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Research Paper

Nutritive and antioxidant evaluation of five leafy vegetables consumed in Western Côte d'Ivoire after refrigeration storage

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Abstract

Leafy vegetables are perishable plants foods subjected to continuous changes which conduct to postharvest losses. So, there is a need to develop preservation methods in order to extent their shelf life and availability for consumers. The main objective of this study was to evaluate the impact of refrigeration storage (4°C) on the nutritive and antioxidant properties of five leafy vegetables (*Abelmoschus esculentus*, *Celosia argentea*, *Ipomea batatas*, *Manihot esculenta* and *Myrianthus arboreus*) consumed in Western Côte d'Ivoire. The result of the study revealed small fluctuations in the proximate composition during refrigeration storage. After 15 days of storage the contents of moisture, ash, proteins and lipids ranged within the following intervals: 66.30 - 83.04%, 5.01 – 16.95%, 8.91 – 19.66% and 0.73 – 4.01%, respectively. The residual contents of minerals at 15 days of storage were: calcium (193.34 – 639.65 mg/100g), magnesium (134.10 – 507.94 mg/100g), potassium (1245.24 – 4606.13 mg/100g), iron (35.22 – 73.30 mg/100g) and zinc (29.60 – 69.67 mg/100g). The registered losses of oxalates, phytates, carotenoids and vitamin C were estimated at 15 days of storage as follow: 8.88 – 15.19%, 23.55 – 31.10%, 8.71 - 13.49%, 19.16 – 21.33%. The total phenolic content of the refrigerated leafy vegetables initially decreased from 0 days to 5 days (164.47 – 264.64 mg/100g to 160.38 – 260.18 mg/100g) and increased (165.77 – 275.10 mg/100g) at 10 days of storage coupled with antioxidant activity retention (68.72 – 75.70%). In view to these results, it would be necessary to preserve fresh leafy vegetables by refrigeration storage within a period not exceeding 7 days in order to contribute efficiently to the nutritional requirement and to the food security of Ivorian population.

Keywords: antioxidant properties, refrigeration storage, leafy vegetables, nutritive value.

Introduction

Sub-Saharan Africa grows an enormous variety of green leafy vegetables (GLVs). These plants have long been recognized as the cheapest and most abundant potential sources of carotenoids, antioxidant compounds, vitamins, pigments and minerals [1]. In addition, the ethno-botanical reports offers information on medicinal properties of GLVs like anti-diabetic, anti-histaminic, anti-carcinogenic hypolipidemic- antibacterial activity [2]. Green leafy vegetables are very popular used as food in many countries of the world. Associated with the new consumer's profile "rich in cash/poor in time", there is a demand for ready-to-eat products, also called ready-to-use, fresh cut or pre cut products [3].Green leafy vegetables are consumed fresh in salads, food dressing and flavoring ingredient for traditional cooking [4]. In many parts of Africa, leafy vegetables are also used as a side dish to accompany a thick starchy gruel that is the primary carbohydrate source [5,6].

Green leafy vegetables are living tissues subject to continuous changes after harvest. These physiological changes have conducted to postharvest losses of leafy vegetables which account for 50% of the total crops in Sub-saharan Africa [7]. The factors that shorten the shelf life of produce are enzymatic browning, microbial spoilage, dehydration, rapid wilting and senescence caused by continued respiration on and ethylene production [8]. Fresh leafy vegetables can be exposed to a variety of conditions which offer the potential for change in quality characteristics, including nutrient content [9]. Indeed, a lot of metabolic alternations, such as higher respiration, turgor loss, pigment oxidation and oxidation of phenolic compounds can affect the sensorial quality and reduce their shelf life and consumer's acceptability [10]. To extend the shelf-life of leafy vegetables, different ways of preserving have been developed. The two main methods are the sun-drying of fresh leaves and the sun-drying of blanched or cooked leaves. Nowadays, electrification of the rural areas has introduced new preservation technologies, including refrigeration and freezing of leaves [11]. These processing technologies transform these perishable leafy vegetables into safe, delicious and stable products. Indeed, refrigeration slows down the respiration of vegetables and helps maintain quality attributes such as appearance, texture mass loss, visual quality and cholorophyll, thereby extending the shelf life [12].

Among the twenty hundred and seven (207) leafy vegetables widely consumed in tropical Africa, about twenty (20) species of leafy vegetables belonging to 6 botanical families, are widely consumed by Ivorian populations after poor postharvest handling techniques [13,14]. Furthermore ethno-botanical studies have stated that most people in Western Côte d'Ivoire consume indigenous green leafy vegetables such as *Abelmoschus esculentus* "gombo", *Celosia argentea* "soko", *Ipomea batatas* "patate", *Manihot esculenta* "manioc" and *Myrianthus arboreus* "tikliti [14,15]. Earlier reports have highlighted the nutritive potential of these fresh leafy vegetables [16] but there is a lack of studies on their refrigeration storage as a preservation method. Thus, this research focused on determining the effect of postharvest preservation method such as refrigeration on the nutritive quality and antioxidant properties of the selected leafy vegetables.

Materials and Methods

Samples collection

Leafy vegetables (*Abelmoschus esculentus*, *Celosia argentea*, *Ipomea batatas*, *Manihot esculenta* and *Myrianthus arboreus*) were collected fresh and at maturity from cultivated farmlands located at Dabou (latitude: 5°19′14″ North; longitude: 4°22′59″West) (Abidjan District). The 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 Houphouët-Boigny, Abidjan-Ivory Coast).

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 [17]. 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. After refrigeration times, the 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 (gallic acid, β-carotene) and reagents (metaphosphoric acid, vanillin, Folin-Ciocalteu, DPPH) were purchased from Sigma-Aldrich. All chemicals used in the study were of analytical grade.

Nutritive properties

Physicochemical analysis

Proximate analysis was performed using official methods [18]. 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 [19]:

Carbohydrates (dry matter basis): 100 – (% proteins + % lipids + % ash + % fibres)

Calorific value (dry matter basis): (% proteins x 2.44) + (% carbohydrates x 3.57) + (% lipids x 8.37)

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

Mineral analysis

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 7500c inductively coupled argon plasma mass spectrometer (ICP-MS) method [20]. Calibrations were performed using external standards prepared from a 1000 ppm single stock solution made up with 2% nitric acid.

Oxalates and phytates determination

Oxalates content was performed using a titration method [21]. One (1) 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 contents were determined using the Wade's reagent colorimetric method [22]. A quantity (1 g) of dried powdered sample was mixed with 20 mL of hydrochloric acid (0.65 N) and stirred for 12 h with a magnetic. The mixture was centrifuged at 12000 rpm for 40 min. An aliquot (0.5 mL) of supernatant was added with 3 mL of Wade's reagent. The reaction mixture was incubated for 15 min and absorbance was measured at 490 nm by using a spectrophotometer (PG Instruments, England). Phytates content was estimated using a calibration curve of sodium phytate (10 mg/mL) as standard.

Antioxidant properties

Vitamin C and carotenoids determination

Vitamin C contained in analysed samples was determined by titration [23]. 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 (10) mL of this mixture was titrated to the end point with dichlorophenol-indophenol (DCPIP) 0.5 g/L.

Carotenoids were extracted and quantified by using a spectrophotometric method [24]. Two (2) 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 β-carotene (1 mg/mL) as standard.

Polyphenols determination

Polyphenols were extracted and determined using Folin–Ciocalteu's reagent [25]. 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.

Antioxidant activity

Antioxidant activity assay was carried out using the 2,2-diphenyl-1-pycrilhydrazyl (DPPH) spectrophotometric method [26]. 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:

Antioxidant activity (%) = 100 – [(Abs of sample – Abs of blank) x 100/Abs positive control]

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 properties

The proximate composition of refrigerated leafy vegetables examined in this study is presented in Table 1. The moisture contents of the selected leafy vegetables ranged from 68.43 ± 1.92 to 84.96 ± 0.44% at 0 days of storage while these values decreased (66.30 ± 0.03 and 83.04 ± 0.05%) at 15 days of refrigeration storage without significance difference ($p>0.05$) between values from 5 to 15 days of storage. During refrigeration storage, small fluctuation in moisture was most likely due to the respiration and others senescence-related metabolic processes [27]. Changes in moisture content in this study were minimized by using of polyethylene bags and cold storage. Indeed, packaging has the potential to reduce moisture loss, restricts the entrance of oxygen, lowers respiration, retards ethylene production, seals in flavor volatiles and retards discoloration [28]. Using of packaging also justifies the fact that it is important for fresh green leafy vegetables to remain well hydrated for maintaining sensory attributes as texture and appearance over period storage [4]. For fresh harvested leaves the contents of ash, proteins and lipids ranged between the following intervals: 9.03 – 23.56%, 9.19 – 23.39% and 1.47 – 4.09%, respectively. After 15 days of refrigeration storage the contents for the same physicochemical parameters were: 5.01 – 16.95%, 8.91 – 19.66% and 0.73 – 4.01%, respectively. The losses rate (28.05 – 44.5%) of ash during refrigeration storage could be explained by the loss of water (transpiration phenomena) which may carry minerals off [4]. In spite of ash losses, the refrigerated leafy vegetables may be considered as good sources of minerals when compared to values obtained for cereals and tubers [29]. As concern proteins and lipids contents, their reductions (3.04 – 15.94% and 1.95 – 50.34%, respectively) could be linked to postharvest metabolic disorders such as protein hydrolysis and lipid oxidation which occur during refrigeration storage[30].

Refrigeration processing of the studied leafy vegetables resulted in a significant increase (1.37 – 21.67 %) in their crude fibres content and this phenomenon may be due to textural changes such as wilting and shriveling [31]. Therefore adequate intake of refrigerated leafy vegetables as desserts could lower the risk of, constipation, diabetes and colon cancer [32]. The estimated calorific values (176.35 – 240.60 kcal/100 g) of the selected refrigerated leafy vegetables (15 days of storage) agree with general observation that vegetables have low energy values due to their low crude fat and relatively high level of moisture [33].

Mineral composition of the refrigerated leafy vegetables is shown in table 2. The residual contents of minerals at 15 days of storage were significantly different ($p < 0.05$): calcium (193.34 – 639.65 mg/100g), magnesium (134.10 – 507.94 mg/100g), potassium (1245.24 – 4606.13 mg/100g), iron (35.22 – 73.30 mg/100g) and zinc (29.60 – 69.67 mg/100g). With regard to these residual values, refrigerated leafy vegetables could be recommended as salads for target populations as infants, pregnant woman and old people in order to cover mineral requirements. Indeed, the standard mineral requirements for human are: calcium (1000 mg/day); magnesium (400 mg/day), iron (8 mg/day) and zinc (6 mg/day) [34]. Calcium and phosphorus play important role in growth and maintenance of bones, teeth and muscles [35]. As regards magnesium, this mineral is known to prevent muscle degeneration, growth retardation, congenital malformations and bleeding disorders [36]. Iron plays important role in prevention of anemia while zinc is important for vitamin A and vitamin E metabolisms [37,34].

The impact of refrigeration storage on anti-nutritional factors (oxalates and phytates) contents is depicted in figure 1. Levels losses at 5 days of storage were 2.89 – 10.04 % and 3.34 – 25.32 % for oxalates and phytates, respectively. At 15 days of storage these values were 8.88 – 15.19 % and 23.55 – 31.10 %, respectively. The reductions in oxalates and phytates contents during refrigeration of leafy vegetables may be due to postharvest physiology which implies transpiration and hydrolytic enzymatic activities [27]. In nutritional point of view, the oxalates and phytates losses 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 [38]. In order to predict the bioavailability of calcium and iron, anti-nutrients to nutrients ratios of roasted leafy vegetables were calculated (Table 3). The calculated [phytates]/[Ca] ratio in all the refrigerated species were below the critical level of 2.5 which is known to impair calcium bioavailability [39].

Antioxidant properties

Total carotenoids and vitamin C decreased during 15 days of refrigeration storage as depicted in figure 2. For carotenoids, losses at 15 days of storage were estimated to 8.71 to 13.49% while for vitamin C content, a reduction (19.16 – 21.33 %) was highlighted at 15 days of storage. Our experiments established that packaging and cold storage were effective in extending the shelf life of fresh leafy vegetables to 15-days, compared to two days under the traditional (ambient) marketing system. However the lowest losses observed for carotenoids and vitamin C contents may be due to auto-oxidation and enzymatic degradation during postharvest handling techniques [4]. The effect of refrigeration storage on polyphenols content and antioxidant activity of the selected leafy vegetables is depicted in figure 3. The total phenolic content of the refrigerated leafy vegetables initially decreased from 0 days to 5 days (164.47 – 264.64 mg/100g to 160.38 – 260.18 mg/100g) and increased (165.77 – 275.10 mg/100g) at 10 days of storage. During storage at 4°C, the trends in phenolic changes appeared similar with a cyclic nature.

This trend was portrayed as an initial decrease in phenolic content, followed by an increase back to original level and subsequent repeat of this trend. This trend is comparable with those obtained in previous studies [40]. The basis of the increase could not be categorically stated, however, it could be attributed to the possible breakdown of the tannins present in the vegetables to simple phenol [41]. In addition, the refrigeration storage highlighted a good impact on antioxidant activity retention (68.72 – 75.70%) of the selected leafy vegetables after 15 days of storage. Thus, the consumption of refrigerated leafy vegetables as salads could be advantageous for lower cellular ageing process in human body because polyphenols are known for their antioxidant and scavenging properties [42,43].

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

	Moisture (%)	Ash* (%)	Fibres* (%)	Proteins* (%)	Lipids* (%)	Carbohydrates* (%)	Calorific energy* (kcal/100g)
A. esculentus							
0 days	83.91 ± 0.08a	11.90 ± 0.10a	15.66 ± 0.05c	9.19 ± 0.15a	3.38 ± 1.59a	59.87 ± 1.90a	264.44 ± 2.51a
5 days	80.02 ± 0.69b	10.90 ± 0.16a	21.37 ± 0.28b	9.02 ± 0.00a	3.07 ± 0.00a	55.64 ± 0.12b	246.33 ± 0.42b
10 days	80.01 ± 0.17b	10.48 ± 0.10a	23.42 ± 0.16a	8.94 ± 0.00b	2.99 ± 0.00b	54.16 ± 0.05c	240.20 ± 0.19c
15 days	80.45 ± 0.01b	10.10 ± 0.05a	24.63 ± 0.02a	8.91 ± 0.00b	2.75 ± 0.00c	53.61 ± 0.03d	236.44 ± 0.10d
M. esculenta							
0 days	68.43 ± 1.92a	9.03 ± 2.12a	26.23 ± 0.31a	23.39 ± 0.71a	4.09 ± 0.02a	37.27 ± 3.16c	224.35 ± 5.67b
5 days	66.34 ± 0.07a	5.68 ± 0.04b	26.45 ± 0.40a	20.39 ± 0.00b	4.01 ± 0.00a	43.46 ± 0.43b	238.48 ± 1.55a
10 days	66.33 ± 0.06a	5.42 ± 0.06c	26.49 ± 0.33a	19.52 ± 0.00c	3.75 ± 0.00b	44.70 ± 0.13b	238.61 ± 0.49a
15 days	66.30 ± 0.03a	5.01± 0.03d	26.52 ± 0.34a	19.66 ± 0.07c	3.60 ± 0.00c	46.20 ± 0.33a	240.60 ± 1.18a
I. batatas							
0 days	79.66 ± 3.57a	23.56 ± 0.26a	21.50 ± 0.82a	15.52 ± 0.40a	2.63 ± 0.06a	36.79 ± 3.41b	232.91 ± 5.78a
5 days	78.28 ± 0.15a	16.28 ± 0.25b	24.60 ± 3.00a	15.23 ± 0.00a	2.22 ± 0.00b	40.95 ± 2.73a	201.95 ± 9.76c
10 days	78.05 ± 0.55a	15.19 ± 0.00c	24.88 ± 1.12a	14.65 ± 0.00b	2.12 ± 0.00c	42.06 ± 1.37a	203.66 ± 4.90b
15 days	78.67 ± 0.64a	15.66 ± 0.24c	24.49 ± 3.85a	14.55 ± 0.50b	1.32 ± 0.00d	43.97 ± 0.09a	203.54 ± 1.18b
C. argentea							
0 days	84.96 ± 0.44a	22.10 ± 0.75a	30.83 ± 1.61b	9.77 ± 0.10a	1.79 ± 0.20a	35.52 ± 0.60b	165.62 ± 6.40b
5 days	83.83 ± 0.69a	17.32 ± 0.08b	31.62 ± 0.49b	9.52 ± 0.00a	1.70 ± 0.00a	39.83 ± 0.57a	179.67 ± 2.05a
10 days	83.19 ± 0.89a	16.95 ± 0.61b	33.58 ± 2.16a	9.45 ± 0.00b	1.65 ± 0.00b	38.36 ± 1.54a	173.83 ± 5.51a
15 days	83.04 ± 0.05a	16.72 ± 0.21b	33.00 ± 1.00a	9.35 ± 0.00b	1.55 ± 0.00c	39.37 ± 1.21a	176.35 ± 4.33a
M. arboreus							
0 days	81.30 ± 0.35a	11.73 ± 0.76a	12.19 ± 0.73b	16.95 ± 0.05a	1.47 ± 0.02a	56.70 ± 1.74a	259.5 ± 5.26a
5 days	80.97 ± 2.32a	9.42 ± 0.15b	13.01 ± 0.00a	15.52 ± 0.19b	1.18 ± 0.00b	54.86 ± 0.10b	243.62 ± 0.83b
10 days	80.41 ± 0.18a	8.44 ± 0.15c	13.83 ± 0.10a	15.41 ± 0.29b	0.91 ± 0.00c	54.40 ± 0.32b	239.45 ± 1.86c
15 days	80.63 ± 0.57a	7.71 ± 0.02d	13.17 ± 0.10a	15.12 ± 0.02b	0.73 ± 0.00d	54.26 ± 0.04b	236.73 ± 0.20c

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.

Table 2
Mineral composition (mg/100g dry matter) of refrigerated leafy vegetables consumed in Western Côte d'Ivoire

	Ca	Mg	P	K	Fe	Na	Zn
A. esculentus							
0 days	468.45 ± 0.55a	364.11 ± 0.43a	671.50 ± 0.79a	1844.25 ± 8.22a	130.95 ± 0.15a	35.76 ± 0.04a	41.45 ± 0.04a
5 days	418.93 ± 8.69b	338.21 ± 3.24b	664.90 ± 3.80b	1801.56 ± 2.10b	85.48 ± 1.77b	32.20 ± 0.15b	40.54 ± 1.09a
10 days	403.15 ± 5.70c	324.05 ± 6.00c	663.73 ± 2.51b	1790.71 ± 4.03c	77.86 ± 1.10c	31.50 ± 0.50b	40.17 ± 0.62a
15 days	391.29 ± 2.73d	308.37 ± 3.36d	628.24 ± 4.81c	1732.86 ± 4.43d	71.40 ± 0.64d	30.25 ± 0.00c	39.70 ± 0.27a
M. esculenta							
0 days	296.66 ± 0.46a	229.45 ± 0.35a	759.81 ± 1.18a	2306.09 ± 3.61a	48.69 ± 0.07a	18.30 ± 0.02a	45.48 ± 0.31a
5 days	217.36 ± 2.43b	157.39 ± 1.76b	717.82 ± 9.15b	2106.57 ± 6.14b	39.59 ± 1.00b	17.15 ± 0.60b	44.79 ± 0.50a
10 days	207.63 ± 3.25c	145.72 ± 2.28c	665.88 ± 1.42c	1994.97 ± 9.68c	38.23 ± 1.64b	17.05 ± 1.00b	44.52 ± 0.41a
15 days	193.34 ± 1.63d	134.10 ± 1.13d	663.46 ± 5.61c	1953.45 ± 5.21d	38.18 ± 0.91b	15.75 ± 0.25c	44.27 ± 0.01b
I. batatas							
0 days	898.83 ± 0.53a	501.75 ± 0.30a	494.76 ± 0.29a	1377.81 ± 0.22a	53.54 ± 0.03a	404.30 ± 3.62a	30.10 ± 0.01a
5 days	674.07 ± 1.35b	441.84 ± 3.67b	384.83 ± 1.00b	1303.50 ± 4.80b	51.65 ± 3.23a	392.55 ± 3.68b	29.71 ± 0.42a
10 days	619.58 ± 3.72c	407.30 ± 1.23c	347.18 ± 0.90c	1251.28 ± 8.71c	48.93 ± 2.32b	375.48 ± 8.31c	29.62 ± 0.03a
15 days	596.45 ± 1.92d	402.65 ± 0.02d	317.05 ± 3.45d	1245.24 ± 5.75c	48.11 ± 2.12b	371.77 ± 8.27d	29.60 ± 0.02a
C. argentea							
0 days	788.02 ± 0.50a	981.31 ± 0.62a	650.37 ± 0.41a	4987.15 ± 3.19a	285.31± 0.18a	42.26 ± 0.02a	62.01 ± 0.03a
5 days	661.43 ± 4.58b	564.61 ± 3.91b	550.87 ± 7.98b	4721.18 ± 9.57b	73.90 ± 0.51b	38.78 ± 1.26b	60.51 ± 0.41b
10 days	652.01 ± 3.44c	563.05 ± 2.88b	528.64 ± 6.20c	4685.08 ± 8.50c	73.38 ± 4.07b	36.79 ± 0.75b	60.31 ± 1.55b
15 days	639.65 ± 1.62d	507.94 ± 9.23c	514.24 ± 0.89d	4606.13 ± 9.40d	73.30 ± 1.27b	32.15 ± 0.40c	60.13 ± 0.32b
M. arboreus							
0 days	436.64 ± 0.52a	354.23 ± 0.42a	283.19 ± 0.34a	2350.58 ± 2.83a	79.54 ± 0.09a	20.83 ± 0.02a	75.20 ± 0.09a
5 days	362.59 ± 8.16b	264.45 ± 5.95b	223.57 ± 1.54b	1947.88 ± 7.00b	35.56 ± 0.80b	17.44 ± 1.29b	70.30 ± 0.66b
10 days	319.23 ± 8.02c	231.25 ± 5.81c	219.81 ± 8.09b	1941.72 ± 5.00b	35.40 ± 0.92b	16.89 ± 1.27c	70.07 ± 0.70b
15 days	292.94 ± 1.07d	241.72 ± 0.88d	204.30 ± 2.84c	1935.61 ± 3.61c	35.22 ± 0.18b	16.85 ± 0.23c	69.67 ± 0.05b

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.

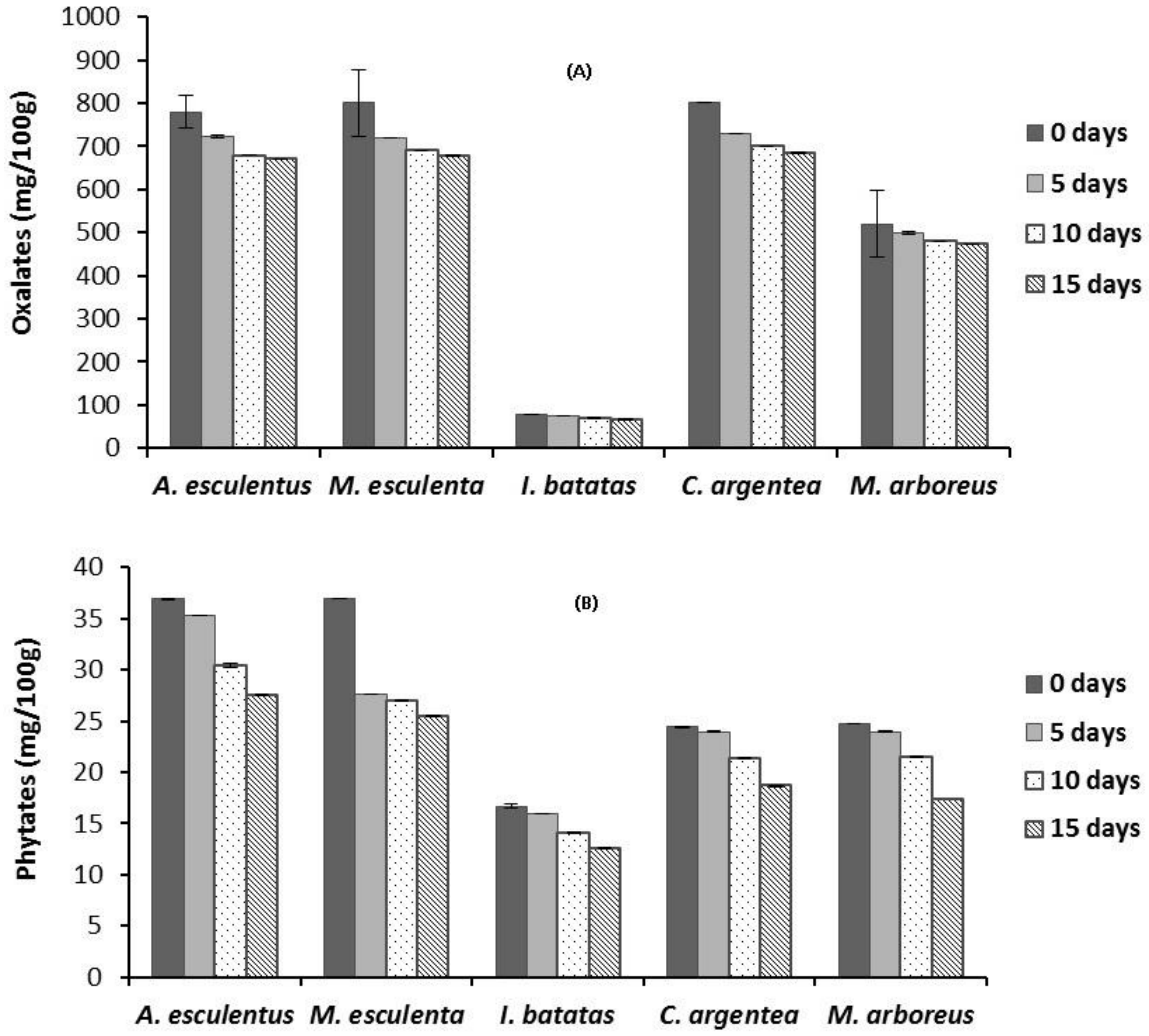
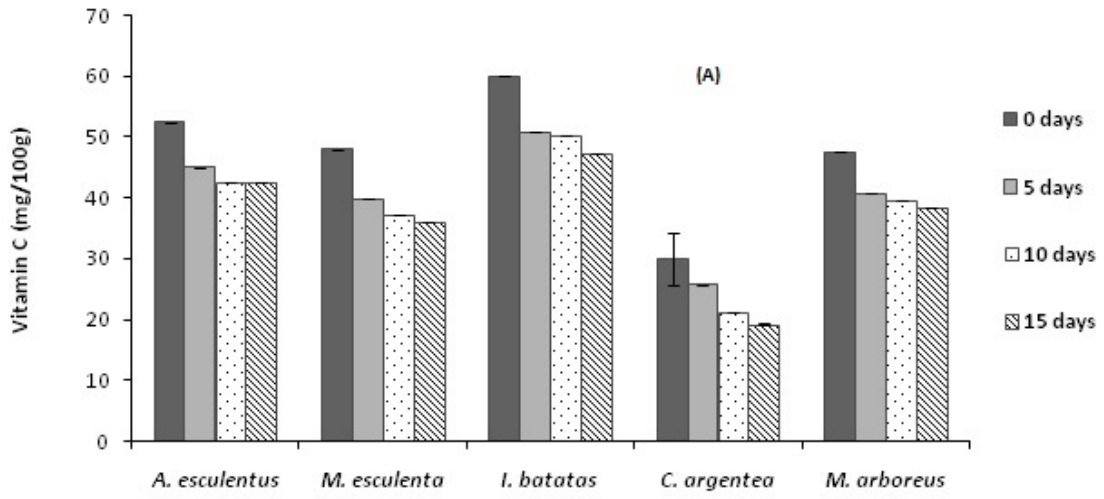


Figure 1: Effect of refrigeration storage (4°C) on oxalates (A) and phytates (B) contents of leafy vegetables consumed in Western Côte d'Ivoire



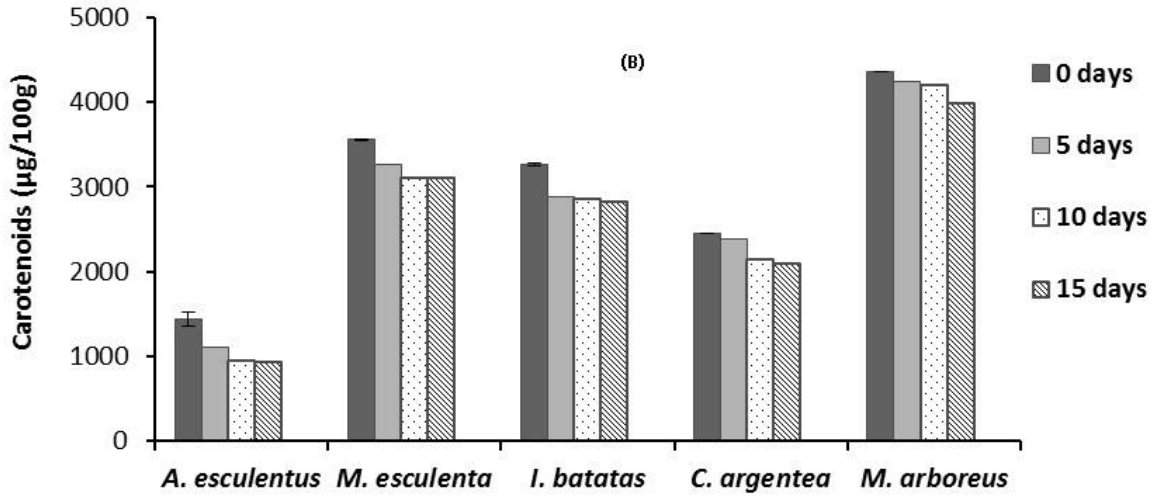


Figure 2: Effect of refrigeration storage (4°C) on vitamin C (A) and carotenoids (B) contents of leafy vegetables consumed in Western Côte d'Ivoire

Table 3: Anti-nutritional factors/mineral ratios of refrigerated leafy vegetables (4°C) consumed in Western Côte d'Ivoire

	Phytates/Ca	Phytates/Fe	Oxalates/Ca
A. esculentus			
0 days	0.07	0.28	1.66
5 days	0.08	0.41	1.72
10 days	0.07	0.39	1.68
15 days	0.07	0.38	1.71
M. esculenta			
0 days	0.12	0.75	2.69
5 days	0.12	0.69	3.31
10 days	0.12	0.70	3.32
15 days	0.13	0.66	3.50
I. batatas			
0 days	0.01	0.31	0.08
5 days	0.02	0.31	0.11
10 days	0.02	0.28	0.11
15 days	0.02	0.26	0.11
C. argentea			
0 days	0.03	0.08	1.01
5 days	0.03	0.32	1.10
10 days	0.03	0.29	1.07
15 days	0.03	0.25	1.07
M. arboreus			
0 days	0.05	0.31	1.19
5 days	0.06	0.67	1.37
10 days	0.07	0.61	1.50
15 days	0.06	0.49	1.61

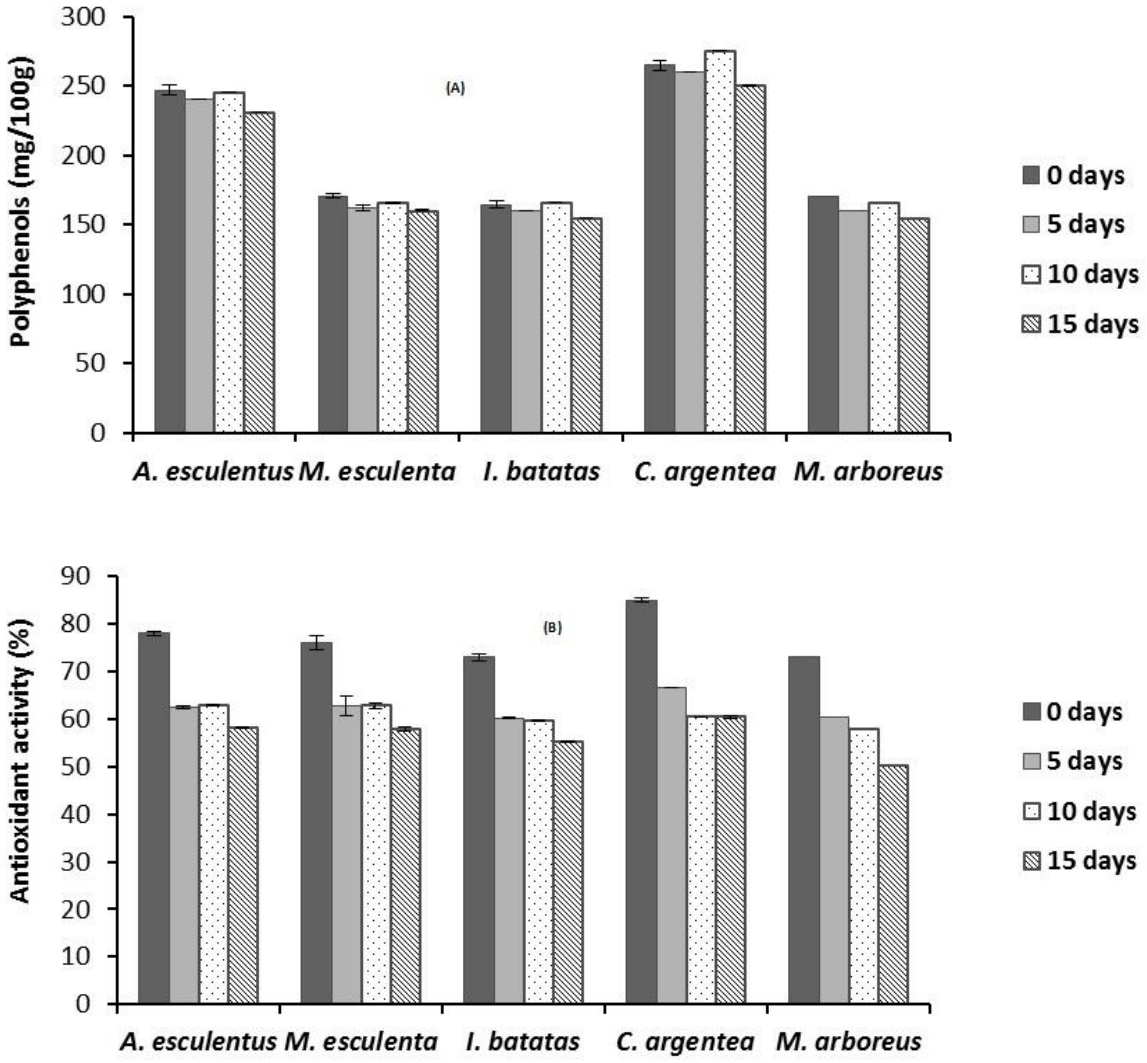


Figure 3: Effect of refrigeration storage (4°C) on polyphenols content (A) and antioxidant activity (B) of leafy vegetables consumed in Western Côte d'Ivoire

Conclusion

Leafy vegetables cultivated and consumed in Sub-saharan Africa contain significant levels of nutrients that are essential for human health. The result of this study revealed that refrigeration storage could enhance the shelf life and nutritional quality of the selected leafy vegetables by slowing down metabolic processes such as respiration, ethylene production and in general, enzyme activity. Indeed, only slight fluctuations in nutrient and antioxidant composition were observed during this experiment. In addition losses in anti-nutrients (oxalates, phytates) contents might have asserted a beneficial effect on bioavailability of minerals like iron and calcium. In view to the results obtained, it would be necessary to preserve fresh leafy vegetables by refrigeration storage within a period not exceeding 7 days in order to contribute efficiently to the nutritional requirement and to the food security of Ivorian population.

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