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Microbial Quality of Tabbouleh, Vegetable Salad Based on Parsley, Commercially Produced in Jordan

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ABSTRACT

This study examined the microbial quality of tabbouleh, a popular Middle Eastern salad, at various restaurants in Amman, Jordan. Sixty samples were collected from 15 restaurants at two different intervals (October (interval I) and December (interval II)), with two samples taken from each restaurant. Additionally, two reference samples were prepared under hygienic conditions and tested for comparison purposes. The moisture content of the samples ranged from 65.2% to 85%, with an average of 76.8%. The pH ranged from 3 to 4.2, with an average of 3.5, and the acidity ranged from 0.5% to 1.1%, with an average of 0.8%. The microbial counts in interval I for aerobic plate counts (APC), coliform counts (CC), and lactic acid bacteria counts (LAB) counts. LAB averaged 5.5, 4.1, and 5.2 log10 CFU/g, respectively. While in the second period, it was 5.3, 3.5, 4.1, and 5.7 log CFU/g, respectively, with a few Staphylococci bacteria (<1 log CFU/g). Reference samples had significantly lower counts of APC, CC, LAB, and yeast counts (YC) 2.7, <10, 1.9, and 2.6 log10 CFU/g, respectively indicating that commercial tabbouleh was generally prepared without following proper hygiene procedures. Staphylococcus aureus counts, which are sensitive to low pH, were < 10 log10 CFU/ g in all the collected samples. Counts of all microorganisms were significantly higher in interval I samples when compared to interval II samples, suggesting that the microbial quality of tabbouleh deteriorated over time as affected by relatively higher seasonal temperatures. This study highlights the importance of proper hygiene practices during the preparation of tabbouleh and the need for regular monitoring of its microbiological quality.

Keywords: Eastern salad, microbiological quality, proper hygiene, tabbouleh.

INTRODUCTION

Traditional food refers to the diet frequently consumed in substantial quantities by the broader populace. Jordan and neighboring countries have a diverse heritage of traditional foods, some of which are internationally spreading, such as hummus and falafel. Vegetables are used as a primary ingredient in many traditional dishes, such as green salads, garnishes, and appetizers in which vegetables are usually eaten raw. These foods are increasingly consumed because of their perceived healthy attributes (Faour-Klingbeil et al., 2016).

Tabbouleh, tabouleh, or tabouli is one of the most common traditional salads in Jordan and neighboring countries, where it is usually consumed as an appetizer. Typically, the ingredients of tabbouleh, include soaked bulgur (partially boiled, dried, and ground whole wheat), tomato, onion, parsley, cucumber, olive oil, the herb mint, lemon juice, and salt. However, some other components like lettuce, garlic, and pomegranate may be added

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differently according to place of preparation. In some Lebanese cities, tabbouleh is prepared by soaking the bulgur in warm water for an hour to soften it; in others, the bulgur is swapped out for the equivalent quantity of quinoa (the seeds of a South American plant that is cooked and eaten as food) (Assaad et al., 2020).

Tabbouleh could be considered ready-to-eat (RTE) food as defined by the Codex Alimentarius Commission (2004): "RTE are raw foods, or any foods manipulated, processed, mixed, cooked or otherwise prepared and consumed without additional process". Since RTE salad vegetables are generally considered healthy and nutritious due to their vitamin, mineral, and antioxidant content, their intake in modern life has expanded in both quantity and diversity in recent years as a result of rising consumer demand (De Giusty et al., 2010; Taban et al., 2013). Ready-to-eat salads retain the indigenous microflora of the raw ingredients from which they are made. The shelf life of these products is primarily determined by their relatively high acidity, which prevents to a marked degree the growth of many spoilage and pathogenic microorganisms since the microbiological quality and safety of RTE foods also are influenced by processing steps and storage conditions that may introduce microorganisms (Tassou et al., 2009). The physical structure of the vegetables and their chemical composition can induce different types of contamination and/or deterioration (Fröder et al., 2007). For this reason, vegetable salads have been identified as the commodity group of highest concern from a microbiological safety perspective (FAO & WHO, 2008) and this probably applies to tabbouleh.

Sources of contamination of tabbouleh: come from fields such as contaminated soil, natural untreated fertilizers, use of contaminated water used in irrigating leafy green vegetables, and from food handlers and contaminated kitchen utensils and food contact surfaces.

Vegetables are often contaminated with microorganisms when they are irrigated with sewage water, especially when irrigating leafy green vegetables and when the soil is fertilized with manure. Furthermore, vegetables and fruits may become contaminated by microorganisms when being washed, peeled, trimmed, packaged, and handled (Oranisi & Olorunfemi, 2011). Also, food handlers and contaminated kitchen utensils and food contact surfaces could serve as a source of contamination. Tabbouleh can become contaminated by many types of microorganisms, possibly including pathogens from the fresh ingredients, or during the preparation steps, and some of the contaminants may grow during storage (Al-Rousan et al., 2018).

So, tabbouleh can pose a risk of food poisoning if prepared in an unsanitary manner. In restaurants, it is expected to contain various types of microorganisms brought from the raw materials and utensils used, as well as from the employees and the environment. Their impact is intensified because neither a heat treatment nor a chemical preservative is used in the preparation. Accordingly, this study was carried out to determine the number of groups of microorganisms that reflect the hygienic conditions prevailing during the preparation of tabbouleh commercially produced in Jordan. A tabbouleh produced under hygienic conditions was used as a reference control for comparison.

Materials and Methods

Sample Collection

Commercial tabbouleh samples were collected from 15 restaurants in Amman, Jordan, at two intervals with two months between the first and second visit. So 60 samples were taken on different days. Two sample units were taken from each restaurant at each visit, so 30 sample units were collected in the first interval (10 October -12 December), at which the range of the average daily temperature was 15.4 to 27.2 °C. Thirty-30 sample units were collected in the second interval (19 December – 10 January), in which the range of the average daily temperature was 5.9 to 14.8 °C. Samples were brought to the laboratory using an icebox within an hour of purchase. Tabbouleh samples were purchased as offered to customers and the weight of each sample unit was ca 200 g.

Reference Tabbouleh

Since no official or standard recipe for tabbouleh specifies the proportion of particular ingredients they were bought at a local market. Tabbouleh was prepared at this department according to the recipe used by Al-Rousan et al., (2018) by mixing 100 g of chopped parsley, 100 g of chopped tomatoes, 10 g chopped onions, 35 g pre-soaked bulgur wheat. Thereafter, lemon juice, 5 g salt, and 30 g olive oil were added. Tabbouleh is acidified with lemon juice, and 5 g salt, 30 g olive oil. Foreign materials and visible dirt were removed from the vegetables. They were then rinsed thoroughly with distilled water. Two batches were prepared at various times and microbiologically examined as described in part 2.4.

Chemical Analysis

Moisture content was determined using the atmospheric oven technique in all samples by the Association of Official Analytical Chemists guidelines (AOAC, 2012).

The titratable acidity was determined according to AOAC (2012) method. Ten grams of the sample were weighed in a 100 ml conical flask, diluted with 90 ml free distilled water, and mixed gently. Phenolphthalein indicator (0.5 ml) was added and the whole mixture was titrated with 0.1N NaOH to the first permanent (30 sec) color change to pink. Titratable acidity is expressed as citric acid. The pH was determined using a Hana instruments pH meter (model HI 8416), by immersing the electrode in tabbouleh diluted with distilled water.

Microbiological Tests

Twenty-five grams of tabbouleh sample were taken from different parts in a salad bowl by pre-sterilized utensils weighed and transferred into a sterile stomacher bag, and 225 ml of peptone water (Oxoid, UK) was added. The bag was placed into a lab blender stomacher and mixed for two minutes. Appropriate serial dilutions were made aseptically as diluents for all microbiological tests. The numbers were calculated and expressed as colony-forming units (CFU/g). All tests are shown in Table 1

Table 1: Details of microbiological enumeration tests for tabbouleh samples by pour plate technique and by streaking method for Staphylococcus aureus

Test	Media	References
Enumeration of aerobic plate count (APC)	Plate count agar (PCA)	FDA/BAM*:2020
Enumeration of coliforms (CC)	Violet red bile (VRB) agar	FDA/BAM:2020
Enumeration of lactic acid bacteria (LAB)	MRS Agar	ISO* 15214:1998
Enumeration of yeast and mold (YAM)	Plate count agar (PCA) +100 mg hloramphenicol /liter	FDA/BAM:2020
Enumeration of *Staphylococcus aureus*	Baird-Parker (BP) agar	FDA/BAM:2019

*BAM: Bacteriological Analytical Manual of the Food and Drugs Administration, USA.
*ISO: International Organization for Standardization

Comparison of Microbiological Findings

A comparative analysis between the microbiological data obtained in this study and similar data existing in the literature about attributes of ready-to-eat foods originating from vegetable sources was carried out (Tables 4, 5, 6, and 7.)

Statistical Analysis

Experiments were conducted in two intervals. Significant differences between treatments were determined by using the SAS program by analysis of variance (ANOVA) at 0.05% level significance.

Results and Discussion

Tabbouleh is a classic Levantine and Middle Eastern salad served as an appetizer, it is a healthful, vegetarian dish that has no cholesterol, low in fat, and high in fiber, vitamins, and other nutrients. This salad could be dealt with as a typical vegan recipe, that may be made gluten-

free by replacing quinoa with bulgur wheat (Assaad *et al*.,2020). According to Hiba Bawadi *et al*., (2009) ½ cup of tabbouleh provides the body with a total of 39 kcal and contains 4.5g carbohydrates, 1.3g protein, and 1.7g fat. Assaad *et al*., (2020) reported that Lebanese tabbouleh contained many minerals including iron, magnesium, zinc, phosphorus, and copper. Tabbouleh had a total of dietary fiber of roughly 7.6 % (Al-Khusaibi, 2019).

The body of published research on the microbiological quality of tabbouleh is limited compared to the papers by Al-Rousan et al., who explored the use of acetic and citric acids as inhibitors of *Escherichia coli* O157:H7, *Salmonella Typhimurium*, and *Staphylococcus aureus* in tabbouleh. Another study by Olaimat *et al.* (2019) focused on the population dynamics of *Salmonella* and *Shigella* in Mediterranean vegetable salads that are ready-to-eat. Their objective was to observe the growth and fate of these pathogens during salad storage. Another work by Olaimat *et al.* (2021) studied the survival and growth of two pathogenic bacteria, *Listeria monocytogenes* and *Staphylococcus aureus*, in ready-to-eat Mediterranean vegetable salads. The study aimed to determine the effects of storage temperature and food matrix on the bacteria. Assaad *et al*., (2020) conducted a study on heavy metal accumulation in edible vegetables of Lebanese tabbouli salad. As a result, it is not possible to make direct comparisons of the current study with local data.

Chemical Analysis

Table (2) shows the moisture content, pH, and acidity of commercial tabbouleh samples, along with a reference sample for comparison purposes. Variations in results were observed among samples from different producers, and even for the same producer tested at two different times. The average moisture content, pH, and titratable acidity (as citric acid) for the tabbouleh samples were found to be 76.8%, 3.5, and 0.8%, respectively, while the reference sample showed a moisture content of 72.8%, pH of 3.48 and an acidity of 0.7%.

It should be noted that the activity of water (a_w) of the tabbouleh samples was not measured, but the moisture content of the tabbouleh samples is relatively high (Table 2) since it is based mainly on vegetables, which could create favorable conditions for the growth of various microorganisms, as evident in the results of microbiological testing.

Differences in pH and titratable acidity among tabbouleh samples were probably due to differences in the amounts of ingredients used, especially salt and lemon juice. The addition of salt and lemon juice might have favored the growth of LAB and other natural microbiota by reduction of pH (Olaimat *et al*., 2019). The relatively low pH and the availability of carbohydrates and some nutrients make tabbouleh an ideal environment for certain microorganisms to grow (Assaad *et al*., 2020).

The majority of tabbouleh samples analyzed exhibited a pH level below 4 (Table 2), resulting in the inhibition of the growth of various spoilage bacteria such as *Staphylococcus*, *Micrococcus*, and *Pseudomonas*, which are typically inhibited at pH levels less than 5.5 (Yamani and Dababseh, 1994). This may have been facilitated by the addition of salt and lemon juice, which helped to lower the pH and potentially promoted the growth of LAB and other natural microbiota (Olaimat *et al*., 2019). Nevertheless, certain microorganisms like LAB and yeast can endure low pH levels and, although not identified as foodborne pathogens, could still proliferate. The citric acid in the undissociated form present in lemon juice, at the low pH of the product, also contributes to the inhibitory effect of low pH. During the production process of tabbouleh, adding an appropriate amount of lemon juice to lower the pH to less than 4 while maintaining an acceptable taste may constitute a critical control point.

It is noteworthy that the reference sample had significantly different results compared to the commercial samples, highlighting the need for consistent quality control in the production process.

Table 2: Averages of moisture, pH, and titratable acidity of tabbouleh samples collected from different food services in Amman, Jordan.

Restaurant	Moisture(%)	pH	%Titratable acidity (as citric acid)
1	83.5^{a*}	3.4^{c}	0.8^{cd}
2	79.7^{bcd}	3.8^{b}	0.7^{def}
3	78.8^{cde}	3.3^{c}	1.1^{a}
4	82.5^{ab}	3.4^{c}	0.9^{bc}
5	69.6^{f}	3.8^{b}	0.7^{de}
6	78.1^{de}	3.5^{c}	1.0^{ab}
7	85.0^{a}	3.0^{d}	0.8^{d}
8	65.2^{g}	3.9^{b}	0.6^{efg}
9	77.4^{de}	3.9^{b}	0.7^{def}
10	77.3^{de}	3.0^{d}	1.0^{ab}
11	76.2^{de}	4.2^{a}	0.5^{g}
12	75.5^{e}	3.8^{b}	0.6^{fg}
13	71.4^{f}	3.5^{c}	1.0^{ab}
14	81.8^{abc}	3.9^{b}	0.5^{g}
15	69.9^{f}	3.5^{c}	0.6^{efg}
Average	76.8	3.6	0.8
Reference samples	72.8^{f}	3.5^{c}	0.7^{def}

*****Figures with different letters in the same column are significantly different ($P < 0.05$)

Microbiological Examination of Tabbouleh

Tabbouleh is distinguished from vegetable salads due to its high quantity of parsley (almost 40%) and bulgur, which reduces the moisture content; however, it can help the growth of some microorganisms as a rich source of nutrients. The high microbial population of tabbouleh reflects its suitability as a medium for microbial growth (its intrinsic factors) as well as the practices applied during preparation and handling. The counts for (APC, LAB, CC, and YAM) of tabbouleh samples are shown in Figure 1. Samples from the same producer and those from separate producers showed remarkable variances in counts.

In the majority of the samples, interval I counts were greater than interval II (Figure 1). Interval I samples were mostly collected and tested in October 2021, while interval II samples were mostly tested in December 2021. In Amman, Jordan, December temperature ranges from 5.9 to 14.8 °C, which is generally lower than in October ranges from 15.4 to 27.2 °C (JMD, 2022).

The lower temperature of December could contribute to the significantly lower numbers of APC.

Notably, the averages of interval I samples for APC, CC, and LAB were 5.5, 4.1, and 5.2 $\log_{10}$ CFU/g, respectively, while these counts were significantly lower in interval II samples (5.3, 3.5, and 4.1 $\log_{10}$ CFU/g). YAM was slightly lower in interval II samples as well, with averages of 5.7 $\log_{10}$ CFU/g compared to 6 $\log_{10}$ CFU/g in interval I samples (Table 3). These findings emphasize the impact of environmental temperature on the microbial load of RTE dishes like tabbouleh.

In comparison, reference tabbouleh samples had significantly lower microbial counts than the commercial samples. The APC, LAB, and YAM averages for reference samples were 2.7, 1.9, and 2.6 $\log_{10}$ CFU/g, respectively, with no coliforms or *Staphylococcus aureus* detected. These results illustrate the potential for improving the microbial quality of commercial tabbouleh products to meet food safety standards and avoid public health risks.

Table 3. Averages and ranges of aerobic plate count (APC) and counts of lactic acid bacteria (LAB), coliforms (CC), yeasts, and molds (YAM) of interval I (10 October -12 December) and II (19 December – 10 January) tabbouleh samples collected from different producers in Amman, Jordan.

Commercial tabbouleh samples				Reference samples
APC	Interval I	Range	3.3^{ef} - 7.2^{a} *	2.7^{e}
		Average	5.5	
	Interval II	Range	3.1^{gh} - 7.8^{a}	2.7^{h}
		Average	5.3	
CC	Interval I	Range	1.5^{gh} - 7.3^{a}	1.0^{h}
		Average	4.1	
	Interval II	Range	1.7^{de} - 6.6^{a}	1.0^{e}
		Average	3.5	
LAB	Interval I	Range	2.9^{hi} - 7.4^{a}	1.9^{i}
		Average	5.2	
	Interval II	Range	2.2^{ef} - 5.8^{a}	1.9^{f}
		Average	4.1	
YAM	Interval I	Range	3.4^{cd} – 8^{a}	2.6^{d}
		Average	6	
	Interval II	Range	3.8^{bc} – 7^{a}	2.6^{c}
		Average	5.7	

*Count expressed as $\log_{10}$ CFU/g, figures with different letters in the same row are significantly different ($P < 0.05$)

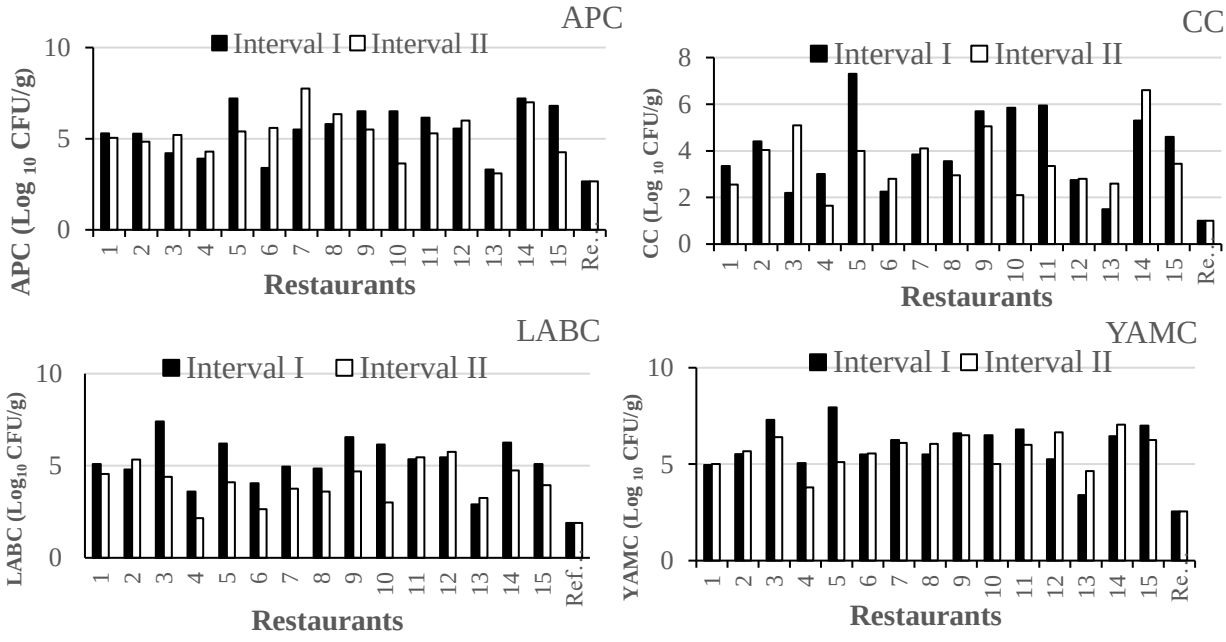

Figure 1. Aerobic plate count (APC) and counts of coliforms (CC), lactic acid bacteria (LAB), and yeasts and molds (YAM) of tabbouleh samples were collected at two intervals from different retailers in Amman, Jordan. Bars with different lowercase letters are significantly different ($P < 0.05$)

Aerobic Plate Count

APC of tabbouleh samples of interval I ranged from 3.3 to 7.2 $\log_{10}$ CFU/g with an average of 5.5 $\log_{10}$ CFU/g; in interval II this count ranged from 3.1 to 7.8 $\log_{10}$ CFU/g with an average of 5.3 $\log_{10}$ CFU/g. In both intervals, APC ranged from 3.1 to 7.8 $\log_{10}$ CFU/g, with an average of 5.4 $\log_{10}$ CFU/g. Notably, the APC values of most of the collected samples were higher in interval I than in interval II. In comparison, the reference tabbouleh sample prepared in our laboratory had an APC value of only 2.7 $\log_{10}$ CFU/g.

APC values are used to assess the presence of microorganisms in a food product and are one of the microbiological indices and markers for food quality (Maturin and Peeler, 2021; Aycicek *et al*., 2006; Halablab *et al*., 2011). High APC loads may indicate that samples were handled improperly from the farm to the consumer. These organisms reflect the food hygiene practices, especially during preparation and handling, and show whether the product was exposed to any contaminants in general and whether there were favorable circumstances for the growth of microorganisms. Furthermore, APC reflects environmental controls, especially refrigeration. So, this measure helps determine if temperature control, cleanliness, and disinfection were adequately handled throughout industrial processing, transportation, and storage (Saranraj *et al*., 2012).

The results of this study demonstrate the importance of monitoring and controlling APC values in RTE products like tabbouleh. These values can help determine whether adequate hygiene measures were taken during the processing, transportation, and storage of the product. High APC values, as observed in this study, indicate a need for increased hygiene measures and the consumption of hygienic food products on a global scale. Similar findings have been reported in other studies about high APC in vegetable salads (Table 4). Furthermore, the comparison between the collected and reference tabbouleh samples demonstrates the need for constant quality control in food processing and preparation to ensure consumer safety and satisfaction

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Table 4. Aerobic plate count (APC) of vegetable salads from different countries.

Country	**APC***		**Type of vegetable salad**	**Reference**
	Range	**Average**		
Egypt	2.0 to 10.4 3.8 to 9.4 2.7 to 5.9	7.08 7.7 3.8	fresh vegetable samples RTE salad samples from restaurants RTES samples purchased from street vendors	(Abaza, 2018)
Ghana	1.0 to 4.7		mixed vegetable salad	(Annor-Gyamfi *et al*., 2020)
Greece	5.1 to 9.8	7.73	Ready-to-eat salads	(Xylia *et al*., 2019).
Iran	5.5 to 7.4 5.3 to 7.5		Ready-to-eat salads Fresh-cut vegetables	(Jeddi *et al*., 2014)
Iran (Mashhad)	4.1 to 8.3 4.3 to 8.3	7 - 8	Mixed fresh-cut salads Mixed green leaves vegetables	(Najafi & Bahreini, 2012)
Italy	4.0 to 8.7		Ready-to-eat vegetable salads	(Caponigro *et al*., 2010)
Italy	6.3 to 6.7	6.5	RTE mixed salad	(Calonico *et al*., 2019)
Korea	7.1 to 9.5	8	Lettuce samples	Tango *et al*., 2018
Korea (Seoul)	5.4 to 8.9		Mixed salads	(Seo *et al*., 2010).

Nigeria	5.5 to 8.8 6.2 to 8.2 3.50 to 7.7		Ready to eat leafy vegetables Vegetable samples Mixed RTES	(Abdullahi and Abdulkareem., 2010) (Itohan *et al*., 2011). (Agu, KC *et al*., 2014)
Pakistan	3 to 8.8		Salad samples	(Hannan *et al*., 2014)
Palestine	3 to 6		Vegetable salad	(Abu Owda, 2014).
Poland	2.4 to 9.3 5.6 to 7.6		30 types of salads Leafy vegetables and their mixes	Łepecka *et al*., 2022) (Berthold-Pluta *et al*., 2017)
Saudi Arabia (Riyadh)	2.8 to 6.4 5 to 5.7	 5.3	Fresh-cut produce and salads Vegetable salads	(Shahbaz et al., 2022). (Khiyami *et al*., 2011)
Serbia	5.8 to 7.1		Vegetable salads	(Cvetković *et al*., 2018).
Singapore	5.8 to 7.3	6.5	Fresh-cut salads	Seow *et al*., 2012
Spain (Catalonia)	4.3 to 8.9		Fresh-cut vegetables	(Abadias *et al*., 2008).
Sudan	5.1 to 7.4		Vegetable samples	(Goja *et al*., 2013).
Turkey	3.3 to7.4 6.3 to 7.6 3.0 to 6.0 3.0 to 7.0 6.0 to 7.0		Raw eaten vegetables Leafy green vegetables Russian salad Sezar salad Mediterranean salad	(Aycicek *et al*., 2006). (Erkan *et al*., 2008). (PAMUK *et al*., 2013)
UAE	5.08		Tabbouleh salad	(Al-Mualla, 2009)

*Microbial count is expressed as Log_{10} CFU/g.

Coliforms Count

The Coliform count (CC) of the tabbouleh samples collected during intervals I and II showed a range of 1.5 to 7.3 log_{10} CFU/g, with an average of 4.1 log_{10} CFU/g and 1.7 to 6.6 log_{10} CFU/g, with an average of 3.5 log_{10} CFU/g, respectively. In most of the samples, the count was higher in interval I than in interval II, and this is possibly because of the relatively high environmental temperatures during interval I compared to interval II (Figure 1). It is important to note that the CC in the collected samples was relatively higher than the reference tabbouleh prepared in our laboratory, where coliforms were not detected (Table 3).

These findings emphasize the importance of monitoring coliform counts as an indicator of hygienic practices and sanitary quality in the food preparation and processing environment. This is consistent with other studies in the literature, as reported in Table 5.

Detection of coliforms is used as an indicator of sanitary quality of water or as a general indicator of sanitary conditions in the food-processing environment (BAM, 2020). Coliforms are commonly used as an indicator of the sanitary quality of foods and water and are considered an indicator of fecal pollution (Saranraj *et al*., 2012). As members of the family *Enterobacteriaceae*, coliforms indicate the possible presence of other enteropathogenic bacteria, such as *Salmonella* as well as viruses and parasites.

The high CC found in this study for tabbouleh salad may be linked to the use of residential sewage or polluted water to irrigate the vegetables in the fields, contaminated water used to clean equipment and cutting/slicing machines leading to cross-contamination as well as handlers not practicing proper sanitation and personal hygiene (Hosein *et al*., 2008) and untreated animal manures, which are frequently used as a fertilizer, as well as contaminated handlers and equipment.

Parsley, being a leafy vegetable used in large amounts in tabbouleh, is especially prone to microbial contamination and requires extra attention in terms of cleaning and disinfection. The comparison between the collected samples and the reference samples further highlights the urgent need for improved hygienic practices and sanitation measures in the food processing and preparation environment.

Table 5. Coliforms count (CC) of vegetable salads from different countries

Country	CC*		Vegetable salad	Reference
	Range	Average		
Greece	1.38 to 7.05		Ready-to-eat salads	(Xylia et al., 2019).
Ghana	<1 to 1.90		Mixed vegetable salad	(Annor-Gyamfi *et al*., 2020
Iran	1.9 to 6 up to 5.5		Ready-to-eat salads Fresh-cut vegetables	(Jeddi *et al*., 2014)
Iran (Mashhad)	3.9 to 7.48 3.2 to 7	5 to 6	Mixed fresh-cut salads Mixed green leaves vegetables	(Najafi & Bahreini, 2012)
Korea (Seoul)	2.2 and 7.9 2.7 to 8.2	4.21	Lettuce samples Mixed salads	(Tango *et al*., 2018) (Seo *et al*., 2010).
Lebanon	2.0 to 10.71		Fresh vegetable samples	(Halablab *et al*., 2011).
Nigeria	5.9 to 9.1 2.60 to 6.73		Ready to eat leafy vegetables Mixed ready-to-eat vegetable salads	(Abdullahi and Abdulkareem., 2010) (Agu, KC *et al*., 2014)
Palestine	2.3 to 6.5		Vegetable salad	(Abu Owda, 2014).
Saudi Arabia (Riyadh)	ND to 4.3 4.3 to 4.9		fresh-cut produce and salads Vegetable salads	(Shahbaz *et al*., 2022) (Khiyami *et al*., 2011).
Singapore	4.2 to 5.9	5.2	Fresh-cut salads	(Seow et al., 2012)
Turkey	3.0 to 6.9 3.2 to 5.9		Vegetable samples Leafy green vegetables	(Aycicek *et al*., 2006). (Erkan *et al*., 2008).

*Microbial count is expressed as Log_{10} CFU/g

Lactic Acid Bacteria Count

The LAB count in the tabbouleh samples collected during the interval I ranged between 2.9 to 7.4 log_{10} CFU/g with an average count of 5.2 log_{10} CFU/g. On the other hand, during interval II, the counts ranged between 2.2 to 5.8 log_{10} CFU/g with an average count of 4.1 log_{10} CFU/g. The LAB ranged from 2.2 to 7.4 log_{10} CFU/g during both intervals, indicating an average count of 4.7 log_{10} CFU/g. Similar findings had been reported in other studies were shown in Table 6. In most of the samples, the LAB count was higher in interval I compared to interval II. These findings were relatively higher than the reference tabbouleh sample that was prepared in our laboratory for comparison purposes, which showed a

count of 1.9 $\log_{10}$ CFU/g. This confirms that the collected samples were not processed under sanitary conditions and were mishandled, especially in terms of cleaning and disinfecting of utensils and apparatus used in the preparation, and also the lack of proper refrigeration.

Moreover, the intrinsic conditions of the tabbouleh, such as the relatively low pH and the availability of readily available carbon sources from the ingredients, might have contributed to the growth of LAB. It has been observed in other studies that several Gram-positive bacteria, particularly LAB can spoil fresh-cut fruits and vegetables that are packaged under a modified atmosphere with <2% and >10% CO_2 and stored at 7°C or above, irrespective of the produce.

It is noteworthy that some of these LABs have inhibitory effects on the growth of certain foodborne pathogens such as *S. aureus* (Abu Owda., 2014). Therefore, the rapid growth of LABs in tabbouleh could outcompete *S. aureus* for nutrients necessary for growth. Additionally, the development of inhibitory chemicals that could go along with the growth of LAB might prevent the growth of other microorganisms.

Overall, the comparison between the collected samples and reference samples highlights the importance of maintaining good hygiene practices during food preparation to prevent the growth of microorganisms that could compromise food safety and quality.

Table 6. Lactic acid bacteria (LAB) count of vegetable salads from different countries.

Country	**LAB**		**Vegetable salad**	**Reference**
	Range	**Average**		
Greece	1.48 to 7.39		Ready-to-eat salads	(Xylia *et al*., 2019).
Iran (Mashhad)	1 to 7.3 less than 5		mixed fresh-cut salads mixed green leaves vegetables	(Najafi & Bahreini, 2012)
Poland	< 1 to 8.43		30 types of salads	(Łepecka *et al*., 2022)
Spain (Catalonia)	< 5		Fresh-cut vegetables	(Abadias *et al*., 2008).

*Microbial count is expressed as Log_{10} CFU/g.

***Staphylococcus aureus* Count**

S. aureus is particularly susceptible to being destroyed by heat treatment and practically all sanitizing chemicals (Tallent *et al*., 2019). But tabbouleh is a product that doesn't receive any kind of heat processing and the pathogen comes mainly from the unhygienic practices of the food handlers. In light of this, the presence of this bacterium or its enterotoxins in prepared meals or on food processing machinery is often a sign of inadequate personal hygiene since the bacterium is indigenous to human skin and nasal cavities. In this study, the absence of *S. aureus* (< 10 $\log_{10}$ CFU/ g), in all tabbouleh samples can be explained by the fact that *S. aureus* is sensitive to the low pH (the minimum pH for growth of *S. aureus* is 4.2 (Tang *et al*., 2015). So, the low pH of tabbouleh, < 4 (Table 1) would inhibit the growth of *S. aureus*. The growth is further inhibited by the fast-growing LAB since *S. aureus* is known as being a poorly competing microorganism. This is supported by the findings of Olaimat *et al*., 2021, which found that tomato-cucumber salad, absent of any added ingredients, facilitated the growth of *S. aureus*, though the populace decreased gradually. The analysis exposed that *S. aureus* flourished in cucumber, but when combined with tomato, the latter's pH level decreased, which made it easier for *S. aureus* to proliferate. Furthermore, integrating lemon juice and salt into the tomato-cucumber salad reduced the initial pH value of the salad. Consequently, the growth of foodborne microorganisms was inhibited, and its population significantly lowered at higher temperatures. Moreover,

the addition of NaCl and lemon juice in the same concentration proved to be an effective inhibitor against foodborne microorganisms within the tomato-cucumber salad.

Yeasts and Molds Count

The YAM count of the tabbouleh samples in interval I ranged from 3.4 to 8.0 $\log_{10}$ CFU/g, with an average of 6.0 $\log_{10}$ CFU/g. In comparison, in interval II, the results ranged from 3.8 to 7.0 $\log_{10}$ CFU/g, with an average of 5.7 $\log_{10}$ CFU/g. Overall, the YAM in both intervals ranged from 3.4 to 8.0 $\log_{10}$ CFU/g, with an average of 5.9 $\log_{10}$ CFU/g.

It is worth noting that the majority of samples had higher counts in interval I compared to interval II. These results were notably higher than the reference tabbouleh prepared in our laboratory as a control, which had a count of only 2.6 $\log_{10}$ CFU/g. Other studies have also reported comparable findings, as shown in Table 7.

Tabbouleh has been identified as an excellent growth medium for yeasts, as these microorganisms are less nutritionally demanding than bacteria and are more tolerant of low pH and other antimicrobial substances produced by LAB (Yamani & Dababseh, 1994). However, the longer generation time of yeasts could explain their relatively low counts in tabbouleh. It is noteworthy that no pathogenic yeasts have been associated with food thus far.

In summary, the collected tabbouleh samples had significantly higher YAM counts compared to the reference sample, indicating potential contamination that could pose a health risk. Therefore, it is essential to implement effective food safety measures to minimize the risk of yeast contamination in tabbouleh.

Table 7. Yeasts and molds (YAM) count of vegetable salads from different countries.

Country	**YAM**		**Vegetable salad**	**Reference**
	Range	**Average**		
Greece	1.63 to 6.68		Ready-to-eat salads	(Xylia *et al*., 2019).
Ghana	ND to 2.48		Mixed vegetable salad	(Annor-Gyamfi *et al*., 2020)
Iran	6.2 to 7.5		Ready-to-eat salads	(Jeddi *et al*., 2014)
Iran (Mashhad)	3.85 1 to 6.7 2.04 to 6.6	5 to 6	Mixed fresh-cut salads Mixed green leaves vegetables	(Najafi & Bahreini, 2012)
Italy	1.8 to 4.2	3.6	RTE mixed salad	(Calonico *et al*., 2019)
Korea (Seoul)	> 5 2.2 to 7.5	6.2	Lettuce samples Mixed salads	Tango *et al*., 2018 (Seo *et al*., 2010)
Poland	< 1 to 7		30 types of salads	Łepecka *et al*., 2022)
Serbia	4.6 to 5.9		Vegetable salads	(Cvetković *et al*., 2018).
Singapore	3.5 to 4.9	4.3	Fresh-cut salads	Seow *et al*., 2012
Spain (Catalonia)	2.0 to 7.8		Fresh-cut vegetables	(Abadias *et al*., 2008).

*Microbial count is expressed as Log_{10} CFU/g.

In the present study, the high range of total microbial flora poses a potential risk of infection to consumers, especially because tabbouleh is an RTE product. According to Olaimat *et al*., (2019), the examination

indicated that the bacterial populations present in salads with tomato, cucumber, and parsley were more substantial than those in salads with lettuce and onion. These elevated microbial counts of the different microbial categories tested in the current study were most likely because tabbouleh salad undergoes several preparation steps such as washing, slicing, chopping, and mixing more than one type of vegetable, which may lead to cross-contamination. These variations might be also attributed to poor adherence of staff to sanitary rules.

Further comparisons between the collected samples and reference samples could provide additional insights into the potential sources of contamination and means for improving food safety. In particular, observations from fine restaurants, where staff are more diligent about wearing gloves and minimizing the time between preparation and serving, could inform best practices for reducing microbial levels in tabbouleh and other ready-to-eat products.

Conclusions

Locally produced traditional tabbouleh has a relatively high microbial load. This is an indication of the unsanitary conditions and procedures used during manufacturing. To have tabbouleh of a satisfactory microbiological quality, it should be mandatory to apply good food hygiene practices. Much attention should be paid to the quality of vegetables used in tabbouleh preparation, parsley, mint, and tomatoes, which should be evaluated since they may play a significant role as a carrier for foodborne diseases.

Following careful personal hygiene, washing, and disinfection methods, as well as keeping the tabbouleh refrigerated until eating, should result in a product of acceptable microbiological quality. Preparing tabbouleh just when needed and storing it in separate containers may also help to reduce microbial populations at the time of consumption.

Future research should focus on identifying the specific pathogens present in traditional tabbouleh and their potential impact on human health. Additionally, studies could investigate the effectiveness of different food hygiene practices and interventions in reducing microbial loads in tabbouleh. Finally, exploring the nutritional and sensory quality of tabbouleh made with alternative ingredients and preparation methods could offer insights to improve the safety and appeal of this popular Middle Eastern dish.

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Conflicts of Interest

We have no conflicts of interest to disclose.

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الجودة الميكروبيولوجية للتبولة، سلطة خضروات تعتمد أساسا على البقدونس، المنتجة تجارياً في الأردن

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ملخص

تمت دراسة جودة 60 عينة تبولة، وهي سلطة شرقية شهيرة، من 15 مطعما في عمان، الأردن؛ بحيث أخذت عينتين من كل مطعم على فترتين ((أكتوبر (الفترة الأولى) وديسمبر (الفترة الثانية)). كما تم تحضير عينتين مرجعيتين من التبولة في ظل ظروف صحية، واختبارهما للمقارنة. تراوح محتوى الرطوبة في العينات من 65.2 إلى 85٪ بمتوسط 76.8٪، وتراوح الحموضة من 3 إلى 4.2 بمتوسط 3.5 وبينما تراوحت الحموضة بين 0.5 إلى 1.1٪ بمتوسط قدره 0.8 ٪. تسمح هذه الظروف الداخلية بنمو العديد من الأحياء الدقيقة. خلال الفترة الأولى كانت متوسطات عدد الطبق الهوائي، أعداد بكتيريا القولون، بكتيريا حمض اللاكتيك و الخمائر (5.5، 4.1، و 5.2، 6 لوغريتم وحدة مكونة للمستعرات/غرام) على التوالي ؛ بينما كانت خلال الفترة الثانية (5,3, 3,5 و 4,1 و5.7 لوغريتم وحدة مكونة للمستعرات/ غرام)، مع عدد قليل من المكورات العنقودية الذهبية (< 1 لوغريتم وحدة مكونة للمستعرات/ غرام) . كانت العينات المرجعية لديها عدد أقل بكثير من عدد الطبق الهوائي، أعداد بكتيريا القولون، بكتيريا حمض اللاكتيك و الخمائر الذي بلغ (2.7، < 10 ،1.9 و 2.6 لوغريتم وحدة مكونة للمستعرات/ غرام) على التوالي. مما يشير إلى أن التبولة التجارية يتم إعدادها عادةً دون اتباع إجراءات النظافة الصحيحة .كانت أعداد جميع الكائنات الحية الدقيقة أعلى بشكل ملحوظ في العينات في الفترة الأولى مقارنة بالفترة الثانية، مما يشير إلى أن جودة التبولة تدهورت مع مرور الوقت بسبب درجات حرارة أعلى نسبيًا. تسلط هذه الدراسة الضوء على أهمية ممارسات النظافة الصحيحة أثناء إعداد التبولة وضرورة مراقبة جودتها البكتيريولوجية بانتظام.

الكلمات الدالة: التبولة، سلطة شرقية، النظافة الصحيحة، الجودة الميكربيولوجية

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