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Research Paper

Effect of keeping time period on acidity of fruit juices and determination of fungal growth in fruit juices

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ABSTRACT : The present investigation was carried out with the objectives to study the effect of keeping time period on acidity of fruit juices and determination of fungal growth in fruit juices. Only those fruit juice samples were taken for study which are mostly consumed by the common people for their health issue. Ten fruit juice samples (orange, pomegranate, mosambi, green grapes, purple grapes, lemon, mango, apple, pineapple and papaya)were taken for study. These fruits were brought to the laboratory for measuring the pH and titratable acidity in fruit juices and also identifying the fungal growth in fruit juices. The results of the present study revealed that that pH of fruit juices increased during storage. Keeping time period affects the titratable acidity of different fruit juices. Titratable acidity increased with increasing keeping time period. Fungi were isolated from all the fruit juices which were stored for specific keeping time periods (0 hrs, 24 hrs, 36 hrs and 48 hrs). The isolates were characterized and identified as *Aspergillus niger, Aspergillus flavus* and species of *Rhizopus* and *Mucor.*

KEY WORDS : Fruit juices, pH, Acidity, Fungal growth, Keeping time period

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Juice is a liquid naturally contained in fruit or vegetable tissue. Juice is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvents. For example orange juice is a liquid extract of one fruit of orange tree. Juice may be prepared in the home from fresh fruits and vegetables using variety of hand or juice extractor. Many commercial juices are filtered to remove fibre or pulp, but high pulp fresh orange juice is a popular beverage. Juice may be marketed in concentrated form, sometimes frozen, requiring the user to add water to reconstitute the liquid back to its original state. However, concentrates generally have a noticeably different taste than their comparable fresh squeezed versions. Other juices are reconstituted before packaging for retail sale. Common methods for preservation and processing of fruit juices include canning, pasteurization, freezing, evaporation and spray drying (Fasoyiro *et al*., 2005).

However, fruit juices are nutritious which offer great taste and health benefits. The 2005 Dietary Guide lines for Americans (2005) recommended consumption of several cups per day of fruits and vegetable. Most fruit juices bought from grocery stores and supermarket shelves are pasteurized. This means that the liquid has been brought to a high temperature that kills harmful bacteria. However, a small percentage of fresh fruit juices are unpasteurized. This means that there is a chance that the product may contain bacteria harmful to our health. Most people can enjoy unpasteurized juice and drinks, however, for young children, the elderly and people with weakened immune systems, the effect can be severe or ever deadly (Fasoyiro *et al*., 2005).

Unpasteurized fruit and vegetable juices have posed serious public health risk in recent years. Seventy people including a child who died-became ill in 1996 after drinking unpasteurized apple juice contaminated by a strain of *Escherichia coli* bacterium (Amato,1999).

Fruit juices are well recognized for their nutritive value, mineral and vitamin content. They are beverages that are consumed for their nutritional value, thirst-quenching properties and stimulating effect or for their medicinal values (Fawole and Osho, 2002). The low pH of fruit juices greatly

limits the number and the type of bacteria that can survive or grow at this low pH but some bacteria that their pH is lower than that of the fruit juice can grow at this condition (Ryu and Beuchat, 1998). Yeast and moulds are also present and can grow when the juice is held at a temperature permitting their growth. Yeasts are primarily responsible for the spoilage of chilled juice that is not sterile and some can withstand the effect of chemicals used to preserve them (Sandeep *et al*., 2001).

In India, there is always a great demand for fresh vegetables and fruit juices. Being tropical in location hot weather continues for a greater part of the year (February-September) increasing the need for these commodities. While most restaurants and cafes serve juices in apparently hygienic conditions in the roadside shops and recreational areas and busy market places, their microbiological quality remains questionable (Sandeep *et al*., 2001). In these shops juices extracted by squeezing from a variety of fresh fruits namely, oranges, grape, pomegranate, apple, pineapple, watermelon, papaya, carrot and soon were served after considerable dilution with water and ice (Splittstosser,1979).

In views of high demand of fresh fruit juices during different diseases, fruit juices was under taken with a view to assess their safety for human consumption (Uljas and Ingham,1998). This study was therefore aimed at : to study the pH of fruit juices, acidity of fruit juices, the effect of keeping period on acidity of fruit juices, and determine the fungal growth in fruit juices.

■ RESEARCH METHODS

The present study was conducted in laboratory of Biophysics Dept., Institute of Medical Sciences, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, and in Department of Home Science, Mahila Maha Vidyalaya, Banaras Hindu University, Varanasi, Uttar Pradesh.

Selection of samples :

Samples were taken whose fruit juices are consumed by more population daily. These fruit juices are consumed by peoples for their health benefit or as prescribed by doctor for therapeutic use. In the present study, the samples were selected : orange, mosambi, lemon, papaya, mango, green grapes, purple grapes, apple, pineapple and pomegranate.

Preparation of sample :

The following procedure was used for preparation of sample. Collect fresh fruits, wash it. Peel and cut it. squeeze the juice by pressing it by hand with the help of sterilized muslin cloth store the fruit juices in sterilized beaker. Use every time clean muslin cloth and sterilized beakers for squeezing and storage of fruit juices.

Storage of sample :

For the storage of sample of fruit juices, 10 sterilized 250 ml beakers were used and these samples were stored in refrigerator for 48 hrs till the whole study was not carried out.

Measuring of pH of fruit juices :

The pH of fruit juices was measured on fresh (0 hrs), 24 hrs, 36 hrs and 48 hrs duration. pH of fruit juice was measured by pH meter (Potentiometer).

Titration of fruit juices :

Titration of fruit juices was done by simple titration method (AOAC, 2000).

formula for calculating titrable acidity:

$$\text{Total acidity (\%age)} = \frac{\text{Titration value x Normality of NaOH x Vol.made up x Equivalent weight of citric acid x 100}}{\text{Volume taken for titration x weight of sample x 1000}}$$

Data analysis :

For statistical analysis ANOVA was used.

■ RESEARCH FINDINGS AND DISCUSSION

Table 1 shows the effect of keeping time period on pH of fruit juices. Mango juice has maximum pH (5.3) decreased with increased keeping time period(24 hrs) whereas,

Table 1: Effect of keeping time period on acidity of fruit juices

No. of sample	Sample of fruit juices	pH reading at			
		0 hr	24 hr	36 hr	48 hr
1.	Orange	4.5	4.5	4.75	4.78
2.	Mosambi	3.95	4.15	4.2	4.25
3.	Green grapes	4.3	4.35	4.45	4.50
4.	Purple grapes	4.05	4.2	4.20	4.2
5.	Lemon	4.25	4.25	4.35	4.38
6.	Papaya	3.5	3.6	3.63	3.67
7.	Mango	5.3	5.2	5.25	5.37
8.	Apple	3.85	3.85	3.9	3.95
9.	Pineapple	3.75	3.75	3.8	3.9
10.	Pomegranate	2.6	2.6	2.8	2.8

pomegranate juice had minimum pH (2.6) and there was no keeping time period effect on pH. Mango juice had maximum pH (5.3) which decreased with increased keeping time period (36 hrs). pomegranate juice had minimum pH (2.6) also decreased with increased keeping time period (36 hrs). Pomegranate juice had minimum pH(2.8) and showed no effect on pH due to keeping time . Similar findings were reported by Zahid *et al*.,(2008) in which pH decreased during storage and the mean values ranged between 3.89 and 2.92. Minimum decrease in the pH of apple juice was observed during storage.

Data pertaining to acidity (%) in different juices at 0 hours have been given in Table 2. Screening of data revealed that the percentage of acidity ranged from 0.48 to 9.56. The lowest total acidity (0.48 %) was found in treatment T_8 (apple juice) at 0 hour. Maximum per cent of total acidity was recorded with treatment T_5 (9.56%), which showed its significant superiority over all other treatments .Treatments T_9 gave significantly more acidity in comparison to treatment T_2, T_3 and T_{10}. Treatment T_6 gave insignificant higher acidity in comparison to T_8. Treatment T_3 also gave insignificantly greater acidity per cent than treatment T_1. Treatment T_2 gave in significantly more acidity per cent than treatment T_3, T_4, and T_{10}.

Data pertaining to acidity (%) in different juices at 24 hours has been given in Table 2. Screening of data revealed that the percentage of acidity ranged from 0.67 to 12.51. The lowest total acidity (0.67 %) was found in treatment T_8 (apple juice) at 24 hour. Maximum per cent of total acidity was recorded with treatment T_5 (12.51%), which showed its significant superiority over all other treatments.Treatments T_9 gave significantly more acidity in comparison to treatment T_2, T_3 and T_{10}. Treatment T_6 gave insignificant higher acidity in comparison to T_8. Treatment T_3 also gave insignificantly greater acidity per cent than treatment T_1. Treatment T_2 gave in significantly more acidity per cent than treatment T_3, T_4, and T_{10}.

Data pertaining to acidity (%) in different juices at 36 hours has been given in Table 2. The data revealed that the percentage of acidity ranged from 0.98 to 13.36. The lowest total acidity (0.98 %) was found in treatment T_8 (apple juice) at 36 hour. Maximum per cent of total acidity was recorded with treatment T_5(13.36%), which showed its significant superiority over all other treatments.Treatments T_9 gave significantly more acidity in comparison to treatment T_2, T_3 and T_{10}. Treatment T_6 gave insignificant higher acidity in comparison to T_8. Treatment T_3 also gave insignificantly greater acidity per cent than treatment T_1. Treatment T_2 gave in significantly more acidity per cent than treatment T_3, T_4 and T_{10}.

Data pertaining to acidity (%) in different juices at 48 hours have been given in Table 2.The data revealed that the percentage of acidity ranged from 1.12 to 16.05. The lowest total acidity (1.12 %) was found in treatment T_8 (apple juice) at 48 hour. Maximum per cent of total acidity was recorded with treatment T_5(16.05%), which showed its significant superiority over all other treatments .Treatments T_9 gave significantly more acidity in comparison to treatment T_2, T_3 and T_{10}. Treatment T_6 gave insignificant higher acidity in comparison to T_8. Treatment T_3 also gave insignificantly greater acidity

Table 2: Effect of keeping time period on titratable acidity of fruit juices samples

Samples (fruit juices)	Treatment	0 hour	24 hours	36 hours	48 hours
Orange	T_1	0.94	0.81	1.11	1.88
Musambi	T_2	1.41	0.94	2.09	2.52
Green grapes	T_3	1.06	1.60	2.05	2.15
Purple grapes	T_4	1.40	1.60	2.50	2.90
Lemon	T_5	9.56	12.51	13.36	16.05
Papaya	T_6	0.56	0.73	1.07	1.24
Mango	T_7	1.92	1.32	2.35	3.12
Apple	T_8	0.48	0.67	0.98	1.12
Pineapple	T_9	2.08	2.37	2.50	2.59
Pomegranate	T_{10}	1.20	1.41	1.96	2.22
	S.E.±	0.19	0.10	0.07	0.06
	C.D. (5%)	0.39	0.21	0.15	0.13

Table 3 : Identification of fungus in different fruit juices

Sample (fruit juices)	Name of fungi
Orange, Pomegranate	*Aspergillus niger, Aspergillus flavus, Mucor sp.*
Mosambi, Green grapes, Purple Grapes, Lemon, Mango, Apple, Pineapple	*Aspergillus niger, Aspergillus flavus*
Papaya	*Aspergillus niger, Aspergillus flavus, Rhizopus sp.*

per cent than treatment T_1. Treatment T_2 gave in significantly more acidity per cent than treatment T_3, T_4 and T_{10}.

In T_1(orange juice) the acidity began to increase in 0- 48 hours. Similar findings were reported by Wissanee (2007) where at 4^0C the orange juice titratable acidity began to increase in 2-6 days. The increase in TA (titratable acidity) indicated fermentation of orange juices. Mean value for titratable acidity of T_8 (apple juice) increased from (0.19 to 0.64 %) during keeping time period. Similar findings were reported by Zahid *et al*.(2008). Mean values for titratable acidity of apple juice increased from 0.34 to 0.53 per cent during storage. T_3 (green grapes),T_4 (purple grapes), T_5 (lemon), T_6 (papaya), T_9 (pineapple) and T_{10} (pomegranate) juices, their mean for titratable acidity regularly increased during the keeping time period, whereas, in T_2 (mosambi) and T_7(mango) juice, their titratable acidity decreased after 24 hrs but after that there was noted regularly increased of titratable acidity during the keeping time period.

The number of micro-organisms in fruit juices can affect the microbial quality of fresh fruit juices. A specific research related to this issue was done to quantify the transfer rates of microorganisms during an extraction process. *Aspergillus niger, Aspergillus flavus* and *species* of *Mucor* and *Rhizopus* were detected from fruit juices of different fruits (Table 3). It was found that about 1.7-2.6 per cent of total aerobic organisms and 2.3- 2.6 per cent of acid uric organisms from the washed fruits were introduced into the fruit juices during extraction process. The quality of fresh fruit juices is essentially depending on careful fruit handling and strict processing sanitation (Steven and Davis,2001).The result in the present study clearly indicated the poor hygienic conditions of these fruit juices and the consumers are at risk of contacting food borne infections.

Conclusion :

It has been concluded that pH of fruit juices decreased during storage. Keeping time period affects the titratable acidity of different fruit juices. Titratable acidity increased with increasing keeping time period. It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils, contaminated water and ice, poor personal and domestic hygiene, peeling of fruits before hand washing, dust particles in the premises. The practice of consuming fresh fruit and juices cannot be stopped on unhygienic grounds . people should avoid street fruit juices. Regular monitoring of the quality of fruit juices for human consumption must be introduced to avoid any future pathogenic outbreak.

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