MICROBIOLOGICAL QUALITY OF FRESH VEGETABLE SALADS FROM THE NOVI SAD MARKET

Dragoljub Cvetković¹*, Dragan Pavlović², Aleksandra Ranitovićⁱ, Siniša Markov¹

¹ University of Novi Sad, Faculty of Technology Novi Sad; Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
² AD Sugar factory Šajkaška, Ćuruški put 3, 21230 Žabalj, Serbia

Increased consumption, big production units and very efficient delivery service networks of fresh vegetables and salads caused a higher number of foodborne illnesses in the last two decades all over the world. In this study 15 fresh vegetable salads from different marketplaces in Novi Sad were tested by reference microbiological methods (detection of Salmonella spp., enumeration of Listeria monocytogenes, mesophilic aerobic bacteria, yeasts and molds, sporogenic mesophilic aerobic bacteria, sporogenic sulfite-reducing bacteria, Bacillus cereus, Enterobacteriaceae, Escherichia coli and coagulase positive staphylococci). Salmonella spp. was found in one sample which, according to the safety criteria for such a product (if treated as ready-to-eat), was considered to be microbiologically unsafe. The number of Listeria monocytogenes in all tested samples was <100 cfu/g, which is a satisfactory result according to the safety criteria. E. coli was detected in only one sample, and the number of Enterobacteriaceae was in the range from 4.4 to 6.9 log CFU/g. The isolated Enterobacteriaceae strains were identified as Enterobacter cloacae, Klebsiella pneumoniae ssp. pneumoniae and Citrobacter spp.

KEY WORDS: food safety, vegetables, salads.

INTRODUCTION

Vegetables are important dietary components because they provide essential nutrients, such as vitamins, minerals and fibers, and have many health benefits. Regular consumption of vegetables is highly recommended since it reduces the risk of certain diseases, namely cardiovascular diseases, obesity, and cancer, among others (1). The recommendations of The World Health Organization (WHO) are to include fresh fruits and vegetables as an indispensable part of human nutrition (2). According to statistical data (3), vegetable production has increased by about 500 billion tons in the last two decades (1990-2011), while in the period from 1970-1990, this growth was nominally around 200 billion tons. Not only did the population increase influenced the global need for vegetables, but also the way of life and nutrition, especially in developed countries, all contributing to a significant change in the habits of the population in terms of food consumption.

* Corresponding author: Dragoljub Cvetković, University of Novi Sad, Faculty of Technology Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia, e-mail: cveled@uns.ac.rs
Despite the health benefits, the risk of microbiological contamination of fresh vegetables is of great concern. Many foodborne illness outbreaks in numerous countries have been associated with consumption of contaminated fresh vegetables (4). According to the European Food Safety Authority (5), food of plant origin is responsible for over 10% of foodborne outbreaks, while this is true in 4.4% of cases for vegetables, juices and similar products. Products which are most related to epidemics are salads consisting of mixed vegetables. WHO categorizes green salads (all types), leafy vegetables (spinach, cabbage) and fresh herbs as primary-value products when it comes to the safety of fresh raw foods from a global point of view (6). During growth, harvesting, further processing and handling, vegetables and vegetable products can be contaminated with pathogenic microorganisms originating from soil, water, animals or the production environment (7). During peeling, cutting and grinding, vegetable surfaces are exposed to the environment and possible additional contamination by bacteria, yeasts and molds. The protective epidermal barrier of plants is disrupted, making nutrients more accessible by facilitating microbiological growth and product impact (8).

Contamination of vegetables by Salmonella spp. and L. monocytogenes, which are among the most important health related species, can occur in the fields or during harvesting, cutting, sorting, or packaging and transport (9). Organic fertilizers often consisting of manure may harbor pathogenic bacteria such as Salmonella spp., L. monocytogenes and E. coli O157:H7 (10). The ability of these microorganisms to survive and/or grow on vegetables and cause diseases depends on the interaction and relation of plant-microorganism and microorganism-microorganism (11), as well as their response to unfavorable conditions during storage or processing (12). Despite the small number of occurrences of L. monocytogenes and Salmonella spp. in vegetables ready for consumption, several studies showed that the total number of its population during storage can reach the values of $10^8$ CFU/g (13). Contamination of fresh fruits and vegetables poses a particular danger due to the products being consumed raw without going through the production process which would reduce or eliminate the microbiological hazard to a satisfactory level (14). The methods which ensure safe production and proper disinfection by the producer are of crucial importance as they allow for safe consumption of fresh fruits and vegetables without endangering the consumers (15).

The aim of this paper was to examine the microbiological quality of salads produced from fresh vegetables. Samples of commercially packaged salads were collected from the Novi Sad market. The obtained results show the extent to which the products offered in the daily market are safe for consumers.

**EXPERIMENTAL**

A total of 15 salads samples were tested (15 salads of three repetitions each). The salads were made from fresh vegetables (not spiced), collected in their original packaging from retail stores in Novi Sad, Republic of Serbia. Vegetables from which the salads were made were produced in a conventional way. The salad samples were marked with numbers 1-15, and their ingredients were: 1-4 (lettuce, peppers, canned red beans, canned...
sweet corn); 5-7 (cabbage, peppers, lettuce, cherry tomatoes); 8, 9 (grated carrots); 10-12 („Vitamins salad” – white cabbage, red cabbage, carrots, sweet corn); 13-15 (“Mix for Serbian salad” - tomatoes, cucumbers, onions, peppers). The preparation of the samples for testing was carried out in accordance with the standard ISO 6887-1:1999 (16). Standard methods were used for the detection of *Salmonella* spp. and enumeration of selected microorganisms, as follows: *L. monocytogenes*, mesophilic aerobic bacteria, yeasts and molds, sporogenic mesophilic aerobic bacteria, *B. cereus*, sporogenic sulfite-reducing bacteria, Enterobacteriaceae, *E. coli*, coagulase positive staphylococci (17-26). The isolated strains of Enterobacteriaceae were identified by Vitek 2 Compact System (bioMérieux, France). The number of microorganisms in the tested samples is expressed as the mean of three measurements with standard deviation. Excel Microsoft XP Office was used to generate statistical results.

**RESULTS AND DISCUSSION**

The microbiological quality of samples was determined by the analysis of *Salmonella* spp. and enumeration of the following microorganisms: *L. monocytogenes*, mesophilic aerobic bacteria, yeasts and molds, sporogenic mesophilic aerobic bacteria, *B. cereus*, Enterobacteriaceae, *E. coli*, coagulase positive staphylococci and sporogenic sulfite-reducing bacteria. *Salmonella* spp. and *L. monocytogenes* were tested as microorganisms of special health importance (microbiological safety criteria). *Salmonella* spp. was detected in one sample of salads (sample 4) which was purchased in a chain store, in its original plastic packaging, stored in a refrigerator and declared with a 3 day expiration date. Due to the presence of *Salmonella* spp., this specimen was health-defective in accordance with the relevant Regulations of the Republic of Serbia (27) in line with Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. According to the results of Jeddi et al. (2014), who analyzed 20 samples of ready-to-eat salads and 32 samples of “mung” bean sprouts, one mixed salad sample and one sprout sample tested positive for *Salmonella* spp. (28). In a study conducted by Najafi et al. (2012), out of 85 tested samples of mixed salads, one sample tested positive for *Salmonella* spp. (29). The presence of *Salmonella* spp. in vegetables is the result of poor hygienic conditions in the entire production chain, while in the case of mixed salads which are made ready for consumption, the conditions in the final processes of preparation such as grinding and packaging must be taken into consideration, including the factors such as the employee and equipment hygiene, which is of key importance. The number of *L. monocytogenes* in salad samples was <1.0 log CFU/g, except for three samples (sample 7, 13 and 15), for which, due to the accompanying bacterial microbiota, it was difficult to determine the number of this bacterium from the base dilution. Therefore, in those cases, the number of *L. monocytogenes* is shown as <2.0 log CFU/g. However, all these results are in accordance with the Regulation on the limit value for the number of *L. monocytogenes*, which is 100 CFU/g for the food that does not support the growth of this bacterium (27). Baumgartner and Schmid (30) published data from the inspection surveillance conducted in Switzerland in the period from 2006 to 2008 on the presence of *L. monocytogenes* in
ready-to-eat products (a total of 13,798 samples). When concerning fresh delicatessen salads, *L. monocytogenes* were detected only in 0.14% of the samples (1/720). Clinical studies in Switzerland showed that fresh vegetable salads are not recognized as high risk products for the occurrence of listeriosis (31). As the most common causes of *L. Monocytogenes* occurrence in these products are insufficient hygiene of equipment and utensils, while in the period prior to vegetable harvesting, contamination by irrigation water is possible (32). In order to reduce the risks for consumers of these products, special attention must be given to expiration dates. It is important to emphasize that an open product must be quickly used and stored at cooling temperatures (≤5 °C), inhibiting the growth of *L. monocytogenes* (33).

The number of mesophilic aerobic bacteria in tested vegetable salads was within the range from 5.8 to 7.1 log CFU/g (Table 1). It is certain that during the salad preparation process (during handling, grinding and packaging) there is a risk of additional contamination by microorganisms from the environment, primarily by the workers, equipment, and even the air itself. According to Maffei et al. (1), who examined the microbiological quality of organic and conventional vegetables sold in Brazil, in over 40% of samples (conventional), or respectively, 55% of samples (organic), the number of mesophilic aerobic bacteria was in the range of 10^6–10^7 CFU/g. Caponigro et al. (34) examined the microbial quality of ready-to-eat vegetable salads, determining that the number of mesophilic aerobic bacteria were in the range of 4.0-8.7 log CFU/g.

The number of yeasts and molds in fresh vegetable salads were in the range from 4.6 to 5.9 log CFU/g (Table 1), and these results are in agreement with those of some other authors. In a paper by Pingulkar et al. (35) the yeasts and molds found in restaurant prepared salads, the reported values were from 4 to 7 CFU/g, while a study of Jeddi et al. (28) showed that the number of yeasts and molds found in salads from chain stores ranged from 6.2 6to 7.5 log CFU/g. In the case of these types of products, despite the high values of yeasts and molds found, the risk of mycotoxins is low, due to the short shelf life, assuming that the products are properly stored at refrigerated temperatures and that upon delivery and inspection of the fresh vegetables, the damaged goods are adequately removed.

The total number of sporogenic mesophilic aerobic bacteria was lower than <1.0, ie, <1.6 log CFU/g for the most number of samples; only two samples showed a slightly higher number (2.0 and 2.9 log CFU/g) (Table 1). Therefore, the sporogenic mesophilic aerobic bacteria and *B. cereus* (which is a significant cause of food intoxication) made up only a small portion of the total microbiota of the tested salad samples. Hildebrandt et al. (36) examined 113 salad samples from fresh mixed vegetables prepared in the restaurant environments and found the same results for *B. cereus* values, <200 CFU/g; coming to the conclusion that this bacterium does not pose a risk to the safety of these products. Sporogenic sulfite-reducing bacteria was not detected in the samples analyzed in this paper.
Table 1. Number (log CFU/g) of mesophilic aerobic bacteria, yeasts and molds, sporogenic mesophilic aerobic bacteria, *B. cereus*, *Enterobacteriaceae* (Ent.), *E. coli* and coagulase positive staphylococci in vegetable salads

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mesophilic aerobic bacteria</th>
<th>Yeasts and molds</th>
<th>Sporogenic mesophilic aerobic bacteria</th>
<th><em>B. cereus</em></th>
<th>Ent.</th>
<th><em>E. coli</em></th>
<th>Coagulase positive staphylococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.2±0.1</td>
<td>5.1±0.2</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>5.1±0.1</td>
<td>&lt;1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>7.1±0.1</td>
<td>4.9±0.2</td>
<td>&lt;1.6</td>
<td>&lt;2.0</td>
<td>6.3±0.1</td>
<td>&lt;1.0</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>3</td>
<td>6.7±0.1</td>
<td>5.0±0.3</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>6.9±0.1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>4</td>
<td>6.0±0.1</td>
<td>5.1±0.1</td>
<td>2.0±0.0</td>
<td>&lt;1.0</td>
<td>5.1±0.2</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>5</td>
<td>7.0±0.2</td>
<td>5.0±0.1</td>
<td>2.9±0.1</td>
<td>&lt;1.0</td>
<td>5.4±0.4</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>6</td>
<td>6.7±0.2</td>
<td>5.9±0.2</td>
<td>&lt;1.6</td>
<td>&lt;2.0</td>
<td>5.7±0.1</td>
<td>&lt;1.0</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>7</td>
<td>6.9±0.3</td>
<td>5.7±0.1</td>
<td>&lt;1.0</td>
<td>&lt;2.0</td>
<td>5.0±0.2</td>
<td>&lt;1.0</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>8</td>
<td>5.8±0.2</td>
<td>4.8±0.2</td>
<td>&lt;1.6</td>
<td>&lt;1.0</td>
<td>5.2±0.1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>9</td>
<td>6.1±0.2</td>
<td>4.6±0.02</td>
<td>&lt;1.6</td>
<td>&lt;1.0</td>
<td>4.9±0.1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>10</td>
<td>6.1±0.01</td>
<td>4.9±0.1</td>
<td>&lt;1.6</td>
<td>&lt;2.0</td>
<td>5.2±0.01</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>11</td>
<td>6.2±0.2</td>
<td>5.0±0.3</td>
<td>&lt;1.0</td>
<td>&lt;2.0</td>
<td>5.3±0.04</td>
<td>&lt;1.0</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>12</td>
<td>6.0±0.3</td>
<td>4.9±0.1</td>
<td>&lt;1.6</td>
<td>&lt;2.0</td>
<td>5.6±0.04</td>
<td>1.0±0.2</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>13</td>
<td>6.1±0.03</td>
<td>5.2±0.04</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>4.6±0.01</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>14</td>
<td>6.0±0.3</td>
<td>4.9±0.1</td>
<td>&lt;1.6</td>
<td>&lt;1.0</td>
<td>4.4±0.07</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>15</td>
<td>5.9±0.1</td>
<td>5.0±0.1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>4.5±0.07</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

The results given in Table 1 show that the number of bacteria from the *Enterobacteriaceae* family (fecal contamination indicators) ranged from 4.4 to 6.9 log CFU/g. In a study by Nguz et al. (37) the number of enterobacteria was in the range of 1.6-6.9 log CFU/g in mixed vegetables and 2.0-9.8 log CFU/g in green beans. The highest overall incidence level was 25.8% for the counts between 3 and 4 log CFU/g. Abadias et al. (38) showed that the number of enterobacteria in 236 fresh salad samples was in the range of <1.0-8.0 log CFU/g, while in most of the tested samples (over 70%) the number was at the level of 6 log units.

*E. coli* was detected in only one salad sample (commercial brand „Vitamins salad”) (Table 1). The levels of *E. coli* are often used for monitoring the sanitary conditions under which foods are processed. The levels found in our study indicate that the vegetables were processed under good sanitary conditions. About 6.7% of the analyzed samples in the study done by Nguz et al. (37) were classified as unacceptably contaminated by *E. coli* with reference to the PHLS guidelines and German criteria. However, in order to get a clearer picture of the dangers of the presence of *E. coli* in fresh vegetables and salads, one should also keep in mind the enterohaemorrhagic *E. coli* O157: H7 as important foodborne pathogens.

The number of coagulase positive staphylococci in salad samples was <1.0 log CFU/g, respectively, <2.0 log CFU/g (Table 1). In the study of Maistro et al. (39), all 172 samples of minimally processed vegetables (MPV) did not present counts of coagulase positive staphylococci over the limit of detection of the method (<1.0 log CFU/g). The
fact that most studies on the microbiological quality of MPV have not studied the population, and the presence of coagulase positive staphylococcus can be explained by their relative weak competitiveness over common microbiota of these products, and by the fact that production of enterotoxins does not take place below 10 °C (according to Oliveira et al., 2010 in 39).

During the detection of *Salmonella* spp. after pre-enrichment and enrichment, and then cultivation on selective media, several selected strains of Enterobacteriaceae were identified by the Vitek 2 Compact System (Table 2). With confirmation of the presence of *Salmonella* spp. in sample 4, previously identified by biochemical tests in accordance with ISO 6579:2008, the remaining isolated enterobacteria strains were identified by Vitek 2 Compact System as *Enterobacter cloacae*, *Klebsiella pneumoniae* ssp. *pneumoniae* and *Citrobacter* spp. The listed bacterial species belong to the coliform bacteria (indicators of fecal contamination) which, through animal feces, can reach and contaminate soil, water and plants.

**Table 2.** Results of the identification of isolated strains of Enterobacteriaceae by Vitek 2 Compact System

<table>
<thead>
<tr>
<th>Sample</th>
<th>Identified organism</th>
<th>Confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td><em>Salmonella</em> spp.</td>
<td>excellent identification</td>
</tr>
<tr>
<td>2</td>
<td><em>Enterobacter cloacae</em></td>
<td>excellent identification</td>
</tr>
<tr>
<td>7</td>
<td><em>Enterobacter cloacae</em></td>
<td>excellent identification</td>
</tr>
<tr>
<td>10</td>
<td><em>Klebsiella pneumoniae</em> ssp. <em>pneumoniae</em></td>
<td>excellent identification</td>
</tr>
<tr>
<td>15</td>
<td><em>Citrobacter</em> spp.</td>
<td>low discrimination</td>
</tr>
</tbody>
</table>

**CONCLUSION**

*Salmonella* spp. was detected in one of tested samples of freshly mixed vegetables salads, which, according to the safety criteria for such a product (if treated as ready-to-eat) is considered to be microbiologically unsafe. The number of *L. monocytogenes* bacteria in all the tested salads was <100 CFU/g, which is a satisfactory result. During the preparation of fresh vegetable salads (especially those prepared for consumption), according to the obtained results, it is a more risky category of food than raw vegetables, therefore the introduction and improvement of the HACCP system with all pre-condition programs, including good manufacturing practice and good hygiene practices, must be fully observed in every step of the production chain.
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22. ISO 7932:2004. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 °C.


**МИКРОБИОЛОШКИ КВАЛИТЕТ САЛАТА ОД СВЕЖЕГ ПОВРЋА СА ТРЖИШТА НОВОГ САДА**

Драгољуб Цветковић¹, Драган Павловић², Александра Ранитовић¹, Синиша Марков¹

¹ Универзитет у Новом Саду, Техношко факултет Нови Сад, Булевар цара Лазара 1, 21000 Нови Сад, Република Србија
² АД Фабрика шећера Шајкашка, Чурушки пут 3, 21230 Жабаљ, Република Србија

У овом раду испитано је 15 узорака свежих салата од поврћа (у три независна понављања свака) прикупљених у различитим продајним објектима на територији Новог Сада. Узорци су испитани референтним микробиолошким методама (откривање *Salmonella* spp., одређивање броја *L. monocytogenes*, мезофилних аеробних бактерија, квасаца и плесни, спорогених мезофилних аеробних бактерија, спороге-

Кључне речи: безбедност хране, салате, поврће

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