

Investigation of Microbiological Quality of Cold Salads Sold in Markets in İstanbul

Mohammed Hachim Nasser¹, Ihab Mohamed Taher²

¹Al - Muthanna Governorate

²Al - Muthanna Health Department

Email: Aabbass12212[at]gmail.com
ehabmuhammed8554[at]gmail.com

Abstract: The aim of the study is to assess the microbial contamination of the cold vegetable salad that is sold to consumers by the supermarket in some regions of İstanbul, many examinations in various nations decide the microbiological nature of cold salad. Tests analyzed in the current examination included various kinds of new cold vegetables salad. A sum of 100 random examples were gathered from İstanbul market. All microbiological assessments were done at the Medical Laboratory Techniques, Altınbaş University. In our study, it was seen that as 65 percent of the cold salad models sold in a supermarket in İstanbul are contaminated with the bacteria that was diagnosed, the rate disseminated as follow; 3% (3) with total Coliform microorganisms, 1% (1) with *E. coli*, 62% (62) with *S. aureus*, and 1% (1) *Salmonella spp.* In this study, the bacterial quality results revealed a relationship between the percentage of contamination causes, the type of cold salad samples and the regions of sample collection sold to consumers in İstanbul markets, where the tables indicated a high bacterial burden and the presence of pathogenic microbes, especially *S. aureus* and *Salmonella spp.* and *E. coli* in a dish of examples of cold vegetable salad. As a conclusion of the findings of this study, it is believed that the hygienic processing of cold salads within the framework of "good manufacturing practices" (GMP) and effective HACCP practices is important in terms of reducing microbiological risks.

Keywords: Cold salad, İstanbul, Bacterial contamination, ready to eat

1. Introduction

Salad dishes are cold prepared to - eat (RTE) dishes that regularly contain crude cuts of vegetables along with other cooked and smoked fixings. As plates of mixed greens contain enormous parts of crude fixings and their arrangement includes broad taking care of cycles (Hannan, et al., 2014). Vegetables act as a significant piece of our daily food supply (Rahman and Noor, 2012). Salad is a general name for a variety of foods numerous food arrangements which have combination of cut fixings which might be generally natural products or veggies. Common salad ingredients include of olives, tomatoes, cucumbers, peppers, onions, red onions, carrot, lettuce, spring onions, and radishes (Osamwonyi et al., 2013). It is well known that vegetables are abundant in nutrients, iron, calcium, proteins, fats also minerals, dietary strands then different supplements universal flavonoids, carotenoids and phenolic intensifies that might bring down the gamble of malignant growth, coronary illness and different sicknesses (Osamwonyi et al., 2013). Crude vegetables contain various pathogenic microorganisms, that might be scattered above the vegetation or show up as micro colonies implanted inside the plant's cells (Rahman and Noor, 2012). During gathering and transit crude vegetables might be wounded bringing about the arrival of plant supplements, and in this manner, giving substrates to microorganisms' existent on the outer layer of the veggies to grow. Also, the handling of new veggies salad might adjust or expand the numbers also sort of microbe's existent on the outer layer of the item (Rahman and Noor, 2012). With a perspective on such openness to microbes, vegetables have been linked to outbreaks of foodborne illness in numerous nations. Food plant diseases can mainly be caused essentially by microorganisms as well as their toxins. Vegetables

development may largely represent such a pathogen. Some of the pathogenic bacteria found in fertilizers include *Salmonella*, *Escherichia coli O157: H7*, *Bacillus anthracis*, *Mycobacterium spp.*, *Brucella spp.*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Klebsiella spp.*, and *Mycobacterium paratuberculosis*. Therefore, the use of natural manures in agriculture represents a remarkable risk with potentially enormous benefits for human health (Rahman and Noor, 2012).

2. Material and Methods

Source and Number of Tests

Test analyzed in the current examination included various kinds of new cold vegetables salad (prepared to - eat servings of mixed greens) involve 50 packaged and 50 non packaged samples. A sum of 100 irregular samples were gathered from markets containers and various market in İstanbul governorates. The review was completed over a time of 35 days began 20 April 2022 to 25 June 2022.

Sample Assortment

All samples were weighed (300 - 400g) appropriately distinguished and marked, put independently in a clean plastic pack and shipped to the research facility in a cooler inside 2 - 4 hours of assortment, at which location ready for bacteriological assessment (Uzeh et al., 2009). Test type, source also other significant information was recorded for each example. Each test was completed at Medical Laboratory Techniques, Altınbaş University.

Sample preparation

Using a stomacher in the lab, we weighed 25 grams of cold vegetable salad, added 225 milliliters of peptone water at a

concentration of 0.1 percent, and mixed the ingredients for two minutes. Under aseptic conditions, ten - fold weakening's were made from every sample utilizing (0.1%) peptone water as diluents; this was followed by consecutive weakness making 10 - 1 and roger weakening's (Andrews et al 2001).

Microbiological Examination

Coagulase positive *staphylococci*

- Inoculate 1 ml of the suspension in the stomacher bag into Baird Parker - RPF agar medium. Inoculate as quickly as possible to the surface of the medium plate, using the loop, without touching the edges of the medium plate.
- Allow the medium plates to dry for approximately 15 minutes at laboratory temperature, with the lids closed and on the upper side.
- Invert the medium plates, incubate at 37°C and incubate for 24±2 hours. After this time (24 ± 2), mark plates with any typical colonies from the bottom.
- There is an opaque zone around the characteristic coagulase - positive *Staphylococcus* colonies. Colonies are usually gray - black in color. Count all colonies and determine the ratio by comparing with the test sample to determine the number of coagulase positive *staphylococci* per gram of food.

Escherichia coli and *Coliform*

Using Chrome ID Coli agar we have Prepared for *E. coli*

- 1 ml of the suspension in the stomacher bag is placed in sterile petri dishes.
- Add 20 ml of the Chrome ID Coli agar medium described in section "A" to the Petri dish. The Petri dish is stirred horizontally.
- The medium plates are left with their lids closed and on the upper side, at laboratory temperature for approximately 15 minutes.
- Invert the medium plates, place them in an oven at 44 °C and incubate for 18 - 24 hours. Care should be taken that this period does not exceed 24 hours and that the incubation temperature does not exceed 45 °C. then the typical colonies are counted.
- Other *coliforms* form colonies 0.5 to 2 mm in diameter and are blue/blue - gray in color.
- The total number of colonies is obtained by counting the pink and purple colonies and all the blue/blue - gray colored colonies.

Salmonella spp.

- Pour the main suspension (225 ml non - selective pre - enrichment=BPW+ 25 g salad) in sterile glass jar, close the bottle. Incubate this jar in an oven at 37°C for 18 hours.
- After this time, take 1 ml of the main suspension and add to 10 ml of MKTT medium and incubate at 37°C for 24 hours.
- At the end of this period, extract a loop of MKTT medium and culture on XLD agar medium. Incubate XLD agar for 24 h ± 3 h at 37°C.
- After this time, mark colonies that may have *Salmonella*. The presence of pink to red colonies (typical colonies) on XLD agar medium, with or without a black center,

provides a very high probability of distinguishing *Salmonella*.

- Biochemical confirmation (one) is made from colonies grown on the Urea medium.
- The slant of the Urea Agar medium in the tube is scratched and incubated for 24 hours ± 3 hours at 37 °C ± 1 °C and examined at intervals.
- All strains of *Salmonella spp.* are negative for Urea medium. In other words, the color of the medium does not change when *Salmonella* is positive.
- Biochemical confirmation (two) is made from colonies grown on the (TSI) Agar.
- TSI Agar is utilized for the hypothetical ID of *Salmonella* in light of the maturation of glucose, lactose, sucrose and the creation of gas and H₂S.

3. Results

The information presented in this part is the primary outcome of the data and the results of the statistical analysis of microbiological tests of cold vegetable salad samples sold in the markets that were collected from 11 region in Istanbul. 100 samples of cold vegetable salad were collected and analyzed during the period which continued the practical part of the study. The aim of examining the samples was to assess the microbiological quality of the salad that is sold to the consumer. Different kinds of media were used as Baird Parker Agar for *Staph. aureus*, XLD agar for *Salmonella spp.*, Chrome ID Coli agar for *E. Coli* and *Coliform* for count and disconnection different kinds of microbes from cold greens salad tests.

S. aureus After 24 Hr.

There is an opaque zone around *S. aureus* states on Baird - Parker agar, regular colonies were generally as dark as displayed in figure (1).



Figure 1: Appearance of *S. aureus* colonies on Baird - Parker agar

Number of *S. aureus* - Positive Samples According to Regions after 24 hr.

The number of samples in which bacteria colonies appeared (3) 3% of all samples tested after (24 hr) incubation period, is much less than the results after 48 hours incubation period 62% of the samples. with *S. aureus* >10³ cfu/ml in 10% of samples (Table 1) this result means that there is critical pollution of salad contents.

Table 1: Number of *S. aureus* positive samples according to regions after 24 - hr.

Regions	Number of sample	10 ⁰ - 10 ¹ cfu/ml	10 ¹ - 10 ² cfu/ml	10 ² - 10 ³ cfu/ml	>10 ³ cfu/ml
Avcılar	10	-	-	-	-
Bakırköy	10	-	-	-	-
Ataköy	8	-	-	-	-
Bahçelivler	10	-	-	-	-
Şirinevler	8	-	-	-	-
Esenyurt	11	-	-	1 (9.09%)	-
Sefaköy	10	-	-	1 (10%)	1 (10%)
Kadıköy	9	-	-	-	-
Cennet Mah.	7	-	-	-	-
Beylikdüzü	10	-	-	-	-
Kağıthane	7	-	-	-	-

Distribution and count of *S. aureus* Colonies in Different Types of Salad after 24 hr.

The distribution of *Staphylococcus aureus* in different types of salad was investigated. The number of *S. aureus* colonies

was counted in different types of salads. It was significantly higher in number of colonies in Yogurt eggplant salad (4000 cfu/ml) and lowest in Olive and Şaksuka salads (170 cfu/ml) (Table 2).

Table 2: Distribution of *S. aureus* colonies in different types of salad 24 hr.

Salads type	Cfu/ml											
	0	0	0	0	0	0	0	0	0	0	0	0
Rus salad	0	0	0	0	0	0	0	0	0	0	0	0
Eggplant salad	0	0	0	0	0	0	0	0	0	0	0	0
Amerikan salad	0	0	0	0	0	0	0	0	0	0	0	0
Olive salad	0	0	0	170	0	0	0	0	0	0	0	0
Italyan salad	0	0	0	0	0	0	0	0	0	0	0	0
Haydari salad	0	0	0	0	0	0	0	0	0	0	0	0
Yogurt and roast hot pepper salad	0	0	0	0	0	0	0	0	0	0	0	0
Yogurt eggplant salad	0	0	0	0	4000	0	0	0	0	0	0	0
Şaksuka salad	0	170	0	0	0	0	0	0	0	0	0	0
Carrot salad	0	0	0	0	0	0	0	0	0	0	0	0

Salmonella spp. Results

Cold salad samples were culture on XLD agar plates were incubated at 37 °C for 24 ± 3 h, the presence of pink to red colonies with or without a black center provides a very high probability of distinguishing *Salmonella* in figure (2a) and figure (2b).

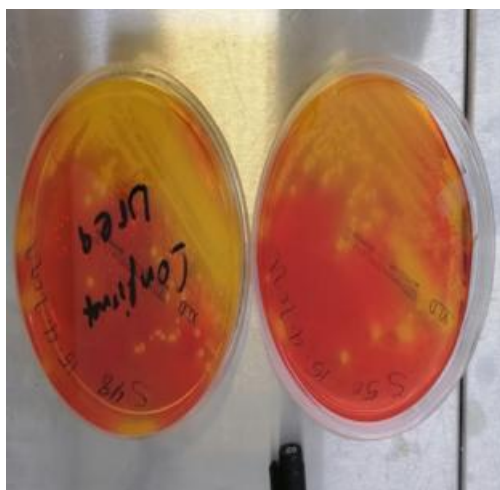


Figure 2a: Appearance of *Salmonella Spp.*

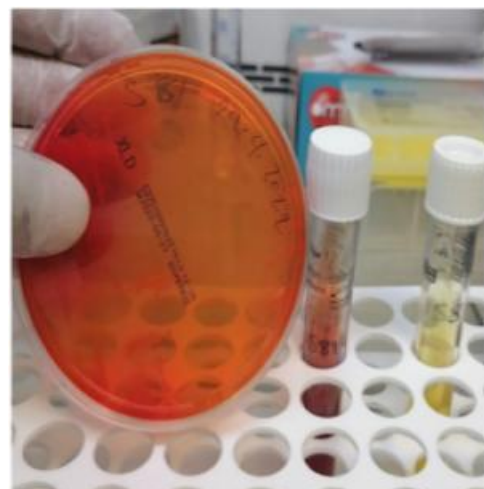


Figure 2b: Appearance of *Salmonella* colonies on XLD Agar colonies on XLD Agar

Salmonella spp was detected in Amerikan salad sample (1 sample) in all samples in Avcilar region, the frequency was 1% in our study, as shown in Table (3), (4), (5). To confirm, a urea test was performed to confirm the results. The urea test was also positive, a TSI agar test was performed to confirm the results, and the test was positive.

Table 3: Number of *Salmonella spp.* colonies by salad type

Varieties of salads	Total number of samples	Number of positive samples	10 ⁰ - 10 ¹ cfu/ml	10 ¹ - 10 ² cfu/ml	10 ² - 10 ³ cfu/ml	>10 ³ cfu/ml
Rus salad	12	-	-	-	-	-
Eggplant salad	10	-	-	-	-	-
Amerikan salad	10	1 (10%)	-	-	Present	-
Olive salad	11	-	-	-	-	-
Italyan salad	10	-	-	-	-	-
Haydari salad	9	-	-	-	-	-
Yogurt roast hot pepper salad	9	-	-	-	-	-
Yogurt eggplant salad	9	-	-	-	-	-
Şaksuka salad	10	-	-	-	-	-
Carrot salad	10	-	-	-	-	-

Table 4: Number of *Salmonella Spp.* colonies by region

Regions	Number of sample	10 ⁰ - 10 ¹ cfu/ml	10 ¹ - 10 ² cfu/ml	10 ² - 10 ³ cfu/ml	>10 ³ cfu/ml
Avcılar	10	-	-	Present	-
Bakırköy	10	-	-	-	-
Ataköy	8	-	-	-	-
Bahçelivler	10	-	-	-	-
Şirinevler	8	-	-	-	-
Esenyurt	11	-	-	-	-
Sefaköy	10	-	-	-	-
Kadıköy	9	-	-	-	-
Cennet Mah.	7	-	-	-	-
Beylikdüzü	10	-	-	-	-
Kağıthane	7	-	-	-	-

Table 5: Distribution of *Salmonella* colonies in different types of salad

Salads type	Cfu/ml										
	0	0	0	0	0	0	0	0	0	0	0
Rus salad	0	0	0	0	0	0	0	0	0	0	0
Eggplant salad	0	0	0	0	0	0	0	0	0	0	0
Amerikan salad	0	Present	0	0	0	0	0	0	0	0	0
Olive salad	0	0	0	0	0	0	0	0	0	0	0
Italyan salad	0	0	0	0	0	0	0	0	0	0	0
Haydari salad	0	0	0	0	0	0	0	0	0	0	0
Yogurt roast hot pepper salad	0	0	0	0	0	0	0	0	0	0	0
Yogurt eggplant salad	0	0	0	0	0	0	0	0	0	0	0
Şaksuka salad	0	0	0	0	0	0	0	0	0	0	0
Carrot salad	0	0	0	0	0	0	0	0	0	0	0

E. coli Results

E. Coli bacteria culture on Chrome ID Agar after incubation period for (18 - 24 hr.) at 44° C, as displayed in figure (3) it have a diameter of 0.5 to 2 mm and are pink to purple in color.

E. coli was detected in Yogurt eggplant salad sample (1 sample) in all samples in Bakırköy region, the frequency was 1% in our study, as shown in Table (6, 7)

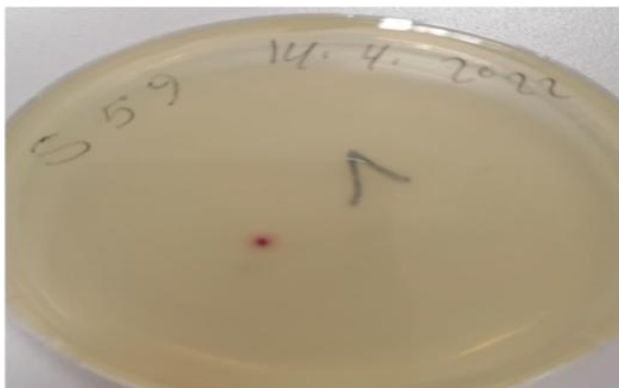


Figure 3: Appearance of total *E. Coli* microorganisms colon culture on Chrome ID agar

Table 6: *E. coli* ratio in different salad samples

Varieties of salads	Total number of samples	Number of positive samples	10 ⁰ - 10 ¹ cfu/ml	10 ¹ - 10 ² cfu/ml	10 ² - 10 ³ cfu/ml	>10 ³ cfu/ml
Rus salad	12	-	-	-	-	-
Eggplant salad	10	-	-	-	-	-
Amerikan salad	10	-	-	-	-	-
Olive salad	11	-	-	-	-	-
Italyan salad	10	-	-	-	-	-
Haydari salad	9	-	-	-	-	-
Yogurt roast hot pepper salad	9	-	-	-	-	-
Yogurt eggplant salad	9	1 (11.11%)	-	1 (11.11%)	-	-
Şaksuka salad	10	-	-	-	-	-
Carrot salad	10	-	-	-	-	-

Table 7: *E. coli* counts of salad samples according to regions in Istanbul.

Regions	Number of sample	10 ⁰ - 10 ¹ cfu/ml	10 ¹ - 10 ² cfu/ml	10 ² - 10 ³ cfu/ml	>10 ³ cfu/ml
Avcılar	10	-	-	-	-
Bakırköy	10	-	1 (10%)	-	-
Ataköy	8	-	-	-	-
Bahçelivler	10	-	-	-	-
Şirinevler	8	-	-	-	-
Esenyurt	11	-	-	-	-
Sefaköy	10	-	-	-	-
Kadıköy	9	-	-	-	-
Cennet Mah.	7	-	-	-	-
Beylikdüzü	10	-	-	-	-
Kağıthane	7	-	-	-	-

Coliform

Coliform colonies appeared in blue color in three different types of salad (3%) of the total samples that were examined. The highest number of colonies was in Yogurt eggplant authority, the result was (11.11%) as shown in Table (8),

while the highest percentage was recorded in Kagithane region (14.28 %) as in Table (9). Bacterial colonies multiplied by the dilution factor. While the distribution of Coliform colonies in different types of salad was 0 CFU/ml

Table 8: *Coliform* count in different types of salads

Varieties of salads	Total number of samples	Number of positive samples	10 ⁰ - 10 ¹ cfu/ml	10 ¹ - 10 ² cfu/ml	10 ² - 10 ³ cfu/ml	>10 ³ cfu/ml
Rus salad	12	-	-	-	-	-
Eggplant salad	10	-	-	-	-	-
Amerikan salad	10	-	-	-	-	-
Olive salad	11	-	-	-	-	-
Italyan salad	10	1 (10%)	-	-	1 (10%)	-
Haydari salad	9	-	-	-	-	-
Yogurt roast hot pepper salad	9	-	-	-	-	-
Yogurt eggplant salad	9	1 (11.11%)	-	-	1 (11.11%)	-
Şaksuka salad	10	1 (10%)	-	-	1 (10%)	-
Carrot salad	10	-	-	-	-	-

Table 9: Coliform counts of salad samples according to regions

Regions	Number of sample	10 ⁰ - 10 ¹ cfu/ml	10 ¹ - 10 ² cfu/ml	10 ² - 10 ³ cfu/ml	>10 ³ cfu/ml
Avcılar	10	-	-	-	-
Bakırköy	10	-	-	-	-
Ataköy	8	-	-	-	-
Bahçelivler	10	-	-	-	-
Şirinevler	8	-	-	1 (12.5)	-
Esenyurt	11	-	-	-	-
Sefaköy	10	-	-	1 (10%)	-
Kadıköy	9	-	-	-	-
Cennet Mah.	7	-	-	-	-
Beylikdüzü	10	-	-	-	-
Kağıthane	7	-	-	1 (14.28%)	-

4. Discussion

This investigation completed the bacteriological concept of a novel cold salad marketed on the Istanbul market, there is

little emphasis on the bacterial concept of cold veggies salad. In this research, eggplant, red pepper, garlic, olive, olive oil, corn, roasted eggplant, mayonnaise, egg yolk, basalia, potatoes, carrot, yogurt, cucumber, onion, tomato, were all

included in this study's cold vegetable salad. In our study, the percentage of examined samples that did not meet the criteria were observed as follows; 3% (3 samples) with *Coliform* bacteria, 1% (1 sample) with *E. coli*, 1% (1 sample) with *Salmonella spp.*, 62% (62 samples) with *S. aureus*. No visible signs of spoilage appeared despite obtaining high microbial contamination for some of the sample results in this study, thus external appearance may not be a good criterion for judging the microbial quality of vegetables. Therefore, before consumption, it is necessary to wash all vegetables well, whether by the processor or the consumer. This study showed that there was an interesting effect of incubation time on the increased contamination of cold salad samples prepared for eating with *Staphylococcus aureus* bacteria. The presence of cut surfaces provides a greater surface area for pollution and growth and permits microbial penetration of the tissues (Osamwonyi et al., 2013), and also releases a great deal of supplements filled fluids that are swiftly consumed by the associated microorganisms (Doyle and Erickson, 2008). This study found that the number of *S. aureus* colonies was counted in different types of salads after 24h from 100: 4000 cfu/ml in 3% (3/100 samples), the number of *S. aureus* colonies was counted in different types of salads after 48h from 10: 20000 cfu/ml in 62% (62/100 samples). This finding exceeds those of a review from India that identified *S. aureus* in 15.1% of a variety of mixed greens vegetables (Tambekar and Mundhada., 2006), and a study from the United Kingdom that found *S. aureus* in 2.7% of ready - to - eat salad vegetables (Meldrum et al., 2009). In Bangladesh, *S. aureus* was found in a range of 2.0×10^5 - 5.95×10^7 CFU/g across many tests (Rahman and Noor., 2012). The nasal regions of food controllers or infected specialists have been connected to the spread of *Staphylococcus aureus*, which has been shown to cause contamination (Tambekar and Mundhada., 2006). *Staphylococcus aureus* contamination of food is typically spread by supervisors, although it can also come through equipment and natural surfaces (Meldrum et al., 2009). Finding *S. aureus* in pre - packaged salad vegetables is a disturbing indication of a lack of cleanliness (Meldrum et al., 2009). *Salmonella spp.* were found to be low level 1% (1/100 samples) in this investigation as table (3, 4, 5), this is a serious health concern for consumers and researchers alike. There were three different types of *Salmonella spp.* found in samples of cold vegetable salad it was detected in 5.8 percent of salad vegetables in India (Tambekar and Mundhada., 2006), and in 6.7% of salad vegetables in Nigeria (also according to multiple studies) (Itohan et al., 2011). *Salmonella* was found in 0.7% (3/398) of tests in the United States (Johnston et al., 2005), and in 5.6% and 9.4% of blended new cut servings of mixed greens and blended green leaves vegetables, respectively, in two separate analyses conducted in Iran (Mohammad et al., 2012). Total *Coliform* levels in all new vegetable samples ranged from 200 to 1071 log₁₀ CFU/g, however a review from Lebanon reported significantly higher levels (Halablal et al., 2011). Furthermore, a review conducted in Nigeria indicated that Total *Coliform* 80000 to 130000 CFU/g in ready - to - eat green vegetables (Abdullahi and Abdulkareem., 2010). The presence of *E. coli* in water and food is widely recognized as a key indicator of the microbiological quality of both (Afolabi and Oloyede., 2010). It is possible that the compost on the ranch is the source of the *E. coli* that has been found in a wide range of foods (Nma and Oruese., 2013). At 132–168 days

after feces application, low amounts of *E. coli* were found in compost - amended soil (Ingham et al., 2004). Evidence of *E. coli* suggests that feces have recently contaminated the food, and that other intestinal microorganisms responsible for food - borne gastroenteritis and bacterial diarrhea may also be present (Nma and Oruese., 2013). the table (6, 7) showed *E. coli* was positive 1% (1/100 samples) in the all salad samples. It low than the number found in the UK study. *E. coli* was found in 1.5 percent (48/3200) of the ready - to - eat natural vegetable samples (Sagoo et al., 2001). Nonetheless, a second Turkish concentration showed that *E. coli* was present at concentrations between 10 and 3800 log₁₀ CFU/g (Aycicek et al., 2006). *E. coli* levels for all green vegetables and spices were below 200 to 3000 cfu/ml, as determined by a study done in the United States (Johnston et al., 2005).

5. Conclusion

A high number of microbes was obtained for some of the instances in this review, it is important to emphasize that none of the samples displayed obvious signs of deterioration, it follows that first impressions might not be a reliable basis for assessing the microbiological quality of a salad made from vegetables.

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