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Research Article

Evaluation of Nutritive and Antioxidant Properties of Frozen Leafy Vegetables Consumed in Western Côte d'Ivoire

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ABSTRACT

This study was conducted to evaluate the impact of freezing storage on the nutritive and antioxidant properties of five leafy vegetables (*Abelmoschus esculentus*, *Celosia argentea*, *Ipomea batatas*, *Manihot esculenta* and *Myrianthus arboreus*) widely consumed in Western Côte d'Ivoire. The selected leafy vegetables were frozen at -18°C for 1, 2 and 3 months and subjected to physico-chemical analysis after defrosting and oven drying. The results of moisture, ash, crude fiber and proteins contents after 3 months of freezing storage were as follow: 67.26-83.27%, 6.09-17.73%, 25.56-32.39%, 8.79-19.45% and 0.81-2.61%, respectively. The residual contents of minerals for this storage period (3 months) were: Ca (152.75-334.73 mg/100g), Mg (96.47-309.00 mg/100g), K (225.13-890.92 mg/100g), Fe (6.05-19.78 mg/100g) and Zn (0.70-4.17 mg/100g). Furthermore losses in oxalates, phytates, carotenoids and vitamin C were registered after 3 months of storage with values as indicated: 9.74-25.64%, 14.11-34.39%, 74.30-90.82%, 25.9-51.52%, respectively. Antioxidant activity of frozen leafy vegetables varied from 69.31 to 80.68% during the 3 months of storage. 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.

Key words: Antioxidant properties, freezing storage, leafy vegetables, nutritive value

INTRODUCTION

Green leafy vegetables (GLVs) have long been recognized as the cheapest and most abundant potential sources of vitamins and minerals and the ethno-botanical reports offer information on their medicinal properties like anti-diabetic, anti-histaminic, anti-carcinogenic hypolipidemic and antibacterial activities (Kubo *et al*., 2004; Raju *et al*., 2007). Among the twenty hundred and seven (207) leafy vegetables widely consumed in tropical Africa, about twenty (20) species belonging to 6 botanical families are widely consumed by Ivorian population (PROTA, 2004; Fondio *et al*., 2007). Furthermore ethno-botanical studies have stated that most people in Western Cote d'Ivoire consume leafy vegetables such as *Abelmoschus esculentus* "gombo", *Celosia argentea* "soko", *Ipomea batatas* "patate", *Manihot esculenta* "manioc" and *Myrianthus arboreus* "tikliti" cooking recipes made of sauces and starchy staples foods (Fondio *et al*., 2007; Soro *et al*., 2012).

Due to their high moisture content, GLVs are highly perishable and are sold at higher prices in the peak season because of non availability and proper processing facilities at the production point (Pande *et al*., 2000). In fact, GLVs are living tissues subject to continuous changes after harvest. The factors that shorten the shelf life of products are enzymatic browning, microbial spoilage, dehydration, rapid wilting and senescence caused by continued respiration and ethylene production (Reyes, 1996). In order to extend the self-life of leafy vegetables different ways of preserving such as sun-drying, solar drying and fermentation have been developed (Muchoki *et al*., 2007). Nowadays, electrification of the rural areas has introduced new preservation technologies including refrigeration and freezing of leaves (Tshikalange *et al*., 2006). Refrigeration slows down the respiration of vegetables and helps maintain quality attributes while freezing processing is generally known as one the best method for long term preservation of foods (Mepba *et al*., 2007). The

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freezing process includes pre-freezing treatments, freezing, frozen storage, and thawing. Even though freezing is regarded as the simplest and most important preservation process for fruits and vegetables, it is not a perfect process since it is well known that some nutritional value (vitamins and minerals) may be lost during the freezing process. The losses of nutrients during freezing can be the result of physical separation (peeling and trimming prior to freezing, or exudates loss during thawing), leaching or chemical degradation such as oxidation (Rickman *et al*., 2007).

Earlier reports have highlighted the nutritive potential of fresh leafy vegetables consumed in Western Côte d'Ivoire (Zoro *et al*., 2013) but there is a lack of studies on using freezing as a preservation method for these leafy vegetables. Thus, this research focused on determining the effect of freezing processing on the nutritive value and antioxidant properties of the selected leafy vegetables in order to predict the delay of post-harvesting preservation and provide nutritional information to consumers.

MATERIALS AND METHODS

Samples collection and processing

Leafy vegetables 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). 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 was stored at -18°C in freezer for one, two and three months (Prabhu and Barett, 2009). After freezing period, the leaves were defrosted at ambient temperature and subjected to drying in oven (Memmert, Germany) at 60°C for 72 h. 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.

Proximate analysis

Ash, proteins and lipids were determined using official methods (AOAC, 1990). For crude fibres, 2 g of dried powdered sample were digested with 0.25 M sulphuric acid and 0.3 M sodium hydroxide 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 fibres content. Carbohydrates and calorific value were calculated using the following formulas (FAO, 2002):

Carbohydrates: 100-(% moisture + % proteins + % lipids + % ash + % fibres).
Calorific value: (% 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 (5g) 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 (CEAEQ, 2013). Calibrations were performed using external standards prepared from a 1000 ppm single stock solution made up with 2% nitric acid.

Anti-nutritional factors determination

Oxalates content was performed using a titration method (Day and Underwood, 1986). 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 (Latta and Eskin, 1980). 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.

Vitamin C and carotenoids determination

Vitamin C content was determined by titration (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 (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 (Rodriguez-Amaya, 2001). 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 quantification

Phenolics were extracted and determined using Folin–Ciocalteu's reagent (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.

Antioxidant activity evaluation

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

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 expressed as means ± standard deviation. One way analysis of variance (ANOVA) was performed by using STATISTICA 7.1 (StatSoft) for significant differences stated at p □ 0.05.

RESULTS AND DISCUSSION

Nutritive properties

The proximate composition of the frozen leafy vegetables is presented in Table 1. The moisture contents ranged from 68.43 ± 1.92 to 84.96 ± 0.44% for raw and fresh samples while these values ranged from 67.26 ± 1.18 to 83.27 ± 0.06% at 3 months of freezing storage. During freezing storage, small fluctuation in moisture content was mostly due to the respiration and other enzymatic processes (Jacxsens *et al*., 2002). After 3 months of freezing storage the physicochemical parameters for ash, proteins and lipids were: 6.09-17.73%, 8.79-19.45% and 0.82-2.61%, respectively. The losses (3.5-6%) in ash during freezing storage could be explained by the transpiration phenomena which may carry minerals off (Maundu, 2006). As concern proteins and lipids contents, the observed 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. However, freezing processing of the studied leafy vegetables resulted in increase (10.70-15.23%) in their crude fiber content and this phenomenon may be due to textural changes such as wilting and shriveling (Ben-Yehoshua and Rodov, 2003). Therefore, appropriate intake of frozen leafy vegetables could lower the risk of, constipation, diabetes and colon cancer (Ishida *et al*., 2000). The estimated calorific values (153.93-218.66 kcal/100 g) of the selected frozen leafy vegetables (3 months of storage) agree with general observation that vegetables have low energy values due to their low crude fat and relatively high level of moisture (Sobowale *et al*., 2011).

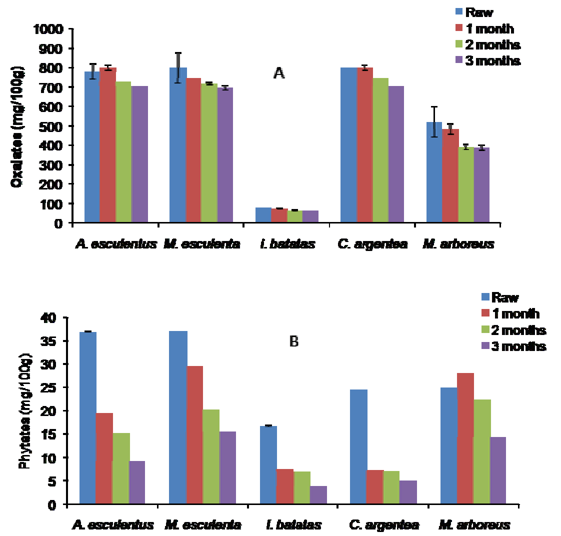

Fig. 1: Effect of freezing on oxalates (A) and phytates (B) contents of leafy vegetables consumed in Western Côte d'Ivoire

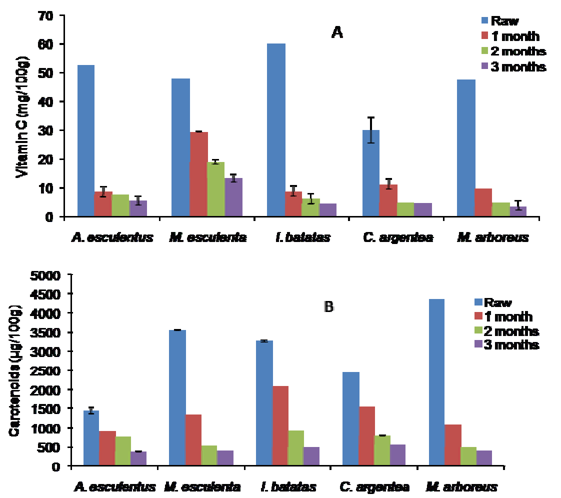

Fig. 2: Effect of freezing on vitamin C (A) and carotenoids (B) contents of leafy vegetables consumed in Western Côte d'Ivoire

Mineral composition of the frozen leafy vegetables is shown in table 2. The residual in minerals at 3 months of storage were significantly different ($p < 0.05$): Ca (152.75-334.73 mg/100g), Mg (96.47-309.00 mg/100g), K (225.13-890.92 mg/100g), Fe (6.05-19.78 mg/100g) and Zn (0.70-4.17 mg/100g). With regard to the mineral profile, the values obtained for magnesium, iron and zinc could cover human mineral requirements (FAO, 2004). Calcium and phosphorus play important role in 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 plays important role in prevention of anemia while zinc is important for vitamin A and vitamin E metabolisms (Trowbridge and Martorell, 2002).

The impact of freezing storage on anti-nutritional factors (oxalates and phytates) contents is depicted in figure 1. Levels losses at 3 months of freezing storage

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

	Moisture (%)	Ash* (%)	Crude fiber* (%)	Proteins* (%)	Lipids* (%)	Carbohydrates* (%)	Calorific value* (kcal /100g)
A esculentus							
Raw	83.91±0.08b	11.90± 0.10a	15.66±0.05b	9.19±0.15a	3.38±1.59a	59.87±1.90a	264.44±2.51a
1 month	87.20±1.22a	10.75±0.01 a	24.84±8.26a	9.09±0.12a	2.88±0.00a	52.00±0.98b	231.20±3.64b
2 months	88.12±1.21a	10.57±3.10a	27.31±1.18a	9.01±0.00a	2.71±0.00a	49.87±1.49c	230.46±4.72b
3 months	88.71±0.27a	10.23±0.18a	24.57±1.02a	8.79±0.05a	2.61±0.00a	48.74±1.00c	218.66±3.58c
M esculenta							
Raw	68.43±1.92b	9.03±2.12a	26.23±0.31a	23.39±0.71a	4.09±0.02a	37.27±3.16a	224.35±5.67a
1 month	85.26±1.32a	4.54±0.16b	26.19±0.77a	22.85±0.41a	3.55±0.00b	36.60±6.55a	212.11±3.82b
2 months	85.31±1.55a	4.35±1.01b	26.49±3.12a	21.07±0.38a	2.56±0.00c	36.53±3.74a	210.08±4.33b
3 months	85.64±1.18a	4.09±0.07c	29.29±6.37a	19.45±0.09b	2.31±0.00c	34.10±1.35a	198.10±3.16c
I batatas							
Raw	79.66±3.57c	23.56±0.26a	21.50±0.82b	15.32±0.40a	2.63±0.06a	36.79±3.41a	232.91±5.78a
1 month	87.95±0.67b	15.02±1.49b	22.26±2.44b	15.31±0.00a	2.27±0.00a	35.54±4.61a	223.75±7.50a
2 months	91.88±1.01a	14.87±0.31b	28.18±1.88a	14.65±0.31a	2.25±0.00a	31.13±1.90a	210.31±8.00b
3 months	92.67±0.33a	14.73±0.15b	27.88±6.11a	14.43±0.16a	2.05±0.00b	25.04±1.88b	183.30±4.08c
C argentea							
Raw	84.96±0.44b	22.10±0.75a	30.83±1.61a	9.77±0.10a	1.79±0.20a	35.52±0.60a	165.62±6.40a
1 month	90.85±0.39a	15.17±0.04b	28.74±4.81a	9.61±0.05a	1.26±0.00b	39.69±9.50a	176.09±3.67a
2 months	91.91±0.15a	12.85±0.24c	31.49±7.67a	9.51±0.05a	1.23±0.00b	33.56±2.16a	163.57±7.86a
3 months	93.27±0.06a	11.28±1.72c	32.39±2.26a	9.30±0.10b	1.22±0.00b	28.68±5.11b	153.93±3.83b
M arboreus							
Raw	81.30±0.35c	11.73±0.76a	12.19±0.73c	16.95±0.05a	1.47±0.02a	56.70±2.71a	259.50±5.26a
1 month	84.76±1.30b	9.89±0.04b	18.76±1.18b	16.35±0.14a	1.45±0.00a	56.94±0.79a	253.42±2.92a
2 months	86.00±0.61a	9.86±0.09b	22.42±8.06a	15.97±0.10a	1.25±0.00b	42.12±2.07b	199.06±4.85b
3 months	89.26±0.81a	9.17±0.17b	25.56±0.50a	15.67±0.17a	0.82±0.00c	38.94±0.60c	188.96±2.39c

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 frozen leafy vegetables consumed in western Côte d'Ivoire

	Ca	Mg	P	K	Fe	Na	Zn
A. esculentus							
Raw	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
1 month	330.24±7.90b	218.69±5.27b	393.10±5.90b	548.27±5.34b	26.95±6.40b	23.77±5.27b	7.69±1.89b
2 months	220.55±8.34c	199.55±1.51c	382.63±4.45c	400.16±6.01c	25.95±0.40b	19.68±3.61c	7.95±0.11b
3 months	193.55±1.11d	154.60±0.66d	362.57±0.43d	225.13±0.27d	19.78±0.02c	16.92±0.35c	4.17±0.00c
M. esculenta							
Raw	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
1 month	290.37±1.85a	155.44±5.81b	270.45±1.10b	348.67±3.03b	9.24±0.34b	13.94±1.64b	3.61±0.08b
2 months	220.18±5.12b	132.56±3.77c	145.58±3.80c	306.59±7.18c	8.41±0.15b	11.38±5.66b	2.86±0.43c
3 months	152.75±2.90c	96.47±1.83d	142.15±2.70c	278.46±5.28d	6.89±0.15c	9.29±0.40c	1.61±0.06d
I. batatas							
Raw	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±0.01
1 month	682.99±1.50b	320.02±3.18b	295.08±4.04b	764.89±7.12b	28.07±4.66b	190.71±7.56b	5.44±0.07b
2 months	410.85±4.41c	269.00±7.92c	216.68±7.60c	520.88±6.55c	20.35±0.55c	105.70±1.95c	4.21±0.00c
3 months	334.73±7.34d	226.23±4.79d	208.27±3.71c	471.30±9.27d	15.75±0.36d	55.31±3.39d	3.57±0.02d
C. argentea							
Raw	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
1 month	615.08±2.50b	594.14±2.41b	328.72±1.33b	1310.03±5.32b	31.16±0.12b	38.14±0.35b	9.83±0.03b
2 months	461.60±1.59c	437.56±1.98c	210.54±4.87c	1024.92±5.73c	20.06±0.50c	26.28±2.92c	3.35±0.08c
3 months	324.44±6.60d	309.00±6.38d	188.65±4.73d	890.92±5.64d	12.38±2.58d	21.66±1.76d	2.70±0.14d
M. arboreus							
Raw	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
1 month	286.93±1.79b	306.42±1.57b	264.03±3.08b	694.76±4.35b	7.14±0.04b	15.79±0.47b	2.46±0.01b
2 months	376.95±3.52c	283.45±3.31c	201.88±1.26c	786.50±9.19c	6.50±0.08c	11.78±0.82c	2.35±0.02c
3 months	301.28±1.13d	254.87±9.07d	167.98±4.96d	719.86±2.29d	6.05±0.17c	10.70±2.12d	2.25±0.06d

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

were 9.74-25.64 % and 7.58-26.26 %, respectively. The reductions in oxalates and phytates contents during freezing of leafy vegetables may be due to postharvest physiology which implies transpiration and hydrolytic enzymatic activities (Jacxsens *et al*., 2002). Indeed, oxalates and phytates are anti-nutrients which chelate divalent cations such as calcium, magnesium, zinc and iron, thereby reducing their bioavailability (Sandberg, 2002). 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 [oxalates]/[Ca] ratio in all the frozen species were below the critical level of 2.5 which is known to impair calcium bioavailability (Hassan *et al*., 2007).

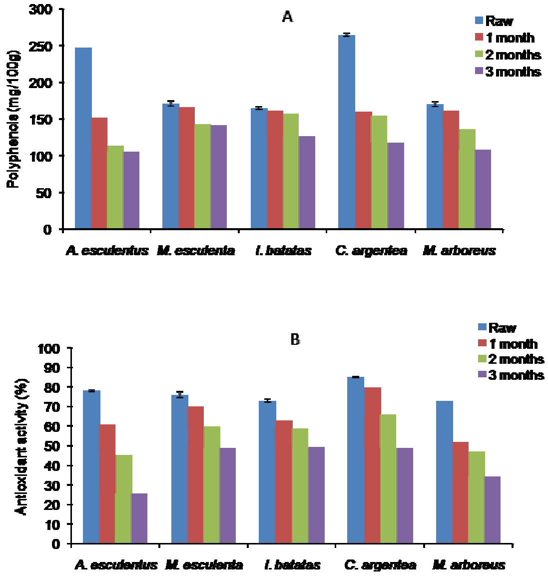

Fig. 3: Effect freezing on polyphenols contents (A) and antioxidant activity (B) of leafy vegetables consumed in Western Côte d'Ivoire

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

	Phytate/Ca	Phytate/Fe	Oxalate/Ca
A. esculentus			
Raw	0.07	0.28	1.66
1 month	0.05	0.72	2.41
2 months	0.06	0.58	3.29
3 months	0.04	0.46	3.63
M. esculenta			
Raw	0.06	0.31	1.19
1 month	0.10	3.20	2.57
2 months	0.09	2.40	3.26
3 months	0.10	2.23	4.56
I. batatas			
Raw	0.02	0.31	0.08
1 month	0.01	0.26	0.10
2 months	0.01	0.33	0.16
3 months	0.01	0.23	0.19
C. argentea			
Raw	0.12	0.79	2.69
1 month	0.01	0.23	1.29
2 months	0.01	0.35	1.62
3 months	0.01	0.40	2.16
M. arboreus			
Raw	0.03	0.08	1.01
1 month	0.07	3.93	1.28
2 months	0.07	3.43	1.28
3 months	0.04	2.36	1.34

Antioxidant properties

Total carotenoids and vitamin C decreased during 3 months of freezing storage as depicted in figure 2. For carotenoids and vitamin C, losses at 3 months of freezing storage were estimated to 3.89 to 11.79% and 7.85-20.27 %, respectively. However the lowest losses observed for carotenoids and vitamin C contents may be due to auto-oxidation and enzymatic degradation during postharvest handling techniques (Souzan *et al*., 2007).The effect of freezing storage on polyphenols content and antioxidant activity of the selected leafy vegetables is depicted in figure 3. The total phenolic content of the frozen leafy vegetables initially decreased from 0 days to 3 months (264.97-156.78 mg/100g) of storage. Generally, phenolic compounds are thus considered a positive for human health due to their substantial antioxidant activity. (Devlieghere *et al*., 2002; Titchenal and Dobbs, 2004). In addition, the freezing storage highlighted a good impact on antioxidant activity retention (69.31-80.68%) of the selected leafy vegetables after 3 months of storage.

Conclusion

African leafy vegetables contain significant levels of nutrients that are essential for human health. The results of this study revealed that freezing storage could enhance the shelf life and nutritional quality of the selected leafy vegetables by inhibition of the metabolic processes. In fact, slight fluctuations in nutrients and antioxidant components were observed during experiment. In view to the results obtained, it would be necessary to preserve fresh leafy vegetables by freezing storage within a period not exceeding 1 month in order to contribute efficiently to the nutritional requirement and to the food security of Ivorian population.

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