Microbiological quality control study of some processed fruit juices by conventional approach

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Abstract: Whilst consumption may occur throughout the year, production and processing of the fruits is seasonal. This necessitates storage and blending of the juices to produce uniform products. The pH range of the apple (3.05), pineapple (3.30), and orange (3.50) juice are within the recommended range (3.0-3.9). The bacteria isolated from the examined fruit juices were *Micrococcus* spp, *Flavobacterium*, *Streptococcus* spp, *Staphylococcus* sp., and *Bacillus* spp. The same type of bacteria *Bacillus* sp, *Streptococcus* spp, *Staphylococcus* spp and *Micrococcus* spp are persistent isolates throughout the period of this study. This indicated that the bacteria are fruit borne rather than contaminants from air water and utensils alone. The isolates could be used as indicators of microbial quality. Further research is necessary to adequately characterise the bacteria isolated. This complex problem of liability of fruit juices to deterioration can be solved by use of healthy fruits for juices processing and proper product storage.
[Babalola Olubukola O, Fagade Obashola E, and Gopane Ramokoni E. Microbiological quality control study of some processed fruit juices by conventional approach. Life Science Journal. 2011; 8(S2):18-24] (ISSN: 1097 – 8135). http://www.lifesciencesite.com.

Keywords: Bacteria, coliform, fruit juice, sachet

Introduction

The soft drinks industry is a rapidly growing aspect of the fast food phenomenon. In line with healthy eating attitudes prevalent in today's western societies the consumption of fruit juices plays significant part in this expansion (Endrizzi et al., 2009). More so it is not only the responsibility of the food industry to provide safe and nutritious food to the public, but also the duty of government to see that industry is meeting its responsibility. Whilst consumption may occur throughout the year, production and processing of the fruits is seasonal. This necessitates storage and blending of the juices to produce uniform products.

From reports (Chen et al., 2010; Guillotin et al., 2009; Petrisor et al., 2010), fruits undergo tremendous chemical changes once separated from the parent plant, until finally spoilage sets in as a result of attack from bacteria, yeasts and fungi. Typical changes may show in texture, colour, flavour and respiratory activity which affect the processed fruits. Among the organisms specified as agents of bacterial food intoxication are *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococci* and with restriction, *Bacillus cereus* (Arnesen et al., 2008; Hunter and Poxton 2002; Pavic et al., 2005; Rodriguez and Vargas 2002). Sachet packed fruit juices are free from a considerable quantity of tin, or iron salts that may be formed or contribute a distinct metallic flavour as in fruits processed or stored in metal containers. The containers are useful for packaging liquid foodstuffs, for example fruit juices which are liable to deteriorate in the presence of air. Aside from packaging materials, additional contamination may come from equipment coming in contact with the juice and from production personnel. Various workers suggest that human beings shed from 10^3 to 10^4 viable organisms per minute. The numbers and type of organism shed is closely related to the subjects working environment. Apart from microbial invasion of plant tissues during various stages of fruit development, a second factor contributing to microbial contamination of fruits pertains to their post-harvest handling (Fernandez- Trujillo et al., 2009) and through enzyme preparation for food processing (Fernandes 2010). Contamination of food stuffs during processing and/or during storage may have various causes. The most frequently occurring contaminants belong to the following classes: microbial toxins substances originating from food processing materials and fermentation products. Detergents, lubricants and any other contaminants may also be encountered (Lawley et al., 2008).

Pasteurization of fruit juice, involve brief exposure to high temperature, for example 2 to 5 minutes at 80°C, which will destroy all vegetative cells, leaving the much more heat-resistant spore unaffected. Pasteurization is used to reduce the bacterial content and therefore to prolong the life of the drink (Gandolfi et al., 1994; Kisko and Roller

2005). Fruit juices are rendered practically sterile by brief pasteurization as the pH is low. Kisko and Roller (2005) reported that heat treatment can be used to destroy all of the microorganisms present (sterilization) or most of them (pasteurization). The types of spoilage associated with fruit drink includes flat sour by *Bacillus thermoacidurans*, butyric anaerobes by *Clostridium butyricum* and nonspore formers (mostly lactic acid types of bacteria) by yeast and moulds. Common food poisoning bacteria *Salmonella breandercup*, *Salmonella enteritidis*, *taphylococcus pyogenes*,*Clostridium welchii*, *Clostridium botulinum*, *Vibrio parahaemolyticus* and *Bacillus cereus*. Acid forming microaerophilic bacteria such as the lactobacilli tolerate lower pH values down to 3.5 in the case of some strains. Weenk et al. (1995) reported that the enumeration of microorganisms in food is by pour plate, surface spread plate, surface drop, agar droplet and micro dilution methods.

According to Kisko and Roller (2005) food poisons are substances that in small amounts are capable of producing serious injury or death. It is a commonplace that flavours and colour additives of unchallenged benefit in appropriate doses may become seriously poisonous where such doses are exceeded. Food poisoning is a painful stomach disorder caused by eating food that contains harmful bacteria or poisonous substances. Nzeako and Al-Hashimi (2006) explain the danger of infection in juice manufactured and objectionable filling into containers. It is important to recognize that much of what happens to food has nothing to do with the materials with which it comes into contact, whether these are plastics, bottle, sachets and thin layers or not (Matanda 1996). Marquenie et al. (2003) and Fiori et al. (2008) emphasized that *Monilia spp* and *Botrytis cinerea* are the most troublesome contaminants of fruit juices.

The foods commonly designated as fruits, are pulpy in character, often juicy, and since they develop from the flowers of plants, they consist of the ripened seed or seeds with some edible tissue attached. Fruit juices are watery mixtures of mostly unstable volatile organic compounds. They are heat sensitive and their colour and flavour deteriorate rapidly as processing temperatures are increased (Belibagli and Dalgic 2007; Yildiz et al., 2009). The solid content of most fruit juices is low, usually in the range of 10-40%. Some types of fruit juices are squashes, cordials, concentrate, fruit-drinks, and crush.

Quality in the context of fruit is the sum total of all those attributes which combine to make fruit acceptable, desirable and nutritionally valuable as human foods (Ha et al., 2007; Jaeger et al., 2009; Schouten et al., 2002). Microbiological quality of drinks is ascertained in order to ensure the safety of the consumer. Traditionally, the detection and enumeration of indicator organisms rather than of pathogens have been used. The coliform is the principal indicator of the suitability of a particular drink for consumption (Derlet 2008). Large numbers of coliforms may cause gastroenteritis, and also streptococci may be a better indicator (Fleisher et al., 1996; Kay et al., 1994). Indicator organism in food serves as a tool to evaluate the microbial quality of the product. The aerobic plate count, with its inherent nutritional limitations which are due to the heterogeneous distribution of bacteria in food, gives a useful measure of the quality of a product.

Microbiological examination of foods may assist in the assessment of hygienic precautions during production, and of the efficacy of a preservation process, and may allow prediction of the potential shelf-life, and also the identification for potential health hazards by the use of suitable indicators or by direct detection of pathogen. However, it has been pointed out that extreme care must be taken in the interpretation of results and in the conclusions drawn from them. Outbreaks of botulism involving acid foods are rare. An acid food is safe from *C. botulinum* if the heat process kills all organisms capable of growth at a pH of 4.6 and there is no post-process contamination. Several authors (Sheth et al., 2008) have reported the problem of botulism in acid foods and indicated that for a botulism hazard to exist in an acid food there must be a number of contributing conditions. These conditions are: presence of viable *C. botulinum* spores, presence of other microorganisms due to a failure in delivery and post processing contamination, composition of the food and storage conditions which are particularly conducive to *C. botulinum* growth and toxin production, and metabiosis. The destruction of microorganisms by heat follows a logarithmic relationship. Thus, there is always a probability that a spore will survive the process. Commercial sterility indicates that conditions in the product after processing are unfavourable for the outgrowth of any surviving spores. More so, obligate anaerobes constitute an important group of food spoilage and food poisoning organisms. These organisms grow in the absence of free oxygen and require a low oxidation-reduction potential in the medium.

It is important that researchers inquire and make recommendations to avert possible outbreak of food poisonings in retailed fruit juices. We envisaged the

need to examine causative agents of deterioration in packed fruit juices. It was hypothesized that fruit juice spoilage sets in as a result of microbial survival and so the fast food phenomenon may not always be a safe eating altitude. The aims of this research are to (1) enumerate, isolate and identify microbiological quality indicators of food drinks and to (2) predict microbiological safety of the samples used.

Materials and Methods

Collection of samples: Sachet packed fruit juices used were pineapple, apple and orange flavour. The samples were bought from retail stores in open market. They were brought to the laboratory for microbiological analysis. Included in the analysis performed were presumptive coliform count and biochemical characterization.

Selection of materials for analysis: The expiry dates of samples used were put into consideration for easy comparison. All the samples selected were examined before they reached their expiry date.

Media: Nutrient agar was used for detection of bacteria. Nutrient broth is a general purpose liquid medium for the cultivation of fastidious organisms. MacConkey No2 and 3 were used for recognition of enterococci. Eosin-methylene blue (EMB) agar was used to differentiate between typical and atypical colonies. Decolourization of the medium occurs during sterilization, but the colour returns after cooling. MacConkey broth (purple) is a sensitive medium for the examination of liquids (e.g. fruit juices). The medium was distributed into test-tubes containing Durham tubes and sterilized by autoclaving at 121^{o}C for 15 minutes. Media were sterilized at 121^{o}C for 15 minutes. On cooling to about 45^{o}C, the medium was aseptically poured into sterile Petri dishes and allowed to solidify.

Isolation of bacteria: The fruit juices were diluted quantitatively so that the total number of colonies on a plate ranged between 30 and 300. For few samples plates suitable for counting were obtained by planting 1ml and 0.1ml of undiluted sample and 1ml of sample diluted (ratio, 1:100). The cover of the sterile Petri dish was lifted just high enough to insert the piper. A sterile pipette was used to transfer measured diluted sample of fruit juices. The pipette was removed without retouching it to the plate; then 15 to 20ml of the melted culture medium of between 44^{o}C was added. The plate was gently rotated for thorough distribution of inoculum through the medium. The plate was incubated in inverted position in an incubator for 24H. The number of colonies counted on plate multiplied by dilution of sample gives the number of bacteria per ml. Representative colonies on the culture plates were successively subcultured onto fresh agar plates of the same medium until pure cultures were obtained. Stock cultures were grown on nutrient agar in McCartney bottles and stored in the refrigerator. All the Petri dishes used were labelled with the sample name, sample number, dilution date and any other desired information prior to preparing the dilutions.

Maintenance of culture: Experimental and stock cultures were grown and maintained in nutrient agar slants. They were kept in the refrigerator (4^{o}C).

Coliform enumeration: The total counts per presumptive test for coliforms by most probable number (MPN) technique was carried out using the five test tube method. An estimation of the number of coliform bacilli in a fruit juice sample was made by adding varying quantities of fruit juices (from 0.01ml to 1ml) to MacConkey broth contained in test tubes with Durham tubes inverted in them to show the formation of gas. Growth of coliform bacilli was indicated by gas formation. The smallest quantity of fruit juice sample containing a coliform bacillus could then be stated and thus used to express the degree of contamination with this group of organisms. An average result was derived through examination by culture of several samples of several different quantities of the fruit juices. The test tubes were incubated at 37^{o}C and were examined after 18 to 24H. Those that showed sufficient gas to fill the concavity at the top of the Durham tube were considered to be "presumptive positive" as a result of the growth of coliform bacilli. Any remaining negative test tubes were reincubated for another 24H, and if gas developed they too were regarded as being positive. The results of the presumptive test were reported with reference made to McCrandy's probability tables. Positive test tubes showed a colour change from purple to yellow or colourless as well as gas formation. Negative tubes showed no change in colour.

Morphological and Biochemical characterization: Each pure isolate of the bacterium was grown on nutrient agar for 24H at room temperature. The growth pattern(s), colonial characteristics, colour, shape and the entire surface were observed by visual examination. The pH determination was done using the pH meter of Kent El 7055 model. Gram's staining; indole production, gelatine hydrolysis, litmus milk test, starch hydrolysis, catalase test and methyl red test were carried out according to standard procedure.

Results and discussion

Table 1 shows the results of the most probable counts for the different samples of fruit juices. In the apple juice, the count had a range of $24x10^3$ CFU/100ml to $2400x10^3$ CFU/100ml (Table 1). In the pineapple at 10^{-1} dilution of 1ml concentration to a tube out of the five tubes (1/5) was positive to coliform test. In all other tubes, no coliform growth was observed. Thus, the pineapple had a mean value of 6.67 CFU/100ml (Table 1) while in orange juice the mean coliform count was 2535 CFU/100ml. The pH of the various samples shows apple juice has pH of 3.05. The pineapple juice has a pH of 3.30 and the orange juice has a pH of 3.59. The approximate pH range recommended by the US Department of Health, Education and Welfare (US Dept of Agriculture and Health 1979) are 3.3-3.5 (apple juice), 3.4-3.9 (pineapple juice), and 3.0-4.0 (orange juice).

Nineteen bacteria spp were isolated from the products. Seven bacteria isolated from apple were labelled CC_1 – CC_7, seven bacteria spp from orange were labelled BB_1 – BB_7 and five bacteria spp from pineapple were labelled AA_1 – AA_5. Cocci species constitute the major contaminants in the juices. The bacteria were tentatively identified using a combination of guides and biochemical tests. The microorganisms isolated from the examined fruit juices were *Micrococcus* spp (BB_1 and BB_6), *Flavobacterium* (BB_2), *Streptococcus* spp (BB_3, BB_5, BB_7), Cocci spp (AA_2, AA_4, CC_5, and CC_7), *Staphylococcus* sp (CC_1 and CC_6), and *Bacillus* spp (AA_1, AA_3 and CC_4) (Table 2). This is in accordance with the work of Sharma and Anand (Sharma and Anand 2002) that made similar observations in the biofilm of pasteurization lines of commercial plant. Several authors have reported that the acid food is safe from *C. botulinum* if the heat process kills all organisms capable of growth at a pH of 4.6 when there is no post-process contamination (Adams and Moss 2008; Sheth et al., 2008).

For every 100ml presumptive coliform count of 0 is excellent. A count range of 1 to 3 is satisfactory and a count of 4 to 10 is suspicious. Any count greater than 10 is unsatisfactory. The table of t distribution shows that at 4 degree of freedom, that is (3-1) + (3-1), when t =0.07, it lies below 0.741. The degree of probability is greater than the conventional level of 50%. The null hypothesis that there is no significant difference between the means is therefore somewhat likely. The total viable counts of the bacteria/ml of the fruit juices at room temperature after 48h of incubation were $1.22x10^6$ (pineapple juice), $2.85x10^5$ (orange juice) and $6.3x10^4$ (apple juice). Table 3 shows the prevalence of the characteristic colour of each bacterium. The colony characteristics of *E. coli* and *Enterobacter aerogene* are differentiated on eosin-methylene blue agar metallic green sheen and pink respectively by pour plate method.

Fruit juices made up of orange, apple and pineapple were examined for microbiological quality. The pH of the sample for orange falls within approved standards. The pH of the sample for apple and pineapple fell below the standard pH range. The same type of bacteria *Bacillus* sp, *Streptococcus* spp, *Staphylococcus* spp and *Micrococcus* spp are persistent isolates throughout the period of this study. Results indicated that the bacteria are fruit borne rather than contaminants from air, water and utensils alone. This complex problem of liability of fruit juices to deterioration can be solved by use of healthy fruits for juices processing and proper product storage.

Packaged fruit juices are usually pasteurized to remove incidence of pathogenic microbes and not to totally remove microbes. Although a large number are eliminated during pasteurization, some still resist the pasteurisation temperature. This in parts accounts for the total viable count of bacteria per millilitre of the fruit juice. From observations in this study, some microorganisms isolated from the examined fruit juice could produce pigments and slime which may also give undesirable characteristics to the fruit juice. The resulting spoilage is due to off- flavour, putrefaction and swelling of container (Adams and Moss 2008).

Gas production in the fermentation tube within 24H at 37^oC is considered a positive reaction indicating the sample probably contained coliforms. Failure to produce gas constitutes a negative reaction (American Public Health Association, APHA) (APHA 1975). The number in the denominator, the total tubes planted; the combination of positives simply represents the total number of positive tubes per dilution. Turbidity of the medium shows that the microorganisms were able to grow in the medium.

According to a recent research finding (Araya et al., 2009) preservative in fruit product is purposely to extend the storage life of the drink by retarding or inhibiting changes in flavour, nutritive value, odour, colour, texture, and other organoleptic properties. In fruit juice the citric acid and ascorbic acid are usually used to stabilize the changes that might occur in the product. With respect to the presumptive tests performed on the fruit juices, the coliforms isolated were *Escherichia coli*, *Enterobacter aerogene* and *Streptococcus* spp. (Table 3). This observation tallies with the report from APHA (APHA 1975). Faecal

coliforms have their way into the fruit juice through faecal contaminated water, the plate count (Table 3) was also of value in judging the efficacy of the pasteurized fruit juice processes and indicating whether a particular supply is suitable for consumption, where a high bacteria content may lead to spoilage. The test for coliform bacilli is of much greater value in assessing the quality of the fruit juice supply. Coliform count as a quality test is internationally accepted (APHA 1975).

From vivid experience, it is known that these products are occasionally temperature abused during distribution, warehousing, marketing (e.g. display products for sale in shopping carts of hawkers and by consumers, storing at ambient temperature). These products may exist in the trade more than expected before they are consumed, that is, after they might have expired. Poor quality fruit juice is probably associated with the higher temperatures (30 to 37^{o}C) which encourage bacteria multiplication. In this study, fruit juice being acid food was expected to be highly favourable for moulds and yeasts to thrive but the interest of this study is bacteria. This is also because with a pH of 4.5, heat treatment will kill most of the microorganisms-yeast, moulds, but bacteria spores may persist. However, such bacteria spores are not able to germinate. This is in accordance with the report of Lund (Lund 1971), who made mention that the low pH of most fruits is an ineffective deterrent to the growth of most kinds of bacteria.

Conclusion

Essentially the maintenance of a very low microbial level of contamination of raw fruits should be desired. Disregard for sanitary practices will result in heavily contaminated drinks that spoil rapidly. However, production of fruit juice performed under hygienic conditions with strict attention to sanitary practices will result in a product with low bacterial content and good keeping quality.

Prevention must be taken to prevent contamination after pasteurization. The finished product should be stored at low temperature to retard growth of microorganisms which survived pasteurization. By and large, a rapid turnover of juice products is necessary to reduce the multiplication of contaminating organisms. The number of coliform bacteria present in a particular fruit drink is an indication of its sanitary quality and so substandard merchandise should be rejected before it enters the processing plant.

The shelf life of processed fruit juice depends on the on the health conditions of the fruit, hygienic standard method of preparation, concentration and the kinds of preservatives used. The shelf life was also affected by the conditions of storage and its microbial loads. By the large, the use of preservatives should not be substituted for proper hygienic and standard method of production of fruit drink.

Acknowledgements:

Authors are grateful to the Faculty of Agriculture, Science and Technology, North-West University for the resources made available to us in the course of this write up.

Table 1. Coliform count in apple, pineapple and orange juice samples (using most probable number technique)

Kind of fruit juice	Dilution	Combination of positive tube	MPN index CFU/100ml	Mean CFU/ml
Apple juice	10^{-2}	5-5-5	24x10	24x10
	10^{-4}	5-5-5	2400x10	2400x10
	10^{-6}	5-5-5	24000x10	24000x10
Pineapple juice	10^{-1}	1-0-0	20	0.2
	10^{-2}	0-0-0	0	0
	10^{-3}	0-0-0	0	0
Orange juice	10^{-2}	5-2-0	490	2.5
	10^{-4}	3-1-1	140	
	10^{-6}	4-0-0	130	

Table 2. Morphological and biochemical characterisation of bacterial isolates from the sachet packed fruit juices samples

Kind of fruit juice	Isolate	Colony colour	Elevation	Edge	Gram staining	Cell shape	Methyl red test	Catalase test	Gelatine hydrolysis	Litmus milk	Indole test	Starch hydrolysis	Tentative identification
Orange juice	BB_1	Pink	spreader	filamentous	+	C	+	+	-	+	-	-	Micrococcus sp
	BB_2	yellow	flat	filamentous	+	R	-	+	-	+	-	+	Flavobacterium
	BB_3	creamy	raised	regular	+	C	+	+	-	+	+	+	Streptococcus
	BB_4	creamy	raised	regular	+	C	+	+	-	+	+	+	Streptococcus
	BB_5	creamy	raised	regular	+	C	+	+	-	+	.	+	Streptococcus
	BB_6	pink	spreader	entire	+	C	+	+	-	+	.	.	micrococcus
	BB_7	pink	raised	regular	+	C	+	+	-	+	.	.	Streptococcus
Apple juice	CC_1	pink	raised	entire	-	C	+	+	+	-	+	.	Staphylococcus
	CC_2	pink	raised	entire	+	C	+	+	+	+	+	.	Cocci
	CC_3	pink	raised	entire	+	C	+	+	+	-	+	.	Staphylococcus
	CC_4	pink	raised	entire	+	R	+	+	+	+	-	+	Bacillus
	CC_5	pink	raised	entire	+	C	+	+	+	+	+	.	Cocci
	CC_6	pink	raised	entire	+	C	+	+	+	-	-	.	Staphylococcus
	CC_7	pink	raised	entire	-	C	+	+	+	+	+	.	Cocci
Pineapple juice	AA_1	pink	raised	entire	+	R	+	+	+	-	.	+	Bacillus
	AA_2	pink	raised	entire	-	C	+	+	+	+	-	-	Cocci
	AA_3	creamy	raised	entire	+	R	+	+	+	-	.	+	Bacillus sp
	AA_4	pink	raised	entire	+	C	+	+	+	-	-	+	Cocci sp
	AA_5	pink	raised	entire	+	C	+	+	-	-	-	.	Cocci sp

Table 3.Total viable counts of each bacterium from the sachets of the presumptive fruit juices at 27^{o}C for 48 h using MacConkey agar*

Bacterium	Total viable count of bacteria 10 dilution/ml		
	Pineapple	Orange	Apple
Pink	0	160	24
Light pink	300	125	28
Cream	10	6	0

*Selective media used for the recognition of enteric bacteria.

References

1. Adams MR, Moss MO. The microbiology of food preservation. Food microbiology. Cambridge, UK: The Royal Society of Chemistry; 2008. p 463.
2. APHA. Multiple-tube fermentation technic for members of the Coliform group. Washington; 1975. 908-926 p.
3. Araya XIT, Smale N, Zabaras D, Winley E, Forde C, Stewart CM, Mawson AJ. Sensory perception and quality attributes of high pressure processed carrots in comparison to raw, sous-vide and cooked carrots. Innov Food Sci Emerg Technol 2009;10:420-433.
4. Arnesen LPS, Fagerlund A, Granum PE. From soil to gut: *Bacillus cereus* and its food poisoning toxins. Fems Microbiol Rev 2008;32:579-606.
5. Belibagli KB, Dalgic AC. Rheological properties of sour-cherry juice and concentrate. Int J Food Sci Technol 2007;42:773-776.
6. Chen SJ, Zhang M, Wang SJ. Physiological and quality responses of Chinese 'Suli' pear (Pyrus bretschneideri Rehd) to 1-MCP vacuum infiltration treatment. J Sci Food Agric 2010;90:1317-1322.
7. Derlet RW. Backpacking in Yosemite and Kings Canyon National Parks and neighboring wilderness areas: How safe is the water to drink? J Travel Med 2008;15:209-215.
8. Endrizzi I, Pirretti G, Calo DG, Gasperi F. A consumer study of fresh juices containing berry fruits. J Sci Food Agric 2009;89:1227-1235.
9. Fernandes P. Enzymes in food processing: A condensed overview on strategies for better biocatalysts. Enzyme Res Fernandez-Trujillo JP, Serrano JM, Martinez JA. Quality of red sweet pepper fruit treated with 1-MCP during a simulated post-harvest handling chain. Food Sci Technol Int 2009;15:23-30. 2010;doi:10.4061/2010/862537.
10. Fiori S, Fadda A, Giobbe S, Berardi E, Migheli Q. *Pichia angusta* is an effective biocontrol yeast against postharvest decay of apple fruit caused by *Botrytis*

cinerea and *Monilia fructicola*. Fems Yeast Res 2008;8:961-963.
11. Fleisher JM, Kay D, Salmon RL, Jones F, Wyer MD, Godfree AF. Marine waters contaminated with domestic sewage: Nonenteric illnesses associated with bather exposure in the United Kingdom. Amer J Public Health 1996;86:1228-1234.
12. Gandolfi I, Palla G, Marchelli R, Dossena A, Puelli S, Salvadori C. D-Alanine in fruit juices - A molecular marker of bacterial-activity, heat-treatments and shelf-life. J Food Sci 1994;59:152-154.
13. Guillotin S, Sanoner P, Renard C. Stabilisation of the colour of anthocyanins in solutions by admixture with phytocomponents from apple. J Hort Sci Biotec 2009:96-99.
14. Ha SY, Hwang YS, Yang YJ, Park YM. Correlation between instrumental quality attributes and consumers' sensory evaluation in refrigerated-stored 'Campbell early' and 'Kyoho' grape. Korean J Hort Sci Technol 2007;25:125-132.
15. Hunter LC, Poxton IR. *Clostridium botulinum* types C and D and the closely related *Clostridium novyi*. Rev Medic Microbiol 2002;13:75-90.
16. Jaeger SR, Axten LG, Wohlers MW, Sun-Waterhouse D. Polyphenol-rich beverages: insights from sensory and consumer science. J Sci Food Agric 2009;89:2356-2363.
17. Kay D, Fleisher JM, Salmon RL, Jones F, Wyer MD, Godfree AF, Zelenauchjacquotte Z, Shore R. Predicting likelihood of gastroenteritis from sea bathing - results from randomized exposure. Lancet 1994;344:905-909.
18. Kisko G, Roller S. Carvacrol and p-cymene inactivate *Escherichia coli* O157: H7 in apple juice. Bmc Microbiol 2005;5.
19. Lawley R, Curtis L, Davis J. The Food Safety Hazard Guidebook. http://www.rsc.org/ebooks/archive/free/BK9780854044603/BK9780854044603-00001.pdf.; 2008. 6 p.
20. Lund BM. Bacterial spoilage of vegetables and certain fruits. J Appl Microbiol 1971;34:9-20.
21. Marquenie D, Geeraerd AH, Lammertyn J, Soontjens C, Van Impe JF, Michiels CW, Nicolai BM. Combinations of pulsed white light and UV-C or mild heat treatment to inactivate conidia of *Botrytis cinerea* and *Monilia fructigena*. Int J Food Microbiol 2003;85:185-196.
22. Matanda M. Marketing of dairy products under a changing economic environment: The case of Zimbabwe. Regional exchange network for market oriented dairy development. http://www.fao.org/DOCREP/004/W3199E/W3199E00.HTM. (Accessed: October, 2010): FAO; 1996.
23. Nzeako BC, Al-Hashmi S. The effect of preservatives on the sterility of microorganisms introduced into different fruit juices. Medical Science Monitor 2006;12:BR179-BR186.
24. Pavic S, Brett M, Petric N, Lastre D, Smoljanovic M, Atkinson M, Kovacic A, Cetinic E, Ropac D. An outbreak of food poisoning in a kindergarten caused by milk powder containing toxigenic *Bacillus subtilis* and *Bacillus licheniformis*. Archiv Fur Lebensmittelhygiene 2005;56:20-22.
25. Petrisor C, Radu GL, Cimpeanu G. Quantification of physico-chemical changes during apricot ripening through non-destructive methods. Revista De Chimie 2010;61:345-350.
26. Rodriguez E, Vargas M. *Clostridium perfringens* in raw and cooked meats and its relation with the environment in Costa Rica. Archivos Latinoamericanos De Nutricion 2002;52:155-159.
27. Schouten RE, Tijskens LMM, van Kooten O. Predicting keeping quality of batches of cucumber fruit based on a physiological mechanism. Postharvest Biol Technol 2002;26:209-220.
28. Sharma M, Anand SK. Biofilms evaluation as an essential component of HACCP for food/dairy processing industry - a case. Food Control 2002;13:469-477.
29. Sheth AN, Wiersma P, Atrubin D, Dubey V, Zink D, Skinner G, Doerr F, Juliao P, Gonzalez G, Burnett C, Drenzek C, Shuler C, Austin J, Ellis A, Maslanka S, Sobel J. International outbreak of severe botulism with prolonged toxemia caused by commercial carrot Juice. Clin Infectious Dis 2008;47:1245-1251.
30. US Dept of Agriculture and Health, Education and Welfare. Nutrition and Your Health: Dietary Guidelines for Americans. Washington, DC: US Government Printing Office; 1979.
31. Weenk GH, Corry JEL, Curtis GDW, Baird RM. Chapter 1 Microbiological assessment of culture media: Comparison and statistical evaluation of methods. Progress in Industrial Microbiology: Elsevier; 1995. pp 1-23.
32. Yildiz H, Bozkurt H, Icier F. Ohmic and conventional heating of Pomegranate juice: Effects on rheology, color, and total phenolics. Food Sci Technol Int 2009;15:503-512.

5/25/2011