The Microbial and Chemical Quality of Ready-to-Eat Olivier Salad in Mashhad, Iran

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A R T I C L E I N F O	A B S T R A C T
	Introduction: Olivier salad is a commonly used cold fast food in Iran, which contains various nutrients. However, this food product is susceptible to bacterial contamination. The present study aimed to evaluate the chemical and microbial quality of the ready-to-eat Olivier salads sold in the groceries in Mashhad, Iran. **Methods:** This study was conducted on 26 samples of two types of Olivier salad containing chicken (n=17) and meat (n=9), which were collected from the local markets in Mashhad city. The samples were transferred to the laboratory in cold flasks to evaluate their microbial quality (coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *clostridium perfringens*, molds, and yeasts) and chemical quality (potassium sorbate, potassium benzoate, and benzoate levels) based on the Iran national standard No. 17813 and 17813-a-1, respectively. **Results:** Chemical tests indicated no significant differences in the levels of potassium sorbate, potassium benzoate, and benzoate (P>0.05) between the samples. On the other hand, 7.7% (2/26) and 23.07% (6/26) of the Olivier salad samples were contaminated with *Salmonella* and *Escherichia coli*, respectively, while all the samples were negative for *Staphylococcus aureus* contamination. **Conclusion:** *Salmonella* and *Staphylococcus aureus* are of particular importance in food contamination. Lack of hygiene during production, contamination of raw materials, and elevated storage temperature are among the key influential factors in the increased contamination of food products. Therefore, the control and monitoring of the food chain must be prioritized.

Introduction

Use of ready-to-eat foods has been on the rise due to lifestyle changes, increased urbanization, and the advantages of such foods as they are easy to use and could be prepared within a short time. Iran is a developing country where the consumption of these food products is highly common, especially Olivier salad (1).

Available data indicate that 60% of Europeans use ready-to-eat (RTE) foods (1, 2). Cold food refers to the food products with the shelf life of more than five days at refrigerated temperatures (3). Since these products are at the risk of corruption, the manufacturers often use preservatives at higher levels than the national standards in order to prevent their corruption due to the inappropriate transport and maintenance of the products at improper temperature (1, 3). Due to the variety of their composition, RTE products constitute a major part of the human needs for carbohydrates, protein, and fat in meals. The preparation process of RTE sandwiches, salads, and meats involves manual interference (e.g., cutting or crushing), which could lead to the contamination of the products (4, 5).

Cold chain is considered to be the only method to control the quality of RTE Olivier salads after production (6). However, various pathogenic psychrotrophic bacteria could grow at refrigerated temperatures, such as *Yersinia enterocolitica*, *Clostridium botulinum* type E, *Listeria monocytogenes,* and *Aeromonas hydrophila* (7, 8)

In appropriate conditions, microorganisms could grow in RTE Olivier salad (9). *Staphylococcus aureus* is a normal flora of the skin and upper respiratory tract in humans, which could be transmitted to food during the preparation chain (9-12). Vegetables are another key ingredient in Olivier salad, which could become a source of *Salmonella* and *Escherichia coli* transmission (13). *Clostridium perfringens* is another bacterium causing food

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poisoning, which is considered to be a spoilage factor in industrial food products. Furthermore, the bacterium causes necrotic enteritis (NE), which is a rare, severe human infection (14, 15). Coliforms are the bacteria that are frequently transmitted to salads through contaminated water with human feces or due to the lack of hygiene in the processing environment of Olivier salad (16, 17). In addition, fungal spores are extremely common in the air and could be transferred to various salads, thereby causing food spoilage and diseases in humans. The presence of molds could also be attributed to improper packaging conditions (18, 19).

Sodium benzoate and potassium sorbate are widely used as preservatives for the prevention of microbial growth and increase the shelf life and quality of food products. However, these compounds have permissible limits and should not be used at higher concentrations (20, 21). Benzoate exposure is inevitable for most individuals as this compound is produced by gut microbes (Beyoğlu and Idle, 2012) and is also found in many berries and milk products (<40 mg/kg) (Rangan and Barceloux, 2009a). Moreover, reports suggest that exposure to sodium benzoate through food could cause asthma (Petrus et al., 1996) or allergic reactions in children (Jacob et al., 2016).

The present study aimed to evaluate the microbial safety and chemical properties of RTE Olivier salad in Mashhad, Iran as a commonly consumed RTE product.

Materials and Methods

Sampling

In this study, Olivier salad samples were aseptically collected from the markets of Mashhad city during the spring of 2019. In total, 26 samples were collected, including two types of Olivier salad containing chicken (n=17) and meat (n=9). All the samples were produced under the supervision of the Iranian Ministry of Health.

Microbial Analysis

Total Count

According to the Iran national standard No. 5272, colony count was performed at the temperature of 30°C using the plate count agar (PCA; Merck, Darmstadt, Germany). After the preparation of a suspension, a serially tenfold dilution was obtained, and 0.1 milliliter of each dilution was streaked on the PCA plates and incubated at the temperature of 30°C.

Enumeration of Coliforms

A colony count technique based on the Iran national standard No. 11166 was performed for the enumeration of the coliform bacteria. After the preparation of the serial dilutions, 0.1 milliliter of each dilution was transferred to the plates containing violet red bile agar (Merck, Darmstadt, Germany) and incubated at the temperature of 30°C or 37°C for 48 or 24 hours.

Enumeration of Staphylococcus aureus

At this stage, 25 grams of each sample was weighed and homogenized with 225 milliliters of buffered peptone water (BPW) (Merck, Darmstadt, Germany) for approximately five minutes. The obtained samples were further diluted with BPW, and 0.1 milliliter of each dilution was spread on the surface of the Baird-Parker media (Merck, Darmstadt, Germany) (12).

Enumeration of Escherichia coli

A specific amount of the test samples was inoculated into lauryl sulfate enrichment broth (Merck, Darmstadt, Germany). Afterwards, the inoculated medium was incubated at the temperature of 37°C for 24-48 hours for gas production. After observing the turbidity or gas in the tube, it was inoculated into a tube containing the *Escherichia coli* broth (Merck, Darmstadt, Germany). Following that, the *E. coli* tube was inoculated at the temperature of 44°C for 48 hours and examined for gas production after 24 hours. Differentiation of *E. coli* was carried out using indole, methyl red, Voges-Proskauer, and citrate utilization test (21).

Isolation of Salmonella spp.

At this stage, 25 grams of each sample was weighed and homogenized with 225 milliliters of BPW and incubated at the temperature of 37 °C for 24 hours. For pre-enrichment, 0.1 milliliter of the sample was transferred to the Rappaport-Vassiliadis enrichment broth (Merck, Darmstadt, Germany) and incubated at the temperature of 42 °C for 24 hours. For enrichment in the selected liquid medium, one milliliter of the obtained sample was transferred to the selenite cystine broth (Sigma-Aldrich, USA) and incubated at the temperature of 37°C for 24 hours. Finally, one loop was streaked onto the Hektoen enteric agar and Brilliant Green agar (Merck, Darmstadt, Germany) and

incubated at the temperature of 37°C for 24-48 hours.

In order to confirm the isolates, one colony was inoculated in tubes containing Triple Sugar Iron agar (Sigma-Aldrich, USA) (22).

Enumeration of Clostridium perfringens

At this stage, 10 grams of each sample was homogenized with 90 milliliters of BPW, and one milliliter was added to a tube containing nine milliliters of fluid thioglycollate (FTG, HiMedia; Merck, Darmstadt, Germany). Afterwards, the tubes were incubated at the temperature of 32°C for 15-24 hours in anaerobic conditions. After the initial enrichment for *C. perfringens*, each tube was cultured linearly on blood agar medium and incubated at the temperature of 45°C for 32 hours in an anaerobic jar (Merck, Darmstadt, Germany).

In order to confirm *C. perfringens*, one loop was cultured on tryptose sulfite cycloserine agar (Sigma-Aldrich, USA) from the colonies with duplicate hemolysis on the blood agar medium. Following that, the plates were incubated at the temperature of 32°C for 24 hours in anaerobic conditions (14).

Enumeration of Molds and Yeasts

After the preparation of tenfold serial dilutions, 100 microliters of each dilution was streaked onto the plates of the potato dextrose agar (Merck, Darmstadt, Germany), and the plates were incubated in aerobic conditions in the dark at the temperature of 22-25°C for 5-7 days.

Chemical Analysis

Extraction of sodium benzoate, potassium sorbate, and benzoic acid salts was performed using high-performance liquid chromatography (HPLC) and through separation using ACE-121-1504 C18 HPLC column (15 cm x 3.9 mm I.D., 5 μm). The acetate buffer was prepared with 0.30 gram of ammonium acetate in 900 milliliters of HPLC grade water and glacial acetic acid in order to adjust the pH at 4.20. Afterwards, the ammonium acetate buffer solution was filtered through a Millipore Millex-HV filter (0.45 μm; Hydrophilic Vinylidene). The mobile phase was degassed for 10 minutes in an ultrasonic bath prior to use. The selected wavelengths to determine sodium benzoate and potassium sorbate were 225 and 255 nanometers, respectively (national standard Numbers 17813-a-1).

Statistical Analysis

All the experiments were carried out in triplicate. Data analysis was performed in SPSS version 16 using one-way analysis of variance (ANOVA) to determine the significant differences between the samples. In all the statistical analyses, P-value of less than 0.05 was considered significant.

Results

According to the information provided in the tables, there were no significant differences between the two sample groups of Olivier salad (meat and chicken) in terms of various preservatives (Table 1). In addition, 3.84% (1/26) of the samples containing meat and chicken had higher sodium benzoate than the permissible limits, while 3.84% (1/26) of the meat and chicken samples had higher potassium sorbate than the permissible limits of the Iran national standards (Table 1).

Table 1. Comparison of different preservatives in meat and chicken Olivier salad.

factors	sample	Mean	Std. Dev.	P value	Permissible limits (mg/L)
Sorbate potassium	chicken	57.03	69.9	0.3	Maximum 150
	meat	64.96	40.7		
Sodium benzoate	chicken	28.96	26.2	0.5	Maximum 150
	meat	61.08	95.9		
Benzoate alone	chicken	61.62	64.9	0.4	Maximum 150
	meat	94.68	137.5		

According to the obtained results, no significant differences were observed in the total counts of coliforms, *E. coli*, *C. perfringens, S. aureus,* molds, yeasts, and *Salmonella* spp. between the Olivier salad samples containing chicken and meat (P>0.05) (Table 2).

None of the chicken and meat Olivier salad samples, had counts above the standard permissible limits, while 11.76% (2/17) of the chicken Olivier salad samples and 44.44% (4/9) of the meat Olivier salad samples were positive for contamination with *E. coli.* Furthermore, *Salmonella* spp. was detected in 11.76% (2/17) of the chicken Olivier salad samples, while the meat Olivier salad samples were negative in this regard (Table 2).

Table 2. Comparison of *Salmonella* spp*, E.coli*, *S. aureus*, *C. perfringens*, mold and yeast, coliforms and total count in meat and chicken Olivier salad.

Microbial Factors	Sample		Positive	Negative	More than 100	Less than 10	Mean	Std. Dev.	P value	Permissible limit
Salmonella spp	Chicken	No.	2	6						Negative in 25 g
		%	25	75					0.3	
	Meat	No.	0	3						
		%	0	100						
E. coli	Chicken	No.	2	15						Negative
		%	12.5	87.5					0.1	
	Meat	No.	4	5						
		%	44.44	55.56						
S. aureus	Chicken	No.			0	0				Maximum 10
		%			0	0			0	
	Meat	No.			0	0				
		%			0	0				
C. perfringens	Chicken	No.			0	17				Maximum 50
		%			0	100			>0.99	
	Meat	No.			0	9				
		%			0	100				
Mold and yeast	Chicken	No.			8	8				Maximum 10^2
		%			47.06	47.06			0.2	
	Meat	No.			1	7				
		%			11.11	77.78				
Coliforms	Chicken						461491	1024729	0.1	Maximum 50
	Meat						29398	84982.31		
Total count	Chicken						304.7	701.9359	0.1	Maximum 10^5
	Meat						75.5	67.47427		

Discussion

Olivier salad is a commonly consumed RTE food, which is used without further processing after preparation. Therefore, the contamination of these products with pathogenic microorganisms could lead to infections or food poisoning in the consumers (1). Refrigeration is the only approach to maintain the health of RTE Olivier salad; otherwise, psychrotrophic microorganisms, such as *Yersinia enterocolitica*, *Clostridium botulinum* type E*, Listeria monocytogenes,* and *Aeromonas hydrophila*, may grow in the products, causing food corruption and food poisoning in humans (7, 8, 22). The microbial tests performed on RTE foods vary in terms of food type, while they mainly involve the total count of bacteria, coliforms, *Staphylococcus aureus*, molds, and yeasts, as well as the detection of pathogenic bacteria, such as *Salmonella, E. coli,* and *Listeria* (23).

Olivier salad contains various ingredients, including chicken, meat, eggs, pasta, potatoes, vegetables, spices, mayonnaise, and flavoring substances. *C. perfringens* is a pathogen that could grow in RTE Olivier salad in the anaerobic condition of the packages (24). According to the results of the present study, *C. perfringens* count was lower than 10 CFU/g in all meat and chicken Olivier salad samples.

Growth of *S. aureus* in food could be due to the non-observance of hygiene by food production personnel. The enterotoxin of this bacterium is highly resistant, affecting the quality of the food at the optimum time and temperature (22, 25, 26). In the present study, *S. aureus* was detected in none of the samples, which is consistent with the findings of Khoramrooz et al. (2014) (27) possibly due to the limited number of the studied samples. In contrast, in the studies by

Tajbakhsh et al. (2015) and Kaseb et al. (2013), 46% and 20% of the samples (total: 200) were reported to be contaminated with *S. aureus* (24, 28).

According to the current research, 7.7% (2/26) of the samples were contaminated with *Salmonella*. Although the estimated rate is rather low, it could still pose health risks to the consumers according to the Iran standard No. 1810 as 25 grams of a sample should be negative for *Salmonella*. In the study by Tajbakhsh et al., 18% of the samples were reported to be contaminated with *Salmonella* (24), while Kaseb et al. stated that *Salmonella* was detected in none of the samples (28). In the present study, *Salmonella* was isolated from the chicken Olivier salad samples, which could be due to the fact that chicken is a major source of *Salmonella* (22, 29, 30).

Bacterial growth prevention in products containing compounds such as starch and mayonnaise has been reported in several studies. For instance, Doyle et al. (1982) prepared meat Olivier salad with variable proportions of mayonnaise, concluding that the presence of mayonnaise in meat Olivier salad delayed the growth of pathogenic *Salmonella* (31).

The human intestinal microbial flora includes microorganisms that could be transmitted to Olivier salad in case of non-hygienic conditions; coliforms are among the intestinal flora that could be transmitted to this type of food (32). The results of the present study indicated that 23.07% (6/26) and 11.53% (3/26) of the samples were contaminated with *E. coli* and coliforms, respectively. In this case, the personal hygiene of the food production staff was considered to be the most important source of contamination. In a study conducted by Wogu and Iwezeua, 60% of RTE salad samples were reported to be contaminated with gram-negative bacteria, including *E. coli* (34).

Among 26 samples in the current research, 23 cases were contaminated with molds and yeasts, while in 47.06% of chicken Olivier salads samples and in 11.11% of meat Olivier salad samples, the contamination rate was higher than the permissible limits proposed by the Institute of Standard and Industrial Research of Iran (ISIRI).

Chemical agents (e.g., sodium benzoate, potassium sorbate, and benzoic acid salts) are the food preservatives that inhibit the growth of microorganisms. In the present study, the levels of these compounds were measured in the Olivier salad samples. According to the Food and Agriculture Organization (FAO) and World Health Organization (WHO), the acceptable daily intake of sodium benzoate and potassium benzoate is 0-5 mg/kg of body weight (36). In the current research, potassium sorbate and benzoate alone were isolated from 53.85% of the samples (14/26), 7.7% (2/26) and 11.53% (3/26) of which contained higher levels than the acceptable limits, respectively. On the other hand, sodium benzoate was isolated from 7.7% (2/26) of the samples, 3.84% (1/26) of which contained higher levels than the permissible limit. In a similar study, Pylypiw et al. (2000) measured the levels of potassium sorbate and sodium benzoate in various food products (e.g., juices, soy sauce, cream cheese, and peanut butter), reporting that these preservatives were present in the studied products (20). Therefore, the evaluation and control of these food additives is of paramount importance since they may cause allergic reactions, such as urticaria, non-immunological contact urticaria, and asthma (38).

Conclusion

According to the results, RTE Olivier salad could be a potential health hazard for the consumers due to the possible contamination with *E. coli* and *Salmonella* spp. Therefore, it is recommended that Good Manufacturing Practice (GMP) and Good Hygienic Practice (GHP) be implemented in food processing plants and food handlers be trained in this regard, so that the secondary contamination of sensitive products (e.g., salads) could be prevented.

Acknowledgments

Conflicts of interest

None declared.

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