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Research Article

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Growth Pattern and the Survival Rate of *E. coli, Salmonella* spp. and *Shigella* spp. in A Commercially Available Juice Products

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ABSTRACT

A brand of commercially available juice products was purchased from the main market of Ado-Ekiti metropolis, Ekiti State, South-West of Nigeria.

A total of three fruit juices of multi fruits juice, orange juice, and coconut-pineapple juice, which are commonly consumed were screened for the survival rate of Escherichia coli, Salmonella species, and Shigella species and the growth pattern of these organisms were also observed. In the fruits analysed; the total plate count were 2.35 × 10^4 Log cfu/ml, 2.44 × 10^4 Log cfu/ml, and 2.57 × 10^4 Log cfu/ml. Escherichia coli range between 0.68 × 10^4 Log cfu/ml, to 0.01× 10^4 Log cfu/ml, Salmonella species range between 0.86 × 10^4 Log cfu/ml, to 0.01× 10^4 Log cfu/ml,, and Shigella species range between 0.84 × 10^4 Log cfu/ml, to 0.03 × 10^4 Log cfu/ml. Among the pathogenic microorganisms used, only Escherichia coli was able to survive after five days, Shigella Species died off after three days, while Salmonella species died off after four days. Many of these organisms can cause diseases in humans which indicate that the fruit juices must full fills the guidelines for the microbiological quality of juices and steps must be taken to improve the microbial quality of products and prompt consumption is recommended not to keep it after lid has been removed.

Keywords: *Fruit juice, Salmonella, E. coli, Shigella.*

INTRODUCTION

Fruit juice is the liquid naturally contained in fruit or vegetable tissues; it denotes a sweetened fruit extract prepared mechanically by squeezing fresh fruit or vegetables flesh without the application of heat or solvent. For example, orange juice is the liquid extract from the fruit of the orange tree.

Juice may be prepared in the home from fresh fruit and vegetable using hand or electric juice extractors juicers. Many commercial juices are filtered to remove fiber or pulp, but high pulp fresh orange juice is a popular beverage. Juice may be marketed in concentrated form sometimes; consumers may need to add water to reconstitute the liquid back to its original state. However, concentrates generally have a noticeable different taste from their freshly squeezed counterparts. Other juices are reconstituted before packaging for retail sales.

The bacteriological examination of juice is important since they are produced commercially to meet the demands of people for consumption. It is known that most types of juices are very high in bacteria load and thus may contribute to the spoilage of juice product in which they are used. It is because of the importance of such spoilage problems that scientists believed that a standard method for the bacteriological examination of juices should be proposed.

The processing of fruit juices is a relatively new industry in many ways but one which will require close supervision is the quality control of the products. Control of quality is essentially a control of cleanliness and handling of the food in the industry.

Foods with a pH lower than 4.0 are considered to be high in acid and are generally regarded as not being susceptible to spoilage by a variety of microorganisms[9]. At this low pH, spoilage is mostly caused by acid

tolerant yeast and mycelia fungi, while bacterial spores will not germinate and grow under these acidic conditions. The acid or acidified juice with a pH below 4.6 are not subjected to a heat treatment at temperature sufficient to destroy all the microbial spores. This will have a detrimental effect on the organoleptic quality of the product.

Traditionally, fruit juices are considered to be susceptible to spoilage only by yeast, mycelia fungi and lactic acid bacteria. The low pH is considered sufficient to prevent the growth of almost all bacteria spore-formers, spores of *Clostridium botulinum* cannot germinate or produce the lethal *botulinum* toxin in an environment with a pH below 4.6 [4]. Another common thermophilic spoilage organism, *Bacillus stearothermophilus*, cannot grow and cause the sour taste type of spoilage below a pH of 5.3.

Other organism of concern are *C. Pasteurianum* and *B. Coagulans*, being the only spore formers able to grow at pH 3.8, [9]. This has allowed the fruit beverage industry to successfully apply a hot fill hold process to pasteurize the products. This pasteurization process holds the products at temperature between 88^{o}C and 96^{o}C for 2minutes and is sufficient to destroy heat liable spoilage organisms such as yeast, lactic acid bacteria and some mycelial fungi. The products are then commercially sterile for duration of the specific shelf life, until the container is opened[2].

Cerney *et al.*,[3] reported the first juice spoilage case of commercially, available pasteurized fruit juice and found shelf – stable, aseptically packaged apple juice to have an off flavour. Following this report, a number of cases were reported from all over the world and almost all these spoilage incidents were caused by the spore- forming, thermo acidophilic bacteria from the genus *Alicyclo bacillus.* The aims and objectives of this research project work were to determine the growth of some selected pathogenic microorganisms in juice product. To know the survival rate of these selected pathogenic organisms in the fruits juice and to know the extent in which preservatives are able to prevent spoilage after removal of cover lids.

MATERIALS AND METHODS

COLLECTION AND PROCESSING OF SAMPLES

A brand of fruits juice which is commonly produced commercially namely: orange juice, multi fruits juice and coconut-pineapple juice were used for the analysis in the study. Three different samples of each of the fruit juice were analyzed. The samples were distributed at some different locations in Ekiti state. They were labeled appropriately and transferred to the laboratory for analysis.

INOCULATION OF MICROORGANISMS

During packaging of the product, contamination can occur (post-processing contamination). Therefore, before the juice sample was used in the investigation it was tested for the presence of any contaminating bacteria. In particular, it was tested for the presence of all the pathogens that were to be used in this study. This was done by plating out 0.1 ml of each sample on selective media and incubated at 37°C for 24 hrs. A pre-inoculum was prepared by inoculating one

loopfull of cells from each pure culture in 10 ml Tryptic Soy Broth (TSB). This was incubated for 6-8 h at 37°C. The pre-inoculum (0.1 ml) was then inoculated into 1 L Erlenmeyer flasks containing 200 ml of TSB and incubated at 37°C for a further 8 h. A cell concentration of 10^4 cfu/ml of each microorganism was inoculated into juice sample.

ENUMERATION OF FOOD-BORNE PATHOGENS AND LACTIC ACID BACTERIA

During each sampling day, 1 ml of the juice samples was aseptically drawn from the test juices and dispensed in 9 ml sterile distilled water. For each test sample, serial dilutions (10^{-1},10^{-2}, 10^{-3}) were carried out for microbial assays in 9 ml sterile distilled water and plated out in duplicate using the spread plate technique onto selective agar 0.1ml of undiluted juice samples was also plated out on the selective agar. The plates were then incubated aerobically at 37°C for 24 h. After incubation, typical colonies of the presumptive pathogens were counted and the results interpreted as cfu/ml. Microbiological analysis of the food borne pathogens was done for five days.

BACTERIOLOGICAL ANALYSIS METHODS; TOTAL PLATES COUNT (TBC)

20ml of the molten nutrient agar cooled at 41^{o}C was poured into each Petri dish. The agar was allowed to solidify and 1ml of the sample was pipetted and spread on the already solidify agar.

The plates were allowed to set after which they were incubated in inverted position at 37^{o}C. The plates were counted after 24 hours of incubation by the means of colony counter.

The agar plate method of examination of the fruit juices may be a method of determining the quality of the products. The method of estimating microorganisms consists of counting the microbial colonies or growth which develops upon a nutrient agar plate after mixing and incubating a suitable dilution of the juice.

RESULTS

From the analysis carried out, the following results were obtained. Table 1 shows the description of the organisms on the selective media. Table 2 shows the presentation of total plate count and *Escherichia coli* count in which there was a drastic reduction in the plate count for the *Escherichia coli* from the initial value of 3.2 logcfu/ml in orange, 0.65 log cfu/ml in multi fruits and 0.68 logcfu/ml in the coconut pineapple. On the first day of inoculation, the cells began to decrease with respects to the numbers of the organisms in the juice samples. This shows that there is highermicrobial count on the first day of inoculation and low count for the subsequent day until the fifth day. Where the cell number reduces to 0.01 log cfu/ml in the multi fruit, 0.13 Logcfu/ml in orange and 0.01 Logcfu/ml in the coconut pineapple.

The result of total plate count and *Salmonella* spp. count are shown in Table 3 in which there was a rapid decrease in the cell number from the initial 4.0 logcfu/ml to 0.86 logcfu/ml in multi fruit juice sample, 0.65 logcfu/ml in orange juice fruit sample, and 0.26 logcfu/ml in coconut pineapple was noticed. On the fourth day of inoculation, the cell population reduced, to 0.01 logcfu/ml, 0.01 logcfu/ml and 0.04 logcfu/ml in multi fruits, orange and coconut pineapple juice respectively. On the fifth day, the cell died off in all the three juice samples.

Table 4shows the result obtained from the total plate count and *Shigella* count in which the strains of *Shigella* species used could no longer be detected after 3 days in the juice sample. A count which showed decreased from an initial value of 3.5 log cfu/ml to 0.52 log cfu/ml in multi fruit, 0.01 log cfu/ml in orange juice sample and 0.8 logcfu/ml in coconut pineapple. The rate at which *Shigella* species died off in the entire juice sample was similar. The microbial count also decreased rapidly from the day of inoculation to the third day of inoculation. There is no significant difference in survival of *Shigella* species in the juice sample.

The total plates count on the samples rangedbetween 0.21×10^4 to 8.10×10^4in all the fruit juice samples.

Table 1: Description of Pathogenic Organisms on Selective Media

Microorganism	Selective Agar	Description of colonies
Escherichia coli	EMB Agar	Green metallic sheen
Salmonella species	SS Agar	Transparent with black center
Shigella species	MacConkey Agar	Colonies translucent with pinkish colour surrounded by yellow zones.

Table 2: Total plate and *Escherichia coli* Count (10^4 log cfu/ml)

Time (day)/hrs	Total plate count			*Escherichia coli*count		
	Multi fruit	orange	Coconut pineapple	Multi fruit	Orange	Coconut pineapple
0/06hrs	-	-	-	-	-	-
1/24hrs	7.33	7.22	7.10	0.65	0.68	0.68
2/48hrs	5.02	5.38	5.57	0.60	0.51	0.51
3/72hrs	4.29	4.47	4.20	0.43	0.43	0.39
4/96hrs	2.47	2.50	2.53	0.28	0.23	0.25
5/120hrs	1.32	1.47	2.30	0.01	0.13	0.01

Table 3: Total plate and *Salmonella species* count (10^4 log cfu/ml)

Time (day)/hrs	Total plate count			*Salmonella species count*		
	Multi fruit	orange	Coconut pineapple	Multi fruit	Orange	Coconut pineapple
0/06hrs	-	-	-	-	-	-
1/24hrs	4.77	4.98	3.80	0.86	0.65	0.26
2/48hrs	2.07	2.36	3.00	0.42	0.32	0.12
3/72hrs	1.86	1.08	1.20	0.03	-	0.06
4/96hrs	1.16	0.66	1.20	0.01	0.01	0.04
5/120hrs	0.78	0.84	0.99	-	-	-

Table 4: Total plate and S*higella* count (10^4 log cfu/ml)

Time (day)/hrs	Total plate count			*Shigella* species count		
	Multi fruit	orange	Coconut pineapple	Multi fruit	Orange	Coconut pineapple
0/06hrs	-	-	-	-	-	-
1/24hrs	2.21	2.17	2.86	0.52	0.644	0.84
2/48hrs	0.72	1.23	2.12	0.34	0.24	0.30
3/72hrs	0.71	1.03	1.92	0.03	0.08	0139
4/96hrs	0.33	0.64	0.55	-	-	-
5/120hrs	0.21	0.53	0.46	-	-	-

DISCUSSION

Fruits juices were until recently considered to be susceptible to spoilage by yeast, mycelia fungi, and lactic acid bacteria. Spoilage by these organisms was prevented by the acidic pH of fruit juices and heat treatment applied during the hot-fill-hold process. Despite these control measures, an increasing number of spoilage causes of fruit juices, fruit juice products and acidic vegetables due to contamination by some organisms have been reported by Suaads and Alwakeel[15].

From the analysis carried out, it was observed that from all juice samples none of the presumptive food borne pathogens being tested was found in any of the uninoculated juice samples (control). There were no bacteria plate count in the control sample which shows that the fruits juice was free from any contaminants and it is said to be fit for consumption as studied by Johnson and Leyer, (2008).

The results showed that the entire juice sample inhibited the growth and the survival of all the tested food -borne pathogen namely E. coli, *Shigella*species and *Salmonella* species the antimicrobial activity increased in the juice samples with increased to the number of days in which the organisms present in the juice samples as stated by Theron and Lues[18]. It has been reported by Tajkarimjji and Ibrahim[17] that *E. coli* survival well at pH values of below 3.4 acidic fruits and juice. Results of the study indicate that the pH (Acidic property) in the juice samples would not be a key factor for influencing the survival of *E. coli*. It was noticed that the antimicrobial activity of the preservation which is sodium sorbate used for the preservation of the juice samples against the Pathogenic micro- organisms which includes *E.coli*, *Salmonella* species, *Staphylococcus aureus* and *Shigella*species leads to the inhibition and reduction in the number of cells in the medium as earlier studied by Cooke and Brook, (2011).

Citric acid and sodium sorbate as preservatives agents in all the juice samples is an antimicrobial agent which inhibits the growth and the survival of microorganism in the juice sample. These preservatives were responsible to kill the vegetative cell of the tested microorganisms in the juice sample and leads to the inability of the organism to multiple. As reported by Badet and Furige[1], the vegetative cells in the bacteria are responsible for their growth and if the vegetative cell in microorganism is damaged. It leads to blockage in the growth cycle and the survival of the organisms will be inhibited.

This results shows that the survival of the presumptive food borne pathogens (*E. coli, Salmonella* species and *Shigella* species). Was very low in all the juice sample and this was as a result of the environment in which these organisms were introduced. Golden[8] reported that organisms will not survive in any

environment which are not favourable for them. And this was observed as non of the tested microorganisms was able to survive in the juice sample. The preservatives was able to kill the vegetative cell of the organisms which were required for the division and growth of the organism and was responsible for the drastic reduction in the number of cell of all the tested microorganisms and in the total plate count in all the juice sample. Low population, reduction in the number of colonies and also the death of the organisms was as a result of inability of the organisms to divide and multiple in the juice samples.

Lewis[12] has reported the need for microbiological examination in this field can be found in the public health aspects of production, distribution and wide spread use of juice products and the need for determination and the causes and prevention of spoilage.

Bottled beverages of juice products are consumed as thirst quenchers. As much they should meet the requirements for drinking water has regards to the various types of organisms. Whenever the furnished juice products or beverages did not meet the requirement for drinking water according to the public health service Drinking water standards[13], there is indication of unsatisfactory raw materials or very faulty processing methods. In most cases, the primary sources and carrier of coliform organisms is the water supply employed.

High bacteria counts and mould may be indicative of poorly cleaned equipment on reflective of the bacteria may be an indication of fermentation of the juice products. The use of good quality product of low microbial count, Suaard and Eman[15] have shown that juices produced from soft rot fruit contains many times the total bacteria number found in juice from sound fruit.

Uchgaonkar and Dahiya[19] have pointed out the vital role of fruits sanitation in maintaining high quality in juice and concentration products.

CONCLUSION

The study carried out shows that food borne pathogen of the Entero-*bacteriaceae* family can survive for long period in the juice sample which shows that the *Escherichia coli* remained viable for longer period compared to other pathogens. This provides clear evidence that this bacteria is slightly tolerant to acidic condition. *Salmonella* species can only survive for three to four days in the juice samples. It was contended that contamination is mainly due to poor quality of water, the raw fruits used for the production of the juice (beverages, improper use of preservatives of the juice products, inadequate sealing and canning of the beverages as well as prevailing unhygienic conditions related to washing of utensils and maintenance of premises.

REFERENCES

1. Badet, A.C., Quinto, E. J. and Mora, M. T. Kinetic parameters of *Escherichia coli* O157:H7 survival during fermentation of milk and refrigeration of home-made yoghurt. *Int. Dairy J.* **16**: 474-481 (2009)
2. Blocher, J. and Busta, L.R., Growth conditions. *Appl. Environ. Intern. Med.* **130**: 202-203 (2010)
3. Cerney E.A., Control bacteria spores of British Medical Bulletin. **56**: 158-121 (1984)
4. Chang, S.S. and Kang, K.Y., Presence and activity of psychrotrophic and survival of *E. coli*under acidic micro organisms in milk and dairy products.A review, *J. Food Prot.* **45**: 172-207 (2004)
5. Clarke, M.A., Yoghurt in the United Kingdom: chemical and microbiological analysis. *Dairy Ind*. **39**: 149-157 (2007)
6. Cooke, K., Cole, M., Jones, M. and Holyoak, C., The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *J. Appl. Bacteriol*. **69**: 63-72 (2000)
7. Dennis K., Beuchat L.R. and Ryu J. H., Produce handling and processing practices. *Emerg. Infect. Dis*. **3**: 359-465 (2010)
8. Golden D.A., Survival of *Escherichia coli* during fermentation of apple cider. *J. Food Prot.* **59**: 1256-1259 (2006)
9. Jay, J.M. Intrinsic and extrinsic parameters of foods that affect microbial growth. *Modern Food Microbiology*, 5th ed. Pp. 38-44 (2008)
10. Johnson, K. Hamann, W. T. and Marth, E.H., Survival of *Streptococcus thermophiles* and *Lactobacillus bulgaricus*in commercial and experimental yoghurts. *J. Food Prot.* **47**: 781–786 (2006)

11. Kenneth, C.A. Benjamin, M.M. and Datta, A.R., Acid tolerance enterohemorrhagic *Escherichia coli. Appl. Environ. Microbial.* **61**: 1669-1672 (2005)
12. Lewis, X. and Alm, L., Survival rate of *Salmonella* and *Shigella*in fermented milk products with and without added gastric juice: an *in vitro* study. *Prog. Food Nutr. Sci*. **7**: 19-28 (2006)
13. PHS. Public Health and non pasteurized fruits juices. *Crit. Rev. Microbial*. **23**:109-119 (2010)
14. Potter, J. F., Water recreation and disease: Plausibility of associated infections: Acute effects, sequelae and mortality. *The Environmentalist* **26(4)**: 329–329 (2006)
15. Suaards, A. and Eman A., Microbial growth and chemical analysis of bottled fruit juices and drinks in Saudi Arabia. *Research Journal of Foods Microbiology.* **3**: 315-325 (2008)
16. Ryan, Kenneth James; Ray, C. George, ed. *Sherris Medical Microbiology: an introduction to infectious diseases* (4 ed.). McGraw-Hill Professional Med/Tech (2004)
17. Tajkarimi, M. and Ibrahim, S.A., Antimicrobial activity of ascorbic acid alone or in combination with Lactic Acid on *Escherichia coli* 0157:H7 in Laboratory Medium and carrot juice. *Food control*. 801-804 (2011)
18. Theron, M.N. and Lues, J.F.R., Organic Acids and Meat Preservation: *A review, Food Reviews International,* 141-158 (2007)
19. Uchgaonkar, P. and Dahiya, Phytochemical Analysis and Antibacterial Activity of juice products. *Journal of pure and Applied Microbiology*. 417-420 (2011)