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Assessment of the Microbial Quality of Industrial Ready-to-Eat Salads Containing Meat Products

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Abstract

Ready-to-eat foods are not usually treated sufficiently to eliminate the existing pathogenic bacteria in them before consumption; therefore, bacterial contamination in these foods requires due consideration. This study aims to detect *Salmonella* and *Escherichia coli* contamination and total microbial count in ready-to-eat salad samples containing meat products in Tehran in 2018. The microbial analysis of 136 samples including Olivier salad, Macaroni salad, and Sausage salad, collected by simple randomized sampling method from chain-stores, grocery and cooperative stores, was done according to the ISO international standards. *Salmonella* was not detected in any of the samples, and only 0.7% of the samples were contaminated with *E. coli*. The total number of microorganisms in 89.6% of the Olivier salad samples, 61.4% of the Macaroni salad samples and 97.7% of the Sausage salad samples was within the permitted limits of the Iranian National Standard. The average total number of microbes in the Olivier salad, Macaroni salad, and Sausage salad samples was obtained as 4.84, 4.23, and 5.34 log CFU/g, respectively. This study confirms the relatively satisfactory microbiological quality of ready-to-eat salads containing meat products in Tehran, Iran.

Introduction

Ready-to-eat foods are usually consumed immediately at the sale place without any preparation or treatment. They include raw, partially cooked, cooked, hot, cold and frozen foods.[1]The Codex Alimentarius Commission (CAC) defines ready-to-

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eat foods, as raw foods,or any foods manipulated, processed, mixed, cooked, or otherwise prepared and consumed without additional process.[2] The presence of pathogens in ready-to-eat foods is a more serious threat to the public health than their presence in raw meat products because ready-to-eat foods do not usually receive additional treatments to eliminate these bacteria;[3] meanwhile, they may contain native microflora including pathogenic bacteria of the raw material from which they are prepared.[4] The most common bacteria in ready-to-eat foods are *Salmonella, Listeria monocytogenes, Campylobacter jejuni, Staphylococcus aureus, Bacillus cereus,* and *Clostridium perfringens.*[5-13]

Since ready-to-eat foods are consumed without any additional treatment, the risk of foodborne outbreaks is high if they are improperly prepared or stored. *Salmonella* is a gram-negative bacilli of the *Enterobacteriaceae* family and one of the most important pathogens transmitted through food to humans. In addition, it is considered as a major cause of death and economic damage worldwide. Annually, 93.8 million cases of gastroenteritis of *Salmonella* types and 155,000 deaths occur throughout the world. It is estimated that 80.3 million of them are foodborne.[14] So far, over 2,500 *Salmonella* serotypes have been identified, half of which are *Salmonella* enterica Serovar Typhimurium. The major transmission ways of *Salmonella* species are through chicken meat, ready-to-eat products, dairy products, fruits, and vegetables.[15-17] Salmonellosis

with acute symptoms emerges with fever, abdominal pain, diarrhea, nausea, and sometimes, vomiting. Symptoms of the disease appear within 6-72 h (usually 12-36 h) after ingesting *Salmonella*, and the disease continues for 2-7 days. Although the clinical symptoms of salmonellosis are relatively mild, in some cases, especially in children and elderly patients, dehydration due to salmonellosis is severe or may even cause death.[18] Recently, the outbreaks due to the consumption of ready-to-eat foods are reported worldwide, most of which are caused by *Salmonella*.[19-22]

Various studies have been conducted on the contamination with *Salmonella* and *Escherichia coli* (*E. coli*) in ready-to-eat foods in different countries,[7,23-33] and different results have been reported. In some studies, *Salmonella* was detected in 39[9], 28.6[28], 16[5], 8[10] and 1.5%[33] of the studied ready-to-eat food samples, while in other studies, *Listeria monocytogenes* and *Salmonella* were not detected in any of the ready-to-eat products.[23, 24, 30, 32] In Iran, the production and consumption of ready-to-eat foods are increasing. Therefore, due attention should be paid to the quality and health of ready-to-eat foods to prevent foodborne diseases. In this study, several types of ready-to-eat salads in the market are examined in terms of contamination to the main food borne pathogens including *Salmonella* and *E. coli* (as an index of fecal contamination of food) and total microbial count.

Table 1: The main ingredients of ready-to-eat salads and their storage conditions at the market

Type	Ingredients	Storage conditions
Olivier salad	Meat (chicken or ham or Persian Mortadella), potato, Mayonnaise, pea, carrot, pickled cucumber, spices	Refrigeration
Macarroni salad	Meat (chicken or ham or Persian Mortadella), cooked Macaroni, Mayonnaise, pickled cucumber, sweet corn, carrot	Refrigeration
Sausage salad	Sausage, tomato paste, bell pepper, spices(potato and mushroom depending on the type of sausage salad)	Refrigeration

Materials and Methods
Samples Collection
Overall,136 packed samples of three types of ready-to-eat salads containing meat products in Tehran food markets were collected. They included 44 Sausage salads (22 Bandari Sausage salads, 18 sausage and potato salads, and 4 sausage and

mushroom salads with tomato sauce), 48 Olivier salads (23 Olivier salads with chicken, 6 Olivier salads with ham and 19 Olivier salads with Persian Mortadella), and 44 Macaroni salads (18 Macaroni salads with chicken, 13 Macaroni salads with ham and 13 Macaroni salads with Persian Mortadella) of 10 different brands. These samples were collected

by simple randomized sampling method from the chain- stores, grocery and cooperative stores in Tehran in 2018. Then they were transported to the laboratory in icebox and refrigerated until the microbial tests were conducted. All salads were packed in polyethylene trays; the packages ranged in weight from 200 to 500 grams. According to the labels, the samples did not receive any special treatment and were certified by the Food and Drug Administeration of Iran. The recommended storage condition for all salad samples was refrigeration temperature. The main ingredients of the ready-to-eat salad samples and their storage conditions at the market on the basis of the labels' information are presented in Table 1.

Microbial Analysis

Microbial tests including *Salmonella* and *E. coli* detection, and total microbial count were conducted according to the methods recommended by the ISO International Standard as follows:

Salmonella Detection

Twenty-five grams of each sample was added to 225 mL of buffered peptone water. After homogenization, the samples were incubated at 37°C for 18-24h, followed by selective enrichment in Rappaport-Vassiliadis medium with soya (RVS) broth at 41.5°C for 24h and Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth at 37°C for 24h. Then the Xylose Lysine Deoxycholate (XLD) agar and brilliant green agar plates were inoculated with the enriched cultures obtained from the RVS and MKTTn broths and incubated at 37°C for 24h. Typical isolated colonies on the XLD and Brilliant green agar plates were further confirmed using biochemical tests by inoculating in Triple Sugar Iron (TSI) agar slope, Urea Agar Christenson, L-Lysine Decarboxylation (LDC) medium and tryptone water (for indole test).[34]

E. coli Detection

E. coli was detected by the most probable number (MPN) technique of ISO by enrichment of the homogenate in Lauryl sulfate broth at 37°C for 24h, followed by inoculating the EC broth tubes containing Durham tubes from previous enriched cultures and incubation at 44°C for 24-48h. The positive EC broth tubes (having turbidity and gas production) were cultured in tryptone water and incubated at 44°C for 24-48h. Then they were examined for indole production using Kovacs reagent.[35]

Total Microbial Count

Aerobic mesophilic bacteria were enumerated by culturing the dilutions ranging from 10^{-2} to 10^{-7} in petri dishes containing plate count agar by pour plate method and incubation at 30°C for 72h.[36]

Duplicate plates were used for microbial enumeration. The calculation of the total number of microorganisms was performed according to the ISO International Standard.[37]

Statistical Analysis

This is a descriptive and cross-sectional study. Statistical analysis was performed on some of the variables based on Kruskal-Wallis test and one-sample t-test with SPSS 21. P-values less than 0.05 were considered significant. The results were interpreted in accordance with the standard limits in the Iranian National Standard (INS).[38-40]

Results and Discussion

Overall, 136 packed ready-to-eat salad samples containing meat products including 48 Olivier salads, 44 Macaroni salads, and 44 Sausage salads were tested for *Salmonella*, *E. coli*, and total number of microorganisms. According to the INS, none of these salads should be contaminated with *Salmonella* and *E. coli*, and the maximum total number of microorganisms in them should be 5, 3 and 6 log CFU/g, respectively.[38-40]

Salmonella Contamination

In this study, none of the salads was contaminated with *Salmonella* (Table 2). As a result, all samples of Olivier, Macaroni and Sausage salads were in accordance with (INS).[38-40] Various factors such as the use of preservatives, type of packaging, cold chain, detection method, and ingredients' antibacterial effect are involved in the detection of microorganisms in food. Therefore, the absence of *Salmonella* in the salad samples of this study does not necessarily mean the absence of *Salmonella*. In studying the survival of *Salmonella* in homemade mayonnaise, lemon juice has a greater inhibitory effect compared to wine vinegar.[41,42] The INS has also authorized the use of some preservatives in Olivier,[38] Macaroni,[39] and Sausage[43] salads. A recent study confirmed the presence of sodium benzoate and potassium sorbate in the Olivier salad and mayonnaise supplied in Kashan, Iran.[44] The results of the present research are in agreement with the studies conducted in

Yazd,[41] and Isfahan,[26] Iran. In another study in the Sharjah markets in the United Arab Emirates, *Salmonella* contamination was not detected in any of the samples.[23] In 2009-2010, in a study on ready-to-eat salads.(120 samples from 34 kinds) in Istanbul, Turkey, *Salmonella* and *Listeria monocytogenes* were not detected.[31] The results of a study in Poland indicated that none of the raw Sausage samples was contaminated with *Salmonella*.[29] In a research in Sweden, *Salmonella* contamination was not detected in 141 ready-to-eat salad samples containing chicken, ham or smoked salmon.[46]

Table 2: Frequency distribution of *Salmonella* contamination in the ready-to-eat salads supplied in Tehran, Iran according to the type of salad (2018)

Type Status	Sausage salad no. (%)	Olivier salad no (%)	Macaroni salad no (%)	All salad types no (%)
Not contaminated	44(100)	48(100)	44(100)	136(100)
Contaminated	0(0)	0(0)	0(0)	0(0)
Total	44(100)	48(100)	44(100)	136(100)

While evaluating the quality of ready-to-eat salads in Turkey, *Salmonella* species were isolated from 8% of 261 samples supplied in the Turkish market.[10] In the study of 50 salad samples (30 industrial samples and 20 traditional samples) presented in the sandwich shops of Shahrekord, Iran, *Salmonella* contamination was reported in 9 samples (18%).[47] In Hong Kong, *Salmonella* isolated from 39% of 115 ready-to-eat samples of Char Sia (Chinese barbecued pork), meaning that secondary contamination is a very serious problem in these shops.[9] In a national survey in China, 0.56% of the 359 sausage samples were contaminated with *Salmonella*.[13]

E. coli Contamination
E. coli contamination was detected in only 0.7% (1 out of 136) of the samples (one sample of Olivier salad with chicken) as shown in Table 3. This result is in agreement with the study of food-borne pathogens in Sweden, in which only 1 out of 141 samples of chicken salad was detected.[46] In testing 634 samples of ready-to-eat foods collected from 47 stores in three different provinces of Korea, *E. coli* and *Listeria monocytogenes* were detected only in two samples.[25] In the study on the microbiological quality of 120 samples of ready-to-eat foods in Barbados, WI, *E.coli* was detected in 1.7% of the samples.[33] When examining the microbial quality of ready-to-eat salads in Turkey, *E. coli* was detected in 4% of the samples.[10] In the United Arab Emirates, 20% of the 120 ready-to-eat food samples including four types of ready-to-eat salads had *E. coli*, though in a low number of 1 log MPN/g.[23] In a national survey in China, none of the 321 sausage samples was contaminated with diarrheagenic *E. coli*.[13]

Table 3: Frequency distribution of *E. coli* contamination in the ready-to-eat salads supplied in Tehran, Iran according to the type of salad (2018)

Type Status	Sausage salad no. (%)	Olivier salad no (%)	Macaroni salad no (%)	All salad types no (%)
Not contaminated	44(100)	47(97.9)	44(100)	135(99.3)
Contaminated	0(0)	1(2.1)	0(0)	1(0.7)
Total	44(100)	48(100)	44(100)	136(100)

Total Microbial Count
As shown in Table 4, the mean total number of microbes in the studied Olivier salad (4.84 log CFU/g) is. in agreement with the standard limit and less than the maximum limit determined by the INS (5 log CFU/g).[38] However, in the Macaroni salad samples, the mean total number of microbes (4.23 log CFU/g) is higher than the maximum limit

determined by the INS(3 log CFU/g).[39] The mean total number of microbes in the studied Sausage salads (5.34log CFU/g) is within the standard range and less than the maximum limit determined by the INS(6 log CFU/g)[40] (p = 0.001).

Table 4: Comparison of the mean total number of micro organisms according to the Iranian National Standard limits in ready-to-eat salads supplied in Tehran, Iran (log CFU/g)

Count / Type	Mean±SD	Standard limit	P value
Olivier salad	4.84±5.36	5	0.361
Macaroni salad	4.23±4.92	3	0.206
Sausage salad	5.34±6.15	6	0.001*
All salad types	5±5.91	-	-

Table 5: Descriptive statistics of the total number of microorganisms in the ready-to-eat salads supplied in Tehran, Iran according to salad type (log CFU/g)

Type Count	Mean±SD	IQR**	Median (Q_1-Q_2)	Range	P value
Olivier salad	4.84±5.36	3.26	2.15-3.95	1.65-6.04	
Macaroni salad	4.23±4.92	2.87	2.58-3.73	1.51-5.74	0.564
Sausage salad	5.34±6.15	2.93	2.45-3.77	1.36-6.97	
All salad types	5±5.91	2.95	2.46-3.81	1.36-6.97	

* shows significant difference (P<0.05).
**Interquartilerange

Various factors such as use of preservatives, type of packaging, cold chain, and food ingredients are involved in the control of microorganisms in food.

Table 5 indicates that the total number of microorganisms in the three studied salad types was not significantly different (p = 0.564). In Hong Kong, in a study on the microbial quality of 115 ready-to-eat food samples, the mean total number of aerobic microbes was 5.05 log CFU/g,[9] which is consistent with the results of this study. In a survey on 634 ready-to-eat food samples collected from 47 stores in three different provinces of Korea, the total number of aerobic microbes had a relatively large range of 1.0-7.9 log CFU/g,[25] which was much more than the presentresearch results.

Table 6: The total number of microorganisms in the studied salads in terms of compatibility with the Iranian National Standard

Type Status	Maximum standard limit† (log CFU/g)	Compatible no (%)	Incompatible no (%)	Total no (%)
Olivier salad	5	43(89.6)	5(10.4)	48(100)
Macaroni salad	3	27(61.4)	17(38.6)	44(100)
Sausage salad	6	43(97.7)	1(2.3)	44(100)

† According to the Iranian National Standard Limits.[38-40]

In the present study, 43 samples (89.6%) of the Olivier salads matched the total number of microorganisms determined bythe INS,[38] and only five samples (10.4%) failed to match the standards. While in the Macaroni salads, 27 samples (61.4%) were compatible with the INS[39] and 17 samples (38.6%) were not compatible. Forty three samples (97.7%) of the Sausage salads matched the total number of microorganisms determined by the INS,[40] and only one sample (2.3%) failed to match the standards (Table 6).

Conclusion

Considering that *Salmonella* was not detected in any of the tested salad samples, and *E. coli* was detected only in 0.7% of the samples, and that most of the Olivier, Macaroni and Sausage salad samples were consistent with the INS limits in terms of the total number of aerobic mesophilic microorganisms, the relatively favorable microbial quality of ready-to-eat salads containing meat products supplied in Tehran is confirmed. It is worth noting that various factors such as use of preservatives, detection method, antibacterial effect of ingredients and cold chain are involved in the growth and detection of microorganisms in food products.

Conflict of Interest

The authors declare that there is no conflict of interest.

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