Comparison of Carotenoids and Vitamin E levels of two varieties of pawpaw (*Carica papaya)* fruits during ripening

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ABSTRACT

Carotenoids and vitamin E levels of two varieties of pawpaw (*Carica papaya*) viz 'Agric pawpaw' (oblong shaped) and 'Local pawpaw' (pear shaped) were determined at various ripening stages. The carotenoids, lycopene and β – carotene were present in both varieties and these antioxidants increased from the unripe to the overripe stage during ripening. The overripe stage of 'Local pawpaw' (pear shaped) was found to contain the highest levels of lycopene (1.937 ± 0.015 µg/g fresh weight) and β – carotene (10.467 ± 0.116 µg/g fresh weight). The ripe stage of the same variety also had the highest level of vitamin E (13.120 ±1.236 µg/g fresh weight). These fruits are not only sources of natural antioxidants they also possess provitamin A activity. The unripe and ripe stages of 'Local pawpaw' (pear shaped) were higher in lycopene and β – carotene than that of 'Agric pawpaw' (oblong shaped); however, the overripe stage of 'Agric pawpaw' (oblong shaped) was higher in vitamin E that in the 'Local pawpaw' (pear shaped).

Key words: Pawpaw, *Carica papaya*, Ripening, Antioxidants, Lycopene, β-Carotene, Vitamin E

INTRODUCTION

Pawpaw (*Carica papaya*), which is also known as papaya, belongs to the family Caricaceae. It is cultivated in most subtropical and tropical countries of the world. Pawpaw is an economically important fruit tree and its fruit is consumed worldwide in the fresh state and also processed into different products (Jaime *et al*., 2007). They are used in making fruit salads, fresh drinks, ice cream, jelly, jam, marmalade, canned fruit balls or cubes in syrup, crystallized fruit, candies, and paste. Unripe, green fruits are usually pickled or used as vegetables. Ripe fresh pawpaw fruits are also commonly eaten for breakfast and as dessert (Geetha and Thirumaran, 2010). The unripe fruit is also a rich source of papain, a proteolytic enzyme that hydrolyses protein and as such it is used in the preparation of food as meat tenderizer and is of industrial importance. Papain is also used in medications for the treatment of hard tissues on the skin (Hewitt *et al*., 2000; Chovatia *et al*., 2010; Da Silva *et al*., 2010; Seenivasan *et al*., 2010). Although the pawpaw fruit is edible, the fruit and other parts of the plant are also being used throughout Africa for their medicinal properties. Practically every part of the plant is of economic value and its use range from nutritional to medicinal (Ahmed *et al*., 2002; Afolabi *et al*., 2011). The fruit has been reported to have antihelminthic activity (Okeniyi *et al*., 2007).

Pawpaw fruit has different shapes; it may be ovoid-oblong, spherical, cylindrical, and pear-shaped or may have grooves (Chen *et al*., 2007). Pawpaw fruit is a climacteric fruit and it exhibits a characteristic rise in ethylene production during ripening; which is accompanied by fruit softening, change in colour, development of a strong aroma and production of reducing sugar from polysaccharides. Genes that are associated with the development of fruit aroma during ripening have been identified in *C. papaya* (Devitt *et al*., 2006).

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 fruit 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 levels thereby lowering the risk of cardiovascular diseases (Rao and Rao, 2007). β - Carotene is a lipophilic caroteniods 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).

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 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 during ripening, in two varieties of pawpaw (*Carica papaya*) fruits that are commonly eaten in Nigeria.

MATERIALS AND METHODS

Plant material

Two varieties of pawpaw (*Carica papaya*) fruits, which are oblong and pear shaped and also known as 'Agric pawpaw' and 'Local pawpaw', respectively (Fig. 1); were purchased in their unripe green state from a local Market in Benin City. These fruits were identified as *Carica papaya* by the Faculty of Agriculture, University of Benin, Benin City. The two varieties of *Carica papaya* were further identified and distinguished based on their shapes and their local names. These fruits were allowed to ripen normally at room temperature (30 ± 2 ^{o}C), while samples were collected from them in the unripe, ripe and overripe stages for the analyses of carotenoids and vitamin E.

Estimation of carotenoids

Carotenoids were extracted from the pawpaw fruits 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 pawpaw fruit 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 Vitamin E

Vitamin E (tocopherol) was extracted and estimated spectrophotometrically by the method of Rosenberg (1992). 2.5 g of each pawpaw fruit 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 supernatant, 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

A one – way analysis of variance (ANOVA) was performed on the ripening effect, using the statistical and presentational system software (SPSS). Tukey- Kramer multiple comparison test was also employed to determine the statistical differences among the means ($p < 0.01$).

RESULTS

Lycopene and β –carotene were present in both varieties of pawpaw fruits. The level of lycopene increased significantly ($p < 0.01$) from the ripe to the overripe stage in the ‘Agric pawpaw’ by 2. 0 fold and in the ‘Local pawpaw’ by 2.6 fold. A little amount of lycopene (0.078 ± 0.001µg/g fresh weight) was detected in the unripe stage of ‘Agric pawpaw’, while lycopene was not detected in the unripe stage of ‘Local pawpaw’ (Fig. 2).

‘Local pawpaw’ (pear shaped) ‘Agric pawpaw’ (oblong shaped)

Fig. 1: The two varieties of pawpaw (*Carica papaya*) fruits that were used in this study.

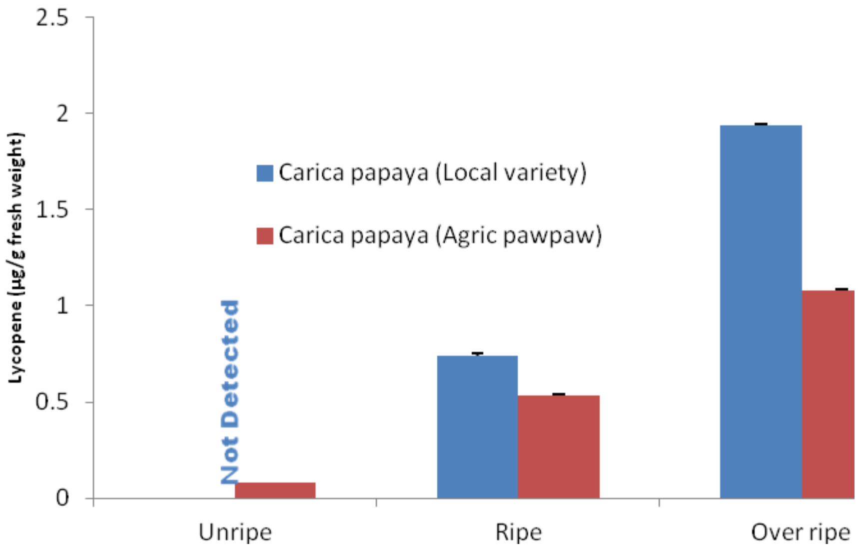

Fig. 2: Lycopene levels of the two varieties of pawpaw (*Carica papaya*) fruits at various ripening stages.

Also, β – carotene increased significantly ($p < 0.01$) from the ripe to the overripe stage in the 'Agric pawpaw' by 2. 4 fold and in the 'Local pawpaw' by 2.5 fold. However, β – carotene was not detected in the unripe stage of both varieties (Fig. 3).

Vitamin E 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 in the 'Agric pawpaw', with the overripe stage having the highest level of vitamin E (7.773 ± 0.227µg/g fresh weight). The vitamin E pattern was reversed in the 'Local pawpaw; the level increased non- significantly ($p>0.01$) from the unripe to the ripe stage and latter decreased significantly ($p<0.01$) from the ripe to the overripe stage; with the ripe stage having the highest level of vitamin E (13.120 ±1.236 µg/g fresh weight). (Fig. 4).

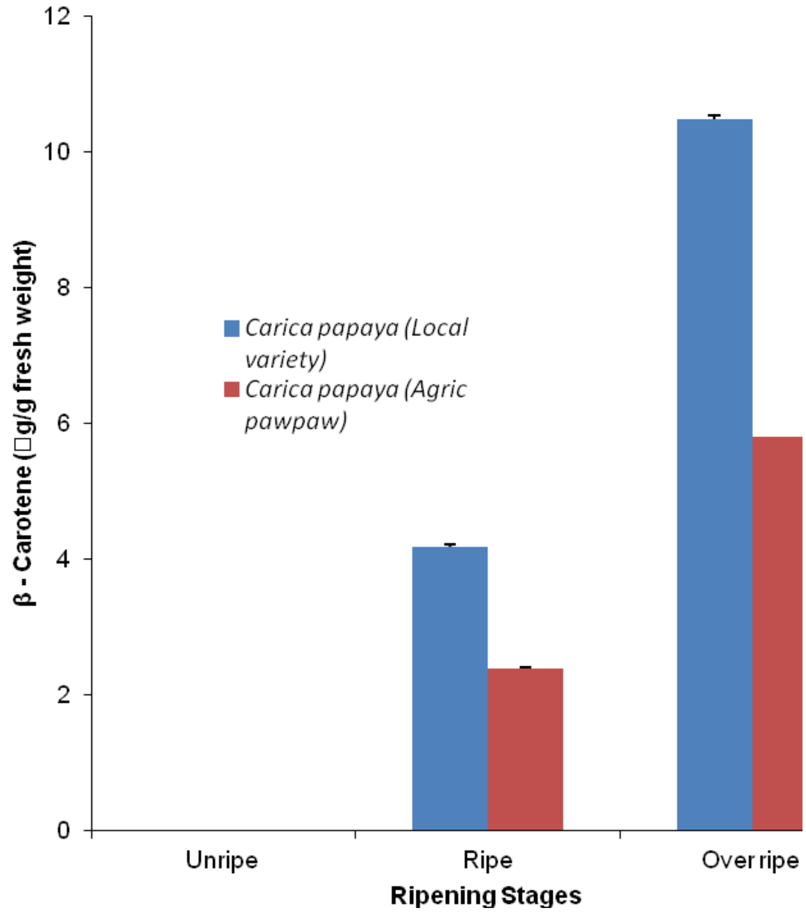

Fig. 3: β - Carotene levels of the two varieties of pawpaw (*Carica papaya*) fruits at various ripening stages.

DISCUSSION

Lycopene is one of the carotenoids that are naturally found in plants. Lycopene plays an important role in human nutrition and health; due to its ability to scavenge singlet oxygen and peroxy radicals, which cause oxidative damage that result in diseases such as cancer (Yeh and Hu, 2000). Analysis of lycopene in two varieties of pawpaw (Carica papaya) fruits viz 'Agric pawpaw' and 'Local pawpaw' showed a significant increase (p<0.01) from the unripe stage to the overripe stage, during ripening. The highest level of lycopene was found in the overripe stage of 'Local pawpaw' and it was 80% higher than that of 'Agric pawpaw (Fig 2). Rivera-Pastrana *et al*. (2010) have also reported an increase in the level of lycopene in 'Maradol' papaya fruit during ripening. Pawpaw (*Carica papaya*) fruits, like

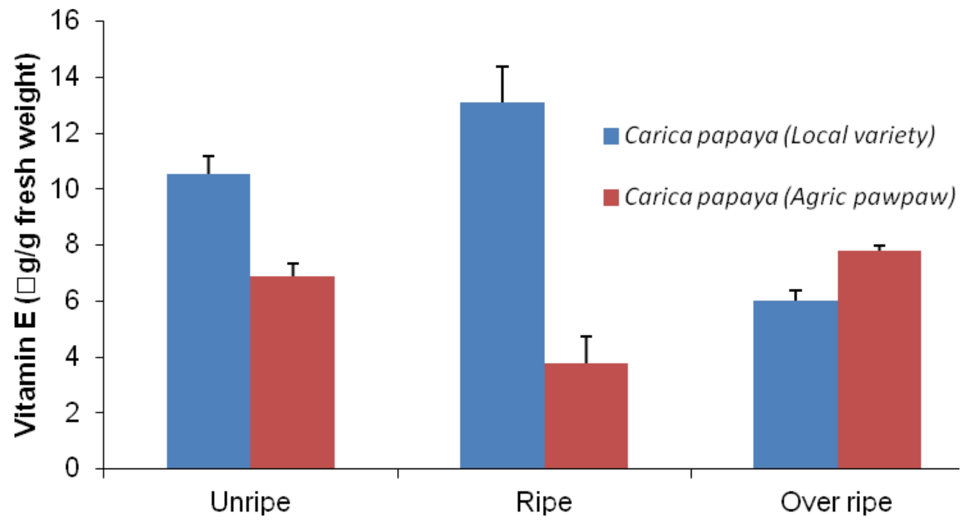

Fig. 4: Vitamin E levels of the two varieties of pawpaw (*Carica papaya*) fruits at various ripening stages.

tomatoes and eggplants are among the few fruits that accumulate the carotenoid, lycopene during ripening.

β – Carotene levels increased significantly (p<0.01) from the unripe to the overripe stage, similar to that of lycopene in the two varieties of Pawpaw (*Carica papaya*) fruits during ripening, and the highest level was found in the overripe stage of the 'Local pawpaw', which was 81% higher than that of the overripe stage of the 'Agric pawpaw' (Fig. 3). The levels of β – carotene in both 'Agric pawpaw' and 'Local pawpaw', were greater than that of lycopene (Figs.2 and 3). In 'Maradol' papaya fruit, β – carotene level was less than lycopene level during ripening; however in 'Formosa' and 'Solo' papaya fruits varieties, lycopene levels were higher than β – carotene levels (Souza *et al*., 2008; Rivera-Pastrana *et al*., 2010). Pawpaw (*Carica papaya*) fruits with high levels of β – carotene during ripennig, have low levels of lycopene, while those with low levels of β – carotene during ripening, have high levels of lycopene. Tomato fruit has high level of lycopene and low level of β – carotene (Bramley, 2002). This inverse relationship between lycopene and β – carotene is due to the fact that they are both synthesized by the carotenoid pathway in which the enzyme β- cyclase converts lycopene to β – carotene. Therefore high level of β – carotene compared to lycopene in a fruit suggests that a substantial amount of the latter has been converted to the former by β- cyclase. In tomato, lycopene accumulation during fruit ripening is due to the decrease in lycopene β-cyclase activity (Devitt *et al*., 2010). The β- carotene contents of 'Agric pawpaw' and 'Local pawpaw' provide good sources of provitamin A activity.

There was a non- significant increase (p> 0.01) in the vitamin E level from the unripe to the ripe stage in 'Local pawpaw' while the level decreased significantly (p< 0.01) from the ripe to the overripe. The pattern was reversed in 'Agric pawpaw' in which there was non- significant decrease (p> 0.01) in the vitamin E level from the unripe to the ripe stage and significant increase (p< 0.01) from the ripe to the overripe (Fig. 4). Although the levels of vitamin E in both unripe and ripe stages of 'Local pawpaw' were quite higher than that of 'Agric pawpaw'; however, that of overripe 'Agric pawpaw' was 29.6% higher than 'Local pawpaw'. 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, while Luo *et al*. (2011) reported 94% decrease in vitamin E content in strawberry during ripening. Vitamin E and carotenoids share the same biosynthetic pathway and are derived from terpenoids. Therefore increase in the level of carotenoid could sometimes cause a decrease in the Tocopherol level (Figs 2 to 4). 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.

Although, the overripe pawpaw fruit could be very soft, but in order to get the full benefit of these antioxidants especially lycopene and β – carotene; the fruit could be employed in the preparation of natural fruit juices.

CONCLUSION

The two varieties of pawpaw (*Carica papaya*) viz 'Agric pawpaw and 'Local pawpaw' were found in this study to be important sources of the natural antioxidants; lycopene, β- carotene and vitamin E, which are nutritionally beneficial in counteracting the effect of oxidative damage. However the levels of lycopene and β-carotene in 'Local pawpaw' were higher than that of 'Agric pawpaw'. The levels of vitamin E in the unripe and ripe stages of 'Local pawpaw' were also higher than that of 'Agric pawpaw' but that of the overripe stage was lower.

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