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RESEARCH ARTICLE

The Microbiological Quality of Ready to Eat Salads Sold in Afyonkarahisar, Turkey

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Summary

This study was designed to evaluate the microbiological quality of RTE salads sold at retail in Afyonkarahisar. Total of 261 Ready-to-eat (RTE) salad samples (58 Russian salad, 52 sesar salad, 45 tuna fish salad, 57 Mediterranean salad and 49 cig kofte) collected from 7 different private restaurants, cafes and shopping centers were microbiologically analysed during 2011-2012. Total viable count (TVC), *Staphylococcus aureus, Enterobacteriaceae* and yeast and mold counts of the samples were determined. Of the total of 261 samples, 55.1% and 54% were found to be contaminated with >6 log cfu/g TVC and >4 log cfu/g *Enterobacteriaceae* respectively whereas 13% of which were found to be contaminated with >2 log cfu/g *S. aureus*. All of Mediterranean salads were contaminated with >6 log cfu/g TVC and >4 log cfu/g *Enterobacteriaceae* and similarly, most of tuna fish and sesar salad samples were found to be contaminated with these agents with the contamination levels of 83.3% and 86.6%, 71.1% and 75% respectively. The results of this study revealed that the high contaminations of these foods may be a potential hazard for public health.

Keywords: *Microbiological Quality, RTE Foods, Salads*

Afyonkarahisar'da Satışa Sunulan Tüketime Hazır Bazı Salata/Mezelerin Mikrobiyolojik Kalitesi

Özet

Bu çalışma Afyonkarahisar'da satışa sunulan tüketime hazır bazı salata/mezelerin mikrobiyolojik kalitesini belirlemek amacıyla planlanmıştır. Çalışma kapsamında 2011-2012 döneminde Afyonkarahisar'da restaurant, cafe ve çeşitli satış merkezlerini içeren 7 farklı özel işletmeye ait 261 salata/meze örneği (58 rus salatası, 52 sezar salatası, 45 ton balıklı salata, 57 akdeniz salatası, 49 çiğ köfte) mikrobiyolojik yönden incelenmiştir. Örneklerde Aerob Mezofil Genel Canlı (AMGC), *Staphylococcus aureus, Enterobacteriaceae* familyasına ait türler ve maya-küf sayıları belirlenmiştir. Bu çalışma sonucunda toplam 261 örneğin %55.1'inin >6 log kob/g düzeyinde AMGC içerdiği belirlenirken, %54'ünün >4 log kob/g düzeyinde *Enterobacteriaceae* familyasına ait türleri ve %13'ünün >2 log kob/g *S. aureus* içerdiği ortaya konmuştur. İncelenen örneklerden akdeniz salatası örneklerinin tamamında >6 log kob/g düzeyinde AMGC ve >4 log kob/g düzeyinde *Enterobacteriaceae* familyasına ait türlerin bulunması benzer şekilde ton balıklı salata ve sezar salatası örneklerinin de söz konusu bakteriyel ajanlarla sırasıyla %83.3 ve %86.6, %71.1 ve %75 oranında kontamine bulunması, çalışma kapsamında incelenen örneklerin halk sağlığı açısından risk teşkil edebileceğini ortaya koymaktadır.

Anahtar sözcükler: *Mikrobiyolojik Kalite, Salata, Tüketime Hazır Gıdalar*

INTRODUCTION

During harvest the superficial microbiota of vegetables comprises mainly Gram negative saprophytes, and pathogenic microorganisms. Vegetables may harbour enteric pathogens involved in foodborne outbreaks world-wide causing symptoms of gastroenteritis and chronic infections [1,2]. Ready-to-eat (RTE) foods can be described as the foods being ready for immediate consumption at the point of sale. RTE foods could be raw or cooked, hot or chilled and can be consumed without heat treatment [3].

Total viable count (TVC) results give knowledge about the food processing conditions. Series of results over time

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generally provide a better understanding. The presence of coagulase positive staphylococci (a subgroup of *S. aureus*), is an indication of human contact. Even minimal handling of foods can result in coagulase positive staphylococci being present in foods at low levels. Extensive handling and/or temperature abuse may result in increased levels and increased food safety risk if toxin production occurs [4].

European Scientific Committee [5] has reported that most of the outbreaks linked to fresh products have been associated with members of *Enterobacteriaceae* group. In RTE foods that are fully cooked, *Enterobacteriaceae* are used as an indication of either post-processing contamination or inadequate cooking [4]. Mycotoxins released by some molds, (*Aspergillus*, *Fusarium*, *Penicillium*, *Chaetomium* and *Stachybotrys*) during their metabolic activities may cause toxic effects ranging from short-term mucous membrane irritation to suppression of the immune system and cancer [6].

In Turkey, large supermarkets are responsible for most of the total sales of the RTE foods. The salads on retail sale in Turkey include fresh or boiled vegetables with or without cooked chicken meat or canned tuna fish and mayonnaise. The vegetables can be obtained from the fresh products through selection, washing, peeling, cutting, rinsing and packaging [2] but these processes may not be enough hurdles for the contamination and growth of pathogens and spoilage microorganisms during storage under refrigeration [7].

This study provides an overview of prevalence data related to some of foodborne pathogens for different categories of RTE foods; Russian salads, sezar salads, tuna fish salads, Mediterranean salads and cig kofte. The major objective of this study was to assess the microbiological safety of these RTE foods and to document the occurrence of some indicator and pathogenic microorganisms. The results can be used for risk assessment and for designing more effective methods.

MATERIAL and METHODS

RTE Food Samples

In this study, a total of 261 samples were collected, from August 2011 to June 2012 in Afyon Turkey. A total of 261 RTE food samples consisting of 58 Russian salad (salad mix containing boiled carrot, potato, peas and mayonnaise), 52 sezar salad (salad mix containing boiled or fried chicken meat, fried bread, lettuce, parsley, tomato, cucumber, boiled corn) 45 tuna fish salad, (salad with canned tuna fish meat, parsley, lettuce tomato cucumber and boiled corn) 57 Mediterranean salad, (salad mix including tomato, Turkish white cheese, olive, boiled corn, lettuce, black cabbage, carrot, cucumber) and 49 cig köfte (with cracked wheat, parsley, fresh onion, tomato paste salt and other spices) samples were randomly acquired from 7 different restaurants, cafes and supermarket chains in city of Afyon located in the middle region of Turkey. Ingredients of RTE foods and their conditions at retail level are presented in *Table 1*.

Microbiological Analyses

- Total Viable Count (TVC)

Total Viable Counts were enumerated by the pour plating method on plate count agar (PCA), followed by incubation at 37°C for 48 h; colonies were recorded as cfu/g [8].

- S. aureus

A direct method was used to determine *S. aureus* counts: 0.1 ml of the appropriate dilution was inoculated on propoured and dried Baird-Parker agar plates supplemented with egg yolk-tellurite emulsion (Oxoid). The plates were then incubated at 37°C for 24 to 48 h. Each typical colony of *S. aureus* (black zone with clearing of egg yolk) was subcultured in tryptone soy agar (37°C, 24 h; Oxoid). Colonies obtained on the last agar were examined microscopically, tested for Gram and catalase reactions, and confirmed by coagulase activity (rabbit plasma-EDTA, MERCK) [9].

- Enterobacteriaceae

The spread plate technique was used to prepare duplicate plates for determination of *Enterobacteriaceae* counts. *Enterobacteriaceae* counts were determined in duplicate in poured plates of violet red bile glucose agar (Oxoid) incubated for 24 hour at 37°C. The typical colonies were confirmed by oxidase test and by the fermentation of glucose in Kligler medium [10].

- Yeast and Mold

Enumeration of yeasts and molds was done using Chloramphenicol glucose yeast extract agar by the pour

Table 1. *Ingredients of RTE foods and their conditions at retail level*
Tablo 1. *Tüketime hazır gıdaların içerikleri ve saklama koşulları*

Samples	Ingredients	Conditions at Retail Level
Russian salad	Boiled carrot, potato, peas and mayonnaise	Stored in fridge
Sezar salad	Boiled or fried chicken meat, fried bread, lettuce, parsley, tomato, cucumber and boiled corn	Stored in fridge
Tuna fish salad	Canned tuna fish meat, parsley, lettuce, tomato, cucumber and boiled corn	Stored in fridge
Mediterranean salad	Tomato, Turkish white cheese, olive, boiled corn, lettuce, black cabbage, carrot and cucumber	Stored in fridge
Cig kofte	Cracked wheat, parsley, fresh onion, tomato paste, salt and other spices	Stored in fridge

plate technique and incubation at 25°C for 3-5 days according to ISO 7954:1999 standard [11].

RESULTS

Table 1 summarizes the ingredients of RTE foods and their conditions at retail level. All of the RTE salad samples were under refrigeration temperature at point of sale. Some of RTE salads contained cooked materials where some of them include mayonnaise, raw vegetables and other raw ingredients.

Table 2 gives the incidences, and the distribution of incidence levels of all analysed parameters (>6 log cfu/g for TVC, >4 log cfu/g for *Enterobacteriaceae* and >2 log cfu/g for *S. aureus*, Yeast and Mold counts) in the analysed RTE food samples.

The TVC of the analysed samples ranged from 3.0 log to 6.0 log cfu/g for Russian salad, 3.0 log to 7.0 log cfu/g for sezar salad, 4.0 log to 7.0 log cfu/g for tuna fish salad, 6.0 log to 7.0 log cfu/g for Mediterranean salad and 3.0 log to 6.0 log cfu/g for cig kofte samples, respectively. The highest proportion of samples (38.69%) fell in the range between 6.0 log and 7.0 log cfu/g for TVC *(Table 3)*. The highest proportion of samples (27.58%) fell in range between 10^5-10^6 log cfu/g for *Enterobacteriaceae* counts.

Regarding the distribution of microbial populations, 55.1% of the samples were found have TVC of >6 log cfu/g, 54% of samples >4 log cfu/g for *Enterobacteriaceae*, 13% of samples >2 log cfu/g for *S. aureus*, 61.3 % of samples >2 log cfu/g yeast and 9.5% of samples with >2 log cfu/g mold *(Table 2)*. The highest incidence of TVC greater than 6 log cfu/g was detected from Mediterranean salad (100%) followed by tuna fish salad (83.3%) and sezar salad (71.1%). Mediterranean salad also had the highest incidence (100%) of *Enterobacteriacea* greater than 4 log cfu/g followed by tuna fish salad (86.6%) and sezar salad (75%). The percentage of samples over contaminated with *S. aureus* (greater than 2 log cfu/g) were, cig kofte samples (20.4%) followed by tuna fish salad (15.55%) and sezar salad (13.46%) *(Table 3)*.

DISCUSSION

Many RTE food products has been developed prepared by various cooking processing and packaging. To provide higher microbiological quality RTE food products, it is important to collect and analyse all the information regarding foodborne outbreak surveys. The principle known factors that contributed to foodborne diseases are reported to be inadequate hand washing (31%) and cross contamination between raw materials and cooked foods.

The presence of high TVC *Enterobacteriaceae*, *S. aureus* and yeast and molds in RTE products are of special concern as RTE products are not usually subjected to sufficient heat treatment before consumption. High counts of these bacteria suggest contamination resulted from poor conditions of processing, insufficient heating or contaminations from contaminated cutting boards, knives and serving wates [12]. Occurrence of *S. aureus* results from poor hygiene practices of operators, cross contamination during preparation or improper storage [13]. High risk conditions during food preparation should be well explained to the operators [14].

Several studies regarding the microbiological quality of various RTE foods have been reported in Brazil [15], in Taiwan [12,16], in Argentina [17], in United Arab Emirates [18], in Italy [19] and in UK [20].

Although TVC does not define the microbiological safety of especially raw products, it is still a very useful tool to monitor the effect of different technologies on the microbiological quality of the products. Also, the determination of TVC should be used as a simple and inexpensive parameter to control the effectiveness of the process in the HACCP monitoring plan [19]. In this study, 55.1% of the RTE samples were found have TVC of >6 log cfu/g which is similar to that reported by Tessi et al.[17] reporting that 74.3% of RTE samples were over contaminated with aerobic counts (>10^5 CFU/g). Likewise, De Giusti et al.[19] also reported high contamination levels (10^6-10^9 CFU/g) from 60.9-82.5% of RTE vegetables in different producers. On the contrary, Patricia and Azanza [21].

Table 2. *Occurrence of TVC, Staphylococcus aureus, Enterobacteriaceae, Yeast and Mold in RTE food samples.*
Tablo 2. *Tüketime hazır gıdalarda TVC, Staphylococcus aureus, Enterobacteriaceae, maya ve küf varlığı*

Sample	Na	TVC (>6 log cfu/g)		*Enterobacteriaceae* (>4 log cfu/g)		*S. aureus* (>2 log cfu/g)		Yeast (>2 log cfu/g)		Mold (>2 log cfu/g)	
		n	(%) b	n	(%) b	n	(%) b	n	(%) b	n	(%) b
Russian salad	58	9	15.5	4	6.8	6	10.34	19	32.7	9	15.5
Sezar salad	52	37	71.1	39	75	7	13.46	44	84.6	9	17.3
Tuna fish salad	45	35	83.3	39	86.6	7	15.55	39	86.6	7	15.5
Mediterranean salad	57	57	100	57	100	4	7.01	57	100	-	-
Cig kofte	49	6	12.2	2	4	10	20.40	1	2.0	-	-
Total	261	144	55.1	141	54	34	13.02	160	61.3	25	9.5

a *Number of samples analysed;* **b** *Number of percentage of positive samples*

Table 3. *Microbiological evaluation and percentage of samples in the following ranges (%)*
Tablo 3. *Örneklerinin mikrobiyolojik olarak değerlendirilmesi ve ilgili kontaminasyon aralıklarında dağılımı (%)*

Sample	Microorganism Levels (cfu/g)	TVC		*Enterobacteriaceae*		*S. aureus*		Yeast		Mold	
		n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Russian Salad (n=58)	$<1.0x10^2$	3	5.3	44	76.0	52	89.7	34	58.9	47	81.0
	10^2 to $<10^3$	-	-	3	5.3	4	6.9	5	8.6	2	3.5
	10^3 to $<10^4$	13	22.5	7	12.1	1	1.7	14	24.1	5	8.6
	10^4 to $<10^5$	18	31.0	2	3.5	1	1.7	4	6.9	4	6.9
	10^5 to $<10^6$	15	25.7	2	3.5	-	-	1	1.7	-	-
	10^6 to $<10^7$	9	15.5	-	-	-	-	-	-	-	-
	10^7 to $<10^8$	-	-	-	-	-	-	-	-	-	-
Sezar Salad (n=52)	$<1.0x10^2$	-	-	2	3.8	45	86.6	8	15.4	41	78.9
	10^2 to $<10^3$	-	-	4	7.7	-	-	-	-	2	3.8
	10^3 to $<10^4$	5	9.6	7	13.5	2	3.8	9	17.3	8	15.4
	10^4 to $<10^5$	3	5.8	7	13.5	4	7.7	15	28.9	1	1.9
	10^5 to $<10^6$	7	13.5	19	36.5	1	1.9	18	34.6	-	-
	10^6 to $<10^7$	26	50.0	13	25	-	-	2	3.8	-	-
	10^7 to $<10^8$	11	21.1	-	-	-	-	-	-	-	-
Tuna Fish Salad (n=45)	$<1.0x10^2$	-	-	5	11.1	38	84.4	6	13.4	38	84.4
	10^2 to $<10^3$	-	-	-	-	-	-	-	-	-	-
	10^3 to $<10^4$	-	-	1	2.2	3	6.7	3	6.7	5	11.1
	10^4 to $<10^5$	1	2.2	10	22.2	4	8.9	14	31.1	2	4.5
	10^5 to $<10^6$	9	20	23	51.1	-	-	14	31.1	-	-
	10^6 to $<10^7$	22	48.9	6	13.4	-	-	8	17.7	-	-
	10^7 to $<10^8$	13	28.9	-	-	-	-	-	-	-	-
Mediterranean Salad (n=57)	$<1.0x10^2$	-	-	-	-	53	93.0	-	-	57	100.0
	10^2 to $<10^3$	-	-	-	-	-	-	-	-	-	-
	10^3 to $<10^4$	-	-	-	-	4	7.0	5	8.8	-	-
	10^4 to $<10^5$	-	-	8	14.0	-	-	16	28.1	-	-
	10^5 to $<10^6$	-	-	28	49.1	-	-	25	43.9	-	-
	10^6 to $<10^7$	38	66.7	21	36.8	-	-	11	19.3	-	-
	10^7 to $<10^8$	19	33.3	-	-	-	-	-	-	-	-
Cig Kofte (n=49)	$<1.0x10^2$	-	-	37	75.5	39	79.6	48	98.0	49	100.0
	10^2 to $<10^3$	-	-	-	-	-	-	-	-	-	-
	10^3 to $<10^4$	2	4.1	10	20.4	7	14.3	1	2.0	-	-
	10^4 to $<10^5$	34	69.4	2	4.1	3	6.1	-	-	-	-
	10^5 to $<10^6$	7	14.3	-	-	-	-	-	-	-	-
	10^6 to $<10^7$	6	12.2	-	-	-	-	-	-	-	-
	10^7 to $<10^8$	-	-	-	-	-	-	-	-	-	-

calculated aerob plate counts for most of the RTE foods as $\geq 10^5$ cfu/unit. Researchers cited the reasons to explain higher aerob plate count values as: use of raw ingredients for the final product, temperature abuse during vending, inadequate cooking and use of leftovers.

In this study, overall *S. aureus* incidence >2 log cfu/g is found to be 13.02%. Among all RTE samples, cig kofte samples showed the highest incidence (20.4%) of *S. aureus* more than 2 log cfu/g possibly associated with the producing method of this product including increased manuel handling compared to other RTE salads analysed. The percentage of samples over contaminated with *S. aureus* following cig kofte samples were 15.55% (tuna fish salad), 13.46% (sezar salad) and 10.34% (Russian salad samples). Similar results were reported by Wei et al.[12] who reported 15.9%. 9.5% and 6.3% of seafood, meat products, and vegetarian food products were over contaminated

with *S. aureus* respectively. In this study 0.38% of samples were habouring more than 10^5 cfu/g *S. aureus* similar to the results of Fang et al.[16] who reported 0.7% of the 18°C ready to eat food samples contained more than 10^5 cfu/g *S. aureus*.

The European Scientific Committee [5] has reported that most the outbreaks linked to fresh produce have been associated with members of *Enterobacteriaceae*. *Enterobacteriaceae* were the second most common contaminating microorganisms for RTE foods in this study, as >4 log cfu/g of these bacteria were isolated from 54% of the RTE foods analysed. Our results are higher than that reported by Little et al.[22] who indicated 39.9% of the samples analysed presented *Enterobacteriaceae* counts higher than 4 log cfu/g but lower than that found by Tessi et al.[17] reporting 63% of analysed RTE samples were over contaminated with these agents.

In this study, higher percentage of *Enterobacteriaceae* >4 log cfu/g were detected in Mediterranean salad (100%) likely due to the use of highly contaminated raw material, lack of good hygienic practices during processing and inadequate storage temperature. Regarding *Enterobacteriaceae* contamination rate, Mediterranean salad were followed by tuna fish salad (86.6%) and sezar salad samples (75%). The microbiological safety and quality of the vegetables used for the preperation of RTE foods depend on the use of appropriate irrigation water and good practices during manipulation but inherent risks of contamination due to cultivation in close contact with soil and organic fertilizers make difficult the control of pathogenic and spoliage microorganisms. Thermal processing and holding temperatures of RTE foods also have critical importance to maintain the microbiological safety. The prevention of contamination and bacterial growth lies in the application of good hygiene practise during growing and processing, effective washing and decontamination, effective temperature control during storage and distribution and the selection of appropriate packaging.

High populations of yeasts and molds were found in this study. High incidence of these bacteria found in this study suggests a short shelf life for the product and poor hygienic quality probably due to highly contaminated raw material, lack of good hygienic practices during preperation and improper storage conditions or different combinations of these factors.

The results obtained within the frame of this study indicates the need of adoption of hygienic practices by food handlers to minimize the risks of transmission of foodborne pathogens through this kind of foods. The results of this investigation revealed that contamination of these foods presented a potential health hazard to consumers. Hygienic rules must be implemented to avoid contamination. Efforts must be employed to ensure that this kind of foods do not became contaminated before final packaging. The expiration dates of the products must accurately reflect the shelf life of product. Better control is needed. Besides some changes in manufacturing practices should be made to enhance the safety.

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