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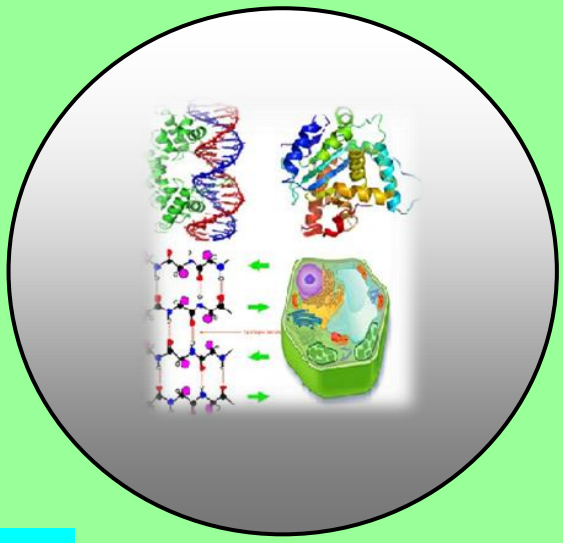

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RESEARCH PAPER

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Effect of Antibacterial Activity of Vinegar on Micro-Organisms Analyzed from Fresh Vegetables and Fruits

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ABSTRACT

Fresh vegetables and fruits are exposed to potential microbial contamination. Consumer demand is to use fresh vegetables and fruits which are bacteriologically safe. Bacteriological survey of 45 samples of fresh vegetables and fruits, (15 collected from 3 different sources) was done. Samples were analyzed to study the density of microorganisms by standard plate count, yeast &mould count, coliform counts. The high contamination and high number of pathogens on the surface of fresh vegetables and fruits in case of road side vendor samples (log 10-11 cfu/ml) and local market samples (log 7-8 cfu/ml) as compared to super market sample (log 5-6 cfu/ml). Various pathogens were identified from the surface of these samples. Escherichia coli, Staphylococcus aureus, Pseudomonas were found to be the pre-dominant species in most of the samples. 5 different concentration of Vinegar (from 0.5% to 2.5%) were studied to decrease the microbial load of these samples. Samples rinsed with these solutions for 5 mins and washed with sterile distilled water; showed 40-80 % reduction in total number of organisms. Vinegar at 2.5% was found to be the most effective, where 80 % samples showed more reduction in total number of organisms. Results indicated that the microbial load in local market samples was very high as compared to super market and Vinegar can be used as rinsing agent to improve the quality of fresh vegetables and fruits.
Key words: Vegetables, Fruits, Foodborne Pathogens, Microbiological Quality and Vinegar.

INTRODUCTION

Fruits and vegetables are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and are thus vital for health and well being. Well balanced

diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are also reported to reduce the risk of several diseases (**Kalia and Gupta, 2006**).

Fruits and vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore harbor a diverse range of microorganisms including plant and human pathogens (**Nguyen-the and Carlin, 1994; Dunn et al. 1995; Carmo *et al.,* 2004**). Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (**Ray and Bhunia, 2007; Ofor *et al*. 2009**). In developing countries such as Nigeria, continued use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contamination (**Amoah *et al.* 2009**).

Fruits and vegetables carry microbial flora while passing from the farm to the table. The produce is exposed to potential microbial contamination at every step including cultivation, harvesting, transporting, packaging, storage and selling to the final consumers. Microbial spoilage and contaminating pathogens pose a serious problem in food safety (**FDA, 2000**). The Centers for Disease Control and Prevention (CDC) estimate that there are 76 million cases of food borne illness each year. An outbreak with identified etiology was predominantly of bacterial origin so that the study about microbial ecosystems on the surface of raw fruits and vegetables are necessary. As consumers, we need to recognize that food safety is important for fresh fruits and vegetables Food from sources like super markets (Spinach, Food land fresh) may be protected from contamination and spoilage (**Ibenyassine, 2007**) during subsequent handling, packaging, storage and while in transit .

Thus comparative study of fresh foods from local market as well as from super market should be done. The lack of an effective antimicrobial treatment at any step from planting to consumption means that pathogens introduced at any point may be present on the final food product, fresh vegetables and fruits must be washed or treated specifically to minimize microbial load (**United States Patent 5869122**).

Water supplemented with varying concentrations of organic acids, such as acetic, citric and sorbic acids, has been shown to reduce microbial populations on fruits and vegetables (**Karapinar and Gonul 1992; Beuchat, 1998**). Previous studies revealed that a vinegar dip resulted in a 3 to 6 log10 decrease in the number of aerobic bacteria on parsley leaves, depending on vinegar concentration used and incubation time (**Beuchat, 1998**).

The present work was carried out to determine the microbial load of fruits and vegetables sold on Nellore market, Andhra Pradesh, India and use of vinegar (acetic acid) as anti-microbial agent to reduce the microbial population in some vegetables.

MATERIAL AND METHODS

Nellore is district headquarter, the Southernmost Coastal District of Andhra Pradesh lies between 13-30’ and 15-6’ of the Northern latitude and 70-5’ and 80-15’ of the Eastern Longitude and extending over an area of 13076 Sq. Kms, accounting for 4.75% of the total area of the state. It is bounded on the north by Prakasam District on the East by Bay of Bengal on the South by Chittoor District and Tiruvallur District of Tamil Nadu and on the West by Veligonda Hills which separate it from Kadapa District.

Sample collection and Preparation

A total of 15 samples comprising ten types of fresh vegetables (Tomato (*Lucopersicon esculentum*), Spinach (*Spina ciaoleracea*), Coriander (*Coriandrum sativum*), Potato (*Solanum tuberosum*), Cabbage (*Brassica oleracea* var. *capitata*), Beet root (*Beta vulgaris*), Capsicum (*Capsicum annuum* var. *grossa*), Cauli- flower (*Brassica oleracea* var. *botrytis*), Brinjal (*Solanum melongena*), Carrot (*Daucus carota*)) and five types of fruits (Lemon (*Citrus limon*), Sweet Lime (*Citrus limetta*), Banana (*Musa paradisiaca*),Guava (*Psidium guajava*), Apple (*Malussy lvestris*)); each collected from a road side vendor, a local market and from a super market (totally 45). The samples were rinsed thoroughly with distilled water. And these were serially 10 folds diluted. Dilution was made depending on cell density. The highest three dilutions were taken for analyzing the total microbial count (Jarvis and Shapton 1986).

Isolation of Bacteria

The pathogens from the surface of samples were enriched and isolated on selective media and identification was done according to "Bergey`s Manual of Determinative Bacteriology."

Enumeration of microbes

The samples were rinsed thoroughly with sterile distilled water and serially diluted up to 10-7. Dilution was made depending on cell density. The highest three dilutions were taken for analyzing the total microbial count by using Nutrient agar medium at 37^{o}C for 24 hours. Standard Plate Count (SPC) was carried out by Spread Plate Technique and Coli form Count (CC) was carried out by Pour Plate Technique.

Isolation and preservation of bacteria

According to Bergey`s Manual of Determinative Bacteriology, the microorganisms were Isolated. In long term preservation, Glycerol stocks were prepared and stored at -800^{o}C where as pure cultures strains were incubated at 50^{o}C for 48 hours. 0.5 ml of each pure culture was transferred into cry tubes accompanied by 40% glycerol. The samples were mixed gently and stored at -80°C.

Nutrient agar plate

Morphological and cultural characteristics such as abundance of growth, pigmentation, optical characteristics, form, size, margin and elevation were studied on Nutrient agar plates.

Gram staining

A loop full of overnight culture was placed on the slide. Smear was prepared by spreading the drop with a toothpick. The heat fixed smear was first stained with crystal violet for 60 sec. After rinse the slide, it was flooded with Grams iodine solution and was kept for 60 sec. Slide was again washed under the tap water and added 95% alcohol for 30sec. After wash the slide, it was stained with safranin for 60 sec. It was again rinsed under tap water and dried on paper towels. The cells were examined under the light microscope.

Motility determination

A small amount of Vaseline was placed at each corner of clean cover glass. Two loopful of the 24 hours culture of the organism was placed at the center of the cover glass. A depression slide was pressed over the cover glass, such that the depressions cover the culture drop and quickly inverted. The completed preparation was observed microscopically.

Examination of endospores

Isolated microorganism grown on Luria Bertani Broth medium for 3-4 days were suspended in 3-5 µl of sterile 0.09% NaCl on a Microscopic slide and covered with a cover slip. Endospores were observed as shiny bodies in the cells under the phase contrast microscope.

Effect of anti-microbial (vinegar) agent

Samples were treated with vinegar (acetic acid) with various concentrations ranging from0.5-2.5% (with 0.5% interval). Samples were washed with these solutions for 5mins and again washed with Sterile Distilled water. 0.1 mL of these was taken (Test) and inoculated in 3 ml sterile nutrient broth. Control samples were washed only with Sterile Distilled water and then further treated as above. They were incubated at room temperature for 24hrs. Density was checked UV-Vis double beam Spectrophotometer (Systronic) at 540 nm and effects of these agents were compared with control. Effect was studied on the basis of % decrease in microbial density, which was calculated by,

Actual cell density decreased = (Cell density of control)

(Cell density at highest concentration) and then % decrease was calculated (**Yueming Jiang, 2004**)

RESULTS

Bacterial load of the fruits and vegetable samples

Standard Plate Count (SPC), Yeast Mold Count, coliform count was in the range of 10-11 log cfu / mL for road side vendor sample and 7-8 log cfu / mL for local market sample. For Super market it was in the range of 5-6 log cfu / mL. The high log cfu / mL of viable counts of Road Side Vendor and Local Markets could be because of unhygienic condition of local market and exposure during transport facility, improper storage condition etc. may add organisms. Generally, there is no pre-treatment given to fresh produce before transporting to retailer and hence number of contaminants is so large. Similar findings were observed by **Richard L. Thunberg et al (2004)**. They have reported total viable count as 8.7, 8.6, 7.5, 7.4, and 6.3 log10 CFU/g for various samples collected from various retail markets. Low occurrence of contaminants on Super Market sample could be because the Fresh produce of Super Markets was treated with chlorinated water before it is transported to retailer (Information given by officials of Super Markets).

Escherichia coli, Staphylococcus aureus, were the common pathogens found on the surface of all the samples collected from all the sources. *Salmonella spp* and *Klebsiella spp* were found in 60 % of the road side vendor and 50 % of samples local Market samples. On the other hand only 20 % of samples of Super Market showed presence of these organisms (Table-1). **Viswanathan and Kaur (2001)** also showed presence of *Salmonella, Serratia, Enterobacter*, *Staphylococcus aureus*, faecal *E. coli* and *P. aeruginosa* in vegetables and fruits. These results indicate that as a consumer, one should buy fresh vegetables and fruits from Super Markets not from road side vendor and Local Market as microbiological quality of these are better in Super market compared to road side vendor and Local Market.

Effect of vinegar on microbial load of vegetable samples

The effect of various vinegar (acetic acid) concentrations and exposure time on microbial load of five vegetable. Increasing the concentration of the acetic acid (vinegar solution) used in washing from 0.5 to 2.5% resulted in 20 - 78% reduction in the microbial loads of the various vegetables (Table 2 and Figure 1).

Highest percentage microbial load reduction due to increase in vinegar concentration was observed in cabbage while lowest reduction was observed in lettuce. Figure 1 also clearly shows that microbial load decreased with increase in the exposure time 24 hrs for all the five vinegar concentration used in this study. Lowest microbial load for all the vegetables were obtained when exposed to 2.5% vinegar solution for 24 hrs.

DISCUSSION

The bacteria present in fruits and vegetables are a direct evidence of the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce (**Beuchat, 1996; Ray and Bhunia, 2007**). All the bacteria isolated in this study have previously been isolated from fruits and vegetables in other studies, both in India and elsewhere (**Dunn *et al*. 1995; Adebolu and Ifesan, 2001; Omemu and Bankole, 2005; Tambekar and Mundhada, 2006; Uzeh *et al*. 2009**). The high microbial contamination observed in the fruits and vegetables in this study may be a evidence of storage conditions and how long these produce were kept before they were obtained for sampling. Bacteria on storage materials may transfer to produce and cross contamination between produce is probable particularly where produce are pre-washed with the same wash water by the vendor or processor. Ore importantly, bacteria on the produce may multiply over time depending on the storage conditions especially those that are phsychro-trophic (**Montville and Matthews, 2008; Abadias *et al*. 2008**). This study aimed at reporting the microbial quality of fruits and vegetables at the point of sale in stalls in Nellore, a district headquarter of Andhra Pradesh State. The disparity observed in the microbial load of sliced ready to eat apples from two different vendors is similar to the disparity observed for the microbial load of carrot from all three shops (Table 1) and could indicate that handling by individual shops significantly affects the level of microbial contamination of fruits and vegetables.

Some of the bacteria isolated in this study may be part of the natural flora of the fruits and vegetables or contaminants from soil, irrigation water and the environment during transportation, washing/rinsing water or handling by processors (**Ofor et al. 2009**). *Pseudomonas* spp. and *Bacillus* spp. are part of the natural flora and are among the most common vegetable spoilage bacteria (**Vanderzant and Splittstoesser, 1992**). The presence of *S. auerus*, a pathogenic organism of public health concern, in most of the samples and the presence of other pathogenic and opportunistic bacteria like *Salmonella* spp. and *Klebsiella spp*, in some of the fruits and vegetables, further highlights the need to safeguard the health of the consumers by proper washing and decontamination of these produce which are consumed without heat treatment.

Results of this study further confirm previous reports of microbial load reduction observed in vegetables washed/rinsed in vinegar (**Karapinar and Gonul 1992; Beuchat, 1998; Amoah *et al.* 2009**). The efficacy of the method used for microbial load reduction is usually dependent on the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of cleaner/sanitizer, pH, and temperature (**Parish *et al.*2003**).

Increases in concentration and exposure time were found to be significant in the role of vinegar as a decontaminant for the reduction of microbial population on vegetables as observed in this study.

The observed proportionate reduction in microbial loads with increase in vinegar concentration can be attributed to the further reduction in pH resulting from increased vinegar concentration. Most bacteria survive in alkaline pH better than acidic pH. Furthermore, the progressive reduction in microbial loads with increase in exposure time may be due to continuous exposure to this unfavorable pH. As vinegar treatment is likely to reduce the risk of food borne illness associated with potentially contaminated vegetables, vinegar may serve as a simple and inexpensive disinfectant for processors of sliced ready to eat vegetables in Nigeria. A possible disadvantage however, is that vinegar may change the taste of the vegetable but this can be overcome if vinegar rinse is followed by rinsing in portable drinking water.

Despite the high microbial counts obtained for some of the samples in this study, it is important to note that these samples did not show any visible signs of spoilage. Thus outward appearance may not be a good criterion for judging the microbial quality of fruits and vegetables. All fruits and vegetables should therefore be adequately washed before consumption either by the consumer or the processor and where possible, decontaminants such as vinegar should be included in the wash water.

To limit the introduction of pathogenic bacteria to vegetables through irrigation, the origin and distribution of irrigation water should be known. Where wells are used, such wells should be well-maintained, and all irrigation sources should be monitored routinely for human pathogens (**Buck et al. 2003**). Manure used as fertilizer should be treated either by composting or aging to eliminate pathogenic microorganisms and farmers should be educated on the need to allow sufficient amount of time between the final manure application and harvest. Fruit and vegetable processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of contamination. Processors/vendors should also observe strict hygienic measures to ensure that they do not serve as source of chance inoculation of microorganisms and contamination.

Table 1. Samples showing presence of various pathogens (in %).

Organisms	Road Side Vendor (%)	Local Market (%)	Reliance Super Market (%)
Escherichia coli	100	100	94
Klebsiellaaerogene	66	52	33
Staphylococcus aurues	100	100	100
Proteus mirabilis	25	30	25
Proteus vulgaris	33	40	18
Salmonella typhi	58	9	20
Salmonella paratyphi	32	15	15
Shigelladysenteriae	40	20	18
Vibrio cholera	85	30	24
Pseudomonas aeruginosa	98	50	27
Bacillus auerus	30	33	25

Total Number of Samples 15

Table 2. Effect of Vinegar (acetic acid) on microbial load (Average cell density for 15 samples).

Concentration (%)	Control	Vinegar (Acetic acid)
0.5	0.65	0.48
1.0	0.65	0.42
1.5	0.65	0.35
2.0	0.65	0.21
2.5	0.65	0.15

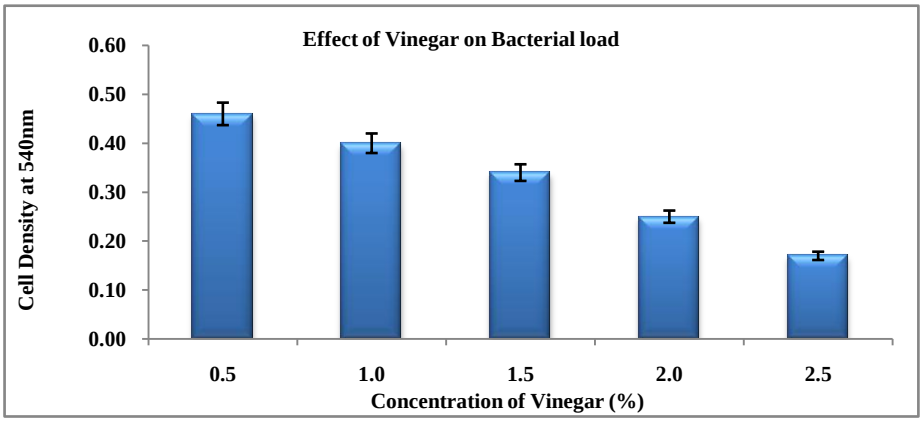

Figure 1. Effect of Vinegar (Acetic acid) on microbial load (Average cell density for 15 samples).

The study also suggests that fresh vegetables and fruits harbor high number of contaminants and pathogen in case of road side vendor and local market compared to super market samples, hence are more prone to spoilage making it necessary to process them before consumption. Thus use of vinegar (As rinsing agent) in fresh vegetables and fruits for reduction of microorganisms can play an important role in food processing.

Although the number of samples studied was small and sample size varied among vendors due to unavailability of some produce, we believe this study provides a general overview of the microbiological quality of fresh-cut fruits and vegetables sold in Nellore, Andhra Pradesh, India.

CONCLUSION

Fresh vegetables and fruits of road side vendor and Local Market are harboring many microbial contaminants and pathogens as compared to of Super Market, indicating that these are protected from contamination while subsequent handling, packaging, storage and transit. Thus, one should buy the Super Market fresh vegetables and fruits, as they are microbiologically safe. In India majority of the people preferred to buy the Local Market's fresh vegetables and fruits even though the quality of Super Market's fresh produce is better. However, the quality of fresh produce of the Local Market must be maintained in hygienic condition and proper handling, transport, storage must be controlled so that risk of contaminants decreases and chances of food borne outbreaks can be minimized. These can be done by pre-treatment of fresh produce by various anti- microbial agents to decrease the density of microbial contaminant from the surface of the fresh produce.

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