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Effects of modified packaging of garden eggs on the total pathogenic aerobic count

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Abstract
Garden eggs (*Solanum aethiopicum)*, like other fruits and vegetables are composed of several vitamins, minerals, and phytochemicals that are recommended for health. They are often consumed fresh for their nutritive values and are often bought directly from open vendors to satisfy immediate hunger. In order to prevent evaporation and dryness of the produce, modified atmosphere packaging is sometimes used to extend their shelf life during sales. However, the surfaces of this important fruit often still get contaminated with pathogens that could be a public health concern even though they are sometimes somewhat protected in polythene. In this research, we examined the surfaces of fresh garden eggs, purchased from Madalla fruit Market and Lugbe main market, Federal Capital Territory (FCT), Abuja for bacteria of public health importance, estimated the total microbial counts and examined the effects of packaging on the quality and microbial loads of the fruit. One Hundred (100) samples of vended garden eggs were collected and tested for the presence of bacteria using standard culturing methods on selective media. The respective isolates were characterized using the biochemical tests, Gram staining and microscopic studies. The results showed the presence of *Salmonella* spp., *Streptococcus spp*., *Pseudomonas spp*., *Staphylococcus aureus*, *Klebsiella spp*. and *E. coli* which ranged from $5.13x10^5$ to $4.21x10^9$ Cfu/g in the samples without packaging, $6.43x10^6$ to $8.21x10^9$ Cfu/g in perforated polythene-packaged samples and $5.23x10^5$ to $8.23x10^9$ Cfu/g in samples kept in totally sealed polythene. Contamination of garden egg were in the following descending order; 37.7% in unpackaged fruits, 36.3% in perforated packaging material and 26.0% from totally sealed packaged fruits. From the types of Bacteria associated with garden eggs in this study, there are public health risks and concerns in the fruit. Also, Packaging offered some protections against bacteria contamination. There is therefore need for urgent approach to educate both the farmers, vendors and the consumers on basic hygiene practice and the implications of failure to do so.

Keywords: garden eggs, pathogenic, packaging, fruits and vegetables

Introduction

The eggplants, popularly refers to as garden egg (Solanum species) is a family of Solanaceae and the plant genus Solanum with about 1,000 species occurring worldwide. About 25 species are known in Nigeria which include the domesticated ones being cultivated for their edible leaves, fruits and sometimes both (Christiane Gebhardt, 2016) [1]. They are known by local names such as gauta in hausa, afufa or anara in igbo or igba in yoruba. They are important both as source of foods (stew and soup dishes) or as local medicinal herbs. It is a curious kind of fruit used for many purposes among which are to achieve weight loss within a short period, to eliminate unnecessary salt in maintaining proper functioning of the heart, to reduce the sugar content level in diabetics because of its low-calorie content and to reduce blood cholesterol. They are cultivated in the north more than other parts of the Country where many varieties are cultivated for both home consumption and on a larger scale for sales (Chinedu *et al*., 2011) [2]. In many parts, the crop is cultivated during raining season but where source of irrigation water exists, they could be produced all-year round. Garden egg fruit is also an important nutritive plant but a very perishable vegetable with a short shelf-life. It is susceptible to fungal and bacteria diseases caused by Phytophthora nicotianea, var. parasitica and if the fruit touches the ground, *Corticium rolfsir* will cause an infection. Also, a lot of other bacteria pathogens are transmitted from the farm along with the fruits to the would-be consumers (Gambari *et al*., 2013) [3].

As a results of lack of naturally fertile soil enough to uphold the life cycle of the crop, fertilizers and organic manure are applied. Poultry, piggery and dairy-derived manure as fertilizers are high in nutrients such as nitrogen and phosphate and can increase organic matter content of the soil thereby improving soil fertility and physical properties such as aggregation, resistance to water or wind erosion, and water-holding capacity. However, application of these manure-derived fertilizers for garden egg production, though can enhance plant root growth and nutrient acquisition, potentially carries different pathogens such as Shiga toxin-producing Escherichia coli (STEC), Salmonella spp., and Listeria spp. (Michael *et al*., 2014) [4] which are three major pathogens frequently involved in fresh produce outbreaks. In general, foodborne diseases associated with fresh fruits and vegetables such as garden eggs are rife in many regions of the world, with at least 1 in 10 people falling ill yearly from consumption of such contaminated stuffs and about 420, 000 deaths occurring as a result (Oluwadara *et al*., 2018) [5]. Such diseases have also exerted pressure on medical services, contributed to economic and political distress, exacerbated malnutrition and led to human suffering due to drug resistance incidences. Both bacteria and fungi contaminants are usually agents that alter foods at different points during food production, packaging and transportation (Buck *et al*., 2003) [6]. Severals of the agents have been characterized and identified extensively. Consequently, many strategies have been proposed by different researchers as to how best to preserve food in order to maintain good qualities in terms of being free from pathogens. One of these methods include fruit packaging to the points of sale and during sales to prevent outbreak of foodborne diseases. Despite the many good and positive progress made so far, there is yet the occurrence of ready to eat fruit associated foodborne diseases with significant cause of mortality and morbidity especially in Nigeria.

Despite the overall importance of garden eggs as a ready-to-eat fresh fruit, the quality is often affected by post-harvest handling, packaging, transportation and storage which may result in early spoilage. Also, because animal manure is directly used as organic fertilizer for many vegetables cultivation most of which is feces, the animal-derived manure used have been regarded as vital reservoirs and sources of transmission of antibiotic resistant bacteria (Séraphin *et al*., 2016). The water used for post-harvest washing of the fruits are usually contaminated. So do the packaging in which the partly processed fruits are transported in also create conducive environment for the activation, incubation and multiplication of the pathogens enroute the consumers. The high-water evaporation from the fresh fruits due to high environmental temperature causes shrinkage and de-freshness of the produce otherwise required to be eaten fresh as such many do not survive the distribution process (Kelly *et al*., 2007) [8].

Garden egg is highly regarded and consumed by all ages and calibers due to their high nutritive and fibre contents. Although, processing of fruits and vegetables like garden eggs generates physiological stresses in the still living cut tissue, leading to quality deterioration, shorter shelf life and exposure to environmental contaminations, several strategies care being implemented with the aim to reduce the rate of deterioration of fresh-cut commodities as well as reduce the microbial burden. Creating low temperature condition coupled with simple packaging maintenance from harvest to the consumers via the retailer (Robert *et al*., 2020) [9]. This work was undertaken specifically to isolate and identify post-harvest bacteria pathogens of garden eggs with a view to determining the effect of protecting the fresh fruits with polyethylene on the bacteria load. The result of this research work will help in determining the types of bacteria associated with garden eggs sold in the studied areas of the Federal Capital Territory (FCT), Abuja, Nigeria with the view to setting up prevention and control strategies in the study area. The study will also enhance human health and secure food safety as well as public enlightenment about food safety and hygiene.

Materials and Methods

Sample Collection

Twenty-seven (27) samples comprising of three different varieties of garden egg at Nine (9) per variety and three per type of packaging viz; a. completely sealed polyethylene (CSP), b. sealed perforated polyethylene (SPP), and c. Unprotected free displayed (UFD) were collected from Madalla fruit Market and Lugbe main market, Federal Capital Territory (FCT), Abuja, Nigeria. The samples were thereafter transported in sterile bags to the Laboratory for further analysis.

Fig 1: The Locational Map of Madalla and Lugbe (Fruit Collection Site)

A B

Fig 2: A-Varieties of Garden Eggs B- Nature of Open Markt, Madalla

Sample Processing

Ten (10g) grams of each set of the collected garden egg were introduced aseptically into Ninety (90) mil of sterile peptone water previously sterilized by autoclaving at 121^0C for 15 mins and allowed to cool. The samples were mixed gently, in a sterile beaker, and 1ml solution was taken and transferred into 9ml sterile peptone water in a test tube and mix properly and the process was repeated until 10^{-5} dilution was obtained (Oludare *et al*., 2020) [11].

Bacteria isolation and Analysis

After each sample treatment, 0.1ml from 10^{-1}, 10^{-3} and 10^{-5} dilutions were taken aseptically with sterile syringe transferred to the central portion of the sterile nutrient agar and was spread carefully with L-bend glass rod until the whole surface of the agar were uniformly inoculated. The inoculated plates were then labelled and (Erin, 2012) [12]. After incubation, discrete colonies on each plate were counted in order to determine the bacteria load from each sample per given area. The distinct colonies were sub-cultured unto fresh sterile nutrient agar plates aseptically using sterile inoculation loop and then incubated for 24 hrs at 37^0C (Prabhusaran *et al.*, 2018) [13].

Identification of Bacterial Isolates

The pure cultures of the bacteria isolates were characterized and identified using basic microbiological procedures including; Gram staining, microscopic examination and biochemical tests (Methyl red- Voges-proskaeur test, Citrate utilization test, Catalase test, H_2S gas production, Mannitol salt, Triple sugar iron test (TSI) and Coagulase test as described by (Nasiru and Dalhatu, 2020) [14].

Results

Given the results of this investigation, Table 1 shows the bacteria mean count from the respective samples. The associated bacteria found on garden egg samples ranged from $53x10^3$ to $43x10^8$ Cfu/ml. The highest count ($43x10^8$ Cfu/ml) was found in sample that was sealed with perforated polyethylene, while the lowest, $53x10^3$ Cfu/ml, was found in sample A1 not wrapped in polyethylene.

Table 2 presents the biochemical characterization of the bacteria isolates which were identified as *Salmonella sp*., *Staphylococcus aureus*; Bacillus sp., Clostridium sp., Lactic acid bacteria and *Staphylococcus epididermis*. The mere presence of most of the organisms in this research shows a serious health concern taking into account the high level of consumption of the produce as well as the mobility of fruits into the city. This calls for urgent attention to avoid foodborne related outbreaks.

Figure 1 represents the occurrence percentage of the bacteria from the perspective of the nature of packaging. From the results, which shows that the highest percentage of bacteria occurrence is found on samples in sealed perforated polyethylene (66%), while the lowest 61%, is found in samples left opened without protecting polythene.

Table 1: Bacteria Colony Count (Cfu/ml) in Garden Egg Samples

Sample	Bacteria CSP	Mean Count SPP	(Cfu/ml) UFD
A1	$21x10^4$	$43x10^8$	$53x10^3$
A2	$33x10^5$	$31x10^7$	$26x10^4$
A3	$22x10^4$	$25x10^7$	$23x10^4$

CSP = completely sealed polyethylene; SPP = Sealed perforated polyethylene; UFD=Unprotected free displayed Cfu/ml = Colony forming unit per mil.

Table 2: Biochemical Identification of Bacteria Isolates

Isolate	Grxn	Cat	Glu	Lac	Suc	Gas	H_2S	Mot	MSA	Coag	Citr	MR	VP	ID
1	-	+	+	-	-	+		+	NA	+	-	+	-	Sal.
2	+	+	-	-	-	-	-	-	+	+	NA	NA	-	Sta.1
3	+	+	-	+	-	-	-	+	NA	NA	+	-	+	Bac.
4	+	+	-	-	-	-	-	-	+	-	NA	NA	-	Sta.2
5	+	-	-	-	-	-	-	+	NA	NA	-	+	-	Clos.
6	+	-	+	+	+	+	-	-	NA	-	-	-	-	LAB

Grxn= Gram's Reaction; Cat.= catalase; Glu.= Glucose; Lac. = Lactose; Suc. = Sucrose; MR=Methyl Red; VP= Voges Proskauer and ID= Identified Organism; Sal.=Salmonella sp.; Sta.1=*Staphylococcus aureus*; Sta.2= *Staphylococcus epididermis*; Bac.= Bacillus sp.; Clos. Clostridium sp. And LAB= Lactic Acid Bacteria

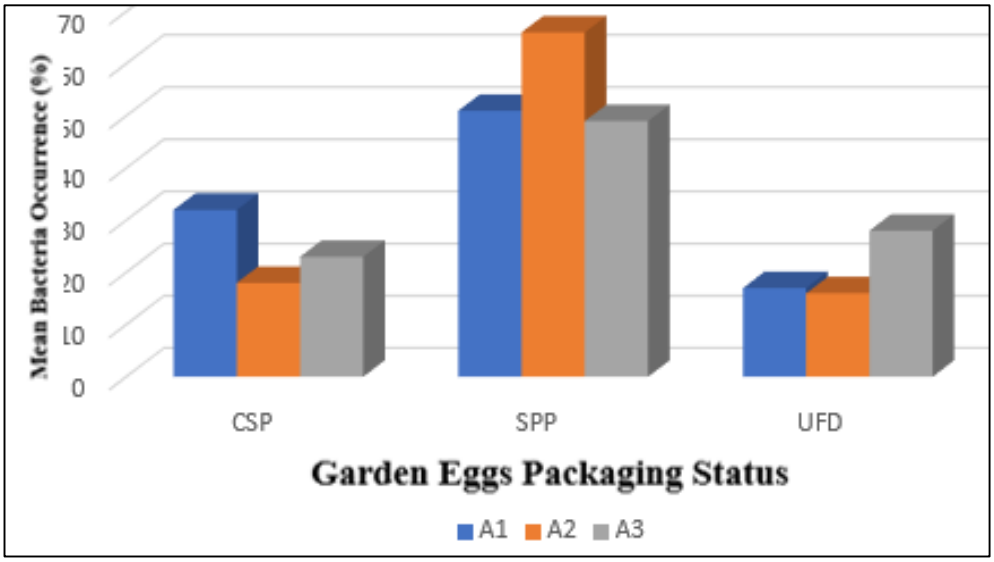

Fig 1: Mean Occurence of Bacteria on Garden Eggs (%)

CSP =Completely sealed polyethylene; SPP = Sealed perforated polyethylene;
UFD=Unprotected free displayed

Discussion

Bacteria associated with garden eggs were studied in respect to the nature in which they are usually made available during sales, that is, whether they are protected in polyethylene or without it, or if the polythene are perforated when wrapped. The bacteria were isolated and identified using standard microbiological techniques including microscopic and biochemical studies respectively (Dronova *et al*., 2016) [15]. From the results, we found that the mean colony count ranged from $53x10^3$ to $43x10^8$ Cfu/ml. The highest count ($43x10^8$ Cfu/ml) was found in sample that was sealed with perforated polyethylene, while the lowest, $53x10^3$ Cfu/ml, was found in sample A1 not wrapped in polyethylene. The highest count recorded in sealed perforated polythene might be due to the favourable condition provided by the wrapping material in that, heat is provided for incubation and cell multiplication while the partial perforation provides oxygen for the cell metabolism thriving on the mineral-rich water occasionally sprinkled on the fruits to keep them in fresh condition. This is in agreement with the work of Fernande *et al*., 2011 [16].

The six bacteria found associated with garden eggs in this study; *Salmonella sp*., *Staphylococcus aureus*; Bacillus sp., Clostridium sp., Lactic acid bacteria and *Staphylococcus epididermis* could be expected because, according to Sollid *et al*., 2014, they are easily and routinely found in the air, water and the soil around the plants used for their cultivation on the field. This observation is in agreement with the work of Ariel *et al*., 2014 who reported that most of the time, due to the low fertility of the soil, manure of animal origin is used directly on the soils without prior treatment which account for high incidence of some bacteria pathogens. The high nutrients in the water used for both irrigation and post-harvest preservation of the fruits also contribute to high bacteria load. This agrees with the work of Oluwadara *et al*., 2018 [5] in their review on the source and contamination routes of microbial pathogens to fresh produce during cultivation.

The result of this study is in agreement with the report of Nasiru *et al*., 2020 [14] who identified bacterial species such as Bacillus spp and Staphylococcus aureus as the bacterial organisms responsible for spoilage of garden eggs.

The mean percentage of occurrence of bacteria isolates as shown on table 3 indicates that while the highest mean total count occurs in the sampled wrapped but perforated (66%), the lowest count (16%) is found in samples left opened on display platform. This is in agreement with the work of Lorenzo *et al*., 2017 [19] in their study on the survival of spoilage and pathogenic microorganisms on cardboard and plastic packaging materials. The presence of *Staphylococcus aureus* might be due to unhygienic handling of fruits, storage and certain environmental factors. *Staphylococcus aureus* is known to be a normal floral of the body; nose, skin, respiratory tract which is in agreement with the work of Tong *et al*., 2015 [20] in their studies on *Staphylococcus aureus* infections epidemiology pathophysiology clinical manifestations, and management. People touching the fruits before and after packaging might be responsible for their horizontal transfer if they fail to wash their hands properly when dealing with this food materials (Jumaa, 2005) [21]. The occurrence of *Clostridium sp* on the fruit might be due to fruits touching the ground because soil is known to be a good reservoir of the organism. This agrees with the findings of Manan and Russell, 2016 in their research on the importance of soil amendment.

In general, from the observation point of view, the status of the results shows that the presence of the bacteria is not surprising owing to the ways fruits and vegetables are treated in Nigeria because it could be seen that the way vegetables were treated in the market which were grossly unhygienic. Also, market is surrounded with displaying platforms that are not clean. All these give room for contamination and re-contamination even after being washed. Transportation from the farms which are usually located in the hinter lands to the markets also gives the bacteria enough incubation time when the pathogens actually accumulate.

Conclusion

The present study showed that pathogenic bacteria of public concerns are harbored and transmitted through garden eggs sold in the market and that the water sprinkled on the fruits to keep their freshness might be responsible for the multiplication of some bacteria during the long time of transporting them from the farm to the market. Also, the

polyethylene material that are used for wrapping the fruits might be creating conducive, micro-environment for the growth of the bacteria. Most of the bacteria isolated are common inhabitant of either soil on which the fruits are grown, or the body of the handlers. Therefore, proper hygiene and education might be able to correct and control possible incidence of outbreak of pathogenic bacteria in Abuja, Nigeria from fruits such as garden eggs.

Recommendations

Since it is agreed that Garden Eggs are one of the healthiest food one can eat due to the presence of carotene, vitamins B6 E, foliate, calcium, iron, magnesium fiber and many essential vitamins and minerals, it is hereby recommended that adequate surveillance be given to the distribution and other post-harvest handling of the fruits because of the possible negative health implications.

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