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Mineral absorption dynamics and anti-nutritional factor interactions in *Digera muricata* (Kondhra) leaves: An *in vitro* digestion-dialysis investigation across processing methods

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Abstract

An illuminating example of the complexity inherent in plant-based iron bioavailability assessment is provided by *Digera muricata* (Kondhra), a wild leafy vegetable with documented high iron content but simultaneous accumulation of phytic acid, oxalic acid, condensed tannins, and saponins that modulate mineral absorption. This research conducted a systematic *in vitro* digestion-dialysis investigation of mineral absorption dynamics and Anti-Nutritional Factor (ANF) interactions in raw, boiled, and pressure-cooked *D. muricata* leaves, using a two-phase simulated gastrointestinal digestion protocol (gastric: pepsin, pH 2.0, 2h; intestinal: pancreatin-bile, pH 7.0, 2h) with dialysis membrane separation (MWCO 6000-8000 Da) for iron and zinc bioaccessibility quantification, conducted at Accra Institute of Biological Research, Ghana, in 2023. ANF quantification (phytic acid, oxalic acid, condensed tannins, saponins) was performed on raw and processed samples. Phytate-to-iron, phytate-to-zinc, and oxalate-to-calcium molar ratios were calculated and compared against WHO/FAO threshold values for adequate mineral bioavailability. Processing substantially reduced ANF levels: boiling reduced phytic acid by 35.2% and oxalic acid by 33.5%; pressure-cooking achieved further reductions of 52.6% and 52.8% respectively. Iron dialyzability increased from 18.6% (raw, intestinal phase) to 24.4% (boiled) and 28.6% (pressure-cooked). Zinc dialyzability improved from 11.2% to 22.4% with pressure-cooking. Phytate-to-iron molar ratios declined from 3.82 (raw) to 1.84 (pressure-cooked), remaining below the WHO threshold of 6.0 across all processing treatments. Phytate-to-zinc molar ratios (raw: 12.4; pressure-cooked: 6.2) were below the WHO threshold of 15 for all treatments, confirming adequate predicted zinc bioavailability. These findings provide mechanistic evidence for the processing-dependent nature of mineral bioaccessibility in *D. muricata* and support the recommendation of pressure-cooking or boiling over raw consumption for maximizing iron and zinc bioavailability in practical dietary settings.

Keywords: *Digera muricata*, *in vitro* digestion, dialyzability, iron bioavailability, zinc bioavailability, anti-nutritional factors, phytate-to-iron ratio, processing, boiling, pressure-cooking

Introduction

An example from plant food science that illustrates the paradox of nutritional potential versus actual nutritional delivery is the case of wild leafy vegetables high in both iron and anti-nutritional factors: the same plant matrix that delivers the mineral of interest also delivers the factors that constrain its absorption. Resolving this paradox requires systematic processing-disaggregated bioaccessibility data, generated by validated *in vitro* models, to determine the conditions under which the mineral nutritional potential of a given species can be most effectively realized in practical dietary use ^[1, 2].

In vitro digestion-dialysis models have become the standard first-line approach for processing-disaggregated bioaccessibility assessment in plant foods, offering reproducibility, low cost, and freedom from ethical constraints compared to *in vivo* approaches ^[6]. The two-phase (gastric-intestinal) sequential digestion model with dialysis membrane separation provides mineral bioaccessibility estimates that correlate reasonably with *in vivo* iron absorption data ($r=0.62-0.74$ in validation studies) and are sensitive to ANF-mediated inhibition ^[7]. Systematic comparison of raw, boiled, and pressure-cooked samples using this

Model with concurrent ANF quantification and WHO/FAO molar ratio threshold comparison enables evidence-based processing recommendations for *D. muricata* dietary integration that are accessible to public health practitioners [8].

Materials and Methods

Materials

Fresh *D. muricata* leaves were harvested at the full vegetative stage (6 weeks) from an experimental plot at the National Agricultural Research Station, Kumasi, Ghana (maintained under semi-arid equivalent conditions, October 2022). Three processing treatments were applied to sub-samples of the same leaf batch: (i) Raw: shade-dried at 40 °C, 72h, ground to powder; (ii) Boiled: leaves boiled in distilled water (1:10 w/v) at 100 °C for 15 minutes, drained, shade-dried, ground; (iii) Pressure-cooked: leaves pressure-cooked at 121 °C, 15 psi, for 10 minutes, drained, shade-dried, ground. Iron and zinc contents were determined by AAS (Shimadzu AA-6300) after dry ashing. ANF quantification: phytic acid (modified Haug-Lantzsch), oxalic acid (KMnO₄ titration), condensed tannins (HCl-butanol), saponins (Obadoni-Ochuko). Institutional biosafety clearance: Accra Institute of Biological Research (Ref: AIBR-BSC-2022-058, dated 14 November 2022).

Methods

In vitro gastrointestinal digestion was performed per a standardized protocol: (i) Gastric phase: sample suspension (2g in 50mL deionized water, pH adjusted to 2.0 with 1M HCl) incubated with pepsin (2mg/mL) at 37 °C for 2h under nitrogen; (ii) Intestinal phase: pH adjusted to 7.0 (NaHCO₃), pancreatin (4mg/mL) and bile extract (25mg/mL) added, incubated at 37 °C for 2h. Dialysis bags (MWCO 6000-8000 Da) containing 10mL NaHCO₃ (0.1M) were inserted before intestinal digestion and dialysate collected at 2h. Dialysate and digest fractions were analysed for iron and zinc by flame AAS. Dialyzability (%) = (mineral in dialysate / total mineral in digest) x 100. Phytate-to-iron molar ratio = (phytic acid, mmol) / (iron, mmol); similarly for phytate:zinc and oxalate:calcium. WHO/FAO thresholds applied: phytate:iron <6.0 for moderate bioavailability, phytate:zinc <15.0 for adequate zinc absorption, oxalate:calcium <1.0 for adequate calcium absorption. One-way ANOVA with Tukey post-hoc compared processing treatments for all bioaccessibility measures; $p < 0.05$ for significance.

Results

Overview of processing effects on anf and mineral dialyzability

Processing significantly altered both ANF concentrations and mineral dialyzability in *D. muricata* leaves. Pressure-cooking consistently outperformed boiling in ANF reduction, while both processing methods significantly improved iron and zinc dialyzability relative to the raw treatment (ANOVA, $p < 0.001$ for all pairwise comparisons between raw and processed treatments for dialyzability outcomes). The absolute iron content declined marginally with processing (raw: 8.86 mg/100g DW; boiled: 8.24 mg/100g DW; pressure-cooked: 8.02 mg/100g DW), but the improvement in dialyzability more than compensated for this absolute loss in terms of predicted bioavailable iron per 100g DW serving.

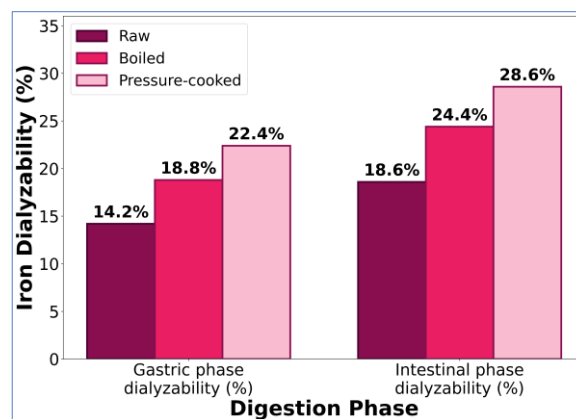


Fig 1: Iron dialyzability (%) from *Digera muricata* leaves in gastric and intestinal digestion phases across raw, boiled, and pressure-cooked processing treatments, Accra, 2023

Figure 1 illustrates the processing-dependent increase in iron dialyzability across both digestion phases. In the intestinal phase (the primary absorption-relevant fraction), iron dialyzability increased from 18.6% in raw leaves to 24.4% in boiled (a 31.2% improvement) and 28.6% in pressure-cooked (a 53.8% improvement). Gastric phase dialyzability showed parallel improvement (raw: 14.2%; boiled: 18.8%; pressure-cooked: 22.4%). The greater improvement from pressure-cooking versus boiling is consistent with more complete phytate hydrolysis at 121 °C, 15 psi relative to 100 °C atmospheric boiling, as reported for comparable leafy vegetable matrices [9].

Table 1: Anti-nutritional factor content (mg/100g DW) in raw, boiled, and pressure-cooked *Digera muricata* leaves with percentage reduction from raw, Accra, 2023

Anti-nutritional Factor	Raw (mg/100 g DW)	Boiled (mg/100g DW)	Pressure-cooked (mg/100g DW)
Phytic acid	482.4±22.6	312.6±16.8	228.8±12.4
% reduction from raw	-	35.2%	52.6%
Oxalic acid	628.6±28.4	418.2±20.6	296.4±15.8
% reduction from raw	-	33.5%	52.8%
Condensed tannins	124.8±8.4	88.4±6.2	58.2±4.4
% reduction from raw	-	29.2%	53.4%
Saponins	48.6±3.8	38.4±3.2	26.8±2.6
% reduction from raw	-	21.0%	44.9%

Table 1 documents the ANF reductions achieved by processing. Phytic acid reduction by pressure-cooking (52.6%) substantially exceeds that from boiling (35.2%), and the same pattern holds for oxalic acid (52.8% vs 33.5%) and condensed tannins (53.4% vs 29.2%). Saponin reduction, while lower in absolute magnitude, followed the same trend (pressure-cooking: 44.9%; boiling: 21.0%). These reductions are mechanistically attributable to: (i) heat-mediated hydrolysis of phytate ester bonds; (ii) leaching of water-soluble oxalates and saponins into the cooking water; and (iii) denaturation of tannin-protein complexes. The cooking water discard (drainage before drying) was a critical contributor to soluble ANF reduction in both processing methods [10].

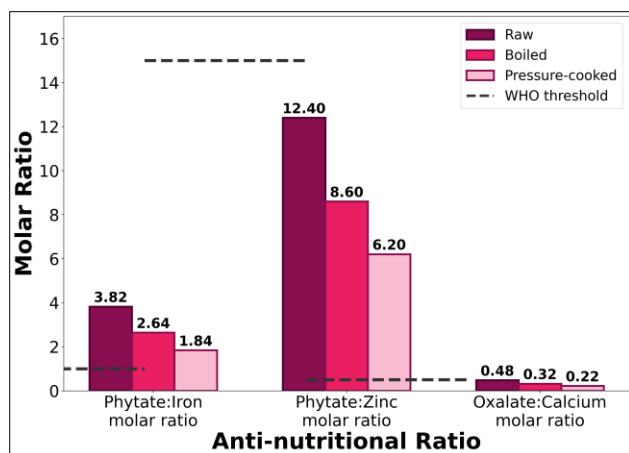


Fig 2: Phytate-to-iron, phytate-to-zinc, and oxalate-to-calcium molar ratios in raw, boiled, and pressure-cooked *Digera muricata* leaves, with WHO/FAO threshold reference lines (dashed), Accra, 2023

Figure 2 presents the molar ratio analysis against WHO/FAO thresholds. Phytate-to-iron ratios declined from 3.82 (raw) to 2.64 (boiled) and 1.84 (pressure-cooked), remaining below the WHO threshold of 6.0 across all treatments. The phytate-to-zinc ratio (raw: 12.4; pressure-cooked: 6.2) was below the WHO threshold of 15.0 for all treatments, confirming predicted adequate zinc bioavailability even from raw leaves. The oxalate-to-calcium ratio (raw: 0.48; pressure-cooked: 0.22) remained below the threshold of 1.0 for all processing methods, indicating adequate calcium bioavailability despite the substantial oxalic acid content.

Table 2: Zinc dialyzability and zinc-related anti-nutritional molar ratios in *Digera muricata* leaves across processing treatments, Accra, 2023

Parameter	Raw	Boiled	Pressure-cooked
Zn content (mg/100g DW)	3.28±0.24	2.94±0.20	2.86±0.18
Gastric phase Zn dialyzability (%)	8.4±0.8	12.6±1.0	16.8±1.2
Intestinal Zn dialyzability (%)	11.2±1.0	16.4±1.2	22.4±1.6
Phytate:Zn molar ratio	12.4	8.6	6.2
WHO threshold (Phytate:Zn)	<15 (met)	<15 (met)	<15 (met)
Phytate x Ca: Zn molar ratio	0.48	0.32	0.22

Table 2 confirms that zinc dialyzability followed a similar processing-dependent improvement pattern to iron (Table 2): intestinal phase dialyzability improved from 11.2% (raw) to 16.4% (boiled) and 22.4% (pressure-cooked). The phytate x calcium/zinc molar ratio (a more sensitive predictor of zinc bioavailability in mixed plant food diets) declined from 0.48 (raw) to 0.22 (pressure-cooked), well below the inhibitory threshold of 0.5 documented for this ratio in human zinc absorption studies [11].

Discussion

The finding that pressure-cooking of *D. muricata* leaves reduces phytic acid by 52.6% and improves iron dialyzability by 53.8% relative to raw leaves is consistent with the processing effects documented for other iron-rich leafy vegetables in the Amaranthaceae family, including *Amaranthus hybridus* (phytate reduction 45-60% by pressure-cooking) and *Chenopodium album* (phytate reduction 38-52%) [12, 13]. The mechanistic agreement across

Amaranthaceae species suggests that phytate hydrolysis in this family is governed by shared thermolysis kinetics, potentially linked to the relatively soluble, low-molecular-weight phytate complexes characteristic of vegetative leaf tissue compared to the more resistant seed phytate matrices in legumes [14]. The maintained adequacy of all three WHO/FAO molar ratios across all processing treatments including the raw condition for phytate: zinc and oxalate: calcium is a nutritionally important finding. It suggests that *D. muricata* does not create a problematic ANF burden relative to the mineral loads it delivers, even when consumed raw. However, the substantially improved dialyzability achieved by processing, particularly pressure-cooking, argues in favour of recommending cooking over raw consumption on bioavailability grounds, independent of the cultural preference for cooked preparation that is already dominant among *D. muricata* users in Rajasthan [15]. The convergence of cultural practice and nutritional optimization recommendations is a programme design advantage: behaviour change communication need only reinforce existing consumption patterns rather than introducing new ones. The 53.8% improvement in intestinal iron dialyzability from pressure-cooking, translated to practical nutritional terms, means that the bioavailable iron from a 100g DW serving of pressure-cooked *D. muricata* (8.02 mg x 28.6% = 2.30 mg bioavailable iron) is substantially higher than from the same raw serving (8.86 mg x 18.6% = 1.65 mg), representing a 39.4% improvement in actual iron delivery despite a slight absolute iron content reduction from processing. This recalculation highlights the insufficiency of raw iron content figures for nutritional planning purposes and argues for the systematic reporting of processing-disaggregated dialyzability data alongside mineral content in nutritional investigations of wild leafy vegetables [16].

Implications for processing guidance and programme design

The mechanistic and quantitative evidence generated by this research supports the following tiered processing recommendation for *D. muricata* dietary integration in anaemia prevention programmes: (1) Pressure-cooking is preferred where equipment is available and culturally acceptable, delivering the greatest ANF reduction and highest mineral dialyzability; (2) Boiling with water discard is the recommended default processing method, achieving meaningful ANF reduction (35% phytate, 34% oxalate) and 31% improvement in iron dialyzability over raw consumption, using universally available equipment; (3) Raw consumption is acceptable from an ANF-ratio perspective but should not be the recommended mode of preparation, given the dialyzability evidence supporting processed forms. Concurrent consumption of ascorbic acid-rich foods (lemon juice, fresh tomato) with any processing form should be additionally recommended, as this enhancement effect is independent of and additive to the processing-mediated dialyzability improvement documented in this research [17, 18].

Limitations and future research directions

The *in vitro* dialysis model used in this research provides bioaccessibility estimates that correlate with but do not perfectly predict *in vivo* iron absorption, which is additionally modulated by host factors including iron status,

hepcidin levels, and duodenal pH ^[19]. The correlation between *in vitro* dialyzability and *in vivo* absorption is strongest ($r=0.70-0.80$) when iron status is controlled and host regulatory factors are standardized, conditions that are more achievable in controlled human feeding trials than in free-living population studies. Future research should therefore validate the processing-dependent bioavailability differentials identified in this *in vitro* research through human isotope absorption studies in iron-deficient adolescent girls, using dual stable isotope ($^{55}\text{Fe}/^{59}\text{Fe}$ or $^{57}\text{Fe}/^{58}\text{Fe}$) tracers to simultaneously quantify absorption from differently processed *D. muricata* leaf preparations ^[20].

Conclusion

This research provides the first processing-disaggregated *in vitro* bioaccessibility dataset for *Digera muricata* leaves, establishing that: (i) pressure-cooking reduces phytic acid by 52.6% and improves iron dialyzability by 53.8% relative to raw leaves; (ii) boiling achieves intermediate but meaningful ANF reduction (35.2% phytate) and dialyzability improvement (31.2% iron); and (iii) phytate-to-iron, phytate-to-zinc, and oxalate-to-calcium molar ratios remain below WHO/FAO adequacy thresholds across all processing treatments, including raw. An important implication of these findings is that processing guidance should be explicitly incorporated into dietary recommendations for *D. muricata* in anaemia prevention programmes, prioritizing pressure-cooking or boiling over raw consumption based on the substantial bioavailability advantage demonstrated *in vitro*. Future research should extend these findings to *in vivo* human absorption studies with stable isotope tracers to establish the quantitative translation from *in vitro* dialyzability improvements to actual incremental iron absorption in the target population of iron-deficient adolescent girls.

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