PJN
ISSN 1680-5194

Pakistan Journal of Nutrition

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 14 (7): 401-408, 2015
ISSN 1680-5194

Effect of Refrigeration Storage on Nutritive and Antioxidant Properties of Five Leafy Vegetables Consumed in Southern Côte d'ivoire

Florentin C. Acho, Lessoy T. Zoue and Sebastien L. Niamke
Laboratoire de Biotechnologies, UFR Biosciences, Université Felix Houphouet-Boigny,
22 BP 582, Abidjan 22, Côte d'Ivoire

Abstract: Leafy vegetables are highly perishable food items and require special processing treatment to prevent postharvest losses. This study was designed to evaluate the effect of refrigeration storage on the nutrient and antioxidant properties of five leafy vegetables (*Colocacia esculenta*, *Basella alba*, *Solanum melongena*, *Talinum triangulare* and *Corchorus olitorius*) commonly used in Southern Côte d'Ivoire. These leafy vegetables were refrigerated for 5, 10 and 15 days at 4°C. Ash, fibre, protein and carbohydrate contents and calorific values varied after 5 days of storage as follow: 7.19±0.28 to 19.16±0.6%, 11.64±0.14 to 24.24±0.12%, 8.05±0.41 to 20.31±1.54%, 47.05±0.49 to 57.86±0.53% and 240.32±6.11 to 286.03±5.68 kcal/100 g, respectively. During 5-days cold storage, the results revealed losses in anti-nutrients factors (oxalates and phytates) ranged from 84.67-742.33 and 15.15-33.27 mg/100 g for oxalates and phytates, respectively. Residual mineral contents after 5 days of refrigeration were: calcium (366.76-705.85 mg/100 g), magnesium (165.96-730.95 mg/100 g), phosphorus (214.45-752.76 mg/100 g), potassium (1046.43-2178.04 mg/100 g), iron (50.16-101.97 mg/100 g), sodium (25.31-368.03 mg/100 g) and zinc (12.43-59.09 mg/100 g). Vitamin C, carotenoid and polyphenols slightly decreased during refrigeration storage. These losses could be attributed to the respiration, transpiration, ethylene production and enzyme activity during storage. The present work showed that refrigeration processing (less than 5 days at 4°C) of leafy vegetables could be the best time of preserving their nutritive value and antioxidant properties.

Key words: Leafy vegetables, refrigeration storage, nutritive value, antioxidant properties

INTRODUCTION

Vegetables serve as indispensable constituents of human diet supplying the body with nutrients (Oyenuga and Fetuga, 1975). Sub-Saharan Africa grows an enormous variety of vegetables (Prabhu and Barrett, 2009) among which green leafy vegetables constitute essential components of human diet particularly in West Africa (Kubmarawa *et al*., 2009).

Particular attention should be paid on leafy vegetables because they contain more nutritionally important chemical constituents than other groups of vegetables (Lisiewska *et al*., 2009). Indeed, leafy vegetables are excellent source of micronutrients and also have many biological activities in the human body (Antonelli *et al*., 2002; Slupski *et al*., 2005). Leafy vegetables are known to contain antioxidants necessary in neutralizing free radicals in human body. These free radicals are generated under a number of conditions such as drinking alcohol and smoking (Akinmoladun *et al*., 2007). The ethno-botanical reports offer information on medicinal properties of green leafy vegetables like anti-diabetic, anti-histaminic, anti-carcinogenic, hypolipidemic, antibacterial activity (Kubo *et al*., 2004). However, green leafy vegetables available to the consumer have typically spent a period of 3-7 days in retail distribution and storage before consumption (Bushway *et al*., 1989). Thus, fresh vegetables can be exposed to a variety of conditions which offer the potential for change in quality characteristics, including nutrient content (Perrin and Gaye, 1986; Shewfelt, 1990). Indeed, the vegetables are typically over 90% water and after harvesting they begin to undergo higher rates of respiration, resulting in moisture loss, quality deterioration and potential microbial spoilage (Rickman *et al*., 2007). Furthermore, the dehydration, the rapid wilting and the senescence lead to their deterioration (Reyes, 1996). To extend the shelf-life, different ways of preserving traditional vegetables have been developed. The four main methods are the sun-drying of fresh leaves, the sun-drying of blanched leaves, the refrigeration and freezing processing. Cooling method helps maintain quality attributes such as appearance, texture mass loss and visual quality, thereby extending the shelf life of vegetables (Vina and Chaves, 2006).

In Côte d'Ivoire, more than twenty six species of leafy vegetables belonging to 6 main botanical families, are widely cultivated and consumed by Ivorian population (Fondio *et al*., 2007). Furthermore, ethno-botanical surveys have revealed that the consumption of these leafy vegetables is linked to cultural regions. Thus, most

Corresponding Author: Lessoy T. Zoue, Laboratoire de Biotechnologies, UFR Biosciences, Université Felix Houphouet-Boigny, 22 BP 582, Abidjan 22, Côte d'Ivoire

people in Southern Côte d'Ivoire consume through sauces preparation, leafy vegetables such as *Basella alba* "epinard", *Colocasia esculenta* "taro", *Corchorus olitorius* "kplala", *Solanum melongena* "aubergine" and *Talinum triangulare* "mamichou" (Fondio *et al.*, 2007; Soro *et al.*, 2012). Earlier reports have also highlighted the nutritive potential of these fresh leafy vegetables (Acho *et al.*, 2014) but there is a lack of scientific data with regards to the effect of refrigeration storage on their physicochemical and nutritive characteristics. Therefore, this paper reports a study of the nutrient changes during the refrigeration storage (4°C) up to 15 days.

MATERIALS AND METHODS

Samples collection: Leafy vegetables (*Basella alba*, *Colocasia esculenta*, *Corchorus olitorius*, *Solanum melongena* and *Talinum triangulare*) were collected fresh and at maturity from cultivated farmlands located at Dabou (latitude: 5°19'141" North; longitude: 4°22'59" West) (Abidjan District). Samples were harvested at the early stage (between one and two weeks of the appearance of the leaves). These plants were previously authenticated by the National Floristic Center (University Felix Houphouet-Boigny, Abidjan-Côte d'Ivoire).

Samples processing: The fresh leafy vegetables were rinsed with deionized water and the edible portions were separated from the inedible portions. The edible portions were chopped into small pieces (500 g) and allowed to drain at ambient temperature. Each sample was subdivided into two parts. One part (fresh and unrefrigerated, 250 g) was dried in an oven (Memmert, Germany) at 60°C for 72 h (Nagy and Smooth, 1977). The dried leaves were ground with a laboratory crusher (Culatti, France) equipped with a 10 µm mesh sieve. The second part (250 g) was packed in airtight polyethylene bags and refrigerated at 4°C for 5, 10 and 15 days (Prabhu and Barrett, 2009). After refrigeration times, samples were subjected to the same treatment (drying and gridding) using for unrefrigerated (control) samples.

Chemicals: All solvents (n-hexane, petroleum ether, acetone, ethanol and methanol) were purchased from Merck. Standards used (glucose, gallic acid, tannic acid, quercetin, beta-carotene) and reagents (metaphosphoric acid, vanillin, Folin-Ciocalteu, DPPH) were purchased from Sigma-Aldrich. All chemicals used in the study were of analytical grade.

Proximate analysis: Proximate analysis was performed using official methods (AOAC, 1990). The moisture content was determined by the difference of weight before and after drying fresh sample (10 g) in an oven (Memmert, Germany) at 105°C until constant weight. Ash fraction was determined by the incineration of dry matter sample (5 g) in a muffle furnace (Pyrolabo, France) at 550°C for 12 h. The percentage residue weight was expressed as ash content. For crude fibres, 2 g of dry matter sample were weighed into separate 500 mL round bottom flasks and 100 mL of 0.25 M sulphuric acid solution was added. The mixture obtained was boiled under reflux for 30 min. Thereafter, 100 mL of 0.3 M sodium hydroxide solution was added and the mixture were boiled again under reflux for 30 min and filtered through Whatman paper. The insoluble residue was then incinerated and weighed for the determination of crude fibres content. Proteins were determined through the Kjeldhal method and the lipid content was determined by Soxhlet extraction using hexane as solvent. Carbohydrates content and calorific value were calculated and expressed on dry matter basis using the following formulas (FAO, 2002):

$$\text{Carbohydrates (dry matter basis): } 100-(\text{proteins}+\text{lipids}+\text{ash}+\text{fibres}\%)$$

$$\text{Calorific value (dry matter basis): } (\%\text{ proteins} \times 2.44)+(\%\text{ carbohydrates} \times 3.57)+(\%\text{ lipids} \times 8.37)$$

The results of ash, fibres, proteins, lipids and carbohydrates contents were expressed on dry matter basis.

Vitamin C determination: Vitamin C contained in analyzed samples was determined by titration using the method described by Pongracz *et al.* (1971). About 10 g of ground fresh leaves were soaked for 10 min in 40 mL metaphosphoric acid-acetic acid (2%, w/v). The mixture was centrifuged at 3000 rpm for 20 min and the supernatant obtained was diluted and adjusted with 50 mL of bi-distilled water. Ten mL of this mixture was titrated to the end point with dichlorophenol-indophenol (DCPIP) 0.5 g/L.

Carotenoids determination: Carotenoids content was carried out according to Rodriguez-Amaya (2001). Two g of ground fresh leaves were mixed three times with 50 mL of acetone until loss of pigmentation. The mixture obtained was filtered and total carotenoids were extracted with 100 mL of petroleum ether. Absorbance of extracted fraction was then read at 450 nm by using a spectrophotometer (PG Instruments, England). Total carotenoids content was subsequently estimated using a calibration curve of beta-carotene (1 mg/mL) as standard.

Polyphenols determination: Polyphenols content was determined using the method reported by Singleton *et al.* (1999). A quantity (1 g) of dried powdered sample was soaked in 10 mL of methanol 70% (w/v) and centrifuged at 1000 rpm for 10 min. An aliquot (1 mL) of supernatant was oxidized with 1 mL of Folin-Ciocalteu's reagent and neutralized by 1 mL of 20% (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at ambient temperature and absorbance was measured at 745 nm by using a spectrophotometer (PG Instruments, England). The polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as standard.

Oxalates determination: The titration method as described by Day and Underwood (1986) was performed. One g of dried powdered sample was weighed into 100 mL conical flask. A quantity of 75 mL of sulphuric acid (3 M) was added and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 mL of the filtrate was titrated while hot against $KMnO_4$ solution (0.05 M) to the end point.

Phytates determination: The method described by Wheeler and Ferrel (1971) was used for determination of phytates content. A quantity (0.5 g) of dried powdered sample was mixed with 25 mL of trichloroacetic acid (3%, w/v) and centrifuged at 3500 rpm for 15 min. The supernatant obtained was treated with $FeCl_3$ solution and the iron content of the precipitate was determined using spectrophotometric method at 470 nm. A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content.

Antioxidant activity: Antioxidant assay was carried out using the 2,2-diphenyl-1-pycrilhydrazyl (DPPH) spectrophotometric method outlined by Choi *et al.* (2002). About 1 mL of 0.3 mM DPPH solution in ethanol was added to 2.5 mL of sample solution (1 g of dried powdered sample mixed in 10 mL of methanol and filtered through Whatman No. 4 filter paper) and was allowed to react for 30 min at room temperature. Absorbance values were measured with a spectrophotometer (PG Instruments, England) set at 415 nm. The average absorbance values were converted to percentage antioxidant activity using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{100-(Abs of sample-Abs of blank)}}{\text{Abs positive control}} \times 100$$

Mineral analysis: Minerals contents were determined by the ICP-MS (inductively coupled argon plasma mass spectrometer) method (CEAEQ, 2013). The dried powdered samples (5 g) were burned to ashes in a muffle furnace (Pyrolabo, France). The ashes obtained were dissolved in 10 mL of HCl/HNO_3 and transferred into 100 mL flasks and the volume was made up using deionized water. The mineral composition of each sample was determined using an Agilent 7500 c argon plasma mass spectrometer. Calibrations were performed using external standards prepared from a 1000 ppm single stock solution made up with 2% nitric acid.

Statistical analysis: All the analyses were performed in triplicate and data were analyzed using EXCELL and STATISTICA 7.1 (StatSoft). Differences between means were evaluated by Duncan's test. Statistical significant difference was stated at $p<0.05$.

RESULTS AND DISCUSSION

Nutritive and anti-nutritive properties: Moisture, ash, fibres, proteins, lipids, carbohydrates and calorific values of refrigerated leafy vegetables are reported in Table 1. The slight decrease in moisture contents (74.38-90.20 to 72.67-88%) during refrigeration storage may be due to the respiration and others senescence related metabolic processes (Souzan and Abd El-AAl, 2007). Ash content of the analyzed samples ranged after 5 days from 7.19±0.28% (*C. olitorius*) to 19.16±0.6% (*T. triangulare*). The highest content of ash was observed for *T. triangulare* (16.31±0.19%) while *C. olitorius* (5.52±0.27%) had the lowest value after 15 days of refrigeration. The proximate values of these common leafy vegetables were closed to other leafy species reported by Aletor *et al.* (2002). The reduced ash contents could be attributed to the loss water carrying off the minerals during refrigeration storage (Souzan and Abd El-AAl, 2007). The decrease in ash contents of processed vegetables could be as a result of processing during which some of inorganic salt in the vegetables might have leached off (Yaciuk and Sofose, 1981). In spite of ash losses, the level of ash in the studied samples may suggest that these leafy vegetables are good sources of minerals. Indeed, it has been reported that leaves should contain about 3% ash for using as human food (Pivie and Butler, 1977). Contrary to the ash contents, the fibres contents slightly increased with refrigeration storage time. The values of fibres contents ranged from 11.59-24.24% at 5 days, from 11.64-24.26% at 10 days and from 11.71-24.90% at 15 days of storage. Increasing in the total fiber of leafy vegetables may be due to increasing amounts of uronic acid in the insoluble fibre fraction (Marlett, 2000). With regard to their fibres content after 15 days of refrigeration storage, adequate intake (200 g/day) of refrigerated leafy vegetables as desserts could lower the risk of constipation, diabetes and colon cancer (Ishida *et al.*, 2000). After 5 days of refrigeration storage, the proteins and lipids contents averaged from 8.05±0.4 to 20.31±1.54% and from 2.46±0.00 to 7.59±0.65%, respectively. The relativity low values of lipids contents at 15 days of refrigeration processing corroborates the finding of many authors which showed that fresh leafy vegetables were found to be poor sources of lipids (Ejoh *et al.*, 1996). The little decrease in proteins and lipids contents caused by refrigerated could be attributed to the fact that some nutrient were leached off by water during refrigerated, but they lose more lipids during storage owing to oxidation (Rickman *et al.*, 2007). Due to

Table 1: Proximate composition of refrigerated leafy vegetables consumed in Southern Côte d'Ivoire

	Moisture (%)	Ash* (%)	Fibres* (%)	Proteins* (%)	Lipids* (%)	Carbohydrates* (%)	Calorific value* (kcal/100 g)
C. esculenta							
0 days	82.35±2.83^{a}	15.03±0.23^{a}	24.00±0.46^{a}	9.80±0.16^{a}	8.35±0.15^{a}	42.85±2.68^{d}	252.34±1.55^{a}
5 days	81.78±0.51^{a}	13.45±0.30^{b}	24.24±0.12^{a}	8.05±0.41^{b}	7.59±0.65^{b}	47.05±0.49^{c}	251.22±8.22^{a}
10 days	80.29±0.33^{b}	12.34±0.03^{c}	24.26±0.24^{a}	7.31±0.00^{c}	6.90±0.32^{c}	49.33±0.20^{b}	251.75±3.41^{a}
15 days	79.22±1.25^{b}	11.31±0.47^{c}	24.90±0.43^{a}	6.75±0.33^{d}	6.05±0.19^{c}	50.95±0.69^{a}	249.07±4.94^{a}
B. alba							
0 days	89.82±1.24^{a}	19.79±0.44^{a}	16.50±0.30^{a}	9.86±0.10^{a}	6.85±0.05^{a}	47.00±0.89^{d}	249.18±2.41^{b}
5 days	88.63±0.48^{a}	17.56±0.00^{b}	16.70±0.23^{a}	8.31±0.11^{b}	6.37±0.07^{b}	51.26±0.32^{c}	256.17±2.09^{a}
10 days	87.55±0.40^{b}	16.36±0.46^{c}	16.81±0.10^{a}	8.09±0.57^{b}	4.92±0.06^{c}	53.73±0.89^{b}	252.81±5.18^{a}
15 days	86.43±0.37^{b}	14.83±0.34^{d}	16.83±0.05^{a}	6.93±0.51^{c}	3.42±0.00^{d}	58.04±0.18^{a}	252.82±1.96^{b}
S. melongena							
0 days	74.38±0.72^{a}	20.32±2.36^{a}	13.70±0.65^{a}	12.34±0.09^{a}	2.73±0.06^{a}	50.91±3.16^{d}	277.57±13.54^{a}
5 days	73.60±0.53^{a}	18.59±0.12^{a}	13.75±0.04^{a}	10.09±0.37^{b}	2.46±0.00^{b}	54.91±0.16^{c}	241.30±1.54^{b}
10 days	72.85±0.50^{a}	16.86±0.48^{b}	13.83±0.01^{a}	9.99±0.57^{b}	2.17±0.02^{c}	57.30±0.32^{b}	247.13±2.80^{b}
15 days	72.67±0.35^{a}	15.90±0.15^{c}	13.85±0.02^{a}	7.68±0.77^{c}	1.95±0.03^{d}	60.54±0.83^{a}	251.24±5.13^{b}
T. triangulare							
0 days	90.20±0.21^{a}	22.20±0.37^{a}	13.98±1.50^{a}	17.18±0.05^{a}	4.90±0.06^{a}	52.77±2.08^{b}	271.32±8.17^{a}
5 days	89.43±0.27^{a}	19.16±0.60^{b}	14.02±0.19^{a}	16.80±1.59^{a}	4.14±0.00^{b}	46.13±0.62^{c}	240.32±6.11^{b}
10 days	88.54±0.28^{b}	18.30±0.06^{b}	14.19±0.30^{a}	15.83±0.75^{b}	3.27±0.00^{c}	48.42±1.06^{c}	238.87±5.66^{b}
15 days	88.00±0.74^{b}	16.31±0.19^{c}	14.27±0.14^{a}	12.77±1.51^{c}	2.12±0.00^{d}	54.43±1.46^{a}	243.25±8.93^{b}
C. olitorius							
0 days	84.28±0.34^{a}	8.53±0.15^{a}	11.49±0.03^{a}	21.12±0.05^{a}	3.28±0.30^{a}	55.58±0.84^{c}	277.40±1.26^{b}
5 days	83.61±0.12^{a}	7.19±0.28^{b}	11.59±0.23^{a}	20.31±1.54^{a}	3.57±0.00^{b}	57.86±0.53^{b}	286.03±5.68^{a}
10 days	81.67±0.91^{b}	6.33±1.03^{b}	11.64±0.14^{a}	19.53±0.50^{b}	3.50±0.00^{b}	59.33±0.81^{a}	288.78±4.13^{a}
15 days	80.48±0.06^{b}	5.52±0.27^{c}	11.71±0.28^{a}	19.50±0.37^{b}	3.27±0.00^{c}	60.10±0.61^{a}	289.51±3.10^{a}

Data are represented as means±SD (n = 3). Means in the column with no common letter differ significantly ($p<0.05$) for each leafy vegetable

*: values given on dry matter basis

the generally low level of crude fat in the vegetable leaves, their consumption in large amounts could be a good dietary habit and may be recommendable to individuals suffering from obesity (Onyeike *et al.*, 2003). The low levels of protein, lipids and total carbohydrate in these leafy vegetables indicate that they are not good sources of calorific foods. These low calorific values could be due to low proteins, lipids and total carbohydrate and relatively high levels of moisture (Onyeike *et al.*, 2003). The result of anti-nutritional factors (oxalates and phytates) contents of the leafy vegetables were presented in Fig. 1. The values ranged within 84.67-742.33 and 15.15-33.27 mg/100 g at 5 days for oxalates and phytates, respectively. These reductions in oxalates and phytates contents during refrigerated storage could be advantageous for improving the health status of consumers. Indeed, oxalates and phytates are anti-nutrients which chelate divalent cations such as calcium, magnesium, zinc and iron thereby reducing their bioavailability (Hassan *et al.*, 2007). Variation in the chemical constituents may be due to species differences, agro climatic conditions, age and stage of the plant.

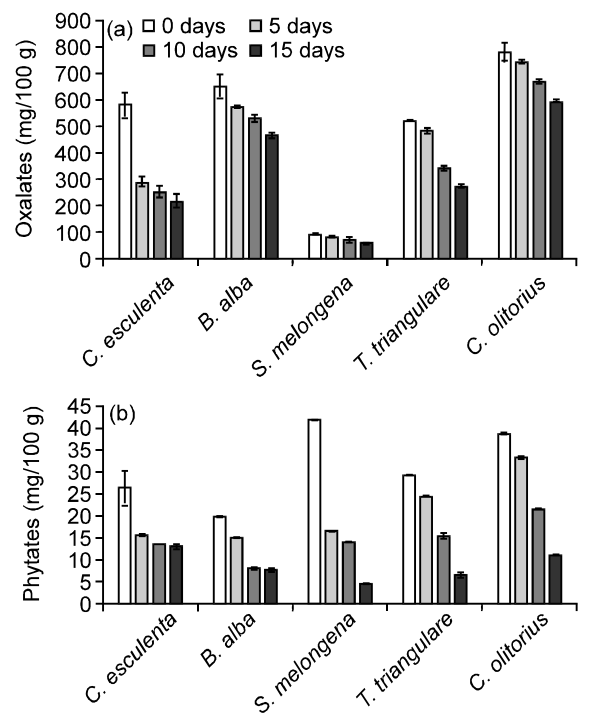

Fig. 1: Oxalate (a) and phytate (b) contents of five refrigerated leafy vegetables consumed in Southern Côte d'Ivoire

Mineral composition: Mineral content is an essential component of the nutritive value of leafy vegetables. Mineral composition of the refrigerated leafy vegetables is shown in Table 2. According to Slupski *et al.* (2005), leaves were characterized by a greater content of mineral constituents than whole plants, petiole, or stems. The residual mineral contents after 5 days of refrigerated were: calcium (366.76-705.85 mg/100 g), magnesium (165.96-730.95 mg/100 g), phosphorus (214.45-752.76 mg/100 g), potassium (1046.43-2178.04

Table 2: Mineral composition of refrigerated leafy vegetables consumed in Southern Côte d'Ivoire

	Ca	Mg	P	K	Fe	Na	Zn
C. esculenta							
0 days	587.24±0.55[a]	347.29±0.32[a]	788.00±0.74[a]	2281.63±2.14[a]	143.37±0.13[a]	39.45±0.16[a]	37.29±0.03[a]
5 days	507.81±5.28[b]	265.91±8.00[b]	752.76±6.47[b]	2114.26±8.67[b]	92.53±2.78[b]	17.46±0.21[b]	29.01±2.67[b]
10 days	470.92±1.34[c]	211.30±0.27[c]	725.61±5.51[b]	2098.20±6.00[c]	74.81±0.21[c]	15.32±0.43[c]	26.06±0.01[c]
15 days	419.91±0.53[d]	209.22±0.59[d]	641.16±2.23[c]	1499.79±1.92[d]	72.99±0.09[c]	11.18±0.15[d]	25.17±0.01[d]
B. alba							
0 days	750.34±0.53[a]	753.88±0.53[a]	390.14±0.27[a]	2709.43±1.93[a]	77.47±0.05[a]	555.01±5.99[a]	67.25±0.72[a]
5 days	668.67±0.26[b]	617.68±0.36[b]	387.44±1.08[a]	1625.76±0.65[b]	50.16±0.02[b]	237.35±0.09[b]	59.08±0.02[b]
10 days	561.73±8.76[c]	432.75±4.45[c]	367.33±9.73[b]	715.38±3.89[c]	41.00±1.03[c]	189.16±2.97[c]	48.79±0.29[c]
15 days	408.53±11.95[d]	255.50±7.47[d]	336.05±1.06[c]	493.67±4.45[d]	31.97±0.05[d]	162.44±1.82[d]	45.63±0.16[d]
S. melongena							
0 days	796.54±0.55[a]	481.90±0.33[a]	374.46±0.26[a]	2256.10±1.57[a]	139.48±0.09[a]	323.13±2.96[a]	64.64±0.04[a]
5 days	705.85±6.98[b]	419.69±4.15[b]	354.01±5.55[b]	2105.53±4.56[b]	101.97±1.00[b]	318.03±3.63[b]	51.31±0.50[b]
10 days	629.12±5.58[c]	341.20±4.26[c]	252.53±4.76[b]	2035.76±3.02[c]	86.83±1.08[c]	313.96±3.17[b]	13.00±0.16[c]
15 days	606.79±7.57[d]	239.93±9.75[d]	233.32±5.39[c]	1186.52±4.82[d]	46.67±1.89[d]	265.70±3.31[c]	12.43±0.50[d]
T. triangulare							
0 days	601.37±0.38[a]	755.97±0.48[a]	239.59±0.81[a]	5053.23±3.21[a]	102.28±0.06[a]	260.25±0.75[a]	36.10±0.02[a]
5 days	592.44±3.74[b]	730.95±7.10[b]	214.45±3.49[b]	2178.04±1.78[b]	101.76±1.45[a]	205.27±2.85[b]	30.93±0.56[b]
10 days	513.35±9.91[c]	636.13±6.68[c]	206.47±9.77[b]	2112.53±9.35[c]	100.96±0.56[a]	150.89±2.29[c]	27.04±0.41[c]
15 days	412.65±9.32[d]	525.21±5.60[d]	174.41±3.97[c]	1708.74±6.01[d]	74.05±1.12[b]	135.27±0.73[d]	22.06±0.11[d]
C. olitorius							
0 days	369.02±2.33[a]	234.51±0.38[a]	316.82±0.52[a]	2622.57±16.56[a]	97.60±0.16[a]	27.75±0.08[a]	24.71±0.04[a]
5 days	366.76±0.81[a]	165.96±0.50[b]	293.89±3.30[b]	1046.43±3.15[b]	56.63±0.17[b]	25.31±0.42[a]	12.43±0.03[b]
10 days	334.51±54.49[b]	117.53±6.98[c]	203.91±8.67[c]	951.58±5.65[c]	39.24±9.11[c]	21.36±1.42[b]	10.81±0.64[c]
15 days	311.67±12.57[c]	105.29±4.46[d]	174.17±4.82[d]	899.51±2.09[d]	36.30±2.15[c]	17.85±2.48[c]	4.11±0.95[d]

Data are represented as means±SD (n = 3). Means in the column with no common letter differ significantly ($p<0.05$) for each leafy vegetable

Table 3: Anti-nutritional factors/mineral ratios of refrigerated leafy vegetables consumed in Southern Côte d'Ivoire

	Phytate/Ca	Phytate/Fe	Oxalate/Ca
C. esculenta			
0 days	0.04	0.18	0.98
5 days	0.03	0.17	0.57
10 days	0.02	0.18	0.53
15 days	0.03	0.17	0.52
B. alba			
0 days	0.02	0.25	0.87
5 days	0.02	0.30	0.85
10 days	0.01	0.19	0.94
15 days	0.01	0.24	1.14
S. melongena			
0 days	0.05	0.29	0.12
5 days	0.02	0.16	0.11
10 days	0.02	0.15	0.11
15 days	0.00	0.09	0.09
T. triangulare			
0 days	0.04	0.28	0.86
5 days	0.04	0.24	0.81
10 days	0.03	0.15	0.67
15 days	0.01	0.08	0.66
C. olitorius			
0 days	0.10	0.40	2.11
5 days	0.12	0.58	2.02
10 days	0.09	0.54	2.00
15 days	0.05	0.3	1.91

mg/100 g), iron (50.16-101.97 mg/100 g), sodium (25.31-368.03 mg/100 g) and zinc (12.43-59.09 mg/100 g). Compared to the values of raw leafy vegetables, these observed reductions in minerals may be due to the losses of ashes. The recommended dietary allowances (RDA) as mg/day/person for calcium, magnesium, iron and zinc are 1000, 400, 8 and 6, respectively (FAO, 2004). The level of iron and zinc in the samples could cover RDA and contribute substantially for improving human diet (FND, 2005). Calcium salts provide rigidity to the skeleton and calcium ions play a role in many metabolic processes (FAO, 2004). However, calcium and phosphorus are associated for growth and maintenance of bones, teeth and muscles (Turan *et al*., 2003). As regards magnesium, this mineral is known to prevent muscle degeneration, growth retardation, congenital malformations and bleeding disorders (Chaturvedi *et al*., 2004). Iron is important in the diet oh the both pregnant and location as well as infant; the convalescent and the elderly to reduce cases of diseases associated with the deficiency of iron such as anemia (Narasinga Rao, 1997) while zinc deficiency results in retarded growth (Barminas *et al*., 1998). To predict the effect of phytates and oxalates on the bioavailability of calcium and iron, phytate and oxalates to nutrients ratios were calculated (Table 3). The calculated ratios of phytates to calcium and iron and oxalates to calcium of the leafy vegetables were below the critical level of 0.5, 0.4 and 2.5, respectively (Hassan *et al*., 2007). This implies that phytates and oxalates may not hinder calcium and iron bioavailability in the refrigerated leaves of *C. esculenta*, *B. alba*, *S. melongena* and *T. triangulare*. However, the calculated ratios of phytate to iron for *C. olitorius* were above the critical level of 0.4 after 5 and 10 days refrigerated storage, resulting that phytates may hinder iron bioavailability (Umar *et al*., 2007).

Antioxidant properties: Many plants have been identified as good sources of natural antioxidants such as tocopherols, vitamin C, carotenoids and polyphenols

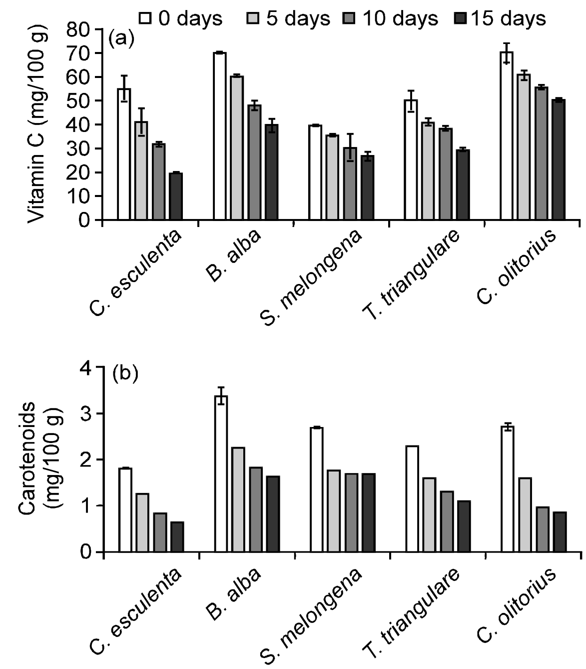

Fig. 2: Vitamin (a) and carotenoid (b) contents of five refrigerated leafy vegetables consumed in Southern Côte d'Ivoire

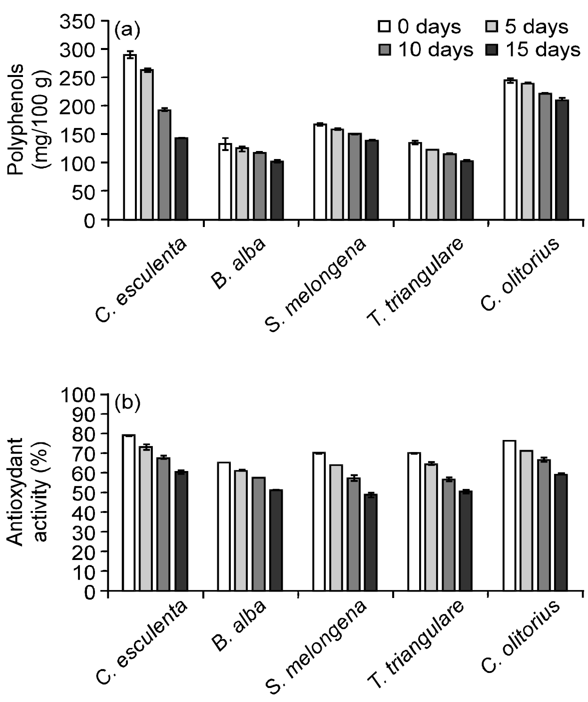

Fig. 3: Polyphenol contents (a) and antioxidant activity (b) contents of five refrigerated leafy vegetables consumed in Southern Côte d'Ivoire

which are responsible for maintaining good health and protect against coronary heart diseases and cancer (Arabshahi *et al*., 2007). Vitamin C and carotenoid contents of the studied leafy vegetables are shown in Fig. 2. Vitamin C losses were estimated to 23.75-28.32%, at 5 days of storage. Indeed, vitamin C is easily oxidized, so it will gradually decrease during refrigerated storage (Howard *et al*., 1999). While the nutritional importance of carotenoids is based primarily on vitamin A activity, carotenoids have also been extensively studied for their potential protection against numerous cancers (Rickman *et al*., 2007). Carotenoids losses at 5 days in the selected leafy vegetables varied from 10.35 to 21.77%. Few studies were found detailing the degradation of carotenoids during fresh storage. Salunkhe *et al*. (1991) indicate that carotenoid degradation during storage is low for intact living tissues. Epidemiological studies show positive correlations vegetables and reduced risk of chronic diseases such as cancer and cardiovascular disease between a diet high in phenolic-rich fruits and vegetables (Rickman *et al*., 2007). The nutritional benefits of phenolic compounds are often attributed to their substantial antioxidant activity (Rickman *et al*., 2007). Antioxidants are compounds used to prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction and thus prevent diseases (Zheng and Wang, 2001). Phenolic also seem to be more affected by storage factors such as temperature, atmosphere and light, than either vitamin C or carotenoids. Low temperatures slow down plant metabolic processes (Ragaert *et al*., 2007). The most frequently used temperature is 4°C, considered the optimal for many leafy vegetables (Jacxsens *et al*., 2002). It has been observed from Fig. 3 that the phenolic compounds gradually decreased during refrigerated storage, with losses at 5 days estimated to 1.76 to 9.45%, 14.02 to 50.20% at 15 days of storage. Indeed, Rickman *et al*. (2007) attributed these losses to the possibility of decreased activity enzymes that cause the oxidation of phenolics. Moreover, this decrease in phenolic contents caused the decrease of antioxidant activity because there is a direct correlation between the concentration of antioxidant compounds and the antioxidant activity (Lan, 2007). Antioxidant activity ranged from 61.45±0.25% (*B. alba*) to 73.15±0.78% (*C. esculenta*) after 5 days of refrigeration storage. Generally, the studied leafy vegetables showed antioxidant activity higher than 50% after 15 days of refrigeration storage. It could be important to reduce the refrigeration storage time to ensure best antioxidant value for these leafy vegetables.

Conclusion: The five leafy vegetables (*Colocasia esculenta*, *Basella alba*, *Solnum melongena*, *Talinum triangulare* and *Corchorius olitorus*) consumed in

Southern Côte d'Ivoire are good sources of nutrients and antioxidant compounds. In this study, refrigeration storage could enhance the shelf life and nutritional quality by slowing down plant metabolic processes such as respiration, ethylene production and enzymes activity. In spite of observed losses, the residual contents in fibres and proteins, minerals (iron and zinc) and antioxidant compounds (vitamin C, carotenoids and polyphenols) as well as the reduction of anti-nutritional factors (oxalates and phytates) could be useful for improving human diet and to contribute to the food security of Ivorian population. In addition, sensorial analysis of refrigerated leafy vegetables must be performed in order to appreciate their palatability. Also a comparison with the major methods as the sun-drying fresh leaves and the frozen must be studied to determinate the best preservation method.

REFERENCES

Acho, F.C., L.T. Zoue, E.E. Akpa, G.V. Yapo and S.L. Niamke, 2014. Leafy vegetables consumed in Southern Côte d'Ivoire: a source of high value nutrients. J. Anim. Plant Sci., 20: 3159-3170.

Akinmoladun, A.C., E.O. Ibukun, E. Afor, B.L. Akinrinlola, T.R. Onibon, A.O. Akinboboye, E.M. Obuotor and E.O. Farombi, 2007. Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotech., 6: 1197-1201.

Aletor, O., A.A. Oshodi and Ipinmoroti, 2002. Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. Food Chem., 78: 63-68.

Antonelli, M.L., G. D'Ascenzo, A. Lagana and P. Pusceddu, 2002. Food analyses: a new colorimetric method for ascorbic acid (vitamin C) determination. Talanta, 58: 961-967.

AOAC, 1990. Official methods of analysis. Association of Official Analytical Chemists Ed., Washington DC. pp: 684.

Arabshahi, D.S., V. Devi and A. Urooj, 2007. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chem., 100: 1100-1105.

Barminas, J.T., M. Charles and D. Emmanuel, 1998. Mineral composition of non-conventional leafy vegetables. Plant Food Hum. Nutr., 53: 29-36.

Bushway, R.J., P.R. Helper, J. King, B. Perkins and M. Krishnan, 1989. Comparison of ascorbic acid content of supermarket versus roadside stand produce. J. Food Quality, 12: 99-105.

CEAEQ, 2013. Determination des metaux. Méthode par spectrometrie de masse a source ionisante au plasma d'argon. MA 200-Met 1.2, Rev., 4. Quebec, pp: 24.

Chaturvedi, V.C., R. Shrivastava and R.K. Upreti, 2004. Viral infections and trace elements: A complex trace element. Curr. Sci., 87: 1536-1554.

Choi, C.W., S.C. Kim, S.S. Hwang, B.K. Choi, H.J. Ahn, M.Z. Lee, S.H. Park and S.K. Kim, 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plant and flavonoids by assay guided comparison. Plant Sci., 163: 1161-1168.

Day, R.A. and A.L. Underwood, 1986. Quantitative analysis. 5th ed. Prentice Hall. pp: 701.

Ejoh, A.R., M.F. Houanguep and E. Fokou, 1996. Nutrient composition of the leaves and flowers of *Colocasia esculenta* and the fruits of *Solanum melongena*. Plant Food Hum. Nutr., 49: 107-112.

FAO, 2002. Food energy-methods of analysis and conversion factors. FAO Ed, Rome. pp: 97.

FAO, 2004. Human vitamin and mineral requirements. FAO. Ed. pp: 361.

FND, 2005. Dietary reference Intake for energy, carbohydrate, fibre, fat, fatty acids, cholesterol, protein and amino acid (micro-nutrients). Food and Nutrition Board, www.nap.edu.

Fondio, L., C. Kouame, J.C. N'zi, A. Mahyao, E. Agbo and A.H. Djidji, 2007. Survey of indigenous leafy vegetable in the urban and peri-urban areas of Côte d'Ivoire. Acta Hort., 752: 287-289.

Hassan, L.G., K.J. Umar and Z. Umar, 2007. Antinutritive factors in *Tribulus terrestris* (Linn) leaves and predicted calcium and zinc bioavailability. J. Trop. Biosci., 7: 33-36.

Howard, L.A., A.D. Wong, A.K. Perry and B.P. Klein, 1999. Beta-Carotene and ascorbic acid retention in fresh and processed vegetables. J. Food Sci., 64: 929-936.

Ishida, H., H. Suzuno, N. Sugiyama, S. Innami, T. Todokoro and A. Maekawa, 2000. Nutritional evaluation of chemical component of leaves stalks and stems of sweet potatoes (*Ipomea batatas*). Food Chem., 68: 359-367.

Jacxsens, L., F. Devlieghere and J. Debevere, 2002. Temperature dependence of shelf-life as affected by microbial proliferation and sensory quality of equilibrium modified atmosphere packaged fresh produce. Postharvest Biol. Technol., 26: 59-73.

Kubmarawa, D., I.F. Andenyang and A.M. Magomya, 2009. Proximate composition and amino acid profile of two nonconventional leafy vegetables (*Hibiscus cannabinus* and *Haematostaphis barteri*), Afr. J. Food Sci., 3: 233-236.

Kubo, I., K. Fijita, A. Kubo, K. Nehi and T. Gura, 2004. Antibacterial activity of coriander volatile compounds against salmonella choleraesuits. J. Agric. Food Chem., 52: 3329-3332.

Lan, W., 2007. Effect of Chlorogenic acid on antioxidant activity of Flos lanicerae extracts. J. Zhejiang Univ. Sci. B., 8: 673-679.

Lisiewska, Z., P. Gebczynski, E. Bernas and W. Kmiecik, 2009. Retention of mineral constituents in frozen leafy vegetables prepared for consumption. J. Food Comp. Anal., 22: 218-223.

Marlett, J.A., 2000. Changes in content and composition of dietary fiber in yellow onions and red delicious apples during commercial storage. J. AOAC Int., 83: 992-996.

Nagy, S. and J.M. Smooth, 1977. Temperature and storage effects on percent retention and percent U.S. recommended dietary allowances of vitamin C in canned single-strength orange juice. J. Agric. Food Chem., 25: 135-138.

Narasinga-Rao, B.S., 1997. Anemia in India: Prevalence, causes, consequences and control. Proceeding of Colloquium on Fortification of wheat flour with iron, UNICEF and C.F.T.R.I. Mysore, India, November 25.

Onyeike, E.N., C.A. Ihugba and G. Chinyere, 2003. Influence of heat processing on the nutrient composition of vegetable leaves consumed in Nigeria. Plant Foods Hum. Nutr., 58: 1-11.

Oyenuga, V.A. and B.L. Fetuga, 1975. First National Seminar on Fruits and vegetables, in process and Recombination by NIHORT, Ibadan, Nigeria, 13-17 Oct., pp: 122-123.

Perrin, P.W. and M.M. Gaye, 1986. Effects of simulated retail display and overnight storage treatments on quality maintenance in fresh broccoli. J. Food Sci., 51: 146149.

Pivie, N.W. and J.B. Butler, 1977. A simple unit leaves. Proc. Nutr. Soc., 36: 136-139.

Pongracz, G., H. Weiser and D. Matzinger, 1971. Tocopherols-Antioxydant. Fat Sci. Technol., 97: 90-104.

Prabhu, S. and D. Barrett, 2009. Effects of storage condition and domestic cooking on the quality and nutrient content of African leafy vegetables (*Cassia tora* and *Corchorus tridens*). J. Sci. Food Agric., 89: 1709-1721.

Ragaert, P., F. Devlieghere and J. Debevere, 2007. Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. Postharvest Biol. Technol., 44: 185-194.

Reyes, V., 1996. Improved preservation systems for minimally processed vegetables. Food Australia. 48: 87-90.

Rickman, C.J., M.D. Barrett and M.C. Bruhn, 2007. Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds. Rev. J. Sci. Food Agric., pp: 15.

Rodriguez-Amaya, D.B., 2001. A guide to carotenoids analysis in foods. ILSI Press, Washington DC., pp: 64.

Salunkhe, D.K., H.R. Bolin and N.R. Reddy, 1991. Storage, Processing and Nutritional Quality of Fruits and Vegetables. Vol. 2: Processed Fruits and Vegetables. CRC Press, Boca Raton, FL, Chem. Comp. Nutr. Quality, pp: 115-145.

Shewfelt, R.L., 1990. Sources of variation in the nutrient content of agricultural commodities from the farm to the consumer. J. Food Quality, 13: 37-54.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxydant substrates and antioxydants by means of Folin-ciocalteu reagent. Methods Enzymol., 299: 152-178.

Slupski, J., Z. Lisiewska and W. Kmiecik, 2005. Contents of macro and microelements in fresh and frozen dill (*Anethum graveolens* L.). Food Chem., 91: 737-743.

Soro, L.C., L.O. Atchibri, K.K. Kouadio and C. Kouame, 2012. Evaluation de la composition nutritionnelle des legumes-feuilles. J. Appl. Biosci., 51: 3567-3573.

Souzan, S. and H.A. Abd El-AAl, 2007. Minerals profile shelf life extension and nutritive value of fresh green leafy vegetables consumed in Egypt. Afr. Crop. Sci. Conf. Proc., 8: 1817-1826.

Turan, M., S. Kordali, H. Zengin, A. Dursun and Y. Sezen, 2003. Macro and micromineral content of some wild edible leaves consumed in Eastern Anatolia. Plant Soil Sci., 53: 129-137.

Umar, K.J., L.G. Hassan, S.M. Dangoggo, M. Inuwa and M.N. Amustapha, 2007. Nutritional content of *Melochia corchorifolia* (Linn.) Leaves. Int. J. Biol. Chem., 1: 250-255.

Vioa, S.Z. and A.R. Chaves, 2006. Antioxidant responses in minimally processed celery during refrigerated storage. Food Chem., 94: 68-74.

Wheeler, E.I. and R.E. Ferrel, 1971. Methods for phytic acid determination in wheat and wheat fractions. Cereal Chem., 84: 312-320.

Yaciuk, G. and J. Sofose, 1981. Food drying proceeding of a not shop held at Edmonton Albeta, 6th-9th July 1981.

Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.