



**EFFECT OF POTASSIUM PERMANGANATE (KMNO<sub>4</sub>) TREATMENT ON THE NUTRITIONAL COMPOSITION, ANTIOXIDANT PROPERTIES AND MICROBIAL COUNT OF AFRICAN STAR APPLE (*Chrysophyllum albidum*- LINN) FRUITS DURING STORAGE**

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**Abstract**

African star apple (ASA) fruit is consumed for its natural antioxidants, believed to fight against oxidative stress-related diseases such as diabetes, cancer, and coronary heart diseases. The seasonality and perishability of the fruit make its storage important to attract so much attention. This study made use of potassium permanganate (KMnO<sub>4</sub>) to monitor changes in the nutritional, antioxidant compositions and microbial count of the fruits. Wholesome ASA fruits were treated with different concentrations of KMnO<sub>4</sub> stored at 28±2°C and 90±5% relative humidity and evaluated for nutritional composition, antioxidant potentials and microbial count. The moisture content, crude fibre, calcium, and potassium contents of the treated fruits increased significantly in storage, however, there were reductions in their protein, carbohydrate, and iron contents. There were no significant (p<0.05) differences in ash content, crude fat, and the sodium contents of the treated samples when compared to the freshly harvested fruits after 15 days of storage. No traces of lead and cadmium were found in any of the samples analyzed. Treated fruits showed greater enzymatic and non-enzymatic activities and with no record of microbial load as compared to untreated fruits. Saturated KMnO<sub>4</sub> treatment proved to be the most efficient in maintaining nutritional and antioxidant qualities of ASA fruits followed by 800 ppm and 400 ppm KMnO<sub>4</sub> treatments relative to the control. Therefore, gaseous molecules of KMnO<sub>4</sub> may be applied for the postharvest treatment of ASA fruits in maintaining their quality attributes for 15 days at ambient temperature and 90 ± 5% relative humidity.

**Keywords:** *Chrysophyllum albidum*, Potassium Permanganate, Postharvest treatment, Antioxidants, Proximate composition, Microbial load.

**Introduction**

African star apple “ASA” (*Chrysophyllum albidum* - Linn) is a genus of about 70-80 species of tropical trees native to tropical regions of the world, with the greatest number of species in the Northern South America and some parts of Africa (Bello and Henry, 2015). It is a seasonal fruit relished

for its tasty fleshy pulp. The pulp is consumed in its natural form by pressing hard and sucking the pulp. This fruit belongs to the class of raw materials that are generally regarded as safe for the production of food and pharmaceuticals for human and animal consumption (Okoye and Ndiwe, 2016). *C. albidum* fruit is of great economic value due to its diverse industrial, medicinal and food

uses (Bello and Henry, 2015). The fruits are not only consumed fresh but also used to produce jam, jellies, stewed fruit, marmalade, syrup and several types of soft drinks (Bello and Henry, 2015). When freshly harvested, the fleshy and juicy fruits can be fermented and distilled for the production of wine, spirits or other alcoholic products. In parts of West Africa, its seeds are occasionally collected and their oil extracted for soap making or cooking (National Research Council, 2008). The fruit pulp is rich in vitamin C and iron and is an excellent source of raw material for industries (Akubugwo and Ugbo, 2007). Its rich sources of natural antioxidants have been established to promote health by acting against oxidative stress related diseases (Ibrahim *et al.*, 2017). It has vast medicinal benefits, which include plasma cholesterol level reduction, rate of sugar uptake as well as its detoxifying action and effectiveness in diarrhea therapy (Ige and Gbadamosi, 2007). Despite the importance of the fruit, it has been greatly neglected and under-utilized due to its seasonality and perishability. With the global focus on increased food production and emphasis on provision of nutritive food for the world's teeming populace, it is therefore very important to make fruits like the African star apple available all year round and extend their storage life long enough to be transported to distant markets (Iro and Ezejindu, 2017). The use of conventional synthetic waxes and/or chemical fungicides as postharvest treatments is becoming increasingly restricted because of concern for the environment and health, as well as the cost of developing new pesticides to overcome resistance developed by pathogens (Palou *et al.*, 2015). Various chemicals generally regarded as safe (GRAS) such as calcium chloride (Mujtaba *et al.*, 2014; Gharezi *et al.*, 2012; Pila *et al.*, 2010),

acetic acid (Gharezi *et al.*, 2012); salicylic acid (Sartaj *et al.*, 2013), Hexanal (Cheema *et al.*, 2018), Gibberlic acid (Pila *et al.*, 2010) are widely used to improve shelf life of perishable commodities. Among various permitted chemicals is the application of Potassium permanganate-'KMnO<sub>4</sub>' (Mujtaba *et al.*, 2014). Potassium permanganate is a strong oxidizing agent that reacts with ethylene and generates carbon dioxide and water as the main end-products. An early use with vegetables was as a disinfectant (Wills, 2015). Its first report was on apple to reduce the ethylene concentration in the atmosphere around produce (Forsyth *et al.*, 1967). KMnO<sub>4</sub> can be used as ethylene absorbent that plays a central role in fruit ripening (Mujtaba *et al.*, 2014). The climacteric burst of ethylene which makes the fruit palatable also triggers senescence and subsequent ripening in the fruits (Isaac *et al.*, 2016). Even very low concentration of ethylene in storage chamber can deteriorate the product quality. The use of ethylene biosynthesis inhibitors leads to a reduction in endogenous ethylene levels in the plant. KMnO<sub>4</sub> treated fruits had shown delayed ripening, reduced respiration, physiological loss in weight and moisture with retention of higher firmness (Sujayasree and Fasludeen, 2017). It is therefore imperative to find a non-damaging consumer and environmentally friendly postharvest treatments that will maintain quality attributes in fresh horticultural products (Ferraz *et al.*, 2013). With only limited information available on the beneficial effects of KMnO<sub>4</sub> on African star apple fruits as efficient postharvest treatment to maintain quality. This study was designed to assess the effect of postharvest KMnO<sub>4</sub> treatment of African star apple fruits on its nutritional, antioxidant qualities and microbial count.

#### **Material and Methods**

All the chemicals used were of analytical grades, KMnO<sub>4</sub> was purchased from BDH

chemical Ltd Poole, England.  $H_2SO_4$  and ascorbic acid was from Avondale laboratories Banbury, Oxon, England, while  $H_2O_2$  was from Eagle scientific Ltd. Nottingham, NG9 6DZ, England. All other chemicals were obtained from other commercial sources (Sigma-Aldrich and Merck). The water used was glass distilled and all glassware was acid washed and rinsed with doubly distilled deionized water ( $ddH_2O$ ).

### **Collection and Preparation**

Fresh, healthy, matured and ripe fruits of ASA were harvested early in the morning from a farm near the Federal University of Technology, Akure, Ondo State, Nigeria. The fruits were transported immediately to the laboratory and sorted for uniformity of maturity, size, shape, peel color, while removing bruised ones. The healthy fruits were surface sterilized with sodium hypochlorite (500 ppm) for 10 min to reduce the microbial infection and air-dried before further treatments. The fruits were identified and authenticated in the herbarium unit of Botany Department, University of Ibadan, Oyo State, Nigeria. The voucher registration number is UIH/2016/22502.

### **Treatment and Experimental Design**

The method of Mujtaba *et al.* (2014) was adopted for the treatment with little modifications. The postharvest treatments were carried out as per completely randomized block design with four treatments. Fruits were divided into 4 treatment groups; containing 40 fruits in each and treatments were applied in the following scheme:  $T_0$  = Fruits rinsed in distilled water (DW) (control),  $T_1$  = fruits treated with gaseous molecules of 400 ppm,  $KMnO_4$ ,  $T_2$  = fruits treated with gaseous molecules of 800 ppm,  $KMnO_4$ ,  $T_3$  = fruits treated with gaseous molecules of saturated  $KMnO_4$ .

Sponge cubes of  $8 \times 8 \times 11$  cubic centimeters cutting were dipped in 400, 800 ppm and saturated solution of potassium permanganate. After that these sponge cuttings were allowed to dry to the extent that no drop of potassium permanganate falls from them. Then one cutting of the sponge from the respective treatments ( $T_1$ ,  $T_2$  and  $T_3$ ) was placed in a sterile aerated pouch at one corner of the storage container to avoid contact with the fruits.

### **Storage of Fruits**

Each set of the treated fruits was stored inside sealed sterile high-density polyethylene (HDPE) plastic transparent containers (10 L) at ambient temperatures ( $28 \pm 2$  °C) and  $90 \pm 5\%$  relative humidity for 15 days and used for further analyses;

### **Determination of proximate and mineral compositions**

Proximate composition (moisture, ash, crude fat, crude fibre, protein and carbohydrate content) was determined according to the method of AOAC (1990). Minerals were analyzed by the method reported by Oseni *et al.* (2011). Minerals were determined by dry-ashing 1 g of the sample at 550 °C in a furnace. The ash obtained was dissolved in 10% HCl, filtered with filter paper and made up to standard volume with deionised water. Flame photometer was used to determine sodium and potassium contents of the sample, while Ca, Fe, Mg, Zn, Cu, Mn, Pb and Cd were determined using atomic absorption spectrophotometer (AAS).

### **Determination of Enzymatic and non-Enzymatic Antioxidant activities**

#### **Phenolic content determination**

The phenolic content was determined according to the method of Singleton *et al.* (1999). Appropriate dilutions of the extracts were mixed with 2.5 ml of 10% Folin-Ciocalteu's reagent (v/v) and neutralised by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was

incubated for 40 min at 45°C and the absorbance was measured at 765 nm in a spectrophotometer (JENWAY 6305). The total phenolic content was subsequently calculated using Gallic acid as standard (phenol standard stock: 20mg/ ml garlic acid) followed by serial dilution. R<sup>2</sup> value was 0.998. The Results obtained were expressed as mgGAE/g.

#### **DPPH free radical scavenging ability**

The free radical scavenging ability using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was determined as described by Singh *et al.* (2002). Different concentrations of the aqueous extract were taken in different test tubes and the volume was made up to 1 ml with distilled water. 4 ml of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature. Changes in absorbance of samples were measured at 516 nm in a spectrophotometer (JENWAY 6305). Free radical scavenging ability was expressed as percentage inhibition and was calculated using the following formula:

Free radical scavenging ability (%) =  

$$\left[ \frac{\text{absorbance of standard} - \text{absorbance of sample}}{\text{absorbance of standard}} \right] \times 100.$$

#### **Total flavonoid content determination**

The total flavonoid content of the extract was determined using a slightly modified method reported by Asare *et al.* (2015). 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 ml of 10% Aluminium chloride (AlCl<sub>3</sub>), 50 ml of 1 mol/l potassium acetate and 1.4 ml water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm using a spectrophotometer (JENWAY 6305). The total flavonoid was calculated using quercetin (5mg/ml) as standard. The total flavonoid content was calculated thus:

(concentration mg/ml) = change in absorbance/slope of standard. The R<sup>2</sup> value was 0.998. The Results obtained were expressed as mgQE/g.

#### **Determination of glutathione activity**

Assay of glutathione peroxidase (GPX) activity was used to determine glutathione activity in the sample. GPX activity in the sample was analyzed according to Rotruck *et al.* (1973). The reaction mixture containing 500 µl phosphate buffer, 100 µl of sodium azide, 200 µl GSH, 100 µl H<sub>2</sub>O<sub>2</sub> were added to 500 µl of the sample, after which 600 µl of distilled water was added and mixed thoroughly. The whole reaction mixture was incubated at 37 °C for 3 min after which 0.5 ml of TCA was added and thereafter centrifuged at 3000 rpm for 5 min. To 1 ml of each of the supernatants, 2 ml of K<sub>2</sub>PO<sub>4</sub> and 1 ml of DNTB was added and the absorbance was read at 412nm against a blank. Glutathione peroxidase activity was obtained by plotting the standard curve and the concentration of the remaining GSH was extrapolated from the curve. GSH activity (IU/ml) = mg/ml x (total volume of assay) dilution factor/ volume of enzyme used x incubation time = (mg/ml x 3).

#### **Determination of catalase activity**

Catalase (CAT) activity was determined according to the method described by Aebi (1974). Sample (70 µl) was mixed with 920 µl Na-PO<sub>4</sub> buffer pH 7 containing 0.1mM EDTA. The reaction started by adding 10 µl of H<sub>2</sub>O<sub>2</sub> (30 mM). The decrease in H<sub>2</sub>O<sub>2</sub> concentration was taken by reading the absorbance at 240 nm (10 seconds intervals) for 180 seconds. Catalase activity (IU/ml) = change in absorbance/slope of standard. Therefore, catalase activity (IU/ml) = mg/ml x (total volume of assay) dilution factor/ Volume of enzyme used x incubation time.

#### **Determination of Superoxide Dismutase (SOD) Activity**

The activity of SOD in the homogenates was determined according to the method

described by Misra and Fridovich (1972). A dilution of 1 ml of the sample was made with 9ml of distilled water to make a 1 in10 dilution. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion (0.2 of sample as well). The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 sec for 150 sec.

Increase in absorbance per minute =  $A_3 - A_0 / 2.5$ . Where,  $A_0$  = absorbance after 150 sec and  $A_3$  = absorbance after 150 sec. % inhibition =  $100 - \text{Increase in absorbance for substrate} / \text{Increase in absorbance of standard} \times 100$ . 1 unit of SOD activity was defined as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 min.

### **Microbiological analysis**

Pour plate, and serial dilution procedures were used to isolate bacteria and fungi. One gram of the infected sections of the fruits was placed into 9 ml sterile distilled water and mixed appropriately for bacterial isolation, after which serial dilutions were performed. To count the microorganisms in each sample, 10-fold serial dilutions of each rinsed water were made, and 1 ml of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$  dilutions were pipetted into sterile Petri-dishes, where molten nutrient agar (NA 45°C) was added and swirled thoroughly to ensure even distribution, and incubated at 37°C for 24 hours. Discrete colonies were counted, documented, and expressed as CFU/g (colony-forming units per gram). 1 ml aliquot dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$  were dispersed on sterilized potato dextrose agar (PDA) with chloramphenicol (30 mg/l) (to limit the

development of bacteria) on Petri plates and cultured for seven days at ambient temperature ( $28 \pm 2^\circ\text{C}$ ) for the isolation of related fungus. The spore-forming units per gram (SFU/g) of the fungal isolates were counted, documented, and expressed. For the total coliform count, MacConkey agar plates were infected. On each plate, the number of colonies was counted and recorded. For each dilution, triplicate plates were made; thus, the total plate count for each dilution was calculated as the average of the three counts (Iro and Ezejindu, 2017). The microbiological analysis of healthy fruits was carried out in the same way.

### **Statistical Analysis**

Statistical analysis was carried out with Statistical Package for Social Sciences Software (SPSS) version 16.0. All the analyses were in triplicate and the standard error of mean (SEM) were calculated. One-way analysis of variance (ANOVA) was used to detect treatment effect. Graph Pad Prism 6 Software was employed for the statistical analysis. Means were separated using Duncan's multiple range test (DMRT). Differences were considered statistically significant at DMRT,  $p < 0.05$ .

### **Results and Discussion**

#### **Proximate composition of African Star Apple**

The effects of postharvest  $\text{KMnO}_4$  treatment on proximate composition of African star apple fruits are shown in Table 1. As the concentration of the  $\text{KMnO}_4$  increased, the moisture contents decreased. Sample treated with saturated  $\text{KMnO}_4$  had about 20% decrease in moisture content as compared to the control fruit with the highest moisture content of  $41.87 \pm 0.01\%$ . However, after 15 d in storage there was increase in moisture content in all the samples investigated when compared to the freshly harvested fruits. This showed that during storage ripening continued and as the storage days progressed

the moisture content of African star apple fruits increased. The increase might be due to metabolic water because during ripening the breakdown of pectic structures by polygalacturonase enzymes usually releases water into the fruit matrix thereby increases the moisture content of fruit. This is in agreement with the results of Lidianys Maria *et al.* (2014) on *Morinda citrifolia* fruits whereby its amount of moisture increased with ripening. The values reported for African star apple in this present study were lower than the moisture content reported by Asare *et al.* (2015); Oloyede and Oloyede (2014); Edem and Dosunmu (2011) and Falegan *et al.* (2017) for ASA fruits. This may be due to some factors, such as location, species, maturity and point at which harvesting was done (Lawal *et al.*, 2016). Moisture content of fruits varies with season (Ige and Gbadamosi, 2007). Low moisture content of any fruits shows that it can be stored for a longer period because it will have better resistance against the growth of undesirable microorganisms. Moisture content of any food material is a measure of the life span of the food. It indicates how long a food material can be stored without becoming mouldy (Ibrahim *et al.*, 2017). There were no significant ( $p < 0.05$ ) differences between the Ash content of the freshly harvested fruits and the treated samples. The lowest Ash content of 1.91% was observed in the control sample at the end of storage period. Ash content indicates the amount of inorganic matter and oxides present in food sample (Bello and Henry, 2015). Ash contents were high in all the samples tested in this investigation compared to the report by Oloyede and Oloyede, (2014); Falegan *et al.* (2017); Lawal *et al.* (2016); Adepoju and Adeniji (2012); Bello and Henry (2015) and Edem and Dosumu (2011) respectively on ASA fruits except for the control value.

The results obtained for fat content analysis showed that there were no significant differences ( $p < 0.05$ ) among the freshly harvested ASA fruits and all the treated fruits. This shows that postharvest  $\text{KMnO}_4$  treatment may be used to maintain fat content of ASA fruits. Fat is an excellent source of energy. It enhances transport of fat soluble vitamins, protects internal tissues and contributes to important cell processes (Bello and Henry, 2015). However, Ibrahim *et al.* (2017) stated in his report that excess of saturated fatty acids is responsible for a tendency to coronary thrombosis and aortic atheroma in men while high level of poly unsaturated fatty acids is important in lowering blood cholesterol level.

Previous studies on ASA fruits showed that the obtained crude fiber contents (Table1) were higher than the values reported by Bello and Henry, (2015) Edem and Dosumu (2011) Oloyede and Oloyede, (2014) and Ibrahim *et al.* (2017) but lower than the value reported by Falegan *et al.* (2017). Fiber decreases the absorption of cholesterol from the gut, delays the digestion and conversion of starch to simple sugars, and also functions in the protection against cardiovascular disease, colorectal cancer and obesity (Ibrahim *et al.*, 2017). Daily soluble fiber intake of 5–10 g from a variety of sources has been found to reduce serum cholesterol by 5–10% (Ibrahim *et al.*, 2017). Crude fibre is the indigestible carbohydrate component that is present in plants. Dietary fiber has recently received much importance, as it is believed to reduce the incidences of colon cancer, diabetes, heart disease and certain digestive diseases (Ingabire and Vasanthakalam, 2011). This implies that the  $\text{KMnO}_4$  treated fruits with the highest fiber fractions could be effectively utilized in the management of diabetes mellitus, colorectal cancers and weight.

As the storage days progressed there was general reduction in the protein and

carbohydrate contents of the  $\text{KMnO}_4$  treated fruits as compared to the freshly harvested and control samples. The values recorded in table 1 were higher than the protein contents earlier reported on ASA fruits (1.71%, 0.59%, 1.71%) by Lawal *et al.* (2016); Oloyede and Oloyede (2014) and Falegan *et al.* (2017). The low protein content in the samples investigated therefore needs to be supplemented from other sources because protein is useful in the repairing of worn out tissues, building up of new ones as well as improving the organoleptic properties of food materials (Bello and Henry, 2015). Protein deficiency causes growth retardation, abnormal swelling of the belly and collection of fluids in the body (Arueya and Ugwu, 2017). The decrease in carbohydrate content of the treated fruits may be as a result of some

enzyme activities on it as the main source of energy during the ripening process (Bello and Henry, 2015). These values were lower than the average values previously reported on ASA fruits by Ukana *et al.* (2012); Bello and Henry, (2015); Asare *et al.* (2015), Edem and Dosumu (2011) but higher than the values reported by Oloyede and Oloyede (2014) and Falegan *et al.* (2017) on ASA fruits. The low carbohydrate contents of ASA fruits according to Adepoju and Adeniji (2012) may be responsible for its lack of sugary taste. The major metabolic role of the carbohydrate in the diets is for energy production (Asare *et al.*, 2015). The carbohydrate contents of the sample investigated were low, but fruits with low contents of carbohydrates are ideal for diabetic and hypertensive patients requiring low sugar diet.

**Table 1 Proximate composition of  $\text{KMnO}_4$ -Treated African star apple fruit [(wet weight basis) %].**

Treatments	Storage Days	Moisture	Ash	Crude Fat	Crude Fibre	Protein	Carbohydrate
At harvest	0	35.03 ± 0.04 <sup>a</sup>	3.24 ± 0.09 <sup>b</sup>	17.75 ± 0.58 <sup>b</sup>	5.59 ± 0.20 <sup>a</sup>	6.97 ± 0.10 <sup>c</sup>	31.42 ± 0.70 <sup>c</sup>
Control	15	41.87 ± 0.01 <sup>e</sup>	1.91 ± 0.00 <sup>a</sup>	9.25 ± 0.43 <sup>a</sup>	5.68 ± 0.17 <sup>a</sup>	8.18 ± 0.00 <sup>d</sup>	33.13 ± 0.58 <sup>d</sup>
400ppm $\text{KMnO}_4$	15	37.64 ± 0.01 <sup>d</sup>	3.26 ± 0.03 <sup>b</sup>	17.88 ± 0.01 <sup>b</sup>	7.08 ± 0.02 <sup>b</sup>	6.28 ± 0.02 <sup>b</sup>	27.87 ± 0.03 <sup>a</sup>
800ppm $\text{KMnO}_4$	15	36.47 ± 0.02 <sup>c</sup>	3.16 ± 0.02 <sup>b</sup>	17.92 ± 0.01 <sup>b</sup>	7.34 ± 0.02 <sup>b</sup>	6.22 ± 0.06 <sup>b</sup>	28.89 ± 0.07 <sup>a,b</sup>
Saturated $\text{KMnO}_4$	15	36.06 ± 0.03 <sup>b</sup>	3.13 ± 0.01 <sup>b</sup>	17.96 ± 0.05 <sup>b</sup>	7.46 ± 0.00 <sup>b</sup>	5.84 ± 0.03 <sup>a</sup>	29.56 ± 0.01 <sup>b</sup>

Means with the same letters within a column are not significantly different at  $p < 0.05$  using DMRT. Values represent Mean ± Standard Error of means on wet weight basis. Each value is the mean for three replicates.

### Mineral composition of African Star Apple

Table 2 presents the results of the mineral analysis. In this present study, it is evident that calcium contents increased significantly ( $P < 0.05$ ) in all the samples investigated in storage relative to freshly harvested fruits. Fruit samples treated with 400 ppm  $\text{KMnO}_4$  had the highest content of

calcium followed by the fruit samples treated with 800 ppm  $\text{KMnO}_4$  and fruit samples treated with saturated  $\text{KMnO}_4$  and the lowest value was observed in the untreated ASA (control) fruits in comparison with that of the freshly harvested fruits. Calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function. In addition, calcium deficiency affects the dentition of

both children and adult (Ukana *et al.*, 2012). Iron functions as haemoglobin in the transport of oxygen. Iron contents of  $KMnO_4$  treated ASA fruits reduced drastically as compared to control sample and the freshly harvested fruits. The highest value was recorded in the control sample and lowest value was observed in the saturated  $KMnO_4$  treated fruits as shown in table 2. Iron content of the treated fruits in this study was low therefore much of it has to be consumed before substantial amount can be obtained because iron deficiency results in anaemia characterized by weakness, dizziness and loss of weight. This observation was in line with the results of Asare *et al.* (2015).

Potassium contents were found to be most abundant. Its contents increased in all the fruit samples as storage days progressed. Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle and cell membrane function. Deficiency disease or symptoms may cause apathy, muscular weakness, paralysis, mental confusion and abnormal heart beat (Ukana *et al.*, 2012). The low sodium content and the high Potassium contents observed

in this study (Table 2) showed that that the consumption of African star apple fruits is suitable for everybody including the hypertensive patients. Sodium is the principle extracellular cation and is used for acid base balance and osmoregulation in inter modular fluid (Ukana *et al.*, 2012). Deficiency will result in nausea, muscle cramps and exhaustion. The mineral content analyzed showed significant difference in most of the elements but not all. The essential metals can also produce toxic effects when the metal intake is excessively high (Asare *et al.*, 2015). Lead is the most recognized toxic environmental pollutant. Maximum permissible limits in fruits and leafy vegetables and herbal or medicinal products are 10 mg/kg and 0.3 mg/kg for Pb and Cd, respectively (Asare *et al.*, 2015). The results obtained indicated that there were no traces of lead and cadmium in any of the fruit samples. Accumulation of lead and cadmium on fruits can cause acute toxicity. That means investigated samples may be free from toxic effects of lead and cadmium. The presence of potassium, calcium, sodium, manganese, copper and iron content in ASA fruits is an indication that the fruits can supply some essential minerals needed for healthy life (Ibrahim *et al.*, 2017).

**Table 2: Mineral contents of Postharvest Potassium permanganate ( $KMnO_4$ ) treated African star apple fruits (mg/g)**

Treatment	Storage Days	Ca	Fe	Mn	Cu	K	Na	Pb	Cd
At harvest	0	1.53±0.02 <sup>a</sup>	2.60±0.06 <sup>b</sup>	ND	ND	4.63±0.03 <sup>a</sup>	0.43±0.02 <sup>b</sup>	ND	ND
Control	15	1.73±0.01 <sup>b</sup>	3.18±0.01 <sup>c</sup>	ND	0.05±0.01	6.35±0.02 <sup>c</sup>	0.33±0.03 <sup>a</sup>	ND	ND
400ppm $KMnO_4$	15	6.75±0.04 <sup>e</sup>	1.33±0.33 <sup>a</sup>	0.02±0.00 <sup>a</sup>	ND	8.35±0.10 <sup>d</sup>	0.42±0.01 <sup>b</sup>	ND	ND
800ppm $KMnO_4$	15	2.30±0.01 <sup>d</sup>	0.90±0.05 <sup>a</sup>	0.04±0.01 <sup>b</sup>	ND	6.66±0.06 <sup>c</sup>	0.47±0.01 <sup>b</sup>	ND	ND
Saturated $KMnO_4$	15	1.89±0.01 <sup>c</sup>	0.88±0.04 <sup>a</sup>	ND	ND	5.30±0.01 <sup>b</sup>	0.45±0.01 <sup>b</sup>	ND	ND

Abbreviations: ND: Not detected, Ca: Calcium, Fe: Iron, Mn: Manganese, Cu: Copper, K: Potassium, Na: Sodium, Pb: Lead, Cd: Cadmium. Means with the same letters within a column are not significantly different at  $p < 0.05$  using DMRT. Each value is the mean for three replicates.

### **Antioxidant activities of potassium permanganate treated African Star Apple**

According to Nirmala and Asiri (2017) an antioxidant can be defined as any substance which significantly delays or prevents oxidation of oxidizable substrate when present at low concentration. Free radicals have very important roles in various pathogenesis, inflammatory diseases and can result in necrosis of the liver. It has been hypothesized that bioactive components with antioxidant capacities present in foods may contribute to lower incidence of cardiovascular disease (Asare *et al.*, 2015). Phenols are secondary metabolites commonly found in plants and are very useful in the defense mechanisms against pathogens and radiation and directly involve in antioxidant activity. These are of major concern in the food industry because they retard oxidative degradation of lipids and hence improve the quality and nutritional value of foods like wise its' nutritional value (Ravimannan and Nisansala, 2017). There was a general increase in total phenolic composition of all the treated samples (Table 3) as compared to the freshly harvested fruit. Natural phenolic compounds have received increasing attention in the last few years, since a great amount of them can be found in plant and plant products, and thus, consumption of these products containing a greater level of such compounds may reduce the risk of the development of several diseases due to their antioxidant activity together with other health-promoting factors (Alam *et al.*, 2016). The high phenolic contents may be due to the liberation of phenolic compounds from the fruit matrix (Mujtaba *et al.*, 2014). Phenols protect plants from oxidative damage. They have also been studied extensively as antioxidant protectants for human beings and play beneficial role in reducing the risk of coronary heart disease,

diabetes, hypertension and some types of cancer (Prakash *et al.*, 2012).

The DPPH activity was found to increase in all the fruit samples compared to the freshly harvested fruits (63.50 %) as the storage days increased. The control fruits had DPPH value of 71.85%, fruits treated with 400 ppm  $\text{KMnO}_4$  had the value of 74.11%, 800 ppm  $\text{KMnO}_4$  treated fruits had mean data of 76.10% and highest DPPH activity of 78.97% was recorded in the fruit treated with saturated  $\text{KMnO}_4$ . The results of antioxidant activities in *C. albidum* showed by Oloyede and Oloyede (2014) was 92.5% which was higher than all the mean data obtained in this study. This is an evidence/ indication that antioxidant activities in *C. albidum* is very high and its radical scavenging ability is very strong. The DPPH assay is commonly used for testing radical scavenging activity of various food products (Arueya and Ugwu, 2017).

The total flavonoid content was highest in the fruit treated with saturated gaseous molecule of  $\text{KMnO}_4$  (3.36 mgQE/g). The total flavonoid increased in all the treated fruit samples including the control sample. The total flavonoid content value was recorded as 0.49 mgQE/g for freshly harvested fruits, 1.51 mgQE/g for the control sample, 2.87 mgQE/g for fruit treated with 400ppm  $\text{KMnO}_4$  and 2.89 mgQE/g for fruit treated with 800 ppm  $\text{KMnO}_4$  as shown in Table 3. Flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and inhibit tumor growth. The most common group of plant phenolics are the flavonoids, the health promoting properties of plant-based foods have largely been attributed to their wide range of plant chemicals (Arshad *et al.*, 2014).

Glutathione (GSH) activities significantly increased during storage in all samples regardless of treatments. The control sample had the lowest value of 40.02 IU/min in

comparison to all the treated samples and the freshly harvested fruits sample (52.5 IU/min). Fruits treated with saturated  $\text{KMnO}_4$  had the highest value of 225.58 IU/min followed by the samples treated with 800ppm  $\text{KMnO}_4$  (221.56 IU/min) and the fruits treated with 400ppm  $\text{KMnO}_4$  (105.49 IU/min). Glutathione (GSH) activities increased at the end of storage period. Glutathione (GSH) is a tripeptide (L- $\gamma$ -glutamyl-L-cysteinyl-glycine) which forms the largest pool of non-protein thiols in cells and it is an important intracellular antioxidant. Under conditions of oxidative stress, GSH reacts either as an electron donor to neutralize hydrogen peroxides and lipoperoxides or as a direct free radical scavenger (Baysar and Karataş, 2018). It is also the most abundant non-enzymatic antioxidant in live cells where it plays an important role against oxidative stress-induced cell injury (Baysar and Karataş, 2018). Glutathione is an antioxidant, which presents in mammalian and known as the most powerful antioxidant. It is called a

“Master Antioxidant” because of its intracellular and possesses the aptitude to exploit the performance of other antioxidants, these include vitamins C and E, CoQ10 (ubiquinone) and alpha-lipoic acid, and is naturally occurring in fresh fruits, and vegetables, and studies suggest that higher intakes of dietary glutathione correlate with a lower risk of some cancers (Sarvananda and Amal, 2019). Furthermore, glutathione is an essential component of the cellular antioxidative defense system which keeps Reactive Oxygen Species (ROS) under control (Baysar and Karataş, 2018). Antioxidative defense and redox reactions play a major role in the acclimation of plants to their environment which make glutathione a suitable candidate as a stress marker (Baysar and Karataş, 2018). The presence of these powerful antioxidants in African star apple fruits may qualify the fruit as one of the natural sources of antioxidants for those that are prone to oxidative stressed related diseases.

**Table 3: Non- Enzymatic antioxidant activities of Potassium permanganate treated African star apple**

Treatments	Storage Days	Total Phenol (mgGAE/g)	DPPH Scavenging Ability (%)	Total Flavonoids (mgQE/g)	GSH (IU/min)
At harvest	0	10.20 ± 0.04 <sup>a</sup>	63.50 ± 3.37 <sup>a</sup>	0.49 ± 0.07 <sup>a</sup>	52.51 ± 0.12 <sup>b</sup>
Control	15	11.06 ± 0.00 <sup>b</sup>	71.85 ± 0.07 <sup>b</sup>	1.51 ± 0.02 <sup>b</sup>	40.02 ± 0.00 <sup>a</sup>
400ppm $\text{KMnO}_4$	15	13.93 ± 0.01 <sup>b</sup>	74.11 ± 0.87 <sup>b,c</sup>	2.87 ± 0.00 <sup>c</sup>	105.49 ± 0.08 <sup>c</sup>
800ppm $\text{KMnO}_4$	15	14.73 ± 0.36 <sup>c</sup>	76.10 ± 0.02 <sup>b,c</sup>	2.89 ± 0.00 <sup>c</sup>	221.56 ± 0.03 <sup>d</sup>
Saturated $\text{KMnO}_4$	15	16.76 ± 0.00 <sup>d</sup>	78.97 ± 0.00 <sup>c</sup>	3.36 ± 0.00 <sup>d</sup>	225.58 ± 0.01 <sup>e</sup>

Means with the same letters within a column are not significantly different at  $p < 0.05$  using DMRT. Each value is the mean for three replicates. GAE – Gallic Acid Equivalent, QE – Quercetin Equivalent.

### Enzymatic Activities

Plants have to counteract unavoidable stress such as oxidative stress to sustain

their lives and serve heterotrophic organisms including humans. Maintenance of an efficient antioxidant system can delay the

senescence process even though anti-oxidative activity in fruits decreases with aging (Cheema *et al.*, 2018). Stress and senescence enhances the generation of reactive oxygen species (ROS) including superoxide radicals ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals (OH) in various plant cell compartments (Cheema *et al.*, 2018). ROS are lethal and can induce oxidative damage to the cellular components. Plants have developed efficient 'ROS' scavenging systems including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX), and glutathione reductase (GR) that are able to eliminate 'ROS' (Cheema *et al.*, 2018).

#### **Catalase (CAT) activity**

Catalase (CAT) activity was significantly higher in all  $KMnO_4$  gaseous molecule treated fruits than control at the end of storage period (Fig. 1). The highest CAT level (8.16 IU/min) was noticed in fruit

treated with Saturated  $KMnO_4$  gaseous molecule when compared to control (2.87 IU/min) and the other  $KMnO_4$  gaseous molecule treatments. Level of CAT activity also increased in fruits treated with 800 ppm  $KMnO_4$  (6.67 IU/min) and 400 ppm  $KMnO_4$  (6.41 IU/min) with respect to storage time. Treated fruits showed enhanced CAT activities than control fruit during postharvest storage. CAT activity of  $KMnO_4$  treated fruits increased with increasing concentration of  $KMnO_4$  gaseous molecule as compared to the control sample (Figure 1). Hexanal treated peppers also showed enhanced CAT activities than control fruit during postharvest storage (Cheema *et al.*, 2018). Catalase decomposes hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular oxygen. Whole of scientific testimonies indicate that catalase plays an important role in plant defence system, aging and senescence (Rabiei *et al.*, 2011).

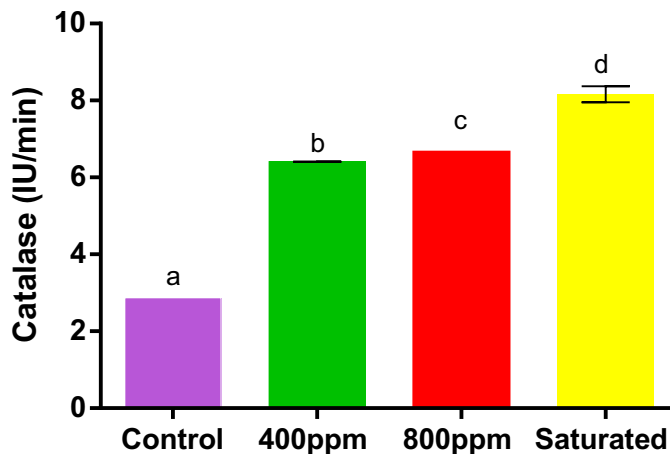


Figure 1: Effect of Postharvest Potassium permanganate treatments of various concentrations (400 ppm, 800 ppm, and Saturated) on the Catalase activity of African star apple fruits stored at  $28 \pm 2^\circ C$  and  $90 \pm 5\%$  Relative humidity. Bars represent Mean  $\pm$  Standard Error of Means. Different Letters on the bars represent significance difference according to Duncan Multiple Range Test at  $p < 0.05$ .

### Superoxide dismutase (SOD) activities

Superoxide dismutase (SOD) activity of the Saturated  $\text{KMnO}_4$  treated fruits (87.50%) were significantly higher than that of the 800 ppm  $\text{KMnO}_4$  (80.15%), 400 ppm  $\text{KMnO}_4$  (80.40%) gaseous molecule treated ASA fruits and control sample (78.10%) (Figure 2). There was no significant difference  $p < 0.05$  between fruits treated with 800 ppm  $\text{KMnO}_4$  and 400 ppm  $\text{KMnO}_4$  gaseous molecule. Increasing SOD activity trend activates other antioxidant enzymes which are very dynamic in  $\text{H}_2\text{O}_2$  scavenging such as catalases (Rabiei *et al.*, 2011). Superoxide dismutases (SODs) is a group of metalloenzymes, they are considered as the first defence against 'ROS', being responsible for the dismutation of  $\text{O}_2^{\cdot-}$  to  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ . The same trend of increase in SOD was reported by Cheema *et al.* (2018) by which SOD activities were significantly enhanced in peppers exposed to hexanal

vapor during the entire storage period as compared with untreated fruit. Similarly, Sharma *et al.* (2010) reported increase in SOD activity in response to hexanal and 1-MCP vapor treatments in sweet cherry fruit. So, higher activities of these enzymes could be considered beneficial because both enzymatic and non-enzymatic antioxidants contribute in the overall antioxidative properties of fruits. In the present investigation, activities of CAT and SOD enzyme were reduced in untreated sample (control), more than in  $\text{KMnO}_4$  treated-stored ASA fruits. CAT and SOD are important antioxidative enzymes that play critical role to combat oxidative damage (Zhang *et al.*, 2015). So, these results indicated that  $\text{KMnO}_4$  gaseous molecules effectively maintained more CAT and SOD enzymes that may be considered highly beneficial to suppress oxidative damage of ASA fruits under ambient storage condition.

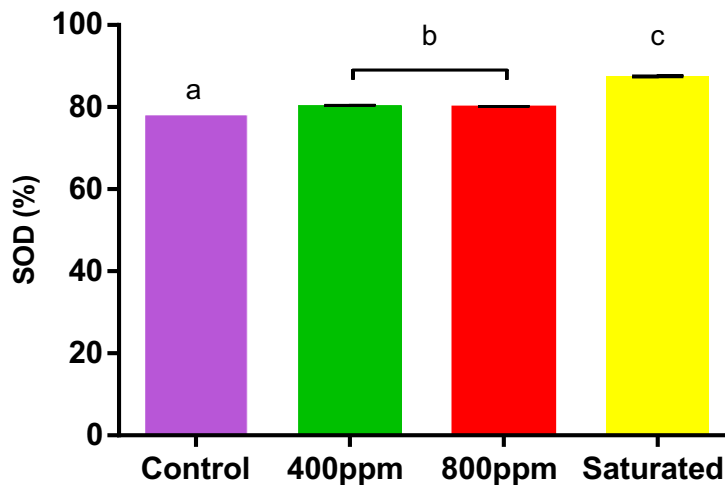


Figure 2: Effect of Postharvest Potassium permanganate treatments of various concentrations (400 ppm, 800 ppm, and Saturated) on the Superoxide Dismutase activity of African star apple fruits stored at  $28 \pm 2^\circ\text{C}$  and  $90 \pm 5\%$  Relative humidity. Bars represent Mean  $\pm$  Standard Error of Means. Different Letters on the bars represent significance difference according to Duncan Multiple Range Test at  $p < 0.05$ .

### Efficacy of Potassium permanganate treatment on microbial count of African star apple

The results of the microbial load of African star apple fruit samples are presented in Table 4. It reveals that the control sample has the highest bacterial count of  $166 \times 10^3$  cfu/g and  $248.33 \times 10^3$  sfu/g fungal count after 15 days of storage. No significant microbial load (including coliforms) was observed in all  $\text{KMnO}_4$  treated fruits as compared to control. The presence of potassium permanganate gaseous molecules with the absence of air in the storage chamber may be responsible for keeping the microbial load in check within

acceptable level. The total microbial counts observed in the fruit samples studied were below the limits ( $1.0 \times 10^2$ ) of the standard board recommendation for fruits (Falegan *et al.*, 2017). According to the HACCP-TQM technical guidelines, raw foods containing  $<10^4$  CFU/g ( $<4 \log_{10}$  CFU/g),  $10^4$ - $5 \times 10^6$  CFU/g ( $4$ - $6.7 \log_{10}$  CFU/g),  $5 \times 10^6$ - $5 \times 10^7$  CFU/g ( $6.7$ - $7.7 \log_{10}$  CFU/g) and  $>5 \times 10^7$  CFU/g ( $>7.7 \log_{10}$  CFU/g) (number of spoilage microorganisms aerobic plate count at  $70^\circ\text{F}$  ( $21.1^\circ\text{C}$ ) are rated as “good”, “average”, “poor” and “spoiled food”, respectively (Serkan *et al.*, 2015). The absence of coliform in the fruits confirms the samples conformity to standards.

**Table 4: Efficiency of Postharvest Potassium permanganate ( $\text{KMnO}_4$ ) treatments on the Microbial Load of African star apple fruit stored for 15 Days**

Treatments	Storage Days	Bacterial Load (CFU/g) $\times 10^3$	Fungal Load (SFU/g) $\times 10^3$
At harvest	0	$1.67 \pm 0.67^a$	$6.00 \pm 1.16^a$
Control	15	$66.33 \pm 3.48^b$	$86.33 \pm 2.33^b$
400ppm $\text{KMnO}_4$	15	ND	ND
800ppm $\text{KMnO}_4$	15	ND	ND
Saturated $\text{KMnO}_4$	15	ND	ND

Means with the same letters within a column are not significantly different at  $p < 0.05$  using DMRT. Each value is the mean for three replicates. Abbreviations: HWT: Hot water treatment; ND: Not detected; CFU/g: colony forming units per gram; SFU/g: spore forming unit per gram.

### Conclusion

Potassium permanganate treatments contributed to maintenance of the nutritional, antioxidant qualities and microbiological attributes of African star apple fruits under storage. The gaseous molecule applied at saturated level proved to be the most efficient in maintaining fruit quality characteristics when compared to the control sample with maximum quality loss during storage at ambient temperature and  $90 \pm 5\%$  relative humidity. Samples treated with 800 ppm  $\text{KMnO}_4$  and 400 ppm  $\text{KMnO}_4$  also retained the nutritional,

antioxidant qualities and reduced microbial loads of the fruits. Higher activities of SOD and CAT were observed in treated fruits and higher activities of antioxidant enzymes lead to successful scavenging of reactive oxygen species that protect cell membranes from damage. Therefore, there is a high potential for African star apple fruits as health promoting and disease preventing source. Gaseous molecules of  $\text{KMnO}_4$  (saturated, 800 ppm and 400 ppm) may be used as postharvest treatment of African star apple fruits to maintain its nutritional, antioxidants composition and reduce

microbial counts at  $28 \pm 2$  °C and  $90 \pm 5\%$  relative humidity for a period of 15 days.

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