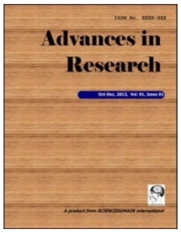

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Effect of Cold Storage on the Concentrations of some Nutrients, Anti-nutrients and Toxic Substances in the Leaves of *Vernonia amygdalina* (Bitter leaf)

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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.

Original Research Article

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ABSTRACT

Aims: In order to maintain the freshness and quality of leafy vegetables after harvesting during glut, vegetables are usually stored in cold condition either in the freezer or refrigerator for certain period as this storage condition is believed to prevent some of the biochemical reactions responsible for food spoilage. This research was carried out to examine the effect of cold storage duration in the freezer at -4ºC on the concentrations of some toxic substances (cyanide, nitrate soluble and total oxalates) and nutrients (β-carotene, vitamin C, Fe, Cu, Mg, Na and K) in the leaves of *Vernonia amygdalina*.
Methodology: Leaves of *Vernonia amygdalina* were washed with distilled water and stored in deep freezer at -4ºC for four-weeks. The concentrations of nutrients, antinutrients and toxic substances were determined at weekly intervals.
Results: The results showed that the concentrations of cyanide, soluble and total oxalates, vitamin C and β-carotene in the leaves of *Vernonia amygdalina* decreased significantly ($p < 0.05$) in the first three weeks of freezing. Significant ($p < 0.05$) decrease in the β-carotene concentration was observed in the fourth week of cold storage. The

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nitrate, Cu and Mg contents in the leaves of *Vernonia amygdalina* decreased significantly ($p < 0.05$) during two weeks of freezing and insignificantly ($P>0.05$) in the third and fourth weeks of cold storage. While freezing has no significant effect on Na content in the leaves of *Vernonia amygdalina* during the storage period, the concentrations of Fe and K in the vegetable decreased significantly ($p < 0.05$) in the third week of freezing.
Conclusion: A maximum of two weeks of cold storage condition in freezer at -4°C significantly reduced the concentrations of antinutrients and toxic substances in the leaves of *Vernonia amygdalina* to tolerable levels without compromising the nutritional benefit in the vegetable.

Keywords: Vernonia amygdalina; cold storage; nutrients; cyanide; nitrate; soluble and total oxalates.

1. INTRODUCTION

Vernonia amygdalina (bitter leaf) is a leafy vegetable which can grow up to a height of 5m, with abovate to oblanceolate leaves with the widest part below the middle. The vegetable is a perennial crop and some are known to have been in continuous production for up to 7 years. *Vernonia amygdalina* is frequently found in gardens in Nigeria, Congo (Democratic Republic), Cameroon and Gabon [1] where it is used to alleviate the problem of micronutrient malnutrition, prominent in tropical Africa [2]. *Vernonia amygdalina* is generally raised by stem cutting and are usually planted at an angle of 45^0 to obtain quicker emergent [1]. This leafy vegetable is relatively inexpensive and rich in several nutrients especially β-cartotene, vitamin C and mineral elements Fe, Cu, Mg, P, Zn, Ca, Na, K [3-5]. *Vernonia amygdalina* is also known to contain some secondary metabolites such as dhurrin which is a cyanogenic glycoside, nitrate, oxalates [4-7]. Bitterness in *Vernonia amygdalina* is partly caused by saponins, which can be poisonous to human and other animals [1]. Generally, the chemical contents and compositions of nutrients and toxic substances in *Vernonia amygdalina* like in any leafy vegetable after harvesting are affected among other postharvest handlings by storage conditions. It is for this reason that this research was conducted to investigate the influence of cold storage condition at -4ºC in freezer for the period of four weeks on the concentrations of some nutrients, antinutrients and toxic substances in the leaves of *Vernonia amygdalina.* This is to determine the fittingness of the storage condition in preserving the vegetable.

2. MATERIALS AND METHODS

2.1 Source of *Vernonia amygdalina*

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

2.2 Chemicals

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

2.3 Cold Storage Condition

The leaves of *Vernonia amygdalina* were washed with distilled water and kept in a well labelled polythene bag and stored in a freezer at the temperature of - 4°C for a period of four weeks. The concentrations of the nutrients, antinutrients and toxic substances 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 of Sjoberg and Alanka [8]. The alkaline picrate method of Ikediobi et al. [9] was used to determine the cyanide concentration in the leaves of *Vernonia amygdalina*. Both soluble and total oxalates in the samples were analysed by titrimetric method [6]. The mineral elements (Fe, Cu, Mg, Na and K) in samples were determined according to the method of Ezeonu et al. [10]. The ascorbic acid concentration in the samples was determined by 2, 6-dichlorophenol indophenols method [11], while β-carotene concentration was determined by ethanol and petroleum ether extraction method [12]

2.3 Statistical Analysis

Analysis of variance (ANOVA) was carried out using statistical package, Minitab, to determine variation of the concentration of nutrients, antinutrients and toxic substances in the leaves of *Vernonia amygdalina* during storage. The DUNCAN's Multiple Range Test (DMRT) was used for comparison of means.

3. RESULTS

3.1 Cyanide Content

The amount of cyanide in fresh leaves of *Vernonia amygdalina* decreased significantly ($p < 0.05$) during first week of freezing from 199.11 to 69.74 mg/kg. In the subsequent second, third and fourth weeks, the decrease was insignificant ($p>0.05$) with time and the values obtained were 58.96, 52.80 and 48.00 mg/kg, respectively (Fig. 1).

3.2 Nitrate Content

The nitrate concentration of *Vernonia amygdalina* also decreased with freezing time. Significant ($p < 0.05$) reduction in the nitrate concentration from 1347.22 mg/kg in fresh sample to 920.57 mg/kg was recorded after three weeks of storage. The mean value obtained in the fourth week (842.26 mg/kg) indicated reduction in nitrate content, which was not significantly ($p>0.05$) different from that of second week (1069.39 mg/kg). However, the concentration of nitrate in the first week (1320.65 mg/kg) of freezing was significantly ($p < 0.05$) higher than those in the third and fourth weeks (Fig. 2).

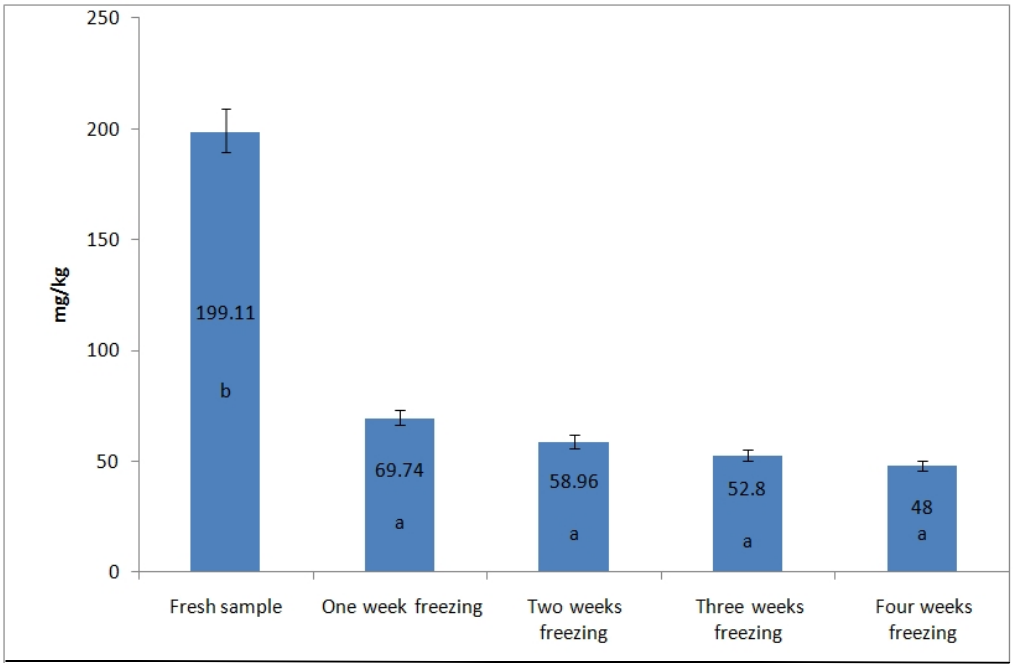

Fig. 1. Effect of freezing on cyanide content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different (p > 0.05)

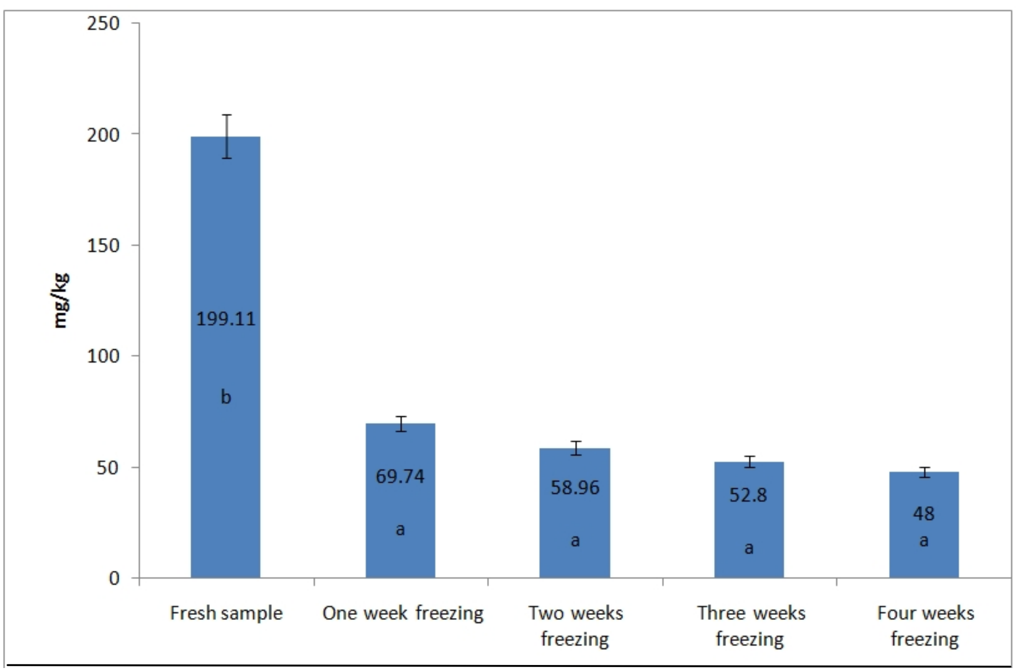

Fig. 2. Effect of freezing on nitrate content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different (p > 0.05)

3.3 Soluble Oxalate Content

The amount of soluble oxalate in the fresh sample of *Vernonia amygdalina* decreased significantly ($p < 0.05$) during one week of freezing from 2.85 to 1.76 g/kg. In the subsequent second, third and fourth weeks of freezing, the oxalate concentration decreased insignificantly ($p > 0.05$) with time and the values obtained were 1.62, 1.50 and 1.31 g/kg, respectively (Fig. 3).

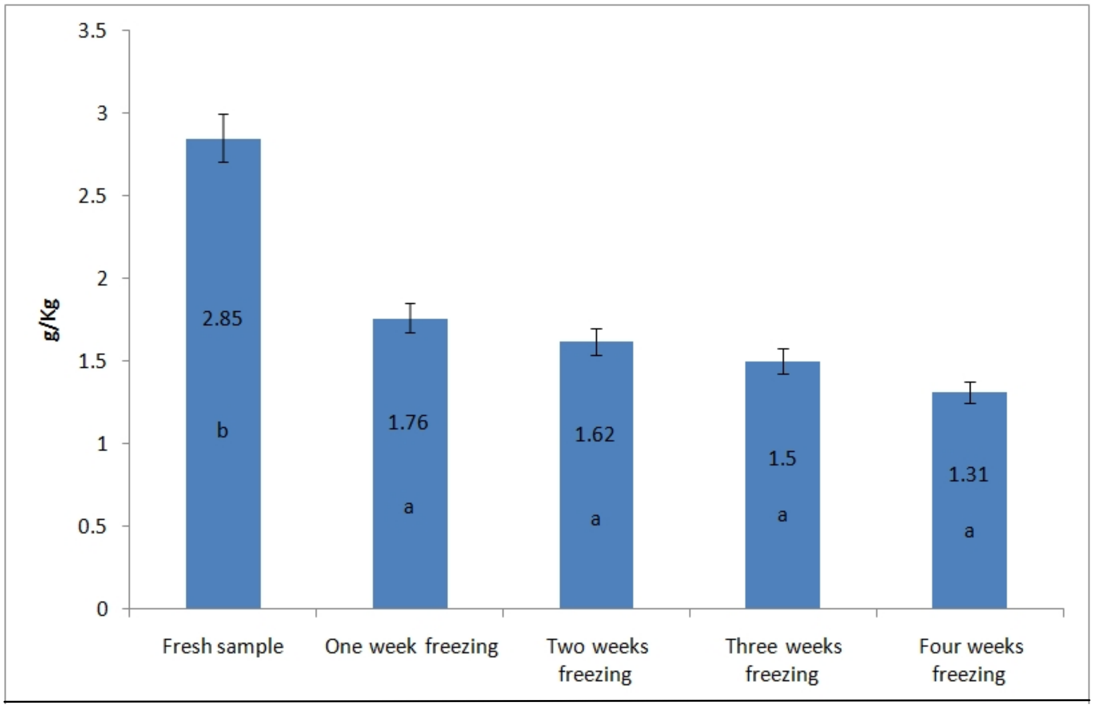

Fig. 3. Effect of freezing on soluble oxalate content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different ($p > 0.05$)

3.4 Total Oxalate Content

The effect of freezing on total oxalate content of *Vernonia amygdalina* leaves is presented in Fig. 4. The concentration of oxalate in the fresh, one, two, three and four weeks frozen samples were 4.76, 3.08, 2.50, 2.50 and 2.26 g/kg, respectively. The reduction in oxalate concentration of fresh sample was significantly ($p > 0.05$) different from the frozen samples. However, the values of total oxalate in the frozen samples was insignificantly ($p>0.05$) different from each other.

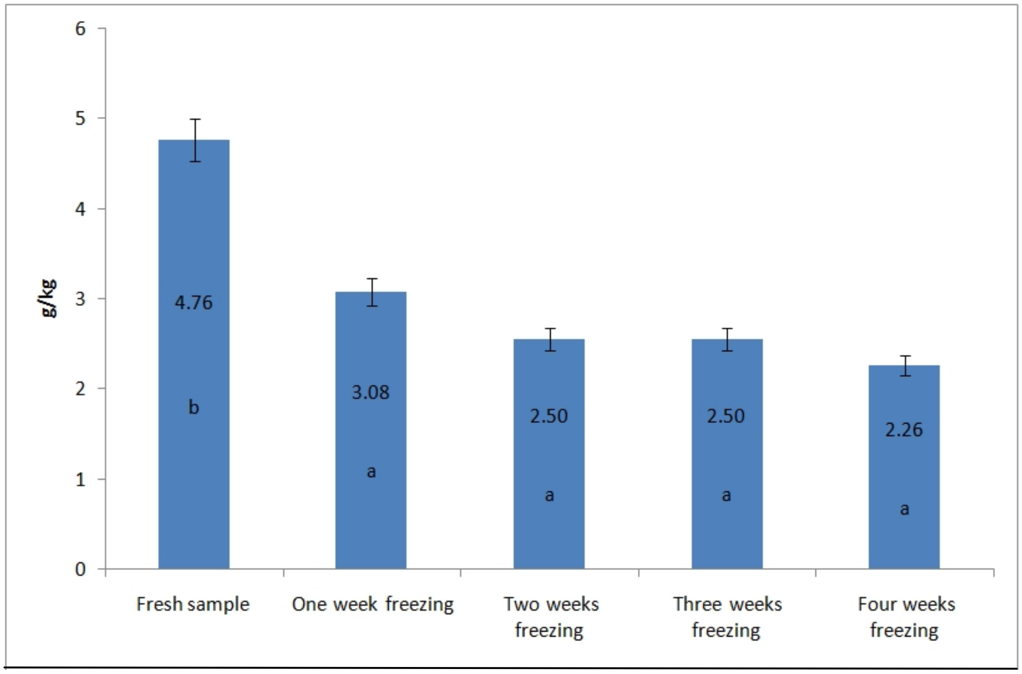

Fig. 4. Effect of freezing on total oxalate content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different (p > 0.05)

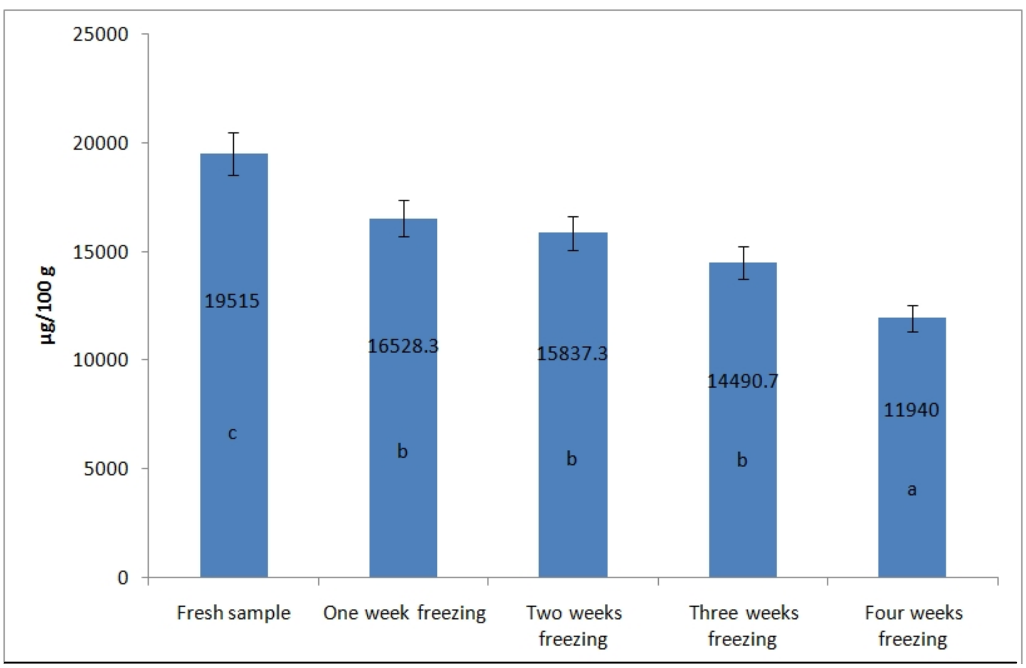

Fig. 5. Effect of freezing on β-carotene content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different (p > 0.05)

3.5 β-carotene Content

Significant reduction of β-carotene concentration from 19515.30 to 16528.30 µg/100 g was observed in one week of freezing (Fig. 5). The mean values obtained for the second (15837.30) and third (14490.70 µg/100 g) weeks were lower but not significantly different from the one week stored sample. However, the concentration of β-carotene obtained in the fourth week of freezing (11940.00 µg/100 g) was significantly lower than those of other frozen samples.

3.6 Vitamin C Content

The vitamin C concentration in fresh sample of *Vernonia amygdalina* decreased significantly ($p < 0.05$) during one week of freezing from 30.68 to 14.25 mg/100 g (Fig. 6). In the subsequent second, third and fourth weeks, the vitamin content decreased insignificantly ($p<0.05$) with freezing time and the values obtained were 10.95, 10.95 and 9.86 mg/100 g, respectively.

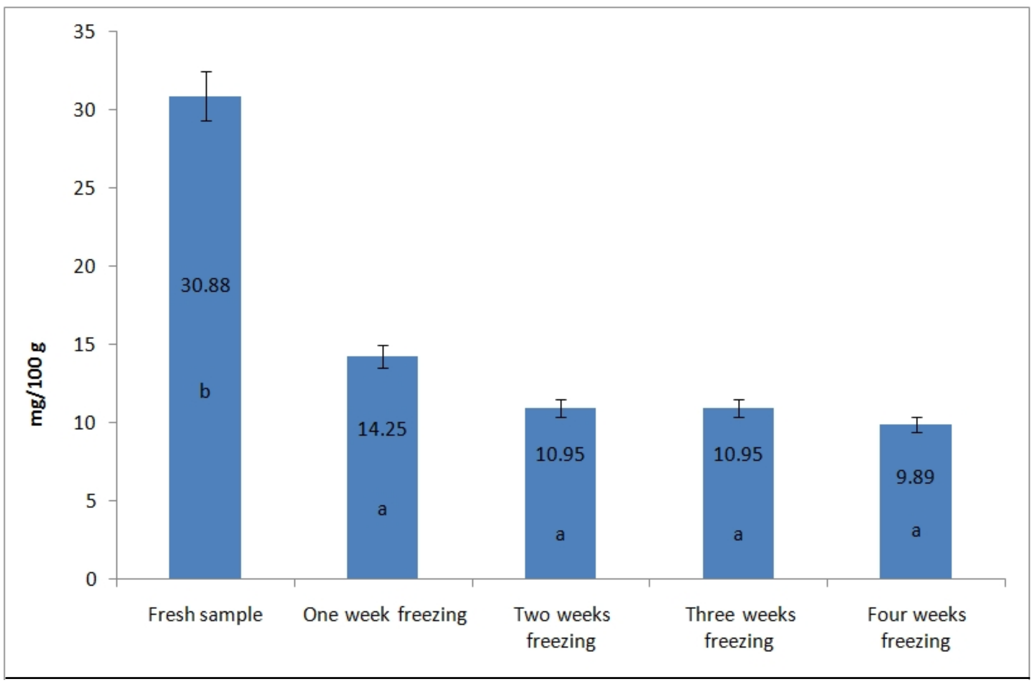

Fig. 6. Effect of freezing on vitamin C content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different ($p > 0.05$)

3.7 Iron Content

The Fe concentration in *Vernonia amygdalina* decreased with freezing time (Fig. 7). Significant ($p < 0.05$) reduction in the concentration of the mineral element from 26.77 to 21.43 mg/kg was observed during third week of freezing. The decrease in the first (23.44 mg/kg) and second (23.03 mg/kg) weeks were, however, not significant. The mean value obtained at the fourth week (18.76 mg/kg) also indicated reduction in concentration which was not significantly different from those of the first, second and third weeks.

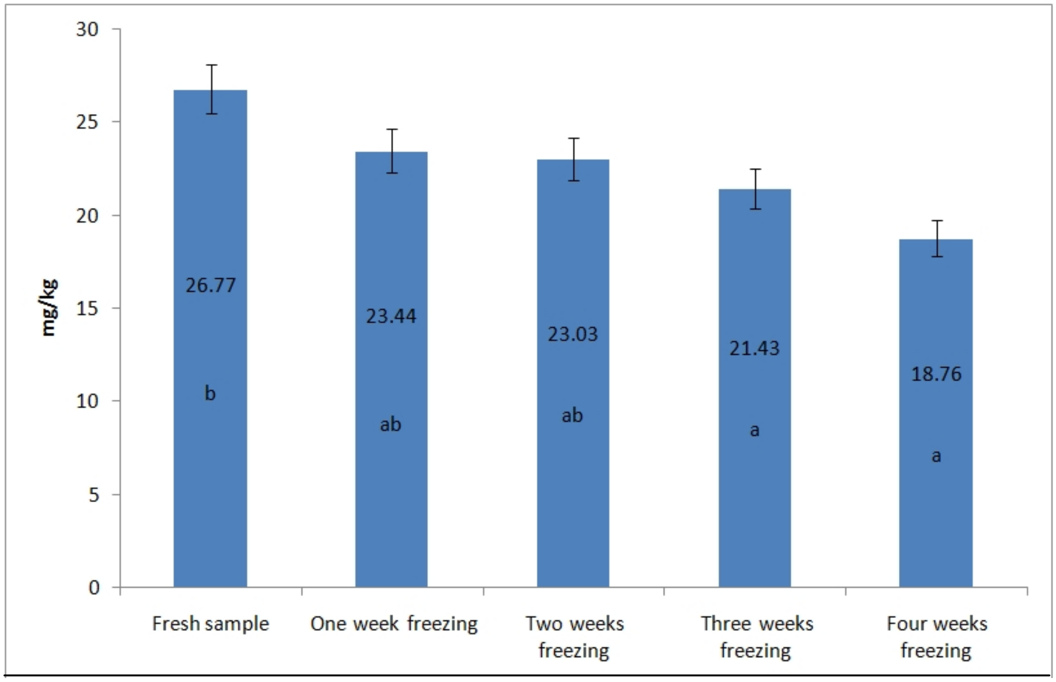

Fig. 7. Effect of freezing on iron content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different ($p > 0.05$)

3.8 Copper Content

The significant ($p < 0.05$) reduction of Cu concentration from 36.70 mg/kg in fresh sample to 26.63 mg/kg was recorded during two weeks of freezing (Fig. 8). The values obtained at the third (24.74 mg/kg) and fourth (22.98 mg/kg) weeks were not significantly ($p>0.05$) different from those of the first and second weeks (Fig. 8).

3.9 Magnesium Content

The concentration of Mg in the fresh sample (55.28 mg/kg) and those of one, two, three and four weeks frozen samples of *Vernonia amygdalina* were 50.49, 37.28, 34.28 and 34.06 mg/kg, respectively. The results showed that the decrease of Mg content in the vegetable was not significant in the first week of freezing. However, in the second week of freezing, the Mg content decreased significantly ($p < 0.05$). The values obtained at the third and fourth weeks of freezing also indicated reduction in concentration of the mineral element which were not significantly different ($p > 0.05$) from the second week (Fig. 9).

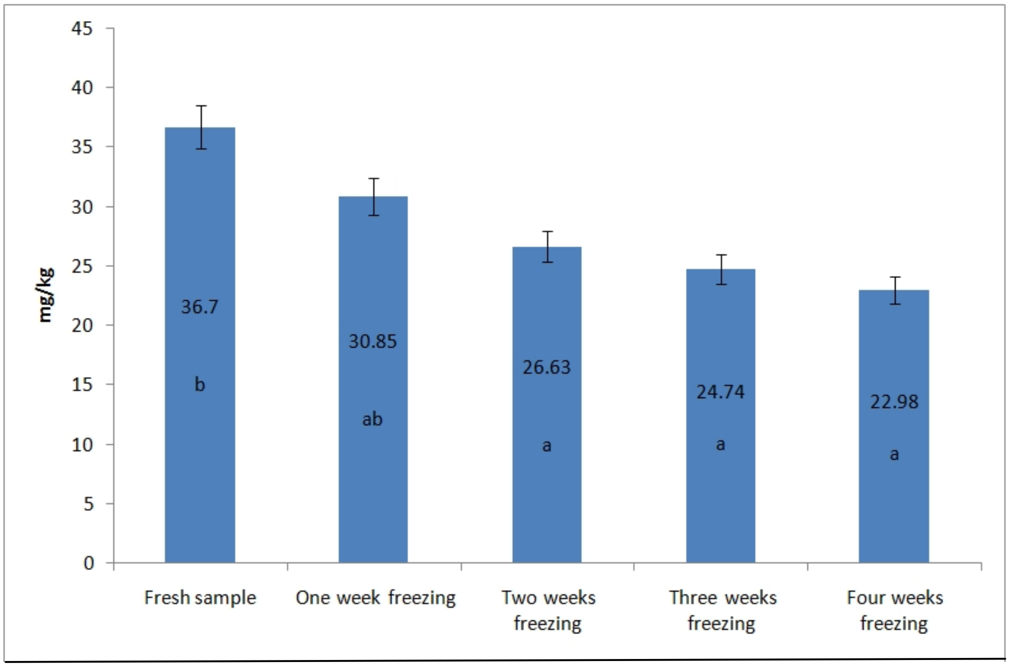

Fig. 8. Effect of freezing on copper content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different ($p > 0.05$)

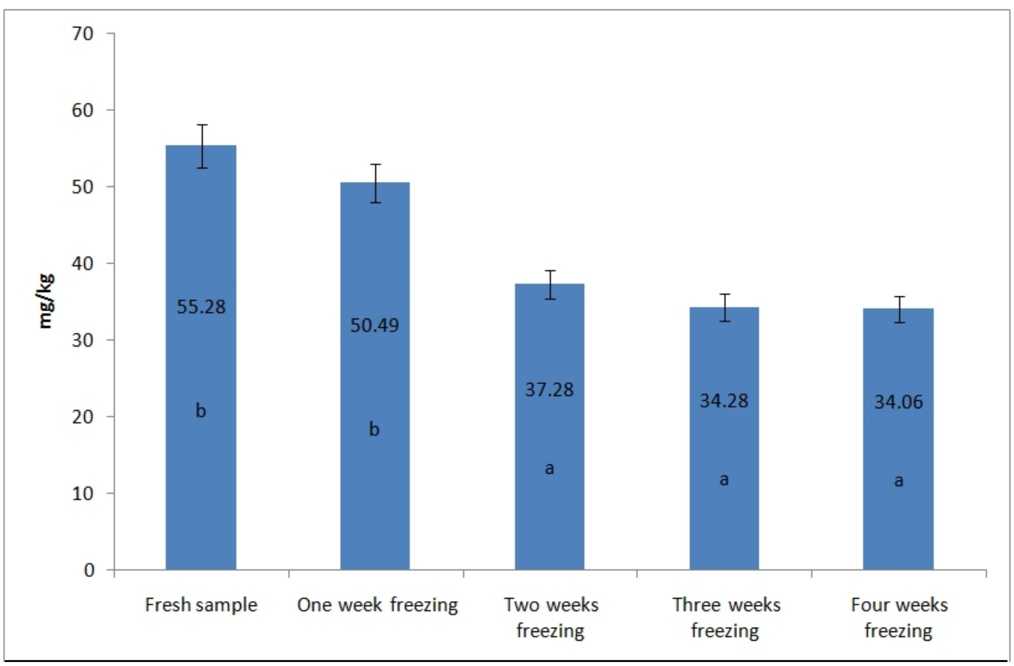

Fig. 9. Effect of freezing on magnesium content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different ($p > 0.05$)

3.10 Sodium Content

The amount of Na in fresh sample (8.03 mg/kg) and samples frozen for one, two, three and four weeks were 7.34, 6.96, 6.45 and 6.44 mg/kg, respectively, (Fig. 10). Results showed that the reduction in the concentration of Na in *Vernonia amygdalina* during freezing period was not significant (p>0.05).

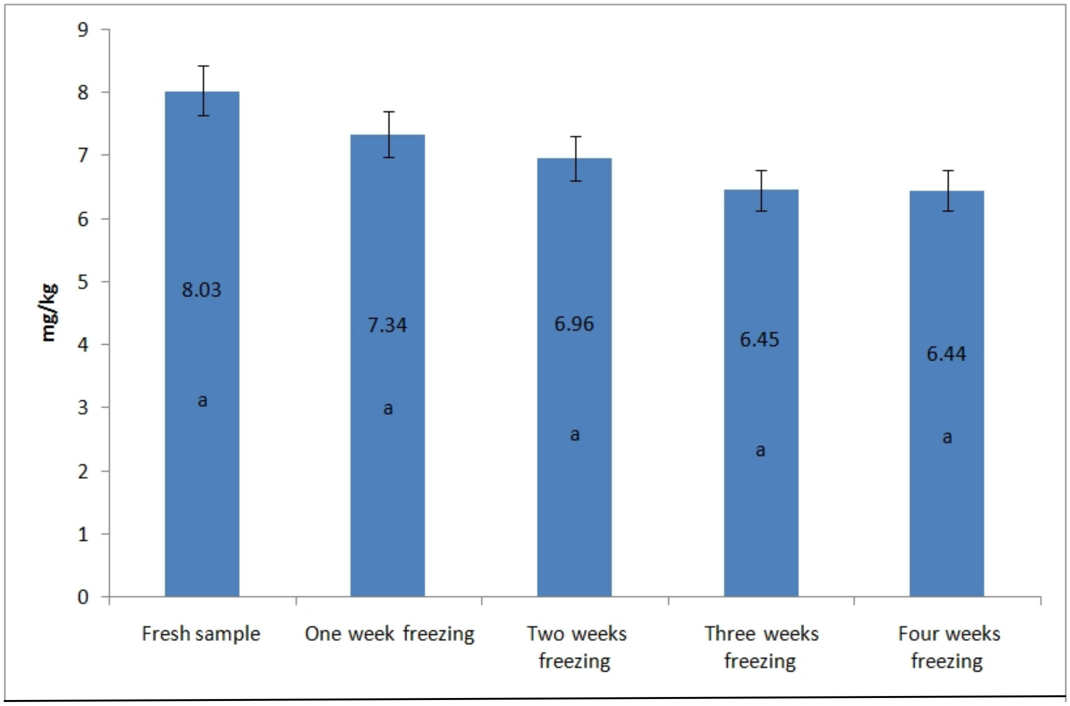

Fig. 10. Effect of freezing on sodium content in *Vernonia amygdalina*. Bars carrying the same letter are not significantly different (p > 0.05)

3.11 Potassium Content

The significant reduction in K concentration from 288.92 mg/kg in fresh sample to 241.29 mg/kg was observed during three weeks of freezing (Fig. 11). The value obtained at the fourth week of cold storage (225.17 mg/kg) indicated reduction in K content which was not significantly (p>0.05) different from that of the second week (260.63 mg/kg). The concentration of the mineral element in the first week (273.75 mg/kg) of freezing was significantly (p < 0.05) higher than those of the third and fourth weeks of freezing.

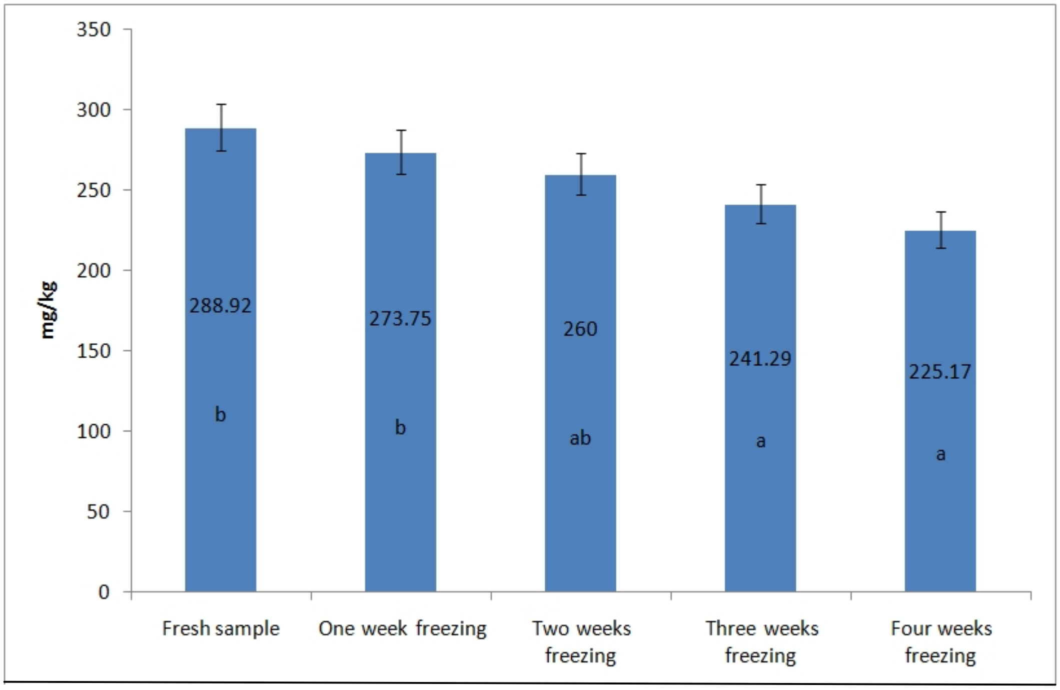

Fig. 11. Effect of freezing on potassium content in *Vernonia amygdaliina*. Bars carrying the same letter are not significantly different (p > 0.05)

4. DISCUSSION

The reduction in the concentration of cyanide in the leaves of *Vernonia amygdalina* during cold storage in the freezer is in accordance with the earlier reports [13-18] that freezing breaks down the plant cells and releases cyanide content of the plant. One of probable reason for the decrease in the concentration of the respiratory poison during cold storage may be due to its high solubility in water [19], thus cyanide may be trapped in the ice and released during defrosting. The cyanide concentration in the fresh leaves of *Vernonia amygdalina* (199.11 mg/kg) is very close to the maximum permissible level of 200 mg/kg [13,20] of cyanide in fresh weight. This was reduced to below half of this value during first and subsequent weeks of freezing. This justifies the hydrophilic nature of this compound. The results therefore, suggest that the concentration of cyanide in the leaves of *Vernonia amygdalina* can be reduced to safe level during cold storage.

The lower concentration of nitrate in frozen samples than in the fresh leaves of *Vernonia amygdalina* corroborates the submission of the following researchers [15-18,21] that freezing decreased the nitrate content in *Vernonia amygdalina*. The authors ascribed the decreased to the leaching of the cell content caused by chilly and defrosting. The generally weekly decline of nitrate concentration in the vegetable during freezing is in agreement with the submission [15,21] to the effect that, losses of nitrate during freezing increased with freezing time. The nitrate concentration in the fresh leaves of *Vernonia amydalina* is within the Acceptable Daily Intake of 220 mg [22] and 219 mg [23] for a 60 kg person (if consumed 100 g/day of fresh vegetable). Thus the nitrate concentration in the fresh and frozen samples of

Vernonia amygdalina is lower than the recommended Acceptable Daily Intake and can be well tolerated in our meal.

The reductions in the soluble and total oxalates concentration in the frozen vegetable during cold storage have been noted [24] in some leafy vegetables. The authors reported that freezing of tissues of 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 previous report [13-18,25] that freezing impart physical damage to the cell, alter the pigments which are sources of phytochemicals, and breaks the plant cells which eventually cause the discharge of the cell content. The results revealed that one and two weeks of cold storage in the freezer at -4^{o}C are require to reduce the soluble and total oxalates content, respectively in the leaves of *Vernonia amygdalina* to tolerable level of 250 mg/100 g [26].

The decrease in the concentration of β-carotene in *Vernonia amygdalina* during cold storage in a freezer observed in this study agreed with the submission [14-18,27-31] that freezing decreases the β-carotene concentration in vegetables. The reduction in the concentration of the provitamin A has been attributed to the physical injury and alteration of the plant pigments that occurs during freezing [14,25]. Booth et al. [27] further stressed that the observed decrease in the β-carotene concentration during cold storage in the freezer may be as a result of enzymatic activity alongside oxidation connected with conjugated double bond in the compound. The fresh and frozen leaves of *Vernonia amygdalina* contained over and above the recommended adult daily allowance of 900 µg vitamin A [32,33]. The implication of the results is that because β-carotene is abundant in *Vernonia amygdalina* and hydrophobic in nature, freezing storage could not reduce the compound to below the normal adult recommended daily allowance. The results obtained therefore imply that freezing the leaves of *Vernonia amygdalina* within the storage period may not require any pharmaceutical supplements for a healthy individual to meet the normal recommended daily allowance.

The observed significantly higher concentration of vitamin C in fresh leaves than various frozen samples of *Vernonia amygdalina* is in line with previous reports [2,14-18,28-31,34,35] to the effect that vitamin C content in fruits and vegetables decreases during freezing. Their observation, however, contradicts that of Matthew and Hall [36], who found that total vitamin C concentration remained relatively constant throughout the frozen storage period for all samples (pepper, strawberries, green beans). Olaofe [34] ascribed the reduction in the concentration of this water soluble vitamin to be partly due to the activities of endogenous enzymes such as vitamin C oxidase, cytochrome oxidase and vitamin C peroxidase that were believed to convert vitamin C (ascorbic acid) to dehydroascorbic acid. This finding agreed with a report [35] that during freezing of vegetables the ascorbic acid content decreased considerably and the dehydroascorbic acid/ascorbic acid ratio increased. McDonald et al. [14] on the other hand stressed that the observed decrease in the vitamin C content during freezing is as a result of defrosting. The observed insignificant difference in the vitamin C content of the frozen samples throughout the storage period may suggest that during the first week of cold storage in the freezer, the physical injury and alteration of pigments, which are sources of phytochemicals [14,25] and the enzymatic conversion of ascorbic acid to dehydroascorbic acid [34,35] may be at acme within one week of storage. However, these processes might have declined considerably as freezing storage progresses. Another probable explanation to this observation could be that the residual vitamin C content after first week of freezing may be confined in different cell compartment, where the factors responsible for reduction of the vitamin during cold storage may perhaps does not have access to the compartment. The concentration of vitamin C in the fresh and frozen leaves of the vegetable is lower than the recommended daily allowance of 60 mg [32, 34] if 100 g of the samples are consumed. Considering the pivotal roles of vitamin C in human health and the remarkable associated ailments resulting from its deficiency, pharmaceutical supplementation of the vitamin will be necessary to augment its low concentration in *Vernonia amygdalina* and losses during cold storage in the freezer.

The observed general declined in the mineral elements (Fe, Cu, Mg, Na and K) concentration in *Vernonia amygdalina* with freezing time signified that freezing decreases the mineral contents in 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 cold storage in the freezer causes the breakage of the cell membranes. Freezing is also known to inflict physical injuries to the cell wall and as well alters plant pigments. These processes led to the discharge of cell content including some of the mineral elements [14-18,25,37-39]. “Freezer burn” which occurs during freezing of vegetables and other soft plant materials is known to decrease the phytochemicals including the mineral elements. The significant reduction in the concentrations of some mineral elements in *Vernonia amygdalina* during cold storage agrees with the report [39] that significant amount of macro and micro mineral elements of vegetables are lost during freezing. Similarly the insignificant decrease in concentration of Na in *Vernonia amygdalina* throughout the freezing time also supports previous reports [37,39] that, even though there was a decrease in the mineral element concentrations in vegetables during freezing, it is not significant because the mineral element concentrations through frozen are approximately the same as via fresh vegetables. These two different reports may suggest that the residual mineral element in the frozen samples during cold storage in the freezer depend immensely on the cultivars and the form in which the mineral element exist in the plant. Some may exist as free ions while others form complexes with compounds [31,39]. It is believed that mineral elements that are chemically bound or form complexes with other compounds may not be easily leached out during freezing when compared with those that exist as free ions [15-18]. *Vernonia amygdalina* is an excellent source of Fe, Cu and K. The concentrations of Fe and Cu in the fresh and frozen leaves of the vegetable are sufficient to meet the adult recommended daily intake of 18 mg/day and 1.5-3.0 mg/day, respectively [40]. However, supplementation from other food sources such as fruits and nuts will be necessary to augment the low concentrations of Mg and Na in the fresh and frozen leaves of *Vernonia amygdalina*.

5. CONCLUSION

It could be concluded that maximum of two weeks of cold storage condition in freezer at -4^{o}C reduced the antinutrients and toxic substances in the leaves of *Vernonia amygdalina* to tolerable levels without compromising the nutrients that abound in the vegetable. It therefore follows that the cold storage condition do not only prevent the leaves of *Vernonia amygdalina* from deterioration during storage, but it also avert the public health problems that might be connected with ingestion of toxic levels of cyanide, nitrate, soluble and total oxalates in the vegetable.

COMPETING INTERESTS

The authors do not have competing interests.

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