

Microbial and geohelminthes population in different parts of *Daucus carota* L. (Carrot)

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ABSTRACT

Daucus carota (Carrot) is a common vegetable often consumed for its enormous nutritional benefits but without thoughts of its possible contamination with disease causing microorganisms. The different parts namely eyes, between buds, leaf stump and body of *Daucus carota* obtained from different spots in Owerri were analyzed using standard microbiological and parasitological methods. The mean heterotrophic bacteria count ranges from 1.28×10^9 cfu/g in between buds to $> 10^{10}$ cfu/g in the leaf stump. Mean coliform count ranges from 1.55×10^7 in the body to $> 10^{10}$ in the eyes, leaf stump and between buds. The mean fungal count was 1.54×10^7 in the body and $> 10^{10}$ cfu/g in the leaf stump and between buds. There was no significant difference at $P = 0.05$ in mean microbial counts in the different parts of *Daucus carota* and from the different sampling spots. Organisms isolated include spp of Staphylococcus, Corynebacterium, Bacillus, Pseudomonas, Micrococcus, Serratia, Escherichia, Enterococcus, Penicillium, Rhizopus, Saccharomyces, Geotrichium, Mucor, and Aspergillus. Geohelminthes detected include *Ascaris lumbricoides*, Hookworms and *Strongyloides stercoralis*. The level of contamination of the carrots calls for urgent need to create awareness on proper hygiene practices, GMP and HACCP application.

KEYWORDS: nutritional benefits, contamination, hygiene, GMP, HACCP

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INTRODUCTION

Daucus carota (carrot) is a delicious root vegetable often consumed raw or cooked. Depending upon the recipe or personal preference, carrots can be left whole or julienned, grated, shredded or sliced into sticks or rounds. It can be eaten in a variety of dishes such as carrots cakes, carrot puddings, snacks, combination with other fruits/vegetables, carrot soup or in salads and as juice. Carrots are rich in phytonutrients including beta-carotene, alpha-carotene, lutein, hydroxycinnamic acids (caffeic, coumaric, ferulic), anthocyanins (cyanidins and malvidins), polyacetylenes (falcarinol, falcarindiol). It is an excellent source of vitamins (vitamins B1, B2, B6, C, E, K, niacin, folate, and vitamin A (in the form of carotenoids)). In addition, they are a very good source of minerals (sodium, potassium, calcium, manganese, iron, zinc, copper, molybdenum, phosphorus and carbohydrates, sugar, dietary fiber (soluble and insoluble fiber), fatty acids, amino acids amongst others [1, 2, 3, 4, 5, 6].

Carrots are known to be one of the healthiest foods consumed by man inadvertently because of its nutrient composition and presence of characteristic phytonutrients. Carrots rich dietary fiber is known to calm irritable bowel and trigger regular bowel movement thereby preventing constipation, diverticulosis and diverticulitis [7, 8].

The lutein content of carrot, geranyl-acetate phytonutrient in carrot seeds (sometimes extracted from purified carrot seed oil), the beta-carotene in carrots (vitamin A precursor) converted to vitamin A and to rhodopsin are known to prevent/reduce the risks of glaucoma, cataract, night blindness,

macular degeneration, dry eyes and thus blindness [6, 9, 10, 11].

Anticancer activities of carrot have been reported to be associated with antioxidant properties of carotenoids- lutein, beta carotene and polyacetylenes (falcariol and falcariindiol) [12, 13].

Cardiovascular diseases prevention has been associated with carrot consumption. The oxidant damages in cardiovascular system due to highly oxygenated blood in the arteries are known to be mitigated by synergistic activities of the antioxidants. Polyacetylenes made from crepenynic acid, stearolic acid and tariric acid are known to contain compounds that have anti-inflammatory and anti-aggregatory properties thus preventing excessive clumping of the erythrocytes, hence its key role in cardiovascular protection [4, 6, 14, 15, 16].

The antioxidants present in carrots have also been reported to play vital roles in the prevention of cell damage as a result, carrot performs anti-aging functions, protects the skin from sun damage and dryness and uneven skin [15].

In Nigeria, carrot is cultivated in few northern states via irrigation and during early rains; thus its availability is seasonal. Extensive handling and transport is needed to move produce to other parts of the country. Like every other vegetable and fruit, carrot is prone to pre and post harvest contamination and thus it is a veritable source of food borne pathogens [17, 18, 19, 20, 21, 22]. Due to the seasonality of carrot, extensive/wild eating of the produce is a common site in season, food vendors hawk this produce all over the streets and school children and the general public buy and eat the produce without washing. The attractive colour of carrot tend to overshadow the thought of it being able to transmit food borne pathogens; this vegetable is known to harbor minute soil particles in different parts (the eyes, between buds/forks and leaf stump). This observation prompted the need to assay for the different microorganisms inherent in the different parts of carrot with a view to educating the public on the need for food safety consciousness.

MATERIALS AND METHODS

Sampling sites and sample collection

Major and busy streets/spots in Owerri metropolis were selected including Douglas road, Wetheral

road, Imo Transport Company (ITC) park, and Okigwe motor park. Ihiagwa was selected as the host community of Federal University of Technology, Owerri.

A total of 100 samples comprising of ten (10) samples from each of the sampling sites, were collected randomly from different vendors on two days interval for a period of three weeks. Samples collected were aseptically wrapped in sterile foil before transporting to the laboratory for investigation within one hour.

Sample analysis

The various parts of the carrots for investigation (eyes, between buds, leaf stump and body of carrots) were aseptically isolated from the carrot using a sterile scalpel blade. About 10 g of each part was weighed out and dispensed into 50 mls of physiological saline to serve as stock solution. Aliquots 10 ml stock solution for various parts was serially diluted and 0.2 ml cultured by spread plate technique onto replicate plates of Nutrient agar (Fluka, Germany), Potato dextrose agar (Oxoid, England) and MacConkey agar (Fluka, Germany) for heterotrophic bacteria, heterotrophic fungi and coliform respectively. Incubation of plates was for 24 to 48 hours at 37°C except however, for PDA that was left for 72 to 120 hours at room temperature 30 ± 2°C. Approximate 1 ml stock solutions were used for coliform test [23]. Lactose broth with inverted Durham's tube was inoculated for 24 to 48 hours incubation at 37°C to 44°C, this is presumptive coliform test. Cultures that produced gas and thus positive for presumptive test, were sub-cultured onto EMB agar (Oxoid, England). Incubation was for 24 to 48 hours at 37°C to 44°C, this is the confirmatory coliform test. Plates with characteristic *E. coli* and or *E. aerogenes* colonies, metallic greenish sheen or mucous colony, Gram positive, non-spore forming constitute positive confirmatory coliform test. Completed coliform test was considered positive if colonies on EMB produce gas when re-inoculated into lactose broth. The remaining stock solution was left overnight for identification of soil helminthes following modified methods of Wafa [24]. The wash water (overnight stock) was sieved to remove debris after which it was

dispensed into centrifuge tubes and centrifuged at 2000 rpm for 20 min. Following centrifugation the supernatant was discarded and the sediments were carefully mixed, a drop was placed on a grease free slide with the addition of a drop of tincture of iodine and was examined for parasite stages under the microscope using the $\times 10$ and $\times 40$ objective lenses. Parasites were identified following the descriptions of Cheesbrough [25].

Enumeration and characterization of isolates

The colonies were counted using a colony counter (Stuart Scientific, UK) after 24 to 48 hours incubation at 37°C. Distinct and discrete colonies on the different media were isolated and purified on nutrient agar by repeated sub-culturing. Further characterization of pure cultures stored on agar slants at 4°C was by the methods as described by Speck [23]; Jolt *et al.* [26]. Cultural characteristics and biochemical tests-catalase, IMVIC test, carbohydrate utilization, reaction on TSI, gelatin liquefaction, starch hydrolysis, nitrate reduction, coagulase, phosphatase production, motility, oxidase and urease production were carried out as preliminary test.

Fungal isolates identification was based on their macroscopic and microscopic characteristics. Reference was made to standard identification keys and atlas [27, 28].

Statistical analysis

Analysis using multi variance two ways ANOVA SPSS 17 statistical tool, gave an insignificant difference at 95% confidence level ($P = 0.05$) for each group of organisms obtained for the different parts of *D. carota* eyes, between, buds, leaf stump and the body and for the various sample locations Wetheral, Douglas, ITC park, Okigwe park and Ihiagwa.

RESULTS

The different parts (eyes, between buds, leaf stump and body) of *Daucus carota*, showed the presence of heavy loads of heterotrophic bacteria, coliforms, heterotrophic fungi and parasitic soil helminthes. Table 1 shows that all the parts had heterotrophic bacterial count above 10^6 cfu/g, the leaf stump is however, more contaminated than other parts. Produce from the different sampling spots are equally contaminated. Table 2 reveals the coliform counts in the different parts of *Daucus carota*. All the parts are heavily contaminated; however, the leaf stump had higher contamination when compared to other parts. Table 3 shows that the leaf stump of *Daucus carota* had relatively higher level of fungal contamination compared to other parts; samples from Douglas road also had higher mean heterotrophic fungal count on the leaf stump than samples from other spots. The geohelminthes

Table 1. Mean heterotrophic bacteria count cfu/g in the different parts of *Daucus carota*.

Parts of Carrot	Sampling Location				
	Wetheral	Douglas	ITC park	Okigwe park	Ihiagwa
Eyes	1.68×10^9	2.81×10^9	2.75×10^9	2.49×10^9	4.80×10^9
Between buds	1.28×10^9	2.78×10^9	1.97×10^9	3.21×10^9	7.00×10^9
Leaf stump	2.72×10^9	$> 10^{10}$	2.80×10^9	$> 10^{10}$	7.75×10^9
Body	1.96×10^9	2.38×10^9	1.85×10^9	1.54×10^9	2.35×10^9

Table 2. Mean coliform count cfu/g in the different parts of *Daucus carota*.

Parts of Carrot	Sampling Location				
	Wetheral	Douglas	ITC park	Okigwe park	Ihiagwa
Eyes	1.37×10^8	$> 10^{10}$	2.19×10^8	3.35×10^8	2.45×10^8
Between buds	8.9×10^7	2.17×10^8	3.04×10^8	$> 10^{10}$	1.78×10^8
Leaf stump	8.9×10^7	2.17×10^8	3.04×10^8	$> 10^{10}$	1.78×10^8
Body	1.96×10^7	2.33×10^7	1.85×10^7	1.55×10^7	2.35×10^7

Table 3. Mean heterotrophic fungal count cfu/g in the different parts of *Daucus carota*.

Parts of Carrot	Sampling Location				
	Wetheral	Douglas	ITC park	Okigwe park	Ihiagwa
Eyes	2.70×10^8	2.98×10^8	2.52×10^8	2.79×10^8	1.35×10^8
Between buds	2.70×10^8	$> 10^{10}$	2.96×10^8	2.21×10^8	1.32×10^8
Leaf stump	$> 10^{10}$	3.12×10^8	$> 10^{10}$	$> 10^{10}$	9.15×10^8
Body	2.11×10^8	2.38×10^8	6.70×10^7	1.54×10^7	1.40×10^8

Table 4. Mean count of geohelminthes (per microscope field) in the different parts of carrot.

Parts of Carrot	Sampling location														
	Wetheral			Douglas			ITC park			Okigwe park			Ihiagwa		
	Al	Hw	Ss	Al	Hw	Ss	Al	Hw	Ss	Al	Hw	Ss	Al	Hw	Ss
Eyes	6	4	-	1	1	-	14	8	-	20	5	-	19	13	3
Between buds	9	1	-	5	3	-	31	9	-	21	19	-	23	16	-
Leaf stump	8	11	-	3	12	-	16	8	-	19	6	-	17	9	-
Body	5	5	-	5	2	-	7	4	-	6	4	-	11	6	-

Key: Al = *Ascaris lumbricoides*, Hw = Hookworm, Ss = *Strongyliode stercoralis*, - = Absent

Table 5. Microbial isolates inherent in the different parts of *Daucus carota*.

Parts of <i>Daucus carota</i>	Microbial isolates
Eyes	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Corynebacterium sp.</i> , <i>Bacillus spp</i> , <i>Micrococcus spp</i> , <i>Serratia marcescens</i> , <i>Bacillus cereus</i> , <i>Enterococcus spp</i> , <i>Saccharomyces spp</i> , <i>Rhizopus spp</i> , <i>Penicillium caseicolum</i> , <i>Mucor sp.</i> , <i>Geotricum sp.</i> , <i>Ascaris Lumbricoides</i> , <i>Hookworm</i> , <i>Strongyliode stercoralis</i>
Between Buds	<i>Bacillus subtilis</i> , <i>Micrococcus spp</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus cereus</i> , <i>Enterococcus spp</i> , <i>Escherichia coli</i> , <i>Corynebacterium sp.</i> , <i>Staphylococcus aureus</i> , <i>Saccharomyces spp</i> , <i>Rhizopus spp</i> , <i>Mucor sp</i> , <i>Geotricum sp.</i> , <i>Rhizopus spp</i> , <i>Aspergillus spp</i> , <i>Penicillium spp</i> , <i>Ascaris Lumbricoides</i> , <i>Hookworm</i>
Leaf St	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Micrococcus spp</i> , <i>Serratia marcescens</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus cereus</i> , <i>Enterococcus faecalis</i> , <i>Saccharomyces cerevisiae</i> , <i>Rhizopus spp</i> , <i>Geotricum sp.</i> , <i>Rhizopus spp</i> , <i>Aspergillus niger</i> , <i>Ascaris Lumbricoides</i> , <i>Hookworm</i>
Body	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Micrococcus spp</i> , <i>Bacillus cereus</i> , <i>Enterococcus faecalis</i> , <i>Escherichia coli</i> , <i>Saccharomyces cerevisiae</i> , <i>Saccharomyces spp</i> , <i>Rhizopus spp</i> , <i>Penicillium caseicolum</i> , <i>Mucor sp</i> , <i>Aspergillus spp</i> , <i>Ascaris Lumbricoides</i> , <i>Hookworm</i> ,

count per microscope field is as shown in Table 4. *Ascaris lumbricoide*, Hookworms and *Strongyliodes stercoralis* were identified, with eyes harboring all three geo-helminthes parasites. *Strongyliodes stercoralis* was absent from between buds, leaf stock and body of carrots analysed. Table 5 shows the microbial isolates to include eight bacteria spp

of *Corynebacterium*, *Staphylococcus*, *Bacillus*, *Escherichia*, *Micrococcus*, *Serratia*, *Pseudomonas* and *Enterococcus*, five fungal spp (*Saccharomyces*, *Rhizopus*, *Mucor*, *Penicillium*, *Aspergillus* and *Geotrichium*), and geohelminthes including *A. lumbricoides*, hookworms and *Strongyliode stercoralis*.

DISCUSSION

The presence of high concentration of heterotrophic bacteria, coliform and fungi in the different parts of *Daucus carota* could be a reflection of the soil from where the root vegetable was harvested. The microorganisms present in raw fruits and vegetables have been reported to be a direct reflection of the environment through which the product has passed. Similarly the extent of microbial contamination of vegetables and fruits have been reported to depend on the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce [18, 29, 30, 31]. Microorganisms form part of the flora and fauna of vegetables and many will be present at the time of consumption [29, 31].

The presence of geohelminthes and counts above 10^6 cfu/g for heterotrophic bacteria, coliform and fungi in *Daucus carota* calls for concern and the urgent need for public enlightenment on the need to wash carrots several times with potable water before consumption. Carrots are often purchased by school children and eaten without washing and this could be a veritable means of transmission of pathogens specifically geohelminthes. Infection with geohelminthes has been associated with blood loss (Anaemia), reduction in the ability to digest lactose, diminishing vitamin A utilization, reduction in growth and cognitive development in children, immunological hypo-responsiveness, intestinal ulceration, peritonitis, enlarged body organs, blockage of blood flow to body organs which could result to organ damage [32, 33, 34, 35, 36, 37].

The relatively higher concentration of microbial contaminants in the leaf stump, eye and between buds could be associated to constant holding/handling of carrot from the leaf stalk by farmers, vendors and consumers. Water, soil, manure and dust particles are easily trapped between the leaf stalks and in the orifice of the eye and between buds and thus these areas present a warm, moist and rich environment for sustenance and proliferation of microorganisms [38, 39, 40].

High level of coliform contamination might be as a result of unhygienic practices by the farmers, vendors and consumers alike. The use of contaminated water for irrigation, contaminated (untreated) manure, non-dedicated transport means

to convey produce and poor sanitary habits have been reported as contributing factors to contamination of vegetables and fruits [24, 30, 31, 41, 42, 43, 44, 45]. Coliforms are indicator organisms and counts of $> 10^6$ cfu/g sample reported in this work are a cause for concern, since the vegetable is usually consumed without further processing.

Pseudomonas, *Staphylococcus aureus* and *B. cereus* are common food contaminants from Man and the environment, their presence in food however, need to be controlled because they have been reported as cause of major food borne illnesses [46, 47, 48, 49, 50].

Penicillium spp, *Mucor* spp, *Rhizopus*, *Aspergillus* spp are common environmental contaminants, they are known to be the major cause of spoilage of fruits and vegetables [51, 52, 53]. Some of these fungi have been reported to produce mycotoxins and are implicated in cases of mycoses [52, 54].

Statistically, the result showed no significant difference between the different parts of *Daucus carota* and the various sampling spots at $P = 0.05$. Carrots sold in Owerri, Imo state showed high contamination rate and this could pose a risk of infection to consumers especially children, the aged, pregnant women and the immunocompromised individuals. Unhygienic practice by famers, middlemen, vendors and consumers are a predisposing factor to the contamination of this produce, there is therefore an urgent need to create awareness on proper hygiene practices, GMP and HACCP application.

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