Effect of boiling times on polyphenol, flavonoid, tannin, vitamin C, and β-carotene contents of African asparagus (*Laccosperma secundiflorum*): their contribution to overall antioxidant activity

ABSTRACT

The effect of boiling on the total polyphenols, flavonoids, tannins, vitamin C and β-carotene contents, and further on the antioxidant activity of the heart of rattan palm (African asparagus) was studied quantitatively at different times. Vitamin C content, which was estimated initially at 37.04 g/100 dry matter, decreased as the cooking time in water increased. Indeed, it is noted 76.80, 78.57, and 84.00 % of loss respectively for the cooking times of 5, 10, and 15 min in the water. Also, these different heat treatments affected caused drastic damage on the β- carotene content of African asparagus with losses ranging from 40 to 80%. Polyphenols were also affected by cooking. The values obtained were 5104.80 mg EAG/100g for fresh African asparagus (FRESH), 5284.09 mg EAG/100g for boiling for 5 min (CE5), 5233.31 mg EAG/100g for boiling for 10 min (CE10), and 3536.44 mg EAG/100g for boiling for 15 min (CE15). The tannin content for fresh asparagus on the other hand was 1954.84 mg/100g DM. However, this rate decreased continuously with heating and was is estimated at 1699.93 mg/100g DM, 1429.977548 mg/100g DM, and 1035.42 mg/100g DM respectively for CE5, CE10, and CE15 samples with losses of 13.04, 26.85 and 47.03% respectively. For flavonoids, the losses were varied from are between 7.41 and 19.92%. Finally, boiling reduces in general the levels of the different parameters studied, but the antioxidant activity of African asparagus increased at the end of the 15 min heat treatment. However, a cooking time of less than or equal to 5 min in water can be advantageous for the consumer.

Keywords : African asparagus, antioxidant activity, boiling.

INTRODUCTION

During this last decade, high consumption of fruits and vegetables associated with decreased risk of diseases such as cardiovascular pathologies, obesity, diabetes, neurodegenerative diseases, and cancer has been observed in numerous epidemiological studies (**Lenoir, 2011**). This high consumption of fruits and vegetables is due to the richness of these foods in antioxidants (ascorbic acid, tocopherols, carotenoids, and polyphenols) which are molecules with preventive effects against these diseases because they participate in the neutralization of free radicals. These free radicals are permanently generated by our body or formed in response to environmental aggressions. Polyphenols are micronutrients that are particularly abundant in cereals, fruits, and vegetables (**Bravo, 1998**). Their interest lies in their antioxidant properties, especially their capacity to trap free radicals (**Mehinagic *et al.*, 2011**). These plants also have multiple properties, among others antioxidants because of antioxidants compounds such as vitamins, carotenoids, phenolic compounds ...in their edible parts (**Minussi *et al.*, 2003; Turkmen *et al.*, 2005**). Several plants, fruits, and vegetables are consumed in Côte d'Ivoire in lean periods (**DaGiau., 2014**), and most of them are cooked before consumption, as in the example of for example the African asparagus. However, cooking culinary practices induce significant changes in their of chemical compositions,

~~which influences~~ the concentration and bioavailability of bioactive compounds in these vegetables. Positive as well as negative effects have been reported based on differences in processing conditions and morphological and nutritional characteristics of vegetable species (**Brou *et al.*, 2018**). Knowing how and why changes occur can help the consumer, the food processor, and even the chef to limit waste and therefore improve the nutritional quality of food. ~~The objective of~~ this study aimed ~~was~~ to evaluate the effect of boiling water cooking on total polyphenols, flavonoids, tannins, vitamin C and β-carotene content, and therefore on the antioxidant activity of African asparagus.

I-MATERIALS AND METHODS

I.1-Sampling

African asparagus *Laccosperma secundiflorum* was harvested in the region of Agneby Tiassa, more exactly in the area of Sikensi, 5°40'40" North latitude 4°34'33" South longitude. African asparagus was transported in polypropylene plastic bags directly to the laboratory for analysis (**Fig.1**).

Fig.1: African asparagus

A: African asparagus enveloped in leaf sheaths, **B**: African asparagus with leaf sheaths removed

I.2-Methods

I.2.1- Heat treatment of samples

The boiling of African asparagus was done according to the method described by **Randrianatoandro (2010)**. 1.5 kg of African asparagus cut into 5 cm "sticks" were immersed in 1 L of boiled water in a stainless steel container for 5, 10, and 15 min. The cooking solution was discarded and the boiled samples were cooled, drained at ambient temperature, and subjected to the same treatment ~~using~~ used for raw samples.
The cooked African asparagus was oven-dried (Biobase, China, Shandong) at 45°C for 48 h. They were then powdered with a Binatone-type blender (BLG-555, China, Hong Kong) and sieved using a sieve with a mesh size of 500 µm (AFNOR -NFX 11504). The obtained

~~resulting~~ powders were stored in stomacher bags and kept at 4°C in a refrigerator (NASCO, DF2-28, China) for further analysis. The fresh sample was used as a control.

I.2.2- Biochemical analysis of the samples

I.2.2.1- Determination of Vitamin C (Vit C) content

The vitamin C content was determined according to the method described by **Pongracz (1971)**, using 2,6-dichlorophenol indophenol. This method involves stabilizing vitamin C with metaphosphoric acid/acetic acid and then oxidizing it with 2,6-dichlorophenol indophenol (2,6-DCPIP) which is then reduced. The vitamin C content was obtained from this mathematical relationship:

$$\text{Vitamin C (mg/100g)} = \frac{(Ve-Vo) \times 20}{(Vs-Vo) \times 10} \times 100$$

Ve: volume of 2,6-dichlorophenol-indophenol poured for the sample ;

V_0: volume of 2,6-dichlorophenol-indophenol poured for the determination of metaphosphoric acid

Vs: volume of 2,6-dichlorophenol-indophenol poured for the determination of the vitamin C stock solution

I.2.2.2- Determination of β-carotene content

The concentration of β-carotene was determined according ~~from~~ to the method described by **Tee *et al.* 1996**. African asparagus samples (10g) were homogenized in ethanol (40 mL). The mixture was introduced into a separatory funnel containing 50 mL of hexane. The hexane phase was evaporated for 24 hours. Another 10 ml of hexane was added to this phase. After the rest of 24 hours, the optical density (OD) was read using a spectrophotometer (MS-V5100 visible spectrophotometer, Germany) at 450 nm against a blank solution. The standard solution was prepared with 10 mg of trans-β-carotene dissolved in pure hexane to obtain a 100 µg/mL solution.

I.2.2.3- Determination of total polyphenol content

~~The method described by **Singleton *et al.* (1999)** using~~ Folin-ciocalteu method was used to determine the total phenols content (**Singleton *et al.* 1999**). To a test tubes were added 1 ml of methanolic extract and 1 ml of Folin-ciocalteu reagent. The tube was left to stand for 3 min and then 1 mL of sodium carbonate solution (20%, w/v) was added. The contents of the tube were made up to 10 mL with distilled water. After 30 min in the dark, the absorbance was read using a spectrophotometer (MS-V5100 visible spectrophotometer, Germany) at 725 nm against a blank. A standard range was performed with a 1 mg/mL gallic acid solution.

I.2.2.4- Determination of total flavonoid content

Flavonoids were determined according to the method described by **Meda *et al.* (2005)**. Into test tubes were successively added, 0.5 mL of methanolic extract, 0.5 mL of distilled water, 0.5 mL of aluminum chloride (10%), and 0.5 mL of potassium acetate (1 M). The final

volume was made up ~~with~~ of 2 mL of distilled water. The test tube was then incubated in the dark for 30 minutes. The absorbance was measured at 415 nm using a spectrophotometer (MS-V5100 visible spectrophotometer, Germany) against a control. A calibration curve was made using a 0.1 mg/mL quercetin standard solution.

I.2.2.5- Determination of total tannin content

Determination of tannins was carried out according to the method described by **Bainbridge *et al.* (1996)**. Into test tube, 1 mL of the methanolic extract was homogenised with 5 mL of vanillin reagent (0.1 mg/mL vanillin in 70% (v/v) sulphuric acid). The mixture was then incubated in the dark for 20 minutes at room temperature. The absorbance was measured at 500 nm using a spectrophotometer (MS-V5100 visible spectrophotometer, Germany) against a blank solution. A calibration range was performed using a 0.1 mg/mL tannic acid standard solution.

I.2.2.6- Measurement of antioxidant activity by DPPH radical

The antioxidant activity of the extract was measured with the DPPH method described by **Choi *et al.* (2002)**. A solution of DPPH was freshly prepared (about 0.3 mM). The extract (2 mL) with varying concentrations (2-20 µg/mL) and DPPH solution (1 mL) were mixed ~~together~~ in each test tube. The test tube was then incubated in the dark for 30 minutes at room temperature. The decrease in absorbance was measured at 517 nm using a spectrophotometer (MS-V5100 visible spectrophotometer, Germany). The percentage inhibition of radicals was calculated using the following formula:

$$\boldsymbol{AA}\ (\%) = \frac{[\boldsymbol{DO_c} - (\boldsymbol{DO_e} - \boldsymbol{DO_b})] \times \mathbf{100}}{\boldsymbol{DO_c}}$$

AA: antioxidant activity

DOc: absorbance of control tube (1 mL DPPH + 2 mL methanol)

DOe: absorbance of test tube (2 mL methanol extract + 1 mL DPPH)

DOb: absorbance of blank tube (1 mL methanol + 2 mL methanolic extract)

I.2.3- Statistical analysis of the results

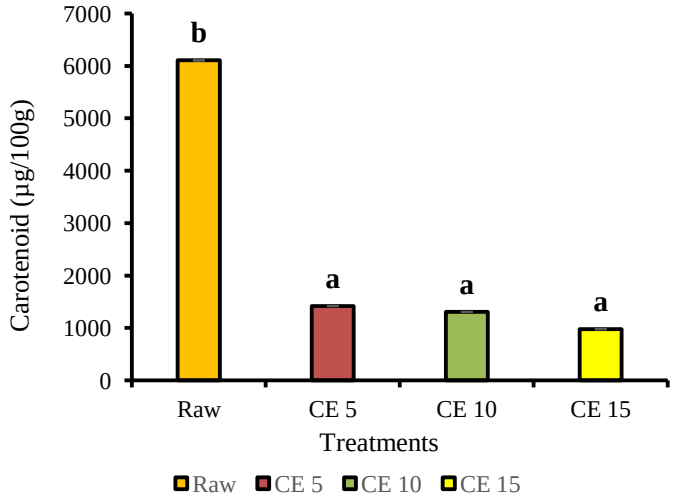
The statistical analysis was applied to the data obtained during the biochemical evaluations. All tests relating to the different analyses were carried out in triplicate and the numerical values obtained were expressed as the arithmetic mean affected by the standard deviation. The one-factor ANOVA variance analysis was performed on all the results obtained to determine the existence of significant differences between the averages calculated according to the DUNCAN test using the STATISTICA software version 7.1. The graphs were built using Excel software.

II. RESULTS AND DISCUSSION

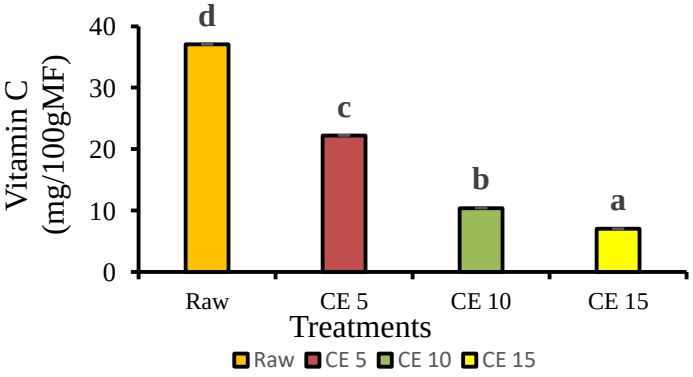
II.1-Results

II.1.1-Vitamin C and β-carotene

Ascorbic acid and β-carotene contents are shown in **Figures 2A and 2B**. A decrease in the contents of these two parameters was ~~is~~ observed with increasing cooking time. As for vitamin C, the losses in content oscillate between 76.80 and 84.~~00~~%. As for β-carotene, ~~they are~~ it was varied from ~~between~~ 40 and 80%. ~~Ascorbic acid and β-carotene contents are shown in Figures 2A and 2B. A decrease in the contents of these two parameters is observed with increasing cooking time. As for vitamin C, the losses in content oscillate between 76.80 and 84.00%. As for β-carotene, they are between 40 and 80%.~~ (Repeated paragraph !)



A



B

Fig.2 : Vitamin C (A) and β-carotene (B) content of raw and water-cooked African asparagus (EC) at different times (5,10 and 15 min)

EC5 : cooking 5 min in water ; EC10 : cooking 10 min in water ; EC15 : cooking 15 min in water.

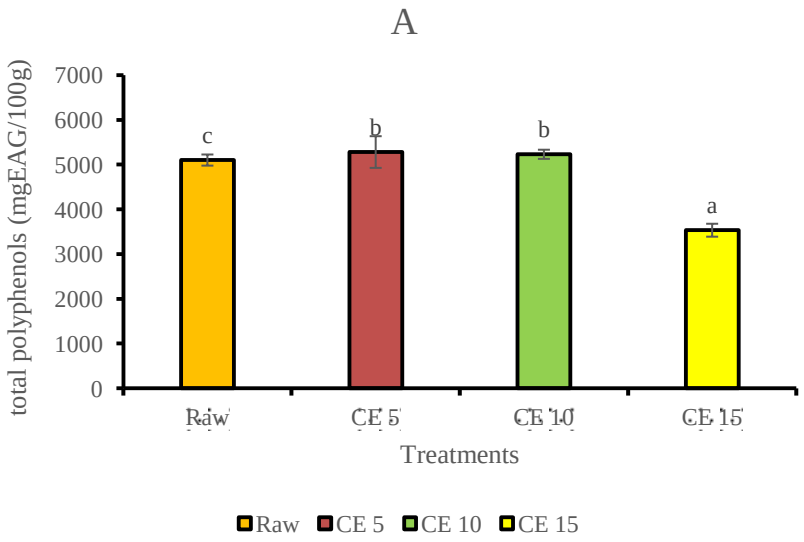
II.1.2-Phytochemical composition of boiled African asparagus

The obtained results for the nutrient parameters (which one of polyphenol ?) were ~~are~~ 5104.80, 5284.09, 5233.31, and 3536.44 mg EAG/100gMS respectively for the FRESH, CE5,

CE10, and CE15 samples (Figure 3A). No significant difference ($p > 0.05$) was ~~is~~ noted between the means of the three cooked samples. However, these averages were ~~are~~ found to be statistically different from those of fresh African asparagus. Furthermore, a slight increase in polyphenol content of ~~2.51% and 17%~~ (please redo the calculation 3.51 and 2.51% for CE5 and CE10, respectively) was observed for CE5 and CE10, respectively. Then, a 30.72% (please redo the calculation, the real percentage was 44.34%, and confirm all percentages given in the whole manuscript, OK !) drop in the averages was recorded at the 15th minute of cooking. Despite this sudden decrease, polyphenol content (3536.44 mg EAG/100gMS) remains high in the samples cooked for 15 min (compared to what ?).

The tannin contents of African asparagus during cooking in water were 1954.84, 1699.93, and 1429.98 mgAT/100gMS for the FRESH, CE5, CE10, and CE15 samples respectively (Figure 3B). In contrast to the polyphenols, cooking in water generally leads to a decrease in the tannin content of the samples with losses of 13.04, 26.85, and 47.03%, respectively for CE5, CE10, and CE15. The analysis of variance shows overall a significant difference ($p < 0.05$) between the cooked samples.

Flavonoid contents ranged from 49.43 mgEq/100gMS (FRESH) to 39.58 mg quercetin/100gMS (CE15) during cooking. At 5 min, 10 min, and 15 min of cooking in water, losses of 7.41 %, 12.15 %, and 19.92 %, respectively were observed. Also, a significant difference ($p < 0.05$) was ~~is~~ observed between fresh and cooked samples (Figure 3C).



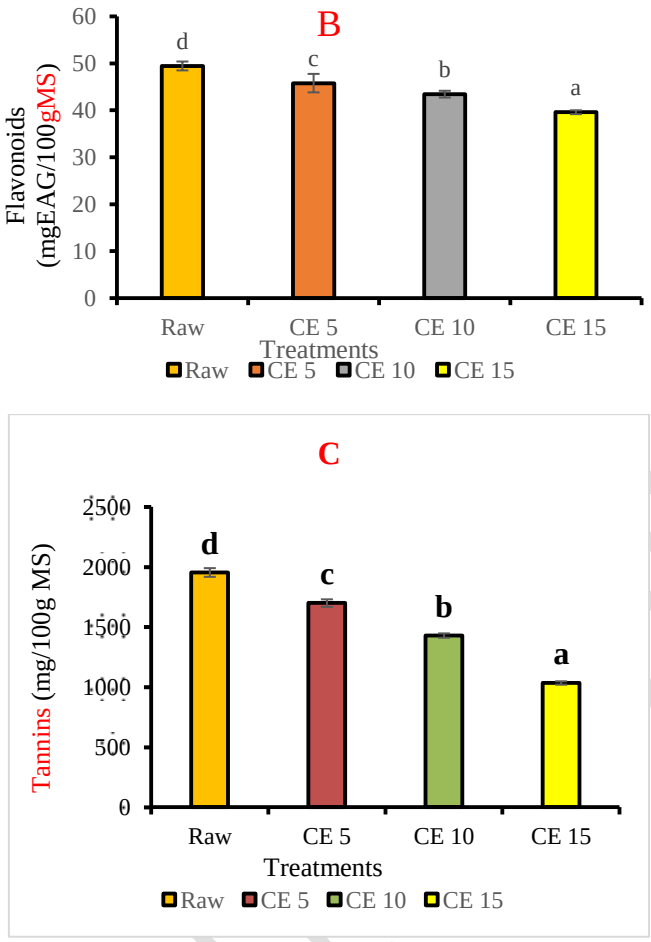


Fig.3 : Total polyphenol (A), flavonoid (B), and tannin (C) contents of fresh and boiled African ~~and boiled~~ asparagus.

EC5: cooking 5 min in water ; EC10: cooking 10 min in water ; EC15: cooking 15 min in water.

II.1.3 Antioxidant activity of water-cooked African asparagus

The percentages of free radical scavenging activity are shown in the figure below. The antiradical activity increases with the concentration of the sample. On the other hand, the activity of cooked samples was ~~is~~ lower than that of fresh samples. Antioxidant activity values vary between 59.81% and 91.16%. They are equivalent to 91.16±0.23%, 84.7±0.5%, 82.59±1.89%, 81.37±0.32% for raw African asparagus and CE5, CE10, CE15 samples, respectively (Figure 4). In contrast to fresh asparagus, a variation in the antioxidant capacity of the boiled samples was observed (Figure 4).

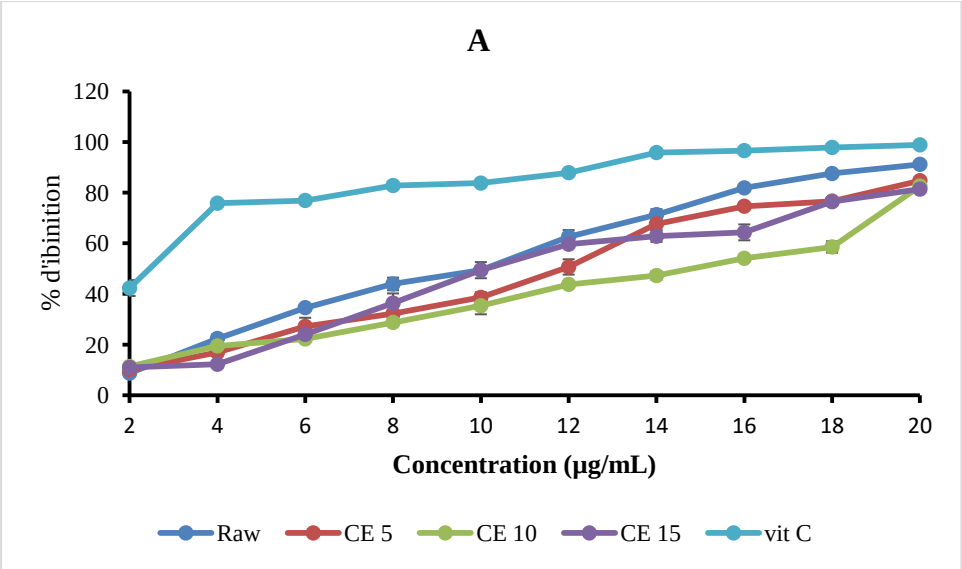


Fig.4 : DPPH free radical scavenging of fresh and water-cooked African asparagus extracts (EC) at different times (5,10 and 15 min).

EC5: cooking 5 min in water ; EC10: cooking 10 min in water ; EC15: cooking 15 min in water ; vit C: vitamin C

An increase in the percentage of DPPH radical inhibition was ~~is~~ observed with the increase of the concentrations of the extracts ~~at increasing concentrations of the extracts of the different samples~~ of fresh African asparagus, cooked in water, and vitamin C used as standard.

Vitamin C and asparagus extracts show good ~~percentages of~~ DPPH radical scavenging activity ~~inhibition~~. The IC50 values of the raw extracts ~~Raw~~, CE5, CE10, and CE15 samples were ~~are~~ 10.07 µg/mL, 12.07 µg/mL, 15.2 µg/mL and 10.33 µg/mL, respectively. These different concentrations were ~~are much~~ higher than that of vitamin C (2.53µg/mL). The different extracts, as well as vitamin C, have significantly different IC50 values at the 5% threshold. The extract of the Raw sample has the highest radical scavenging activity followed by CE5 and CE15 samples (it's the opposite, isn't it? Followed by CE15 and CE5 samples. Since when the value of IC50 is low, therefore the anti-free radical activity will be considered important). On the other hand, under the same conditions, CE10 sample shows the lowest inhibitory activity (Table).

Table : IC50 values of the different samples tested

Treatments	IC50 (mg/mL)
RAW	10.07
CE 5	12.07
CE 10	15.20
CE 15	10.33
Vit C	2.53

CE5 : cooking 5 min in water ; CE10 : cooking 10 min in water ; CE15 : cooking 15 min in water.

II.2-Discussion

The results analysis of the heat treatment shows the effect of cooking in water on the nutritional composition of African asparagus. The different cooking times to which the African asparagus studied are subjected significantly influence their nutritional parameters studied. Boiling significantly reduces the vitamin C content. ~~In fact,~~ This method of processing caused more than 20-70% loss, with the greatest loss (70%) occurring in samples cooked for 15 minutes. Earlier studies, including those cited by **Yadav and Sehgal (1995, 1997),** have shown that boiling results in huge losses of vitamin C in spinach and fenugreek in the range of 36-83%. The level of β-carotene also decreases during heat treatment and losses vary between 76 and 84%. **Tessier (2012**), attributes these losses to the sensitivity of β-carotene to oxygen and oxidation catalysts (lipoxygenase enzyme) or occasioned by the leaching of nutrients from the vegetables during blanching or boiling. Raw and cooked African asparagus contains over 900 µg of β-carotene per 100g of flour, which is more than the recommended daily intake for adults (**George, 1999; Akanya, 2004**). β-carotene deficiency remains a public health problem affecting 19 million pregnant women in Africa (**WHO, 2009**) where the estimated requirement for this vitamin is 800 µg/day (**WHO, 2011**). Consumption of African asparagus would therefore be beneficial for this segment of the population. The susceptibility of African asparagus to browning depends on the relative concentration of the different groups of phenolic compounds. Boiling resulted in losses of up to 9.94% (total polyphenols), 19.92% (flavonoids), and 47.03% (tannins) after 15 min of cooking.

The decrease in flavonoid and tannin content is attributed to the leaching of phenolic compounds during heat treatment (**Wong *et al.*, 2006**). The analysis of nutritional properties reveals that fresh African asparagus was ~~is very~~ rich in tannins with ~~a~~ content equal to (2091.66 mgAT/100gMS). However, this content decreases with increasing cooking time. Similar results were observed in comparison with the study ~~with the work~~ carried out by **Zhang and Hamauzu (2004)** and **Brou *et al.* (2018)** who showed a loss of tannic acid after different cooking processes due not only to the degradation of tannins, but also to the formation of insoluble complexes. Tannins are involved in tissue regeneration. They contribute to stopping hemorrhages and help fight infections (**Khanbaba and Ree, 2001**), particularly in pre-and post-natal care, as is the case in southern Côte d'Ivoire. Regular consumption of African asparagus in households could thus prevent ~~a number of~~ several diseases such as gastrointestinal disorders, high blood pressure, and malaria (**Sereme *et al.*, 2008**). Total polyphenol contents, on the other hand, undergo a slight increase at 5 min of cooking in water. **Kao *et al.*, (2014)**, in their work on Thai basil and potato leaves, observed a more or less significant increase in total polyphenol content during the first five (5) minutes of boiling. **Turkmen *et al.*, (2005)** reported that cooking for a short time (5 min) increased the polyphenol content of some green vegetables such as green beans, spinach, and pepper. Indeed, studies have shown that the increase in polyphenol content is due to the degradation of complex phenolic compounds present in vegetables into simple polyphenols but also, the decomposition of dietary fiber-bound polyphenols into free phenolic compounds under the effect of heat (**Stewart *et al.*, 2000**). These simple or free phenolic compounds would have subsequently migrated into the cooking water due to prolonged exposure to heat, resulting in a reduction in total polyphenol content. Increased antioxidant activity is closely related to vitamin C and total phenolic compounds. Indeed, **Montoro *et al.*, (2006b)** have shown that the antioxidant activity of plant extracts is strongly correlated to ~~its~~ their polyphenol composition. Moreover, studies carried out on the antioxidant activity of plant extracts

(*Nigella sativa* L.) have shown the capacity of these extracts to trap radical species and reactive oxygen forms of phenolic compounds (**Talbi *et al.*, 2015**). Furthermore, the antioxidant activity of phenolic compounds would ensure better preservation of food products by preventing the oxidation of lipids. Thus, the high content of total polyphenols found in both fresh and cooked African asparagus would be useful for children, who are subject to allergies caused by anti-nutritional substances. They could also be involved in the prevention of cardiovascular disease and cancer in adults (**Talbi *et al.*, 2015**).

CONCLUSION

This study found that African asparagus is a true source of antioxidants. Boiling increases total polyphenol levels when the cooking time is short but decreases them when the cooking time is long. Also, tannins, flavonoids, vitamin C and β-carotene contents all decreased with increasing cooking time. Finally, it is noted that cooking in water decreases the contents of the different parameters studied, but the antioxidant activity of African asparagus increased at the end of the 15 min heat treatment. However, a short cooking time of less than or equal to 5 min can be beneficial for the consumer, who is also recommended to consume the water used for cooking.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

Akanya, H. O. (2004). Retinol: The vitamin of life. Federal University of Technology, Minna. Inaugural Lecture Series No. 5. Scan Prints Nig. Ltd. p. 12.

Baindridge Z., Tomlins K. & Westby A. (1996). Analysis of condensed tannins using acidified vanillin. *Journal of Food Science*, 29, 77-79.

Bravo, L. (1998). Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. Nutrition Reviews. 56 (11) : 31 7-333.

Brou M. R., Ekissi G. S. E., Fagbohoun J. B., Faulet M. B., Kouamé P. L. (2018). Impacts of Boiling Times on Physicochemical and Nutritive Composition from Heart of Oil Palm Tree (Elaeis guineensis Jacq.) Consumed as Vegetable in Côte d'Ivoire. *Advances in Research* 16(5) : 1-16

Choi C. W., Kim S. C., Hwang S. S., Choi B. K., Ahn H. J., Lee M. Z., Park S. H. & Kim S. K., (2002). Antioxidant activity and free radical scavenging capacity between

Korean medicinal plant and flavonoids by assay guided comparison. *Plant Sciences*. 163: 1161-1168.

DaGiau. S. (2014). "Ethnobotany, morpho-anatomy and phytogeography of useful palm (*Arecaceae*) species from Ivory Coast, with emphasis in rattan general. Master in *plant biology*, Faculty of Sciences (University of Geneva) 171 pages.

FAO/OMS (2004). Vitamin and mineral requirements in human nutrition. Report of a Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements, Bangkok, Thailand, 21–30 September 1998. 2nd ed. Genève, Organisation mondiale de la Santé.

George, D. P. R. (1999). Newlife style : Enjoy it. Editorial Safeliz. Spain. p. 39-100.

Kao F.J., Chiu Y.S., Chiang W.D. (2014). Effect of water cooking on the antioxidant capacity of carotenoid-rich vegetables in Taiwan. J. Food Drug Anal. 2014; 22:202–209. doi: 10.1016/j.jfda.2013.09.010.

Khanbaba K. & Ree T. R. (2001). Tannins: classification and Definition. ~~*Journal of royal society of chemistry*~~ Natural Product Reports, 18, 641-649 ~~page~~.

Lenoir, L. (2011). Effet protecteur des polyphénols de la verveine odorante dans un modele d'inflammation colique chez le rat. Médecine humaine et pathologie. Thèse de Doctorat de Nutrition. Université d'Auvergne. Science de la vie et de la sante, 1.

Meda A., Lamien C.E., Romito M., Millogo J. & Nacoulma O.G. (2005). Determination of total phenolic, flavonoid and proline contents in Burkina Faso honeys as well as their radical scavenging activity. Food Chemistry, 91, 571-577.

Mehinagic, E., Bourles, E. et Jourjon, F. (2011). Composés des fruits d'intérêt nutritionnel : impact des procédés de transformation sur les polyphénols. Revue suisse Viticulture, Arboriculture, Horticulture. 43 (6) : 364–368.

Minussi, R. C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastore, G. M., et al. (2003). Phenolic compounds and total antioxidant potential of commercial wines. Food Chemistry. 82 : 409–416.

Montoro P., Braca A., Pizza C., De Tommasi N. (2005). Structure-antioxidant activity relationships of flavonoids isolated from different plant species. Food Chemistry, **92**(2): 349-355.

OMS, (2009). Global prevalence of vitamin A deficiency in population at risk 1995 – 2005. WHO Global Database on vitamin A Deficiency. Genèse, Suisse

OMS (2011). Directive sur l'enrichissement des aliments en micronutriments, Italie. 412 P

Pongracz G., Weiser H. & Matzinger D. (1971). Tocopherols-Antioxidant. *Fat Science Technology*, 97, 90-104.

Randrianatoandro V. A. (2010). Identification et caractérisation des plates sources en micronutriments consommés en milieu urbain (Manjakaray, Madagascar) : étude des plats à base de légume-feuilles, thèse, Madagascar, Manjakaray, 150 p.

Sereme A., Millogo-Rasolodimby J., Guinko S., Nacro M. (2008). Propriété thérapeutique des plantes à tanins du Burkina Faso. *Pharmacopée et Médecine traditionnelle Africaines* 15: 41-49.

Singleton (1999). Analysis of total phenols and other oxydant substrates and antioxydants by means of Follin-ciocalteur eagent. Methode in Enzymology, 299:152-178.

Stewart AJ, Bozonnet S, Mullen W, Jenkins GI, Lean MEJ, Crozier A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *J Agric Food Chem*. ; 48(7) : 2663–2669. doi: 10.1021/jf000070p.

Talbi H., Boumaza A., El-mostafa K., Talbi J. & Hilali A. (2015). Evaluation de l'activité antioxydante et la composition physico-chimique des extraits méthanolique et aqueux de la *Nigella sativa* L. (Evaluation of antioxidant activity and physico-chemical composition of methanolic and aqueous extracts of *Nigella sativa* L.). *Journal of Materials and Environmental Science*, 6 (4): 1111-1117.

Tee. E. S, Kuladevan R., Young S. I, Khor S. C. & Zakiyah H. O. (1996). Nutrient analysis of foods. In Amin I., Cheah S. F., 2003. Determination of Vitamine C, β-carotene and Riboflavin Contents in Five Green Vegetables Organically and Conventionally Grown. Malaysian Journal of Nutrition, 9 (1) : 31-39.

Tessier F. J. (2012). Effet de la cuisson des aliments sur les pertes en vitamines. In : Correspondances en Métabolismes Hormones Diabètes et Nutrition - Vol. XVI - n^{os} 5-6

Turkmen, N., Sari Fet Velioglu, Y.S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry. 93 : 713–718.

Wong SP, LP Leong, JHW Koh (2006). Antioxidant activities of aqueous extracts of selected plants. Alimentary Chemistry, food and sciences technology programs, department of chemistry, National University of Singapore 117543, Singapore.

Yadav, S.K., Sehgal, A. (1995). Effect of home processing on ascorbic acid and beta carotene content of spinach and amaranth leaves. *Plant Food for Human Nutrition*, 47, 125-131.

Yadav, S.K., Sehgal, A. (1997). Effect of home processing on ascorbic acid and beta carotene content of bathua (Chenopyadavodium album) and fenugreek (*Trigonella foenun graecum*) leaves. *Plant Food for Human Nutrition*. Vol. 50, 239-247.

Zhang D. et Hamauzu Y. (2004b). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry. 88 : 503-509.