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Evaluation of the viability and survival rates of some selected pathogens on artificially inoculated fruit juice in Iwaro & Ikare Akoko market store, Ondo state, Nigeria

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Abstract
Artificially inoculated fruit juice is a very viable profit making sole business in Nigeria, the product is locally available in all market in different parts of the country, especially in Ondo state, using Ikare and Iwaro Akoko market, as the focal point of this present study. Fifty (50) samples of the inoculated fruit juice were purchase from the market in Iwaro and Ikare Akoko, Ondo State. The test organisms used for this research work are *Staphylococcus aureus* (ATCC) 25923, *Salmonella spp* (ATCC) 43971, *and Escherichia coli 0157:H7* (ATCC) 27325, *Pseudomonas aeruginosa* (ATCC) 9027 *and Aspergillus flavus* (ATCC) 9804, 83435. Sterility test and survival rate of the test organisms were carefully carried out into the inoculated fruit juice which were purchase from the two market. It was observed that the survival rate of *Staphylococcus aureus (*ATCC) 25923, *Pseudomonas aeruginosa* (ATCC) 9027, *Escherichia coli 0157:H7* (ATCC) 27325, *Salmonella spp* (ATCC) 43971 and *Aspergillus flavus* (ATCC) 9804, 83435 were conspicuously very high, *Staphylococcus aureus(*ATCC) 25923 were completely inhibited within ten(10) hours contact time and none of these microbes were viable for a period of hundred (100) hours in the fruit juices with preservatives except *Aspergillus flavus* (ATCC) 9804, 83435. It was also observed that the viability of of *Aspergillus flavus* (ATCC) 9804, 83435 may be due to presence of spores formation within the inoculated fruit juice. The purpose of this present study is to investigate the viability and survival rates of the microorganism in artificially inoculated fruit juice in Iwaro and Ikare market of Ondo state, Nigeria

Keywords: Sterility test, Survival rates, Artificially Inoculated fruit juice,

1. Introduction
The conversion of fruits into juice was originally developed as a method for making use of supplies surplus to the fresh fruits market, but, while it still fulfils this function, juice production is now firmly established in its own right. A fresh juice may be defined as the liquid expressed by pressure or mechanical means from the edible portion of the fruit. It will frequently by turbid, containing cellular components in colloidal suspension with variable amount of finely divided tissue. It may also contain only or waxy and carotene pigments derived from the skin of the fruit. Some juices, for example orange juice, are consumed in their naturally state [8, 11, 2]. All types of juice are inherently unstable; they rapidly undergo microbiological attack by organism already present on the fruit or gaining access to the produce during processing; they are also subjected to enzymic and non-enzymic changes.

Micro-organisms are present in the air, in dust, soil, sewage and on the hands and other parts of the body. They are so widely distributed that their presence in or on food is inevitable unless special steps are taken to kill them. If food is to be kept in good condition for any length of time, it is essential that the growth of micro-organisms be prevented. This can be done either by killing them and then storing the food in conditions where further infection is impossible or by creating an environment, which slows, down or stops their growth [2, 10, 15, 23].

Juices are often consumed for their perceived health benefits. For example, orange juice is rich in vitamin C, folic acid, potassium, is an excellent source of bioavailable antioxidant phytochemicals [12] and significantly improves blood lipid profiles in people

affected with hypercholesterolemia (Kurowska *et al.*, 2005). Prune juice is associated with a digestive health benefit. Cranberry juice has long been known to help prevent or even treat bladder infections, and it is now known that a substance in cranberries prevents bacteria from binding to the bladder [7].

Many fruit juices have a higher sugar (fructose) content than sweetened soft drinks; e.g., typical grape juice has 50% more sugar than Coca-Cola. While soft drinks (e.g. Coca-Cola) cause oxidative stress when ingested and may even lead to insulin resistance in the long term, the same thing cannot be attributed to fruit juices. On the contrary, fruit juices are actually known for their ability to raise serum antioxidant capacity and even offset the oxidative stress and inflammation normally caused by high-fat and high-sugar meals (Ghanim 2010). However, frequent consumption of fruits and fruit juice causes dental decay, and may be a more significant factor in the development of dental caries (cavities) than eating candy. Fruit juice causes dental decay because it naturally contains acids, which chemically dissolve the enamel off the surface of the tooth, and sugars that the bacteria in the mouth ferment to create even more tooth-destroying acids [13].

Fruit juice consumption overall in Nigeria has increased in recent years, probably due to public perception of juices as a healthy natural source of nutrients and increased public interest in health issues. Indeed, fruit juice intake has been consistently associated with reduced risk of many cancer types, might be protective against stroke and delay the onset of Alzheimer's disease [7, 17, 26].

Some fruit juices have filtered out the dietary fiber present in the fruit. In other cases, other ingredients are added. High-fructose corn syrup, an ingredient in many juice cocktails, has been linked to the increased incidence of type II diabetes. High consumption of juice is also linked to weight gain in some studies, but not in others. In a controlled clinical study, regular consumption of grape juice for 12 weeks did not cause any weight gain in volunteers, but consumption of a soft drink did. Fruit juice in moderate amounts can help children and adults meet daily recommendations for fruit consumption, nutrient intake and calories [1, 22, 14].

2. Materials and Methods

This research work involve fifty(50) samples from the two market in Ikare and Iwaro Akoko, Ondo state.and five different microorganism were used for this research work. The five microbes are *Staphylococcus aureus* (ATCC) 25923, *Salmonella spp* (ATCC) 43971, *and Escherichia coli 0157:H7* (ATCC) 27325, *Pseudomonas aeruginosa* (ATCC) 9027 *and Aspergillus flavus* (ATCC) 9804, 83435.

i , Standardization of the Test Microorganisms Used

The test microorganisms used were obtained from the Department of Microbiology, College of Natural Science, University of Agriculture Abeokuta, Ogun State. They include *Staphylococcus aureus (*ATCC) 25923, *Salmonella spp* (ATCC) 43971, *and Escherichia coli 0157:H7* (ATCC) 27325, *Pseudomonas aeruginosa* (ATCC) 9027 *and Aspergillus flavus* (ATCC) 9804, 83435. The organisms were maintained on Nutrient Agar and Sabouraud Agar for bacteria and fungi respectively.

The test organisms used were standardized by transferring 40µl of an overnight broth culture of the test organism into 20ml molten Nutrient Agar and molten Potato Dextrose Agar for bacteria and fungi respectively. The molten agar was mixed by rolling between palms, poured into plate, allowed to set and incubated for 24 hours at 37 ºC for bacteria and 72 hours at room temperature for fungi respectively. This was performed in triplicate. All colonies that appeared on the plate after incubation were counted using colony counter and the average count was noted [3].

ii. Sterility Test on the Juices

The top of the juice container was swabbed with 70% alcohol and allowed to dry, the lid was opened and 100 µl of the fruit juice was transferred into universal bottles containing 20 ml of Nutrient broth and 20 ml of Sabouraud broth respectively before subculturing on Nutrient Agar and Sabouraud Agar to dilute out the effect of the preservatives, which may inhibit the growth of the organisms present and to isolate the organisms. Forty microliters was taken from the content of the bottle and transferred onto Nutrient Agar and Sabouraud Agar plates. The bottle, Nutrient broth and agar were incubated at 37 ºC for 24 hours for the presence of bacteria; the Sabouraud broth and Agar were incubated at room temperature for 72 hours for the presence of fungi. The growth organisms were subcultured onto blood and Sabouraud Agar plates, which were incubated for 24 hours at 37 ºC for bacteria and 72 hours at room temperature for the fungi respectively. After incubation, all the fruit juices that were sterile were tested further for rate of survival and effect of storage on some selected organisms while those that were non- sterile were enumerated for microorganisms [6, [18]

iii. The Survival Rate of Test Microorganisms Inoculated Into Fruit Juices

The fruit juice container was swabbed with 70% alcohol and allowed to dry. The lid was opened, 20 ml was especially dispensed into sterile universal bottle and was inoculated with 500 µl of standardized test organisms. An aliquot of (40 µl) of the inoculated sample was immediately transferred into 10 ml of 0.1% peptone water to obtain a (1:250 dilutions), which dilute the effect of the preservatives and raise the pH of the juices) and 40 µl of was plated in duplicate on Agar at different time intervals (10, 20, 40, 60, 80, and 100 hours). The plates and the peptone water were incubated at 37 ºC for 24 hours for bacteria and 72 hours at room temperature for the mold. The plates were examined for growth and counted. The peptone water was checked for turbidity. Growths in peptone water were confirmed by subculturing onto Nutrient Agar and Sabouraud Dextrose Agar plates [5, 19].

3. Result and Discussion

Table 1: Comparing the Viability of Some Selected Microorganisms in the Juices after 24 hours of Artificial Inoculation.

TYPE CFU/ML VIABLE OF FRUIT SD JUICE	INOCULATED MICROBES	LOG CFU/ML MEAN ± SD	F	P-VALUE	MOST ORGANISM
ZO	*Staphylococcus aureus*	0.00 ± 0.00			
	Pseudomonas spp	0.86 ± 2.27			
	Escherichia coli	0.86 ± 2.27	12.94	< 0.05	*Aspergillus*
	Salmonella spp	0.86 ± 2.27			*flavus*
	Aspergillus flavus	5.92 ± 0.08			
UB	*Staphylococcus aureus*	0.00 ± 0.00			
	Pseudomonas spp	2.51 ± 3.13			
	Escherichia coli	1.67 ± 2.86	6.16	<0.05	*Aspergillus*
	Salmonella spp	2.44 ± 3.05			*flavus*
	Aspergillus flavus	6.00 ± 0.00			
DO	*Staphylococcus aureus*	0.00 ± 0.00			
	Pseudomonas spp	0.86 ± 2.27			
	Escherichia coli	0.86 ± 2.27	41.14	< 0.05	*Aspergillus*
	Salmonella spp	0.86 ± 2.27			*flavus*
	Aspergillus flavus	6.00 ± 0.00			
UA	*Staphylococcus aureus*	0.00 ± 0.00	34.74	< 0.05	*Aspergillus*
	Pseudomonas spp	1.66 ± 2.86			*flavus*
	Escherichia coli	3.31 ± 3.10			
	Salmonella spp	3.30 ± 3.10			
	Aspergillus flavus	6.00 ± 0.00			
MB	*Staphylococcus aureus*	0.00 ± 0.00	35.78	< 0.05	*Aspergillus*
	Pseudomonas spp	3.32 ± 3.11			*flavus,*
	Escherichia coli	3.28 ± 3.07			*Salmonella*
	Salmonella spp	4.87 ± 2.16			*spp*
	Aspergillus flavus	6.00 ± 0.00			
MO	*Staphylococcus aureus*	0.00 ± 0.00	40.22	< 0.05	*Aspergillus*
	Pseudomonas spp	4.12 ± 2.82			*flavus,*
	Escherichia coli	4.12±2.8			*Salmonella,*
	Salmonella spp	5.81 ± 0.14			*Pseudomonas,*
	Aspergillus flavus	5.92 ± 0.08			*Escherichia*

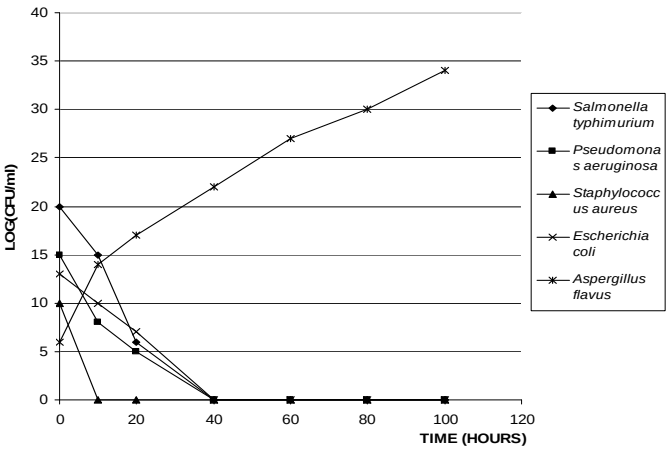

Fig. 4.1: SURVIVAL RATE OF VARIOUS ORGANISMS INOCULATED INTO ZO JUICE

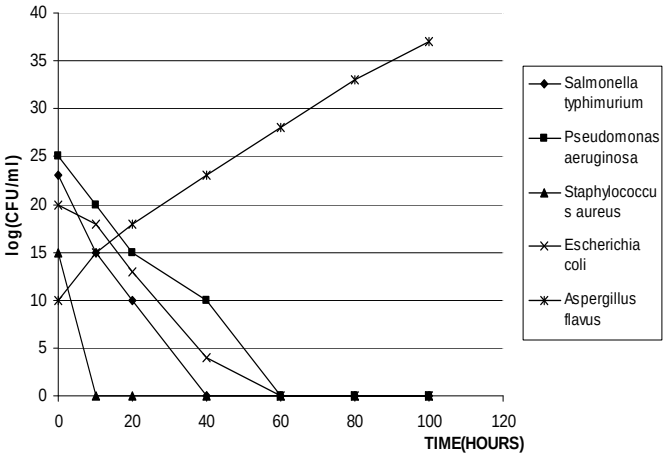

Fig. 2: SURVIVAL RATE OF VARIOUS ORGANISMS INNOCULATED INTO U B JUICE

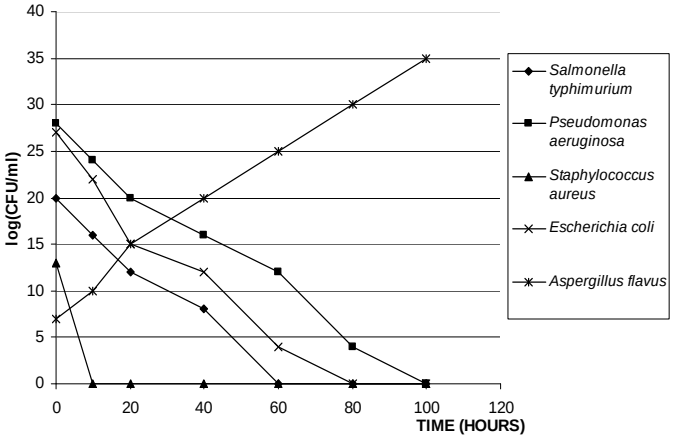

Fig.4 3: SURVIVAL RATE OF VARIOUS ORGANISMS INOCULATED INTO DO JUICE

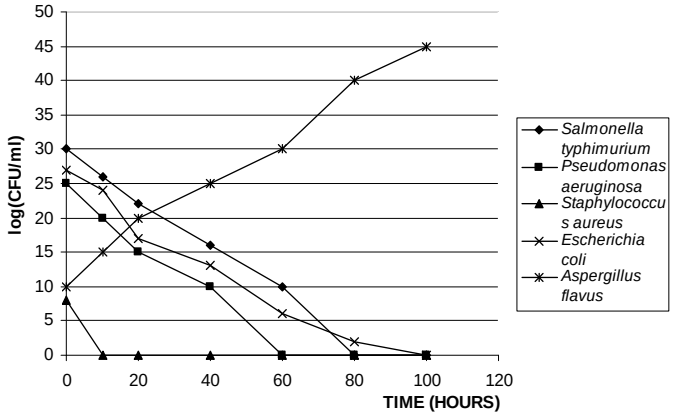

Fig. 4.4: SURVIVAL RATE OF VARIOUS ORGANISMS INOCULATED INTO UA JUICE

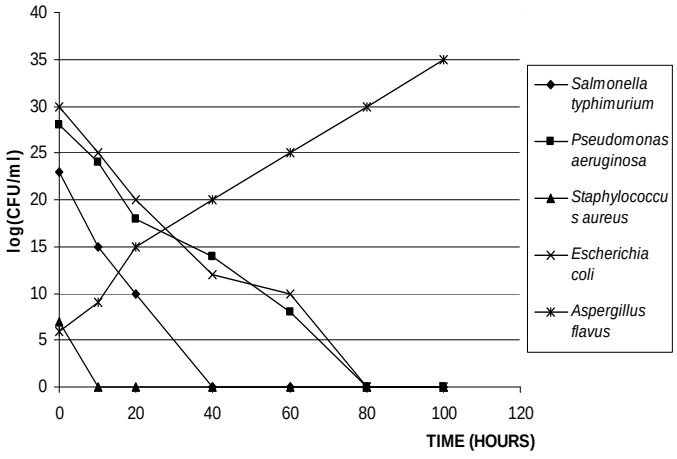

Fig. 4.5: SURVIVAL RATE OF VARIOUS ORGANISMS INOCULATED INTO MB JUICE

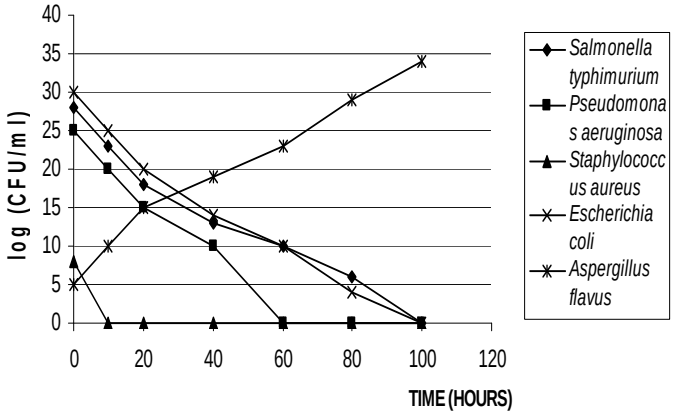

Fig. 4.6: SURVIVAL RATE OF VARIOUS ORGANISMS INOCULATED INTO MO JUICE

4. Discussion

The comparison between comparing the viability of some selected microorganisms in the fruit juices with preservative agents after 24 hours of artificial inoculation showed that there were significant differences among their growths ($p<0.05$) (Table 6).

The survival rate of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium* and *Aspergillus flavus* when they were artificially inoculated into ZO, DO, UA, UB, MB and MO packaged juices with preservative agents at different time intervals showed that *Staphylococcus aureus*, did not survive beyond 10hours while *Aspergillus flavus* survived beyond 100 hours (Figure 1, 2, 3, 4, 5 and 6).This finding were corroborated by buster 1983, which stated that the microbe, *Aspergillus flavus* was able to survive due to the fact of spore formation and other environmental factors like pH, water availability and moisture content.

On assessment of the survival rates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium* and *Aspergillus flavus* in ZO, DO, UB, UA, MB and MO fruit juices showed that *Staphylococcus aureus* was completely inhibited 10 hours contact time. None of these microbes was viable for a period of 100 hours in these fruit juices with preservatives except *Aspergillus flavus*. The viability of *Aspergillus flavus* may be due to presence of spores. Sporulated organisms are resistant to environmental factors such as temperature, heat etc (Michael *et al*. 2000). On comparing the average microbial density over the whole period, the most viable organism in ZO, UB, DO, and UA was *Aspergillus flavus* while *Aspergillus flavus* and *Salmonella typhimurium* were the most viable in MB ($p<0.05$) Davidson (1997) [9].

5. Conclusion

It can be clearly stated that inoculated fruit juice production is another small scale business in Nigeria, and also a fast moving trade, but caution must be taking by the regulatory agency on the scourge of pathogenic organism which is associated with the production and hardly of this importance essential commodity. The use of preservatives and other fruit juice enhancers must be adequately controlled. Quality control and assurance must be set up in order to control the quality of this useful product in the society where health is paramount to us and the physical well -being of the people is will not be compromised.

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