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Antioxidant Activities, Phenolic Compounds and Organic Acids of Raw and Boiled Berries of *Solanum Torvum* Swartz From Eastern Côte d'Ivoire

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***Abstract* — Berries of *solanum torvum*, referred to as wild eggplant, are widely consumed in eastern Côte d'Ivoire in boiled form in sauces or soups. This study aimed to evaluate the antioxidant potential of these fruits through the estimation of antioxidant capacities of the extracts, identification and quantification of phenolic compounds and organic acids of raw berries and boiled berries to gauge the influence of this cooking. Results showed that boiling caused a considerable decrease in the antioxidant capacity of *S. torvum* berry extracts. By DPPH scavenging, the effective concentration (EC_{50}) value of extract increased from 20.00 to 42.20 µg/mL, respectively for raw and boiled berries. Regarding EC_{50} values for antioxidant activities via FRAP, the values of 75.10 and 87.20 µg/mL were obtained, respectively for raw and boiled berries extract. In terms of phenolic compounds, the most predominant were catechin and gallic acid with respective contents of 1.06 and 0.86 mg/kg in the raw berries; 0.86 and 0.71 mg/kg in boiled berries, demonstrating the decrease in levels of these compounds by boiling. Organic acids showed the same decreasing trend during boiling. However, using cooking water when preparing soups or sauces could minimize these losses. In addition to this, one could consider other cooking methods such as steaming to better ensure nutrient retention.**

***Keywords* — *Solanum torvum* berries; boiling; antioxidant capacities; phenolic compounds; organic acids**

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I. INTRODUCTION

Solanum torvum Swartz is a wild plant species of the solanaceae family which produces berries referred to as turkey berries or wild eggplant. This solanaceae is essential plant in many tropical areas of the world as Africa and West Indies [1,2**]**, Malaysia, China and Philippine [3**]**, Thailand [4**]**, where it constitutes a traditional vegetable and a great tool in traditional medicine for the natives. Although growing spontaneously on moist and fertile soils, *S torvum* is nowadays cultivated in Africa and the West Indies [5**]**. It is also cultivated as a small-scale vegetable in southern and eastern Asia, and is especially popular in Thailand [4**]**. In terms of traditional medicine, the berries of *S. torvum* have been widely investigated in many countries [2,6,7,8,9,10,11**]**. Extracts from these berries have generally been shown to be antioxidants, antimicrobials, anti-inflammatories, cardioprotectors, nephroprotectors, hepatoprotectors and cough suppressants. In terms of food, according to several authors [6,12,13**]**, cooked berries of *S. torvum* are important ingredients in soups, sauces and some stews. The proximate composition of the raw berries indicated significant contents of certain nutrients, as ash, fibers and carbohydrates [12,14**]**. A recent study indicated that the effectiveness of nutritional quality has been demonstrated by the highest amounts of protein, total carbohydrate, almost all minerals, vitamins C, D and E in comparison to others Solanum species [15]. In addition, many phytochemical compounds well-known for their antioxidant properties, as phenolic compounds, flavonoids, carotenoids and ascorbic acid was quantified with the significant levels in the extracts of raw berries [12,14,16,17]. Due to its consumption in the

cooked state, it is timely to explore the impact of cooking on the nutritional quality of *S. torvum* berries.

Hence, this work aimed to explore antioxidant properties and identify phenolic compounds and organic acids in methanolic extracts from raw and boiled berries of *S. torvum* in order to appreciate the effects of cooking by boiling on antioxidant potential of these fruits widely consumed in eastern Côte d'Ivoire.

However, scant publication on comparison of numerous carbohydrate sources on the microbial survival and gac yoghurt quality. Therefore, this research aimed to monitor the lactic acid survival during the storage gac yoghurt enriched with different carbohydrate sources (inulin, honey, fruit syrup, sucrose) and analyze the product quality in terms of chemical, physical, and sensory profiles during a chilled storage controlled for 28 days.

II. Material and Methods

A. Materials

Standards for phenolic compounds (gallic acid, caffeic acid, Tannic acid, cinnamic acid, and catechin) and acetonitrile were provided from Merck (Darmstadt, Germany). Organic acids standards (citric acid, malic acid, fumaric acid, tartric acid, tartaric acid, lactic acid, acetic acid and butyric acid), trolox and Folin-Ciocalteu were obtained from Sigma-Aldrich (Steinheim, Germany).

B. Sample preparation

The berries of *S. torvum* harvested at the mature stage were obtained from Tanda in eastern Côte d'Ivoire. The fruits were then transported directly to the laboratory. The selected fruits were washed thoroughly with sterilized water to remove foreign matter and dried on blotting paper. These fruits were then subdivided into two parts of 250 g, one remaining raw and the other boiled in 1L of water for 25 min. To prepare the raw berry powder, the fruits were cut manually using a stainless knife and put in an oven at 45 °C for 72 hours. Then, the dried fruits were crushed in a mill type Mill IKA (Germany/Deutschland). The powder of raw berries obtained was stored in airtight containers for analysis. As for the powder from the boiled berries, it was obtained after cooking these berries in a stainless-steel container for 25 minutes, followed by draining for a few minutes, drying and grinding under the same conditions as for raw berries. The powder of boiled berries obtained was also stored in airtight containers for analysis.

C. Evaluation of antioxidant properties

In the present study, the antioxidant activities of raw and boiled samples extract of *S. torvum* berries was evaluated in terms of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging and ferric ion reducing antioxidant power (FRAP).
DPPH radical scavenging activity - The free radical scavenging activities of each *S. torvum* berry sample extract (raw and boiled) was measured by using DPPH according method of Hatano *et al.* [18]. Briefly, 2.5 mL of each extract at various concentrations (0 to 100 µg/mL) was added to 1 mL of DPPH solution (3 mM), vigorously shaken and maintained for 24 h at room temperature in the dark. Methanol was used instead of berries extract as a control. Then the absorbance was measured at 515 nm. Scavenging percentage of DPPH radical by *S. torvum* berry sample extracts was calculated using the following equation:
DPPH scavenging (%) = $[(A_0 - A_1/A_0) \times 100]$, where A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample.
The concentration of berry sample extract producing 50% scavenging (EC_{50}) was calculated from the graph of percentage DPPH scavenging versus extract concentration. Trolox was used as a standard

Ferric ion reducing antioxidant power (FRAP) - The reducing powers of the *S. torvum* berry sample extracts were determined according to the method described by Ferreira *et al*. [19]. In a test tube containing 0.1 mL of sample extract prepared at different concentrations (0 to 100 µg/mL), was added 2.0 mL of phosphate buffer (0.2 M, pH 6.6), followed by 2 mL of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$] (w/v). The whole was incubated at 50 ° C in a water bath for 20 min, and then cooled. A volume of 2 mL of 10 % (w/v) trichloroacetic acid (TCA) was then added and the mixture was centrifuged at 3000 rpm for 10 min. Finally, 2 mL of the supernatant was mixed with 2 mL of distilled water and 0.4 mL of ferric chloride [$FeCl_3$] (1g/L). A blank without sample extract was prepared under the same conditions. Absorbance was measured at 700 nm against blank; the increase in absorbance indicated higher reducing power. The extract concentration of samples giving an absorbance of 0.5, i.e., the value of the EC_{50} was estimated from the graph of the absorbance at 700 nm versus the concentration of extract. Trolox prepared as above at different concentrations was used as a standard.

D. Identification and quantification of individual phenolic compounds by HPLC

A sample (10 g) of each fine *S. torvum* powder previously prepared was extracted by stirring with 50 mL of methanol 80 % (v/v) at 25 ˚C for 24 hours and filtered through Whatman No 4 paper. The residue was then extracted with two additional 50 mL portions of methanol. The combined methanolic extracts were evaporated at 35 ˚C (rotary evaporator HEILDOLPH Laborata 4003 Control, Schwabach, Germany) until 25 mL. These 25 mL of each phenolic extract

were diluted in 50 mL of distilled water and 20 µL of each sample was analyzed using an analytical HPLC unit (HPLC (Shimadzu Corporation, Japan) equipped with a binary pump (LC-6A) coupled to a UV-VIS detector (SPD-6A). Phenolic compounds were separated on a column ICSep ICE ORH-801 (length 25 cm) at a temperature set at 30 ˚C. The mobile phase consisted of 50 mM $NaH_4H_2PO_4$ to pH 2.6 (eluent A), a solution of acetonitrile/$NaH_4H_2PO_4$ (80:20, v/v) (eluent B) and 200 mM acid o-phosphoric pH 1.5 (eluent C). The operating time was 70 min with a flow rate of 1 mL/min. Phenolic compounds in methanolic extract of *S. torvum* berry samples were identified through comparison of their retention times and UV-visible spectra with those obtained by injection of the standard solutions under the same conditions. Peak area was used for quantification purposes, using external calibration with standards.

E. Identification and quantification of organic acids by HPLC

Organic acids of *S. torvum* berry samples were extracted according to Hasib *et al*. [20]. Two (2) g of raw or boiled berry powder previously described were added to 20 ml of distilled water, and then centrifuged at 4000 rpm for 30 min. The supernatant was collected and filtered through Whatman No 4 paper and then through a 0.45 µm Millipore filter (Millipore; Sartorius AG, Goettingen, Germany). The separation of the organic acids was performed with a system consisting of an analytical HPLC unit (Shimadzu Corporation, Japon) in conjunction with a column heating device set at 35 °C with the aid of an oven Meta Therm TM (Interchrom, France), with an ions exclusion column ICSep ICE ORH-801 (40 cm x 5 µm, Interchom, France). The system was also coupled to a pump (Shimadzu LC-6A Liquid Chromatograph), a UV detector (Shimadzu SPD-6A UV Spectrophotometric Detector) and an integrator (Shimadzu Chromatopac CR 6A). Elution was carried out isocratically with sulphuric acid 0.04 N, at a solvent flow rate of 0.6 mL/min and detection was performed at 210 nm. The different organic acids from *S. torvum* berry extracts were identified by comparing the retention times and spectral data obtained from standards under the same conditions. Quantification was performed by comparing the peak areas with those of the respective external standards.

F. Statistical analysis

All chemical analyses and assays were carried in triplicate. Results *were* expressed as mean values ± standard deviation (SD). Analysis of variance (ANOVA) followed by Duncan's test was performed to test for differences between means by employing Statistica 7.1 software. Significance of differences was defined at the 5 % level ($p < 0.05$).

III. Result and Discussion

A. Evaluation of antioxidant properties

The antioxidant activities via DPPH radical scavenging of the extracts from S. torvum raw and boiled berries Shown in the **Fig. 1**. The analysis of this figure indicates that the percentage scavenging of DPPH increased with the concentration of the extract. The order of scavenging was as follows: trolox > raw berry extract > boiled berry extract, indicating that raw berries have a higher DPPH scavenging activity than boiled berries. At 100 mg/mL, values of percentage scavenging of DPPH were respectively 97; 90 and 80 % for trolox, raw berries extract and boiled berries extract. These results showed the same trend as those of other authors [21,22] who indicated that antioxidant activity via DPPH scavenging of tomato extract and that of Sonneratia apetala fruit significantly decreased from raw to boiled fruit.

The same trend has been reported for three varieties of green bean [23] and some green vegetables consumed in the Mediterranean diet [24]. On the other hand, Naveena et al. [21] obtained the opposite trend with the extract of carrot with a significant increase in the scavenging of the DPPH from raw to boiled carrot. An increase of antioxidant activity via DPPH scavenging was also obtained during boiling of various fruits and vegetables such pumpkin [25], pepper, broccoli, spinach and green beans [26].

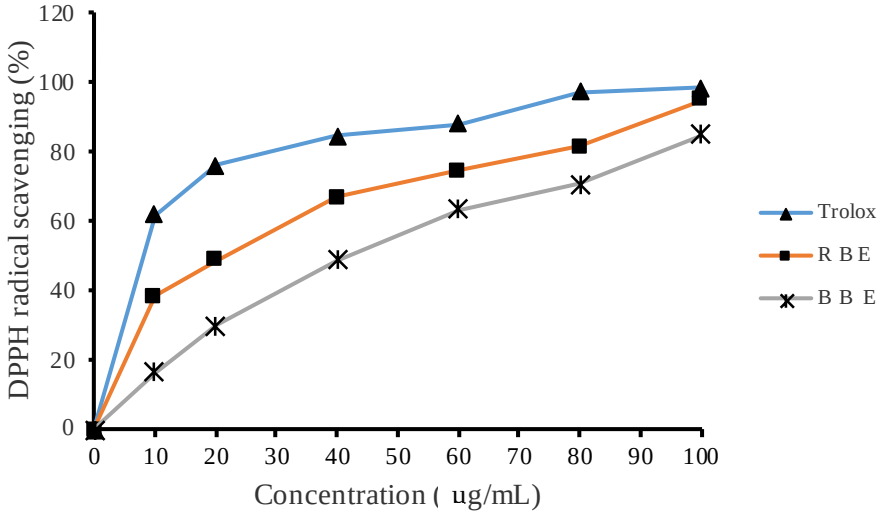

Fig. 1. Antioxidant activities via DPPH radical scavenging depending of *S. torvum* raw and boiled berry extract concentrations. Trolox (standard of antioxidant), R B E (raw berry extract), B B E (boiled berry extract)

Via ferric ion reducing antioxidant power (FRAP) **(Fig. 2)**, we also observed that the antioxidant activities of extracts of both raw and boiled fruits of S *torvum* increased with the concentration of the extract. The order of ferric reducing was similar to that of DPPH scavenging, i.e. trolox > raw berry extract > boiled berry extract. So, extract of raw berries exhibited a higher ferric reducing than that of boiled berries. At 100 µg/mL, the extracts of raw and boiled berries respectively caused values of ferric ion reducing of 0.59 and 0.64 against 0.80 for trolox. Similar results were obtained during the investigation devoted to many species of edible mushrooms from Thailand [27]. Findings of these investigation indicated that the boiling process significantly decreased antioxidant activities via FRAP. In contrast, other authors [28] reported that extracts of fruits and vegetables such as carrot, beet root, tomato and bitter gourd exhibited considerable increase of FRAP values during cooking by boiling

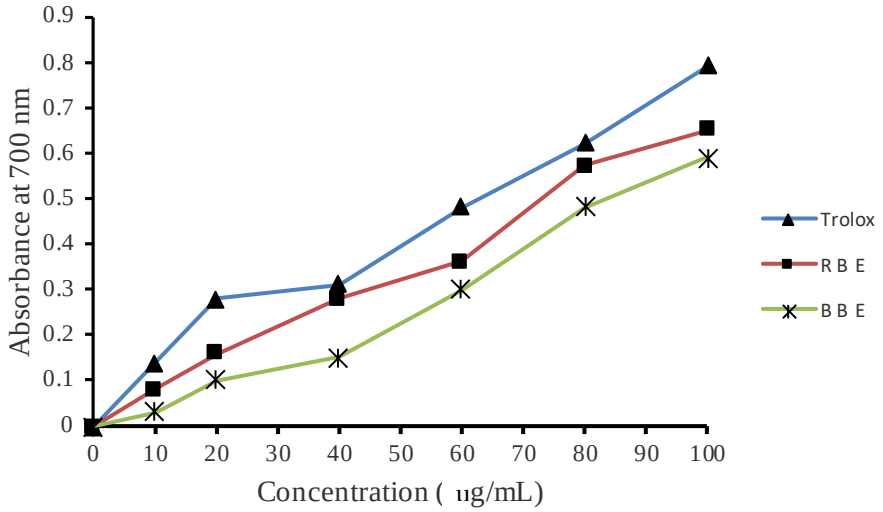

Fig. 2. Antioxidant activities via FRAP depending of *S. torvum* raw and boiled berry extracts concentrations. Trolox (standard of antioxidant), R B E (raw berry extract), B B E (boiled berry extract)

To better appreciate the effectiveness of the antioxidant properties of the extracts of the extracts of raw and boiled berries, the effective concentrations of each extract (EC_{50}) were estimated graphically. The effective concentration EC_{50} corresponds to the concentration of the antioxidant extract which caused an antioxidant activity of 50 %. The EC_{50} values are presented in **Table 1**. Regarding DPPH scavenging, extracts of raw and boiled berries of *S. torvum* respectively exhibited EC_{50} values of 20.00 and 42.20 µg/mL against 8.60 µg/mLfor trolox. The EC_{50} value (20µg/mL) of raw berries methanolic extract for DPPH scavenging was comparable to those obtained for methanolic extracts of several varieties of *Solanum* spp. berries from Ghana which ranged from 17 to 28 µg/mL [29]. However, these EC_{50} value was significantly lower than that from methanolic extract of *S. torvum* berries from India which was 92 µg/ml [30]. The increase of the value of the effective concentration EC_{50} from 20.00 µg/ml (for the raw berries extract) to 42.20 µg/mL (for the boiled berries extract) reflected a significant decrease in antioxidant activity via the scavenging of free radicals during boiling. In terms of EC_{50} values for antioxidant activities via FRAP, the values of 64.80; 75.10 and 87.20 µg/mL were respectively obtained for raw and boiled berries extract against 64.80 20 µg/mL for standard antioxidant. This also reflected the significant decrease of antioxidant activity via FRAP during boiling. The effective concentration EC_{50} of our raw berries extract via FRAP was higher than that obtained for ethanolic extract of raw berries harvested in Indonesia which was 41.32 µg/mL [31]. This could be attributed to the fact that the berries were harvested from different ecological zones and the extractions were carried out with different solvents [32].

TABLE 1
VALUES OF EFFECTIVE CONCENTRATION (EC_{50}) EXPRESSED IN µG/ML OBTAINED FOR TROLOX, RAW AND BOILED BERRY EXTRACTS OF *S. TORVUM*

Method Type	**DPPH**	**FRAP**
Trolox (standard antioxidant)	8.60	64.80
Raw berry extract	20.00	87.20
Boiled berry extract	42.20	87.20

These results highlighted the decrease in the antioxidant potential of *S. torvum* berries during cooking by boiling. This is in accordance with recent results which indicated that boiling of *S. torvum* berries caused the decrease of antioxidant compounds such as total phenolic compounds, flavonoids, carotenoids and ascorbic acid [14]. Similar results have been reported for edible plants in Mexico after short process of boiling [33]. According to Nicoli *et al.* [34], one of the causes of antioxidant depletion in heat-treated fruits and vegetables is represented by the consumption of ascorbic acid and phenolic compounds as reactants in the Maillard reaction. As for some authors, they suggested the use of a short boiling time for certain fruits and vegetables in order to minimize the losses of water-soluble antioxidant compounds such as phenolic compounds and ascorbic acid [35]. In contrast, several studies have indicated that certain fruits and vegetables have seen their antioxidant potential unaffected or increased with boiling [21,25,36,37]. This increase of the antioxidant potential would be due to the softening of the food matrix which would promote the extraction of compounds, which would be converted into more antioxidant chemical species [36,37]. According to other authors, this increase could be attributed to the increase of phenolic compounds during heat-treatment and this due to softening or disruption of plant cell walls and the destruction of complex phenolic compounds [38,39]. In sum, we retained that the effects of cooking by boiling depend on several factors including the boiling time, the fruit or vegetable in question, the structure of the food matrix, the bioaccessibility of antioxidant compounds. Regarding in particular, the berries of *S. torvum*, empirically, the Ivorian consumers practice cooking by boiling in water for about 25 min to soften the matrix and these boiled berries are then crushed in this water for preparing soups or sauces. Under these conditions, the water-soluble antioxidant compounds (phenolic compounds and ascorbic acid) not used as reactants in the Maillard reaction could be retained in the soup or sauce. Indeed, According to Sun *et al.* [40] boiling could cause phenolic compounds to leak into the cooking water.

B. Identification and quantification of individual phenolic compounds by HPLC

In this work, we identified and quantified the individual phenolic compounds in extracts of raw and boiled berries of *S. torvum* by HPLC-UV-Vis, in order to get an idea of the effects of boiling undergone by these compounds which constitute a large part of the main antioxidants in fruits and vegetables [39,41,42]. **Fig. 3** shows the chromatographic profile of the phenolic compounds. We noted that gallic acid, caffeic acid, catechin and tannic acid were detected in both extracts. The most preponderant were catechin and gallic acid with respective contents of 1.06 and 0.99 mg/kg in raw berries; 0.86 and 0.71 mg/kg in boiled berries **(Table 2)**. This could be beneficial for consumers because phenolic acids including gallic acid and flavonoids including catechin, are well documented for their powerful antioxidant properties [43,44]. The same phenolic compounds, except gallic acid were previously identified in ethyl acetate fraction of *S. torvum* raw berries harvested in Daloa province (in the center west of Côte d'Ivoire) [45]. Gallic acid was previously detected in methanolic extract of *S. torvum* raw berries from Malaysia

[16] and *S. ferrugineum* from Mexico [46**].** Caffeic acid was detected in *S. ferrugineum* raw berries from Mexico [46]. These variations observed in terms of individual phenolic compounds could be attributed to genetic and environmental factors. Indeed, it is well known that cultivars, culture conditions, and harvest time influence phenolic compounds of fruits and vegetables [47,48,49]. Regarding the contents, the results presented in **Table 2** indicate that the four phenolic compounds detected in the extracts of raw berries experienced a significant decrease after cooking in water, which is in accordance with the decrease in the content of total phenolic compounds observed previously [14]. During boiling of sweet potato leaves, it was noted significant decrease in contents of several individual phenolic compounds including caffeic acid [40]. According to these authors, this suggests that boiling, induced leaching or degradation of caffeic acid. Significant decrease in contents of caffeic acid was also observed during boiling of fruits and vegetable such as carrot, tomato and Beetroot [21]. In contrast, some authors have reported that boiling did not significantly affect the caffeic acid content of pulps (with or without peel) of several plantain cultivars [50]. Cooking xoconostle by boiling caused also significant decrease in gallic acid and catechin contents [51]. Despite these losses of phenolic compounds, the residual contents could be considerable due to the fact that the cooking water of the berries of *S. torvum* is used for the preparation of sauces and soups in Côte d'Ivoire. Indeed, some of these compounds were lost by leaching into the cooking water [40].

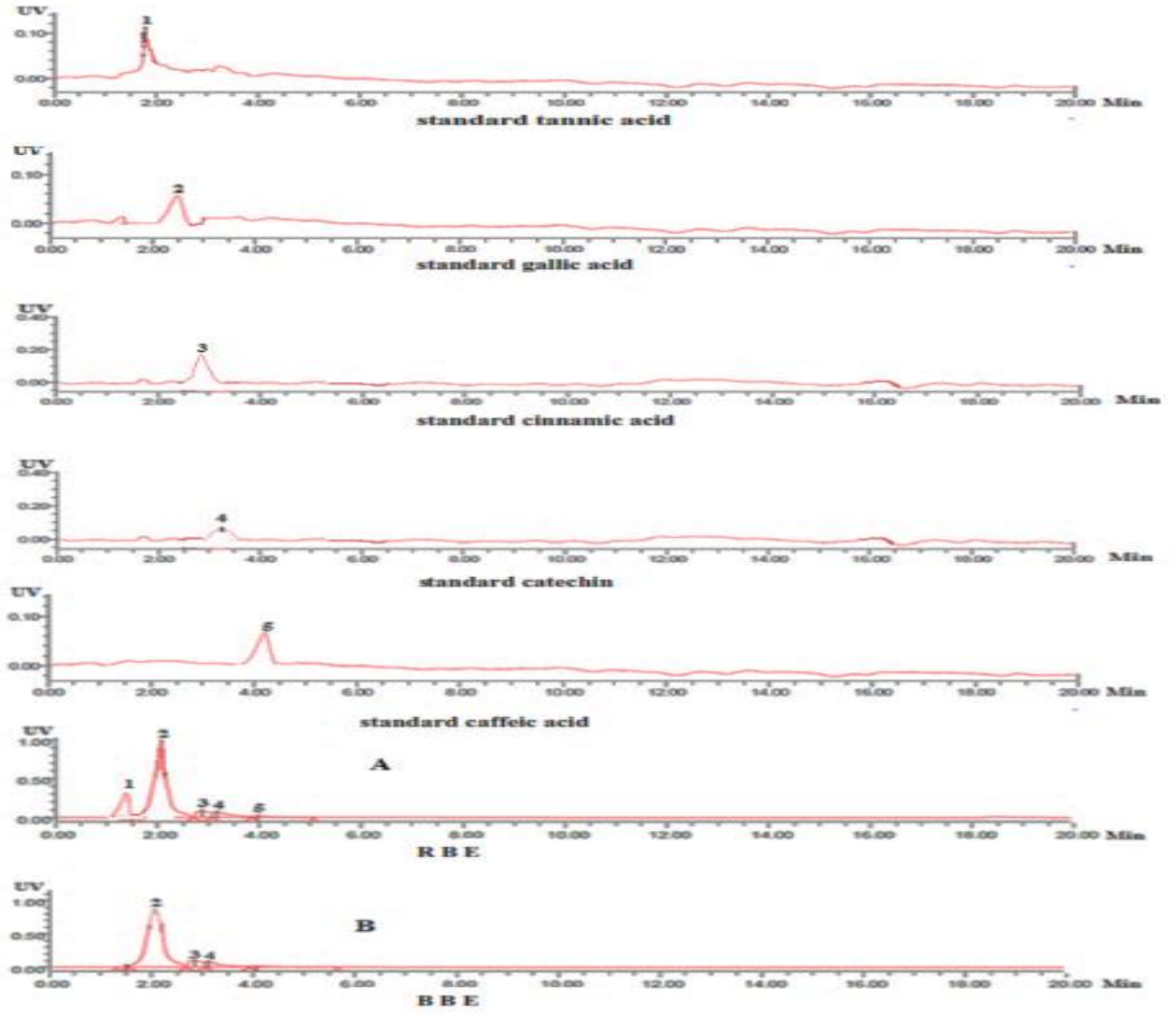

Fig. 3. HPLC profiles of phenolic compounds of raw and boiled berry extracts of *S. torvum*; detection at 210 nm; standard phenolic

compounds used: tannic acid, gallic acid, cinnamic acid, catechin, caffeic acid; samples: R B E (raw berry extract and B B E (boiled berry extract); detected peaks: 1 at 1.51 min (tannic acid), 2 at 2.11 min (gallic acid), 3 at 2.86 min (cinnamic acid), 4 at 3.16 min (catechin), 5 at 4.06 min (caffeic acid)

TABLE 2
DIFFERENT PHENOLIC COMPOUNDS CONTENTS (MG/KG DW) IN RAW AND BOILED BERRIES OF *S.TORVUM*

Phenolic compound (mg/kg DW)	**Extract of *S. torvum* berry**	
	Raw berry	**Boiled berry**
Tannic acid	0.09±0.01[a]	0.03±001[b]
Gallic acid	0.86±0.01[a]	0.71±0,04[b]
Caffeic acid	0.04±0.01[a]	0.01±0.01[b]
Catechin	1.06±0.02[a]	0.99±0.06[b]

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different $p \leq 0.05$ as assessed by the test of Duncan.

C. Identification and quantification of organic acids by HPLC

Organic acids are naturally found in vegetables and fruits and may be formed during processes like fermentation [52,53]. They are known to influence the organoleptic and sensory properties of foodstuffs, and are also been used for their quality control [54,55]. **Fig. 4** shows the chromatographic profile of the organic acids in extracts of raw and boiled berries of *S. torvum*. Seven organic acids were detected and identified as fumaric acid, tartaric acid, citric acid, lactic acid, adipic acid, acetic acid and butyric acid. **Table 3** displays the levels of these organic acids in the extracts of raw et boiled berries The most preponderant were lactic acid and tartaric acid in extract of raw berries with respective contents of 9.81 and 7.74 mg/Kg. Comparing the amounts of each organic acid in extracts of raw and boiled berries, we observed that boiling process significantly affected all the organic acids by decreasing their contents. Other authors obtained similar decreases of organic acids content during boiling of various fruits and vegetables [55,56,57,58]. Armesto *et al.* [55] reported that this was because cooking with water caused leaching of organic acids into the cooking water. In this case, concerning the berries of *S. torvum*, the water of boiling being taken into account in the preparation of sauces and soups by the Ivorian consumers, the losses of organic acids could be minimized. However, Silva *et al.* [56] had instead reported that boiling resulted in the degradation of organic acids including citric acid in quince. The presence of citric acid in raw berries with an acceptable content of 3.59 mg/kg could be beneficial since this acid is well known as a preservative in fruits and vegetables due to its antibacterial activity [59]. In addition, it is endowed with antioxidant activities and its presence added to that of tartaric acid determines the tartness and flavor of fruits [60]. The high content of lactic acid could also be beneficial because it is well established that lactic acid plays an important role in the preservation of fruits and vegetables through lactic fermentation [61].

TABLE 3
DIFFERENT ORGANIC ACID CONTENTS IN RAW AND BOILED BERRIES OF *S. TORVUM*

Organic acid (mg/kg DW)	**Extract of *S. torvum* berry**	
	Raw berry	**Boiled berry**
Fumaric acid	1.44±0.01[a]	0.87±0.01[b]
Tartaric acid	7.74±0.01[a]	5.99±0.01[b]
Citric acid	3.59±0.01[a]	0.15±0.02[b]
Lactic acid	9.81±0.04[a]	0.79±0.01[b]
Acetic acid	1.28±0.01[a]	0.37±0.01[b]
Adipic acid	2.14±0.06[a]	1.61±0.02[b]
Butyric acid	0.07±0.01[a]	0.02±0.01[b]

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different $p \leq 0.05$ as assessed by the test of Duncan.

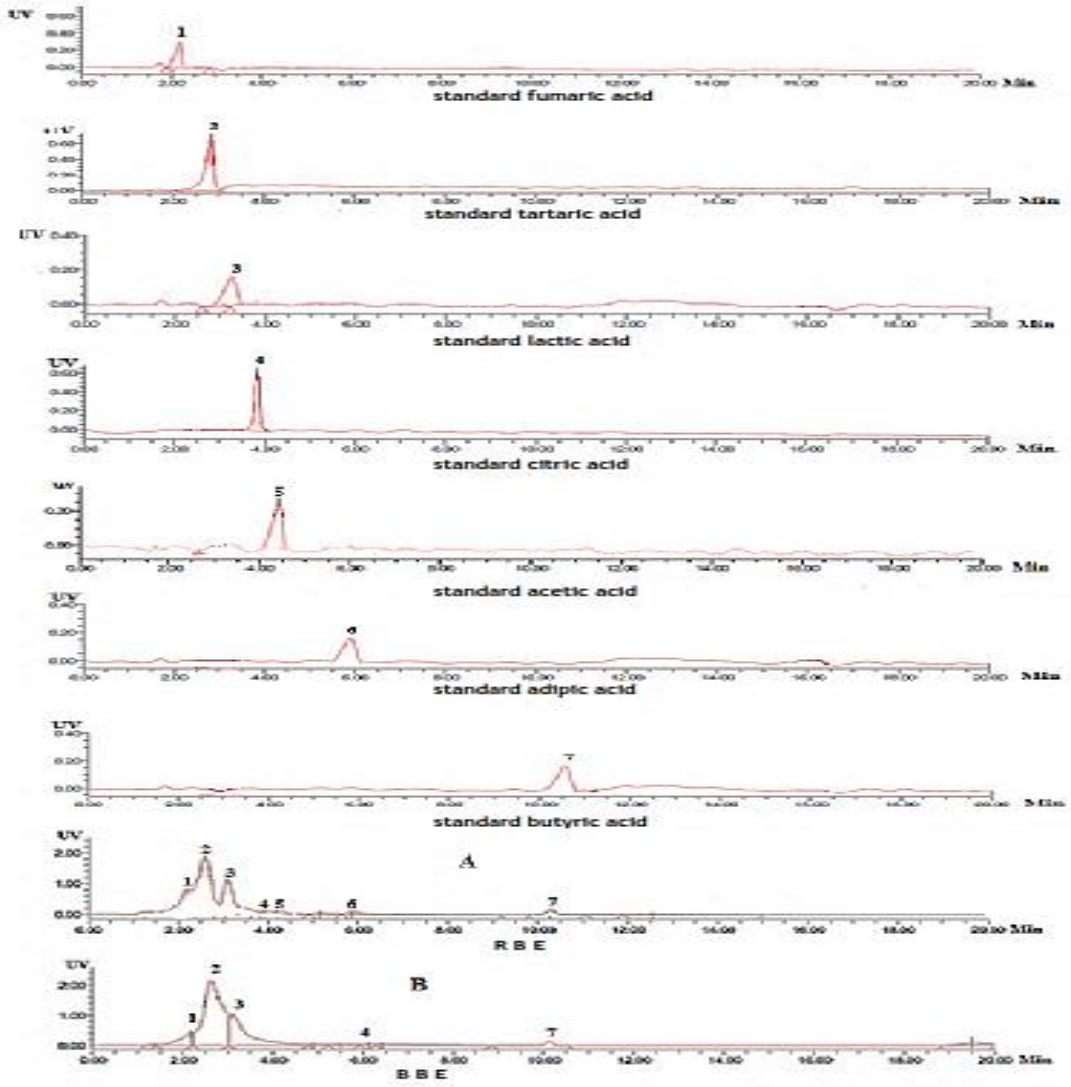

Fig. 4. HPLC profiles of organic acids of raw and boiled berry extracts of *S. torvum*; detection at 210 nm; standard organic acids used: fumaric acid, tartaric acid, lactic acid, citric acid, acetic acid, adipic acid, butyric acid, ; samples: R B E (raw berry extract and B B E (boiled berry extract); detected peaks: 1 at 2.37 min (fumaric acid), 2 at 2.81 min (tartaric acid), 3 at 2.94 min (lactic acid), 4 at 3.91 min (citric acid), 5 at 4.22 min (acetic acid), 6 at 5.96 min (adipic acid), 7 at 10.23 (butyric acid)

IV. Conclusion

In conclusion, the first intriguing findings were the high DPPH scavenging and ferric reduction activities of methanolic extracts of raw and cooked *S. torvum*. Boiling, on the other hand, dramatically inhibited these actions. This indicated that these berries had an acceptable antioxidant capacity, as evidenced by low effective concentrations EC50 and the presence of particular phenolic components known to be powerful antioxidants. Furthermore, organic acids that contribute to organic and sensory qualities, as well as antioxidant capabilities, have been discovered and quantified in these berries. However, cooking with water has a significant impact on the contents. Because the cooked berries are crushed in the cooking water, these losses are minimised during the creation of sauces and soups.

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