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Hygienic Quality and nutrient characterization of three fruits juices sold in Lome Schools

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ABSTRACT

Objectives: The objective of this study was to evaluate the microbiological quality and physico-chemical parameters of fruit juices served to the students in several schools in Lomé town, following standard procedures.

Results: All of juices analyzed were contaminated by total aerobic count. 79.31% of lemon juices samples were infected by total coliforms, 41.38% by fecal coliforms and 31.03% by *E. coli*. The same bacteria had contaminated respectively 73.53%, 26.7% and 26.47% of orange juices samples and 48.48%, 11.76% and 11.76% of Bissap juices samples. Yeast contaminated 82.76%, 85.29% and 78.78% of samples of Lemon, Orange and Bissap juices respectively. Sulfite-reducing clostridium and molds were found in samples of juices. Salmonella and *Staphylococcus aureus* were not found in all of juices samples analyzed. The pH average was 3.56 for orange juice, 3.51 for bissap juice and 2,81 for lemon juice. Soluble Solid content (SSC) in juices was 7, 8, 11, 16 and 9.8 respectively in Orange, Bissap and Lemon juices.

Conclusion: All of juices samples analyzed were contaminated by several microorganisms. Whatever fruits juice contains minerals and vitamins components which can contribut to its nutritional qualities.

Key words: Hygienic quality, Fruits juices, pH, %Brix

INTRODUCTION

Fruit juices are well recognized for their nutritive values, minerals and vitamin contents. In many developing countries they are common beverages and are sold at all public places, along roadsides shops and schools.

Fruit juices and nectars are food products of great nutritional value, rich in vitamins, mineral salts, simple sugars and organic acids which are easily assimilated by the human organism (de Donno and al, 1998). The consumption of fruit juices could have both positive and negative effect on the part of consumers. Fruit juices processed under hygienic conditions could play important role in enhancing consumer's health through inhibition of breast cancer, congestive heart failure, and urinary tract infection (Buchanan, 1999; Bhaskar, 2004). But under poor conditions, fruit juices could likely to be a high risk of chemical and microbial contamination (Essien and al. 2011).

Many case of food borne illness associated with the consumption of fruit juices are reports at several places in India, in USA, Australia and elsewhere (Chumber and al. 2007; Lewis and al. 2006, Ghosh and al. 2007; Mosupye and Holy, 2000).

Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* O157:H7, species of Salmonella, Shigella and Staphylococcus aureus (Buchmann and al., 1999; Ryu and al., 1998; Uljas and al., 1998; Sandeep and al., 2001).

The sources of contamination were however vary. Most fruits contain bacterial counts up to 10^5 CFU/cm^2 on their surface (Splittstosser, 1979; Harrigan 1998). Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination.

In 1995, more than 60 visitors were affected in Florida (USA), by fresh orange juice contaminated with Salmonella (Schmidt et al., 1997). 427 cases of salmonellosis were reported in 1999 in Australia after drinking fresh orange juice (Victorian Government Department of Human Services). Case of cholera epidemic was related in Pune city, India, after consumption of sugarcane juice cooled by ice contaminated with *Vibrio cholerae* (Mosupye and al., 1999). In Togo, street food safety was not assured. Microbial studies on such foods samples and well's water in Lomé have revealed there contamination by *Escherichia coli, Staphylococcus*

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aureus, Salmonella and *Clostridium perfringence* (SADAOC, 2002).

In view of the threat posed by the pathogenical bacteria in street foods and juices, the present work was undertaken to assess the microbiological quality of fruit juice sold in twelve schools in Lomé during Mars to June 2013. The objective was also to evaluate the sanitary quality of this fruit juices often demanded by young pupils in Lomé.

MATERIAL AND METHODS

Twelve schools in Lomé City were chosen for the study and 96 samples of fresh fruit juices were collected from Mars to June 2013.

The juice samples were bought randomly on the basis of their sale in the schools.

Three types of fruit juices, i.e. orange, lemon and juices of *Hibiscus sabdariffa* (bissap) were collected. All samples were collected with here plastic containers (sachet or useless bottle of water) and kept in the ice box at 4ºC during transportation to the laboratory. Juices samples, get at 25°C, were removed aseptically in sterile bottles for microbiological analysis and 50 ml portion of all juice samples removed for titratable acidity and brix determination.

For microbiological analysis, decimal dilutions of juices sample were prepared and spread plate technique was used on appropriate selective media.

Normalizes methods of Normalization Association for French (AFNOR) was used to enumerate bacterial and identify potential pathogens.

Total viable count were enumerated on plate count agar, total coliforms and fecal coliforms using violet red bile agar, and for staphylococci using Baird Parker and mannitol salt agar, yeasts and molds using Sabouraud + chloramphenicol agar and Sulphito-reducing Clostridium using Tryptone Sulfite Neomycin agar. Inoculated plates were incubated at the requisite time- temperature combination. The mean number of colonies counted was expressed as colony forming units (cfu)/ml.

The molds and yeasts isolates were stained using lactophenol cotton blue and examined under the microscope.

For detecting the presence of Salmonella, portions (25ml) of juice samples were enriched in peptone and in Rappaport broths and insulated on hektoen and Salmonella/Shigella agar.

Microbial counts were analyzed according to the Kenya Bureau of Standard (KEBS, 1999) criteria who indicated that fruits juices must contain: aerobic micro-organisms <100000/ml, Total coliform <100/ml, E. coli <10/ml, Yeasts and moulds <100/ml, Sulphito-reducing Clostridium: 0/10ml and Salmonella absent/25ml.

The Soluble Solid content (SSC) of fruit juices was determined using a refractrometer whereby a drop of pulp solution was placed on its prism. The percentage of SSC was obtained from direct reading of the refractometer (Euromex, Holland).

pH was measured using digital pH meter (WTW pH330i, Germany) after homogenizing 10ml of the fruit juices in 90 ml of distilled water (Erkmen and Bozkurt, 2004). Standard method was used to measure Titratable acidity (TA) (Antony and Chanrda, 1997). The fruit juice sample (10ml) was homogenized in distilled water (40ml). Two to three drops of phenolphthalein were added to the solution as indicator and titrated against 0.1M NaOH to the end point of phenolphthalein control at pH 8.1 with digital pH meter (WTW pH330i, Germany). Titratable acidity was expressed as g lactic acid/100g of juice and calculated using the formula:

TA = MNaOH x ml NaOH x 0.064 x 100 /ml juice sample

Where, TA = titratable acidity; MNaOH = Molarity of NaOH used; ml NaOH = amount (in ml) of NaOH used; 0.064 = equivalent weight of citric acid.

The significance of differences ($P<0.05$) of the mean microbial count among the fruit juices was evaluated with one-way ANOVA using SPSS for windows version 10.0.

RESSULTS AND DISCUSSION

Results

All the fruit juice makers were females. None of them had any training related to their career.

Fruits were washing with simple water (Lemon and orange). Fruit juices were the unfermented natural juices obtained by a mechanical process of extraction from mature fruit. Fruit nectar have been often diluted with water and added of sugar. Juice of bissap is a petal's decoction of *Hibiscus sabdariffa*. Then juices packaged in sachet or retrain bottles of water are frozen and sold after.

Twenty nine samples of orange juices, thirty four of lemon juices and thirty three of bissap juices were analyses. Samples of juice were taken in plastic sachets or in retrain bottles of mineral water. The boatels of juice was sold, package in a cold-box and covered with very uses bags or crouched.

Physico-chemical control showed that pH of juices is acidic average was 3.42 (3.23 to 3.71) for orange juice, 2.82 (2.56 to 3.02) for bissap juice and 2.76 (2.52 to 3.03) for lemon juice. The average values of Titratable acidity was 0.20 (0.101 to 0.352), 0.25 (0.121 to 0.512) and 0.42 (0.192 to 0.768) respectively in orange, bissap and lemon juices.

Table 1: Microbiological contamination of juices samples

Bacteria sought		Lemon juices	Orange juices	Bissap juices
Total viable count batteries	% of contamination	100	100	100
	Value extreme/ml	1000	200	50
		$6,85.10^7$	$6,95.10^7$	8.10^6
Total coliforms	% of contamination	79,31	73,53	48,48
	Value extreme/ml	0	0	0
		$4,1.10^4$	$1,1.10^5$	43000
Fecal coliforms	% of contamination	41,38	26,47	11,76
	Value extreme/ml	0	0	0
		3350	$3,35.10^4$	5200
Yeast	% of contamination	82,76	85,29	78,78
	Value extreme/ml	0	0	0
		99300	99300	21600
Escherichia coli	% of contamination	31,03	26,47	11,76
	Value extreme/ml	0	0	0
		950	300	305
Sulfite-reducing Clostridium	% of contamination	10,34	5,88	0
	Value extreme/ml	0	0	0
		100	100	

Note: (a) - The values express the colony forming units (cfu)/ml counted.
(b) – *Salmonella* and *S. aureus* are free to the all of samples.

Measuring of sugar content (%Brix) or the total soluble solids in juices was 7.8(4 to 11.8), 11.16 (7 to 17.6) and 9.8 (8 to 13.8) respectively in orange, bissap and lemon juices. The SSC and TA ratio were 22.84, 39 and 44.64 respectively in Lemon, orange and bissap juices.

Total coliform and fecal coliform contamination under 10^2cfu/ml concerned respectively 44.13% and 85.29% of Lemon, 27.58% and 72.41% of Orange, and 84.36% and 96.88% of Bissap juices. *E. coli* was isolated in 31.03%; 26.47% and 11.76% of Lemon, Orange and Bissap juices respectively. Yeasts were found in 82.29% of juices samples tested. The highest yeasts contamination was found in orange juices (85.3%) followed by lemon juices (82.76%) and Bissap juices (78.78%).

Molds were isolated in sixteen samples (four in orange, nine in lemon and three in bissap juices). *Aspergillus niger, Aspergillus flavus* and *Mucor spp* were the main species identified. The number of molds colonies counted was from 10 to 380cfu/ml.

Discussion:

Fruits in general, though very rich in vitamins and mineral elements, could pose a danger to human health if they are not properly treated prior to consumption as a result of the presence on them of contaminating microbial agents (Al-Jedah et al, 2001). Indeed, most fruits contain bacterial counts up to 1.0×10^5 CFU/cm2 on their surface (Splittstosser, 1979; Harrigan, 1998). Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with houseflies and fruit flies and airborne dust can also act as sources of contamination (Lewis et al., 2006; Poonam, 2013). The juices are sold preserved in refrigerators and exposed in the course of the schools to the sun and dust. This situation can involve the multiplication of the bacteria within the products. In addition to the favorable conditions of pH and temperature, contaminant micro-organisms used sugar in juices like energy source in their metabolism. The producers always do not have hygienic water to wash the fruits before pressing. Study realized in Lomé show that well's water was contaminated by fecal coliforms and *Escherichia coli* (SADAOC, 2002). These bacteria were counted in most of juice samples. Over 10^4ufc of Total viable count were present in 69% of juices. It was used, as the best indicator of fecal contamination, to evaluate sanitary condition of waters and of food preparation (Naseem et al. 2009) and in particular of fruit juices. Total viable count bacteria (100%), Total coliforms (66.31%) and yeast (82.10%) were the most microbial count in juices samples. The results were consigned in the table 1. This contamination is mainly due to

Table 2: Physico-chemical characteristics of juices samples

Physico-chemical parameters		Lemon juices	Orange juices	Bissap juices
pH	Average values	2.76	3.42	2.82
	Value extreme	2.52 – 3.03	3.23 – 3.71	2.56 – 3.02
Titratable acidity	Average values	0.42	0.20	0.25
(g citric acid/100ml)	Value extreme	0.192 – 0.768	0.101 – 3.352	0.121 – 0.512
%Brix	Average values	9.8	7.8	11.16
	Value extreme	8 – 13.8	4 – 11.8	7 – 17.6
Ratio %Brix / Titratable acidity		22,84	39	44,64

unhygienic conditions, poor maintenance of remises and poor personal hygiene (Adams and Moss, 1995). Results revealed that pH of the analyzed juices varying between 2.52 and 3.50 depending on the type of the fruit (table n°2). The presence of yeast (respectively in 82.76% of lemon, 85.29% of orange and in 78.78% of bissap juices) was expected due to its preference for sugar and low pH, which highly favour her proliferation (Adams and Moss, 1995). Lewis and all (2006) showed that the major microflora found on fruits includes fungi, bacteria and yeast. Such fruits are sources of bacterial pathogens notably *E.coli*. 057:H7, specie of Salmonella, Shigella and *Staphylococcus aureus*. *Escherichia coli* were present in 31.03% of lemon, 26.47% of orange and 11.76% of bissap juices in spite of their pH acid. Poonam (2013) counted *E. coli* in 33% of fruit juices being served in various cities of Vidarbha.

The low pH of fruit juices in principle limits the number and types of bacteria that can survive or grow. The spoilage of acidic foods is most often due to contamination of the foods with aerobic acid tolerant bacteria as well as yeasts and moulds (Hatcher et al. 1992). The mean microbial counts of bissap juices were significantly different ($P<0.05$) from that of both lemon and orange. KEBS (1999) criteria for microbiological quality of juice indicated the presence of *Escherichia coli* <10CFU/ml, of Total coliform, yeasts and moulds $<10^2$CFU/ml, of Total count bacteria $<10^5$CFU/ml and Sulfito-reduction Clostridium and Salmonella must be absented. By considering KEBS criteria, Bissap juices have the lower percentage of samples non-conform (Figure 1).

Bissap juices have lower microbial loads than the other two. This could be attributed mainly to the very low pH observed (pH: 2.82) and because of heat treatment in the process of this juice. It is observed that yeasts induced more non-conformity at the tested samples. At the juices of bissap which are contaminated than the other juices, more than 54% of samples are beyond the value fixed by KEBS criteria. Yeasts grow in sugar and low pH condition (Adams and Moss, 1995). There SSC and acid ratio are highest (44.64) than orange (39) and lemon (22.84) juices. SSC and titrable acidity are important for gustatory characteristics of juices (Garg et al., 2008). It used as index of maturity of fruits. There are higher soluble solids content and lower titratable acidity in mature fruits when compared to young fruits (Kader, 1999). Studies by FAO (1995) recorded poor knowledge, practices in food handling in the assessment of microbial contamination of food sold by vendors. In fact, conditions under which the juice was processed contribute to the betterment of the product. The low values of pH fruit juices (2.5-3.8) inhibit most of the bacteria, but don't affect any yeast (Ashurst, 2005). But presence of *Escherichia coli* was due to her resistance to low pH (Youssuf and Munir, 2007). Heat pasteurization used in conventional juice production increases shelf life by inactivating certain enzymes and microorganisms (yeasts, molds, bacteria). However, heat processing also results in flavor loss and other changes which detract from the fresh, natural quality of the juice (Carter, 1989). The juice of better microbiological and organoleptic quality is that resulting from the cooled fruits and immediately put at the expenses (Schmidt et al, 1997).

CONCLUSION

The study indicated that all fruit juices samples collected in many schools of Lomé city were contaminated. It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of fruits and utensils, contaminated water and ice, poor personal and domestic hygiene, squeezing of fruits with hand, shop in crowed place.

In the school, fruit juices was the second drink witch were consumed by many of students without water. Fruit juices processed in unhygienic condition could be health problem often to the young students. The occurrence of pathogenic *E. coli* and importance of the contamination of the

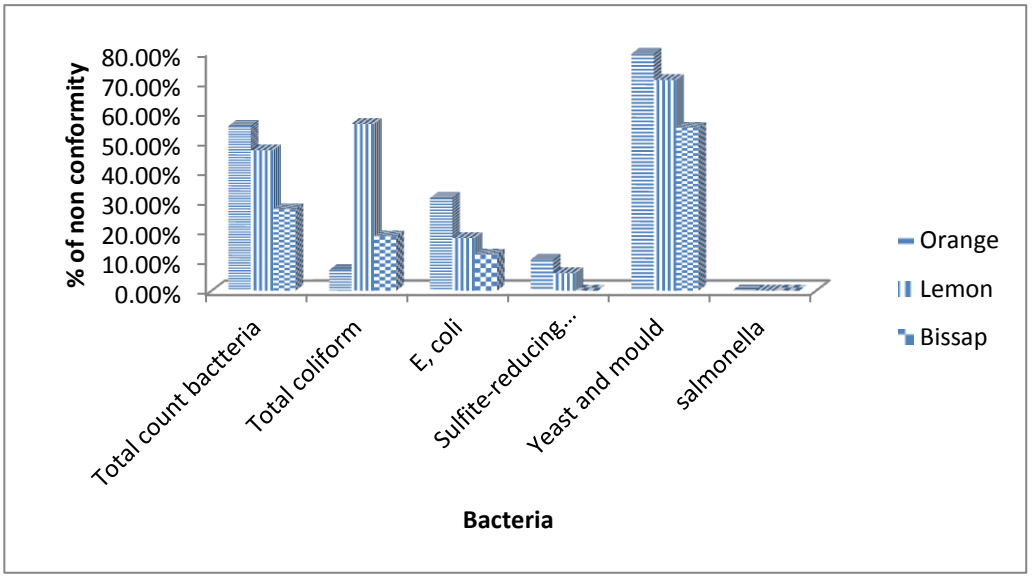

Figure1: Appreciation of the non-conformity of the juices compared to KEBS criteria

juices samples were alarming enough for an immediate action by the suitable agency. Health education of the vendors and implementation of standard hygienic protocols may reduce contamination of fruit juices. Regular monitoring of the quality of fruit juices for human consumption is necessary to avoid any future bacterial pathogen outbreak.

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