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The effect of *Aloe vera* Gel on Microorganisms Associated with the Deterioration of Sweet Orange Fruits (*Citrus sinensis*)

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors OOO and AOO conceived and designed the study. Author OOO carried out the bench work. Authors AOO and OBB managed the literature searches and wrote the first draft of the manuscript. Authors OOO, AOO and OBB analyzed the data the study, agreed with manuscript results and conclusions. Author OBB made critical revisions and approved final version. All authors read and approved the final manuscript.

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ABSTRACT

The effect of *Aloe vera* gel on microorganisms associated with the deterioration of sweet orange (*Citrus sinensis*) fruits was investigated. Sweet orange fruit was obtained from selected markets and farm in Akure, Nigeria. Microorganisms associated with the deterioration were identified using microbiological techniques. A total of nine fungi and six bacteria were isolated from the orange fruits. Fungal isolates include *Aspergillus flavus*, *A. niger*, *Fusarium oxysporium*, *Penicillum digitatum*, *Rhizopus stolonifer*, *Penicillium italicum*, *Mucormucedo*, *Saccharomyces cerevisiae* and *Geotrichium candidum*, while the bacterial isolates were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *B. substilis*, *Serratia marcesens* and *Pseudomonas aeruginosa*. The average fungal counts ranged from 2.4×10^3 cfu/g to 5.3×10^3 sfu/g and bacterial count ranged from 1.4×10^5 cfu/g to 3.6×10^5 cfu/g. Pathogenicity test revealed that *Rhizopus stolonifer, Penicillum*

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digitatum, Penicillum italicum, Aspergillus flavus, Fusariun oxysporium were able to induce spoilage in apparently healthy orange fruits. At 100% concentration of *Aloe vera* gel, *E. coli* and *Saccharomyces cerevisiae* demonstrated the highest susceptibility among bacterial and fungal isolates respectively. This study revealed that *Aloe vera* gel was partially effective in controlling the growth of bacterial and fungal isolates associated with the deterioration of sweet orange. Good agricultural practices, adequate storage facilities and good handling practices must be put in place to reduce the incidence of microbial spoilage of sweet oranges to increase agricultural output, profits and maintain food security.

Keywords: Orange fruits; microorganisms; Aloe vera gel; food preservation; food security.

1. INTRODUCTION

Citrussinensis also known as sweet orange is one of the fruits of the citrus species in the family *Rutaceae*. It originated in Southeast Asia but consumed all over the world as an excellent source of vitamin C, a powerful natural antioxidant that builds the body immune system. Important phytochemicals like liminoids, synephrine, hesperidin flavonoid, polyphenols, pectin, and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium are also present in orange fruits [1]. These biologically active compounds prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health. Citrus is widely grown in Nigeria and many other tropical and subtropical regions [2].

Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in the human daily diet to maintain a good health [3]. The exposure of food to oxygen, light, warmth or even small amounts of moisture can often trigger a series of damaging chemical and or microbial reactions. Sweet orange fruits, however, have severe challenges to their existence and these include changes in climatic condition, pests, inadequate rainfall and fungal attack [4].

The improper handling, packaging,storage and transportation may result in decay and growth of microorganisms [5]. Sweet orange fruit, due to their low pH, higher moisture content and nutrient composition are very susceptible to attack by pathogenic fungi, which in addition to causing rots, may also make them unfit for consumption by producing mycotoxins [6].

However, it is essential to preserve fruits properly to avoid microbial deterioration, and it has been estimated that around 25% to 80% of harvested fresh fruits are wasted due to spoilage [7]. (*Aloe vera* is one of the natural substances that contain antibacterial anti-inflammatory, anti-viral, antioxidant [8], (and antifungal substances [9,10]. The *Aloe vera* gel is made up of water, amino acids, vitamins, lipids, sterols, tannins, and enzymes and contains phenol, saponin, anthraquinones components [11]. *Aloe vera* gel can be applied as edible coatings for fruits as its biological activities prevent loss of moisture, firmness, control respiration rate and maturation development, delay oxidative browning, and reduce microorganism proliferation [12,13].

This study was aimed at determining the effect of *Aloe vera* gel on bacterial and fungal isolates associated with the deterioration of sweet orange fruits. This is to gain a better understanding of how the shelf-life of orange fruits may be improved in order to ensure the availability of sweet orange all year round especially in low-income countries where there are inadequate storage facilities.

2. MATERIALS AND METHODS

2.1 Collection of Orange Fruit Samples

A total of 123 orange fruit samples were obtained from the Federal University of Technology, Akure, 23 orange fruit were collected from research farm, 40 fruit samples were obtained from fruit vendors and 60 fruit samples were collected from two open markets (Oja-Oba and Isikan) in Akure, Ondo State. The fruits were separately packaged, labelled and carefully transported immediately to the laboratory for analysis within an hour.

2.2 Isolation of Bacteria from Sweet Orange Fruits Using the Pour Plate Method

Infected portions of the fruits were sliced into pieces, transferred into the sterile distilled water

from which serial dilutions were carried out. 1g of each sample was suspended into 90 ml of sterile water to make a stock and serially diluted. A 0.1 ml aliquot of the serially diluted sample was pour plated in triplicate with freshly prepared Nutrient Agar media and incubated at 37˚C for 24 h. Discrete colonies were observed, counted, recorded as colony forming unit per gram (CFU/g) and further sub-cultured using streak plate technique to obtain pure cultures. The pure isolates were characterised and identified using the methods described by Zimbro et al. [14].

2.3 Isolation of Fungi from Sweet Orange Fruits pour Plate Method

The samples were serially diluted using sterile distilled water and homogenized. 0.1 ml aliquot of the third dilution (10^{-3}) was dispensed on sterilized potato dextrose agar (PDA) in Petri dishes and incubated for seven days at ambient temperature. Mycelia of the isolated fungi were inoculated on a slide, two drops of lactophenol cotton blue were added. Fungi isolates were characterized and identified based on their colonial morphology and microscopic characteristics at a magnification of ×40 objective lens [15].

2.4 Pathogenicity Tests of Bacterial and Fungal Isolates on Sweet Orange Fruits

The bacterial and fungal strains were tested on healthy orange fruits for their ability to induce spoilage. The fruits were washed with tap water and rinsed with distilled water after which they were surface sterilized with 75% ethanol and then inoculated with10^{-2} of 24 h old culture of bacterial isolates whereas the spores of 48 h old fungal isolates were injected into the fruits and the control fruits were injected with water. The various points of inoculation were sealed with petroleum jelly to prevent contamination and the inoculated fruits were observed for symptom development. The etiological agents were re-isolated from the infected orange fruits and compared with the original isolates using standard methods [6].

2.5 *Aloe vera* gel Extraction and Determination of Phytochemicals in the *Aloe vera* Gel

Freshly harvested leaves of *Aloe* leaves were washed with distilled water. The matrix was separated from the outer cortex of the leaves and the colourless liquid was homogenized in a blender. The resulting mixture of the solution was filtered appropriately according to the method described [7]. The fresh *Aloe vera* gel was pasteurized at 70ºC for 45 min and cooled immediately to an ambient temperature. Phytochemicals such as tannins, anthraquinones, phlobatannins, steroids, tarpenoids, flavonoids, alkaloids, saponins and cardiac glycosides in the *Aloe vera* gel were determined using standard methods.

2.6 Coating of Sweet Orange Fruits with Varying Concentrations of *Aloe vera* Gel and Determination of Antibacterial and Antifungal Activities of the Gel

Fresh, mature, green orange fruits without any visible blemish were dipped completely into the coatings solutions of *Aloe vera* gel (100%, 75% and 50%) at room temperature for 25 min. The fruits were allowed to drain and then dried at room temperature to allow a thin film layer to be formed on the fruits. The fruits were then stored at room temperature and at 13ºC. Orange fruits that served as control were not dipped into the coating solutions *Aloe vera* gel. Thereafter, weight loss of the orange fruits, antibacterial and antifungal activities of the *Aloe vera* gel were determined using standard methods.

2.7 Proximate and Mineral Composition of Sweet Orange Fruits and those Coated with *Aloe vera* Gel

In the proximate analysis, parameters such as the percentage of moisture, ash, protein, carbohydrate, fat and fibre content of the orange fruits were determined using standard method. Minerals such as sodium and potassium were determined by flame photometry while calcium, magnesium and iron were determined by atomic absorption spectrophotometer [7].

2.8 Statistical Analysis of Data

Data was presented as mean ± standard error (SE). The significance of differences between different treatment groups was tested using one-way analysis (ANOVA) using SPSS (Statistical Package for Social Science) version 20 software. For all test, the significance was determined at the level of $P<0.05$.

3. RESULTS

3.1 Characterization and Identification of Bacteria from Sweet Orange Fruits

The bacteria isolatedfrom sweet orange fruits were *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Serratia marcescens* and *Bacillus cereus* (Table 1). The mean bacterial count from sweet orange fruits ranged from 1.40×10^3 CFU/g to 3.60×10^3 CFU/g. Sweet orange fruits obtained from Oja Oba had the highest bacterial count while those obtained from the Federal University of Technology, Akure, Research farm had the least bacterial count. *Bacillus subtilis* had highest percentage occurrence (19.3%) while *Serratia marcescens* had least percentage occurrence (12.9%) (Table 2).

3.2 Characterization and Identification of Fungi from Sweet Orange Fruits

The fungi isolated from sweet orange fruits were *Aspergillus flavus, A. niger, Penicillium digitatum, Rhizopus stolonifer, Mucor mucedo, Fusarium oxysporium, Saccharomyces cerevisiae, P. italicum, Geotrichum candidum* and *Alternaria* spp. (Table 3). The mean fungal count from sweet orange fruits ranged from 2.40×10^3 sfu/g to 5.36×10^3 sfu/g. Similarly, sweet orange fruits obtained from Oja Oba had the highest fungal count while those obtained from the Federal University of Technology, Akure, Research farm had the least fungal count. *Aspergillus niger* had the highest percentage occurrence (25.3%), while *Saccharomyces cerevisiae* had the least percentage occurrence (2.9%) (Table 4).

3.3 Pathogenicity Test of Bacterial and Fungal Isolates on Fresh Sweet Orange Fruits

The pathogenicity test of bacterial and fungal isolates on fresh sweet orange fruit samples carried out over a five-day period showed that fungal isolates demonstrated the higher magnitude of deterioration compared with bacterial isolates. *Alternaria, Candida,* and *Saccharomyces cerevisiae* were not able to grow on the fresh sweet orange. However, from day 1 to day 5, *Rhizopus stolonifer, Penicillium digitatum, Penicillium italicum, Aspergillus flavus, Fusarium oxysporium* were able to grow with similar growth characteristic features to the original deteriorated orange fruit samples. On the other hand, *E. coli*, *Bacillus cereus* and *Serratia marcescens* only appeared on the sweet orange on the fifth day whereas *Pseudomonas aeruginosa* was not isolated from the sweet oranges throughout the period of test (Table 5).

3.4 Phytochemical Composition of *Aloe vera* Gel

The qualitative phytochemical constituents of the *Aloevera* gel showed that tannins, flavonoids, alkaloids, anthraquinones, terpenoids and saponin were present while cardiac glycoside, phlobatannin and steroids were absent (Table 6).

3.5 Effect of *Aloe vera* Gel on the Weight of Sweet Orange Fruits

There was a significant decrease in the weight of sweet orange fruits and those coated with *Aloe vera* gel over a period of 18 days. However, the rate of weight loss in sweet orange fruits without *Aloe vera* gel was greater than those coated with *Aloe vera* gel (Fig. 1).

3.6 Antibacterial Activity of *Aloe vera* gel on Isolates from Deteriorated Sweet Orange

The total count of bacteria in sweet orange fruits coated with *Aloe vera* gel was observed to be lower than those in orange fruits without *Aloe vera* gel over a 30-day period (Table 7). In addition, *E. coli* had the highest zone inhibition followed by *B. subtilis*, *B. cereus* and *Pseudomonas aeruginosa* at 100% concentration of *Aloe vera* gel (Fig. 2).

3.7 Antifungal Activity of *Aloe vera* Gel on Isolates from Deteriorated Sweet Orange

The total count of fungi in sweet orange fruits coated with *Aloe vera* gel was observed to be lower than those in orange fruits without *Aloe vera* gel over a 30-day period (Table 8). In addition, *Saccharomyces cerevisiae* had the highest zone inhibition followed by *A. flavus*, *Fusarium oxysporium*, *Penicillium digitatum*, *Geotrichum candidum* and *Penicillium italicum*at 100% concentration of *Aloe vera* gel (Fig. 3).

3.8 Proximate and Mineral Composition of Sweet Orange Fruits and those Coated with *Aloe vera* Gel

The percentage of moisture, protein, ash, fibre and fat content in fresh orange fruits appeared to

Table 1. Cultural, microscopic and biochemical characteristics of bacteria isolated from sweet orange fruits

Isolate code	Cultural characteristics			Microscopic characteristics		Biochemical characteristics													Probable identity
	Colour	Shape	Elevation	Gram reaction	Cell shape	Spore	Motility	Catalase production	Coagulase production	Oxidase production	Citrate utilization	Starch hydrolysis	Maltose	Mannitol	Glucose	Galactose	Sucrose	Lactose	
O^1	Cream	Irregular	Flat	+	Rod	+	+	+	-	-	+	+	+	+	+	-	+	-	*Bacillus subtilis*
O^2	Cream	Irregular	Flat	+	Rod	+	+	+	-	-	+	+	+	-	+	-	+	-	*Bacillus cereus*
O^3	Green	Round	Flat	-	Rod	-	+	+	-	+	+	-	-	+	+	-	+	-	*Pseudomonas aeruginosa*
O^4	Cream	Round	Raised	-	Rod	-	+	+	-	-	-	-	+	+	+	+	+	+	*Escherichia coli*
O^5	pink	Flat	Irregular	-	Rod	+	+	+	-	-	+	+	-	-	+	-	+	-	*Serratia marcesens*
O^6	Cream	Round	Raised	+	Round	-	-	+	+		+	+	+	+	+	+	+	+	*Staphylococcus aureus*

Key: + = Present/ Positive, - = Absent/ Negative

Table 2. Distribution of bacterial isolates from orange fruits collected from different locations

Fermentation of:

Bacterial isolates	LA	LB	LC	LD	Number of isolates	% of occurrence
Bacillus subtilis	+	+	+	+	12	19.3%
Staphylococcus aureus	+	-	+	+	11	17.7%
Escherichia coli	+	-	+	+	10	16.1%
Serratia marcescens	+	+	+	+	8	12.9%
Bacillus cereus	+	+	+	+	11	17.1%
Pseudomonas aeruginosa	+	-	+	+	10	16.1%

Key: + = Present, - = Absent, LA = Location A (Oja Oba); LB = Location B (FUTA Research Farm); LC = Location C (Fruit vendor); LD= Location D (Oja Isikan).

Table 3. Micro-morphological characteristics of fungi isolated from sweet orange samples

Probable fungi	Cultural characteristics and microscopic appearance
Aspergillus flavus	The colonies were green when observed under the microscope, the conidia were globose in shape, the hyphae branched and septate conidiophores were long, rough, septate and granular. The conidia heads were compact, radiate and unseriate. Each conidium was yellowish green, globose to subglobose.
Aspergillus niger	Appeared dark brown. Upon microscopic observation, the mycelium appeared whitish at first, frequently developed areas which were bright yellow. Dark brown to blackheads were compact radiate, dark brown and biseriated. The vesicles were globose, ranged from colourless to grey. The primary sterigmata in immature heads were shorter while the secondary were dark and were in short chain of two to four spores.
Saccharomyces cerevisiae	White colour raised smooth colony. Microscopic morphology showed large ellipsoidal budding cells or blastoconidia.
Rhizopus stolonifer	The colonies appeared whitish fluffy and cottony in texture. The colony turned brown as it aged. Microscopic examination revealed erect sporangiophores were smooth walled, aseptate and light brown in colour. The sporangia were globose.
Mucor mucedo	The colonies appeared black cotton-like at 24 h turning dirty with the development of black spore on mycelium, non-septate hyphae, thin sporangiospore with a sporangium in a club-like form.
Fusarium oxysporium	White mycelium which later turned pink. Hyphae were septate and hyaline. The conidiophores were short and simple. Macroconidia were slightly sickle-like shaped, thin walled with attenuated apical cell and a foot-shaped basal cell.
Penicillium italicum	Blue colonies on potato dextrose agar. It has a long slender septate branch above, above ending in hyphae which bear simple conidiopore
Penicillium digitatum	Colonies appeared yellow, green conidiophores smooth, relatively short. Penicillia mycelia arranged very irregularly and asymmetrical with branches of various lengths. Sparse and irregular metulae with phialides on them, conidia smooth and ellipsoidal.
Alternaria spp.	Dark brown blackish hyphae branched conidia formed in a long chain, ovoid, oblavate
Geotrichium candidum	Creamy and white anamorph state is characterized by hyphae that appear creeping, mostly submerged and septate. Conidia appear arthrosporous, terminal or intercalary, aerial on an agar surface.

be higher than those in the orange fruits coated with *Aloe vera* gel. Only the percentage of carbohydrate content in orange fruits coated with *Aloe vera* gel was higher than those in fresh orange fruits (Table 9). The concentration of potassium and iron in the orange fruits coated with *Aloe vera* gel appeared to be higher than those in fresh orange fruits, whereas the concentration of magnesium, sodium and calcium in fresh orange fruits appeared to be higher than those in the orange fruits coated with *Aloe vera* gel (Table 10).

Table 4. Distribution of fungal isolates from orange fruit collected from different locations

Fungal isolates	LA	LB	LC	LD	Number of isolates	% of occurrence
Aspergillus flavus	+	+	+	+	68	18.3%
Aspergillus niger	+	+	+	+	94	25.3%
Penicillium digitatum	+	+	+	+	36	9.7%
Rhizopus stolonifer	+	-	+	+	34	9.2%
Mucor mucedo	+	-	-	+	17	4.6%
Fusarium oxysporium	+	-	+	+	23	6.2%
Saccharomyces cerevisiae	+	-	-	+	11	2.9%
Penicillium italicum	+	+	+	+	48	12.9%
Geotrichium canditum	+	+	-	+	12	4.0%
Alternaria spp.	+	+	-	+	25	6.7%

Key: + = Present, - = Absent, LA = Location A (Oja Oba); LB = Location B (FUTA Research Farm); LC = Location C (Fruit vendor); LD= Location D (Oja Isikan).

Table 5. Pathogenicity test on fresh and healthy citrus fruit samples

Isolates	Day 1	Day 2	Day 3	Day 4	Day 5
Aspergillus flavus	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Penicillum italicum	+	+	+	+	+
Rhizopus stolonifer	+	+	+	+	+
Fusarium oxysporium	+	+	+	+	+
Alternaria alternata	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	-
Mucor mucedo	-	-	-	+	+
Candida	+	-	-	-	-
Penicillium digitatum	+	+	+	+	+
Escherichia coli	-	-	-	-	+
Pseudomonas aeruginosa	-	-	-	-	-
Bacillus cereus	-	-	-	-	+
Serratia marcescens	-	-	-	-	+

Key: + = Detected; - = Not detected

Table 6. The qualitative phytochemical constituents of *Aloe vera* gel

Phytochemical constituents	Inferences
Tannins	+
Flavonoid	+
Cardiac glycoside	-
Alkaloids	+
Anthraquinones	+
Phlobatannin	-
Terpenoids	+
Steroids	-
Saponin	+

Key: + = Present, - = Absent

Table 7. Concentration of bacteria in sweet orange fruits and those coated with *Aloe vera* gel

DAY	Coated orange fruits (CFU/g)	Fresh orange fruits (CFU/g)
Day 1	1.1×10^5	1.5×10^5
Day 5	1.1×10^5	3.3×10^5
Day 10	1.5×10^5	3.9×10^5
Day 15	2.0×10^5	4.3×10^5
Day 20	2.2×10^5	9.6×10^5
Day 25	3.2×10^5	8.1×10^5
Day 30	5.0×10^5	7.2×10^5

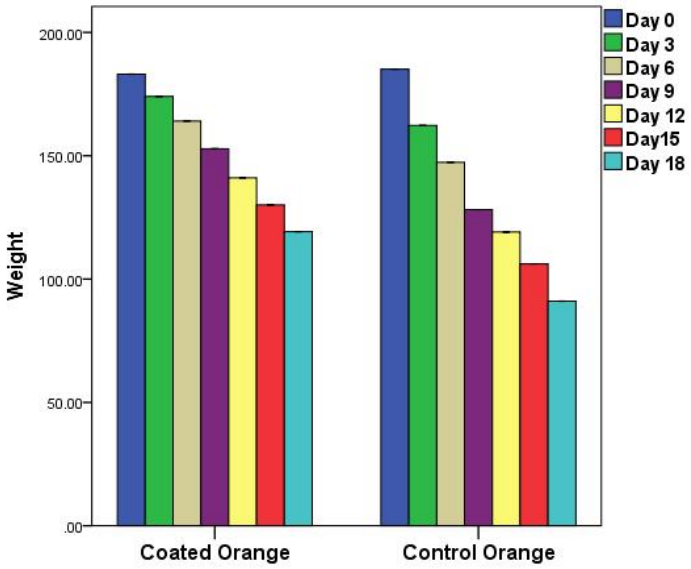

Fig. 1. Weight of sweet orange fruits and those coated with *Aloe vera* gel

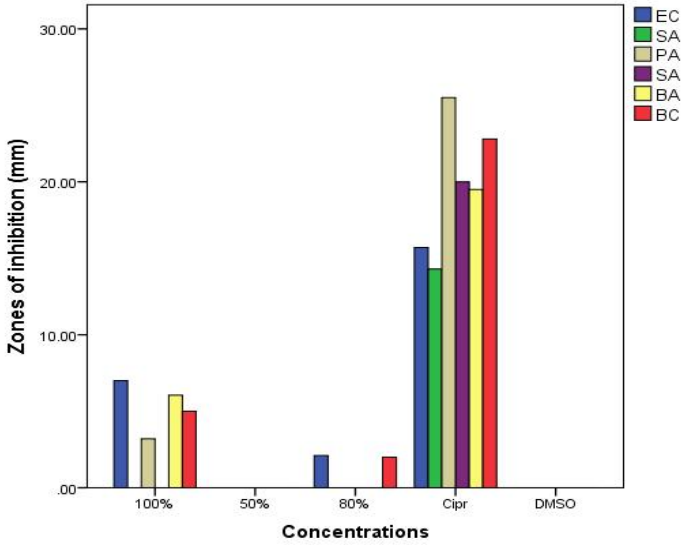

Fig. 2. Effect of *Aloe vera* gel on isolated bacteria from deteriorated sweet orange samples

Key: EC- Escherichia coli; SA- Staphylococcus aureus; PA- Pseudomonas aeruginosa; SA- Serratia marcescens; BA- Bacillus subtilis; BC- Bacillus cereus; Cipr - Ciprofloxacin; DMSO – Dimethyl Sulfoxide

Table 8. Concentration of fungi in sweet orange fruits and those coated with *Aloe vera* gel

DAY	Coated orange fruits (sfu/g)	Fresh orange fruits (sfu/g)
Day 1	2.00×10^3	3.00×10^3
Day 5	2.40×10^3	4.10×10^3
Day 10	1.20×10^3	3.20×10^3
Day 15	1.16×10^3	1.40×10^3
Day 20	1.10×10^3	1.30×10^3
Day 25	1.20×10^3	1.80×10^3
Day 30	1.03×10^3	1.27×10^3

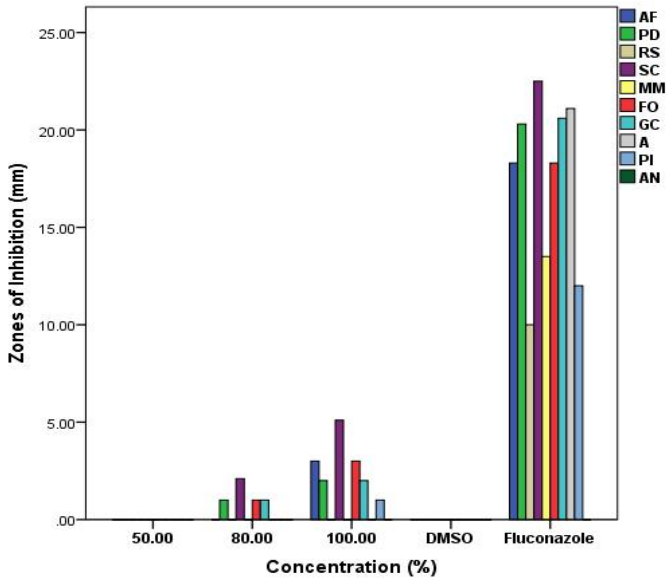

Fig. 3. Effect of *Aloe vera* gel on isolated fungi from deteriorated sweet orange samples
Key: AF - Aspergillus flavus; A - Alternaria spp.; PD - Penicillium digitatum; PI - Penicillium italicum; RS - Rhizopus stolonifer; AN - Aspergillus niger; SC - Saccharomyces cerevisiae; MM - Mucor mucedo; FO - Fusarium oxysporium; GC - Geotrichum candidum; DMSO – Dimethyl Sulfoxide

Table 9. Proximate composition of fresh sweet orange fruits and those coated with *Aloe vera* gel

Proximate composition (%)	Coated orange fruits	Fresh orange fruits
Moisture content	85.18 ± 0.08^a	89.22 ± 0.04^b
Protein content	10.05 ± 0.04^a	11.25 ± 0.03^b
Ash content	1.81 ± 0.03^a	2.08 ± 0.04^b
Carbohydrate content	9.02 ± 0.00^b	8.75 ± 0.00^a
Fibre content	11.30 ± 0.00^a	13.60 ± 0.00^b
Fat content	3.35 ± 0.00^a	3.94 ± 0.0^b

Legend: Data are presented as Mean ± SD (n=2) from triplicate determinations, different superscripts in the same column are significantly different (P< 0.05)

4. DISCUSSION

The microorganisms associated with the deterioration of sweet orange fruits sold in two major markets (Oja Oba and Isikan) in Akure, fruit vendor and FUTA Research farm were studied and the result revealed the presence of fungi and bacteria. The array of these microorganisms observed in the orange fruit samples may be as a result of the nutrient rich nature of the fruits, thus supporting the growth and proliferation of the microorganisms. The

Table 10. Mineral composition of fresh sweet orange fruits and those coated with *Aloe vera* gel

Mineral composition (mg/100g)	Coated orange fruits	Fresh orange fruits
Potassium	192.16± 0.05[b]	185.08±0.07[a]
Magnesium	10.11 ± 0.02[a]	13.86±0.05[b]
Sodium	25.38±0.13[a]	27.38±0.09[b]
Calcium	23.17±0.04[a]	25.15±0.04
Iron	1.24±0.04[b]	0.85±0.03[a]

Legend: Data are presented as Mean ± SD (n=2) from triplicate determinations, different superscripts in the same column are significantly different (P< 0.05)

orange fruits from Oja Oba had the highest bacterial and fungal count, while those from FUTA Research farm had the least bacterial and fungal count. The high microbial count in orange fruits from Oja Oba may be as a result of improper sanitary and hygienic practices in the market.

The percentage frequency of occurrence showed that *Aspergillus niger* (25.3%) occurred in all the orange fruits from all the sampling locations. This observation is in agreement) where the authors reported that *Aspergillus* spp. is the predominant microorganism associated with the spoilage of orange. Webber and Reuther [16] also reported that deteriorated oranges sampled from Na'ibawa yan Lemu Market in Kano were observed to be massively infected with six genera of fungi namely *Fusarium, Aspergillus, Candida, Rhizopus, Penicillium* and *Mucor*. The occurrence of these organisms may be attributed to their ability to produce resistant spores, as reported by West and West [17] that *Aspergillius* generally grow at higher temperatures Similarly, Wilson et al. [18] reported that *Alternaria* sp. causes black rot in citrus fruits, *Aspergillus* species causes brown rot of citrus fruits and pineapple, *Penicillium* species causes blue and green mould rots of citrus fruits, apples, grapes, pears and also brown rot of pineapple, *Aspergillus* species and *R. stolonifer* causes watery, soft rot of apples, pears, stone fruits and grapes. The principle of the spread of fungal infection in fruits supports that a single infected orange can be the source of infection to other oranges during storage and on transit [19]. The presence of the fungi or their resistant spores is most likely to have originated from the farms where the fruits were harvested and some from the stores due to horizontal contamination by the already spoilt fruits.

The incidence of *Pseudomonas aeruginosa* could be adduced to its nutritional versatility [20]. *Pseudomonas aeruginosa, E. coli,* and *Staphylococcus aureus* in fruits may be as a result of human contamination during handling. The occurrence of *Staphylococcus aureus* in orange fruits contamination has been reported to be associated with faecal matters through inadequate human handling processes [21]. While the incidence of *B. subtilis* and *B. cereus* are indicative of environmental contamination of the fruits as the fruits are constantly exposed to air, aerosols and dust particles during the course of selling the fruits which in most cases take days to weeks. The poor hygienic standard and improper handling of the fruits may be responsible for the occurrences of bacteria in fruits [21]. *P. digitatum, R. stolonifer,* and *A. niger* were found to be associated with spoilage or deterioration of orange fruits in Ibadan [22].

The pathogenicity test of bacterial and fungal isolates on sweet orange fruits during the five-day deterioration process that demonstrated fungal isolates recording higher magnitude compared with bacteria may be a result of the ability of the fungi species to survive in the oranges especially when the environmental conditions are favourable, producing spores, toxins and enzymes. Fungal pathogens, *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Penicillium italicum, Fusarium oxysporium* were present from day one to five while other fungi and bacteria recorded slow growth at the early days of culturing. *Saccharomyces cerevisiae, Alternaria alternata* and *Pseudomonas aeruginosa* were unable to induce spoilage. Different spoilage types were observed on re-infection of healthy oranges with a pure isolate of fungi species. This observation is similar to [10] who reported that different spoilage types when healthy oranges were re-inoculated with the pure isolates of the pathogens.

The phytochemical composition of the extracts of *Aloe vera* indicated the presence of alkaloids, saponin, terpenoids, Anthraquinones, flavonoids, tannins and saponin. *Aloe vera* gel is rich in a wide variety of secondary metabolites, such as anthraquinone glycosides, glycoproteins, gamma-lanoline acid, prostaglandins and

mucopolysaccharides, these are mainly responsible for the antimicrobial activity (Adetunji, 2000). Anthraquinones presented antimicrobial activity against *Staphylococcus aureus, Escherichia coli*, through inhibition of solute transport in membranes [1].

The coated orange fruit showed a rapid decrease in weight than the control orange. Weight loss occurred due to water loss by transpiration and loss of carbon reserves due to respiration [10]. The rate at which water is lost depends on the water pressure gradient between the fruit tissue and the *Aloe vera* gel (100%) has been used to preserve papaya fruit at room temperature 25˚C-29˚C and 82-84% relative humidity. Throughout storage, the weight loss of uncoated fruit (sample) was significantly greater than that of *Aloegel* coated fruit. The reduction in weight loss for coated oranges was probably due to the effects of these coatings as a semi-permeable barrier against oxygen carbon-dioxide, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates [12]. Postharvest weight changes in fruits and vegetables are usually due to the loss of water through transpiration [17]. This loss of water can lead to wilting and shriveling which both reduce a commodity's marketability.

The total plate counts of uncoated samples increased than the coated orange during deterioration. The decrease in the plate count of coated fruit with *Aloe vera* compared to the uncoated fruit may be as a result of the antimicrobial properties of the *Aloe vera* which inhibited the growth of microorganisms. *Aloe vera* gel-based edible coatings have been shown to prevent loss of moisture and firmness, control respiratory rate and maturation development, delay oxidative browning, and reduce microorganism proliferation on sweet cherries [2]. As with other edible coatings, *A. vera* gel prevented moisture loss and controlled respiratory gases exchange. *Aloe vera* gel combined with calcium chloride was effective in reducing microorganism proliferation [4].

The result of the proximate analysis of healthy sweet orange revealed an increase in moisture content, crude protein and ash content along with variation in other nutrients. This may be attributed to the degrading activity of different microorganisms during deterioration of the orange fruit [11]. The decrease in carbohydrate content of deteriorated orange fruit stored at room temperature might be due to fermentation caused by microbes and the utilization of carbohydrate by the microorganism during metabolism [19] have also reported that fermented shea butter fruit is low in carbohydrate content. Tsuda et al. [11] attributed the steady decline in starch contents of stored *Dioscorea rotundata* tubers to the respiratory loss of sugars as carbon dioxide.

Mineral content analysis showed higher calcium, magnesium sodium in deteriorated fruits than healthy fruits. The availability of these minerals in the fruits is an indication of the rich nature of the fruits with the essential elements. Agrio [3] posited that infection and deterioration of the fruits by pathogens may lead to an increase in mineral content and decrease in metabolic synthetases of African star apple fruits.

5. CONCLUSION

This study has demonstrated that various microorganisms involved in the deterioration of sweet orange. It showed that the samples contained a considerable amount of protein, fat, moisture, carbohydrate, ash and crude fibre which made the samples a potential food supplement. It also contains certain mineral such as calcium, sodium, magnesium, iron and potassium. To effectively reduce the deterioration of orange fruit, *Aloe vera* gel-based coating can be used as a green alternative to synthetic coatings and other postharvest chemical treatments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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