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Nano Biofortification of Australian Purple Rice Using Electrochemical Behavior to Optimize Zinc Retention and Anthocyanin Bioavailability

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ABSTRACT

Zinc deficiency is a serious problem in cereal dependent populations and the use of purple rice is a potential carrier matrix for combined micronutrient delivery and polyphenol based functionality. This review aims to summarize available evidence on nano biofortification of rice via zinc (mainly ZnO and other nano zinc systems) and explore the potential of electrochemical methods to optimize the biofortification of zinc in Australian purple rice and anthocyanin bioavailability. Literature suggests that grain mineral can be significantly enriched by agronomic means, particularly via genotype selection, although this is severely constrained by the association of these minerals with the bran (localized mineral deposition in the grain), by polishing losses and by binding to phytate and by processing-induced re-distribution [1] to [6], [18] to [25]. Meanwhile, the anthocyanins of purple rice—mainly Cyanidin 3-O-glucoside and Peonidin-3-O-glucoside—are present in the outermost parts of the rice grain, and are unstable to degradation under heat treatment, neutral to alkaline digestion and upon storage for a long period [8] to [14], [40] to [50].

On one hand, nano zinc systems provide a mechanistic opportunity that can change the dissolution process, adhesion to the leaf surface, leaf cell wall, root uptake, xylem loading and sink allocation, but on the other hand, raise unanswered questions regarding particle transformation, zinc ion release, oxidative stress, and food safety [26] to [39]. The particular relevance of electrochemical methods here is due to the redox activity of anthocyanins and the related phenolics, and the ability to determine zinc in a sensitive manner using stripping methods. Thus, the combined electrochemical platform could be used for assessment of antioxidant potential and changes in zinc speciation, matrix

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interaction, and digestion – all at rapid speed for treatment screening and formulation optimization to [51] to [60]. The review suggests an integrated framework to link nano zinc treatment, grain micro distribution, polishing severity, digestion stage, cooking and electrochemical fingerprint in a multi objective optimization workflow. A similar scheme might speed up the process of establishing Australian purple rice as a functional grain with an even higher retention of zinc and a higher concentration of bioavailable anthocyanin metabolites.

Keywords—

Introduction and literature search strategy

Rice remains one of the most important staple foods globally, and its centrality to human diets makes it the most consequential cereal platform for micronutrient improvement [1], [2], [26]. However, the zinc deficiency remains due to the unabsorbable nature of zinc in the cereal based diets and also the relatively excessive level of zinc inhibitors such as phytate, as well as the removal of the bran and embryo (which are rich in zinc) from the edible part of rice when it is polished [2] to [6] and [18] [19]. Biofortification can thus have two related targets in rice: one, total zinc concentration; and two, the proportion of the zinc that is found in common rice products and that is available during digestion. Conventional, genetic, and transgenic biofortification techniques have significantly progressed to include manipulation of transport and chelation traits [2] to [7] and the breeding of high zinc genotype [20] to [25].

The shift from micronutrient enhancement to multi nutrient quality design, as in the case of purple rice. The color of pigmented rice is largely attributed to the presence of anthocyanins in the pericarp and bran, and in most cases, the major anthocyanins are Cyanidin 3-O-glucoside and Peonidin-3-O-glucoside [8] to [14]. These compounds supply high radical scavenging and reducing properties, and have led to increased interest in black and purple rice as functional grain, rather than simply a carbohydrate source [10] – [14] and [40] – [45]. However, purple rice's anthocyanin-richness is also due to its localization, making it vulnerable. Polishing will take off the pigmented layers. Labile flavylium species will be destroyed by cooking. Anthocyanins turn into chalcone and phenolic degradation products in the gastrointestinal pH changes. Therefore, a review up to and including grain composition is insufficient. The appropriate endpoint is still in place and bioaccessibility functions [40] to [50] and [66] to [72] are still operating.

Despite a lack of specifically published research on Australian purple rice, there is an increasing strengthening towards Australian relevance. Cultivation of rice takes place in southern NSW irrigated rice systems and there is viable infrastructure for rice and rice-related research and development via industry and breeding partnerships in the Riverina region. Australian wild Oryza resources also offer a valuable local scientific background: they exhibit region specific adaptations, important grain properties, and a good abiotic stress biology that will be important for future breeding and pre breeding activities [15] to [19]. The immediate translation gap does not mean that it is not

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possible to grow purple rice in Australia. It is the lack of a research plan that links agronomic zinc enrichment, pigmented grain chemistry, electrochemical fingerprinting, processing retention, digestion and feasibility in the Australian regulatory system.

This review was prepared as an Integrative Literature Synthesis. Peer-reviewed primary research publications from 2016 to May 2026 were prioritized and older foundational papers which were methodologically or mechanistically central were retained. Searches were carried out with Web of Science, Scopus, PubMed, AGRICOLA, CAB Abstracts, Google Scholar and the publishers ACS, Elsevier, Springer Nature, Wiley, MDPI and Taylor & Francis. The official sources from Australia included were also strong, particularly FSANZ and APVMA. Some typical search terms were: “purple rice anthocyanin zinc”, “rice zinc biofortification foliar”, “ZnO nanoparticles rice uptake”, “anthocyanin bioaccessibility digestion black rice”, “cyclic voltammetry food antioxidants”, “anodic stripping voltammetry zinc food”, “electrochemical impedance spectroscopy polyphenols”, “Australian rice breeding pigmented rice”, and “nanotechnology food regulation Australia”. Studies that reported rice zinc accumulation, nano zinc synthesis and characterization, plant uptake and transport mechanisms, anthocyanin profile, anthocyanin stability, electrochemical method development, digestion or bioaccessibility, changes due to processing, or regulatory issues were included. Studies on non food nanotoxicology, poorly characterized nanomaterials, or which were on other crops for which there were no transferable mechanisms were not included in the main analytical narrative.

Figure 1. Conceptual framework for optimizing zinc retention and anthocyanin bioavailability in Australian purple rice

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Table 1. Conventional biofortification strategies in rice and their relevance to purple rice

Strategy	Main mechanism	Strengths	Main limitations for purple rice	Representative references
Genetic selection and breeding	Select high grain zinc genotypes,	Durable, seed based	Slow cycle, genotype by environment	[1], [5], [6], [18], [25]

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	favorable transporters, lower antagonists	deployment, scalable	effects, anthocyanin and zinc traits not always co selected	
Agronomic soil zinc fertilization	Increase plant available Zn in rhizosphere	Familiar practice, field ready	Soil fixation, low recovery, uncertain transfer to endosperm	[2], [3], [4]
Foliar zinc fertilization	Direct delivery to canopy during reproductive stages	Often higher grain Zn response than soil only	Response depends on timing, formulation, humidity, genotype	[2], [3], [7]
Seed priming and soaking	Improve early vigor and local Zn availability	Low cost, simple handling	Usually weaker grain enrichment than late foliar delivery	[2], [27], [31]
Transgenic or genome directed enrichment	Modify chelation, loading, transport, storage	Can target endosperm deposition and dietary targets	Regulatory burden, deployment complexity, public acceptance	[5], [6], [25]
Integrated N and Zn management	Strengthen sink formation and Zn remobilization	Often improves both yield and grain concentration	N effects on anthocyanin accumulation can be genotype dependent	[2], [7], [10]

Agronomic and physicochemical basis

Conventional zinc biofortification in rice has established several principles that remain essential even Enrichment of zinc is genotype response as well as influenced by soil chemistry, irrigation regime, nitrogen application and stage of application. Often foliar application of zinc around the heading or grain filling stage is more effective to enrich grain, than the basal soil application due to avoiding bigger soil sorption limitation and more appropriate timing with grain loading [2,3]. But, enriching brown rice does not necessarily mean enriching polished rice. However, zinc is taken up mainly by the embryo, aleurone and outer parts of endosperm and hence with milling its uptake may be depleted considerably [4] to [6], [18] and [23]. This is particularly significant to purple rice as anthocyanins are also present in the outer layers of the grain. That is, if a

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significant amount of zinc is retained after processing, a considerable amount of pigment is removed, as well.

Now that the molecular basis of accumulation in rice is known it can be used in an optimization framework. ZIP family transporters are involved in zinc uptake, YSL transport metal complexes bound to nicotianamine, HMA are involved in long distance transport, and others are responsible for transport around nodes that regulate the allocation of zinc to vegetative and reproductive sinks [20]-[25]. This points to a significant design opportunity for purple rice that needs to be explored. Nano zinc delivery will be of real consequence only if it begins to affect not only uptake into the plant, but deposition into edible grain domains which come through the polishing process and survive digestion to become bioavailable. The critical end point is thus not just the total concentration [4], [18], [23], [24] but microdistribution.

Nano zinc systems are technically fascinating, as the properties and behaviour of the nanoscale materials can be altered in terms of: - high apparent reactivity, - surface adhesion properties, - dissolution properties, - and controlled delivery properties, when compared with bulk salts from [26] to [35]. In the field of agriculture, ZnO remains the most popular candidate since it is easily available in the market, inexpensive, and also it is used in other industrial and biomedical applications [31], [32]. The synthesis of ZnO nanoparticles is possible by various methods such as precipitation, sol gel, hydrothermal, combustion, sonochemical, microwave assisted synthesis and green biological synthesis. The most pertinent parameters used during the characterization for agri food applications are primary particle size, hydrodynamic size in the working suspension, aggregation behavior, zeta potential, crystallinity, morphology, surface area, zinc release kinetic and impurity profile [27] to [35]. Otherwise, agronomic comparisons may be unhelpful as nominally the same “ZnO nanoparticles” may behave in very different ways in water, on leaf surfaces and in soil solution.

The literature on nano fertilizers is still emerging and the reported agronomic benefits are attributed to a number of overlapping processes instead of one ‘nano effect' [26] to [31]. Some formulations are primarily slowly dissolving sources of ions; some improve adhesion and cuticular residence time following foliar spraying and some change the chemistry of the roots or rhizosphere. The biological response is observed in many plant systems, including the plant model of cereals, and is likely a combination of the behavior of nanoparticles and the presence of released Zn2+. This distinction is important for the review level analysis, as it has an impact on the interpretation of efficacy, toxicity and regulation [26], [28], [29], [31], [32]. Therefore, a promising future research is the comparison of the true nano specific performance with the soluble zinc with the same concentration of ion (nonspecific) with the same environmental conditions in the case of purple rice. Iron is a vital microelement that plays an important role in plant metabolism. Consuming a large amount of chemical fertilizers increases the risk factors of neoplastic diseases such as heavy metals and harmful components in crops edible parts. Therefore, utilizing novel technologies to increase yields without requiring more chemical fertilizers seems essential.

Although published rice studies are still heterodox, there are some patterns that are sufficiently regular to warrant the synthesis of a mechanism. One, seed priming and

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hydroponic seedling experiments have often shown a better germination response, root vigor, chlorophyll content or stress tolerance at lower levels with a reduction in root elongation at higher levels, which may be a combination of oxidative stress or excessive zinc exposure [31]–[39]. Second, foliar application is more likely to be successful for biofortification than soil application as there is less loss due to fixation prior to entry to plant [2, 27, 28]. Third, there is still overwhelming literature reporting the use of small zinc nanoparticles in seedling and pot studies while the literature relevant to the use of nano zinc and the retention and digestion of polished grains in mature rice plants is still limited. The difference is one of the most obvious ways in which electrochemical screening might be valuable. It could shorten the treatment decision making process prior to field validation.

Figure 2. Mechanistic pathway for nano zinc uptake and grain deposition in rice

One of the major questions yet to be answered is whether or not nano zinc can help to modify the metabolism of anthocyanin in purple rice. It is known that zinc may be affecting anthocyanin accumulation indirectly via its ability to activate various enzymes, enhance nitrogen metabolism, limit oxidative damage, and/or adjust source sink relations [2, 7, 10, 20]. However, zinc also interacts with the flavonoids system, membrane stability and signaling of reactive oxygen species in ways that can stabilize, or destabilize pigment-rich matrices, depending on dose and environment [40] to [45]. In the case of purple rice, optimizing the zinc can't be separated from optimizing the anthocyanin chemistry. Again, the result, likely to be a nonlinear response surface, is not the usual "more zinc is better.

The anthocyanins in purple rice primarily are of flavylium type anthocyanidins which are glycosylated. Typically, Cyanidin 3-O-glucoside is the primary compound, with Peonidin-3-O-glucoside being a common second product, but the profile can be changed due to cultivar, environment, extraction solvent and processing history [8] to [14]. The structural interconversion, a pH dependent phenomenon governs their chemical behavior. At lower pH values the flavylium cation produces the most intense color and often the larger electrochemical oxidation signals such as at higher pH when the hydrated and chalcone forms predominate, with the attendant diminution of color and alteration of redox signals [40] to [45]. This is more than chemistry for chemistry's sake for a rice biofortification plan. It implies that the nutritional interpretation and electrochemical readout will be altered due to the change in pH during cooking and during digestion.

Figure 3. Anthocyanin state transitions and possible zinc interactions in purple rice

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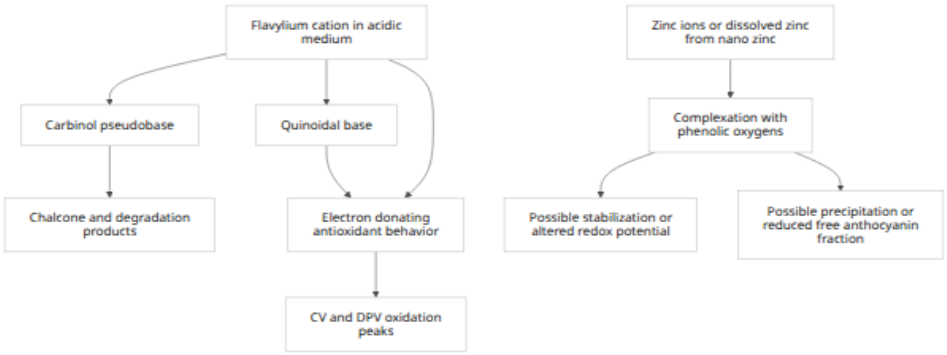

Table 2 summarizes representative nano zinc studies in rice and rice seedling systems. Because the literature is still uneven, incomplete characterization remains a recurring theme.

Table 2. Representative nano zinc studies in rice and rice seedling systems

Study focus	Zinc material	Reported size range	Dose range	Application route	Main reported outcome	Key limitation	References
Seedling vigor and germination	ZnO nanoparticles	about 20 to 50 nm	about 10 to 100 mg L^{-1}	Seed priming	Faster emergence and early vigor at low doses	Benefit declines at higher dose	[31], [37]
Hydroponic uptake and phytotoxicity	ZnO nanoparticles	about 20 to 30 nm	sub mg L^{-1} to low mg L^{-1}	Root exposure	Mixed dissolution and particle effects, root growth sensitivity	Difficult separation of NP and ionic effects	[26], [39]
Foliar nutritional enhancement	ZnO nanoparticles	about 20 to 60 nm	tens to hundreds of mg L^{-1}	Foliar spray	Improved Zn loading efficiency	Field rice data still limited	[27], [28], [29]

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			equivalent		compared with soil only in many cereal contexts		
Stress physiology in rice seedlings	ZnO nanoparticles	nanoscale, formulation dependent	low to moderate ppm equivalent	Foliar or hydroponic	Increased antioxidant enzymes and chlorophyll under stress at low dose	Stress mitigation not equal to grain biofortification	[36], [39]
Green synthesized ZnO for crop delivery	Biogenic ZnO nanoparticles	about 10 to 70 nm	formulation dependent	Soil or foliar	Improved colloidal stability and public acceptance potential	Agricultural reproducibility remains under tested	[33], [34], [35]
Grain enrichment goal	Nano zinc formulations versus soluble Zn	variable	variable	Mostly foliar	Promising but still insufficient mature grain retention data in rice	Need polishing and digestion endpoints	[2], [27], [28], [38]

Table 3 lists the anthocyanin compounds most relevant for electrochemical and nutritional interpretation.

Table 3. Major anthocyanins reported in purple and black rice

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Compound	Typical occurrence in pigmented rice	Analytical significance	Nutritional and electrochemical significance	References
Cyanidin 3-O-glucoside	Usually dominant anthocyanin	Major HPLC peak, strong UV and MS signal	Strong reducing capacity, often low oxidation potential relative to less hydroxylated analogues	[8], [10], [11], [40], [43]
Peonidin-3-O-glucoside	Common secondary anthocyanin	Supports cultivar discrimination	Slightly different redox behavior and stability relative to cyanidin analogue	[8], [10], [11], [40]
Cyanidin derivatives with acylation or additional glycosylation	Cultivar dependent, often lower abundance	Shifts retention time and molecular ion pattern	Can improve color stability but may alter bioaccessibility	[8], [12], [40], [45]
Peonidin derivatives	Cultivar dependent	Relevant for metabolomic fingerprinting	May contribute to sustained color in processed matrices	[8], [11], [12]
Co existing phenolic acids and flavonols	Widely present in bran	Broaden electrochemical response beyond anthocyanins	Important for total reducing capacity and possible zinc complexation	[9], [10], [11], [51], [52]

Electrochemical interrogation and optimization

The chemistry of purple rice is particularly suitable for application to electrochemistry as the analytes of interest are redox active and sensitive to environment with regards to structure. The voltammetric signatures contain information on the quantity and reactivity of anthocyanins, phenolic acids, flavonoids and related antioxidants as they transfer electrons at inert electrodes [51] to [60]. Voltammetry has several advantages over colorimetric antioxidant assays, which typically are reduced to a single endpoint; voltammetry can differentiate multiple oxidation processes and easier and harder oxidizing fractions and yield kinetic information from the shape of the peaks and dependence on the scan rate [51, 52, 53, 54, 55]. For a complex food matrix as purple rice, it is valuable since the electrochemical reducing activity of total anthocyanin content does not necessarily predict the electrochemical reducing activity following cooking and digestion.

The best place to start is cyclic voltammetry, it is the most conceptually informative.

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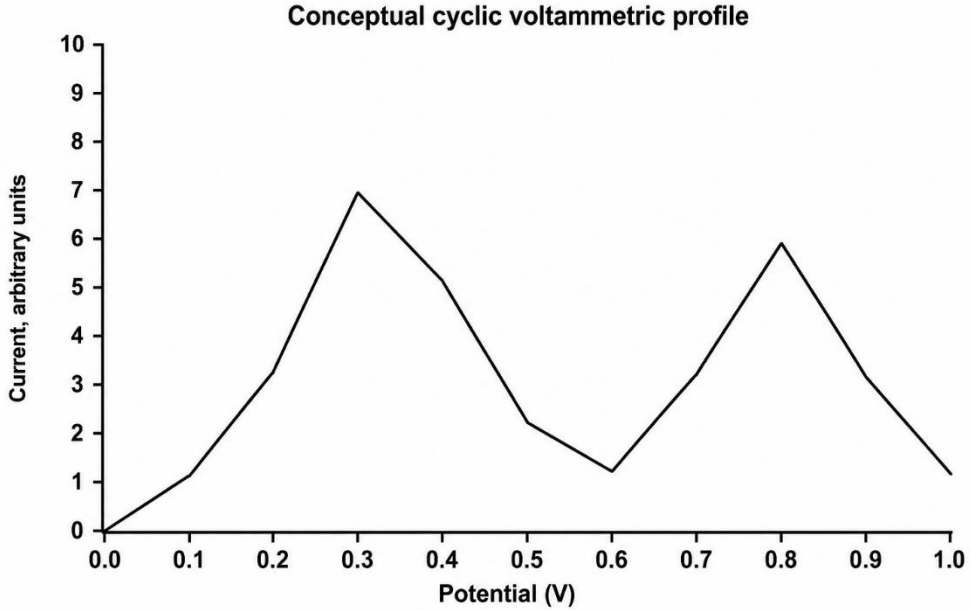
For anthocyanin rich extracts, usually the forward scan will show oxidation waves corresponding to phenolic hydroxyl groups and conjugated flavylium related groups. As the current in a CV varies approximately linear with the concentration of the electroactive species (under diffusion controlled conditions) [51] to [55] and lower anodic peak potentials usually show that the relative concentration of the species is more reducing and thus more easily oxidized. Within a treatment screening framework, CV might thus be able to differentiate between nano zinc enriched samples because of a higher number of extractables or samples which have a more reactive antioxidant pool preserved. This distinction is important because the occurrence of bioactive potential depends not only on its concentration, but also on its chemical accessibility.

Figure 4. Conceptual cyclic voltammetric response of a purple rice extract



When careful discrimination between antioxidant pools is the analysis objective, differential pulse voltammetry may be the preferred method. As pulsed waveform is more efficient in suppressing capacitive current than simple CV, DPV can be used to detect less changes in phenolic composition, depleted anthocyanin pools and digestion-induced changes in the types of electroactive fractions [51, 55, 59]. DPV seems to be the most feasible technique to rank the purple rice after processing, and after in vitro digestion. A probable pathway is to apply DPV to acidified grain extracts prior to cooking, following cooking, following gastric and following intestinal digestion and to use low PEAs as an operational indicator of preserved functionality of phenols.

The electrochemical logic is different for the trace amount of zinc. The concentration of zinc is usually not measured directly in foods by voltammetry to the same extent as anthocyanins. Anodic stripping voltammetry is rather the fundamental technique. The labile zinc is first reduced and accumulated at the working electrode and then stripped anodically to generate a current peak which is proportional to the concentration of labile

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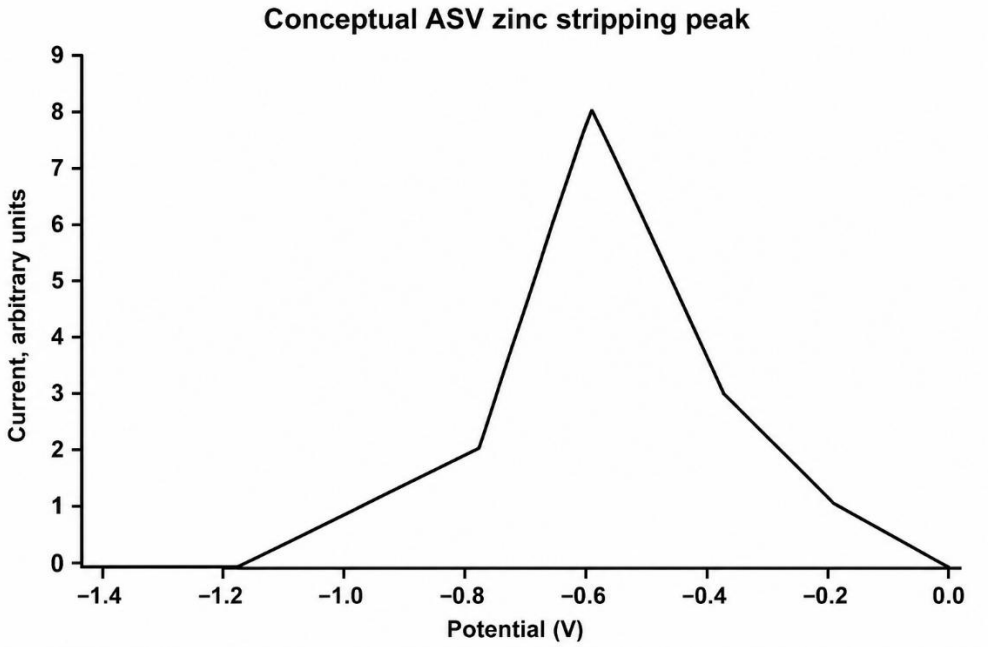
zinc [56-58, 63-65]. Bismuth film and screen printed carbon electrodes are emerging as attractive electrodes because they help alleviate the need for mercury use, and allow for sensitive multielement analysis [56] to [58]. The ASV has no ambitions to replace total elemental analysis by ICP based methods as a means of reference quantification in purple rice systems, but it could be used for a different purpose – estimation of the readily electroactive potentially more labile zinc fraction in digests or extracts of purple rice. That's the fraction most appropriate to Bioaccessibility screening.

Figure 5. Conceptual anodic stripping voltammetric signal for labile zinc



A 4th dimension is provided by electrochemical impedance spectroscopy. The emphasis of CV, DPV and ASV is on the generation of faradaic signals, whereas EIS is concerned with the interfacial resistance, capacitance and diffusion type behaviour over a frequency domain [57,58]. In food systems this is helpful when electrode fouling, adsorption, polymerization or matrix binding are a concern. Bran rich extracts are particularly susceptible to such complications including proteins, polysaccharides, lipids and polymerizable phenolics, especially in the case of purple rice extracts. EIS can then be used to identify whether a decreased voltammetric signal after cooking is due to the loss of the compound or an impact on the electron transfer as a result of the altered matrix [51].

The selection of the material of the electrodes is not simply a technical issue; rather it is a determinant of interpretability. Glassy carbon is still the workhorse for plant phenolics as it provides wide potential windows and has good reproducibility post surface renewal [52], [55]. For portable field deployable or processing line applications,

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the screen printed carbon electrodes are more promising, particularly when functionalized with graphene, reduced graphene oxide (rGO), metal nanoparticles or functional polymers, as described in the following references [57] through [60]. Bismuth modified carbon surfaces are still an option for zinc stripping because they are sensitive and have a much safer regulatory profile to mercury [56] to [58]. The most robust publishable workflow thus would employ a benchtop reference system based on the glassy carbon and bismuth film electrodes and extrapolate the most effective designs to the disposable, screen printed versions for use in high throughput screening. Electrochemistry should not be considered as a replacement for the other analytical techniques. It is valuable but an adjunct. Molecularly specific profiling of anthocyanins still needs to be performed by HPLC DAD or LC MS. ICP MS/ICP OES is still the primary technique for the determination of the total zinc. Spatial distribution requires X ray fluorescence, or synchrotron. But, these endpoints can be brought together via electrochemistry into a screening logic. Samples that contain high amounts of zinc but don't contain a high proportion of labile zinc in the digests or have high concentration of anthocyanin and low signals in the low potential anodic part of the signal after cooked samples can be deprioritized before any costly confirmatory analyses are conducted [4], [18], [23] and [46] to [60].

Table 4. Electrochemical methods applicable to zinc enriched purple rice

Method	Primary target	Typical readout	Main strength	Main weakness	Recommended use in this topic	References
Cyclic voltammetry	Total electroactive phenolic pool	Peak potential, peak current, charge	Rapid redox fingerprint, mechanistic insight	Lower sensitivity in complex matrices	First pass screening of raw and processed extracts	[51] to [55]
Differential pulse voltammetry	Sensitive phenolic and anthocyanin profiling	Peak count, height, potential shift	Better signal to background than CV	Requires careful matrix matching	Compare treatment effects after processing and digestion	[51], [55], [59]
Anodic stripping voltammetry	Labile zinc and other trace metals	Stripping peak current and area	High sensitivity, lower cost than ICP for screening	Susceptible to matrix interferences	Screen dialyzable or soluble zinc fractions	[56] to [58], [63] to [65]
Electrochemical impedance	Interfacial behavior	Charge transfer resistan	Useful for sensor develop	Indirect, model	Evaluate electrode fouling and	[57], [58]

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spectroscopy	and fouling	ce, diffusion elements	ment and matrix effects	dependent	extract interactions	
Amperometry with modified electrodes	Targeted sensing	Steady state current	Portable and real time potential	Less structurally informative	Later stage sensor translation	[57], [60]

Processing bioaccessibility and regulatory translation

Unless rice biofortification can stand the test of the consumer pipeline, no strategy is nutritionally successful. This pipeline is unusually bad in purple rice, since the nutrient-rich tissues are on the outside. Whilst, highly polished products lose a significant amount of zinc and anthocyanins [4], [6], [10], [11], [66] to [72] which are retained in whole grain or very little polished products. Not all of the distribution is congruent. Zinc continues to be present beyond the most heavily pigmented layers and anthocyanins are even more abundant in the pericarp and bran. However, the net effect is equal: the extent of the processing can greatly influence whether agronomic improvements are achieved in dietary improvements.

There is a second loss mechanism when the plant is thermally processed. Zinc itself is relatively stable chemically under normal cooking conditions, but the content in the edible portion may reduce due to washing, soaking, leaching into excess cooking water and discarding the outer portions of the particles rich in the tissue before cooking [4] [66] [70] [71] [72]. Anthocyanins are less stable. Release kinetics and apparent extractability is affected by hydration, cleavage, and oxidation (promoted by heat, oxygen, and near neutral pH) and starch gelatinization and protein interactions. It is important to note that a cooked product containing purple rice may have a lower concentration of native anthocyanin, yet yield an electrochemical signal if the low molecular mass degradation products are able to reduce. Therefore, the result of the endpoint cannot only be pigment concentration.

Figure 6. Conceptual relation between polishing intensity and zinc retention in edible rice

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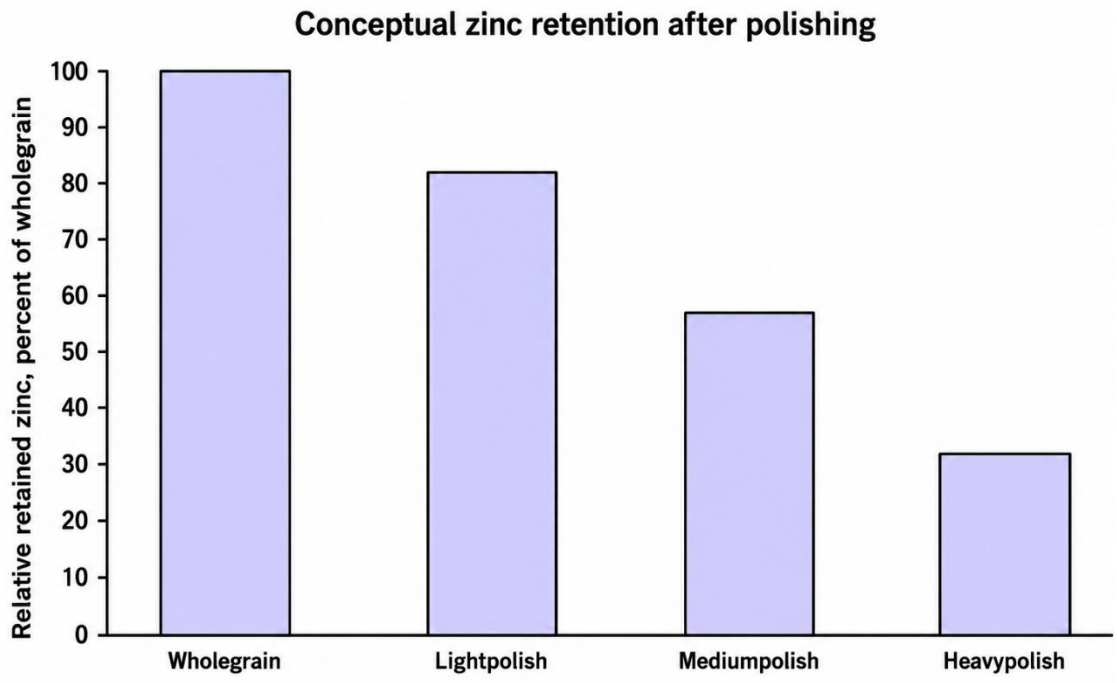

In vitro digestion models can supply the connection between food processing and probable food nutrition. The INFOGEST static model is widely adopted as the main harmonized model as it standardizes both the oral, gastric and intestinal phases and allows for the comparability of study results. [46, 47]. In such models, typically the soluble or dialyzable fraction after digestion is considered as bioaccessible zinc, and non-reversibly degraded or electrochemically non-respondent anthocyanin after gastrointestinal simulation is considered as bioaccessible anthocyanin. The two phytate and anthocyanins play a central role in antagonism to zinc in rice, while factors such as pH, enzymatic exposure and colonic transformation affect anthocyanins. Meaning, there is no correlation between the bioaccessibility of zinc and anthocyanin. An example of this is a formulation or crop treatment that increases zinc deposition into bran, but does not lower phytate, will improve total zinc, but not dialyzable zinc.

The digestion of anthocyanin is especially complicated as the parent glycosides frequently exhibit a low apparent intestinal stability, which does not necessarily mean that they are not bioactive [43] to [50]. In human and model studies, protocatechuic acid and other phenolic metabolites have been found to be formed early in the process, and the plasma levels of the intact anthocyanins are only detectable for short periods and at very low levels [43, 44]. The question of whether nano zinc enrichment leads to a reduction of digestible phenolic redox system becomes relevant to the nutritional value of purple rice even if the parent anthocyanins are reduced. Here the use of electrochemistry can be helpful, as it will be able to monitor the retained electron donating ability throughout digestion without having to first structurally identify all products at the start of the digestion process.

A suitable optimization framework for publication should thus include the assessment of at least five interdependent end points: zinc concentration in whole grains,

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concentration of zinc in polished grains, retained zinc in cooked grains, soluble zinc after digestion via INFOGEST, and electrochemical antioxidant fingerprints after different digestion stages. These should not be interpreted independently, but in conjunction with each other. For instance, a treatment that would result in an increase of 40 percent in the zinc content of the wholegrain but would not change the zinc content of the polished grain should not be given first consideration. Similarly, if a treatment can maintain a high concentration of anthocyanin in the raw grain, but yield weak DPV signals after intestinal digestion, it may not be of high functional value. The ultimate one is going to be found on the one that provides a well-balanced profile between processing and digestion. The principle processing effects are listed in Table 5.

Table 5. Processing effects relevant to zinc retention and anthocyanin preservation in purple rice

Step	Effect on zinc	Effect on anthocyanins	Practical implication	References
Dehusking and light polishing	Moderate loss from outer tissues	Marked loss from bran pigments	Preserve as brown or lightly polished product when possible	[4], [10], [11], [66], [67]
Heavy polishing	Major loss from embryo and aleurone associated Zn	Severe loss, often near complete pigment depletion	Contradicts functional positioning of purple rice	[4], [6], [66], [67]
Washing and soaking	Possible leaching of loosely associated Zn into water	Extractable pigment loss into soaking medium	Minimize soak time if retention is primary endpoint	[66], [68], [69]
Absorption cooking	Better mineral retention than excess water boiling	Moderate heat loss but less leaching	Preferred for retention studies	[66], [68], [70]
Excess water boiling	Lower mineral retention because of discard water	Greater anthocyanin leaching and degradation	Should be tested but not preferred	[66], [68], [70]
Storage under light, oxygen, and heat	Minor direct Zn change but possible matrix redistribution	Progressive anthocyanin decline and oxidation	Use oxygen and light protective packaging	[41], [42], [45], [71], [72]

Ultimately the Australian translation of nano zinc biofortification has to go through regulation. Currently, FSANZ reports that there is not widespread use of

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nanotechnologies in the food sector; that if new food is made using nanotechnologies which might raise safety concerns it would need to undergo detailed scientific assessment before it could legally be added to the food supply; and that if the particle size is relevant to the functionality or toxicology of the food to the human body then information on particle size would be required. FSANZ also reports that work on understanding the uptake, distribution and elimination of nanoscale materials is ongoing and it continues to be vigilant on commercialization of manufactured nanoscale materials. This is significant for pigmented rice since there is need for a different evidentiary set up for any deliberate nano encapsulated zinc application along a food facing pathway.

APVMA is the determining regulator of agricultural chemical products, for field side applications, in Australia. According to APVMA, agricultural and veterinary chemicals are regulated based on risk, products are only registered if they are not likely to cause harm when used as directed; and hazard and exposure are evaluated together to complete a risk assessment. APVMA also highlights that products that contain nanomaterials could have different behaviour than the conventional products, nanotechnology in agvet chemicals remains in relatively early stages of development and there is a need to provide more data regarding nanomaterials and additional risks when agvet products with nanomaterials are applied. This guidance now suggests, then, that a case-by-case assessment be made instead of accepting or rejecting doctrines by category. [5]

The implication is clear in analysis. When developing a serious program of purple rice in Australia, any development from the outset should be geared towards regulatory grade evidence generation, including full nanoparticle characterization, ionic control treatments, field appropriate rates, residue and fate studies, grain fraction analysis, digestion studies and risk aware product claims. More than agronomic benefit in greenhouse pots, is necessary. For the viability of the transfer, a distinction between particle effects, dissolved zinc effects and matrix mediated food quality effects must be analytically defendable.

Figure 7. Proposed optimization workflow for Australian purple rice

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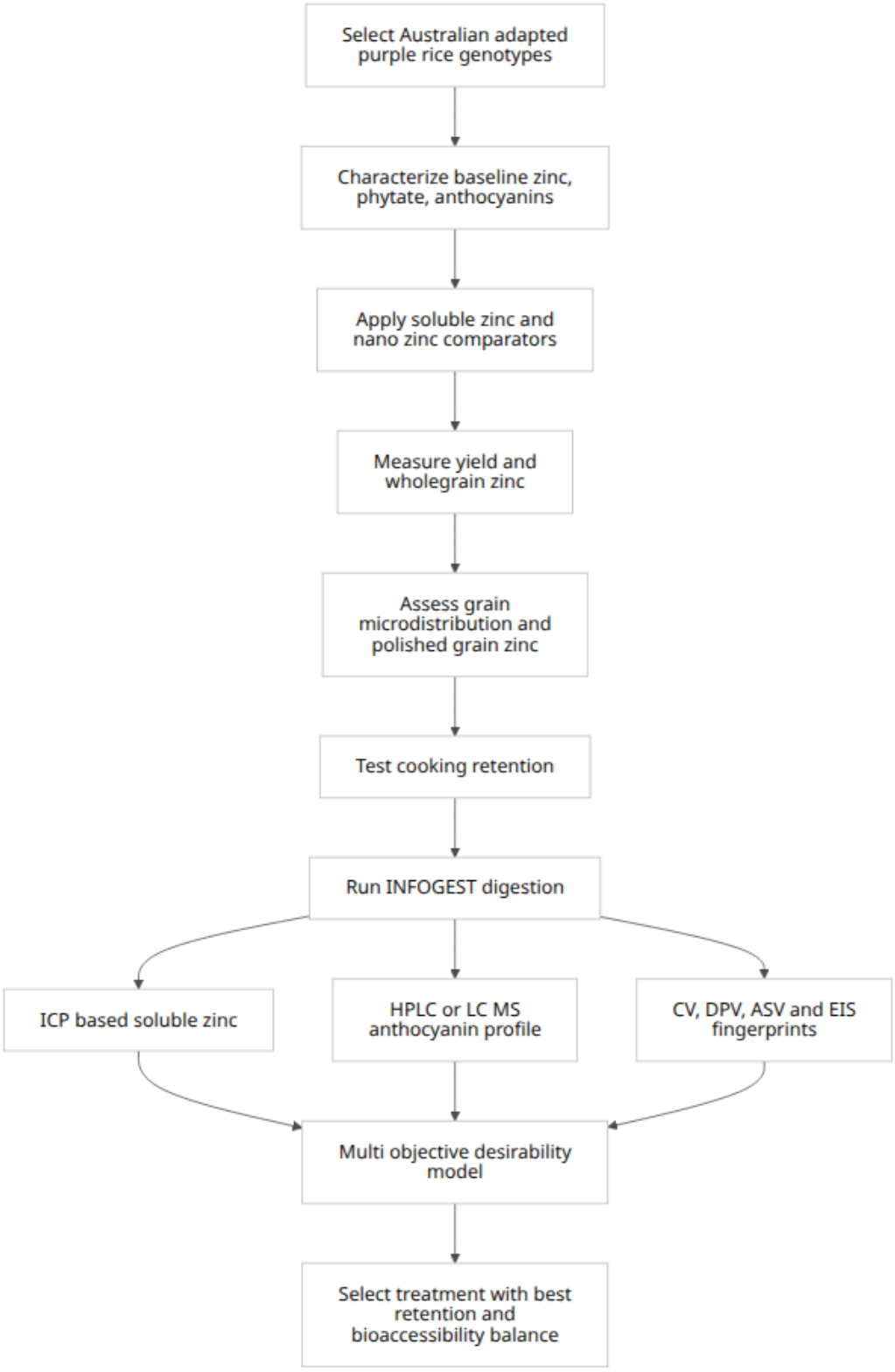

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Table 6 closes the analytical section by identifying the principal gaps that currently prevent a decisive evidence base.

Table 6. Research gaps and priority studies

Gap	Why it matters	Priority study design	Priority references
Lack of mature grain rice nano zinc field data	Seedling responses do not predict edible grain retention	Multi site Australian field trials with soluble and nano zinc comparators	[2], [27], [28], [29]
Poor reporting of nanoparticle characterization	Prevents cross study comparison	Minimum reporting standard for size, zeta potential, crystallinity, dissolution, impurities	[27] to [35], [73], [74]
Few studies combining zinc and anthocyanin endpoints	Purple rice requires dual optimization	Joint Zn, anthocyanin, phytate, and electrochemical profiling in same experiment	[7] to [14], [40] to [45]
Limited polished grain and cooking retention data	Total brown rice zinc can overestimate dietary benefit	Fraction specific retention studies under household cooking methods	[4], [6], [66] to [70]
Limited digestion stage electrochemistry	Missing fast functional screening step	INFOGEST plus CV, DPV, ASV, and EIS at sequential digestion phases	[46] to [60]
Limited Australian regulatory pathfinding	Translation requires early compliance alignment	Pre regulatory data package modeled on FSANZ and APVMA expectations	[76] to [79]

Conclusions and future research agenda

The literature provides evidence of a strong but incomplete case for nano biofortification of purple rice. The introduction of zinc into rice is already possible using existing agronomic and genetic approaches, while the bran chemistry of purple rice is scientifically interesting as it combines the delivery of minerals with anthocyanin rich bran chemistry [1] to [14]. The review also, however, reveals why many current studies are not adequate for making decisions in the translation field. These stress the total zinc accumulation, initial vegetative response and raw grain composition, and underlook polishing loss, cooking loss, digestion and the chemical form of retained antioxidants. Those omissions are important for a crop where the premiums are based on the outer layers.

A possible way of breaking this deadlock is provided by electrochemical behaviour. CV and DPV are effective tools to profile the retained redox active phenolic fraction, ASV can be used to measure labile zinc in digests and extracts, whilst EIS can be used for diagnosis of matrix and sensor behaviour [51] to [60]. Elemental analysis or

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chromatographic profiling should never be substituted with any of these methods. They have power due to integration and throughput. They have the ability to transform a wide, and various, treatment area right into a workable list of candidates for more research. From an applied point of view, electrochemistry can serve as an integrating screening language between the fields of agronomy, food chemistry and nutritional science.

The evidence leads to a prioritised research agenda. A genotype x formulation x timing of application experiment in Australian adapted pigmented rice is the first priority, with at least one soluble zinc control and well characterized nano zinc comparator. The second is spatial grain analysis, differentiating between wholegrain, lightly polished and heavily polished grains. The third is a standardization of a housewife cooking and storage study. The fourth one is INFOGEST digestion with parallel ICP based zinc quantification, anthocyanin profiling and integrated voltammetric measurements. The fifth is regulatory pathfinding, which incorporates discussions informed by the present requirements and perspective of FSANZ and APVMA for nanomaterials characterization and risk assessment. [6]

The current evidence base has the disadvantage that so far, studies on purple rice, particularly those in Australia are still limited, while the number of studies on nano zinc in mature grain rice is relatively small when compared to the entire nano fertilizer literature. This does not detract from the research program outlined in this paper. It sharpens it. Widespread speculation of nano enabled rice is the rational next step. It's an integrated Australian discipline program which optimises the presentation of zinc retention, bioavailability of anthocyanin, electrochemical response and regulatory feasibility.

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