Microbiological Safety Assessment of Fresh Fruits and Vegetables Collected from Main Markets of Multan, Pakistan

Bina Rida
The Women University,Multan, bina.rida12@gmail.com

Farah Deeba
The Women University, Multan, farah.9003@wum.edu.pk

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MICROBIOLOGICAL SAFETY ASSESSMENT OF FRESH FRUITS AND VEGETABLES COLLECTED FROM MAIN MARKETS OF MULTAN, PAKISTAN

BINA RIDA* **AND FARAH DEEBA**

The Women University Multan, Multan, Pakistan

*Corresponding Author: bina.rida12@gmail.com

ABSTRACT

Most of the research related to food-borne human pathogens is conducted on the transmission of pathogens from foods of animal origin. However, recent studies showed that fruits and vegetables are the sources of many disease outbreaks. This study was carried out to assess the current knowledge and future developments for the microbial safety of fresh fruits and vegetables. Eight different fruits and vegetables, i.e., grapes (*Vitis vinifera*), banana (*Musa acuminate*), orange (*Citrus sinensis*), apple (*Malus domestica* Borkh), carrott (*Daucus carota*), cucumber (*Cucumis sativus*), green chili (*Capsicum annuum*) and onion (*Allium fistulosum*) were collected from two localities to determine the frequency of microorganisms using standard plate count method. The effect of varying concentration of acetic acid on the microbial load of vegetables was evaluated at different time intervals. *E. coli, Pseudomonas* spp.*, Salmonella, Shigella, Klebsiella* spp., *Enterobacter* spp. and *Bacillus* spp. were identified in this study. Highest (23.3%) frequency of occurrence was observed in case of *E. coli*, followed by *Pseudomonas* and *Enterobacter* spp. (16.6%), *Salmonella* (16%), *Shigella* (13.3%), *Klebsiella* spp. (10%) and *Bacillus* spp. (3.3%). This study showed that increasing vinegar concentration from 0.5 to 2.5% reduces microbial load. The risk of foodborne illness associated with consumption of fresh produce can be minimized by controlling the potential contamination.

Keywords: fruits and vegetables, microbial quality, food borne pathogens, acetic acid

INTRODUCTION

Vegetables and fresh fruits are vital part of a nutritious and healthy diet because they promote a healthy body and mind. According to researchers, the people who intake five or more serving of fruits and vegetables in a day have 20% lesser chances of developing stroke (He et al.*,* 2006) and the coronary heart disease (He et al.*,* 2007). Due to their dietary values, fruits and vegetables also harbor a variety of elevated microbial contaminants (Eni et al.*,* 2010). Toxins produced by variety of microorganisms play major role in contaminating the food products (Angulo et al.*,* 2008).

Disease causing microorganisms like *E. coli, Rhizopus, Staphylococcus aureus, Salmonella, Clostridium botulinum, Bacillus cereus, Pseudomonas aeruginosa*, *Mucor* species*, Aspergillus* and *Candida* species can contaminate fresh produce (Pandey, 2016). The number of reported outbreaks both in European countries and United States are less than the actual number of outbreaks that occur (Arendt et al.*,* 2013). In September 2006, a large outbreak occurred across 26 states of USA. The outbreak was caused by *E. coli* and resulted in 3 deaths and 183 infections. This outbreak was found to be related with the pre-packaged spinach consumption (Grant et al.*,* 2008; Wendel et al.*,* 2009). In 2008, multiple *Salmonella*

outbreaks were found to be associated with serrano peppers and jalapeno (Centers for Disease Control and Prevention, 2008).

According to standard plate counts study, there are four level of microbiological quality, including satisfactory, marginal, unsatisfactory and potentially hazardous. Satisfactory levels are those which show good microbiological quality. In this case further processing is not required. Marginal levels are those which show border line quality and require further consideration to solve possibility of various hygiene problems occurred in the processing of the fruits and vegetables. Unsatisfactory levels are those levels which are beyond the acceptable microbiological limits, which are resulted due to poor hygienic conditions or handling practices. Potentially hazardous level may cause food borne illness and further processing is immediately done to initiate remedial action (Zwietering, 2002).

The consumers in Pakistan usually get fruits and vegetables from vendors and open stalls which are not properly covered. Microbial contamination of fruits and vegetables can be minimized by following precautionary measures during picking, packaging, transportation and storage (Park et al., 2012). The main objective of this study was to check the quality of products prior to eating, to assess the risk of contamination of raw consumed fruits and vegetables and to provide the scientific information for development of hygienic field for public health and safe handling practices of fresh products being sold in the markets of Multan, Pakistan.

MATERIALS AND METHODS

Sample Collection

Samples of eight different fruits and vegetables including grapes (*Vitis vinifera*), banana (*Musa acuminate*), apple (*Malus domestica* Borkh), orange (*Citrus sinensis*),

onion (*Allium fistulosum*), carrot (*Daucus carota*), green chili (*Capsicum annuum*) and cucumber (*Cucumis sativus*) were obtained from a local market of fruits and road side vendors. All fruits and vegetable samples were brought to the Microbiology Lab within one hour and stored at 4℃. 10g of each fruit/vegetable was mixed with 90 ml of the distilled water in the sterile container and centrifuged at 10,000 rpm for 2 minutes prior to make dilutions. Then 1ml of the homogenate mixture was taken to make serial dilutions up to 10^{-6}.

Isolation of Microorganisms

For the isolation of microbes EMB (Eosin Methylene Blue) agar, SS (Salmonella-Shigella) agar and MacConkey agar were used. The agars were prepared and autoclaved for sterilization at 121° C for 15 minutes. For SS agar autoclaving is not required so it was simply boiled for 15 minutes at hotplate. All agars were plated by pour plate method and were kept for solidification at room temperature. After solidification, media was inoculated with 1 ml of sample from dilution 10^{-2} and 10^{-6}, followed by incubation for 24 hours at 37° C. The isolated and distinct colonies were selected and further streaked on separate plates for formation of pure isolated colonies for identification.

Total Plate Count

For microbial load determination, serial dilutions of each homogenized mixture were prepared upto 10^{-6} and dilutions 10^{-2} and 10^{-4} were used for inoculation on nutrient agar media. The inoculated plates were incubated at 37° C for 24 hours. Duplicate plates with 25 to 250 colonies were selected for total count. The number of colonies was multiplied to dilution factor (reciprocal of dilution) in order to find the microbial load.

Effect of Concentration of Acetic Acid and Exposure Time

The activity of different concentrations of vinegar solution and exposure time on microbial load of samples was determined. Approximately, 10 g of each sample was washed in 10 ml of three different acetic acid concentrations, i.e., 0.5, 1.5 and 2.5%. Then 0.1 ml of rinsed solution from each concentration was used to spread over the nutrient agar at different time intervals, for instance, at initial time of washing, then after 5 minutes and 10 minutes followed by statistical analysis.

RESULTS

Biochemical Characterization

Biochemical tests were performed for species confirmation and biochemical characterization. Some bacterial species showed both indole and gas production e.g., *E. coli* and *Shigella*, while some species showed positive results for only gas production test. On the basis of these biochemical tests, seven bacterial species were identified (Table 1).

Table 1: Biochemical test for bacterial isolates.

Bacterial isolates	Gas Production	Indole Production
E. coli	+	+
P. aeruginosa	-	-
Salmonella	-	-
Shigella	+	+
Klebsiella	+	-
Enterobacter spp.	+	-
Bacillus spp.	-	-

Total Plate Count

In case of vegetables, highest number of microorganisms was isolated from carrot, i.e., 10.9×10^{11}, followed by cucumber (9.6×10^{11}), while green chili showed the lowest growth (1.8×10^4). In case of fruits, apple showed maximum (8×10^{11}) while banana showed least number (1.5×10^4) of bacterial load. Microbial loads on differential media were also varied. Results are shown in table 2 and table 3.

Effect of Concentration of Acetic Acid and Exposure Time

Microbial load of samples were reduced considerably after treatment of vegetables samples with acetic acid. The highest concentration of acetic acid, i.e., 2.5% for 10 minutes was found to be effective in maximum elimination of microorganisms. The results are shown in figure 1 and figure 2.
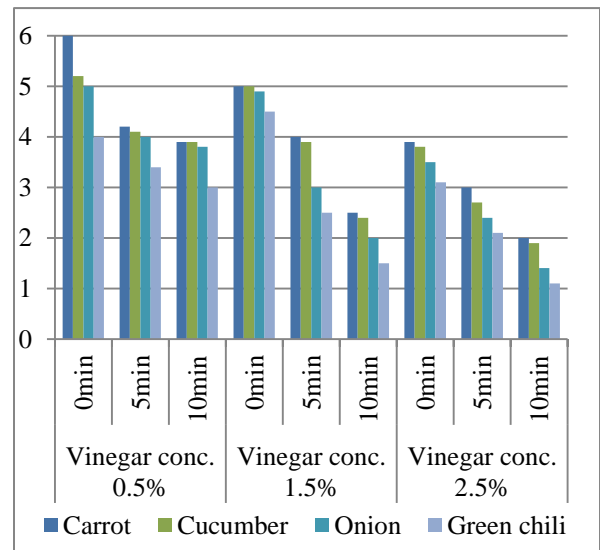

Figure 1: Effect of acetic acid concentration (0.5-2.5%) and incubation time (0-10min) on microbial flora of vegetable samples.
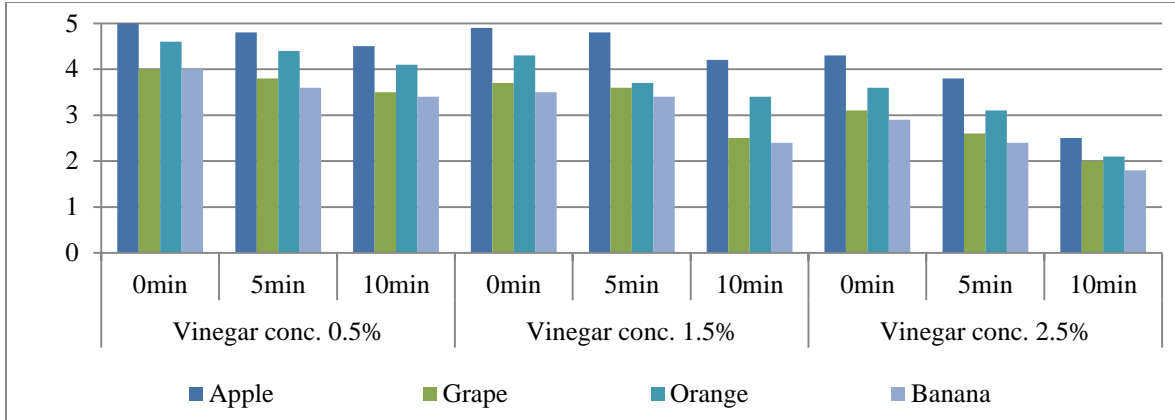
Figure 2: Effect of acetic acid concentration and incubation time on microbial flora of fruit samples.

Table 2: Comparison of total plate count of fruits and vegetables collected from two different localities.

S. No.	Samples	Microbial Load (CFU)	
		Locality 1	**Locality 2**
1	Apple	$8 \times 10^{11} \pm 0.03$	$5 \times 10^{6} \pm 0.01$
2	Grape	$2 \times 10^{7} \pm 0.04$	$2.5 \times 10^{7} \pm 0.04$
3	Orange	$4.6 \times 10^{6} \pm 0.03$	$8.2 \times 10^{9} \pm 0.01$
4	Banana	$1.5 \times 10^{4} \pm 0.01$	$1.8 \times 10^{4} \pm 0.01$
5	Carrot	$10.9 \times 10^{11} \pm 0.03$	$10.8 \times 10^{11} \pm 0.03$
6	Cucumber	$9.6 \times 10^{11} \pm 0.02$	$9.8 \times 10^{11} \pm 0.02$
7	Onion	$5.3 \times 10^{4} \pm 0.04$	$7.4 \times 10^{8} \pm 0.02$
8	Green chili	$2 \times 10^{4} \pm 0.01$	$1.8 \times 10^{4} \pm 0.01$

Table 3: Microbial load detected on MacConkey, EMB and SS agars.

S. No.	Samples	MacConkey Agar	EMB Agar	SS Agar
1	Apple	$2.3 \times 10^{5} \pm 0.01$	$1.4 \times 10^{2} \pm 0.03$	$1.2 \times 10^{2} \pm 0.01$
2	Grape	$2.1 \times 10^{4} \pm 0.05$	$3.1 \times 10^{3} \pm 0.03$	$1.1 \times 10^{2} \pm 0.01$
3	Orange	$3.8 \times 10^{4} \pm 0.03$	$3.1 \times 10^{4} \pm 0.02$	$3.0 \times 10^{4} \pm 0.01$
4	Banana	$1.1 \times 10^{2} \pm 0.01$	$1.4 \times 10^{2} \pm 0.06$	$1.5 \times 10^{2} \pm 0.02$
5	Carrot	$5.4 \times 10^{5} \pm 0.01$	$4.7 \times 10^{11} \pm 0.01$	$4.8 \times 10^{11} \pm 0.01$
6	Cucumber	$2.1 \times 10^{4} \pm 0.07$	$2.9 \times 10^{3} \pm 0.04$	$3.1 \times 10^{4} \pm 0.01$
7	Onion	$1.2 \times 10^{4} \pm 0.02$	$1.4 \times 10^{3} \pm 0.01$	$2.2 \times 10^{3} \pm 0.05$
8	Green chili	$1.1 \times 10^{3} \pm 0.05$	$1.5 \times 10^{4} \pm 0.03$	$1.3 \times 10^{3} \pm 0.1$

DISCUSSION

All the microorganisms isolated in this study seem to be related to fruits and vegetables contamination as seen from previous studies (Adebolu and Ifesan, 2001; Omemu at al., 2005; Tambekar and Mundhada, 2006; Uzeh et al., 2009). Bacterial population in fruits and vegetables was as high as 10^{10} to 10^{11} CFU/g. The causes of such high microbial contamination in fruits and vegetables could be poor or unfitting storage conditions and prolonged storage of fruits and vegetables before transportation to the market. Cross contamination between produce may also occur if they are washed with the same water. Moreover, psychrotrophic microbes can multiply after a while if the storage conditions are favorable (Montville and Matthews, 2007; Abadias et al., 2008).

Some bacteria are present naturally in fresh fruits, as they are said to be the part of the natural flora. The other bacteria may be the result of contamination with irrigation, water, soil or they may be added in produce from environment during washing, transportation or storage (Ofor et al., 2009). *Pseudomonas* and *Bacillus* species, isolated from both fruit and vegetable samples in current study are most common among the natural flora of fresh produce and are considered as vegetable spoilage bacteria (Vanderzant and Splittstroesser, 1992). Some Bacillus species especially *B. cereus* can cause food borne sickness. Some other pathogenic species like *Klebsiella* spp*., Salmonella* spp. and *E. coli* were also found in study subjects during current study. Presence of these microbes shows the contamination of market products and unhygienic storage conditions (Zwietering, 2002; Grant et al., 2008).

The microbial populations can invade and grow on the fresh produce during pre-harvest season. The chances of invasion are increased during harvesting along with direct contamination and propagation of previous species during post-harvesting dealings. Water is said to be the major source of contamination as waste water from houses, industries and other sources is being used to grow fruits and vegetables in many areas of Punjab, Pakistan. Contaminated soil could be another source of bacterial contamination of fruits and vegetable because it harbors a variety of pathogenic and non-pathogenic bacteria (Steele and Odumeru, 2004; Ijabadeniyi et al., 2011; Rasool and Irum, 2014). The presence of *E. coli* and *Salmonella* species in produce showed that there must be fecal contamination of soil and water as these organisms are mostly isolated from fecal samples. Insects are the other possible reason of contamination because different flies sit on products at uncovered stalls and vendors, thus transferring the microorganisms to these products (Sela et al., 2005).

In this study, acetic acid was used in order to reduce the microbial load. The acetic acid concentration and exposure time was varied accordingly. No significant reduction was observed with in lower concentration at initial time of incubation. With increasing acetic acid concentration and exposure time, significant reduction of microbial load was observed. The reason for microbial reduction with acetic acid can be attributed to increase in pH, as most of the neutrophilic bacteria residing the produce surface can easily grow at neutral pH (7). For microbial growth pH plays a vital role. As the amount of acid increases, it changes the pH from neutral to acidic (<7), leading to slow or no growth of microorganisms (Berry and Cutter, 2000; Ryssel et al., 2009). As it is obvious from this study that vinegar treatment of contaminated fresh produce is significant to decrease the chances of food contaminating microbes, so it can be used as a simple, useful and cheap disinfectant, in local markets.

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