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MICROBIOLOGICAL ANALYSIS OF SOME VEGETABLE SPECIES COMMERCIALIZED ON LOCAL AND SUPERMARKET MARKETS

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ABSTRACT

Fruits and vegetables are an important part of the human diet, providing essential vitamins, minerals and fiber and adding variety to the diet. In the Food Guide Pyramid, the US Department of Agriculture encourages eating 3-5 servings of vegetables and 2-4 servings of fruit per day (León J.S, 2009). The present study aims at microbiological analysis of frequently consumed vegetables. It was found that the highest loading was on cucumbers and lettuce and the lowest on peppers and tomatoes. Refrigerated storage for 7 days resulted in an increase in the number of microorganisms
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INTRODUCTION

Contamination of fruit and vegetables occurs at every stage of the food chain, from cultivation to processing. Consumers in developing countries have become more concerned about the nutritional and sensory aspects as well as the safety of the food they eat due to growing health awareness (Qadri O.S., 2015). Polluted environments during cultivation or poor hygienic conditions in processing increase the risk of contamination with food-borne pathogens. In recent decades, there has been a dramatic increase in outbreaks of food-borne diseases mainly caused by *Escherichia coli* and *Salmonella* through the consumption of fresh and minimally processed fruits. The minimum processing required for fresh and fresh-cut produce, which omits any effective microbial elimination step, results in food products that naturally would carry microorganisms, some of which may be potentially hazardous to human health (Harris L.J., 2003). Beuchat, L.R., et al. (2003) have highlighted the presence and resistance of some pathogens in the soil in which tomatoes were grown in order to determine if it is a vector in the transmission of microorganisms to plant. Islam M et al. (2004) studied the presence of *E.coli* in soil in which manure was applied. Solomon B.E. et al (2003) studied the effect of irrigation water on lettuce leaf contamination. Guo, X. (2002) studied the evolution of a *Salmonella* population on tomatoes kept at room temperature. Food spoilage refers to several changes that make food toxic and less palatable to consumers, and these could be associated with changes in appearance, texture, taste or odor. Microbial spoilage of vegetables leads to a reduction in their market value and nutritional qualities and sometimes made them unfit for consumption. This is due to either mycotoxin or pathogen contamination. Vegetables are often marketed in the open (unpacked), which makes it possible to expose them to micro-organisms present in the marketing premises, on the skin of handlers or consumers. Fruits and vegetables,and in particular leafy greens that are consumed raw,are increasingly being recognized as important vehicles for transmission of human pathogens that were traditionally associated with foods of animal origin (Cedric N. Berger et al, 2010). Fruits and vegetables minimally procesed fresh can harbor psychrotrophic microorganisms such as fluorescent pseudomonads or *Listeria monocytogenes*, good refrigeration temperature control limits the growth of spoilage and pathogenic microorganisms (Christophe Nguyen-the et al. 2009). The microbiology of fruits and vegetables must be approached from the point of view of product contamination by examining both pre-harvest and post-harvest sources and practices (Sapers G.M., 2005). The economic value of fresh-cut products is affected by the proliferation of micro-organisms, as it can lead to a decrease in the shelf life of the product through spoilage and also poses a risk to public health by causing food-borne diseases. F. Devlieghere (2004) studied the effect of carbon dioxide-rich packaging on microbial spoilage of fresh-cut yellow melon and suggested that 50% O_2 + 50% CO_2 is a potential gas composition in a modified atmosphere for shelf-life extension of fresh-cut yellow melon. The specialized literature cites that 10^2-10^7 UFC/g can be found on the surface of vegetables (Dan V. 2000). Of the total coliform bacteria found on the surface of vegetables, about 30% belong to the *Escherichia coli* species, which is responsible for the outbreak of numerous epidemics. Considering the above, we considered it appropriate to study the microflora present on the surface of some vegetables sold both in supermarkets and in the market, in order to identify them (categorization of colonial isolates into genera) and to determine their quantity (number of UFC/g product).

MATERIAL AND METHODS

Plant material

Various vegetable species purchased from two different places namely: local market (local producers) and supermarket were taken in the study. The vegetable species were: bell peppers, gherkin type cucumber, tomato, green lettuce.

Growing media

The culture media used were:

- nutrient agar for bacteria
- PDA (potato-dextrose agar) for fungi

Working mode

Nutrient agar (20 g) was dissolved in 1 litre of distilled water, homogenized for 10 minutes and then sterilized by autoclaving for 15 minutes at 121 ^{0}C. The medium was poured into Petri dishes and allowed to solidify under sterile conditions. PDA medium (30 g) was also dissolved in 1 liter distilled water and boiled to dissolve completely before sterilization at 121 ^{0}C for 15 minutes. The pH was adjusted to 3.5 after addition of 10 ml lactic acid solution to facilitate microbial growth. The medium was thereafter allowed to cool at 55 ^{0}C and poured into Petri dishes. For each sample, the number of viable germs was determined by UFC method. For this purpose, 25 g of each sample were weighed sterilely, homogenized with 225 ml sterilized distilled water. Sterile bags and the Bigmixer stomacher type Bigmixer in the microbiology laboratory were used for homogenization. Homogenization was carried out for 1 minute at maximum speed. For each sample, decimal dilutions were made, using a 10^{-3} dilution for seeding. Seeded plates were thermostated for 48 hours at 30 ^{0}C for bacteria and 25 ^{0}C for fungi. First observations were made after 24 hours.

For each sample, two plates were seeded by averaging the number of colonies between the two plates. The number of UFC/g produced was determined taking into account the dilution factor and loop calibration. After growth, the colonies were counted and described macroscopically and microscopically. Macroscopic description involved noting the shape, color, edge and surface appearance of the colony, consistency, degree of transparency. Under the microscope, in Gram-stained smears for bacteria and freshly prepared flattened smears for yeasts and moulds, morphological aspects of the cell (shape, grouping mode, staining affinities) were observed. On the basis of these cultural and morphological characters it was possible to classify the colonial isolates into genera. The vegetable samples taken in the study were kept under refrigerated conditions for 7 days when they were reanalyzed in terms of total aerobic mesophilic bacterial counts.

RESULTS AND DISCUSSIONS

Microbiological analysis of vegetables and fruits involves the determination of the total number of aerobic mesophilic germs to which in cases of suspicion is added the determination of bacteria from the coliform group, the determination of *Escherichia coli* species, *Salmonella*, coagulase-positive *Staphylococcus aureus*, as well as the determination of yeasts and molds. The samples taken in the study were analyzed in terms of the determination of the total number of aerobic mesophilic germs as well as the number of yeasts and molds. Figure 1 shows the total count of aerobic mesophilic aerobic germs for each sample taken in the study. From the graph it can be seen that the highest counts of aerobic mesophilic germs were recorded in the samples commercialized in the market (Fig.1).

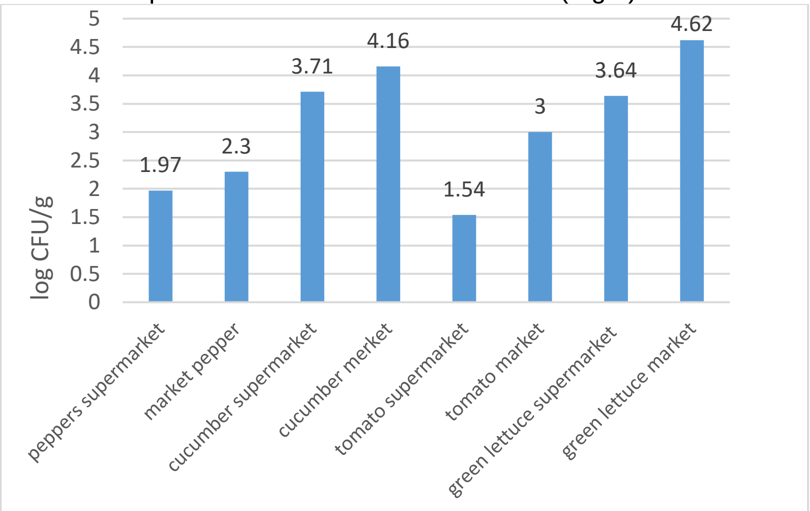

Figure 1.Graphical representation of the total number of aerobic mesophilic germs in the studied vegetable samples (log CFU/g)

Comparing the vegetables studied, the highest number of aerobic mesophilic bacteria was recorded in lettuce, followed by cucumber. This can be explained by the rough surface of cucumber and a larger contact surface of lettuce.

In addition, lettuce grows very close to the soil surface, so it is in contact with many more germs. Tomatoes and bell peppers had lower total aerobic mesophilic bacteria counts, which can be explained by the shiny surface, less adherent for microorganisms. Analyzing the results as a whole, it can be seen that limits noted in the literature (10^2-10^7 UFC/g). Vegetables present a very high load of microorganisms, which, however, is within the Figure 2 shows the percentage distribution of the germs isolated from sample I.

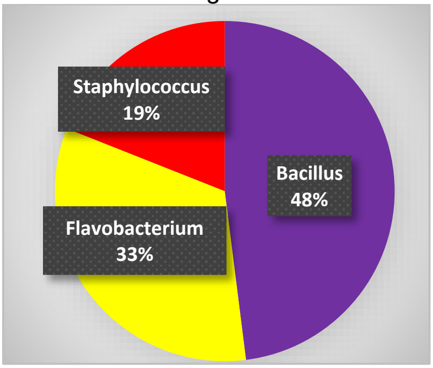

Figure 2. Percentage distribution of isolated sprouts from supermarket peppers

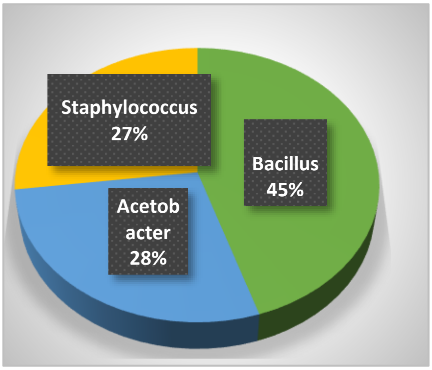

Figure 3. Percentage distribution of isolated sprouts from market peppers

In the sample of peppers from the market, the presence of bacteria of the genera *Bacillus, Acetobacter, Staphylococcus*, with a predominance of sporulated bacteria as in the first sample analyzed (Figure 3). The presence of bacteria of the genus *Acetobacter* can be explained by the aerobic metabolism of these bacteria. The total number of aerobic mesophilic bacteria in the cucumber samples analyzed ranged from 3.71 to 4.16 log CFU/g, with the highest values being recorded in cucumbers sold on the market. As regards the qualitative analysis, in sample I of cucumbers it was found that most of the microorganisms belong to microaerophilic bacteria of the genus *Pediococcus*, with the presence of bacteria of the genus *Leuconostoc*, but also *Listeria* and *Enterobacteriaceae* (Figure 4).

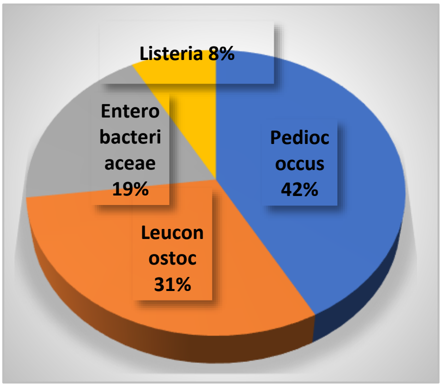

Figure 4. Percentage distribution of germs isolated from supermarket cucumbers

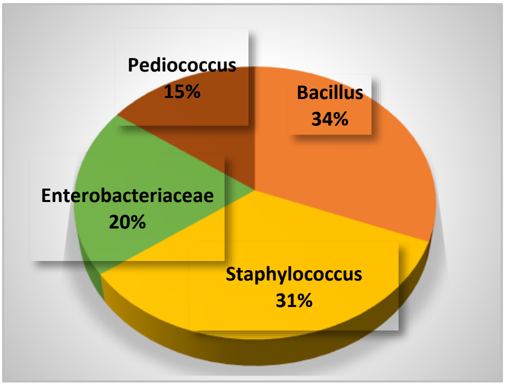

Figure 5. Percentage distribution of germs isolated from market cucumbers

In the market cucumber sample, it was found that the number of mesophilic bacteria was much higher compared to the supermarket cucumber sample. This can be explained on the one hand by the contact with the microaerophlora in the storage and marketing premises, but also by human vectors that can transmit the microorganisms present on the teguments. With regard to the type of microorganisms present in this sample, the genus *Staphylococcus*, the genus *Bacillus* and bacteria of the family *Enterobacteriaceae*. Bacteria of the genus *Pediococcus* were also isolated, but in a much lower percentage (Figure 5). In the supermarket tomato sample, the lowest load of aerobic mesophilic germs was found, 1.54 log CFU/g. This was represented by aerobic and facultatively anaerobic bacteria, i.e. *Bacillus, Staphylococcus* and *Pediococcus* (Figure 6).

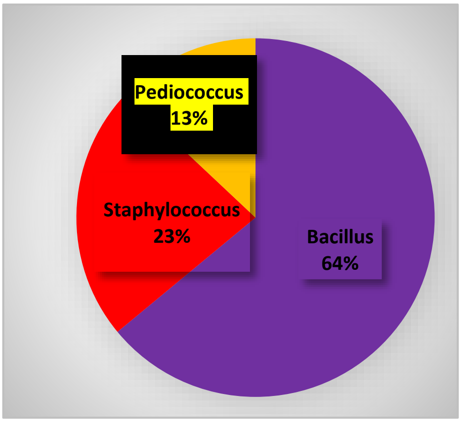

Figure 6. Percentage distribution of sprouts isolated from supermarket tomatoes

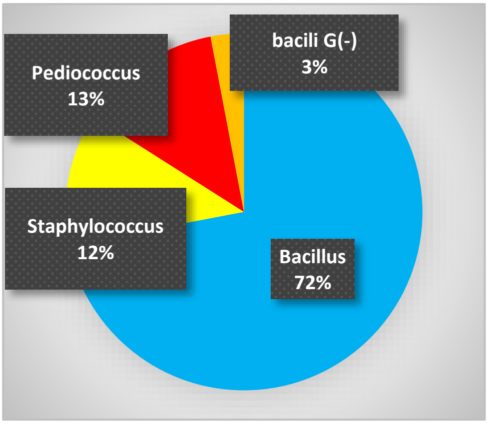

Figure 7. Percentage distribution of sprouts isolated from market tomatoes

In tomatoes sold in the market, the number of aerobic mesophilic bacteria was found to be much higher compared to those sold in the supermarket. However, there were no major differences in the type of microorganisms present in these samples, with *Bacillus* bacteria predominating (Fig. 7). The highest number of aerobic mesophilic bacteria was found in the lettuce samples. The percentage distribution of aerobic mesophilic bacteria is shown in Figures 8 and 9.

After 7 days of storage by refrigeration of the samples taken in the study were determined again, the results obtained being presented in Figure 10. As can be seen from the above figure, as in the initial analysis, the highest number of aerobic mesophilic bacteria was present in lettuce samples and the lowest in tomatoes. In all analyzed samples, an increase in the number of aerobic mesophilic bacteria was observed after 7 days of storage (Fig.11). From the presented data it can be observed that the number of aerobic mesophilic germs increased in all analyzed samples.

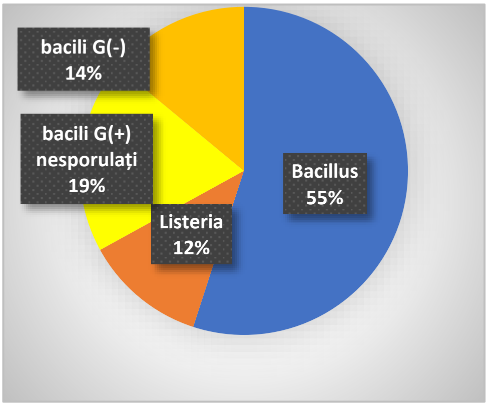

Figure 8. Percentage distribution of germs isolates from supermarket lettuce

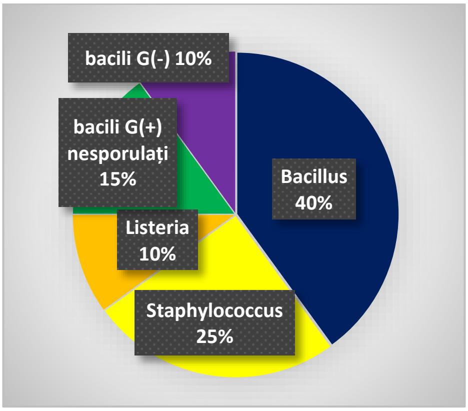

Figure 9. Percentage distribution of germs isolates from market lettuce

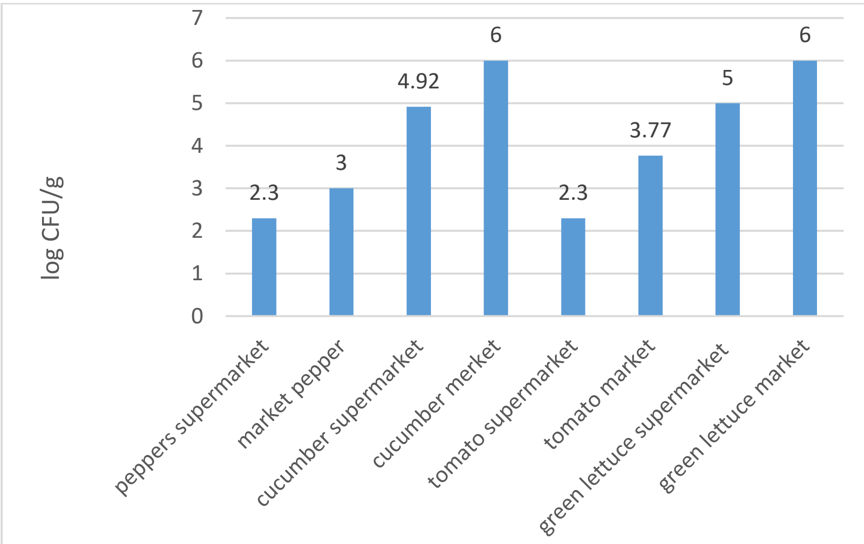

Figure 10. Graphical representation of the total number of aerobic mesophilic germs after 7 days of storage in the studied vegetable samples (log UFC/g)

The highest increases were observed in cucumbers (1.21 log UFC/g in cucumbers sold in supermarket system and 1.84 log CFU/g in cucumbers sold in the market) and lettuce (1.36 log CFU/g in lettuce sold in supermarket system and 1.38 log UFC/g in lettuce sold in the market). This can be explained by the rough surface in cucumber, which allows the installation and multiplication of microorganisms, while in lettuce we can talk about a large leaf surface and soil contact, which in lettuce is much higher compared to the rest of the analyzed samples.

The smallest increases were found in tomatoes commercialized in the market system (0.77 log UFC/g) and peppers commercialized in the supermarket system (0.7 log UFC/g). The shiny surface of tomatoes and peppers prevents the growth of microorganisms. Therefore, in all the analyzed cases, the drying of the vegetables led to an increase in the number of bacteria, which implicitly leads to the deterioration of these products.

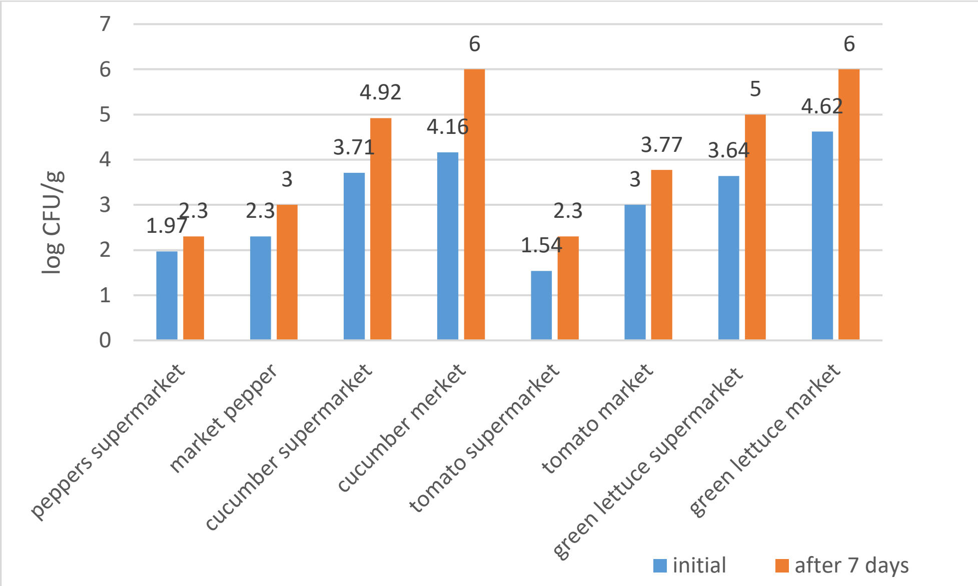

Figure 11. Graphical representation of the total number of aerobic mesophilic germs, initially and after 7 days of storage, in the studied vegetable samples (log CFU/g)

CONCLUSIONS

The global production and consumption of fresh vegetables has increased over the last three decades, at the same time, foodborne outbreaks linked to fresh vegetables have been reported in higher numbers;

The pathogens most implicated in these vegetable-related outbreaks include *Norovirus*, *Salmonella* spp., *Escherichia coli* and *Shigella* spp. To minimize these vegetable-related outbreaks internationally, guidelines such as those from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have been developed to prevent or control conditions or factors that lead to microbial contamination, survival or growth along the "farm to fork" route.

The microflora of vegetables is very rich in both soil-derived and microaerophilic germs. To this autochthonous microflora must be added the microflora of association from human vectors or from storage.

The highest load of aerobic mesophilic bacteria was found in lettuce and cucumber traded under market conditions.

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