SURVEY OF BIOLOGICAL HAZARDS IN THE LETTUCE USED IN COMMERCIAL SNACKS (SANDWICHES) FROM CURITIBA, PR, BRAZIL

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ABSTRACT: Over the last decades, lettuce has been associated to several foodborne outbreaks. The present study surveyed the microbiological and parasitological contamination of green leaf lettuce used in sandwiches sold in snack bars in Curitiba, Paraná, Brazil. Lettuce was collected before and after commercial washing. Such procedure was made by holding and rotating the whole lettuce or separated leaves under the faucet of a sink with cold water for 10-30s. Three samples of 25g of lettuce leaves from each snack bar were assessed for mesophilic aerobic bacteria, molds and yeasts, Escherichia coli, incidence (%) of general parasites, Salmonella spp, Staphylococcus aureus. The obtained mean counts, expressed as Colony Forming Units per gram of lettuce, were respectively: 5.9 x 10^6; 6.4 x 10^5; 2.1 x 10^5; 73%, for non-washed samples; and 7.3 x 10^4; 5.9 x 10^3; 1.0 x 10^2; 18%, for washed samples. The water washing, as it has been performed in the food establishments investigated, showed to be inefficient for sanitizing lettuce. Though Salmonella and Staphylococcus aureus were not found, the results reflect unsanitary production practices and show that the assessed lettuce has been a source of biological hazard.

KEYWORDS: Microbiology; lettuce; parasitology; sandwiches; hamburgers.

INTRODUCTION

Consumption of meals prepared away from home have been increasing worldwide. Among the two hundred primary food commodities officially reported last year, the lettuce is included. It has been consumed mainly raw, as salads.9 Thus, identifying the biological risks associated to the consumption of lettuce-added commercial meal is an important step to avoid foodborne illnesses.

Lettuce contamination can be minimized by using safe practices during the whole production chain, i.e., harvesting, transportation, processing and consumption. Food safety public authorities should work as guiders for lettuce producers towards its safety assurance in order to avoid outbreaks.25 Sandwiches containing lettuce are widespread prepared foods. At some extent, their safety-for-consumption assessment on food service level can be made by evaluating microorganisms and parasites in lettuce handlers, in the washing water and in the lettuce itself.26 Assessment of lettuce sanitation has been usually made by performing microbiological and parasitological evaluation, mostly Salmonella spp, Staphylococcus aureus, mesophilic aerobic bacteria, molds and yeasts and Escherichia coli counts and checks for generic parasites.17, 19, 23, 32

The presence of Salmonellas in food suggests that it is unhygienic, since such bacteria are frequently found in animal and human feces. Furthermore, food contaminated by Salmonellas usually represents a biological hazard, since the vast majority of human isolates are disease-causing.8 The foodborne pathogens responsible for production of toxins in foods include Clostridium botulinum and Staphylococcus aureus. The latter includes strains capable of both infection and intoxication.10 Aerobic mesophilic organisms in food can infer its microbioblogical contamination and usually indicate that there are favorable conditions for microbial multiplication. For various reasons, this parameter is useful to indicate if cleaning and disinfection during the production chain have been performed sufficiently.5 Mesophilic aerobic bacteria are frequent and prevalent microbes in lettuce. Together with small yeasts, they represented the majority of the total microbial flora of fresh-aseptically cut lettuce.25 There are favorable conditions for growth of mesophilic aerobic bacteria when lettuce leaves are added to hot sandwiches, where their temperature reaches the optimum growth range for mesophiles, i.e., from 34 to 38°C.15 Molds and yeasts are fungal microorganisms widespread in the environment. Frequent sources include air streams, soil, water, insects, animals and decaying organic matter. In food, the molds and yeasts count provide several information about the way it was handled along the production chain, e.g. the hygienic conditions of the appliances used.21

Escherichia coli (E. coli) are part of the group of fecal coliforms and inhabit exclusively animals and humans’ intestines. For this reason, these species of bacteria are commonly used as an indicator of sanitary hygiene of food establishments.14 Recent foodborne outbreaks have linked infection by E. coli to the consumption of contaminated lettuce. Contamination via food handler error and on-the-farm contamination are thought to be responsible for
several outbreaks.3 The presence of E. coli in food is also evidence that it might be a source of parasitological risk. In fact, it seems to exist a direct relationship between the presence of E. coli and parasites in leafy vegetables. A recent study showed that among thirteen farms studied, all of them presented leafy vegetables infected by parasites and, at the same time, by fecal coliforms, such as E. coli.

Previous researches have been evidencing that raw lettuce is a frequent source of biological hazards. Therefore, the ingestion of sandwiches containing lettuce is a risk for a broad consumer public. Although, surveys of the biological safety of lettuce used by retailers in the preparation of snacks have not been found. In this report, we obtain a microbiological and parasitological profile of the lettuce used by retailers in the preparation of sandwiches in Curitiba, Brazil.

MATERIAL AND METHODS

Raw Materials and Sampling

The study was carried out in snack bars around the Campus III of the Federal University of Paraná (UFPR). The UFPR is located in Curitiba, a city in Southern Brazil inhabited by around 1.8 million people. Samples of green leaf lettuce (Lactuca sativa L., Verônica variety) were collected in eleven randomly chosen snack bars (coded as B1, B2, ..., B11) located in Jardim das Américas district, which hosts the Polytechnical Center of that institution. Analyses were performed at the Labs of the Food Processing Research Center (CEPPA) and Health Sciences Dept. Six 25g samples of lettuce were collected in each snack bar: three of them before and three of them right after water washing, which is a fixed procedure in sandwiches preparation at the snack bars evaluated. The non-washed samples can be compared to lettuce usually sold in vegetable markets. The washed samples can be compared to ready-to-eat lettuce which is added to commercial meals like hamburgers and salads. The water washing was made by holding and rotating the whole lettuce, or separated leaves, under the faucet of a food service sink with cold water, for ten to thirty seconds, depending on the respective snack bar. Once collected, each sample was immediately transported to the lab in a sterile 23 x 15 cm zip-lock polyethylene bag, which was properly labeled with a sample code, date and time of collection, according to the procedures recommended by Castro & Pouzada.9

Microbiological Analysis

Lettuce samples were prepared for the microbiological assessment according to the standards recommended by APHA.2 The detailed procedure was as follows: three non-washed and three washed lettuce samples were transferred to a flask containing 225mL of 0.1% peptonated water and the mixture was homogenized in a stomacher® 400 circulator (Seward, West Sussex, United Kingdom) homogenizer for 30s. The resultant solution was immediately diluted 1000-fold with water and used in the inoculation of hydrated Petrifilm™ (3M, Saint Paul, USA) plates. To quantify microorganisms that expressed microbiological contamination, two different methods were used. A method reported by APHA2 for quantifying Salmonella spp was used. The quantification of Staphylococcus aureus, mesophilic aerobic bacteria, molds and yeasts and Escherichia coli followed the methods of AOAC.3 Microbiological counts were expressed in Colony Forming Units per gram of material (CFU/g).

Since lettuce was sampled in triplicate, mean values of the microbiological counts were generated. Analysis of variance (ANOVA) was applied to these means in order to identify if there were statistically significant differences from one sample to another regarding their microbiological counts. When ANOVA pointed out differences between means, the Tukey’s Test of Means was performed in order to identify which lettuce samples were more or less contaminated than the others. The 5% statistical confidence level was used.

Parasitological Analysis

Evaluations of the presence of parasites in the lettuce samples studied were carried out on the basis of a method related by Rey.29 The detailed procedure was as follows: Four grams of lettuce were exhausted with 100mL of distilled water. The resultant mixture was transferred through a piece of gauze to a decantation cup and the filtered solution was left immovable for two hours. Then, the sediment was removed with a pipette, transferred to slides, added with two drops of Lugol’s solution and analyzed in an optical microscope with a 10x magnification objective lens in order to identify the presence of parasites eggs. In case one or more eggs were visually detected, the lettuce sample was regarded as infected by parasites.

RESULTS AND DISCUSSION

The absence of Salmonella spp in raw vegetables is a current requirement of the Brazilian law.7 Both non-washed and washed raw lettuce was found to be free of all Salmonella. Such results suggest that the lettuce hereby evaluated did not have contact with feces, where such bacteria is frequently found, but it would be better to check the result of the tests for E. coli, a fecal bacteria, before making a conclusion on this issue. Previous studies, namely Johannessen et al.22 and Nascimento et al.27 did not detect Salmonella in raw lettuce either. More recently, barely significant incidences of these bacteria – ranging from 0.3%6 to 1.5%30 have been detected in the same food. Another bacteria surveyed hereby, Staphylococcus aureus, was not detected in any of the samples (non-washed or washed). This results was not expected, because masks and gloves, which represent a physical barrier for this nose-and-skin-inhabiting bacteria, were not wore by lettuce handlers of the snack bars evaluated. Mesophilic aerobic bacteria counts, frequently used as an indication of a food sanitary quality, are showed in Figure 1. The non-washed lettuce mean count was 5.9 x 106 CFU/g, which is within the range observed by Goulart27 and slightly below the 8.7 x 106 CFU/g level observed by Nascimento et al.27
Concomitantly, results were above that detected by Aycicek et al., i.e., $1.4 \times 10^6$ CFU/g. Regarding washed lettuce, mean count of mesophilic aerobic bacteria was $7.3 \times 10^5$ CFU/g. Therefore, the average decrease seen due to the water washing was of $5.8 \times 10^2$ CFU/g. This value is greater than the minimal reduction ($2.5 \times 10^1$ CFU/g) obtained by Smith et al. after washing one third of a lettuce head with running water for a period of 20s. As confirmed by Figure 2, the non-washed samples molds and yeasts mean count was $6.4 \times 10^3$ CFU/g. When compared to non-washed lettuce previously evaluated, such value is far below the $4.0 \times 10^5$ CFU/g level observed by Nascimento et al. for Brazilian samples, but far above the $8.0 \times 10^0$ CFU/g level and the $1.3 \times 10^1$ CFU/g observed by Ongeng et al. and Gómez-López et al. for Belgian samples, respectively. On initial consideration, such a huge difference between foreigner and local lettuce regarding molds and yeasts content seem to be a clear indication that the production chain abroad is the most hygienic. Although, it must be taken into account that the European authors analyzed samples of iceberg lettuce. Such sort of lettuce present inner leaves that clearly seem to be more protected against contamination by molds and yeasts from the environment due to its head-like shape, when compared to leafy lettuce, which constitute the majority of the varieties consumed in Brazil. With regard to the washed lettuce, molds and yeasts mean count was $5.9 \times 10^3$ CFU/g. Other authors, namely Jacxens et al. found a similar amount of yeasts ($6.3 \times 10^3$ CFU/g) in a mixture of minimally processed endive, curled endive, radicchio lettuce, red and green leafy lettuce (20% of each vegetable) commercially available in Belgium. Similar levels ($4 \times 10^4$ CFU/g) were detected by Abadias et al. in a ready-to-eat mixed salad made from different varieties of lettuce, including both leafy and iceberg types.

On the other hand, authors that assessed washed iceberg lettuce detected a much lower level of molds and/or yeasts. Gómez-López et al. detected a level of $1.3 \times 10^0$ CFU/g of yeasts after cutting and immersing iceberg lettuce in tap water for one minute. Ongeng et al. found a small content of molds and yeasts ($4.0 \times 10^0$ CFU/g) in iceberg lettuce after washing it in tap water for one minute. The same tendency ($9.3 \times 10^0$ CFU/g) was observed by Gómez-López et al. after washing pieces of iceberg lettuce in tap water for one minute. This group of results reinforce the hypothesis mentioned above: leafy varieties of lettuce, such as Verônica, present a shape that makes them more exposed to contamination from the environment than the iceberg varieties of lettuce. Regarding the reduction in molds and yeasts count observed after water washing, the maximum levels achieved were of $1.5 \times 10^1$ CFU/g. Results indicate a far less severe reduction by washing than that observed by Berbari et al. of $8.0 \times 10^3$ CFU/g, suggesting that the procedures observed in the studied food establishments were not efficient. It is important to mention that not even the well-known sanitizing properties of the chlorine contained in tap water were able to promote a satisfying reduction in molds and yeasts count. Such result was expected, since the washing treatments applied to lettuce in the snack bars studied were carried out during 30s with running tap water, which usually presents a chlorine level of 4mg/L at most, and authors like Berbari et al. have showed that it is necessary to immerse lettuce leaves for at least 15min in solutions of 100mg/L of chlorine, to bring numbers of molds and yeasts below $1.0 \times 10^2$ CFU/g, which represent a good level of microbiological hygiene.

With concern to E. coli counts, Figure 3 shows the comparison between non-washed and washed samples. Units of E. coli were found in 100% of the samples. Though
the absence of Salmonella spp, reported above, have suggested that lettuce samples had not had contact with feces, the 100% incidence of E. coli observed confirms the opposite. Therefore, the practices used in lettuce production chain that preceded the water washing step at food service level were somehow unhygienic. Besides the hygiene abnormality, samples hereby assessed could also be a source of biological hazards, since some strains of E. coli are pathogenic. Aycicek et al. found out that 11.7% of the samples of non-washed lettuce contained E. coli. Abadias et al. did not detect any E. coli in 25g of of non-washed leafy lettuce of several different varieties. In this study, the level of E. coli was statistically similar in all of the non-washed samples. The observed mean count was 2.1 x 10^2 CFU/g. Washed lettuce, on the other hand, presented statistically higher levels of E. coli in snack bars B6 and B10 than in the others, except from those collected in B11, which were similar (Figure 3). Furthermore, the levels observed in washed samples collected in snack bars B6, B10 and B11 were above the recommended level suggested by the Brazil Health Surveillance Agency, which is of E. coli counts less than or equal to 1.0 x 10^2 CFU/g. Thus, the washing used for sanitizing lettuce in snack bars B6, B10 and B11 was not satisfying as regards removal of these fecal bacteria. It is important to mention, though, that the average decrease in lettuce E. coli content achieved by washing procedures at the assessed snack bars (1.1 x 10^2 CFU/g) was ten times more efficient than the reduction promoted by others authors in the population of Enterobacteriaceae, which includes E. coli, through a washing under similar conditions. Therefore, it can be said that the procedures hereby evaluated were as efficient as a water washing should be. Hence, one or more extra sanitizing processes, such as chemical treatments, should be introduced in the snack bars studied, in order to assure the safety for consumption of the lettuce used in sandwiches preparation. For instance, Kondo et al. studied the effect of different washing/sanitizing treatments in killing native microflora and some foodborne pathogens attached to fresh-cut lettuce. They found that the combination of 200 ppm of sodium hypochlorite and mild heat treatment at 50 degrees C for 1 min reduced the pathogen populations, including pathogenic E. coli, by 94 to 98% without impairing the visual quality of lettuce (due to enzymatic browning). Comparing the incidence, in percentage, of E. coli in the washed lettuce assessed hereby to previous ones, Abadias et al. detected E. coli in only 3.4% of the samples (versus 100% here). With regard to the E. coli level observed in washed samples, 1.0 x 10^2 CFU/g was the mean count. The presence of E. coli in washed lettuce can be related to handling without gloves, which makes food more susceptible to contamination through human excrements. When it comes to lettuce, other possible reasons for contamination by E. coli were pointed out in previous studies. Islam et al. relate that contaminated manure and polluted irrigation water are probable vehicles for pathogens in many outbreaks. In addition, they found that E. coli serotype O157:H7 is capable of persisting in soil for at least five months after application of contaminated compost or irrigation water. Solomon et al. found that cells of E. coli O157:H7 are capable of entering the roots of mature lettuce plants, and can be transported upwards to locations within the edible portions of the plant. Furthermore, the inaccessibility of a large number of organisms, as a consequence of their subsurface location, is said by the authors to be the possible reason for a certain lack of effectiveness of surface-sanitizing treatments.
**FIGURE 3** – *Escherichia coli* count in lettuce used for sandwiches prepared in the snack bars (B1-B11) studied, both before washing (BW) and after washing (AW) with running water. Statistically significant difference between mean counts on 5% level is denoted by different letters.

**FIGURE 4** – Incidence of parasites (%) in lettuce used in sandwiches before washing (BW) and after washing (AW) with running water.
Figure 4 shows the results of the parasitological evaluation carried out in lettuce. Samples collected in all of the snack bars, except from B5, B8 and B11, presented parasites eggs or oocysts before water washing, which represent an incidence of 73%. Such value is slightly lower than the 78% incidence observed by Falavigna et al.11 in samples harvested in the same state (Paraná), but much higher than the incidence of 15% observed by Takayanagui et al.32 in samples harvested in another Brazilian state. Here, the majority of the parasites eggs and oocysts found were identified as members of the family Ancylostomatidae (hookworms). Such result confirms the previous findings of Falavigna et al.11 for raw lettuce. Ascaris lumbricoides, which was the species most detected hereby, are found in insufficiently treated sewage-fertilizer and in soils where they embryonate. Therefore, the target population, who is subjected to contract the illness called Ascariasis, is composed particularly by consumers of uncooked vegetables and fruits grown in or near soil fertilized with sewage. In addition, infected food handlers may contaminate a wide variety of foods.34 Almost the totality of washed samples were freed of worms – two snack bars, B7 and B10, still contained them, which gives an after-washing-incidence of 18%.

CONCLUSION

Results confirm that the production chain of the lettuce used in the preparation of sandwiches at some snack bars of an important Brazilian city needs several improvements with concern to biological safety. Finally, the real data hereby generated can be comprehensively used as a guidance for people involved in lettuce production toward its microbiological and parasitological safety assurance.

REFERENCES


