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The Influence of Storage Conditions on the Microbial Quality of *Daucus carots* (Carrots) and *Capsicum annuum* (Green Pepper)

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ABSTRACT

The influence of different storage conditions on the microbial quality carrot (*Daucus carots*) and green pepper (*Capsicum annuum*) was determined using standard microbiological method from day zero to day ten. Total bacteria count for carrot stored at room temperature ranged from Log₁₀Cfu/g 3.22 to 7.45 and for carrot stored at refrigeration temperature ranged from Log₁₀ Cfu/g 2.13 - 3.14. Total bacteria count for green pepper stored at room temperature ranged from Log₁₀Cfu/g 4.22 to 7.45 and for green pepper stored at room temperature ranged from 1.12 to 4.14 for refrigeration temperature. Bacteria isolated includes *E.coli* (4%), *Bacillus sp.* (8%), *Pseudomonas* (16%), *Proteus vulgaris* (4%), *Staphylococcus sp.* (28%), *Klebsiella* (8%), *Salmonella* (12%), *Micrococcus sp.* (12%) and *Acinetobacter* (8%). Fungal count for carrot at room temperature ranged from Log₁₀ 2.22 to 2.54 Cfu/g and 2.01 to 2.34 Cfu/g for refrigeration temperature. Fungal count for green pepper at room temperature ranged from Log₁₀ Cfu/g 3.02 to and 7.45, Log₁₀1.81 Cfu/g to 3.34 for refrigeration temperature. Fungal isolated includes *Penicillium* (33.3%), *Aspergillus* (53.3%), and *Candida* (13.4%). Proximate composition indicates that moisture, ash, carbohydrates, lipid and fibre are lower at room temperature compared to refrigeration temperature. Temperature and storage duration have been said to affect the content of fruits and vegetables, therefore constant temperature and appropriate storage condition should be maintained.

1. Introduction

In recent years, outbreaks linked with fresh produce have emerged as an important public health concern and reported illnesses following consumption of raw produce or related products have been linked to bacteria, parasites and viruses^[1]. Fresh produce can be contaminated

with pathogens not only in the field, but also by several postharvest conditions such as wash and rinse water, unhygienic human handling, transport vehicles, cross contamination, improper storage, processing and packaging^[2]. The low sanitation standards especially during postharvest handling and an increased consumption of raw produce and related products have generated heightened concerns

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for food safety in developing countries^[3-5]. Fresh produce means fruits and vegetables that have not been processed in any manner. Vegetables are necessary in our daily diet. They bestow not simply the key nutritional fibre component of our rations but a choice of micronutrients, together with minerals, vitamins and antioxidant compounds^[6].

Green-Pepper (*Capsicum annuum*) is an essential agricultural crop, not only because of its economic usefulness, but because of its high content of ascorbic acid. Green-Pepper is a warm season annual crop which can be grouped to the family Solanaceae. It is regarded as “sweet” since they do not possess the pungent chemical (capsaicin) present in hot peppers. It is one of the common and highly used vegetable crops cultivated in tropical and subtropical parts of the world^[7]. There are many important vegetable crops in thy world and green pepper happen to be one of them, it is perishable in nature and this result to its quick deterioration after harvest under poor post harvest management^[9]. It is known for its water loss, sunscald and heat destruction. Fresh green chilies losses moisture very easily after harvest and starts to wrinkle accompanied with change in colour within a few days without proper storage condition^[8,10]. Strong physiological activities, shriveling, wilting and fungal diseases are the most common post harvest problems associated with green pepper.

Carrot is a well known vegetables with functional food compositions such as minerals and vitamin. Carotenoids and other antioxidants in carrot are useful in the interference of oxidation processes, as well as in equalizing free radical activities. Therefore, carrots and their fresh produce may shield humans against different kinds of cancer and cardiovascular diseases^[11].

Time and temperature are one of the most important factor that enhances the quality and increase the shelf life of most vegetables and fruits. Refrigeration is also vital in controlling spoilage. It is of vital importance to prevent temperature fluctuations because this can cause chilling injury, irregular softening, and spoilage. However storage conditions for both vegetables in this study are kept at refrigeration and room temperature since one of the factors increasely influencing individual health and longevity and safe high-quality food. The poor shelf-life of vegetables had led to it’s increased number of spoilage, huge losses and market lost during harvest as observed by large heaps of unsold rotten vegetable in the refuse dumps of rural and urban markets. The study aim at determining the effect of storage on the microflora and physicochemical quality of green pepper and carrot.

2. Materials and Methods

2.1 Sample Collection

Carrot and green pepper samples were purchased from the market. The samples were firm, undamaged and fresh. The samples were brought in clean bags to the laboratory.

2.2 Methods of Storage

Two storage methods were adopted. These included standard refrigeration at 4 °C and on a clean concrete floor (under room temperature). The refrigeration was done using Haier Thermocool. Storage of pepper on concrete floors is mainly practiced in rural areas but also in cities. The samples were maintained under the different storage conditions for 10 days. They were taken out briefly to make observations and to collect samples for microbial assessment (composition and load analysis) at an interval of 2 days. An initial microbial assessment was conducted prior to storage (day 0), then on day 2, 4, 6, 8 and finally the 10th day. All materials (media, glass ware) used in this study were sterilized in an autoclave at 121 °C at 15 psi for 15 minutes.

2.3 Microbiological Analysis of Samples

2.3.1 Total Bacteria Count

10 g of carrot and green pepper both at refrigerated and room temperature was weighed into 90 mL of peptone water under aseptic condition. It was then placed in a stomacher for 2 minutes and homogenize and diluted serially, then 0.1 mL aliquot of the dilutions 10^{-2} and 10^{-3} using a sterile syringe and inoculate on a Plate Count Agar (PCA) surface was done as described by Afam-ezeaku *et al.*^[12].

2.3.2 Total Fungi Count

10 g of carrot and green pepper both at refrigerated and room temperature was weighed into 90 mL of peptone water under aseptic condition. It was then placed in a stomacher for 2 minutes homogenize and dilute serially, an aliquot (0.1 mL) was transferred into the test tubes and diluted serially. From the dilutions of 10^{-1} , 0.1 mL aliquot was transferred aseptically into freshly prepared potato dextrose agar media plate and also 10^{-2} and were spread for both carrot and green pepper was done as described by Afam-ezeaku *et al.*^[12].

2.3.3 Purification of Bacteria Isolates

With a sterile wireloop, a loopful of each distinct colony was picked up and transferred to the edge of a freshly prepared Nutrient Agar plate to make a smear and streaked. Streaked plates were incubated at 37 °C for 24 hours. After that, colonies that grew on the streaked plates were transferred on agar slants and incubated at 37 °C for 24 hours to obtain stock culture. Isolates were identified based on their morphological and cultural characteristics on growth media. Identification materials, reagents and protocols according to [53] were used to identify discrete colonies from the bacteriological media of sub-cultured isolates. The isolates were characterized and identified based on their colony characteristics and subjected to a series of biochemical tests for confirmation.

2.3.4 Purification of Fungi Isolates

With a sterile needle, distinct colony was cut out and transferred to the edge of a freshly prepared Potato Dextrose Agar plate and placed in a reversed form and incubated at 37 °C for 2-5 days. After which, distinct colonies that developed from the plates were transferred on agar slants and incubated at 37 °C to obtain stock culture. The cultural characteristics of each fungi isolates were identified according to their colour, shape and the cell morphology was done based on mycelia, hyphae, septate, spore formation using lactophenol blue. A piece of the mycelium from the Petri plates was mounted on a clean grease free slide using a sterile wire loop and covered with a cover slip, after which a drop of lactophenol cotton blue was added and examined with the microscope.

3. Results and Discussion

Figure 1 presents the total bacteria count for carrot at room temperature ranged from $\text{Log}_{10}\text{Cfu/g}$ 3.22 to 7.45 and for refrigeration temperature it ranged from $\text{Log}_{10}\text{Cfu/g}$ 2.13 - 3.14. Figure 3 shows the Total Bacteria count for green pepper at room temperature ranged from $\text{Log}_{10}\text{Cfu/g}$ 4.22 to 7.45 and 1.12 to 4.14 for refrigeration temperature. Figure 2 presents the Fungal count for carrot at room temperature ranged from Log_{10} 2.22 Cfu/g to 2.54 Cfu/g and 2.01 Cfu/g to 2.34 Cfu/g for refrigeration temperature. Figure 4 shows the Fungal count for green pepper at room temperature ranged from Log_{10} 3.02 Cfu/g to and 7.45, Log_{10} 1.81 Cfu/g to 3.34 Cfu/g for refrigeration. Figures 5 - 8 show the comparison of different storage temperature on the total bacteria and fungal counts of carrot and green pepper.

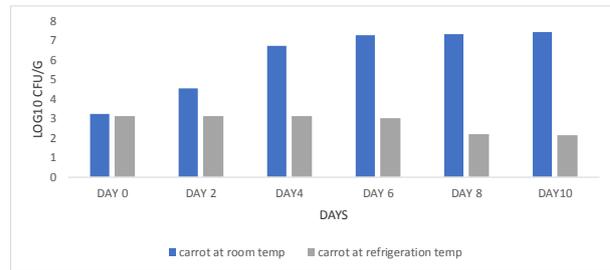


Figure 1. Total bacteria count of carrot at different storage conditions

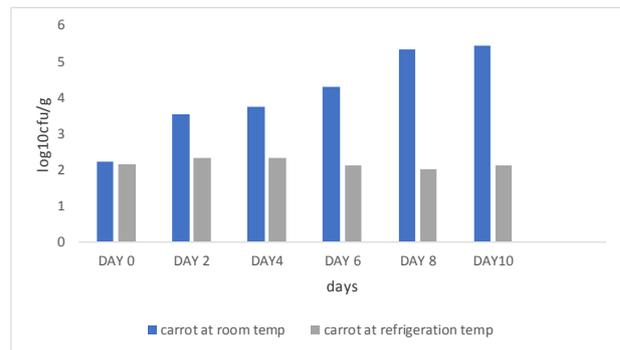


Figure 2. Total fungi count for carrot at different storage conditions

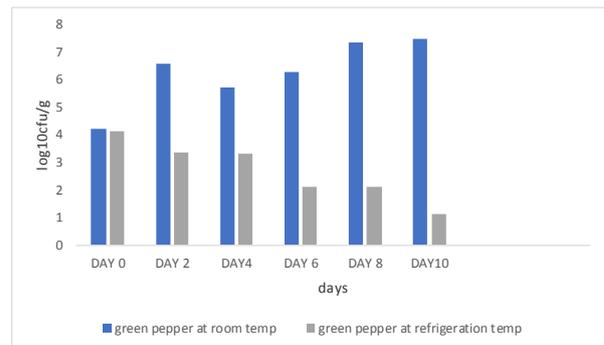


Figure 3. Total bacteria count for green pepper at different storage conditions

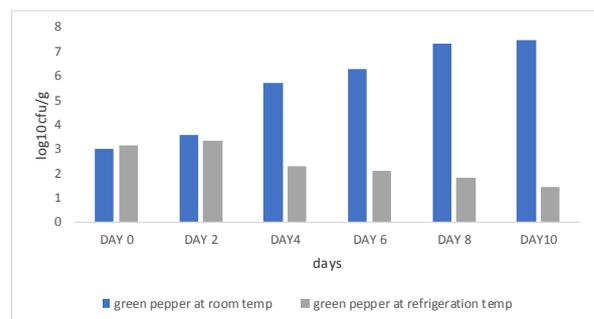


Figure 4. Total fungi count for green pepper at different storage conditions

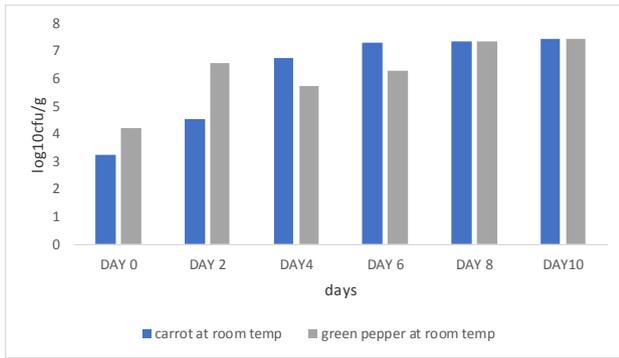


Figure 5. Total bacteria count for carrot and green pepper stored at room temperature

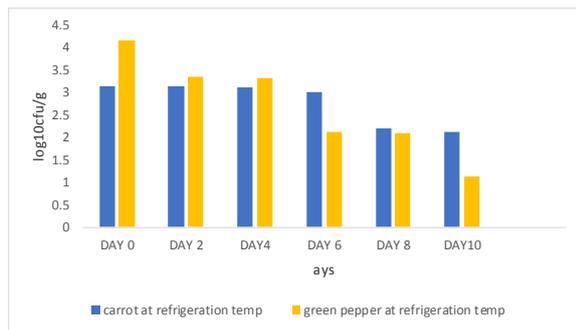


Figure 6. Total bacteria count for carrot and green pepper at refrigeration temperature

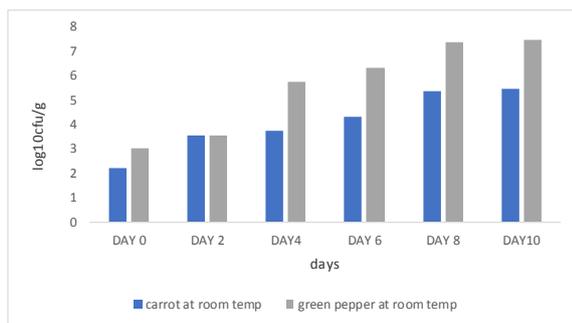


Figure 7. Total fungi count for carrot and green pepper at room temperature

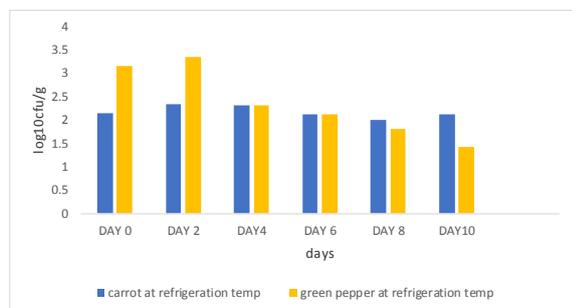


Figure 8. Total fungi count for carrot and green pepper at refrigeration temperature

3.1 The Effect of Storage Condition on the Microbial Quality of Carrot and Green Pepper

The degree of contamination in vegetables and fruits has been known to depend on the clean water, harvesting, transportation, storage temperature and processing of the produce [13]. The total bacteria count for carrot at room temperature ranged from Log₁₀Cfu/g 3.22 to 7.45 and for refrigeration temperature ranged from Log₁₀Cfu/g 2.13 - 3.14. While the total bacteria count for green pepper at room temperature ranged from Log₁₀Cfu/g 4.22 to 7.45 and 1.12 to 4.14 for refrigeration temperature. Mritunjay and Kuma [14] reported that most of these microorganisms managed to grow in the storage temperature. Therefore, high counts are an indication of exposure to contaminants because of the existence of favorable conditions [15]. The Hazard Analysis and Critical Control Points-Total Quality Management (HACCP-TQM) Technical Guidelines states the microbial limits for raw foods, where the food samples grouped based on colony forming units as expressed in Log₁₀ per gram; less than 4, 4–6.69, 6.69–7.69 and greater than 7.69 log CFU g⁻¹ (aerobic plate count) is termed as good, average, poor, and spoiled food, respectively [16]. According to the guideline the mean counts of green pepper and carrot stored at room temperature are log₁₀ 7.5 and 7.3 respectively and are regarded as poor according to the stated guideline. Green pepper and carrot stored at refrigeration temperature is log₁₀ 3.5 and 3.3 respectively is regarded as good according to the stated guideline. Aerobic organisms reflect level of contamination and microbiological indicator for food quality [15,17]. Foods are considered as harmful when they possess high number of aerobic mesophilic microorganisms, even if the organisms are not known to be pathogenic [18]. The fungal count for carrot at room temperature ranged from Log₁₀ 2.22 Cfu/g to 2.54 Cfu/g and 2.01 Cfu/g to 2.34 Cfu/g for refrigeration temperature. Fungal count for green pepper at room temperature ranged from Log₁₀ 3.02 Cfu/g to and 7.45, Log₁₀ 1.81 Cfu/g to 3.34 Cfu/g for refrigeration. Fungi isolated includes *Penicillium* (33.3%), *Aspergillus* (53.3) and *Candida* (13.4%). Hameed *et al.* [19] reported that the storage temperatures of 0 °C and 10 °C had reduced respiration rate at removal day which may be linked to fruits being kept under low temperature, the respiration rate reduces and as temperature increases, the rate of respiration is faster because every 10 °C increase the rate of respiration is roughly doubled [9]. After a week of shelf-life the fruit kept at 10 °C showed the lowest rate of respiration while the 0 °C storage showed the maximum rate, significantly different from all other storage temperatures. This result is similar with the studying of [20] who discovered

that respiration rates of peppers stored at 10 °C lowered over the storage period of 20 days. And also freshly harvested chili or other hot peppers should be stored at 10 °C with 80-90% RH [21-23].

It can be stated that the variation in the temperature range played a great role in fastening the decay of the stored samples [24,25]. An increase in the temperature affected the respiration of the green pepper and carrot, which yielded to weight loss and softening of the outer layer of the green pepper and carrot. Nevertheless, the sample stored in the refrigerator retained its firmness and weight for a longer period before showing any signs of spoilage.

Bacteria isolated includes *E.coli* (4%), *Bacillus sp.* (8%), *Pseudomonas* (16%), *Proteus vulgaris* (4%), *Staphylococcus sp.* (28%), *Klebsiella* (8%), *Salmonella* (12%), *Micrococcus sp.* (12%) and *Acinetobacter* (8%). Organisms isolated is similar to these authors [12,27]. An increased bacteria counts obtained for the fruits and vegetables in this study are in accordance to these authors [12,26-28,31]. The high level of microbial contamination observed in the fruits and vegetables in this study may be a reflection of storage conditions and how long these produce were kept before they were obtained for sampling. bacteria on the produce may multiply over time depending on the storage conditions especially those that are psychro-trophic [32,33].

The occurrence of *E.coli* in both carrot and green pepper is indicative of faecal contamination. Some strain of *E.coli* are known to cause of diarrhea, gastroenteritis and other urinary tract infection. *Staphylococcus aureus*, *Pseudomonas*, and *Bacillus sp.* are food contaminants from humans and the surroundings, its occurrence in food however, need to be put in check because they have been reported as known cause of the major food borne diseases [34].

In the present study, *Micrococcus sp.* was among the most occurring organism this is as a result of its presence in wastewater and soil [35]. This is in accordance to the study of Guchi and Ashenafi [36] who reported *Micrococcus sp.* is one of the most occurring microflora isolated from lettuce and green pepper in Addis Ababa, Ethiopia. *Micrococcus sp.* which are common environmental bacteria that can be present in fresh vegetables through cross-contamination, for example, from wastewater used by the grower during irrigation. *Micrococcus* is thought to be a saprotrophic organism, thought it can be an opportunistic pathogen, especially with compromised immune individuals, such as HIV patients [38]. The prevalence of *Staphylococcus aureus* (28%) in this study was lower than that report in the study of Halablab *et al.* [38] who stated higher prevalence of *Staphylococcus aureus* (51.5%) from Lebanon. The presence of *Staphylococcus aureus* (28%)

in study was similar to those obtained by Ikpeme *et al.*, 2011 (25%-33%). In this study, the prevalence of *Samo-nella sp.* in carrot and green pepper was higher than that reported [36,39] who indicated 10% in lettuce and green pepper and 11% in broccoli and cauliflower respectively.

The fungi isolates from both carrot and green pepper include *Candida sp.* (13.4%), *Aspergillus sp.* (53.3%) and *Penicillin notatum* (33.3%). These partly similar to the findings of Li - cohen and Bruhn [41] who discovered that species of fungi linked with the spoilage of some edibles fruit include species of *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*. *Penicillium and Mucor sp.* *Aspergillus sp.* are environmental contaminants, which can cause deterioration of fruits and vegetables [42]. Mycotoxins are produced by some these fungi and are implicated in cases of mycoses [43]. *Aspergillus sp.* which was isolated fungi in this study is known to produce aflatoxins which is associated with liver cancer [44].

Significant change was obtained from the room temperature compare to the reduction in cold storage. This indicates that cold storage could decrease the rate of respiration and loss of energy substrate and this significant decline could be contributed to usage of sugar respiration process [50]. Temperature is a foremost reason affecting microbial growth. Refrigeration is deal for storage of nearly all perishable fresh produce. Mould growth and chilling injuries ought to be taken into account as well as the length of storage [51].

The greater diversity of bacteria was obtained when compare to fungi which could be attributed to the relative high moisture content of fruits which subjects them to more bacteria that fungal attacks [52] in both vegetables a great number of pathogens are known to exert effects on the microbial load acquired during the period of study. As much as these pathogen levels are not detrimental to human health, it is however an indication that proper care should be taken in handling vegetables and fruits which include thorough washing of fruit and vegetables before consumption.

3.2 Effect of the Storage Conditions on the Proximate Composition of Carrot and Green Pepper

The proximate composition of carrot and green pepper at different storage temperature as shown in Table 1. The value of moisture content to be 85.19% and 95.02% for carrot and green pepper respectively and this is in accordance with the study [46]. At room temperature, a reduction of moisture content occurred in both carrot and green pepper. In many horticultural products, a reduction of more than 5% would cause loss of freshness, witting appearance and even loss of commodity values [45].

The value of protein for carrot and green pepper shows that carrot and green pepper does not contain much protein but can still be used as protein food supplement. The value for carrot ranged from 1.02% - 2.64% for carrot and 0.98% - 2.80% for green pepper, which is higher than the result obtained [47]. The lipid content at room temperature reduced significantly while it showed a slight reduction at cold storage. The losses might be narrowed to the fact that fats are rich in unsaturated fatty acids which is liable to oxidation degradation [48]. In this study the ash content for carrot is 3.030% and 1.33% for green pepper which is higher compare to the Brazilian Table of Food Composition 2001. Carbohydrates and fibre are relatively low (6.71% and 2.89%) compared to the work reported [49]. The value of carbohydrates was 31 g and fibre was 29.3 g.

Table 1. Proximate composition of the various samples stored at different condition

Sample identity	Moisture (%)	Ash (%)	Carbohydrates (%)	Protein (%)	Lipid (%)	Fibre (%)
Carrot room temperature on day 2	82.54	1.21	4.62	1.07	1.21	2.37
Carrot room temperature on day 8	73.69	1.18	4.22	1.02	0.26	1.98
Green pepper RM temp on day 2	95.02	1.33	6.10	1.13	0.37	1.17
Green pepper RMemp on day 8	87.97	1.29	6.06	2.80	0.28	1.16
Carrot refrigeration temperature on day 2	85.19	3.030	3.26	2.64	1.52	2.89
Carrot refrigeration temperature on day 8	83.62	3.03	2.93	2.51	0.90	1.59
Green pepper refrigeration temperature on day 2	91.10	1.030	6.71	1.19	0.75	1.59
Green pepper temperature on day 8	91.50	1.03	5.59	0.98	0.64	1.53

3.3 Effect of Storage Temperature on the Quality of Carrots and Green Pepper

Quality of carrot and green pepper is affected by water loss during storage, which depends on the temperature

and RH of the storage conditions and this is similar to the study [54,55]. Hardenburg *et al.* [56] stated that storage under minimum temperature is the most efficient way to maintain quality of fruits and vegetables as a result of its effects on reducing respiration rate, ethylene production, ripening, senescence, and rot development. Higher temperature increases the vapour pressure difference between the fruit and the surrounding, which is the driving potential for faster moisture transfer from the fruit to the surrounding air and this is in accordance to this present study.

4. Conclusions

Based on the findings of this study, storage temperature has a better impact in retarding the respiration rate, weight loss and decay, while maintaining the fruit firmness and general quality. The higher the temperature ranges the faster the rate of spoilage. It can as well be stated that refrigeration storage condition is a better method of storing green-pepper and carrot.

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Conflict of Interest

There is no conflict of interest.

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