

RESEARCH ARTICLE

(Open Access)**Evaluation of Freezing Effect on Nutritive Value and Antioxidant Properties of Leafy Vegetables Consumed in Northern Côte d'Ivoire**PATRICIA OULAI^{1*}, LESOY ZOUE¹, NIAMKEY ADOM¹, SEBASTIEN NIAMKE¹¹Biotechnology Laboratory, Biosciences Faculty, University Félix Houphouët-Boigny, 22 BP 582 Abidjan 22**Abstract**

Postharvest handling is one of the major factors affecting the nutritive value of leafy vegetables and it is important to preserve these nutrients by appropriate processing and storage. Thus, this study was mainly conducted to determine the influence of freezing (-18°C) during 1, 2 and 3 months on the nutrients contents of five leafy vegetables (*Hibiscus sabdariffa*, *Amaranthus hybridus*, *Andersonia digitata*, *Vigna unguiculata* and *Ceiba pentandra*) widely consumed in Northern Côte d'Ivoire. The results showed that the longer period (more than 1 month) of freezing affected the nutritional value and the registered losses at 3 months of freezing were as follow: ash (4.21 - 16.86%), proteins (3.39 - 13.42%), vitamin C (9.56 - 24.18%), carotenoids (15.38 - 34.19%), total phenolics (2.06 - 19.67%), oxalates (16.41 - 31.28%), phytates (25.12 - 51.26%). The residual contents of minerals were: Ca (360.52 - 824.02 mg/100g), Mg (193.11 - 503.45 mg/100g), K (107.69 - 387.39 mg/100g), Fe (46.84 - 137.18 mg/100g) and Zn (15.05 - 26.45 mg/100g). In view to some essential nutrients losses it would be necessary to preserve traditional leafy vegetables by freezing storage within a period not exceeding 1 month in order to contribute efficiently to the nutritional requirements and to the food security of Ivorian population.

Keywords: Antioxidant properties, freezing processing, leafy vegetables, nutritive quality.

1. Introduction

African Leafy Vegetables (ALVs) are herbaceous plants whose parts are eaten as supporting food or main dishes and they may be aromatic, bitter or tasteless [1]. The utilization of leafy vegetables is part of Africa's cultural heritage and they play important roles in the customs, traditions and food culture. There are more than 45,000 plant species in sub-Saharan Africa of which about 1000 can be eaten as green leafy vegetables [2]. In Côte d'Ivoire (Ivory Coast), twenty (20) species of leafy vegetables belonging to 6 botanical families are widely consumed and cultivated [3, 4]. Furthermore, the consumption of these leafy vegetables is linked to the region and ethno-botanical studies have stated that most people in Northern Côte d'Ivoire (Ivory Coast) consume indigenous leafy vegetables such as *Amaranthus hybridus* "boronbrou", *Andersonia digitata* "baobab", *Ceiba pentandra* "fromager", *Hibiscus sabdariffa* "dah" and *Vigna unguiculata* "haricot" by cooking recipes made of sauces and starchy staples foods [3, 5].

Leafy vegetables are important protective foods and highly beneficial for the maintenance of health and prevention of diseases. Indeed, they contain non-nutrient bioactive photochemical that have been linked to protection against cardiovascular and other degenerative diseases [6]. In addition, ALVs play a key role in income generation and subsistence because they are cheap, available and accessible and provide millions of African consumers with health promoting compounds such as vitamins, minerals and antioxidants [7 - 9]. They are also compatible in use with starchy staples and represent a quality food to the poor segment of population both in urban and rural areas where malnutrition is widespread [10].

Despite these enormous potentialities, fresh leafy vegetables have a short shelf life and they become after several days unsafe or undesirable for consumption. In fact, leafy vegetables are highly perishable in nature and subjected to rapid deterioration by microorganisms, enzymes, or oxidation reactions. Storage and processing technologies have been utilized for centuries to transform these perishable vegetables into safe, delicious and stable products. Refrigeration and

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(Accepted for publication on December 15, 2015)

freezing slow down the respiration of fruits and vegetables and allows for longer shelf lives while canning and drying transform perishable fruits and vegetables into products that can be consumed and transported safely to consumers all over the world [11].

Considering that the concentration of the nutrients and anti-nutrients in vegetables are mostly affected by post-harvest treatments, this research focused on determining the effect of freezing processing on the nutritive value and antioxidant properties of five leafy vegetables viz, *Hibiscus sabdariffa*, *Amaranthus hybridus*, *Adansonia digitata*, *Vigna unguiculata* and *Ceiba pentandra* in order to predict the delay of post-harvesting preservation and provide nutritional information to consumers.

2. Material and Methods

2.1. Samples Collection

Leafy vegetables (*Amaranthus hybridus*, *Adansonia digitata*, *Ceiba pentandra*, *Hibiscus sabdariffa* and *Vigna unguiculata*) 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-Côte d'Ivoire).

2.2. Samples Processing

The fresh leafy vegetables were destalked, washed with deionized water and edible portions were separated from non edible portions. The edible portions were allowed to drain at ambient temperature and separated into two portions of 250 g each. The first portion (250 g) was packed in polyethylene bags and stored at -18°C in freezer for one, two and three months. After freezing period, the leaves were defrosted at ambient temperature and subjected to drying in oven (Memmert, Germany) at 60°C for 72 h [12]. The dried samples were ground with a laboratory crusher (Culatti, France) equipped with a 10 µm mesh sieve and stored in air-tight containers for further analysis. The second 250 g portion of fresh leafy vegetables was used as the control (raw) and subjected to the same treatment of drying and gridding.

2.3. Nutritive properties

Ash, proteins and lipids were determined using official methods [13]. For crude fibres, 2 g of dried powdered sample were digested with 0.25 M H₂SO₄ and 0.3 M NaOH solution. The insoluble residue obtained was washed with hot water and dried in an oven (Memmert, Germany) at 100 °C until constant weight. The dried residue was then incinerated, and weighed for the determination of crude fibers content. Carbohydrates and calorific value were calculated using the following formulas [14]:

Carbohydrates: $100 - (\% \text{ moisture} + \% \text{ proteins} + \% \text{ lipids} + \% \text{ ash} + \% \text{ fibres})$.

Calorific value: $(\% \text{ proteins} \times 2.44) + (\% \text{ carbohydrates} \times 3.57) + (\% \text{ lipids} \times 8.37)$. The results of ash, fibers, proteins, lipids and carbohydrates contents were expressed on dry matter basis.

Mineral analysis was performed as follow: the dried powdered samples (5g) 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 7500c inductively coupled argon plasma mass spectrometer (ICP-MS). Calibrations were performed using external standards prepared from a 1000 ppm single stock solution made up with 2% nitric acid.

For antinutritional factors, oxalates and phytates contents were determined. Oxalates contents was performed using a titration method [15]. For this, 1 g of dried powdered sample was weighed into 100 mL conical flask. A quantity of 75 mL of H₂SO₄ (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 KMnO₄ solution (0.05 M) to the end point. Phytates contents were determined using the Wade's reagent colorimetric method [16]. A quantity of 1.0 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.

2.4. Antioxidant properties

Vitamin C content was determined by titration [17]. About 10 g of ground 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 [18]. For this, 2 g of ground 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 α -carotene (1 mg/mL) as standard.

Polyphenols were extracted and determined using Folin-Ciocalteu's reagent [19]. A quantity of 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 of 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 assay was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method [20]. 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), filtered through Whatman No.4 filter paper and 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:

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

2.5. Statistical Analysis

All the analyses were performed in triplicate and data were expressed as means \pm standard deviation. One way analysis of variance (ANOVA) was performed by using STATISTICA 7.1 (StatSoft) for significant differences stated at $p < 0.05$.

3. Results and Discussion

3.1. Nutritive Value

The proximate composition of leafy vegetables stored at -18°C is presented in Table 1. During freezing storage, slight variation in ash, fibers, protein, lipids and carbohydrates were observed compared to fresh samples. The values of ash contents were in the range of 8.43 – 24.35% after 1 month of storage and the interval for 3 months were 7.82 – 23.09%. Besides the decrease rate of ash contents from 1.16 - 5.14% to 4.21 - 16.86% at 1 and 3 months respectively, the studied leafy vegetables may be considered as a good source of minerals when compared to values (2 – 10%) obtained for cereals and tubers [21]. As concern protein contents, freezing at -18°C caused 0.04 to 1.45% reduction after 3 months of storage with residual values ranged from 12.80 to 20.92%. It's important to indicate that plant foods which provide more than 12% of their calorific value from proteins could be considered as source of proteins for human nutrition [22]. This suggests that all the studied leafy vegetables could provide cheap and available proteins for rural communities by replacing animal proteins. The relatively low values of lipids contents (1.06 – 4.70%) in the frozen samples corroborate the findings of many authors which showed that leafy vegetables are poor sources of fat [23]. The slight decrease observed in ash, protein and fat contents could be due to the formation of ice crystals which are capable of lacerating the cell membranes resulting in cell leakage and its contents [24]. Freezing storage at -18°C of the selected leafy vegetables resulted in small increase (0.28-1.11%) to (1.59-4.17%) of their fiber contents after 1 and 3 months respectively. This increase in total dietary fiber may be due to wilting caused by lower temperature and the relatively high level (12.62 – 32.00%) of crude fibers in these leafy vegetables would be advantageous for their active role in the regulation of intestinal transit [25].

Table 1. Proximate composition of frozen leafy vegetables consumed in Northern Côte d'Ivoire

	Moisture (%)	Ash*	Fibres*	Proteins*	Lipids*	Carbohyd*	Energy* (kcal/100g)
<i>H. sabdariffa</i>							
Raw	86.05±0.35b	10.30±0.10a	14.27±1.70a	14.47±0.10a	4.75±0.15a	56.21±1.78a	275.71±0.55a
1 month	89.07±0.39a	10.03±1.06a	14.31±2.80a	14.30±2.44a	4.70±0.04a	56.66±3.90a	276.50±3.64a
2 months	91.19±1.11a	9.75±0.15a	14.53±3.53a	14.04±0.00a	4.56±0.01a	57.12±3.40a	276.34±2.02a
3 months	91.53±0.19a	9.22±0.08b	14.68±2.82a	13.66±1.36a	4.39±0.03b	58.05±2.71a	277.32±9.94a
<i>A. hybridus</i>							
Raw	72.98±0.10b	8.59±1.34a	17.80±0.30a	13.25±0.13a	2.15±0.01a	58.21±1.78a	305.19±7.73a
1 month	87.44±0.44a	8.43±0.36a	17.08±4.94a	13.17±6.75a	2.06±0.01a	59.26±4.55a	260.95±6.42b
2 months	88.45±0.20a	8.07±1.30a	17.23±7.07a	13.04±0.00a	1.97±0.03a	59.69±5.80a	261.40±2.46b
3 months	88.60±1.44a	7.82±0.15b	17.62±1.49a	12.80±1.28a	1.86±0.03b	55.02±2.24a	243.22±4.29c
<i>A. digitata</i>							
Raw	77.63±0.15c	10.97±0.40a	12.56±0.45a	18.08±0.10a	2.18±0.03a	56.23±1.25a	267.03±4.00a
1 month	79.25±0.19b	10.62±0.22a	12.62±4.94a	17.90±4.14a	2.00±0.07b	57.74±5.23a	259.20±8.36a
2 months	80.48±0.56b	10.11±0.23a	12.80±1.06a	17.24±2.87a	1.70±0.04c	59.70±0.75a	271.93±3.30a
3 months	84.79±0.27a	9.12±0.19b	13.01±1.20a	17.19±3.94a	1.12±0.03d	51.49±2.86b	240.00±6.31b
<i>V. unguiculata</i>							
Raw	80.04±0.56b	11.17±0.25a	18.00±0.92a	21.96±0.30a	4.23±0.25a	44.64±1.72a	248.35±10.33a
1 month	87.59±0.19a	11.04±0.90a	18.20±1.06a	21.95±0.00a	4.08±0.11b	44.73±0.30a	247.35±0.11a
2 months	88.72±0.55a	10.91±0.58a	18.52±2.15a	21.30±1.26a	3.90±0.06b	45.36±1.61a	246.56±5.34a
3 months	89.07±0.00a	10.70±0.0b2	18.75±2.82a	20.92±3.57a	3.46±0.06c	46.17±2.93a	244.83±4.33a
<i>C. pentandra</i>							
Raw	70.45±0.52a	25.67±1.12a	31.50±1.50a	15.20±0.05a	1.39±0.22a	26.30±0.11b	142.61±7.74b
1 month	71.12±1.81a	24.35±0.36a	31.65±1.48a	14.98±1.29a	1.22±0.03b	27.80±2.09b	127.72±5.98c
2 months	72.34±0.44a	23.33±0.30b	31.75±0.70a	13.85±4.69a	1.06±0.07c	44.99±1.11a	203.29±3.30a
3 months	74.78±1.62a	23.09±0.13b	32.00±3.07a	13.16±3.56a	0.96±0.08c	45.30±3.03a	201.82±4.42a

Data are represented as means ± SD (n=3). Means in the column for each leafy vegetable with no common letter differ significantly (p<0.05). *: values given on dry matter basis.

The mineral profile of the frozen leafy vegetables is given in Table 2. The mineral composition of vegetables depends on species, cultivar, plant age, production techniques, postharvest handling and other environmental factors [11, 26, 27]. The residual contents of minerals after one month of freezing at -18 °C were: Ca (360.52 - 824.02 mg/100g), Mg (193.11 - 503.45 mg/100g), K (107.69 - 387.39 mg/100g), Fe (46.84 - 137.18 mg/100g) and Zn (15.05 - 26.45 mg/100g). The declined in the minerals viz. Ca, Mg, K, Fe and Zn contents could be attributed to the physical injuries of vegetable cell wall which lead to the release of cell content including some of the minerals [28 - 32].

The freezing processing used in this study slightly decreased the contents of oxalates and phytates compared to the contents of fresh leafy vegetables (Figure 1). Oxalates and phytates are

considered as anti-nutritional factors which reduce minerals bioavailability [33]. In order to predict the bioavailability of Ca and Fe, anti-nutrients to nutrients ratios of frozen samples were calculated (Table 3). It was observed a decrease of anti-nutritional factors in the stored samples at -18 °C compared to fresh green leafy vegetables. Excepted *H. sabdariffa* leaves, the calculated [oxalates]/[Ca] ratios in all the species were below the critical level of 2.5 known to impair calcium bioavailability [33]. It was also observed that the calculated [phytates]/[Fe] ratios of *H. sabdariffa* leaves were above the critical level of 0.4. This implies that the phytates of these leafy vegetables may hinder iron bioavailability [34]. However, the [phytates]/[Fe] ratios could be considerably reduced after processing such as soaking, boiling or frying [35].

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

	Ca	Mg	P	K	Fe	Na	Zn
<i>H. sabdariffa</i>							
Raw	402.21±0.55a	295.93±0.41a	407.59±0.00a	816.19±1.12a	102.08±0.14a	23.46±0.03a	26.06±0.04a
1 month	390.79±4.69b	248.38±3.78b	240.09±9.15b	718.17±3.74b	53.49±2.15b	21.61±3.95a	21.21±0.66b
2 months	370.01±3.21c	229.65±2.60c	168.09±4.72c	639.08±2.22c	52.14 ±0.20b	18.89±1.04a	18.33±0.01c
3 months	360.52±5.85d	217.93±1.27d	107.69±2.60d	600.68±1.72d	47.10±0.09c	14.98±0.57b	15.05±0.19d
<i>A. hybridus</i>							
Raw	932.6 ±0.55a	497.75±0.49a	368.69±0.00a	1989.32±2.12a	77.88± 0.05a	94.39±0.04a	31.73±0.04a
1 month	906.01±3.22b	472.21±3.74b	354.34±2.45b	1895.89±8.94b	69.39±0.37b	90.64±5.15a	28.34±0.20b
2 months	892.26±7.57c	469.33±3.32b	346.46±2.71c	1745.95±7.88c	63.71±0.96c	78.46±4.49b	23.81±0.38c
3 months	824.02±5.97d	443.44±3.09c	220.93±3.69d	1736.62±1.76d	59.65±0.08d	72.83±0.15c	18.25±0.16d
<i>A. digitata</i>							
Raw	496.26±2.20a	264.36±1.17a	761.63±0.00a	1856.90±8.23a	106.27±0.47a	37.13±0.12a	22.61±0.10a
1 month	454.72±7.65b	216.97±3.23b	480.49±5.84b	1776.40±3.12b	66.70±0.22b	36.03±1.81a	20.76±0.28b
2 months	437.37±4.89c	210.69±3.04b	440.43±3.85c	1646.95±9.52c	60.60±0.21c	30.90±1.34b	17.98±0.08c
3 months	412.22±2.76d	193.11±2.50c	330.34±5.39d	1494.53±2.54d	57.69±0.15d	28.82±1.39b	16.34±0.01d
<i>V. unguiculata</i>							
Raw	439.54±0.56a	341.34±0.18a	309.04±0.00a	718.11± 0.91a	91.45± 0.12a	33.32±0.02a	40.83±0.04a
1 month	415.23±8.86b	318.61±8.53b	288.91±8.46b	688.24±5.12b	59.89±2.55b	31.11±7.03a	37.34±0.15b
2 months	405.70±9.38b	276.98±1.69c	179.07±7.66c	555.81±7.69c	48.92±1.43c	27.64±2.98a	33.05±0.15c
3 months	377.25±0.86c	239.03±0.36d	171.21±0.31c	527.12±0.23d	46.84±0.05d	26.83±0.07b	26.45±0.01d
<i>C. pentandra</i>							
Raw	997.02±0.55a	773.55±0.43a	570.85±2.11a	1585.58±0.87a	219.84±0.12a	42.69±0.02a	35.68±0.02a
1 month	804.38±6.63b	555.70±9.74b	505.33±7.83b	1101.11±9.53b	144.19±0.67b	36.79±3.73b	31.80±0.13b
2 months	727.09±7.43c	527.10±9.19c	457.41±5.74c	1036.40±8.61c	139.06±0.55c	35.82±3.27b	28.12±0.11c
3 months	651.27±8.40d	503.45±3.33d	387.39±2.09d	1011.39±2.61d	137.18±0.37d	34.47±0.59b	25.60±0.02d

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

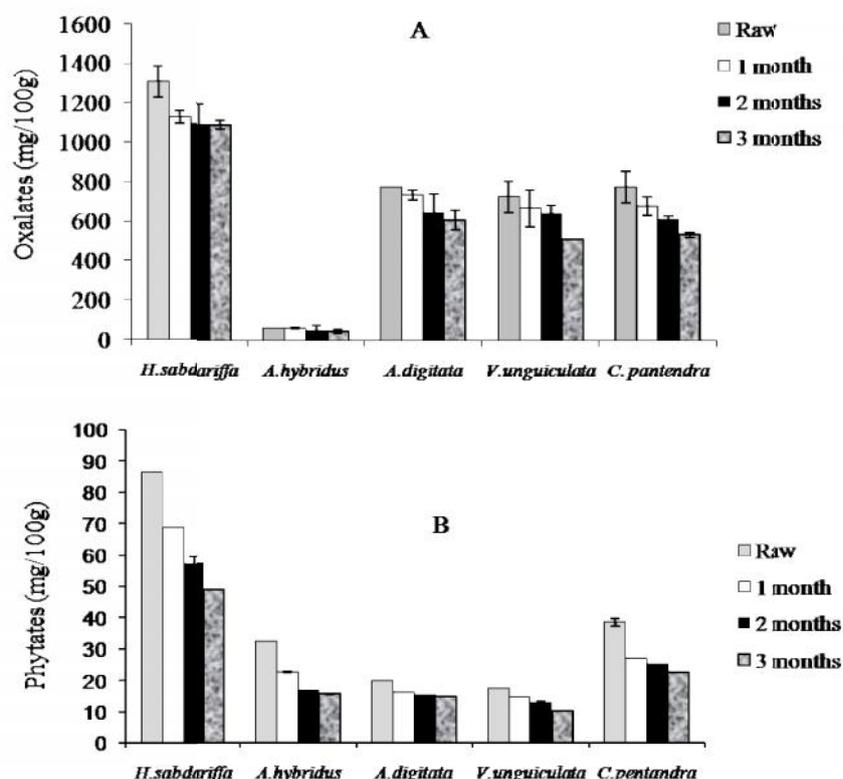


Figure 1. Oxalates (A) and phytates (B) contents of frozen leafy vegetables consumed in Northern Côte d'Ivoire

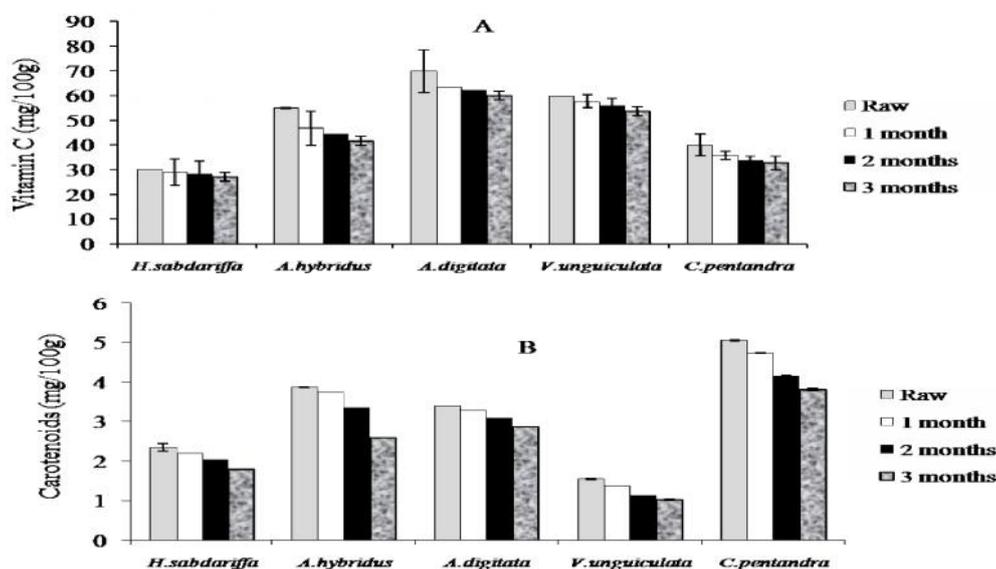
Table 3. Anti-nutritional factors/mineral ratios of frozen leafy vegetables consumed in Northern Côte d'Ivoire.

	Phytates/Ca	Phytates/Fe	Oxalates/Ca
<i>H. sabdariffa</i>			
Raw	0.21	0.85	3.26
1 month	0.17	1.31	2.89
2 months	0.15	1.07	2.97
3 months	0.13	1.03	3.03
<i>A. hybridus</i>			
Raw	0.03	0.41	0.07
1 month	0.02	0.32	0.07
2 months	0.02	0.27	0.06
3 months	0.02	0.26	0.06
<i>A. digitata</i>			
Raw	0.04	0.19	1.57
1 month	0.03	0.24	1.62
2 months	0.03	0.25	1.50
3 months	0.03	0.26	1.48
<i>V. unguiculata</i>			
Raw	0.04	0.19	1.66
1 month	0.03	0.24	1.62
2 months	0.03	0.28	1.59
3 months	0.03	0.22	1.36
<i>C. pentandra</i>			
Raw	0.04	0.17	0.78
1 month	0.03	0.18	0.85
2 months	0.03	0.18	0.85
3 months	0.03	0.16	0.82

3.2. Antioxidant Properties

Freezing processing of the studied leafy vegetables resulted in decrease of vitamin C and carotenoids contents (Fig. 2). This decrease effect on vitamin C and carotenoids content corroborates have already been reported by researchers [36 - 38]. Losses in vitamin C ranged from 3.23 to 14.90% and 9.56 to 24.18% respectively at 1 and 3 months of freezing respectively. Vitamin C is a soluble antioxidant component which is sensitive to oxidative phenomenon and serves as appropriate marker for

monitoring quality change during, transportation, processing and storage [39, 40]. Carotenoids play an important potential role in human health by acting as biological antioxidants [41]. The registered losses of carotenoid contents were estimates to 2.96 - 11.61% after 1 month of freezing storage. Loss of carotenoids during storage of food has been reported in numerous papers. The observed decrease in the β -carotene concentration could be as a result of enzymatic activity coupled with oxidation of double bonds in the compound [42, 43].

**Figure 2.** Vitamin C (A) and carotenoids (B) contents of frozen leafy vegetables consumed in Northern Côte d'Ivoire

The effect of freezing on polyphenols content and antioxidant activity of the selected leafy vegetables is depicted in Fig. 3. Phenolic compounds were also affected by storage factors such as temperature, atmosphere and light, than either vitamin C or carotenoids [44]. The total phenolic content of

the five fresh green leafy vegetables ranged from 135.21 to 293.08 mg/100g. At the end of the freezing period, total phenolic decreased and ranged from 121.53 to 280.69 mg/100g. Generally, freezing causes minimal destruction to phenolic compounds in vegetables due to enzymatic action [44].

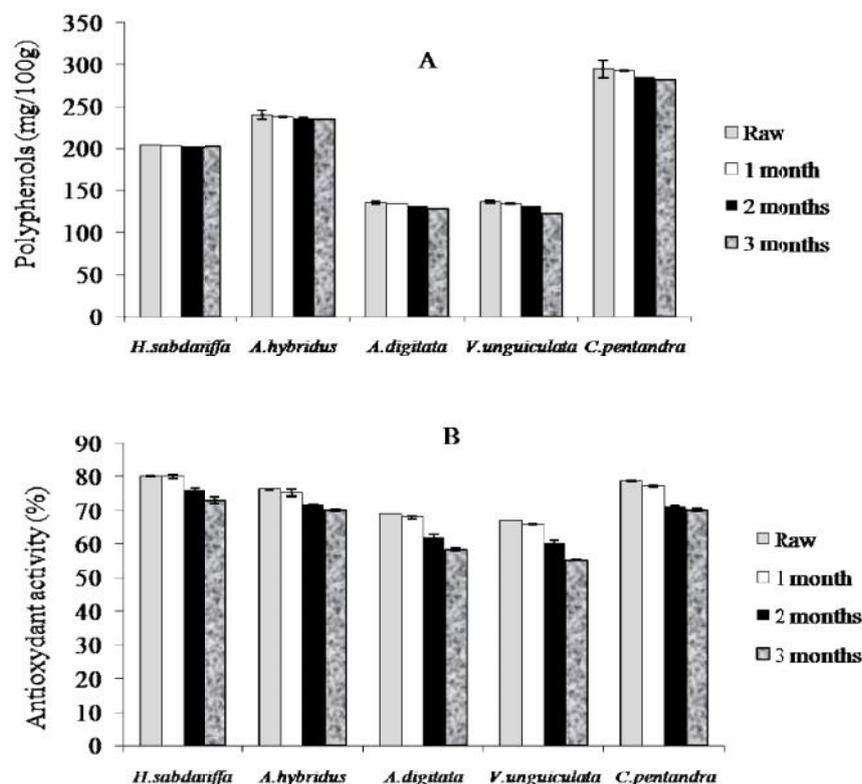


Figure 3. Polyphenols content (A) and antioxidant activity (B) of frozen vegetables consumed in Northern Côte d'Ivoire

4. Conclusions

Leafy vegetables consumed in Northern Côte d'Ivoire contain significant levels of nutrients that are essential for human health. The use of freezing technologies allows the retention of freshness qualities of these vegetables for long periods, extending their availability. This study revealed that, slight fluctuation in ash; crude fibers and protein contents were observed after one, two and three month of freezing storage. It was also observed a minimal destruction of vitamin C, carotenoids and phenolic compounds. Even if the losses observed were lesser, a longer period of freezing (more than 1 month) could significantly reduce the nutrient quality of leafy vegetables.

6. References

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