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Effect of Increasing Acidity on Shelf-Life of Bottled Mixed Fruit Juice Drink

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Abstract: **With the growing concern of the public on health effect by consumption of artificial drinks, there is a general tendency of increasing consumer preference for fruit drink preparations. However public is also hesitant in consuming such preparations as those contain chemical preservatives. This study was done in order to find out an alternative preservation method avoiding the use of chemical preservatives for mixed fruit juice drink packaged in PET bottles. Treatments with different acidity and sugar levels were used to find the best acid and sugar levels at which shelf-life can be extended with minimum effects on sensory and nutritive properties. The initial acid percentage and brix values of each treatment were 0.26, 0.28, 0.30 and 0.32 and 15, 16.1, 17.2 and 18.4 respectively. pH of fruit juice drink samples were in the range of 2.86 to 3.04, whereas for the vitamin C ranged between 6.75 × 10^{-4} mol/dm^3 and 9.12 × 10^{-4} mol/dm^3. Considerable microbial growth was observed in fruit drink sample with 0.26% acidity by fifth week and 0.26% acidity sample (with preservatives) by seventh week. The highest sensory score was obtained for fruit drink sample with 0.30% and 0.32% acidity During the storage period, pH increased, acidity slightly decreased, brix level slightly increased and vitamin C content decreased in fruit drink samples.**

It was evident that preservation of mixed fruit juice drink can be increased by slightly increasing the acid and sugar levels without using chemical preservatives without affecting sensory properties while minimizing the effect on nutritional values as indicated by vitamin C content.

Keywords: **juice, pH, microbial growth, preservation.**

1. INTRODUCTION

As Sri Lanka is a tropical country there is a wide variety of seasonal fruits available with high nutritional value and delicious in taste. Most of these fruits are wasted without use because of their perishability. However, these losses can be minimized by subjecting these fruits to some kind of processing, such as production of fruit juice drink where these products can be preserved for few months.

Fruit juices are important trade commodities in most countries (Vasavada, 2003). Health conscious public show a growing trend in consumption of fruit juices obtained by natural fruits over other carbonated soft drinks because fruit juice drinks consist of high nutritive values and pose considerably less harmful effects on health.

According to Franke *et al.* 2005 Juices are fat-free, nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health. Consumption of natural juices can provide health benefits due to the antioxidants and high content of vitamins and minerals (Edwards *et al.*2003). Fruit juice is important in human nutrition. Most fruits contain carbohydrates, proteins and minor nutrients such as minerals and vitamins.

Thermally Processed Fruit Beverages / Fruit Drink/ Ready to Serve Fruit Beverages (Canned, Bottled, Flexible Pack And/ Or Aseptically Packed) means an unfermented but fermentable product which is prepared from juice or Pulp/Puree or concentrated juice or pulp of sound mature fruit, by blending with nutritive sweeteners and water or milk and processed

by heat, in an appropriate manner, before or after being sealed in a container, so as to prevent spoilage (Prevention of food adulteration (ii Amendment) rules, 2005).

Fruit juice drink is a ready to serve drink.

Ready to serve drink is a fruit drink intended for consumption without dilution. (SLS 729: 2008)

Spoilage of fruit juices is mainly due to the presence of osmophillic microflora (Tahiri *et al.*, 2006). This microflora (yeasts) causes fermentation and produces a buttermilk-like off-flavour (Tournas *et al*., 2006). Adding chemical preservatives is the most frequently used method for preservation of fruit juice drinks. Chemical preservatives, such as sodium benzoate and potassium sorbate, are commonly used in fruit juices and beverages to extend their shelf life (Walker and Phillips, 2008). Potassium metabisulphite and sodium metabisulphite are the next common chemical preservatives. Shelf life of fruit juice drink packaged in PET (Polyethylene terepthalate) bottles is about three months when recommended amount of chemical preservatives are added. There is a concern among consumers that these chemical preservatives have negative effects on health and there is a consumer demand for beverages where there is no or less amount of chemical preservatives added.

In Sri Lanka, commercially produced fruit juices are most commonly packaged in PET bottles and glass bottles. There is a high consumer and manufacturer attraction for beverages packaged in PET bottles over glass bottles. That is because of the disadvantages of glass bottles such as weight and brittleness that leads to problems during transportation and storage. PET is the most popular food grade plastic used for packaging of beverages. PET has properties like those of glass; but it lacks the disadvantages of weight and brittleness. PET bottles can also be laminated with other plastics to give good barrier properties.

Presently, thermal pasteurization is considered as the most effective technology in inactivating microorganisms and enzymes to extend product shelf life (Noci *et al*., 2008). Heat treatment is critical for microbiological stability of fruit juice drinks. PET bottles are not subjected to heat treatment before or after filling. If subjected to higher heat than the bottle can withstand, then when the product cools the bottle contracts. Typically the bottles can withstand temperatures around 50°C, some recent types can be heated to 85°C (Ashurst, 2005).These bottles may also be permeable to oxygen, allowing the growth of aerobic spoilage agents (Ashurst, 2005).Therefore microbial growth is higher in fruit juices packaged in PET bottles limiting their shelf life. Most spoilage is by yeasts, moulds and also some acid tolerant bacteria. Consequently, there is a need for finding a method for increasing their shelf life without increasing the addition of chemical preservatives.

The acidity can be increased by the addition of citric acid. The pH of the product should be less than 4.2. The acidity should be less than 1%. The acidity can be increased, in order to prevent the growth of microorganisms in the product, thereby increasing their shelf life. The excellent keeping quality of fruits and soft drinks is influenced by low pH (Bates *et al*., 2001). The extension of shelf life can be resulted primarily from the inhibition of spoilage microorganisms. The acid percentage of the mixed fruit juice drink can be increased slightly than the normally recommended value. This should be done without having any undesirable effects on the sensory properties and nutritive values of the product. It has been found that destruction of vitamin C occurs at higher acid levels. At low pH vitamin C is a more powerful antioxidant (Gramlich *et al.*, 2002), hence degrades more quickly at low pH. To have increased acid percentage without altering the taste the sugar level also has to be increased. This study is done to find out whether increasing acidity helps to extend the shelf life of mixed fruit juice drink without having additional chemical preservatives.

2. MATERIALS & METHODS

2.1 Sample Collection:

Four types of fruits (Mango, pineapple, papaya, and passion fruit) were used in the preparation of mixed fruit juice drink. Fully ripened and fresh fruits of mango (*Mangifera indica*), papaya (*Carica papaya*), pineapple (*Ananas comosus*) and passion fruit (*Passiflora edulis* f. *flavicarpa*) of sound condition were selected from the local market of Kandy.

2.2 Preparation of mixed fruit juice drink:

The fully ripened fruits were washed thoroughly with clean water. Each type of fruit was measured separately and made into a pulp by blending. Total fruit pulp percentage was taken as 10% by weight. Sugar solution was prepared by adding

half of required amount of water that is required to make the desired concentration to sugar and heating. The rest of water was added to the mixed pulps after filtering them through the strainer and heat was given. Then sugar solution and citric acid were added to the mixture and heated until boiling. Then heat was removed. SMS (sodium metabisulphite) was added (to the control sample (70mg)). Each sample was filled into 200 ml PET bottles while keeping in a water bath. Sample was filled to the top of the bottle without keeping any airspace and the lids were fixed. Four samples were selected with differing percentages of citric acid and sugar for mixed fruit juice drink.

Table 1 Mixed fruit juice drink samples with different acidity and brix value

Sample	Acidity (%)	Brix value (%)
J_0 (preservative added)	0.26%	15.0
J_1	0.26%	15.0
J_2	0.28%	16.1
J_3	0.30%	17.2
J_4	0.32%	18.4

2.3 Chemical analysis:

Acidity of the fruit juice samples was determined by titrating with standard sodium hydroxide solution. Total soluble solids (TSS) were measured using a refractometer. pH was measured using pH meter. Vitamin C content was determined by titrating with iodine solution. The chemical tests were conducted for all the mixed fruit juice drink samples at one week interval for a period of two months.

2.4 Microbiological Analysis:

Serial dilutions of each fruit drink sample were prepared. Yeast and mold count of each sample was determined by plating on Potato Dextrose Agar (PDA) medium. Standard plate count was determined using nutrient agar (NA) medium. Detection of Coliforms was done using EC broth. All the microbiological tests were conducted once in every week for a period of two months.

2.5 Sensory Evaluation:

Sensory evaluation was conducted using 5- point hedonic scale test. Twenty semi-trained panelists were provided with each of the four samples. The samples were coded with three digit random numbers. Sensory ballot papers were provided to each panelist to mention their responses regarding, colour, odor, taste, mouth feel and overall acceptability. Sensory evaluation was conducted once in two weeks for a period of two months.

2.6 Storage study:

Mixed fruit juice drink samples were stored at room temperature for two month period. Chemical analysis, microbiological analysis and sensory evaluation were performed once a week for a period of two months.

2.7 Statistical Analysis:

The data obtained were analyzed and interpreted by analysis of variance (ANOVA), Friedman test and Polynomial Regression Analysis using pre-packaged computer statistical software MINITAB version 14.

3. RESULTS & DISCUSSION

3.1pH:

The initial pH of the fruit drink samples were found in the range of 2.86 to 3.04, with the maximum pH in J_1 sample and the minimum pH in J_4 sample. The pH of all the five samples increased during second and third weeks. During the fourth week the pH of J_3 sample increased and pH was decreased in J_4 sample, while it remained constant in other samples. The pH remained constant in all the five samples during the fifth week. Only the pH of J_2 increased during the sixth week. The pH of J_1 decreased during the seventh week, while it remained constant in other samples. During the last week of storage the pH of J_2 increased again, while it remained constant in other samples. The increase of pH at the end of storage was observed in the decreasing order J_2, J_0, J_3, J_4, J_1. The highest pH was recorded in J_2 sample and lowest in J_4 sample at the

end of storage. ANOVA test results indicates that the pH values of five samples were significantly different during each week ($p<0.05$). Mean separation was done using the method of least significant difference. It was noticeable that the pH remained constant or increased slightly after the 3rd week. It remains to observe what the trend would be during further storage. The increase in pH is due to the decrease in acidity. The Pearson correlation value for J_0, J_1, J_2, J_3 and J_4 are -0.781, -0.604, -0.310, -0.573, -0.722, respectively. P-values are 0.022, 0.013, 0.140, 0.001, and 0.000, respectively. Negative value means that one variable tends to increase as the other decreases. According to p-values a good correlation was found only between pH and acidity except in J_0, J_1 and J_2 samples.

3.2 Acidity:

The initial acidity of the fruit juice drink samples were in the range of 0.26% and 0.32%. The minimum acidity was in J_0 and J_1 samples. The maximum acidity was in J_4 sample. Only the acidity of J_0 sample decreased during the second week. During the third week acidity of J_1 and J_2 decreased, while it remained constant in other samples. During the fourth week the acidity decreased in J_2, J_3 and J_4 samples and it remained constant in J_1 and J_2 samples. During the fifth week the acidity of all the samples decreased except in J_3 sample. The acidity of all the samples remained constant during sixth, seventh and the eighth week of storage. The decrease of acidity at the end of storage was observed in the decreasing order J_1, J_2 and J_3 and J_4 and J_0. The lowest acidity was recorded in J_1 sample and the highest was in J_4 sample in the eighth test. ANOVA test results indicates that the acidity of five samples were significantly different during each week ($p<0.05$). Mean separation was done using the method of least significant difference.

Sensory data shows that high acidity samples have high consumer preference. The sensory preference was decreased in lowest acidity sample with decreasing acidity over time. The consumer preference of high acidity samples remained same with time even though the acidity slightly decreased. With decreasing acidity an increase of microbial load was observed in the sample with lowest acidity and in control sample. A change in colour was observed in the sample with lowest acidity, but no relationship was observed in decreasing acidity with colour change. With decreasing acidity, increase in pH and brix value and decrease in vitamin C content was noted.

3.3 Brix value:

The initial brix values of the nectar samples range from 15.0% to 18.2%. The minimum brix value was in J_0 and J_1 samples, while the maximum brix value was in J_4 sample. The brix value J_0 and J_4 decreased during the second week. During the third week only the brix value of J_0 increased while it remained constant in other samples. During the fourth week only the brix value of J_1 sample increased. During the fifth week the acidity of J_0 and J_4 increased, in J_1 it was decreased and remained constant in J_2 and J_3. During the sixth week the brix value only increased in J_1 sample. During the seventh week the brix value of all the five samples remained constant. The brix value of J_0 and J_4 samples increased during the last week of storage, while it remained constant in other samples. The increase of brix value at the end of storage was observed in the decreasing order J_0, J_1, J_4 and no increase in J_2 and J_3. The highest brix value was recorded in J_4 sample and the lowest was in J_0 and J_1 samples at the end of storage. ANOVA test results indicates that the brix value of five samples were significantly different during each week ($p<0.05$). Mean separation was done using the method of least significant difference. The highest acidity sample shows the highest brix value and it doesn't change with time. Sensory data shows that high brix value samples have high consumer preference. The consumer preference didn't change with the increasing brix value.

3.4 Vitamin C content:

The initial vitamin C content of the fruit juice drink samples was found in the range of 6.75×10^{-4}mol/dm³ and 9.12×10^{-4}mol/dm³, with highest vitamin C concentration in J_1 and lowest in J_2 samples. The vitamin C content of all the samples reduced during the second week. During the third week the vitamin C content of all the samples reduced except in J_0 sample. During the fourth week the vitamin C content of all the samples decreased except in J_4 sample. . During the fifth week the vitamin C content of all the samples decreased. . During the sixth week the vitamin C content of all the samples decreased except in J_0 sample. . During the seventh week the vitamin C content of all the samples decreased except in J_1 sample. The vitamin C content of all the samples except in J_4 was decreased during the eighth test. The decrease of vitamin C content at the end of storage was observed in the decreasing order J_1, J_2, J_0, J_3, J_4. The lowest vitamin C content was recorded in J_2 sample and the highest was in J_1 and J_3 samples at the end of storage. ANOVA test results indicates that the vitamin C content of five samples were significantly different during each week ($p<0.05$). Mean

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separation was done using the method of least significant difference (Appendix 11). Table 4.18 shows the p- values and F-values obtained for each ANOVA test.

A correlation of acidity with degradation of vitamin C content was not observed. The initial drop of vitamin C content may be due to the high pasteurization temperature. Degradation of vitamin C over time was noticed.

Table 2- Results for chemical tests of mixed fruit juice drink samples

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
pH of fruit juice drink samples during storage at ambient temperature								
J_0	2.89^a	3.09^a	3.12^a	3.12^a	3.12^a	3.12^a	3.12^a	3.12^a
J_1	3.04^b	3.10^b	3.14^b	3.14^b	3.14^b	3.14^b	3.13^b	3.14^b
J_2	2.98^c	3.13^c	3.14^b	3.14^b	3.14^b	3.24^c	3.24^c	3.24^c
J_3	2.89^a	3.01^d	3.05^c	3.06^c	3.06^c	3.06^d	3.06^d	3.06^d
J_4	2.86^d	2.97^e	3.01^d	3.00^d	3.00^d	3.00^e	3.00^e	3.00^e
Acidity of the fruit juice drink samples during storage at ambient temperature (%)								
J_0	0.26^a	0.25^a	0.25^a	0.25^a	0.24^a	0.24^a	0.24^a	0.24^a
J_1	0.26^a	0.26^b	0.24^b	0.24^b	0.22^b	0.22^b	0.22^b	0.22^b
J_2	0.28^b	0.28^c	0.27^c	0.26^c	0.25^c	0.25^c	0.25^c	0.25^c
J_3	0.30^c	0.30^d	0.30^d	0.27^d	0.27^d	0.27^d	0.27^d	0.27^d
J_4	0.32^d	0.32^e	0.32^e	0.31^e	0.30^e	0.30^e	0.30^e	0.30^e
Brix value of the fruit juice drink samples during storage at ambient temperature (%)								
J_0	15.0^a	14.2^a	15.0^a	15.0^a	15.6^a	15.6^a	15.6^a	15.6^a
J_1	15.0^a	15.0^b	15.0^a	15.2^b	15.0^b	15.2^b	15.2^b	15.6^a
J_2	16.0^b	16.0^c	16.0^b	16.0^c	16.0^c	16.0^c	16.0^c	16.0^b
J_3	17.1^c	17.0^d	17.0^c	17.0^d	17.0^d	17.0^d	17.0^d	17.0^c
J_4	18.2^d	18.1^e	18.1^d	18.1^e	18.2^e	18.2^e	18.2^e	18.4^d
Vitamin C content of the fruit juice drink samples during storage at ambient temperature ($\times 10^{-4}$) (mol/dm^3)								
J_0	9.12^a	5.63^a	5.63^a	5.00^a	4.75^a	4.75^a	4.13^a	4.08^a
J_1	11.8^b	7.75^b	6.75^b	5.38^b	4.88^b	4.50^b	4.50^b	4.10^b
J_2	8.25^c	6.87^c	5.38^c	4.50^c	4.38^c	4.25^c	4.00^c	3.16^c
J_3	8.13^d	6.75^d	5.13^d	5.12^d	4.38^c	4.25^c	4.13^d	4.10^b
J_4	6.75^e	6.25^e	4.75^e	4.75^e	4.25^d	4.00^d	3.75^e	3.75^d

Values are means of duplicate determinants. Values within the column with the same superscript indicate that there is no significant difference. ($p < 0.05$)

3.5 Microbiological Analysis:

The initial colony count of all the five samples is within the acceptable range according to Sri Lanka standards (i.e. less than 50 in 1ml). 50 CFU were observed in J_2 sample and 100 CFU were observed in J_3 sample during the second week. Considerable growth was not observed in other samples. During the third week 50 CFU were formed in J_0 and J_2 which is within the acceptable limit according to SLS standards, while in other samples no considerable growth was observed. During the fourth week 50 CFU were observed in J_3 sample, while no colonies were formed in other samples. During the fourth week 50 CFU were observed in J_0, J_3 and J_4 samples, which is within the excepted microbiological limit. 300 CFU were observed in J_1 sample while no colonies were observed in J_2 sample. During the sixth week 50 CFU were observed in J_0 and J_3 samples, which is within the acceptable limit, 100 CFU were observed in J_1 sample and no growth in J_2 and J_4 samples. During seventh week 50 CFU were observed in J_0 and J_1 sample. During the last week of storage no growth was observed in all the samples. Standard plate count of samples J_0, $J_2$and J_4 is within the acceptable microbial limit at the end of storage period. J_1 sample exceeded the acceptable standard plate count by fifth week, except in last week. J_3

sample showed colony formation which was within the acceptable limit except in fifth week. Only J_1 showed increasing microbial growth and it was unsuitable for consumption by fifth week.

The initial yeast and mold count of all the five samples is within the acceptable range according to Sri Lanka standards (i.e. absent in 1ml). Considerable fungal growth was observed in J_1 sample during second week. During third week considerable fungal growth was observed in J_0 and J_1 samples and no growth was observed in other samples. Only J_2 sample showed fungal growth during fourth week. During fifth week fungal growth was observed only in J_1 and J_3 samples. Only J_1 sample showed considerable fungal growth during sixth week. J_0 and J_1 samples showed considerable fungal growth during seventh week. During the last week of storage only J_0 and J_1 samples showed fungal growth. Increasing fungal growth was observed in J_0 and J_1 samples during the storage period. All the colonies were obtained within 48 hours of incubation, indicating the presence of yeast.

Coliform bacteria were absent in all the five samples during the two-month storage period, and hence the samples were found to be within the acceptable limit for Coliforms, i.e. absent in 1 ml of the sample.

Table 3- Microbiological results of mixed fruit juice drink samples

Sample	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8
standard plate count of fruit juice drink samples (CFU in 1ml sample)								
J_0	nil	nil	nil	nil	5.0×10^1	5.0×10^1	5.0×10^1	nil
J_1	nil	nil	5.0×10^1	nil	3.0×10^2	1.0×10^2	5.0×10^1	nil
J_2	nil	5.0×10^1	5.0×10^1	nil	nil	nil	nil	nil
J_3	nil	1.0×10^2	nil	5.0×10^1	5.0×10^1	5.0×10^1	nil	nil
J_4	nil	nil	nil	nil	5.0×10^1	nil	nil	nil
yeast and mold count of fruit juice drink samples (CFU in 1ml sample)								
Sample	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8
J_0	nil	nil	5.0×10^1	nil	nil	nil	5.0×10^1	1.0×10^2
J_1	nil	5.0×10^1	5.0×10^1	nil	5.0×10^1	5.0×10^1	5.0×10^1	4.0×10^2
J_2	nil	nil	nil	5.0×10^1	nil	nil	nil	nil
J_3	nil	nil	nil	nil	5.0×10^1	nil	nil	nil
J_4	nil	nil	nil	nil	nil	nil	nil	nil

3.6 Sensory Evaluation:

Friedman analysis of sensory data had shown that there is a significant difference in sensory acceptance in four samples in first, second and third sensory evaluation ($p< 0.05$). The p-value changed from 0.011, 0.070, 0.215 and 0.000. The highest estimated median was obtained for J_3 and J_4 and lowest in J_1 and J_2 samples during the first sensory evaluation. During second sensory evaluation the order of estimated median was in the decreasing order J_1, J_4, J_3, J_2. The preferences of the panelists were observed to be closer to intermediate. During the third sensory evaluation the order of estimated median was in the decreasing order J_1, J_4, J_3, J_2. During the last week of sensory evaluation highest estimated median was obtained for J_3 and J_4 samples, J_1 and J_2 samples were not preferred by panelists. The most preferred sample was J_4 for first and fourth sensory evaluation and J_1 for second and third sensory evaluation.

3.7 Shelf life of mixed fruit juice samples:

In order to determine the shelf-life of mixed fruit juice drink samples regression analysis was done and the best model out of linear, quadratic and cubic models were selected. The pH value of 3.8 was selected as the end of shelf-life and time taken to attain this pH was determined.

Cubic model was selected as the best model to determine the behavior of J_0, J_1, J_2, J_3 and J_4 samples.

According to the model shelf-life of J_0 sample was determined as 11 weeks (pH = 2.664 + 0.2987 Week - 0.05953 Week**2 + 0.003687 Week**3), shelf-life of J_1 as 13 weeks (pH = 2.940 + 0.1218 Week - 0.02321 Week**2 + 0.001389 Week**3), shelf-life of J_2 as 13 weeks (pH = 2.886 + 0.1397 Week - 0.02318 Week**2 + 0.001439 Week**3), shelf-life

of J_3 as 13 weeks (pH = 2.736 + 0.1955 Week - 0.03686 Week**2 + 0.002197 Week**3) and shelf-life of J_4 as 13 weeks (pH = 2.716 + 0.1859 Week - 0.03687 Week**2 + 0.002273 Week**3).

But according to microbiological results both J_0 and J_1 samples were unsuitable for consumption by seventh week and second week respectively.

4. CONCLUSION

According to results of mixed fruit juice drink samples the sample with lowest acidity (0.26%) and sugar level (15%) was unable to preserve more for than two weeks. The sample with preservatives was unable to preserve more than seven weeks. Growth of yeasts was observed in these two samples. Samples with higher acidity has shown increased shelf-life and shown to discourage bacterial and fungal growth. Sensory evaluation indicates that samples with high acidity and sugar level were preferred by the panel. The preservation of mixed fruit juice drink can be achieved even by slightly increasing the acid and sugar level (to 0.28% and 16% respectively).

Following patterns were observed in mixed fruit juice drink. pH of all the samples increased with time corresponding to the decrease in acidity. Slight increase in brix value of samples was observed with time. Vitamin C content of all the samples was shown to decrease with time.

Considering both shelf-life study by increasing acidity, preservation of mixed fruit juice drink by increasing acidity without the addition of chemical preservative can be considered a viable method. This may be applicable in industrial scale fruit juice drink processing as it is a cost effective preservation technique. For the best results all the critical control points from fruit harvesting to storage conditions have to be maintained.

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