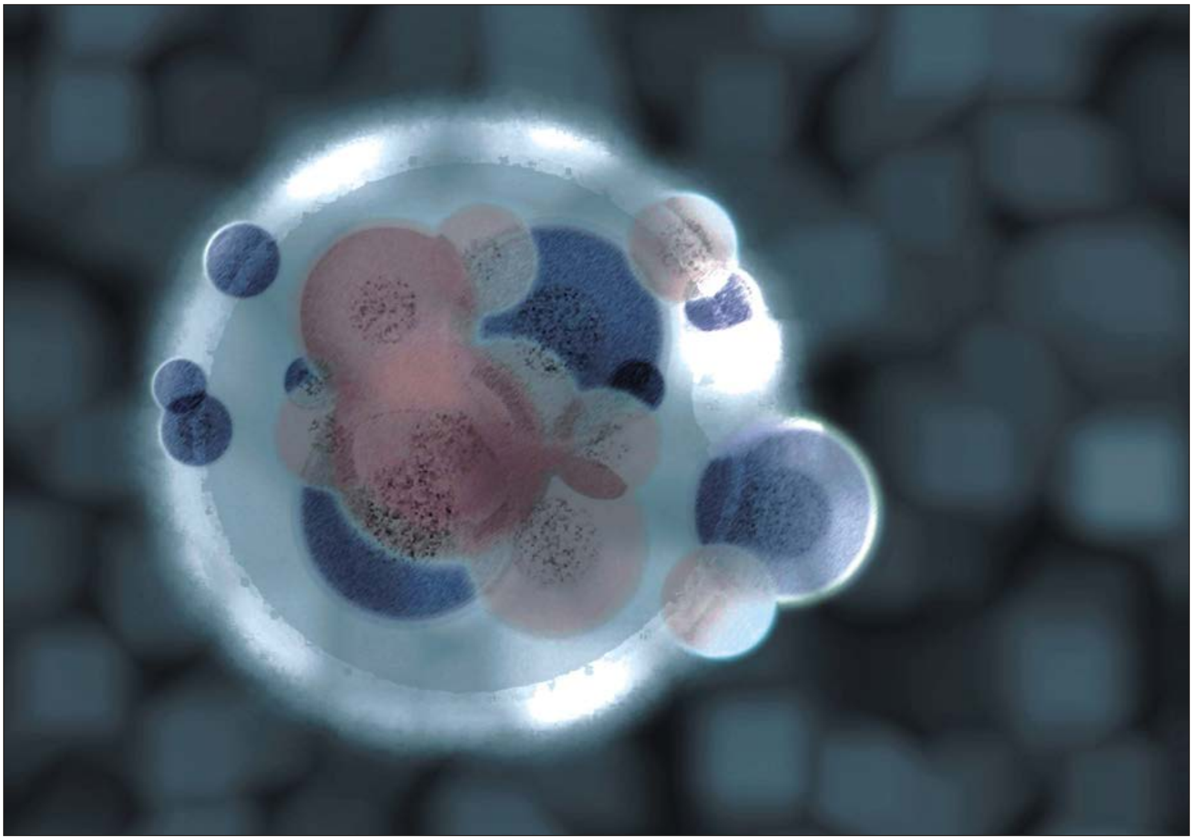

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Influence of Freezing on Some Phytotoxins and Micronutrients in the Leaves of *Telfairia occidentalis* (Fluted Pumpkin)

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Abstract: Cold storage is one of the methods that is used to safeguard the quality of fresh foods (especially vegetables and fruits) and prevent food spoilage as the method reduces the activities of some microorganisms and enzymes that are involved in the sequence of some biochemical invents leading to deterioration reactions. Therefore, it is common practice to store fresh foods in refrigerator and freezer for some period of time. This study investigates the effect of freezing duration as it affects the concentrations of some inherent phytotoxins (cyanide, nitrate, soluble and total oxalates) and micronutrients which include vitamins (vitamin C and β-carotene; provitamin A) and mineral elements (Fe, Cu, Mg, Na and K) in the leaves of *Telfairia occidentalis*. The washed leaves of *Telfairia occidentalis* were stored in a deep freezer at-4°C for the period of four weeks and chemical analyses were done at weekly intervals. The result obtained showed that the concentrations of cyanide and Fe decreased significantly ($p<0.05$) with freezing time in the first and second weeks and their values remained significantly unchanged in the third and fourth week of storage. The nitrate, soluble and total oxalates and vitamin C contents in the leaves of *Telfairia occidentalis* decreased significantly ($p<0.05$) in the first week of freezing and remained significantly the same in the subsequent second, third and fourth weeks of storage. Similarly, the concentrations of β-carotene and Na in the vegetable were significantly ($p<0.05$) reduced in the second weeks of storage and their values obtained in the third and fourth weeks also indicated reduction which was not significantly different from the second weeks of freezing. While freezing had no significant effect on the Cu content in *Telfairia occidentalis*, the concentrations of Mg and K decreased significantly ($p<0.05$) in the third weeks and changed insignificantly in the fourth weeks of freezing. The result concludes that short period of freezing (especially one week of freezing) significantly reduced the plant toxins in *Telfairia occidentalis* and safeguard most of the micronutrients.

Key words: *Telfairia occidentalis*, freezing duration, phytotoxins and micronutrients

INTRODUCTION

Telfairia occidentalis (Fluted pumpkin) is one of the leafy vegetables that are grown across the low land humid tropic in West Africa (Nigeria, Ghana and Sierra Leone being the major producers). Fluted pumpkin is a creeping vegetative shrub that spread low across the ground with large lobed leave and long twisting tendrils (Syndenham, 1985; Horsfall and Spiff, 2005; Agatemor, 2006; Ojiako and Igwe, 2008). *Telfairia occidentalis* prefers a loose, friable ample humus and shaded position. Nitrogen is vital for sufficient vegetation and should ideally be given in the form of manure (Schippers, 2000). Harvesting of fluted pumpkin takes place 120-150 days after planting (Horsfall and Spiff, 2005). The vegetable is of commercial importance in West African because of the high nutrient contents abound in it (Nkang *et al*., 2003).

The leaves and seeds are widely eaten as they are good sources of micronutrient such as mineral elements (Fe, Mg, Cu, Ca, Zn K, Na and P), vitamins (such as vitamin C, β-carotene), fibres, fats (Schippers, 2000; Nkang *et al*., 2003; Agatemor, 2006; Ogbadoyi *et al*., 2011; Musa *et al*., 2011; Musa and Ogbadoyi, 2012a). *Telfairia occidentalis* also accumulates various antinutrients and toxic substances such as oxalate, phytate, cyanide and nitrate. These plant secondary metabolites have been known to have deleterious effect on human and animals' health at high concentrations (Ogbadoyi *et al*., 2011; Musa *et al*., 2011; Musa and

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Ogbadoyi, 2012a). In order to maintain the quality of fresh foods and avert food spoilage, it is a common practice to store the fresh foods in refrigerator and freezer as this practice is known to reduce the activities of microorganisms and enzymes that are associated with biochemical reactions leading to deterioration of food substances. Thus this research was conducted to evaluate the influence of freezing duration on the concentrations of some plant toxins and micronutrients in the leaves of *Telfairia occidentalis* with a view to determine the suitability of the storage conditions for the vegetable.

MATERIALS AND METHODS

Source of *Telfairia occidentalis*: The fresh samples of *Telfairia occidentalis* was bought in three sets at different time from Maikunkele, Bosso and Chanchanga markets in Minna town, Nigeria.

Chemicals: Except otherwise stated, all the chemicals used were of analytical grade and were purchased from Sigma and BDH chemical companies, both of England.

Freezing: The leaves of *Telfairia occidentalis* 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 4 weeks. The concentrations of the nutrients, antinutrients and toxic substances were determined at weekly intervals over the four-weeks period.

Sample analysis: The nitrate concentration in the samples was determined by the colourimetric method as described by Sjoberg and Alanko (1994). While the alkaline picrate method of Ikediobi *et al.* (1980) was used to analyse the cyanide content in the leaves of *Telfairia occidentalis*. Both soluble and total oxalates in the samples were determined by titrimetric method of Oke (1966). The mineral elements (Fe, Cu, Mg, Na and K) in samples were determined according to the method of Ezeonu *et al.* (2002). The ascorbic acid concentration in the samples was determined by 2, 6-dichlorophenol indophenols method of Jones and Hughes (1983). While β-carotene concentration was determined by ethanol and petroleum ether extraction method as described by Musa *et al.* (2010).

Statistical analysis: Analysis of variance (ANOVA) was carried out using statistical package Minitab to determine variation between different freezing time on the concentration of nutrients, antinutrients and toxic substances in the leaves of *Telfairia occidentralis*. The Duncan's Multiple Range Test (DMRT) was used for comparison of means.

RESULTS

Cyanide concentration: Cyanide concentration was found to decrease with freezing in *Telfairia occidentalis*. Significant ($p<0.05$) reduction of cyanide content from 170.83-99.45 mg kg^{-1} was observed in the first week of freezing. Value obtained in the second weeks (84.51 mg kg^{-1}) also indicated significant reduction of cyanide content which was not significantly different from the mean values obtained for the third (70.66 mg kg^{-1}) and fourth weeks (69.04 mg kg^{-1}) (Fig. 1).

Nitrate concentration: Significant ($p<0.05$) decreased in nitrate content in *Telfairia occidentalis* from 2799.04 mg kg^{-1} in fresh sample to 1514.83 mg kg^{-1} was observed during two weeks of freezing. The mean values obtained for the second (1339.83 mg kg^{-1}), third (1072.22 mg kg^{-1}) and fourth weeks (1035.17 mg kg^{-1}) also indicated reduction in concentration of nitrate which was not significantly ($p>0.05$) different from the value obtained from the first week (Fig. 2).

Soluble oxalate concentration: The soluble oxalate concentration in *Telfairia occidentalis* also decreased with freezing storage. Significant reduction ($p<0.05$) in the parameter from 2.85 g kg^{-1} in fresh sample to 2.40 g kg^{-1} was recorded in one week of freezing. The mean values obtained in second weeks (2.29 g kg^{-1}), third weeks (2.07 g kg^{-1}) and fourth weeks (1.84 g kg^{-1}) frozen leaves of *Telfairia occidentalis* were significantly the same with that of the first week (Fig. 3).

Total oxalate concentration: Analysis of total oxalate content in fresh samples of *Telfairia occidentalis* showed that the oxalate concentration decreased significantly

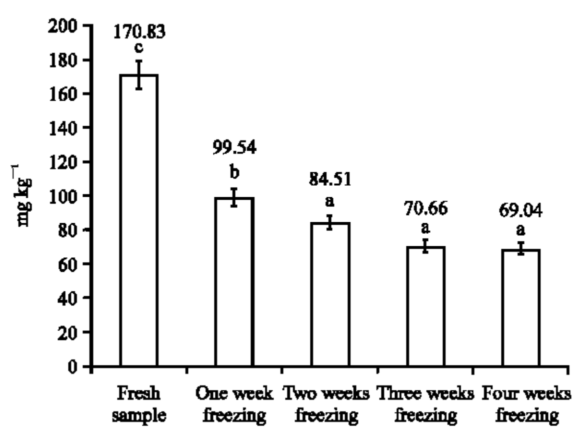

Fig. 1: Effect of freezing on cyanide content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different ($p>0.05$)

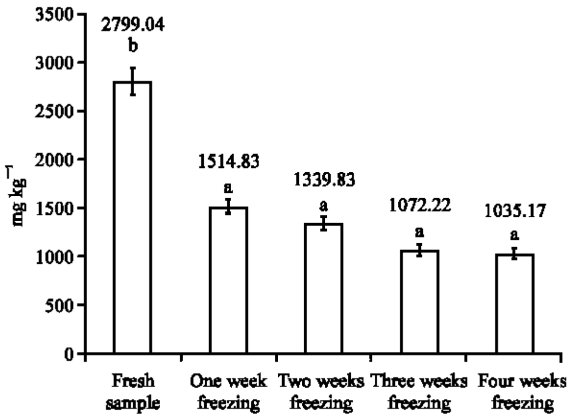

Fig. 2: Effect of freezing on nitrate content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

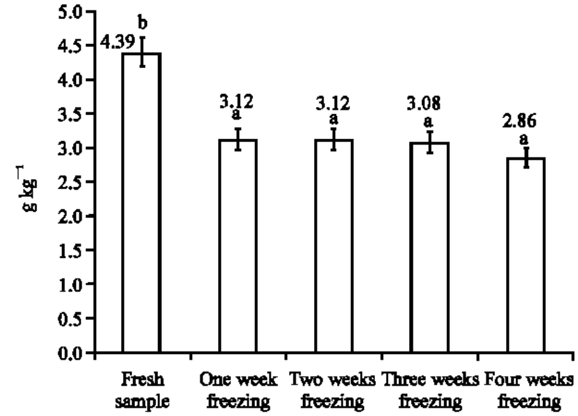

Fig. 4: Effect of freezing on total oxalate content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

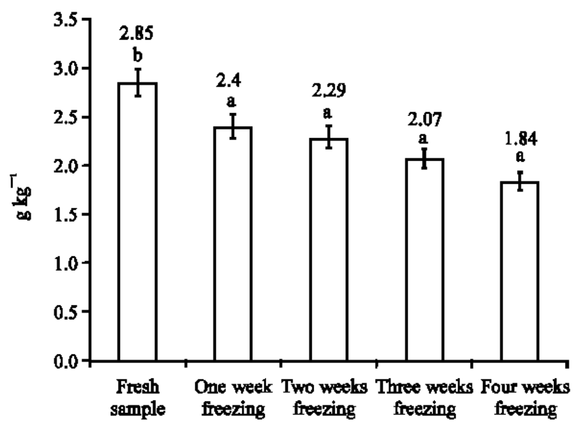

Fig. 3: Effect of freezing on soluble oxalate content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

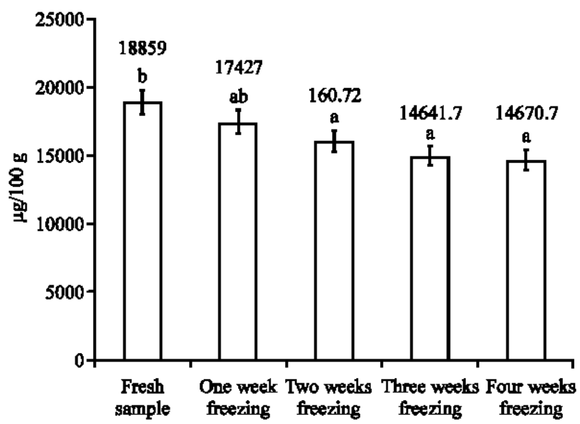

Fig. 5: Effect of freezing β-carotene content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

($p<0.05$) in one week of cold storage from 4.39 to 3.12 g kg^{-1}. In the subsequent second, third and fourth weeks, the oxalate content remained significantly unchanged and the values obtained were 3.12, 3.0 8 and 2.86 g kg^{-1}, respectively (Fig. 4).

β-carotene concentration: The β-carotene content in the fresh sample (18859.00 µg/100 g) of *Telfairia occidentalis* decreased significantly ($p<0.05$) in the second weeks of freezing to 16072.00 µg/100 g. The decreased in the first week (17427.00 µg/100 g) was however, not significant. The values obtained for the third (14941.70 µg/100 g) and fourth weeks (14690 µg/100 g) also indicated reduction in the concentration of β-carotene which was not significantly different from those of the first and second weeks (Fig. 5).

Vitamin C concentration: The concentration of vitamin C in fresh sample of *Telfairia occidentalis* decreased significantly ($p<0.05$) during one week of freezing from 192.28 to 19.15 mg/100 g. The concentrations obtained in the second, third and fourth weeks of freezing also indicate decreased in the vitamin content which were insignificantly different from the first week and the values obtained were 14.24, 12.60 and 11.50 mg/100 g, respectively (Fig. 6).

Iron concentration: The results obtained from the analysis of Fe content in *Telfairia occidentalis* showed that it's decreased significantly ($p<0.05$) from 23.43 mg kg^{-1} in fresh sample to 19.55 mg kg^{-1} in one week of freezing. The mean value obtained in the second weeks (16.41 mg kg^{-1}) was significantly the same with

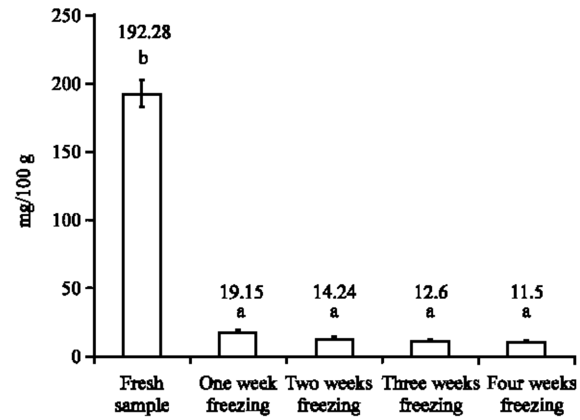

Fig. 6: Effect of freezing on vitamin C content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

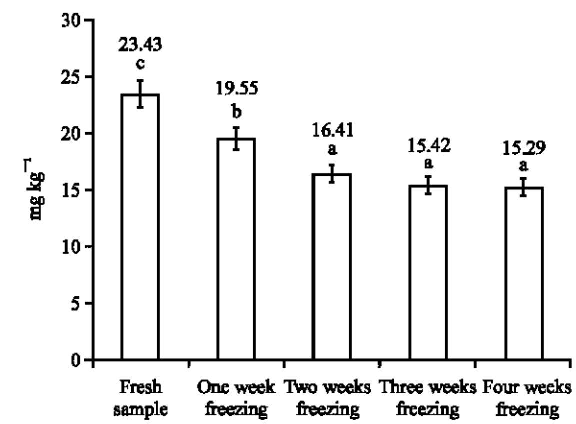

Fig. 7: Effect of freezing on iron content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

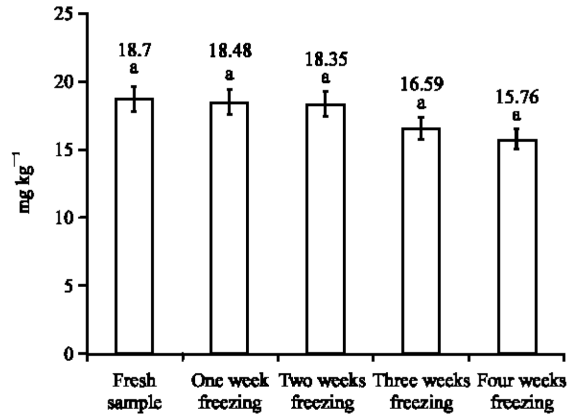

Fig. 8: Effect of freezing on copper content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

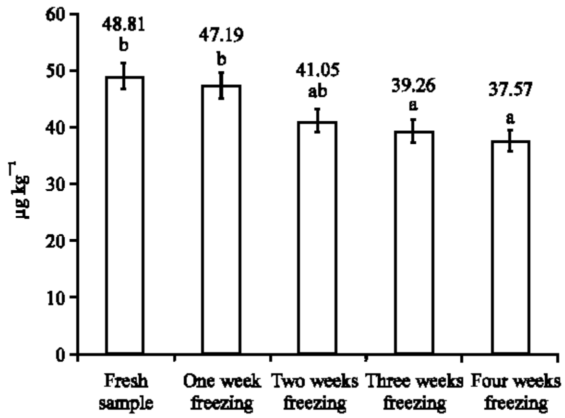

Fig. 9: Effect of freezing on magnesium content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different (p>0.05)

the third weeks (15.42 mg kg^{-1}) and fourth weeks (15.29 mg kg^{-1}) of freezing, however, these values were significantly ($p<0.05$) lower than the first weeks of freezing (Fig. 7).

Copper concentration: Freezing had no significant effect on Cu concentration in *Telfairia occidentalis* during the storage period. The mean values obtained in fresh and those frozen samples for one, two, three and four weeks were 18.70, 18.48, 18.35, 16.59 and 15.7 6 mg kg^{-1}, respectively (Fig. 8).

Magnesium concentration: The Mg concentration in *Telfairia occidentalis* also decreased with freezing duration. Significant reduction ($p<0.05$) in the mineral concentration from 48.81 mg kg^{-1} in fresh sample to 39.26 mg kg^{-1} was observed in the third weeks of storage in deep freezer. The mean value obtained in the fourth weeks (37.57 mg kg^{-1}) indicated reduction in Mg content which was not significantly different from second weeks (41.05 mg kg^{-1}). The mineral concentration in first week (47.19 mg kg^{-1}) was however significantly higher compared to those in the third and fourth weeks (Fig. 9).

Sodium concentration: Significant reduction ($p<0.05$) of Na concentration in *Telfairia occidentalis* during freezing from 11.48 mg kg^{-1} in fresh sample to 8.76 mg kg^{-1} was observed in second weeks of storage. The decreased in the first week (9.86 mg kg^{-1}) was however, not significant. The mean values obtained for the third (8.48 mg kg^{-1}) and fourth (8.10 mg kg^{-1}) weeks also

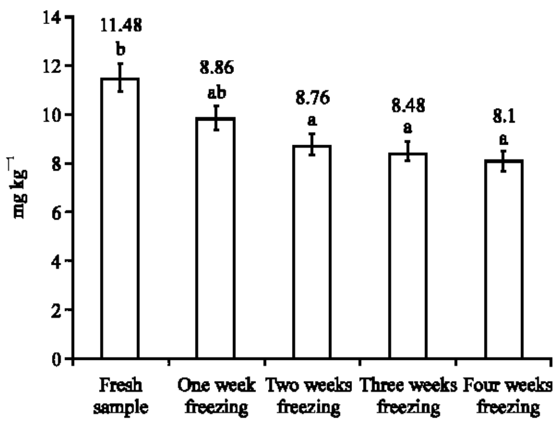

Fig. 10: Effect of freezing on sodium content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different ($p>0.05$)

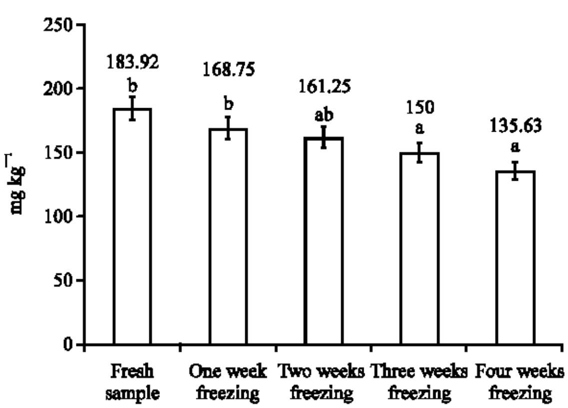

Fig. 11: Effect of freezing on potassium content in *Telfairia occidentalis*. Bars carrying the same letter are not significantly different ($p>0.05$)

indicated reduction in concentration which was not significantly different from those of the first and second weeks (Fig. 10).

Potassium concentration: The concentration of K in *Telfairia occidentalis* also decreases with freezing time. Significant decreased ($p<0.05$) in the mineral concentration from 183.92 mg kg^{-1} in fresh sample to 150.00 mg kg^{-1} was recorded in three weeks of storage in the deep freezer. The mean value obtained in the fourth weeks (135.63 mg kg^{-1}) indicated reduction in mineral content which was not significantly different from second weeks (161.25 mg kg^{-1}). The K content in the first week (168.75 mg kg^{-1}) of storage was however, significantly higher ($p<0.05$) than those in the third and fourth weeks (Fig. 11).

DISCUSSION

The decreasing effect of freezing on cyanide content in *Telfairia occidentalis* agreed with the reported of Richard (1991) and McDonald *et al.* (2006) to the effect that freezing ruptures the plant cells which resulted in the release of cell contents including cyanide. The solubility of cyanide in water was also responsible for the decreased of its concentration during freezing (Budavari *et al.*, 1989). Thus cyanide may be trapped in the ice and released during thawing. The cyanide concentration of 170.83 mg kg^{-1} in the fresh sample of *Telfairia occidentalis* is very close to the maximum permissible level of 200 mg kg^{-1} of cyanide in fresh weight (Everist, 1981; Richard, 1991) were reduced more than half of this value after one and subsequent weeks of freezing. The result obtained indicates that the cyanide content in the vegetable could be reduced to safe level by freezing.

The results in this study which show that there was a lower concentration of nitrate in frozen samples than in the fresh sample of *Telfairia occidentalis* is in line with the findings of Bakr and Gawish (1997), who showed that freezing reduced the nitrate content in the vegetables. The authors ascribed the decrease to the leaching of the cell content caused by freezing and defrosting. The general weekly decline of nitrate content in the vegetable during freezing is also in agreement with the submission of Bakr and Gawish (1997), who reported that losses of nitrate during freezing storage increase with freezing time. The nitrate concentration in fresh sample of *Telfairia occidentalis*, was higher than the Acceptable Daily Intake of 220 mg (Macrae *et al.*, 1997) and 219 mg (Anjana *et al.*, 2007) for a 60 kg person (if 100 g day^{-1} is consumed). Thus consumption of raw samples of the vegetable could lead to nitrate overload and could subject the body to disease conditions associated with nitrate toxicities such as metheamoglobinaemia and cancer (Galler, 1997; Macrae *et al.*, 1997; Mevissen, 1997; Mozolewski and Smoczynski, 2004; Anjana *et al.*, 2007). However, one week of freezing is required to reduce the nitrate concentration in *Telfairia occidentalis* to the recommended Acceptable Daily Intake.

The decreased in soluble and total oxalates concentration in the frozen leaves compared to fresh leaves of *Telfairia occidentalis* recorded in this work corroborates with the report of Ogbadoyi *et al.* (2006). 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 shredding the cell membranes resulting in cell leakage. This finding is in conformity with the report of Richard (1991), Fellow (2000) and McDonald *et al.*

(2006) that freezing imparts physical injury to the cell, alters the pigments which are sources of bioactive The soluble and total oxalates concentration in fresh leaves of *Telfairia occidentalis* are more than the permissible level of 250 mg kg^{-1} fresh weight as reported by Oguchi *et al.* (1996). Thus regular consumption of fresh leaves of the vegetable can cause oxalate toxicosis with attendant health problems of kidney stone, reduction of bioavailability of elements, electrolyte imbalance and irritation of digestive system (Antia *et al*., 2006; Prohp *et al*., 2006; Musa *et al*., 2011; Ogbadoyi *et al*., 2011; Musa and Ogbadoyi, 2012a, b).Fortunately one and two weeks of freezing reduced the soluble and total oxalates content in *Telfairia occidentalis* to tolerable level, respectively.

The reduction of β-carotene content in *Telfairia occidentalis* during freezing has been attributed to the physical injury and alteration of the plant pigments (Fellow, 2000; McDonald *et al*., 2006). Booth *et al.* (1992) stated that the observed decrease in the β-carotene concentration in the vegetable during storage in the freezer may be as a result of enzymatic activity coupled with oxidation associated with conjugated double bond in the compound. Dutta *et al.* (2005) observed that the loss of β-carotene in frozen vegetables could be due to non-oxidative changes (cis-trans isomerisation, epoxide formation) or oxidative changes on exposure light and oxygen. The insignificant difference recorded in the β-carotene concentration in second to fourth weeks of cold storage could be seen as a result of decrease in endogenous enzymatic activity and reduction of oxidation of the provitamin A as freezing storage progresses. The β-carotene concentration in the fresh and frozen leaves of *Telfairia occidentalis* contained over and above the recommended adult daily allowance of 900 µg of vitamin A (George, 1999; Akanya, 2004). The implication of this is that because β-carotene is abundant in *Telfairia occidentalis* and non-hydrosoluble, its residual concentration in the frozen samples are high enough to meet the normal adult recommended daily allowance. The results obtained therefore imply that freezing the leaves of *Telfairia occidentalis* within the studied periods may not require any pharmaceutical supplements of β-carotene.

The significant higher vitamin C concentration in the fresh sample of *Telfairia occidentalis* than its frozen leaves is in agreement with findings of Olaofe (1992), Yadav and Sehgal (1995), Yadav and Sehgal (1997), Lisiewska and Kmiecik (2000), Ejoh *et al.* (2005), Bergquist *et al.* (2006), McDonald *et al.* (2006) and Gebczynski (2006). The observation made in this study and those of the above researchers contradicts the observations of Mathew and Hall (1987), who found that total vitamin C concentration remained relatively constant throughout the frozen storage period for all samples (pepper, strawberries, green beans, etc). Olaofe (1992) attributed the reduction of the vitamin concentration to be 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 Bergquist *et al.* (2006) that during freezing of vegetables the ascorbic acid concentration decreased considerably and the dehydroascorbic acid/vitamin C ratio increased. This finding could suggest that during cold storage, ascorbic acid may undergo oxidation to dehydroascorbic acid by some of the endogenous enzymes. Tosun and Yucecan (2007) added that the chemical, biochemical and physical reactions that occurred in vegetables after harvesting do not completely stop during frozen storage and this may lead to the oxidation of susceptible molecules like ascorbic acid (vitamin C) while McDonald *et al.* (2006) stressed that the observed decrease in the vitamin C content during freezing is as a result of thawing. The insignificant difference in the vitamin C content in first to fourth week of storage may be that during the first week of freezing, the physical injury and alteration of pigments reported by Fellow (2000) and McDonald *et al.* (2006) and the enzymatic oxidation of ascorbic acid to dehydroascorbic acid (Olaofe, 1992; Bergquist *et al*., 2006) may be at the peak within one week of storage. However, these processes might have declined considerably as freezing storage progresses. The fresh leaves of *Telfairia occidentalis* could supply enough of vitamin C to meet the recommended daily allowance of 60 mg (Olaofe, 1992; George, 1999) if 100 g of the samples are consumed. However, *Telfairia occidentalis* contained some plant toxins that could be reduced to tolerable level through freezing. Although, the residual vitamin C concentration in the frozen leaves of the vegetable was lower than the recommended daily allowance. Since vitamin C play important roles in human health and diseases associated from its deficiency, pharmaceutical supplementation of the vitamin will be necessary to augment its losses during freezing storage. This will enable the body to meet the dietary requirement of this water soluble vitamin.

The reduction in mineral element (Fe, Cu, Mg, Na and K) concentrations in *Telfairia occidentalis* with freezing time in the current study may be as results of infliction of physical injuries to the cell wall of the leaves of the vegetable by ice crystals that were formed during freezing which eventually leads to cell leakage (Polo *et al*., 1992; Pruthi, 1999; Fellow (2000); Hui *et al*., 2004;

McDonald *et al.* (2006). "Freezer burn" which also occurs during cold storage of vegetables and other soft plant materials at the temperature of -4°C is known to decrease the bioactive compounds including the mineral elements. The significant reduction in the mineral elements content in some of the vegetables during freezing agrees with the report of Hui *et al.* (2004), who reported that significant amount of macro and micro mineral elements concentration in vegetables are lost following freezing. Similarly the insignificant effect of freezing on Cu concentration in the vegetable throughout the freezing duration also support the finding of Polo *et al.* (1992) and Hui *et al.* (2004). The authors stressed that, even though there was a decreased in the mineral element in vegetables during freezing, it is not significant. These two factions of the results obtained may suggest that the retention of mineral elements in frozen samples or reductions 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 (Hui *et al.*, 2004). It is believed that mineral elements that exist as free ions can easily be leached out during freezing when compared with those that are chemically bond or form complexes with other compounds.

CONCLUSION

The results of this present study revealed that freezing the leaves of *Telfairia occidentalis* at -4°C in a deep freezer for one week significantly reduced the concentrations of the plant toxins to tolerable levels and safeguard the micronutrients content in the vegetable. This finding thus imply that short time freezing do not only maintaining the quality of fresh vegetable, but it also reduces the phytotoxins content in the vegetable and thereby reduce the incidence of disease conditions associated with ingestion of high concentration of the phytotoxins.

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