The Effect Of Ph And Chemical Preservatives On The Growth Of Bacterial Isolates From Commercial Samples Of Fruit Juices Sold In South Eastern Nigeria

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Abstract: Five bacterial pathogens were isolated from commercial samples of fruit juices sold in South Eastern Nigeria. The isolates were characterized and identified as *Bacillus*, *Staphylococcus, Psuedomonas, Lactobacillus* and *Gluconobacter* species. The effect of pH, benzoic acid and Sodium chloride concentration on the growth rate of isolates was investigated. The result was that as the pH of the growth medium increased from 3 to 7, the rate of growth of the isolates increased. As the concentration of Sodium chloride increased from 2 to 4%, the rate of growth of the isolated decreased. As the concentration of benzoic acid increased from 250 to 1000mg/L, to growth rate of all the isolates decreased. Also as the concentration of Sodium chloride increased from 2 to 5% the growth rate of all the isolates decreased. The higher the concentration of the preservatives the lower the rate of growth of the isolates.
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Key words: Preservatives, *Bacillus, Staphylococcus, Psuedomonas, Lactobacillus* and *Gluconobacter* species.

Introduction

Fruit juice is extractable liquid naturally contained in fruit or vegetable tissue. Fruit juices are essentially products from fresh fruits such as orange, mango, pineapple, grapes etc. Juice is prepared by mechanically squeezing fresh fruits or vegetable without the application of heat or solvents. Juice may be prepared in the home from fresh fruits and vegetables using variety of land or juice extractor. The liquid expressed from lemons, limes and excessively acid fruits are certain by fruit juice, but the liquid is sour to consume directly addition of sugar and water to produce lemonade (Salunkle and Kadan, 1996). Many commercial juices are filtered to remove fiber or pulp, but a high pulp fresh orange juice is a popular beverage. Juice may be marketed in concentrated form, sometimes frozen, requiring the user to add water to reconstitute the liquid back to its original state (Frazier and Westhoff, 2002). Common methods for preservation and processing of fruit juices include freezing, pasteurization, evaporation and spray drying (Fasoyiro *et al*., 2005). Fruit juices are nutritious which offer great taste and health benefits. The 2005 Dietary Guidlines for Americans (2005) recommended consumption of several cups per day of fruit juices. Juices normally have a standard defined level of purity, which is 100% in many countries. To be labeled as a fruit juice, the Food and Drug Administration (FDA) mandates that a product should be 100% fruit juices, constituted from concentrate (FAO, 1992). Fruit juices are often consumed for their health benefits, for example orange juice is rich in vitamin C while prune juice is associated with digestive health benefits. Cranberry juice has long been known to help prevent or event treat bladder infections. However, the high amounts of fructose in fruit juice when not consumed with fiber have been suggested as a contributor to the growing diabetes epidemic in the West (Zarate-Rodriguez *et al*., 2000). Most fruit juices bought from supermarket shelves are pasteurized while a small percentage of fresh fruit juices are unpasteurized. Most people can enjoy unpasteurized juice and drink, but the effect can be severe or even deadly for young children, the elderly and people with weakened immune systems (Fasoyiro *et al*., 2005). Fruit juices are spoiled by *Bacillus lincheniformis*, *Aeromonas hydrophila, Bacillus circulans, Proteus morganni, Pseudomonas chloroaphis, Bacillus alvei* etc.

Their presence may pose risk to consumer's health (Hatcher *et al.,* 1992). They are highly resistant to the commonly used antibiotics which are a reflection of misuse or abuse of these antibiotics in the environment (Abbar and Kaddar, 1991; Malik and Ahmad, 1994). The low pH of fruit juices greatly limits the number and type of bacteria that can survive or grow at this low pH. However, there are a few bacteria that can tolerate and grow at such pH (Ryu and Beuchat, 1998). Most industrial juice concentrators use a high temperature evaporation and microbes are killed during this process, thus frozen concentrated fruit juice should have few if any microbes (Parish, 1997). It is possible to reduce the growth of bacteria in fruit juices by addition of chemical preservatives (IPAN, 1992). However, the use of unhygienic water for dilution, dressing with ice, prolong preservation without refrigeration while unhygienic surroundings can also act as sources of

contamination and such juices have shown to have short shelf lives and potential source of bacterial pathogens, notably *E. coli, Salmonella, Shigella* and *Staphylococcus aureus* etc. (Buchannan *et al*., 1999). This work was aimed at determining bacteria associated with commercially packaged fruit juices sold in South Eastern Nigeria as well as the effect of pH and chemical preservatives on the isolates.

Collection of Samples:

A total of hundred and thirty (130) packaged fruit juice samples consisting of thirteen different brands/manufacturer were purchased from main markets in Enugu, Awka, Owerri, Abakiliki and Umuahia (Capital cities) of South Eastern Nigeria. The samples included packaged: 'Five alive citrus burst', 'Dansa apple', 'Dansa orange', 'Dansa mango', 'Frutta mango', 'Frutta apple', 'Frutta orange', 'Chivita mango, Chivita orange', Five alive apple splash', and Fumman orange juices. The juices had at least 4 months to their expiry date from the period of analysis. The sampled fruit juices are registered food products approved by National Agency for Food and Drug Administration and Control (NAFDAC).

Isolation and Characterization of microorganisms: Nutrient agar (NA) was used for Total Heterotrophic Bacterial Count (THBC). The spread plate method of Cruickshank *et al*., (1982) was adopted. A O. 1ml aliquot of an appropriate dilution of the sample was used to inoculate the nutrient agar petri-dish in duplicates and incubated at 37^0C for 24h, after which the total colony counts and mean, was determined as described by Cheesebrough, (2003). The colonies were screened and identified as described by Buchanan and Gibbons, (1978). The results are shown in Table 1.

Isolation of Coliform

Coliform was detected using most probable number (MPN) technique. Their ability to ferment lactose with acid and gas production within 48 hours of incubation at 37^0C distinguished them. Three-tube procedure using lactose broth (Fawole *et al*., 2002; Bakare, 2003) to detect the coliform and determine most probable number (MPN) of coliform bacilli was adopted. A 0.1ml, 1ml and 10ml of each undiluted sample were used to inoculate Lauryl sulphate Tryptose Broth (LSTB) in three replicates. The inoculated LSTB tubes were incubated at 37^0C for 48h and the MPN determined in accordance with standard method (APHA, 1985) for detection of faecal coliform becteria. Positive reactions were indicated by turbidity and presence of acid and gas in Durham tubes. Most probable number (MPN) table was read for the tubes showing positive reactions and were cultured into MacConkey broth and incubated at 37^0C for 48hours. The tubes were plated on Eosin Methylene Blue agar (EMB) and incubated as before. Colonies on EMB plate were selected based on colour changes from dark colony to metallic Sheen. They were finally identified based on their cultural, morphological and biochemical characteristics, after isolation (Buchana and Gibbons, 1978).

Isolation and detection of *E. coli* was based on LSTB Enteric Enrichment (EE) broth and Streaking on Eosin Methylene Blue Agar (EMB). Each gassing tube was gently agitated and a loopful of suspension transferred to tube containing EE medium, which was incubated for 48hours at 45 (+ or – 2)^{o}C. The tubes were examined for gas production at 48hours. The MPN was calculated using MPN table and based on the proportion of confirmed gassing on EE medium for three (3) consecutive days. The presence of *E. coli* was further confirmed by biochemical characteristics (Buchanan and Gibbon, 1978).

Growth of Isolates at Different pH ranges:

Nutrient broth was prepared and the pH adjusted using 0.1M phosphate buffer to 3.0, 5.0 and 7.0. It was then dispensed into screw-capped bottles and then sterilized by autoclaving at 121^0C for 15min. After cooling, the various test isolates were inoculated into it and incubated at 30^0C for 48h. Growth was detected by increased turbidity using Cecil 2031 (automatic) spectrophotometer. Uninoculated tubes serve as control. The best pH that favours the bacterial growth and metabolism as indicated by the increased turbidity is determined (Schillinger and Lucke, 1989).

Evaluation of the effect of chemical preservatives on the growth of isolates: Growth in different concentrations of Benzoic acid.

Nutrient broth containing 250, 750 and 1000mg/l of benzoic acid was prepared and 10ml of the broth was dispensed into sterile screw capped bottles and then sterilized by autoclaving at 121^0C for 15min. after cooling, the bottles were inoculated with the test organisms and incubated for 24h at 37^0C. Growth was detected using an automatic spectrophotometer. Increase in turbidity of the medium was recorded as positive for growth while a negative result shows no turbidity. Uninoculated tubes serve as control.

Growth of isolates in Different Concentration of Sodium Chloride:

Nutrient broth containing 2% (w/v), 3% (w/v) Nacl was prepared and sterilized at 121^0C for 15min. 20ml of the broth was dispensed into sterile screw capped vials aseptically. After cooling, the tubes were inoculated with the test organisms and incubated for 24h at 30^0C. Increased turbidity of the medium was recorded as positive for growth while a negative result shows no turbidity. After cooling, the tubes were inoculated with the test organisms and incubated for 24h at 30^0C. Increased turbidity of the medium was recorded as positive for growth while a negative result

shows no turbidity Uninoculated tubes serve as control (Shillinger and Lucke 1989).

Results

A total of five (5) organisms were isolated from the packaged fruit juice samples. The isolates were subjected to cultural, morphological and biochemical tests and they were identified to be *Bacillus, Staphylococcus, Pseudomonas, Lactobacillus* and *Gluconobacter* species (Table 1).

Table 1: List of sources of Isolates:

Sample Code	Isolates
B1	*Bacillus* species
B2	*Staphylococcus* species
B3	*Pseudomonas* species
B4	*Lactobacillus* species
B5	*Gluconobacter* species

No coliform bacteria or *E.coli* was detected in any of the fruit juices analysed.

The growth of the isolates at different pH ranges was monitored using automatic spectrophotometer at wave length of 560nm. It was observed that as the pH of the growth medium was tending from acidic to basic, the growth rate of almost all the organisms increased (i.e pH increased from 3 to 7), the optical density (OD) readings for *Bacillus* spp. increased from 0.095 to 1.266nm, *Staphylococcus spp* increased from 0.098 to 1.121nm, *Pseudomonas* spp. increased from 0.156 to 0.882nm, *Lactobacillus* spp. increased from 0.54 to 1.340 and *Gluconobacter* spp. increased from 0.092 to 1.269nm (Table 2).

The growth rate of isolates in different concentrations of Benzoic acid decreased as the concentration of Benzoic acid increased from 250mgl^{-1} to 1000mg/l^{-1}, *Bacillus* species decreased from 1.352 to 0.178nm, *Staphylococcus* spp. decreased from 1.256 to 0.156nm, *Pseudomonas* species decreased from 1.283 to 0.125nm, *Lactobacillus* species decreased from 1.205 to 0.118nm and *Gluconobacter* species decreased from 1.298 to 0.10nm respectively (Table 3).

The rate of growth of isolates was also observed in different concentrations of Sodium chloride.As it increased, the rate of growth of the isolates decreased as indicated by the optical density readings; *Bacillus* species reduced from 0.902 to 0.132nm, *Staphylococcus* species reduced from 0.849 to 0.089nm, *Pseudomonas* species reduced from 0.311 to 0.032nm, *Lactobacillus* spp. reduced from 1.203 to 0.298nm, *Gluconobacter* species reduced from 1.016 to 0.417nm (Table 4).

Table 2: Rate of Growth of Isolates at Different pH (OD at 560nm)

Isolates	pH3	pH5	pH7
Bacillus spp	0.095	0.518	1.266
Staphylococcus spp	0.098	0.790	1.121
Pseudomonas spp	0.156	0. 780	1.882
Lactobacillus spp	0.054	0.870	1.340
Gluconobacter spp	0.092	0.945	0.269

Table 3: Rate of Growth of Isolates in Different Concentrations of Benzoic Acid (OD at 560nm)

Isolates	250mg/L	500mg/L	1000mg/L
Bacillus spp	1.352	0.451	0.178
Staphylococcus spp	1.256	0.408	0.156
Pseudomonas spp	1.283	0.427	0.127
Lactobacillus spp	1.205	0.392	0.118
Gluconobacter spp	1.298	0.501	0.109

Table 4: Rate of Growth of Isolates in Different Concentrations of Sodium chloride (OD at 560nm)

Isolates	2%	3%	5%
Bacillus spp	0.902	0.484	0.132
Staphylococcus spp	0.849	0.527	0.089
Pseudomonas spp	1.1.32	0.986	0.311
Lactobacillus spp	1.203	1.054	0.298
Gluconobacter spp	1.016	0.812	0.417

Discussion

The presence of different bacteria in commercially available fruit juices is of serious public health concern and may pose risks to consumer's health. The high acidity of fruit juice could have contributed for low number and few types of organisms isolated, although the isolates have been found to be associated with food spoilage (Prescott *et al*., 2002).

Results obtained from the effect on isolates in different pH ranges indicated that when these microorganisms are in acidic medium, their growth rate was reduced but as the pH tends from acidic medium to basic medium, the growth rate of all the isolates increased. These show that acidic medium greatly reduced their growth while in basic medium their growth was favored. The result for effect of chemical preservative against the microorganisms is that as the concentration of Benzoic acid increased from 250 to 1000mgL^{-1}, the growth rate of all the microorganisms decreased.

Also as the concentration of Sodium chloride (NaCl) increased from 2 to 5%, the rate of growth of all the isolates decreased. Preservatives have been used to store food substances and they act by inhibiting, retarding or arresting the growth of microorganisms or they may be microbiostatic in which case they simply prevent them from growing, thus improving the shelf-life of the product (Fawole and Osha, 2002). To encourage improved quality and good manufacturing practices, the use of some chemical preservatives which are Generally Regarded As Safe (GRAS) would be put into consideration and these preservatives should not permit the growth of food poisoning organism while suppressing the growth of others that would make spoilage evident (Oladipo *et al*.,2010). The absence of *Salmonella* and *Shigella* species showed improved hygienic sanitary conditions (Ashbolt *et al*., 2002).

Fruit juices are well recognized for their nutritive value, mineral and vitamin contents. They are beverages that are consumed for their nutritional value, thirst-quenching properties and stimulating effect or for their medicinal values. Contamination of fruit juices by these bacteria may occur when the organisms enter the processing plant or on the surface of the fruit having originated from soil, untreated surface water, dust and decomposing fruit. However, it is contended that contamination is mainly due to poor quality of water used for dilution, prevailing unhygienic conditions related to washing of utensil, maintenance of the premises and location by the side of waste disposal system or overcrowding.

Conclusion

The occurrence of pathogenic bacteria in fruit juices is a serious public health concern and needs immediate action by the suitable agencies. It is necessary that regular monitoring of the quality of fruit juices for human consumption be introduced to avoid any future pathogen out breaks. Also regulation in the issuance of permit to produce and sell these products should be under strict quality control to reduce and mitigate exposure to harmful microbes deleterious to consumers' health.

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