British Journal of Pharmaceutical Research
4(10): 1217-1227, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Determination of Survival Rates of Bacteria in Contaminated Fresh Fruit Juice Samples

Olufunmiso O. Olajuyigbe[1*], Olujimi M. Akinwande[1] and Otunola Adedayo[1]

[1]Department of Biosciences and Biotechnology, Babcock University, Ilisan Remo, Ogun State, Nigeria.

Authors' contributions

Author OOO wrote the first draft of the manuscript. Author OMA carry out the study, collate and analyzed the data. Author OA designed the research and reviews the final manuscript. All authors approved the submission of the manuscript

Original Research Article

Received 6th October 2013
Accepted 23rd February 2014
Published 12th May 2014

ABSTRACT

Aims: To determine the survival rates of bacteria in contaminated fresh fruit juice samples
Place and Duration of Study: Biosciences and Biotechnology Department, Babcock University, Ilisan Remo, Ogun State, Nigeria, between November, 2012 and May, 2013.
Methodology: Freshly extracted juice samples were obtained from intact pineapple (*Ananas comosus* Merr.) and watermelon (*Citrillus lanatus* Thunb.) were pasteurized before being contaminated with *Escherichia coli* ATCC 25922 and *Lactobacillus acidophilus*. While the pH and the sugar contents were determined at interval after being contaminated with the bacterial strains, the contaminated juice samples were sampled for 150 min to determine colony forming unit per milliliter (cfu/ml) at different sampling time.
Results: In pineapple juice, the log of concentration of *E. coli* was reduced from 6.452 at 0 min to 5.079 at 150min. In watermelon, the log of concentration of *E. coli* was reduced from 6.301 at 0min to 5.954 at 150min. While the log of concentration of *L. acidophilus* in pineapple juice was between 6.204 at 0 min and 6.262 at 150min, its log of concentration in watermelon juice ranged between 6.228 at 0 min and 6.291 at 150min. The pH was reduced to 2.9 and 3.7 by *E. coli* while *L. acidophilus* reduced the pH to 2.5 and 3.0 for pineapple and watermelon juices respectively. After 150min, the sugar contents of pineapple and watermelon juices decreased from 1.181 and 1.060mg/ml to 0.011 and 0.004mg/ml by the *E. coli* while *L. acidophilus* reduced the sugar contents to 0.003mg/ml

**Corresponding author: Email: oadedayo@gmail.com;*

for pineapple juice and 0.018mg/ml for watermelon juice. The reduction in the pH values of each of the fruit juices showed that the activities of each bacterial strain resulted in increase in the production of acid in the growth medium.
Conclusion: The inability of coli form (*E. coli)* to survive in the fruit juices suggested that the fruit juices may not harbor and/or disseminate enteric pathogens if allowed to stay for a while before packaging.

Keywords: Bacterial contamination; pineapple juice; watermelon juice; rate of kill; sugar content.

1. INTRODUCTION

Juice is a liquid that is naturally contained in fruits and vegetables. It can also refer to liquids that are flavoured with these or other biological food sources such as meat and seafood commonly consumed as a beverage or used as an ingredient or flavouring in foods. It is one of the most popular drinks prepared mechanically by squeezing or macerating fruits or vegetable flesh without the application of heat or solvents using a variety of techniques such as using hand or electric juicers [1]. While the final product is an unfermented, un clarified, untreated juice, ready for consumption [2], the fresh fruit juices always have natural sweetness and no artificial colour and that is why they are preferred over bottled or canned juices [3].

Juices are often consumed for their perceived health benefits. Many fruit juices have higher sugar (fructose) content than sweetened soft drinks. For instance, typical grape juice can be 50 calories more sugar than cola soda drink [4]. Being known to be a rich source of vitamin C, folic acid and potassium, juices are an excellent source of bio available antioxidant phytochemicals [5] and significantly improve blood lipid profiles in people affected with hypercholesterolemia [6]. They have digestive health benefit and their phytochemicals are known to prevent bacteria from binding to the bladder. Hence, fruit juices hygienically produced have been reported to prevent urinary tract infections [7]. Contrary to soft drinks such as Coca Cola capable to cause oxidative stress and even lead to insulin resistance after long term consumption, fruit juices have the ability to raise serum antioxidant capacity and offset the oxidative stress and inflammation normally caused by high-fat and high-sugar meals [8]. Moreover, fruit juices might be protective against stroke and delay the onset of Alzheimer's disease [9] and their intake has been consistently associated with reduced risk of many cancer types [6] and promotion of detoxication in human body [10].

Although fruit juice in moderate amounts can help children and adults meet daily recommendations for fruit consumption, nutrient intake and calories [8], the acceptance of commercial fruit juices as being equal in health benefit to fresh fruit has been questionable due to its lack of fibre and high degree of processing. Consequently, high-fructose corn syrup, an ingredient of many juice cocktails, has been linked to the increased incidence of type II diabetes and weight gains in some studies [1] as observed earlier with the consumption of soft drinks [9]. Since excessive juice consumption can lead to poor nutrition, diarrhoea, gas, abdominal pain, bloating and tooth decay [6], The American Academy of Paediatrics indicated that fruit juices should not be given to infants before 6 months of age and its intake by children ages 1–6 years should be limited to 4 to 6 ounces per day [1].

Even though intake of fruit juices can be beneficial, its contamination is inevitable. The contamination can result from improper washing of fruits, use of unhygienic water for

dilution, prolonged preservation without refrigerator, unhygienic atmosphere for juice preparation and improper handling [11]. The added water used for juice preparation could be a major source of microbial contaminants while fomites such as utensils may play significant roles in spreading *Salmonella spp., Shigella spp., Vibrio spp., E. coli* 0157:H7 and other disease-causing and fruits spoilage organisms [12,13]. While microbial spoilage of fruit juices may be caused by microorganisms capable of multiplying at pH<4.6 [14,15], Spotti et al. [16] and Vicini et al. [17] indicated that the presence of asporogenous bacteria and thermolabile fungi could be due to contamination after the heat treatment. Thus, since consumption of contaminated fruit juices can cause significant health problems and their outbreaks have been linked to a wide range of microorganisms, food borne illnesses associated with the consumption of fruit juices have been widely investigated and reported [18-20]. Consequently, to ascertain the self purification ability of fruit juices contaminated during the extraction procedures over a period of time, this study was designed to determine the rate of survival of pathogenic bacteria *E. coli* and *L. acidophilus* in different juices made for home consumption.

2. MATERIALS AND METHODS

2.1 Source of Bacteria

Cultures of *E. coli* ATCC 25922 and *Lactobacillus acidophilus* LA-5 were obtained from the Department of Biosciences and Biotechnology, Babcock University, Ilisan Remo, Ogun State, Nigeria. The *E. coli* was used because of its pathogenic potential in faecal contaminants while the *L. acidophilus* was considered as a control for its potential to survive in acidic environment.

2.2 Collection of Fruit Samples

Five fruits of pineapple (*Ananas comosus* Merr.) and watermelon (*Citrillus lanatus* Thunb.) respective samples were obtained from the market in Ilisan Remo, Ogun State, Nigeria. The pineapple fruits were washed and peeled while the watermelon was washed and sliced into pieces. The fruit juices extracted with a juice extractor were filtered to obtain pulp-free juice samples. The seeds of the watermelon were removed along with the pulps. The extracted juice samples were pasteurized at 63°C for 30min in a water bath before being stored at 4°C. Their sterility was confirmed by introducing 1ml of each of the fruit juices into 9ml sterile McConkey and nutrient broths before being incubated overnight at 37°C.

2.3 Fehling's Sugar Test

Different concentrations of glucose used as standard were prepared from 5g of D-glucose dissolved in 45ml of distilled water. The sugar contents of the D-glucose and fruit samples concentrations were determined, in duplicate, using Fehling's sugar test method. Here, 20ml of prepared Fehling solution "A" were mixed with Fehling solution "B". 2ml of the Fehling solution mixture were added to ten test tubes containing the different concentrations of D-glucose used as standard and another set of 10 test tubes containing the fruit juice samples at different concentrations. The test tubes were placed in a water bath at 60°C for 15min after which the mixtures were filtered with Whatman No. 1 filter paper to separate the precipitates before the absorbance was read by spectrophotometer at a wavelength of 500nm at an interval of 30min. The pH of undiluted fruit juice samples was measured simultaneously with the pH meter as the sugar contents were being determined.

2.4 Bacterial Inoculum Preparation

For the bacterial inocula preparation, the inocula of each test bacterial strain were prepared using the colony suspension method [21]. Colonies picked from 24h old cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600nm. The suspension was then diluted 1:100 by transferring 0.1ml of the bacterial suspension to 9.9ml of sterile nutrient broth before being used. The density of bacterial suspension for susceptibility test was finally determined by comparison with 0.5 McFarland standard of Barium sulphate solution [22].

2.5 Determination of Rate of Kill

Assays for the rate of killing bacteria by the different fruit juices were carried out using a modified plating technique of Eliopoulos and Eliopoulos [23] and Eliopoulos and Moellering [24]. 100µl of approximately 10^7cfu/ml further verified by total viable count, was used to inoculate 10 ml volumes of each of the fruit juices and peptone water in McCartney bottles. The bottles were incubated at 37°C on an orbital shaker at 120 rpm. A 100µl aliquot was removed from the culture medium at 0, 30, 60, 90, 120 and 150min for the determination of cfu/ml by the plate count technique [25] by plating out 25µl of each of the dilutions. Considering that the juices are prepared and served immediately, the juices were sampled for 150min to evaluate changes in their colony forming units during consumption. Experiments were performed in duplicate. After incubating at 37°C for 24h, emergent bacterial colonies were counted, average cfu/ml calculated and compared with the count of the culture control without the extract.

2.6 Statistical Analysis

All the data were subjected to one way analysis of variance (ANOVA) and the mean values were separated at (P=.05) using Duncan's Multiple Range Test. The one way ANOVA test was used to determine if there was any statistically significant difference in the log of concentration of each bacterial isolate sampled over a period of time in the juice samples. All statistical analyses were done using SAS software (1999) model.

3. RESULTS AND DISCUSSION

The spectrophotometric determination of the concentration of sugar in prepared solution of D-glucose showed that the sugar contents increased with increase in the concentration of the solutions Fig. 1. The graph illustrated absorbance against different concentrations in mg/ml and gives an idea that the concentration of sugar contents in fruit juices treated with Fehling solution and read under the spectrophotometer could be traced on the standard curve. Comparing the sugar contents of the fruit juices with those obtained from D-glucose used as standards, it was observed that the sugar contents in the juices were within the range of calibration.

Comparative analysis of the survival of *E. coli* and *L. acidophilus* in the different fruit juices showed that there is a reduction in the log of concentration of these bacterial strains when compared with the observed increase in their log of concentrations when cultured in peptone water. In peptone water, the log of concentration of *E. coli* was between 5.748 at 0min and 6.212 after 150min of incubation. In pineapple juice inoculated with *E. coli*, the log of concentration of *E. coli* was reduced from 6.452 at 0min to 5.079 at 150min while that of *E. coli* in watermelon juice was reduced from 6.301 at 0min to 5.954 at 150min. Comparing the

survival of *L. acidophilus* in peptone water, pineapple juice and watermelon juice, it was recorded that the log of concentration of *L. acidophilus* increased from 5.839 at 0 min to 6.418 at 150min in peptone water. While the log of concentration of *L. acidophilus* was 6.204 at 0min and 6.262 at 150min in pineapple juice, its log of concentration in watermelon juice ranged between 6.228 at 0min and 6.291 at 150min Table 1.

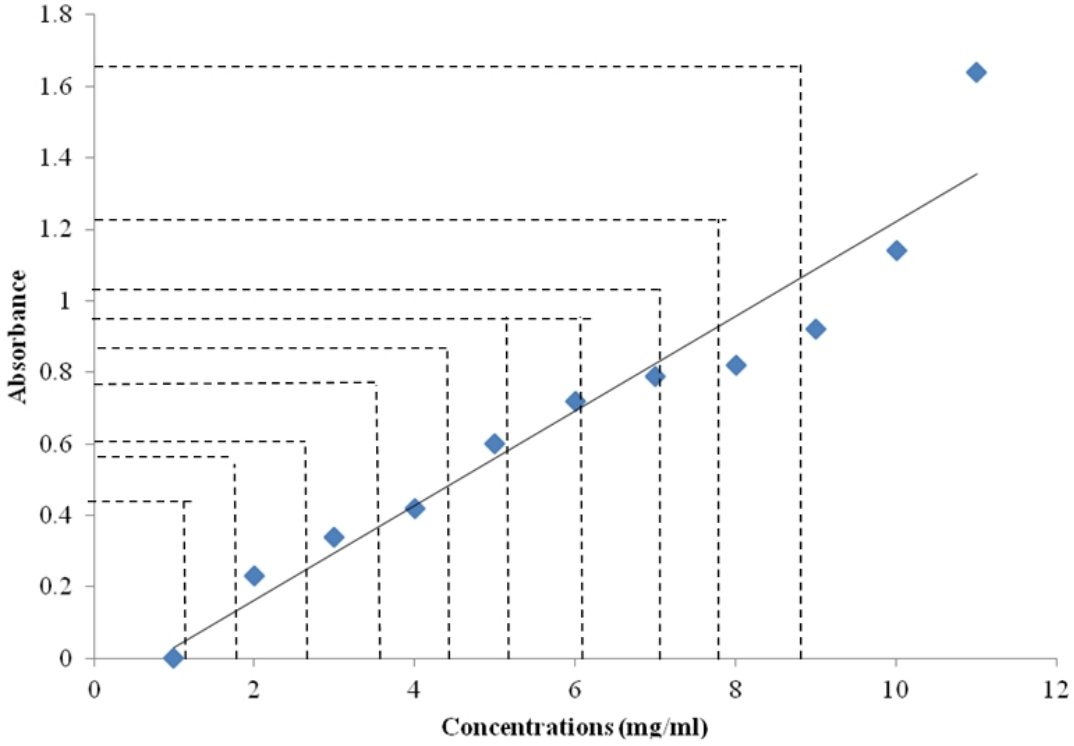

Fig. 1. Concentration (mg/ml) of D-glucose determined spectrophotometrically from different concentrations prepared as standards

Comparative analysis of the survival of *E. coli* and *L. acidophilus* in the different fruit juices showed that there is a reduction in the log of concentration of these bacterial strains when compared with the observed increase in their log of concentrations when cultured in peptone water. In peptone water, the log of concentration of *E. coli* was between 5.748 at 0min and 6.212 after 150min of incubation. In pineapple juice inoculated with *E. coli*, the log of concentration of *E. coli* was reduced from 6.452 at 0min to 5.079 at 150min while that of *E. coli* in watermelon juice was reduced from 6.301 at 0min to 5.954 at 150min. Comparing the survival of *L. acidophilus* in peptone water, pineapple juice and watermelon juice, it was recorded that the log of concentration of *L. acidophilus* increased from 5.839 at 0min to 6.418 at 150min in peptone water. While the log of concentration of *L. acidophilus* was 6.204 at 0min and 6.262 at 150min in pineapple juice, its log of concentration in watermelon juice ranged between 6.228 at 0min and 6.291 at 150min Table 1.

Table 1. Comparative analysis of the survival of *E. coli* and *L. acidophilus* in peptone water, pineapple and watermelon juices

Sampling time (min)	Log of concentration of *E. coli* in different fruit juices			Log of concentration of *L. acidophilus* in different fruit juices		
	Peptone water	Pineapple Juice	Watermelon	Peptone water	Pineapple juice	Watermelon
0	5.748^{f}	6.452^{a}	6.301^{a}	5.839^{a}	6.204^{f}	6.228^{a}
30	5.813^{e}	6.143^{b}	6.143^{b}	6.017^{b}	6.207^{e}	6.230^{b}
60	5.949^{d}	6.017^{c}	6.130^{c}	6.286^{c}	6.215^{d}	6.233^{c}
90	6.079^{c}	5.839^{d}	6.017^{d}	6.301^{d}	6.243^{c}	6.241^{d}
120	6.185^{b}	5.491^{e}	6.013^{e}	6.330^{e}	6.246^{b}	6.248^{e}
150	6.212^{a}	5.079^{f}	5.954^{f}	6.418^{f}	6.262^{a}	6.291^{f}

The log of concentrations of each bacterium in each juice samples with different superscript along the same column are significantly different (P=.05)

Table 2. pH values and sugar contents of pineapple and watermelon juices after being inoculated with *E. coli* and *L. acidophilus*

	pH and sugar contents of different juices contaminated with *E. coli*				pH and sugar contents of different juices contaminated with *L. acidophilus*			
	Pineapple juice		Watermelon juice		Pineapple juice		Watermelon juice	
Sampling time (min)	pH values	Sugar content (mg/ml)	pH values	Sugar content (mg/ml)	pH values	Sugar content (mg/ml)	pH values	Sugar content (mg/ml)
0	4.3	1.181^{a}	5.1	1.060^{a}	4.3	1.181^{a}	5.1	1.060^{a}
30	4.0	0.722^{b}	4.7	0.795^{b}	3.7	1.019^{b}	4.8	0.891^{b}
60	3.9	0.032^{c}	4.5	0.647^{c}	3.4	0.847^{c}	4.5	0.626^{c}
90	3.4	0.020^{d}	4.2	0.131^{d}	3.0	0.032^{d}	4.0	0.433^{d}
120	3.1	0.015^{e}	3.9	0.089^{e}	2.9	0.015^{e}	3.8	0.147^{e}
150	2.9	0.011^{f}	3.7	0.004^{f}	2.5	0.003^{f}	3.0	0.018^{f}

The pH and sugar contents of each contaminated juice sample with different superscript along the same column are significantly different (P=.05)

Sampling these juices immediately after being inoculated with *Escherichia coli* showed that the pH were 4.3 and 5.1 for pineapple and watermelon juices respectively. However, during the 150min sampling period, the pH was reduced to 2.9 and 3.7 for pineapple and watermelon juices respectively. At 0min, the sugar contents of pineapple and watermelon juices were 1.181 and 1.060 mg/ml respectively. After inoculating with *E. coli* and sampling for 150min, the sugar contents were drastically reduced to 0.011 and 0.004mg/ml for the respective fruit juices. In the juices inoculated with *L. acidophilus*, the pH of the pineapple juice was reduced to 2.5 and that of watermelon juice was reduced to 3.0 while the sugar content of pineapple juice was reduced to 0.003mg/ml and that of watermelon juice was reduced to 0.018mg/ml Table 2. The reduction in sugar contents of each of the fruit juices indicated that the sugar in the fruit juices were adequately utilized by the two organisms and corresponded with the decreases observed in their pH values. A comparison of the sugar utilization by the two organisms showed that the sugar contents of the pineapple juice were almost exhausted within 90 min of incubation compared to that of watermelon juice sample while *L. acidophilus* was slower in its sugar utilization and had an increase in the log of concentration when compared to that of *E. coli*. The reduction in the pH values of each of the fruit juices, however, indicated that the activities of each bacterial strain resulted in increase in the production of acid in the growth medium.

In the recent times, the demand for freshly squeezed juices has increased as unpasteurized juices are preferred by the consumer because of the fresh flavour attributes while the growth in the fruit drink market has been attributed to promotional activity, product innovation and the move to larger pack sizes. Despite the relevance of fruit juices as food accompaniment, freshly squeezed fruit juices do not have process steps that reduce pathogen levels if contaminated. Consequently, *Salmonella spp.* and verotoxin producing *E. coli* had been associated with outbreaks that resulted from the consumption of unpasteurized fruit juices [26-29]. Although most fruit juices are acidic enough to prevent the growth of pathogens and producers of unpasteurized juices have traditionally relied upon a juice's inherent acidity to render their product microbiologically safe, Food and Drug Administration [30] indicated that a number of pathogenic organisms can be present and survive in a wide range of fruit and vegetables while documented outbreaks of *Salmonella spp.* and *E. coli* O157:H7 associated with unpasteurized juices have negated this belief. Hence, the necessity to investigate the potential of pathogens to survive in peptone water and freshly extracted watermelon and pineapple juices.

Though analyses of fruit juice samples are often directed towards determining the presence of potential pathogens and estimation of nutrient compositions, there is a dearth of information on survival rates of these potential pathogens in juice samples. From this study, the sugar contents of pineapple and watermelon juices were 1.181 and 1.060mg/ml respectively. This is in disagreement with 95mg/ml recorded by Dizy et al. [31] and 3.9mg/ml for glucose and 1.41mg/ml for fructose recorded by Okonkwo et al. [32] in pineapple juices samples. While the differences in the sugar content might be due to the maturity stages of the fruits at harvesting time, species, soil condition and season of the year, the amount of sugar may also be correlated with the fruit dehydration [33], the ripening of the fruits [34] and the percentage composition of the fructose, glucose and sucrose making up the sugar content [35]. Also, there was increase in population of *E. coli* cultured in peptone water. On the other hand when *E. coli* was introduced to the pineapple and watermelon samples, there was a decrease in its initial population as a result of the reduction in the pH values and increased acidic nature of the fruit juices that discouraged the multiplication of the organism. From fruit juices contaminated with *L. acidophilus*, there was an increase in the population of the *L. acidophilus* inoculated in the pineapple and watermelon juices. At the onset, the

growth was stable to indicate that the organism was in its lag growth phase and adjusting to the medium environment before the increase in population as compared to the decreases observed in colonies formed by *E. coli* with time. Subject to further study, the lag phase of the *L. acidophilus* may have been delayed by the presence of these fruit juices. Although the growth of this organism was slow at the lag phase, it was metabolically active and utilized the sugar contents of the fruit juices in the production of acid as indicated by the reduction in the pH of the fruit juices. In *E. coli*, there is a correlation between the decrease in the pH values of the fruit juices and the reduction in *E. coli* population. The decrease in pH signifies increase in acidity. The increase in acidity is inhibitory to the *E. coli*. This implies that fruit juices contaminated with pathogenic bacteria could be self purifier as pathogenic organism may not be able to survive in such an environment with increasing level of acidity over a period of time. The observed decline in the population of *E. coli* (coli form) may, therefore, suggest that enteric pathogens are not likely to survive in fruit juice because of the low pH value.

Although pasteurization is a reliable method for pathogen reduction, economic and sensory concerns for fruit juices make it undesirable for many processors. While flavour and aroma profiles of the fruit juices may be affected, the costs of pasteurizing juices at lower production scales may also be challenging [36]. A possible alternative, however, may be the use of chemicals such as hydrogen peroxide, ethanol and organic acids generated as a result of fermentation by the *E. coli* as well as the low pH. The bactericidal efficacy of hydrogen peroxide earlier showed that Gram negative organisms were more susceptible than Gram positive [37]. The antimicrobial action of the hydrogen peroxide stemmed from its ability to form reactive oxygen species such as the hydroxyl radical and singlet oxygen which can damage DNA and membrane constituents [38]. It rapidly degrades into oxygen and water upon contacting organic material, thus having no long term residual activity. While the residual peroxide could be removed by adding catalase [37], hydrogen peroxide generally regarded as safe is currently being used as an antimicrobial in starch processing and in milk for cheese manufacturing. In agreement with the finding of Nagy et al. [39], as natural components of fruits, organic acids lower the pH and help to alter the proper sugar/acid balance in fruit juices as observed in this investigation. These are in addition to the anti oxidative activities of bioactive compounds [40,41] and phenolic compounds [41,42] in these juice samples.

4. CONCLUSION

Although food borne illness associated with the consumption of contaminated juices is rarely reported, producers of freshly squeezed juices should know that preventative measures through food safety control strategies is important. The raw materials to be used should be of a good quality and fruits that are badly damaged or bruised should be sort and discarded. Although the concentration of *E. coli* cells in pineapple and watermelon juices were reduced with time contrary to what was obtained with *L. acidophilus* which increased in population, it may be concluded that the fruit juice environments was not favourable for *E. coli* and its growth was inhibited. The inability of coli form (*E. coli)* to survive in these fruit juices suggested that the fruit juices may not habour and/or disseminate enteric pathogens. The removal of badly damaged or bruised fruits will, however, decrease the risk of microbial transfer from the raw materials to equipment and the final products.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Uckoo RM, Jayaprakasha GK, Balasubramaniam VM, Patil BS. Grapefruit (*Citrus paradisi* Macfad) Phytochemicals Composition Is Modulated by Household Processing Techniques. J Food Sci. 2012;77(9):C921–C926.
2. Victorian Government Department of Human Services, Food Safety Unit, Melbourne, Victoria. Microbiological survey of freshly squeezed juices from retail businesses across Victoria-2005. Accessed 27 July 2013. Available:http://www.health.vic.gov.au/archive/archive2011/foodsafety/archive/downloads/fruit_juice_survey_report_aug05.pdf.
3. Addo MG, Akanwariwiak WG, Addo-pordjour P, Obiri–Danso K. Microbiological and sensory analysis of imported fruit juices in Kumasi, Ghana. Res J Microbiol. 2008;3(8):522-28.
4. SDWF - Safe Drinking Water Foundation. Liquid Candy and Its Health Effects.2014;1-3. Accessed 30 January, 2014. Available:http://www.safewater.org/PDFS/resourcesknowthefacts/LiquidCandyandItsHealthEffects.pdf.
5. Franke AA, Cooney RV, Henning SM, Custer LJ. Bioavailability and antioxidant effects of orange juice components in humans. J Agric Food Chem. 2005;53(13):5170-78.
6. Menezes EW, Lajolo FM. Markers *In vivo* and *In vitro* carbohydrate In: Lajolo, F. M., Menezes, E. W. (ed.) Carbohydrates Latin American regional foods. Proyecto CYTED/CNPq XI.18 Composition, Structure, Biological Properties of Carbohydrates and Their Utilization in Food . EDUSP, São Paulo, Brazil. 2006:309-334.
7. Ketema T, Gaddisa T, Bacha K. Microbiological safety of fruit juices served in cafes/restaurants, Jimma town, Southwest Ethiopia. Ethiopia J Health Sci. 2008;18(3):98-100.
8. Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaysheh S, Mohanty P, Feldman EB. Fruits and vegetables and the risk of stroke. Nutr Rev. 2001;59(1):24-27.
9. Dai Q, Borenstein AR, Wu Y, Jackson JC, Larson EB. Fruit and vegetable juices and Alzheimer's disease: The Kame Project. Amer J Med. 2006;119(9):751-59
10. Minich D, Bland J. Acid-alkaline balance: role in chronic disease and detoxification. Review. Altern. Ther Health Med. 2007;13(4):62-65.
11. Lewis JE, Thompson P, Rao B, Kalavati C, Rajanna B. Human Bacteria in Street scale fruit juice processing. A Case Study of Visakhapatnam City, India. Internet J Food Safety, 2006;8:35-38.
12. Doyle MP, Beuchat LR, Montville TJ. Food Microbiology. Washington DC: American Society for Microbiology, ASM Press; 2001.

13. Barro N, Bello AR, Savadogo A, Ouattara CAT, Ilboudo AJ, Traoré AS. Hygienic status an assessment of dishwashing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). Afr J Biotechnol. 2006;5(11):1107-12.
14. De Donno A, Montagna MT, Erroi R, Liaci D, Sanapo S, Caggiano G. Food products and fungal contamination. Note II. Study on moulds presence in pasteurized and fresh fruit juices. J Preventive Med Hyg. 1998;39:71-73.
15. Jackson GJ, Merker RI, Bandler R. Food & Drug Administration (FDA), U.S. CFSAN. Bacteriological Analytical Manual Online. Chapter 2001;12:1-6. Accessed 30 July 2013.
Available:http://www.911emg.com/Ref%20Library%20ERG/FDA%20Bacteriological%20Analysis.pdf.
16. Spotti E, Quintavalla S, Mutti P. Contamination by fungal spores heat-resistant fruit, tomato and their derivatives. Ind Conserve. 1992;67:421-25. Italian
17. Vicini E, Barbuti S, Spotti E, Campanini M, Castelvetri F, Gola S, et al. Alterazione di succhi di frutta causata da muffe gasogene. Ind Conserve. 1992;59:4-7. Italian
18. Muinde OK, Kuria E. Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. Afr J Food Agric Nutr Dev. 2005;5(1):1-13.
19. Chumber, SK, Kaushik K, Savy S. Bacteriological analysis of street foods in Pune, Indian. J Public Health. 2007;51(2):114-6.
20. Ghosh M, Wahi S, Kumar M, Ganguli A. Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella* spp. in some raw street vended Indian foods. Int J Env Health Res. 2007;17(2):151-56.
21. EUCAST (European Committee for Antimicrobial Susceptibility Testing). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clin Microbiol Infect. 2000;6(9)509-15.
22. Cheesbrough M. Medical laboratory manual for tropical countries. ELBS edition. Tropical health technology publications, UK. 2002;2:2-392.
23. Eliopoulos GM, Eliopoulos CT. Antibiotic combinations: Should they be tested? Clin Microbiol Rev. 1988;1(2):139–56.
24. Eliopoulos GM, Moellering RC. Antimicrobial combinations. In: Antibiotics in Laboratory Medicine, 4th ed; Lorain, V. Ed.; The Williams & Wilkins Co.: Baltimore, MD, USA. 1996;330–96.
25. Cruishank R, Duguid JP, Marmion BP, Swain RHA. Medical Microbiology. The Practice of Medical Microbiology, 12th ed.; Churchill Livingstone: London, UK; 1975.
26. Anonymous. CDCP (Centers for Disease Control and Prevention): Outbreak of *Escherichia coli* 0157:H7 infections associated with drinking unpasteurized commercial apple juice - British Columbia, California, Colorado, and Washington, 1996. MMUT 1996;45(44):975. In: O'Carrol P. Beyond Odwalla: Epidemic Investigation in an On-line World. Washington Public Health vol. 15/Fall 1997. Accessed 30 July 2013.
Available: http://www.nwpublichealth.org/docs/wph97/odwalla.html.
27. Anonymous. FSACSIRO (Food Science Australia CSIRO). How safe are fruit juices and acid foods. Food Safety and Hygiene Bulletin, March 1997. Accessed 31 July 2013. Available: http://www.foodscience.csiro.au/fshbull/fshbull8a.htm.
28. Schmidt RH, Sims CA, Parish ME, Pao S, Ismail MA. A model HACCP plan for Small-Scale, Fresh-Squeezed (Not Pasteurised) Citrus Juice Operations, 1997;1-20. Accessed 19 May 2010.
Available: http://university.uog.edu/cals/people/Pubs/FS07500.PDF.

29. Anonymous. DHA (Department of Health and Ageing). Salmonellosis outbreak, South Australia. Communicable Diseases Intelligence 1999;23(3):18. Accessed 31 July 2013. Available: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi2303-pdf-cnt.htm/$FILE/cdi2303b.pdf.
30. United State Department of Health and Human Services. Food and Drug Administration (FDA), Potential for Infiltration, Survival and Growth of Human Pathogens within Fruits and Vegetables. 1999; Accessed 15 August 2013. Available: http://www.fda.gov/Food/GuidanceRegulation/HACCP/ucm082063.htm.
31. Dizy M, Martin-Alvarez PJ, Cabezudo MD, Polo MC. Grape, apple and pineapple juice characterization and detection of mixtures. J Sci Food Agric. 1992;60(1):47-53.
32. Okonkwo SI, Ogbuneke RU, Uyo BK. Elucidation of sugar in edible fruit–pineapple (*Ananas comosus*). Research J Chem Sci. 2012;2(1):20-24.
33. Nagar, P.K. Effect of some ripening retardants on fruit softening enzymes of Kinnow mandarin fruits, Indian J Plant Physiol. 1994;37:122.
34. Ersoy N, Gozlekci S, Kaynak L. Changes in sugar contents of fig fruit (*Ficus carica* L. Cv. Bursa Siyahi) during development. Suleyman Demirel Universitesi Ziraat Fakultesi Dergisi. 2007;2(2):22-26.
35. Zhang XM, Dou MA, Yao LY. Dynamic analysis of sugar metabolism in different harvest seasons of pineapple (*Ananas comosus* L. (Merr.)). Afr J Biotechnol. 2010;10(14):2716-23..
36. Ozempel M, McAloon A, Yee W. The cost of pasteurizing apple cider. Food Technol. 1998;52(1):50-52.
37. Davidson MP, Branen AL. Antimicrobials in Foods. 2nd ed. Marcel Dekker, Inc., New York; 1993.
38. Juven BJ, Pierson MD. Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation. J Food Prot. 1996;59(11):1233-41.
39. Nagy S, Chen CS, Shaw PE. Fruit Juice Processing Technology. Agscience, Inc., Auburndale, Florida; 1993.
40. Sánchez-Moreno C, Plaza L, De Ancos B, Cano MP. Nutritional characterisation of commercial traditional pasteurised tomato juices: Carotenoids, vitamin C and radical-scavenging capacity. Food Chem, 2006;98**(**4):749-56.
41. Zulueta A, Esteve MJ, Frasquet I, Frígola A. Vitamin C, vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain. Food Chem, 2007;103(4):1365-74.
42. Vinson JA, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods: Fruits. J Agric Food Chem. 2001;49**(**11):5315-21.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=508&id=14&aid=4535