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Assessment of microbial load of some common vegetables among two different socioeconomic groups

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Abstract

Microbiological control is very important in food industry to prevent foodborne diseases. Therefore, the present investigation was undertaken to assess the microbiological quality of fresh vegetables collected from several regions of Ropar, Punjab, India. A total of 36 vegetable samples were collected and examined for their microbial load. Contamination was mainly found in cauliflower, peas, cabbage, and potato. The microbial load in vegetables procured from low economic area was significantly higher in comparison to vegetables from high economic areas. In low economic area maximum total plate count was observed in onion followed by carrot and radish while in high economic area it was recorded in peas followed by potato. Similarly, in low economic area maximum yeast and mold count was recorded in radish, cauliflower while in high economic area maximum yeast and moulds count were recorded in radish, onion and cauliflower, followed by cabbage. Maximum coliform count was observed in low economic areas in cauliflower, followed by onion. Considerable numbers of microbes were also detected in carrot, peas, cabbage and potato in the area. Maximum coliform count in high economic area was recorded in radish followed by carrot. *Eshcerichia coli* were detected only in onion procured from shops of low economic area. This study demonstrated that vegetables that are sold in the open market are usually having higher microbial load that may represent a risk for human health. Hence, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these vegetables and infect susceptible host.

Introduction

The increasing number of outbreaks of food poisoning has highlighted the importance of microbiological control in the food industry. Consumption of raw vegetables contaminated with harmful microorganisms may result in food poisoning. Contamination of vegetables may take place at all stages during pre and post-harvest techniques (De Roever, 1999). Raw fruits and vegetables are known potential for a wide range of microorganisms, including human pathogens (EC-SCF, 2002). Food-borne bacterial pathogens commonly detected in fresh vegetables are coliform bacteria, *E. coli*, *Staphylococcus aureus* and *Salmonella* sp. (Tambekar and Mundhada, 2006). Microorganisms capable of causing human illness and others whose food-borne disease potential is uncertain, such as *Aeromonas hydrophila*, *Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella* sp. have been isolated in lettuce and salad vegetables (Francis *et al*., 1999). Numerous food-borne molds can produce mycotoxins, and some yeasts and molds are responsible for human and animal infections (Beuchat and Cousin, 2001). Contaminated food is a common source of human infections. Microbiological risk assessment is an emerging tool for the evaluation of the safety of food and water supplies. Different organizations have suggested that microbiological risk assessment should be carried out so that appropriate remedial measures can be adopted to curtail the episodes of food-borne illness as a result of consumption of contaminated foods. Microbes, mainly the coliforms group has been used extensively as an indicator of the main indicators of microbiological quality of water and food. Their presence indicates improper treatment or post-disinfection contamination. There may be significant differences in the microbiological quality of food products at the market level sold in different socioeconomic areas. Hence, a qualitative survey of two common market foods based on microbiological criteria was envisaged. The aim was to get baseline data on microbial load of vegetables at market level, and to compare the microbial load of vegetables

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between low and high economic areas. The present paper deals with the microbiological quality of fresh vegetables.

Materials and Methods

Study area

The study was carried out in Ropar, Punjab, India. In the execution of present research work, two areas were selected: high economic area, Ropar main city (HIG) and low economic area, Asron village (LIG) of Ropar city (Punjab, India).

Sample collection and processing

Random sampling procedure was adopted to collect the sample materials. In total eight vegetable shops were selected, four from HIG and four from LIG respectively. Sampling details are given in Table 1. Shops serving a substantial portion of the population were selected. Nine commonly used vegetables namely potato, cabbage, cauliflower, peas, onion, garlic, carrot, radish and ginger, were selected for the present investigation. Vegetables were procured and the purchasing individuals were instructed to select vegetables items as if these vegetables were being purchased for their own consumption. Sterile polythene zip bags were used for collection of samples. Immediately after collection of the sample, temperature of the vegetables was noted down. Samples were carried to the laboratory in aseptic condition. The polythene bags with vegetables were kept in an ice box maintained at 6–10°C and processed within 2–4 hrs.

Media used

Different media were used. All media used were of analytical reagent grade. Plate count agar (Oxoid CM463) was used for detecting total aerobic bacterial count after incubation at 30°C for 48 h. Total coliforms were determined by using Mac Conkey agar medium (Oxoid) (Tambekar and Mundhada, 2006). *Escherichia coli* cells were enumerated using trypton bile X-glucouronide (TBX) agar followed by incubation at 30 and 44°C. Only blue/green colonies were included in the calculations (Tendekayi *et al.*, 2007). Typical colonies on the plates were enumerated and colony counts per 1 g sample were determined. Colony counts were converted into log10 CFU g^{-1}.

Microbial analysis of vegetables

25 g of each vegetable sample were weighed and blended in 100 mL of sterile saline solution for 2 min under sterile conditions. The blender was carefully disinfected to prevent any cross contamination. The homogenates were collected in sterile bottles and stored at -20°C until needed. Aliquots (0.5 mL) of each homogenate were serially diluted in sterile saline solution. The diluent of buffered peptone water was then inoculated on to the respective media. Total plate count in vegetables was determined by the procedure described by IS 5402:2002. Yeast and Mould count in vegetables was determined by the procedure described by IS 5403: 1999 and Rediff 2005. *E. coli* in vegetables was determined by the procedure described by IS 5887(I):1976 and Rediff (2005). Coliforms bacteria in vegetables were determined by the procedure described by IS 5401(II):2002.

Table 1. Sampling details of vegetables from LIG and HIG

S. No.	Samples	LIG Samples*	HIG Samples*
1	Potato	4	4
2	Cabbage	4	4
3	Cauliflower	4	4
4	Peas	4	4
5	Onion	4	4
6	Garlic	4	4
7	Ginger	4	4
8	Radish	4	4
9	Carrot	4	4

*A total of eight shops (four from HIG and four from LIG) were selected for sampling of vegetables; LIG, Low economic area, HIG, High economic area

Statistical analysis

Mean of the vegetable samples and standard deviation was calculated using Microsoft Excel 2007 program. The graphs were prepared using Microcal™ Origin version 6.0. In figure, error bars indicate standard error of the mean, where error bars are not visible; they are smaller than the marker.

Results and Discussion

A total of nine vegetables were selected for the study namely potato, cabbage, cauliflower, peas, onion, garlic, ginger, radish and carrot. Two samples of each vegetable were taken from two different shops in both high as well as low economic areas i.e., a total thirty-six samples were taken. Total plate count (TPC), yeast and moulds count, *E. coli* count and coliform bacteria count were determined. Figure 1 depicts the TPC count among the various vegetables in high and low economic areas. The microbial load in vegetables procured from low economic area was significantly higher in comparison to vegetables from high economic areas. Maximum TPC (46.6 ± 0.47 cfu/g) was observed in onion from low economic area followed by carrot (23.3 ± 0.47 cfu/g) and radish, cauliflower, and peas (20.0 ± 0.00 cfu/g each). Maximum TPC in high economic area

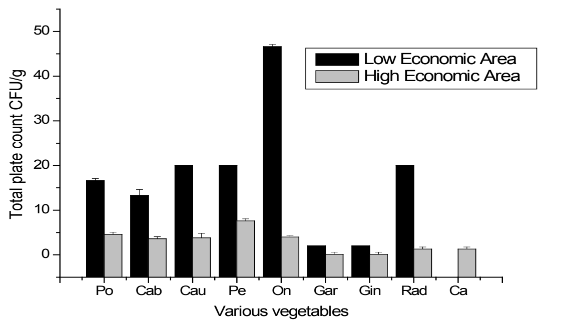

Figure 1. Total Plate Count of various vegetables from Low and high economic areas; (CFU/g; $\times 10^3$); each value is mean of triplicates ± standard deviation; Po, potato; Cab, cabbage; Cau, cauliflower; Pe, peas; On, onion; Gar, garlic; Rad, raddis; Ca, carrot; bar indicates the ± standard deviation.

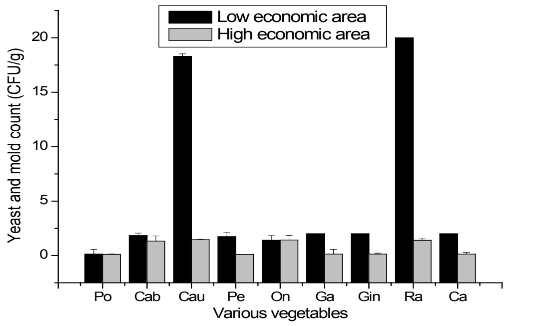

Figure 2. Yeast and Mould Count in various vegetables from low and high economic areas (CFU/g; $\times 10^3$); each value is mean of triplicates ± standard deviation; Po, potato; Cab, cabbage; Cau, cauliflower; Pe, peas; On, onion; Gar, garlic; Ra, radish; Ca, carrot; bar indicates the ± standard deviation.

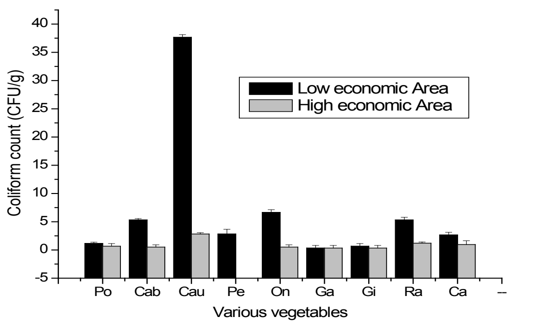

Figure 3. Coliforms count in various vegetables from low and high economic areas (CFU/g; $\times 10^1$); each value is mean of triplicates ± standard deviation; Po, potato; Cab, cabbage; Cau, cauliflower; Pe, peas; On, onion; Gar, garlic; Ra, radish; Ca, carrot, bar indicates the ± standard deviation.

was recorded in peas (07.6 ± 0.47 cfu/g) followed by potato (04.6 ± 0.47 cfu/g) whereas minimum for garlic and ginger (Figure 1). Plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality (Aycicek *et al*., 2004). These microorganisms reflect the exposure of the sample to any contamination and

Table 2. Depicts the vegetables contamination with *E. coli*

Food items	*E. coli* (cfu/gm)	
	Low economic area	High economic area
Potato	Absent	Absent
Cabbage	Absent	Absent
Cauliflower	Absent	Absent
Peas	Absent	Absent
Onion	Present	Absent
Garlic	Absent	Absent
Ginger	Absent	Absent
Radish	Absent	Absent
Carrot	Absent	Absent

Experiment was performed in triplicates

in particular, the existence of favorable conditions for the multiplication of microorganisms. For many reasons, this parameter is useful to indicate whether cleaning, disinfection and temperature control during industrial processing, transportation and storage, have been performed sufficiently (Tortora, 1995). Most of the vegetables with higher microbial load grow inside soil (radish, potato and carrot) or near to soil (cauliflower, cabbage) with the exception of peas. This may be responsible for their higher count. Beside this, other sources of contamination are improper handling and improper storage and transportation conditions. Ginger, garlic and radish grow under the soil but still microbial count in these three vegetables was found to be very low. This may be attributed to the antimicrobial activity of these vegetables.

In general, the yeast and mold count in vegetables procured from low economic area was higher. Maximum yeast and mold count (20.00 ± 0.00 cfu/gm) in vegetables procured from low economic areas were recorded for radish, followed by cauliflower (18.3 ± 0.23 cfu/gm) (Figure 2). Maximum yeast and moulds count in vegetables purchased from high economic area were recorded in radish, onion and cauliflower followed by cabbage. Minimum yeast and mold count was detected in potato and peas (Figure 2). Similarly, Figure 3 depicts the *E. coli* among the selected vegetables. The data reveals the presence and absence of *E. coli* between high and low economic areas. *E. coli* were absent in potato, cabbage, cauliflower, peas, garlic, ginger, radish and carrot of low and high economic areas. *E. coli* was detected only in onion procured from shops of low economic area. Presence of *E. coli* may be attributed to unhygienic conditions that are prevalent among low socioeconomic areas. Table 2 shows the coliform bacteria count among the selected vegetables. An analysis of the data reveals difference in coliform bacteria count between the two socio-economic areas. Maximum microbial count was observed in low economic areas for cauliflower (37.66 ± 0.47 × 10^1), followed by onion (6.66 ± 0.47

× 10[1]). Considerable numbers of microbes were also detected in carrot, peas, cabbage and potato in the area. Maximum coliform count in high economic area was recorded in cauliflower (2.83 ± 0.23 ×10[1]), radish (1.2 ± 0.21 × 10[1]) followed by carrot (0.96 ± 0.68 × 10[1]). The microorganism was also detected in cauliflower, ginger, onion and carrot. Coliforms are commonly-used bacterial indicator of sanitary quality of foods and water and considered as an indicator of microbial pollution and they are common inhabitants of animal and human guts (Tortora, 1995). Our results are in concurrence with those of Tambekar and Mundhada (2006). They reported the presence of various food-borne bacterial pathogens in fresh vegetables. They reported that coliform bacteria, *E. coli*, *Staphylococcus aureus* and *Salmonella* species were commonly detected in fresh vegetables.

The survival or growth of these organisms on intact fruit and vegetable surfaces is dependent on the extrinsic factors of available nutrient, temperature, mechanical handling and moisture. The vegetables on display for sale are often touched by many hands of the customers and by the vendors. The customers pick and drop as many vegetables as are available, to enable them make a choice. Frequent handling by unhygienic hands is a factor contributing to the high microbial load. The dusty environments of the roads, busy roads and campuses/institutions, coupled with water of questionable quality that often is used to sprinkle the vegetables to keep it fresh are contributing factors that could aid the survival and possible multiplication of microorganisms on vegetables' surfaces.

Many vegetables grow low to the ground where they are likely to come in contact with the soil. If the soil has been treated with improperly treated animal manure as fertilizer or irrigated with contaminated waters, vegetables are also likely to be contaminated. It may also be attributed to the unhygienic handling practices by farm and food factory workers. Using unhygienic water for rinsing and sprinkling the vegetables to keep them fresh is also another potential source of contamination. Our results are in agreement with that reported by Mensah *et al.* (2002). The results of the present study are also in accordance with the findings of Halablab *et al.* (2011). They reported presence of microbial contamination in vegetables. Other possible sources of microorganisms in fresh agricultural produce may be soil, faeces (human and animal origin), water (irrigation, cleaning), animals (including insects and birds), handling of the product, harvesting and processing equipment and transport. Johannessen *et al.* (2002) also opined that these may be potential source of microbial contamination in fruits and vegetables.

Conclusion

Microbiological control is very important in food industry to prevent food poisoning and other health hazards. In the present paper, the authors assessed the microbial load of nine different vegetable samples from low and high economic areas. Contamination was mainly found in cauliflower, peas, cabbage, and potato. Beside soil microbes, other sources of their contamination are improper handling, unhygienic storage and transportation conditions. Vegetables may be contaminated with pathogenic microorganisms during growing in the field or during harvesting, post harvesting, handling, processing and distribution. Therefore, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these vegetables and infect susceptible host.

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