



Determination of Quality Control of Fresh Salads in IBB City, Yemen

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ABSTRACT

This study aims to determine the quality control of fresh salads which sold in Ibb city, Yemen in a street shops, cafeterias, and restaurants by analyzing the microbial parameters and focusing on the coliform bacteria group. Total samples determined reached to fifty samples collected randomly through standard procedure from different sources. Samples analyzing was carried out in the laboratories of Ibb university in 2013, and the results could be summarized as the following: Total bacteria count ranged from Zero to 33.9×10^4 CFU and total coliform count ranged from Zero to 21.7×10^4 CFU. Meanwhile, total fungi count ranged from Zero to 12.3×10^2 CFU. On the other hand species of the coliforms were Enterobacter, klebsiella, E.coli and Citrobacter by percentages 24, 22, 20, and 16 %, respectively. Meanwhile, anaerobic bacterial growth in some samples were 10% and 8% of the samples were no growth has occurred.

Keywords: Fresh Salads - Quality Control - Coliform Bacteria.

INTRODUCTION

Salads can be defined as a food made primarily from a mixture of raw vegetables. Health benefits of salads are many, owing to the various vegetables present in them. Vegetables are a good source of antioxidants and phyto-nutrients. They are low in calories and are rich in complex carbohydrates, vitamins and minerals. Salads should be cleaned properly, as they are generally eaten raw or partially cooked (Bartz and Wei, 2003). Fresh cut vegetables are minimally processed by washing, slicing, dicing, peeling and / or shredding prior to sale. In Australia, leafy salad vegetables such as lettuce, rocket and baby spinach are the most common products in the fresh cut vegetable category, contributing towards an estimated national production value of \$44 million for the year 1997/98 (Szabo and Coventry, 2001).

According to data from the Centers for Disease Control and Prevention in the US (CDC), food borne illness due to contaminated vegetables is on the rise, with products implicated including baby spinach, lettuce, seed sprouts and green onion (FDA, 2004). Investigations into outbreaks have identified issues such as agricultural water quality, the use of manure as fertilizers, the presence of animals in fields or packing areas, and the health and hygiene of workers handling the fresh produce during production, packing, processing, transportation, distribution, or preparation. Many of these

products are often consumed raw which can contribute to their potential as a source of food-borne illness. Raw vegetables can harbor many microorganisms, which may be dispersed over the plant or appear as micro-colonies embedded in the plant tissue (Szabo and Coventry, 2001). The majority of microorganisms associated with raw vegetables are non-pathogenic and gram negative organisms tend to dominate the bacterial population including *Enterobacter* spp., and other coliforms group. Vegetables are highly exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing they therefore harbor adverse range of microorganisms including plant and human pathogens (Nguyen-the and Carlin, 1994 ; Dunn *et al.*, 1995 and Carmo *et al.*, 2004). Differences in microbial profiles of various vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007 and Ofor *et al.*, 2009). Using of untreated waste water and manure as fertilizers for the production of vegetables is a major contributing factor to contamination (Amoah *et al.*, 2009 and Olayemi, 2007). The term coliform represents a group of species from several genera, namely, *Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter* and probably *Aeromonas* and *Serratia* (Bibek, 2005). The main reason for grouping them together is their many common characteristics. They are all gram negative, motile, non-sporulating, rod-shaped, facultative anaerobic bacterium, a normal inhabitant of the intestinal tract of humans and warm-blooded animals and birds, resistant to many surface-active agents and ferment lactose to produce acid and gas within 48 h at 32°C or 35°C (Bibek, 2005). Some species can grow at higher temperature (44.5°C), whereas others can grow at 4°C to 5°C. All are able to grow in foods except in those that are at pH \leq 4.0 (a few that are acid resistant can grow or survive) and $A_w \leq$ 0.92 (Bibek, 2005). Microbes associated with fresh-cut vegetable products can vary greatly in accordance with the produce type and storage conditions. Temperature plays a significant role in determining the nature of the microflora associated with refrigerated fresh-cut vegetables (James and Ngarmak, 2011). Microbial hazards continue to be one of the biggest threats to food safety (Al-Binali *et al.*, 2006). The implications of the microbial contamination and growth on vegetable produce include spoilage, decreased sensory appeal and decreased shelf life. However coliforms are expected to be present in many raw foods and food ingredients of animal and plant origin (Bibek, 2005). Also, the occurrence of some coliforms of non fecal origin and their ability to grow in many foods reduces the specificity of coliforms as an indicator of fecal contamination in raw foods (Bibek, 2005). However, coliforms are commonly used bacterial indicator of sanitary quality of foods and water and are also utilized as indicators of microbial pollution as they are common inhabitants of both animal and human guts (Tortora, 1995). So, our study aims to determine the quality control of fresh salads which sold in Ibb city, Yemen in a street shops, cafeterias and restaurants by analyzing the microbial parameters and focusing on the coliforms bacteria group.

MATERIALS AND METHODS

Study area: IBB city is situated in the republic of Yemen. IBB province located central of the Yemen, geographically its mountain area and also its well known it rained in April to November, nature of the people are farmers and there are depending on the rain and occasionally to the wells and other sources. Peoples of IBB city, are well known they cultivate large amount of vegetables, fruits and other cereals. It is also good to mention some farmers irrigate there vegetables by sewage water and some of the sellers sold there, mixed salads near to the rubbish and unclean places in the streets.

Sampling of raw mixed-vegetable salads:

The total number were 50 samples, ten street vendors were randomly selected at the winter and repeated at summer season, because we think that, the risk and hazards from street foods is very highly. The remnant samples were taken from popular restaurants, large restaurants and cafeterias equally at one season. According to the time, we collect the samples at 8 Am to 12 Pm. Samples were placed in separate, sterilized pre-labeled containers, sealed, placed on crushed ice during time not exceeded one hour and sent to the laboratory for microbiological examination, according to the methods of (Canadian Food Inspection Agency, 2001).

Microbiological analysis:

Stock preparation:

A stock solution of 10^{-1} dilution was prepared by taking 30g of sample and mixed with 270 ml of sterile normal saline and homogenized in a sterilized Warring blender for 5 min. The suspension was filtered through sterile filter paper (What-man No. 2) and the filtrate received in a sterilized labeled conical flask, and used for subsequent tests.

Culture and enumeration:

Aerobic plate count (APC) was done by preparing serial dilutions of the samples, ranging from dilutions of 10^{-2} to 10^{-6} from the stock. Computing and recording count of bacteria, fungi, and cliforms were carried out according to (FDA, 2001) by using this formula:

$$N = \frac{\Sigma c}{[(1 \times n_1) + (0.1 \times n_2) \times (d)]}$$

Where:

N = Number of colonies per ml or g of product

ΣC = Sum of all colonies on all plates counted

n_1 = Number of plates in first dilution counted

n_2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

Culture and isolation of microorganisms:

About 2 ml from each of the prepared dilution was inoculated into two plates, one of them considered as original plate, other as repeated plate to all media, Potatoes Dextrose Agar (PDA) to estimation of load of the fungi and the incubation period for three to five days at room temperature and we added to the (PDA) weak acid especially citric acid to inhibit bacterial growth. Nutrient agar (NA) for total bacterial count, incubated at 37°C overnight. MacConkey agar (MA) to estimation coliforms load and incubated at 37°C overnight. Growth was determined by appearing colonies on the surface of the medium. The pure isolates were identified by their colonial, cell morphology, gram reaction and a combination of standard biochemical tests (Benson, 2001).

The coliform bacteria groups were classified by biochemical tests which reported in table (1).

Table (1) : The biochemical tests which were used to classify coliforms bacteria:

Name of bacteria	Biochemical test				
	Indol	Urease	S.C.A	Motility test	K.I.A
<i>E.coli</i>	+	-	-	+	Y/Y with gas production and no H ₂ S production
<i>Klebsiella</i>	-	+	+	-	
<i>Enterobacter</i>	-	-	+	+	
<i>Citerobacter</i>	-	+	+	+	

RESULT AND DISSCUSION

Table (2) : Total bacteria, coliform and fungi counts in fresh salads samples collected from popular restaurants, cafeterias and large restaurants in lbb city, Yemen.

Samples No.	Samples sources	Total bacteria count CFU	Total coliform count CFU	Total fungi count CFU
1	Popular restaurants	1.9 x 10 ⁴	Zero	1.6 x 10 ²
2		7.3 x 10 ⁴	0.9 x 10 ⁴	0.7 x 10 ²
3		1.1 x 10 ⁴	0.4 x 10 ⁴	4.8 x 10 ²
4		26.9 x 10 ⁴	21.7 x 10 ⁴	0.8 x 10 ²
5		2.6 x 10 ⁴	4.6 x 10 ⁴	1.3 x 10 ²
6		3.9 x 10 ⁴	0.2 x 10 ⁴	0.5 x 10 ²
7		1.9 x 10 ⁴	0.2 x 10 ⁴	0.1 x 10 ²
8		8.8 x 10 ⁴	0.3 x 10 ⁴	0.5 x 10 ²
9		2.5 x 10 ⁴ ×	0.5 x 10 ⁴	Zero
10		0.5 x 10 ⁴	0.2 x 10 ⁴	0.4 x 10 ²
11	Cafeterias	Zero	0.6 x 10 ⁴	0.2 x 10 ²
12		15 x 10 ⁴	2.3 x 10 ⁴	Zero
13		19.4 x 10 ⁴	2.6 x 10 ⁴	11.7 x 10 ²
14		3.5 x 10 ⁴	1.8 x 10 ⁴	2.3 x 10 ²
15		4.3 x 10 ⁴	0.7 x 10 ⁴	5.1 x 10 ²
16		3.8 x 10 ⁴	1.2 x 10 ⁴	0.8 x 10 ²
17		5.1 x 10 ⁴	2 x 10 ⁴	1.9 x 10 ²
18		Zero	Zero	2 x 10 ²
19		11.5 x 10 ⁴	4.7 x 10 ⁴	2.9 x 10 ²
20		20.6 x 10 ⁴	Zero	3.6 x 10 ²
21	Large restaurants	Zero	0.5 x 10 ⁴	Zero
22		Zero	0.3 x 10 ⁴	Zero
23		3 x 10 ⁴	0.2 x 10 ⁴	0.1 x 10 ²
24		Zero	0.6 x 10 ⁴	Zero
25		25.6 x 10 ⁴	4.4 x 10 ⁴	Zero
26		26.1 x 10 ⁴	4.2 x 10 ⁴	0.04 x 10 ²
27		10.5 x 10 ⁴	5.8 x 10 ⁴	2.1 x 10 ²
28		2.4 x 10 ⁴	4.6 x 10 ⁴	0.3 x 10 ²

29		16.3 x 10 ⁴	Zero	Zero
30		3.8 x 10 ⁴	0.1 x 10 ⁴	Zero

These are 50 samples as total of fresh salads, which were collected from popular restaurants, cafeterias, large restaurants and street shops in two different seasons winter and summer in Ibb City, Yemen, 10 samples from each source.

Data in Tables (2) showed that, the total bacteria count ranged between Zero to 26.9×10^4 CFU in fresh salads in cafeterias, popular and large restaurants in Ibb City, Yemen, meanwhile total coliform count ranged between Zero to 21.7×10^4 CFU, on the other hand, total fungi (mold and yeast) counts range between Zero to 11.7×10^2 CFU in the same samples.

Data in table (3) showed that, total bacterial counts ranged between 0.04×10^4 to 11.1×10^4 CFU, in winter season. Meanwhile, in summer season it ranged between 1.2×10^4 to 33.9×10^4 CFU. On the other hand total coliform counts ranged between 0.4×10^4 to 5.5×10^4 CFU in samples of winter, whereas in summer it ranged between Zero to 12.2×10^4 CFU. And finally, the total fungi counts in the samples which were collected in winter season ranged between 0.3×10^2 to 12.3×10^2 CFU. Meanwhile, in other samples which were collected in summer season it was ranged between Zero to 3.6×10^2 CFU in fresh salads samples which were collected from different 10 street shops in Ibb City, Yemen, in two seasons winter and summer, we think that, the risk and hazards from street foods is very highly, especially fresh salads which are normally contaminated by several kinds of microorganisms.

Table (3) : Total bacteria, coliform and fungi counts in fresh salads in winter and summer seasons samples collected from street shops in Ibb city, Yemen.

Seasons	Winter samples			Summer samples		
Street shops	Total bacteria counts CFU	Total coliform count CFU	Total fungi counts CFU	Total bacteria counts CFU	Total coliform count CFU	Total fungi counts CFU
1	4.7×10^4	5.5×10^4	1.5×10^2	17×10^4	Zero	2.9×10^2
2	0.8×10^4	0.1×10^4	0.8×10^2	7.7×10^4	0.4×10^4	Zero
3	2.7×10^4	4.8×10^4	4.5×10^2	13.5×10^4	7.2×10^4	0.6×10^2
4	1.1×10^4	0.4×10^4	0.5×10^2	1.2×10^4	1.6×10^4	Zero
5	1.9×10^4	5.5×10^4	2.3×10^2	21.7×10^4	1.6×10^4	3.6×10^2
6	1×10^4	3.1×10^4	0.5×10^2	12.6×10^4	6.5×10^4	3.1×10^2
7	11.1×10^4	5.1×10^4	4.5×10^2	33.9×10^4	6×10^4	2.4×10^2
8	3.3×10^4	1.6×10^4	0.3×10^2	17×10^4	2.6×10^4	0.5×10^2
9	7.7×10^4	2.1×10^4	2.4×10^2	3.9×10^4	0.4×10^4	Zero
10	0.04×10^4	0.7×10^4	12.3×10^2	3.6×10^4	12.2×10^4	Zero

Vegetable salads are composed of different range of plant parts (leaves, roots, tubers, fruits, and flowers). Production practices, growth conditions and the location of the edible part during growth (soil, soil surface, aerial part) will in combination with intrinsic, extrinsic, harvesting and processing factors affect their microbial status at the time of consumption (Anonymous, 2002 and Beuchat, 2002).

Fresh vegetables are essential components of the human diet and there is considerable evidence of the health and nutritional benefits associated with the consumption of fresh vegetables. In the USA, Canada, New Zealand and several European Union states, health institutions have run campaigns recommending the daily consumption of at least five daily servings of vegetables. As well as a

significant rise in the consumption of fresh produce for health benefits, there have also been significant changes in lifestyles and major shifts in consumption trends (Anonymous, 2007). During harvest and transport raw vegetables may be bruised resulting in the release of plant nutrients, providing nutrients microorganisms present on the surface of the vegetable to grow. In 1995, twenty one persons associated with a hospital in Canada became infected with *E. coli* O157:H7. Although no food samples were available for testing, epidemiological studies concluded that the vehicle of infection was imported iceberg lettuce. On receipt of the lettuce, kitchen staff had filed an incident report citing heavy spoilage (Preston, 1997). *Listeria monocytogenes*, *Salmonella* and *Escherichia coli*, have been isolated from raw vegetables, which can become contaminated while growing or during harvesting, postharvest, (Nguyen-the and Carlin, 1994). Tomato, Cucumber, Carrot, Green chili, Lemon, Coriander leaf, Pepper, mint and Beet root are some of the vegetables that are normally consumed raw in order to obtain their valuable nutrients in best form and their traditional use in best form and their traditional use in preparing salads is familiar throughout the world in the same manner (Wells and Butterfield, 1997). Pathogens isolated from vegetable salad include; *Staphylococcus aureus*, *Enterobacter* spp, *Klebsiella* spp, *Salmonella typhi*, *Serratia* spp, *Providencia* spp, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Shigella sonnei*. The number and kinds of microorganisms associated with fresh-cut produce are highly variable. Mesophilic bacteria from plate count studies typically range from $10^3 \times 10^1$ to $10^9 \times 10^1$ CFU/g (James and Ngarmsak, 2011). Total counts of products after processing range from $10^3 \times 10^1$ to $10^6 \times 10^1$ CFU/g (Nguyen-the and Carlin, 1994). It is well known that most of coliform bacteria group were grow in mixed vegetable salads and these coliform can cause food-borne diseases to consumer. Therefore, the present work objective was to do the isolation and identification of these coliforms. Isolation of coliform was done by using MacConkey agar as a growing medium and biochemical tests as confirmed identification. So, more studies are necessary to determine the species of pathogenic bacteria such as *Salmonella* SPP. and *E. Coli*. O157:H7 and others. Microorganisms such as coliforms group are pathogenic now and many scientists in the world reported that, these genus of bacteria caused food-borne diseases.

Table (4): Percentage of Coliform according to its sources and total samples:

Coliform and other	Ratio %					According to total samples
	According to samples sources					
	Street(winter)	Street(summer)	cafeterias	Popular restaurant	Large restaurant	
<i>E.coli</i>	60%	-	20%	20%	40%	20%
<i>Klebsiella</i>	-	10%	10%	20%	-	22%
<i>Citrobacet</i>	20%	30%	20%	40%	-	16%
<i>Enterobacter</i>	10%	50%	20%	10%	30%	24%
No growth	-	10%	20%	10%	-	8%
Anaerobic	10%	-	10%	-	30%	10%

Finally data in Table (4) showed that, the percentage of *E.coli* was highly represented in the samples from street shops (winter) and large restaurants sources by percentages reached to 60 and 40 %, respectively and it is more than the other sources. Meanwhile, *Klebsiella* was in a medium percentage 20% in popular restaurants and low percentage 10% in the street (summer) and cafeterias. On the other hand, *Citrobacter* was in higher percentage in popular restaurants, more than in street shops (summer), street shops (winter) and cafeterias by percentages were 40, 30, 20 and 20 %, respectively. Meanwhile, *Enterobacter* were more represented in the samples from street

shops (summer), large restaurants, cafeterias, popular restaurants and street shops (winter) sources by percentages reached to 50, 30, 20, 10 and 10 %, respectively. Meanwhile, represented that, from 50 samples the percentages of *E.coli*, *Klebsiella*, *Citrobacter* and *Enterobacter* were 20, 22, 16 and 24 %, respectively. Meanwhile, some samples showed non growth or anaerobic growth samples represented 8 and 10 % percentage, respectively. In discussion we can assume that, our results are similar with the trend of all authors who worked in the microbial quality control of fresh vegetable salads in any place in our world such as a study by (Osamwonyi *et al.*, 2013) in Nigeria, their results showed that HPC and TCC of vegetable salads obtained from restaurant A at both the morning (10:00 am) and evening (4:00pm) periods during the sampling months (May-July, 2012) ranged from 2.80×10^4 to 1.46×10^4 CFU/g and 1.46×10^4 to 2.84×10^4 CFU/g, respectively. So, the very important notes that, fresh salads in Ibb City, Yemen has contaminated by a highly numbers of microorganisms and this is very dangerous for human health according to the standers that accommodated by some countries as the limit of total coliform not exceeded (100 CFU/gm), especially we know that, fresh salads ready to eat without any cooking or other processing. So, microorganism such as coliform bacteria groups which is pathogenic now and many scientists in the world reported that, these genus of bacteria caused food-borne diseases.

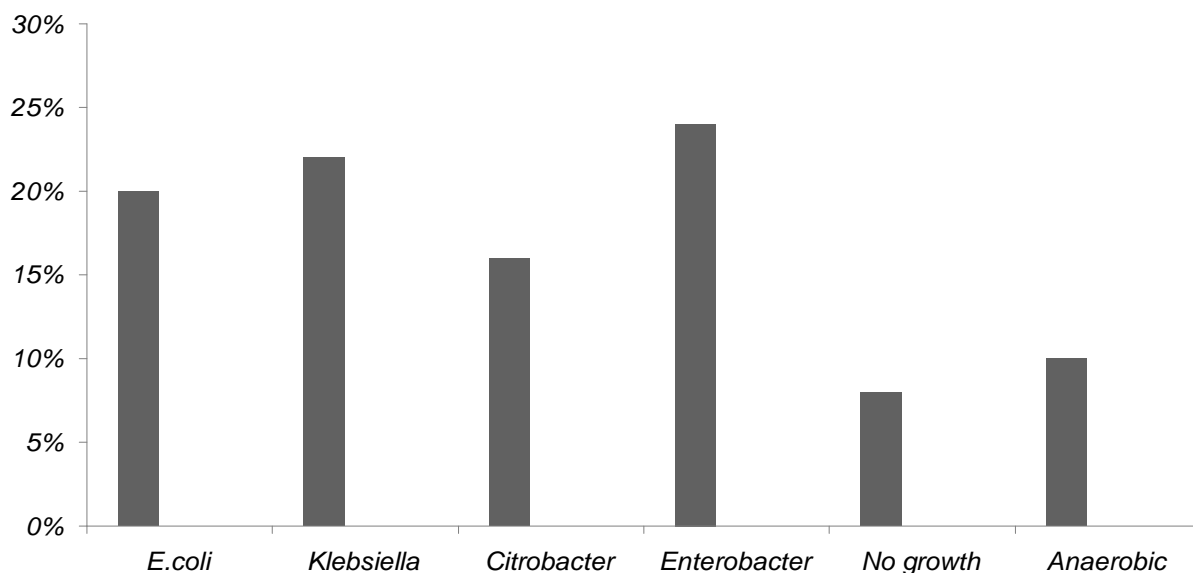


Figure (1) : Total percentages of coliform bacteria in salad samples.

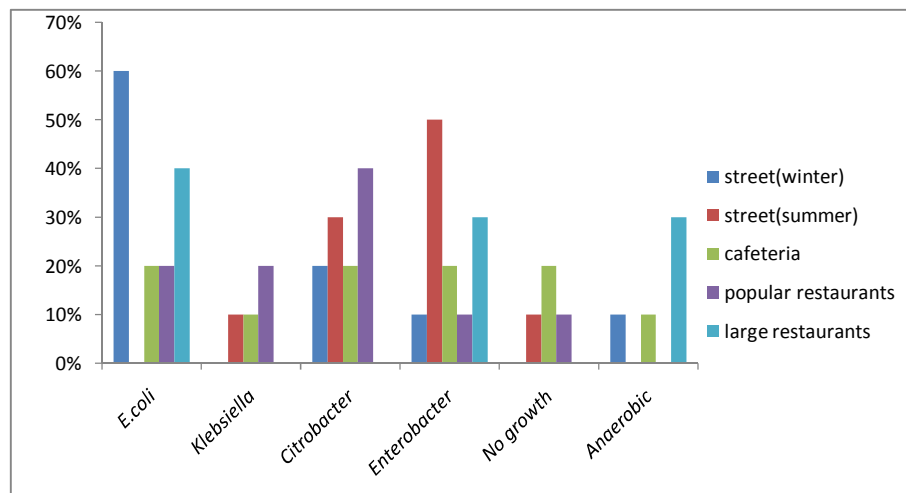


Figure (2): percentage of coliform in salad samples according to samples sources.

CONCLUSION

We think that, the fresh salads which sold in Ibb City, Yemen contaminated by highly numbers of microorganisms. Including these microorganism coliform groups bacteria in most samples. There are some differences between the sources of fresh salads but it is still in the same grade. Fresh salads in Ibb City, Yemen, are dangerous for human health because, fresh salads are ready to eat without any cooking or other processing.

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