Survey of microbial quality of plant-based foods served in restaurants

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A B S T R A C T

This study was carried out to evaluate the microbiological quality of plant-based foods obtained from foodservice establishments. The samples included cereals, legumes, fruits and vegetables. According to the European Commission Regulation (No. 2073/2005 and No. 1441/2007) and Spanish microbiological criteria (No. 3484/2000), vegetables were the plant-based dishes where more samples exceed the adopted limits of mesophilic aerobic counts and Enterobacteriaceae. Furthermore, Staphylococcus aureus and Escherichia coli were also found in several vegetable dishes. E. coli and Salmonella spp. were detected in 6.6% and 0.7% of lettuce samples, respectively. However, all the samples were negative for Listeria monocytogenes. Salads ingredients are eaten as fresh vegetables without cooking processes and lettuce was one of the most contaminated samples. As ready-to-eat salads which have been previously cleaned, this means that contamination might be caused by incorrect cleaning practices or inadequate hygienic handling. These results emphasize the necessity to apply and to maintain good hygienic practices in the restaurants.

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1. Introduction

The consumption of fruits and vegetables becomes a global priority, as well as it is vital in a healthy diet. The daily fruit and vegetable intake of at least 400 g is recommended by the World Health Organization of the United Nations (WHO, 2003). It is known that Mediterranean diet is characterized by abundant plant foods, so Spanish population consumes a high quantity of plant-based foods (Willett et al., 1995).

The mean consumption in a year of cereals, legumes, fruits and vegetables is 7.7, 3.2, 102.2 and 10.2 kg, respectively (MARM, 2011). Since these products are often consumed it is essential to guarantee food safety to prevent consumer’s diseases.

Foodborne disease has emerged as an important and growing public health and economic problem in many countries during the last decades. A wide range of foods can be involved as sources of foodborne diseases in restaurants because of incorrect food processing operations, including plant-based foods. The infection agent may be directly or by cross-contamination transferred to food, so hygienic food preparation and education of those involved in preparation, processing and serving of meals are crucial lines of defense in the prevention of most types of foodborne illness.

Microbiological control allows for identifying, evaluating and preventing contamination risks to the end consumer, through food products, and it is essential to guarantee food safety.

There are few reported incidents of food poisoning resulting from contaminated cereals and legumes. Unprocessed products (grains and flours) may contain high bacteria levels, however this contamination is low in processed and end products (Berghofer, Hocking, Miskelly, & Jansson, 2003). The process of cooking should kill the bacteria but some bad practices of handling or storage can even increase the bacterial load of the initial product.

Generally, heat-treated foods have a good safety record, cooked foods are subjected to heat treatment and when these processes are properly applied, heat can eliminate biological agents that spoil or compromise food safety. The applied treatment factors (time/temperature regime) can vary to accomplish almost any degree of microbial inactivation, ranging from limited reductions of microbial load to complete sterilization (Juneja & Novak, 2003; Rajkovic, Smigic, & Devlieghere, 2010; Yousef & Courtney, 2003). However, it is essential that fresh food be produced safe. Good agricultural practices should be applied during growing, harvesting, sorting, packaging, and storage of fresh fruits and vegetables. It is important to prevent microbial contamination. Prevention is the first and most important component of a food safety program. Because contamination may occurs at any point along the farm-to-fork continuum. Most human pathogens associated with consumption of fresh fruits and vegetables are transmitted via the fecal-oral
pathway. Most often, it occurs when an infected individual handles food without properly washing his/her hands (Barinas et al., 2010, pp. 1–7).

In a botanical definition, the term "vegetables" refers to edible part of a plant. This definition includes fleshy fruits and dry fruits such as cereals, tubers, legumes and pulses. However, the definitions of fruits and vegetables vary considerably between countries and dietary guidelines. In spite of the precision of botanical definitions, culinary definitions are commonly preferred. In order to perform this work, samples of plant-based foods collected from restaurants were divided in four groups, cereals, legumes, fruits and vegetables, the latter including edible part of plants commonly considered as vegetables, as well fresh green pulses and sprouts, fresh sweet corn, potatoes, botanical fruits used as vegetables such as tomatoes, peppers, cucumbers or eggplants, as well as mushrooms.

The aim of this work is to evaluate the microbiological quality of plant-based food dishes of the most consumed products in Spanish restaurants.

2. Material and methods

2.1. Food samples

Food samples were purchased over a period of three years from restaurants in Valencia (Spain). Analyzed products include raw and processed plants-based foods that are commonly consumed in these establishments (Mendonça, Sospedra, Sanchis, Mañes, & Soriano, 2011).

2.2. Sampling plan

A total of 781 batches of plant-based dishes were collected randomly and aseptically in sterile bags and bottles (VWR Int. Eurolab, Barcelona, Spain) (555 vegetables–, 161 cereals–, 43 legumes– and 22 fruit-based dishes). A fundamental principle of sample acceptance sampling plans is that the samples collected will reflect the batch as a whole. For this reason, it is critical that the samples be collected at various points throughout the entire lot. Immediately after collection, samples were chilled to 4 °C and transported to the laboratory for analysis. The microbiological analysis was done on the same day.

2.3. Legislation

The microbial quality of the food was evaluated attending to the specifications of the Spanish Regulation No. 3484/2000 (Anonymous, 2001), Commission Regulation No. 2073/2005 (European Union, 2005) and Commission Regulation No. 1441/2007 (European Union, 2007). According to these legislations and ICMSF (1986), two-class attributes plan is preferred when the microorganism of concern is not permitted in the food (Listeria monocytogenes, and Salmonella spp.). However, if the number of microorganisms must be counted (aerobic plate counts, Enterobacteriaceae, Staphylococcus aureus, Escherichia coli), a three-class attributes plan is adopted.

2.4. Sample preparation

25 g or 25 ml of each of the samples collected were suspended in 225 ml of buffered peptone water (BPW) (Oxoid, Unipath, Hampshire, United Kingdom) and aseptically homogenized in a stomacher (Classic; JUL, Barcelona, Spain). Serial decimal dilutions were prepared from this initial dilution. Each of the different dilutions (1 ml or 0.1 ml) was transferred onto the surface of the plates containing appropriate cultures media for each analyzed microorganism.

2.5. Microbiological analyses

Four 10-fold dilutions were made for each sample. 1 ml of each step was inoculated into duplicate plates of standard Plate Cont Agar (PCA) (Oxoid) and incubated at 30°C ± 1 °C for 72 ± 3 h, according to the International Organization for Standardization (ISO, 2003) to determine the aerobic plate counts (APC).

Enterobacteriaceae were determined according to the ISO 21528-2 (2004), using duplicate poured plates of Violet Red Bile Glucose agar (VRBG) (Oxoid). The plates were incubated at 37 °C for 24 h and typical colonies were counted on all plates having not more than 150 typical colonies.

Attending to ISO 16649-2 (2001), to isolate E. coli, the previous BPW tubes were inoculated onto CHROMagar ECC (CHROMagar Microbiology, Paris, France). After incubation at 37 °C for 48 h, colonies were confirmed using Rapid ONE System (REMEL Inc. Santa 108 Fe, USA).

For enumeration of S. aureus, a 0.1 ml of the inoculated BPW was surface plated on Baird-Parker agar containing egg-yolk tellurite emulsion (Oxoid), and incubated at 37 °C ± 1 °C for 24 + 24 h (1999). Typical colonies (i.e., black, convex and with or without halo on BP agar) were counted and examined microscopically. Colonies were also tested for catalase reaction and confirmed with agglutination Staphytest Plus test (Oxoid).

Isolation and identification of Salmonella spp. was done according to the ISO 6579 (2002) and performed using the homogenate in BPW. Quantities of 1 and 0.1 ml of the BPW were inoculated into Tetrathionate broth with Novobiocin (Oxoid) and Rappaport-Vassiliadis broth (Oxoid), respectively. The enrichment broths were incubated for 24 ± 2 h respectively at 37°C ± 1 °C (for TTn broth) and 42 °C (for RV broth). The positive cultures were streaked onto XLD Salmonella agar (Oxoid) at 37 °C ± 1 °C for 24 h, and the confirmation was done using the Rapid ONE System (REMEL Inc. Santa Fe, USA). L. monocytogenes was detected according to the ISO 11290-1 (1996). Samples (25 g) were weighed into sterile stomacher bags, diluted and homogenized with 225 ml of Fraser broth (Oxoid). After homogenizing and preculturing at 37°C ± 1 °C for 48 ± 2 h, the positive broth was streaked onto Listeria Palcam agar (Oxoid) and incubated at 37°C ± 1 °C for 24 ± 2 h. Characteristic colonies were Gram stained, tested for motility, oxidase and catalase activity followed by identification with the API Listeria system (BioMérieux, Mancy l’Etoile, France).

3. Results and discussion

3.1. Cereals

Samples collected were divided into 2 types, wheat and rice-based dishes, according to their main component. Results obtained reflect that only 12 out 102 wheat samples were above legal limits for aerobic colony count. In the case of Enterobacteriaceae, 40 wheat-based dishes presented an unacceptable quality (Table 1).

Most wheat samples were pasta dishes cooked as salads and some with sauces as meat with tomato or with milk cream. Macaroni and spaghetti with tomato sauce were the pasta dishes most consumed and also most contaminated (8 out 12were unacceptable for total aerobic mesophilic bacteria and 23 out 40 for Enterobacteriaceae), followed by pasta with milk cream and salad pasta. Our results are according to other studies (Mensah, Yeboah-Manu, Owusu-Darko, & Ablordey, 2002; Yeboah-Manu, Kpeli, Akyeh, & Bimi, 2010) about ready-to-eat pasta dishes. Yeboah-Manu et al. (2010) studied the bacterial quality of ready-to-eat
foods sold on and around the university of Ghana campus. They found macaroni samples had very high levels of bacterium. Possibly, it could be because of the steps of cooking process. Macaroni are prepared by boiling in hot water for few minutes. After that, macaroni are drained from the water. In this point, though the water is drained from the food, it still has a moist appearance and this provides a good environment for bacterial growth. Although pasta is an excellent growth medium for bacteria, these sauces can contribute and increase the level of growth. Possibly, it could be because of the steps of cooking process. After that, macaroni are drained from the water. In this point, though the water is drained from the food, it still has a moist appearance and this provides a good environment for bacterial growth. Although pasta is an excellent growth medium for bacteria, these sauces can contribute and increase the level of growth. Possibly, it could be because of the steps of cooking process.

Table 1 Microbial quality, according to the European and Spanish legislations, for total aerobic mesophilic bacteria and Enterobacteriaceae in cereal, legumes and fruit dishes samples collected from studied foodservice establishments.

<table>
<thead>
<tr>
<th>Source (no. of lots)</th>
<th>Microbial quality (%)</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total aerobic mesophilic bacteria</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Cereals</td>
<td>Wheat (n = 102)</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Rice (n = 59)</td>
<td>49</td>
</tr>
<tr>
<td>Legumes</td>
<td>Beans (n = 14)</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Lentils (n = 21)</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Chickpeas (n = 8)</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Melon (n = 4)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Coconut (n = 5)</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Baked Apple (n = 9)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Others (n = 4)</td>
<td>100</td>
</tr>
</tbody>
</table>

According to other studies (ACMSF, 2000; McMeekin et al., 1997) long time between preparation and consumption or an inappropriate storage temperature are critical control points that can contribute to food contamination.

3.3. Fruits

To prevent or reduce risk of foodborne illness or injection by contaminated fruits an important step is to wash all raw fruits before serving or combining with other ingredients. Usually, fresh fruits are served whole and uncut. For this reason unpeeled fresh fruits have a smaller microbial risk than fruits served cut into pieces or fruits that have undergone heat treatment or other processing to make other products such as juices. The samples collected were peeled or cut fruit and also fruits treated to cooking were analyzed. “Cut product” is defined as “Any product where the intact protective surfaces of the plant have been breached or removed”. The process of peeling could have some potential consequences; it could remove the pathogen if it is present on the outside of the fruit but only if the process is carried out in a proper hygienic manner.

It could give the pathogen access to the nutrients available on and from the inside of the fruit. It can lead to multiplication of certain pathogens during storage. It can spread the pathogen from contaminated to uncontaminated product as a result of inappropriate hygiene of large batches of the product during processing (ACMSF, 2000). The results reflect a good fruit quality for aerobic colony count. We have not found any fruit sample contaminated at
unacceptable levels by these microorganisms (Table 1). However, some samples exceeded legislated levels for Enterobacteriaceae. Even so some reports about outbreaks associated with fruit products have shown that fruits such as melon or cantaloupe can act as a vehicle for food-borne pathogens as Salmonella (CDC, 2002; Institute of Food Technologists, 2001; Sivaplasingham, Friedman, Cohen, & Tauxe, 1997), fortunately, none of the fruit samples analyzed was contaminated by pathogens as E. coli, S. aureus, Salmonella spp. or L. monocytogenes. Wilson and Mackenzie (1955) also reported typhoid fever and salmonellosis illness in Australia caused by the ingestion of contaminated coconut. According to them, other authors have also shown coconut as cause of salmonellosis in England (Ward, Duckworth, & O’Brien, 1999).

3.4. Vegetables

Out of 555 samples analyzed, about 13% had unsatisfactory levels for total aerobic mesophilic bacteria and 23% for Enterobacteriaceae (Table 1). However, despite this expected frequency of contamination, high levels of microorganisms found indicate poor handling practices.

According to the results reported by several reviews (Heaton & Jones, 2008; Little & Gillespie, 2008) consumption of fresh fruits and vegetables could be a risk factor for infection with enteric pathogens. Recent examples of outbreaks related to fresh produce include cases of E. coli (spinach, lettuce) and many species of Salmonella. In 2008 a nationwide outbreak of Salmonella Enterica occurred in Finland. A total of 77 culture-con

<table>
<thead>
<tr>
<th>Source (no. of lots)</th>
<th>Microbial quality (%)</th>
<th>Total aerobic mesophilic bacteria</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acceptable</td>
<td>Marginal</td>
<td>Unacceptable</td>
</tr>
<tr>
<td>Lettuce (n = 137)</td>
<td>70</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Tomato (n = 77)</td>
<td>80</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>French beans (n = 72)</td>
<td>89</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Potatoes (n = 80)</td>
<td>86</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Zucchini (n = 44)</td>
<td>91</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Pepper (n = 30)</td>
<td>73</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Green peas (n = 28)</td>
<td>96</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Cauliflower (n = 21)</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Spinaches (n = 11)</td>
<td>82</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Broccoli (n = 10)</td>
<td>30</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Eggplant (n = 10)</td>
<td>60</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Mushrooms (n = 10)</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Onion (n = 9)</td>
<td>78</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Lima beans (n = 6)</td>
<td>83</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Artichokes (n = 5)</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Corn (n = 5)</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2
Microbial quality according to the European and Spanish legislations, for total aerobic mesophilic bacteria and Enterobacteriaceae in vegetable samples collected from studied foodservice establishments.

In the harvest, transport and/or storage of foods, bacteria can adhere and also propagate on the surface of the foods. Bacteria could adversely affect consumers’ health and it should therefore be removed from the surface of foods before ingesting. It has long been a practice to wash foods such as vegetables and fruits with chlorinated water, a saline solution or appropriate detergents to remove bacteria. In restaurant establishments studied, 14 samples, including both cooked and fresh vegetables (11 lettuce, 2 French beans and 1 potato) were found to be contaminated by S. aureus.

In the literature, some comparisons between raw and cleaned vegetables can be found. Soriano, Rico, Moltó, and Mañes (2001b) studied microbial contamination in raw and “ready-to-eat” lettuces and they found the same percentage of samples contaminated by S. aureus in both products. Ready-to-eat salads have been previously cleaned and it means that contamination might be caused by incorrect cleaning practices or inadequate hygienic handling. The lettuce samples could be contaminated by these reasons. Considering that French beans and potato meals are cooked dishes, it is more likely that contamination occurred after food processing. Cross contamination is reported by a contributory factor outbreak associated with the consumption of fruits and vegetables. Infected food handlers are often implicated in outbreaks of known or suspected viral or bacterial etiology and might well have been the cause of many of these outbreaks. There are some cases of foodborne diseases in which the food handling faults have contributed to food contamination. It is recorded in one outbreaks report from England and Wales that a restaurant chef was suddenly taken ill while preparing a meal. He vomited over the salad he was preparing. He rinsed the salad in cold water and it was then served to the customers. Many of these customers subsequently became ill. In other outbreak cause by E. coli, restaurant staff revealed that the salad items were stored in plastic containers prior to serving. These containers were also used for storing raw beef and were rinsed out before re-using for salad vegetables (ACMSF, 2000).
4. Conclusions

In percentage, regarding to mesophilic aerobic counts, vegetables are the most contaminated products, mainly broccoli (50%) and eggplant (40%). Cereals-based (rice; 27%) dishes are the following products with high percentage of unacceptable microbial quality. Furthermore, attending to Enterobacteriaceae microbial quality, broccoli-based dishes are the most contaminated (70%).

On the other hand, S. aureus and E. coli were only found in several vegetable dishes (lettuce, French beans and potato in a range from 1.2 to 8%). E. coli and Salmonella spp. were detected in 6.6% and 0.7% of lettuce samples, respectively. L. monocytogenes was not found in any plant-based sample. Salads ingredients are eaten as fresh vegetables without cooking processes and lettuce was one of the most contaminated samples. Because of ready-to-eat salads have been previously cleaned, this means that contamination might be caused by incorrect cleaning practices or inadequate hygienic handling. The presence of some studied microorganisms has shown that several handling practices require more attention, as is the incidence of S. aureus which indicates cross-contamination. For this reason, our results emphasize the importance of strict hygiene during handling practices in order to avoid contamination of the food products.

References

- Berghofer, L. K., Hocking, A. D., Miskelly, D., & Jansson, E. (2003). Microbiology of fresh vegetables without cooking processes and lettuce was one of the most contaminated products, mainly broccoli (50%) and eggplant (40%). Cereals-based (rice; 27%) dishes are the following products with high percentage of unacceptable microbial quality.