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

SAMPLE ABSTRACT:

Aims: *Laccosperma secundiflorum* is a very important rattan species for certain populations in the Ivory Coast. They use the apical meristem in their food and the stems to make various items such as furniture, carpets and baskets etc. However, there is a gap in the study of its nutritional value and its nutritional potential before and after boiling.
Study design: Mention the design of the study here.
Place and Duration of Study: Department of Food Science and Technology (UFR-STA), University Nangui Abrogoua, between December 2019 and November 2020.
Methodology: African asparagus was boiled for 5, 10 and 15 minutes respectively. 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 this different times. Standardized methods were adopted for phytochemical, antioxidant activity of this samples.
Results: 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 40, 72, and 80% of loss respectively for the cooking times of 5, 10, and 15 min in the water. Also, these different heat treatments affected the β-carotene content of African asparagus with losses ranging from 76.80 to 84%. Polyphenols were also affected by cooking. The values obtained were 5104.80 mg GAE/100g for fresh African asparagus, 5284.09 mg GAE/100g for boiling for 5 min (CE5), 5233.31 mg GAE/100g for boiling for 10 min (CE10), and 3536.44 mg GAE/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 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 7.41 and 19.92%. In our study, the scarving activity was found to be highest in the fresh sample, followed by CE15 and CE5.
Conclusion: Finally, boiling reduces 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.

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Keywords: African asparagus, Laccosperma secundiflorum, antioxidant activity, boiling, palm heart.

1. 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 [1]. 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 [2]. Their interest lies in their antioxidant properties, especially their capacity to trap free radicals [3]. These plants also have multiple properties, among others antioxidants because of antioxidants compounds such as vitamins, carotenoids, phenolic compounds ...in their edible parts [4,5]. Several plants, fruits, and vegetables are consumed in Côte d'Ivoire in lean periods [6], and most of them are cooked before consumption, for example the African asparagus. However, culinary practices induce significant changes of chemical compositions, 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 [7].

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. this study aimed 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.

2. MATERIAL AND METHODS

2.1 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

2.2 Method

2.2.1 Heat treatment of samples

The boiling of African asparagus was done according to the method described by **Randrianatoandro** [8]. 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 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 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.

2.2.2 Biochemical analysis of the samples

2.2.2.1 Determination of Vitamin C (Vit C) content

Vitamin C contained in analysed samples was determined by titration using 2,6-dichlorophenol indophenol [9]. 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:

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

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

2.2.2.2 Determination of β-carotene content

The β-carotene were extracted and quantified by using a spectrophotometric method [10]. 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.

2.2.2.3 Determination of total polyphenol conent

Folin-ciocalteu method was used to determine the total phenols content [11]. 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 of gallic acid as standard and the methanolic extract was measured at 725 nm using a spectrophotometer (MS-V5100 visible spectrophotometer, Germany) against a blank. A standard range was performed with a 1 mg/mL gallic acid solution.

2.2.2.4 Determination of total flavonoid content

Flavonoid quantification was carried out using aluminium chloride colorimetric method [12]. 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 of 2 mL of distilled water. The test tube was then incubated in the dark for 30 minutes. The absorbance of standard (quercetin) and the methanolic extract was measured spectrophotometrically at 415 nm. A calibration curve was made using a 0.1 mg/mL quercetin standard solution.

2.2.2.5 Determination of total tannin content

Tannins of samples were quantified using vanillin reagent method [13]. 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.

2.2.2.6 Measurement of antioxidant activity by DPPH radical

Antioxidant activity assay was carried out using the 2,2-diphenyl-1-pycrilhydrazyl (DPPH) spectrophotometric method [14]. A solution of DPPH was freshly prepared (about 0.3 mM). The methanolic extract (2 mL) with varying concentrations (2-20 µg/mL) and DPPH solution (1 mL) were mixed 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). Vitamin C was used as the standard. The values of the inhibitory concentrations (IC50) of the different extracts were obtained by projection from the graph of percentage inhibition versus extract concentrations and are expressed in mg/ml. 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)

2.2.3 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.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 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 observed with increasing cooking time. As for vitamin C, the losses in content oscillate between 40 and 81%. As for β-carotene, it was varied from 76.80 and 84%.

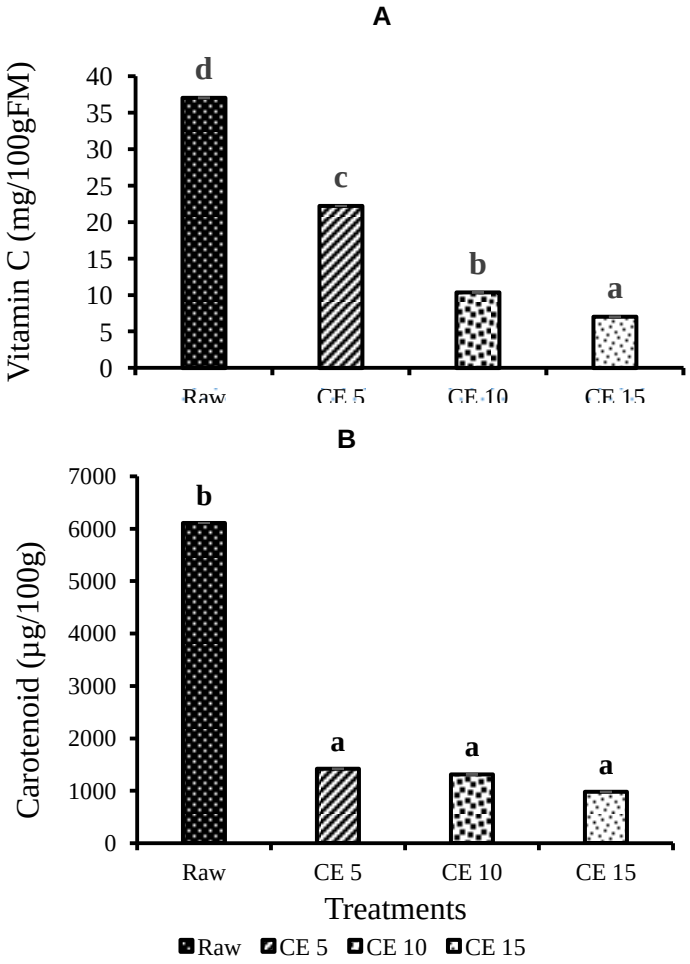


Fig.2 : Vitamin C (A) and β-carotene (B) content of raw and water-cooked African asparagus (EC) at different times (5, 10 and 15 min)

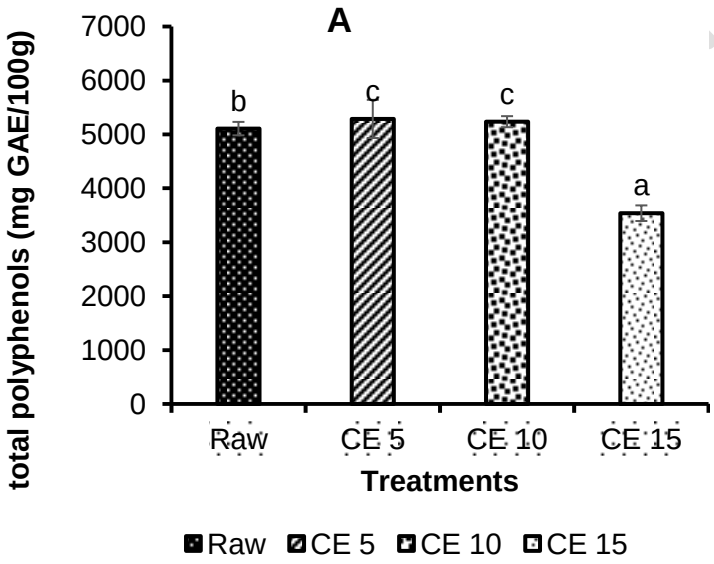
EC5 : cooking 5 min in water ; EC10 : cooking 10 min in water ; EC15 : cooking 15 min in water

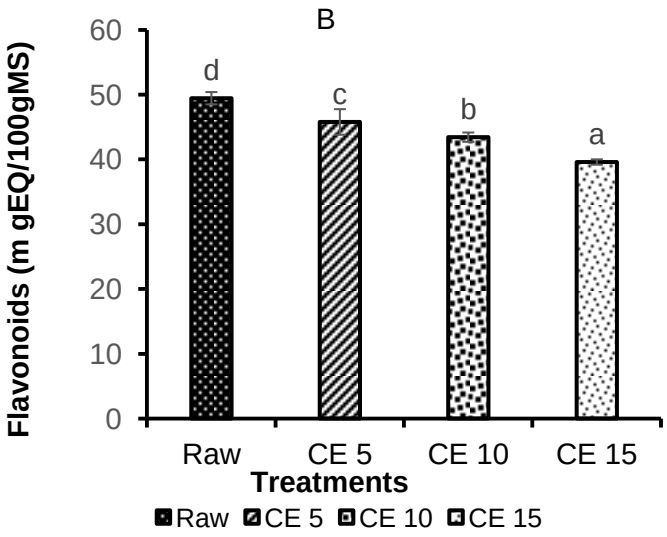
3.1.2 Phytochemical composition of boiled African asparagus

The obtained results for the polyphenol content were 5104.80, 5284.09, 5233.31, and 3536.44 mg GAE/100gMS respectively for the FRESH, CE5, CE10, and CE15 samples (Figure 3A). No significant differences ($p > 0.05$) was noted between the means of the cooked samples at 5 and 10 min. However, these means were found to be statistically different from those of the fresh African asparagus and the CE15 sample. Furthermore, a slight increase in polyphenol content of 3.51% and 2.51% for CE5 and CE10, respectively. Then, a 30.72% drop in the averages was recorded at the 15th minute of cooking.

The tannin contents of African asparagus during cooking in water were 1954.84, 1699.93, 1429.98, and 1035.42 mgTA/100gMS for samples FRESH, CE5, CE10, and CE15, respectively (Figure 3C). Cooking with water results in 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. Analysis of variance shows a significant difference ($p < 0.05$) between the four samples.

Flavonoid contents ranged from 49.43 mg QE/100g (FRESH) to 39.58 mg QE/100g (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 observed between fresh and cooked samples (Figure 3B).





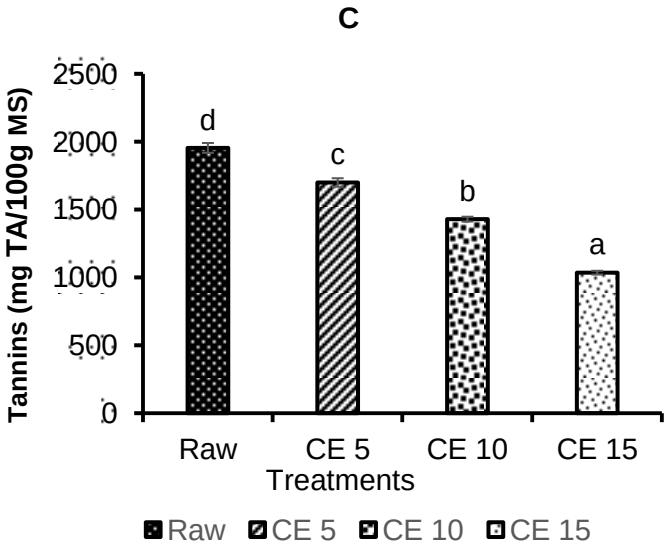


Fig.3 : Total polyphenol (A), flavonoid (B), and tannin (C) contents of fresh and boiled African asparagus.

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

2.1.3 Antioxidant activity of water-cooked boiling 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 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).

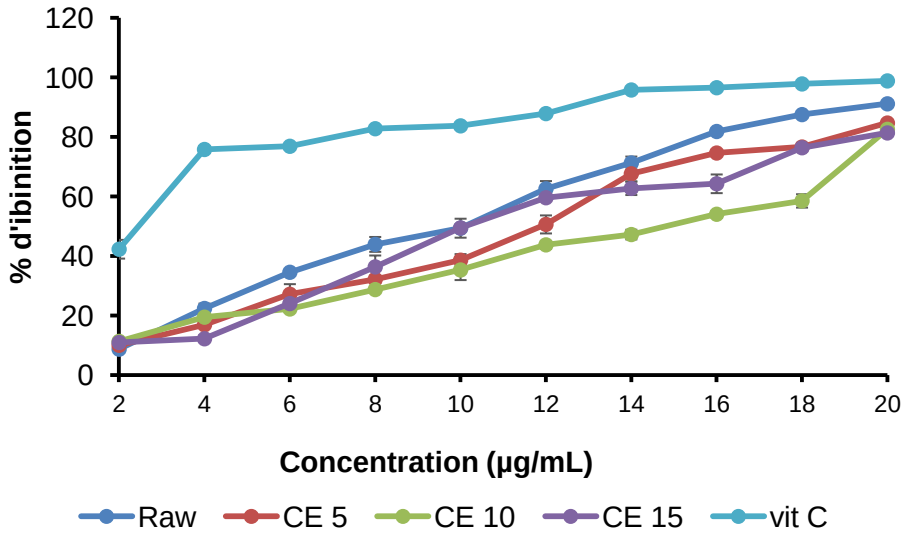


Fig.4 : DPPH free radical scavenging of fresh and water-cooked African asparagus extracts (EC) at different times (5,10 and 15 min).

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

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 observed with the increase of the concentrations of the extracts of fresh African asparagus, cooked in water, and vitamin C used as standard.

Vitamin C and asparagus extracts show good DPPH radical scavenging activity. The IC50 values of the raw extracts, CE5, CE10, and CE15 samples were10.07 µg/mL, 12.07 µg/mL, 15.2 µg/mL and 10.33 µg/mL, respectively. These different concentrations were 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 CE15 and CE5. 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
RawCE5	10.07
CE10	12.07
CE15	15.20
Vit C	10.33
	2.53

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

2.2 Discussion

The analysis of the heat treatment results shows the effect of cooking in water on the nutritional composition of African asparagus. The different cooking times to which the studied African asparagus are subjected significantly influence their studied nutritional parameters. Indeed, boiling resulted in a loss of more than 20-70% of the vitamin C content of African asparagus with the greatest loss (70%) observed in samples cooked for 15 minutes. These results corroborate those of Acho [15] who recorded significant vitamin C losses ranging from 28.61 to 78.02% at 15 min of boiling in five of the leafy vegetables consumed in southern Côte d' Ivoire. The level of β-carotene also decreases during heat treatment and losses vary between 76 and 84%. These losses are attributed to the sensitivity of β-carotene to oxygen or leaching of nutrients from the vegetables during blanching or boiling [16]. Raw and cooked African asparagus contains more than 900 µg⁄100g of β-carotene, which is higher than the recommended daily intake for adults [17,18]. (George, 1999; Akanya, 2004). Indeed, β-carotene deficiency remains a public health problem affecting 19 million pregnant women in Africa [19] where the estimated requirement of this vitamin is 800 µg/day [20]. Thus, the consumption of African asparagus would be beneficial for this segment of the population. Boiling resulted in losses of up to 9.94% (total polyphenols), 19.92% (flavonoids) and 47.03% (tannins) after 15 minutes of cooking. The decrease in flavonoid and tannin content is attributed to leaching of phenolic compounds during heat treatment [21]. The analysis of nutritional properties reveals that fresh African asparagus was rich in tannins with a content equal to (2091.66 mgAT/100gMS). However, this content decreased with increasing cooking time. Similar results were observed in relation to the study carried out by some authors that showed a loss of tannic acid after different cooking processes due not only to the degradation of tannins [22,7]. Tannins are involved in tissue regeneration. They help stop bleeding and help fight infections [23], especially in pre- and post-natal, as is the case in southern Ivory Coast. Regular consumption of African asparagus in households could thus prevent several diseases such as gastrointestinal disorders, high blood pressure and malaria [24].

As for the total polyphenol content, it increases slightly during the first 10 minutes of cooking. This result is in agreement with those of Kao et al [25] obtained during their work on the boiling of Thai basil leaves and potatoes. Similarly, Turkmen et al [5] have shown that short cooking time increases the polyphenol content of some green vegetables such as green beans, spinach and peppers. This increase in polyphenol content in the first few minutes of cooking is thought to be due to cell wall disruption, which released soluble phenolics from insoluble ester bonds, or to the heat-induced breakdown of dietary fiber-bound polyphenols into free phenolics [26, 27, 28]. But, the decrease in total phenolics after 10 minutes of cooking, could be explained according to leaching of soluble or free

polyphenols in the cooking water due to prolonged exposure to heat. However, these levels remain higher than those of Brou et al [7] (291,43 to 909,79 mg/100g) recorded during the cooking of oil palm heart (*Elaeis guineensis jacq.*) Thus, the high content of total polyphenols found in fresh and cooked African asparagus would be useful for children, prone to allergies caused by anti-nutritional substances. They could also participate in the prevention of cardiovascular diseases and cancer in adults [29]. The decrease in antioxidant activity of African asparagus during the first 10 minutes of cooking is thought to be due to a leaching of some compounds such as tannins, β-carotene, vitamin C and flavonoids possessing high antioxidant activity [7]. However, the increase in antioxidant activity of African asparagus after 10 minutes of cooking is thought to be due to the synthesis of new compounds such as Maillard reaction products with antioxidant activity [30].

4. CONCLUSION

African asparagus (Laccosperma secundiflorum) is a food rich in polyphenols, tannin and flavonoids, vitamin C and beta carotene which are compounds known for their antioxidant potential and health benefits. However, cooking significantly reduces the content of these compounds. However, after less than 10 minutes of cooking, more than half of almost all the compounds are still present in African asparagus. Therefore, to benefit from the antioxidant potential of African asparagus, it would be advisable to cook them in boiling water at times between 5 and 10 minutes.

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