Effect of *Xylopia aethiopica* aqueous extract on antioxidant properties of refrigerated Roma tomato variety packaged in low density polyethylene bags

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Abstract Effects of *Xylopia aethiopica* (Dunal) A. Richard aqueous extract on the antioxidants of matured tomato fruits at red stage were investigated at 13 ± 2 °C and 80 ± 5 % relative humidity. A sample treated with sodium bicarbonate and untreated samples were included. Samples packaged in low density polyethylene (30 μ m thickness) bags were analysed at intervals of 5 days. The treatments revealed statistically significant differences in ascorbic acid content of stored tomato fruits. Fruits treated with 5 % *X. aethiopica* on day 5 of storage had 21.0 mg/100 g which was significantly ($p < 0.05$) higher than 18.2 mg/100 g in untreated control samples. At 15th day of storage, ascorbic acid was 10.0 and 14.2 mg/100 g in tomato fruits treated with sodium bicarbonate and 5 % *X. aethiopica* respectively. The carotenoid and lycopene contents were lower in sodium bicarbonate-treated and the untreated control samples than in *X. aethiopica*-treated sample. The total phenolic contents were better retained in *X. aethiopica*-treated tomato than in control. Treatment of tomato fruits with *X. aethiopica* at 4 & 5 % levels significantly retained the qualities evaluated.

Keywords Tomato · *Xylopia aethiopica* · Low density polyethylene · Antioxidant properties

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Introduction

Tomato (*Lycopersicon esculentum* Mill) is one of the most commonly consumed vegetable crops (Sahlin et al. 2004) which belongs to family Solanaceae. It contains a variety of natural antioxidants including lycopene, ascorbic acid and phenolic compounds (Giovannelli et al. 1999). Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress (Dai and Mumper 2010). Some of these compounds possess the ability to trap peroxyl radicals (Kuhad et al. 2008). For instance in lycopene, the singlet quenching ability is twice as high as that of β -carotene and 10 times higher than that of α -tocopherol and butylated hydroxyl toluene (BHT) (Agarwal and Rao 2000). Phenolic compounds also suppress reactive oxygen species formation by inhibiting some enzymes or chelating trace metals involved in free radical production. The aforementioned antioxidants are still under considerable investigation on their ability to treat cancer, cardiovascular diseases, diabetes and osteoporosis (Rao 2007).

Despite the various health benefits, utilization and potentials of tomato, its fruits are highly perishable and thus have an inherently short shelf life. The fruits encounter substantial post harvest qualitative losses (Nasrin et al. 2008). Measures taken so far to reduce post-harvest losses in tomato include controlled and modified atmosphere packaging and chemical control. Prevalence of synthetic chemical has generated a lot of human health problems. Fasoyiro et al. (2001) reported that some chemical residues left on food components are carcinogenic. Application of chemical pesticides like fungicides poses a risk to the health of human beings and animals (Amati et al. 1989); hence it is expedient to proffer an alternative to over-dependence on chemicals in post harvest treatment of vegetables.

Crude extracts, essential oils and powders of *Xylopia aethiopica* had been used by several authors to protect grains against stored products (Babarinde et al. 2008; Babarinde and Adeyemo 2010). Its candidacy for incorporation into edible products is supported by the fact that it is a spice used as condiment in many African dishes. Hence, it is relatively safe and has not been reported to pose human toxicological problems. Therefore this research was designed to evaluate the effect of *X. aethiopica* aqueous extract and modified atmosphere packaging (low density polyethylene bags) on the quality and shelf stability of Roma tomato variety.

Materials and method

Freshly harvested matured Roma tomato variety at red stage was obtained from Ireesadu in Surulere Local Government, Ogbomoso, Nigeria (USDA 1997). Fruit diameters were between 50 and 60 mm. Dried seeds of *X. aethiopica* were procured from Bode Market, Ibadan, Nigeria. Analytical grade sodium bicarbonate was obtained from Department of Food Science and Engineering, Ladoko Akintola University of Technology (LAUTECH), Ogbomoso Nigeria. Low density polyethylene of 30 µm thickness with 250×150 mm dimension was obtained from a private company in Lagos, Nigeria.

Spice extraction and tomato treatment Aqueous extracts (1, 2, 3, 4 and 5 %) w/v of *X. aethiopica* were prepared. The extract was kept in the refrigerator for 5 days followed by centrifugation according to the method of Adegoke et al. (2002). Tomato fruits were sorted to eliminate bruised and damaged ones. Two hundred grams each of the fruits were dipped in the aqueous extract at different concentrations for 10 min, drained at 26±2 °C for 30 min and packaged in low density polyethylene bags. Another batch of 200 g each was dipped into sodium bicarbonate and the untreated samples were packaged as control. Packaged samples were stored at refrigerated condition, 13±2 °C and 80±5 % relative humidity. Samples were packaged in triplicates and were taken for analysis at 5 days interval.

Analyses

Determination of Lycopene Ten grams of tomato fruits were homogenized and added to 50 ml mixture of hexane: methanol: acetone (2:1:1), containing 2.5 % butylated hydroxy toluene (BHT) according to the method of Perkins-Veazie et al. (2001). It was centrifuged at 14,000 rpm for 20 min at 4 °C. The extraction continued until the residue became colourless and was purified using 1 M Sodium chloride and 10 % aqueous potassium carbonate. Sodium sulphate was

used to remove the moisture content. Optical density of the hexane extract was measured spectrophotometrically (Hitachi Model 550, Japan) at 503 nm against hexane blank. Concentration of lycopene was calculated as described by Opiyo and Ying (2005). All analyses were done in triplicates.

Ascorbic acid determination Ascorbic acid content in tomato fruits was estimated by macerating the sample with 20 % metaphosphoric acid as described by Kirk and Sawyer (1991). 0.05 g of 2, 6- dichlorophenol indophenols was dissolved in water and diluted to 100 ml. Standard ascorbic acid was prepared by dissolving 0.05 g pure ascorbic acid in 60 ml of 20 % metaphosphoric acid and was diluted with water to exactly 250 ml in a volumetric flask. 10 ml of standard ascorbic acid solution was pipetted in a flask and titrated with indophenols until a faint pink colour persist for 15 s. The concentration was expressed as mg ascorbic acid equivalent to 1 ml dye solution. 50 ml of juice was extracted from macerated fruits and 25 ml of 20 % metaphosphoric acid was added. 10 ml of the mixture was withdrawn into a small flask and 2.5 ml of acetone was added. This was titrated with indophenols solution until a faint pink colour persists for 15 s.

$$\text{Vitamin C in mg/100 g} = (\text{Titre value} \times 0.212 \times 100) / \text{Weight of sample}$$

Total phenol determination In the extraction of phenol for all tomato samples, methanol and 1 % HCl in the ratio 8:2 were used as solvent system. Standard was prepared by dissolving 10 mg of Gallic acid in 100 ml of solvent. Tomato fruits were finely crushed in laboratory mortar and pestle using the above mentioned solvent. It was centrifuged in a Uniscope laboratory centrifuge (model SM902B, England) at 14,000 rpm for 20 min. Using Folin-Ciocalteu reagent, total phenol was estimated at 725 nm. Total phenols were determined as gallic acid equivalents (mg gallic acid/ g in fruits), and the values were presented as means of three replicates (Dai and Mumper 2010).

Total carotenoids Total carotenoids were analyzed according to Moretti et al. (2002). Fifteen grams of fresh tomato tissue were homogenized with 30 ml of acetone for 1 min at a speed setting of 5 so as to extract pigment. The acetone pigment extract and 45 ml of hexane were mixed in a separatory funnel and after phase separation, the lower phase was discarded and the pigment-hexane extract was washed three times with 100 ml of deionized water. After the final wash, the extract was transferred to a 100 ml volumetric flask and the volume was brought up with hexane. Absorbance was read in a spectrophotometer (Hitachi Model 550, Japan) at 451 nm and 503 nm. Total carotenoids were expressed as mg per 100 g of fresh tissue. The analysis was carried out in triplicate.

Table 1 Influence of *Xylopi aethiopica* on lycopene contents (mg/100 g) of tomato fruit packaged in LDPE during storage at 13 °C

Treatment*	Storage time (days)						
	0	5	10	15	20	25	30
1 %	2.5±0.20a	2.2±0.06b	2.2±0.06b	2.1±0.00b	1.5±0.06bc	1.4±0.06b	1.2±0.06b
2 %	2.5±0.20a	2.2±0.10b	2.2±0.00b	2.2±0.06bc	1.7±0.12 cd	1.6±0.10bc	1.5±0.06c
3 %	2.5±0.20a	2.2±0.00b	2.2±0.00b	2.2±0.00bc	1.8±0.06 cd	1.6±0.06bc	1.5±0.06c
4 %	2.5±0.20a	2.3±0.12b	2.3±0.06c	2.2±0.10bc	1.9±0.10d	1.7±0.06c	1.6±0.06 cd
5 %	2.5±0.20a	2.2±0.12c	2.3±0.00c	2.2±0.06c	2.0±0.00d	1.9±0.06c	1.8±0.06d
NaHCO ₃	2.5±0.20a	1.5±0.00a	1.5±0.00a	1.5±0.00a	1.2±0.29a	1.2±0.29a	1.2±0.29ab
Control	2.5±0.20a	1.5±0.00a	1.5±0.00a	1.5±0.06a	1.3±0.29ab	1.2±0.29a	1.0±0.00a

Means (±SD) with the same letters within a column are not significantly different at 5 % probability level using Duncan multiple range test ($n=3$)

LDPE Low density polyethylene

*Percentage concentration (w/v) of *Xylopi aethiopica* aqueous extract used for storage of tomato fruit

Statistical analysis The results obtained from laboratory analyses were expressed as means of three replicates ± standard deviation and data were subjected to one way analysis of variance. Where there was significant treatment effect, comparisons of the treatment means were done using Duncan's multiple range test at 5 % probability level using SPSS Software Version 15 (SPSS 2006).

Results and discussion

Effect of treatments on lycopene The lycopene content of the fresh sample prior to storage was 2.5 mg/100 g (Table 1). Lycopene progressively decreased during storage and values decreased from 2.5 mg/100 g on the first day of storage to 1.0 mg/100 g on day 30 in untreated samples. The values recorded in treated samples ranged from 1.2 to 1.8 mg/100 g. The lycopene contents of *X. aethiopica*-treated samples were

significantly ($p \leq 0.05$) higher than values obtained from sodium bicarbonate-treated and untreated tomato samples. Factors that affect isomerization and auto oxidation of lycopene include extreme pH, lipid degrading enzyme, heat, light and oxygen. After oxidation, lycopene molecule split which causes loss of colour and off-flavour. Lycopene contains no oxygen and is usually red or orange in colour. Because it is highly saturated, it is particularly susceptible to oxidation (Xianquan et al. 2005). The oxidation of lycopene could be responsible for the lower values recorded in untreated samples. This shows that the degradation of lycopene of tomato at the red stage was slowed down when the fruits were treated with *X. aethiopica* aqueous extract. Aneesh et al. (2007) reported an initial increase in lycopene of tomato which later decreased at the end of storage when treated with ionising radiation and modified atmosphere packaging at a low temperature. In tomato treated with hot water and packaged in low density polyethylene, Akbudak and Akbudak (2007) recorded a decrease in the lycopene contents. Toor and Savage (2006)

Table 2 Influence of *Xylopi aethiopica* on ascorbic acid (mg/100 g) of tomato fruit packaged in LDPE during storage at 13 °C

Treatment*	Storage time (days)						
	0	5	10	15	20	25	30
1 %	22.0±0.00a	19.3±0.29b	17.9±0.12b	14.3±0.10b	13.8±0.06c	11.6±0.10b	10.9±0.10c
2 %	22.0±0.00a	19.3±0.29b	18.4±0.06c	14.4±0.06c	14.4±0.10d	12.4±0.15c	11.3±0.12d
3 %	22.0±0.00a	20.2±0.29c	18.8±0.00d	15.7±0.06d	14.8±0.06e	13.0±0.00d	12.6±0.15e
4 %	22.0±0.00a	20.2±0.29c	19.4±0.21e	16.3±0.23e	15.3±0.12f	13.8±0.10e	10.2±0.12b
5 %	22.0±0.00a	21.0±0.00d	19.8±0.06f	16.7±0.10f	15.7±0.06 g	14.2±0.06f	14.2±0.06f
Na HCO ₃	22.0±0.00a	18.2±0.29a	17.3±0.15a	12.1±0.15a	11.4±0.1b	10.2±0.12a	10.0±0.15a
Control	22.0±0.00a	18.2±0.29a	17.3±0.06a	12.1±0.1a	11.2±0.17a	10.4±0.10a	10.1±0.06a

Means (±SD) with the same letters within a column are not significantly different at 5 % probability level using Duncan multiple range test ($n=3$)

LDPE Low density polyethylene

*Percentage concentration (w/v) of *Xylopi aethiopica* aqueous extract used for storage of tomato fruit

Table 3 Influence of *Xylopi aethiopica* on total phenolic contents (mg GAE/g) of tomato fruit packaged in LDPE during storage at 13 °C

Treatment*	Storage time (days)						
	0	5	10	15	20	25	30
1 %	1.5±0.00a	1.5±0.00bc	1.5±0.00b	1.5±0.10b	1.2±0.29ab	1.2±0.29ab	1.2±0.29ab
2 %	1.5±0.00a	1.8±0.29c	1.3±0.29b	1.5±0.10b	1.3±0.29ab	1.3±0.29ab	1.3±0.29ab
3 %	1.5±0.00a	1.5±0.00bc	1.5±0.00b	1.5±0.10b	1.3±0.29ab	1.3±0.29ab	1.3±0.29ab
4 %	1.5±0.00a	1.7±0.29c	1.5±0.00b	1.5±0.10b	1.3±0.29ab	1.3±0.29ab	1.3±0.29ab
5 %	1.5±0.00a	1.5±0.00b	1.5±0.00b	1.5±0.10b	1.5±0.00b	1.5±0.00b	1.5±0.00b
NaHCO ₃	1.5±0.00a	1.2±0.29ab	1.1±0.00a	1.00±0.00a	1.0±0.00a	1.0±0.00a	1.0±0.00a
Control	1.5±0.00a	1.1±0.29a	1.0±0.00a	1.00±0.00a	1.0±0.00a	1.0±0.00a	1.1±0.00a

Means (±SD) with the same letters within a column are not significantly different at 5 % probability level using Duncan multiple range test ($n=3$)

LDPE Low density polyethylene

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observed insignificant reductions after storage of tomato fruits for 10 days compared with initial values. In the same study, the mean lycopene contents of tomatoes stored at 15 and 25 °C was 1.8 fold higher than that of refrigerated tomato; this implies that refrigeration also hinders the synthesis of lycopene. Batu and Thompson (1998) reported that lycopene constitutes the main red pigments of tomatoes and their concentration increase steadily through ripening. Formation of lycopene depended on the presence of oxygen. Its formation however can be inhibited by low O₂ atmosphere storage; the lower the O₂ concentration the higher the rate of inhibition. Batu and Thompson (1998) reported that at 1 % O₂ and 99 % N₂ storage, lycopene formation was completely inhibited. This could also be responsible for the decline in lycopene content of tomato during storage since they were packaged in LDPE bags. The higher losses of lycopene recorded in the untreated and sodium bicarbonate treated samples can affect the overall acceptability and general marketability of tomato after storage.

Ascorbic acid There was a gradual decline in ascorbic acid contents of tomato throughout the storage period. Values obtained for varying concentration of spices ranged from 19.3 to 21.0 mg/100 g on day 5 while 18.2 mg/100 g (Table 2) was recorded in the sodium bicarbonate-treated and untreated control samples. A sharp reduction in ascorbic acid was recorded on day 15 for all samples; untreated fruits and sample treated with sodium bicarbonate had the least values which were significant at 5 % probability level. Ascorbic acid was best retained in samples treated with 3–5 % *X. aethiopica* and 5 % *X. aethiopica* samples had the highest value (14.2 mg/100 g) at the end of the storage period. Better retention of ascorbic acid in spice-treated samples confirmed the report of Adegoke and Gopala-Krishna (1998) who reported that spices have antioxidant properties. Nasrin et al. (2008) reported an initial value of 12.3 mg/100 g of ascorbic acid in tomato treated with chlorine and packed in varying packaging materials stored in refrigerator which is lower than the value obtained in this study. A significant loss was reported by the authors at the end of the

Table 4 Influence of *Xylopi aethiopica* on total carotene contents (mg/100 g) of tomato fruit packaged in LDPE during storage at 13 °C

Treatment*	Storage time (days)						
	0	5	10	15	20	25	30
1 %	42.7±2.87a	41.3±0.58b	41.0±0.00b	40.7±2.89ab	40.5±0.00ab	40.3±2.89ab	40.3±2.89ab
2 %	42.7±2.87a	41.7±2.52d	41.5±0.00c	40.7±2.89ab	40.7±2.89b	40.5±0.00ab	40.5±0.00ab
3 %	42.7±2.87a	41.7±1.16d	41.3±2.89bc	40.5±0.00a	40.5±0.00ab	40.5±0.00ab	40.5±0.00ab
4 %	42.7±2.87a	41.6±0.00c	41.3±2.89bc	40.8±2.89b	40.7±2.89b	40.7±2.89b	40.7±2.89b
5 %	42.7±2.87a	42.0±0.00e	41.5±0.00c	41.5±0.00ab	40.7±2.89b	40.5±0.00ab	40.0±0.00ab
NaHCO ₃	42.7±2.87a	41.0±0.00a	40.5±0.00a	40.5±0.00a	40.7±2.89a	40.2±2.89a	40.7±2.89
Control	42.7±2.87a	41.0±0.00a	40.7±2.89a	40.5±0.00a	40.3±2.89ab	40.3±2.89a	40.3±2.89a

Means (±SD) with the same letters within a column are not significantly different at 5 % probability level using Duncan multiple range test ($n=3$)

LDPE Low density polyethylene

*Percentage concentration (w/v) of *Xylopi aethiopica* aqueous extract used for storage of tomato fruit

storage period. The values reported at the end of storage ranged from 4.1 to 5.3 mg/100 g in both untreated control and samples treated with hypochlorite.

The value recorded for ascorbic acid content of tomato by Akbudak and Akbudak (2007) was 28.1 mg/100 g. This value was higher than the one reported in this study. The reason for variation could be due to varietal differences and stage of maturity (Leonardi et al. 2000). Reductions were also observed in ascorbic acid values during storage of tomato treated with hot water and packaged in 100 µm polyethylene. The lowest value of 20.5 mg/100 g were recorded at the end of the storage in hot water treated-tomato, packaged in 100 µm polyethylene, while the highest value of 24.7 mg/100 g was recorded in treated tomato packaged in 50 µm polyethylene (Akbudak and Akbudak 2007). It was further reported that CO₂ treatment retarded the loss in ascorbic acid content. In this study however, better retention of ascorbic acid in treated samples was due to the anti oxidative property of *X. aethiopica* which slowly prevented degradation of vitamin C.

Total phenolic contents

Table 3 shows the result of phenolic contents of tomato during storage. The phenolic content increased in samples treated with 2 and 5 % *X. aethiopica* on day 5 which later decreased as storage time progressed. On the thirtieth day, a constant value was recorded in samples treated with 2–4 % *X. aethiopica* while lowest value of total phenol was recorded in sodium bicarbonate-treated and untreated samples. Phenolic compounds are one of the main antioxidants in tomato (Giovanelli et al. 1999). Phenolic compounds are also water-soluble and oxygen labile, but changes during processing, storage and cooking appear to be highly variable by commodity (Rickman et al. 2007). The presence of phenolics in the fruit cells is essential because it has been reported to help maintain the ascorbic acid content (Pila et al. 2010). In this work, slight increase in total phenolic content was observed till day 5 in treated samples and the values later decreased. Phenolic compounds are one of the main antioxidants in tomato and are water-soluble and oxygen labile (Giovanelli et al. 1999). Vinha et al. (2013) reported initial increase in phenolic content which later decreased after day 9 in the stored tomato fruits. He explained the decrease to be due to damage caused by reduced temperature (6 °C) particularly in the vacuoles of cells where phenolic compounds are accumulated. Excess rate of fruit maturation when stored at higher temperature also affect phenolic content. In this study however, the initial increase could be due to the presence of phenolic compounds in *X. aethiopica* (Abdou et al. 2010) and significant higher values were obtained from sample treated with 2 and 4 % *X. aethiopica*.

Total carotenoids The carotene content of the fresh tomato was 42.7 mg/100 g. The highest carotene values were

obtained from samples treated with 4 % *X. aethiopica* and the sample treated with sodium bicarbonate at the end of storage which was significantly higher than the value obtained in untreated sample (Table 4). In the experiment conducted by Akbudak and Akbudak (2007) on postharvest storage of tomato fruits, 45 % reductions were obtained at the end of 28 days of storage. Moretti et al. (2002) treated tomato with 1-methycyclopropene (MCP) and observed that as 1-MCP dose increased, pigment synthesis and expression was further delayed. At the end of the storage period, control fruits contained around 190 % more total carotenoids than fruits treated with 1,000 mL.L⁻¹ of 1-MCP. Uneven de-greening in individual fruits treated with 1-MCP was however observed at the early stages of ripening. In some cases, colour development started first at the stem end and then gradually shifted to the blossom end. Jiang et al. (1999) observed that bananas treated with 1-MCP showed uneven skin de-greening. They attributed this to positional differences in the rate of new synthesis of ethylene binding sites. Gradual reduction of total carotene in *X. aethiopica* treated samples will prevent uneven de-greening or ripening and the problem of positional differences in the rate of new synthesis of ethylene binding sites when tomatoes are removed from packs.

Conclusion

This work evaluates the effect of varying concentration of aqueous extract of *X. aethiopica* on antioxidant properties of tomato fruits. Samples treated with 5 % *X. aethiopica* had better retention of ascorbic acid and phenolic contents, compared with untreated control. Gradual reduction in lycopene and carotenoid contents during storage will also improve the overall acceptability and general marketability by preventing uneven ripening that could occur when tomato fruits are removed from packs. The potential of *X. aethiopica* to retain antioxidant properties of tomato promises to reduce the problems of synthetic preservative in post harvest handling.

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