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Levels of Phytotoxins and Nutrients in *Hibiscus sabdariffa* as Influenced by Freezing Storage

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Authors' contributions

This work was carried out in collaboration between all authors. The work was part of author AM research work and author EOO designed and supervised the work. Both authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The biochemical changes affecting the quality and chemical contents (which include nutrients and phytotoxins) in leafy vegetables during storage depend on the storage conditions. It is in line with this that the work was designed to investigate the effect of freezing time on some nutrients, namely, vitamins (β-carotene and vitamin C) and mineral elements (Fe, Cu, Mg, Na and K) and plant toxins (cyanide, nitrate, soluble and total oxalates) in *Hibiscus sabdariffa.*
Methodology: The leaves of the vegetable were stored in a deep freezer at - 4ºC for the period of four weeks and chemical analyses were conducted at weekly intervals.
Results: The concentrations of cyanide, nitrate, oxalates (soluble and total), vitamin C and Mg in *Hibiscus sabdariffa* decreased significantly ($p < 0.05$) during one week of freezing and remained significantly the same throughout the storage duration, except that the soluble oxalate concentration further decreased significantly in the fourth week of freezing. β-carotene content decreased significantly ($p < 0.05$) in the first and second weeks of freezing, however, in the third and fourth weeks, the provitamin A concentration remained significantly unchanged. Freezing had no significant effect on Cu content in *Hibiscus sabdariffa*. While freezing beyond two weeks significantly ($p < 0.05$) decreased the Fe and K contents, the concentration of Na in *Hibiscus sabdariffa* was reduced

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significantly ($p < 0.05$) only in the fourth week of freezing.
Conclusion: The results thus suggest that one week of freezing at - 4ºC significantly reduced the concentrations of the phytotoxins in *Hibiscus sabdariffa* and preserved most of the micronutrients in amount required for the normal metabolic activity of a healthy individual.

Keywords: Hibiscus sabdariffa; freezing time; nutrients; phytotoxins.

1. INTRODUCTION

Roselle (*Hibiscus sabdariffa*) is a popular leafy vegetable in Indonesia, India, West Africa and many tropical regions [1,2]. It belongs to the family of Malvaceace. This plant has been found to flourish on a wide range of soil conditions. The vegetable is commonly grown in the North-Eastern and middle belt regions of Nigeria [3]. The plant can perform satisfactorily on relatively infertile soils but for economic purposes, a soil well supplied with organic materials and essential nutrients is important in the productions [1,4]. It can tolerate relatively high temperature throughout the growing and fruiting periods. *Hibiscus sabdariffa* requires an optimum rainfall of about 45 - 50cm distributed over a 90 - 120 day growing period [1]. Two botanical varieties were recognised in Nigeria, the red variety in which the calyx is used for the preparation of "sobo" drink and the green variety which calyx and leaves are used in soups [4,5]. The leaves and calyx of the green variety are rich in β-carotene, vitamin C and riboflavin with some mineral elements [6-8]. Roselle also contains some plant toxins such as phytic acid, oxalates, cyanide, nitrate, tannin and glucoside which are toxic to animal and human tissues at high concentrations [7-10]. Tannins form complexes with protein [11]. Phytic acid chelates minerals and form complexes with proteins, and thereby affects their nutritive value [12]. Cyanogenic glucosides also found in this plant are inhibitors of cytochrome oxidase enzyme [13]. The quality and chemical content of the vegetables including the nutrients started depreciating after the harvest due to biochemical changes associated with cell death. It is therefore, a common practice to store vegetables in a refrigerator or freezer in order to preserve the vegetable and the contents. Thus this research is set out to determine the effect of freezing on the concentrations of some nutrients and toxic substances in *Hibiscus sabdariffa*.

2. MATERIALS AND METHODS

2.1 Source of Samples

The fresh samples of *Hibiscus sabdariffa* were bought in three sets at different time from Chanchanga, Bosso and Maikunkele markets in Minna town, Nigeria.

2.2 Chemicals

All the chemicals used for this research were of analytical grade and were purchased from Sigma and BDH chemical companies, both of England.

2.3 Freezing Storage

The leaves of *Hibiscus sabdariffa* were washed with distilled water and kept in a well labelled polythene bag and stored in a freezer at the temperature of - 4ºC for a period of four weeks. The concentrations of the plant toxins and micronutrients in the vegetable were determined at weekly intervals over the four - weeks period.

2.4 Sample Analysis

The nitrate concentration in the samples was determined by the colourimetric method as described by [14]. While the alkaline picrate method of [15] was used to analyse the cyanide content in the leaves of *Hibiscus sabdariffa*. Both soluble and total oxalates in the samples were determined by titrimetric method of [16]. The mineral elements (Fe, Cu, Mg, Na and K) in samples were determined according to the method of [17]. The ascorbic acid concentration in the samples was determined by 2, 6-dichlorophenol indophenols method of [18]. While β-carotene concentration was determined by ethanol and petroleum ether extraction method as described by [19].

2.5 Statistical Analysis

Analysis of variance (ANOVA) was carried out using statistical package Minitab to determine variation among different freezing time on the concentration of phytotoxins and micronutrients in the leaves of *Hibiscus sabdariffa*. The DUNCAN's Multiple Range Test (DMRT) was used for pair-wise comparison of means.

3. RESULTS

3.1 Cyanide Concentration

The cyanide concentration decreased with freezing in *Hibiscus sabdariffa*. Significant ($p < 0.05$) reduction of cyanide concentration from 63.98 mg/kg in fresh to 57.17 mg/kg frozen samples was observed in one week of freezing. The concentration of cyanide in the second week (54.26 mg/kg), third week (50.42 mg/kg) and fourth week (47.01 mg/kg) also indicated reduction in the cyanide content which were not significantly different from that of the first week of freezing (Fig. 1).

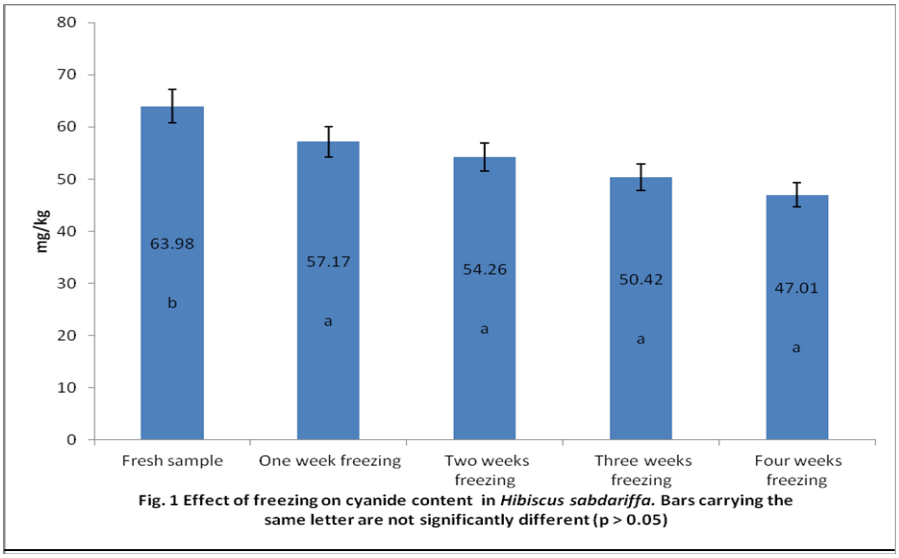

Fig. 1 Effect of freezing on cyanide content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.05)

3.2 Nitrate Concentration

The significant ($p < 0.05$) reduction of nitrate concentration in *Hibiscus sabdariffa* from1281.50 to 878.72 mg/kg was observed during first week of storage. The mean values obtained for the second (800.96 mg/kg), third (678.13 mg/kg) and fourth (661.93 mg/kg) weeks also indicated reduction in nitrate content and they were not significantly ($p > 0.05$) different from each other (Fig. 2).

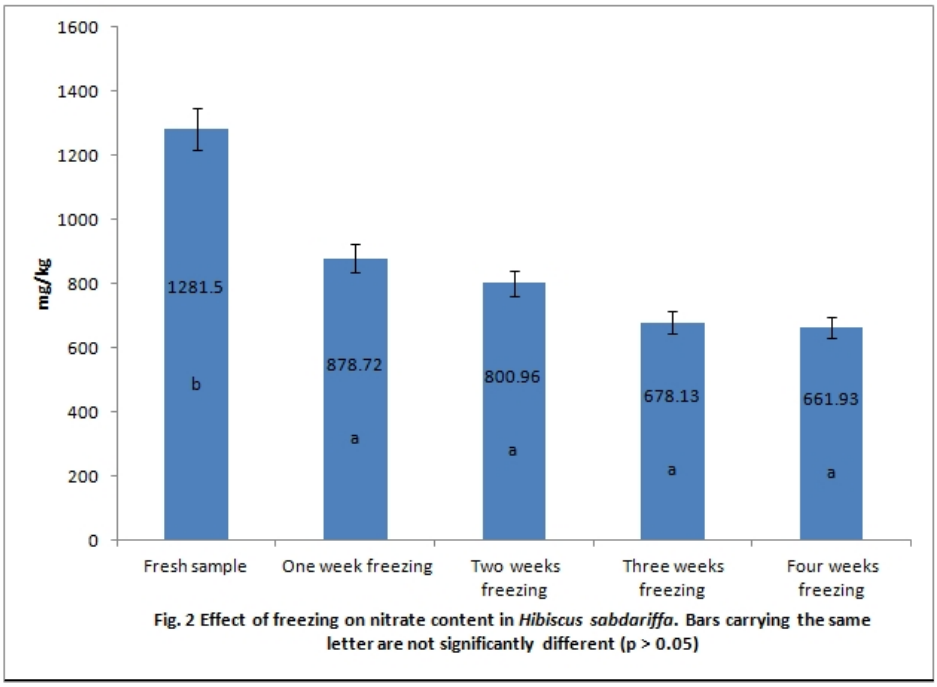

Fig. 2 Effect of freezing on nitrate content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.05)

3.3 Soluble Oxalate Concentration

Similarly, the soluble oxalate concentration was found to decrease with freezing of *Hibiscus sabdariffa*. The reduction of soluble oxalate content from 1.91 to 1.61 g/kg was significant ($p < 0.05$) in the first week of storage. In the subsequent second and third weeks, the soluble oxalate also decreased insignificantly with freezing and the values obtained were 1.60 g/kg and 1.58 g/kg respectively. The fourth week of freezing led to a significant reduction ($p < 0.05$) of the oxalate concentration to 1.39 g/kg (Fig. 3).

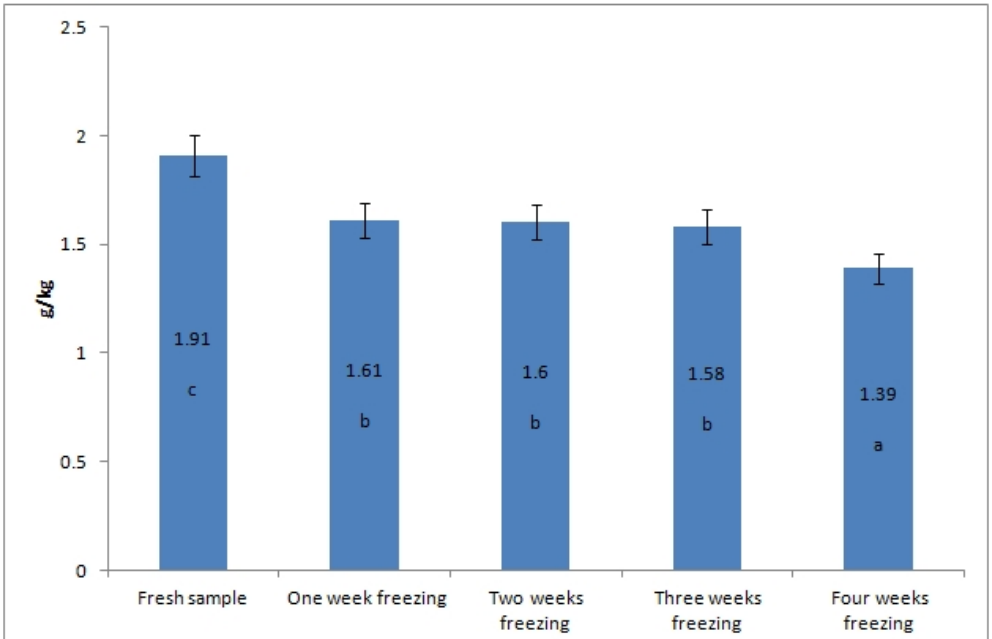

Fig. 3 Effect of freezing on soluble oxalate content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different ($p > 0.05$)

3.4 Total Oxalate Concentration

The decreasing effect of freezing on total oxalate concentration was observed in *Hibiscus sabdariffa.* The total oxalate in fresh, and those frozen samples for one, two, three and four weeks were 4.35, 3.56, 3.52, 3.26 and 3.22 g/kg, respectively. The decreased in the total oxalate concentration was significant ($p < 0.05$) in the first week of freezing. However, the values obtained in the frozen samples were significantly the same (Fig. 4).

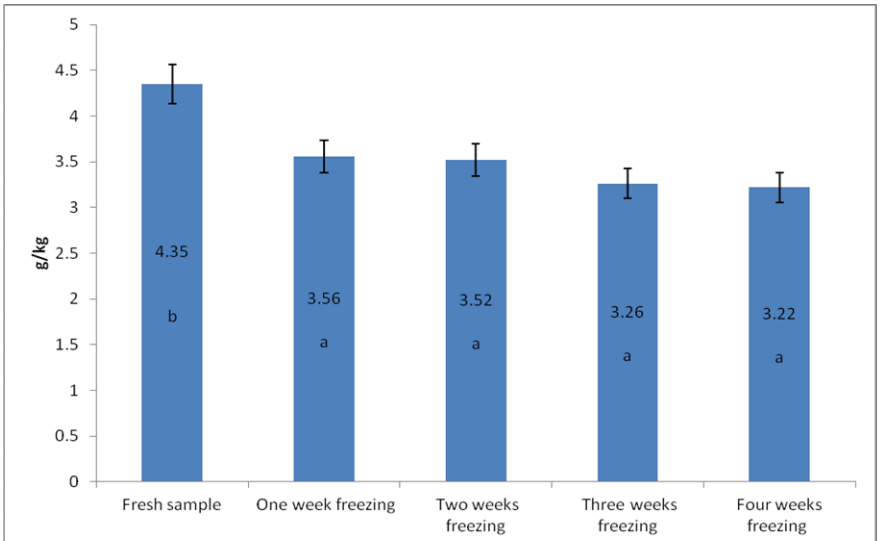

Fig. 4 Effect of freezing on total oxalate content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.005)

3.5 β-Carotene Concentration

The β-carotene concentration in *Hibiscus sabdariffa* decreased significantly in the first and second weeks of freezing and the mean values obtained were 6249 and 4551 µg/100g, respectively. However, the mean values of β-carotene obtained in the subsequent third (3891.63 µg/100g) and fourth (3814.67 µg/100g) weeks of freezing were significantly the same (Fig. 5).

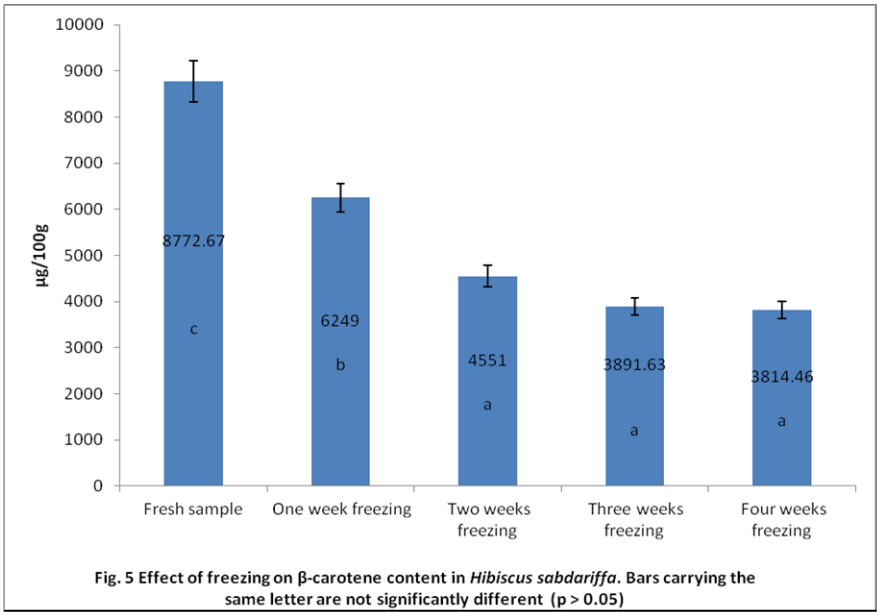

Fig. 5 Effect of freezing on β-carotene content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.05)

3.6 Vitamin C Concentration

The decreasing effect of freezing on vitamin C content was also observed in *Hibiscus sabdariffa.* The concentration of vitamin C in fresh, and frozen samples for one, two, three and four weeks were 27.44, 12.91, 11.06, 10.49 and 9.46 mg/100g, respectively. The vitamin C concentration in the fresh sample of *Hibiscus sabdariffa* was significantly ($p < 0.05$) higher than frozen samples. However, the concentrations of this vitamin in the frozen samples were significantly the same (Fig. 6).

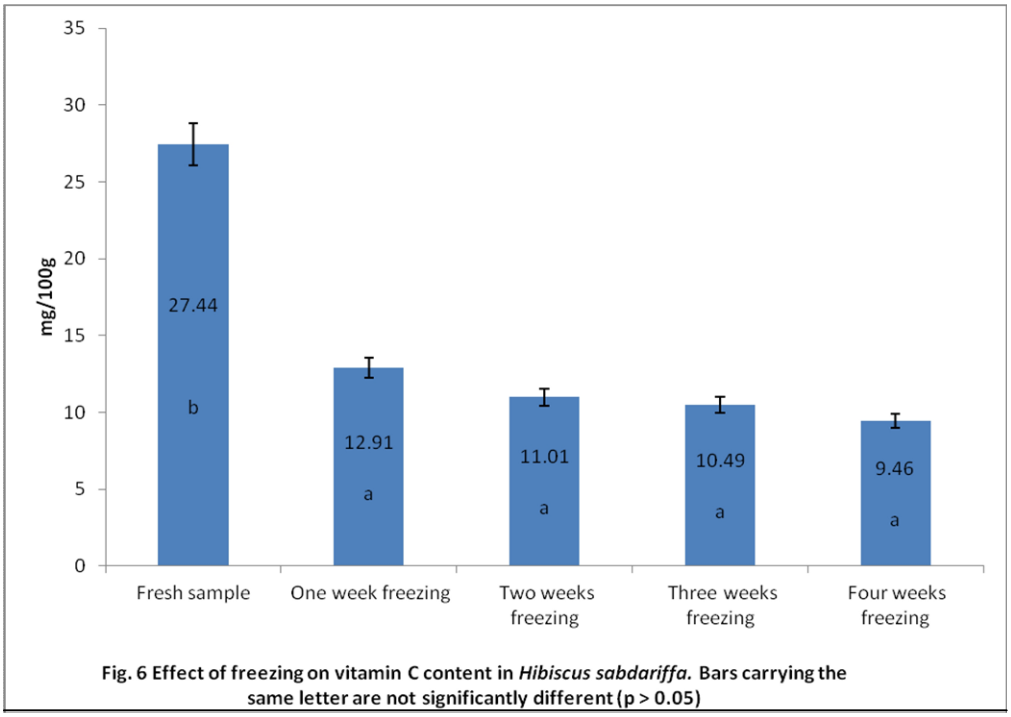

Fig. 6 Effect of freezing on vitamin C content in *Hibiscus sabdariffa.* Bars carrying the same letter are not significantly different ($p > 0.05$)

3.7 Iron Concentration

The Fe content in *Hibiscus sabdariffa* also decreases with freezing time. Significant ($p < 0.05$) reduction in the mineral concentration from 18.51 mg/kg in fresh sample to 15.55 mg/kg was recorded in the third week of storage. The mean value obtained from the fourth week (14.45 mg/kg) indicated reduction in Fe content which was not significantly different from second week (16.91 mg/kg). The mineral content in first week (17.56 mg/kg) was however, significantly ($p < 0.05$) higher than those in the third week and fourth week (Fig. 7).

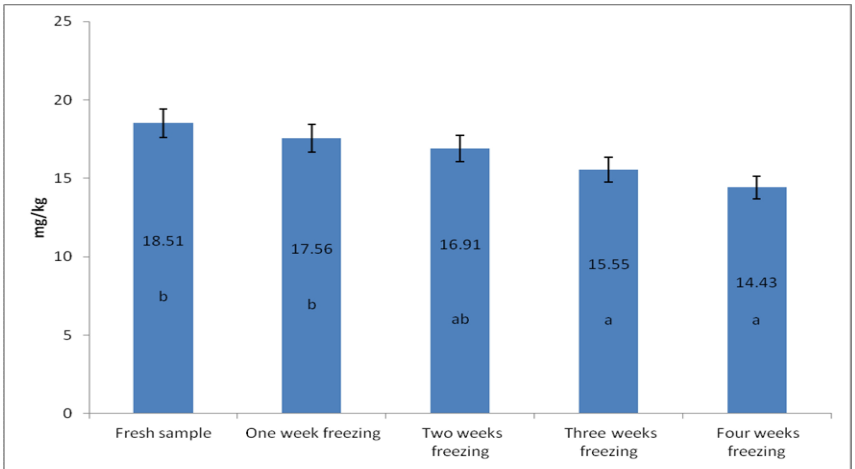

Fig. 7 Effect of freezing on iron content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.05)

3.8 Copper Concentration

The concentration of Cu in fresh, and frozen samples for one, two, three and four weeks were 26.67, 23.89, 22.83, 22.34 and 20.84 mg/kg, respectively. Data analysis showed that the reduction of the mineral content in the vegetable during freezing period was not significant (Fig. 8).

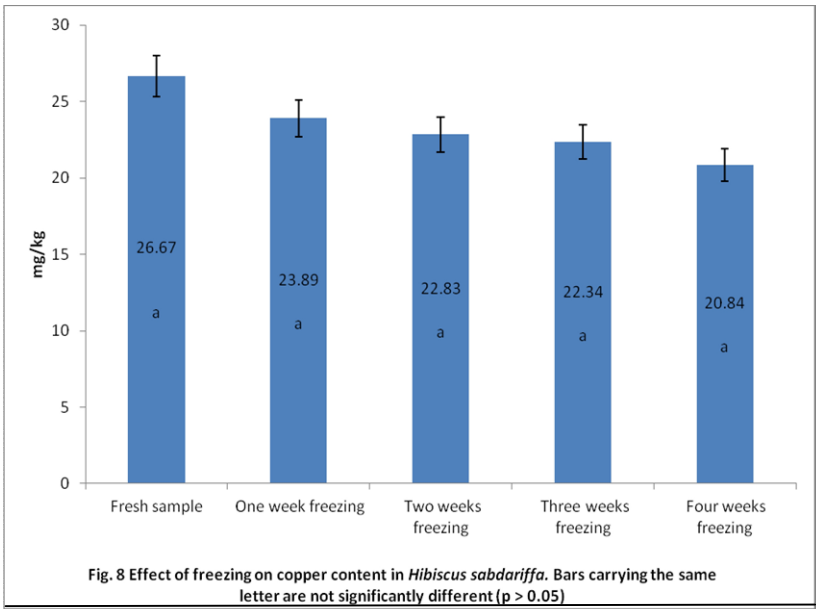

Fig. 8 Effect of freezing on copper content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.05)

3.9 Magnesium Concentration

The concentration of Mg in fresh samples of *Hibiscus sabdariffa* decreased significantly after a week of freezing from 21.69 to 17.09 mg/kg. In the subsequent second, third and fourth

weeks, the Mg content also decreased insignificantly with time and the values obtained were 16.50, 15.39 and 14.73 mg/kg, respectively (Fig. 9).

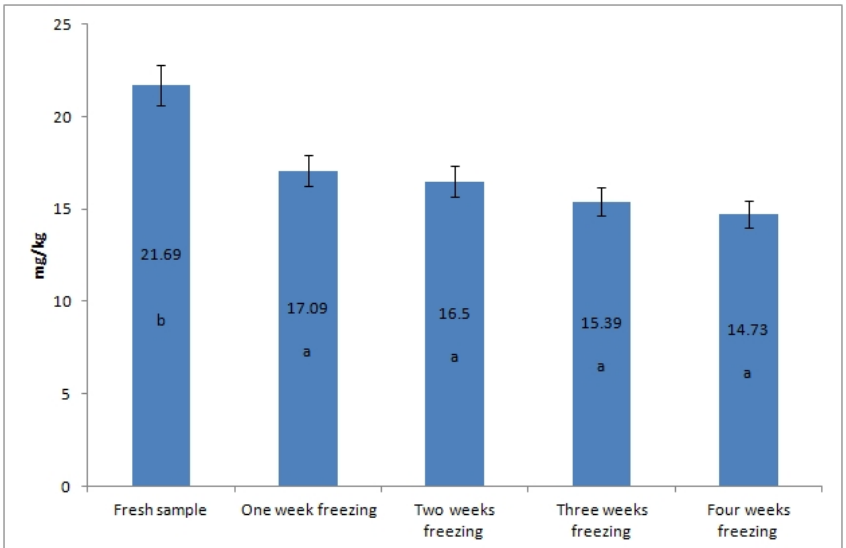

Fig. 9 Effect of freezing on magnesium content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.05)

3.10 Sodium Concentration

The significant ($p < 0.05$) decreased in the Na content from 6.11 mg/kg in fresh sample of *Hibiscus sabdariffa* to 4.41 mg/kg was observed only in four weeks of freezing. The reduction in the mineral content recorded in the first week (5.40 mg/kg), second week (5.36 mg/kg) and third week (4.80 mg/kg) of freezing were not significant (Fig. 10).

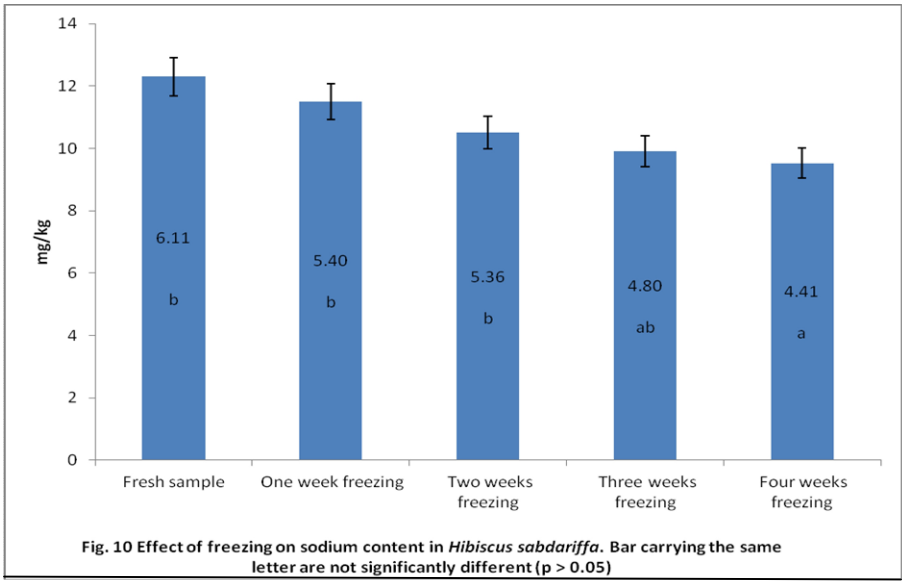

Fig. 10 Effect of freezing on sodium content in *Hibiscus sabdariffa*. Bar carrying the same letter are not significantly different (p > 0.05)

3.11 Potassium Concentration

The decreasing effect of freezing on K concentration was also observed in *Hibiscus sabdariffa* during storage. The concentration of K in fresh, and frozen samples for one, two, three and four weeks were 61.88, 61.86, 58.13, 50.46 and 47.68mg/kg, respectively. Data analysis showed that the decreased in the mineral concentration in the vegetable during freezing was significant in the third week which remains significantly same in the fourth week of storage (Fig. 11).

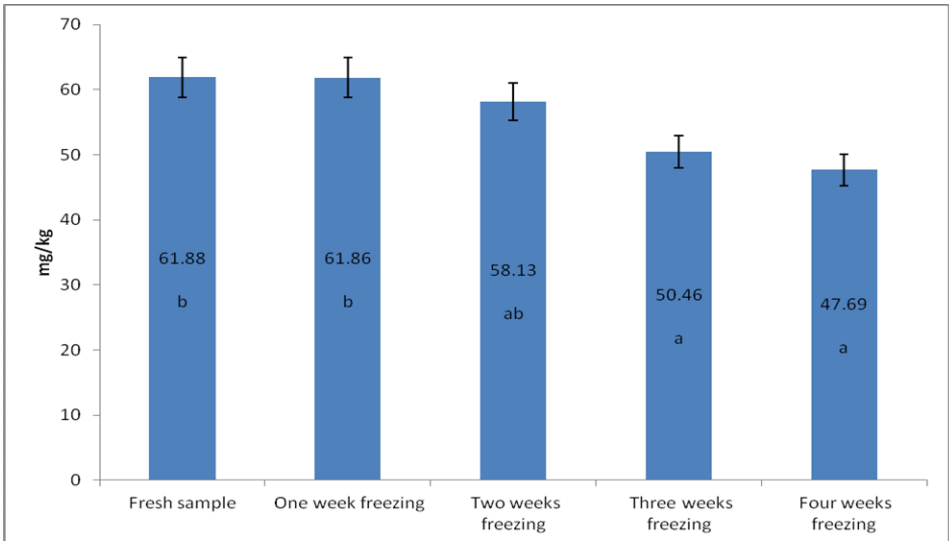

Fig. 11 Effect of freezing on potassium content in *Hibiscus sabdariffa*. Bars carrying the same letter are not significantly different (p > 0.05)

4. DISCUSSION

The decreasing effect of freezing on cyanide concentration in *Hibiscus sabdariffa* is in accordance with the results of [20,21] that freezing raptures the plant cells and releases cyanide content. Another reason that may be adduced for the decreased of the respiratory poison during freezing can be attributed to the solubility of the compound in water [22], thus cyanide may be trapped in the ice and released during thawing.

The lower concentration of nitrate in frozen samples than in the fresh sample in the vegetable is in line with the submission of [23] that freezing of the vegetable decreased their nitrate content. The authors ascribed the decreased to the leaching of the cell content caused by freezing and thawing. The generally weekly decline of nitrate concentration in the vegetable during freezing is in agreement with the submission of [23], to the effect that lost of nitrate during freezing increase with freezing time. The cyanide and nitrate concentrations in the fresh and frozen samples of *Hibiscus sabdariffa* are within the acceptable daily intake of 200 mg of cyanide [24] and 219 mg of nitrate [20] for a 60 kg person (if consumed 100 g/day). Thus consumption of fresh and frozen leaves of the *Hibiscus sabdariffa* may not cause any negative health effect arising from high intake of cyanide and nitrate in the vegetable.

The decreased in soluble and total oxalates concentration in the frozen samples compared to fresh leaves of *Hibiscus sabdariffa* agreed with the findings of [25] in some leafy vegetables. The authors observed that freezing of tissues at high moisture content results in the formation of ice crystals within the cells. The sharp edges of the crystals so formed are capable of lacerating the cell membranes resulting in cell leakage. This finding is in agreement with the report of [20,21,26] that freezing; imparts physical injury to the cell, alteration of the pigments which are sources of bioactive compounds, and ruptures the plant cell which ultimately causes the release of the cell content.

The decreasing effect of freezing on β-carotene concentration in *Hibiscus sabdariffa* is in line with the submission of [21,27-31] that freezing decreases the β-carotene content in vegetables. The reduction of the provitamin A has been attributed to the physical injury and alteration of the plant pigments, which are sources of bioactive compounds, that occurs during freezing [21,26]. [27] stated that the observed decreased in the β-carotene concentration during storage in the freezer could be as a results of enzymatic activity coupled with oxidation associated with conjugate double bond in the compound. The generally insignificant differences recorded in the β-carotene content in second to fourth weeks of storage could be seen as a result of decreased in endogenous enzymatic activity and reduction of oxidation of the provitamin as freezing storage progresses. The fresh and one week frozen leaves of *Hibiscus sabdariffa* contained enough of β-carotene that will meet the recommended adult daily allowance of 900µg vitamin A [32,33]. The results obtained therefore implies that freezing of the vegetable for one week may not require any pharmaceutical supplements for a healthy individual to meet the normal recommended daily allowance.

The significant higher vitamin C concentration in the fresh leaves than frozen samples of *Hibiscus sabdariffa* corroborates with the report of the following authors [21,28-31,34-36] to the effect that vitamin C content in fruits and vegetables decreases during freezing. Their observation contradicts the observations of [37], who found that total vitamin C content remained relatively constant throughout the frozen storage period for all samples (pepper, strawberries, green beans etc). [34] attributed the reduction in the vitamin C concentration partly due to the enzymatic activities of vitamin C oxidase, cytochrome oxidase and vitamin C peroxidase that were endogenously present. The results agreed with the report of [36] that during freezing of vegetables the ascorbic acid content decreased considerably and the dehydroascorbic acid/vitamin C ratio increased. This observation could suggest that during cold storage in refrigerator, ascorbic acid may undergo oxidation to dehydroascorbic acid by some of the endogenous enzymes. [21] on the other hand stressed that the observed decreased in the vitamin C concentration during freezing is as a result of thawing. The observed insignificant difference in the vitamin C content in first to fourth weeks of storage could be seen that during the first week of freezing, the physical injury and alteration of pigments, which are sources of bioactive compounds, reported by [21,26] and the enzymatic oxidation of ascorbic acid to dehydroascorbic acid [34,36] may be at peak within one week of storage. However, these processes might have declined considerably as freezing storage progresses. Another possible explanation to this observation could be that the residual vitamin C content after first week of freezing may be localized in different cell compartment, where the known factors responsible for reduction of the vitamin during cold storage could not have the asses to the compartment.

The concentration of vitamin C (27.44 mg/100g) in the fresh sample of *Hibiscus sabdariffa* is lower than the recommended daily allowance of 60 mg [32,34] if 100g of the vegetables were consumed. With frozen samples even lower. Considering the important roles of this

water soluble vitamin in human health and the associated diseases resulting from its deficiency, pharmaceutical supplementation of the vitamin will be necessary to augment its low level in the vegetable and losses during freezing storage. This will enable the body to meet the dietary requirement of the vitamin.

The decreased in the minerals (Fe, Cu, Mg, Na and K) concentration in *Hibiscus sabdariffa* with freezing time indicated that freezing generally decreases the mineral content of the vegetable. The reason for this could be attributed to the fact that freezing can damage some foods (especially salad vegetable, mushrooms and soft fruits) because the formation of ice crystals during freezing causes the breakage of the cell membranes. Freezing is also known to inflict physical injuries to the cell wall as well as alteration of plant pigments, which are sources of bioactive compounds. All these processes lead to the release of cell content including some of the mineral content [21,26,38-40]. “Freezer burn” which occurs during freezing of vegetables and other soft plant materials is known to decrease the bioactive compounds. The significant reduction of the mineral elements concentration in *Hibiscus sabdariffa* during freezing period agrees with the report of [40], to the effect that considerable amount of macro and micro minerals content of vegetables are lost following freezing. Similarly the insignificant decreased in some mineral elements in *Hibiscus sabdariffa* during two weeks and throughout the storage duration also support the finding of [38,40]. The authors stressed that, even though there was a decreased in the mineral content of the vegetable during freezing, it is not significant because the mineral content via frozen is approximately the same as via fresh vegetables. These two factions of the results obtained could imply that the retention of the mineral element in the frozen samples or decrease of mineral in vegetables during freezing storage depend to a great extend on the plant species/ cultivars and the form in which the mineral exist; such as in chemical compound, molecular complexes and some may even exist in as a free ion [31,40]. It is believed that mineral elements that are chemically bond or form complexes with other compounds may not be easily leach out during freezing when compared with those that exist as free ions.

5. CONCLUSION

Freezing the leaves of *Hibiscus sabdariffa* at - 4ºC for one week significantly reduced the concentration of phytotoxins and still conserves most of the micronutrients, especially the β-carotene and some mineral elements in amount that will meet the nutritional requirements of a healthy body.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tindal HD. Vegetable in the tropics. Macmillan Edu. Ltd; Hampshire; 1986.
2. Babatunde FEF. Intercrop productivity of roselle in Nigeria. Afri Crop Sci J. 2003;11(1):43-47.
3. Akanya HO, Oyeleke SB, Jigam AA, Lawal FF. Analysis of sorrel drink. Nig J Bioch. 1997;12:77–79.
4. Adanlawo IG, Ajibade VA. Nutritive value of the two varieties of roselle (*Hibiscus sabdariffa*) Calyces soaked with wood ash. Pak J Nutr. 2006;5(6):555-557.

5. Ojokoh AO. Roselle (*Hibiscus sabdariffa*) calyx diet and histopathological changes in lives of Albino rats. Pak J Nutr. 2006;5(2):110-13.
6. Babalola SO. Chemical analysis of roselle leaf (*Hibiscus Sabdariffa*), In Proceeding of 24th annual conference of NIFST. 2000;228-229.
7. Musa A, Ogbadoyi EO. Effect of processing methods on some micronutrients, antinutrients and toxic substances in *Hibiscus sabdariffa*. Asian J Biochem. 2012;7(2);63-79. ISSN 1815 - 9923/DOI: 10.3923/ajb.2012.63.79.
8. Musa A, Ogbadoyi EO. Effect of nitrogen fertilizer on the levels of some nutrients, antinutrients and toxic substances in *Hibiscus sabdariffa*. Asian J Crop Sci. 2012;4(3):103-112. ISSN 1994-7879 / DOI:10.3923/ajcs.2012.103.112.
9. Morton J. Roselle in fruits of warm climates. Florida Flair Books; Florida; 1987.
10. Ojokoh VO. Adetuye FA, Akinyosoye E, Oyetayo O. Fermentation studies on roselle (*Hibiscus Sabdariffa*) calyces neutralized with trona. J Food Technol Afr. 2002;7:75-78.
11. Goldstein L, Swain T. Change of ripening fruits. Phytochemical. 1963;2:271-283.
12. Evans RJ, Badndemer SL. Nutritive value of legume seed proteins. J Agric Food Chem. 1967;15:439-443.
13. Aletor VA. Allelochemical in plant foods and feeding stuffs; Nutritional, biochemical and physiological aspect in animal production. Vet Human Toxicol. 1993;35:57-67.
14. Sjoberg AMK, Alanka TA. Spectrophotometric determination of nitrate in baby food: Collaborative study. J AOAC Int. 1994;77(2):425-430.
15. Ikediobi CO, Onyia GOC, Eluwah CE. A rapid and inexpensive enzymatic assay for total cyanide in cassava (*Manihot esculenta crantz)* and cassava product. Agric Biol Chem. 1980;44:2803-2808.
16. Oke LO. Chemical Composition of some Nigeria leafy vegetables. J Am Dietetic Assoc. 1966;53:130-132.
17. Ezeonu FC, Musa A, Stanly CD, Oswald CE. Iron and zinc status in soils, water and stable food cultivars in Itakpe, Kogi state of Nigeria. Environmentalist. 2002;22;237-240.
18. Jones E, Hughes RE. Foliar ascorbic acid in some Angiosperms. Phytochemistry. 1983:2(11):2493.
19. Musa A, Ezenwa MIS, Oladiran J A, Akanya HO, Ogbadoyi E.O. Effect of soil nitrogen levels on some micronutrients, anti-nutrients and toxic substances in *Corchorus olitorius* grown in Minna, Nigeria. Afr J Agric Res. 2010;5(22):3075–3081. Available: http://www.academicjournals.org/AJAR.
20. Richard DW. Cooperative Extension Service: Cooperative Extension work acts may 8 and June 30, 1914, as amended, Kansas State University, County Extension Councils. Extension Districts and U.S. Department of Agriculture Cooperating; 1991.
21. McDonald JK, Caflin NA, Sommano S, Cocksedge R. The Effect of post harvest handling on selected native food plant; A report for the rural Industries Research and Development Corporation; 2006. Available: http://www.rirdc.gov.au/reports/NPP/06-021.pdf.
22. Budavari S, Oneil MJ, Smith A. The merck index. Merck and Co. Inc. Rahway; New Jersey; 1989.
23. Abakr A, Ragaa A. Trials to reduce nitrate and oxalate content in some leafy vegetables; Interactive effect of manipulating of the soil nutrient supply, different blanching media and preservation methods followed by cooking process. J Sci Food Agric. 1996;97:169–178.
24. Anjana SU, Muhammed I, Abrol YP. Are nitrate concentrations in leafy vegetables within safe limits? Current Sci. 2007;92(3):355–360.

25. Ogbadoyi EO, Makun AH, Bamigbade OR, Oyewale OA, Oladiran JA. The effect of processing and preservation methods on the oxalate levels of some Nigeria leafy vegetables. Biokemistri. 2006;18(2):121–125.
26. Fellow PJ. Food processing technology; Principles and practice. Wood Publishing Limited; 2000.
27. Booth LS, Johns T, Kuhnlein HV. Natural food sources of vitamin A and provitamin A; Difficulties with the published values. United Nations University Press; Food Nutr Bull. 1992;14(1):2–13.
28. Yadav SK, Sehgal S. Effect of home processing on ascorbic acid and beta-carotene content of spinach (*Spinacia oleracia*) and amaranth (*Amaranthus tricolor*) leaves. Plant Foods Human Nutr. 1995;47(2):125–131.
29. Yadav SK, Sehgal S. Effect of home processing and storage on ascorbic acid and beta – carotene content of bathua (*Chenopodium album*) and fenugreek (*Trigonella foenum graecum*) leaves. Plant Foods Human Nutr. 1997;50(3):239–247.
30. Lisiewska Z, Kmiecik W. Effect of storage period and temperature on the chemical composition and organoleptic quality of frozen tomato cubes. Food Chem. 2000;70:167-173.
31. Piotr G. Content of selected antioxidative compounds in raw carrot and in frozen product prepared for consumption. Electr J Polish Agric. Unin. 2006. Available: http://www.Ejpau.Media.Pl/volume9/issue3/art--03-html.
32. George DPR. Newlife style: Enjoy it. Editorial Safeliz; Spain; 1999.
33. Akanya HO. Retinol: The vitamin of life. Federal University of Technology, Minna. Inaugural lecture series No. 5. Scan Prints Nig Ltd; 2004.
34. Olaofe O. Vitamin C content of Nigerian food – stuffs. Nig J Nutr. Sci. 1992;13(1& 2):1–7.
35. Ejoh AR, Tanya, AN, Djuikwo NA, Mbofung CM. Effect of processing and preservation methods on vitamin C and total carotenoid levels of some *Vernonia* (bitter leaf) species. Afr J Food Agric Nutr Dev. 2005;5(2):105-117.
36. Bergquist SAM, Gertsson UE, Olsson ME. Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (*Spinacia oleracea L.*). J Sci Food Agric. 2006; 86(3): 346-355.
37. Mathew RF, Hall IW. Ascorbic acid, dehydroascorbic acid and diketogluonic in frozen peppers. J Food Sci. 1978;43:532–534.
38. Polo MV, Lagarda MJ, Farre R. The effect of freezing on mineral element content of vegetables. J Food Compos Anal. 1992;5(1):77–83.
39. Pruthi JS. Quick freezing preservation of foods; Principles, practices. R and D needs. Allied Publishers; New Delhi Idian; 1999.
40. Hui YH, Legarretta IG, Lim MH, Murrell KD, Nip WK. Hand book of frozen foods. Marcel Dekker Incorporated; New York; 2004.

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