Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Mayıs/May 2022, 19(2)
Başvuru/Received: 14/09/21
Kabul/Accepted: 18/01/22
DOI: 10.33462/jotaf.994068

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Multi-criteria Decision-making Technique Approach to Assess the Microbial Quality and Safety of Fresh-cut Salads Sold at Retail in Istanbul, Turkey

İstanbul, Türkiye'de Perakende Olarak Satılan Taze Kesilmiş Salataların Mikrobiyal Kalitesini ve Güvenliğini Değerlendirmek İçin Çok Kriterli Karar Verme Tekniği Yaklaşımı

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Abstract

The goal of this study was to investigate the microbial quality and safety of fresh-cut salads sold in restaurants that were located at Esenler, Fatih, Besiktas, Uskudar, Kadikoy and Umraniye in Istanbul, Turkey. In total, 180 samples were assayed for microbiological analysis including enumeration of total aerobic mesophilic (TMAB), aerobic psychotropic (AP) bacteria, yeast and mold (YM), fecal coliforms bacteria, *Escherichia coli*, *Staphylococcus aureus*, isolation of *Salmonella* spp. and *Listeria monocytogenes*. Meanwhile, a coagulase test was conducted out for *S. aureus*. Technique for order preference by similarity to ideal solution (TOPSIS)-based multi-criteria decision-making technique (MCDM) approach was used to rank six districts in terms of microbial count. The enumeration of TMAB in Esenler, Fatih and Besiktas ranged from 4.34 to >7.48 log CFU g^{-1}, 3.69 to >7.48 log CFU g^{-1} and 4.05 to >7.48 log CFU g^{-1}, respectively. However, the enumeration of TMAB in Uskudar, Kadıkoy and Umraniye ranged from 2.57 to >7.48 log CFU g^{-1}, 4.01 to >7.48 log CFU g^{-1} and 4.24 to >7.48 log CFU g^{-1}, respectively. The enumeration of AP, YM and *S. aureus* ranged from 2.00 to >7.48 log CFU g^{-1}, 2.00 to >7.48 and 2.00 to 6.13 log CFU g^{-1}, respectively. Coagulase-positive staphylococci were found in 43 samples. When total coliforms counts were calculated for the samples, Esenler had the highest count (3588.1 MPN/g). The highest and lowest count of fecal coliform was found in Esenler and Fatih, respectively. *E. coli* was isolated 7.22% in samples. *Salmonella* spp. and *L. monocytogenes* were not found in any of the samples. According to TOPSIS, Esenler had very bad values of TMAB and AP, *S. aureus*, coliform and fecal coliform. As for YM, Fatih had the worst scores. Uskudar was found as the best district considering the count of all microbial groups. On the other hand, it has been discovered that the microbial safety of fresh-cut salads sold in Istanbul is adequate for consumption.

Keywords: Fresh-cut salads, Microbial quality, Microbial safety, Multi-criteria decision technique, TOPSIS

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Atıf/Citation: Ucak Ozkaya G., Gecgel U., Durak M.Z. . Multi-criteria Decision-making Technique Approach to Assess the Microbial Quality and Safety of Fresh-cut Salads Sold at Retail in Istanbul, Turkey *Tekirdağ Ziraat Fakültesi Dergisi,* 19 (2), 366-379.

*Bu çalışma Yüksek Lisans tezinden özetlenmiştir.

Öz

Bu çalışmanın amacı İstanbul, Türkiye'de Esenler, Fatih, Beşiktaş, Üsküdar, Kadıköy ve Ümraniye'de bulunan restoranlarda satılan taze kesilmiş salataların mikrobiyal kalite ve güvenliğini belirlemektir. Toplam aerobik mezofilik (TMAB), aerobik psikrotrofik (AP) bakteriler, maya ve küf (YM), fekal koliform bakterileri, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. ve *Listeria monocytogenes* izolasyonu dahil olmak üzere mikrobiyolojik analiz için toplam 180 numune test edilmiştir. Bu arada, *S. aureus* için koagulaz testi gerçekleştirilmiştir. Altı ilçeyi mikrobiyal sayı açısından sıralamak için çok kriterli karar verme tekniği (MCDM) yaklaşımına dayalı ideal çözüme benzerlik bakımından sıralama performansı tekniği (TOPSIS) kullanılmıştır. Esenler, Fatih ve Beşiktaş'ta TMAB sayımı sırasıyla 4.34−>7.48 log KOB g^{-1}, 3.69−>7.48 log KOB g^{-1} ve 4.05−>7.48 log KOB g^{-1} arasında değişmektedir. Ancak Üsküdar, Kadıköy ve Ümraniye'de TMAB sayımı sırasıyla 2.57−>7.48 log KOB g^{-1}, 4.01−>7.48 log KOB g^{-1} ve 4.24−>7.48 log KOB g^{-1} arasında değişmektedir. AP, YM ve *S. aureus* sayımı sırasıyla 2.00 ->7.48 log KOB g^{-1}, 2.00-7.48 log KOB g^{-1} ve 2.00-6.13 log KOB g^{-1} arasında değişmiştir. Örneklerin 43'ü koagülaz pozitif stafilokok olarak tespit edilmiştir. Örnekler için toplam koliform sayıları hesaplandığında en yüksek sayıya (3588.1 EMS/g) Esenler'in sahip olduğu tespit edilmiştir. En yüksek ve en düşük fekal koliform sayısı sırasıyla Esenler ve Fatih'te bulunmuştur. Örneklerin %7.22'sinde *E. coli* izole edilmiştir. Örneklerin hiçbiri *Salmonella* spp. ve *L. monocytogenes* için pozitif olarak tespit edilmemiştir. TOPSİS'e göre, Esenler TMAB, AP, *S. aureus*, koliform ve fekal koliform bakımından en kötü değerlere sahip olduğu bulunmuştur. YM'de ise en kötü skoru Fatih ilçesi almıştır. Tüm mikrobiyal grupların sayısı göz önüne alındığında, Üsküdar en iyi ilçe olarak bulunmuştur. Öte yandan İstanbul'da satışa sunulan taze kesilmiş salataların mikrobiyal güvenliğinin tüketilmek için yeterli olduğu çalışmanın sonuçları ile ortaya çıkmıştır.

Anahtar Kelimeler: Taze kesilmiş salatalar, Mikrobiyal kalite, Mikrobiyal güvenlik, Çok kriterli karar tekniği, TOPSIS

1. Introduction

Fresh vegetables are significant ingredients in the human diet, which boost the nutritional content and quality of all diets by supplying important vitamins, minerals, fiber, and biopsychological agents, which assist the body function (Niyaz and Demirbas, 2018; Saini et al., 2017; Slavin and Lloyd, 2012). The WHO (2002) states that a daily consumption of at least 400 g of fruit and vegetables is required. It is predicted to be able to save up to the lives of 2.7 million individuals a year by sufficient intake of fruits and vegetables (FAO, 2004). Vegetable consumption on a regular basis is widely recommended since it lowers the risk of certain ailments like cardiovascular disease, obesity, and cancer (CDC, 2018).

Depending upon an increase in the quantity of vegetable consumption, it has been observed that outbreaks linked with these products have increased (Bennett et al., 2018; de Oliveira Elias et al., 2018). Vegetables may threaten public health if they are contaminated with the most common pathogenic microorganisms including *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* or others (CRS, 2019; Faour-Klingbeil et al., 2016; Korir et al., 2016; Losio et al., 2015). The majority of pathogenic bacteria have existed in animal source foods rather than raw vegetables. However, processes like simply trimming, peeling, slicing/shredding, and washing which are carried out on raw vegetables are able to lead to contamination of these products, which in turn might give rise to serious problems threatening public health such as foodborne diseases, poisoning, and death. Therefore, researchers have conducted various studies on ready-to-eat foods specific to their own countries and have reported different results (Allen et al., 2013; Cardamone et al., 2015; De Giusti et al., 2010; Fröder et al., 2007; Gao et al., 2018; Graça et al., 2017). In our country, various studies have been also conducted on the microbiological quality of ready-to-eat foods, which are served hot and cold, and researchers generally report low microbiological quality (Gurler et al., 2015; Pamuk et al., 2013; Şenses-Ergül et al., 2015; Gumus et al., 2005; Temelli et al., 2005).

The presence of various microorganism groups is being studied in order to evaluate the microbial quality and safety of food. Multi-criteria approaches can be used to combine all of the microbiological analysis criteria. Multi-criteria decision-making (MCDM) enables deciding between alternatives using qualitative and quantitative data (Kumar et al., 2017). The technique for order preference by similarity to ideal solution (TOPSIS) which is the one of the MCDM techniques might be used to facilitate the comparison of the samples according to the results (Ozturk and Batuk, 2011). Even though MCDM techniques are implemented in various areas such as integrated manufacturing systems, evaluation of technology investment, water and agriculture management and energy planning (Erdin and Ozkaya, 2019, 2020a, 2020b; Ozkaya, 2017), there has not been a lot of research done in the food field (Dogan et al., 2016; Hosseinzadeh Samani et al., 2020; Şimşek, 2019).

The primary aim of present study is to assess the microbial properties of fresh-cut salads at the different districts of Istanbul and to rank those so as to find the best district by TOPSIS techniques based on microbial analysis results. To the best of our knowledge, multi-criteria decision approaches have not been employed in the literature to rank regions based on microbiological quality and safety. The findings of this research will provide an overview of the prevalence of some indicators and pathogenic microorganisms that are commonly found in salads and threaten human health, as well as data that can be used for risk assessment.

2. Materials and Methods

2.1. Sample Collection

A total of 180 vegetable salads were gathered from various restaurants in Esenler, Fatih and Besiktas (European side), Uskudar, Kadikoy and Umraniye (Asian side) in Istanbul, Turkey. All samples were immediately transported to the laboratory in insulated boxes with ice bags and kept in freezers at -18 °C to be tested on the following days. All materials for microbiological analysis were purchased from Merck (Darmstadt, Germany).

2.2. pH Value and Vegetable Content

A 10 g portion of each sample was placed in a stomacher bag and homogenized using a stomacher (VWR Star Blender LB 400) with 90 ml of distilled water for 1 min (Reale et al., 2019). The pH of each sample was then

measured by pH meter (Hanna HI 2211) as four parallel. The pH values of samples were determined by averaging the obtained results (Şimşek and Esmer Kizilirmak, 2017). The vegetable content of each sample was also specified.

2.3. Microbiological Analysis

All the 180 fresh-cut salads samples were analyzed for each of the following microorganisms or microbial groups: total aerobic mesophilic (TMAB), psychrotrophic bacteria (AP), yeast and mold (YM), fecal coliforms bacteria, and *E. coli*, coagulase-positive staphylococci, *Salmonella* spp., and *L. monocytogenes*.

2.3.1. Enumeration of Total Aerobic Mesophilic, Psychotropic Bacteria and Yeast and Molds

Total aerobic mesophilic (TMAB), aerobic psychotropic (AP) bacteria and yeast and mold (YM) were enumerated by the spread plate method on Plate Count Agar (PCA), followed by incubation for 48 h at 37 °C (Rosmini et al., 2004), on PCA, followed by incubation for 10 days at 7 °C (Silva et al., 2007) and on Dichloran Rose Bengal Chloramphenicol Agar (DRBC), followed by incubation for 5-7 days at 25 °C (Beuchat and Mann, 2016), respectively. The colonies were expressed as colony-forming units per gram (CFU g^{-1}).

2.3.2. Isolation and Enumeration of Fecal Coliform Bacteria and E. coli with MPN Method

Most Probable Number (MPN) method was used to enumerate fecal coliform bacteria and *E. coli*. LST (Lauril Sulfat Triptoz), BGBB (Brilliant Green Bile Broth) and EC (*E. coli*) broth were mediums used to carry out the analysis after which results were recorded and examined based on the MPN table. The culture-produced gas in EC tubes was streaked onto EMB agar plate for 24 h at 35 °C and confirmed as *E. coli* after being treated with Gram stain and IMViC biochemical tests (Cakir, 2000; Erkmen, 2000).

2.3.3. Determination of S. aureus

Determination of *S. aureus* was done by the spread plate method on Baird Parker Agar (BPA) supplemented with egg yolk Tellurite enrichment suspension, followed by incubation at 35 °C for 24 h; the results were expressed as colony-forming units per gram (CFU g^{-1}) (Ayçıçek et al., 2004; Meldrum et al., 2009). The colonies with typical *S. aureus* morphology (i.e., black, convex and with or without light halo on BP agar) were evaluated as a suspicious coagulase-positive colony. Up to 3 typical colonies were tested for coagulase production (Ghosh et al., 2007).

2.3.4. Isolation and Identification of Listeria monocytogenes

Twenty-five grams of each sample was weighed aseptically into a sterile stomacher and homogenized using a stomacher (VWR Star Blender LB 400) with 225 ml of sterile Listeria Enrichment Broth (LEB) for 2 min and incubated for 24 h at 30 °C. After the incubation period, the resultant homogenate of 0.1 ml was transferred into tubes containing 10 ml LEB and incubated for 48 h at 30 °C. All tubes were evaluated and a loopful suspension from positive tubes was streaked on Listeria Selective Agar (LSA) with Listeria-selective supplement and incubated up to 48 h at 37 °C. Brownish green or black halo of colonies breeding on LSA was considered as presumptive colonies. Then, the suspected colonies of loopful were inoculated on Trypticase Soy Agar supplemented with 0.6% yeast extract (TSAYE) plates and incubated for 24 h at 37 °C (Öğüt and Polat, 2009).

Colony PCR technique was used for screening the presence of the Listeriolysin O (*hlyA*) gene which is present at *L. monocytogenes*. Forward *hlyA* 634F: 5'-ACTTCGGCGCAATCAGTGA-3′ and Reverse *hlyA* 770R: 5′-TTGCAACTGCTCTTTAGTAACAGCTT-3′ were the oligonucleotide primer sets. The extraction of DNA and cycling conditions were performed according to Al-Ashmawy et al. (2014). PCR-amplified DNA fragments were run with ethidium bromide-stained 1.2% agarose gel electrophoresis. The separated DNA fragments were then visualized under UV light and photographed.

2.3.5. Isolation and Identification of Salmonella spp.

Twenty-five grams of each sample was homogenized with 225 ml of sterile 1% (w/v) buffered peptone water (BPW) for 2 min and incubated at 37 °C for 24 h. After the completion of the pre-enrichment process, 0.1 ml aliquots were transferred into the Rappaport-Vassiliadis (RV) Broth for the enrichment step and incubated for 48 h at 42 °C (Gurler et al., 2015; Nguz et al., 2005). Then, the positive cultures were finally plated onto Bismuth Sulfite Agar, Xylose Lysine Desoxycholate Agar and Hectoen Enteric Agar and incubated for 24-48 h at 37 °C. Triple Sugar Iron Agar (TSIA) was used for presumptive confirmation of colonies (Sant'Ana et al., 2011).

2.4. Statistical Analysis

Excel software (Microsoft Office 2016) was used to calculate arithmetic averages and standard deviations. The average results collected from the microbiological assessment for fresh-cut salads were analyzed using a Windows-based JMP statistical analysis software (SAS Institute. Inc. Cary, NC, USA) with one-way and two-way analysis of variance (ANOVA) and subjected to Tukey's multiple comparison test to ascertain any statistically significant difference ($p<0.05$) among the means. All counted data were converted to log values before statistical analysis.

2.5. Application of Multi-Criteria Decision-Making Method

Determining the microbial quality and safety of fresh-cut salads and comparing them according to districts is a multi-criteria decision-making (MCDM) problem and requires evaluation of many conflicting criteria (Forman and Selly, 2001). When the MCDM methods are classified with regard to their different aims, the TOPSIS method is applied to choose the best options and ratings.

TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) was developed by Yoon and Hwang (1995). It involves a 5-step solution process and calculation was conducted using equation (1-9). The steps of the TOPSIS method are described below.

Step 1: Normalization of the decision matrix

The R matrix is built by employing the *rij* values computed in this stage:

$$r_{ij} = \frac{x_{ij}}{\sqrt{\sum_{k=1}^{m} x_{kj}^2}}, i = 1, \dots, m; j = 1, \dots, n \quad \text{(Eq.1)}$$

Step 2: Computing *vij* matrix using the *vij*-weighted normal values. *wj* defines the weight of the *jth* criterion or indicator.

$$v_{ij} = w_j r_{ij}, \sum_{j=1}^{n} w_j = 1 \quad \text{(Eq. 2)}$$

Step 3: Positive (*A**) and negative (*A*-) ideal solutions:

$$A^* = \left\{\left(\max_i v_{ij} \,\middle|\, j \in C_b\right), \left(\min_i v_{ij} \,\middle|\, j \in C_c\right)\right\} = \left\{v_j^* \,\middle|\, j = 1,2, \dots, m\right\} \quad \text{(Eq. 3)}$$

$$A^- = \left\{\left(\min_i v_{ij} \,\middle|\, j \in C_b\right), \left(\max_i v_{ij} \,\middle|\, j \in C_c\right)\right\} = \left\{v_j^- \,\middle|\, j = 1,2, \dots, m\right\} \quad \text{(Eq. 4)}$$

If the j is a benefit indicator,

$$v_j^+ = max\{v_{ij}, i = 1, \dots, m\}, v_j^- = \min\left(v_{ij}, i = 1, \dots, m\right) \quad \text{(Eq. 5)}$$

If the j is a cost indicator,

$$v_j^- = max\{v_{ij}, i = 1, \dots, m\}, v_j^+ = \min\left(v_{ij}, i = 1, \dots, m\right) \quad \text{(Eq. 6)}$$

Step 4: Calculation of deflections of all options from positive and negative solutions employing the m-dimensional Euclidean distance:

$$S_i^* = \sqrt{\sum_{j=1}^{m} \left(v_{ij} - v_j^*\right)^2}, j = 1,2, \dots, m \quad \text{(Eq. 7)}$$

$$S_i^- = \sqrt{\sum_{j=1}^{m} \left(v_{ij} - v_j^-\right)^2}, j = 1,2, \dots, m \quad \text{(Eq. 8)}$$

Step 5: Determination of relative proximities to the A*. Then the relative closeness (RCi) of the alternative defined as Ai to the ideal solution is obtained. Then these values are ordered from largest to smallest.

$$RC_i^* = \frac{S_i^-}{S_i^* + S_i^-}, i = 1, \dots, m \quad \text{(Eq. 9)}$$

3. Results and Discussion

3.1. Vegetable Content and pH Values

Table 1 displays the pH values of salad samples used in this study. The mean pH of vegetable salads ranged between 2.87 and 6.17. The highest pH values were obtained from Esenler, Kadikoy, and Umraniye, respectively ($p<0.05$). The European side had a lower mean pH value compared to the Asian side ($p<0.05$). The average pH values of the European and Asian sides were 4.37±0.03 and 4.60±0.32, respectively. Salads were largely composed of lettuce, iceberg lettuce, tomato, carrot, kale, pepper, onion, parsley, and cucumber.

Table 1. pH values of samples

Regions	Number of samples	Minimum pH	Maximum pH	Mean pH
Esenler	30	3.39	5.63	4.67 ± 0.71^{A}
Fatih	30	2.87	4.77	3.96 ± 0.38^{CD}
Besiktas	30	3.62	6.17	4.47 ± 0.54^{AB}
Uskudar	30	3.39	5.23	4.15 ± 0.50^{BC}
Kadıkoy	30	3.53	5.63	4.72 ± 0.69^{A}
Umranıye	30	3.86	5.38	4.79 ± 0.48^{A}

Means with various letters ($^{A-C}$) according to ANOVA ($p<0.05$) are substantially different. Mean pH values in table are given as average values ± standard deviations.

3.2. Microbial Flora

The incidence and levels of foodborne pathogen contamination in food products at the retail level are crucial for the development of risk assessment models, particularly for estimating consumer exposure to diverse microorganisms. At any point of the process, from agriculture to consumption, pathogenic bacteria can contaminate fresh vegetables and fruits. Salads available for purchase in Turkey comprise fresh or boiled vegetables, cooked chicken meat, tinned tuna fish, and mayonnaise. However, in our study, 180 fresh cut vegetable salads without any meat product sold in Esenler, Fatih, Besiktas, Uskudar, Kadikoy and Umraniye in Istanbul were analyzed for total aerobic mesophilic (TMAB), aerobic psychotropic (AP) bacteria, yeast and mold (YM), fecal coliforms bacteria, *Escherichia coli*, *Staphylococcus aureus*, isolation of *Salmonella* spp. and *Listeria monocytogenes*.

The results of TMAB, AP bacteria, YM, and *S. aureus* counts were shown in *Table 2-5*, respectively. The mean total aerobic mesophilic bacteria (TMAB) count of the salads was ~5.85 log CFU g^{-1}, with a range of 2.57->7.48 log CFU g^{-1} *(Table 2)*. The enumeration of TMAB in Esenler, Fatih and Besiktas ranged from 4.34 to 7.48 log CFU g^{-1}, 3.69 to >7.48 log CFU g^{-1} and 4.05 to 7.48 log CFU g^{-1}, respectively. However, the enumeration of TMAB in Uskudar, Kadikoy and Umraniye ranged from 2.57 to 7.48 log CFU g^{-1}, 4.01 to >7.48 log CFU g^{-1} and 4.24 to 7.21 log CFU g^{-1}, respectively. According to ANOVA results, the highest count of TMAB was recorded in Esenler with the number of 6.42 log CFU g^{-1}, whereas the lowest count was found in Uskudar with the number of 5.32 log CFU g^{-1} ($p<0.05$). On the other hand, the TMAB count on the European side had a lower count with the mean 5.69 log CFU g^{-1} compared to the Asian side which had the mean 6.01 log CFU g^{-1} count. The levels of aerobic bacteria counts obtained from fresh-cut salads in this study are comparable to findings of previous studies.

Table 2. Results of TMAB count in the analyzed samples

Regions	Number of samples	Percentage (%) of samples in the indicated interval						Range[a]	Mean[a]
		2-3	3-4	4-5	5-6	6-7	7-8		
Esenler	30	0.0	0.0	10.0	26.6	23.3	40.0	4.34-7.48	6.42 ± 1.01^{A}
Fatih	30	6.6	0.0	23.3	26.6	23.3	20.0	3.69-7.48	5.61 ± 1.17^{BC}
Besiktas	30	0.0	0.0	16.6	36.6	20.0	26.6	4.05-7.48	6.32 ± 1.04^{BA}
Uskudar	30	5.0	10.0	20.0	35.0	25.0	5.0	2.57-7.48	5.32 ± 1.19^{C}
Kadıkoy	30	0.0	0.0	40.0	12.0	32.0	16.0	4.01-7.48	5.64 ± 1.17^{BC}
Umranıye	30	0.0	0.0	15.0	20.0	50.0	15.0	4.24-7.21	6.13 ± 0.93^{BA}

Means with various letters ($^{A-C}$) according to ANOVA ($p<0.05$) are substantially different. [a] The measurement unit is log CFU g^{-1}.

This study showed a mean TMAB count for freshly cut salads similar to the recent study by Seow et al. (2012). Furthermore, a study in Turkey by Aycicek et al. (2006) reported that a total of 180 samples (including lettuce, parsley, dill and carrots) were found to have a mean TMAB of 5.8 log CFU g^{-1}. Similarly, another study found that TMAB levels in 140 ready-to-eat lettuce samples from 16 university restaurants ranged from 3.01 to 7.81 log CFU g^{-1} (Soriano et al. 2000) and a research performed by Szabo et al. (2000) reported that TMAB of 120 ready-to-eat lettuce samples were found to range from 3.00 to 9.00 log CFU g^{-1}. The findings of another study also showed that TMAB was present in the between 4.00-7.00 log CFU g^{-1} (Ahmed et al., 2019).

The mean count of psychotropic bacteria (AP) in all collected samples was ~6.10 log CFU g^{-1}, with a range of 2.00->7.48 log CFU g^{-1} *(Table 3)*. While the highest AP count was obtained from Esenler with the number of 6.41 log CFU g^{-1}, the samples collected in Uskudar had the lowest value with the number of 5.66 log CFU g^{-1}. In terms of AP counts, there were no statistically significant differences between the samples obtained from the European side (Esenler, Fatih, and Besiktas) ($p>0.05$). The mean AP counts of the European and Asian sides were 6.36 and 5.86 log CFU g^{-1}, respectively, which means there were significant differences in the AP microbial counts ($p<0.05$). The means of aerobic psychotropic bacteria of fresh-cut salads varied from 2.00 to >7.48 log CFU g^{-1}. The results of an investigation performed by Silva et al. (2007) revealed that AP count was found as ranging from 6.89 to 8.43 log CFU g^{-1}. The outcomes of this study were significantly higher than the results obtained in this present study. Regardless of whether the count of these bacteria is low or high, the ability of aerobic psychotropic microorganisms to proliferate at refrigerated temperatures promotes the spoiling of vegetables, with the exception of a few psychotropic species (Graça et al., 2017).

Table 3. Results of AP count in the analyzed samples

Regions	Number of samples	Percentage (%) of samples in the indicated interval						Range[a]	Mean[a]
		2-3	3-4	4-5	5-6	6-7	7-8		
Esenler	30	0.0	2.66	10.0	13.3	26.6	43.3	3.85-7.48	6.41 ± 1.17^{A}
Fatih	30	0.0	0.0	3.3	36.6	36.6	23.3	4.16-7.48	6.34 ± 0.88^{A}
Besiktas	30	0.0	0.0	13.3	23.3	30.0	33.3	4.38-7.48	6.32 ± 0.95^{A}
Uskudar	30	5.0	5.0	10.0	35.0	35.0	10.0	2.00-7.48	5.66 ± 1.27^{B}
Kadıkoy	30	0.0	0.0	20.0	24.0	28.0	24.0	3.47-7.48	5.91 ± 1.21^{BA}
Umranıye	30	0.0	0.0	20.0	25.0	35.0	20.0	4.14-7.48	5.98 ± 1.04^{BA}

Means with various letters ($^{A-C}$) according to ANOVA ($p<0.05$) are substantially different. [a] The measurement unit is log CFU g^{-1}.

The mean count of yeast and molds (YM) for salads was ~4.77 log CFU g^{-1}. The count of YM in Esenler, Fatih and Besiktas ranged from 2.47 to >7.48 log CFU g^{-1}, 2.84 to >7.48 log CFU g^{-1} and 3.60 to >7.48 log CFU g^{-1}, respectively, while the enumeration of YM in Uskudar, Kadikoy and Umraniye ranged from 2.00 to 6.34 log CFU g^{-1} 2.00 to 5.85 log CFU g^{-1} and 3.43 to 7.37 log CFU g^{-1}, respectively (*Table 4*). These findings found no statistically significant differences between samples ($p>0.05$). The samples collected from the European side had the higher YM counts, with the mean 5.06 log CFU g^{-1} count, and the mean YM count of the Asian side was 4.47 log CFU g^{-1}. YM counts of samples collected from two sides were significantly ($p<0.05$) different.

Table 4. Results of YM count in the analyzed samples

Regions	Number of samples	Percentage (%) of samples in the indicated interval						Range[a]	Mean[a]
		2-3	3-4	4-5	5-6	6-7	7-8		
Esenler	30	3.3	23.3	23.3	36.6	6.6	6.6	2.47-7.48	4.82 ± 1.29^{A}
Fatih	30	3.3	0.0	36.6	33.3	20.0	6.6	2.84-7.48	5.30 ± 1.06^{A}
Besiktas	30	0.0	20.0	33.3	30.0	13.3	3.3	3.60-7.48	5.05 ± 1.07^{A}
Uskudar	30	10.0	30.0	20.0	35.0	5.0	0.0	2.00-6.34	4.41 ± 1.09^{A}
Kadıkoy	30	16.0	20.0	44.0	20.0	0.0	0.0	2.00-5.85	4.16 ± 1.04^{A}
Umranıye	30	0.0	30.0	25.0	25.0	15.0	5.0	3.43-7.37	4.91 ± 1.12^{A}

Means with various letters ($^{A-C}$) according to ANOVA ($p<0.05$) are substantially different. [a] The measurement unit is log CFU g^{-1}.

In our study, YM showed their incidence level between 2.00 and >7.48 log CFU g^{-1}. Fruits, raw and ready-to-eat vegetables were collected in a study carried out by Badosa et al. (2008) and the YM count of these samples was found as ranging from 5.00 to 6.00 log CFU g^{-1}. In another study, a total of 116 samples were collected by Pingulkar (2001) to determine the microbial quality of salad samples and YM counts ranged from 2.00 to 5.00 log CFU g^{-1}. As for a study conducted in Brazil (Dos Santos et al., 2021) researchers found that the count of YM was between 3.40 and > 6.30 log CFU g^{-1}. A lower count of YM with a mean of 3.69 log CFU g^{-1} was observed in another study (Schuh et al., 2019) Mold growth in fresh-cut salads, in fact, is not the cause of microbial degradation in these foodstuffs. Some molds, on the other hand, may be linked to health hazards in fruits and vegetables due to the production of mycotoxins and the induction of allergies (Seow et al., 2012).

S. aureus is a frequent foodborne pathogen that has been isolated from a variety of foods. In the food sector, the agent exploits a variety of contamination sources (Fetsch and Johler, 2018). *S. aureus* in food is usually caused by direct or indirect contact with infected food. *S. aureus* has identified contaminated hands, nostrils, mouths of personnel, contaminated food contact surfaces, and equipment. Humans play an important role in the food-human chain (Bilgin et al., 2019). Hence, the existence of *S. aureus* in our salads was also investigated. The enumeration of *S. aureus* ranged from 2.00 to 3.29 log CFU g^{-1} *(Table 5).* The enumeration of *S. aureus* in Esenler, Fatih and Besiktas ranged from 2.00 to 6.13 log CFU g^{-1}, 2.00 to 4.60 log CFU g^{-1} and 2.00 to 4.86 log CFU g^{-1}, respectively. However, the enumeration of *S. aureus* in Uskudar, Kadikoy and Umraniye ranged from 2.00 to 3.29 log CFU g^{-1}, 2.00 to 5.30 log CFU $g^{-1}$and 2.00 to 4.54 log CFU g^{-1}, respectively. Fatih was the lowest *S. aureus* count with 1.73 log CFU g^{-1} ($p<0.05$). However, the European and Asian sides had no substantial ($p>0.05$) differences. In contrast to our study, a survey conducted in Norway by Johannessen et al. (2002) found no *S. aureus* in the samples collected. In contrast, 50 vegetable salads samples sold in India were collected and the results revealed that *S. aureus* was detected in 15.1% of vegetable salads (Tambekar and Mundhada, 2006). Interestingly, the results of a study revealed no *S. aureus* in salad samples (Aghalari et al., 2021). In our study, coagulase test was also applied to some picked *S. aureus* colonies (3-5 colonies from each sample). A total of 43 samples were determined by coagulase-positive staphylococci. Of these, 51.2% belonged to the samples collected in Esenler, Besiktas and Fatih while 48.8% belonged to Uskudar, Kadikoy and Umranıye. On the contrary to our results, Becker et al. (2019) found the level of coagulase-positive staphylococci strains undetectable.

Table 5. Results of S. aureus count in the analyzed samples

Regions	Number of samples	Percentage (%) of samples in the indicated interval						Range[a]	Mean[a]
		2-3	3-4	4-5	5-6	6-7	7-8		
Esenler	30	60.0	20.0	16.6	0.0	3.3	0.0	2.00-6.13	2.23 ± 1.93^{BA}
Fatih	30	86.6	10.0	3.3	0.0	0.0	0.0	2.00-4.60	1.73 ± 1.36^{B}
Besiktas	30	80.0	16.6	3.3	0.0	0.0	0.0	2.00-4.86	2.51 ± 0.73^{A}
Uskudar	30	80.0	20.0	0.0	0.0	0.0	0.0	2.00-3.29	2.44 ± 0.47^{A}
Kadıkoy	30	84.0	8.0	4.0	4.0	0.0	0.0	2.00-5.30	2.50 ± 0.88^{A}
Umranıye	30	85.0	5.0	10.0	0.0	0.0	0.0	2.00-4.54	2.26 ± 0.62^{BA}

Means with various letters ($^{A-C}$) according to ANOVA ($p<0.05$) are substantially different. [a] The measurement unit is log CFU g^{-1}.

MPN method was used to determine the count of coliform and fecal coliform. *Table 6* shows the results of coliform and fecal coliform count in the samples analyzed with MPN.

On the European side, the highest number of coliform was found in Esenler, whereas the lowest number was in Fatih. Likewise, the highest and lowest count of fecal coliform was found in Esenler and Fatih, respectively. A verification test was performed to the colonies which were thought to be probable *E. coli*. Of collected samples, 7 samples from the European side verified as *E. coli*.

On the Asian side, Umraniye scored the highest coliform count, while Kadikoy had the lowest coliform count (*Table 6*). As for the count of fecal coliform on the Asian side, the highest and lowest count of fecal coliform was found in Umraniye and Kadikoy, respectively. As mentioned above, the verification test was performed on samples considered to be possible *E. coli*. After all, *E. coli* was found positive in a total of 6 samples on the Asian side. As a result, *E. coli* was found in 7.2% (13 of 180) of all samples. Of the positive *E. coli*, 53.8% were found on the European side and 46.2% on the Asian side. The presence of *E. coli* is comparable to those found by other researchers. A survey performed by Mukherjee et al. (2004) was reported that *E. coli* was present in 9.7% of the samples. In India, Tambekar and Mundhada (2006) examined a total of 50 samples of salad and found *E. coli* in

38.2% of the analyzed samples. Regarding the survey carried out in Pakistan, *E. coli* had been found in positive nine of the twelve types of samples tested (Ejaz et al., 2020). In a previous conducted study, *E. coli* count of salads was found to be 11.57% (Xylia et al., 2019). Compared to the above-mentioned surveys, our results were found to be lower.

Table 6. Results of coliform and fecal coliform count in the analyzed samples with MPN

Regions	Number of samples	Coliform			Fecal Coliform		
		Minimum	Mean	Maximum	Minimum	Mean	Maximum
Esenler	30	92	3588.1	>11000	92	2790.8	>11000
Fatih	30	<30.0	1046.5	>11000	<30.0	955.5	>11000
Besiktas	30	<30.0	2745.2	>11000	<30.0	25.19.8	>11000
Uskudar	30	<30.0	1650.1	>11000	<30.0	1205.5	>11000
Kadıkoy	30	<30.0	1520.08	>11000	<30.0	1019.04	>11000
Umranıye	30	<30.0	1917.2	>11000	<30.0	918.50	>11000

On the European side, *Listeria* spp. was found in 46.6%, 30.0% and 36.3% of the samples analyzed in Esenler, Fatih and Besiktas, respectively. On the Asian side, *Listeria* spp. was determined in 10.0%, 16.0% and 25.0% of the samples analyzed in Uskudar, Kadikoy and Umraniye, respectively (*Table 7*). In the present study, 45 of the collected 180 samples were found to be positive for *Listeria* spp. PCR was applied to presumptive colonies to find out the presence of some genes that determine whether the bacterium is associated with Listeriosis and none of them were identified as *L. monocytogenes.* In the current study, 45 of the collected 180 samples were found to be positive for *Listeria* spp. The high counts of presumptive *Listeria* spp. were reported in a study, as well (Ssemanda et al., 2017). Nevertheless, *Listeria* spp. was not confirmed as *L. monocytogenes*. Our results were similar to an investigation conducted by De Giusti et al. (2010). Contrary to our results, *L. monocytogenes* were isolated from conventionally produced salad samples in a study (Becker et al., 2019). Besides, some contrary results were also obtained from researches performed by other scholars (Cardamone et al. 2015; Gurler et al. 2015).

Table 7. The incidence of pathogens in the analyzed samples

Regions	Number of samples	Percentage (%) of samples with presumptive colonies	
		Salmonella spp.	***Listeria spp.***
Esenler	30	[a]ND	46.6
Fatih	30	ND	30.0
Besiktas	30	ND	36.3
Uskudar	30	ND	10.0
Kadıkoy	30	ND	16.0
Umranıye	30	ND	25.0

a ND: not detected.

Salmonella spp. may contaminate fruits and vegetables throughout the harvesting, processing, and transportation and preparation steps. *Salmonella* spp. were not isolated from any of the samples in our investigation. Therefore, no identification test such as PCR was applied for the detection of *Salmonella*. This is supported by the findings of various investigations. No *Salmonella* spp. were discovered in a study on Brazilian origins (Maffei et al. 2013). A similar investigation was carried out in Portugal, and *Salmonella* spp. were not found in any of the samples (Santos et al., 2012). This is supported by a previous study of Xylia et al. (2019) who reported no prevelance of *Salmonella* spp. in vegetables salads.

In our study, the microbiological quality and safety along with some physicochemical attributes of the salads were analyzed. The effects of season, salad producer and type of salad and/or their interactions with the tested parameters were not investigated as other researchers (Xylia et al., 2019). Later studies may conduct on these attributes, as well.

3.3. TOPSIS results

One of the most preferred MCDM methods is TOPSIS. With the use of this method in this regard, it is aimed to provide a new contribution to the field. In the present study, the ranking of the districts was made with the help of ANOVA and TOPSIS. However, comparison of the data can also be made by using other MCDM methods.

According to the ANOVA results, while one district is superior based on one criterion, another district is superior based on a different criterion. As a result, comparing alternatives or samples is quite difficult. The TOPSIS approach was used as an MCDM strategy to determine district ranking based on microbiological quality and safety of fresh-cut salads in order to make comparisons easier. The criteria used in TOPSIS analysis were TMAB, AP, YM, *S. aureus*, coliform, and fecal coliform bacteria count. Before TOPSIS analysis, the count of bacteria (log values for TMAB, AP, YM, *S. aureus* and MPN results for coliform, and fecal coliform bacteria) was normalized to evaluate criteria with different units together. Therefore, the values of each criteria were converted to be between 0 and 1. This also prevents the analysis from being affected by extreme values. In the evaluation, districts are required to have minimum values for each criteria.

Table 8 summarizes the TOPSIS analysis results for microbial safety and quality by district. *Table 9* shows the microbial count values for each district at general criteria level. According to the results (*Table 8*), Uskudar was defined as the most suitable district for TMAB and AP count. Based upon *Table 8*, Kadikoy was found to be the best district in terms of YM count. In terms of *S. aureus*, coliform and fecal coliform criteria, Fatih had the best values with 0.642598, 0.696173 and 0.760257, respectively. Esenler had very bad values of TMAB and AP, *S. aureus*, coliform and fecal coliform. As for YM, Fatih had the worst scores. Upon determining the general comparison of districts in *Table 9*, Uskudar was at the top of the ranking followed by Kadikoy, Fatih, Besiktas, Umraniye. There were no significant differences between Uskudar (0.622451) and Kadikoy (0.611779). Esenler had the worst scores (0.469538) considering all microorganism count.

Table 8. Comparison of districts with TOPSIS method in terms of TMAB, AP, YM, S. aureus, coliform and fecal coliform bacteria

Raking	TMAB		AP		YM	
1	Uskudar	0.624761	Uskudar	0.556921	Kadikoy	0.695579
2	Fatih	0.558363	Kadikoy	0.526103	Uskudar	0.593599
3	Kadikoy	0.550451	Umraniye	0.48182	Umraniye	0.525903
4	Besiktas	0.447955	Besiktas	0.439515	Esenler	0.501697
5	Umraniye	0.399797	Fatih	0.406436	Besiktas	0.492622
6	Esenler	0.398304	Esenler	0.390423	Fatih	0.435144
Raking						
1	Fatih	0.642598	Fatih	0.696173	Fatih	0.760257
2	Umraniye	0.612393	Uskudar	0.690001	Umraniye	0.704425
3	Uskudar	0.588401	Kadikoy	0.688461	Kadikoy	0.687015
4	Kadikoy	0.556822	Besiktas	0.650184	Uskudar	0.649603
5	Besiktas	0.544825	Umraniye	0.571863	Besiktas	0.570672
6	Esenler	0.439811	Esenler	0.554388	Esenler	0.53177

Table 9. General comparison of districts based on TMAB, AP, YM, S. aureus, coliform and fecal coliform bacteria with TOPSIS method

	Districts	Ranking by TOPSIS
1	Uskudar	0.622451
2	Kadikoy	0.611779
3	Fatih	0.575374
4	Besiktas	0.536703
5	Umraniye	0.533344
6	Esenler	0.469538

4. Conclusions

This study focused on comparing the microbial quality and safety of fresh-cut salads according to different districts in Istanbul. The ANOVA and TOPSIS results were found to be comparable. In the TOPSIS method, equal

weighting was applied to the criteria to avoid subjective evaluation. While there was no difference among Uskudar, Kadikoy and Umraniye in TMAB results obtained from the ANOVA, Uskudar was found as the best district in TMAB results obtained from the TOPSIS. The best district for AP was found compatible with the TOPSIS and ANOVA. According to the ANOVA, no difference was found among the districts in the YM results but the best district for YM results was Kadikoy according to the TOPSIS. In the ANOVA, Esenler, Fatih, and Umraniye had close values for *S. aureus*, while Fatih was at the top of list in the TOPSIS. As the general comparison could not be made in the ANOVA, districts have been ranked by using the TOPSIS method in terms of all criteria including TMAB, AP, YM, *S. aureus*, coliform, and fecal coliform. Based on the TOPSIS results, the best and worst district were found to be Uskudar and Esenler, respectively.

As a conclusion, the findings of this study gave information on the microbiological quality of fresh-cut salads marketed commercially in Istanbul, Turkey. Although the results of the current study show that fresh-cut salads could be conveniently consumed in Istanbul, the execution of the cold chain and the correct storage and sanitation condition of the fresh products should be maintained in order to ensure acceptable quality.

Acknowledgment

This research was financially assisted by Namik Kemal University Scientific Research Projects Coordination Department, project number NKUBAP.00.24.YL.13.06. This article is produced from M.S. thesis of Gulsum UCAK.

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