Evaluation of Microbiological Quality of Ready to Eat Salads in Alexandria City, Egypt

Nahla Gaber Zeid

High Institute of Hotels & Tourism
King Marriott, Alexandria

Abstract

Microbial hazards continue to be one of the biggest threats to food safety. Ready to eat salads are one of the minimally processed foods that are widely served in restaurants, homes and hotels and should be evaluated to check there safety for human consumption.

This study was carried out to evaluate the microbiological quality of ready to eat salad served in most restaurants in Alexandria city, Egypt. A total of 448 samples were collected from different restaurants at different districts of the city.

Seven types of salads were analyzed for Total Viable Count Enterobacteriacea, Staphylococcus aurous, Molds and yeasts.

The results revealed that most of the collected samples were highly contaminated with total aerobic bacteria. Different contamination levels of the salad with S. aurous, Enteobacteriaceae، Molds and yeasts were noticed to be dependent on the salad type and food hygiene level .Relevant recommendations are given.

Key words: Ready- to- eat salad- microbial- Quality-Enterobacteriacease

……...

INTRODUCTION

Today, the diseases resulting from food are considered one of the major health problems and are occurring repeatedly in advanced and industrial countries (Razavilar, 2010)

Ready to eat foods (RTE) can be described as the foods that are ready for immediate consumption at the point of sale .These foods could be raw or cooked , hot or chilled and can be consumed without heat treatment (Tsang,2002)

Salad is a term broadly applied to many food preparations that have a mixture of chopped or diced ingredients mostly fruits or vegetables. Among vegetables used are cucumber, pepper, tomatoes, onions carrots and radish, which are well known sources of useful nutrients, vitamins, minerals, dietary fibers and other phytonutrients including flavonoids, carotenoids and phenolic compounds . Other ingredients such as olives, mushrooms, hard – boiled egg, green beans, cheese, meat or seafood are sometimes added to salads.

In spite of the health benefits of vegetables and fruits , they may harbor enteric pathogens involved in food borne outbreaks worldwide causing symptoms of gastroenteritis and chronic infections.

European Scientific Committee has reported that most of outbreaks linked to fresh products have been associated with members of Enterobacteriaceae , thereby they are used as an indication of either post processing contamination or inadequate cooking .Mycotoxins released by some molds,(Aspergillus , Fusarium , Penicillium) during their metabolic activities may cause toxic effects ranging from short-term mucous membrane irritation to suppression of the immune system and cancer (European Commission,2002 ; Tourmas,2005)

Total viable count (TVC) results give knowledge about the food handling and processing conditions. A series of results from time to time generally provides a better understanding. The presence of coagulase positive staphylococci is an indication of human contact. Even minimal handling of foods can result in contamination with coagulase positive staphylococci at low levels , extensive handling and /or temperature abuse may result in higher levels and diminution of food safety if toxin production occurs.

The outbreaks vary in size from a few persons affected to many thousands. Contamination of vegetables may take place at all stages during pre and post harvest techniques (Halablab et. al 2011).

Unsafe water used for rinsing the vegetables and sprinkling to keep them fresh is also a source of contamination (Mensah et. at. 2002). Other possible sources of microorganisms include soil feces (human

and animal origin), handling of the product, harvesting and processing equipments and transport (Johannessen et. al 2002).

Microbes associated with fresh-cut fruit and vegetable products can vary greatly in accordance with the product type and storage conditions (James and Ngormsak, 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 vegetables produce include: spoilage, decreased sensory appeal and decreased shelf life. However, coliform are expected to be present in many raw foods and food ingredients of animal and plant origin (Bibek, 2005). Coliforms are commonly used as a bacterial indicator of either sanitary quality or pollution of food and water as they are common inhabitants of both animal and human guts (Tortora, 1995).

According to the standard , the microbiological tests which usually conducted on ready to eat foods vary according to the type of food, but mostly include total bacterial count, Coliform ,Staphylococcus aureus ,mold and yeasts, beside searching for some pathogenic bacteria like Salmonella ,Escherichia coli and Listeria monocytogenes (Chapman,et al ,2010)

According to many researchers, one important way to ensure food safety, is the implementation of HACCP system in food preparation and distribution centers, including restaurants which are supplying foods that in order to ensure consumer health, the emphasis is on the implementation of these controlling systems (Vanderzant and splittstoesser,(1992) and Rocesvalles et al,2011)

With the above in view the aim of this study is to evaluate the bacteriological quality of ready to eat salads presented in some restaurants located in different districts of Alexandria city, Egypt.

MATERIALS & METHODS

A- Collection of salad samples: seven types of salads, a total of 448 samples were collected from different restaurants located in different districts of Alexandria city, Egypt through the period from February to August 2015. The samples were collected twice a day at 11.0 am and 4.0 pm from each restaurant in cooled sterile containers and analyzed within an hour at the department of Food Science and Technology, Faculty of Agriculture Alexandria University.

The different types of ready to eat salad used in this study and their ingredient are listed in table (1).

B- Microbiological Analysis

- Total viable count (TVA):

Total viable count was carried out by the pour plating method or plate count agar (PCA), followed by incubation at 37^{o}C for 48h ; colonies were recorded as cfu /g according to ISO, 2003.

- **Staphylococcus aureus**:

A direct method was used to determine S.aureus counts: 0.1 ml of the appropriate dilution was inoculated into prepared and dried Baird-Parker agar plates supplemented with egg yolk-telluriteemulsion. The plates were then incubated at 37^{o}C for 24 – 48h. Each typical colony of S-aureus (black zone with clearing of egg yolk) was subcultured in tryptone soy agar (37^{o}C, 24h). Colonies obtained were examined microscopically and tested according to the method of De Glustle et. al.(2010).

- Enterobacteriaceae

The spread plate technique was used to determine Enterobacteriaceae counts in poured plates of violet red bile glucose agar and incubated at 37^{o}C for 24 hr according to ISO standard 2004.

- **Yeasts and Molds**

Enumeration of yeasts and molds was carried out according to ISO standard 1999.

Results and Discussion

Table (1) illustrate the ingredient of ready to eat salad under investigation. As shown in the table, some of these ingredients were cooked, while others include raw materials as lettuce, tomato, cucumber in addition to mayonnaise. Cooking increase the palatability of the salad and guarantees a higher safety.

The results in tables (2&3), show the incidence and its levels for all analyzed parameters (> 6 log cfu/g for TVC, > 4 log cfu/g for Enterobacteriaceae and > 2 log cfu/g for S.aureus, yeasts and molds counts) in collected ready to eat salad samples.

Table (1) Ingredient of Ready to Eat Salad Served in Restaurants

Salad	Ingredients
Tuna fish salad	Canned tuna fish meat, parsley, lettuce , tomato, cucumber and boiled corn.
cezar salad	Boiled or fried chicken meat, fried bread, lettuce, parsley, tomato, cucumber and boiled corn.
Mediterranean salad	Tomato, Turkish white cheese, olive, boiled corn, lettuce, black cabbage, carrot and cucumber.
Russian Salad	Boiled carrot, potato, peas and Mayonnaise.
Hommos salad	Chickpeas, tahini, olive oil, lemon juice salt, garlic.
Tahini salad	Tahini, lemon juice, salt and water.
Mutabble salad (papa khanog)	Eggplant, tahini, salt, olive oil, garlic.

Table (2) Occurrence of TVC, Enterobaceriacea, Staphylococcus aureus, yeast and mold in ready to eat salad samples

Salad	No	TVC > 6 log cfu/g		Enterobacter-iaceae> 4 log cfu/g		s. aureus > 210g cfu/g		Yeasts> 2 log cfu/g		Molds > 2 log cfu/g	
		a	b%	a	b%	a	b%	a	b %	a	b %
Hommos salad	64	24	37.5	13	20.31	3	4.69	12	18.75	7	10.94
Tahini salad	64	32	50	18	28.13	3	4.69	15	23.44	9	14.06
Mutabble salad (papa khanog)	64	25	39.06	16	25.0	2	3.13	20	31.25	12	18.75
Tuna Fish salad	64	22	34.38	7	10.94	3	4.69	2	3.13	3	4.69
cezar Salad	64	20	31.25	6	9.38	-	0.0	2	3.13	-	0.0
Mediterranean salad	64	18	28.13	10	15.63	5	7.81	4	6.25	6	9.38
Russian Salad	64	15	23.45	2	3.13	-	0.0	3	4.69	4	6.25
Total	448	156	34.82	72	16.07	16	3.57	58	12.95	41	9.15

a= Number of sample analyzed b= Percent of positive samples

The results illustrated in Table(2) revealed that an average of 34,82% of the analyzed samples contain > 6 log cfu/g TVC, 16,07% > 4 log cfu/g Enterobacteriaceae, 3,57% > 2 log cfu/g S.aureu,12,95% > 2 log cfu/g yeast and mold 9,15% > 2 log cfu/g.

The high bacterial count in Hommos and Tahini salads, despite their acidity might be due to inadequate amount of added acids or to cross contamination through handling and preparation.

Table (3) Microbial evaluation and percentage of positive ready to eat salad samples

Salad	Micro organism level cfu/g	TVC > 6 log cfu/g		Enterobacteriaceae> 4 log cfu/g		s. aureus > 210g cfu/g		Yeats> 2 log cfu/g		Molds > 2 log cfu/g	
		a	b%	A	b%	a	b%	a	b%	a	b%
Hommos(check peas) salad n=64	< 1 $\times 10^2$	-	-	40	62.5	61	95.31	52	81.25	57	89.06
	10^2 to < 10^3	5	7.81	8	12.5	3	4.68	7	10.93	5	7.81
	10^3 to < 10^4	10	15.62	2	3.13	-	-	3	4.68	2	3.13
	10^4 to < 10^5	15	23.44	7	10.94	-	-	2	3.13	-	-
	10^5 to < 10^6	10	15.62	4	6.25	-	-	-	-	-	-
	10^6 to < 10^7	18	28.13	2	3.13	-	-	-	-	-	-
	10^7 to < 10^8	6	9.38	-	-	-	-	-	-	-	-
Tahinic n=64	< 1 $\times 10^2$	4	6.25	45	70.31	61	95.31	49	17.95	55	85.93
	10^2 to < 10^3	8	12.50	6	9.38	2	3.13	8	12.5	6	9.38
	10^3 to < 10^4	-	-	-	-	1	1.56	3	4.68	3	4.68
	10^4 to < 10^5	20	31.25	6	9.38	-	-	4	6.25	-	-
	10^5 to < 10^6	22	34.38	6	9.38	-	-	-	-	-	-
	10^6 to < 10^7	10	15.62	1	1.56	-	-	-	-	-	-
	10^7 to < 10^8	-	-	-	-	-	-	-	-	-	-
Mutabble (papa khanog) n=64	< 1 $\times 10^2$	13	20.31	3.8	59.38	62	69.87	44	68.75	41	64.06
	10^2 to < 10^3	10	15.62	10	15.63	2	3.13	10	15.63	10	15.63

Salad	Micro organism level cfu/g	TVC > 6 log cfu/g		Enterobacteriaceae> 4 log cfu/g		s. aureus > 210g cfu/g		Yeats> 2 log cfu/g		Molds > 2 log cfu/g	
		a	b%	A	b%	a	b%	a	b%	a	b%
	10^3 to < 10^4	-	-	-	-	-	-	7	10.94	3	4.68
	10^4 to < 10^5	2	3.13	10	15.63	-	-	3	4.68	-	-
	10^5 to < 10^6	4	6.25	4	6.25	-	-	-	-	-	-
	10^6 to < 10^7	18	28.13	2	3.13	-	-	-	-	-	-
	10^7 to < 10^8	7	10.94	-	-	-	-	-	-	-	-
Tuna Fish n=64	< 1 $\times 10^2$	19	29.69	47	73.44	61	95.31	62	96.87	61	95.31
	10^2 to < 10^3	15	23.44	10	15.63	2	3.13	2	3.13	3	4.68
	10^3 to < 10^4	-	-	-	-	1	1.56	-	-	-	-
	10^4 to < 10^5	-	-	6	9.38	-	-	-	-	-	-
	10^5 to < 10^6	8	12.5	1	1.56	-	-	-	-	-	-
	10^6 to < 10^7	15	23.44	-	-	-	-	-	-	-	-
	10^7 to < 10^8	7	10.94	-	-	-	-	-	-	-	-
cezar salad n=64	< 1 $\times 10^2$	34	53.12	58	90.62	64	100	62	96.87	64	100
	10^2 to < 10^3	5	7.81	-	-	-	-	2	3.13	-	-
	10^3 to < 10^4	2	3.13	-	-	-	-	-	-	-	-
	10^4 to < 10^5	-	-	4	6.25	-	-	-	-	-	-
	10^5 to < 10^6	3	4.68	1	1.56	-	-	-	-	-	-

Salad	Micro organism level cfu/g	TVC > 6 log cfu/g		Enterobacteriaceae> 4 log cfu/g		s. aureus > 210g cfu/g		Yeats> 2 log cfu/g		Molds > 2 log cfu/g	
		a	b%	A	b%	a	b%	a	b%	a	b%
	10^6 to < 10^7	14	21.87	1	1.56	-	-	-	-	-	-
	10^7 to < 10^8	6	9.37	-	-	-	-	-	-	-	-
Mediterranean salad n=64	< 1 $\times 10^2$	31	48.43	48	75	59	92.18	60	93.75	58	90.62
	10^2 to < 10^3	-	-	-	-	3	4.68	3	4.68	4	6.25
	10^3 to < 10^4	8	12.5	6	9.37	1	1.56	1	1.56	2	3.13
	10^4 to < 10^5	4	6.25	7	10.93	1	1.56	-	-	-	-
	10^5 to < 10^6	3	4.68	2	3.13	-	-	-	-	-	-
	10^6 to < 10^7	9	14.06	1	1.56	-	-	-	-	-	-
	10^7 to < 10^8	9	14.06	-	-	-	-	-	-	-	-
Russian salad m=64	< 1 $\times 10^2$	-	-	59	92.18	64	100	61	95.31	60	93.75
	10^2 to < 10^3	40	62.50	3	4.68	-	-	3	4.68	2	3.13
	10^3 to < 10^4	-	-	-	-	-	-	-	-	2	3.13
	10^4 to < 10^5	4	6.25	2	3.13	-	-	-	-	-	-
	10^5 to < 10^6	5	7.81	-	-	-	-	-	-	-	-
	10^6 to < 10^7	9	14.06	-	-	-	-	-	-	-	-
	10^7 to < 10^8	6	9.38	-	-	-	-	-	-	-	-

The distribution of microbial population is shown in table (3), the TVC of the analyzed samples ranged from < 2 log to 8 log cfu/g for Hummus, 2 log to 7 logcfu/g for tahini salad, 2 log to 7 log for mutabbel salad (papa khanog), 2 log to 8 log cfu/g for tuna fish salad, cezar salad, Mediterranean salad and Russian salad.

Although TVC does not define the microbiological safety of raw products , it is, beside its simplicity a very useful tool to monitor the effect of different technological processes on the microbiological quality of the products, and to control the HACCP plan (De Glustle el al 2010).

The latter authors reported that 62.9 to 82% of ready to eat samples were over contaminated with aerobic count at levels of > 10^6 to 10^9cfu/g.

Mention should be made that the results obtained in this study for TVC were lower than those obtained by Tessi.et al.(2002).

In the present study (table 2) the overall S.aureus > 2 log cfu/g is found to be 3.57%. Among all studied samples, the Mediterranean salad showed the highest incidence 7.81% may be as a result of manual handling during the preparation. Again the obtained results in this study are lower than those obtained by Fang et al (2003) and Wel,et al (2006).

The European scientific committee has reported that most of the outbreaks linked to fresh produce have been associated with members of Enterobacteriaceae that actually showed to be the second most common contaminating microorganism in this study: Unfortunately, Tahini, Mutabbe (papa khanog) and Hommos showed the highest incidence 28.13%, 25.0 and 20.31 consequently (table 2). These types of salad are widespread in all Alexandria restaurants. The lowest incidence 3, 13% was noticed in Russian salad.

The prevention of contamination and bacterial growth in ready to eat salads lies in the application of good hygienic practice during growing of vegetables and processing, effective washing, effective temperature control during storage and distribution.

The populations of Yeasts and Molds were found to be low in this study.

The presence of high TVC, Enterobacteriaceae, S.aureus, Yeasts and Molds the salad samples is of special concern as these products are not usually subjected to sufficient heat treatment before consumption.

Conclusion

The result of these study revealed that many samples of the collected ready to eat salads were highly contaminated and may be a potential hazard for public health.

The reasons behind this problem may be lack of controlling and inspection of the premises that present these foods.

Also using low quality water for cleaning and processing of such foods and low efficiency of hygienic system in most restaurants are sources of contamination. We noticed that most of the people working in these premises have insufficient background about food safety, optimum storage conditions and safer method of handling such foods.

Recommendation

1- The authorized agencies must pay more attention to controlling and inspection of the places that serve foods especially minimally processed foods like salads.
2- All restaurants must qualify their employee through short courses or lectures and workshops concerning food safety and hygiene.
3- Ready – to – eat salads must be kept refrigerated from preparation to serving and storage.

References

1-Abdulsudl Issa,Z.,Kamltani,Y.,Muhlmbula,H.S. and Ndablkuze,B,K.(2010).A review of microbiological safety of fruits and vegetables and the introduction of electrolyzed water as an alternative to sodium hypochlorite solution.African Journal of Food Sci.4,778-789.

2-Al – Binali, A.M., Bello, C.S.;El-Shewy, K. and Abdulla, S.G (2006). The prevalence of parasites in commonly used leafy vegetables in south western Saudi Arabia. Saudi Medical Journal 27 : 613 – 616.

3-Al-Haq,M.I.,Sugiyama,J. and Isobe,S.(2005).Application of electrolyzed water in Agricultural and food Indusries.Food Sci. and Technol.Res.11,135-150.

4-.Bibek, R. (2005). Fundamental food microbiology. CRC press, London, pp. 625.

5-Chapman,B.E.,T.;Fillion,K.,Maclaurin,T and powell,D.(2010).Assessment of food safety practices of food service and food handlers :Testing a communication .J .Food Pro.73:1101-1107.

6-De Glustle M., Aurlgemma, C., Marinell, L., Tufl, D., De Medlcl, D., Dipasquala, S., De Vlto, C. and Boccla, A (2010). The evaluation of the microbial safety of fresh ready to eat vegetables produced by different technologies in staly. J. Appl. Microbiol, 109 – 996 – 1006.

7- European Commission(2002) Risk profile on the microbiological contamination of fruits and vegetables eaten raw.Report of the Scientific Committee on Food,Health and consumer Protection Directorate General.Brussels,Belgium.

8-Fang, T.J., Wel, O.K., Liao, C.W., Hung, M.U., and Wang, T.H. (2003). Microbiological quality of 10^{o}C ready to eat food products sold in Taiwan. Int. J. Food Microbiol. 80: 241 – 250.

9-Halablab, M.A., Sheet and Holail H.M. (2011). Microbial quality of raw vegetables grown in bakka valley, Lebanon. American Journal of Food Technology 6 : 129 – 139.

10- ISO, (1999).I nternational Organization for StandardizationISO 7954: General guidance for enumeration of yeasts and moulds colony count technique.

11 -ISO (2003)International Organization for Standardization, ISO 4833 Microbiology of food and animal feeding stuffs-horizontal methods for the enumeration of microorganism. Colony count technique at 30^{o}C.

12- ISO, (2004). International Organization for Standardization, ISO 2152: Microbiology of food and animal feeding stuff-horizontal methods for the detection and enumeration of Enterobacteriaceae – part – 2: Colony count method.

13-Johannessen, G. S., Loncarevic, S. and Kruse, H (2002). Bacteriological analysis of fresh produce in Norway. International Journal of Food Microbiology 77 : 199 – 204.

14-.Mensah, P. D., Yeboah – Manu, K., Owusu. Darko, S. and Ablordey, A. (2002). Street foods in Accra, Ghana: How safe are they? WHO bulletin, 80 : 546 – 554.

15- Razavilar,V.(2010).Pathogenic bacteria in food.3rd ed. Tahran:Tahran University Publication.

16- Rocesvalles,G.Leturia,D.M. and Jalon,G.M.(2011)Food safety and the contract catering companies:Food handlers,facilities and HACCP evaluation.Food Control,22,206-211dlers:Testing a communication intervention.J. Food pro.,73,1101-1107.

17-Tsang,D.(2002).Microbiological Guidelines for ready to eat Food.Food and Environmental Hygiene Department,Queensway,115-116,Centre for Food Safety,Hong Kong.

18-Tessi, M. A., Aringoll, E.E., Pirovani, M.E. Vincenzini, A. Z., sabrag, N. G., Costa, S. C., Garcia, C. C., Zanier, M. S., Silva, ER. and Moguilevsky, M. A. (2002). Microbiological quality and Safety of ready – to – eat cooked foods from a centralized school kitchen in Argentina. J. Food protection 65, 636 – 642.

19-Tortora, G. (1995). Microbiology. The Benjamin publishing co. Inc. New York pp. 532.

20-Tourmas,V.H.(2005).Moulds and Yeasts in fresh and minimally processed vegetables and sprouts.Int.J.Food Microbiology 99,71-77.

21-Vanderzant,C.,and Splittstoesser,D.F.(1992). Compendium of methods for the microbiological examination of foods. 3 rd ed. Washington:American public Health Association.

22-Wel, O.K., Hwang, S. L. and Chen, T. R. (2006). Microbiological quality of ready - to - eat food products in Southern Taiwan. J. Food Drug Anal. 14, 68 – 73.

تقييم الجودة الميكروبية للسلاطات الجاهزة للأكل في مدينة الإسكندرية - جمهورية مصر العربية

نهلة جابر زيد

المعهد العالي للسياحة والفنادق- كنيج مريوط

الملخص العربي

تعتبر الأخطارالميكروبية أحد أكبر المخاطر التى تهدد سلامة الغذاء وبالتالي الإنسان . و السلاطة الجاهزة للتقديم تعتبرأحد أكثر الأغذية التى تقدم فى المطاعم والمنازل والفنادق إذ تتعرض لمعاملات حرارية قليلة ولذلك فهي من أكثر الأغذية التى يجب تقييمها للتأكد من أنها آمنة للمستهلك.

ولقد أجريت هذه الدراسة لتقييم الجودة الميكروبية للسلاطات التى تقدم فى معظم مطاعم مدينة الاسكندرية ، حيث تم جمع 448 عينة من مناطق مختلفة وتم تحليل سبعة أنواع من السلاطات لتقدير العد الميكروبى الكلى TVC, Enterobacteriaceae ,S.aureus, Molds and Yeasts . أوضحت النتائج أن معظم العينات كانت ملوثة بدرجة كبيرة بالنسبة للعد الكلى، بينما اختلفت درجة تلوثها بكل من الأنواع الميكروبية الأخرى على حسب نوع السلاطة ومستوى الشروط الصحية فى المطعم.

وتبعا للنتائج المتحصل عليها فإن معظم السلاطات التى تقدم فى تلك المطاعم قد تمثل خطرا على صحة المستهلك ،ولذلك يجب على الجهات المعنية بالمدينة أن تعطى مزيدا من الاهتمام لاتباع نظام الهاسب فى المطاعم ورفع مستوى الشروط الصحية فيها لمنع أو تقليل مخاطر الأمراض التى تنتقل من خلال الغذاء.