Research Article

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Nutritional and antioxidant properties of *Moringa oleifera* leaves are affected by harvest season and drying techniques

Les propriétés nutritionnelles et antioxydantes des feuilles de *Moringa oleifera* sont affectées par la saison de récolte et les techniques de séchage

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Abstract

Moringa oleifera is a rapid growing tree with leaves, harvested several times each year. These leaves are generally consumed in the dry powdered form. It is well known that preservative methods are able to modify food composition. It had then been hypothesised that the harvest season and drying technique can affect the composition of *Moringa oleifera* leaves. This research was carried out to investigate the effect of harvest season and drying techniques on the nutritional and antioxidant quality of *M. oleifera* leaves. Four leaves samples were collected (during the long and small rainy and dry seasons) and subsamples were dried under sun, shade, in room and in an oven at 45°C and 55°C to obtained a constant weight. Proximate analysis, total phenolic compounds, ascorbic acid, minerals and reducing power were done to assess the nutritional and antioxidant values. The result of the proximate analysis revealed that the harvest season as well as drying technique uncertainly affected *Moringa oleifera* composition. The long rainy season was the one that best preserved antioxidant activity. Surprisingly, sun drying was the better drying technique during rainy seasons. Oven drying at 45°C had positive effect on protein and mineral content although microbial contamination could be of significance.

Keywords: *Moringa oleifera*, harvest season, drying techniques, nutritional and antioxidant properties

Résumé

Moringa oleifera est un arbre à croissance rapide dont les feuilles peuvent être récoltées plusieurs fois par an. Ces feuilles sont généralement consommées sous forme de poudre sèche. Il est bien connu que les méthodes de conservation peuvent modifier la composition des aliments. L'hypothèse selon laquelle la saison de récolte et les techniques de séchage affectent la composition des feuilles de *Moringa oleifera* a alors été émise. Cette recherche visait à étudier l'effet de la saison de récolte et de la technique de séchage sur la qualité nutritionnelle et antioxydante des feuilles de *M. oleifera*. Quatre échantillons de feuilles ont été collectées (pendant les longues

et petites saisons pluvieuses et sèches) et les sous-échantillons séchés au soleil, à l'ombre, en chambre et à l'étuve (45°C et 55°C) jusqu'à obtention d'un poids constant. La composition proximale, les composés phénoliques totaux, l'acide ascorbique, les minéraux et le pouvoir de piégeage ont été déterminés afin d'évaluer la valeur nutritionnelle et antioxydante. Les résultats d'analyse ont révélé que la saison de récolte ainsi que la

technique de séchage affectaient de manière aléatoire la composition des feuilles de *Moringa oleifera*. La longue saison de pluie est celle qui a le mieux préservé l'activité antioxydante. Le séchage au soleil de façon surprenante a été la meilleure technique de séchage pendant les saisons de pluie. Celui à l'étuve à 45°C a eu un effet positif sur les teneurs en protéines et minéraux quoiqu'une contamination microbienne puisse être la cause.

Mots-clés : *Moringa oleifera*, saison de récolte, techniques de séchage, propriétés nutritionnelle et antioxydante

1. Introduction

Living organisms require nutrient to pursue essential functions such as growth, development and reproduction. Plants are an efficient nutrient source but harvesting and post-harvesting steps are essential to obtain higher nutrient content and better quality. The main factors to be accounted on harvesting are the harvesting time and season, drying temperature, and period of drying (Filho *et al*., 2006). In many tropical countries, there are often only two seasons a wet season and a dry season. In other countries or towns, it can vary and lead to four seasons (as it is the case in Yaoundé, Centre region of Cameroon). The need to meet nutritional requirement through adequate food supplies and proper selection of diet has been a basic determinant of stability and progress (Onimawo, 2001).

Moringa oleifera or the horseradish tree (one of the most useful tropical trees), is a pan-tropical species that is known by such regional names as "benzolive", "drumstick tree", "kelor", "marango", "saijhan", and "sajna" (Fahey, 2005). It is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. Today it has become naturalized in many locations in the tropics and is widely cultivated in Africa (Fahey, 2005). It is one of the newly discovered vegetable which is gaining wide acceptance. From the appearance of long, slender, triangular seed pods, it is also known as drumstick tree (Fahey, 2005; Mishra *et al.*, 2012). The tree is slender and with drooping branches that grow to approximately 10 m in height. In cultivation, it is often cutback annually to 1-2 m and is allowed to re-grow, so the pods and leaves remain within arm's reach (Fuglie, 2001). Cited as one of the world's most useful plants (almost every part of *M. oleifera* is of value, no part of the plant is useless), *Moringa leaves* are edible and are of high nutritive value. Fahey (2005) considered them to be a significant source of essential nutrients such as beta-carotene, vitamin C, protein, iron, potassium, calcium and phosphorus and are commonly dried and crushed into a powder and stored without refrigeration for months without loss of nutritional values. *M. oleifera* leaves have been used successfully in its dried state or powdered form to augment and make delicious meals and porridge diets for pregnant women, nursing mothers, infants and young children, as well as adults of all age groups. In Africa, nursing mothers have been statistically shown to produce far more milk when they add *M. oleifera* leaves to their daily diets and malnourished children have made significant weight gains when nursing mothers and care-givers add them to their diets as well (Duke, 1982).

For purposes of preservation, packaging, transportation and distribution, *Moringa oleifera* leaves as many others vegetable is most commonly dried, frozen, fermented, pasteurized or canned (William & Dennis, 2004; Prabhu *et al*., 2011). Generally, the process of preservation is classified as drying, heating, refrigerating, irradiating, and the use of chemicals or natural agents but Moringa leaves are most available and consumed in the dried form. Dehydration is one of the most possible strategies for preservation of green leafy vegetables, which are highly seasonal and perishable too.

It is largely believed and advised that the way to preserve *Moringa oleifera* leaf nutrient content is to dry them under shade (Olushola, 2006). This method is generally adopted by local holder of the material. However, with the renewed campaign and interest in *M. oleifera* consumption, some people

think that it may become difficult to produce sufficient leaf powder by drying naturally under the shade to meet the growing demand. Therefore, it is needed to conduct a laboratory study on the effect of drying temperature on the nutrient content of Moringa leaves according to harvest season. This study will be useful when considering industrial drying of the leaves for large scale production of *M. oleifera* leaf powder. It will serve as a guide to industrialist or intensive producers to select optimum drying temperature to maximize nutrient retention. During the drying process lot of losses takes place like nutritional, physical and chemical composition of leaves.

The objective of this work was therefore to evaluate the influence of harvest season and drying techniques on nutritional and antioxidant value of *Moringa oleifera* leaves.

2. Material and Methods

2.1. Sample collection

Large quantity of fresh *Moringa oleifera* leaves (Figure 1) were collected (in Ngousso, Yaoundé) every three months from November 2015 to October 2016.

Figure 1: Fresh collected *Moringa oleifera* branches

The harvest period matched with Yaoundé different seasons that are small rainy and dried seasons and long rainy and dried seasons. Figure 2 indicates the exact period of sample collection. Sample were randomly collected on ten five years trees to constitute a composite sample.

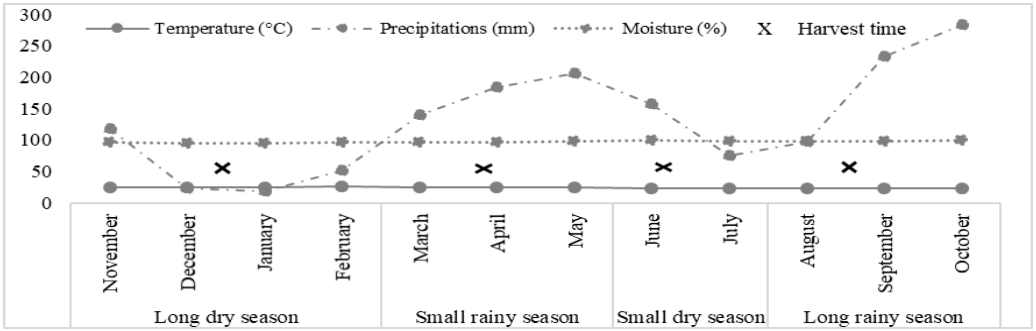

Figure 2: Different temperatures, precipitations and moisture in the town of Yaoundé during harvest period of *Moringa oleifera* leaves (data from Yaoundé airport meteorological station, 2017)

2.2. Preparation of *Moringa oleifera* leaves

The leaves were washed with tap water to remove dirt and other impurities. The excess water was drained out from leaves. After complete removal of water drops, the leaves were kept in thin layer in grid for actual drying process (Figure 3). The leaves were divided into five portions according to applied treatments. The various types of drying techniques that were used in this study were sun, shadow and hot oven (at 45°C and 55°C).

Figure 3: *Moringa oleifera* leaves prepared for drying

2.3. Drying

The following types of drying process were employed:

a) Sun drying: Sun drying was done by exposing the sample to the sun (adequate

amount of sunlight) between day times from 10.00 am to 5.00 pm daily till the sample attained constant weight.

b) Shadow drying: sample was exposed under trees between day times from 10.00 am to 5.00 pm daily till sample attained constant weight.
c) Room drying: sample was spread in a room which was well ventilated and allowed to dry till constant weight was obtained.
d) Oven drying: The oven was preheated to 55°C and 45°C. The temperatures were maintained and the process was carried out until leaves were completely dried.

The different dried samples were then ground into powder using kitchen blender, and screened through a sieve (0.4 cm) and stored in freezer at -12°C until chemical analysis.

2.4. Water content and moisture analysis

Moisture and dry matter contents were carried out using hot air oven. About 5g of sample was stored at 105°C for 24 hours, and difference in weight between the dry condition and wet (initial weight) condition was reported as water and moisture content (AOAC, 1980; Ranganna, 1986).

2.5. Determination of proximate composition

The proximate analysis of *Moringa oleifera* leaves was carried out by standard method.

2.5.1. Determination of ash content

Ash content of material represents inorganic residue remaining after destruction of organic matter present in the sample. The silica dish was kept in a muffle furnace at not more than 525°C for 4-6 hours. The ash weight was taken and the % ash determined by formula, as given in standard method (AOAC, 1980).

2.5.2. Determination of crude fat

Hexane soluble material in food was extracted from dried sample using a Soxhlet Extraction apparatus. The hexane was evaporated and residue was weighed (Bourely, 1982).

2.5.3. Determination of crude fibres

Crude fibres are organic residues which remain after food sample has been treated under standardized conditions with standard boiled acid and alkali solutions. The crude fibres were determined by standard method (AOAC, 1990).

2.5.4. Determination of crude protein

The crude protein content was measured by Kjeldahl method based on the conversion of organic nitrogen to ammonium sulphate. The nitrogen content (N) was converted into protein using 6.25 as the converting factor (AOAC, 1980).

2.5.5. Total carbohydrate content

The total carbohydrates of the samples were determined by subtraction of total content of water, protein, lipid, fibre and ash to 100.

Carbohydrate (%) = 100 – (Water + Protein + Lipid + Fibre + Ash)

2.6. Determination of some mineral composition

The mineral (Ca, Mg, P, K and S) composition was determined using the atomic absorption spectrophotometry as described by AOAC (2005).

2.7. Determination of antioxidant potential

2.7.1. Preparation of the hydro-methanolic extracts

Each sample powder (2 g) was extracted with 20 mL of hydro-methanol (30:70 v/v), stirred on a stirring plate at room temperature for 24 h. Contents were filtered through N° 1 filter paper (Whatman). The filtrate was kept in a refrigerator (4°C) and used to determine the scavenging effect on α,α-diphenyl-β-picrylhydrazyl (DPPH) radicals. The hydro-methanolic extract was also used to determine the total phenolic compounds.

2.7.2. Total phenolic compounds

The amount of phenolic compounds in the extracts of leaves was estimated by using Folin–Ciocalteau reagent using gallic acid as a standard (Vinson *et al.*, 1998). In a series of test tubes, 2 ml of the extract in water-methanol was taken, mixed with 2 ml of Folin–Ciocalteau reagent and 2 ml of sodium carbonate. After shaking, it was kept for 15 min and the absorbance was measured at 765 nm. Using the standard curve, the total phenolic content was

calculated and expressed as gallic acid equivalent in mg/g of extracts.

2.7.3. Ascorbic acid (Vitamin C)

Ascorbic acid was measured by titration using DCPIP (2,6-dichlorophenol-indophenol) (Negi & Roy, 2004). Extraction was done in acetic acid as extraction solvent. 5ml of extract was pipetted into a boiling tube and 1ml of glacial acetic acid was added and titrated with the dye solution to a faint permanent pink colour. The titre (T) was recorded. The titration was repeated with 5ml of water for the blank (B1) and 5ml of ascorbic acid standard solution (st) and vitamin C content of the test sample was calculated using the relationship:

Vitamin C (mg/100 ml) = [(T - B1) / (st - B1)] x Dilution factor

2.7.4. Determination of the scavenging effect on DPPH (α,α-diphenyl-β picrylhydrazyl) radicals

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free DPPH radical. Thus, 200 µL of extract in water/methanol was added to 0.5 mL of 0.04% (w/v) DPPH solution in methanol. The content was mixed vigorously and allowed to stand at 20°C for 30 min (Molyneux, 2004). The absorbance was read at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = [$((A_0 - A_1)/A_0) \times 100$] Where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample.

2.8. Statistical analysis

Data were analysed by analysis of variance (ANOVA) using general linear model. Tukey's multiple range test was used to determine the differences among samples. Significant levels were defined as probabilities of 0.05 or less. All processing tests were done at least in triplicate.

3. Results and Discussion

3.1. Water content

The way harvest season affected the water content of fresh *Moringa oleifera* leaves has been determined and results are presented in Table 1. Values were 33%, 56%, 61% and 73% respectively during the long dry, small rainy, small dry and long rainy seasons. Previous studies reported that *Moringa oleifera* leaves contain large quantity of water with the content ranging from 67 to 88% (Prabhu *et al.*, 2011; Alakali *et al.*, 2015; Djouhou *et al.*, 2019a).

From the results, it can be concluded that season significantly influenced the water content of leaves. The highest value was obtained during the long rainy season and the lowest during the long dry season. From Figure 1, precipitation level was very high reaching 206 mm to 284 mm during rainy seasons while it remained very low during dry seasons (23 mm).

Table 1: Effect of season on water content of *Moringa oleifera* leaves

Harvest season	Water content (%)
Long dry season	32.70 ± 0.56^{a}
Small rainy season	55.96 ± 1.46^{b}
Small dry season	61.25 ± 2.18^{b}
Long rainy season	72.62 ± 0.49^{c}

Values are means of triplicate determinations. Means with different superscripts within the same column are significantly different from each other ($p<0.05$).

3.2. Proximate composition of *Moringa oleifera* leaves

Table 2 presents the proximate composition of different sample of *Moringa oleifera* leaves collected during the four main seasons of the town of Yaoundé and submitted to five different dehydration techniques.

As long as sun drying is concerned, moisture content decreased significantly ($p<0.05$) from 32.7% to 7.6%, 56.0% to 7.5%, 61.3% to 8.0% and 72.6% to 5.6% respectively during long dry, small rainy, small dry and long rainy seasons. It can be observed that in rainy seasons the end product is better dried than in dry seasons. The wind effect on the relative humidity could explain that effect. Higher residual moisture content of 9.95% was observed in room drying during rainy season where the relative humidity could be high and the wind effect negligible. Moisture values were within the interval reported by Esper & Muhibauer, 1998 and Satwase *et al.*, 2013.

When observing crude fibre content, it seemed as there were two major groups of samples: one from

long seasons and the other from small seasons. Fibre content was higher in long season sample than in small season one. Values, ranging from 14.92 ± 0.48 to 17.72 ± 0.22 were lower than the recommended average daily requirement for adults (16-32 g). During the long seasons, sun-dried, room-dried or oven-dried (45°C) samples were equivalent to end products more digestible than shadow-dried and oven-dried samples. Conversely, during small seasons, shadow-drying or oven-drying (55°C) were techniques recording best digestible products (less rich in crude fibres). *Moringa oleifera* leaves, harvested from August to February (long rainy and drying seasons) had least crude fibre content (average of 15%) than those harvested out of this period (17%). Drying techniques slightly modified sample crude fibres with highest value recorded by shadow-drying and oven-drying techniques (long seasons) and sun-drying and oven-drying (45°C) techniques (small seasons).

Harvest season and drying techniques did not significantly affect the ash content of *Moringa oleifera* leaves. The obtained values were lower than those of Shih *et al.*, 2011 and Alakali *et al.* (2015).

Crude protein content of leaves was 28.87 ± 0.99 - 39.45 ± 0.61 suggesting that leaves are a good source of protein. These values were higher than those obtained by Prabhu *et al*. (2011); Satwase *et al*. (2013); Djouhou *et al.* (2019a) with values ranging from 14 to 26%. In all the four harvest seasons, protein contain were significantly higher in room and oven-dried (45°C) *Moringa oleifera* leaves. As the drying temperature decreased from sun drying to room-drying, crude protein increased significantly ($p<0.05$). The high results obtained at 45°C could be due to a microbial contamination. Actually, main microbes have their optimum growth temperature at 45°C. They grow best in moderate temperature and can contaminate food and vegetable. What was done in this study was to determine the total nitrogen content of the samples (Kjeldahl method). The nitrogen content (N) was converted into protein using 6.25 as the converting factor. The obtained values could then be an approximation because if a sample contains contaminants, the later could increase the protein content.

The harvest season affected the protein content with higher value during the small rainy season and the lowest during the long dry season. In general, protein content was low during the dry seasons than rainy season. This can be due to the fact that dry seasons present more rough conditions, the later can induced the use of proteins to cover other plant functions. Proteins are macromolecules involved is processes such as catalysing chemical reactions (enzymes), facilitating membrane transport, intracellular structure and energy generating reactions involving electron transport, just to name a few. These results are different from those obtained by Shih *et al*. (2011) who showed that proteins may not be affected by season.

The fat content of different dried leave samples ranged from 5.03 ± 0.08 - 4.13 ± 0.14 % (long dry season), 3.96 ± 0.26 - 4.29 ± 0.39 (long rainy season), 4.66 ± 0.11 - 4.04 ± 0.37 (small dry season) and 4.96 ± 0.32 - 4.06 ± 0.53 (small rainy season). The values were more important during the long dry season than others and drying technique did not significantly affect this lipid content. The moderate amount of fat at each harvest season indicates that the vegetable is not a source of lipid accumulation which can cause arteriosclerosis, aging (Antia *et al*., 2006). The high protein and low-fat characteristic of Moringa leaves has been previously reported by Makkar & Becker (1996).

The oven-dried (at 55°C) most preserved the carbohydrate content of *Moringa oleifera* leaves with lowest results belonging to room-dried samples. Season did not significantly affect carbohydrate content and values were higher than those of Satwase *et al*. (2013) and similar to those of several others authors (Prabhu *et al*., 2011; Alakali *et al*., 2015). As carbohydrates were evaluated as complement to 100 after subtracting other constituents, the low value observed in oven-dried at 45°C treatment is consistent with the hypothesis of product contamination by microorganisms during this treatment.

Table 2: Effect of harvest season and drying techniques on proximate composition of dried *Moringa oleifera* leaves

	Long dry season	Long rainy season	Small dry season	Small rainy season
		Moisture (%)		
Sun	7.61 ± 0.26^{b}	5.58 ± 0.09^{ab}	7.99 ± 0.28^{b}	7.45 ± 0.11^{b}

Shadow	8.05 ± 0.17^{b}	6.59 ± 0.11^{b}	9.12 ± 0.17^{c}	9.10 ± 0.24^{c}
Room	7.44 ± 0.16^{b}	9.95 ± 0.27^{c}	9.34 ± 0.17^{c}	8.62 ± 0.22^{c}
Oven 55°C	5.72 ± 0.08^{a}	4.86 ± 0.49^{a}	6.34 ± 0.14^{a}	6.56 ± 0.24^{a}
Oven 45°C	5.16 ± 0.21^{a}	6.07 ± 0.30^{ab}	6.01 ± 0.16^{a}	7,03 ± 0,02^{ab}
		Crude fibres (%)		
Sun	15.11 ± 0.24^{ab}	15.12 ± 0.19^{a}	17.25 ± 0.07^{b}	17.29 ± 0.10^{a}
Shadow	16.51 ± 0.40^{b}	17.01 ± 0.07^{b}	15.49 ± 0.27^{a}	16.96 ± 0.51^{a}
Room	14.92 ± 0.48^{a}	15.36 ± 0.20^{a}	15.72 ± 0.30^{a}	17.72 ± 0.22^{a}
Oven 55°C	16.52 ± 0.31^{b}	17.05 ± 0.10^{b}	15.47 ± 0.13^{a}	16.86 ± 0.11^{a}
Oven 45°C	15.93 ± 0.07^{ab}	15.48 ± 0.30^{a}	17.20 ± 0.51^{b}	17.41 ±0.36^{a}
		Ash (%)		
Sun	3.07 ± 0.25^{a}	3.13 ± 0. 19^{a}	3.38 ± 0.12^{b}	3.53 ± 0.23^{a}
Shadow	3.63 ± 0.06^{a}	2.79 ± 0.02^{a}	3.58 ± 0.22^{b}	3.31 ± 0.08^{a}
Room	3.12 ± 0.11^{a}	2.72 ± 0.12^{a}	3.34 ± 0.00^{b}	3.33 ± 0.20^{a}
Oven 55°C	2.97 ± 0.39^{a}	2.64 ± 0.11^{b}	3.78 ± 0.05^{b}	3.02 ± 0.02^{a}
Oven 45°C	3.36 ± 0.09^{a}	3.67 ± 0.15^{a}	2.81 ± 0.12^{a}	3.62 ± 0.01^{a}
		Protein (%)		
Sun	28.87 ± 0.99^{a}	34.58 ± 0.87^{ab}	31.77 ± 0.63^{ab}	34.33 ± 0.81^{a}
Shadow	29.73 ± 1.40^{a}	33.15 ± 0.73^{ab}	31.46 ± 1.35^{ab}	34.45 ± 1.02^{a}
Room	34.36 ± 1.37^{ab}	34.37 ± 0.96^{ab}	35.48 ± 0.85^{b}	38.52 ± 1.21^{b}
Oven 55°C	33.11 ± 2.00^{ab}	31.79 ± 0.81^{a}	29.95 ± 0.97^{a}	33.96 ± 0.67^{a}
Oven 45°C	36.56 ± 0.80^{ab}	35.93 ± 0.69^{b}	34.03 ± 0.96^{ab}	39.45 ± 0.61^{b}
		Lipids (%)		
Sun	4.63 ± 0.13^{b}	3.96 ± 0.26^{a}	4.30 ± 0.18^{a}	4.49 ± 0.14^{a}
Shadow	4.89 ± 0.13^{b}	4.29 ± 0.39^{a}	4.66 ± 0.11^{a}	4.58 ± 0.10^{a}
Room	5.03 ± 0.08^{b}	4.29 ± 0.21^{a}	4.04 ± 0.37^{a}	4.96 ± 0.32^{a}
Oven 55°C	4.98 ± 0.17^{b}	4.18 ± 0.17^{a}	4.26 ± 0.49^{a}	4.06 ± 0.53^{a}
Oven 45°C	4.13 ± 0.14^{a}	4.24 ± 0.20^{a}	4.15 ± 0.29^{a}	4.76 ± 0.06^{a}
		Carbohydrates (%)		
Sun	39.55 ± 0.58^{b}	36.56 ± 0.47^{c}	35.96 ± 0.42^{b}	32.38 ± 0.10^{c}
Shadow	38.42 ± 1.45^{ab}	35.28 ± 0.71^{bc}	34.46 ± 0.17^{ab}	30.90 ± 1.20^{bc}
Room	33.84 ± 1.06^{a}	32.80 ± 0.63^{a}	31.35 ± 1.15^{a}	25.88 ± 1.23^{a}
Oven 55°C	35.14 ± 1.25^{ab}	39.15 ± 0.06^{d}	39.99 ± 0.70^{c}	35.64 ± 0.09^{d}
Oven 45°C	34.13 ± 0.57^{a}	33.87 ± 0.54^{ab}	34.76 ± 1.39^{ab}	28.23 ± 0.71^{ab}

Values are means of triplicate determinations. Means with different superscripts within the same column are significantly different from each other ($p<0.05$).

3.3. Mineral composition of *Moringa oleifera* leaves

Tables 3 and 4 present the results of the effects of drying techniques and harvest season on the mineral (P, K, Ca, Mg and S) composition of *Moringa oleifera* leaves. To determine the effect of season, Moringa leaves were oven-dried at 45°C because this was the best drying technique obtained in this study (Table 3).

Table 3: Effect of drying techniques on mineral composition of dried *Moringa oleifera* leaves (mg/100g)

	P	K	Ca	Mg	S
Sun	918.00 ± 3.00^{a}	638.00 ± 7.00^{b}	1906.50 ± 11.50^{b}	695.50 ± 6.50^{b}	721.50 ± 3.50^{c}
Shadow	885.00 ± 7.00^{a}	395.00 ± 6.00^{a}	1339.50 ± 16.50^{a}	633.50 ± 9.50^{ab}	648.50 ± 18.50^{a}
Room	888.50 ± 1.50^{a}	374.50 ± 7.50^{a}	1321.50 ± 23.50^{a}	584.50 ± 16.50^{a}	628.00 ± 7.00^{a}

Oven 55°C	907.50 ± 4.50^{a}	1169.50 ± 17.50^{c}	1974.00 ± 6.00^{bc}	772.50 ± 16.50^{c}	700.50 ±2.50^{bc}
Oven 45°C	1722.50 ± 11.50^{b}	1284.00 ± 20.00^{d}	2008.00 ± 7.00^{c}	938.50 ± 17.50^{d}	749.50 ± 3.50^{c}

Values are means of triplicate determinations. Means with different superscripts within the same column are significantly different from each other (p<0.05).

Table 4: Effect of harvest season on mineral composition of dried *Moringa oleifera* leaves (mg/100g)

Seasons	P	K	Ca	Mg	S
Long dry	1722.50 ± 11.50^{b}	1284.00 ± 20.00^{b}	2008.00 ± 7.00^{d}	938.50 ± 17.50^{b}	749.50 ± 3.50^{b}
Long rainy	1579.50 ± 23.50^{a}	1100.50 ± 13.50^{a}	1652.50 ± 20.50^{a}	751.00 ± 18.00^{a}	792.00 ± 9.00^{d}
Small dry	1899.50 ± 4.50^{c}	1057.00 ± 53.00^{a}	1870.50 ± 8.50^{c}	763.50 ± 18.50^{a}	772.50 ± 8.50^{bc}
Small rainy	1769.00 ± 17.00^{b}	1306.00 ± 15.00^{b}	1765.50 ± 20.50^{b}	874.00 ± 15.00^{b}	630.00 ±12.00^{a}

Values are means of triplicate determinations. Means with different superscripts within the same column are significantly different from each other (p<0.05).

Oven-dried and sometimes sun-dried samples presented high P, K, Ca, Mg and S content. Drying techniques significantly ($p<0.05$) affected mineral content. Between room-dried and shadow-dried sample, there are slight variations with no significant difference ($P>0.05$). For the same mineral, the content generally increased as a function of increase temperature except for oven-dried samples.

From one season to another, there are slight variations with significant difference ($p<0.05$) among them. Long dry season presented the highest mineral content and long rainy season the lowest. The trend of the effect of harvest season on mineral composition was long dry season > small dry season > small rainy season > long dry season.

From season to drying techniques, *Moringa oleifera* leaves contained high amount of sulphur with values ranging from 792.00 ± 9.00 to 630.00 ±12.00 mg/100g. Sulphur (S) is one of six macro-elements which are essential for proper plant growth and development. It forms part of amino acids and proteins and plays an important role in redox control of cellular processes (Foyer & Noctor, 2009) and in plant defence mechanisms (Rausch & Wachter, 2005; Noctor, 2006).

Calcium is a macro-element that builds healthy, strong bones and teeth and also assists blood clotting (Gordon, 1999). Deficiency can cause rickets, bone pain and muscle weakness. *Moringa oleifera* leaves presented considerable amount of Ca with values ranging from 1321.50 ± 23.50 to 2008.00 ± 7.00 mg/100g; with the highest content obtained when drying samples at 45°C.

The high phosphorus content of *Moringa oleifera* leaves (885.00 ± 7.00 - 1722.50 ± 11.50) suggests that their consumption could help in the process of children tooth and bone formation and their healthy development (Olaofe *et al.*, 2009).

From Tables 3 and 4, it can be concluded that modification in drying techniques and harvest season did not deplete the mineral content of samples (even though there were some modifications in the mineral content) and therefore did not have negative effect on the nutritional content of the samples because in general, values were above recommended daily allowance (Gamman & Sherrington, 1990).

3.4. Antioxidant composition of *Moringa oleifera* leaves

Hydro-methanol was chosen as extraction solvent for both phenolic content and scavenging activity because of its wide solubility properties for low molecular and moderately polar substances, including the antioxidant-active phenolic compounds. The total phenolic compounds and scavenging effect on DPPH radicals of hydro-methanolic extracts of Moringa samples from different harvest season and drying techniques are shown in Figures 4 and 5 as well as the vitamin C content (Figure 6).

In this study, the total phenolic content of the selected samples was determined, however, the phenolic composition of the extracts was not analysed as it was not within the scope of the present investigation. In general, the trend of total phenolic compounds as a function of the season was long rainy season > small rainy season > long dry season > small dry season. The low total phenolic compounds of dry seasons compared to rainy seasons could be an evidence that biotic stress is the main factor of phenolic compound production

by plants. The rainy season offers microbial development conditions in plants, inducing plant synthesis of protective molecules like phenolic compounds.

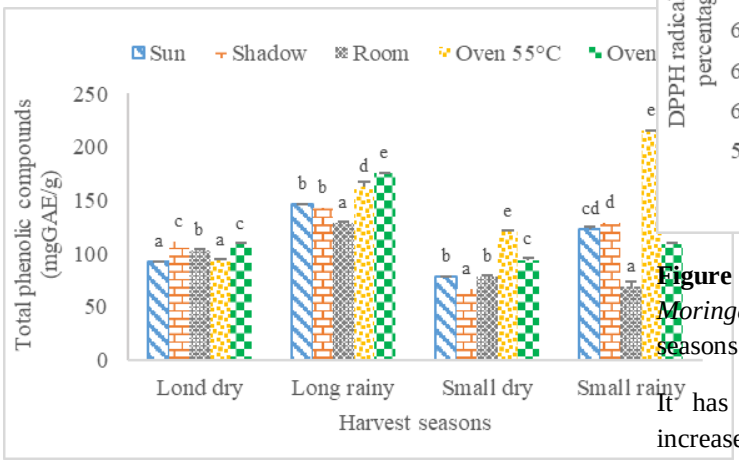

Figure 4: Total phenolic compounds of *Moringa oleifera* samples from different harvest seasons and drying techniques

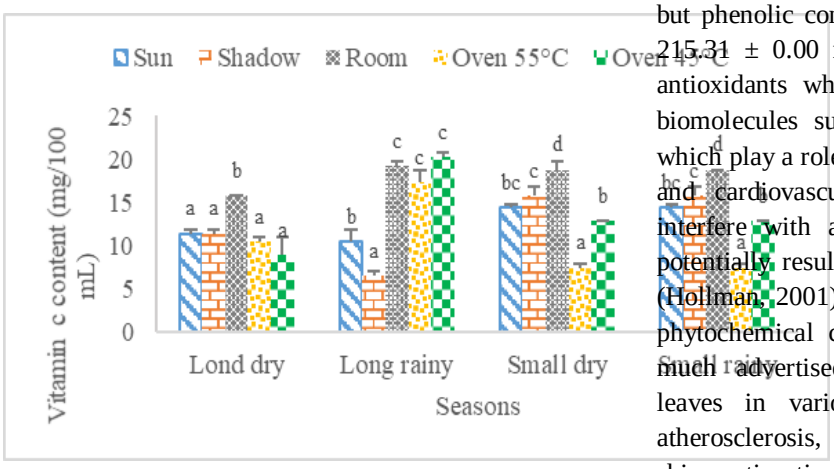

Figure 5: Vitamin C content of *Moringa oleifera* samples from different harvest seasons and drying techniques

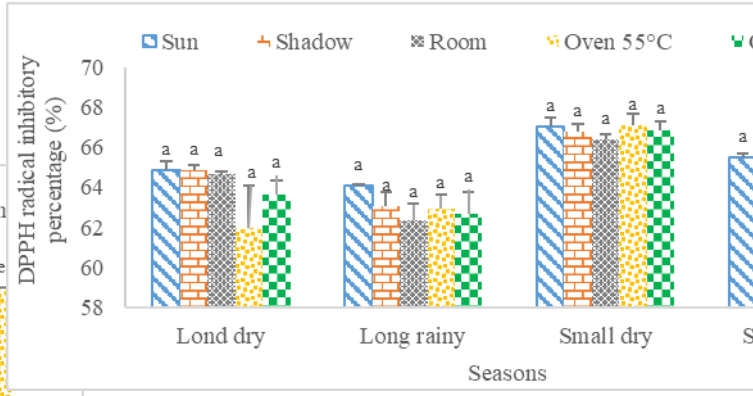

Figure 6: Scavenging effect on DPPH radical of *Moringa oleifera* samples from different harvest seasons and drying techniques

It has been stated that total phenolic content increases gradually with the maturity of leaves (Iqbal & Bhanger, 2006) but what was obtained in this study cannot help to confirm this affirmation. In fact, Djouhou *et al*. (2019a) working on newly opened leaves obtained a phenolic content ranging from 160 to 260 mEGA/100 g and in this study, *Moringa oleifera* trees were at least five years old but phenolic content ranged from 66.29 ± 0.29 to 215.31 ± 0.00 mEGA/100 g. Phenols are strong antioxidants which prevent oxidative damage to biomolecules such as DNA, lipids and proteins which play a role in chronic diseases such as cancer and cardiovascular disease. Plant phenols may interfere with all stages of the cancer process, potentially resulting in a reduction of cancer risk (Hollman, 2001). In general, the presence of these phytochemical compounds could account for the much advertised medicinal properties of these leaves in various disease conditions such as atherosclerosis, arthritis, diabetes nausea, asthma, skin antiseptic, diarrhea, dysentery, colitis and cancer (Bamishaiye *et al*., 2011).

Drying sample at 55°C was the worst treatment to maintain the vitamin C content of *Moringa oleifera* leaves and the content in dried leaves was lower than the value obtained by Shih *et al*. (2011) in fresh leaves. The reduction in vitamin C content of the leaves could be due to drying temperature. This is consistent with the reports of Duke (1983), Olushola (2006), Mbah *et al*. (2012) and Gernah & Ajir (2007), that high temperature can cause huge losses of vitamin C.

The result also showed that the scavenging effect on DPPH radicals was higher in all samples apart

from harvest season and drying techniques. One important mechanism of antioxidation involves the scavenging of hydrogen radicals. DPPH has a hydrogen free radical and shows a characteristic absorption at 517 nm. After encountering the proton-radical scavengers, the purple colour of the DPPH solution fades rapidly. The extracts of Moringa are able to reduce the unstable radical DPPH to the yellow coloured diphenylpicrylhydrazine. Neither the harvest season nor the drying technique affected the inhibitory effect of *Moringa oleifera* extracts on DPPH radical.

The involvement of free radicals, especially their increased production, appears to be a feature of many diseases including cardiovascular disease and cancer (Deighton *et al*., 2000). Phenolic compounds as well as other bioactive compounds of the extracts are probably involved in their antiradical activity. Polyphenols are phytochemical compounds that have common structure, a flavone backbone. Phenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity because of their scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989). These results indicated that there was no correlation between antioxidant activity and total phenolic content ($p>0.05$). However, different results were reported on this aspect; some authors found correlation between phenolic content and antioxidant activity (Yang *et al*., 2002), whereas the others found no such relationship, since other compounds are responsible for the antioxidant activity (Kahkonen *et al*., 2009; Shih *et al*., 2009; Djouhou *et al*., 2019a, 2019b). Although the phenolic compounds are believed to be the major phytochemicals responsible for antioxidant activity of plant material (Kuo *et al*., 2002), *Moringa oleifera* is a rich source of ascorbic acid which also has the antioxidant activity (Arabshahi *et al*., 2007).

Antioxidants are phytochemicals, vitamins and other nutrients that protect systems against damage caused by free radicals. They retard oxidative processes through several mechanisms: hydrogen donation, singlet oxygen quenching; enzyme inhibition, UV absorption, peroxide decomposition and metal chelation (Pisoschi & Pop, 2015).

Differences between the results obtained in this study and others can be explained by the age of the plant (stage of maturity), soil physicochemical composition and nature, harvest time and climate. The alternation of the four seasons of the town of Yaoundé directly or indirectly affected the nutritional and antioxidant potential of *Moringa oleifera* leaves dried under different techniques. During the dry seasons, average of temperature is generally high and can be responsible for plant metabolism modification to adapt itself to rough conditions. Sometimes, these strong conditions can lead to accumulation of nutrient with an increase of their content as a consequence; but during rainy seasons the variability of many microorganisms infecting plants can also lead to the synthesis of secondary metabolites.

4. Conclusion

In summary, it can be concluded that *Moringa oleifera* leaves, which are normally consumed as vegetables and as food supplement, were rich in macronutrients and had stronger antioxidative activity required for proper growth and good health for human. Harvest season and drying techniques affected the nutrient content of *Moringa oleifera*. There is more water in leaves collected during the long rainy season and less in leaves from the long dry season. Proteins are more present in small dry and long rainy seasons *Moringa oleifera* leaves. The results of this study also showed that *Moringa oleifera* leaves can be used as an easily accessible source of natural antioxidants in the food and pharmaceutical industries. The abundantly available inexpensive leaves of *Moringa oleifera* can serve as a pool house of nutrients and can be used in the developing countries to combat micronutrient deficiencies. Although, the leaves of all the seasons have varying percentages of nutritional and antioxidant composition tested for, the small rainy and dry seasons recorded the highest nutritional composition and the long rainy season the highest antioxidant potential. The drying technique uncertainly influenced the content of nutrients and antioxidants. Therefore, the choice depends on the individual as it is a potential leaf source of food that is suitable for fortification of foods and their use as nutritional supplements is highly promising.

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Conflict of interests

The author(s) did not declare any conflict of interest related to this work.

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