Trop J Nat Prod Res, December 2021; 5(12):2051-2056
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Review Article

Food Biofortification: A Transition from Nutrient Enrichment to Physiological Significance

Ini-Oluwa A. Akindoyeni[1,2], Stephen A. Adefegha[1], Sunday I. Oyeleye[1,3], Ganiyu Oboh[1]*

[1]*Functional Foods, Nutraceuticals and Phytomedicine Laboratory, Department of Biochemistry, Federal University of Technology, Akure, Nigeria*
[2]*Department of Science Laboratory Technology, Federal Polytechnic, Ile-Oluji. 200223, Ondo, Nigeria*
[3]*Department of Biomedical Technology, Federal University of Technology, P.M.B. 704, Akure 340001, Nigeria*

ARTICLE INFO

Article history:
Received 12 March 2021
Revised 19 November 2021
Accepted 28 December 2021
Published online 03 January 2022

ABSTRACT

The benefits of food surpassed the traditional role of providing energy. Foods are being researched and channelled toward the treatment of nutrient deficiencies and the management of certain diseases. This is due to the presence of micronutrients and phytochemicals, which confer a variety of health benefits. The concentrations or levels of these phytochemicals and micronutrients vary in food crops, particularly in the edible portion, which can lead to nutrient deficiency. Nutrient deficiency may pose a threat to life and also lead to the degeneration of some disease conditions. This review aims purposely to throw more light and encourage the use of biofortified foods in the treatment of certain diseases. This review was conducted based on previous scholarly works, which were accessible online and published in English. The source includes Google Scholar, PubMed, ResearchGate and Scopus, and other platforms for reviews and studies related to the aim of this work. The search terms included biofortification, selenium, Nutrient Deficiency, Micronutrient, Phytochemicals; Biofortification; Diseases and other related terminologies. The advent of biofortification has helped to promote the availability of essential nutrients and phytochemicals in the edible portion of crops, thereby reducing micronutrient deficiency in world populations. Diseases caused by nutrient deficiencies, such as goitre, night blindness, and anaemia, have been addressed with the help of biofortification programs.

Keywords: Nutrient Deficiency, Micronutrient, Phytochemicals, Biofortification, Diseases.

Introduction

One of the major challenges that have been plaguing populations especially in low-income countries is the issue of micronutrient deficiencies and diseases (endemic, epidemic, and chronic). This has put vulnerable groups: children, pregnant women, and the elderly at risk. These micronutrient (vitamin A, selenium, zinc, folate, iron) deficiencies could progress to the development of certain diseases, even worsening some infectious and chronic disease conditions, thereby increasing morbidity and mortality rates.[1] Micronutrient deficiency leads to disorders such as night blindness (Vitamin A deficiency), goiter (iodine deficiency), anaemia (iron deficiency), decreased resistance to infectious diseases, stunted growth, impaired cognitive function, xerophthalmia, etc.[2] There is a positive correlation between the spread of infectious diseases such as HIV, tuberculosis, measles, Keshan diseases, Kashin- Beck disease, and micronutrient deficiencies.[3] In fact, degeneration of some chronic diseases such as cardiovascular diseases (CVDs), Osteoporosis, Osteomalacia, thyroid deficiency, colourectal cancer, etc have also been linked to this challenge. All these chronic diseases are global burdens that reduce life quality and lead to the death of millions yearly all over the world. To combat this challenge on a large scale, strategies that have been put in place include the promotion of food diversification, dietary supplementation, and food biofortification.[4]

*Corresponding author. E mail: ganiyu.oboh@gmail.com
Tel: 07031388644

Citation: Akindoyeni IA, Adefegha SA, Oyeleye SI, Oboh G. Food Biofortification: A Transition from Nutrient Enrichment to Physiological Significance. Trop J Nat Prod Res. 2021; 5(12):2051-2056. doi.org/10.26538/tjnpr/v5i12.1

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Food diversification is described as the consumption of a variety of food and different food groups over a given period.[5] Although the method makes use of the traditional foods of the population, the plausibility of making available all the essential micronutrients, especially folate, vitamin A, selenium, zinc, and iron is not guaranteed. This method, therefore, might not be effective in the management of some diseases as it is limited with low productive returns as well as issues of affordability for developing countries.[6] Dietary supplements on the other hand are substances that are produced in the form of tablets, capsules, and gels are taken to provide nutrients. They are not taken with the intention of prophylaxis or treatment of diseases.[7] Examples are protein, fibre, vitamins, calories, minerals herbal supplements, and body-building supplements. Although dietary supplements help to provide the body with the needed essential micronutrients, when taken in excess, they can bring upside effects, proving toxic to the users. For example, vitamin A toxicity can lead to liver damage, nausea, headaches, blurred vision, etc. Supplements can also lead to drug-drug or food-drug interactions, leading to the inhibition of certain drugs. Food biofortification, which is the third strategy for combating nutrient deficiency is the process of improving the nutritional profile of plant-based foods via agronomic interventions, conventional cross-breeding, and biotechnology.[8]

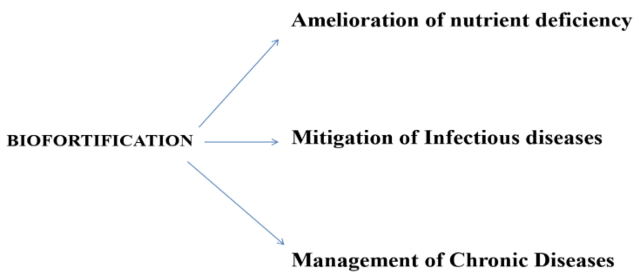

Figure 1: Health benefits of biofortification

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

It is also known as the nutritional breeding of desirable nutritional genes into staple or local food crops.[9] Biofortification aims to fortify the edible portion of plants with micronutrients, making them available during consumption (Figure 1).
Biofortification of food crops especially cereals (maize, rice, and wheat), pulses, and cassava in diet peculiar to a certain group of people has been shown to provide these populations with nutrients lacking in their staple foods. Biofortification of essential micronutrients such as zinc, iodine, iron, selenium, folate, and vitamin A into staple food of some regions has helped these populations to meet the recommended dietary intake of these micronutrients.[9,10] This strategy is sustainable and provides the micronutrients to the body in the organic form. Plants absorb and transform the inorganic form of these micronutrients and metabolize them to the organic form which is best utilized by the human body. An example is the transformation of selenite by plants to the organic form selenomethionine and selenocysteine by plants.

Methodology

This review was conducted based on previous scholarly works, which were accessible online and published in English. The source includes Google Scholar, PubMed, ResearchGate and Scopus, and other platforms for reviews and studies related to the aim of this work. The search terms included biofortification, selenium, Nutrient Deficiency, Micronutrient, Phytochemicals; Biofortification; Diseases and other related terminologies.

Results and Discussion

Techniques used in biofortification
The techniques used in biofortification include agronomic techniques, biotechnology, and conventional cross-breeding (Figure 2).

Agronomic technique
This method of biofortification is simple and sustainable. Mineral fertilizers are used, and the soil is inoculated with beneficial microorganisms.[11,12] The mineral fertilizer of choice is applied to the soil, increasing the availability of the mineral in the soil, thus increasing its level in the plant. An important factor to be considered when choosing the element is its mobility in the soil and in the food crop to be planted. This method has been successful in the biofortification of selenium, iodine, and zinc as they have good mobility and accumulation in the plants.[13,14] The country of Finland made use of sodium selenate supplemented fertilizers and this increased the levels of selenium in the food crops and, consequently, the intake of selenium in the population.[15] China and Thailand also successfully biofortified plants with iodine and zinc via the use of inorganic fertilizers.[16]
Micronutrients with poor mobility in the soil and plants are fortified via *foliar fortification*. The fertilizers are sprayed on the leaves of the plants to increase the level of the preferred micronutrient in edible parts of the plant.[10] Foliar application of Iron, Zinc, and Selenium in pulses increased the concentration of these nutrients in the grains of the plants. [17] Countries that record low levels of these essential micronutrients in their soil make use of this method because it is cheap and easily sustainable.

Biotechnology
Biofortification via the use of genetic engineering is another technique of biofortification. The desired traits for a specific micronutrient are incorporated into the staple food. This method is efficient, cost-effective, and sustainable. The genes of interest which are not expressed in the plant are directly inserted into the plant genome resulting in the expression of the desired trait by this plant. This method is not limited by the plant gene pool or the accumulation of nutrients in the non-edible parts of the plant.[18] The Golden Rice, for instance, derived its name from its high b-carotene level. It has a golden colour and was produced via biotechnology by the research group of Ingo Potyrus. As the majority of grains and cereals lack the precursor molecule for vitamin A biosynthesis, the transgenic crop was engineered with the use of two genes from daffodil and the Bacterium *Ermina uredovoia*. These genes reconstitute the carotenoid biosynthetic pathway within the rice genome.[19] This method has enhanced the reduction and elimination of Vitamin A deficiency in rice-consuming populations.[20,21] Other crops which have been fortified via this method include maize (biofortified with beta carotene), cassava (beta carotene).[22] Recently, the use of artificial nucleases such as Transcription Activator Like Effector Nucleases (TALENS) and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) [23] have been used to modify genes of interest, making them more applicable in crops such as rice, wheat, and tomatoes.

Conventional cross breeding: this is the process of selecting plants that naturally contain high levels of the desired nutrients and cross-breeding them to produce staple crops with desirable nutrient traits. The genetic variations of beta-carotene, iron, zinc, and functional carotenoid level varied among cultivars, and this makes it possible to select nutritionally appropriate cultivars. Beans, for instance with improved iron content have been bred through conventional means.[24] This method relies entirely on the genetic pool of the plant crops and genetic control is used to improve the level of micronutrients.

Micronutrient deficiencies properly managed by biofortification
1. Vitamin A deficiency: Vitamin A is a fat-soluble vitamin that is needed by the body for growth, vision, the integrity of the immune system, reproduction, cell proliferation, etc. Vitamin A is essential for vision because it is a main component of rhodopsin, the protein responsible for the absorption of light in the retinal receptors. The Recommended Daily Allowance for men is 900ug, while that of women is 700uug retinol activity equivalents.[25,26] The RDI for children however ranged from 400 to 600 retinol activity equivalents.[26] Vitamin A deficiency is common in developing countries, especially among infants and children, with symptoms such as night blindness and xerophthalmia. Over three million children develop eye problems due to vitamin A deficiency, with more than half becoming blind.[27] The availability of Vitamin A to humans is in two major forms; provitamin A and preformed vitamin A. Provitamin A is the carotenoids gotten from plants while the preformed vitamin A is gotten from animal sources. However, of all the carotenoids, beta carotene is the only provitamin that can be transformed to vitamin A by the human body (Figure 3). Hence, the gene encoding for the beta carotene pigment is inserted into the genetic pool of some food crops.
Some food crops which have been successfully biofortified with beta carotene include rice (the golden rice), cassava [28,] and maize.[29] Tang *et al* in 2009 proved that when ingested, the beta carotene is duly converted to Vitamin A and that the golden rice could provide about 55% to 70% of the recommended dietary allowance RDA of vitamin A.

2. Zinc deficiency: Zinc is an essential micronutrient, being the second most abundant trace element in the human body after iron.[30]

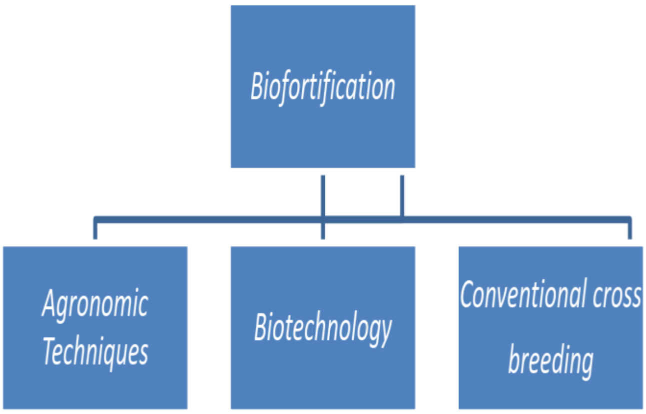

Figure 2: Techniques used in biofortification

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

It exists as a co-factor to various enzymes and as a structural stabilizer to various proteins. Zinc-containing proteins play major roles in the body such as signal transduction, transcription, and cellular metabolism.[31,] Zinc is essential for wound healing, the integrity of the immune system, apoptosis, blood clotting, DNA synthesis, etc.[31] Zinc deficiency could lead to anemia, loss of appetite, loss of the immune system integrity, stunted growth, and infertility.[31] Some diseased conditions such as HIV, cancer, renal diseases, gastrointestinal disorders, could also lead to zinc deficiency.[32] The recommended dietary intake for zinc is 15 mg/ day.[32]

Nearly one-third of the world's population suffers from Zinc deficiency with over 450,000 children dying, as a result, every year. The deficiency of this micronutrient has been linked to the lack of the micronutrient in the diet. The major diet of populations happens to be cereals which contain low or no levels of zinc. As a result, there have been various biofortification programs aimed at fortifying cereals with zinc, via the use of zinc fertilizers. Food crops that have been successfully fortified with zinc include maize and wheat.[32]

3. Iodine deficiency: Iodine is another essential micronutrient that is needed by the body for the production of thyroid hormones. The recommended dietary intake ranges between 90- 250ug/ day. The major source of iodine is kinds of seafood, hence groups with great distance away from seas have a greater risk of iodine deficiency. The main symptom of iodine deficiency is the enlargement of the thyroid, commonly known as goiter.[33] Although, due to the prevalence of iodized salt in the early 2000s, the epidemiology of goiter was reduced, iodine deficiency remains prevalent in areas where iodized salt is not accessible, making it apparent that reliance on iodized salt is not enough to combat iodine deficiency.[34] Biofortification of vegetables with iodate has thus taken precedence in the bid to increase iodine levels in populations. Studies have shown the biofortification of leafy vegetables such as spinach, lettuce, and cabbage.[35, 36]

Biofortified foods in endemic diseases

1. Keshan disease: Keshan disease is an endemic myocardial disease that was first discovered in the Keshan country of Heilong- Jiang province in 1935.[37] The disease was later discovered in other parts of China. It is characterized by cardiogenic shock, enlarged heart, congestive heart failure, cardiac arrhythmias, and ECCG changes. The various hypothesis was postulated concerning the etiology of the disease and they include viral infection, environmental detoxification, mycotoxin intoxication, nutritional deficiency, etc. Only two however proved reasonable after various experiments were conducted. First, the enterovirus, Coxsackie B virus was isolated from the tissues of patients with the disease[38], establishing the fact that the disease was caused by a virus. Secondly, the diseases were found to be endemic in areas with low soil selenium concentration, creating a correlation between selenium deficiency and the disease.[39] Clinical trials using oral supplementations of selenium were successful in the prevention and management of the disease, establishing the fact that selenium deficiency plays a role in the progression of the disease. Cermelli *et al.*, in the year 2000[40] in an *in vitro* study showed that selenite was able to inhibit the replication of the coxsackie B virus while selenate and selenomethionine did not possess and anti-retroviral activity.

Although inorganic selenite is often used as a dietary supplement, the human body makes use of organic selenocysteine as the biologically active selenium form. This is because the biological essentiality of selenium has to do with its occurrence in enzymes and proteins, where it is incorporated in the organic form selenocysteine. This might help to promote the use of selenium biofortified foods in the prevention and management of the disease as plants absorb the inorganic selenite from the soil and transform it into the organic forms selenomethionine and selenocysteine. This was first observed in wheat by Horn and Jones in 1941.[41]Selenocysteine is an important amino acid in the active site of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, and thioredoxin reductase. Selenium is a structural component of the antioxidant enzyme glutathione peroxidase which protects the cells against the accumulation of hydrogen peroxide and organic peroxides (Figure 5).

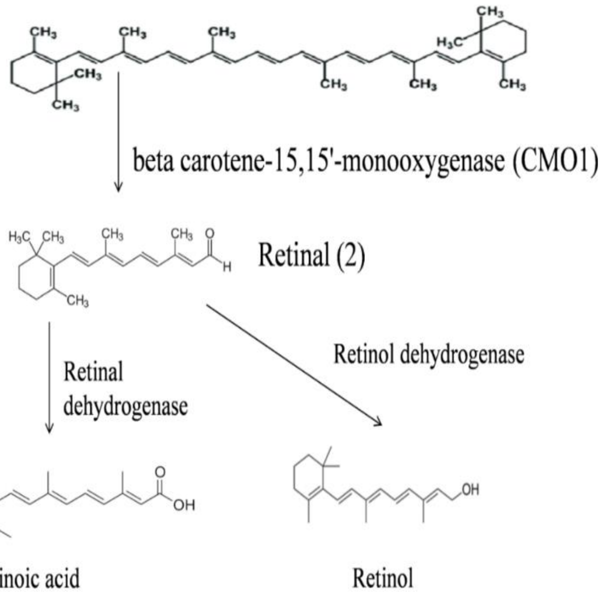

Figure 3: Schematic diagram showing the metabolism of Vit A in the Human body

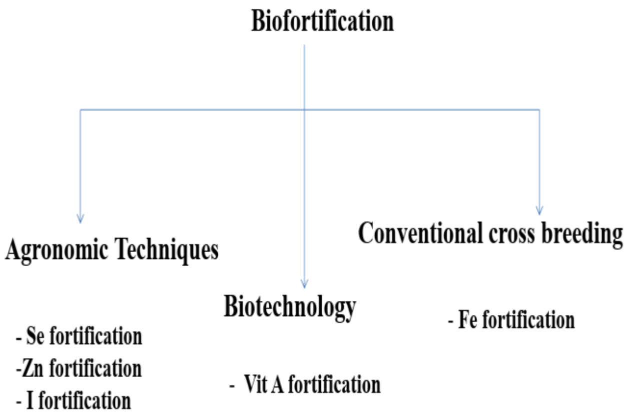

Figure 4: Micronutrients that have been biofortified with the different biofortification techniques

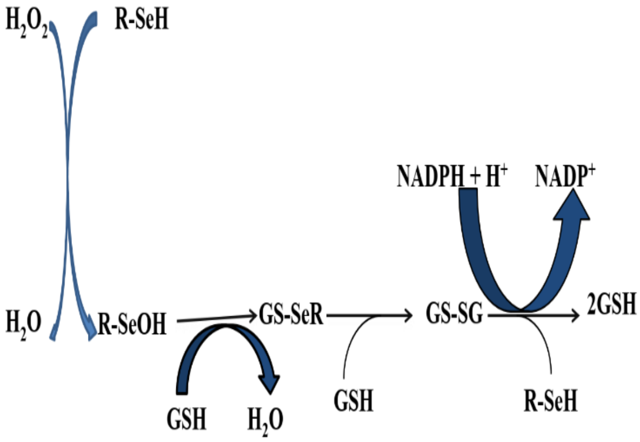

Figure 5a: Mechanism of action of selenium in the peroxidation of hydrogen peroxide via glutathione peroxidase pathway and glutathione reductase pathway.[42]

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The glutathione peroxidase enzyme possesses a selenol/ selenolate group (Figure 6) which gives them a strong reducing power, enabling them to act as reducing agents in antioxidant enzymes.[42]
Hence for selenium to carry out its functions, it must be incorporated into selenoproteins. The use of selenium biofortified foods might have a greater chance in the management of the disease over dietary supplements as the biofortified food contain the organic form of selenium compound selenocysteine. Selenocysteine is more biologically active in the human body when compared to inorganic selenite. It also helps to eliminate selenium toxicity which can occur as a result of dietary supplementation.

Possible roles of biofortified foods in chronic diseases
1. Cancer: cancer is the uncontrolled growth and division of cells. It can kill an organism by crowding out the normal cells resulting in loss of tissue function.[43] Oncological therapies seem to be effective, but they lead to the damage of both tumour cells and the normal tissue. These therapies- radiotherapy and chemotherapy due to their side effects (nausea and vomiting) make the balanced nutritional status of cancer patients hard to accomplish and maintain. Supplements are thus administered to cancer patients to facilitate better tolerance of chemo and radiotherapy.
Selenium supplements were shown to mitigate the side effects of conventional cytotoxic therapies e.g. nephrotoxicity by Cisplatin and mucositis by radiotherapy.[44] Although, selenium confers these beneficial roles in the system, inorganic forms of selenium such as selenite or selenate produces genotoxic effects via single-strand breaks of the DNA. However, due to the metabolic process of selenium by plants, selenite is converted to the organic form of selenium yielding selenomethionine and selenocysteine which can be absorbed by the body. These organic forms are infused into general body proteins and function in redox regulation, antioxidant protection, reproduction, and thyroid function.[45] Hence, foods biofortified with selenium could prove to play beneficial roles in cancer management.
2. HIV/AIDS: Ever since the eruption of this disease, a total of 25 million people have died (UNAIDS). Many patients suffer from chronic malnutrition and antioxidant drugs are prescribed alongside antiretroviral drugs in the management of the disease. Malnutrition has been shown to speed up the progression of HIV to AIDS making nutrition an important strategy of HIV therapy. Hence biofortified foods could be required to help in boosting the immune system, fighting opportunistic infections, increasing the efficacy of antiretroviral drugs, and enhancing the antioxidant system. In HIV/AIDS infection, accumulation of reactive oxygen species up-regulates viral replication leading to the production of more viral genome via the activation of transcription factors nuclear factor-kappa b NF-KB and activator protein 1 AP1.[46]
One major mechanism of defence is the negative regulation of HIV replication by the enzyme thioredoxin reductase (TR1).[47] Thioredoxin reductase is an enzyme belonging to the pyridine nucleotide-disulfide oxidoreductases family. It is a flavoprotein with each monomer known to contain a FAD prosthetic group, an NADPH binding site, and a selenylsulfide active site.[48] In the predominant cell types affected with HIV, the $CD4^+$ cells and the monocytes/ macrophages, HIV replication is mainly regulated via the Transactivator of transcription (TAT) pathway. TaT is a transactivating protein encoded by HIV, which is required for viral replication. It is a protein containing 86 to 101 amino acids depending on the location and contains a cysteine-rich region which is required for its activity.[49] The protein is sensitive to the redox state of the cell and reducing agents inhibit its activity.
Upon the entry of HIV into the host cell, the viral RNA is transcribed to complementary DNA by the action of reverse transcriptase, which is then infused into the cellular chromosome. The Tat protein then translocates into the nucleus and binds RNA stem-loop structure (TAR) and also recruits positive transcription elongation factor (P-TEFb) to the HIV long terminal repeat. All these increase the activity of the RNA polymerase II, increasing HIV replication and activation (Figure 7a). This whole process is weakened in the absence of Tat protein, resulting in the amassing of permanently prune transcripts (Figure 7b).[49]

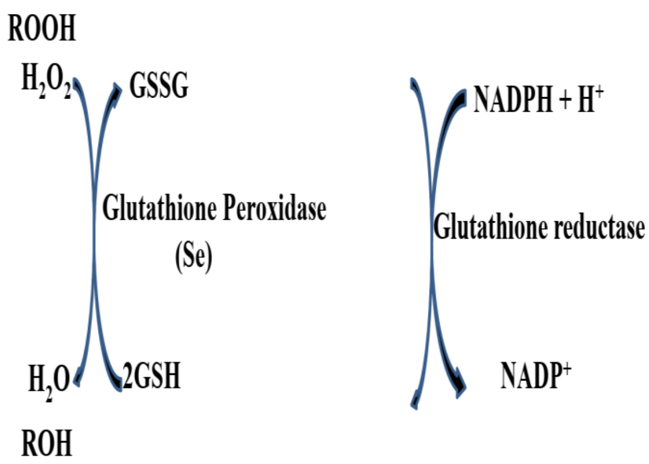

Figure 5b: Antioxidant activity of glutathione peroxidase and glutathione reductase[42]

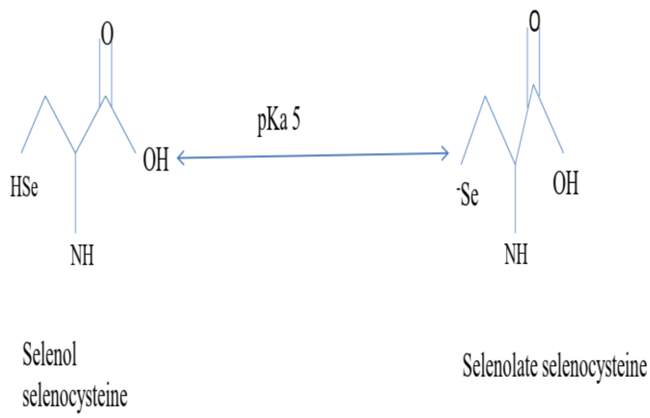

Figure 6: Selenohydryl group of selenium (a powerful nucleophile)[42]

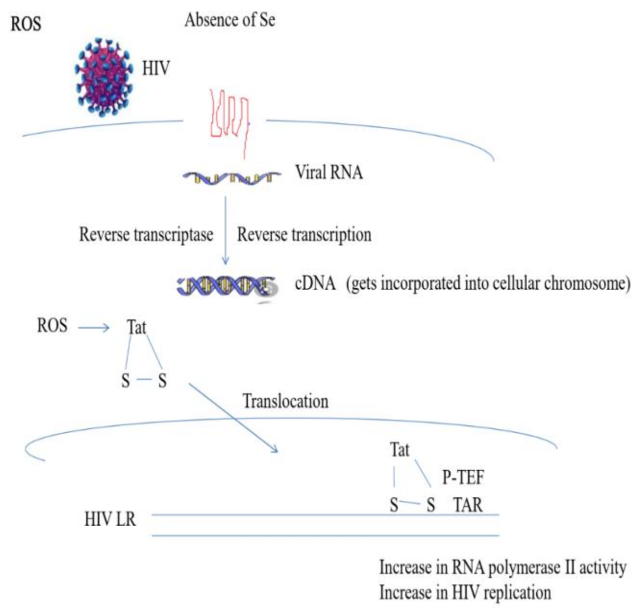

Figure 7a: Proposed mechanism showing the replication of HIV via the Tat pathway in the absence of selenium[49]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

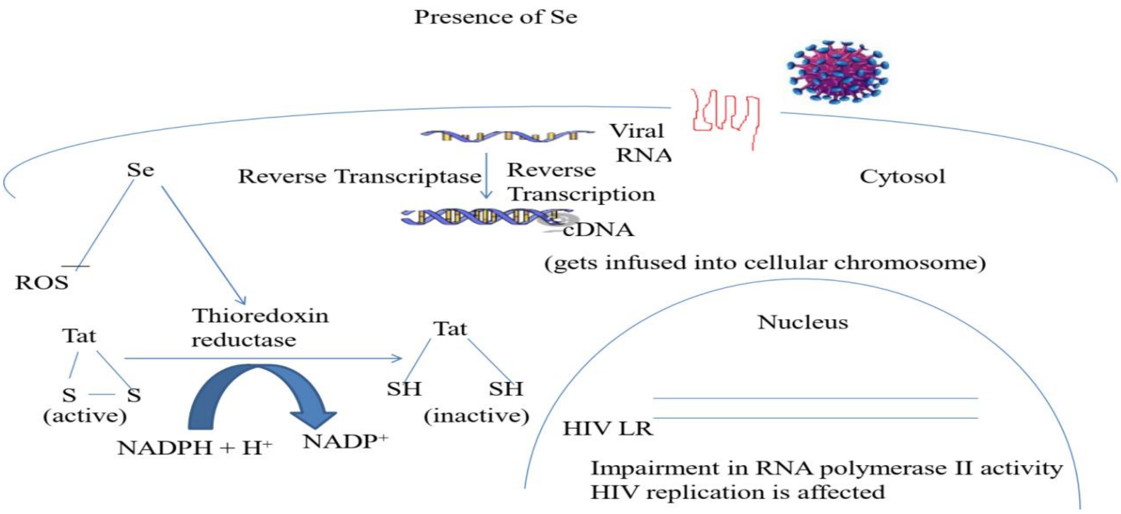

Figure 7b: Proposed mechanism of selenium inhibition of HIV transcription via Tat pathway[49]

Kalantari *et al.*[47] reported that selenium inhibited HIV-1 transcription via the Tat-dependent HIV transcription. They showed that HIV-1 could target selenoproteins including TR-1 to generate free radicals and consequently create an oxidative stressed environment to effectively carry out replication. However, in a physiologically stable environment, thioredoxin reductase acts as a reducing agent and reduces the cysteine-rich motif of Tat protein which is required for its activity, rendering it inactive, leading to a truncation in the replication of the viral genome. Biofortified foods could therefore be effective in the management of HIV/AIDS as they would provide the patients with the essential micronutrients needed as well as present it to the body in the much-needed organic form.

Conclusion

This review unravels the importance and application of food biofortification most especially in the treatment of certain diseases, as biofortified foods have helped to improve the nutritional status of populations. The utilization of biofortified food in the management of diseases could be linked to the improved required micronutrients and pigments (beta-carotene) in foods, which are needed in disease management.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

Acknowledgements

The authors wish to appreciate the Tertiary Education Trust Fund (TETFUND) of the Nigeria Federal Government for supporting this research work TETFund/DR&D/CE/NRF/CC/11/VOL1.

References

1. Margulies SL, Kurian D, Elliott MS, Han Z. Vitamin D deficiency in patients with intestinal malabsorption syndromes–think in and outside the gut. J Digestive Dis. 2015; 16(11):617-633.
2. Olson R, Gavin-Smith B, Ferraboschi C, Kraemer K. Food Fortification: The Advantages, Disadvantages and Lessons from Sight and Life Programs. Nutr. 2021; 13(4):1118.
3. Godswill AG, Somtochukwu IV, Ikechukwu AO, Kate EC. Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: A systematic review. Int J Food Sci. 2020; 3(1):1-32.
4. Mejia LA, Kuo WY, Beltran-Velazquez F. Provision of micronutrients in coexisting public health programs and risk of excessive intake: regulatory considerations. Anal New York Acad Sci. 2019; 46(1):66-80.
5. Birru SM, Tariku A, Belew AK. Improved dietary diversity of school adolescent girls in the context of urban Northwest Ethiopia: 2017. Italian J Ped. 2018; 44(1):1-6.
6. Titcomb TJ and Tanumihardjo SA. Global concerns with B vitamin statuses: biofortification, fortification, hidden hunger, interactions, and toxicity. Compr Rev Food Sci Food Saf. 2019; 18(6):1968-1984.
7. Ghosh S, Pahari S, Roy T. An updated overview on food supplements. Asian J Res Chem. 2018; 11(3):691-697.
8. Jha AB and Warkentin TD. Biofortification of Pulse Crops: Status and Future Perspectives. Plants. 2020; 9(1):73-102.
9. Mayer JE, Pfeiffer WH, Beyer P. Biofortified crops to alleviate micronutrient malnutrition. Curr Opin Plant Biol. 2008; 11(2):166-170.
10. Bouis HE and Welch RM. Biofortification- A Sustainable Agricultural Strategy for Reducing Micronutrient Malnutrition in the Global South. Crop Sci. 2010; 50(1):20-32.
11. Gómez-Galera S, Rojas E, Sudhakar D, Zhu C, Pelacho AM, Capell T, Christou P. Critical Evaluation of strategies for mineral fortification of staple food crops. Transgenic Res. 2010; 19(2):165-180.
12. Ali B, Ali A, Tahir M, Ali S. Growth, Seed Yield and Quality of Mungbean as Influenced by Foliar Application of Iron Sulphate. Pak J Life Soc Sci. 2014; 12(1):20-25.
13. Hossain A, Skalicky M, Brestic M, Maitra S, Sarkar S, Ahmad Z, Vemuri H, Garai S, Mondal M, Bhatt R, Kumar P. Selenium biofortification: roles, mechanisms, responses and prospects. Molecules. 2021; 26(4):881-899.
14. Hefferon KL. Nutritionally enhanced food crops; progress and perspectives. Int J Mol Sci. 2015; 16(2):3895-3914.
15. Alfthan G, Eurola M, Ekholm P. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland. From deficiency to optimal selenium status of the population. J Trace Elem Biol. 2015; 31(1):42-47.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

16. Winkler JT. In Access Not Excess; C. Pasternak, Ed.; Smith-Gordon Publishing: St Ives, UK. 2011; 100-112.
17. Márquez-Quiroz C, De-la-Cruz-Lázaro E, Osorio-Osorio R, Sánchez-Chávez E. Biofortification of cowpea beans with iron: Iron's influence on mineral content and yield. J Soil Sci Plant Nutr. 2015; 15(4):839-847.
18. Jha AB and Warkentin TD. Biofortification of pulse crops: Status Future Perspectives Plants. 2020; 9(1):73-79.
19. Van Der Straeten D, Bhullar NK, De Steur H, Gruissem W, MacKenzie D, Pfeiffer W, Qaim M, Slamet-Loedin I, Strobbe S, Tohme J, Trijatmiko KR. Multiplying the efficiency and impact of biofortification through metabolic engineering. Nature Comm. 2020; 11(1):1-10.
20. Tang G, Hu Y, Yin S, Wang Y, Dallal G, Grusak M, Russell R. β-Carotene in Golden Rice is as good as b-carotene in oil at providing vitamin A to children. Am J Clin Nutr. 2015; 102(3):715-715.
21. Tang G, Hu Y, Yin SA, Wang Y, Dallal GE, Grusak MA, Russell RM. Retracted: *β*-Carotene in Golden Rice is as good as β-carotene in oil at providing vitamin A to children. The Am J Clin Nutr 2012; 96(3):658-664.
22. Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, Perez Conesa D, Ros G, Sandmann G, Capell T. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci USA. 2009; 106(19):7762-7767.
23. Curtin SJ, Xiong Y, Michno JM, Campbell BW, Stec AO, Čermák T, Starker C, Voytas DF, Eamens AL, Stupar RM. Crispr/cas9 and talens generate heritable mutations for genes involved in small rna processing of glycine max and medicago truncatula. Plant Biotechnol J. 2018; 16(6):1125-1137.
24. Nestel P, Bouis H, Meenakshi J, Pfeiffer W. Biofortification of Staple Foods. J Nutr 2006; 136(4):1064-1067.
25. Solomons NM. Vitamin A. In: Bowman B, Russell R, eds. Present knowledge in Nutrition. 9th ed. Washington DC: Int Life Sci Inst. 2006; (1)57-183.
26. Ross CA. Vitamin A. 2nd ed. London and New York: Informa Healthcare. 2010; 778-791p.
27. Hefferon K. Nutritionally Enhanced Food Crops; Progress and Perspectives. Int J Mol Sci 2015; 16 (2):3895-3914.
28. Frano M, Woodhouse L, Burnett D, Burri B. Biofortified cassava increases beta carotene and Vitamin A concentration in the TAG-rich plasma layer of American women. Br J Nutr. 2013; 110(2):310-320.
29. Pillay K, Siwela M, Derera J. Provitamin A carotenoids in biofortified maize and their retention during processing and preparation of South African maize foods. J Food Sci Technol. 2014; 51(4):634-644.
30. Chasapis CT, Ntoupa PS, Spiliopoulou CA, Stefanidou ME. Recent aspects of the effects of zinc on human health. Arch Toxicol. 2020; 94(5):1-18.
31. Gooding AJ, Packer CD, Pensiero AL. Zinc deficiency-induced hypogeusia in a patient with refractory iron-deficiency anemia: a case report. Cureus. 2019; 1(12):1-6.
32. Ivanović D, Dodig D, Đurić N, Kandić V, Tamindžić G, Nikolić N, Savić J. Zinc biofortification of bread winter wheat grain by single zinc foliar application. Cereal Res Comm. 2021; 49(1):673-679
33. Cakmak I, Prom-u-thai C, Guilherme LRG, Rashid A, Hora K, Yazici A. Iodine biofortification of wheat, rice and maize through fertilizer strategy. Plant Soil. 2017; 418(1):319-335.
34. Cakmak I, Guilherme LRG, Rashid A, Hora KH, Yazici A, Savasli E, Kalayci M, Tutus Y, Phuphong P, Rizwan M, Martins FAD. Iodine biofortification of wheat, rice and maize through fertilizer strategy. Plant Soil. 2017; 418(1):319-335.
35. Smoleń S, Kowalska I, Kováčik P, Sady W, Grzanka M, Kutman UB. Changes in the chemical composition of six lettuce cultivars (*Lactuca sativa* L.) in response to biofortification with iodine and selenium combined with salicylic acid application. Agron. 2019; 9(10):660-678.
36. Smoleń S, Baranski R, Ledwożyw-Smoleń I, Skoczylas Ł, Sady W. Combined biofortification of carrot with iodine and selenium. Food Chem. 2019; 300(1):125-202.
37. Zhou H, Wang T, Li Q, Li D. Prevention of Keshan disease by selenium supplementation: a systematic review and meta-analysis. Biol Trace Elem Res. 2018; 186(1):98-105.
38. Peng T, Li Y, Yang Y. Characterization of Enterovirus Isolates from Patients with Heart Muscle Disease in a Selenium-Deficient Area of China. J Clin Microbiol. 2000; 38(10):3538-3543.
39. Stone R. Diseases. A medical mystery in middle China. Sci. 2009; 324(5933):1378-1381.
40. Cermelli C, Vincetti M, Scaltriti E, Bazzani E, Beretti F, Vivoli G, Portolani M. Selenite inhibition of Coxsackie virus B5 replication: implications on the etiology of Keshan Disease. J Trace Elem Med Biol. 2002; 16(1):41-46.
41. Horn MJ and Jones D. Isolation of a crystalline selenium containing organic compound from plant material. J Am Chem Soc. 1940; 62(1):234-234.
42. Zwolak I. The role of selenium in arsenic and cadmium toxicity: an updated review of scientific literature. Biol Trace Elem Res. 2020; 193(1):44-63.
43. Ni HK, Huang RL, Zhou W. The relationship between gastric cancer and Helicobacter pylori cytotoxin-related gene A genotypes. Cellul Mol Biol. 2020; 66(7):1-4.
44. Hwalla N, Al Dhaheri AS, Radwan H, Alfawaz HA, Fouda MA, Al-Daghri NM, Zaghloul S, Blumberg JB. The prevalence of micronutrient deficiencies and inadequacies in the Middle East and approaches to interventions. Nutr. 2017; 9(3):229-256.
45. Kieliszek M. Selenium–fascinating microelement, properties and sources in food. Mol. 2019; 24(7):1298-1311.
46. Das AT, Harwig A, Berkhout B. The HIV-1 TaT Protein has a versatile role in activating viral transcription. J Virol. 2011; 85:9506-9516.
47. Kalantari P, Narayan V, Natarajan S, Muralidhar K, Gandhi U, Vuntra H, Henderson A, Sandeep Prabhu K. Thioredoxin reductase-1 negatively regulates HIV-1 transactivating protein Tat-dependent Transcription in Human Macrophages. J Biol Chem. 2008; 283(48):33183-33190.
48. Turanov A, Su D, Gladyshev VN. Characterization of alternative cytosolic forms and cellular targets of mouse mitochondrial thioredoxin reductase. J Biol Chem. 2006; 281(32):22953-22963.
49. Jiang Y, Chai L, Fasae MB, Bai Y. The role of HIV Tat protein in HIV-related cardiovascular diseases. J Trans Med. 2018; 16(1):1-7.