# Changes in Nutrient Composition, Antioxidant Properties, and Enzymes Activities of Snake Tomato (*Trichosanthes cucumerina*) during Ripening

Adebanjo Ayobamidele Badejo, Adeyemi Philips Adebowale, and Victor Ndigwe Enujiugha

Department of Food Science and Technology, Federal University of Technology, Akure 340001, Nigeria

**ABSTRACT:** Snake tomato (*Trichosanthes cucumerina*) has been cultivated and used as a replacement for *Lycopersicum esculentum* in many Asian and African diets. Matured *T. cucumerina* fruits were harvested at different ripening stages and separated into coats and pulps for analyses to determine their suitability for use in culinary. They were analyzed for the nutritional composition and antioxidant potential using different biochemical assays [1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activities, and ferric reducing antioxidant power] and antioxidative enzymes activities. The nutritional composition revealed that *T. cucumerina* contains over 80% water and is very rich in fiber, thus it can serve as a good natural laxative. The lycopene and β-carotene contents were especially high in the ripe pulp with values of  $21.62\pm1.22$  and  $3.96\pm0.14$  mg/100 g, respectively. The ascorbic acid content was highest in the pulp of unripe fruit with a value of  $56.58\pm1.08$  mg/100 g and significantly (P<0.05) decreased as ripening progressed. The antioxidant potential of the fruits for the 3 assays showed that unripe pulp > ripe coat > ripe pulp > unripe coat. There were decreases in the antioxidant enzymes (superoxide dismutase, ascorbate peroxidase, and glutathione reductase) activities, with the exception of catalase, as ripening progressed in the fruits. These decreased activities may lead to the softening of the fruit during ripening. Harnessing the antioxidative potential of *T. cucumerina* in culinary through consumption of the coats and pulps will alleviate food insecurity and help maintain good health among many dwellers in sub-Saharan Africa and Southeast Asia.

Keywords: snake tomato, antioxidative potential, enzyme activity, nutrient composition

## INTRODUCTION

The major staple foods in sub-Saharan Africa are mainly starch-derived, especially from cassava (Manihot esculentum), yam (Discorea sp.), and maize (Zea mays), while rice (Oryza sativa) is predominant in Asia. Regular inclusion of fruits and vegetables in daily meals has been strongly encouraged to promote healthy living. Snake tomato (Trichosanthes cucumerina) is an underutilized plant in Asia and Africa, which has gained popularity for its low cost of cultivation, rapid growth, health promotion, and as suitable replacement for Lycopersicum esculentum in many countries of Africa (1). L. esculentum is the second most consumed vegetable in the world. The metabolism of *T*. cucumerina is similar to that of L. esculentum. Being climacteric, harvested matured fruits continue to ripen after detachment from the main plant and during storage. The edible portions of *T. cucumerina* include the mature unripe coat and the red fleshy pulp covering the seeds in the ripened fruit. The fully mature fruit contains a soft, red, and tomato-like pulp that is used in stews or sauces as a substitute for tomato puree or paste. During the ripening process, the fruit undergoes several changes such as synthesis of pigments, development of compounds responsible for flavor and aroma, and carotenoids (especially lycopene) biosynthesis (2).

The vitamin C contents of fruits and vegetables have been found to vary depending on the ripening stages. For most climacteric fruits, the vitamin C content correlates positively with the ripening stages (3); however, for the tropical fruits like acerola (*Malpighia glabra*) the reverse was observed to be the case (4). Phytochemicals in fruits and vegetables have been associated with reduced risk of cardiovascular diseases. The potential health benefits have led to a number of research works on many underexploited plants. Different parts of *T. cucumerina* have been

reported to have nutritional and health benefits (5).

Reactive oxygen species (ROS) in the form of hydroxyl, superoxide, and hydrogen peroxide are constantly being generated as the plant matures and ripens. Plants have also developed antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) to quench these radicals (6). When a plant is undergoing senescence, several changes take place in these enzymes, and also during fruit ripening.

Since *T. cucumerina* is consumed at the unripe (coat) and ripened (fleshy pulp) stages, it is imperative to examine the changes in the nutritional composition during this crucial transition for optimal health benefits when consumed. The present study seeks to determine the changes in the proximate composition, major antioxidant compositions, and activities of antioxidant enzymes in the coats and pulps of the fruit during ripening. It is expected that the information provided will encourage wider cultivation, processing, consumption, and utilization of *T. cucumerina*.

## **MATERIALS AND METHODS**

# Source of plant materials

The seeds of *T. cucumerina* were planted in a local garden near the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. They were planted in April 2013 at the beginning of the rainy season. The plant was nurtured to maturity and harvested at two different stages of ripening according to local consumers' criteria. Harvested plants were washed, sliced, and the fleshy parts were separated from the seeds. The outer coats and the succulent fleshy parts (pulp) from both ripe and unripe fruits were stored at 4°C for further analyses.

# **Proximate composition**

Each of the samples was blended to form a paste and then analyzed for proximate chemical composition (moisture, protein, fat, carbohydrates, crude fibre, and ash) using the Association of Official Analytical Chemists (AOAC) procedures (7). The macro-Kjeldahl method was used for the determination of the crude protein content (N× 6.25) of each sample while the crude fat was determined using the Soxhlet apparatus by extracting a known weight of the paste sample with petroleum ether. The ash content was determined by incineration at  $600\pm15^{\circ}$ C for 8 h, and the total carbohydrate contents were calculated by difference. The Energy value was calculated as follows:

Energy (kJ) =  $[(g \text{ fat} \times 9) + (g \text{ carbohydrates} \times 4) + (g \text{ protein} \times 4)] \times 4.184$ 

# Carotenoid (lycopene and $\beta$ -carotene) and ascorbic acid contents

T. cucumerina coat and fruit were extracted in hexane: acetone: ethanol (2:1:1) and the lycopene content was determined as described by Toor and Salvage (8). β-Carotene content was determined according to the method of AOAC (7). Ascorbic acid content was determined as previously described (9).

## Phenolic and flavonoid content

The phenolic content was determined as described by Meda et al. (10) with slight modifications. Briefly, an aliquot of 0.2 mL of the sample extracted in ethanol was thoroughly mixed with 2.5 mL 10% Folin-Ciocalteau's reagent (Merck, Darmstadt, Germany) and 2 mL of 7.5% sodium carbornate. The mixture was incubated in the dark for 30 min at 45°C and the absorbance was read at 750 nm. Gallic acid was used as the standard and data were expressed as milligram gallic acid equivalents (GAE) per g sample.

The aluminum chloride colorimetric method described by Meda et al. (10) was used in the determination of the total flavonoid content. An aliquot of 0.2 mL of the extract was added to 0.3 mL of 5% NaNO<sub>3</sub>. After 5 min, 0.6 mL of 10% aluminum chloride was added and after 6 min, 2 mL of 1 M NaOH was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510 nm against the reagent blank. Rutin was used as the standard and data expressed as mg rutin equivalent per g of the sample.

## **Evaluation of non-enzymatic antioxidant potentials**

Two grams of the paste was weighed into a Corning tube and 10 mL of methanol added. The mixture was shaken gently on a laboratory mixing rotator for 12 h. The mixture was then centrifuged at 1,000 g for 1 min. The extracts obtained with aqueous methanol were used to evaluate the antioxidant potentials of T. cucumerina.

The Trolox equivalent antioxidant capacity (TEAC) was determined as described by Re et al. (11). A stock solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L  $K_2S_2O_8$  and the mixture bottle was wrapped with aluminum foil and kept in the dark at room temperature for 16 h. A working solution was then prepared, immediately before the analysis, by diluting the stock solution in ethanol until the absorbance at 734 nm was  $0.70\pm0.02$ . An aliquot of  $200~\mu L$  of the extract was mixed with 2 mL of the ABTS working solution and after 5 min of reaction the absorbance was read at 734 nm. The results were expressed as mmol of Trolox per g sample.

The ferric reducing property of the extracts was determined as described by Pulido et al. (12). An aliquot of

0.25 mL of the extract was mixed with 0.25 mL of 200 mM of sodium phosphate buffer pH 6.6 and 0.25 mL of 1%  $K_3Fe(CN)_6$ . The mixture was incubated at  $50^{\circ}C$  for 20 min, thereafter 0.25 mL of 10% trichloroacetic acid was also added to the mixture which was centrifuge at 2,000 g for 10 min. Then, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.1 mL of 0.1% of  $FeCl_3$  solution and the absorbance was measured at 700 nm. Ferric reducing antioxidant power (FRAP) values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of  $Fe^{2+}$ , and expressed as mmol of  $Fe^{2+}$  equivalents per gram of the sample.

The free radical scavenging ability of the extract against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was carried out using the method described by Gyamfi et al. (13). An aliquot (1 mL) of the extract was mixed with 1 mL of 0.4 mM methanolic solution of the DPPH reagent and left in the dark for 30 min before measuring the absorbance at 515 nm. The DPPH radical scavenging activity was calculated as percentage of DPPH discoloration using the equation:

% DPPH inhibition=
$$\frac{control_{Abs} - sample_{Abs}}{control_{Abs}} \times 100$$

where, sample  $_{Abs}$  is the absorbance of the sample when the extract was added, and control $_{Abs}$  is the absorbance of the DPPH solution without the extract.

# **Colour analyses**

The paste from each of the samples was placed in a cuvette and a CM-700d spectrophotometer (Konica Minolta Sensing, Inc., Tokyo, Japan) was used to determine the colour parameters. The  $L^*$ ,  $a^*$ , and  $b^*$  values were read from the spectrophotometer after it was calibrated against a white plate.

# **Enzyme assays**

The extraction buffer [0.1 M phosphate buffer, pH 7.5, containing 5% (m/v) polyvinylpyrrolidone, 1 mM ethyl-

enediaminetetraacetic acid, and 10 mM  $\beta$ -mercaptoethanol] was prepared and stored at refrigeration temperature. *T. cucumerina* was harvested and 1 g was ground in a mortar with pestle in 1 mL of cold extraction buffer. Soluble protein was obtained after 10 min centrifugation at 10,000 g and used immediately or stored at  $-20^{\circ}$ C for the determination of enzyme activity. SOD activity was determined by measuring the ability of the extracts to inhibit conversion of nitroblue tetrazolium to formazan (14). GR activity was determined as described by Mondal et al. (6). CAT and APX were determined as described by Aebi (15) and, Nakano and Asada (16), respectively.

# Statistical analyses

All the samples were prepared and analyzed in triplicates. All data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test using the Statistical Package for the Social Sciences (SPSS) version 17.0 software (SPSS Inc., Chicago, IL, USA). A *P*-value less than 0.05 was considered statistically significant.

## **RESULTS AND DISCUSSION**

# Nutritional composition of T. cucumerina

*T. cucumerina* is a climacteric fruit that continues to ripen after harvesting at the matured stage. The harvested fruits were separated into the peel (referred to as coat) and pulp (the fleshy part that covers the seed). The fruits were carefully selected at the matured stage when the coat and pulp are still at the green unripe, and the ripened stage (where the coat and pulp have changed to red colour). At these two stages no obvious change in fruit size was observed. The moisture contents ranged from  $88.34\pm0.39$  to  $90.95\pm0.22$  g/100 g. Apart from the moisture content, carbohydrate and crude fibre were the other major components of *T. cucumerina* with values of  $3.72\pm0.13\sim6.11\pm0.17$  g/100 g and  $3.32\pm0.08\sim4.67\pm0.09$  g/100 g, respectively (Table 1). The protein, crude fat, and ash contents were very low. The nutritional com-

Table 1. Proximate compositions of unripe and ripened Trichosanthes cucumerina

	Unripe		Ripened	
	Coat	Pulp	Coat	Pulp
Moisture (g/100 g)	90.95±0.22°	90.04±0.21 <sup>b</sup>	88.34±0.39 <sup>d</sup>	89.08±0.82 <sup>c</sup>
Ash (g/100 g)	0.32±0.03 <sup>c</sup>	0.73±0.06 <sup>b</sup>	$0.24\pm0.02^{d}$	$0.84\pm0.07^{a}$
Crude fat (g/100 g)	0.19±0.01 <sup>c</sup>	0.29±0.02 <sup>b</sup>	0.29±0.01 <sup>b</sup>	0.51±0.06°
Protein (g/100 g)	$0.37\pm0.02^{c}$	0.39±0.01 <sup>b</sup>	0.31±0.01 <sup>d</sup>	0.51±0.02°
Crude fibre (g/100 g)	4.45±0.02 <sup>b</sup>	3.34±0.01 <sup>c</sup>	4.67±0.09 <sup>a</sup>	$3.32\pm0.08^{c}$
Carbohydrates (g/100 g)	3.72±0.13 <sup>d</sup>	5.21±0.10°	6.11±0.17 <sup>a</sup>	5.72±0.09 <sup>b</sup>
Energy (kJ/100 g FW)	71.83 <sup>c</sup>	104.64 <sup>b</sup>	118.37 <sup>a</sup>	123.46°

Values are mean±SD of 3 replicates.

Different letters (a-d) within same row are significantly different (P<0.05) according to Duncan's multiple range test.

Table 2. Antioxidant compositions of coat and pulp Trichosanthes cucumerina at unripe and ripened stages

	Unripe		Ripened	
	Coat	Pulp	Coat	Pulp
Lycopene (mg/100 g)	10.82±0.96 <sup>d</sup>	12.64±0.44 <sup>c</sup>	18.28±0.91 <sup>b</sup>	21.62±1.22°
β-Carotene (mg/100 g)	1.75±0.17 <sup>d</sup>	2.19±0.06 <sup>c</sup>	3.22±0.21 <sup>b</sup>	3.96±0.14°
Phenolics (mg/GAE g) <sup>1)</sup>	0.81±0.06°	1.85±0.13 <sup>a</sup>	0.97±0.08 <sup>b</sup>	0.74±0.11 <sup>c</sup>
Flavonoids (mg/RE g) <sup>2)</sup>	1.36±0.09°	2.04±0.07°	1.35±0.09°	1.84±0.11 <sup>b</sup>
Ascorbic acid (mg/100 g)	39.32±0.88 <sup>c</sup>	56.58±1.08 <sup>a</sup>	32.36±1.77 <sup>d</sup>	51.09±0.61 <sup>b</sup>

Values are mean±SD of 3 replicates.

Different letters (a-d) within same row are significantly different (P<0.05) according to Duncan's multiple range test.

position of the fruit is similar to those of *T. anguina* and *L. esculentum* (17-19). The carbohydrate, protein, crude fat, and crude fibre contents of *T. cucumerina* compared well with the values reported for *L. esculentum* (17). The crude fibre was particularly higher in the coats of both the ripe and unripe *T. cucumerina*. The energy value ranged from 71.83 kJ/100 g in the unripe coat to 123.46 kJ/100 g fresh weight in the pulp of the ripened *T. cucumerina* (Table 1). Similar energy values had been reported for *L. esculentum* (17) and other vegetables commonly consumed in tropical and temperate countries (20).

## Antioxidant composition and colour analysis

L. esculentum is a major source of several antioxidants such as carotenoids (lycopene, β-carotene, and lutein), flavonols, and flavonones (21). The lycopene contents of the ripened *T. cucumerina* ranged from  $18.28\pm0.91\sim21.62$ ±1.22 mg/100 g. These values are significantly higher than those of the unripe coat and pulp (Table 2). The β-carotene contents of the coat and pulp of ripened *T. cu*cumerina are 3.22±0.21 and 3.96±0.14 mg/100 g, respectively. This shows a positive correlation between the lycopene and  $\beta$ -carotene in ripened T. cucumerina. The antioxidant capacity of tomato fruit has been found to correlate positively with the lycopene content (22). Lycopene is a fat soluble pigment responsible for the red colour of L. esculentum and T. cucumerina which accumulates in the phytochromes. The phytochrome organelles determine the colour development by controlling the amount of accumulated lycopene. The lycopene content increased in T. cucumerina as the fruit ripened. This was evident from the colour analysis which showed the ripened pulp with very high  $a^*$  value (Table 3). The  $a^*$  represents the redness to greenness level of the sample. There was about 70% increase between the lycopene content in the coats of unripe and ripened fruit. Lycopene is increasingly synthesized as the fruit ripens and this correlates with the degree of maturation. The pulps also showed similar differences in the lycopene content (Table 2). The β-carotene value was least in the coat of unripe fruit and highest in the ripened fruits. The  $\beta$ -carotene values are high-

**Table 3.** Colour analysis of different fractions of *Trichosanthes cucumerina* 

	Unripe		Ripened		
	Coat	Pulp	Coat	Pulp	
L* a* b*	33.34±0.60 <sup>c</sup> -1.52±0.08 <sup>d</sup> 3.08±0.39 <sup>d</sup>	50.95±1.57 <sup>a</sup> -1.11±0.04 <sup>c</sup> 8.12±0.49 <sup>b</sup>	36.06±0.21 <sup>b</sup> 3.17±0.04 <sup>b</sup> 11.71±0.29 <sup>a</sup>	27.96±0.33 <sup>d</sup> 14.35±0.26 <sup>a</sup> 4.85±0.15 <sup>c</sup>	

Values are mean±SD of 3 replicates.

Different letters (a-d) within same row are significantly different (P<0.05) according to Duncan's multiple range test.

er than 0.51 mg/100 g reported for *L. esculentum* (19). A difference of over 80% was observed between the  $\beta$ -carotene contents of the pulps of ripened and unripe fruit. Increase in the daily consumption of *T. cucumerina* will contribute to the total antioxidants available within the body, which is associated with several health benefits. Daily intake of antioxidants have been strongly associated with higher plasma concentrations of  $\beta$ -carotene (23).

The total phenolic contents ranged from 0.74±0.11 to 1.85±0.13 mg/g GAE with the highest value in the unripe pulp. This correlates strongly with the flavonoid content (Table 2). The phenolic contents of fruits and vegetables have been associated with their antioxidant activities, which may be due to their redox properties, which allow them to inhibit atherosclerosis and cancer due to their ability to scavenge free radicals (24). The values obtained in the present study are lower than those reported for melon concentrate (25). Rutin is among the most abundant flavonoids in tomato fruit (26). The flavonoid content of T. cucumerina was measured as rutin equivalent per gram sample. There was no significant difference between the flavonoid contents of the coats in ripened and unripened T. cucumerina; however, the flavonoid content of the fruit decreased by just 10%.

The ascorbic acid content was inversely correlated with ripening. The ripened pulp had ascorbic acid content greater than 50 mg/100 g (Table 2). Ascorbic acid is one of the major hydrophilic antioxidants in tomato, contributing greatly to its antioxidant capacity (27). The role of

<sup>&</sup>lt;sup>1)</sup>GAE: gallic acid equivalent.

<sup>&</sup>lt;sup>2)</sup>RE: rutin equivalent.

94 Badejo et al.

ascorbic acid in the prevention of diseases related to oxidative damage is largely due to its ability to neutralize the action of free radicals in biological systems. The ascorbic acid content of the unripe pulp was the highest with a value of 56.58±1.08 mg/100 g. Consuming a little over 100 g of T. cucumerina will meet the 60 mg daily vitamin C requirement. The ascorbic acid content of the ripe and unripe pulps were significantly higher than the values recorded for the coats of either (Table 2). The values are higher than the 25 mg/100 g previously reported (28). This may be due to the differences in the variety as well as planting and harvest seasons. The coat also contained considerably high ascorbic acid content. There was a significant decrease of about 18% in the ascorbic acid content of the coat as the fruit ripened (Table 2). Trends like this have been reported for the tropical fruit acerola, where the ascorbic acid content decreased as the fruit ripened (4) and inversely correlates with the observation in L. esculentum where the ascorbic acid contents increased with ripening (3,26).

# Non-enzymatic antioxidant potential

The percentage DPPH inhibition ranged from 16.57± 1.23 to 58.24±2.53% with the lowest value in the unripe coat and highest in the unripe pulp. There was a significant difference between the DPPH inhibition of the coat and the pulp of the ripened T. cucumerina (Table 4). A positive correlation was observed among all the three parameters used to determine the antioxidant activity. The unripe pulp had the highest FRAP value of 3.29±0.24 mmol Fe<sup>2+</sup>/100 g while the coat had the lowest. There was no significant difference between the TEAC values for the unripe pulp and the ripe coat. The TEAC value of the ripened pulp was the lowest (Table 4). Reactive oxygen species are generated in plants during metabolic processes within the cellular compartments (29). Fruit ripening is an oxidative phenomenon which is characterized by the breakdown of cell wall polymers and cellulose thus resulting in the softening of the fruits. Plants have therefore developed various defense mechanisms to protect them against oxidative stress damages. The antioxidant potential of any fruit or vegetable is the ability of the different antioxidants (independent of their bioavailability) present in the samples in scavenging preformed free radicals. In evaluating the antioxidant potential of any food sample, it is imperative to use two or more radical systems as the solvent and substrate do impact on the values obtained (30). In this study, DPPH, ABTS, and FRAP assays were used in evaluating the antioxidant potentials of the coat and pulp of T. cucumerina. High antioxidant activities were obtained for the coat and pulp of the ripe and unripe *T. cucumerina* (Table 4). Although the green fruit had the highest antioxidant potential as measured by the percentage DPPH inhibition, FRAP, and TEAC, the pulp also had high value that correlates with the antioxidant composition (Table 2). The DPPH, FRAP and TEAC showed positive correlations across the various organs analyzed in the order unripe pulp> ripe coat> ripe pulp> unripe coat (Table 4).

# Antioxidant enzymes activities

SOD activity in T. cucumerina ranged from 18.7±0.3 to 22.2±1.2 U/g protein. The coat of the unripe fruit had the highest value while the pulp of the ripened fruit had the lowest (Table 5). The CAT activity was lowest in the coat of the unripe fruit with a value of 148.3±4.8 nmol H<sub>2</sub>O<sub>2</sub>/g protein/min. As ripening progressed, CAT activity also increased with the pulp of the ripened fruit having an activity of 222.3±5.7 nmol H<sub>2</sub>O<sub>2</sub>/g protein/min. In contrast, APX and GR activities showed an inverse correlation with ripening. APX activity was lowest in the pulp of the ripened fruit with a value of 193.7±8.3 nmol ascorbic acid/g protein/min and highest in the coat of the unripe fruit with a value of 443.5±11.5 nmol ascorbic acid/g protein/min (Table 5). GR activity was also highest in the coat of the unripe fruit [70.6+2.6 nmol nicotinamide adenine dinucleotide phosphate hydrate (NADPH) /g protein/min] and lowest in the pulp of the ripened fruit with a value of 45.9±1.8 nmol NADPH/g protein/ min (Table 5). Some of the antioxidative enzymes that contribute to the protective system in plants include SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), APX (EC 1.11. 1.11), and GR (EC 1.6.4.2). All these enzymes contribute to minimizing the levels of oxidative damage within the plant tissues by keeping ROS at low levels. The activities of SOD, APX, and GR were high in T. cucumerina

Table 4. Antioxidant activities of Trichosanthes cucumerina at unripe and ripened stages

	Unripe		Ripened	
	Coat	Pulp	Coat	Pulp
DPPH inhibition (%)	16.57±1.23 <sup>d</sup>	58.24±2.53 <sup>a</sup>	45.79±1.13 <sup>b</sup>	42.27±1.33 <sup>c</sup>
FRAP (mmol Fe <sup>2+</sup> /100 g) <sup>1)</sup>	2.05±0.06 <sup>d</sup>	3.29±0.24°	3.07±0.23 <sup>b</sup>	2.58±0.18 <sup>c</sup>
TEAC (mmol Trolox/100 g) <sup>2)</sup>	1.86±0.09 <sup>b</sup>	2.34±0.17 <sup>a</sup>	2.24±0.07 <sup>a</sup>	1.17±0.10 <sup>c</sup>

Values are mean±SD of 3 replicates.

Different letters (a-d) within same row are significantly different (P<0.05) according to Duncan's multiple range test.

<sup>&</sup>lt;sup>1)</sup>FRAP: ferric reducing antioxidant power. <sup>2)</sup>TEAC: trolox equivalent antioxidant activity.

**Table 5.** SOD, CAT, APX, and GR enzymes activities in coat and pulp of unripe and ripened *Trichosanthes cucumerina* 

	Unripe		Ripened		
	Coat	Pulp	Coat	Pulp	
SOD	22.2±1.2 <sup>a</sup>	20.1±0.8 <sup>b</sup>	20.7±0.4 <sup>b</sup>	18.7±0.3 <sup>c</sup>	
CAT	148.3±4.8 <sup>d</sup>	172.3±4.3 <sup>c</sup>	180.8±5.8 <sup>b</sup>	222.3±5.7 <sup>a</sup>	
APX	443.5±11.5°	404.2±8.7 <sup>b</sup>	257.8±10.7°	193.7±8.3 <sup>d</sup>	
GR	70.6±2.6 <sup>a</sup>	65.7±2.8 <sup>b</sup>	58.6±1.9°	$45.9 \pm 1.8^{d}$	

Values are mean±SD of 3 replicates.

Different letters (a-d) within same row are significantly different (P<0.05) according to Duncan's multiple range test. SOD, superoxide dismutase (U/g protein); CAT, catalase (nmol H<sub>2</sub>O<sub>2</sub>/g protein/min); APX, ascorbate peroxidase (nmol ascorbic acid/g protein/min); GR, glutathione reductase (nmol NADPH/g protein/min).

coats and pulps of the unripe fruit with a significant decrease as it transitioned during ripening (Table 5), thus showing that oxidative stress has been elicited in the process. Only CAT showed an increase in activity as the fruit ripened. Similar observations have been reported in ripening of L. esculentum and peach fruit (Prunus persica) (6,31). SOD is a metalloenzyme that catalyzes the dismutation of superoxide anion to oxygen and H<sub>2</sub>O<sub>2</sub> thus, protecting plant tissues from oxidation of lipids and DNA damages. CAT (an oxidoreductase enzyme) and APX are responsible for the removal and conversion of the H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O (29). The ascorbate-glutathione cycle, where GR plays a role, is responsible for the efficient removal of excess ROS (32). Thus, a decrease in GR activity will increase the susceptibility of T. cucumerina to oxidative stress, which may lead to the softening of the fruit. Also, decreased activities of SOD and APX during ripening are evidence that there is a built-up of oxidative stress and a decreased ability to catabolize the generated reactive oxygen species.

# **CONCLUSION**

*T. cucumerina* is a fruit that is low in energy value but very rich in crude fibre, which is beneficial to humans as natural laxative. It is also very rich in ascorbic acid and lycopene. The combined effect of the non-enzymatic and enzymatic antioxidant compounds give *T. cucumerina* the protective support from the ROS generated during the development and ripening stages. In all, *T. cucumerina* will significantly contribute to solving the problem of food insecurity that is ravaging sub-Saharan Africa and beyond if the cultivation is encouraged on a large scale. Hence, there is the need to further investigate the impact of processing on the nutritional composition of the fruit and its nutrients' bioavailability.

# **AUTHOR DISCLOSURE STATEMENT**

The authors declare no conflict of interest.

## **REFERENCES**

- 1. Adebooye OC, Oloyede FM, Onagoruwa OO. 2006. Fruit characteristics and nutrient composition of landrace morphotypes of snake tomato. *J Veg Sci* 11: 5-16.
- 2. Toor RK, Savage GP. 2006. Changes in major antioxidant components of tomatoes during post-harvest storage. *Food Chem* 99: 724-727.
- 3. Badejo AA, Wada K, Gao Y, Maruta T, Sawa Y, Shigeoka S, Ishikawa T. 2012. Translocation and the alternative D-galacturonate pathway contribute to increasing the ascorbate level in ripening tomato fruits together with the D-mannose /L-galactose pathway. *J Exp Bot* 63: 229-239.
- 4. Badejo AA, Fujikawa Y, Esaka M. 2009. Gene expression of ascorbic acid biosynthesis related enzymes of the Smirnoff-Wheeler pathway in acerola (*Malpighia glabra*). *J Plant Physiol* 166: 652-660.
- Arawwawala LDAM, Thabrew MI, Arambewela LSR. 2012. Lipid lowering effect of hot water extract of *Trichosanthes cu-cumerina* Linn. and antihyperglycemic activity of its fractions on streptozotocin-induced diabetic rats. *Isr J Plant Sci* 60: 447-455
- Mondal K, Sharma NS, Malhotra SP, Dhawan K, Singh R. 2004. Antioxidant systems in ripening tomato fruits. *Biol Plant* 48: 49-53.
- 7. AOAC. 2005. Official method of analysis. 18th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- 8. Toor RK, Savage GP. 2005. Antioxidant activity in different fractions of tomatoes. *Food Res Int* 38: 487-494.
- Awe FB, Fagbemi TN, Ifesan BOT, Badejo AA. 2013. Antioxidant properties of cold and hot water extracts of cocoa, *Hibiscus* flower extract, and ginger beverage blends. *Food Res Int* 52: 490-495
- 10. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem* 91: 571-577.
- 11. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26: 1231-1237.
- Pulido R, Bravo L, Saura-Calixto F. 2000. Antioxidant activity
  of dietary polyphenols as determined by a modified ferric
  reducing/antioxidant power assay. *J Agric Food Chem* 48: 33963402.
- 13. Gyamfi MA, Yonamine M, Aniya Y. 1999. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *Gen Pharmacol* 32: 661-667.
- Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 44: 276-287.
- Aebi H. 1985. Catalase. In Methods of Enzymatic Analysis. 3rd ed. Bergmeyer HU, Bergmeyer J, Grassl M, eds. Verlag Chemie GmbH, Weinheim, Germany. Vol 3, p 273-286.
- Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22: 867-880.
- 17. Guil-Guerrero JL, Rebolloso-Fuentes MM. 2009. Nutrient composition and antioxidant activity of eight tomato (*Lyco-*

96 Badejo et al.

persicon esculentum) varieties. J Food Compos Anal 22: 123-129.

- 18. Ojiako OA, Igwe CU. 2008. The nutritive, anti-nutritive and hepatotoxic properties of *Trichosanthes anguina* (snake tomato) fruits from Nigeria. *Pak J Nutr* 7: 85-89.
- 19. Pinela J, Barros L, Carvalho AM, Ferreira ICFR. 2012. Nutritional composition and antioxidant activity of four tomato (*Lycopersicon esculentum* L.) farmer' varieties in Northeastern Portugal homegardens. *Food Chem Toxicol* 50: 829-834.
- Salazar J, Velàsquez R, Quesada S, Piccinelli AL, Rastrelli L.
   2006. Chemical composition and antinutritional factors of Lycianthes synanthera leaves (chomte). Food Chem 97: 343-348.
- 21. Rao AV. 2004. Processed tomato products as a source of dietary lycopene: bioavailability and antioxidant properties. *Can J Diet Pract Res* 65: 161-165.
- 22. Martínez-Valverde I, Periago MJ, Provan G, Chesson A. 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *J Sci Food Agric* 82: 323-330.
- Del Rio D, Valtueña S, Pellegrini N, Bianchi MA, Ardigò D, Franzini L, Scazzina F, Monti L, Zavaroni I, Brighenti F. 2009. Intervention study with a high or low antioxidant capacity diet: effects on circulating β-carotene. Eur J Clin Nutr 63: 1220-1225.
- 24. Ma X, Wu H, Liu L, Yao Q, Wang S, Zhan R, Xing S, Zhou Y. 2011. Polyphenolic compounds and antioxidant properties in mango fruits. *Sci Hortic* 129: 102-107.
- Carillon J, Del Rio D, Teissèdre PL, Cristol JP, Lacan D, Rouanet JM. 2012. Antioxidant capacity and angiotensin I

- converting enzyme inhibitory activity of a melon concentrate rich in superoxide dismutase. *Food Chem* 135: 1298-1302.
- Moco S, Capanoglu E, Tikunov Y, Bino RJ, Boyacioglu D, Hall RD, Vervoort J, De Vos RCH. 2007. Tissue specialization at the metabolite level is perceived during the development of tomato fruit. *J Exp Bot* 58: 4131-4146.
- Raffo A, Leonardi C, Fogliano V, Ambrosino P, Salucci M, Gennaro L, Bugianesi R, Giuffrida F, Quaglia G. 2002. Nutritional value of cherry tomatoes (*Lycopersicon esculentum Cv.* Naomi F1) harvested at different ripening stages. *J Agric Food Chem* 50: 6550-6556.
- Adebooye OC. 2008. Phyto-constituents and anti-oxidant activity of the pulp of snake tomato (*Trichosanthes cucumerina L.*).
   Afr J Tradit Complement Altern Med 5: 173-179.
- 29. Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48: 909-930.
- 30. Badejo AA, Damilare A, Ojuade TD. 2014. Processing effects on the antioxidant activities of beverage blends developed from *Cyperus esculentus*, *Hibiscus sabdariffa*, and *Moringa oleifera* extracts. *Prev Nutr Food Sci* 19: 227-233.
- Zheng X, Tian S, Meng X, Li B. 2007. Physiological and biochemical responses in peach fruit to oxalic acid treatment during storage at room temperature. *Food Chem* 104: 156-162.
- 32. Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol* 141: 391-396.