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Insight of microgreens biofortification and its nutraceutical perspective

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Abstract
Microgreens are outstanding greens that are prized for their high nutritional content since they contain more chemicals that are good for you than mature greens. These exceptional greens offer a concentrated dosage of vitamins and crucial phytocompounds, making them suitable replacements and alternatives to mature veggies. A major issue is soil depletion, which causes absorbed microelements to become scarce and reduces microgreen yields. In order to solve this, biofortification can play a crucial role in enhancing the selenium or iodine content of microgreens, hence boosting their total yield and microelement content. In order to get the greatest results, the fortification process should be supported by a combination of balanced nutrient fertilization, the presence of advantageous microbes, and careful selection of plant species. This in-depth review article focuses on examining the health advantages of microgreens, their potential as nutraceuticals, and the benefits of biofortification. Countries like India and other developing nations may successfully reduce the risk of chronic illnesses and improve microelement availability by putting these techniques into practice. Additionally, this strategy offers promising chances for commercializing microgreens and promoting their growth on homesteads to benefit from the accompanying health benefits.

Keywords: Microgreens, biofortification, nutraceutical, selenium, iodine

Introduction
Microgreens are immature, fragile, and fragile plants made up of different cereals, vegetables, herbs, and flowers that are well known for their beneficial effects on human health. Due to the rising interest in microgreens as a freshly developed superfood, their consumption and popularity have dramatically expanded over the past ten years [1]. Microgreens have become very essential due to their remarkable nutritional value and delectable flavour [2] and they are now regarded as a unique food category that improves the sustainability and variety of the global food chain. Data from the USDA's National Nutrient Database show that microgreens are more nutrient-rich compared to their mature equivalents, making them a highly advised dietary source for human health.

In recent years, there has been a growing public awareness of the importance of leading a healthy lifestyle, leading to a search for nutrient-dense food sources that can benefit human health. This has resulted in increased interest in fortified foods and nutraceuticals, as they offer not only basic nutrition but also potential health advantages in reducing the risk of chronic diseases. Moreover, scientific initiatives and research teams are actively working on repurposing food wastes to create high-value byproducts that can be utilized as functional components in the development of new products [4]. Among the promising goals is the enhancement of the nutritional value and concentrations of essential elements in microgreens. To achieve this, biofortification has emerged as a viable strategy to increase the micronutrient levels during the growth of microgreens, consequently enhancing their nutritional value. For instance, biofortification with selenium (Se) has shown to be beneficial for sprouts and microgreens by significantly improving their antioxidant defense capabilities [4]. This approach holds the potential to further elevate the health benefits of microgreens and promote their utilization as a valuable dietary component.

An effective and secure source of organic selenium supplements for supporting human nutrition and health is found in plant-based meals. The biological action of selenium (Se), an essential element, is mostly carried out by selenoproteins. Instead of essentiality, different plant species show varied degrees of Se tolerance. Se biofortification aims to raise the Se content without significantly lowering crop production. Other dietary minerals and antioxidant substances, like polyphenols, which have anticancer and cardioprotective characteristics, may impacted by se biofortification [5].

This review covers practically every aspect of microgreens belonging to the various family including their nutraceutical properties. The review outlines the uses for microgreens, including some advantages for human health, as well as the selenium biofortification to increase microgreens growth. Additionally, it contains certain bioactive mechanism regarding the potential of microgreens in a health promoting contexts. In this review, we speculate and consider the possibility that microgreens could provide a full nutritional package and hence be employed as a nutraceutical for nutraceutical for improving human health more naturally and successfully.

Variety of Microgreens

The vegetable species that are most frequently used to make microgreens come from a number of botanical families, including the Brassicaceae (such as cauliflower, cabbage, tatsoi, Chinese cabbage, mustard, rappini, Savoy cabbage, watercress, or kale, brassica raab, radish, mizuna, broccoli, and arugula), Apiaceae (notably celery, dill, fennel, carrot), Asteraceae (such as lettuce, endive, escarole, chicory), Amarillydaceae (garlic, onion, leek), Cucurbitaceae (such as cucumber, melon, squash) and Amaranthaceae (especially red orach, amaranth, Swiss chard, spinach, beet). Microgreens can be produced from a diverse range of herbaceous species such as oats, durum wheat, legumes, corn, barley, soft wheat, rice, oleaginous plants like sunflower, as well as fiber plant species like flax. Additionally, numerous aromatic species like basil, chives, cilantro, and cumin are also frequently used for microgreens production [6]. These microgreens can be cultivated using either commercial cultivars or local varieties, each possessing distinct characteristics in terms of seedling form, color, texture, and flavor. Moreover, they boast high levels of phytonutrients that offer various health benefits. Furthermore, beyond the commonly cultivated species, there is potential to explore microgreens production using wild edible species that have been traditionally employed in folk cooking. By utilizing these wild species for microgreens, an array of colors, shapes, and flavors can be introduced, along with an abundance of essential nutrients beneficial for consumers' health [7]. The versatility and nutritional value of microgreens derived from both cultivated and wild species make them a promising addition to the culinary and dietary landscape.

Biofortification

Selenium biofortification

Selenium is a crucial trace mineral essential for maintaining human health, as its deficiency can lead to severe ailments like cardiovascular diseases, cancer, viral infections, and diabetes. However, it's important to note that there is a narrow range between the required intake level (55 μg day−1) and the level at which it becomes toxic (400 μg day−1). The Mediterranean diet, which includes cereals, vegetables, fruits, meat, and dairy products, typically provides an average total selenium intake of about 80 μg day−1 per person [8]. This dietary pattern has traditionally been sufficient to meet selenium needs. However, the dietary habits of Mediterranean populations are evolving, potentially leading to deficiencies in certain vitamins and minerals, including selenium, due to changes in food consumption and choices. Consequently, this shift in eating habits introduces the risk of nutrient imbalances and deficiencies.

The available literature does not provide specific data on the average daily consumption of microgreens. However, considering their typical use as a garnish to enhance the taste of dishes like salads, meat, or fish, it can be assumed that the average daily serving size of microgreens is approximately 10 grams fresh weight [9]. In selenium biofortification programs, it is essential to select crops that have the capacity to assimilate and accumulate this element in the parts of the plant that are consumed. The low selenium content in plants is not always due to a deficiency in the soil; rather, it can result from its presence in forms that are not readily available for plant uptake and utilization. To enhance the bioavailability of selenium, bio-fertilizers containing microorganisms are utilized. These microorganisms, such as Bacillus bacteria (e.g., Bacillus cereus, Bacillus licheniformis, and Bacillus pichinotyi), produce secondary metabolites, including inorganic and organic acids, which promote the solubilization of selenium compounds present in the soil. This approach has been used to enrich wheat, resulting in increased selenium content in the seeds compared to control groups [10]. Additionally, fungi have also been studied as microorganisms that can enhance the uptake of selenium from the soil, leading to biofortification of plants with selenium [11].

These endeavors aimed at enhancing the availability of selenium in crops offer potential in addressing deficiencies and promoting healthier selenium intake in human diets. Studies suggest that both phenotype and genotype play significant roles in the biofortification of plants with selenium. Research on spinach has shown varying degrees of microelement enrichment based on the plant's sex [12]. In this context, male plants exhibited more improvement and achieved higher selenium content in leaves compared to females after the application of selenium and selenite. On another front, numerous researchers emphasize the importance of plant genotype selection to achieve higher micronutrient uptake, as demonstrated in wheat, rice, lentils, and peas [13]. These studies suggest the potential existence of genes that regulate the selenium uptake process from the soil.

Iodine Biofortification

Adequate and regular iodine supply to the human body is crucial to combat deficiencies that affect a considerable number of people worldwide. Insufficient iodine levels, depending on age and gender, can lead to various health issues, including thyroid enlargement (goitre), abnormal thyroid functioning (hyperthyroidism/hypothyroidism), and, in the case of women, even miscarriage and mental disability [18]. To address this concern, the recommended dietary allowance (RDA) of iodine is set at 150–200 μg/day for adolescents and adults, while lactating/pregnant women require 230–260 μg/day. Traditionally, iodination of table salt has been widely used to prevent iodine deficiency. However, excessive salt consumption may lead to hypertension or heart disease, prompting the need for new solutions. A potential alternative approach to overcome iodine deficiency in both humans and animals is the biofortification of plants [19]. Sophisticated forms of iodine or combinations of iodine with supportive additives are commonly used to achieve specific effects. Salicylic acid is the most frequently used additive in combination with iodine, and when used at an appropriate dose (typically 0.1 mg/L), it does not increase plant yield but enhances the uptake of iodine, leading to the biofortification of edible plant parts [20]. Similarly, humic and fulvic acids have a similar effect, where they do not increase plant yield but promote iodine uptake, resulting in enriched plants. Researchers have explored various forms of iodine beyond KI (potassium iodide) and KIO3 (potassium iodate). These include 5-iodosalicylic acid, 3.5-diiodosalicylic acid, 2-iodo-benzoic acid, 4-iodobenzoic acid, and 2.3.5-triiodobenzoic acid [21].

Table 1: Effect of biofortification of microgreens with selenium

Microgreen variety	Biofortification material	Effect of biofortified microgreen	Reference
Lettuce	Selenium along with salicylic acid	• Addition of salicylic acid resulted in a slight decrease in nitrates and an increase in ascorbic acid and sugars. Additionally, the microelements iron (Fe), zinc (Zn), and copper (Cu) also showed an increase. • The effect of salicylic acid on selenium content exhibited a slight increase.	[14]
Broccoli	Selenium	• The correlations observed align with those from the initial test cycle. • In broccoli, the lowest dose yielded the highest fresh weight, while the highest dose resulted in the greatest concentration of Se in broccoli florets.	[15]
Cabbage	Selenium along with betaine	• Incorporating betaine showed a beneficial impact on leaf count, plant height, and weight. • When compared with the control group fertilized solely with sodium selenate, the addition of betaine resulted in an insignificant reduction in Se content.	[16]
Lentil	Selenium along with salicylic acid	• The introduction of salicylic acid did not influence the plant's fresh weight; however, it led to a slight rise in Se, ascorbic acid, flavonoids, and anthocyanins in lettuce leaves. • A substantial rise in proline content was observed.	[17]

The substance that yielded the best results in iodine enrichment was 2-iodobenzoic acid, which led to a significant increase in iodine levels in tomato plants. When compared to soil applications, foliar fertilizers proved to be more effective, increasing iodine concentration by 5–10 times without adversely affecting the yield. For optimal iodine enrichment in all tested plants, the recommended form and dose were foliar application of KIO3 at a concentration of 0.05% w/v[22]. In the case of pepper fruits [22], Li *et al*. conducted research on iodine biofortification and its impact on fruit quality. Fertilization with KI solutions in concentrations ranging from 0.25 to 5.0 mg/L resulted in the accumulation of 350–1330 μg/kg of iodine in broccoli. Thus, biofortified pepper fruits can fulfill the daily iodine requirement recommended by the WHO. Even the application of a low iodine dose (0.25–1.0 mg/L) contributed to the enhancement of pepper quality, including reduced fruit acidity and increased vitamin C content.

Table 2: Effect of biofortification of microgreens with iodine

Microgreen variety	Biofortification material	Effect of biofortified microgreen	Reference
Radish	Iodate	• The biofortified radish exhibited superior iodide content, resulting in a higher yield, while iodate led to greater iodine enrichment in the plants.	[23]
Cabbage	Iodine	• An elevation in the dosage led to an increase in both dry matter and iodine content. • The most noteworthy results were obtained when using the highest dosage.	[24]
Lettuce	Iodine	• The iodine content in the sprouts rises with an increase in the dosage (reaching its highest level at the highest dose). • Conversely, there is a decline in both yield and chlorophyll content as the dosage is increased.	[25]
Cowpea	Potassium iodide	• Following the application of potassium iodide, cowpea exhibited elevated iodine content. • A noteworthy rise in iodine levels in plant tissues was observed, corresponding to the dosage applied. • The most substantial enrichment was achieved with the highest dose.	[26]

Microgreens: A health promoting nutraceutical

Microgreens possess an assortment of bioactive elements known for their potential in enhancing health. Researchers have documented their capabilities such as anti-cancer, anti-microbial, antioxidant, anti-inflammatory, anti-obesity, anti-diabetic properties, and other health benefits [27] (Fig 1). Both in vivo and in vitro investigations have proposed the potential use of microgreens as nutraceuticals. The association between chronic inflammation and the advancement of various chronic illnesses, like cardiovascular disease (CVD), obesity, atherosclerosis, and cancer, has been established [28]. It is widely acknowledged that an abundance of liver lipids induced by a high-fat diet can elicit inflammatory reactions. However, the intake of red cabbage microgreens has shown potential in reducing liver C-reactive protein (CRP) and TNF-α levels, thus mitigating the risk of an inflammatory response.

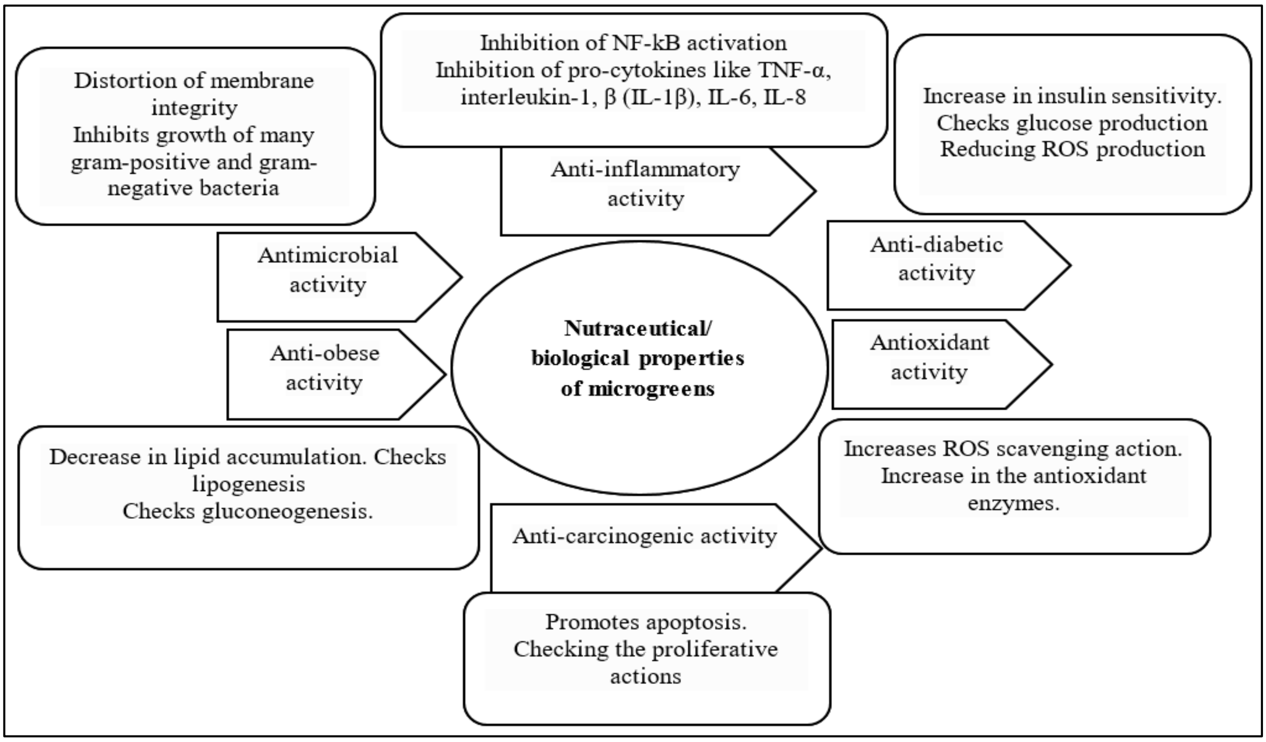

Fig 1: Mechanism and Nutraceutical activities of Microgreens [28, 29, 30, 31]

Microgreens possess the ability to influence various immunological pathways that can lead to diverse consequences. In particular, red cabbage microgreens have a significantly higher glucosinolate content of 17.15 mol/g dry weight, compared to mature red cabbage (8.30 mol/g). Glucosinolate is believed to inhibit the catabolic process of the nuclear factor of light chain gene enhancer in B cell inhibitor (IB), which in turn inhibits NF-B activation [29]. This suggests that dietary glucosinolates from microgreens can serve as an effective anti-inflammatory nutraceutical. Another key enzyme involved in the regulation of prostaglandin production and inflammatory responses is COX-2 (cyclooxygenase-2), situated downstream of NF-κB. Interestingly, microgreens contain flavonoid components such as kaempferol, isohamnetin, and quercetin, which have demonstrated the ability to inhibit COX-2 activity [30]. This further supports the potential anti-inflammatory properties of microgreens.

Numerous recent studies have shown that adopting a cruciferous vegetable diet can be highly effective in providing protection against several types of cancers, such as those affecting the colon, rectum, pancreas, thyroid, and more. Although the exact protective mechanism is not yet fully understood, researchers speculate that the bioactive components present in this diet may play a preventive role against various cancers, including breast, prostate, and colon cancers [32]. Given the public's growing concern about cancer prevention, the concept of maintaining a healthy diet has gained significant importance in disease management. Microgreens, as part of a functional diet, have emerged as potent anti-cancer agents, largely due to their rich content of vitamins, carotenoids, polyphenols, and glucosinolates. These bioactive constituents contribute to their potential in combatting cancer effectively.

The consumption of Brassicaceae microgreens, including broccoli, kale, mustard, and radish, has been found to possess inhibitory effects on the proliferation of colon cancer cells. One important bioactive component present in microgreens, particularly in broccoli microgreens, is sulforaphane, which has shown promising cancer-preventive properties[33]. Microgreens are known to contain a diverse array of chemical compositions. Compounds such as indoles and flavonoids present in microgreens play a role in regulating processes mediated by steroid hormones. Among these bioactive chemicals, indole-3-carbinol (I3C) is found in higher concentrations in cruciferous vegetables and has been demonstrated to offer protection against various malignancies, including breast cancer[34]. The abundant presence of these beneficial substances in microgreens contributes to their potential in promoting health and preventing cancer.

Recent advancements in technology, along with a deeper understanding of the gut microbiome, have brought to light the functional relationship between the human host and gut bacteria. The gut microbiota plays a crucial role in various functions, impacting both the overall health of the host and the development of chronic illnesses. An interesting potential approach to impeding disease progression as a preventive measure involves diversifying and modifying the gut microbiome[35]. Moreover, the composition of bacteria in the human intestine has been found to be linked to numerous phytoactive substances. In a study conducted by Ni *et al.* [36], bioinformatics was utilized to identify more than 400 chemicals present in a plant-based diet, which were associated with approximately 609 target microorganisms in the gut. In the study, various flavonoids like kaempferol, quercetin, and apigenin were identified. It was found that consuming higher amounts of flavonoids derived from cocoa could lead to an increase in the presence of bifidobacteria and lactobacilli in the human gut, while also lowering plasma triacylglycerol levels. Microgreens, known for their richness in flavonoids, are considered an excellent dietary addition for regulating gut microbiota.

The research indicates that microgreens offer cardiovascular protection. In particular, red cabbage microgreens have a noteworthy sinapine (hydrocinnamic acid/phenolic acid) content, which surpasses that of mature red cabbage. Furthermore, red cabbage microgreens contain bioactive substances like glucosinolates, indoles, isothiocyanates (glucosinolate breakdown products), as well as specific flavonoids and non-flavonoid phenolic compounds. These potent substances play a crucial role in safeguarding against cardiovascular and metabolic issues, including cancer[37]. Sulforaphane, an isothiocyanate known for its antioxidant and anti-inflammatory properties, has proven to be highly effective in combating cardiovascular disorders. It achieves this by activating the leucine zipper transcription factor Nrf-2, as demonstrated in the study by Bai *et al*. [38]. Once activated, Nrf-2 triggers the activation of downstream target genes, leading to the initiation of a protective mechanism against oxidative stress and harmful electrophilic substances. This mechanism involves the production of various cytoprotective proteins, including antioxidants and detoxification enzymes. On the other hand, flavonoids play a crucial role in scavenging free radicals and activating cellular antioxidants, which effectively shields low-density lipoproteins from oxidation, as highlighted in the study conducted by Sohel *et al*. [39].

Commercial prospects of biofortification and nutraceutical activity of microgreens

Commercially grown microgreens serve a dual purpose: they not only offer high-value and nutritious food for human consumption but also provide significant health benefits owing to their enhanced nutritional content. This increased nutritional profile has the potential to generate substantial income for farmers, particularly in the post-pandemic landscape of 2020 [28]. However, a significant challenge in the cultivation of microgreens lies in the lack of standardization of mass/volume units for the fertilizers utilized during their growth. This lack of uniformity makes it difficult, and sometimes even impossible, to compare the effectiveness of different cultivation strategies. To address this issue and facilitate the statistical advancement of future applications, it is crucial to establish standards for biofortification in microgreens. Doing so will enable better evaluation and optimization of their nutritional value and overall benefits. Consumers have turned to microgreens as a solution to incorporate bioactive compounds and essential food components into their diets without the need to travel to supermarkets, as highlighted in the study by Gupta *et al*. [40]. This trend is particularly relevant in the current era of heightened interest in biofortification, where the focus is on achieving a slower release of micronutrients for long-term benefits. However, there remains a lack of discussion concerning the assimilability of specific forms of micronutrients. While recommended intake standards for selected ions are relatively low, the economic constraint lies not in the concentration of a given ion but rather in its availability for absorption. Soil conditions play a crucial role in this regard. For instance, Selenium (Se) and iodine can be easily absorbed by humic acids and reduced to forms that are not taken up by plants. On the other hand, Se, due to its higher mobility, is readily absorbed by plants under suitable conditions. This information is essential to ensure effective biofortification strategies and maximize the nutritional benefits of microgreens.

Greater emphasis should be placed on enhancing the activation and solubility of ions in the soil. In recent years, microgreens have garnered significant attention due to their potential profitability [41]. The ultimate profitability of microgreens depends on the market and the specific sector in which farmers intend to sell their products. Additionally, in the context of commercial microgreens production, it is crucial to take customer preferences into account, as they heavily rely on overall sensory evaluation. Consequently, the future success of microgreens is contingent upon catering to customer preferences. The research findings indicate that while the aesthetic aspect holds considerable value, the flavor and texture play a pivotal role in determining their approval. The preference for customer acceptance lies with microgreens that possess a milder, less astringent, sour, and bitter flavor. Among the microgreens evaluated, Swiss chard and coriander emerged as the most favorable choices for introduction to Western markets, while mibuna and cress were found to be the least popular options [42]. Continued research is essential to deepen our understanding of the phytochemicals and bioactivity of each microgreen, as this knowledge can expedite their entry into the market. Additionally, in developing nations like India, it becomes crucial to promote the scientific and health benefits of microgreens to generate increased consumer interest and foster a positive perception of the product.

Conclusion

Biofortification involves increasing the mineral content of crops through plant breeding, transgenic techniques, or agronomic practices. The primary purpose of this process is to address health issues caused by deficiencies in essential microelements. By enhancing the forms of various microelements and effectively supplementing plants with micronutrients, biofortification aims to combat microelement deficiencies in the human population. The implementation of biofortification in major crops can significantly improve the intake of essential microelements by individuals. Agronomic biofortification, which involves the efficient use of microelements like Selenium (Se) and Iodine (I), leads to the accumulation of higher microelement densities in the edible parts of plants. Simultaneously, genetic biofortification focuses on developing genotypes that accumulate microelements through overexpression approaches. Despite the potential health benefits of microgreens, the exact mechanisms through which they promote well-being remain unknown. It is crucial to establish how microgreens interact with various pathways, including inflammatory, molecular, and cellular processes, to influence human health positively. Further research and evidence are required to substantiate the health-promoting properties of microgreens thoroughly. To fully comprehend and establish the potential of microgreens in preventing chronic illnesses and inflammatory disorders, comprehensive research using higher animal models is imperative. Although there are various challenges to overcome, considering microgreens as a "Super food" makes them a promising nutraceutical crop that can supplement the diet with essential nutrients, offer energy, and contribute to reducing protein malnutrition.

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