EFFECT OF WASHING AND PREVALENCE OF BACTERIA IN LEAFY VEGETABLE FOODS COLLECTED FROM IRAQI MARKETS

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Abstract

In this research, the effect of repeated washing with water and bacterial contamination had been examined on leafy vegetablesthat are used as healthy food for human, twenty-five bundles had been collectedrandomly from different vegetable stores and itinerant's vendor in Baghdad;washing process, cultivation, counting and identificationof viable bacteria had been done. A total of 25 bundles had been collected from different Vegetable stores and itinerant's vendor in Baghdad, leafy vegetables had been washed three times and in each time washing water had been cultured on nutrient agar for bacterial isolation and identification. It had been found that viable bacterial count was less with repeated washing and the best result was after the third wash; this should be done with soaking and vigorous shaking. Different genera of bacteria had been collected in this study and identified by different methods depending on colonial morphologyin different types of media, Gram's stain and biochemical activity of isolated colonies. Some bacterial isolates had been identified by Vitek 2 System Version 08.01. *Staphylococcus* spp., *Bacillus* spp. and some coliforms were found in high incidence, while some expected bacteria had not been isolated. Washing of leafy vegetables playing an important role in reduction of bacterial viability count.Different types of bacteria can be isolated from leafy vegetables that may cause serious illnesses for human. This research spotted light on types of bacteria that can be isolated from leafy vegetables and the effect of repeated washings in viable bacterial count reduction.

Key words: leafy vegetables, water washing, bacterial contamination

Introduction

Leafy vegetables are important part of healthy food and used widely as main meal for most people because it filled with vitamins and minerals that play an important role in human health, green leaves and stalks should be eaten raw or lightly cooked to preserve the nutrients. Exposure to heat during cocking reduces the nutritional value of these vegetables. Bacteria can reach leafy vegetables easilyduring and after harvest, from handling, storing, transporting and at the grocery store,some of them are pathogens others are potential pathogen or non-pathogen(Barrera *et al.*, 2012; Martínez-Vaz *et al.*, 2014; Nüesch-Inderbinen and Stephan 2016), when these vegetables eaten, illnesses caused by these microorganisms may be occur. Washing with water can help in reduction of bacterial viable count even if it did not cause them to be completely killed but it of course leading to less harmful effect of these contaminants.

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Repeating this step causes the microbial content to be reduced each time, especially if it is done with soaking and shaking, which helps to transfer the largest amount of microbes to the water that will be disposed of (Tomás-Callejas *et al.*, 2011; Barrera *et al.*, 2012). Washing with water is helpful not only for bacterial reduction but also to get rid of dirt and pesticide residues and useful to preserve more of the nutrients (Gil *et al.*, 2009).

E.coli, Salmonella spp., *Shigella* spp. were responsible for many deaths all over the world (Tomás-Callejas *et al.,* 2011; Nüesch-Inderbinen and Stephan 2016); *E. faecalis, Pseudomonas* spp. *Enterobacter spp., Mycobacterium* spp., *Listeria monocytogenes* also detected in leafy vegetables worldwide and responsible for many illnesses (Karlo Malavé Llamas, 2015; Murray *et al.,* 2017). Illnesses represented by gastrointestinal tract infections, nervous system infections, bacteremia and many other illnesses can be caused by these bacteria (Martínez-Vaz, *et al.,* 2014; Nüesch-Inderbinen and

Table 1: General description of viable count.

Washing steps	N	Mean± Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
control	25	3.2933±.04933	.02848	3.1708	3.4157	3.26	3.35
Wash1	25	2.8500±.02646	.01528	2.7843	2.9157	2.83	2.88
Wash2	25	1.6000±.10000	.05774	1.3516	1.8484	1.50	1.70
Wash3	25	.4000±.10000	.05774	.1516	.6484	.30	.50
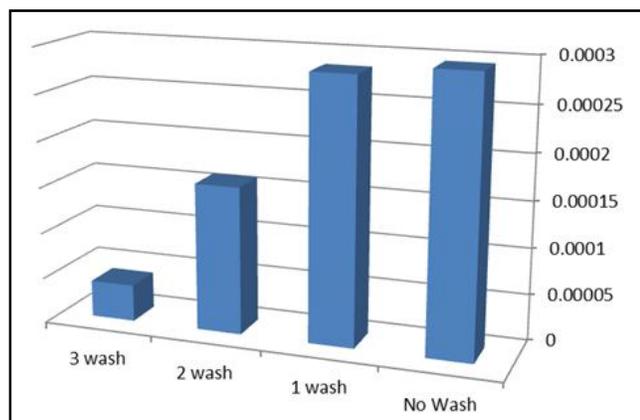
Fig. 1: General description of viable count with repeated washings.

Stephan 2016).

Materials and Methods

Sampling

During the period from November 2019 to February 2020, samples had been collected from different Vegetable stores and itinerant's vendor in Baghdad Karkh

Table 2: One way a nova (LSD) showing the differences in viability among different group of washing steps.

(I) group	(J) group	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
control	Wash1	.44333*	.05239	.000	.3225	.5641
	Wash2	1.66000*	.05239	.000	1.5392	1.7808
	Wash3	2.89333*	.05239	.000	2.7725	3.0141
Wash1	control	-.44333-*	.05239	.000	-.5641	-.3225
	Wash2	1.21667*	.05239	.000	1.0959	1.3375
	Wash3	2.45000*	.05239	.000	2.3292	2.5708
Wash2	control	-1.66000-*	.05239	.000	-1.7808	-1.5392
	Wash1	-1.21667-*	.05239	.000	-1.3375	-1.0959
	Wash3	1.23333*	.05239	.000	1.1125	1.3541
Wash3	control	-2.89333-*	.05239	.000	-3.0141	-2.7725
	Wash1	-2.45000-*	.05239	.000	-2.5708	-2.3292
	Wash2	-1.23333-*	.05239	.000	-1.3541	-1.1125

*The mean difference is significant at the 0.05 level.

and Risafa, a total of 25 bundles had been collected randomly and transported with minimum delay to the microbiology laboratory without cooling by fridge.

Method

1. In this research, 20 g of leafyvegetable had been soaked in 800 ml of sterile distilled water and manual shaking for 3 minutes.

2. 1ml of soaked water had been added to 9 ml of peptone water to make serial dilutions (1:10–1:10,000) with sterile peptone water.

3. 0.1 ml of each dilution had been inoculated onto nutrient agar media plates for determining the bacterial contents and CFU/ml of the sample.

4. The previously washed leafy vegetables had been soaked three times again in 800 ml of sterile distilled water and in each time the same method had been repeated.

5. The inoculated plates of the stock and the three washes had been incubated in 37°c for 18 -24 hr.

Notes

The total bacterial colonies had been counted and Identification of different genera was depended on colonial morphology in different types of media, Gram's stain and biochemical activity of isolated colonies. Some bacterial isolates had been identified by Vitek 2 System Version 08.01.

All steps of experiments had been conducted at room temperature and use sterile distilled water.

Data analysis

Data were analyzed by using (ANOVA 1) method for multiple comparisons. Numeric data were expressed as (mean +SD), P-value (p < 0.01) was considered significant when it was (P ³ 0.05) and highly significant when it was (P ³ 0.001). To generalize the mean count, 95% confidence interval had been used.

Results

Washing steps

Viable bacterial count of control and three steps of washings had been explained in Fig. 1 and table 1. Table 2 showing differences in viability among different steps of washings.

Bacterial isolates

In 25 runs of this research 11 genera of bacteria had been identified and one isolate could not be

Table 3: Number of bacterial isolates from 25 runs.

Bacteria	No. of isolates N=25 (%)
Staphylococcus spp.	25 (100)
Bacillus spp.	25 (100)
Enterobacter spp.	25 (100)
Shigella spp.	25 (100)
Escherichia coli	25 (100)
Klebsiella spp.	23 (92)
Salmonella spp.	23 (92)
Acinetobacter spp.	20 (80)
Pseudomonas spp.	18 (72)
Leclerciaa decarboxylata	15 (60)
Neisseria spp.	3 (12)
Unidentified bacterial isolate	25 (100)

identified table 3.

Discussion

The high prevalence of bacteria in raw food is recognized in developing countries, this is due to unhealthy irrigation system and handling, in addition to human and animal waste, which comes in direct contact with leafy vegetables (Alimi and Buliyaminu Adegbemiro 2016). In this research, using samples immediately with no refrigerating had been done in order to prevent effect of cold in some of bacterial isolates (Ríos-Castillo *et al.,* 2020), samples washed three times with sterilized distilled water, viable bacterial count reduced with repeated washings. In table 1, bacterial viable count was 3.2933*10t with a control, then reduced to 2.8500*10t , 1.6000*10t, .4000*10t after wash 1, wash2, wash3 respectively, this explain that repeated washing was significantly important and was almost acceptable method, this result is in agreement with Subramanya *et al.*, (Subramanya *et al.*, 2018). An important factor may affect this process representing by the presence of clean water for washing; using impure or unclean water may play a role in presence of other microorganisms and impurities. In this research distilled water had been used in order to obtain a typical result, but in fact this is difficult to provide at home permanently for daily use, so using tap water is enough to get a good result (Camelo, 2004; Amoah *et al.*, 2007; Gombas *et al.*, 2017). Shaking and soaking of samples for a period of time help in removing of bacteria and all types of impurities to water leaving leafy vegetable clean and less contaminated (Subramanya *et al.,* 2018; Wu *et al.*, 2019).

Tested samples had a high content of bacteria belonging to many genera. Although many of these genera have nonpathogenic species or strains with no harmful effect in human health, but pathogenic ones are very danger and many of them responsible for serious illnesses especially in gastrointestinal tract. High incidence of *Staphylococcu*s and coliforms *Shigella* spp., *Escherichia coli* (100% of samples), *Klebsiella* spp. and *Salmonella* spp. *(92% of samples), Acinetobacter* spp *(*80% of samples).*, Pseudomonas* spp. (72% of samples) is in agreement with many studies (Tomás-Callejas *et al.,* 2011; Denis *et al.,* 2016; Balali *et al.,* 2020). *Leclerciaa decarboxylata* had been isolated from 60% of samples, it isnormal flora in the gut ofanimals including human stool andis a rare human pathogen most commonly affecting immunocompromised individuals (Matsura *et al.,* 2018; Merza *et al.,* 2019). More studies suggested other genera like *Serratia* spp., *proteus* spp., *Listeria monocytogenes, Citrobacter* spp., *Aeromonas spp., Enterococcus faecalis* (Lee *et al.,* 2014; Karlo Malavé Llamas, 2015; Subramanya *et al.,* 2018), which had not been isolated from samples of this study may be this is related to fluctuate over time with seasonal changes (Camelo, 2004, Denis *et al.,* 2016).

Although the different methods for identification of the isolates, one bacterial isolate could not be identified even with VITEK and gave unknown results in repeating test defined as a slash line. This may be due to variation in bacterial behavior or bacterial species is not yet known.

Conclusion

This work gives a good image for the importance of washing of leafy vegetables and its role in reducing number of bacteria of different genera which may cause serious illnesses for consumers when eaten directly without any treatment.

Limitations

This study had been done only in vitro cultivable bacteria. Bacterial isolates had not been further characterized for species, properties or drug resistance.

MacConeky agar had been used with crystal violate in order to differentiate between lactose fermenter and non-lactose fermenter. S-S agar and TCBS agar had been also used for differentiation of *Salmonella, Shigella* and for *Vibrio* spp. respectively.

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