Research article

The Effect of Storage and Water Rising of Microbial Contamination of Carrots and Cucumbers

Ibrahim F. Ahmed

Department of Microbiology, Faculty of Pure and Applied Sciences
International University of Africa, Khartoum Sudan

Abstract

In this work the Effect of storage and water rinsing in reducing the microbial contamination of carrots and Cucumbers and the time of spoilage was evaluated. In storage experiment done, washed and unwashed of carrots and cucumbers samples were stored at room and refrigerator temperatures. Washing with running tap water resulted in big reduction of the microbial load of carrots and cucumbers. The reduction ranged between 85 – 99 % for the total bacterial count, 92 % for moulds, and between 74 and 90 % for coliform bacteria. Unwashed samples and samples stored at room temperature spoiled more quickly than the washed samples and the sample stored in the refrigerator. In general carrots samples stored at room temperature spoiled in six to seven days and those store in the refrigerator spoiled in nine to ten days, with respect to cucumbers spoilage occurred often six days at room temperature and after seven days in the refrigerator. From this experiment we found that carrots was more resistant to spoilage than the cucumbers. Carrots and cucumbers spoilage was eminent when the microbial load reached about 10^6 CFU/g. It was concluded that, although the thorough cleaning of salad Carrots and Cucumbers with water and their storage in refrigerator would greatly reduce their microbial load, elimination of all microbial would not be achieved.

Keywords: Vegetables, Wash – Tapwater, Raw- carrots, cucumbers, Corynebacterium, Micrococcus, Bacillus-Staphylococcus

Introduction

Vegetables are rich in carbohydrates, ant – oxidants, minerals, vitamins and fibers (said, 2012). In contrast to their health benefits, the consumption of fresh vegetables has also been associated with risk for consumers (Weldezigina and Muleta, 2016).

Cucumbers

Common name : Cucumber .Latin name : *Cucumis sativus* . Family : Cucurbitaceae (Phillips and Rix, 1993). Cucumbers (*C. sativus*) has been an article of food from hundreds of

years . it grow on sprawling medium lent length vine clothed with rough , dark green 3pointed leaves, which are fuzzy.

Although cucumbers are produced at various times of the year and imported during the colder months, the supply is most plentiful in the summer months (Agricultural Marketing Services, 1994). (Suleiman, 2010,)

Carrots

Common Name : Carrot. Latin Name : *Daucus carota* Family Name : Umbelliferae (Apiaceae) (Nonnecke, 1989). Carrot (*Daucus carota*) is one of the most important vegetable crops used worldwide for human and animal consumption, as an excellent source of sugars, vitamins A and C and fiber in the diet (Gibberd et al, 2002). Freshly harvested carrots are available year round.

The ideal stage for use raw carrot sticks when they are relatively young, tender, well-colored, and mild flavored . Larger carrots are packed separately and used primarily for cooking or shredding (Vince , et al, 2009).

Source of contamination of vegetables

The phytonutrient can act as effective media for the transmission of pathogens (Khan et al, 1992; Abougrain et al 2010.).

Raw salad vegetables can become contaminated with pathogenic micro organism whilst growing in fields or during harvesting , Post-harvest handling , processing and distribution . Different agronomic practices can contaminate vegetables in various stages. Contamination occurs mostly before harvesting, either by contaminated manure , sewage irrigation water and waste water from livestock operations or directly from wild and domestic animals or during harvesting, processing-distribution and marketing or even at home (Eraky et al 2014; Rhman et al; 2014; Pagadala et al ; 2015; Mattei et al ; 2016) the predominant bacterial genera and species of the vegetables .

There has been an increasing number of fresh produce –associated foodborne illnesses identified internationally and efforts are being made to resolve these food safety problems (Denis et al ; 2016).

The objectives of this study were to determine the effect of storage and effect of water rising of microbial contamination of Carrots and Cucumbers.

Materials and Methods

Sampling:

Samples of carrots and cucumbers were obtained from local vegetable markets (also known as markazi) located at the northern part of Khartoum city . They were put separately into plastic bags and were analysed immediately after arrival to the laboratory for microbiological analysis.

The storage experiment of carrots and cucumbers in the laboratory

The experiment was done on carrots and cucumbers the samples were divided into two portions, one washed and the other not. They were stored at room temperature (25°C – 35°C) and in the refrigerator at 4°C.

The microbiological analysis was carried out daily till apparent spoilage occurred and the parameters studied were as follows:

- 1- Total viable count using plate count agar (oxide)
- 2- Yeast and moulds count using potato dextrose agar (oxide)
- 3- Count of coliform , faecal coliforms and faecal streptococci

Isolation, purification and identification of the dominant microorganism

3.7 Microbial Group Studied

(i) Total viable count was carried out using the pour plate technique as described by Harrigan and McCance (1976).

One ml of samples from suitable dilution were transferred aseptically into sterile petri-dishes, each dilution was examined in duplicate. Approximately ten ml of melted plate count agar (45-56°C) were poured into the dishes. The dishes were then thoroughly mixed to facilitate distribution of the sample throughout the medium. The medium was allowed to solidify, The plates were then incubated in an incubator (Heraeus) at 37°C for 48 hours. A colony counter (Quebec colony counter at and hand-Tally) was used for the determination of the total bacterial counts.

(ii) Moulds and yeast count

0.1ml samples were aseptically surface plated potato dextrose agar medium (PDA) with 40 ppm chloramphenicol added for bacterial growth- inhibition. For each series of tests control plates were included. The plates were incubated at 25°C for 48 hours, as described by Harrigan and McCance (1976). Colony forming units (CFU) were counted using colony counter and the results were presented as CFU/g.

(iii) Presumptive coliform test

One ml of each dilution was inoculated in triplicates into nine ml MacConkey broth in test tubes with Durham tubes. The tubes were incubated at 37°C for 48 hours. The most probable number (M.P.N) was recorded.

(iv) Confirmed coliform test

All tubes of the two highest dilutions showing gas fermentation in 24 hours, were submitted to the confirmed test using Brilliant green bile lactose broth fermentation tubes. All tubes of all dilutions in which gas was produced only at the

end of 48 hours, were submitted to the confirmed test, then incubated at 37°C for 48 hours. The most probable number (M.P.N) was recorded.

(v) Faecal coliform test

At least 3 loopfuls of each confirmed positive tube were subcultured into EC broth medium and then incubated at 44.5°C for 24 hours. Tubes showing any amount of gas production were considered positive. The most probable number (M.P.N) was recorded.

(vi) Differentiation of faecal coliform test

For further confirmation of faecal coliforms, tubes giving positive reaction at 44.5°C for 24 hours were streaked on Eosin Methylene Blue (E.M.B) agar.

(vii) Faecal streptococci test

Inoculation of samples was carried out as in the coliform presumptive test. Glucose azide broth was used and the fermentation tubes were incubated at 37°C and examined for turbidity using electric light at the end of 48 hours.

Purification and Identification of Isolates

The predominant microorganisms from morphologically different colony types were selected from plate count agar plates. These isolates were purified by subculturing part of a well isolated and typical colony into 5 ml sterile nutrient broth. The tubes were incubated into 37°C for 18-24 hours and then streaked onto nutrient agar plates. The process was repeated twice to ensure pure culture. The cultures were then kept in a refrigerator at 4°C until used for further tests.

The identification of the purified isolates were done according to Cowan and Steel (1974).

Examination of Cultures

Growth on solid medium was examined visually with naked eye for morphology and any changes in the medium. Growth on liquid media was examined for colour turbidity.

Biachemical Tests

(i) Gram's reaction

This was done as described by Harrigan and McCance (1976). A film was made on a clean slide by emulsifying part of a colony in a loopful of distilled water. The film was then air dried and fixed by slight flaming and stained as follows:

1. The smear was stained with crystal violet solution for 1-2 minutes.
2. The smear was rinsed rapidly with water and Gram's iodine solution was added and left for 1-2 minutes.
3. The iodine was poured off and the slide was washed with 95% ethanol for 5-15 seconds.

Results and Discussion

Microbial contamination of carrots and cucumber was Stored in the Lab at Room Temperature and in the Refrigerator. The experiment was done on carrots and cucumbers.

The samples were divided into two portions; one washed and the other not. Both were kept at room and refrigerator temperatures, and their microbial load was followed till complete spoilage occurred. The results are presented in (Tables 1, 2, 3 and 4).

Washing with running tap water resulted in a marked reduction in the microbial load of the samples (Das *et al*; 2016). The reduction ranged between 85 and 99% for the total bacterial count, 92 % for the moulds and between 74 and 90% for the coliforms, the faecal coli forms and the faecal streptococci (Tables 1, 2, 3 and 4).

In storage, the microbial load started to increase. For the samples stored at room temperature all unwashed cucumbers showed signs of complete spoilage at day six, where the microbial load was about 1×10^6 CFU/g bacteria, 8×10^5 CFU/g yeasts and 6×10^4 CFU/g in moulds.

The washed samples showed signs of complete spoilage at day seven and their microbial load was about 10^6 CFU/g bacteria, 7×10^4 CFU/g yeast and 2×10 CFU/g moulds. In both cases, the loads of coliforms, faecal streptococci reached 1100 MPN/g at day five. The microbial load of the samples increased further with time and at day ten all carrots were completely destroyed and covered with a massive growth of moulds. At that stage the bacterial load was between 10^7 and 10^8 CFU/g and the load of yeasts and moulds was between 10^5 and 10^6 CFU/g.

Table 1. The microbial contamination of carrots stored at refrigerator

Count	Colony b.		Yeast		Mould		MPN/g		MPN/g		MPN/g	
Period of Storage	Count /g		Count /g		Count /g		Coliform		Faecal coliform		Faecal streptococci	
	*1	*2	*1	*2	*1	*2	*1	*2	*1	*2	*1	*2
Zero hour	3.6x10 ⁴	5x10 ⁴	-	-	-	-	150	40	150	32	32	4
One day	9x10 ⁴	2.9x10 ⁴	-	-	-	-						
Two days	3x10 ⁵	2.5x10 ⁴	7x10 ⁴	-	7x10 ⁴	2.4x10 ⁴						
Three days	4x10 ⁵	3.2x10 ⁴	-	9x10 ⁴	2.7x10 ⁴	2.7x10 ⁴						
Four days	4.2x10 ⁵	3.5x10 ⁴	7.7x10 ⁴	1.5x10 ⁴	1.6x10 ⁵	2.9x10 ⁴						
Five days	4.3x10 ⁵	6.6x10 ⁴	8.9x10 ⁴	1.6x10 ⁴	1.9x10 ⁵	3x10 ⁴						
Six days	6.1x10 ⁵	3x10 ⁵	9.2x10 ⁴	1.9x10 ⁴	1.4x10 ⁵	6x10 ⁴						
Seven days	7.9x10 ⁵	4x10 ⁵	2.1x10 ⁵	2.1x10 ⁴	2x10 ⁶	2.5x10 ⁵						
Eight days	8.1x10 ⁵	4.3x10 ⁵	4.9x10 ⁵	3.2x10 ⁴	-	2.9x10 ⁵						
Nine days	9.2x10 ⁵	8.4x10 ⁵	5.2x10 ⁵	8x10 ⁵	2.6x10 ⁶	-						
Ten days	9.7x10 ⁵	1.1x10 ⁶	5.8x10 ⁵	1.1x10 ⁵	-	3x10 ⁵						
Eleven days	9.9x10 ⁵	1.5x10 ¹⁶	6x10 ⁵	1.2x10 ⁵	5.2x10 ⁶	-						
Twelve days	7.8x10 ⁶	1.6x10 ⁶			8.5x10 ⁶	4x10 ⁵						

*1 = Unwashed .

*2 = Washed .

Table 2. The microbial contamination of cucumbers stored at refrigerator

	COUNT	COLONY B.	YEAST	MOULD	MPN/G	MPN/G	MPN/G
Period of:	Count/g	Count/g	Count/g	Count/g	Coliform	Faecal coliform	Faecal streptococci
Storage		*1 *2	*1 *2	*1 *2	*1 *2	*1 *2	*1 *2
Zero hour		2x10 ³ 2x10 ²	- -	5x10 ³ 4x10 ²	200 20	200 20	1100 150
Three days		2.4x10 ⁴ 5x10 ⁴	- -	7x10 ³ 6x10 ³			
Six days		3x10 ⁴ 7x10 ⁴	- -	9x10 ⁶ 8x10 ³			
Seven days		3.3x10 ⁶ 1.2x10 ⁶	- -	3x10 ⁴ 9x10 ³			

*1 = Unwashed .

*2 = Washed .

Table 3. The microbial contamination of carrots stored at room temperature

	COUNT	COLONY B.	YEAST	MOULD	MPN/G	MPN/G	MPN/G
Period of:		Count/g	Count/g	Count/g	Coliform	Faecal coliform	Faecal streptococci
Storage		*1 *2	*1 *2	*1 *2	*1 *2	*1 *2	*1 *2
Zero hour		3.6x10 ⁴ 5x10 ²	- -	- -	150 40	150 23	23 4
One day		1.2 x10 ⁵ 7.2x10 ⁴	- 1.5x10 ³	- -			
Two days		1.4x10 ⁵ 1.1x10 ⁵	1.7x10 ⁴ 1.3x10 ⁴	1x10 ⁴			
Three days		1.7 x10 ⁵ 1.5x10 ⁵	2 x10 ⁴ -	4x10 ⁴ 5x10 ³			
Four days		1.8 x10 ⁵ 1.7x10 ⁵	2.1 x10 ⁴ 5x10 ⁴	5.2 x10 ⁴ 1.1x10 ⁴			
Five days		2.9 x10 ⁵ 2.6x10 ⁵	2.9 x10 ⁴ 5.8x10 ⁴	5.9 x10 ⁴ 3.6x10 ⁴	1100 1100	1100 1100	1100 1100
Six days		1.1 x10 ⁶ 3.6x10 ⁵	8.1 x10 ⁵ 6.2x10 ⁴	6 x10 ⁴ 9x10 ⁴			
Seven days		7.5 x10 ⁶ 9.8x10 ⁵	9.7 x10 ⁵ 7x10 ⁴	- 2x10 ⁵			
Eight days		1.5 x10 ⁷ 2x10 ⁶	- 8.3x10 ⁴	1.1 x10 ⁵ -			
Nine days		2x10 ⁷ 2.9x10 ⁶	1.2 x10 ⁶ 9.3x10 ⁴	2.9 x10 ⁶ 2.1x10 ⁵			
Ten days		3 x10 ⁸ 2x10 ⁷	7 x10 ⁶ 1.6x10 ⁵	3.6 x10 ⁶ 2.2x10 ⁵	1100 1100	1100 1100	1100 1100

*1 = Unwashed . *2 = Washed .

Table 4. The microbial contamination of cucumbers stored at room temperature

	COUNT	COLONY B.	YEAST	MOULD	MPN/G	MPN/G	MPN/G
Period of:		Count/g	Count/g	Count/g	Coliform	Faecal coliform	Faecal streptococci
Storage		*1 *2	*1 *2	*1 *2	*1 *2	*1 *2	*1 *2
Zero hour		2x10 ³ 3x10 ²	- -	5x10 ³ 4x10 ²	200 20	200 20	1100 150
Three days		8x10 ⁴ 1.5x10 ⁴	- -	3x10 ³ 5x10 ³			
Six days		6 x10 ⁶ 4.4x10 ⁶	- -	5 x10 ³ 6x10 ³			
Nine days		7.7x10 ⁷ 7x10 ⁷	- -	3.5 x10 ⁴ 6.6x10 ³	1100 150	1100 200	150 20

*1 = Unwashed . *2 = Washed .

For the samples of carrots stored in the refrigerator (Table 1), signs of complete spoilage were observed at day nine for the unwashed samples and at day ten for the washed ones. In both cases, the bacterial load was about 10^6 CFU/g. The yeast count was 5.8×10^5 CFU/g for the unwashed and 1.1×10^5 CFU/g for the washed. The mould count was 2.6×10^6 CFU/g for the unwashed and 3×10^5 CFU/g for the washed samples.

The microbial load did not increase significantly on the next two to three days and remained in the range of 10^6 CFU/g for bacteria and moulds and 10^5 CFU/g for yeasts (Table 1). The count of the coliforms, the faecal coliforms and the faecal streptococci remained in the range between 150 and 210 MPN/g.

With respect to the other cucumbers the initial bacterial load was relatively low amounting to about 2×10^3 CFU/g. This is lower than the initial load of carrots by more than one decimal (Tables 2 and 3), still the samples stored at room temperature were completely spoiled after six days of storage, and the bacterial load was then 6×10^6 CFU/g for the unwashed and 4.4×10^6 CFU/g for the washed samples. The samples, stored in the refrigerator (Table 2) were spoiled were completely after seven days of storage where the bacterial load was 3.3×10^6 CFU/g for the unwashed and 1.2×10^6 CFU/g for the washed samples. These results indicated that bacterial growth rates on cucumbers were much higher than on carrots.

The results of this study also indicate that spoilage of carrots and cucumbers were eminent when the microbial load reaches 10^6 CFU/g.

It, seems also that moulds play important role in carrots and cucumber spoilage and their counts increase in late stage of spoilage when the bacteria destroy the protective skin of carrots and cucumber (Tables 1, 2, 3 and 4). These spoiled carrots and cucumbers could be a source of pathogens since the load of enteric bacteria was found to be very high in the spoiled samples. Washing with running tap water resulted in a prolongation of the shelflife of the carrots and cucumbers by at least one day. Also storage in the refrigerator prolongs the shelflife by up to three days compared to room temperature.

Identification of Bacteria and Moulds from carrots and cucumbers

According to their colony forms, the dominant bacteria and moulds in any sample analysed were isolated, purified and identified using morphological and physiological typing methods. A total of eight pure isolates of bacteria and three isolates of moulds were isolated.

The results are presented in Table (5). The predominant bacteria, were identified tentatively as *Bacillus* making about 33.3% of isolates, *Micrococcus* about 25%, *Staphylococcus* also about 25 % and *Corynebacterium* about 12.5 % of the isolates (Eniet *al.*, 2010).

Table 5. Types of genera isolated from stored carrots and cucumbers

Test	Gram stain	Growth in air	Oxide test	Catalase Test	Q/F test	Motility	Spore forming	Shape	%*	Genus
Unknown Isolates										
1	+	+	-	-	O	-	-	Rod	12.5	Corynebacterium
2	+	+	-	+	-	-	-	Cocci	25	Micrococcus
3	+	+	-	+	-	-	-	Cocci	25	Micrococcus
4	+	+	-	+	-	+	+	Rod	33.3	Bacillus
5	+	+	-	+	F	-	-	Cocci	25	Staphylococcus
6	+	+	-	+	F	-	-	Cocci	25	Staphylococcus
7	+	+	-	+	-	+	+	Rod	33.3	Bacillus
8	+	+	-	+	F	+	+	Rod	33.3	Bacillus

The mould isolates were also tentatively identified as *Rhizopus*, *Penicillium* and *Aspergillus* (R. Barkai-Golan and N. Paster, 2008).

Conclusion

It was concluded that, although the thorough cleaning of salad Carrots and Cucumbers with water and their storage in refrigerator would greatly reduce their microbial load, elimination of all microbial would not be achieved.

Acknowledgement My deep thanks to the members of department of microbiology I.U.A for their assistance during the study.

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