ANTIMICROBIAL POTENTIAL OF EDIBLE CHITOSAN-STARCH BASED COMPOSITE ON SOME BACTERIA AND FUNGI ISOLATED FROM DETERIORATED EGGPLANT AND TOMATO FRUITS

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Abstract
In developing countries like Nigeria, post-harvest loss of fruits and vegetables have been a serious problem as they become unavailable out of season due to poor preservative techniques. The study aimed at evaluating the antimicrobial potency of edible chitosan**-**starch based composite on some bacteria and fungi isolated from deteriorated eggplant and tomato fruits. The tomato and eggplant fruits were coated using dipping method while the microorganisms were isolated and identified using standard laboratory procedures. The microbial load of deteriorated fruits showed that tomato had the highest total bacterial, fungal and coliform counts (6.01±0.26 x 10^4, 2.83±0.07 x 10^4 and 3.27±0.11 x 10^4 cfu/g respectively). The bacteria isolated from deteriorated tomato and eggplant were *Bacillus megaterium, Staphylococcus. aureus, Escherichia. coli* and *Klebsiella* sp. while fungi were *Aspergillus* spp, *Fusarium* spp., *Rhizopus* spp. and *Saccharomyces* spp. However, *S. aureus* and *Aspergilus* spp. had the highest percentage occurrence (56.52 and 50.00 %, respectively). The zone of inhibition of *B. megaterium* growth increased with increase in the volume (thickness) of the film with the highest (40 ml) being 30 mm. Coat volumes of 25 and 30 ml showed the highest activity of 15 mm zone of inhibition against *Rhizopus* spp. The antimicrobial propertis of the film showed activity against the microorganisms except for *S. aureus* and *Aspergilus* spp. respectively.

Keywords: Chitosan, Starch, eggplant, tomato, coat/film, *Aspergilus, S. aureus*

Introduction
Fruits and vegetables are vital to the health and well-being of man as they are good reserve of essential vitamins, minerals, fibers and other health - promoting phytochemicals (Saranraj *et al.,* 2016). Their consumption has dramatically increased in Nigeria by more than 30 % during the past few decades. It is also estimated that about 20 % of all fruits and vegetables produced are lost each year due to activities by spoilage microbes. These spoilage microorganisms can be introduced in the crop during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution. Both bacteria and fungi have been isolated from fruits and fruit products (Saranraj *et al.,* 2012).

The preservation of fruits is a continuous fight against microbes spoiling the food or making it unsafe for consumption. Several food preservation system such as heating, refrigeration and addition of synthetic antimicrobial compounds have been used in recent times to reduce the effects of these microorganisms on the fruits. However, these techniques have associated shortcomings, which may be in the form of characteristics loss of nutrients. As a result, this calls for urgency in the need for other friendly alternative measures that can effectively preserve these fruits and retain their characteristic features (Seema, 2015).
It has been reported that chitosan has strong antimicrobial effects that could effectively control fruit decay due to its coating forming feature on fruit and vegetable. By this, there is reduced respiration in the fruits as a result of regulation of the permeability of carbon dioxide and oxygen (Elsabee *et al*., 2013). Considering the superior properties of chitosan, it has been successfully used in preserving and extending the shelf life of many postharvest fruits and

vegetables, such as garden eggs, berry, tomatoes, jujube and fresh-cut lotus root (Jianglian, and Shaoying, 2013). Despite several reports of the antimicrobial properties of chitosan and its other composites, only scanty reports on these applications has been reported in Nigeria. This study therefore attempts to show the antimicrobial property of edible chitosan-starch based composite in fruit preservation.

Materials and Methods

Collection of Samples

The deteriorating fruit samples (eggplant and tomato) were obtained from Kure market, Minna, Niger State, Nigeria for the isolation and identification of bacterial species. The samples were transported to the laboratory of the Department of Biochemistry, Federal University of Technology, Minna and stored for further analysis. Cassava roots were procured from the local market in Bosso, Minna, Niger State, Nigeria while Oyster mushroom chitosan was obtained from Beijing, China (Wisapple Biotech. Co., Ltd).

Extraction of Starch

The cassava tubers were peeled and cut into small pieces. They were steeped in water for 24 hours. The solution was decanted, and the cut cassava ground into a slurry. The slurry was screened through a muslin cloth and washed thoroughly with distilled water. After 6 hr, the supernatant was decanted from the filtrate and the settled starch layer, re-suspended in distilled water and centrifuged at 2800 rpm for 10 min. The upper non-white layer of the residue was scraped off and the white layer re-suspended in distilled water, centrifuged and the starch was collected and dried at 50 ^{o}C in the oven (Gallenkamp Hotbox 2, England) (Kaur *et al.,* 2007).

Preparation of Edible Composite Coatings

The composite film was prepared using a modified method of Singh *et al*. (2015). To 100 ml of 2 % acetic acid, 1.0 g of chitosan was added and allowed to properly dissolve. Starch gel was made by dissolving 0.5 g of cassava starch into 100 ml of boiled distilled water. To the chitosan and starch solution, 0.5 g of glycerol was added with continuous stirring and heating (60 ^{o}C) for 45 minutes using a magnetic stirrer. The resulting solution was then filtered through a sterile cheese cloth and then poured into different glass petri dishes at varying volumes (20,30, 40, 50, 60 and 70 ml) and allowed to dry at 40 °C in an incubator.

Enumeration of Microbial Load

A ten-fold serial dilution of each of the samples was carried out. Spread plate technique was employed by inoculating 0.1 ml aliquot aseptically from the 10^{-3} and 10^{-4} dilutions onto nutrient agar plates for enumeration of bacteria count. The agar plate was incubated at 37 °C for 24 - 48 hours for bacterial count. Each sample was inoculated in duplicate agar plates and the mean values of bacterial was recorded as colony forming unit per gram (cfu/g).

Enumeration of Coliform Bacteria from the Deteriorated Fruits

This was carried out as described by Food Safetey and Standards of India (2012). Three tubes containing 10 ml pre-autoclaved MacConkey broth (MCB) and an inverted Durham tubes were inoculated with 1 ml of the liquid homogenate (1:10). Similar procedures were carried out for the first (1:100) and the second (1:1000) dilution tubes using a sterile pipette. The MCB tubes were then incubated at 37 °C for 48 hours. Afterwards, the tubes were checked for colour change of the indicator (bromocresol purple) from purple to cream and for gas production in the inverted Durham tubes.

Bacterial isolation from Fruit and Vegetable
A sterile blade and forceps were used to cut small section of the tissue containing the rotten portion. The cut potions were pounded with pestle and mortar to make paste. 0.1 milliliter aliquots of the serially-diluted samples were introduced into plates containing sterile nutrient agar and spread uniformly with a sterile glass rod. The plates also had ketoconazole at a concentration of 0.05 mg/ml to inhibit fungal growth (Samuel *et al.,* 2016). Incubation was carried out in an inverted position at 28^{o}C for 24 hours for the development of the bacterial colonies.

Isolation of Coliform Bacteria
0.1 milliliter aliquots of the serially-diluted samples were introduced into culture plates of Eosin Methylene Blue (EMB) Agar containing Ketoconazole at a concentration of 0.05 mg/ml to inhibit fungal growth and spread evenly with a sterile glass rod. Incubation was carried out in an inverted position at 28 ^{o}C for 48 hours for the development of the coliform bacteria (Samuel *et al.,* 2016).

Characterization of Bacterial Isolates
The various colonies observed in the plates were distinguished on the basis of their cultural characteristics such as colony size, shape, color, consistency and hemolytic characteristics. Bacterial growth was sub-cultured on nutrient agar and was preserved in nutrient agar slants. The representative bacterial colonies distinguished on the basis of their cultural characteristics were smeared thinly on grease free microscopic slide and Gram-stained. The stained smears were then examined microscopically under the oil immersion objectives lens (Bergey *et al.,* 1994).

Isolation of Fungi from Deteriorated Fruits
Spoilt fruits surface was washed with distilled water to enhance removal of dirt. A small portion of the spoilt area of the fruits was cut in and out using a sterile scalpel and inoculated onto a freshly prepared nutrient agar plate. The inoculated plates were incubated at 30 ^{o}C for 5 days and were observed for fungal growth and later sub-cultured for another 10 days at 30 ^{o}C on nutrient agar. Resulting colonies were then sub-cultured onto Potato dextrose agar (PDA) until pure isolates were obtained Udoh *et al.,* 2015).

Morphological identification of the Isolates from Deteriorated Fruits
The organisms were identified based on their morphological characteristics and was examined for colony colour, shape, texture and elevation according to the method described by Etok *et al.* (2004) and Tankeshwar (2013). Their characteristics were compared with known taxa (Bergey *et al.,* 1994)

***In vitro* antibacterial (Zone of Inhibition)**
Antibacterial activity test on coatings was carried out using the agar diffusion method (Balouiri *et al.,* 2016). The zone of inhibition assay on solid media was used for determination of the antibacterial effects of the film against the isolated bacteria and fungi. The edible coat was cut into 6-mm diameter discs and then placed on nutrient agar plates, which had been previously seeded with 0.2 ml of inoculums containing approximately 10^{5}-10^{6} CFU/ml of tested bacteria. The plates were then incubated at 37 ^{o}C for 24 h. After that, the plates examined for "zone of inhibition" on the coat discs. Inhibition zones were measured on bases of the average diameter of the clear area, directly on the dishes.

Results
Table 1 presents the microbial load of deteriorated eggplant and tomato fruits. The total bacterial count was observed to be higher in eggplant (8 x 10^{3} cfu/g) than in the tomato fruit

(6 x 10^3 cfu/g). Similarly, eggplant had the highest total coliform count (4.3 x 10^3 cfu/g) than was observed in tomato (2.83 x 10^3 cfu/g). However, for total fungal count, tomato fruit was higher (3.27 x 10^4 cfu/g) than eggplant (2.12 x 10^4 cfu/g). Characterization and biochemical identification of bacteria from deteriorated tomato and eggplant is presented in Table 2. The identified bacterial isolates from tomato fruit were *Bacillus megaterium, Staphylococcus aureus* and *E. coli* while isolates from spoilt eggplant were *Bacillus megaterium, Staphylococcus aureus* and *Klebsiella* spp. Identification of fungi from spoilt eggplant and tomato fruits (Table 3) showed that *Aspergillus* spp. and *Fusarium* spp. were present in the tomato while *Rhizopus* spp., *Saccharomyces* spp. and *Fusarium* spp. were present in deteriorated eggplant samples.

The frequency and percentage occurrence of the isolated bacteria is as shown in Table 4. A total of 23 bacteria were isolated from both fruit samples of which *Staphylococcus aureus* had the highest occurrence 13(56.52 %) while the lowest was *E. coli* (8.70 %). The bacterial isolates *Bacillus megaterium* and *Klebsiella* spp. had occurrences of 5(21.74 %) and 3(13.04 %). The fungal occurrence as presented in Table 5, showed that 33(50 %) of the total population of fungi isolated from the fruits was *Aspergillus* spp. The fungal isolates: *Fusarium* spp., *Saccharomyces* spp. and *Rhizopus* spp. had occurrences of 15(22.72 %), 13(19.70 %) and 5(7.58 %) respectively. The antibacterial activity of the edible composite film with varying coat volumes is presented in Table 6. The result showed that the zone of inhibition of *B. megaterium* growth increased with increase in the volume (thickness) of the film with 40 ml showing the highest zone of inhibition (30 mm). However, decrease in zone of inhibition set in with increase in coat volume. Similar trend was observed for *Klebsiella* spp. with 40 ml coat showing the highest activity (19 mm) although 70 ml coat showed no activity. The coat (25-40 ml) showed no activity on *S. aureus* except for coat volume of 50-70 ml with 50 and 60 ml having the same activity (13 mm) while 70 ml coat showed the least zone of inhibition. The zone of inhibition (antibacterial activity) decreased with increase in coat volume with the lowest coat volume (25 ml) having the highest activity (25 mm) on *E. coli.* However, coat volumes of 50 and 60 ml showed similar zones of inhibition (15 mm).

The antifungal activity of edible chitosan-starch based film is presented in Table 7. The film showed no activity on *Aspergillus* spp. in all the various volumes of coat except for 40 ml. The film's activity against *Fusarium* spp. decreased with increase in coat volume from 30 ml which showed the highest activity with 20 mm zone of inhibition. Coat volumes of 25 and 30 ml showed the highest activity of 15 mm zone of inhibition against *Rhizopus* spp. while 70 ml coat produced the lowest zone of inhibition (10 mm) against the same fungi. Similar trend was observed for *Saccharomyces* spp. except that 40 and 50 ml coat showed similar zone of inhibition (16 mm), however 25 ml coat showed the highest activity (20 ml) while 70 ml coat displayed the least activity (11 mm).

Table 1: Total Microbial Load of Deteriorated Eggplant and Tomato Fruits

Sample	Eggplant	Tomato
Total bacterial count (CFU/g)	8.00±0.18 x 10^3	6.01±0.26 x 10^4
Total coliform count (CFU/g)	4.30±0.25 x 10^3	2.83±0.07 x 10^4
Total fungal count (CFU/g)	2.12±0.23 x 10^4	3.27±0.11 x 10^4

Table 2: Biochemical Identification of Bacteria from Deteriorated Eggplant and Tomato Fruit

Sample code	Gram reaction	Shape	Catalase test	Coagulase test	Starch Hydrolysis	Mannitol Salt Agar	Citrate	M. R	V. P	Probable Organisms
T1	**+**	Rods	**+**	**-**	**+**	**-**	**+**	**-**	**+**	*Bacillus megaterium*
T2	**+**	Cocci	**+**	**+**	**-**	**+**	**+**	**+**	**-**	*Staphylococcus aureus*
T3	-	Rod	**+**	**-**	**-**	**-**	**-**	**+**	**-**	*E. coli*
Eg1	**+**	Rods	**+**	**-**	**+**	**-**	**+**	**-**	**+**	*Bacillus megaterium*
Eg2	**+**	Cocci	**+**	**+**	**-**	**+**	**+**	**+**	**-**	*Staphylococcus aureus*
Eg3	**-**	Rod	**+**	**-**	**-**	**-**	**+**	**-**	**-**	*Klebsiella* spp.

T1-3 represents different randomly selected tomato fruit, **Eg1-3** represents different randomly selected eggplant

Table 3: Macroscopic and Microscopic Identification of Fungal Isolates from Deteriorated Fruits (Eggplant and Tomato)

	Macroscopy	**Microscopy**	**Fungal isolates**
T1	Greenish, filamentous with profuse proliferation of black velvety spores	Septate hyphae, branched condiophore with secondary branches. The condiophore is enlarged at the tip forming rounding vesicle-like chains	*Aspergillus* spp.
T2	Greenish, filamentous with profuse proliferation of black velvety spores	Septate hyphae, branched condiophore with secondary branches. The condiophore is enlarged at the tip forming rounding vesicle-like chains	*Aspergillus* spp.
T3	Initially white and cottony but later develop pink centre with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia, condiophores bear conidia singly or in cluster.	*Fusarium* spp.
Eg1	Cottony white, filamentous, coenocytic, stolons, rhizoids	Ovoid sporangiospores, tall sporangiospores in groups	*Rhizopus* spp.
Eg2	Colonies of Saccharomyces sp. grow rapidly. They are flat, smooth, moist glistening or dull, and cream to tannish cream in color	Multilateral budding is typical Pseudohyphae, if present are rudimentary. Hyphae are absent. Saccharomyces sp. produces ascospores.	*Saccharomyces* spp.
Eg3	Initially white and cottony but later develop pink centre with a lighter periphery.	Septate hyphae with canoe-shaped macroconidia, condiophores bear conidia singly or in cluster.	*Fusarium* spp.

T1-3 represents different randomly selected tomato fruit, **Eg1-3** represents different randomly selected eggplant

Table 4: Percentage Occurrence of Bacterial Isolates

Bacterial Isolates	Frequency (Total number of colonies)	Occurrence (%)
Bacillus megaterium	5	21.74
Staphylococcus aureus	13	56.52
E. coli	2	8.70
Klebsiella spp.	3	13.04
Total	**23**	**100**

Table 5: Percentage Occurrence of Fungal Isolates

Fungal Isolates	Frequency (Total number of colonies	Occurrence (%)
Aspergillus spp.	33	50.00
Fusarium spp.	15	22.72
Rhizopus spp.	5	7.58
Saccharomyces spp.	13	19.70
Total	**66**	**100**

Table 6: Antibacterial Activity (Zone of Inhibition, mm) of Varying Volume (Thickness) of Composite Film against isolated Bacteria from Deteriorated Fruits

		Volume of coat (Thickness)					
	Volume (ml)	25	30	40	50	60	70
Bacteria	Thickness (mm)	(0.115)	(0.121)	(0.127)	(0.131)	(0.135)	(0.138)
B. megaterium		16	21	30	26	19	16
S. aureus		NA	NA	NA	NA	13	10
E. coli		25	23	20	17	15	15
Klebsiella spp.		13	15	19	12	10	NA

NA- No Activity

Table 7: Antifungal Activity (Zone of Inhibition, mm) of Varying Volume (Thickness) of Composite Film against isolated Fungi from Deteriorated Fruits

		Volume of coat (Thickness)					
	Volume (ml)	25	30	40	50	60	70
Fungi	Thickness (mm)	(0.115)	(0.121)	(0.127)	(0.131)	(0.135)	(0.138)
Aspergillus spp.		NA	NA	15	NA	NA	NA
Fusarium spp.		17	20	18	17	12	12
Rhizopus spp.		15	15	13	14	12	10
Saccharomyces spp.		20	17	16	16	14	11

NA- No Activity

Discussion

Microorganisms are basic part of the fruit and vegetable epiphytic flora as such they will always be present even during consumption. Several studies have both reviewed and reported different bacteria and fungi of diverse strain isolated from eggplant and tomato fruits. Some of the isolated bacteria include *Bacillus megaterium* and *Bacillus Laterosporus*, while on the other hand some researchers found bacteria *Bacillus subtilis*, *Bacillus cereus, Bacillus aureus, Lactobacillus fermenti, Pseudomonas stutzeri, Leuconostoc* spp and *Rothia* spp. as well as fungi such as *Aspergillus flavus, Aspergillus fumigatus, Penicillium expansum, Penicillium notatum, Mucor mucedo, Monilia spp* (Chinedu and Enya, 2014; Wogu and Ofuase, 2014).

The microbial load in eggplant and tomato fruits observed in the present study is in agreement with the report of Saranraj *et al.* (2016) whose findings on the prevalence of bacterial and fungal pathogens in fruits and pickles showed high fungal and bacterial population. Also similar to the identified organisms isolated in the present work, the authors reported *S. aureus, E. coli, K. pneumonia, B. cereus* among other bacteria and fungi which included *Rhizopus, F. oxysporum, M. racemosus.* Mbajiuka *et al.* (2014) who isolated microorganisms associated with tomato and pawpaw reported a lower total bacterial count which was half that obtained in this study. However, their report on total fungal count was similar to that of this work. Although, the species of fungi isolated (*Rhizopus, Fusarium* spp*., Mucor mucido, Aspergillus* spp.) were similar to those in the present study. Ghosh (2009) reported that all the spoilt tomato samples obtained from different locations were noticed to be severely infected with fungi with *Aspergillus niger* and *Fusarium* sp. being predominant. Obunukwu *et al.* (2018) reported similar counts of total coliform, bacteria and fungi in deteriorated tomato fruit with *E. coli, Klebsiella* spp*., Bacillus* spp., and *Pseudomonas* spp. being the probable bacteria and this concurs with the findings in the present research. Tsado *et al.* (2015) who evaluated the effect of postharvest handling of selected fruits and vegetables in markets from Abuja, Nigeria reported similar bacteria and fungi loads in deteriorating eggplant during rainy season as obtained in this study. The authors reported *P. aeuriginosa* and *E. coli* as probable bacteria present in deteriorating eggplant which were similar to those isolated in the present study.

The higher microbial load observed in tomato than in eggplant may be due to several factors such as its high moisture content which is very essential for microbial growth. The soft tissue membrane of tomato may also be a possible cause for the observed higher microbial load (Montville and Mathews, 2007: Bello *et al.,* 2016). The age and storage time of the fruit or vegetable could also predispose them to microbial infestation as time would allow ageing influence the breakage or deterioration of their structural integrity. Other possible cause of microbial infestation may be from the soil, inoculation through irrigation water, through the environment, while in transportation, inoculation from poor washing/rinsing of the produce or during handling by poor uneducated processers (Ofor *et al.,* 2009).

The occurrence of bacteria and fungi showed that *Staphylococcus aureus* and *Aspergillus* spp. were predominant, however, this was not in agreement with the report of Obeng *et al.* (2018) who had *Klebsiella* spp. as the predominant bacteria in their study. Partly in contrast to the current finding, Obunukwu *et al.* (2018) who isolated and identified microbial deteriogens of some fruits reported *Bacillus* spp. as the predominant bacteria *Aspergillus* spp. was the predominant fungi. This variations may be due to several factors such as environmental, postharvest conditions, species variation among others (Ofor *et al.,* 2009).

The presence of *E. coli* signifies a probable association with faecal contamination which may be due to poor sanitation and hygienic conditions around and this pose a serious threat to public health (Saranraj *et al.,* 2012). *Staphylococcus aureus* have been reported to be the third most common cause of food poisoning in the world of which its related illnesses are as a result of the consumption of enterotoxins that it produces in foods (Andrews and Harris*,* 2000). *Fusarium* are filamentous fungi widely distributed in plants and in the soil and found in normal mycoflora infested commodities such as maize, rice, sorghum, millet bean as well as some fruits and vegetables (Chilaka *et al.,* 2018). The fungi *Fusarium* spp. produces toxins such as trichothecenes, deoxynivalenol (DON) and nivalenol among others which may give rise to allergic symptoms or become carcinogenic after long-term exposure. *Rhizopus* are termed weak parasitic fungi that inhabit a wide variety of plant and plant products such as matured fruits, vegetables, bread and cereals amongst others (Leyva-Salas *et al.,* 2017, Muhammad *et al*., 2019). They are linked to severe complications of diabetic ketoacidosis.

The antimicrobial effect of edible chitosan-starch composite observed in the present study agrees with the report of several authors who reported that chitosan as well as several of its modifications have the potential of inhibiting microbial growth (Severino *et al.,* 2015; Malinowska-Pañczyk *et al.,* 2015; Jovanović *et al.,* 2016; Escárcega-Galaz *et al.,* 2018). Unlike several reports which considered several factors/parameters of chitosan in eliciting antimicrobial effect, the present study focused on the effect of film thickness (varying volumes of coat) at a fixed concentration. There is a dearth of information on the influence of film thickness or coat volume on the antibacterial property of chitosan based films. The higher the volume of coat (with uniform concentration), the less viscous it becomes with time as well as the thicker it becomes.

The observed decrease in zone of inhibition with composite coat volume (thickness), may therefore be due to poor diffusion of the composite coat through the agar medium on which the microorganisms were inoculated (Coma *et al.,* 2002; Zivanovic *et al.,* 2005; Malinowska-Pañczyk *et al.,* 2015). Coma *et al*. (2002) and Abdollahi *et al.* (2012) in their studies reported that there was no zone of inhibition on inoculating selected microorganisms onto a chitosan solution. It may also be proposed that reduced antimicrobial activity of chitosan-starch film may be due to interactions between chitosan and starch molecules which could alter the chelating property that chitosan possesses as it aids the destabilization of outer membrane of the microorganisms (Malinowska-Pañczyk *et al.,* 2015). Another possibility may be that higher volume of the coat makes available more the starch as well as water which are vital ingredients for the growth of microorganisms as starch may serve as carbon for them and water being necessary for metabolic activities. Lozano-Navarro *et al.* (2017) who investigated the antimicrobial, optical and mechanical properties of chitosan–starch films with natural extracts reported that chitosan-starch coat exhibited only little effect on coliform, however, on incorporation with other extracts, there was greater inhibitory effect.

The antibacterial effect of the composite coat as seen against *B. megaterium, E. coli* and *Klebsiella* spp. may be solely attributed to the presence of chitosan and possibly the organic acid used in its dissolution (Jovanovi *et al.,* 2016). Chitosan has been reported to be either bactericidal (kill bacteria) or bacteriostatic (hinders bacterial growth) (Goy *et al.,* 2009). Chitosan and its other modified forms may elicit their influence on bacteria by the interaction mediated by the electrostatic force between protonated NH^{+}_{3} and negative residues from the microbial cell membrane. This may lead to or promote alteration in the bacterial cell membrane integrity as well as its permeability, as a result provoke an unstable internal osmotic balances which subsequently inhibit microbial growth (Shahidi *et al.,* 1999). There is also the possibility of this interaction to cause the hydrolysis of peptidoglycans which is a basic composition of the bacterial cell wall, leading to the leakage of intracellular electrolytes such as potassium ions and other vital constitutions in the cell (Chen *et al.,* 1998; Escárcega-Galaz *et al.,* 2018). The inactivity of the composite (25-50 ml) on *S. aureus* as shown in the present study, agrees with the reports of some authors who claimed that some bacteria are resistant to the antibacterial effect of chitosan as well as some of its modifications (Goy *et al.,* 2009; Malinowska-Pañczyk *et al.,* 2015; Singh *et al*., 2015). In contrast to this was the report of Adila *et al.* (2013) who investigated the effect of solvent types on the antimicrobial and physical properties. However, this may be due to some other factors which may include the molecular weight, degree of deacetylation as well as microbial strain amongst others (Andrews, 2001).

Chitosan activity against fungi is assumed to be fungistatic rather than fungicidal with a potential to communicate regulatory changes in both the host and fungus (Raafat *et al.,* 2008; Goy *et al.,* 2009). In the present study, the composite coat showed antifungal activity against all the fungi except *Aspergillus* spp. However this agrees with the report in the review of Goy

et al. (2009) who shortlisted *Aspergillus* spp. as a fungi resistant to chitosan fungistatic or fungicidal effect. It was reported to have a mean inhibition concentration >2000 mg/ml. This resistance may be due to the barrier system present in most chitosan resistant fungi. Palma-Guerrero *et al*. (2010) reported that the plasma membrane of such fungi form a barrier to chitosan. However, they reported a striking observation which is presently under study, the authors reported that the plasma membranes of chitosan-sensitive fungi were shown to have more polyunsaturated fatty acids than chitosan-resistant fungi. As a result we may draw a conclusion that their permeability by chitosan may be changed depending on the membrane fluidity.

Antifungal activity showed by the chitosan-starch coat in this study may be due to the diffusion of chitosan oligomers into the fungal hyphae which in turn interferes with several enzymatic and metabolic activities of the microbe and therefore inhibit spore germination, germ tube elongation and radial growth (El Ghaouth *et al.,* 1992; Goy *et al.,* 2009). In general, it is worthy of note that absence of zone of inhibition should not be an outright conclusion of inactivity. Malinowska-Pańczyk *et al.* (2015) reported in their finding that although there were no zones of microbial inhibition, growth of microbes under chitosan discs was drastically reduced.

Conclusion

On isolation of microorganisms from deteriorated tomato and eggplant, the isolated bacteria include *B. megaterium, S. aureus, E. coli* and *Klebsiella* sp. while fungi were *Aspergillus* spp, *Fusarium* and *Saccharomyces* spp. although, *S. aureus* and *Aspergilus* spp. were the predominant bacteria and fungi respectively. Chitosan-starch composite film developed in this study, showed antimicrobial activities however, *S. aureus* and *Aspergilus* spp. showed resistance. The antimicrobial activity of the film was not basically on the basis of it being a covering that shields the fruits from the microorganisms but majorly due to the effect of its composition.

Conflict of Interest

There was no conflict of interest amongst the authors

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