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Influence of Different Storage Conditions on the Postharvest Microbial Spoilage of Green-Pepper

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To Evaluate the Effects of Different Storage Conditions on the Storability of Green-Pepper (*Capsicum Annum*) Fruits, the Effects of three Storage Conditions on the Fruits, Isolate and Identify The Microorganisms Present In Each Storage Conditions Using Suitable Means.
Study Design: Biochemical Test Was Carried out
Place and Duration of Study: Department of Botany Laboratory, Nnamdi Azikiwe University, Awka, Anambra State of Nigeria Between December 2019 and June 2021.
Methodology: This Work Shows the Effects of Three Different Storage Methods on Fresh Green-Pepper Which were Purchased from Two Different Markets (First And Second Market) Within Awka Metropolis, Anambra State under Aseptic Conditions And Were Properly Analyzed Using Three Different Storage Methods Which Included: Refrigeration (Refrigerator), Open Space (Floor) and in a Sterile Plastic.
Results: The Results Showed Total Bacterial Colony Count Values in the Range of 83.6×10^{-5} – 40.3×10^{-5}Cfu/Ml in the Different Storage Conditions Having Samples Stored In The Fridge With The Lowest Bacterial Count And Samples Stored On Dried Floor Recorded With The Highest Bacterial

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Count. The Spoilage Microorganisms Observed from the Study Included: *E. Coli, Staphylococcus Aureus, Streptococcus Spp., Lactobacillus Spp., Bacillus Spp., Micrococci Spp., Klebsiella Pneumonia And Staphylococcus Saprophyticus.* Variation In The Temperature Range Played a Great Role In Fastening the Decay of the Stored Samples. The Higher the Temperature Ranges the Faster the Rate of Spoilage. It Can As Well Be Stated That Fridge Storage Condition is a Better Method of Storing Green-Pepper Vegetables.

Conclusion: On the Basis of The Findings in this Study, it Can Be Concluded that Storage Temperature Has a Better Impact In Slowing Down The Respiration Rate, Weight Loss and Decay, While Maintaining the Fruit Firmness and Overall Quality. The Higher the Temperature Ranges the Faster the Rate of Spoilage. It Can as Well Be Stated That Fridge Storage Condition Is a Better Method of Storing Green-Pepper Vegetables. Therefore Prior to the Storage, Measures to Avoid Mechanical Damage Should Be Taken and the Firmness, Color, Weight Should be Observed for Each Green Pepper to Avoid Cross-Contamination During Storage.

Keywords: Green-pepper; spoilage; refrigeration; E. Coli; staphylococcus aureus; and Streptococcus Spp.

1. INTRODUCTION

Green-Pepper (*Capsicum annuum*) is an important agricultural crop, not only because of its economic importance, but also mainly due to the fact that they are an excellent source of ascorbic acid. Green-Pepper is a warm season annual crop which belongs to the family Solanaceae. Green-Peppers are considered "sweet" since they lack the pungent chemical (capsaicin) present in hot peppers. It is one of the most popular and highly valued vegetable crops grown in tropical and subtropical parts of the world [1]. Green-Pepper (Capsicum annuum) is one of the important vegetable crops in the world. Due to perishable nature, it is liable to fast quality changes and spoilage (through wilting, shriveling, pathogenic disorder, water loss, etc.) after harvest under improper post-harvest management [2]. Green-peppers are highly susceptible to water loss, sunscald and heat damage. Fresh green chilies losses water very quickly after harvest and begin to wrinkle and change colour within a few days without refrigerated storage [3]. Darkening, shriveling or rotting of stem indicates that green chilies were not harvested recently [4]. The most encountered postharvest problems for green-peppers are strong physiological activities, shriveling, wilting and fungal diseases. Moreover, these are sensitive to chilling injury if they are stored at or below 7 °C. Cold storage permits fruit and vegetables to be offered for consumption or for processing in fresh condition over a long period [2]. For postharvest handling of green-peppers, higher temperature is recommended while on the other hand, as temperature is increased, the rate of water loss is also increased [5]. Proper storage temperature selection is the most important factor for storage of the peppers. Optimum storage temperature range for the peppers is reported between 7 °C to 13 °C for 2-3 weeks [6].

Lowering of water activity in the product reduces microbial growth and enzyme activity, giving a product with an extended shelf-life [7]. The benefits of dehydration, however, are nullified if the product is not protected from reabsorbing moisture, which may result in discoloration and rapid deterioration in flavor.

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Proper storage system reduces wastes, adds value and makes the product qualitatively and quantitatively acceptable [6]. Respiration rate and gas exchange through the package material are the processes involved in creating a modified atmosphere inside a package that will extend shelf life of fresh green-pepper [2]. Green-Peppers are not suitable for long term cold storage; the recommended range of storage temperatures for green-peppers is from 7 to 13˚C, depending on the variety and the maturity stage [4].

Green-pepper is an economically important crop for both local and export market (Shehata *et al.,* 2013). It is known for its antioxidant properties as it is a good source of vitamins A and C as well as phenolic compounds (Shotorbani *et al.,* 2013).

With its nutritional contribution, it is believed to prevent certain types of cardiovascular diseases, atherosclerosis, cancer, and haemorrhage [8]. Unlike dried grains and legumes, fresh fruits and vegetables such as peppers have an extremely low level of natural protection against biochemical and physiological deterioration in warm and humid places [6].

During prolonged storage, the main factors for the quality degradation of sweet pepper include poor external appearance, decay development, shriveling associated with water loss and its high susceptibility to chilling injury (Shehata *et al.,* 2013). Temperature management during storage period is the most effective tool in maintaining the quality and extending the shelf life of fresh horticultural crops such as sweet pepper (Leon *et al.,* 2013). However, refrigeration requires high initial cost and power sources which cannot be afforded by most small-scale farmers, retailers and wholesalers in developing countries such as the Philippines [9-14]. Evaporative cooling is a postharvest treatment that is usually done in rural areas. It is a physical process wherein evaporation of a liquid cools an object in contact with it. It is far less expensive than the usual refrigeration cooling. Bautista *et al.,* (2007) explained that the heat of respiration of the produce evaporates the water that is applied to its immediate surroundings.

When it is windy or when air movement is greater, there is also faster evaporation. Compared to the surroundings, it maintains a lower temperature and a higher relative humidity in the storage chamber (Dadhich *et al.,* 2008).

1.1 Objectives of the Study

- Evaluate the effects of different storage conditions on storability of green-pepper (*Capsicum annum*) fruits.
- Evaluate the effects of at least two or three storage conditions on the storability of green-pepper (*Capsicum annum*) fruits
- Isolate and identify the microorganisms present in each storage conditions of the above using suitable means.
- Possible solutions to reduce spoilage.

2. MATERIALS AND METHODS

2.1 Collection of Green-pepper

Fresh green peppers were bought from two different markets (first and second market) within Awka metropolis, Anambra state, Nigeria. The samples were transported on a clean polythene bag to the laboratory. At the time of collection, the peppers were characteristically green in colour, apple in shape; fresh, undamaged and firm.

2.2 Methods of Storage

For the purpose of this work, there are three storage methods that have been adopted. These included standard refrigeration at 4^{o}C, storage in a plastic basket and on a clean concrete floor (under room temperature). The refrigeration was done using Haier Thermocool. Storage of pepper in plastic baskets is widely practiced in urban and suburban Nigeria whereas storage of pepper on concrete floors is mainly practiced in rural areas but also in cities. The fresh pepper was stored in an open plastic basket as well as on the concrete floor at room temperature. The samples were maintained under the different storage conditions for two weeks [15-17]. They were taken out briefly (for 20 mins) to make observations and to collect samples for microbial assessment (composition and load analysis) at an interval of five days. An initial microbial assessment was conducted prior to storage (day 1), then on 5th day and finally the 10th day.

2.3 Microbial Assessment

A small portion of each of the grinded pepper samples weighing 1 g was transferred aseptically into 9 ml of distilled water in a test tube. Samples were serially diluted into 4 fold using 1:10 dilution. 1 ml of the dilution was taken from the 4th test tube into the sterile petri dishes with labeling. Nutrient agar was added into the Petri dishes accordingly, mixed and allowed to gel together. The nutrient agar was then incubated at 37^{o}c for 24 hour.

2.3.1 Plate reading and bacterial isolation

After 24-hour incubation, bacterial growth on nutrient agar was observed and counted. Different bacterial colonies were isolated and sub-cultured according to their colony morphology. The isolates were characterized and identified based on their colony characteristics and biochemical reaction as follows:

2.3.2 Biochemical tests

The following biochemical test were done on the isolate for further identification; gram staining,

catalase, urease, coagulase, citrate, motility, sugar fermentations

2.3.3 Gram-staining technique

A drop of distilled water was placed on a clean grease free glass slide and a colony of the isolate was picked with a sterilized wire loop and emulsified. The glass slide was passed over the flame four times to heat fix. The smear was flooded with crystal violet for 60 seconds and rinsed with distilled water. Lugol's iodine was added for 60sec and then decolorized with acetone for 10secmediately with distilled water. The smear was counter-stained with safranin for 30sec and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under microscope using x100 objective lens. Result gram positive appears purple color while gram negative is pink/red in color.

2.3.4 Catalase test

This test is used to detect the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide when broken down. One drop of hydrogen peroxide solution was dropped on a clean glass slide, followed by the inoculation of a 24 hour old culture on the slide. The presence of gas bubbles indicates a positive test while the absence of gas bubbles indicates negative reaction [18].

2.3.5 Methyl red test

This test determines whether the organism performs mixed acid fermentation when supplied glucose. Tubes of MR broth were inoculated with a pure culture of the isolates and incubated at 35^{O}C for 4 days. 5 drops of methyl red reagent was added to the culture,positive test indicated by red colour formation while no change denotes negative [19].

2.3.6 Citrate utilization test

Simmon's citrate agar was prepared in accordance with manufacturer's manual, 38g of the media was weighed and dissolved in 100ml of water. Media was boiled for 15 min and autoclave for 15 min. Media was allowed to cool to a temperature of about 40^{o}c before pouring it. After pouring, the media was allowed to gel and isolates inoculated on it [20].

2.3.7 Urease TEST

Urease agar was prepared in accordance with manufacturer's manual; 24g of the media was weighed and dissolved in 100ml of water. Media was boiled for 15 min and autoclave for 15 min. Media was allowed to cool to a temperature of about 40^{o}c before pouring its test tubes. Media was allowed to gel and isolate inoculates on it. Color change was observed after 24 hours [21].

3. RESULTS AND DISCUSSION

3.1 Results

Upon assessment in this study, a considerable number of bacteria were isolated and identified from the samples with eight (8) different species of bacteria isolated.

Table 1 shows the morphological features of the bacteria isolates. These were observed upon physical assessment, the different colonies of various colors and forms growing on the Nutrient Agar plates after 24 hours of incubation. The growth type, texture, shape, and patterns were seen and recorded. Data in Table 2 shows the total bacteria count of the various samples.

The bacteria count was further represented on a bar graph as seen in Fig. 1.

The frequency of occurrence of each isolates in the different storage methods were given in Table 3. These isolates were further subjected to series of biochemical tests such as the gram stain, catalase, coagulase, citrate, indole, urease, motility, for identification to genus level. The probable organisms are as shown in Table 4.

Different storage conditions of Green pepper were carried out and duly observed based on room temperature. During the duration of the different storage conditions, the rate of spoilage (decay/change in color, texture and firmness), presence of spoilage microorganisms and frequency of occurrence of these organisms were recorded. The results showed total bacterial colony count values in the range of 83.6×10^{-5} – 40.3×10^{-5}Cfu/ml in the different storage conditions as shown in Table 2 above having samples stored in the fridge with the lowest bacterial count and samples stored on dried floor recorded with the highest bacterial count. It can be stated that the variation in the temperature range played a great role in fastening the decay of the stored samples [22-24]. An increase in the temperature affected the respiration of the green pepper which yielded to weight loss and softening of the outer layer of the green pepper

as weighed subsequently during the days of examination. Nevertheless, the sample stored in the fridge retained its firmness and weight for a longer period before showing any signs of spoilage.

This result also agrees with the one given according to Hameed *et al.,* (2013), his experimental results indicated that the storage temperatures 0°C (1.67mgkg^{-1}h^{-1}) and 10°C (1.75mg kg^{-1}h^{-1}) had minimum respiration rate at removal day. It may be due to fruits under low temperature, the respiration rate is slow and as temperature increases the rate of respiration is fastened because every 10°C increase the rate of respiration is roughly doubled (Jobling, 2012). Meanwhile, after one week of shelf–life the fruit stored at 10°C slowed the lowest rate of respiration (2.61mg kg-1h-1) while the 0°C storage exhibited the maximum rate (6.28mgkg^{-1}h^{-1}), significantly different from all other storage temperatures. This is in accordance with the findings of Mercado *et al*. [25] who found that respiration rates of peppers stored at 10°C lowered over the storage period of 20 days. The fruit firmness progressively decreased with increase in storage time. This result is consistent with reports of Lahay *et al.* [26] who found a reduction in firmness of fruits during prolonged storage periods. This could be due to high respiration rate and weight loss as supported by Cantwell *et al.* [27].

Table 1. Morphological characteristics of Isolates

Isolates	Sh	Pgm	Txt	Elev
1	Circular	White	Smooth	Raised
2	Circular	Milky	Smooth	Raised
3	Irregular	Dark-brown	Rough	Raised
4	Circular	Brownish	Undulated	Raised/rough
5	Circular	Colorless	Undulated	Flat
6	Circular	Dark-brown	Rough	Convex
7	Irregular	Colorless	Undulated	Convex

Key: Sh - Shape, Pgm - Pigmentation, Txt - Texture, Elev - Elevation.

Table 2. Total bacteria count of three different storage conditions of green pepper

Samples	1st day(x10^{-5})	5th day (x10^{-5})	10th day (x10^{-5})	Total count(cfu/ml)
Fridge	1.5	2.8	36	40.3
Floor	2.4	5.2	76	83.6
Plastic	2.9	6.0	60	68.9

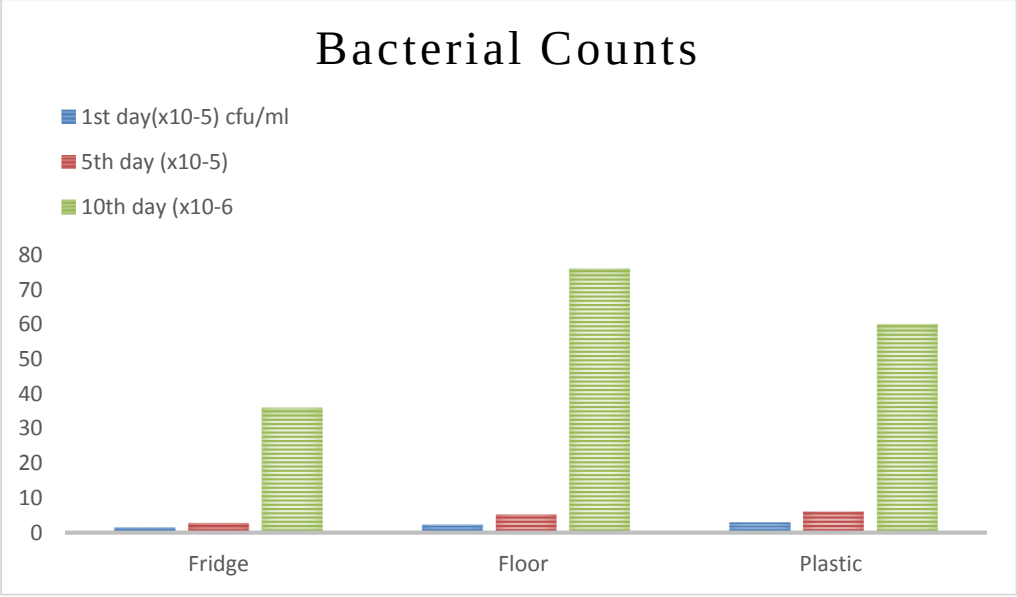

Fig. 1. Bar graphical representation of the Bacteria count of the three different storage conditions of the green pepper

Table 3. Frequency of occurrence of probable bacteria samples obtained from the three different storage conditions

Samples	Suspected Bacteria	Frequency
Floor	*E. coli*	1
	Staphylococcus aureus	1
	Streptococcus spp.	1
	Lactobacillus spp.	1
	Bacillus spp.	0
	Micrococci spp.	0
	Klebsiella pneumonia	0
	Staphylococcus saprophyticus	0
Fridge		
	E. coli	2
	Staphylococcus aureus	0
	Streptococcus spp.	0
	Lactobacillus spp.	0
	Bacillus spp.	1
	Micrococci spp.	1
	Klebsiella pneumonia	0
	Staphylococcus saprophyticus	0
Plastic		
	E. coli	0
	Staphylococcus aureus	1
	Streptococcus spp.	1
	Lactobacillus spp.	0
	Bacillus spp.	0
	Micrococci spp.	0
	Klebsiella pneumonia	1
	Staphylococcus saprophyticus	1

Table 4. Biochemical results of the bacterial isolates

Isolates/Label	Gm	Ca	Co	In	Ur	Cit	Mo	P. org
F_1	-	-	-	+	-	+	+	*E.coli*
F_2	+	-	-	-	-	+	+	*Bacillus spp.*
P_4	+	+	-	-	-	-	-	*Streptococcus spp.*
F_4	+	+	-	-	-	+	-	*Lactobacillus spp.*
P_1	-	-	-	-	+	+	+	*Klebsiella spp.*
F_2	+	+	+	-	-	-	-	*Staphylococcus aureus*
F_3	+	-	-	-	-	+	-	*Staphylococcus saprophyticus*
F_3	+	-	-	-	-	+	-	*Micrococcus spp.*
F_1	-	+	-	+	-	-	-	*Proteus vulgaria*

Key; Gm = Gram staining, Ca = Catalase, Co = Coagulase, In = Indole, Ur = Urease, Cit = Citrate utilization test, Mo = Motility, - = Negative, + = Positive, P. org = Probable organisms, F_1 = Fridge sample1, F_2 = Fridge sample2, F_3 = Fridge sample3, P_1 = Plastic sample1 and P_4 = Plastic sample4.

The bar graphical representation shows the progressive deterioration and spoilage of the green pepper with respect to the three different storage conditions. The floor samples had the highest frequency of spoilage followed by the plastic samples and finally the fridge samples. This therefore depicts that the fridge storage method is the best way to preserve green pepper depending also on the period of usage.

The spoilage microorganisms observed from the study included: *E. coli, Staphylococcus aureus, Streptococcus spp., Lactobacillus spp., Bacillus spp., Micrococcus spp., Klebsiella pneumoniae and Staphylococcus saprophyticus.* The introduction of these spoilage microorganisms could have been from the surfaces of raw, whole produce which appears to be the major source of microbial contamination and consequent spoilage of fresh-cut fruits and vegetables.

Sapers *et al.* [28] reported that, compared with good surface sanitization practices, no decontamination treatment or an ineffective antimicrobial treatment on whole cantaloupe resulted in premature microbiological spoilage of fresh cut cantaloupe. Studies have also revealed over a 1-year period of sampling that there is a close relationship between the total mesophilic aerobic counts on lettuce raw material and those on finished shredded lettuce products. Robbs *et al.* [29], determined that the most common bacteria on raw celery plants, including fluorescent *Pseudomonas* spp. and *Aeromonas* spp., were also the most common bacteria on cut celery products. Boyette *et al.,* [30] reported that the microbial decay of fresh-cut lettuce is largely due to the growth of microorganisms originating from preharvest environments. Delaquis *et al.,* [31] determined that the types of microorganisms found on shredded lettuce were highly associated with the microorganisms detected on lettuce before shedding. Several studies have revealed that yeast species identified on fresh-cut produce can also be isolated from raw materials prior to processing. Garg *et al.,* [32] concluded that peel is the major source of microbial contaminants on carrot sticks. Several outbreaks of salmonellosis that were associated with cut cantaloupe and watermelon have resulted from *Salmonella* present on the rind contaminated in the field or packinghouse [33]. Inoculation of *Listeria monocytogenes* and *Salmonella* on the surface of entire cantaloupes resulted in the contamination to fresh-cut pieces during cutting [34,35]. These results indicate that the bacteria on the surface of whole produce are the same as those on freshcut produce and can contaminate finished product through processing. Fresh cut products can also be contaminated by spoilage microorganisms through contact by people or equipment during processing, possibly by air during processing and packaging steps, especially in facilities that have been used for produce processing over an extended period of time.

The frequency of occurrence was recorded in Table 3. The presence of *E. coli* and *Streptococcus spp.* was predominant in the storage conditions of the floor and plastic while that storage condition of the fridge had more of *E. coli, Bacillus spp. and Micrococcus spp.* From assessment, the presence of these spoilage microorganisms could be majorly through mechanical damage during postharvest and storage process. Others could be due to poor sterilized storage environments which upon variation in temperature range would create a conducive atmosphere for the spoilage organisms to carry out their activities. The major organisms found in the fridge are extremophiles which are able to withstand higher temperatures. This is simply in coherence with Moneruzzaman *et al.* [36] who also determined that fruit spoilage increases when fruits are harvested at early maturity stage due to poorly developed fruit cuticular wax layer. The increment in spoilage during a prolonged period of time could be due to the influence of high respiration rate, fruit senescence and enzymatic degradation of fruits' cell wall [37].

Colonization and lesion development more typically and more rapidly occurs within damaged or otherwise compromised plant tissue. External damage such as bruising, cracks, and punctures creates sites for establishment and outgrowth of the spoilage microbes. Lesion development can be relatively rapid, occurring within days or weeks [38,39]. This presents the risk that rapidly reproducing spoilage microorganisms will arrive within open wound sites at the packing facility, and thereby, through shedding from the asymptomatic wound, present the potential for cross contamination within the facility during handling, culling, washing, sorting, and packing before storage [40-42]. Such cross-contamination to some degree is inevitable and, if not carefully managed with a robust facility sanitation program, could lead to the establishment of a population of spoilage microbes endemic to the facility that may be difficult to eradicate [43-45].

4. CONCLUSION

Based on the findings of this study, it can be concluded that storage temperature has a better impact in slowing down the respiration rate, weight loss and decay, while maintaining the fruit firmness and overall quality. The higher the temperature ranges the faster the rate of spoilage. It can as well be stated that fridge storage condition is a better method of storing green-pepper vegetables. Therefore prior to the storage, measures to avoid mechanical damage should be taken and the firmness, color, weight should be observed for each green pepper to avoid cross-contamination during storage.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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