

Full Length Research Paper

Hygienic and sanitary evaluation of minimally processed vegetables sold in public fairs in the Western Region of Paraná State, Brazil

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The microbiological quality of minimally processed vegetables sold in the public fairs of the numerous towns in the Western Region of Paraná State, Brazil, was studied. Lettuce, salad rocket, cabbage and chicory randomly collected were sent to the Food Analysis Laboratory (Lanali) in Cascavel, Brazil. The vegetables were analyzed for total and thermotolerant coliforms, aerobic mesophilic bacteria and *Salmonella* sp. Total coliforms were detected at different rates in all samples of vegetables analyzed. Cabbages from town 5 had the highest contamination rate (3.6×10^5 UFC/g) and the highest mean contamination rate (4.1×10^4 UFC/g) in terms of thermotolerant coliforms in 147 samples, at different count rates. In all the samples collected and analyzed, *Salmonella* sp. occurred in 10. Results show that vegetables sold in the public fairs in the Western Region of Paraná, Brazil were not good for human consumption due to feces-originated bacteria or high mesophilic counts. In fact, they are the possible sources of toxin and infections caused by pathogens.

Key words: Coliforms, *Salmonella*, vegetables.

INTRODUCTION

The consumption of vegetables by humans is highly relevant for a balanced diet as they contain vitamins, have health benefits and lead subsequently to healthier life style. In fact, their intake does not only reduce the development of disease, but also their fibers regulate the digestive functions of the human body (Germano and Germano, 2003). Vegetables are also a source of vitamins, especially vitamin C, B complex and A (β -carotene); while minerals, such as iron, calcium, potassium and magnesium are highly important components found in them (Philippi, 2003).

The consumption of minimally processed vegetables is highly relevant for the transmission of several infectious diseases especially those caused by irrigation with contaminated water or through the farm workers' lack of hygiene (Takayanagui et al., 2001). Since the culture of vegetables demands a moist environment, the constant irrigation of vegetables mainly leads to the formation and development of pathogenic microorganisms (Germano and Germano, 2003).

Food-transmitted diseases are mainly due to inadequate handling, lack of personal hygiene, dirtiness of the

person who is supposed to clean the places where vegetables are prepared and sold and improper cleaning of the equipment and utensils before and after food manipulation (Vitti et al., 2010).

Since most vegetables may be consumed raw up to seven days after harvest, they may also cause many diseases. In fact, microorganism may be viable for a period between 7 and 40 days and may thus become a source of public health concern (Mogharbel, 2007).

Microbiological evaluations of minimally processed vegetables are required to verify the hygiene and sanitary conditions of food consumed by people. Verifying the presence of certain micro-organisms in vegetables is mandatory within the context of public health (Franco and Landgraf, 2005; Mogharbel, 2007).

MATERIALS AND METHODS

Current investigation was conducted between March and June 2012 at all fair stalls which sold vegetables in five different towns in the western region of Paraná. Sixty samples of all available vegetables, namely, lettuce, salad rocket, cabbage and chicory were collected in triplicates. Specific fairs were selected owing to the population's interest in consuming products originating from these markets. The vegetables were conditioned individually in sterilized plastic bags, identified and sent to the Food Microbiology Laboratory (LANALI) of Cascavel PR, Brazil, for microbiological analysis.

Further, 25 g aliquots of each vegetable collected were retrieved and microbiologically evaluated to determine total and thermotolerant coliform counts (UFC/g), following methodology of Norm 62 of August 2003 (Brasil, 2003).

Total coliforms were counted in Violet Red Bile Agar and the counting of suspected colonies was done at a later phase, according to legislation. They were then inoculated in Brilliant Green Bile Lactose Broth at 2% and incubated (48h/36±1°C) to confirm total coliforms. Thermotolerant coliforms were inoculated in EC broth and tubes were incubated (48h/44.5°C) in a warm bath under stirring (68rpm/min) for confirmatory proof.

In the case of *Salmonella* sp., the materials were pre-heated in buffered peptonated water (24h/35°C) and selectively enriched in Rappaport-Vassiliadis and Selenito-Cistina broth (24h/42°C); they were then isolated in agar XLD and brilliant-green phenol-red lactose sucrose (BPLS). Biochemical identification was confirmed by tests for urease, fermentation of glucose, sucrose and lactose in TSI medium, de-carboxylation of lysine in LIA medium, H₂S production, motility and production of indol by SIM medium.

Total counting of mesophilic micro-organisms was undertaken by serial dilutions in plate count agar (PCA) with plates incubated at 35°C for 24-48 h for reading and counting of viable colonies.

Statistic analysis of variance of data evaluated was done by ANOVA and media comparison by Tukey's test, both at 5% significance. All variables at 5% significance did not have a normal distribution but variance heterogeneity among treatments.

Box-Cox transformation was conducted with these variables to obtain normal distribution and homogeneity of variances. Results were compared with rates given by current ANVISA¹ legislation, according to RDC n. 12 of 2nd January, 2001.

RESULTS AND DISCUSSION

Whereas total coliforms at different rates were reported in

11 samples, thermotolerant coliforms were registered in 147 samples of all the samples, at different rates. *Salmonella* sp. occurred in 10 samples of the total samples evaluated

Cabbages from town 5 registered the highest contamination rate (3.6x10⁶ UFC/g), followed by lettuce, chicory and salad rocket, when total coliform rates in the different vegetables were analyzed.

Analysis of variance for total coliforms at 5% significance showed that interaction was not significant with an influence of vegetables according to their origin, except in the case of cabbages. Results were obtained by Tukey's test of mean multiple comparison (Table 1) with a significant difference of 5% in town 4 compared to the others.

Further, significant differences (5%) were detected among the vegetables from towns 1 and 4. Tukey's test at 5% significance (Table 1) showed that only lettuce in towns 1 and 2 differed from the others. The vegetable group of cabbage and lettuce differed from chicory and salad rocket group in town 3; only lettuce and salad rocket from towns 4 and 5 were statistically different.

Similar results were reported by Silva et al. (2007) when they analyzed the microbiological quality of minimally processed vegetables sold in Porto Alegre RS Brazil. High coliform counts were registered. Only two samples had low total coliform rates. In fact, total coliforms may be very common in food and may originate from several contamination sources such as soil and irrigation water (Mogharbel, 2007).

Analysis of thermotolerant coliforms in different vegetables revealed that cabbages had the highest mean contamination rate (4.1x10⁴ UFC/g) for town 5, followed by lettuces. Contamination data mainly occurred as a probable result of lack in personal hygiene, handling, transport and inadequate storing of the vegetables.

In fact, there were significant differences between the towns for all the vegetable types under analysis. Results were also given by Tukey's mean multiple comparison test, especially with regard to cabbages from town 4 when compared to the others. A statistical difference for lettuce was reported between towns 3 and 5; whereas for cabbages significant difference was reported in town 4 when compared to towns 2, 3 and 5 (Table 2).

According to Mogharbel (2007), poor hygiene conditions during food processing, production, storage and handling, coupled with lack of treatment of irrigation water from streams and rivers and transport to the consumer market are factors for the occurrence of food contamination.

Similar results were reported by Takayanigui et al. (2001) who observed high concentrations of thermotolerant coliforms in vegetables sold without any sort of treatment and related to lack of hygiene during the collection process and food selling.

Results from the microbiological evaluation of minimally processed lettuces (*Lactuca sativa* L.) and cabbages

Table 1. Comparison for the presence *Escherichia coli* (in 10,000 units) in minimally processed vegetables between the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	55.80 ^{aA}	84.70 ^{aA}	230.00 ^{aA}	0.19 ^{bAB}	41.2 ^{abA}
2	0.83 ^{aB}	0.24 ^{aB}	5.90 ^{aA}	1.28 ^{aA}	1.63 ^{aAB}
3	15.00 ^{aB}	0.06 ^{aB}	0.05 ^{aB}	0.03 ^{aAB}	0.25 ^{aAB}
4	0.03 ^{aB}	0.02 ^{aB}	0.03 ^{aB}	0.005 ^{aB}	0.04 ^{aB}

Small letters represent comparison between lines; capital letters represent comparison between columns at 5% probability.

Table 2. Comparison for the presence of *Escherichia coli* (in 10,000 units) in minimally processed vegetables between the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	55.80 ^{aA}	84.70 ^{aA}	230.00 ^{aA}	0.19 ^{bAB}	41.2 ^{abA}
2	0.83 ^{Aab}	0.24 ^{aB}	5.90 ^{aAB}	1.28 ^{aA}	1.63 ^{aA}
3	15.00 ^{Aab}	0.06 ^{aB}	0.05 ^{aBC}	0.03 ^{aAB}	0.25 ^{aA}
4	0.03 ^{aB}	0.02 ^{aB}	0.03 ^{aC}	0.005 ^{aB}	0.04 ^{aA}

Small letters represent comparison between lines; capital letters represent comparison between columns at 1% probability

(*Brassica oleracea* L.) sold in Brasília DF Brazil identified thermotolerant coliforms in some samples analyzed. In fact, 19% of the 36 lettuce samples were contaminated by thermotolerant coliforms, whereas 81% were free from any contamination, according to current legislation. Further, 22% of 60 raw lettuces and cabbages analyzed were contaminated by thermotolerant coliforms and 78% were free from any contamination.

However, a study undertaken in the Brazilian Federal District revealed that all minimally processed lettuce, cabbage and turnip samples were contaminated by thermotolerant coliforms (Almeida et al., 2012). The same result was verified in another study in Uberlândia MG Brazil, where all raw food samples were contaminated at rates above those allowed by current legislation (Bonnas et al., 2005). *Escherichia coli* was reported in 8 out of 56 vegetable samples in Porto Alegre RS Brazil (Silva et al., 2007).

Vegetables actually free from thermotolerant coliforms indicated good sanitary conditions during processing and compliance with the microbiological standards of ANVISA (Sasaki et al., 2006). The contaminated vegetables were not fit for consumption due to fecal contamination.

Table 3 shows a descriptive analysis for data on *Salmonella* sp. Cabbages and lettuces had the highest contamination (approximately 66.67%), especially those from towns 2 and 3.

Contamination by *Salmonella* sp. is a public health concern due to the possibility of infection by toxins.

However, its low contamination rate when compared to that by coliforms may be related to competition by other bacteria in the environment as well as by producers' hygiene conditions when dealing with their products (Marques et al., 2006). Takayanagui et al. (2001) reported similar results, with only 9% of vegetables contaminated by *Salmonella* sp.

Mean mesophile counts in UFC / g varied between 2.5x10 and 3.6x10⁶. The farmer from town 5 not only had the highest mesophile rates in harvested vegetables corresponding to data on total and thermotolerant coliforms but also showed contamination related to lack of hygiene. Perhaps due to their greater leaf surface, cabbages had the highest mesophilic count when compared to rates of the other vegetables.

Interaction was not significant at 5% for mesophile counting (Table 4). Consequently, the behavior of the variable may be analyzed separately with regard to each factor (town and vegetable type) significant at 1% probability. Mesophile count at 1% significance is influenced by town and vegetable type. Tukey's test of mean multiple comparison for mesophiles (Table 5) shows that, at 1% significance, there were significant differences among all types of vegetables and between town 4 and towns 1 and 5.

Even after minimal processing, raw vegetables may still retain most of their previous microbiota. This is a serious health issue since pathogens may be included in the microbiota. In fact, they should be stored at proper

Table 3. Comparison of minimally processed vegetables as presence of *Escherichia coli* (in 10,000 units), the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	6.73 ^{aA}	1.09 ^{aA}	1.22 ^{aA}	0.02 ^{bA}	1.03 ^{aA}
2	0.61 ^{abA}	0.05 ^{bB}	0.03 ^{bB}	0.36 ^{abA}	2.38 ^{aA}
3	0.004 ^{abB}	0.009 ^{aB}	0.02 ^{aB}	0.00 ^{bB}	0.05 ^{aB}
4	0.001 ^{abB}	0.007 ^{aB}	0.03 ^{abB}	0.00 ^{bB}	0.003 ^{abB}

Small letters represent comparison between lines; capital letters represent comparison between columns at at 5% probability.

Table 4. Comparison of minimally processed vegetables as presence of *Escherichia coli* (in 10,000 units), the cities of origin.

Vegetable	Town				
	1	2	3	4	5
1	6.73 ^{aA}	1.09 ^{aA}	1.22 ^{aA}	0.02 ^{bA}	1.03 ^{abAB}
2	0.61 ^{aA}	0.05 ^{aAB}	0.03 ^{aAB}	0.36 ^{aA}	2.38 ^{aA}
3	0.004 ^{abB}	0.009 ^{abB}	0.02 ^{aB}	0.00 ^{bB}	0.05 ^{abBC}
4	0.001 ^{aB}	0.007 ^{aB}	0.03 ^{aB}	0.00 ^{aB}	0.003 ^{aC}

Small letters represent comparison between lines; capital letters represent comparison between columns at 1% probability.

Table 5. Comparison of minimally processed vegetables as the presence of *Salmonella* in the cities of origin.

Vegetable	Town					Total (%)
	1	2	3	4	5	
Cabbages	1	2	2	0	1	6 (54.55)
Lettuce	0	2	2	0	1	5 (45.45)
Chicory	0	0	0	0	0	0 (0.00)
Salad rocket	0	0	0	0	0	0 (0.00)
Total (%)	1 (0.09)	4 (36.36)	4 (36.36)	0	2 (18.18)	11

temperatures so that the growth of pathogens could be inhibited.

Conclusion

Vegetables sold in the public fairs of the main towns of the western region of Paraná are improper for natural human consumption since they contain fecal bacteria or high levels of mesophiles and certain quantity of *Salmonella*; and have the possibility of causing toxin and infections.

Cabbages had the highest contamination rate compared to the other vegetables, due to their larger leaf surface.

Vegetables from town 5 had the highest contamination, with the least hygiene and sanitary care.

Effective supervision by the health authorities should be warranted to ensure that agricultural products that are consumed raw could be sold in good conditions without the addition of any special treatment.

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