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

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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 (Colocasia 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 (Busway 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
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°19’14″ 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 grinding) 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):

\[
\text{Carbohydrates (dry matter basis)}: \frac{100 \cdot (\text{proteins} + \text{lipids} + \text{ash} + \text{fibres})}{\text{w/v}}
\]

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

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.

Oxidant activity: Antioxidant assay was carried out using the 2,2-diphenyl-1-pycrilhydrozayl (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:

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

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/HNO₃ 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 EXCEL 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 EI-AAI, 2007). Ash content of the analyzed samples ranged after 5 days from 7.19±0.28% (C. ollitorius) to 19.16±0.6% (T. triangulare). The highest content of ash was observed for T. triangulare (16.31±0.19%) while C. ollitorius (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 EI-AAI, 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
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 caloric foods. These low caloric 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.

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 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)

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
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., 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 

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

<table>
<thead>
<tr>
<th>C. esculenta</th>
<th>Ca</th>
<th>Mg</th>
<th>P</th>
<th>K</th>
<th>Fe</th>
<th>Na</th>
<th>Zn</th>
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<tr>
<td>0 days</td>
<td>587.24±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>347.29±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>788.00±0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2281.63±2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143.73±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.45±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.29±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 days</td>
<td>507.81±5.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>265.91±8.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>752.76±6.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2114.26±8.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.53±7.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.46±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.01±2.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 days</td>
<td>470.92±1.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>211.30±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>725.61±5.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2098.20±6.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.81±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.32±0.43</td>
<td>26.06±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 days</td>
<td>419.91±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>209.22±0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>641.16±2.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1499.79±1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.99±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.18±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.17±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

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

<table>
<thead>
<tr>
<th>C. esculenta</th>
<th>Phytate/Ca</th>
<th>Phytate/Fe</th>
<th>Oxalate/Ca</th>
<th>Phytate/Mg</th>
<th>Phytate/P</th>
<th>Phytate/K</th>
<th>Phytate/Zn</th>
<th>Phytate/Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days</td>
<td>0.04</td>
<td>0.18</td>
<td>0.98</td>
<td>0.03</td>
<td>0.17</td>
<td>0.57</td>
<td>0.02</td>
<td>0.18</td>
</tr>
<tr>
<td>5 days</td>
<td>0.02</td>
<td>0.25</td>
<td>0.87</td>
<td>0.02</td>
<td>0.20</td>
<td>0.85</td>
<td>0.01</td>
<td>0.19</td>
</tr>
<tr>
<td>10 days</td>
<td>0.03</td>
<td>0.24</td>
<td>1.14</td>
<td>0.03</td>
<td>0.09</td>
<td>0.09</td>
<td>0.05</td>
<td>0.04</td>
</tr>
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</table>

### Antioxidant properties: Many plants have been identified as good sources of natural antioxidants such as tocopherols, vitamin C, carotenoids and polyphenols
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 performed in order to appreciate their palatability. Also a comparison with the major methods as the sun-drying.

**REFERENCES**


