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EFFECT OF DIFFERENT DRYING METHODS ON THE ANTIOXIDANT PROPERTIES OF 4 LEAFY VEGETABLES CONSUMED IN NORTHERN CÔTE D'IVOIRE

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ABSTRACT

In tropical Africa, reports of the antioxidant potential of consumed leafy vegetables are rare. In order to contribute to their valorization, leafy vegetables consumed in the north of Côte d'Ivoire (*Cerathoteca sesamoïdes*, *Leptadenia hastata*, *Ocimum gratissimum* and *Portulaca oleracea*) were studied. The objective of this study was to evaluate the effect of three drying treatments (sun, oven and shade) on the antioxidant properties of these leafy vegetables. These leafy vegetables were collected in the cities of Korhogo and Dabakala located in the North and North-central of Côte d'Ivoire, respectively and then were subjected to different drying methods, namely shade, sun and oven before their antioxidant properties were determined. The results show that shade drying resulted in less loss of vitamin C (37 to 66%) and carotenoids (93 to 100%). Leaves of *L. hastata* have the residual vitamin C (23.96±0.28 mg/100g) and carotenoids (0.32 ± 0.01 mg/100g) levels. This drying also resulted in a better concentration of phytochemicals. Leaves of *O. gratissimum* recorded the highest contents of total polyphenols (1118.19±0.74 mg/100g) and tannins (69.75 ± 0.43 mg/100g) while the leaves of *C. sesamoïdes* have the highest flavonoid content (99.44 ± 0.28 mg/100g). The concentration of phenolic compounds increases an antioxidant activity in three drying modes. This increase is greater during shade drying. *O. gratissimum* has the highest antioxidant activity (86.78%) during shade drying. Based on their antioxidant properties, *L. hastata* and *O. gratissimum* leaves could be considered good ingredients in the formulation of dietary supplements.

KEYWORDS: Leafy vegetables, antioxidant activity, sun drying, shade drying, oven drying.

INTRODUCTION

Most diseases are caused by oxidative stress, which is prompting the search for new antioxidant cures. Several situations of daily life lead to the production of free radicals responsible for oxidative stress. It results from environmental exposure to prooxidants such as tobacco, alcohol, drugs and pesticides (Sohal *et al*., 2002; Magder, 2006; Gbohaïda *et al*., 2015). Oxidative stress causes significant damage such as cell aging. This aging leads to serious pathologies such as cardiovascular and neurodegenerative diseases, cancer, diabetes, metabolic syndrome and digestive diseases (Aseervatham *et al*., 2013; Gbohaïda *et al*., 2016). In developing countries, these pathologies are responsible for at least 40% of deaths (Abdou, 2009). Moreover, the monitoring and drug management of these diseases are a real economic problem for the populations of developing countries (Le Quellec-Nathan, 2002; Kreatsoulas and Anand, 2010; Agban *et al*., 2013).

However, the use of available synthetic antioxidant molecules is currently questioned due to the potential health risks and toxicity they are capable of causing (Kicel *et al*., 2016; Liu & Yang, 2018). It is with this in mind that emphasis is placed on the search for new sources of antioxidants, including food and medicinal plants (Liu *et al*., 2017; Wang *et al*., 2018). Many studies have shown that these have antioxidant properties (Bokhari *et al*., 2013; Konan *et al*., 2014; Afsar *et al*., 2018). Indeed, these plants are full of significant quantities of phytochemical compounds (antioxidants) whose intake in adequate quantities through food or

enriched supplements constitutes a way against these pathologies raised whose effectiveness is today recognized (Salem, 2018). According to Pousset (2006), the use of natural plant products (fruits, vegetables) rich in phytochemicals could play an important role in the prevention of diseases linked to oxidative stress. On this subject, Owen *et al*. (2000) showed that plant extracts rich in phytochemical compounds and presenting a very marked antioxidant power could play an interesting role in the prevention of cancer by stabilizing free radicals. Among phytochemicals, vitamins C and E, carotenoids as well as phenolic compounds are the most studied and are linked to human health and well-being (Liu, 2003; Liu, 2004; Salem, 2018).

In foods of plant origin like leafy greens, these phytochemicals have remarkable biological activities, which enables these leafy greens to be used as medicinal ingredients and food additives for therapeutic, flavoring and culinary (Sinsin and Kampmann, 2010; Gouwakinnou *et al*., 2011; Al-Snafi, 2018). According to some authors (Alexander, 2016; Bayala *et al*., 2018; Diarra *et al*., 2020) these leafy vegetables are very rich in micronutrients and bioactive compounds and could therefore constitute an ideal alternative to so-called conventional drugs (Effoé *et al*., 2020).

Despite all the recognized potential of traditional leafy vegetables, they are still underexploited and underutilized due to a lack of information on their antioxidant properties.

The objective of this study is to evaluate the effect of different drying methods on the phytochemical and antioxidant properties of the leaves of *Cerathoteca sesamoïdes*, *Leptadenia hastata*, *Ocimum gratissimum* and *Portulaca oleracea* with a view to their valorization in the dietary field.

MATERIAL AND METHODS

1- Material

1.1- Biological material

Leafy vegetables (*Cerathoteca sesamoïdes*, *Leptadenia hastata*, *Ocimum gratissimum* and *Portulaca oleracea* were collected fresh at Dabakala (latitude: 08°23' North; longitude: 04°26'West) and Korhogo (Latitude North: 09°27'41''; longitude west: 05°38'19''). These plants were previously authenticated by the National Floristic Center (University Felix Houphouët-Boigny, Abidjan- Côte d'Ivoire).

1.2- Chemicals

Standards used (gallic acid, tannic acid, quercetin, beta-carotene, dichlorophenol-indophenol) and reagents (metaphosphoric acid, vanillin, Folin-Ciocalteu, DPPH) were purchased from Sigma Aldrich.

2- Methods

2.1- Leafy vegetables processing

The fresh leafy vegetables were destalked, washed with deionized water and edible portions were separated from the inedible portion. The edible portions were allowed to drain at ambient temperature and separated into 4 portions of 250 g each

2.1.1- Oven drying

Oven drying was carried out according to the method of **Chinma and Igyor (2007)**. 250 g of leaves were dried in an oven (MEMMERT) at 60°C for 3 days. The dried leafy vegetables were ground with a laboratory crusher (Culatti, France) equipped with a 10 µm mesh sieve and stored in air-tight containers for further analysis.

2.1.2- Sun drying

The fresh leafy vegetables (250 g) was spread on black polythene sheet and dried under the sun (35-38°C) for 5 days during 10 hours per day **(Mepba *et al*., 2007)**. The leaves were constantly turned to avert fungal growth. After drying period, the dried leaves were ground with a laboratory crusher (Culatti, France) equipped with a 10 µm mesh sieve and stored in air-tight containers for further analysis.

2.1.3- Shade drying

250 g was spread on clean filter paper and kept in a well-ventilated room of the laboratory at 25°C for 15 days. Natural current of air was used for shadow drying and the leaves were constantly turned to avert fungal growth **(Vanderhulst *et al*., 1990)**. After drying period, the dried leaves were ground with a laboratory crusher (Culatti) equipped with a 10 µm mesh sieve and stored in air-tight containers for further analysis.

2.2- Antioxidant Compounds Analysis

Vitamin C contained in analysed samples was determined by titration **(Pongracz *et al*., 1971)**. 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 **(Rodriguez-Amaya, 2001)**. Two (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 β-carotene (1 mg/mL) as standard.

Polyphenols 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 total flavonoids content was evaluated using the method reported by **Meda *et al.* (2005)**. Briefly, 0.5 mL of the methanolic extract was mixed with 0.5 mL methanol, 0.5 mL of AlCl3 (10%, w/v), 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water. The mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was measured at 415 nm by using a spectrophotometer (PG Instruments, England).

Tannins of samples were quantified according to **Bainbridge *et al.* (1996)**. For this, 1 mL of the methanolic extract was mixed with 5 mL of vanillin reagent and the mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was read at 500 nm by using a spectrophotometer (PG Instruments, England).

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 methanol 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 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:

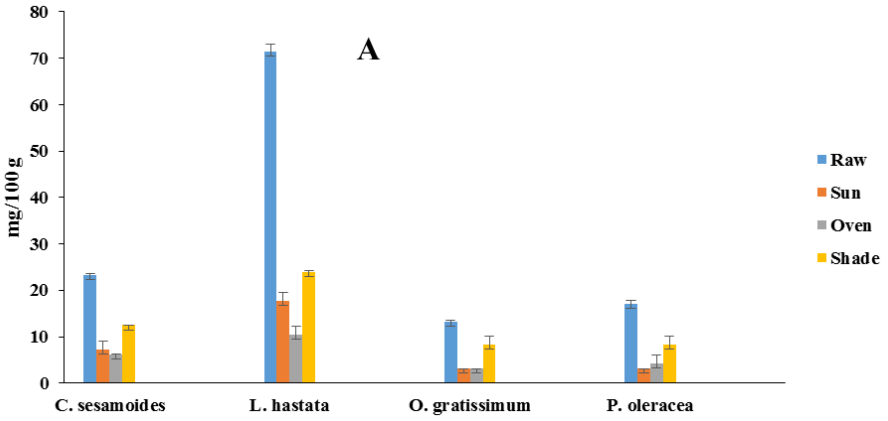
Antioxidant activity (%) = 100 – [(Abs of sample – Abs of blank) x 100/Abs positive control].

2.3- Statistical analysis

The statistical analyses were performed with Graph Pad Prism software version 8.0.2 (263). The variance analysis (ANOVA) was performed to determine differences between the averages according to method of Turkey at the 5% threshold ($p<0.05$ was considered significant). The results were expressed as averages with standard error on mean (mean ± SEM).

RESULTS AND DISCUSSION

Figure 1 shows the effect of drying on vitamin C and carotenoid contents of the leafy vegetables studied. Statistical analyzes of these parameters showed a significant difference ($p \leq 0.05$) in the 4 dried leafy vegetables. In general, losses of vitamin C and of carotenoids are observed varying from 37 to 85% and 93 to 100% respectively during the different drying modes. These losses of vitamin C and carotenoids are greater during oven during (72 to 85% and 98 to 100%) followed by sun drying (67 to 82% and 97 to 100%) and finally shade drying (37 to 66% and 93 to 100%). Vitamin C losses were most pronounced in the leaves of *L. hastata* (66–85%), followed by *P. oleracea* (51–82%), *C. sesamoïdes* (44–72%) and finally *O. gratissimum* (37 to 76%) while those of carotenoids were recorded in the leaves of *P. oleracea* (100%) followed by *O. gratissimum* (96 to 100%), *L. hastata* (93 to 97%) and finally in *C. sesamoïdes* (93 at 98%). These losses in vitamin C and carotenoids were also observed by **Zoro *et al.* (2015)** during sun-drying of leafy vegetables consumed in western Côte d'Ivoire. Similarly, the work of **Oulai *et al.* (2016)** showed that vitamin C and carotenoids losses were 35.52 – 70.50% and 22.82 – 45.63% respectively after 15 days of drying in the shade of leafy vegetables consumed in the North from Côte d'Ivoire. These losses of vitamin C and carotenoids could be due to the action of heat, oxygen, light and UV rays, which are factors that accelerate their oxidation **(Turkyilmaz *et al.*, 2014; Garcia -Martinez *et al.*, 2013; Gumusay *et al.*, 2015)**. Despite these losses, consumption of the leaves could cover half of the daily vitamin C requirements (40 mg/day) **(FAO, 2004)**. While supplementation of these leaves with fat could contribute significantly to improving vitamin A intake **(Takyi, 1999)**.



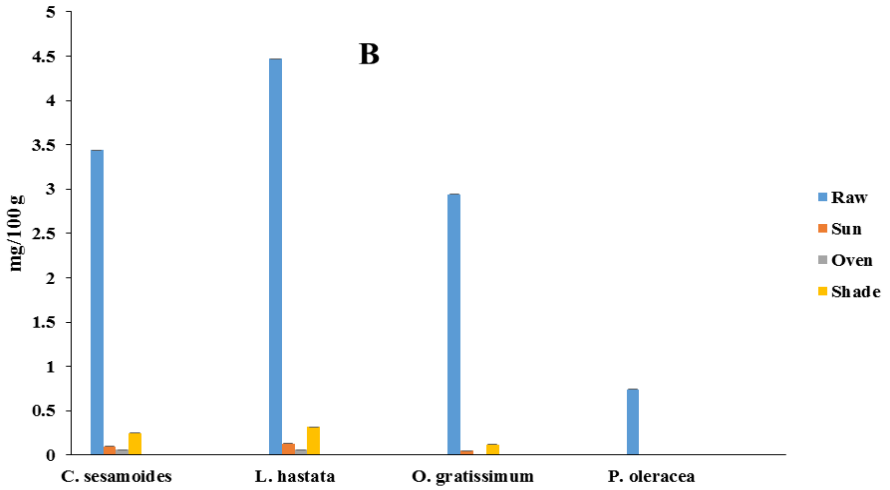


Figure 1: Vitamin C and Carotenoid contents of leafy vegetables studied at different drying times.

The table below presents the flavonoid and tannin contents of the 4 leafy vegetables studied according to the different drying methods. Statistical analysis shows a significant difference ($p \leq 0.05$) in the two parameters studied. Unlike carotenoids and vitamin C, there is a concentration of flavonoids and tannins. For flavonoids, compounds such as myricetin, quercetin, kaempferol, isorhamnetin and luteolin have been reported in leafy vegetables by **Trichopoulou *et al*. (2000)**. They are diphenols with vitamin properties and antioxidant action by capturing free radicals in the body **(Perez-Viscaino *et al*., 2010)**. They are also involved in the prevention of cardiovascular diseases. According to **Scalbert *et al*. (2005a)**, flavonoids also act in the body by inhibiting the platelet aggregation involved in thrombosis, which can lead to artery occlusion. Flavonoid contents of the leaves studied ranged from 10.86±0.35 (*P. oleracea*) to 99.44±0.88 (*C. sesamoïdes*). The leaves of *P. oleracea* had the highest concentrations (15 to 30 times) followed by leaves of *L. hastata* (7 to 8 times) then of *C. sesamoïdes* (5 to 9 times) and finally of *O. gratissimum* (4 to 6 times). Shade drying allowed the highest concentrations (6 to 30 times) followed by sun (4 to 18 times) and oven (4 to 15 times). Tannins are phenolic compounds of plant origin that are widely used in human food **(Hemingway, 1989; Muanda, 2010)**. They have the property of binding to macromolecules such as proteins. Tannins play a major role in the treatment of inflamed tissues, in diarrhea and in the prevention of cancer **(Okwu and Emenike, 2006)**. The tannin contents of the leaves studied vary between 21.61±0.22 (*L. hastata*) and 69.75±0.43 (*O. gratissimum*). Leaves of *P. oleracea* had the highest concentrations (9 to 22 times) followed by leaves of *L. hastata* (7 to 12 times) then leaves of *C. sesamoïdes* (8 to 10 times) and finally *O. gratissimum* (4 to 5 times). Unlike flavonoids, shade drying has the highest concentrations (5 to 22 times) followed by oven drying (4 to 16 times) and finally sun drying (5 to 9 times). This concentration of flavonoids and tannins could be due to evaporation of water from the leaves during drying. This concentration could be advantageous for the consumer since flavonoids and tannins are involved in the prevention of cardiovascular diseases.

Table I: Flavonoids and tannins content of leafy vegetables studied in different drying modes.

Leafy vegetables	Flavonoids (mg/100g)	Tannins (mg/100g)
C. sesamoïdes		
Raw	15.36±0.28^{k}	6.02±0.06^{j}
Sun	81.12±0.58^{b}	50.43±0.36^{c}
Oven	76,65±0.58^{c}	48.75±0.28^{d}
Shade	99.44±0.28^{a}	57.42±0.28^{b}
L. Hastata		
Raw	2.28±0.05^{m}	3.37±0.15^{k}
Sun	19.09±0.68^{h}	21.61±0.22^{h}
Oven	18.23±0.84^{h}	25.79±0.61^{g}
Shade	25.23±0.57^{e}	37.03±0.22^{f}
O. gratissimum		
Raw	5.41±0.95^{l}	13,80±0.21^{i}
Sun	22.17±0.48^{f}	68.63±0.65^{a}
Oven	21.14±0.61^{fg}	50.33±0.46^{c}
Shade	30.84±0.27^{d}	69.75±0.43^{a}
P. oleracea		

Raw	0.68±0.01^{n}	2.65±0.19^{l}
Sun	12.55±0.94^{i}	24.75±0.92^{g}
Oven	10,86±0.36^{j}	41.08±0.49^{e}
Shade	20.35±0.17^{g}	57.21±0.57^{b}

Values given are the averages of at least three experiments ±SE. Values followed by different superscript on the same column are significantly different (P=0.05).

Figure 2 shows the change in total polyphenol content and antioxidant activity with different drying modes. Statistical analyzes showed significant differences ($p \leq 0.05$) among the 4 leafy vegetables studied. The total polyphenols ranged from 258.11±0.71 (*P. oleracea*) to 1118.47±0.49 (*C. sesamoïdes*). Leaves of *C. sesamoïdes* recorded the highest concentrations followed by leaves of *O. gratissimum* then *L. hastata* and finally *P. oleracea*. Drying in the shade allows a better concentration of total polyphenols (7 to 23 times) followed by the sun (5 to 22 times) and oven (4 to 16 times). These results are in agreement with the work of **Park *et al.* (2006)** which recorded a concentration of total polyphenol in dried khaki (*Diospyros kaki*). This high concentration during shade drying could be explained by the fact that the high drying temperature decreased phenolic compounds due to oxidation and thermal degradation of these compounds **(Galaz *et al.* (2017); Asami *et al.* (2003))**. This concentration of total polyphenols is correlated with the increase in antioxidant activity. Thus, shade drying shows a better increase in antioxidant activity (5–9 times), followed by sun drying (4–8 times) and finally shade drying (3–8 times). The antioxidant activity of the studied leaves varied from 50.27±0.13% (*P. oleracea*) to 86.78±0.10% (*O. gratissimum*). The antioxidant activity of the leaves studied ranged from (39.15±1.39% - 91.31±1.01%) obtained by **Mohammad *et al.* (2020)** on the study of total polyphenols, carotenoids and antioxidant activity of selected unconventional vegetables in Bangladesh. Leaves of *O. gratissimum* showed the highest increases, followed by *C. sesamoïdes*, *L. hastata* and *P. oleracea*. Leaves of *O. gratissimum* and *C. sesamoïdes* could be used for medicinal purposes.

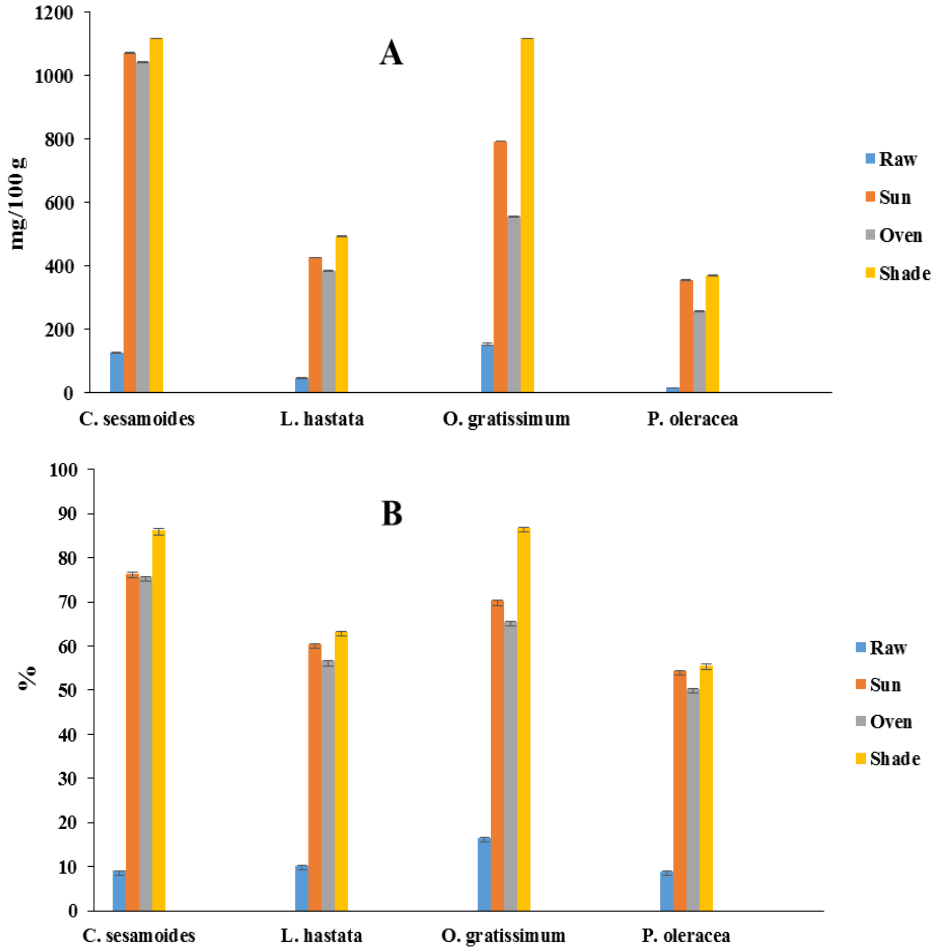


Figure 2: Total polyphenol content (A) and antioxidant activity (B) of leafy vegetables studied according to drying method.

CONCLUSION

In view of the results of the present study, the different drying modes led to an increase in the concentration of phenolic compounds (polyphenols, flavonoids and tannins). In contrast, they led to a decrease in vitamin C and carotenoids concentrations in the leafy vegetables studied. However, shade drying has resulted in a higher concentration of phytochemicals in leafy vegetables. This method of drying resulted in lower losses of vitamin C and carotenoids compared to sun and oven drying. Moreover, on the basis of their antioxidant properties, the leaves of *L. hastata* and *O. gratissimum* could contribute to the prevention and improvement of the health of Ivorian populations.

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