Bio-Deterioration, and Antioxidant Activities of *Chrysophyllum Albidum*-Linn Fruits Treated with Acetic Acid

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ABSTRACT

Chrysophyllum albidum fruits also known as African star apple (ASA), face limited availability throughout the year due to biochemical and microbial alterations. This study aimed to explore the impact of specific postharvest techniques on the antioxidant activities and microbial decay of African star apple fruits during storage. Fresh, Ripe and healthy fruits were treated with Acetic acid (AA) and then stored under controlled conditions for 15 days. The assessment encompassed microbial counts and antioxidant capacity. The outcomes revealed that fruits treated postharvest with varied concentrations of acetic acid exhibited decreased microbial counts compared to untreated counterparts. Certain microbial strains were observed on healthy and deteriorated African star apple fruits. The identified genera consist of *Aspergillus, Rhizopus, Fusarium, Mucor, Cladosporium, Saccharomyces, Alternaria, Trichophyton, Rhodotorula, Penicillium, Bacillus, Micrococcus, Proteus, Pseudomonas, and Staphylococcus.* Some of these microorganisms are pathogenic and if present in high numbers, can lead to food spoilage and potentially cause foodborne illnesses. Treated fruits demonstrated enhanced antioxidant activities and higher levels of total phenols, flavonoids, and ascorbic acid compared to untreated fruits. Variations were observed in carotenoid contents of fruits treated while glutathione activities increased in fruits treated with acetic acid, indicating varied responses to different treatments. Overall, it was concluded that all acetic acid postharvest treatments, especially at higher doses and immersion times, possess the potential to uphold the quality attributes of *Chrysophyllum albidum* fruits compared to the control groups when stored under ambient conditions of 28 ± 2 ^{o}C and 90 ± 5% relative humidity for 15 days.

Keywords: *Chrysophyllum albidum*, Acetic acid, Antioxidant, Microbial decay, Postharvest treatment

INTRODUCTION

Fruits are highly perishable, non-staple foods that account for approximately 39% of the food intake (whether fresh or processed) in developing countries of Africa [1]. They play a crucial role in nutrition, being rich sources of vitamins, minerals, dietary fibres, essential carotenoids (such as lycopene, beta-carotene, xanthophyll), flavonoids, phenolics, and other beneficial phytochemicals [2]. Fruits are integral components of a balanced diet, providing natural nutrients like protein, carbohydrates, minerals, and dietary fiber that are essential for both humans and animals [3; 4]. Regular consumption of fruits and vegetables has been linked to reduced risks of diabetes, hypertension, coronary heart disease, cancer, and stroke. Fruits offer carbohydrates in the form of soluble sugars, cellulose, and starch, serving as valuable sources of nutrients, appetizers, and food supplements in a world grappling with food scarcity issues [5].

The lack of these essential nutrients can result in widespread diseases and, ultimately, lead to fatalities. Findings from the Global Burden of Disease project in 2000 reveal that inadequate consumption of fruits and vegetables may be linked to up to 2.7 million deaths globally and account for 1.8 percent of the total global disease burden [6]. Despite the abundance of fruits and vegetables during their respective seasons, more than 50% of them are lost to wastage due to factors like deterioration in tropical conditions, high temperatures, humidity, pests, diseases, inadequate handling, and storage facilities [7]. A report by the World Resources

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Institute in 1998 highlighted that these losses not only waste food but also squander human effort, agricultural inputs, livelihoods, investments, and precious resources like water. This inefficiency often means that fruits fail to reach their full market value, resulting in reduced returns for growers individually and economic losses for the nation as a whole [8].

Storage plays a crucial role in ensuring a continuous supply of raw materials to processing lines, extending the processing season, and safeguarding specific commodities. The primary objective of postharvest technology is to develop methods that minimize product deterioration between harvest and end-use [9]. Therefore, the focus of storage studies is to achieve maximum storage capacity, often utilizing combinations of treatments [10].

Microorganisms, particularly fungi, are notorious for causing fruit spoilage, leading to reduced quality, quantity for consumption, and profits from fruit sales [11]. Fungi have been linked to postharvest decay of agricultural products in Nigeria [12]. The freshness of *Chrysophyllum albidum* fruits typically lasts only 4-8 days before becoming unsuitable for consumption due to changes in aroma, colour, taste, and appearance. Their natural microbiological quality deteriorates during storage [13]. In Africa, inadequate storage of *Chrysophyllum albidum* fruit results in significant wastage, evident in refuse dump sites and markets in Nigeria during the fruit's ripening season [14]. The deterioration of African star apple fruits is visually identifiable by discolouration at the stem end, which can spread to the edible pulp, leading to loss of firmness and fermentation [15].

Research indicates that over 30% of postharvest losses of *Chrysophyllum albidum* fruits occur within five days due to high tropical temperatures, humidity, poor postharvest handling practices, and inadequate processing and preservation techniques [16]. Preserving the freshness of *Chrysophyllum albidum* fruits for extended periods poses a significant challenge for farmers and traders [17]. To mitigate these losses, further research is needed to explore postharvest treatments that can prolong shelf-life and maintain fruit quality after harvest. Postharvest rot remains a major obstacle in extending the storage life of many freshly harvested fruits and vegetables ([18; 19].

Acetic acid, also known as vinegar, has been shown to have various beneficial effects on preserving fruits and preventing decay caused by fungi [20; 21]. Studies have demonstrated its effectiveness in inhibiting the growth of common storage fungi and reducing postharvest diseases in fruits like stone fruits, strawberries, and apples [22; 23]. Acetic acid vapour is particularly effective in killing spores of postharvest fungi, making it a valuable tool for food preservation [24]. Additionally, treatments with acetic acid have shown promising results in reducing the severity of infections in tomato fruits caused by fungi like *Alternaria alternata* and *Botrytis cinerea* [21].

This study aims to further explore the potential benefits of acetic acid postharvest treatments on *Chrysophyllum albidum* fruits, to improve storage stability, extend shelf life, and reduce wastage.

RESEARCH METHOD

Materials

All chemicals and reagents used in this study were sourced from reputable suppliers, including Sigma Aldrich (St. Louis MO), Merck (England), Avondale Laboratories Banbury, Oxon, England, and Eagle Scientific Ltd. Nottingham, NG9 6DZ, England. Key chemicals such as NaOH (Merck) (0.1 M), Hydrochloric acid (0.1 M), and 2.2-diphenyl-1-picrylhydrazyl hydrate (DPPH) 0.2 mM (Sigma-Aldrich, USA) were among the materials utilized. Additionally, essential laboratory supplies like Gram stain reagents, Oxidase strips, and various indicator solutions were included. HiMedia Laboratories GmbH, Einhausen, Germany provided the nutrient agar, MacConkey agar, and Potato-Dextrose agar for the experiments. Glass-distilled water was used throughout the procedures, and all glassware was meticulously cleaned with acid and doubly distilled deionized water (ddH_2O). The *Chrysophyllum albidum* fruit sample was officially registered with the voucher number UIH/20I6/22502 at the Botany Department, University of Ibadan, Oyo State, Nigeria.

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Procurement of *Chrysophyllum albidum* and sample preparation

This process involved harvesting fresh, mature, and ripe fruits from a local farm in Akure, Ondo State, Nigeria, early in the morning. The fruits were promptly transported to the laboratory where they underwent sorting, grading, and surface sterilization with sodium hypochlorite (500 ppm) to ensure their suitability for the research.

Application of Acetic Acid (AA) Solution and Storage of Fruits

The method described by [25] was utilized with minor adjustments for the application of Acetic Acid (AA) Solution in this study. The fruits were categorized into four treatment groups, each containing 40 fruits, and subjected to specific treatments as follows: T_0 = Unprocessed fruits were used as the negative control group, T_1 = Fruits immersed in distilled water (DW) for durations of five, ten, and 15 minutes, acting as the experimental (positive) control, while the remaining fruits were immersed in varying concentrations of acetic acid (AA) – T_2 = 1% AA, T_3 = 3% AA, T_4 = 5% AA – for dip times of 5, 10, and 15 minutes, respectively.

After treatment, all fruits were allowed to air-dry at room temperature for one hour before being placed in sealed sterile high-density polyethene (HDPE) plastic containers (10 L). These containers were stored at ambient temperatures (28 ± 2 °C) and 90 ± 5% relative humidity for a period of 15 days for further analysis. To maintain the desired humidity level, approximately 30 ml of sterile water was added to the bottom of each container to provide water vapour. Sterile plastic trays and cups were used as supports for the fruit samples inside the chamber. The rims of the container covers were sealed with petroleum jelly and covered with aluminium foil to ensure an airtight environment.

Preparation of fruit extract

Fruits were randomly chosen from both the control and treated groups. Fifty grams (50 g) from each fruit were blended in a Fisons Scientific equipment whirl mixer with the addition of 50 ml of distilled water to achieve a consistent mixture. The resulting slurry was transferred to a measuring cylinder and diluted to 100 ml using distilled water. Subsequently, the slurry samples were centrifuged for 10 minutes using an Eppendorf AG centrifuge 5418R. The supernatant was then refrigerated at 0 °C to serve as the fruit extract for future analyses, referred to as "extract" in subsequent mentions according to the procedure detailed by [26].

Quality parameters of the fruits were assessed at day zero (before treatment), at seven-day intervals, and some on the 15th day of storage to evaluate the effects of the acetic acid treatments on fruit quality over time.

Analysis of Quality Parameters

Microbiological analyses

Nutrient Agar (NA), Mannitol Salt Agar (MSA), Potato Dextrose Agar (PDA), and MacConkey Agar were prepared following the manufacturer's instructions, utilizing the methods described in references [27; 28; 29; 30; 31].

Preparation and inoculation of samples

The pour plate method, as described by [27], was utilized for culturing the fruit samples. Approximately 1.0 grams of each fruit sample was aseptically weighed using a sterile spatula and transferred into individual test tubes containing 9.0 ml of cooled sterilized water. The fruit samples in different test tubes were thoroughly mixed to ensure the even distribution of microorganisms in the suspended sterile water. A 10-fold serial dilution of each 1ml homogenate was prepared, and 1.0 ml of dilution factors 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were inoculated into sterile Petri dishes for culture. Incubation took place at 37 °C for 24 hours for bacterial growth and 25 °C for 2-4 days for fungi growth. Colonies were counted to determine the total viable count, and discrete colonies were purified by sub-culturing into new agar media. Growth was observed under a microscope and characterized using standard methods.

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Enumeration of Microbial Colonies

After the designated incubation periods, colonies that grew on Nutrient agar, Potato Dextrose agar, and MacConkey agar media were counted using a Gallenkamp Electronic Colony Counter and expressed as Colony Forming Units per gram (CFU/g) of the samples. Counts were recorded from plates supporting less than 300 colonies.

The mean count was calculated by multiplying with the appropriate dilution factor using the formula:

$$\text{Total viable count } \left(\frac{\text{CFU}}{\text{g}} \text{ or } \frac{\text{SFU}}{\text{g}}\right) = \frac{(Number\ of\ colonies) \times (dilution\ factor)}{Inoculum\ size}$$

Enumeration of Coliform Bacteria

The enumeration of total coliform bacteria was conducted following the method outlined by [28]. MacConkey agar was prepared according to the manufacturer's instructions and cooled to (45 ± 2 °C). One millilitre aliquots of each dilution were transferred onto corresponding MacConkey agar plates and spread using sterile glass spreaders. The plates were then inverted and incubated at 37 °C for 24 hours. Pinkish colonies indicated lactose-fermenting bacteria, while colourless colonies represented non-lactose-fermenting bacteria. Colonies within the range of 25-250 were counted and presented as colony-forming units per gram (CFU/g).

Identification of Isolates

Isolates from the fruit samples were identified using cultural, morphological, and biochemical characteristics observed on incubated Nutrient agar, Mannitol Salt agar, and Potato Dextrose agar.

Identification of bacterial isolates involved characterizing the isolates based on their cultural, morphological, and biochemical traits. Cultural and morphological assessments were conducted by observing the colonies directly on plates for various characteristics like size, elevation, consistency, shape, and colour, and through Gram's staining and microscopic examination. Biochemical tests on the pure isolates included the catalase test, spore staining test, citrate test, hydrogen sulphide production test, nitrate test, indole production test, motility test, sugar fermentation test, and carbohydrate utilization test. The organisms' identity was confirmed by analyzing their morphological, cultural, and biochemical features [29].

Gram's staining involved preparing a smear of each bacterial isolate on a clean slide, staining it with crystal violet, Gram's iodine, and safranin, and examining it under a microscope with oil immersion [30]. The catalase test was performed by observing bubbling and frothing in test tubes within 5 minutes of inoculation, indicating a positive result for catalase activity [31].

The hydrogen sulphide production test involved inoculating organisms into labelled tubes and observing black colouration in semi-solid agar tubes to confirm hydrogen sulphide production [32; 33].

The nitrate test included adding nitrate reagents to test tubes after incubation to detect colour changes, indicating nitrate positivity [32; 33].

The indole test was conducted by adding Kovac's reagent to culture broths and observing colour changes to determine indole production [32; 33].

The citrate utilization test involved streaking pure isolates on citrate agar and observing colour changes to differentiate between positive and negative results [32; 33; 34].

The motility test assessed the ability of bacteria to migrate away from the line of inoculation on nutrient agar, indicating motility if migration was observed [32; 33].

The sugar fermentation test involved inoculating pure cultures into test tubes containing different sugars and observing colour changes and gas production to determine sugar fermentation capabilities [30].

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Spore Staining

To perform spore staining, a heat-fixed smear of the organisms was prepared on a sterile slide. Malachite green solution was added and steamed for five to ten minutes without allowing the stain to dry out. The slide was then washed with cold water and counterstained with Safranin solution for 15 seconds. After washing and drying, the slide was examined under a microscope with an oil immersion objective of ×100. Spores appeared green, while bacteria cells stained red [34].

Carbohydrate Utilization

Bacteria produce acidic products when fermenting certain carbohydrates. Carbohydrate utilization tests detect pH changes indicating fermentation. The acid lowers the pH, turning the pH indicator (phenol red) yellow. If no fermentation occurs, the medium remains red. Gas production during fermentation is indicated by bubbles in the Durham tube. Tests include Glucose, Lactose, Sucrose, Mannitol, Raffinose, Arabinose, Xylose, among others. The procedure involves adding sterile sugar to peptone water, inoculating test tubes, and incubating for 48 hours at 37°C to observe colour changes and gas production.

The determination of vitamin C in *Chrysophyllum albidum* fruit

The vitamin C concentration in the aqueous extract was analyzed using the procedure outlined by [35]. To 500 µl of the extract mixture (comprising 300 µl of a proper dilution of the extract with 100 µl of 13.3% trichloroacetic acid (TCA) and water), 75 µl of DNPH solution (consisting of 2 g dinitrophenyl hydrazine, 230 mg thiourea, and 270 mg copper sulfate ($CuSO_4.5H_2O$) in 100 ml of 5 ml/L H_2SO_4) were added. The reaction mixture was then incubated at 37°C for 3 hours, followed by the addition of 0.5 ml of 65% H_2SO_4 (v/v). The absorbance was measured at 520 nm using a UV spectrophotometer (JENWAY 6305, Barloworld Scientific Ltd., Dunmow, Essex, UK). The vitamin C content of the extracts was determined based on ascorbic acid as a standard. The results were calculated as described below:

$$\text{Vitamin C}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{Absorbance\ of\ test\ samples\ x\ Concentration\ of\ standard}{Absorbance\ of\ standard\ x\ Concentration\ of\ samples}$$

Evaluation of overall carotenoid levels

To determine the total carotenoid content in the sample, an acetone-petroleum ether extraction method was employed, in line with the adapted technique detailed by [36]. Ten African star apple fruits were randomly chosen from both the control and treated groups. After removing the seeds, the pulp was diced into small sections for analysis. The carotenoid extraction process involved multiple steps: initially, 10 g of fruit pulp was extracted with acetone using a mortar and pestle until the solution became colourless. The acetone was then combined, extracted, and transferred to a separating funnel with approximately 20 ml of petroleum ether, gently mixed. To ensure a clear separation of the layers, 20 ml of a 5% sodium sulfate solution was added and mixed thoroughly. The aqueous and petroleum layers were separated, and the aqueous phase was re-extracted with additional petroleum ether until it became colourless. A small amount of distilled water was used to cleanse the pooled petroleum ether extract, which contained carotenoids. This extract was then combined with anhydrous sodium sulfate in a brown bottle and left to settle for roughly 30 minutes. The petroleum extract was decanted into a 100 ml volumetric flask through a cotton wool-containing funnel. Further washing of the sodium sulfate slurry with petroleum ether was done until it was colourless, and the rinse was added to the volumetric flask. The total carotenoid concentration was gauged using a spectrophotometer (General Scientific. GS-UV32PCS) at an absorbance of 503 nm, utilizing petroleum ether as a reference. The outcomes were presented in milligrams per kilogram of fresh weight (mg/kg FW) [26].

Measurement of total phenolic content in *Chrysophyllum albidum* fruit

The total phenolic content was assessed following the protocol outlined by [37]. Various dilutions of the extracts were combined with 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) and then neutralized with 2.0 ml of 7.5% sodium carbonate. The mixture underwent a 40-minute incubation at 45 °C, with the absorbance being

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evaluated at 765 nm using a spectrophotometer (JENWAY 6305). Total phenolic content was later determined using Gallic acid as a reference standard (phenol standard stock: 20 mg/ml Gallic acid) through a series of dilutions. An R_2 value of 0.998 was obtained. The total phenolic content in all samples was computed using the formula:

$$C = c\frac{V}{m}$$

where C represents the total phenolic content in mg/g in GAE (Gallic acid equivalent), c is the concentration of Gallic acid derived from the calibration curve in mg/mL, V stands for the volume of the extract in ml, and m indicates the mass of the extract in grams. The outcomes were expressed in milligrams of gallic acid equivalent (GAE) per gram of body weight [26].

Assessment of total flavonoid content in African star apple fruit

The total flavonoid content in the extract was determined with a slightly adjusted method as detailed by [38]. Specifically, 0.5 ml of suitably diluted sample was mixed with 0.5 ml of methanol, 50 ml of 10% Aluminum chloride ($AlCl_3$), 50 ml of 1 mol/l potassium acetate, and 1.4 ml of water. This mixture was then left to incubate at room temperature for 30 minutes. Subsequently, the absorbance of the reaction mixture was measured at 415 nm utilizing a spectrophotometer (JENWAY 6305). The total flavonoid content was calculated using quercetin stock (5 mg/ml) as the standard, followed by progressive dilution. The total flavonoid content was computed as follows:

$$\text{The total flavonoid content} = \frac{Absorbance\ of\ test\ samples\ x\ Concentration\ of\ standard}{Absorbance\ of\ standard\ x\ Concentration\ of\ samples}$$

The concentration of total flavonoid content in the test samples was calculated from the calibration plot (Y = 0.0162x + 0.0044, R^2 = 0.998). The results obtained were expressed as mgQE/g. (QE – Quercetin equivalent) [26].

Assessment of DPPH free radical scavenging capacity in African star apple fruit

The evaluation of free radical scavenging capacity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) was conducted following the methodology outlined by [39]. Various concentrations of the aqueous extract were placed in separate test tubes, and the volume was adjusted to 1 ml with distilled water. Subsequently, 4 ml of 0.1 mM methanolic DPPH solution was added. After vigorous shaking, the tubes were left to stand for 20 minutes at room temperature. A standard was prepared similarly as described above, minus the sample, and distilled water was utilized for baseline correction. Alterations in sample absorbance were gauged at 516 nm through a spectrophotometer (JENWAY 6305). The free radical scavenging capacity was articulated as percentage inhibition and was computed using the subsequent formula:

$$\text{Free radical scavenging ability } (\%) = \frac{[(absorbance\ of\ standard) - (absorbance\ of\ sample)]}{absorbance\ of\ standard} \text{x } 100$$

Assessment of glutathione activity in African star apple fruit

To determine the glutathione activity in the sample, an assay of glutathione peroxidase (GPX) activity was conducted, following a method adapted from [40]. The GPX activity was assessed by mixing 500 µl of phosphate buffer, 100 µl of sodium azide, 200 µl of GSH, and 100 µl of H_2O_2 with 500 µl of the sample. Subsequently, 600 µl of distilled water was added and thoroughly mixed. The entire reaction mixture was then incubated at 37 °C for 3 minutes, followed by the addition of 0.5 ml of Trichloroacetic acid (TCA) and centrifugation at 3000 rpm for 5 minutes. To 1 ml of each supernatant, 2 ml of K_2PO_4 and 1 ml of DNTB (5,5'-dithiol-bis- [2-nitrobenzoic acid]) were added, and the absorbance was read at 412 nm against a blank. The glutathione peroxidase activity was determined by constructing a standard curve, from which the concentration of the remaining GSH was extrapolated.

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GSH activity $\left(\frac{\text{IU}}{\text{ml}}\right) = mg/ml x \frac{(total\ volume\ of\ assay)\ dilution\ factor}{volume\ of\ enzyme\ used\ x\ incubation\ time}$

Statistical Analysis

Statistical analysis was conducted using Statistical Package for Social Sciences Software (SPSS) version 16.0. All analyses were performed in triplicate, and the standard error of the mean (SEM) was calculated. One-way analysis of variance (ANOVA) was utilized to identify the treatment effect. Microsoft Excel 2016 was utilized for creating graphs and charts. Means were distinguished using Duncan's multiple range test (DMRT), with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Impact of using acetic acid after harvesting on the microbial quality of *Chrysophyllum albidum* fruits

Influence of different concentrations of acetic acid on the microbial count of *C. albidum*

The average microbial loads of *Chrysophyllum albidum* fruits treated with acetic acid are detailed in Table 1. The range of microbial counts observed in the treated samples was notably lower compared to the microbial loads found in the control samples. Alawlaqi and Alharbi (2014) observed that acetic acid's inhibitory effect on microorganisms is likely attributed to its impact on the cell membrane, disrupting the transport of metabolites and the maintenance of membrane potential. Fungal loads ranged from 0.01 x 10^3 SFU/g to 0.01 x 10^3 SFU/g, while bacterial loads varied from 0.01 x 10^3 CFU/g to 0.03 x 10^3 CFU/g. Elevated fungal loads of 248.33 x 10^3 SFU/g and bacterial loads of 166.0 x 10^3 CFU/g were observed in the negative control samples compared to other investigated samples. *Escherichia coli,* serving as an indicator organism, was undetected in all treatment groups.

Table 1: Illustration of the influence of postharvest treatment with acetic acid (AA) on the microbial count of *Chrysophyllum albidum* fruit stored under conditions of 28 ± 2 ºC temperature and 90 ± 5% relative humidity for 15 days.

Treatments	Storage (Days)	Bacterial Count (CFU/g) x 10^3	Fungal Count (SFU/g) x 10^3
At harvest	0	1.67 ± 0.67^{b}	6.00 ± 1.16^{b}
Control (untreated sample)		166.00 ± 9.85^{f}	248.33 ± 4.66^{f}
DWT (5 min)	15	66.33 ± 3.48^{e}	86.33 ± 2.33^{e}
DWT (10 min)	15	16.33 ± 2.33^{d}	69.00 ± 2.08^{d}
DWT (15 min)	15	8.33 ± 0.88^{c}	47.33 ± 4.33^{c}
1% Acetic Acid (5 min)	15	0.03 ± 0.01^{a}	0.01 ± 0.01^{a}
1% Acetic Acid (10 min)	15	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}
1% Acetic Acid (15 min)	15	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}

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3% Acetic Acid (5 min)	15	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}
3% Acetic Acid (10 min)	15	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
3% Acetic Acid (15 min)	15	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
5% Acetic Acid (5 min)	15	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
5% Acetic Acid (10 min)	15	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
5% Acetic Acid (15 min)	15	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}

The average ± standard error. Dissimilar superscript letters in the same column indicate significant differences at $p<0.05$ according to Duncan's multiple range test (DMRT). Each value represents the average of three replicates. Abbreviations used: DWT: Distilled water treatment; (CFU/g): colony forming units per gram; SFU/g: spore-forming unit per gram.

Microorganisms obtained from *Chrysophyllum albidum* fruits in various conditions, including healthy, deteriorated, and acetic acid treated.

Table 2 to Table 9 present the various fungi and bacteria extracted from *Chrysophyllum albidum* fruits, whether healthy, deteriorated, or treated with acetic acid. Identified genera include *Aspergillus, Rhizopus, Fusarium, Mucor, Cladosporium, Saccharomyces, Alternaria, Trichophyton, Rhodotorula, Penicillium, Bacillus, Micrococcus, Proteus, Pseudomonas,* and *Staphylococcus*, with distinctions made based on cultural, morphological, and biochemical traits. Twenty microorganism species were identified, with ten being fungal and ten bacterial isolates. The bacterial isolates are predominantly rod-shaped, except for *Staphylococcus aureus* and *Micrococcus* spp, which exhibit cocci shapes. Gram-positive bacterial isolates include *Bacillus, Micrococcus,* and *Staphylococcus*, while the Gram-negative ones are *Pseudomonas aeruginosa* and *Proteus mirabilis.*

Fungal isolates present on *Chrysophyllum albidum* fruits at harvest include *Aspergillus fumigatus*, *Rhizopus stolonifer*, and *Aspergillus niger*. Bacterial isolates found on these fruits are *Bacillus tequilensis, Micrococcus* spp, *Proteus mirabilis, Bacillus licheniformis, Pseudomonas aeruginosa*, and *Bacillus cereus*. On untreated (negative control) African star apple fruits, microbial isolates encompass *Rhizopus stolonifer, Fusarium solani, Mucor hiemalis, Aspergillus fumigatus, Aspergillus niger, Bacillus tequilensis, Staphylococcus gallinarum, Proteus mirabilis, Bacillus licheniformis, Pseudomonas aeruginosa,* and *Bacillus cereus.*

Mucor hiemalis, Rhizopus stolonifer, Cladosporium herbarum, Fusarium verticilliodes, Alternaria tenuissima, Aspergillus fumigatus, Aspergillus niger, Bacillus korlensis, Bacillus badius, and *Staphylococcus* spp were the microbial isolates derived from distilled water-treated African star apple fruits (positive control). Among the commonly detected microbes extracted from spoiled African star apple fruits post-acetic acid treatment were *Alternaria tenuissima, Aspergillus niger, Fusarium verticilliodes, Penicillium chrysogenum, Saccharomyces* spp, *Rhizopus stolonifer, Cladosporium herbarum, Bacillus korlensis,* and *Paenibacillus macquarieensis.*

Table 2: Features of moulds obtained from *Chrysophyllum albidum* fruits at the point of harvest, including cultural and morphological traits.

Growth Rate	The colony's size after a week on PDA.	Colour changes as growth progresses.	Hyphae Form	Conidium Form	Probable Organisms
Average	7 mm	Teal	Vertical flask-shaped	Extending in a branched manner from the tips of the	*Aspergillus*

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			structure.	phialide.	*fumigatus*
Picky	8 mm in 4 days	Shifting from white to a brownish hue.	Vertical flask-shaped structure.	Short ellipsoidal sporangiophores originating from the tapered tip of the phialide.	*Rhizopus stolonifer*
Average	7 mm	Transitioning from a brownish shade to a powdery black.	Vertical flask-shaped structure.	Conidiophores emerging from the elongated, wide, and sturdy tip of the phialide.	*Aspergillus niger*

Key: PDA = Potato Dextrose Agar

Table 3. Characteristics of bacteria obtained from *Chrysophyllum albidum* fruits at the time of harvest, including cultural, morphological, and biochemical traits.

Spore	Gram Reaction	Shape	Catalase	H_2S Productio	Lactose	Sucrose	Glucose	Nitrate	Motility	Indole	Citrate	Arabinose	Xylose	Mannitol	Raffinose	Probable Organisms
+	+	Rod	+	-	-	+	+	+	+	-	+	+	+	+	+	*Bacillus tequilensis*
-	+	Cocci	+		-		+	+	-	-	-			+		*Micrococcus* spp
-	-	Short rod	+	+	-	-	+	+	+	-	+	-	+	-	-	*Proteus mirabilis*
+	+	Rod	+	-	-	+	+	+	+	-	+	+	+	+	+	*Bacillus licheniformis*
-	-	Short rod	+	-	-	-	-	+	+	-	+	+	+	+	+	*Pseudomonas aeruginosa*
+	+	Rod	+	-	-	+	+	+	+	-	+	+	+	+	-	*Bacillus cereus*

Key: - = Negative to the test, + = Positive to the test

Table 4. Traits of moulds separated from natural *Chrysophyllum albidum* fruits (utilized as a negative control), encompassing cultural and morphological features.

Growth Rate	Diameter of Colony after 7 Days on PDA	Colour changes as growth progresses.	Hyphae Form	Conidium Form	Probable Organisms
Fussy	8 cm in 4 days	From a white hue to a brownish colour.	Upright phialide.	Sporangiophores which are short ellipsoidal arise from the pointed end of the phialide.	*Rhizopus stolonifer*
Average	7 cm	Transitioning from white to pink.	Short, broadly ending non-	Conidiophores richly branched but not in spirodochia usually one-	*Fusarium solani*

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			proliferating phialides.	celled macro-conidia.	
Picky	At 25 ^{o}C on PDA 8 cm in 3 days	White to yellow tater dark grey black sporangia.	Upright phialide.	Branched out from the phialide.	*Mucor hiemalis*
Average	7 cm	Bluish-green.	Upright phialide.	Conidiophores branched out from the end of the phialide.	*Aspergjllus fumigatus*
Average	6 cm	Brownish to black powdery.	Upright phialide.	Conidiophores arise from the long broad thick – walled end of the phialide.	*Aspergillus niger*

Key: PDA = Potato Dextrose Agar

Table 5. Cultural, Morphological and Biochemical Characteristics of Bacteria Isolated from Untreated *Chrysophyllum albidum* Fruits (Negative Control)

Spore	Gram Reaction	Shape	Catalase	H_2s Productio	Lactose	Sucrose	Glucose	Nitrate	Motility	Indole	Citrate	Arabinose	Xylose	Mannitol	Raffinose	Probable Organisms
+	+	Rod	+	-	-	+	+	+	+	-	+	+	+	+	+	*Bacillus tequilensis*
-	+	cocci in clusters	+	+	-	+	+	+	-	-	+	+	+	+	+	*Staphylococcus gallinarum*
-	-	Short rod	+	+	-	-	+	+	+	-	+	-	+	-	-	*Proteus mirabilis*
+	+	Rod	+	-	+	+	+	+	+	-	+	+	+	+	+	*Bacillus licheniformis*
-	-	Short rod	+	-	-	-	-	+	+	-	+	+	+	+	+	*Pseudomonas aeruginosa*
+	+	Rod	+	-	-	+	+	+	+	-	+	-	-	-	-	*Bacillus cereus*

Key: - = Negative to the test, + = Positive to the test

Table 6. Cultural and Morphological Characteristics of Moulds Isolated from *Chrysophyllum albidum* Fruits Treated with Distilled Water (Positive Control)

Growth Rate	Diameter of Colony after 7 Days on PDA	Pigmentation During Growth	Hyphae Form	Conidium Form	Probable Organisms
Fastidious	At 25 ^{o}C on a PDA 8mm in 3 days	White to yellow tater dark green black sporangia.	Upright phialide.	Branched out from the phialide.	*Mucor hiemalis*
Fastidious	8 cm in 4 days	White to	Upright	Sporangiophores which	*Rhizopus*

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		brownish.	phialide.	are short ellipsoidal arise from the pointed end of the phialide.	*stolonifer*
Slow growing	At 25 ^{o}C on a PDA 27-30 mm in 7 days	Velvet and olive green to olive-yellow.		Condiophores are light brown and smooth at the basal end turning olive brown and finely verrucose at the top.	*Cladosporium herbarum*
Moderate	7 cm	White to pink	Short, broadly ending non-proliferating phialides.	Conidiophores richly branched but not in spirodochia usually one-celled macro-conidia.	*Fusarium verticilliodes*
Slow Growing	At 25 ^{o}C on PDA 38-40 mm in 7 days	Cool white fluorescent light/darkness		Conidia are produced in long, unbranched chains via short, apical secondary conidiophores.	*Alternaria tenuissima*
Moderate	7 cm	Bluish green	Upright phialide	Conidiophores branched out from end of the phialide	*Aspergjllus fumigatus*
Moderate	6 cm	Brownish to black powdery.	Upright phialide	Conidiophores arising from long broad thick – walled end of the phialide.	*Aspergillus niger*

Key: PDA = Potato Dextrose Agar

Table 7. Cultural, Morphological and Biochemical Characteristics of Bacteria Isolated from *Chrysophyllum albidum* Fruits Treated with Distilled water (Positive Control)

Spore	Gram Reaction	Shape	Catalase	H_2s Productio	Lactose	Sucrose	Glucose	Motility	Indole	Arabinose	Xylose	Mannitol	Gas	Raffinose	Probable Organisms
+	+	Long Rod	+	+	+	+	+	+	-	A/G	A/G	A/G	-	A/G	*Bacillus korlensis*
+	+	Long Rod	+	+	-	-	-	+	-	-	A+/-	-	-	A+/-	*Bacillus badius*
-	+	Cocci	+	-	A/G	A/G	A	-	-	A/G	A/G	A/G	-	A/G	*Staphylococcus* spp

Key: - Negative to the test, + Positive to the test, A = Acid, G = Gas, AG = Acid and Gas

Table 8: Cultural and Morphological Characteristics of Moulds Isolated from *Chrysophyllum albidum* Fruits Treated with Different Concentrations of Acetic Acid.

Growth Rate	Diameter of Colony after 7 Days on PDA	Pigmentation During Growth	Hyphae Form	Conidium Form	Probable Organisms
Slow	At 25 ^{o}C on PDA 38-40	Cool white fluorescent		Conidia are produced in long, unbranched chains via	

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Growing	mm in 7 days	light/darkness		short, apical secondary conidiophores.	*Alternaria tenuissima*
Moderate	6 cm	Brownish to black powdery	Upright phialide	Conidiophores arise from the long broad thick –walled end of the phialide.	*Aspergillus niger*
Moderate	7 cm	White to pink	Short, broadly ending non-proliferating phialides	Conidiophores richly branched but not in spirodochia usually one-celled macro-conidia.	*Fusarium verticilliodes*
Slow	3 mm in 7 days	Grey–green reverse intensely yellow	Upright phialide which forms basipetal chains of dry conidia.	Penicillate conidiophores branched out as verticillate phialide.	*Penicillium chrisogenum*
Moderate	5 mm	Creamy	Pseudohypha formed	The ovid shape produced laterally mycelium	*Saccharomyces* spp
Fastidious	8 cm in 4 days	White to brownish	Upright phialide	Sporangiophores which are short ellipsoidal arising from the pointed end of the phialide.	*Rhizopus stolonifer*
Slow Growing	At 25 ^{o}C on a PDA 27-30 mm in 7 days	Velvet and olive green to olive yellow		Conidiophores are light brown and smooth at the basal end turning olive brown finely verrucose at the top.	*Cladosporium herbarum*

Table 9: Cultural, Morphological and Biochemical Characteristics of Bacteria Isolated from *Chrysophyllum albidum* Fruits Treated with Different Concentrations of Acetic Acid

Spore	Gram Reaction	Shape	Catalase	H_2S Production	Lactose	Sucrose	Glucose	Motility	Indole	Arabinose	Xylose	Mannitol	Gas	Raffinose	Probable Organisms
+	+	Long Rod	+	+	+	+	+	+	-	A/G	A/G	A/G	+	A/G	*Bacillus korlensis*
+	+	Long Rod	+	-	+	+	+	-	-	-	A+/-	A+/-	-	A+/-	*Paenibacillus macquarieensis*

Key: - Negative to the test, + Positive to the test, A = Acid, G = Gas, AG = Acid and Gas

Antioxidant properties of *Chrysophyllum albidum* Fruits treated with acetic acid (AA)

Results from figure 1 to 6 revealed that after treatment with 1% acetic acid for 5 and 10 minutes, there was a boost in total phenol (mgGAE/g), vitamin C (mg/g), total flavonoids (mgQE/g), and GSH (IU/min) contents. However, the DPPH scavenging ability, and carotenoid content decreased in the fruits following acetic acid postharvest treatments for 15 days of storage at room temperature.

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Treatment with 1% acetic acid for 10 minutes resulted in a notable increase in the average total phenol content (16.97 mgGAE/g). The highest vitamin C levels (47.34 mg/g) were found after using 3% acetic acid for 5 minutes. The greatest DPPH scavenging ability (82.76%) was observed in fruits treated with 1% acetic acid for 15 minutes. Total flavonoids (2.95 mgQE/g) peaked at 3% acetic acid for 15 minutes. The highest carotenoid content (0.79 mg/kg) was detected in fruits treated with 5% acetic acid for 10 minutes, while the maximum GSH level (325.42 IU/min) was seen with 5% acetic acid for 15 minutes compared to the control group.

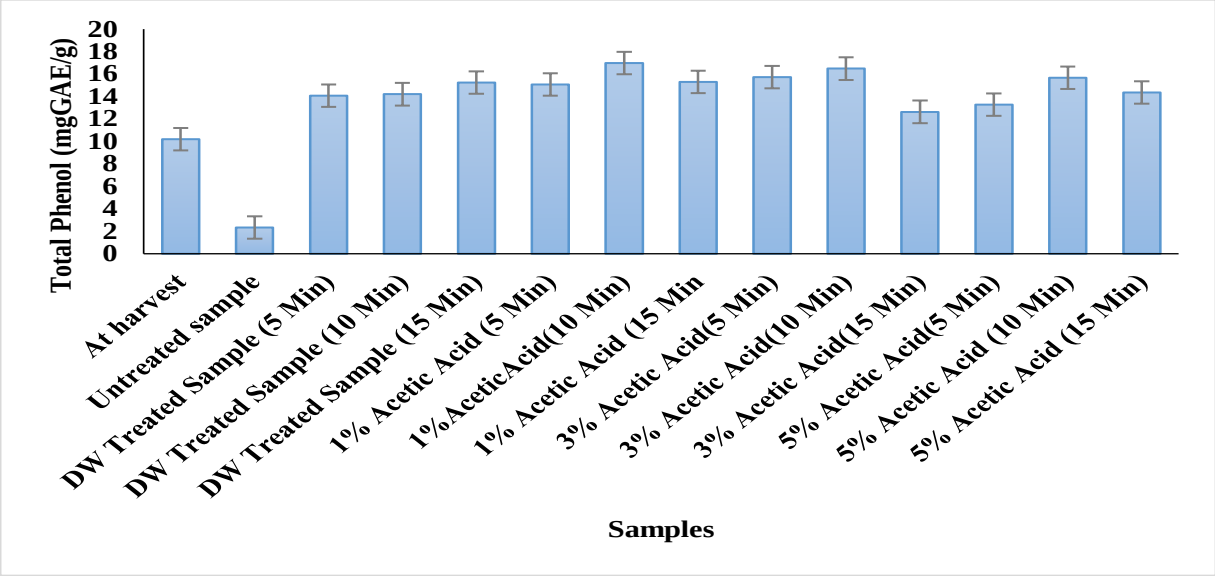

Figure 1: The impact of applying acetic acid (AA) after harvest on total phenol activities of African Star Apple (*Chrysophyllum albidum*-Linn) fruits stored at 28 ± 2 °C and 90 ± 5% relative humidity for 15 days is shown. The bars indicate the mean ± standard error of mean.

* DW: Distilled water

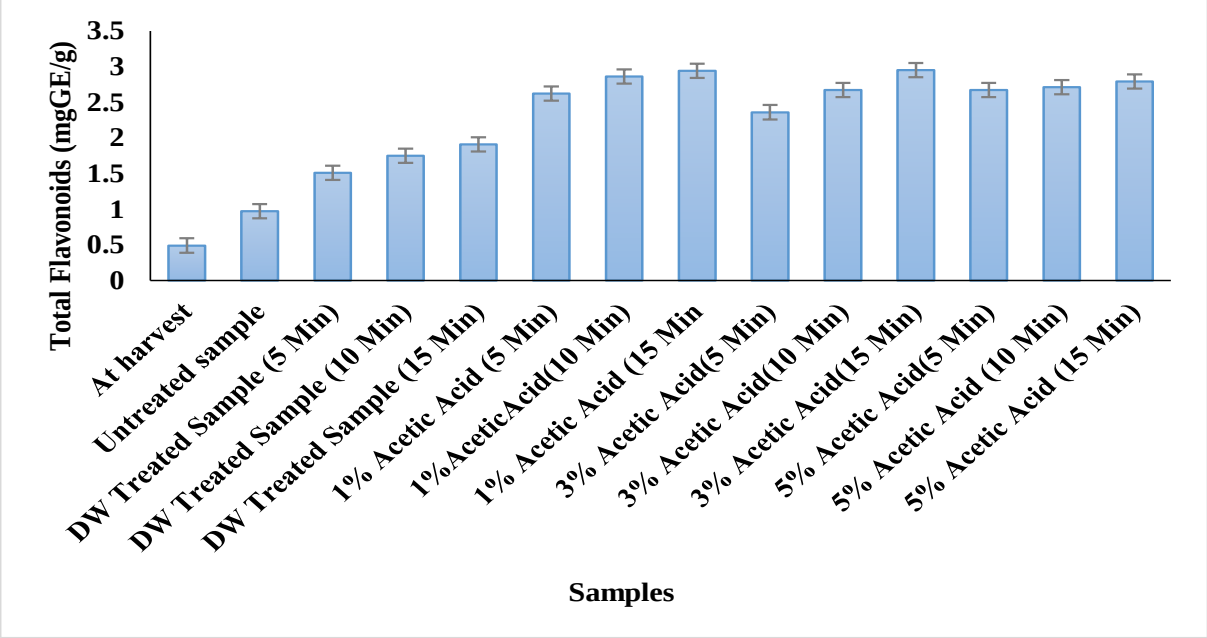

Figure 2: The impact of applying acetic acid (AA) after harvest on total flavonoids activities of African Star Apple (*Chrysophyllum albidum*-Linn) fruits stored at 28 ± 2 °C and 90 ± 5% relative humidity for 15 days is shown. The bars indicate the mean ± standard error of mean.

* DW: Distilled water

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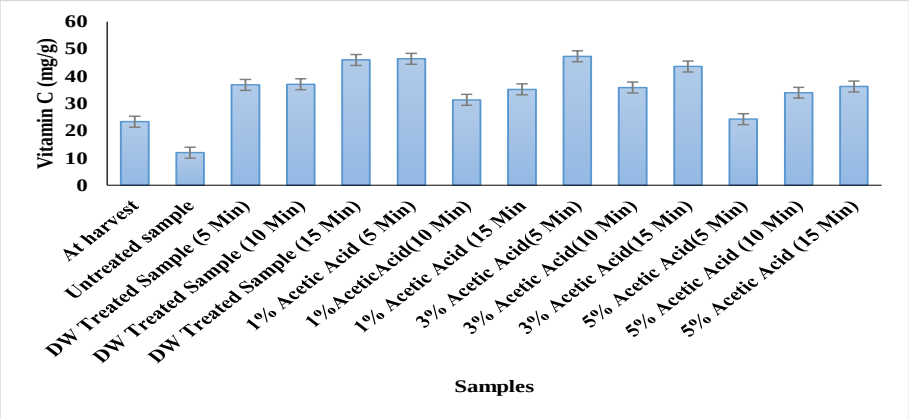

Figure 3: The effect of applying acetic acid (AA) after harvest on the vitamin C content of African Star Apple (*Chrysophyllum albidum* Linn) fruits stored at 28 ± 2 °C and 90 ± 5% relative humidity for 15 days. The bars represent the mean values with their corresponding standard error of the mean.

*DW: Distilled water

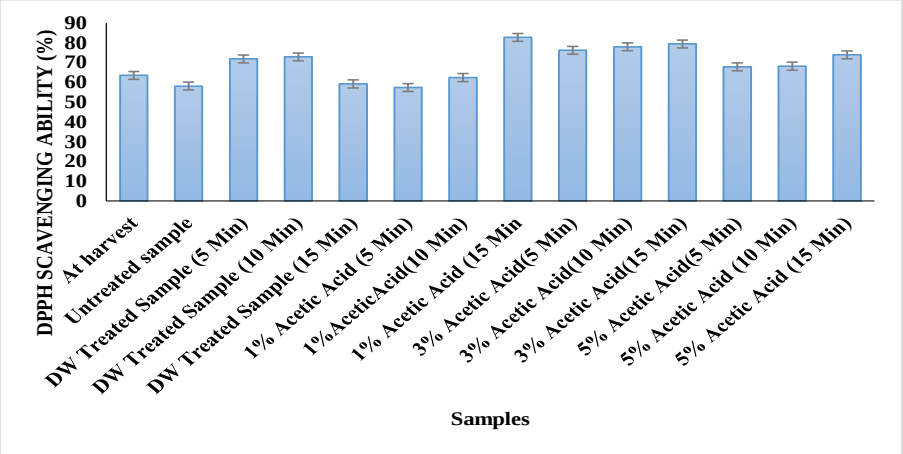

Figure 4: The impact of applying acetic acid (AA) after harvest on DPPH Scavenging Ability of African Star Apple (*Chrysophyllum albidum*-Linn) fruits stored at 28 ± 2 ºC and 90 ± 5% relative humidity for 15 days is shown. The bars indicate the mean ± standard error of mean.

* DW: Distilled water

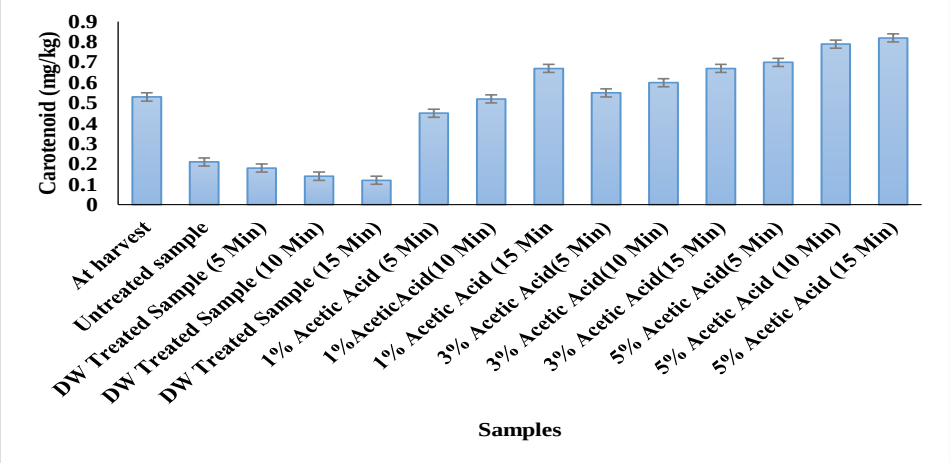

Figure 5: The impact of applying acetic acid (AA) after harvest on the Carotenoid content of African Star Apple (*Chrysophyllum albidum*-Linn) fruits stored at 28 ± 2 ºC and 90 ± 5% relative humidity for 15 days is shown. The bars indicate the mean ± standard error of mean.

* DW: Distilled water

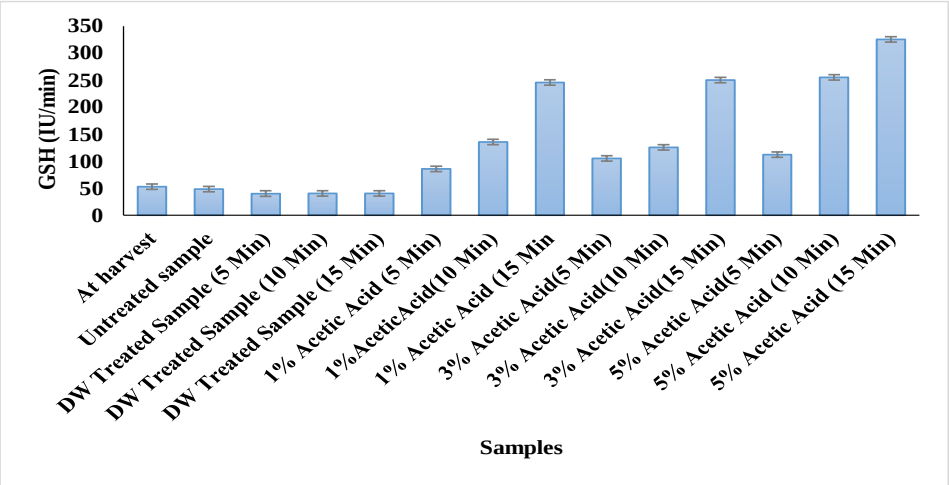

Figure 6: The impact of applying acetic acid (AA) after harvest on the Carotenoid content of African Star Apple (*Chrysophyllum albidum*-Linn) fruits stored at 28 ± 2 ºC and 90 ± 5% relative humidity for 15 days is shown. The bars indicate the mean ± standard error of mean.

* DW: Distilled water

DISCUSSION

This study demonstrated that different concentrations of acetic acid significantly reduced the microbial counts of *Chrysophyllum albidum* fruits. These findings align with [41]'s research, which illustrated that treatment with varying concentrations of acetic acid (5%, 10%, and 15%) for holding times of 5, 10, and 15 minutes led to a substantial decrease in microbial populations on certain sliced fruits and vegetables.

The microorganisms identified on *Chrysophyllum albidum* fruits at harvest, the deteriorated control samples, and the acetic acid-treated samples were consistent with those documented by [42], [43], [12], and [17], except for *Aspergillus tamari, Botryodiplodia theobromae, Lasiodiplodia theobromae, Aspergillus repens, Mucor mucedo, Penicillium citrinum, Bacillus polymyxa,* and *Escherichia coli.*

The majority of microorganisms identified in this research have previously been documented as pathogens causing fruit rot [12]. The presence of these microorganisms in African star apple fruits could lead to diminished market value, compromised quality, and fruit safety concerns, ultimately resulting in significant economic losses.

Food manufacturers commonly utilize acetic acid as an antimicrobial preservative or acidulant in various food items, deemed environmentally safe [21]. Acetic acid vapour has been proven highly effective in eradicating spores from postharvest fungi responsible for the rot in different fruits and grains. [44] noted that acetic acid vapour, at proper concentrations, significantly reduced or completely halted mycelial growth and spore germination in prevalent storage fungi such as *Alternaria* spp., *Aspergillus flavus, A. niger, A. terreus, Botrytis cinerea, Fusarium moniliforme,* and *Penicillium* spp. Additionally, fumigation utilizing acetic acid vapour has been implemented to combat tomato fruit rots induced by *Alternaria alternata, A. niger*, and *B. cinerea* [21].

Acetic acid's inhibitory impact on microorganisms surpasses that of pH alone, as the undissociated form of acetic acid can infiltrate microbial cells, eliciting its harmful effects [45]. Out of the range of organic acids available, acetic acid is relatively safe, cost-effective, and easily accessible, rendering it a potent and budget-friendly antimicrobial agent for eradicating foodborne pathogens [46]. Fruits commonly face microbial contamination due to exposure to soil, water, and handling during harvesting.

As stated by [38], the antioxidants found in *Chrysophyllum albidum* fruits could enhance the fruit's ability to scavenge free radicals, which play crucial roles in different disease processes and inflammatory conditions,

potentially leading to liver necrosis. The presence of these bioactive compounds with antioxidant properties in dietary sources might help reduce the risk of cardiovascular diseases, as indicated by [47].

Flavonoids, potent water-soluble antioxidants and radical scavengers play vital roles in preventing oxidative cell damage, exhibiting strong anti-cancer properties, and inhibiting tumour growth [38]. The presence of phenolic compounds in *C. albidum* fruits suggests the presence of antimicrobial agents [38]. With its high levels of ascorbic acid and other potent antioxidants, this fruit stands out as having significant health-promoting potential [38]. Phenolic compounds are known for their bacteriostatic and fungistatic properties [48]. The fruit's high ascorbic acid content contributes to its tart flavour, while low levels of ascorbic acid have been linked to fatigue and increased susceptibility to respiratory infections [49]. [50] noted that the abundance of phenolic substances in the fruit is crucial for maintaining the stability and accumulation of ascorbic acid.

The use of acetic acid in postharvest storage has economic implications primarily due to its questionable effectiveness and potential negative effects on fruit quality. Research indicates that repeated treatments with acetic acid may not preserve fruit adequately, leading to potential financial losses for producers [51]. (Venditti et al., 2017).

Environmentally, acetic acid alters the storage atmosphere by decreasing oxygen levels and increasing carbon dioxide, which could have complex ecological impacts [52]. (Zhao et al., 2024). While this method can prevent postharvest decay, it also raises health concerns and could negatively affect the quality of the fruit [53]. (Thewes et al., 2021). The accumulation of compounds like ethanol during storage has been linked to acetic acid usage, which may further complicate the environmental assessment of this practice.

Correct application of acetic acid may significantly extend the shelf life of fruits by inhibiting microbial growth, which can lead to reduced waste and spoilage. Properly preserved fruits may maintain quality, which may command higher prices in the market. Minimising losses due to spoilage can lead to substantial financial savings, particularly for high-value crops. Using acetic acid can be more environmentally friendly than synthetic preservatives, appealing to a growing market of health-conscious consumers. While, Incorrect application may lead to fruit spoilage or reduced quality, resulting in financial loss.

CONCLUSION

The findings of this study highlight the potential of acetic acid postharvest treatments in mitigating the deterioration of *Chrysophyllum albidum* fruits. By inhibiting the growth of common storage microbes and reducing postharvest rot, acetic acid offers a promising solution to prolonging the storage life of these fruits. Further research and application of acetic acid treatments could significantly reduce wastages and losses, ultimately benefiting both farmers and consumers. This study underscores the importance of exploring innovative postharvest preservation methods to enhance food security and economic sustainability in agricultural practices. If the cost of implementation (material, labour, among others) is less than the benefits gained from reduced spoilage, increased sales, and market access, then large-scale application of acetic acid can be economically beneficial. The specific figures will depend on local market conditions, fruit types, and operational efficiency. While acetic acid has been used as a postharvest treatment for fruit storage, its economic viability is challenged by its effectiveness and potential adverse effects on fruit quality. Environmentally, it modifies storage conditions which can lead to further complications. Continuous research is essential to ensure the safe and effective use of acetic acid in postharvest processes.

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