Isolation, Characterization and Identification of Microorganisms from Spoilt Carrots Obtained from Ose Market Onitsha, Nigeria

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Abstract Studies on the microorganisms associated with spoilt carrots obtained from Ose Market, Onitsha, Nigeria were carried out using standard cultural techniques. Nutrient agar, sabouraud dextrose agar and Eosin methylene blue agar were the growth media for the isolation of the heterotrophic bacteria, fungi and coliforms. The bacteria were identified as *Serratia marcescens*, *Escherichia coli* and *Corynebacterium bovis* while the fungi was identified on the basis of their colonial and microscopic characteristics as *Penicillium digitatum*, *Rhizopus stolonifer*, *Aspergillus niger* and *Alternaria alternata*. *Escherichia coli* was predominantly isolated among the bacterial isolates (50%) while *Aspergillus niger* occurred most frequently than the other fungal species (40%). These organisms may have been introduced to the carrots during growth, harvesting, handling, storage and distribution. The presence of the organisms is a public health risk because of the diseases known to be caused by them. It is therefore imperative that adequate hygienic practices must be put in place during the storage and handling of carrots. Spoilt carrots must also not be consumed as they contain a teeming population of bacteria and fungi, some of which are pathogenic to humans.

Keywords Identification, Microorganisms, Spoilt Carrots, Ose Market, Nigeria

1. Introduction

Vegetables are considered as leafy out-growth of plants or plant shoots used as food. These include those plants or plant parts used in different ways such as in soup or served integral as part of main meal [1]. They can also be regarded as the edible components of plants, which include leaves, stalks, roots, tubers, bulbs, flowers and seeds [2]. They are important protective foods and are highly beneficial for the maintenance of health and prevention of diseases [2]. They

offer the most rapid and lowest cost method of providing adequate supplies of vitamins, minerals, and fibers. These ingredients are essential for proper function of the body [2].

The incidence of microorganisms in vegetables may be expected to reflect the microbiological condition of the raw products at the time of processing [3]. These vegetables are frequently consumed without being exposed to those processes that reliably eliminate pathogens. Many viruses, bacteria and protozoans on vegetables which can cause food poisoning are derived from human faeces [3]. Certain fungi such as *Aspergillus*, *Fusarium* and *Penicillium* are commonly occurring filamentous fungi found in vegetables and their growth may result in production of mycotoxins, which can cause a variety of illnesses in humans, from allergic response to immune suppression and cancer [3].

Carrot (*Dacus carota*) is a root vegetable which contains the important biological active compound carotenoid [4]. It is one of the major vegetable crops cultivated worldwide and is susceptible to microbial spoilage. The consumption of carrots in Nigeria has increased tremendously in the recent years due to increased awareness of its health importance [2]. Local utilization of carrots is limited to direct unprocessed eating either wholly or as salads. It is estimated that 20% of carrots harvested for human consumption are lost through microbial spoilage, though other factors such as enzymatic spoilage and insect attacks are also implicated.

The primary agents of microbial spoilage are bacteria and molds [5]. These organisms can be introduced to the crop during growth in the field, during harvesting and post-harvest handling or during storage and distribution [6]. Many of these organisms are pathogenic and have been known to cause diseases in humans and animals; therefore it is imperative that the microorganisms associated with the spoilage of this vegetable in the biological environment characteristic of Nigeria are known. Thus in this work, the microorganisms associated with spoilt carrots obtained from Ose Market Onitsha, Anambra State, Nigeria, were isolated, characterized and identified.

2. Materials and Methods

2.1. Samples Collection and Processing

Thirty samples of carrots with signs of spoilage were purchased from different vendors at Ose Market, Onitsha, Anambra State, Nigeria. The samples were placed in separate sterile plastic containers and transported in ice packs to the microbiology laboratory of Nnamdi Azikiwe University, Awka, for analysis. Segments of tissues (1g) from the spoilt areas of each carrot were cut out with a sterile scapel and introduced into flat-bottomed flasks containing 100 ml of previously prepared nutrient broth. The flasks were incubated at 28°C for 24 hours, after which tenfold serial dilutions of the enriched culture were made.

2.2. Isolation of Heterotrophic Bacteria

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 0.05mg/ml to inhibit fungal growth [7]. Incubation was carried out in an inverted position at 28°C for 24 hours for the development of the bacterial colonies.

2.3. 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 0.05mg/ml to inhibit fungal growth and spread evenly with a sterile glass rod. Incubation was carried out in an inverted position at 28°C for 48 hours for the development of the coliform bacteria.

2.4. Isolation of Fungi

0.1 milliliter aliquots of the serially-diluted samples were introduced into culture plates containing sterile Sabouraud Dextrose Agar (SDA), with Chloramphenicol at a concentration of 0.05mg/ml to inhibit bacterial growth [8]. The samples were uniformly spread on the surface of the medium with a sterile glass rod. Incubation was carried out in an inverted position at 28°C for five days for the development of the fungal colonies.

2.5. Purification and Maintenance of the Microbial Isolates

The bacterial colonies which developed on the plates were randomly picked and purified by subculturing on nutrient agar (NA) plates before transferring onto NA slants. The fungal isolates were also purified using the same method as for the bacterial isolates but they were subcultured onto SDA plates before transferring onto SDA slants. These isolates were stored at 4°C in the refrigerator as stock cultures for characterization and identification.

2.6. Characterization and identification of the bacterial isolates

The isolates were subjected to morphological and biochemical tests and were identified by comparing their characteristics with those of known taxa as described by Holt et al [8]. Gram staining, motility test, sugar fermentation test (glucose, raffinose, lactose, sucrose, maltose and fructose), catalase test, indole test, methyl red test, citrate utilization test, spore test, hydrogen sulphide production test and voges proskauer test were carried out as done by Onuorah et al [9].

2.7. Characterization and Identification of the Fungal Isolates

The colonial and microscopic characteristics of the fungal isolates were determined using the lactophenol cotton blue staining method and the slide culture test:

2.7.1. Lactophenol Cotton Blue Staining

A solution of lactophenol cotton blue was prepared. Using a straight wire, a fragment of a fungal isolate was placed on a clean grease-free slide. Two drops of the lactophenol cotton blue solution were added and the stain allowed to penetrate. The slide was then viewed under the microscope. The isolates were identified following the description of Oyeleke and Manga [10].

2.7.2. Slide culture test

A fragment of the aerial mycelia was inoculated on a slide containing sterile prepared sabouraud dextrose agar with a sterile inoculating loop. The slide was thereafter incubated at 28°C for 24 hours, then stained with lactophenol cotton blue dye, covered with a coverslip, and examined under the x 100 objective lens of the microscope.

3. Result

The microorganisms isolated from the spoilt carrots are presented in Table 1. Fifty bacterial isolates were recovered from the samples and were identified as *Serratia marcescens*, *Escherichia coli* and *Corynebacterium bovis* while twenty five moulds were isolated from the samples which were identified as *Penicillium digitatum*, *Rhizopus stolonifer*, *Aspergillus niger* and *Alternaria alternata*. The predominant bacteria isolated were the gram-negative rods while moulds were the fungi isolated.

Table 1. Microorganisms Isolated from the Spoilt Carrots

Bacteria	Fungi
<i>Serratia marcescens</i>	<i>Penicillium digitatum</i>
<i>Escherichia coli</i>	<i>Rhizopus stolonifer</i>
<i>Corynebacterium bovis</i>	<i>Alternaria alternata</i>
	<i>Aspergillus niger</i>

The frequency of occurrence of the bacterial isolates in the spoilt carrots is shown in Table 2. *Escherichia coli* occurred most frequently (50%) while *Serratia marcescens* had the lowest frequency of occurrence (20%).

Table 2. Frequency of Occurrence of the Bacterial isolates in the Spoilt Carrot

Bacterial Isolates	Number Isolated	Frequency of Occurrence (%)
<i>Serratia marcescens</i>	10	20
<i>Escherichia coli</i>	25	50
<i>Corynebacterium bovis</i>	15	30

The frequency of occurrence of the moulds in the spoilt carrots is presented in Table 3. *Aspergillus niger* had the highest occurrence of 40% while *Alternaria alternata* had the lowest frequency of occurrence of 12%.

Table 3. Frequency of Occurrence of the Moulds in the Spoilt carrots

Fungal Isolates	Number Isolated	Frequency Of Occurrence (%)
<i>Penicillium digitatum</i>	4	16
<i>Rhizopus stolonifer</i>	8	32
<i>Aspergillus niger</i>	10	40
<i>Alternaria alternata</i>	3	12

4. Discussion

Three species of bacteria were isolated from the spoilt carrots obtained from Ose Market, Onitsha, Anambra State. They were characterized and identified as *Escherichia coli*, *Serratia marcescens* and *Corynebacterium bovis* (Table 1). *Escherichia coli* was the most dominant (50%) of the three (Table 2). This result agreed with the report of Adebayo et al (11) on the study of microorganisms associated with spoilt vegetables in Uyo metropolis. However the frequency of occurrence of *Escherichia coli* was higher than that reported by Adebayo et al (11). The presence of *E. coli* is an indication of faecal contamination. Probably, human or animal faeces were used as manure for the production of the crop. Its presence also suggested the possible presence of other enteric pathogens known to cause food-borne gastroenteritis (12). *Pectobacterium carotovorum* is the most common spoilage organism of carrot and other fruits and vegetables (2). The absence of the organism might be due to the nature or kind of the spoilt carrot samples or the methods of analysis used in this study.

All the bacteria isolated in this work are inhabitants of the soil. *Serratia marcescens* and *Corynebacterium bovis* may come from the soil or from faecally-contaminated water used for irrigation. *Serratia* is an opportunistic pathogenic bacteria capable of causing diseases in diverse organisms including humans. The presence of *Corynebacterium bovis* may be as a result of contamination with animal faeces. Some *Corynebacterium* species are known for their

pathogenic effects in humans, particularly *Corynebacterium bovis*. More bacteria were isolated from the samples than moulds indicating that the bacteria are the principal agents of spoilage of carrots.

The moulds isolated from the spoilt carrots were *Penicillium digitatum*, *Rhizopus stolonifer*, *Aspergillus niger* and *Alternaria alternata* (Table 1). Among the moulds, *Aspergillus niger* occurred most frequently (Table 3). This agreed with the findings of Adebayo et al (11) who reported that the species of *Aspergillus* and *Rhizopus* occurred most frequently, though their frequency of occurrence was slightly lower than that obtained in this work. These pathogens have been reportedly isolated from fruits in Nigeria (13). *Aspergillus sp* were found to be the most common fungi responsible for post-harvest-loss of fruits in South-Western Nigeria (14).

Generally, spoilage fungi are considered toxigenic or pathogenic (15). *Aspergillus spp.* are known to produce several toxic metabolites such as malformins, nathopyrones [16] and they can produce ochratoxin, a mycotoxin which is a very important toxin worldwide because of the hazard it poses to human and animal health [17,18].

The contamination of carrots by pathogenic bacteria and moulds could also be as a result of poor storage distribution and marketing practices. Post-harvest handling and transport of these products are inadequate, therefore most of them do not usually get to the major cities in time due to the nature of the transport system and poor road networks existing in the rural areas while products with bruises are also not isolated from unbruised ones, thereby causing cross infections.

Reduction in the rate of spoilage of carrots can be achieved by observing good agricultural practices. This may include protecting surface and ground water from uncontrolled live stock or wildlife access to limit the extent of faecal contamination. Properly treated manure can be used to improve soil quality while the harvest and storage facilities should be cleaned and disinfected prior to harvest. In addition, clean containers should be used for the transport of fresh carrots and dirt and adhering mud should be removed. Finally spoilt carrots should be sorted out to avoid cross infections.

5. Conclusions

Microorganisms are naturally present on all food stuffs including carrots and can be brought in by wind, soil, water, animals and humans. Carrots can become contaminated during their growth, harvesting and transportation, eventually leading to their spoilage. It is therefore necessary that the farmers and marketers should take necessary precautions in preventing the contamination of the produce to reduce their spoilage and the health risk their consumption pose to the consuming public. In addition, spoilt carrots should be discarded and not be consumed by humans.

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