

Short Communication

Determination of bioload of commercially available brands of fruit juice in Uyo, Nigeria

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Accepted 28 June, 2010

Six brands of fruit juice preparations made up of three different batches per brand were used for the study. The purpose of the experiment was to identify and characterize the microbial load (bioload) in these products using standard procedures. At the end of the study, none of the samples was found to contain any viable microorganism. These findings showed that the samples used in the study were of high quality standard, and therefore fit for human consumption. Foods with a pH lower than 4 are considered as high in acid and are generally regarded as not being susceptible to spoilage by a variety of microorganisms. At this low pH, acid – tolerant yeasts and mycelial fungi mostly cause spoilage, while bacterial spores will not germinate and grow under these acidic conditions. The acid or acidified foods with a pH below 4.6 are not subjected to heat – treatments at temperatures sufficient to destroy bacterial spores.

Key words: Bioload, fruit juice, viable, microorganism.

INTRODUCTION

Traditionally, fruit juices are considered susceptible to spoilage only by yeast, mycelial fungi and lactic acid bacteria (Chang and Kang, 2004). Foods with a pH lower than 4 are considered as high in acid and are generally regarded as not being susceptible to spoilage by a variety of microorganisms (Jay, 1998). The low pH is considered sufficient to prevent the growth of almost all bacteria spore – formers. Spores of *Clostridium botulinum* can not germinate or produce the lethal *botulinum* toxin in an environment with a pH below 4.6 (Chang and Kang, 2004).

The microorganisms present in fruit juice often originate from the natural flora of the raw materials used for the preparation and those introduced during the course of the processing (Splittstoesser et al., 1994). The number and types of organisms are determined by the properties of the food product and activity of the organisms in the product. In some cases, the micro-organisms have no discernable deleterious effects and the food is consumed without harm (Yeh et al., 2004). In other cases, however, the presence of microorganisms have manifested in form

of spoilage, food-borne illness and fermentation (Yeh et al., 2004). During the heat – treatment of foods, pathogens and most non – spore – forming micro – organisms are killed, but a heat process sufficient to destroy all the microbial spores will have a detrimental effect on the organoleptic quality of the product (Walls and Chuyata, 2000). The purpose of this study is to evaluate the purity of some brands of fruit juice in Uyo Nigeria. The presence of Registration Number from Regulatory Agencies may not necessarily give assurance of quality, as these can be faked.

MATERIALS AND METHODS

Brands of fruit juice used

The fruit Juices employed in this study were locally manufactured by companies in Nigeria and were purchased at Uyo market. A total of 6 brands were used in the study and each brand contains three samples of different batches (a, b and c). The various fruit juice preparations are shown on Table 1.

Microbial count

The experiment was carried out in three different phases. Each batch of the different brands of the fruit juice constituted a phase

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Table 1. Various fruit juice preparations used.

Product code	Name of product	Manufacturer's address	Container type	Volume	NAFDAC reg. No	Batch No.
1a.	Apple extract drink (Chivita®)	9A, Block E Ind Estate, Badagry Express way, Amuwo Odofin Lagos Nigeria.	X	50 cl	01-4278	521032388
b.	"	"	"	"	"	732433210
c.	"	"	"	"	"	313489021
2a.	Yoghurt fruit drink	Chi Ltd, Chivita Avenue Ajao Estate Akpakun Oshodi Lagos	Y	250 ml	Nil	18;45;26
b.	"	"	"	"	"	"
c.	"	"	"	"	"	"
3a.	Orange fruit drink (Jucee®)	3 Ladipo Oluwole Street, Ikeja, Lagos, Nig.	Y	1 L	Nil	09.06
b.	"	"	"	"	"	09.04
c.	"	"	"	"	"	09.01
4a.	Blackcurrant Fruitdrink (Ribena®)	Igbesa Rd, Agbara, Ogun State.	Y	200 ml	01-3699	01:39:02
b.	"	"	"	"	"	01:41:21
c.	"	"	"	"	"	02:04:31
5a.	Citrus drink (Tampico®)	Eleyele Ind. Layout, Ibadan, Nigeria.	Y	200 ml	01:3699	01:39:02
b.	"	"	"	"	"	01:32:11
c.	"	"	"	"	"	01:34:05
6a.	Greatson immense flavoured orange drink (Dansa®)	KM 10 Onitsha Expressing, Abeokuta	X	1 L	01-75163	001
b.	"	"	"	"	"	002
c.	"	"	"	"	"	003

X = plastic container, Y = paper pack, a, b and c = different batches.

made up of both positive and negative controls. For the positive control, a mixture of culture containing *Escherichia coli* and *Candida albicans* was inoculated on an over-dried agar plate and incubated in the same environment conditions as test. The negative control is made up of only the over-dried agar on which nothing was inoculated and was also placed at the same environmental conditions as the test.

For phase A, the experiment was carried out without further diluting of the fruit juice. This was to determine the presence or otherwise of viable microorganisms in the fruit juices at the concentration at which they were consumed. In phase B, the diluent used was sterile water while normal saline was used as the diluent in phase C. The use of both sterile water and normal saline are known to encourage growth of microorganisms in fruit juice under storage. The underside of an over-dried agar plate was divided into eight segments using a marker. 5 ml of the fruit juice was withdrawn from each sample in phase A and transferred to sterile test tubes. A sterile rubber teat was attached to a sterile pipette and used to suck up and down twice from the samples in the test tubes. The sample was finally collected and at a constant rate of one drop per second, one drop each was placed at the centre of each marked segment of the plates.

Bacterial numbers were estimated using the Pour Plate Technique. Using sterile distilled water, serial dilutions of (10⁻¹-10⁻⁶) were prepared from each of the fruit juice samples and incubated at respective temperatures for 48 h. Total viable counts were determined on Plate Count Agar, *E. coli* on Macconkey medium, and *C.*

albicans on Sabouraud medium. Identification of microorganisms was done using the API 20E system (Analytical Profile Index, Biomérieux, Durham, NC, USA). The pH determinations were done using a pH meter (Orion Model 420A).

The plates were covered and kept until the drops permeated into the medium. The plate were labeled for the particular sample and incubated in an inverted position for 24 h at 37°C alongside of the positive and negative controls. For phase B, the diluent used was sterile water. A sterile pipette was used to aseptically transfer 1 ml of each sample in this group into sterile test tubes containing 9 ml of sterile water making a 10-fold serial dilution. The same procedure as in phase A was applied in the inoculation of the plates in phase B with samples and same environmental conditions were applied. For phase C, the diluent used was normal saline. A 10-fold serial dilution of the samples in this group was made using the normal saline. The same procedures as in A and B above were strictly followed also allying the positive and negative controls.

RESULTS

After a period of 24 h, no growth was seen on any of the test groups. There was also no growth on the negative control groups while there were some visible growths on positive groups which has plates containing the microorganisms (*C. albicans* = 2.3 × 10³, *E. coli* = 3.4 × 10³ for

Apple drink, *C. albicans* = 2.1×10^3 , *E. coli* = 3.5×10^3 for orange drink, *C. albicans* = 1.75×10^3 , *E. coli* = 3.0×10^3 for black currant drink and *C. albicans* = 2.8×10^3 , *E. coli* = 5.4×10^3 for yoghurt). The plates were left in the incubator for further 48 h. After the 72 h period, there were still no growths on any of the test groups as well as the negative controls.

Since there were no visible growths on any of the test samples, it therefore showed that, they do not contain any viable micro-organisms. They could contain dead microorganisms, however, but this did not show in form of growth. For the group in phase A which was undiluted and showed no visible growths, it implied that, either the preservative used did not allow the growth of the micro-organisms at that concentration or the manufacturers observed strict aseptic techniques to eliminate microbes at the time of production. The difference in packaging did not affect the quality of the six brands of fruit juice analyzed.

DISCUSSION

In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised. In the present investigation, there was visible growth only on the positive control. The results from positive control group showed that black currant has the least microbial count while yoghurt has the highest. Milk is an essential component; this might be responsible for the microbial count on positive control.

Overall, the preservatives used in the preparations could have put the microorganisms in their dormant stage which resulted in the lack of growth in the above two experiments. The effect of introduction of water and normal saline which has been shown to support the growth of micro-organisms was also checked. After 72 h of incubation, there were still no growths on the test samples. The result of this work has gone a long way in assuring the public of the safety of the six brands of the fruit juices produced and marketed in Nigeria.

In conclusion, it can be categorically stated that, within the limits of experimental error, the above brands of fruit beverages marketed in Uyo, Nigeria are pure and free from microbial contamination and, therefore, fit for human and animal consumptions.

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