

Figure 1. Calibration curve for zinc determination by AAS using the recorded standard solutions.

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Zinc leaching during soaking

Table 2. Recorded filtrate volume, zinc concentration, reported zinc recovery and reported zinc loss.

Soaking time (h)	Filtrate volume (mL)	AAS concentration in final digest (mg/L)	Final digest volume (mL)	Zn in 1-mL aliquot (mg)	Total Zn recovered in filtrate (mg)	Zn loss (%)	Apparent retention (%)
2	364	0.0003	100	0.000030	0.01092	0.40	99.60
4	349	0.0015	100	0.000150	0.05235	1.94	98.06
8	339	0.0020	100	0.000200	0.06780	2.51	97.49
16	332	0.0045	100	0.000450	0.14940	5.53	94.47
24	331	0.0051	100	0.000510	0.16881	6.25	93.75

AAS concentrations represent concentrations measured in the final 100-mL digest prepared from a 1.0-mL aliquot of each soaking filtrate. Zinc recovered was calculated as

[AAS concentration × 0.100 L] × total filtrate volume in mL. This calculation is valid under the stated 1-mL aliquot and 100-mL final-digest scheme.

Zinc Recovery and Apparent Zinc Loss during Soaking

The zinc concentrations measured by AAS in the final 100-mL digests increased progressively with soaking duration, from 0.0003 mg/L at 2 h to 0.0051 mg/L at 24 h. After accounting for the 1.0-mL aliquot-to-100-mL digestion dilution and scaling the measured zinc concentration to the total volume of each soaking filtrate, calculated zinc recovery increased from 0.01092 mg at 2 h to 0.16881 mg at 24 h. The corresponding percentage zinc loss increased from 0.40% at 2 h to 6.25% at 24 h, while apparent zinc retention decreased from 99.60% to 93.75% as shown in Table 2.

At 4 h, zinc recovery was 0.05235 mg, corresponding to 1.94% zinc loss and 98.06% apparent zinc retention. Overall, the results indicate a progressive increase in reported zinc release into the soaking filtrate with increasing soaking duration as revealed in Figure 2.

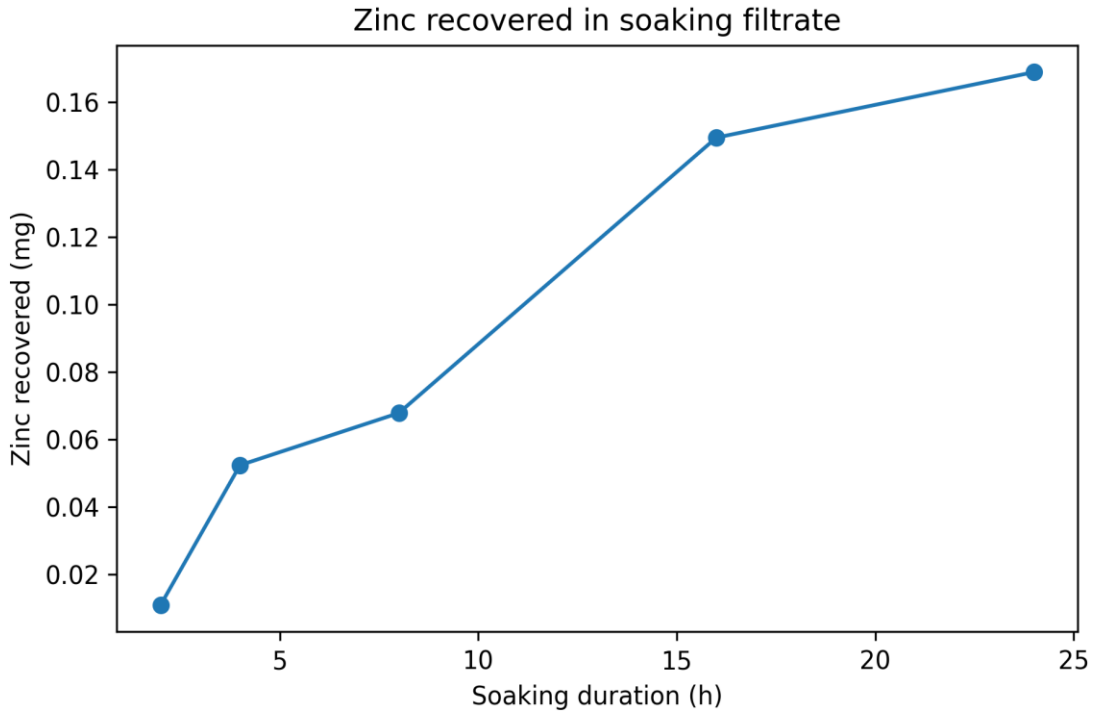

Figure 2. Calculated amount of zinc recovered in soaking filtrate as a function of soaking duration.

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Apparent zinc retention

Table 3. Apparent zinc retention calculated from the reported zinc-loss values.

Soaking time (h)	Reported Zn loss (%)	Apparent Zn retention (%)
2	0.40	99.60
4	1.94	98.06
8	2.51	97.49
16	5.53	94.47
24	6.25	93.75

Apparent retention decreased from 99.60% at 2 h to 93.75% at 24 h as shown in Table 3. A descriptive regression of apparent retention against soaking duration gave $R^2 = 0.9372$ using the corrected 4-h value of 98.06% as illustrated in Figure 3. Because retention was calculated as 100 minus the reported loss, the two measures necessarily display complementary trends.

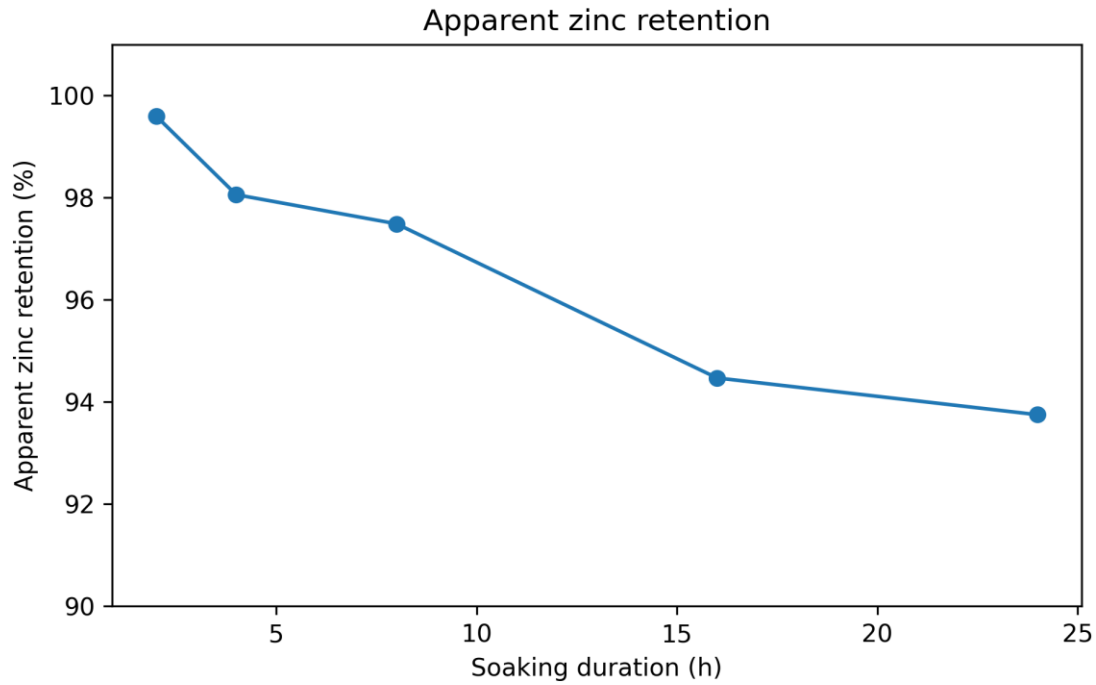

Figure 3. Apparent zinc retention calculated from reported zinc loss as a function of soaking duration.

DISCUSSION

The central pattern in the supplied dataset is a progressive increase in reported zinc recovery with increasing soaking duration. Reported recovery rose from 0.01092 mg at 2 h to 0.16881 mg at 24 h, while reported zinc loss increased from 0.40% to 6.25% and apparent retention decreased from 99.60% to 93.75%. The direction of this trend is consistent with the expectation that longer contact between hydrated cereal tissues and water can increase mineral transfer to the soaking medium.

Lestienne et al. (2005) directly demonstrated mineral leaching during 24-h soaking of whole cereals and legumes. Their study found that zinc, although less readily leached than iron, decreased in several cereal grains following soaking. The present reported pattern is therefore consistent with an established processing phenomenon. Direct quantitative comparison should nevertheless be avoided because the previous investigation involved different crops and experimental conditions.

Luo and Xie (2013) showed why mineral retention should not be considered synonymous with nutritional availability. In faba beans, soaking reduced zinc content but also reduced phytate and increased in-vitro zinc

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availability. The present study did not measure phytate, phytase activity, phytate:zinc molar ratio or in-vitro bioaccessibility. It therefore establishes, at most, reported total zinc release and apparent retention; it does not establish the net nutritional effect of soaking on zinc absorption.

The increase in zinc recovered with soaking duration may plausibly reflect continued hydration and increased opportunity for soluble or weakly associated zinc fractions to migrate from grain tissues into the surrounding water. However, zinc speciation, mineral localization, membrane permeability and diffusion were not measured. These mechanisms should therefore be regarded as plausible interpretations rather than demonstrated mechanisms.

The recorded initial zinc concentration was 2.70 mg/100 g. Wheat grain zinc concentration varies with genotype, soil and environmental conditions and agronomic management. Gashu et al. (2021) demonstrated geospatial variation in cereal nutrient concentrations, while Hui et al. (2025) showed that zinc fertilization can substantially alter wheat grain zinc concentration. Zulfiqar et al. (2020) similarly reported effects of zinc management on grain zinc concentration and zinc use efficiency. These studies provide context for the starting zinc concentration but do not establish the agronomic history of the present sample.

Production-stage zinc enrichment and post-harvest retention should therefore be viewed as complementary stages of the wheat zinc pathway. Ali et al. (2023) demonstrated the potential of zinc-solubilizing bacteria to increase zinc availability and wheat zinc accumulation, while Makhdum et al. (2024) reported enhanced wheat growth and zinc uptake following zinc fertilization. The present work addresses the subsequent processing stage by examining endogenous zinc release during soaking.

The broader evidence synthesized by Huey et al. (2023) indicates that post-harvest processing can materially influence iron and zinc retention in biofortified crops. The present study contributes a focused examination of soaking duration in wheat. Its practical implication is that, if the reported values are confirmed, unnecessarily prolonged soaking may increase mineral loss. However, no universal maximum soaking time can be prescribed from this experiment because temperature, water-to-grain ratio, pH, variety and subsequent processing were not systematically varied.

Nutritional significance of observed zinc losses

The reported zinc losses of 0.40–6.25% are relatively modest and may not have a substantial impact on the zinc contribution of wheat to the diet, particularly when wheat is consumed as part of a mixed diet containing other zinc sources. However, the nutritional significance of these losses depends on the zinc status of the population and the contribution of wheat to total zinc intake. In populations with marginal zinc status and high wheat consumption, even modest losses could be of concern. Conversely, if soaking reduces phytate content and improves zinc bioaccessibility, the net effect on zinc absorption could be neutral or even positive. Future research should measure phytate and zinc bioaccessibility to evaluate the net nutritional effect of soaking. The phytate:zinc molar ratio, which is a commonly used indicator of zinc bioavailability, would be a useful parameter to include in such studies. Wheat with a phytate:zinc molar ratio >15 is generally considered to have low zinc bioavailability, and soaking may be beneficial if it reduces the ratio below this threshold. This study did not measure phytate, so it cannot determine whether the zinc losses observed are offset by improved bioavailability.

Possible mechanisms of zinc leaching during soaking

The progressive increase in zinc release with longer soaking duration likely reflects several concurrent processes. First, hydration of the grain tissues increases the permeability of cell membranes and cell walls, facilitating the movement of soluble zinc ions from the grain interior to the surface and into the soaking water. Second, the dissolution of zinc-containing minerals or complexes on the grain surface may contribute to the observed release. Third, the activity of endogenous phytase, which can hydrolyse phytate and release bound zinc, may increase during soaking, particularly at the longer durations used in this study. Zinc that is bound to phytate in the grain may become more soluble as phytate is hydrolysed, potentially increasing the amount of zinc available for leaching. The relative importance of these mechanisms is not known from the present data,

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and future research could investigate zinc speciation in the soaking water and grain residue to better understand the leaching process. Differences in zinc localization within the grain—with higher concentrations in the bran and germ compared to the endosperm—may also influence the extent of leaching, as the bran is more exposed to the soaking water.

CONCLUSION

Within the conditions represented by the supplied dataset, longer soaking was associated with progressively greater reported zinc release into soaking water and lower apparent zinc retention in wheat. Reported zinc recovery increased from 0.01092 mg at 2 h to 0.16881 mg at 24 h, while calculated zinc loss increased from 0.40% to 6.25% and apparent retention decreased from 99.60% to 93.75%. The recovery calculations are arithmetically consistent when the stated 1.0-mL aliquot, 100-mL final digest and complete filtrate volumes are applied. The remaining pre-submission verification items are the original calculation supporting the 2.70 mg/100 g initial zinc value, confirmation that the AAS concentrations are final-digest concentrations with no additional dilution, the original replicate records if available, and the instrument-specific quality-control parameters. The study therefore supports a cautious conclusion that soaking duration is associated with zinc release, while the exact magnitude of loss should be considered confirmed only after those laboratory records are checked.

Limitations of the Study

Several limitations of this study should be acknowledged. First, the experiment was conducted using a single wheat sample purchased from one location, and the findings may not be generalizable to other wheat varieties, growing conditions, or geographic origins. Wheat grain zinc concentration and the distribution of zinc within the grain vary with genotype and environment, and these factors could influence leaching behaviour. Second, the study was conducted with a single water-to-grain ratio and at room temperature; the results may differ under other soaking conditions. Third, the study measured total zinc release but did not assess zinc speciation, phytate content, or zinc bioaccessibility, which are important for interpreting the nutritional significance of the observed losses. Fourth, the analysis was conducted without independent experimental replicates, which limits the assessment of variability and the application of inferential statistics. Fifth, the study did not evaluate the effects of soaking on other nutritional parameters such as protein quality, starch digestibility, or vitamin content. Despite these limitations, the study provides useful preliminary evidence on the relationship between soaking duration and zinc leaching in wheat, and the findings can inform the design of future research.

RECOMMENDATIONS

- Use independent experimental replicates in future experiments rather than relying only on repeated instrumental readings from one preparation.
- Measure phytate, phytate:zinc molar ratio and, where feasible, in-vitro zinc bioaccessibility.
- Evaluate soaking temperature, water-to-grain ratio, pH and soaking-water composition.
- Test multiple wheat cultivars and independent sample sources.
- Investigate soaking together with milling, germination, fermentation and cooking to better represent real processing chains.
- Where soaking is necessary, evaluate the shortest duration that achieves the required processing function while maintaining food quality and safety.

Practical implications for food processing

The findings of this study have practical implications for food processing and household preparation of wheat products. Soaking is often used to soften grains, reduce cooking time, or improve digestibility, and the duration of soaking can vary considerably in practice. The results suggest that prolonged soaking (≥16 hours) may lead to measurable zinc losses, and that the shortest soaking duration that achieves the desired processing function should be used. For example, a 2-hour soaking period resulted in minimal zinc loss (0.40%) and may be sufficient for many purposes. However, the optimal soaking duration depends on the specific processing

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objective, and the findings of this study should be considered alongside other factors such as food safety, sensory quality, and cooking efficiency. In practice, soaking water is often discarded, and the zinc that has leached into the water is lost from the food. If the soaking water is used in cooking (e.g., for soups or stews), the zinc may be retained in the final dish. The recommendations for soaking duration should therefore consider the entire food preparation process, including the use of soaking water.

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