

Microbiological Quality of Fresh Vegetables and Fruits Collected from Supermarkets in Istanbul, Turkey

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Abstract: Two hundred sixty one samples, collected from supermarkets in Istanbul, Turkey. All samples were analysed for aerobic mesophilic bacterial counts (AMC), aerobic psychrotrophic bacterial counts (APC), enumeration of yeasts and moulds (YM), coliforms, *Escherichia coli* and detection of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, thermotolerant *Campylobacter* spp. AMC ranged from 2.95 to 3.75 log₁₀ CFU/g. APC ranged from 0 to 3.55 log₁₀ CFU/g. The highest counts of coliforms were found in carrot, spinach, green leaf lettuce, cos lettuce and iceberg lettuce. The highest counts of YM were found in tomato, spinach, green leaf lettuce, cos lettuce and iceberg lettuce. Green leaf lettuces, cos lettuces, iceberg lettuces, spinach and carrot were highly contaminated with aerobic mesophilic (3.6 log₁₀ CFU/g), psychrotrophic microorganisms (3.4 log₁₀ CFU/g), and showed a high incidence of *E.coli* (41.77% of samples). Of the samples analysed, 10 (3.83%) were *Salmonella* spp. positive and 17 (6.51%) were thermotolerant *Campylobacter* spp. positive. None of the samples was positive for *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Fresh vegetables and fruits, sold in Istanbul, Turkey, are needed to control foodborne pathogens especially *Salmonellosis* and *Campylobacteriosis*.

Keywords: Food Safety, Fresh Fruit, Microbiological Quality, Raw Vegetable

1. Introduction

Hygienic fresh vegetables and fruits are important components of the human diet and there is strong relationship between fresh vegetable and fruit consumption and health [1]. Raw vegetables and fruits provide proteins, carbohydrates and many vitamins, minerals which are essential in healthy human life. These commodities are convenient meal for contemporary lifestyles. Various types of fruits and raw eaten vegetables and salads are also very popular due to their attributes. In many countries, including Turkey, consumption rates of raw fruits and vegetables is increasing day by day. To prevent cancer, diabetes, heart disease, obesity and micronutrient deficiencies, joint FAO/WHO Expert Consultation Panel recommends an adequate daily intake of 400–500 g of fruit and vegetables [2].

Fresh produce may be contaminated with a different kind of microorganisms [3-5]. Microbial contamination can occur

during any steps of the farm-to-table and this contamination can arise from environmental, animal, human sources and technological applications. Microorganisms could spread not only by direct contact, but also through air and water [6-9]. Raw eaten vegetables and fruits are consumed without enough heating process, and therefore the possibility of food poisoning and food-borne infections always exists [10]. The number of reported food-borne outbreaks associated with raw fruits and vegetables has increased in the late years. Identified outbreaks etiology has primarily bacteriological origin [11]. However, most of farmers don't still have enough information about hygienic production and good agricultural practices. This is a highly potential risk for public health. Increasing consumption of fresh produce has associated outbreaks of foodborne illnesses [5, 12, 13]. Fresh produce can be a vehicle for the contamination of enterotoxigenic and enterohemorrhagic *Escherichia coli* [1, 10, 14-16], *Salmonella* spp. [1, 14, 17], *Listeria monocytogenes* [1, 17, 18, 19], thermotolerant *Campylobacter* spp. [20, 21],

parasitic and viral pathogens capable of causing human illness and a number of reports refer to raw vegetables and fruits harbouring potential foodborne pathogens [3, 13, 16, 22-25].

The Centers for Disease Control and Prevention has reported an increase of fresh produce related foodborne disease outbreaks between 1995 and 2005 around the world [26].

Istanbul which has a geographic and geopolitics importance due to representing a transit corridor between Europa and Asia is a rapidly developing market cause of high population growth rate and continuous migration from various regions of Turkey and the neighboring countries. This high density of the population is regarded as a comprehensive supply of foods. Especially, the rapid growth of supermarkets chains in Turkey over the past ten years and also the subsequent development of quality requirements like volume, regularity, quality homogeneity, range of varieties, packaging have given rise to new market opportunities for local agriculture [27].

The present survey was intended to supply some assessment on the microbiological quality of marketed fresh vegetables and fruits in Istanbul, Turkey [28].

2. Materials and Methods

2.1. Collection of Samples

Two hundred sixty one fresh vegetables and fruits samples were analyzed between the period May 2012 and January 2013. The samples analyzed included: 161 samples of whole fresh vegetables, 100 samples of fresh fruits. Samples of all of the fresh produce sold in Istanbul were randomly purchased from different major supermarkets and local chain markets, in their original, individual packages. Production data of the samples are shown in table 1.

2.2. Preparation for Microbial Analysis

All samples were analysed as soon as possible after purchase. Before samples were taken out of their original packaging, the possible contact surfaces were carefully sterilised using polyurethane sponges to prevent cross-contamination. Damaged samples were discarded before analysis. For lettuce, spinach and purslane approximately 25 g of each sample were placed in a sterile stomacher bag and homogenized using a stomacher (Interscience - BagMixer 400 P, France) with 225 ml of sterile 0.1% buffered peptone water (BPW) (Oxoid, Cambridge, UK) for 2 min. For all other commodities, i.e. cucumber, tomato, green bean, squash, carrot, plum and peach each sample (whole) was aseptically transferred into a stomacher bag filled with equal weight of BPW. Each whole sample was then agitated and rubbed by hand in the stomacher bag for 2 min to suspend surface microbes [23, 29]. Appropriate 1:10 dilutions of the resultant homogenate or the rinse fluid were prepared using BPW.

2.3. Aerobic Mesophilic Plate Count and Aerobic Psychrotrophic Plate Count

Laboratory analyses to enumerate of aerobic mesophilic plate count and psychrotrophic plate count were performed in accordance with the ISO 4833-1:2003 standard [30] and ISO 17410:2001 [31], respectively.

2.4. Enumeration of Yeasts and Moulds

Laboratory analyses to enumerate of yeasts and moulds were performed in accordance with the ISO 7954:1987 standard [32].

2.5. Enumeration of Coliforms and *Escherichia coli*

Samples were prepared as described above. Homogenate or the rinse fluid was prepared using BPW. For each selected dilution, 0.1 ml of sample was spread-plated onto brilliance *E. coli*/coliform agar (Oxoid, Cambridge, UK). The plates were incubated at 37 °C for 24 h, following which, the number of pink (coliform) and purple (presumptive *E. coli*) colonies was counted [33]. Identification of *E. coli* was carried out with IMVIC tests [34].

2.6. Isolation of *E. coli* O157:H7

Samples were prepared as described above. Homogenate or the rinse fluid was prepared using BPW. Laboratory analyses to detect of *E. coli* O157:H7 were performed in accordance with the ISO 16654:2001 standard [35].

2.7. Isolation of *Salmonella* Spp.

Samples were prepared as described above. Laboratory analyses to detect of *Salmonella* spp. were performed in accordance with the ISO 6579:2002 standard [36].

2.8. Isolation of *Listeria monocytogenes*

Samples were prepared as described above. Laboratory analyses to detect of *L. monocytogenes* were performed in accordance with the ISO 11290 method [37].

2.9. Isolation and Confirmation of Thermotolerant *Campylobacter* spp.

Laboratory analyses to detect thermotolerant *Campylobacter* spp. were performed in accordance with the ISO 10272-1:2006 standard (qualitative analysis) [38]. To confirm suspect isolates polymerase chain reaction (PCR) methods [39] and a commercially available real-time PCR kit (Taq Man *Campylobacter* spp. Kit, AB Applied Biosystems) were applied. For quality control, the *C. jejuni* ATCC 33560 reference strain was used.

2.10. Confirmation of Presumptive Colonies

API® (bioMérieux, Marcy-l'Étoile, France) was used for the confirmation of presumptive colonies. The biochemical tests were carried out according to the manufacturer's instructions.

2.11. Statistical Analysis

Colony counts were converted into \log_{10} CFU/g. The mean values obtained from the microbiological evaluation of fruits and vegetables were analysed by independent samples *t*-test

and to determine any statistically significant difference ($P < 0.05$) among the all commodities means by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test using SPSS 17.0 software (SPSS Inc. Chicago, IL, USA).

Table 1. Data on production of the samples.

| samples | production region | production area | source of water | method of irrigation |
|---------------------------------|------------------------------|-----------------|-----------------|----------------------|
| Green leaf lettuce ^a | Antalya ^b | greenhouse | ground water | drip |
| Iceberg lettuce ^a | Antalya ^b | greenhouse | ground water | drip |
| Cos lettuce ^a | Antalya ^b | greenhouse | ground water | drip |
| Spinach ^a | Eskişehir ^c | greenhouse | ground water | drip |
| Purslane ^a | Eskişehir ^c | greenhouse | ground water | drip |
| Cucumber | Antalya-Demre ^b | greenhouse | ground water | drip |
| Tomato | Antalya-Demre ^b | greenhouse | ground water | drip |
| Green bean | Antalya-Serik ^b | greenhouse | ground water | drip |
| Kidney bean | Antalya-Serik ^b | greenhouse | ground water | drip |
| Squash | Antalya-Demre ^b | greenhouse | ground water | drip |
| Eggplant | Antalya-Aksu ^b | greenhouse | ground water | drip |
| Pepper | Antalya-Demre ^b | greenhouse | ground water | drip |
| Carrot | Sakarya ^c | field | river | aerosolization |
| Plum | Antalya-Serik ^b | field | ground water | drip |
| Apricot | Malatya-Arapgir ^c | field | ground water | drip |
| Peach | Antalya-Serik ^b | field | ground water | drip |
| Apple | Antalya-Serik ^b | field | ground water | drip |
| Pear | Antalya-Serik ^b | field | ground water | drip |
| Grape | Antalya-Serik ^b | field | ground water | drip |
| Strawberry | Antalya-Serik ^b | greenhouse | ground water | drip |

^aUntreated bovine or ovine originated manure are applied during pre-harvest on the greenhouse

^bMediterranean climatic condition (mildly during spring, autumn and winter seasons, hot and dry during summer season)

^cColdly during spring, autumn and winter seasons, hot and dry during summer season)

3. Results

AMC, APC and YM counts are shown in table 2. Coliform and *Escherichia coli* counts and percentages are shown in table 3. Results of incidence of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and thermotolerant *Campylobacter* spp. in the samples analysed are shown in table 4.

AMC and APC were up to 3.75 and 3.55 \log_{10} CFU/g. Coliform and *Escherichia coli* counts were up to 3.45 and 2.65 \log_{10} CFU/g. *Escherichia coli* contamination was highest in green leaf lettuce (80%). The highest YM counts

were detected in tomatoes (3.15 \log_{10} CFU/g). Statistically, whole fresh vegetable samples had significantly higher microbial loads than fresh fruit samples ($p < 0.05$) and green leaf lettuce, iceberg lettuce, cos lettuce and spinach had significantly higher microbial loads than other commodities ($p < 0.05$). No pathogenic bacteria could be isolated from fresh fruit samples, purslane, cucumber, tomato, green bean, kidney bean, squash, eggplant, and pepper. *Salmonella* spp. was isolated from 10 whole fresh vegetable samples. Thermotolerant *Campylobacter* spp. was isolated from 17 whole fresh vegetable samples.

Table 2. The presence of aerobic mesophilic count (AMC), aerobic psychrotrophic count (APC) and yeasts and moulds (YM) in the samples analyzed (\log_{10} CFU/g).

| Type of samples | n | AMC/g | | APC/g | | YM/g | |
|--------------------|----|-----------|--------|-----------|--------|-----------|--------|
| | | range | median | range | median | range | median |
| Green leaf lettuce | 15 | 3.6-3.75 | 3.7 | 3.4-3.45 | 3.45 | 2.95-3.05 | 3.0 |
| Iceberg lettuce | 15 | 3.5-3.65 | 3.5 | 3.3-3.5 | 3.35 | 2.9-3.05 | 3.0 |
| Cos lettuce | 16 | 3.5-3.6 | 3.6 | 3.35-3.5 | 3.4 | 2.95-3.05 | 2.95 |
| Spinach | 19 | 3.3-3.6 | 3.5 | 3.1-3.55 | 3.4 | 2.9-3.05 | 2.95 |
| Purslane | 14 | 3.4-3.45 | 3.45 | 3.25-3.35 | 3.3 | 2.8-2.95 | 2.85 |
| Cucumber | 10 | 3.2-3.35 | 3.3 | 2.95-3.2 | 3.05 | 2.6-2.8 | 2.65 |
| Tomato | 11 | 3.35-3.45 | 3.4 | 3.1-3.25 | 3.2 | 3-3.15 | 3.1 |
| Green bean | 11 | 3-3.2 | 3.1 | 2.6-2.85 | 2.8 | 2.5-2.8 | 2.6 |
| Kidney bean | 11 | 2.95-3.1 | 3.05 | 2.55-2.8 | 2.65 | 2.5-2.8 | 2.65 |
| Squash | 10 | 3-3.2 | 3.1 | 2.55-2.65 | 2.6 | 2.5-2.8 | 2.55 |
| Eggplant | 10 | 2.95-3.1 | 3.05 | 2.55-2.65 | 2.6 | 2.5-2.8 | 2.6 |
| Pepper | 11 | 3.05-3.2 | 3.1 | 2.55-2.65 | 2.6 | 2.5-2.8 | 2.55 |
| Carrot | 14 | 3.6-3.75 | 3.7 | 3.4-3.45 | 3.45 | 2.4-2.8 | 2.65 |
| Plum | 12 | 2.95-3.1 | 3.1 | 0-2.5 | 2 | 2.55-2.8 | 2.55 |
| Apricot | 12 | 3.05-3.35 | 3.3 | 0-2.7 | 2 | 2.5-2.8 | 2.6 |

| Type of samples | n | AMC/g | | APC/g | | YM/g | |
|-----------------|------------|------------------|-------------|---------------|------------|---------------|-------------|
| | | range | median | range | median | range | median |
| Peach | 12 | 2.95-3.35 | 3.1 | 0-2.85 | 2 | 2.5-2.8 | 2.6 |
| Apple | 12 | 3.3-3.5 | 3.35 | 0-3.45 | 2 | 2.3-2.7 | 2.5 |
| Pear | 14 | 3.25-3.5 | 3.3 | 0-3.5 | 2.3 | 2.3-2.7 | 2.4 |
| Grape | 14 | 3.3-3.35 | 3.3 | 0-2.7 | 1 | 0-2.2 | 0.7 |
| Strawberry | 18 | 3.3-3.35 | 3.3 | 2-2.65 | 2.3 | 2-2.65 | 2.2 |
| Total | 261 | 2.95-3.75 | 3.45 | 0-3.55 | 2.8 | 0-3.15 | 2.65 |

Table 3. The presence of coliform and *E.coli* in the samples analyzed.

| Type of samples | n | Coliform count (log ₁₀ CFU/g) | | <i>E.coli</i> count <i>E.coli</i> presence (log ₁₀ CFU/g) | | |
|--------------------|------------|--|-------------|--|----------|-------------------------------|
| | | range | median | range | median | number of positive samples(%) |
| Green leaf lettuce | 15 | 3.25-3.45 | 3.3 | 0-2.65 | 2.5 | 12 (80) |
| Iceberg lettuce | 15 | 3.25-3.45 | 3.35 | 0-2.55 | 2.25 | 7 (46.67) |
| Cos lettuce | 16 | 3.2-3.4 | 3.3 | 0-2.6 | 2.35 | 9 (56.25) |
| Spinach | 19 | 2.95-3.45 | 3.2 | 0-2.5 | 0 | 4 (21.05) |
| Purslane | 14 | 2.95-3.05 | 3 | 0 | 0 | 0 |
| Cucumber | 10 | 2.7-2.85 | 2.8 | 0 | 0 | 0 |
| Tomato | 11 | 2.9-3.1 | 2.95 | 0 | 0 | 0 |
| Green bean | 11 | 2.3-2.8 | 2.7 | 0 | 0 | 0 |
| Kidney bean | 11 | 2.5-2.8 | 2.55 | 0 | 0 | 0 |
| Squash | 10 | 2.5-2.8 | 2.7 | 0 | 0 | 0 |
| Eggplant | 10 | 2.5-2.8 | 2.55 | 0 | 0 | 0 |
| Pepper | 11 | 2.5-2.8 | 2.7 | 0 | 0 | 0 |
| Carrot | 14 | 3.25-3.45 | 3.25 | 0-2.3 | 0 | 1 (7.14) |
| Plum | 12 | 0-2.3 | 0 | 0 | 0 | 0 |
| Apricot | 12 | 0-2.3 | 0 | 0 | 0 | 0 |
| Peach | 12 | 0-2.3 | 0 | 0 | 0 | 0 |
| Apple | 12 | 2.5-2.9 | 2.7 | 0 | 0 | 0 |
| Pear | 14 | 2.6-2.9 | 2.65 | 0 | 0 | 0 |
| Grape | 14 | 0-2.3 | 0 | 0 | 0 | 0 |
| Strawberry | 18 | 0-2.3 | 0 | 0 | 0 | 0 |
| Total | 261 | 0-3.45 | 2.75 | 0-2.65 | 0 | 33 (12.64) |

Table 4. The incidence of *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and thermotolerant *Campylobacter* spp. in the samples analysed.

| Type of samples | n | Number and percentage (%) of positive samples | | | |
|--------------------|------------|---|------------------------|-------------------------------|---------------------------|
| | | <i>E.coli</i> O157:H7 | <i>Salmonella</i> spp. | <i>Listeria monocytogenes</i> | <i>Campylobacter</i> spp. |
| Green leaf lettuce | 15 | nd | 4 (26.67) | nd | 5 (33.33) |
| Iceberg lettuce | 15 | nd | 1 (6.67) | nd | 4 (26.67) |
| Cos lettuce | 16 | nd | 3 (18.75) | nd | 4 (25) |
| Spinach | 19 | nd | 1 (5.26) | nd | 3 (15.79) |
| Purslane | 14 | nd | nd | nd | nd |
| Cucumber | 10 | nd | nd | nd | nd |
| Tomato | 11 | nd | nd | nd | nd |
| Green bean | 11 | nd | nd | nd | nd |
| Kidney bean | 11 | nd | nd | nd | nd |
| Squash | 10 | nd | nd | nd | nd |
| Eggplant | 10 | nd | nd | nd | nd |
| Pepper | 11 | nd | nd | nd | nd |
| Carrot | 14 | nd | 1 (7.14) | nd | 1 (7.14) |
| Plum | 12 | nd | nd | nd | nd |
| Apricot | 12 | nd | nd | nd | nd |
| Peach | 12 | nd | nd | nd | nd |
| Apple | 12 | nd | nd | nd | nd |
| Pear | 14 | nd | nd | nd | nd |
| Grape | 14 | nd | nd | nd | nd |
| Strawberry | 18 | nd | nd | nd | nd |
| Total | 261 | nd | 10 (3.83) | nd | 17 (6.51) |

Abbreviations: n, number of samples; nd: not detected

4. Discussion

Fresh vegetables and fruits can be contaminated with pathogenic bacteria in any steps of all the process from

cultivation to consuming. These bacteria cause major public health concern worldwide in terms of human illnesses. In addition, food-borne diseases have a large economic loss. Different studies on this subject are available in the world.

But in our country, number and content of the study is not satisfactory. Our study was planned for this reason. The AMC for the whole vegetables and fresh fruits examined was around $3.35 \log_{10}$ CFU/g, with a range of 2.95 - $3.75 \log_{10}$ CFU/g and $3.30 \log_{10}$ CFU/g, with a range of 2.95 - $3.50 \log_{10}$ CFU/g, respectively. Pianetti *et al.* [40] reported that aerobic colony count does not relate to food poisoning and infections, generally. However, it acts as an indicator for food quality and shelf life.

According to the HACCP-TQM technical guidelines, raw foods containing $<10^4$ CFU/g ($<4 \log_{10}$ CFU/g), 10^4 - 5×10^6 CFU/g (4 - $6.7 \log_{10}$ CFU/g), 5×10^6 - 5×10^7 CFU/g (6.7 - $7.7 \log_{10}$ CFU/g) and $>5 \times 10^7$ CFU/g ($>7.7 \log_{10}$ CFU/g) (number of spoilage microorganisms aerobic plate count at 70°F (21.1°C)) are rated as "good", "average", "poor" and "spoiled food", respectively (41). In our study, the quality of 261 samples was regarded as "good".

Aycicek *et al.* [10] reported some raw eaten vegetables (lettuce, cos lettuce, iceberg lettuce, parsley, dill, carrot) total aerobic counts between 0 and $7.4 \log_{10}$ CFU/g. Abadias *et al.* [1] found the AMC ranged from 4.3 to $8.9 \log_{10}$ CFU/g in fresh-cut vegetables, from 2.0 to $7.1 \log_{10}$ CFU/g in fresh-cut fruits, from 7.1 to $9.2 \log_{10}$ CFU/g in sprouts and from 2.7 to $8 \log_{10}$ CFU/g in whole vegetables. APC counts were similar to those of mesophilic microorganism, with carrot ($7.9 \log_{10}$ CFU/g) and spinach ($7.4 \log_{10}$ CFU/g) being the vegetables with the highest mean counts. Badosa *et al.* [17] reported fruits had AMC ranging from 1 to $8 \log_{10}$ CFU/g, most of them ranging between 3 and $4 \log_{10}$ CFU/g. Oliveira *et al.* [15] noted that the AMC ranged from <3 to $7 \log_{10}$ CFU/g in conventional and from 5 to $>7 \log_{10}$ CFU/g in organic lettuce. Psychrotrophic microorganism is very similar to those of mesophilic microorganisms, with ranges between 3 to $>7 \log_{10}$ CFU/g in organic lettuce and between <3 to $7 \log_{10}$ CFU/g in conventional lettuce. Seow *et al.* [23] reported that AMC of fresh vegetables and fruits counts ranged from 1.6 to $9.1 \log_{10}$ CFU/g with the lowest counts recorded for orange and highest counts recorded for bean sprouts. Fresh-cut salads had the highest mean APC of $4.9 \log_{10}$ CFU/g. In another study done by Viswanathan and Kaur [14], it was reported that AMC for raw salad vegetables, fruits and sprouts were in the range between 5 - $10 \log_{10}$ CFU/g, 6 - $8 \log_{10}$ CFU/g and 9 - $12 \log_{10}$ CFU/g, respectively. These findings were higher than our study. These differences may be originated from regions, geographical and climatical conditions, irrigation techniques and post-harvest processes.

Regarding the YM range of fresh produces, our findings is similar to other author's studies. Generally, lettuces, tomatoes and carrots contamination is higher than the other commodities for YM. Abadias *et al.* [1] found that the ranges for YM in fresh-cut vegetables, fresh-cut fruit, sprouts and whole vegetables were 2.0-7.8, 1.7-4.9, 2.8-7.6 and 2.2- 6.1 \log_{10} CFU/g, respectively. Grated carrot was the highest mean counts for YM ($6.1 \log_{10}$ CFU/g). Oliveira *et al.* [15] reported that 51.4% of the organic lettuce, 52.8% conventional lettuce was found in the range between 4 and $5 \log_{10}$ CFU/g, respectively. The YM mean was 4.7 and $4.2 \log_{10}$ CFU/g.

Tournas (4) noted that YM counts ranged between 3.1 - $5.95 \log_{10}$ CFU/g in lettuce, <2 - $5.3 \log_{10}$ CFU/g in carrots, 2 - $3.8 \log_{10}$ CFU/g in cucumbers, 4 - $4.1 \log_{10}$ CFU/g in iceberg lettuce, 3.4 - $3.6 \log_{10}$ CFU/g in spinach, 2 - $6.2 \log_{10}$ CFU/g in different kind of tomatoes (cherry, grape, Roma). Tournas [4] and Tournas and Katsoudas [42] declared the some health problems related with the presence of yeasts and moulds in vegetables and fruits. Some of these may produce mycotoxins and others are known to cause allergic reactions.

Aycicek *et al.* [10] found coliform counts up to $6.9 \log_{10}$ CFU/g, *E.coli* counts up $3.8 \log_{10}$ CFU/g. Microbial loads of outer leaves of lettuce, cos lettuce and iceberg lettuce, parsley and dill samples detected the same levels. *E.coli* positive sample percentages of raw eaten vegetable was among 10% (iceberg lettuce and carrot)-70% (parsley). Seow *et al.* [23] noted that highest level of coliforms was found in bean sprouts and fresh-cut salads. Viswanathan and Kaur [14] found coliform counts ranged between 6 - $9 \log_{10}$ CFU/g in raw salad vegetables, 4 - $7 \log_{10}$ CFU/g in fruits and 8 - $11 \log_{10}$ CFU/g in sprouts, respectively. Also, it was reported that prevalences of *E.coli* 50% of lettuces and 10% of carrots in their study. Abadias *et al.* [1] noted that *E.coli* was not detected in fresh-cut fruit, but was present in 7.1% whole vegetable samples and 11.4% fresh-cut vegetable samples. Our findings about *E.coli* prevalences showed similarity with these results. Especially, lettuces varieties, spinach, purslane, carrot etc. are risky products. Applying agricultural techniques should be reviewed for hygienic conditions.

HACCP-TQM technical guidelines give threshold and quality levels for food-borne illness hazards according to the volunteer feeding test of healthy people groups. For, *E.coli*, the estimated illness dose is 6 - $10 \log_{10}$ CFU/g and suggested level of *E.coli* for purchasing $1 \log_{10}$ CFU/g (41). In our study, purchasing limit for *E.coli* was exceeded 19.76% of vegetable samples (33 of the 167). On the other hand, none of the fruit samples were exceeded this level.

A lot of studies were undertaken about food-borne disease related to consumption of fresh fruit and vegetable showed that the number of the outbreaks has increasing day by day. Each year millions of cases occur that most of this *E.coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp. etc. Infections cause mild illness, severe infections and serious complications-including death [43]. Abadias *et al.* [1] noted that none of the fresh, minimally-processed fruit and vegetables, and sprouts samples was positive for *E.coli* O157:H7 and thermotolerant *Campylobacter*. Of the samples analyzed 1.3% was *Salmonella* spp. and 0.7% were *L.monocytogenes* positive. Seow *et al.* [23] reported that *E.coli* O157:H7 and *Salmonella* spp. was not detected in the analyzed of total 125 fresh fruits and vegetables samples. Al-Hindawi and Rished [44] reported that 7% of 43 vegetables samples were detected positive for *Salmonella* spp. Ercolani [45] noted that 68.3% rate of *Salmonella* in lettuces. Garcia-Villanova Ruiz *et al.* [46] reported that analyzed of 80 lettuce samples, 6.3% were positive and 5.2% of analyzed 28 spinach samples were positive for *Salmonella* spp. Garcia-Villanova Ruiz *et al.* [47]

at the another study reported that analyzed 46 of 849 vegetables samples were detected positive *Salmonella* spp. Jerngklinchan and Saitanu [48] reported 8.7% rate of *Salmonella* in bean sprouts. Viswanathan and Kaur [14] tested of 72 vegetables and 33.3% of the samples were *Salmonella* spp. positive; tested of 24 fruits and 37.5% of the samples were *Salmonella* spp. detected positive.

In the present study *E.coli* O157:H7 was not detected. *E.coli* O157:H7 presents sporadically at very low levels together with very high levels of competitor organisms that's why it is very difficult to detect. *Salmonella* spp. contamination in lettuces, spinach and carrots may cause by the use of contaminated irrigation water and untreated bovine or ovine originated manure.

Arumugaswamy *et al.* [49] reported of the analyzed 22 lettuce, 5 cucumber, 7 bean sprouts sample, 22.7%, 80%, 85% were positive for *L.monocytogenes*, respectively. Breer and Baumgartner [50] noted 2.3% of 263 salad vegetable samples were detective *L.monocytogenes* positive. De Simon *et al.* [51] reported the *L.monocytogenes* prevalence rate of 7.8% of vegetables samples in Spain. Gunasena *et al.* [18] reported 10 of 20 lettuce sample were positive for *L.monocytogenes*. Harvey and Gilmour [52] reported 25% *L.monocytogenes* prevalence rate in salad vegetables in Northern Ireland. In another study was undertaken Hesick *et al.* [53], *L.monocytogenes* contamination were detected in 2 of 92 eggplant samples. MacGowan *et al.* [54] noted that 6.2% *L.monocytogenes* prevalence rate in vegetables in UK. Wong *et al.* [55] reported that 6 of 49 vegetable samples were detective *L.monocytogenes* positive. Our findings in this study showed contrary with these studies results.

Chai *et al.* [56] reported that 153 of 309 vegetables samples were detected *Campylobacter* spp. by polymerase chain reaction (PCR) techniques. Kumar *et al.* [20] reported that out of 56 vegetables samples examined, two (3.57%) revealed the presence of *Campylobacter* spp. Park and Sanders [57] reported that 1 of 40 leafy vegetables samples, 2 of 67 lettuce samples, 1 of 63 pepper and 2 of 60 spinach samples were positive for *Campylobacter* spp., respectively. Verhoeff-Bakkenes *et al.* [21] noted that 10 of 4691 (0.21%) vegetables samples, 2 of 790 (0.25%) fruits samples were positive for *Campylobacter* spp. Whyte *et al.* [58] reported that 2 of 279 (0.72%) vegetables samples were positive for *Campylobacter* spp.

The most important source of the *Campylobacter* species is birds, rodents, sheep and cattle intestinal tracts. Agricultural areas may attack by birds during the cultivation and/or storage; use of untreated manure may be source of this contamination [59].

5. Conclusion

In conclusion, considering the public health, fresh fruits and vegetables are common sources for various microorganisms and also pathogenic bacteria. Therefore, it is essential to ensure applying good agricultural practices GAP(s) and good manufacturing practices GMP(s) during

production. Farmers should be informed about the sources of microbial contamination and should be trained in hygienic production. It is emphasized that whole fresh vegetables and fresh fruits should be protected from contamination by human, animal and other wastes which may constitute a hazard to health of the consumer through fresh produces. The use of poor microbiological quality of irrigation water should be avoided. On the other hand, post-harvest washing of fresh vegetables and fruits with organic antimicrobial agent and use of edible coatings containing antimicrobial is an important method for pathogen reduction. Transport vehicles and warehouse should be designed specifically for the transport and storage of fruits and vegetables. Also, strict temperature control from harvesting to consumption by farmer, handler, transporter, warehouseman etc. and consumers is highly important.

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