

## Antioxidant Content in *Solanum Anguivi* Lam Berries as Affected by Cooking at Different Stages of Ripening

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### Authors' contributions

This work was carried out in collaboration among all authors. Authors CYA and PAA designed the study and managed the literature searches. Then, authors CYA and NA performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** In this research changes in vitamin C, flavonoids, polyphenols, tannins contents and antioxidant activity in *Solanum anguivi* Lam berries during ripening and heat treatment have been studied.

**Place and Duration of Study:** Department of Food Science and Technology (UFR-STA), University Nangui Abrogoua, between May 2017 and August 2018.

**Methodology:** The fresh and boiled berries (at 10 and 15 min) were dried and ground to obtain powders. Then, phenolic compounds (tannins, flavonoids and polyphenols), vitamin C were investigated using standard methods. Antioxidant properties were determined by reducing power (RP), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

**Results:** Green berries had the highest content in vitamin C ( $32.23 \pm 0.03$  mg / 100 g FW), tannins ( $0.19 \pm 0.02$  mg tannic acid / 100 g DW), polyphenols ( $1162.33 \pm 0.02$  mg GAE / 100 g DW) and antioxidant activity ( $86.96 \pm 0.49\%$ ). As far as the berries were ripened, its contents of tannins,

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vitamin C and polyphenols decreased, like its antioxidant activity. Registered losses were polyphenols (61.2%) > vitamin C (59.8%) > tannins (52.6%) > antioxidant activity (17.88%). While, total flavonoids content increased in red berries. Under boiling times, phenolic compounds, vitamin C and antioxidant activity decreased gradually in the berries. Registered losses in green berries were antioxidant activity (97.86%) > vitamin C (59.78%) > polyphenols (52.39%) > tannins (31.57%).

**Conclusion:** *Solanum anguivi* Lam berries consumed in Côte d'Ivoire contain significant levels of phenolic compounds, vitamin C and antioxidant activity that are essential for human health but no more 10 min of boiling, this nutritive value were well-preserved.

**Keywords:** *Solanum anguivi* Lam; stage of ripening; boiling time; phenolic compounds; antioxidant activity.

## ABBREVIATIONS

DPPH: 2, 2-diphenyl-1-picrylhydrazyl

## 1. INTRODUCTION

*Solanum anguivi* Lam (African eggplant) belongs to the *Solanaceae* family and can be found throughout the non-arid parts of Africa [1]. It's one of the non-tuberous *Solanum* species, which is widely distributed in non-arid areas of Africa notably in West, Central, East and South Africa [2].

In Côte d'Ivoire, *Solanum anguivi* Lam is locally named "Gnagnan" [3]. This plant is widely cultivated due to its high demand and cultural importance. So, it's used in the central of that country to welcome visitors. Currently, this preference extends increasingly in all regions of the country [4]. Traditionally, "Gnagnan" is used as an herbal remedy for several diseases such as diarrhoea, malaria and prostate diseases [3]. The roots are used as carminative and in the treatment of nasal ulcers, asthma, difficult parturition, toothache, cardiac disorder, worm expeller, nervous disorder and fever. The leaves and the fruits rubbed up with sugar are used as external application against itching [5].

*S. anguivi* Lam berries are especially characterized by their bitterness due to the presence of various phenolic compounds conferring them an antioxidant property [4, 6]. "Gnagnan" berries contain several phytochemical substances such as polyphenols, flavonoids, tannins ect [4]. Otherwise, Aberoumand and Deokule [7] indicated that these berries had the highest content in phenolic compounds compared to some edible plants from Iran and India.

Phenolic compounds are secondary metabolites synthesized by the plant during growth and

reproduction. It is also produced in biotic (infection by pathogens) and abiotic (UV radiation, environment conditions ect) [8,9]. Different groups of phenolic compounds have been classified according to the number of phenol rings and moieties attached to these rings. Most common phenolic compounds in fruits and vegetables are flavonoids and phenolic acids [10].

"Gnagnan" is consumed fresh or after sun dried in Côte d'Ivoire. Berries are usually used alone or mixed with other vegetables to prepare a soup [3]. But, the preparation methods, mostly boiling time may affect the nutritional value of many nutrients in vegetables [11,12]. Earlier reports have highlighted the nutritive and phytochemical potential of these fresh berries but, there is a lack of scientific data with regards to the effect of cooking (boiling) on their nutritive values. The aim of this study was to determine the effect of boiling on some phytochemical compounds and antioxidant activity of these berries during ripening.

## 2. MATERIALS AND METHODS

### 2.1 Samples Preparation

Berries were harvested as follow [3]: Green berries at 90 days after growth, Yellow berries about 6 days after green stage, Orange berries about 3 days after yellow stage, and red berries about 2 days after Orange stage.

Color analysis was done using a Chroma meter (Konica Minolta, Inc. Color Reader CR\_10 (Japan)).

After harvesting at different stages of ripening fresh berries were washed with deionised water and allowed to drain at ambient temperature. Each sample was divided into two lots. The first lot (raw) was dried in an oven (Mettler,

Germany) at 45°C for 72 h according to Chinma and Igyor, [13]. Then, the dried berries were ground with a Moulinex-type mixer. The powder obtained was sieved (100 µm) and samples were stored in clean dry air-tight bottles at 4°C until required for analyses. The second lot was cooked by using the method of Randrianatoandro, [14] modified as follow: 250 g of berries were immersed in 1.5 L of boiled water in stainless steel container for 10 and 15 min. The boiling solution was discarded and the boiled samples were cooled, drained at ambient temperature and subjected to the same treatment using for raw samples.

## 2.2 Chemicals

Ascorbic acid (AA) was purchased from Riedel-de Haën (Sigma-Aldrich, St. Louis, MO, USA).

Gallic acid, tannic acid (2 mg/mL), quercetin, dichlorophenol-indophenol (DCPIP) 0.5 g/L and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA).

## 2.3 Methods

### 2.3.1 Vitamin C determination

Vitamin C content in analyzed samples was determined by titration [15]. About 10 g of ground berries 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.

### 2.3.2 Total phenolics determination

Polyphenols content was determined using the method reported by Singleton and al., [16]. A

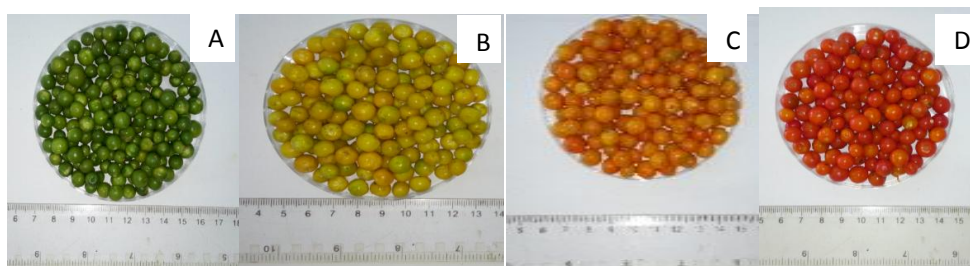
quantity (1 g) of dried powdered sample was soaked in 10 mL of methanol 70% (v/v) and centrifuged at 1000 rpm for 10 min. An aliquot (1 mL) of supernatant was oxidized with 1 mL of quantity (1 g) of dried powdered sample was soaked in 10 mL of methanol 70% (v/v) and centrifuged at 1000 rpm for 10 min. 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 (spectrophotometer invisible Model MS-A 5100). The polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as standard.

### 2.3.3 Total flavonoids determination

The total flavonoids content was evaluated using the method reported by Meda and al., [17]. 0.5 mL of the methanolic extract was mixed with 0.5 mL methanol, 0.5 mL of AlCl<sub>3</sub> (10%, w/v), 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water. The mixture was allowed to incubate at room temperature for 30 min. Thereafter, the absorbance was measured at 415 nm by using a spectrophotometer (spectrophotometer invisible Model MS-A 5100, Espagne, Europe). The total flavonoids were determined using a calibration curve of quercetin (0.1 mg/mL) as standard.

### 2.3.4 Tannins determination

For this, 1 mL of the methanol 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 (spectrophotometer invisible Model MS-A 5100). Tannins content of samples was estimated using a calibration curve of tannic acid (2 mg/mL) as standard.



**Fig. 1. Photographs of fresh fruit of *Solanum anguivi* Lam ; Green berries (A), Yellow berries (B), Orange berries (C) and Red berries (D)**

### 2.3.5 Antioxidant activity

Antioxidant assay was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method outlined by Choi et al., [18]. About 1 mL of DPPH solution (0.3 mM) in methanol was added to 2.5 mL of sample solution (1 g of dried powdered sample mixed in 10 mL of methanol and 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 (spectrophotometer invisible Model MS-A 5100; Espagne, Europe) set at 517 nm. The average absorbance values were converted to percentage of antioxidant activity using the following formula:

$$\% \text{ inhibition} = (\text{Absorbance of control} - \text{Absorbance of sample}) \times 100 / \text{Absorbance of Control}$$

Tannins of samples were quantified according to Bainbridge et al. [19].

### 2.4 Stastical Analysis

All experiments were carried out in triplicate and data were expressed as mean  $\pm$  standard deviation (SD) or standard error of mean (SEM). Two ways analysis of variance (ANOVA) was conducted on each of the variables and the Duncan test at significant level  $P < 0.05$  was performed using STATISTICA 7.1 software to compare the difference between treatment means.

## 3. RESULTS AND DISCUSSION

### 3.1 Colour Indices

Colorimetric indexes are presented in Table 1.  $L^*$  value increased to green berries for yellow berries and decreased to red berries.  $a^*$  values increased to green berries for red berries ( $-7.067 \pm 0.37 - 15.73 \pm 3.82$ ).  $b^*$  value increased to green berries for yellow berries and decreased to red berries.

Similar observations was made by Dan et al., [4] on the same berries.

### 3.2 Vitamin C Content

Fig. 2 showed the evolution of vitamin C content of berries during ripening and after boiling times. Vitamin C content decreased significantly ( $p < 0.05$ ) from green berries ( $32.23 \pm 0.03 \text{ mg} / 100$

g) to red berries ( $12.96 \pm 0.01 \text{ mg} / 100 \text{ g}$ ). Also, when the berries were boiled at 10 and 15 min, vitamin C content decreased significantly ( $p < 0.05$ ) in the green ones (from  $32.23 \pm 0.03$  to  $07.15 \pm 0.02 \text{ mg} / 100 \text{ g}$ ), in the yellow ones (from  $27.04 \pm 0.02$  to  $06.01 \pm 0.01 \text{ mg} / 100 \text{ g}$ ), in the orange ones (from  $19.98 \pm 0.01$  to  $04.39 \pm 0.02 \text{ mg} / 100 \text{ g}$ ) and in the red ones (from  $12.96 \pm 0.01$  to  $02.43 \pm 0.03 \text{ mg} / 100 \text{ g}$ ).

Vitamin C content decreased significantly ( $p < 0.05$ ) during the berries ripening (Fig. 2). A similar decrease in ascorbic acid content was reported by Dan et al., [4] and N'Dri et al., [3] in the same berries and by Msogoya et al., [20] in three African eggplants. This significant loss of ascorbic acid content is due to increasing rate of cell respiration during ripening, inducing its oxidative degradation [3]. For Pila et al. [21], ascorbic acid decrease is an indicator of fruit senescence. However, our values obtained ( $32.23 \pm 0.03 - 12.96 \pm 0.01 \text{ mg} / 100 \text{ g}$ ) at different stages of ripening in *Solanum anguivi* Lam berries were higher than those obtained by N'Dri et al. [3] ( $8.46 - 6.67 \text{ mg} / 100 \text{ g}$  of fresh weight). These differences may be due to variability of extraction methods and different ways of expressing the results [22]. In this study, only green berries non-boiling value ( $32.23 \pm 0.03 \text{ mg} / 100 \text{ g}$ ) is closed to the standard value ( $40 \text{ mg} / \text{day}$ ) recommended by FAO [23]. The other values ( $27.04 \pm 0.02 - 12.96 \pm 0.01 \text{ mg} / 100 \text{ g}$ ) were widely under the standard.

In the case of boiled berries, values were too low ( $11.08 \pm 0.03 - 02.43 \pm 0.03 \text{ mg} / 100 \text{ g}$ ) compared to the reference. Boiling caused a higher decrease of vitamin C contents in the berries (Fig. 2). Losses were estimated from 59.65 to 65.90% at 10 min and from 77.77 to 81.25% at 15 min. These results were supported by Zoro et al. [24] who also found a significant reduction (50.73 – 84.21%) of vitamin C content in cooked vegetables after 15 min boiling. With regard to the drastic decrease of vitamin C during boiling, consumption of cooked berries may be supplemented with other sources of vitamin C such as tropical fruits to cover the daily need for humans ( $40 \text{ mg} / \text{day}$ ) as recommended by Food and Agriculture Organization [23] or increase the quantity of edible berries e.g. 200 g.

### 3.3 Total Flavonoids Content

The results of combined effects of ripening and boiling on flavonoids content in the berries are

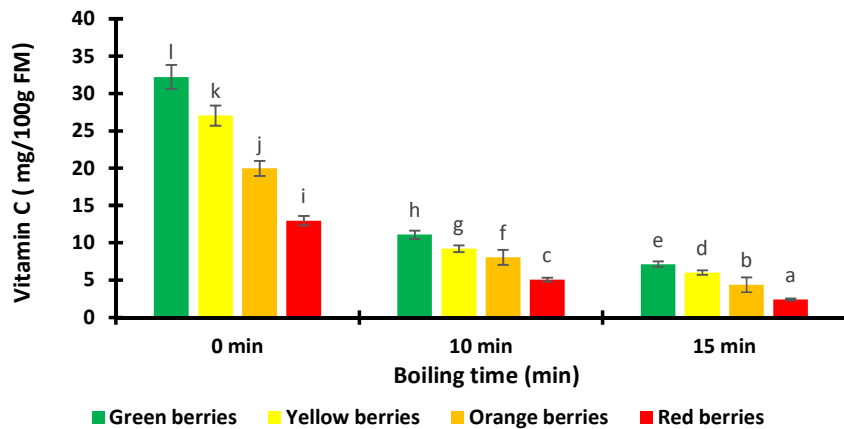
presented by Fig. 3. When berries were ripened, its content of flavonoids increased significantly ( $p < 0.05$ ) from  $141.33 \pm 0.02$  mg / 100g (in green berries) to  $455.00 \pm 2.00$  mg / 100 g (in red berries). But, whatever the stage of ripening, Boiling induced significant ( $p < 0.05$ ) decrease of flavonoids content in the berries. This content

ranged from  $141.33 \pm 0.02$  to  $93.10 \pm 0.01$  mg quercetin / 100g DW) in green berries, from  $153.00 \pm 1.00$  to  $87.33 \pm 0.01$  mg quercetin / 100 g DW) in yellow berries, from  $406.33 \pm 0.02$  to  $113.00 \pm 1.00$  mg quercetin / 100g DW) in orange berries and from  $455.00 \pm 2.00$  to  $179.17 \pm 0.03$  mg quercetin / 100 g DW) in red berries.

**Table 1. Colour indices of green, yellow, orange and red *Solanum anguivi* L. berries**

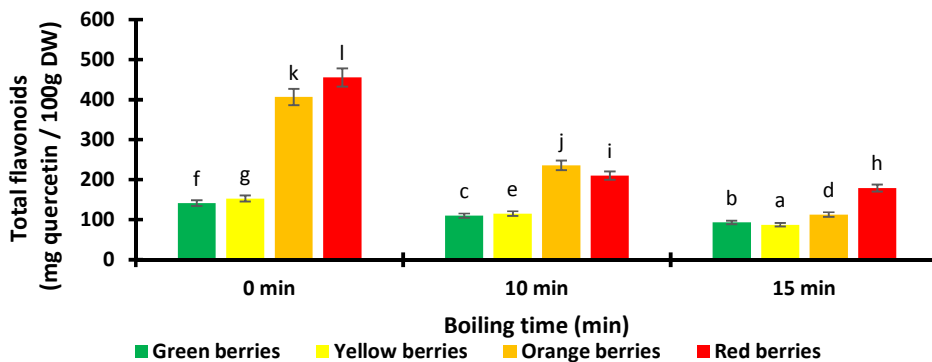
	Green berries	Yellow berries	Orange berries	Red berries
L*	$43.36 \pm 0.75^a$	$51.63 \pm 0.86^c$	$46.26 \pm 0.20^b$	$47.5 \pm 2.38^b$
a*	$-7.067 \pm 0.37^a$	$-0.56 \pm 0.23^b$	$8.33 \pm 0.25^c$	$15.73 \pm 3.82^d$
b*	$17.7 \pm 0.87^a$	$28.96 \pm 0.70^c$	$23.96 \pm 0.77^b$	$23.53 \pm 1.19^b$

Values are expressed in colorimetric units and presented as mean  $\pm$  SD ( $n = 10$ ). Means in line for each berry followed by different letters differed significantly ( $p \leq 0.05$ )



**Fig. 2. Effect of boiling time on vitamin C content (mg / 100 g FM) in *Solanum anguivi* Lam berries at different stages of ripening**

Values represent Mean  $\pm$  SD ( $n = 3$ )



**Fig. 3. Effect of boiling time on flavonoids content (mg quercetin / 100g DW) of *Solanum anguivi* Lam berries at different stages of ripening**

Flavonoids are potent antioxidants in lipid systems where they reduce oxidative modifications of membranes by restricting the access of oxidants to the bilayer and the propagation of lipid oxidation in the hydrophobic membrane matrix [25]. In this study, flavonoids content increased during berries ripening. Similar observations are reported by Dan et al. [4] on *Solanum anguivi* Lam berries. In the case of boiling, flavonoids content dropped in the berries. Luzia et al. [26] observed that flavonoids levels decreased in organic broccoli after boiling. These losses of flavonoids content depend on their chemical nature. Ioku and al. [27] attributed the decrease of vegetable's flavonoids content to their significant transference into boiling water, particularly quercetin 3,4'-diglucoside molecular. In spite of this molecular transfer, all berries would be considered as good source of flavonoids after 15 min of boiling when compared to the values obtained by Haddadi [28] for grapefruit (7.12 mg of quercetin /100 g MS) and strawberry (17.53 mg of quercetin /100 g DW).

### 3.4 Tannins Content

Tannins content in the berries under ripening and boiling conditions are presented in Fig. 4. As the berries ripened, tannins content decreased significantly ( $p < 0.05$ ) from  $0.19 \pm 0.02$  mg / 100 g (green berries) to  $0.09 \pm 0.02$  mg tannic acid / 100 g DW (red berries). In the same way, whatever the ripening stage, tannins content decreased significantly ( $p < 0.05$ ), when berries

are boiled from 10 to 15 minutes. The values dropped in green berries (from  $0.19 \pm 0.02$  to  $0.09 \pm 0.02$  mg tannic acid / 100 g DW), in yellow berries (from  $0.13 \pm 0.01$  to  $0.07 \pm 0.01$  mg tannic acid / 100g DW), in orange berries (from  $0.11 \pm 0.01$  to  $0.07 \pm 0.02$  mg tannic acid / 100 g DW) and in red berries (from  $0.09 \pm 0.02$  to  $0.06 \pm 0.01$  mg tannic acid / 100 g DW).

Tannins content found in *S. anguivi* Lam berries were widely below the critical level of 760 - 900 mg/100 g DW found by Aletor and Adeogun [29]. For these authors, it's above the critical value that tannins are considered as an anti-nutritional factor. Meanwhile, underneath critical value, tannins are known to possess antioxidant, antibacterial and as well as anti-inflammatory properties [30,31]. With regard to tannins level in this berries subjected to ripening and boiling conditions ( $\leq 0.19$  mg /100 g DW), *S. anguivi* Lam could protect human body against ageing and bacterial infections.

### 3.5 Total Phenolics Content

The Fig. 5 exhibited polyphenols content in the berries of *S. anguivi*. During the ripening of the berries, polyphenols content decreased significantly ( $p < 0.05$ ) from  $1162.33 \pm 0.02$  mg GAE / 100 g DW (in green berries) to  $451.50 \pm 0.01$  mg GAE / 100g DW (in red berries). In the case of boiling, polyphenols content dropped significantly ( $p < 0.05$ ) at all stages of ripening.

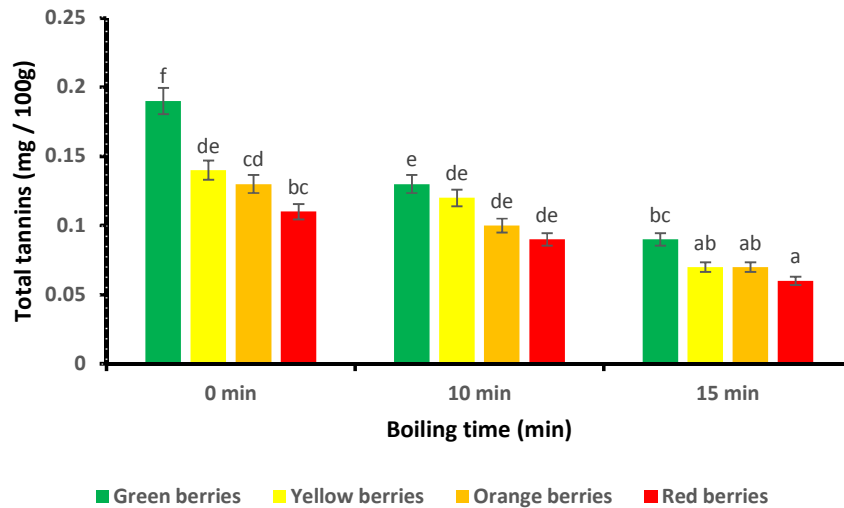
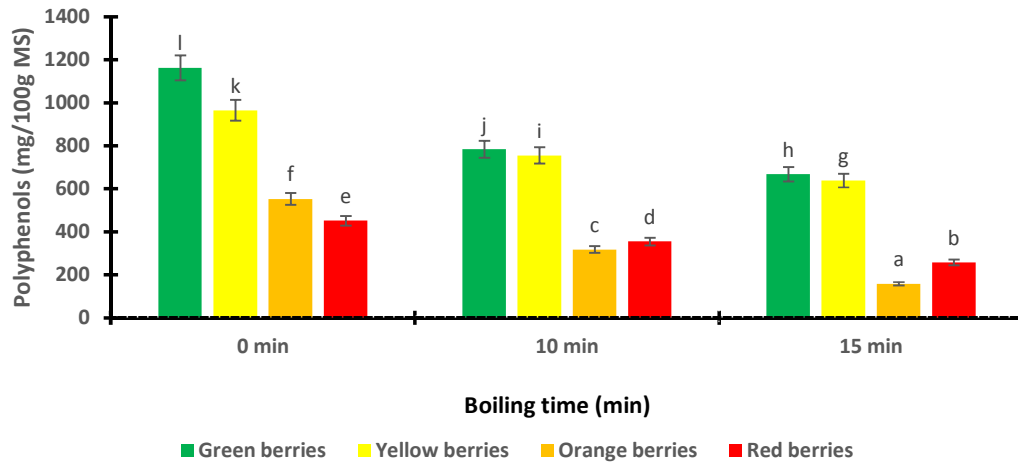


Fig. 4. Combined effect of boiling time on tannins content (mg /100 g DW) in *Solanum anguivi* Lam. berries at different stages of ripening



**Fig. 5. Effect of boiling time on polyphenols (mg GAE / 100 g MS) content in *Solanum anguivi* Lam berries at different stages of ripening**

This content decreased in green berries (from  $1162.33 \pm 0.02$  to  $667.33 \pm 0.01$  mg GAE / 100 g DW), in yellow berries (from  $965.33 \pm 0.03$  to  $638.67 \pm 0.02$  mg GAE / 100 g DW), in orange berries (from  $553.33 \pm 0.02$  to  $158.33 \pm 0.02$  mg GAE / 100 g DW), in red berries (from  $451.50 \pm 0.01$  to  $257.83 \pm 0.03$  mg GAE / 100 g DW).

The polyphenol content decreased as far as berries ripened. This observation has already been made by Dan et al. [4] who also evaluated nutritional value of *Solanum anguivi* Lam berries. The decrease of polyphenols content in *S. anguivi* Lam berries during ripening could be attributed to enzymatic reaction. In fact, Kadam and Salunkhe [32], found that insoluble polymers from polyphenols polymerization and condensation reactions which occurred in the berries led to its ripening. For this reason, the green berries of *S. anguivi* Lam contain more phenolic compounds than the red ones, proved by their deep bitterness [4].

Boiling at 10 and 15 min resulted in higher decrease of polyphenols contents in the berries, whatever the stage of ripening. However, at the end of that boiling time, the values were higher than those obtained from leafy *Solanum melongena* ( $115.74$  mg / 100 g) consumed in southern of Côte d'Ivoire by Acho et al. [33] and from *S. anguivi* Lam berries cultivated in India and Iran were  $702$  mg / 100 g [34]. Even cooked or no, the studied berries may be considered as good sources of polyphenols and for human consumption. They have been shown that

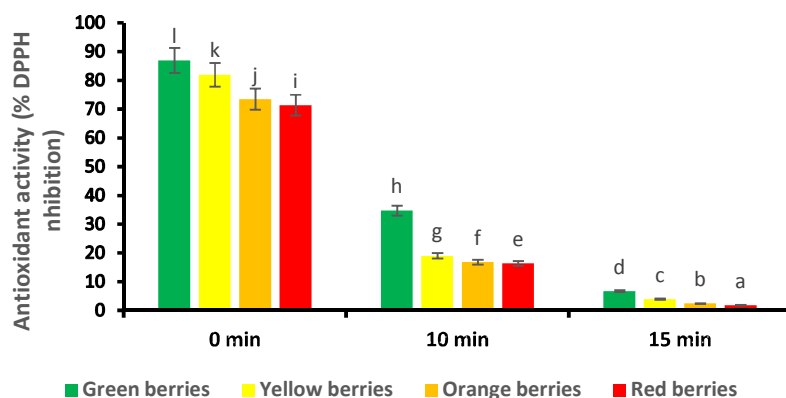
polyphenols possess antibacterial, antiviral, anti-mutagenic and anticarcinogenic properties [35, 36]. Phenols had the ability to delay lipid oxidation in oils and fatty foods according to Rumbaoa et al. [37] thereby reducing cardiovascular diseases. Phenols could as well reduce cancer risk by interfering with all stages of the cancer process [38].

### 3.6 Antioxidant Activity

Antioxidant activity expressed in *S. anguivi's* berries is shown in Fig. 6. A significant ( $p < 0.05$ ) decreases of antioxidant activity were observed as the berries ripened. Antioxidant activity decreased from  $86.96 \pm 0.49\%$  (in green berries) to  $71.41 \pm 0.39\%$  (in red berries). Under the effect of boiling, the antioxidant activity decreased significantly ( $p < 0.05$ ) at all of ripening stages. This content decreased in green berries (from  $86.96 \pm 0.49$  to  $6.77 \pm 0.10\%$ ), in yellow berries (from  $81.96 \pm 0.52$  to  $3.97 \pm 0.59\%$ ), in orange berries (from  $73, 44 \pm 0.85$  to  $2.49 \pm 0.19\%$ ) and in red berries (from  $71.41 \pm 0.39$  to  $1.86 \pm 0.28\%$ ).

Antioxidant activity decreased during ripening. Several studies have also reported a good correlation between the total phenolics content and antioxidant activity [39]. According to several authors, the antioxidant activity can be affected by many factors: the structure of phenolic compounds in particular the degree and position of the hydroxyl groups on the aromatic ring of the molecule [40]. Antioxidant activity can vary





**Fig. 6. Effect of boiling time on Antioxidant activity in *Solanum anguivi* Lam berries at different stages of ripening**

considerably with the changes in environmental conditions within the same maturity stage [41]. Gandhiappan and Rengasamy [42] evaluated and compared the antioxidant properties of six different plant's ethyl acetate extracts of *Solanaceae* family. Strong inhibitions of free radicals were caused by the ethyl acetate extract of *S. anguivi*. Thus, *S. anguivi* could be considered as a potential source of natural antioxidants.

Antioxidant activity decreased after boiling times of 10 and 15 minutes. The results were in agreement with the report of Mazzeo et al. [43]. These authors showed that antioxidant of cauliflower inflorescences was lower than that of the fresh vegetables after boiling and steaming. Also, Wachtel-Galor et al. [44] analysed the antioxidant activity of broccoli and cauliflower using the FRAP method for fresh samples and after boiling for 5 or 10 min. The authors observed that vegetables boiled for 10 min showed lower antioxidant capacity than those boiled for 5 min.

#### 4. CONCLUSION

This study investigated the change in vitamin C, flavonoids, polyphenols, tannins contents and antioxidant activity in *Solanum anguivi* Lam or "Gnagnan" berries at different stage of ripening and after boiling times of 10 and 15 minutes. The results of this study revealed that fresh berries, in case of the green ones, contained appreciable amount of vitamin C, polyphenols, tannins and antioxidant activity. While, for the flavonoids, it was the red berries that had the higher content. Furthermore, boiling at 10 and 15 minutes strongly reduced the content of these

phytochemicals. Thus, the recommended time of domestic boiling of berries must be about 10 minutes, in order to contribute efficiently to the nutritional requirement and to the food security of Ivorian population.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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