Nyerhovwo *et al. (2015).* Effect of preservation on the chlorophyll content, phytochemicals, and antioxidant capacity of two different *varieties of pumpkin (Telfairia occidentalis) leaves. Vol 10 no. 1:9-16.*

Effect of preservation on the chlorophyll content, phytochemicals, and antioxidant capacity of two different varieties of pumpkin (*Telfairia occidentalis*) leaves

Nyerhovwo J. Tonukari*, Oghenetega J. Avwioroko, and Akpovwehwee A. Anigboro. Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Nigeria.

Abstract

The aim of the present study was to investigate the effect of two preservation techniques (refrigeration and freezing) on the chlorophyll content, phytochemicals, and antioxidant capacity of two different varieties of pumpkin (Telfairia occidentalis) leaves over a period of two weeks. The biochemical parameters assessed include chlorophyll content, total soluble protein, reducing sugar, total phenolics, total flavonoids and ferric reducing antioxidant power (FRAP). The results of the study show that in both broad and slender leaves pumpkin varieties investigated, refrigeration and freezing for two weeks led to a significant decrease ($p<0.05$) in their chlorophyll contents. Total chlorophyll a and b content in the preserved broad leaf pumpkin variety (BLP) decreased by an average of 49.12%, whereas the average percentage decrease in total chlorophyll a and b observed in the preserved slender leaf variety (SLP) was 79.01% compared to their respective fresh leaves controls. Refrigeration and freezing for two weeks also resulted in a significant decrease ($p<0.05$) in total soluble protein and reducing sugar contents of both broad leaf (BLP) and slender leaf (SLP) varieties of pumpkin. However, the preservative methods did not significantly ($p>0.05$) affect the level of phenolics in the broad leaf pumpkin variety, although it was significantly decreased in the slender leaf pumpkin variety preserved by refrigeration. There was no significant difference ($p>0.05$) in flavonoid contents among the refrigerated, frozen and fresh leaves of the broad pumpkin leaf variety (BLP). However, the preserved slender pumpkin leaves variety (SLP) again suffered a significant decrease. Though the ferric reducing antioxidant power (FRAP) of broad leaf pumpkin variety (BLP) preserved by refrigeration (4°C) and freezing (-20°C) were slightly decreased by 20.9% and 16.1%, respectively, compared to their fresh broad leaf control, the decrease was not significant ($p>0.05$). On the contrary, the FRAP of the preserved slender leaf pumpkin variety (SLP) was significantly decreased ($p<0.05$) relative to their fresh slender leaf control. It could be inferred from the findings of this research that the broad leaf pumpkin variety (BLP) can be preserved by freezing (temperature -20°C) for a period of two weeks without any significant ($p>0.05$) loss of its valuable phytochemicals and antioxidant capacity compared to the slender leaf pumpkin variety.

Key words: Pumpkin (*Telfairia occidentalis*) leaves, preservation, freezing, refrigeration, chlorophyll, phenols, flavonoids, free radicals scavenging activity.
E-mail: tonukari@gmail.com
Received: 2014/06/24
Accepted: 2015/03/20
DOI: http://dx.doi.org/10.4314/njtr.v10i1.3

Introduction

Food preservation usually involves preventing the growth of bacteria, fungi (such as yeasts), or any other micro-organisms (although some methods work by introducing benign bacteria or fungi to the food), as well as retarding the oxidation of fats that cause rancidity (Abdulmumeen *et. al.*, 2012). It can also include processes that inhibit visual deterioration, such as the enzymatic browning reaction in apples after they are cut, which can occur during food preparation (Lee, 2004).

The use of pumpkin (*Telfairia occidentalis*) leaves as vegetables, in preparation of many African dishes has been on the increase since the last three decades. This may probably be due to its rich contents of vitamin K, vitamin Bs, minerals as well as its high contents of phenolic compounds, flavonoids, protein and low sugar levels (Onoja and Nnam, 2014; Gupta *et.. al.*, 1986; González *et. al.*, 2014).

Pumpkin leaves are commonly known as fluted gourd, fluted pumpkin and Ugu in Nigeria and as 'Eberu me' among the Urhobo tribe in Delta State, Nigeria; belong to the genus Telfairia and family Cucurbitaceae of the plant kingdom. They are frequently used in cooking of egusi (melon) vegetable soup and in making of vegetable sauces in the Niger-Delta region of Nigeria (Tonukari *et. al.*, 2013).

The leaf is of high nutritional, medicinal and industrial values rich in protein (29%), fat (18%) and minerals and vitamins (4%) (Akanbi *et al.*, 2006). The leaf extract of pumpkin alone is useful in the management of hypercholesterolaemia, liver problems and impaired immune system (Eseyin *et. al.*, 2005; Adaramoye, 2007) but the oil from seed could result to hyperlipidermia and hyperglycaemia if consumed excessively. Protein Energy Malnutrition (PEM) is rarely seen among the dwellers where *Telfairia* is consumed in large proportion daily (Dike, 2010).

In Nigeria the fresh leaves are ground and the juice used as tonic by women that have just

given birth; its high iron content assists in the replenishment of lost blood; being used for treatment of anaemia, chronic fatigue and diabetes (Alada, 2010; Dina *et. al*., 2006; Aderibigbe *et. al.*, 1999). The blood schizontocidal activity of the root of *Telfairia* is comparable to that of chloroquine (Okokon *et. al*., 2007). The extract also shows inhibitory effect on growth of some bacteria (Oboh *et. al.*, 2006; Oluwole *et. al.*, 2003). In spite of the frequent use of pumpkin leaves (*T. occidentalis*) in food preparations both in rural and urban areas as well as during ceremonies, this vegetable has always been utilized in its fresh state.

Freezing is a common process used for both commercial and domestic preservation of very wide range of foods (Li and Sun, 2002). There are various traditional methods of food preservation like salting, drying, refrigeration, freezing, smoking, sugaring, pickling and blanching (Petzold *et al*., 2014; Lee, 2004). Though, similar in mechanism to refrigeration, but differs by exerting extreme coldness on the food samples stored by it (Petzold *et. al.*, 2014).

In this study, we investigated the effect of two preservation techniques (refrigeration and freezing) on the chlorophyll content, phytochemicals, and antioxidant capacity of two different varieties of pumpkin (*T. occidentalis*) leaves over a period of two weeks.

Materials and Methods

Experimental design

Broad and slender pumpkin leaves' varieties (Figure 1) were collected from HarmonyPath Farms in Otorho-Agbon, Delta State, Nigeria and put in well labeled polythene containers (two for each variety). The samples were then preserved in a refrigerator (4°C) and freezer (-20°C) for 2 weeks before leaf extraction. Fresh leaves (broad and slender) were collected from the same pumpkin plants on the day of extraction to serve as control for the preserved ones.

Figure 1. Two varieties of pumpkin (*Telfairia occidentalis*) leaves. BLP denotes broad leaf pumpkin (BLP) and SLP, slender leaf pumpkin.

Aqueous leaf extraction

0.5 g of sliced broad and slender pumpkin leaf varieties were weighed with an electronic balance (SPEC 21, England) and homogenized in a cold medium using 0.1 M phosphate buffer (pH 7.2) and the final volumes made to 50 ml with same buffer making a 1%w/v of the leaf extracts. The homogenates were filtered with a sieve cloth and the filtrate further centrifuged at 3000g for 10 min. The later filtrates were used for subsequent analyses as aqueous leaf extracts of both pumpkin leaf varieties (carefully labeled).

Estimation of chlorophyll content

Chlorophyll content of the leaves was determined according to the method described by Arnon (1949) and Reezi *et. al*. (2009) with some modifications. About 500 mg of leaf samples were weighed and ground with 50 ml of distilled water in a pestle and mortar. It was centrifuged at 4000 rpm for 10 min till a clear supernatant was obtained. Absorption was measured, using a spectrophotometer at 645 and 663 nm and chlorophyll content (mg/g fw) was calculated using the equation: chlorophyll a + b = $8.02(A_{663}) + 20.21(A_{645})$. Three replicates were made of each treatment.

Estimation of total protein content

Protein concentration of the pumpkin leaf aqueous extracts was determined by means of the Biuret method as described by Soliman *et. al*. (2013) with some modifications. One milliliter (1 ml) of the diluted sample was taken and added to 3 ml of Biuret reagent. The mixture was incubated at room temperature for 30 min after which the absorbance was read at a wavelength of 540 nm using distilled water as blank. A solution of bovine serum albumin (10mglmi) was used as standard protein and

subjected to the assay conditions as described. Thereafter, protein contents of the extract was calculated as follows: Protein content (mg/ml) = [Asample/ Astandard] x concentration of standard.

Estimation of total reducing sugar content

The reducing sugar content of each pumpkin leaf extract was determined according to the method of Miller (1959) with some modifications. Three milliliters (3 ml) of DNS reagent was added to 3 ml of pumpkin leaf extract and then, test tubes were tightly caped to avoid loss of liquid due to evaporation. Test tubes contents were heated at 90°C for 5 -15 min to develop a red/brown colour. One milliliter (1 ml) of a 40% potassium sodium tartrate (Rochelle salt) was added to stabilize the colour. After cooling to room temperature in a cold water bath, absorbance was read in a spectrophotometer at 575 nm wavelength against reagent blank.

Estimation of total phenol content

This was carried out according to the method described by Singleton and Rossi (1965). One milliliter (1 ml) of Folin C reagent was added to 1ml of the sample. After 3 min, 1 ml of saturated Na_2CO_3 solution was added and the solution was made up to 10ml with distilled water. The reaction mixture was kept in the dark for 90 min before reading the absorbance at 725 nm. Ascorbic acid (AA) was used as the standard.

Estimation of total flavonoid content

The flavonoid content was determined by colorimetry using a method described by Jia *et. al.* (1999). Two hundred and fifty microliter (250 µl) of the extract was added to 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$. After 5 min 150 µl of 10% $AlCl_3.H_2O$ was added, followed by 500 µl of 1M NaOH and 275 µl of distilled water after 6 min. The solution was properly mixed and the colour intensity of the mixture was read at 510nm. Ascorbic Acid (AA) was used as the standard and the flavonoid content expressed in milligram ascorbic equivalent per milliliter of extract (mgAA eq/ml).

Ferric reducing antioxidant power (FRAP) assay

This was estimated using the method of Oyaizu (1986). 2.5ml of 200 mM of phosphate buffer (pH 6.6) and 2.5ml of 1% K_3FeCN were added to various concentrations of the extract. The mixture was incubated for 2 min at 50°C and then centrifuged at 1000 g for 8 min. Five milliliter (5 ml) of the supernatant was then mixed with 5 ml of distilled water and 1 ml of 0.1% $FeCl_3$. The absorbance of the mixture was measured at a wavelength of 700 nm. Ascorbic acid (AA) was used as the standard.

Results

Chlorophyll content

Chlorophyll contents of two varieties of pumpkin leaves preserved by refrigeration and freezing for two weeks are as shown in Figure 2. The total chlorophyll (a+b) content of fresh broad pumpkin leaves (BLP) was 87.688 mg/g FW compared to 55.593 and 44.585 mg/g of the same leaf preserved by refrigeration (4°C) and freezing (-20°C), respectively. Also, the fresh slender pumpkin leaves (SLP) had 143.421 mg/FW total chlorophyll (a+b) compared to 26.721 and 30.141 mg/g total chlorophyll of the same leaf preserved by refrigeration and freezing, respectively. The average percentage decrease in total chlorophyll (a+b) content (49.12%) in the preserved broad leaf pumpkin variety (BLP) is significantly lower than the percentage decrease in total chlorophyll a and b (79.01%) observed in the preserved slender leaf variety (SLP) compared to their respective fresh leaves controls.

Total protein and reducing sugar content

The results of preservation on the total soluble protein and reducing sugar of the two varieties of pumpkin studied are shown in Figures 3 and 4, respectively. Total protein content of fresh broad pumpkin leaves (BLP) aqueous extract was 96.32 mg/ml compared to 13.079 and 9.748 mg/g of the same leaf preserved by refrigeration (4°C) and freezing (-20°C), respectively; that of the fresh slender pumpkin leaves (SLP) aqueous extract was 29.448 mg/ml compared to 6.986 and 5.402 mg/ml protein of the same leaf preserved by refrigeration and freezing, respectively (Figure 3). Refrigeration and freezing for two weeks resulted in a significant decrease ($p<0.05$) in total soluble protein content of both broad leaf (BLP) and slender leaf (SLP) varieties of pumpkin compared with their respective fresh leaf varieties (Figure 3). A similar trend was

observed in their reducing sugar contents as revealed by Figure 4.

Total phenol content

Figure 5 shows the effect of preservation by refrigeration and freezing on the phenolic content of the broad (BLP) and slender (SLP) leaf varieties of pumpkin. Total phenolic content of fresh broad pumpkin leaves (BLP) aqueous extract was 7.685 mg AAEq/ml compared to 5.496 and 6.74 mg AAEq/g of the same leaf preserved by refrigeration (4°C) and freezing (-20°C), respectively; that of the fresh slender pumpkin leaves (SLP) aqueous extract was 10.252 mg AAEq/ml compared to 3.984 and 6.598 mg AAEq/ml of the same leaf preserved by refrigeration and freezing, respectively (Figure 5). The preservative methods did not significantly ($p>0.05$) affect the level of phenolics in the broad leaf pumpkin variety. A similar result was obtained for slender leaf pumpkin variety preserved by freezing (Figure 5). However, a significant decrease in total phenol was observed in the slender leaf pumpkin variety preserved by refrigeration.

Total flavonoid content

The results of the effect of the preservation techniques on the flavonoid contents of the pumpkin leaves are as shown in Figure 6. The flavonoid content of fresh broad pumpkin leaves (BLP) aqueous extract was 8.266 mg AAEq/ml compared to 5.424 and 7.557 mg AAEq/g of the same leaf preserved by refrigeration (4°C) and freezing (-20°C), respectively; that of the fresh slender pumpkin leaves (SLP) aqueous extract was 23.026 mg AAEq/ml compared to 3.579 and 6.015 mg AAEq/ml of the same leaf preserved by refrigeration and freezing, respectively (Figure 6). There was no significant difference ($p>0.05$) in flavonoid among the refrigerated, frozen and fresh leaves of the broad pumpkin leaf variety (BLP). However, the preserved slender pumpkin leaves variety (SLP) again suffered a significant decrease ($p<0.05$) in flavonoid content compared to their fresh leaf control (Figure 6).

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) of the fresh and preserved pumpkin leaf varieties are as shown in Figure 7. The FRAP of fresh broad pumpkin leaves (BLP) aqueous extract was 0.62 compared to 0.497 and 0.523 in the same leaf variety preserved by refrigeration (4°C) and freezing (-20°C), respectively; that of the fresh slender pumpkin leaves (SLP) aqueous extract was 0.327 compared to 0.021 and 0.095 in the same leaf preserved by refrigeration and freezing, respectively (Figure 7). Also, 10 mg/ml of standard Ascorbic acid solution expressed a FRAP of 0.539. Although the ferric reducing antioxidant power of broad leaf pumpkin variety (BLP) preserved by refrigeration (4°C) and freezing (-20°C) decreased by 20.9 and 16.1%, respectively, compared to their fresh broad leaf control, the decrease was not significant ($p>0.05$). Also, the FRAP of the preserved broad leaf pumpkin variety compared favourably ($p>0.05$) with that of the standard antioxidant (10 mg/ml ascorbic acid) used as a positive control. On the contrary, the ferric reducing antioxidant power of the preserved slender leaf pumpkin variety (SLP) significantly decreased ($p<0.05$) relative to their fresh slender leaf control.

Discussion.

Leaf relative chlorophyll content has been reported as an indicator parameter to predict nitrogen content of plant leaves (Schlemmer *et. al.*, 2005) which, in turn, is used in estimating the crude protein content or level of other nitrogen-based organic compounds found in green leaves (Sara *et. al*., 2013). In this study, the chlorophyll contents of both fresh and preserved leaves of the two pumpkin varieties were determined. The results of the study show that in both broad and slender leaves pumpkin varieties investigated, refrigeration and freezing for two weeks led to a significant decrease ($p<0.05$) in their chlorophyll contents (Figure 2). The average percentage decrease in total chlorophyll a and b content (49.12%) in the preserved broad leaf pumpkin variety (BLP) is significantly lower than the percentage decrease in total chlorophyll a and b (79.01%) observed in the preserved slender leaf variety (SLP) compared to their respective fresh leaves controls. According to Schlemmer *et al.* (2005), a loss in chlorophyll content of the leaves may be due to a loss in nitrogen content of the leaves as a result of metabolic protein degradation, or other spoilage-related microbial activities not fully arrested by the applied preservation technique. Hence, the results may be an indication that less chlorophyll (and possibly

less of other nutritive and functional constituents of the vegetable) is retained in the slender leaf pumpkin (SLP) after the period of preservation than in the broad pumpkin leaf variety (BLP). Also, the decrease in chlorophyll contents of the vegetables suggests that the preservation methods used (refrigeration and freezing) were not able to completely curb the activity of microorganisms (microflora) on the pumpkin leaves and that was probably why chlorophyll was partly degraded or metabolized even during the preservation period.

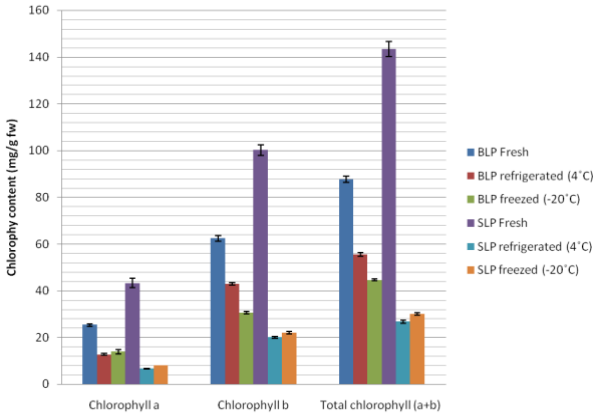

Figure 2. Effect of preservation on chlorophyll a and b contents (mg/g fw) of two varieties of pumpkin leaves. BLP denotes pumpkin leaves variety 1 (broad leaf) and SLP, pumpkin leaves variety 2 (small leaf); both preserved for two weeks at -20°C and 4°C.

The results of the effect of preservation on the total soluble protein and reducing sugar of the two varieties of the pumpkin studied are respectively shown in Figures 3 and 4. Refrigeration and freezing for two weeks resulted in a significant decrease ($p<0.05$) in total soluble protein and reducing sugar contents of both broad leaf (BLP) and slender leaf (SLP) varieties of pumpkin. This presupposes, as inferred by Wu *et. al.* (2013), that the observed decrease in the protein and reducing sugar contents of the preserved pumpkin leaves may eventually lead to a reduction in their overall nutritional value (Wu *et. al*., 2013). However, since Nigerian diets or soups are usually prepared with other rich sources of proteins like meat, chicken, and or fish, and also usually eaten with staple carbohydrates like garri, Akpu and or yam (*Dioscorea rotundata*) (Tonukari *et. al*., 2013), the overall effect of the decreased protein and reducing sugar contents of the preserved pumpkin leaves of both varieties will be highly negligible in diets.

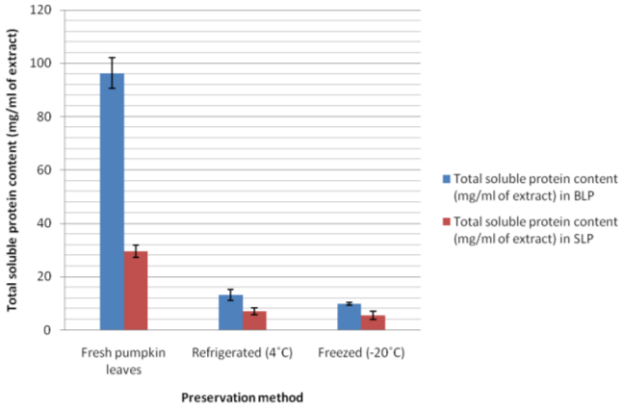

Figure 3. Effect of preservation on the total soluble protein content (mg/ml) of two varieties of pumpkin leaves. BLP denotes pumpkin leaves variety 1 (broad leaf) and SLP, pumpkin leaves variety 2 (small leaf); both preserved for two weeks at -20°C and 4°C.

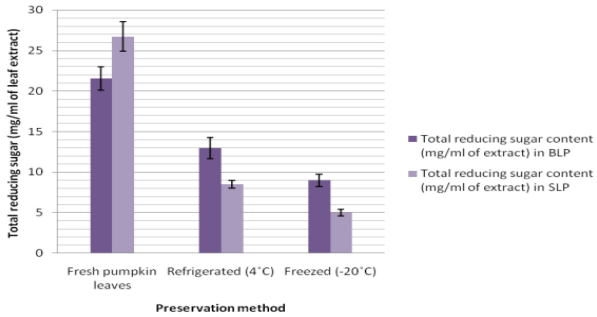

Figure 4. Effect of preservation on total reducing sugar content (mg/ml) of two varieties of pumpkin leaves. BLP denotes pumpkin leaves variety 1 (broad leaf) and SLP, pumpkin leaves variety 2 (small leaf); both preserved for two weeks at -20°C and 4°C.

Research on the effects of dietary polyphenols on human health has developed considerably in the past 10 years (Scalbert *et. al.*, 2005a). Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular diseases, cancers, and osteoporosis; and also suggests a role in the prevention of neurodegenerative diseases and diabetes mellitus (González *et al*., 2014; Scalbert *et. al*., 2005b). Amidst other possible mechanisms, the polyphenols have so far been implicated in chemoprevention of such diseases through the modulation of oxidative stress by their antioxidant properties. Figure 5 shows the results of the effect of preservation by refrigeration and freezing on the phenolic content of the broad (BLP) and slender (SLP) leaf pumpkin varieties. The preservative methods did not significantly ($p>0.05$) affect the level of phenolics in the broad leaf pumpkin variety. This implies that the broad leaf pumpkin variety (BLP) can be safely preserved for a period of two weeks by

freezing without any significant detriment to its phenolic content. A similar result was obtained for slender leaf pumpkin variety preserved by freezing (Figure 5). However, a significant decrease in total phenol was observed in the slender leaf pumpkin variety preserved by refrigeration. These observations suggest that preservation of pumpkin leaves by freezing is better than by refrigeration since a good number of microbial activities in the vegetables would be highly arrested by freezing than by refrigeration thus preventing drastic loss of polyphenols as well as antioxidant capacity of the pumpkin leaves preserved by freezing (Haiying *et al*., 2007).

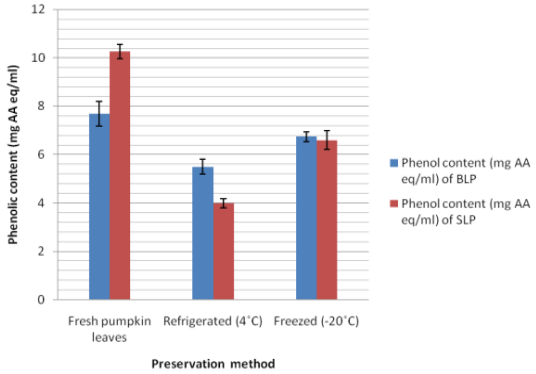

Figure 5. Effect of preservation on total phenol content (mg AA eq/ml) of two varieties of pumpkin leaves. BLP denotes pumpkin leaves variety 1 (broad leaf) and SLP, pumpkin leaves variety 2 (small leaf); both preserved for two weeks at -20°C and 4°C. mg AA eq/ml = mg Ascorbic acid equivalent/ml of extract.

Synonymous to the observed results of total phenols in the preserved and unpreserved pumpkin leaves are the results shown in Figure 6, depicting the effect of the preservation techniques on the flavonoid contents of the pumpkin leaves. There was no significant difference ($p>0.05$) in flavonoid among the refrigerated, frozen and fresh leaves of the broad pumpkin leaf variety (BLP). However, the preserved slender pumpkin leaves variety (SLP) again suffered a significant decrease ($p<0.05$) in flavonoid content compared to their fresh leaf control (Figure 6). Antioxidants play a major role in the protection of cells from lethal effects of free radicals and their derivatives (Majewska *et al*., 2011) and phenolic compounds and flavonoids have long been established to exhibit antioxidant properties. A diet rich in antioxidant compounds (like phenols and flavonoids) therefore helps to strengthen the antioxidant-based defence system in the human body (Murugan *et. al*., 2013; Stangeland *et. al*., 2009). Oxidative damage occurs, if there is an imbalance between the levels of antioxidants and oxidants in the system. The results of the phenolic and flavonoids content suggest a level of success in the attempt to preserve pumpkin leaves, especially the broad leaf variety (BLP). Also, aside the total soluble protein and reducing sugar levels that were seemingly reduced in both varieties of the preserved pumpkin leaves, the preservation methods appear not to have significantly affected the antioxidant status or capacity of the preserved pumpkin leaves (especially the broad leaf variety, BLP) since their total phenol and flavonoid contents were not significantly affected.

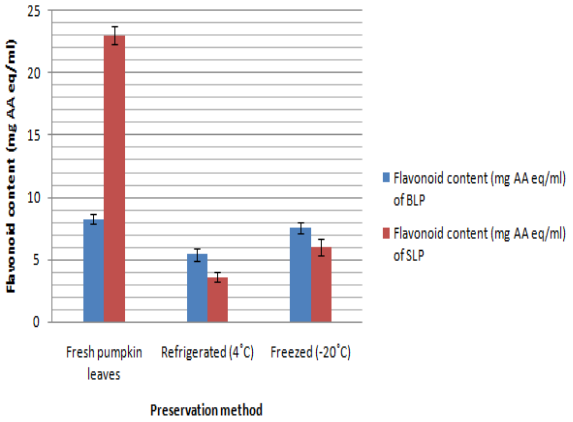

Figure 6. Effect of preservation on total flavonoid content extract (mg AA eq/ml) of two varieties of pumpkin leaves. BLP denotes pumpkin leaves variety 1 (broad leaf) and SLP, pumpkin leaves variety 2 (small leaf); both preserved for two weeks at -20°C and 4°C. mg AA eq/ml = mg Ascorbic acid equivalent/ml of extract.

The ferric reducing antioxidant power of a plant extract is often used as an index of its antioxidant capacity because it shows the potential of the plant extract to donate electron(s) to free radicals or reactive oxygen species (ROS), a reaction called reduction. A free radical is any species capable of independent existence (hence the term free) that contains one or more unpaired electrons (Halliwell and Gutteridge, 2006). An unpaired electron is one that occupies an atomic or molecular orbital by itself. When free radicals, reactive oxygen or nitrogen species, are reduced (i.e gain electrons), they lose their highly reactive nature and thus become unable to cause cellular oxidative damage (Halliwell and Gutteridge, 2006). In this study, the ferric reducing antioxidant power (FRAP) of the fresh and preserved pumpkin leaves was determined and reported (Figure 7). Although

the ferric reducing antioxidant power of broad leaf pumpkin variety (BLP) preserved by refrigeration (4°C) and freezing (-20°C) decreased by 20.9 and 16.1%, respectively, compared to their fresh broad leaf control, the decrease was not significant ($p>0.05$). Also, the FRAP of the preserved broad leaf pumpkin variety compared favourably ($p>0.05$) with that of the standard antioxidant (10 mg/ml ascorbic acid) used as a positive control. This again points to refrigeration and freezing as suitable preservation techniques for the broad leaf pumpkin variety (BLP) for a period of two weeks since the vegetable's free radical reducing power, a measure of its antioxidant capacity, is relatively conserved with these preservation techniques (especially the freezing method). On the contrary, the ferric reducing antioxidant power of the preserved slender leaf pumpkin variety (SLP) significantly decreased ($p<0.05$) relative to their fresh slender leaf control. A number of factors may be responsible; it could be possible that the slender leaf pumpkin variety (SLP) carries a higher load of microorganisms than the broad leaf variety (BLP) or that degradating enzymes in SLP are active even at low temperatures (4 and -20°C) than those in BLP, thereby leading to a breakdown of phytochemicals in SLP (Majewska *et. al*., 2011) and the eventual loss of free radical reducing power (Pulido *et. al*., 2000).

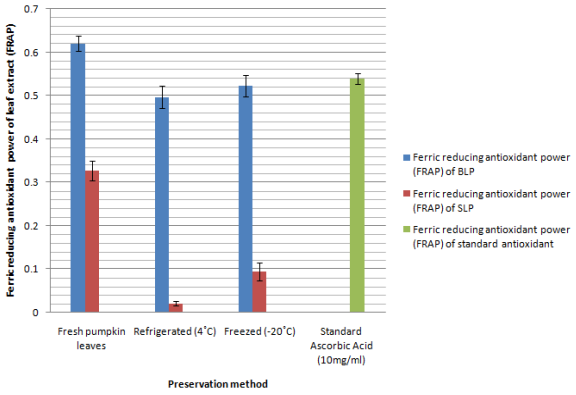

Figure 7. Effect of preservation on free radical reducing power (FRAP) of two varieties of pumpkin leaves. BLP denotes pumpkin leaves variety 1 (broad leaf) and SLP, pumpkin leaves variety 2 (small leaf); both preserved for two weeks at -20°C and 4°C.

Conclusion

It could be inferred from this study that broad leaf pumpkin variety (BLP) can be preserved by freezing (temperature -20°C) for a period of two weeks without any significant ($p>0.05$) loss of its valuable phytochemicals and antioxidant capacity.

References

Abdulmumeen, H. A., Risikat, A. N., and A. R. Sururah (2012). Food: Its preservatives, additives and applications. International Journal of Chemical and Biochemical Sciences, 1:36-47.

Adaramoye, O. A., Achem, J., Akintayo, O. O., and M. A. Fafunso (2007). Hypolipidaemic effect of *Telfairia occidentalis* (fluted pumpkin) in rat fed a cholesterol-rich diet. J. Med. Food 10: 330-336.

AderIbigbe, A. O., Lawal, B. A. S., and J. O. Oluwagbemi (1999). The antihyperglycaemic effect of *Telfairia occidentalis*. in mice. Afr. J. Med. Sci. 28: 171-175.

Akanbi, W. B., Adebooye, C. O., Togun, A. O., Ogunrinde, J. O. and S. A. Adeyeye (2006). Growth, herbage and seed yield and quality of *Telfairia occidentalis* qualityas influenced by cassava peel compost and mineral fertilizer. World J. Agric. Sci. 3(4): 508-516.

Alada, A. R. A. (2000). The haematologic effect of *Telfairia occidentalis* in diet preparation. Afr. J. Biomed. Res. 3: 185-186.

Arnon, D. (1949). Copper enzymes in isolated chloroplasts. Phenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.

Augustin Scalbert, Ian T Johnson, and Mike Saltmarsh (2005a). Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr., 81(1): 215S-217S.

Dike, M. C. (2010). Proximate phytochemical and nutrient compositions of some fruits, seed and leaves of some plant species at Umodike, Nigeria. ARPN J. Agric. Biol. Sci. 5:7-16.

Dina, O. A., Adedapo, A. A., Oyinloye, O. A. and A. B. Saban (2006). Effect of *Telfairia occidentalis* extract on experimentally induced anaemia in domestic. Afr. J. Biomed. Res. 3: 181-188.

Eseyin, O. A., Igboasoiyi, A. C. Oforah, E., Mbagwu, H., Umoh, E., and J. F. Ekpe (2005). Studies of the effect of alcohol extract of *Telfairia occidentalis* on alloxan induced diabetic rats. Global J. Pure and Applied Sci. 11: 85-87.

Farkas, J., Doyle, M. P., and L. R. Beuchat (2007). Physical methods of food preservation. Food microbiology: fundamentals and frontiers, (Edn. 3), 685-712.

González, S., Fernández, M., Cuervo, A. and C. Lasheras (2014). Dietary intake of polyphenols and major food sources in an institutionalised elderly population. Journal of Human Nutrition and Dietetics, 27(2): 176-183.

Gupta, K., Barat, G. K., Wagle, D. S. and H. K. L. Chawla (1989). Nutrient contents and antinutritional factors in conventional and non-conventional leafy vegetables. Food Chem. 31(2): 105–116.

Halliwell, B. and J. M. C. Gutteridge (2006). Free Radicals in Biology and Medicine, Ed 4. Clarendon Press, Oxford.

Jia, Z., Tang, M. and J. Wu (1999.) The determination of flavonoid contents of murlberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555- 559.

Lee S (2004). Microbial Safety of Pickled Fruits and Vegetables and Hurdle Technology. Internet Journal of Food Safety, 4: 21–32.

Li, B., and D. W. Sun (2002). Novel methods for rapid freezing and thawing of foods–a review. Journal of food engineering, 54(3), 175-182.

Majewska, M., Skrzycki, M., Podsiad, M. and H. Czeczot (2011). Evaluation of antioxidant potential of flavonoids: An *in vitro* study. Acta Pol. Pharm. 68:611-615.

Miller, G. C. (1959). Use of the dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. 31: 420 -428.

Murugan, S. B., Reshma, A., Deepika, R., Balamurugan, S., and R. Sathishkumar (2013). Antioxidant capacities of *Amaranthus tristis* and *Alternanthera sessilis*: A comparative study. J. Med. Plants Res. 7(30): 2230-2235.

Oboh, G., Nwanna, E. E., and C. A. Elusiyan (2006). Antioxidant and antmicrobial properties of *Telfairia occidentalis* (fluted pumpkin) leaf extract. J. Pharmacol. Toxicol. 1: 167-175.

Okokon, J. E., Ekpo, A. J., and O. A. Eseyin (2007). Antiplasmodial activity of ethanolic toot extract of *Telfairia occidentalis*. Res. J. Parasitol. 2: 94-98.

Oluwole, F. S., Falode, A. O., and O. O. Ogundipe (2003). Anti inflammatory effect of some common Nigeria vegetables. Niger. J. Physiol. Sci.18: 35-38.

Onoja, I. U., and N. N. Nnam (2014). Evaluation of beta-carotene, iron and other micronutrient status of rats fed sun or shade-dried fluted pumpkin (Telfariaoccidentalis) leaves and its product, the leaf curd. Science, 2(5): 454-460.

Oyaizu, M. (1986.) Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307- 315.

Petzold, G., Caro, M., and J. Moreno (2014). Influence of blanching, freezing and frozen storage on physicochemical properties of broad beans (Vicia faba L). International Journal of Refrigeration, 40, 429-434.

Pulido, R., Bravo, L., and F. Saura-Calixto (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 48 (8): 3396–3402.

Reezi, S., Babalar, M., and S. Kalantari (2009). Silicon alleviates salt stress, decreases malondialdehyde content and affects petal color of salt-stressed cut rose (*Rosa xhybrida* L.) 'Hot Lady'. Afr. J. Biotechnol. 8 (8): 1502-1508.

Sara, S., Morad, M. and C. M. Reza (2013). Effects of seed inoculation by Rhizobium strains on chlorophyll content and protein percentage in common bean cultivars (Phaseolus vulgaris L.). International Journal of Biosciences (IJB), 3(3), 1-8.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., and L. Jiménez (2005b). Dietary polyphenols and the prevention of diseases. Critical reviews in food science and nutrition, 45(4), 287-306.

Schlemmer, M. R., Francis, D. D., Shanahan, J. F. and J. S. Schepers (2005). Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. Agronomy Journal, 97(1), 106-112.

Singleton, V. L., and J. A. Rossi (1965.) Colorimetry of total phenolics with phophomolibdic-phophotungstic acid reagents. Am. J. Enol. Vitic. 16:144- 158.

Soliman, A. M., Abu-El-Zahab, H. S., and G. A. Alswiai (2013). Efficacy evaluation of the protein isolated from *Peganum harmala* seeds as an antioxidant in liver of rats. *Asian Pacific journal of tropical medicine*, *6*(4), 285-295.

Stangeland, T., Remberg, S. F., and K. A. Lye (2009). Total antioxidant activity in 35 Ugandan fruits and vegetables. Food Chem. 113:85–91.

Tonukari, N. J., Avwioroko, O. J., Seitonkumoh, G., Enuma, C. C., Sakpa, S. O., Eraga, L., Ezedom, T., Edema, U., Odiyoma, E., and A. A. Anigboro (2013). Nutritional compositions and antioxidant properties of typical Urhobo Nigerian soups. Niger. J. Technol. Res. 8(2): 1-9.

Wang, H., Zhang, S., and G. Chen (2007). Experimental study on the freezing characteristics of four kinds of vegetables. LWT-Food Sci. Technol. 40(6):1112–1116.

Wu, Guoyao, Zhenlong Wu, Zhaolai Dai, Ying Yang, Weiwei Wang, Chuang Liu, Bin Wang, Junjun Wang, and Yulong Yin (2013). Dietary requirements of "nutritionally non-essential amino acids" by animals and humans. Amino Acids 44(4): 1107-1113.