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Carotenoids, Glutathione and Vitamin E contents of Eggplants (*Solanum spp.)* during ripening

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ABSTRACT

Eggplants (*Solanum spp.*) are important fruit vegetables that are mostly eaten in the unripe stage. Analyses of lycopene, glutathione and vitamin E contents of Solanum *melongena* (round and oval varieties) and *Solanum aethiopicum*, showed that these antioxidants increased from the unripe to the overripe stage during ripening. The overripe stage of *Solanum melongena* (round variety) was found to contain the highest level of lycopene (0.717 ± 0.016 µg/g fresh weight), while highest levels of glutathione and vitamin E (105.00 ± 02 nmole/g fresh weight and 12.867 ±2.433 µg/g fresh weight respectively) occurred in *Solanum melongena* (oval variety). The overripe stages of these fruits are good sources of these important natural antioxidants that have nutritional and health benefits.

Key words: Antioxidants, Eggplants, *Solanum spp.*, Ripening, Lycopene, Glutathione, Vitamin E

INTRODUCTION

Eggplants (Solanum spp.) belong to the family of Solanaceae and the plant genus Solanum. They exhibit different sizes, shapes and colours. The colours of most eggplants become yellow and red, when they are ripe and overripe, respectively. Eggplants are mostly cultivated in the tropical and subtropical zones, where they are used both as food and as medicine (Magioli and Mansur, 2005; Chinedu et al., 2011; Eun-Ju et al., 2011). Eggplants are eaten raw and also, when boiled or fried as ingredient of stews, soups and vegetable sauces. The unripe fruit of eggplant is primarily used as a vegetable in cooking in various dishes all over the world (Nisha et al., 2009).

Carotenoids are pigmented compounds that are synthesized by plants. They are responsible for the yellow, orange and red colours of fruits and vegetables. Fruits and vegetables constitute the major sources of carotenoids in human diet. Caroteniods such as lycopene and β – carotene, also known as carotenes, contribute to the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular disease, cancer and other chronic diseases (Rao and Rao, 2007). Lycopene is a lipid soluble, potent antioxidant responsible for the vibrant red colour of many fruits and vegetables such as tomatoes, watermelon, guava, papaya and pink grapefruits (Rao and Rao, 2007; Hamid et al., 2010). Lycopene serves as an intermediate for the biosynthesis of other caroteniods like β – carotene. Unlike β - carotene, lycopene lacks the terminal β-ionone rings in its structure; it also lacks provitamin A activity and therefore does not act as a precursor of vitamin A (Rao and Rao, 2007).

Lycopene as an antioxidant acts as a free radical scavenger; counteracting the damaging effects of oxidative stress (Wayne et al., 2002). It also reduces the levels of oxidized LDL and total cholesterol, thereby lowering the risk of cardiovascular diseases (Rao and Rao, 2007). β - Carotene is a lipophilic careteniods that has provitamin A activity due to the presence of terminal β-ionone rings in its structure (Bast and Haenen, 2002). It is the most potent dietary precursor of vitamin A (Bramley, 2002). β - Carotene functions as an efficient singlet oxygen quencher, a radical trapping antioxidant, which reduces the extent of nuclear damage and inhibits lipid peroxidation (Gupta and Sharma, 2006).

Glutathione is a cysteine containing tripeptide that is found in most forms of aerobic life (Hamid et al., 2010). It is an important low molecular weight non-protein thiol, which plays a crucial role in intracellular defence against reactive oxygen species (Pallavi et al., 2012). Glutathione is one of the most important cellular antioxidant, since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced (Hamid et al., 2010). Glutathione is part of the glutathione - ascorbate cycle that helps to prevent or minimize damage caused by reactive oxygen species (Mendoza et al., 2008). It is a highly reducing chemical barrier that prevents excessive oxidation of sensitive cellular components. Glutathione is maintained in predominantly reduced state (GSH) by the enzyme glutathione reductase, which has high affinity for GSSG (oxidized glutathione) and most importantly NADPH (Noctor et al., 2011). Glutathione as an antioxidant plays an important protective role against oxidative stress by scavenging hydroxyl radical and singlet oxygen directly, detoxifing hydrogen peroxide and lipid hydroperoxides (intermediates of lipid peroxidation) by catalytic action of glutathione peroxidase. It also regenerates important antioxidants such as vitamins E and C back to their active forms and is a cofactor of several detoxifying enzymes that act against oxidative stress (Masella et al., 2005; Pallavi et al., 2012).

Vitamin E, which is also known as tocopherol is a fat-soluble vitamin with antioxidant property (Herrera and Barbas, 2001). It is the most important lipid-soluble antioxidant. Tocopherol is a strong lipophilic antioxidant with a protective function in the preservation of cell membrane during oxidative stress (Falk and Munne-Bosch, 2010). It protects membranes from oxidation by reacting with lipid radicals that are produced in the lipid peroxidation chain reaction. This reaction with lipid radicals produces oxidized α-tocopheroxyl radicals that are recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate and glutathione. Vitamin E is efficient in inhibiting the development of conditions such as heart disease, cancer, cataract, neuropathies and myopathies, due to its activity as a major free radical chain breaking antioxidant (Hamid et al., 2010).

Several fruits such as mango have been reported to accumulate high levels of tocopherol and carotenoid during ripening (Burns et al., 2003; Ajila et al., 2007; Rajesh et al., 2011). Since lycopene, β-carotene, glutathione and vitamin E are very beneficial in countering the harmful effect of free radicals or reactive oxygen species (ROS) (Pallavi et al., 2012); this study was carried out to determine the levels of these antioxidants in eggplants (Solanum spp.) during ripening.

MATERIALS AND METHODS

Plant material

Unripe fruits of Solanum melongena L. (round and oval varieties) and Solanum aethiopicum L. (Fig. 1) were purchased from a local market in Benin City, Nigeria. These eggplants were allowed to ripen normally at room temperature (30 ± 2 oC). Samples were obtained from the eggplants at various ripening stages for carotenoids, glutathione and vitamin E analyses. Unripe fruits of Solanum melongena L. (round and oval varieties) are white in colour, while those of Solanum aethiopicum L. are also white but with green stripes. These fruits all turned yellow when ripe and red when overripe.

Estimation of carotenoids

Carotenoids were extracted from the Solanum spp. with a mixture of acetone and hexane (4:6 v/v) by the method of Bortolotti et al., 2003 with slight modifications. Ten grammes of each Solanum spp. was ground and extracted with a mixture of 20 ml of acetone and 30 ml of hexane (4/6 v/v). The mixture was poured into a separatory funnel and shaken until the content became homogenous. The mixture was allowed to separate and also protected from light; after which the top layer was taken with a pipette and the optical density was measured at 453 nm for β-carotene and 503nm for lycopene. Lycopene has a maximum absorbance at 503 nm, while β-carotene has only negligible absorbance (Zakaria et al., 1979).

Extraction of glutathione

Glutathione (GSH) was extracted by the method of Moron et al., (1979). One gramme of each Solanum spp. was homogenized with 5 ml of 5% trichloroacetic acid in a mortar containing acid-washed sand. The homogenate was immediately acidified by adding 250 µl of 25% trichloroacetic acid to prevent aerial oxidation of glutathione. The homogenate was filtered through a double layer of cheesecloth and was centrifuged at 1000 g for 10 min. The supernatant obtained was cooled on ice and used for the estimation of glutathione.

Estimation of glutathione

Glutathione (GSH) was measured by its reaction with DTNB (5, 5′ dithiobis-2-nitrobenzoic acid) (Ellman's reaction) to give a yellow coloured product that absorbs at 412 nm (Moron et al., 1979). An aliquot of 0.1ml of the supernatant that was obtained from the extraction of glutathione was used for the estimation. The supernatant was made up to 1 ml with 0.2 M sodium phosphate buffer (pH 8.0). 2 ml of freshly prepared 0.6 mM 5, 5′ - dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate buffer (pH 8.0) were added and the intensity of the yellow colour formed was read at 412nm after 10 min of incubation; against a blank containing 0.1 ml of distilled water, 0.9 ml of 0.2M sodium phosphate buffer (pH 8.0) and 2 ml of DTNB. The amount of glutathione was extrapolated from a glutathione standard curve and expressed as nmoles glutathione/g fresh weight.

Extraction of Vitamin E

Vitamin E (tocopherol) was extracted and estimated spectrophotometrically by the method of Rosenberg, (1992). 2.5 g of each Solanum spp. was homogenized in a mortar containing acid-washed sand, with 0.14 ml of 0.05 M sulphuric acid, which was finally made up to 50 ml by adding 0.05 M sulphuric acid slowly and the homogenate was allowed to stand overnight. The homogenate was stirred vigorously on the next day and filtered through Whatman No. 1 filter paper. Aliquot of the filtrate was used for the estimation of vitamin E.

Estimation of vitamin E

The estimation of vitamin E was done by the Emmerie-Engel reaction; based on the reduction of ferric to ferrous ions by tocopherols, which with 2, 2 - dipyridyl forms a red colour (Rosenberg 1992). Aliquot of 3ml of the filtrate that was obtained from the extraction of vitamin E was used for the estimation. 3ml each of absolute ethanol and xylene were added to the filtrate, which was thoroughly mixed for 2 min and centrifuged at 1000 g for 10 min. After centrifugation, 2.0 ml of 120 mg/100 ml of 2, 2 - dypyridyl in propanol were added to 2.0 ml of the supernant, which is the xylene layer. Absorbance was read at 480 nm after which 0.66ml of 120 mg/100 ml of ferric chloride in ethanol was added to the reaction mixture and the absorbance was measured again after 30sec at 520 nm. 1 mg/100 ml of vitamin E and distilled water were used as standard and blank respectively.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) of data was evaluated by the statistical and presentational system software (SPSS). Tukey- Kramer multiple comparison test was employed to determine the statistical differences among the means.

RESULTS

The overripe stage of all the Solanum spp. had the highest level of lycopene. Lycopene in the overripe stage of Solanum melongena (round variety) was 1.2 fold higher than that of Solanum melongena (oval variety) and 2.1 fold higher than that of Solanum aethiopicum. The content of lycopene increased significantly ($p< 0.01$) from the unripe to the overripe stage during ripening in all the Solanum spp. However in the unripe stage of Solanum melongena (round variety) and Solanum aethiopicum, lycopene was not detected (Fig. 2). Also, β – carotene was not detected in any of the ripening stages of all the Solanum spp. that were used in this study.

Glutathione level decreased non- significantly ($p>0.01$) from the unripe to the ripe stage and latter increased significantly ($p<0.01$) from the ripe to the overripe stage; with the overripe stage having the highest level of glutathione in both Solanum melongena (oval variety) and Solanum aethiopicum. In Solanum melongena (round variety) the same pattern was observed except that the increase from the unripe to the ripe stage was not significant ($p>0.01$) and the unripe stage has a slightly higher level of glutathione than the overripe stage (Fig. 3).

Vitamin E level decreased non- significantly ($p>0.01$) from the unripe to the ripe stage and latter increased non - significantly ($p>0.01$) from the ripe to the overripe stage; with the overripe stage having the highest level of vitamin E in both Solanum melongena (oval variety) and Solanum aethiopicum. In Solanum melongena (round variety) the same pattern was observed except that the unripe stage has a slightly higher level of vitamin E than the overripe stage (Fig. 4).

Solanum melongena L. (round variety) *Solanum melongena* L. (oval variety)

Fig. 1: The various eggplants (*Solanum spp.*) that were used in this study.

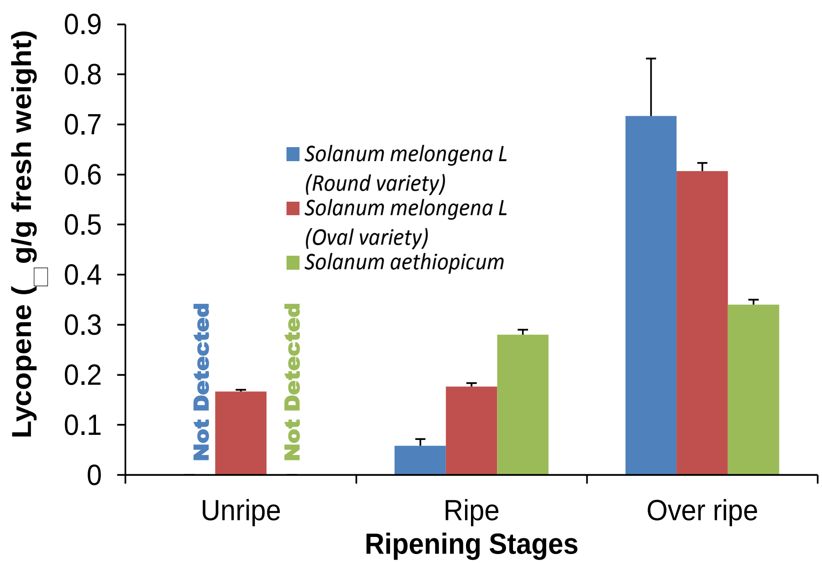

Fig. 2: Lycopene levels of the different eggplants (*Solanum spp*.) at various ripening stage

DISCUSSION

Carotenoids are important in human nutrition and health, due to their provitamin A and antioxidant activities. Carotenoids are also used in cosmetics, pharmaceuticals or as food colorants, (Tanaka et al., 2008).

In this study, lycopene an important carotenoid was found to increase significantly ($p<0.01$) in eggplant (Solanum spp.) as ripening progressed. The highest level of lycopene was found in the overripe stage of the Solanum spp (Fig 2). The concentration of carotenoids in tomato has also been reported to increase during ripening due to the accumulation of lycopene (Bramley, 2002). Bramley, (2002) also reported that tomato is usual in its ability to accumulate high amount of lycopene during ripening since only very few plants have this ability. Consequently, eggplants (*Solanum spp*.) are among the few plants that accumulate lycopene like tomato during ripening;

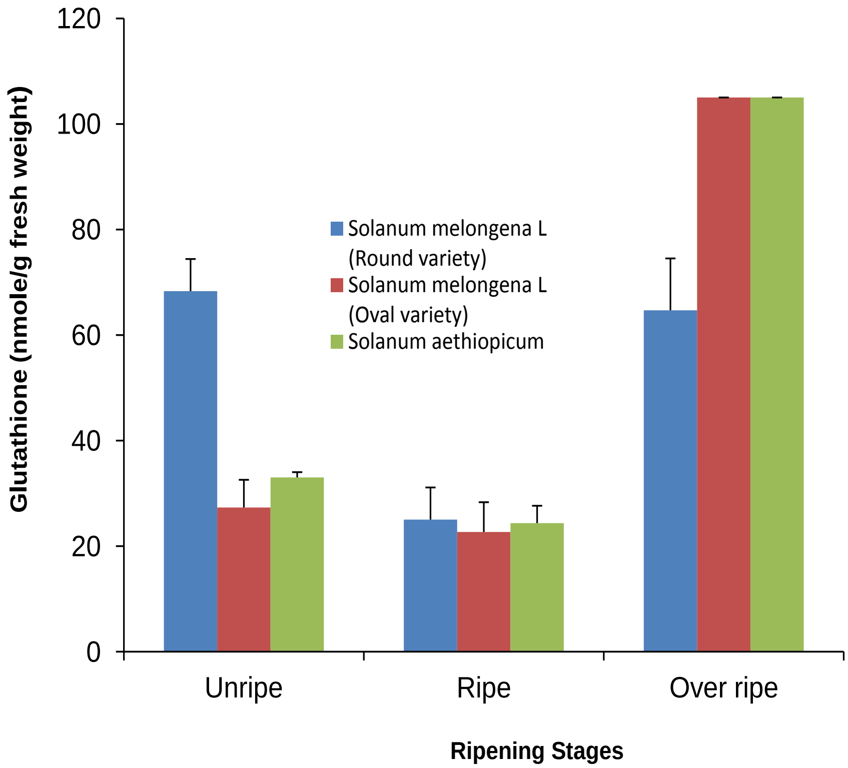

Fig. 3: Glutathione levels of the different eggplants (*Solanum spp*.) at various ripening stages

moreover eggplants and tomato belong to the same family and genus, solanaceae and solanum, respectively. Lycopene has been shown to alleviate age-related diseases when taken in sufficient quantities in the diet, presumably because of its powerful property as a lipophilic antioxidant. Recently, evidence has been presented to show that tomato sauce reduces the amount of DNA damage in white blood cells and prostate tissues of prostate cancer victims (Chen *et al*., 2001). Since tomato fruit is considered the sole dietary source of lycopene, its formation in the tomato has been the subject of great attention and attempts have been made to increase the levels of lycopene by genetic manipulation (Fraser *et al*., 2002) or conventional plant breeding. This study therefore suggests that the addition of overripe eggplants (*Solanum spp*.) to the diet will also help to increase the quantity of lycopene consumed, thereby preventing cancer. β - Carotene was however not detected in the *Solanum spp.*that were used in this study; probably the level was below detectable quantity. Kozukue and Friedman (2003) have also reported that tomato contains high level of lycopene and low level of β – carotene.

Glutathione is one of the major contributors to the antioxidant status in cells, due to its protective role in scavenging free radicals, which would otherwise oxidize proteins, lipids, and nucleic acids; resulting in oxidative damage (Moskaug et al., 2005). In this study, a significant increase ($p < 0.01$) was observed in the level of glutathione in Solanum melongena (oval variety) and Solanum aethiopicum from the unripe to the overripe stage.

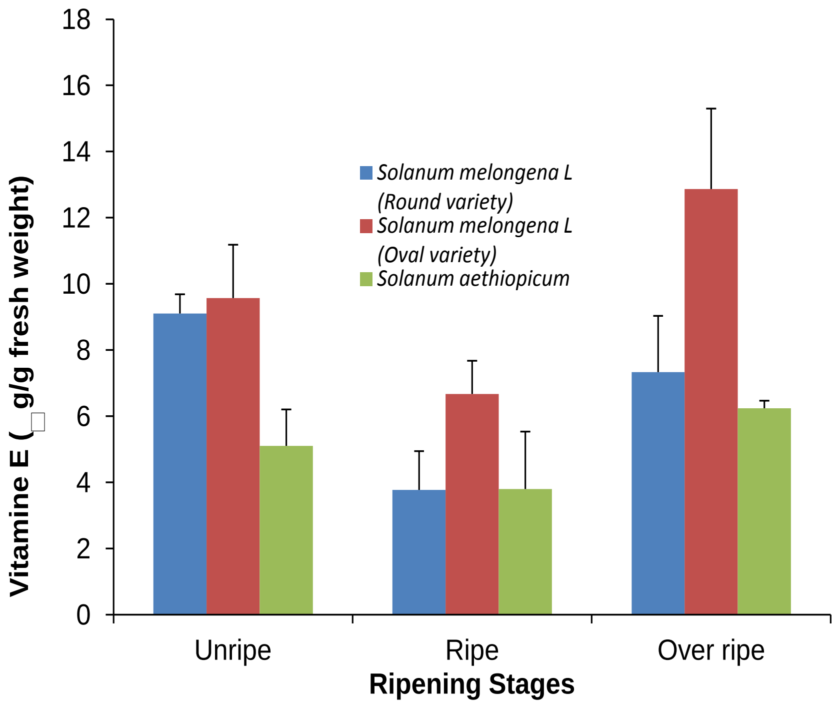

Fig. 4: Vitamin E levels of the different eggplants (*Solanum spp*). at various ripening stages

The glutathione of Solanum melongena (oval variety) and Solanum aethiopicum increased by 3.8 and 3.2 folds respectively (Fig. 3). Jiménez et al. (2002) have also reported an increase in glutathione level in tomato during ripening. Glutathione level of Solanum melongena (round variety) decreased non- significantly ($p < 0.01$) by 1.1 fold (5.4%) (Fig. 3). Decrease in glutathione levels have also been reported in some fruits like apples, oranges, peaches and Japanese plums during ripening (Wang and Jiao, 2001; Huang et al., 2007; Wang et al., 2006; Singh et al., 2012)

Moreover, the level of glutathione in fruits has been reported to be influenced by cultivar type or species (Wang and Jiao, 2001; Davey and Keulemans, 2004). Also, Noctor et al. (2011) reported that in some cases in plants, accelerated synthesis of glutathione (GSH) may be accompanied by unchanged or decreased glutathione levels due to increased consumption during oxidative stress; while sometimes, when there is a very high oxidative stress in plants, increased synthesis of glutathione results in the accumulation of glutathione, which is a response that balances the change in redox potential caused by increased oxidized glutathione (GSSG). Glutathione regenerates vitamin E back to its active form and was found to show almost similar pattern with vitamin E in this study (Figs. 3 & 4). There was a non- significant increase ($p < 0.01$) in the vitamin E level from the unripe to the overripe stage in Solanum melongena (oval variety) and Solanum aethiopicum by 34.5% and 22.25% respectively , while that of Solanum melongena (round variety) decreased non- significantly ($p < 0.01$) by 19.4%. (Fig. 4). Increase or decrease in the level of vitamin E (tocopherol) has been reported in various fruits during ripening. Rajesh et al., (2011) reported an increase in vitamin E level of mango during ripening; similar to what was observed in this study in Solanum melongena (oval variety) and Solanum aethiopicum. On the other hand, Luo et al., (2011) reported 94% decrease in vitamin E content in strawberry during ripening, in this study only 19.4% decrease was observed in Solanum melongena (round variety) . Vitamin E and carotenoids share the same biosynthetic pathway and are derived from terpenoids. Therefore increase in the level of carotenoid as seen in the ripe stage of the Solanum spp. could sometimes cause a decrease in the tocopherol level (Figs 2 & 3). Burns et al., (2003) also reported that since tocopherol is derived from terpenoids, alterations in the carotenoids contents of fruits can have effect on the levels of tocopherol.

CONCLUSION

Overripe eggplants (Solanum spp.) were found in this study, to be important sources of the natural antioxidants; lycopene, glutathione and vitamin E. Therefore, these overripe eggplants are nutritionally beneficial in counteracting the effect of oxidative damage.

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