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Phytochemical and Vitamin Contents of *Mangifera indica* (Mango) Fruits Subjected to Ripening by Artificial Methods

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Abstract *-- Phytochemical and vitamin contents of ripe, unripe as well as unripe mango (Mangifera indica) fruits subjected to different ripening methods (use of dark polybag, calcium carbide and hot water) were investigated. The Phytochemicals namely carotenoids, phenols, terpenes, alkaloids, tannins, saponins, phytosterols, flavonoids and glycosides were quantified. Natural ripening (control) increased carotenoids (6.53±0.02mg/100g to 11.46±0.04mg/100g), phenols (15.13±0.02 mg/100g to 25.27±0.02 mg/100g), terpenes (0.08±0.02 mg/100g to 1.77±0.02 mg/100g) but decreased alkaloids (0.61±0.00 mg/100g to 0.53±0.02 mg/100g), tannins (2.06±0.02 mg/100g to 1.08±0.02 mg/100g) and flavonoids (56.80±0.01 mg/100g to 35.88±0.02 mg/100g) among others. Among the naturally ripe (control), unripe and artificially ripened mango fruits, there were no significant differences ($p<0.05$) in vitamins A, B1, B2, and B3. No significant difference ($p<0.05$) was obtained in the values of B6 for the naturally and artificially ripened mangoes which ranged from 0.20±0.01 mg/100g in calcium carbide group to 0.28±0.06 mg/100g in hot water group, but each was significantly higher than the value for the unripe mango group (0.13±0.02 mg/100g) The levels of vitamin C (mg/100g) was highest in the unripe fruits (51.06±0.05 mg/100g) followed by hot water treated fruits (50.06±0.05 mg/100g) which did not differ significantly ($p<0.05$) from the values for polybag treated fruits (49.54±0.19 mg/100g) but each was significantly higher than the value for the naturally ripe fruits (30.90±0.14 mg/100g). In general, it may be concluded that artificial ripening methods increased the phytochemical constituents and vitamin levels in the fruits investigated.*

Keywords— Phytochemical, vitamin, artificial ripening, mango fruits, unriped.

I. INTRODUCTION

The role of plants in folklore medicine is ascribed to the presence of various phytochemicals like carotenoids, phenols, flavonoids, alkaloids, tannins, saponins, glycosides and phytosterols (Schreiner and Huysken-keil, 2006; Basu *et al*., 2007) which are non-nutritive plant chemicals that have disease averting and curative properties (Duyn & Pivonka, 2000).

Information from emerging data posits that consumption of phytochemical rich foods such as fruits may provide protection against neurodegenerative diseases such as Alzheimer's, and Parkinson's diseases (Davinelli *et al*., 2012; Gao *et al*., 2012; Jones *et al*., 2012), promote cardiovascular health, (Dauchet *et al*., 2006), lower the risk of breast, colon and lung cancer among others (Hung *et al*., 2004) and may improve insulin sensitivity leading to decreased risk of type 2 diabetes (Arts & Holman, 2005).

Vitamins which might be water soluble (C, B) or fat soluble (A, D, E, K) are vital food nutrients, critical for sustaining cellular function. Vitamins are vital for the progression and support of most biological processes fundamental for human survival (Rossato *et al*., 2009) as they promote wellbeing in various ways including the enhancement of cell capacities, mediating an immense number of biological processes, disease prevention, promoting bone structure and strength, reducing inflammation, promoting cardiovascular health and improving endothelial cell function (Naidu, 2003).

Example of fruits include; mango, apple, cashew, orange, grapes, water melon, lemon and pineapple. Among the fruits, mango (*Mangifera indica*) in the *Anacardiaceae* family broadly found in tropical and subtropical districts is known as the lord of fruits (Onyeani *et al*., 2012) for its revivifying and exciting savour (Zewter *et al*., 2012; Farina *et al*., 2013).

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Fruit intake is not as high as it ought to be because of unavailability during off seasons for reasons such as rapid ripening, vulnerability to diseases (Onyeani *et al*., 2012) and rapid post-harvest decay because of ripening and softening which limit the storage, handling and transportation of the fruit (Amarakoon *et al*., 1999). In other to minimize post harvest loss, fruit vendors therefore harvest them prior to ripening and ripening is induced artificially (Goldman *et al*., 1999) by the use of various chemicals such as ethylene gas, ethephon and calcium carbide (Singal *et a.l*, 2012; Sogo –Temi *et al*., 2014; Gbakon *et al*., 2018). Iroka *et al*., (2016) has also reported other methods such as dipping into hotwater and wrapping in dark polyethylene bags. The use of these ripening agents and ripening techniques may successfully reduce or minimize post harvest losses but such activities may lead to the exposure of these fruits to food contamination (Orisakwe *et al*, 2012) thereby exposing consumers to numerous health conditions such as diarrhea,, digestive disorders, dementia, oedema, liver and kidney dysfunction, as well as cardiovascular diseases (Kader, 2007; Kjuus, *et al*., 2007; Pandarinathan and Sivakumar, 2010; Dhembare *et al*., 2013). The peremptory request for food safety (Ruchitha 2008) has inspired this research work which is geared toward exploring possible hazards associated with artificial ripening of fruits. More so, considering the importance of plant constituents to the overall wellbeing of man, this investigation was conducted to ascertain possible changes in the phytochemical and vitamin contents of mango fruit traceable to these artificial ripening methods.

II. MATERIALS AND METHODS

Collection and preparation of samples

Ripe and unripe mango fruits were sourced from Ibeku Community in Aboh Mbaise Local Government in Imo State, Nigeria. The ripe and a set of the unripe mango fruits were left untreated while the remaining unripe mango fruits were cleaned and given the following treatment; a set of ten was left under the sun for 4 hours after which the fruits were tied in a clean empty dark poly bag for 3 days. A second set was soaked in hot water (100^{o}C) for five minutes and was covered with a thin cloth for 2 days while the third set was placed in a plastic bucket containing ground calcium carbide (2g/100g of mango fruit) for twenty-four hours. After ripening was induced in all treated sets, fruits were sliced, air dried, ground and used for the various analysis (Iroka *et al*., 2016).

Phytochemical analysis: Calibration, identification and quantification

Standard solutions were prepared in methyl alcohol for alkaloids, flavonoids and simple phenolics; acetone for carotenoids; dichloromethane for phytosterols and simple terpenes; ethanol for, glycosides and saponins. The linearity of the dependence of response on concentration was confirmed by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for every compound determined, using the standards.

Phytochemical tests were conducted on the dry ground sample using standard methods to quantify carotenoids (Rodriguez-Amara and Kimura, 2004), phenols (Provan *et al*., 1994), terpenoids (Ortan *et al*., 2009), alkaloids (Tram *et al*., 2002), tannins (Luther 1992), saponins (Guo *et al*., 2009), Phytosterols (AOAC International, 2006), flavonoids (Millogo *et al*., 2009) and glycosides (Oluwaniyi and Ibiyemi 2007).

Vitamin analysis

Samples were extracted following the method of Zhao *et al*., (2004)

Procedure

The weights of the samples were determined and samples were ground into powder, using Janice and Kunkel grinder. One gram of the sample was homogenized in I ml of ethanol, and separated by refluxing with 10 ml of re-refined methanol, for 6 hours at very low temperature. To guarantee removal of the pulverized vitamins, this procedure was duplicated using new solvents. Another 1g of the pulverized sample was homogenized in I ml of ethanol and extracted by refluxing with 10 ml of Chlorofoam for an additional six hours still at very low temperature. After which the two extracts were evaporated to dryness with the aid of a rotary evaporator and their residues were then combined. 4.00 ml of 7% BF3 Reagent was included into the blend which was then warmed in the oven for 45 minutes at 100^{o}C after which it was cooled to room temperature before 1.0g of anhydrous Na2SO4 was added so as to guarantee the removal of water. It was then exposed to gas chromatographic investigation using pulse fire photometric detector for Vitamin determination.

Standard solutions were prepared and the linearity of the dependence of response was checked by regression analysis. Identification was based on comparison of retention times and spectral data with standards.

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Quantification was performed by setting up calibration curves for each compound determined, utilizing the standards.

Statistical analysis

The data were analyzed by the analysis of variance (ANOVA). The differences between the groups were compared using the Duncan multiple range test. The results are expressed as mean ± standard deviation. Significance was accepted at $p \leq 0.05$.

III. RESULTS AND DISCUSSION

The concentrations of carotenoids, phenols and terpenes were significantly ($p>0.05$) higher (11.46±0.04mg/100g), (25.27±0.02 mg/100g) and (1.77±0.02 mg/100g) in the naturally ripe (control) but lower in the unripe (6.53±0.02mg/100g), (15.13±0.02 mg/100g) and (0.08±0.02 mg/100g) groups respectively whereas values obtained for alkaloids, tannins, phytosterol, flavonoids and glycosides were higher in the unripe group (0.61±0.00 mg/100g), (2.06±0.02 mg/100g), (85.77±0.23), (56.80±0.01 mg/100g) and (3.84±0.02) but lower (0.49±0.06 mg/100g), (0.93±0.02 mg/100g), (54.30±0.07), (36.09±0.02 mg/100g) and (2.61±0.02) in the hot water groups respectively. The concentration of Saponins ranged from (0.19±0.02mg/100g) in hot water group to (0.47±0.03mg/100g) in polybag group.

Table 2 shows the vitamin contents of mango (*mangifera indica)* subjected to different ripening methods. The concentrations of vitamins A, B1, B2 and B3 followed the same trend and revealed that there were no significant ($p \leq 0.05$) differences in all groups under comparison and values were higher in the hot water group but lowest in the unripe group. No significant difference ($p<0.05$) was obtained in the values of B6 for the control and the artificially ripe mangoes which ranged from 0.20±0.01 mg/100g in calcium carbide group to 0.28±0.06 mg/100g in hot water group, but each was significantly higher than the value for the unripe mango group (0.13±0.02 mg/100g). The levels of vitamin C mg/100g was highest in the unripe fruits (51.06±0.05 mg/100g) followed by hot water treated fruits (50.06±0.05 mg/100g) which did not differ significantly ($p<0.05$) from the values for polybag treated fruits (49.54±0.19 mg/100g) but each was significantly higher than the value for the naturally ripe fruits (30.90±0.14 mg/100g)

Table.1: Phytochemical composition (mg/100g) of mango (Mangifera indica) subjected to different methods of ripening.

	Carotenoids	Phenols	Terpenes	Alkanoids	Tannins	Saponins	Phytosterols	Flavonoids	Glycosides
Naturally (Ripe)	11.46±0.04^{a}	25.27 ±0.02^{a}	1.77±0.02^{a}	0.53±0.02^{c}	1.08±0.02^{c}	0.37±0.02^{b}	59.13±0.02^{c}	35.88 ±0.02^{c}	2.90 ±0.20^{b}
Unripe	6.53±0.02^{d}	15.13 ±0.02^{d}	0.08±0.02^{c}	0.61±0.00^{a}	2.06±0.02^{a}	0.19 ±0.02^{d}	85.77 ±0.23^{a}	56.80 ±0.01^{a}	3.84 ±0.02^{a}
Polybag	9.73±0.04^{c}	16.27 ±0.02^{c}	0.17±0.02^{b}	0.57±0.02^{b}	1.38±0.02^{b}	0.47 ±0.03^{a}	65.26 ±0.12^{b}	41.03 ±1.71^{b}	2.89 ±0.02^{b}
Carbide	9.94±0.06^{c}	16.37 ±0.04^{c}	0.18±0.14^{b}	0.51±0.00^{b}	1.28±0.05^{b}	0.46 ±0.06^{a}	66.28 ±0.03^{b}	41.72 ±0.57^{b}	2.88 ±0.05^{b}
Hot Water	8.90±0.21^{b}	17.30 ±0.20^{b}	0.18±0.03^{b}	0.49±0.06^{d}	0.93±0.02^{d}	0.24 ±0.02^{c}	54.30 ±0.07^{d}	36.09 ±0.02^{c}	2.61 ±0.02^{c}

Values are Mean ± standard deviations of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level.

Table.2: Vitamin contents (mg/100g) of mango fruits (mangifera indica) subjected to different methods of ripening.

Groups	Vitamin A	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B6	Vitamin C
Naturally Ripe	0.30±0.06 ^{a}	0.06±0.06 ^{a}	0.07±0.03 ^{a}	0.76±0.04 ^{a}	0.26±0.04 ^{a}	30.90±0.14^{a}
Unripe	0.26±0.03 ^{a}	0.05±0.02 ^{a}	0.04±0.01 ^{a}	0.65±0.04 ^{a}	0.13±0.02 ^{b}	51.06±2.00^{b}
Polybag	0.33±0.05 ^{a}	0.06±0.07^{a}	0.06±0.03 ^{a}	0.68±0.11 ^{a}	0.28±0.14 ^{a}	49.54±0.19 ^{b}
Carbide	0.29±0.06 ^{a}	0.06±0.03 ^{a}	0.05±0.02 ^{a}	0.67±0.10 ^{a}	0.20±0.01 ^{a}	41.54±0.16^{c}
Hot water	0.34±0.06 ^{a}	0.08±0.03 ^{a}	0.09±0.04 ^{a}	0.78±0.09 ^{a}	0.28±0.06 ^{a}	50.06±0.05^{b}

Values are Mean ± standard deviations of triplicate determinations. Values in the same column having the same superscript letters are not significantly different at the 5% level.

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IV. DISCUSSION

Phytochemical investigation revealed that carotenoids, phenolics, terpenes decreased significantly ($p<0.05$) in the artificially induced ripening groups compared with the control. Carotenoids have antioxidants, anti-carcinogenic and anti-mutagenic properties, giving protection against various diseases such as different types of tumors, cardio vascular diseases as well as age related illnesses. (Dauchet *et al*., 2006; Davinelli *et al*., 2012). Carotenoids are vital in their role as precursors of important vitamins such as vitamin A (Schreiner and Huysken-keil, 2006). They also play extremely vital role as immune system booster aiding the body's ability to combat diseases and infections (Basu *et al*., 2007; Blanchflower *et al*, 2013). Menichini *et al*., (2009) in a similar work using *capsicum Chinese pepper* submitted an increase in carotenoids with ripening but Alagbaoso *et al*., (2017) submitted an insignificant difference in the carotenoid content of Avocado pear with ripening. Difference may be ascribed to the fruit type or maybe due to the ripening methods employed.

Polyphenols enhance insulin sensitivity thereby lowering the risk of type 2 diabetes by inhibiting carbohydrate digestion and glucose absorption in the intestine; stimulation of insulin secretion from the pancreas and the modulation of glucose release from the hepatic cells (Carter *et al*., 2010). The observed increase in the phenol content of the naturally ripe compared with the artificially ripened mango fruit, does not agree with earlier report (Rodríguez *et al*., 2016) who submitted a decrease in the phenolic contents of palm fruit with ripening. Variation might be ascribed to differences in the fruits used.

Terpenes are the main constituents of the essential oils of many plants products. They function as natural flavor additives for food, as fragrances in perfumery, and in medicine and alternative medicines such as aromatherapy.

The observed decrease in the contents of carotenoids, phenols and terpenes in the artificially ripened mango fruit, in the current study connotes a likely reduction in disease prevention, antioxidant and flavor enhancing potentials of the fruit.

Flavonoids were observed to be higher in the unripe mango group compared to the naturally ripe as well as the artificially ripened mango groups suggesting that ripening depleted this bioactive compound. According to Herrmann (1991), changes in the amount of flavonoids during fruit ripening might be connected to the development of anthocyanin pigment in the fruit. The antioxidant property of plant products is thought to correlate with the absolute flavonoid content in the fruits, demonstrating that flavonoids could be the determinants of the anti-oxidant capacity of the fruit as reported by Izundu *et al*., (2016). In recent times, there has been a surge of interest in bioactive constituents like flavonoids, owing to their antioxidant capacity which is associated with the redox properties of their hydroxyl side chain as well as the structural relationship between their different functional groups thereby empowering them to function as metal chelators, reducing agents, hydrogen donors, (Amic *et al*., 2003), singlet oxygen quenchers (Gomez-Alonso *et al*., 2003, Materska & Peruka, 2005) and free radical chain breakers (Oboh *et al*., 2008) Flavonoids decreased in the artificially ripened groups thus connoting a decrease in the antioxidant capacity of the fruit. The fact that unripe mango fruit has higher flavonoid content suggests that it might be recommended for diabetic and hypertensive patients as dietary intervention in the management of their pathological conditions (Ibukun *et al*., 2012).

Present study revealed significantly lower concentrations of flavonoids, tannin, alkaloids, phytosterol and glycosides in the artificially ripened mango groups compared with the unripe but the values for each (except glycosides) were significantly higher in the calcium carbide and the polybag ripened mangoes compared to the naturally ripe mangoes. The hotwater ripened mangoes were significantly lower in alkaloids, tannins, saponins, phytosterols and glycosides which agree with earlier report (Negi, 2012) that many phytochemicals are lost by heat processing. Increase in tannin content of the artificially ripened mangoes compared with the naturally ripe ones may be connected to their role as flavor contributors (Aina, 1990) adduced by the observed higher tannin content of the unripe mangoes compared to the rest of the group. Information from emerging data suggest that tannin depletes blood cholesterol (Basu *et al*., 2007) connoting that the consumption of unripe mangoes might be better preferred for the management of hypertension and Ischemic diseases. Saponins are high molecular weight plant constituents that have a sugar moiety associated with a steroid glycone (Price *et al*., 1987). They have cleansing, pesticidal, antilipideamic and anticancer properties (Gurfinkel & Rao, 2003; Kim *et al*., 2003) and are believed to be valuable in the treatment of polluted water (Hall & Walker, 1991). Alkaloids are low atomic weight basic (nitrogenous) compounds. They exert strong defensive impact against pathogens and herbivores and are thought to have pain relieving, antimalarial, antibacterial and antihypertensive properties (Dangi *et al*., 2002). Glycosides are triterpenoids which have cardio active properties (Brian *et al*., 1985). They exert various

impacts on the mechanical and electrical activities of the heart just as on the neural tissue (Olaleye, 2007). Present study revealed that flavonoids, tannins, alkaloids, phytosterols and glycosides contents were higher in the artificially ripened mangoes compared to the naturally ripe ones.

Vitamin analysis showed a significantly higher value of vitamin C in the artificially ripened mango fruits compared with the control which did not vary with the unripe mango group. This report agrees with previous report (Mamiro *et al*.,2007, Appiah, *et al*., 2011) that presented significant increase in Vitamin C with induced ripening but disagrees with the report of (Ralman *et al*., 2007) who reported that fruit nutrients like Vitamin C were higher in naturally ripened fruits. Variations might be attributed to ripening stage or method of artificial ripening employed in the fruit ripening processes (Gbakon *et al*., 2016). Subjection of fruits to light directly impacts on the chemical composition of fruits implying that the higher the light intensity, the higher the vitamin C content in the fruits since light is required for energy generation during photosynthesis which is later used as glucose to synthesize more ascorbic acids in the fruits (Stumpf *et al*., 1988), this agrees with the report of lower vitamin C content observed in the polybag group in current study.

The observed increase in the concentration of vitamin C in the artificially ripened mango fruits is important since vitamin C is regarded as essential to human health (Appiah *et al*., 2011) for various reasons, including, prevention of scurvy and cancer, relief from common cold, formation of bile salt from cholesterol, stimulation of collagen synthesis and also for its critical role in the wound healing process (Iqbal *et al*., 2004). More so, vitamin C improves absorption of iron from non-heme sources (Teucher, *et al*., 2004). Vitamin C is a water-soluble, free radical scavenging antioxidant found in the cytoplasm of cells, (Mckee and Mckee 1999). Production of free radicals in living organisms gives rise to oxidative stress in the living cells (Nwaogu, *et al*., 2011). Vitamins A, C, and E and some enzymes such as catalase, glutathione peroxidase, and superoxide dismutase help to terminate free radical chain reactions in a living cell which if left unchecked, may prompt onset of numerous diseases (Nwaogu, *et al*., 2008). Vitamin C is commonly utilized as food additive acting as antioxidant (Whitney & Rolfes, 2008). Humans are unable to synthesize their very own vitamin C since human cells cannot perform the last but crucial step in vitamin C biosynthesis which is the change of L-gulono-g-lactone into ascorbic acid catalyzed by gulonolactone oxidase enzyme (Muhamed *et al*., 2014; Eze *et al*., 2017). Therefore, vitamin C is required for maintaining physiological capacities and to meet this requirement, vitamin C must be consumed from diet.

The adverse effect of calcium carbide as a ripening agent has long been established by various researchers (Singal *et al*., 2012; Dhembare *et al*., 2014). Also, the migration of lead from black polythene bag into cooked green banana has been reported by Banadda *et al*., (2011). They reported that lead (Pb) and cadmium (Cd) which are residues from the polymerization process of polythene can migrate into food materials at high temperature and under long storage period. It is therefore, pertinent to note that though artificial ripening of fruits may increase vitamin C and many of the disease preventing phytochemical content of the fruit than the naturally ripe fruits, such practice may increase the risk of contamination of fruit with toxicants such as arsenic and lead found in the chemical materials utilized in these artificial ripening processes (Sogo-Temi *et al*., 2014.

V. CONCLUSION

Findings have shown that artificial ripening of fruits increased many of the Phytochemicals investigated and also increased vitamin C content of the mango fruits. Owing to the importance of these food constituents in maintaining optimal health, biological methods, which would not pose any threat to human health should be discovered and encouraged.

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