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Microbiological and Sensory Analysis of Imported Fruit Juices in Kumasi, Ghana

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Abstract: A study conducted on some imported fruit juices on microbiological and sensory analysis showed a significant increase in microbial load in the apple and mango fruit juices as they stay over the period in the shelves from June, 2007 to February, 2008. Though significant, the orange juice showed the lowest microbial count of 3.1×10^3 and 9.5×10^3 in June, 2007 and February, 2008, respectively in terms of real numbers. Apple and mango fruit juices showed insignificant increases over the period in yeast growth ($p = 0.062$ and $p = 0.093$, respectively). In general, yeast numbers were relatively lower than bacterial counts in both apple and mango fruit juices. The genus *Bacillus* was the most diverse organism although other Gram positive cocci of the genera *Micrococcus*, *Leuconostocs*, *Lactobacillus* and the yeast *Saccharomyces* were also isolated. Sensory analysis also showed that, consumers preferred the product which had been on the shelf for the shortest period of time (i.e., June, 2007 and October, 2007 products) in terms of aroma, taste and colour.

Key words: Microbial count, real numbers, significant

INTRODUCTION

Worldwide, health concerns have led to the popularization of natural fruit juices as a healthy alternative to other beverages. Fruit juices and drinks are nutritious which offer great taste and health benefits (Suaad and Eman, 2008). In recent times, the consumption of fruit juices has also become a relatively common phenomenon in Ghana. These juices have gained popularity among workers, students and other economic classes. For many, fruit juices have come to represent an alternative to carbonated soft drinks, which have high sugar level. Fruit juices are consumed for their characteristic aroma and are also considered sources of vitamins, minerals and soluble and insoluble fibres (Righetto *et al*., 1999; Wardlaw, 2000).

In spite of the above, there have been many reported cases of fruit juices having different aroma, taste and colour when kept over a period of time. Many of the problems in the shelf life of fruit juices stem from storage. Locally, fruit juices are stored mostly on shelves in the supermarket until they are purchased by consumers. Storage on shelves in supermarkets is not extremely detrimental to the quality of the product, but when the fruit juices which are often in paper cartons are exposed to direct sunlight for extended periods of time, the increase in temperature enhances the growth of certain thermophilic microorganisms in the fruit juice. This is characterized by the production of unpleasant flavors, colour change and product deterioration which is often caused by yeast (Parish, 1991; Corrèa de Souza *et al*., 2004).

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Freitas *et al*. (2006) reported that fruit juices processed by hot fill method and stored for 350 days in the same conditions as market showed a reduction of the overall acceptance and appearance. The stability of fruit juices depend on the raw material, processing conditions, packaging material and the storage conditions. These factors can cause microbiological, enzymatic, chemical and physical alterations that will affect the sensorial and nutritional characteristics (Corrèa and Faria, 1999). Although fruit juice is pasteurized under high temperatures and pressures, pressure-resistant and temperature-resistant microorganisms can remain in the juice. These products therefore undergo spoilage under certain conditions and in their recommended validity period, may show varying changes in their sensorial and microbiological properties (Stone and Sidel, 1995).

This study was carried out to assess the effect of prolonged shelving on the microbiological and sensorial changes that occur during the validity period (shelf life) of three different flavours (orange, mango and apple) of imported juices produced by the Ceres Fruit Juice Company of South Africa and sold in the Kumasi Metropolis, Ghana.

MATERIALS AND METHODS

Study Area

The study was carried out in Kumasi, Ghana (between latitude 6.35-6.40° and longitude 1.30-1.35°) from June 2007 to February 2008. Kumasi has a population of 1,517,000 and it is the second largest city in Ghana. The centrality of the city as a traversing point from all parts of the country makes it a special place for many to migrate to, especially students due to increased student population.

Sampling

A total of 162 one-litre boxes of fruit juices made up of orange (54 boxes), mango (54 boxes) and apple (54 boxes) juices produced in June, 2007 by the in South Africa were bought from twenty different supermarkets across the Kumasi Metropolis. Samples from each of the three different fruit flavours (orange, mango and apple) were taken periodically at random at four months interval in June 2007, October 2007 and February 2008 for microbiological and sensorial examination at the Microbiology and Quality Assurance Laboratories of the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. All samples were bought with their security seals untampered and ensured were in their required storage temperatures with their expiry dates checked. All the samples had a validity period of one year.

Microbial Counts

Bacterial numbers were estimated in triplicates 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, Coliforms on Lauryl Sulphate Agar, Lactic Acid Bacteria on de Man Rogosa Sharpe and yeast and mould on Suboround. S-S agar on the other hand, was used for *Salmonella* sp. Identification of microorganisms was done using the API 20E system (Analytical Profile Index, Biomerieux, Durham, NC, USA). Monthly pH determination were done using a pH meter (Orion Model 420A).

Sensory Evaluation

Sensory analysis was carried out on the aroma, taste and colour of the products. A 50 member panelists, who are all regular users of imported fruit juices and therefore familiar with its sensory characteristics were used. Questionnaire for panelists using a 9-point Hedonic scale to indicate the various characteristics as acceptable, neither acceptable nor unacceptable and unacceptable were used in the evaluation.

Statistical Analysis

Statistical analysis (ANOVA) was carried out on the data obtained using Graph Pad Prism version 4.03 for Windows (Graph Pad Software, San Diego California, USA). p-values of <0.05 was considered to be significant.

RESULTS AND DISCUSSION

Microbiological Assessment

There was a general increase in microbial numbers in the different fruit juices with increasing time spent on the shelf. This increase was different for all the fruit juices. This increase is supported by the study of Suaad and Eman (2008), who showed that, there is the presence of different species of bacteria in supposedly bacteria-free commercially available fruit juices over time. The increase is partly due to the high moisture content in fruit juices which has been found to promote the growth of yeast and bacteria (MacRae *et al*., 1993). There were however, no *Salmonella* sp. and coliforms in any of the samples. Initial microbial counts in the juices were 3.2×10^3, 5.7×10^3 and 3.1×10^3 for apple, mango and orange, respectively. However, after staying in the shelves for eight months, the microbial load increased to 2.4×10^4, 2.5×10^4 and 9.5×10^3 for apple, mango and orange, respectively (Table 1). This observation is confirmed by Furuhata *et al*. (2005), who isolated Thermotolerant Acidophilic Bacteria (TAB) from mango, apple, grape and pineapple juices. Grande *et al*. (2005) also reported that after 90 days of preservation of fruit juices with enterocin AS-48 preservative, viable cells were still detected in apple, peach and grapefruits. These increases were significant in apple ($p = 0.042$) and orange ($p = 0.039$) juices but not the case in mango juice ($p = 0.156$). Though significant, the orange juice showed the lowest microbial counts, with a value of 3.1×10^3 in June 2007 and 9.5×10^3 in February 2008, for the eight month period. This may be due to the comparatively lower pH recorded in orange juice as compared to apple and mango juices (Table 1).

The total microbial counts observed in all the fruit juice samples tested were above the limits (1.0×10^2) of the Ghana Standards Board recommendation for fruit juices (www.ghanastandardsboard.org). This finding raises questions about the wholesomeness of these fruit juices on the Ghanaian market.

The pH of fruit juices often shows a noticeable decline towards acidity during storage (Nanos and Kader, 1993). This was evident in all the results obtained with the mango juice recording the least noticeable change in pH (from 5.63 in June, 2007 to 5.17 in February, 2008). The pH changes were significant in apple ($p = 0.004$) and orange ($p = 0.003$) but insignificant in mango ($p = 0.226$).

Although, Mendoza *et al*. (1982) have stated that the predominance of yeasts over bacteria in almost all juices is evident, our results were on the contrary. Bacteria of the genus *Bacillus* was the most predominant and common isolate in all the fruit juices. This is supported by Suaad and Eman (2008), who stated that *Bacillus cereus* was the most common isolate in all types of fruit juice. Yeast counts were also seen to rise as the time spent on the shelf increased, an occurrence also observed by Corrèa de Souza *et al*. (2004), who stated that the yeast count in fruit juices increased over time. Orange juice showed the most significant increase in total yeast count over time ($p = 0.032$), with initial values of 2.3×10^3 in June 2007 to 2.2×10^4 in February 2008 (Table 2). This may be due to the

Table 1: Comparison of total viable count in all the fruit juices from June, 2007 to February, 2008

	Period		
Fruit juice	June 2007	October 2007	February 2008
Apple	$3.2\times10^3{}_{(5.93)}$	$4.2\times10^3{}_{(5.86)}$	$2.4\times10^4{}_{(4.71)}$
Mango	$5.7\times10^3{}_{(5.63)}$	$6.2\times10^3{}_{(5.21)}$	$2.5\times10^4{}_{(5.17)}$
Orange	$3.1\times10^3{}_{(5.03)}$	$4.2\times10^3{}_{(4.82)}$	$9.5\times10^3{}_{(4.39)}$

Values are averaged from twenty different supermarkets, pH values are in parenthesis

Table 2: Comparison of yeast count in all the fruit juices from June, 2007 to February, 2008

	Period		
Fruit juice	June 2007	October 2007	February 2008
Apple	$1.6\times10^{2}_{(5.93)}$	$2.1\times10^{2}_{(5.86)}$	$2.6\times10^{2}_{(5.42)}$
Mango	$1.2\times10^{2}_{(5.63)}$	$2.5\times10^{2}_{(5.21)}$	$4.5\times10^{2}_{(5.17)}$
Orange	$2.3\times10^{3}_{(5.03)}$	$5.1\times10^{3}_{(4.82)}$	$2.2\times10^{4}_{(4.39)}$

Values are averaged from twenty different supermarkets, pH values are in parenthesis

continual increase in acidity (5.03 to 4.39) over the period of storage, as yeasts thrive well in acidity medium. Though apple and mango juices showed a consistent increase in yeast count, their increase were not significant ($p = 0.062$ and $p = 0.093$, respectively). These insignificant increases are probably due to the comparably slight decrease in acidity over time. Like the total microbial count, all yeast counts were also above the recommended limits (5.0×10^{1}) by the Ghana Standards Board and therefore raise concerns about the safety of the products.

In general, yeast numbers were relatively lower than bacteria in both the apple and mango fruit juice samples but a little higher in the orange juice (Table 1, 2).

Microbial Flora

Using the API 20E system in the identification and characterization of the isolates, both Gram positive and catalase positive rods of the genus *Bacillus* were mostly identified. The presence of large numbers of *Bacillus* sp. can be explained by the fact that endospores of *B. stearothermophilus* can survive pasteurization and may thereafter cause spoilage in foods stored above temperatures of 30°C (Singleton, 1999; Bergey *et al*., 1984). Collado *et al*. (2003) also reported that the species of this genus can survive, grow and sporulate despite changes in water activity, pH and temperature. Gram positive and catalase positive cocci of the genera *Micrococcus*, *Leuconostoc* and *Lactococcus* were also isolated and identified. The genus *Lactobacillus* was the only Gram positive and catalase negative organism isolated. In fruits and fruit juices, this genus has been found to be common (Suaad and Eman, 2008). The only yeast isolate was of the genus *Saccharomyces* although Mendoza *et al*. (1982) reported that *Candida*, *Rhodotorula*, *Saccharomyces* and *Pichia* are the genera commonly present in fruit juices. The occurrence of these different microorganisms in these commercially imported fruit juices is a matter of great concern as they may pose health risk to consumers (Suaad and Eman, 2008).

Sensory Properties

Sensory characteristics of any food item contribute significantly to its consumer acceptance or rejection. Thus sensory evaluation of food using panelists is routinely carried out by food scientists to help evaluate the acceptability or otherwise of any new food product (Dzogbefia and Djokoto, 2006).

Appearance, flavour and texture are the most important attributes considered by consumers when making food choices. The frequency distribution of panelists' responses for aroma, taste and colour and overall acceptability or otherwise are shown in Fig. 1-3.

Most panelists could tell a vast difference between products that had been on the shelf for eight months (i.e., February, 2008 samples). Though there were changes in all the fruit juices that had stayed in the shelves for only four months (June, 2007-October, 2007), they were not that significant in all the sensory properties tested ($p = 0.065$, $p = 0.061$ and $p = 0.058$ for apple, orange and mango respectively). This indicates that panelists could not detect any significant differences between the June and October products that had stayed in the shelves for only four months. In fact, most panelists rated them as moderate. The difference was however significant in the February, 2008 samples ($p = 0.032$) suggesting that the sensory parameters changed significantly beyond four months. Panelists easily detected significant changes in all the parameters studied. They preferred in general the product which had been on the shelf for the shortest period of time, in terms of aroma, taste and colour and gave such products the highest ranking.

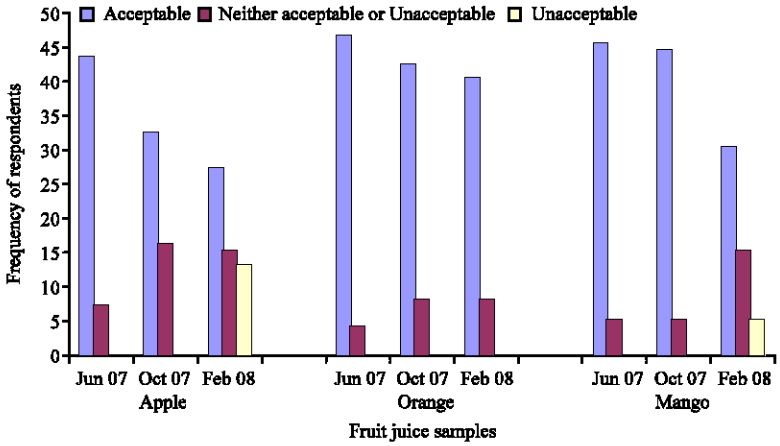

Fig. 1: Frequency distribution of panelists' response to aroma in different fruit samples

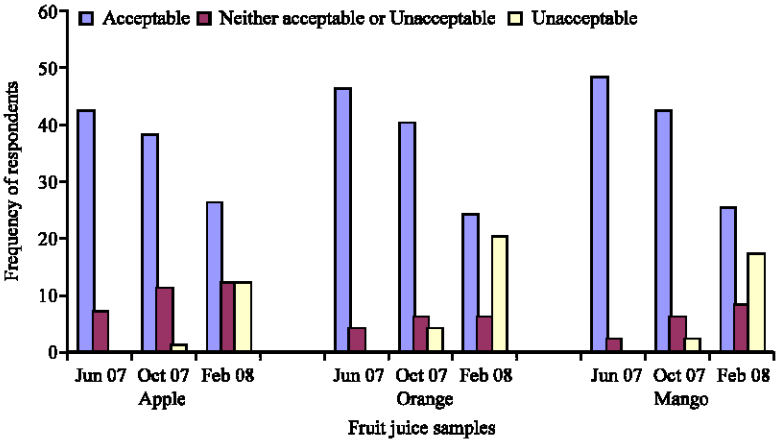

Fig. 2: Frequency distribution of panelists' response to taste in different fruit juice

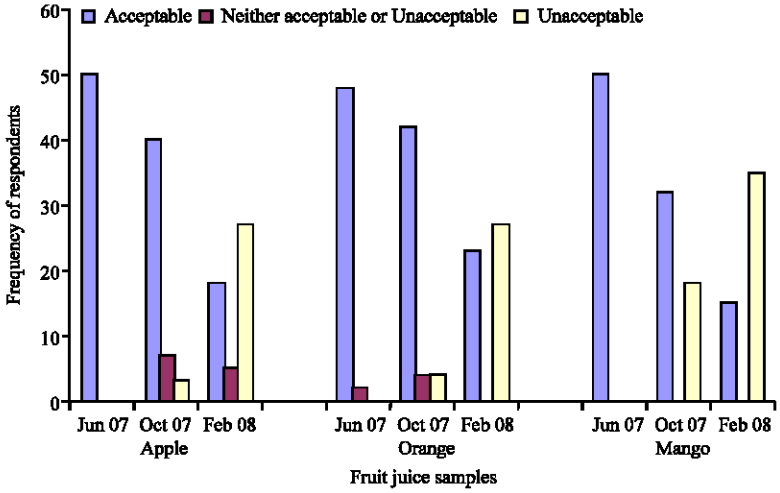

Fig. 3: Frequency distribution of panelists' response to colour in different fruit juice

Microbial growth in fruit juices is often characterized by the production of unpleasant flavours and product deterioration that is commonly caused by yeasts (Parish, 1991). This probably resulted in client dissatisfaction in this study (Fig. 1-3). The February, 2008 samples were observed to be darker in its colour than the June, 2007 samples. This colour change may be due to the enzymes in fruit juices that cause enzymatic browning. Maurice *et al*. (2000) reported that the understanding and control of the mechanism(s) of browning in fruits and vegetables may be applied in either preventing the browning reaction or slowing its rate and thus extending the shelf life of the products. It may also be influenced by naturally occurring pigments or by pigments resulting from both enzymatic and non-enzymatic reactions. The colour differences were however not of a bother to the panelists as the differences in aroma and taste.

The results of this study have several implications for the consumption of these commercial fruit juices in Ghana. Esteve and Frigola (2007) reported that, although most people enjoy fruit juice and drinks, the effect on young children, the elderly and people with weakened immune systems can be severe or even deadly. The difference in the microbial content should be further investigated since the issue of potability, acceptability and safety may inadvertently harm consumers.

CONCLUSION

Though the picture is far from complete, the study has shown that imported fruit juices which are most patronized by workers, students and the economic class in the Kumasi Metropolis undergo gradual deterioration and loss of storage stability. Its microbial load and sensory characteristics all change as they stay on the shelves for a longer period though they may not have expired. Apple and mango fruit juices showed significant increase in total microbial load whereas orange on the other hand showed significant yeast growth as they stay for longer periods. Consumers also preferred products which had been on the shelf for shorter periods in terms of aroma, taste and colour. It is therefore recommended that a further study of imported fruit juices should be done in relation to the storage temperature and the validity period. It is also important that further studies should be conducted to find the health risk associated with these products since the microbial load in most cases were significant. Corresponding authorities should also be vigilant to reduce and mitigate exposure to harmful microorganisms that will finally affect consumers' health.

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