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Microbiological quality of ready-to-eat salads and handling practices in some local restaurants in Erbil

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Abstract:

The demand for eating ready-to-eat (RTE) vegetables in restaurants are increased, which is one of the reasons for maintaining a healthy lifestyle. This study aimed to assess the microbiological quality of RTE salads and handling practices during working in restaurants such as; Total count bacteria (TCB), coliform bacteria, *Staphylococcus aureus*, *Salmonella* sp., and fungi. Total of RTE salad samples (e.g. tomato, cucumber, lettuce, purple cabbage, white cabbage, Sellery, and mixed salad) and swab samples from utensil and place of working with RTE salads were collected from some local restaurants in central of Erbil. The samples were microbiologically analyzed. TCB ranged from 1.33 to 5.93 Log10CFU/g, which the highest TCB growth found in cucumber. The highest number of coliform bacteria was found in the lettuce samples. While, the highest growth of *Staphylococcus aureus* was found in sellery samples. *Salmonella* sp. found in two samples of tomato and three samples of sellery. Also, the results show that the growth of mold and yeast ranged from 0.74 to 3.25 Log10CFU/g, according to the Iraq standard specifications for foods, some of the samples are unacceptable from the standpoint of microbiological safety; therefore, these data indicate that food handlers may contribute to pathogens contamination and that there are some handling practices that require more attention. The results of this study revealed that the high contaminations of these foods may be a potential hazard for public health.

INTRODUCTION

Fruits and vegetables are essential components of a human healthy diet and nutrition (Campos *et al.*, 2013). Leafy green vegetables are an important component of a healthy diet, which providing important vitamins, minerals, and phyto-nutrients. Ready-to-eat (RTE) salads are low calorie, convenience foods of good dietary value. RTE salads technology are obtaining of raw vegetables from vertically coordinated farms, washing, cutting, sorting and serving with other food products in local restaurants. While, after the previous processes, they might be drying, packaging and storing in permeable plastics for retailing in cold place (2-4°C) (Caponigro *et al.*, 2010).

Consumers in developing countries have become more concerned about the nutritional and sensory aspects of food products as well as the safety of the food they eat due to growing health awareness. As a result of these changes, consumer demand for fresh agricultural products is increasing and, in particular, non-heated food that is consumed directly without cooking, so is the demand for RTE fruits and vegetables is increasing (Stephan *et al.*, 2015; Mir *et al.*, 2018; Gullino *et al.*, 2019).

Contamination of RTE vegetable salads may occur through various routes from farm to fork. The contamination may occur from human, animal and environmental sources. The unit operations of vegetable salads are also responsible for the contamination of these products which may affect the quality and lead to safety threats (Mir *et al.*, 2018)

Ferguson et al. (2005) reported that the number of gastroenteritis outbreaks caused by food-borne pathogens, including diarrheagenic *E. coli* pathotypes (DEPs), has increased worldwide after consumption of raw vegetables salads. However, safety and quality of such products are an issue of concern as these products can act as vehicles for transmitting infectious diseases. Additionally, RET produce is more susceptible to spoilage and can facilitate rapid growth of spoilage microorganisms as well as the microorganisms of public health significance (Qadri *et al.*, 2015).

The aim of this study was to evaluate the microbiological quality of ready-to-eat salads which are available in some local restaurants in Erbil-Iraq and also to investigate the cross contamination during handling practices.

MATERIALS AND METHODS

Culture media

Media were prepared according to the manufacturer's instructions (Oxoid Ltd., Basingstoke, Hampshire, England) and were sterilised by autoclaving at 121°C for 15min 15 psi pressure. Nutrient agar was used for enumeration of total count bacteria (TCB). MacConkey agar and violet red bile agar (VRB agar) were used for enumeration of coliform bacteria. Mannitol salt agar was used for *Staphylococcus aureus* count. xylose lysine deoxycholate agar (XLD agar) was used for *Salmonella* sp. count. Sabouraud dextrose agar and potato dextrose agar were used for fungi (moulds and yeasts). Peptone water was prepared for serial dilutions. One g of peptone was added to 1.0L distilled water. Peptone water was mixed thoroughly and boiled. Then, it was sterilised by autoclaving at 121°C for 15min 15 psi pressure.

Sampling procedure

Salad samples (e.g. tomato, cucumber, lettuce, purple cabbage, white cabbage, sellery, and mixed salad) were collected from some local restaurants in central of Erbil at the morning around 9:00 to 11:00 am. They were put into sterilized bags. Then, the samples were transported to the microbiology lab to evaluate the microbiological quality of the RTE salad samples. Also swab samples from knife, chopping board and bowels were collected from the restaurants to evaluate the cross contamination during handling practices.

Microbiological quality analysis

The RTE salad samples were taken for microbiological analysis at the morning. Ten g of each sample was homogenised with 90 ml of peptone water for 3min. Aliquots were serial diluted in maximum recovery diluent and plated out following National Standard Methods (HPA, 2004, 2009). Total count bacteria (TCB) was determined on nutrient agar. The inoculated nutrient agar was incubated at 37°C for 48h. Coliform bacteria and *E. coli* counts were determined on MacConkey agar and VRB agar. *Staphylococcus aureus* count was determined on Mannitol salt agar. *Salmonella* sp. count was determined on XLD agar. Mould and yeast counts were determined on sabouraud dextrose agar and PDA. The inoculated media were incubated at 37°C for 48h except the inoculated sabouraud dextrose agar and PDA which were incubated at 25°C for five days.

Statistical analyses: All data were analyzed by One-way Analysis of Variance (ANOVA) by applying SPSS version 16.0.

RESULTS AND DISCUSSIONS

Microbial quality of the RTE salad samples was determined by microbial growth at the day of sample collections as the results are shown in Table 1 and 2. The growth of TCB, coliform bacteria and *Staphylococcus aureus* were observed in all samples except Coliform bacteria and *Staphylococcus aureus* were not found in the white cabbage. TCB ranged from 1.33 to 5.93 Log_{10}CFU/g, which the highest TCB growth found in cucumber. The highest number of coliform bacteria was found in the lettuce samples. While, the highest growth of *Staphylococcus aureus* was found in sellery samples. No *Salmonella* sp. found in the RTE salad samples except two samples of tomato and three samples of sellery as the result shown in Table 1.

The results of Table 2 show that the growth of mould and yeast ranged from 0.74 to 3.25 Log_{10}CFU/g, which the highest mould and yeast growth found in mixed salad when tested on sabourad agar media. In addition, there was no growth of mould and yeast detected in any lettuce, purple cabbage and white cabbage when tested on PDA media.

The results were similar with the finding of Vescovo, *et al.* (1995) and Kaneko, *et al.* (1999) who reported that the microbial growth found in raw vegetables and ready-to-use mixed salad respectively. Also, Al Saif

and Brazier (1996) analysed 300 raw vegetables on sale in retail premises in Cardiff/ Wales by direct plating and found a 2.4% contamination rate. The reason for the growth of microorganisms is probably due to irrigation of vegetable with dirty water. On the other hand, handling practice is one of the reasons for contamination of RTES samples.

This suggested that post-harvest cross-contamination occurs predominantly in the washing stage.

Table 1: Bacterial growth counts* ($Log_{10}CFU/g$) of RTE salads in some local restaurants in Erbil

Sample	N**	Bacterial growth ($Log_{10}CFU/g$)				
		TCB	Coliform bacteria on VRB	Coliform bacteria on Mackonkey agar	*Staphylococcus aureus*	*Salmonella* sp.
Tomato	9	4.34±0.06	3.59±0.29	3.15±0.39	2.35±0.05	0.54±0.09 (2)
Cucumber	8	5.93±0.04	3.85±0.05	3.95±0.03	2.26±0.02	n.d.
Lettuce	8	5.29±0.03	3.89±0.05	3.97±0.04	2.69±0.07	n.d.
Purple cabbage	6	2.54±0.05	1.12±0.08	1.30±0.04	1.24±0.18	n.d.
White cabbage	6	1.33±0.12	n.d. ***	0.56±0.17	n.d.	n.d.
Sellery	6	4.41±0.06	2.63±0.05	2.91±0.09	3.10±0.13	0.78±0.16 (3)
Mixed Salad	8	4.71±0.09	2.77±0.05	3.46±0.01	2.17±0.02	n.d.

* Values are mean $Log_{10}CFU/g$ ± standard deviations from three replicates for each collected sample (ANOVA was followed by Turkey's test)

** Number of the samples

*** n.d.: not detected, the detection limit was <10

Table 2: Mould and yeast growth counts * ($Log_{10}CFU/g$) of RTE salads in some local restaurants in Erbil

Sample	N**	Mould and yeast growth counts ($Log_{10}CFU/g$)	
		Sabourad	PDA
Tomato	9	2.42±0.24	1.94±0.04
Cucumber	8	2.31±0.11	2.10±0.03
Lettuce	8	1.81±0.08	n.d. ***
Purple cabbage	6	1.94±0.03	n.d.
White cabbage	6	0.74±0.13	n.d.
Sellery	6	2.37±0.08	2.03±0.03
Mixed Salad	8	3.25±0.07	2.88±0.04

* Values are mean $Log_{10}CFU/g$ ± standard deviations from three replicates for each collected sample (ANOVA was followed by Turkey's test)

** Number of the samples

*** n.d.: not detected, the detection limit was <10

Table 3 and 4 show the results of bacteria and moulds and yeasts growth on utensils such as; knife and chopping boards which used daily for cutting vegetables in the restaurants in Erbil. The results of Table 3 show that the growth of TCB was 2.46 and 2.21 Log_{10}CFU/g on the knife and chopping board respectively. The growth of coliform bacteria and *Staphylococcus aureus* lower on the knife and chopping board. From the results, *Salmonella* sp. found in two samples of knife, while there was no growth of *Salmonella* sp. on the chopped board. The Table 4 shows the growth counts of moulds and yeasts which was 0.61and 1.32 Log10CFU/g on the knife in both Sabourad and PDA respectively. Whie, the growth ranges were increased on the chopping board in both Sabourad and PDA.

The reason for growing microorganisms might be due to left the knife or chopping board with the residue of leaves and vegetables after working or cleaning them poorly.

The washing methods for utensils were limited the growth of microorganisms which may reduce the contamination of vegetables with microorganisms. In general, the pre-wash knife and chopping board reduced the contamination of vegetable as the results similar with findings of Faour-Klingbeil (2017) who reported that cleaning the utensils and also vegetables reduced the growth of microorganisms by 1.3 log_{10}CFU/g.

Table 3: Bacterial growth counts* (Log_{10}CFU/g) of utensils used for RTE salads in some local restaurants in Erbil

Sample	N**	Bacterial growth (Log_{10}CFU/g)				
		TCB	Coliform bacteria on VRB	Coliform bacteria on Mackonkey agar	*Staphylococcus aureus*	*Salmonella* sp.
Knife	8	2.46±0.13 (4)	1.74±0.35 (2)	1.91±0.21 (3)	1.32±0.01(3)	0.48±0.01 (2)
Chopping Board	8	2.21±0.17 (2)	1.69±0.22 (2)	1.96±0.05 (2)	1.63±0.01 (2)	n.d. ***

* Values are mean Log_{10}CFU/g ± standard deviations from three replicates for each collected sample (ANOVA was followed by Turkey's test)

** Number of the samples

*** n.d.: not detected, the detection limit was <10

Table 4: Mould and yeast growth counts * (Log_{10}CFU/g) of utensils used for RTE salads in some local restaurants in Erbil

Sample	N**	Mould and yeast growth counts (Log_{10}CFU/g)	
		Sabourad	PDA
Knife	8	0.61±0.28 (4)	1.32±0.12 (4)
Chopping Board	8	0.77±0.10 (2)	1.42±0.07 (3)

* Values are mean Log_{10}CFU/g ± standard deviations from three replicates for each collected sample (ANOVA was followed by Turkey's test)

** Number of the samples

CONCLUSION

In conclusion, the results of this study demonstrate that the increasing numbers of microbes in RTE salads in the restaurants is might be due to the use of poor-quality vegetables, disinterest good hand washing vegetables and poor-quality of working place with vegetables. Therefore, it is recommended to use the best vegetables or raw material for RTE salads, and use the of sanitary conditions for workers in restaurants, as well as clean the utensils before use, and must keep RTE salads under cooling temperature in refrigerators isolated from the outside air show.

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