IODINE FORTIFICATION STUDY OF SOME COMMON NIGERIAN VEGETABLES USING COPPER COMPLEX.

Abstract

Iodine deficiency (ID) is the result of insufficient dietary iodine intake in humans, the deficiency not only leads to goiter formation but also to severe retardation of growth, development and maturation of nearly all the tissues of the body, especially those that are fast developing. The aim of this research is to evaluate levels of iodine intake by ten Nigerian vegetables using copper complex. Dry alkaline ash method was used in the determination. The results obtained showed that all the ten vegetables used absorbed high concentrations of iodine when inoculated with 0.09 M and 0.14 M $[Cu(I)_4]^{2-}$. Iodine phytotoxicity was observed in all the plants when inoculated with 0.2-0.8 M of the copper complex as all the plants died. Increase in the concentration of copper complex from 0.09-0.14 M increased the absorption of iodine by *Amaranthus hybridus, Corchorous olitoruis, Gongronema lotifolium* and *Solanum melongena*. The reverse was observed with *Murraya koenigii, Ocimum gratissimum, Talinium triangulare, Abelmoschus esculentus, Cucurbita pepo* and *Telfairia occidentalis. Ocimum gratissimum* absorbed the iodine at 0.09M with value of 164.79 mg/kg, the vegetable also absorbed least at 0.14 M with a dose of 61.80 mg/kg. The intake of the iodine by the plants showed that the method can be used in the fortifying these Nigerian vegetables with iodine, thereby reducing the incidence of Iodine deficiency disorder in humans.

Keywords: Iodine; Biofortification; Copper complex; *Murraya koenigii; Ocimum gratissimum; Cucurbita pepo; Amaranthus hybridus; Abelmoschus esculentus; Corchorous olitoruis; Gongronema lotifolium; Telfairia occidentalis; Talinium triangulare; Solanum melongena*

Introduction

Increasing population, inadequate food and nutrition, hunger, malnourishment of vitamins and micronutrients etc. are the biggest challenges to address most of the nations across the world [1]. Humans require at least 22 mineral elements for their wellbeing. These can be supplied by an appropriate diet. However, it is estimated that over 60% of the world's 6 billion people are iron (Fe) deficient, over 30% are zinc (Zn) deficient, 30% are iodine (I) deficient and 15% are selenium (Se) deficient [2]. Deficiencies in vitamins and minerals in our diets create major public health problems, mental as well as physical, especially in developing countries [3]. Micronutrient malnutrition affects more than one-half of the world's population, especially women and preschool children. Reaching the Millennium Development Goals to reduce the under-5 child mortality ratio by two-thirds and the maternal mortality ratio by three-quarters between 1990 and 2015 will require additional technologies and approaches to improving nutritional status, which is an important determinant of these mortalities [4]. Iodine is a trace element that is fundamental for human health: its deficiency affects about two billion people worldwide [5-7]. It is a component of the thyroid hormones which determines the metabolic rate of the body [8-11]. Iodine deficiency (ID) is the result of insufficient dietary iodine intake in humans, resulting in multiple adverse effects due to inadequate thyroid hormone production, globally named iodine deficiency disorders IDDs [12-14]. Despite myriad improvements to global food production systems in the context of human nutrition, nearly two billion people worldwide still receive insufficient dietary iodine such that iodine deficiency disorder (IDD) still persists as one of the world’s most intractable micronutrient-related diseases [15]. To counteract the IDD, one of the most effective way is the iodization of kitchen salt but, even so, a third of the global population is still unprotected from iodine deficiency [16]. The IDD is a major problem even in the developed countries, and biofortification with iodine is a good alternative to iodized salt to cope with such problem [17]. Usually fruits and vegetables are usually poor sources of iodine; however, plants can accumulate iodine if it is either present or exogenously administered to the soil [5]. The biofortification of crops with iodine is a recent strategy to further enrich the human diet with a potentially cost-effective, well accepted and bioavailable iodine source [11]. Since salt iodisation is insufficient to ensure global iodine adequacy, with an estimated one-third of humanity at risk of hypothyroidism and associated iodine deficiency disorders (IDD) [18]. In most industrialized countries, excess consumption of salt has become recognized as a health risk [19], enrichment of crops with iodine would be able to address negative economic and health consequences of iodine deficiencies in humans. Vegetables have shown to have the potentials of absorbing both heavy metals [20-27] and essential elements [28-30] from the soil.

Ujowundu et al, 2009 studied Iodine fortification of Selected Plants Using Potassium Iodide and found that iodine biofortification of *Telfairia occidentalis, Talinium trangulare and Zea mays* if properly harnessed, may improve the iodine content of plants [31]. In our previous work, we also studied Iodine Fortification Study of Some Common African Vegetables using potassium iodide and potassium iodate and found out *that Murraya koenigii , Ocimum gratissimum, Solanum nigrum, Zingiber officinale* can be used in iodine biofortification using KI and KIO_3 at concentration < 0.5 M [32]. Caffagni et al., 2011 studied the iodine fortification of *Hordeum vulgare , Zea mays , Nicotiana tabacum , Solanum tuberosum and Solanum lycopersicum* using potassium iodide and potassium iodate and reported that *Solanum tuberosum and Solanum lycopersicum* were shown to be good targets for a fortification-rate study among the species screened [33]. Another study of Iodine fortification of *Solanum lycopersicum* using potassium iodide and potassium iodate also suggested that tomato was a particularly suitable crop for iodine biofortification programs [6,10]. Iodine fortification of spinach, cabbage, coriander, potherb mustard, tomato, cucumber, long cowpea, eggplant, and hot pepper using algal organic iodized fertilizer showed that both leaf vegetables and fruit vegetables can absorb exogenous iodine from soil. The uptake amounts increase with the application intensity of algal organic iodized fertilizer [34]. Work that was carried out to enrich Polignano Carrot with iodine showed that the concentrations of iodine into biofortified carrots in open field can allow to satisfy the recommended daily allowance (RDA) by consuming 100 and 200 g of fresh product [35].

Methodology

The seeds of ten African vegetables- *Murraya koenigii* , *Ocimum gratissimum, Cucurbita pepo, Amaranthus hybridus* , *Abelmoschus esculentus* , *Corchorous olitoruis* , *Gongronema lotifolium, Telfairia occidentalis* , *Talinium triangulare* , *Solanum melongena* were bought from Eke Awka market in Awka, the State Capital of Anambra State, Nigeria. The seeds were planted in nurseries and the seedlings transplanted to seventy (70) beds. Each plant was planted in seven beds, 20 mL of each of 0.09M, 0.14M, 0.2M, 0.33M, 0.4M, 0.8M of $[Cu(I)_4]^{2-}$ was added to each of the plant. One each of the plants was left uninoculated (control). The iodine content was evaluated using Dry Alkaline Ash Method as described by Anarado *et al., (*2019*)* [32], was used after 14 days. This was done by adding 0.5 g of each of the samples into a nickel crucible. Exactly 1 mL of a mixture 0.5 M sodium hydroxide and 0.1 M potassium nitrate was added to the samples, mixed and allowed to dry. The containers were covered, placed in a muffle furnace, heated to 250˚C, held, for 15 minutes, and heated further to 480˚C, held for 15 minutes, and finally brought to 580˚C. The temperature was maintained for three hours, allowed to cool to room temperature. The resultant ashed was extracted with three successive 2 mL portions of a 1.0 mM NaOH, made up double-distilled water. The solution was centrifuged at 2500 g for 20 minutes using polypropylene centrifuge tubes and the supernatant solution collected for iodine determination. I mL of the sample solution was added to a cuvette at 35˚C and 1 mL sodium arsenite, and 1 mL of ceric ammonium sulphate. A standard curve containing 0.5 to 10 ppm iodine was used. The intensity of the developed colour was measured at 420 nm. The iodine concentration in the samples was calculated from the standard curve. Concentrations of the control were deducted from the concentrations of the inoculated.

Table 1. Result of absorbed I_2 by the plants (mg/kg).

Plant	0.09M$[Cu(I)_4]^{2-}$	0.14M$[Cu(I)_4]^{2-}$	0.2M$[Cu(I)_4]^{2-}$	0.33M$[Cu(I)_4]^{2-}$	0.4M$[Cu(I)_4]^{2-}$	0.8M$[Cu(I)_4]^{2-}$
Murraya koenigii	74.87	72.33	died	Died	Died	died
Ocimum gratissimum	164.79	61.80	Died	Died	Died	died
Cucurbita pepo	78.68	65.99	died	Died	died	died
Amaranthus hybridus	63.45	67.26	died	Died	died	died
Abelmoschus esculentus	71.06	65.53	died	Died	died	died
Corchorous olitoruis	62.18	69.79	died	Died	died	died

Gongronema lotifolium	73.60	77.41	Died	Died	died	died
Telfairia occidentalis	74.33	68.80	Died	Died	Died	Died
Talinium triangulare	72.21	67.26	died	Died	died	died
Solanum melongena	63.45	65.99	died	Died	died	died

Results & Discussion

The result of the analysis (Table 1) showed that all the ten plants used suffered iodine phytotoxicity when inoculated with $[Cu(I)_4]^{2-}$ at concentration above 0.14M. It was observed that most of the plants' leaves turned yellow after few hours of inoculation at higher concentrations and died within twenty-four hours. Though there was phytotoxicity, the vegetables showed very good absorption potential of I_2 with $[Cu(I)_4]^{2-}$ at lower concentrations since all the plants absorbed more than 6000 mg/kg of I_2 at lower concentrations. The inability of the plants to survive at higher concentrations is against the report of Kiferle *et al.,* (2019) [36]. They revealed that in open field, the use of increasing concentrations of both iodine salts (KI and KIO_3) gradually enhanced iodine accumulation in leaves of *Ocimum basilicum.* At concentration of 0.09 M$[Cu(I)_4]^{2-}$, *Ocimum gratissimum* absorbed most of the I_2(164.79 mg/kg), followed by *Cucurbita pepo*(7867.80 mg/kg) and the least *Corchorous olitoruis*(62.18 mg/kg).As the concentration of $[Cu(I)_4]^{2-}$ increased from 0.09 M to 0.14 M, the concentration of I_2 absorbed by *Murraya koenigii(*74.87-72.33 mg/kg*), Ocimum gratissimum(*164.79-61.80 mg/kg*)*, *Cucurbita pepo*(78.68-65.99 mg/kg), *Abelmoschus* esculentus(71.06-65.53 mg/kg), *Telfairia* occidentalis(74.33-68.80 mg/kg) and *Talinium* triangulare(72.21-67.26 mg/kg) decreased, but the concentration of I_2 absorbed by *Amaranthus* hybridus(63.45-67.26 mg/kg), *Corchorous* olitoruis(62.18-69.79 mg/kg), *Gongronema lotifolium*(73.60-77.41 mg/kg) and *Solanum* melongena(63.45-65.99 mg/kg) increased. When compared with the results of our previous research; *Murraya koenigii* absorbed 6.90 mg/kg and 5.70 mg/kg of I_2 when inoculated with 0.3 M KI and 0.2 M KIO_3 respectively. Increase in the concentrations of both KI and KIO_3 at 0.5 M resulted in the death of the plants, which is in line with the results of the present study. However, the increase in the absorption using $[Cu(I)_4]^{2-}$ at and 0.09 M and 0.14 M was more than 1000% . *Ocimum gratissimum* also absorbed very high concentration of I_2 with $[Cu(I)_4]^{2-}$ at 0.09 M when compared with 2.35 M and 2.24 M using 0.3 M KI and KIO_3 respectively. The increase in absorption was more than 7000% when compared with its absorption at 0.09 M$[Cu(I)_4]^{2-}$. Salau *et al.,* (2011) [37] had earlier reported that *Talinium* triangulare absorbed very little amount of I_2(0.49 + 0.01 µg / 100 g) hen compared with the I_2 absorption *Telfairia occidentalis* *(*23.94 ± 1.88 µg / 100 g*)*. Our result showed that even at low concentration, the absorption of iodine by the vegetables was higher than the needed and sufficient levels of iodine consumption in humans (Adults- 200 µg)[38].

Conclusion

The vegetables used showed very good iodine absorption capacity using $[Cu(I)_4]^{2-}$ even at very low concentrations. When compared the results from our previous work, the copper complex has shown to be a better way of introducing iodine into our common Nigerian vegetables. Consequently, could be a very good way of addressing iodine deficiencies in humans. However, lower concentrations of the copper complex should be employed to reduce the doses of iodine in the vegetables.

References

1. Prasad, B.V.G., Mohanta, S., Rahaman, S. and Prerna, B. (2015) Bio-Fortification in Horticultural Crops. Journal of Agricultural Engineering and Food Technology, 2, 95-99.

2. Philip, J.W. and Martin, R.B. (2009) Biofortification of Crops with Seven Mineral Elements Often Lacking in Human Diets—Iron, Zinc, Copper, Calcium, Magnesium, Selenium and Iodine. New Phytologist , 182, 49-84. https://doi.org/10.1111/j.1469-8137.2008.02738.x
3. Winkler, J.T. (2011) Biofortification: Improving the Nutritional Quality of Staple Crops. 100-112.

4. Penelope, N., Howarth, E.B., Meenakshi, J.V. and Wolfgang, P. (2006) Symposium: Food Fortification in Developing Countries Biofortification of Staple Food Crops. American Society for Nutrition, Rockville, 1064-1067. https://doi.org/10.1093/jn/136.4.1064
5. Khalid SMN (2017) Iodine Bio-fortification of Crops for Solving Iodine Deficiency Disorder Problem in Afghanistan. J Food Nutr Disord 6:3. doi: 10.4172/2324-9323.1000228

6. Kiferle, C., Gonzali, S. and Holwerda, H.T. (2013) Tomato Fruits: A Good Target for Iodine Biofortification. Frontiers in Plant Science , 27, 205. https://doi.org/10.3389/fpls.2013.00205.
7. Olum, S., Gellynck, X., Okello, C., Webale, D., Odongo, W., Ongeng, D. and De Steur, H. (2018) Stakeholders' Perceptions of Agronomic Iodine Biofortification: A SWOT-AHP Analysis in Northern Uganda. Nutrients , 10, 407. https://doi.org/10.3390/nu10040407
8. Hetzel B.S (1989). The story of iodine deficiency. An international challenge in nutrition. Oxford University Press. Delhi.

9. Grasberger H, Refetoff S. Genetic causes of congenital hypothyroidism due to dyshormonogenesis. *Curr Opin Pediatr*. 2011;23:421–428.

10. Landini, M., Gonzali, S. and Perata, P. (2011) Iodine Biofortification in Tomato. Journal of Plant Nutrition and Soil Science , 174, 480-486. https://doi.org/10.1002/jpln.201000395

11. Gonzali, S., Kiferle, C. and Perata, P. (2017) Iodine Biofortification of Crops: Agronomic Biofortification, Metabolic Engineering and Iodine Bioavailability. Current Opinion in Biotechnology, 44, 16-26. https://doi.org/10.1016/j.copbio.2016.10.004

12. Tonacchera, M., Dimida,A., De Servi, M., Frigeri,M., Ferrarini, E., De Marco, G., Grasso,L., Agretti,P., Piaggi, P., Aghini-Lombardi,F., Perata,P., Pinchera,A. and Vitti, P.(2013). Iodine Fortification of Vegetables Improves Human Iodine Nutrition: In Vivo Evidence for a New Model of Iodine Prophylaxis. jcem.endojournals.org J Clin Endocrinol Metab, April 2013, 98(4):E694–E697. doi: 10.1210/jc.2012-3509
13. Weng, H.X., Hong, C.L., Xia, T.H., et al . (2013) Iodine Biofortification of Vegetable Plants—An Innovative Method for Iodine Supplementation. Chinese Science Bulletin, 58, 2066-2072. https://doi.org/10.1007/s11434-013-5709-2
14. Aliasgharpour, M. & Farzami, M. (2013). Trace Elements in Human Nutrition: A Review. Inter J Med Invest. 2. 115-128.

15. Mottiar Y. (2013). Iodine biofortification through plant biotechnology. Plant biofortification as potential strategy o address iodine deficiency. *J. Nutrition*. 29, Issues 11-12, Page 1431. DOI: https://doi.org/10.1016/j.nut.2013.04.009

16. Lyons, G. (2018) Biofortification of Cereals with Foliar Selenium and Iodine Could Reduce Hypothyroidism. Frontiers in Plant Science , 9, 730. https://doi.org/10.3389/fpls.2018.00730

17. Signore, A., Renna, M., D'Imperio, M., Serio, F. and Santamaria, P. (2018) Preliminary Evidences of Biofortification with Iodine of "Carota di Polignano": An Italian Carrot Landrace. Frontiers in Plant Science , 9, 170. https://doi.org/10.3389/fpls.2018.00170

18. World Health Organization (2005). Preventing Chronic Diseases: A Vital Investment. Available online at: http://www.who.int/entity/chp/chronic_ disease_report/contents/part1.pdf?ua=1

19. Tonacchera, M., Dimida, A., De Servi, M., Frigeri, M., Ferrarini, E., De Marco, G., Grasso, L., Agretti, P., Piaggi, P., Aghini-Lombardi, F., Perata, P., Pinchera, A., Vitti, P.,(2013). Iodine Fortification of Vegetables Improves Human Iodine Nutrition: In Vivo Evidence for a New Model of Iodine Prophylaxis, *The Journal of Clinical Endocrinology & Metabolism*, 98(4): E694–E697, https://doi.org/10.1210/jc.2012-3509

20. Anarado, C.E., Anarado, C.J.O., Okeke, M.O., Ezeh, C.E., Umedum, N.L. and Okafor,P.C. (2019) Leafy Vegetables as Potential Pathways to Heavy Metal Hazards. Journal of Agricultural Chemistry and Environment , 8, 23-32. https://doi.org/10.4236/jacen.2019.81003

21. Ali, M.H.H., Al-Qahtani, K.M., (2012). Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets, *The Egyptian Journal of Aquatic Research*, 38(1): 31-37. https://doi.org/10.1016/j.ejar.2012.08.002.

22. Elbagermi, M. A., Edwards, H. G. M. and Alajtal, A. I., (2012). Monitoring of Heavy Metal Content in Fruits and Vegetables Collected from Production and Market Sites in the Misurata Area of Libya. *International Scholarly Research Notices*. https://doi.org/10.5402/2012/827645

23. Sharma, A., Katnoria, J. K., & Nagpal, A. K. (2016). Heavy metals in vegetables: screening health risks involved in cultivation along wastewater drain and irrigating with wastewater. *SpringerPlus*, *5*, 488. https://doi.org/10.1186/s40064-016-2129-1

24. Bilal, M., Asghar, W., Azeem, M., Ahmad, M., Asad Abbas, A. A. & Zulfiqar A. M. & Toufeeq, S. (2018). Heavy Metal Accumulation in Vegetables and Assessment of their Potential Health Risk. *Journal of Environmental Analytical Chemistry*. 5(1). DOI: 10.4172/2380-2391.1000234

25. Bağdatlıoğlu, N., Nergiz, C. & Ergonul, P., (2010). Heavy metal levels in leafy vegetables and some selected fruits. Journal für Verbraucherschutz und Lebensmittelsicherheit. 5: 421-428. DOI: 10.1007/s00003-010-0594-y

26. Guerra, F., Trevizam, A. R., Muraoka, T., Marcante, N. C., & Canniatti-Brazaca, S. G., (2012). Heavy metals in vegetables and potential risk for human health. *Scientia Agricola*, *69*(1):54-60. https://dx.doi.org/10.1590/S0103-90162012000100008

27. Schaeffer, R., & Esbenshade, J. (2018). A quantitative analysis of the uptake of heavy metals into common garden vegetables from contaminated soils. *Journal of the Pennsylvania Academy of Science, 92*(2), 111-135.

28. Adotey, D., Serfor-Armah, Y., Fianko, J. R. & Yeboah, P. (2009). Essential elements content in core vegetables grown and consumed in Ghana by instrumental neutron activation analysis. African Journal of Food Science. 3:243-249.

29. Alzahrani,H.R., Kumakli, H., Ampiah, E., Mehari, T., Thornton, A.J., Babyak, C.M., Fakayode, S.O.,(2017). Determination of macro, essential trace elements, toxic heavy metal concentrations, crude oil extracts and ash composition from Saudi Arabian fruits and vegetables having medicinal values, *Arabian Journal of Chemistry*, 10(7): 906-913, https://doi.org/10.1016/j.arabjc.2016.09.012.

30. **Njinga**, **R. L.,** Moyo, M. N. and Abdulmaliq, S. Y. (2013). Analysis of Essential Elements for Plants Growth Using Instrumental Neutron Activation Analysis. *International Journal of Agronomy*. https://doi.org/10.1155/2013/156520

31. Ujowundu, C.O., Ukoha, A.I., Agha, N.C., Nwachukwu, N. and Igwe, K.O. (2009) Iodine Biofortification of Selected Plants Using Potassium Iodide. Nigerian Journal of Biochemistry and Molecular Biology, 24, 17-21.

32. Anarado, C.J.O., Anarado, C.E., Areh, R.I., Ifoh, N., Eze, E.O. and Ikeakor, E. (2019) Iodine Fortification Study of Some Common African Vegetables. Journal of Agricultural Chemistry and Environment, 8, 172-183. https://doi.org/10.4236/jacen.2019.83014

33. Caffagni, A., Arru, L., Meriggi, P., Milc, J., Perata, P., Pecchioni, N., (2011). Iodine Fortification Plant Screening Process and Accumulation in Tomato Fruits and Potato Tubers. *COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS*. 42: 706-718. 10.1080/00103624.2011.550372.

34. Weng, H. X., Hong, C. L., Xia, T. H., (2013). Iodine biofortification of vegetable plants—An innovative method for iodine supplementation. Chin Sci Bull, 58: 2066-2072, doi: 10.1007/s11434-013-5709-2

35. Signore, A., Renna, M., D'Imperio, M., Serio, F., & Santamaria, P. (2018). Preliminary Evidences of Biofortification with Iodine of "Carota di Polignano", An Italian Carrot Landrace. *Frontiers in plant science*, *9*, 170. https://doi.org/10.3389/fpls.2018.00170

36. Kiferle, C., Ascrizzi, R., Martinelli, M., Gonzali, S., Mariotti, L., Pistelli, L., Flamini, G., & Perata, P. (2019). Effect of Iodine treatments on Ocimum basilicum L.: Biofortification, phenolics production and essential oil composition. *PloS one*, *14*(12), e0226559. https://doi.org/10.1371/journal.pone.0226559

37. Salau, B.A., Ajani, E.O., Soladoye, M.O. and Bisuga Nurudeen, B. (2011). Evaluation of iodine content of some selected fruits and vegetables in Nigeria. African Journal of Biotechnology. 10(6): 960-964. DOI: 10.5897/AJB10.1121

38. Golubkina, N., Moldovan, A., Kekina, H., Kharchenko, V.; Sekara, A.; Vasileva, V.; Skrypnik, L.; Tallarita, A.; Caruso, G. Joint Biofortification of Plants with Selenium and Iodine: New Field of Discoveries. *Plants* **2021**, *10*, 1352. https://doi.org/10.3390/plants10071352