Effect of Refrigeration on Microbial Load in Raw Fruits & Preserved Fruit Juices

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Abstract—*Consumption of fruits and vegetable products has increased tremendously in the last few decades. It has been calculated that about 20% of these products are lost every year due to spoilage. Recently there is surge in the consumption of fresh products stored in refrigeration. This work reports about the effect of refrigeration on fruits and fruit juices (Apple, Mausami, Guava, Pineapple and Pomegranate). Based on the microbiological analysis this paper highlights the striking difference between fresh and preserved fruits and its juices. Contamination was found to be caused by both bacteria and fungi. Among bacteria, both rods and cocci were observed. Species of fungus such as Aspergillus, Penicillium, Mucor and Rhizopus have shown predominance in the both raw fruits and fresh juices.*

It was found that among the fresh and preserved and unpreserved fruits, fresh fruits are better for consumption i.e. it shows less contamination. Among fresh and preserved fruit juices it was observed that preserved juices showed less contamination and can be stored longer than the fresh ones.

Keywords*: Microbial load, Fruit, Fruit juices, Refrigeration, Contamination*

1. INTRODUCTION

Fruit is one of the major components of healthy foods. It consists of water, sugar, fruit acids, vitamins and minerals. The main nutrients provided by fruits are vitamin C, carotenes, foliates carbohydrate and dietary fiber [1]. Fruits can be processed into various products, namely, fruits juices, fruit salads, wines etc, amongst which fruit juices are the most common product. Any fruit can be used to make fruit juice, but the most common ones include pineapple, orange, grapefruit, apple, mango, pomegranate and passion fruit [2]. Some juices, such as guava juice, are not filtered after extraction and are sold as fruit nectars. A wide range of drinks can be made using extracted fruit juice or fruit pulp as the base material. Many are drunk as a pure juice without the addition of any other ingredients, but some are diluted with sugar syrup. The types of drink made from fruit can be separated into two basic types: a)Those that are drunk straight after opening b) Those that are used little by little from bottles which are stored between uses. Juice is a liquid naturally contained in fruit tissue. Fruit juice consists of 85.4% moisture, 10.6% total sugars, 1.4% pectin, 0.1 g/100 ml total acidity (as citric acid), 0.7 mg/100 ml ascorbic acid, 19.6 mg/100 ml free amino nitrogen and 0.05 g/100 ml ash [3].

Juice is prepared by either mechanically squeezing or macerating fresh fruits without the application of heat or solvents. Juice may be prepared in the home from fresh fruits using variety of hand or electric juicers. Juice may be marketed in concentrate 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[4].

Fruits and their juices are good source of phytochemicals. The demand for lightly processed foods that preserve their fresh like qualities without compromising their safety has increased rapidly in the past few years [5].Owing to recent consumer preferences, impetus has been given to the development of concept-driven novel technologies that provide the required processing through non- or mildly thermal means[6]. Therefore, the food industry is expected to prevent or reduce negative changes in food quality over time to provide a wide variety of food rich in color, spoilage texture and flavor and to adapt and develop new food processes to satisfactorily meet the requirements of a wide demographic within different cultures [7].

The quality of the fruit and fruit juices could be adversely affected by both bacterial and fungal growth. Despite the high water activity of most fruits, the low pH leads to their spoilage being dominated by fungi, both yeasts and moulds but especially the latter. The most common pathogens encountered are *Aspergillus*, *Penicillium italicum, Penicillium expansum, Venturia inaequalis, Monilinia Fructigena* and *P. digitatum.*

Scientists at the University of Manitoba have found that some pesticides actually encourage the growth of life threatening bacteria on fruit. As a result, microbes like *Salmonella, E.coli* & *Shigella* could pose a threat to people eating raw fruit.

If fruit is contaminated due to any reason during any process of preservation, it produces some food borne diseases. The most commonly recognized food borne infections are those caused by the bacteria *Campylobacter, Salmonella, and E. coli* O157:H7, and by a group of viruses called calicivirus, also known as the Norwalk and Norwalk-like viruses.

There are reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere [8]. Sources of contamination however vary. Most Fruits contain bacterial counts up to 1.0×105 CFU/cm^2 on their surface.

Improper washing of fruits add these bacteria to extracts leading to contamination. In addition, use of unhygienic water for dilution, dressing with ice, prolonged preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* O157:H7, species of *Salmonella*, *Shigella* and *Staphylococcus aureus* [9]. Although the infectious dose for these contaminating bacteria in fruit juices is not yet well established, based on the standards provided for drinking water, the numbers required to cause illness could be low particularly with reference to faecal coliforms and streptococci.

The storage life of a commodity is drastically affected by the temperature and humidity of its surroundings. The most common and widely used method of storage is refrigeration. The refrigeration of fruits and vegetables retards respiratory heat generation, wilting due to moisture loss, and spoilage caused by the invasion of bacteria, fungi and yeasts. Refrigeration also retards undesirable growth or sprouting by the commodity itself [10].

This study was therefore carried out to compare the effect of refrigeration on the microbial load of fresh and preserved fruits and fruit juices.

2. MATERIALS AND METHODS

Samples

Fresh fruits Apple, Pomegranate, Orange, Guava, and Pineapple were obtained from the local fruit market of Delhi. To obtain fresh juices, these fruits were washed thoroughly with water and the juices were extracted by mechanical pressure. Each type of juice samples was filtered to remove pulp and seeds and stored in already labeled glass test tubes. Preserved juices of these fruits were obtained from the local grocery shop after their expiry dates checked.

Microbiological Analysis of Fresh and preserved Fruits

The fresh fruits were mashed to make a pulp.1 g of pulp was mixed with 10ml of sterile distilled water, and shaken vigorously for 2 min to make the stock solution. These suspensions were serially diluted in sterile distilled water. 100µl of the samples from the stock as well as from the dilutions were plated on nutrient agar medium [consisting of (g L^{-1}): peptone- 5; Beef extract- 3; NaCl 5; Agar – 20g]. The plates were incubated at 37°C for 24hr.The numbers of colonies were counted on the next day using a colony counter. For fungal analysis similar dilutions were plated on Potato Dextrose Agar plates [consisting of (g L^{-1}): Potato infusion – 200, Dextrose- 20, Agar- 20]. The plates were incubated at 28^{o}C for 4-5 days.

Four sets of fresh fruits were taken; two sets each were kept under preserved and unpreserved conditions. For preserved conditions the fruits were kept in a refrigerator at 4^{o}C. For the unpreserved conditions the fruits were kept at room temperature. On 8th and 15th days respectively, the samples were subjected to microbiological analysis by the process as discussed above.

Microbiological Analysis of Fresh Juices.

1 ml of the juice was taken and was serially diluted in sterile distilled water. 100µl of the samples from was taken from neat juice and from the dilutions 10^{-1}and 10^{-2} and plated on nutrient agar medium and PDA plates for the microbiological analysis. The juices samples were preserved in refrigerator at 4^{o}C. The microbiological analysis was performed by withdrawing the sample on day 3, day 5, day 7 and day 9 respectively.

Microbiological Analysis of Preserved Juices

Microbiological Analysis of Preserved Juices was performed in the above discussed procedure. The neat samples as well as diluted samples were used for the analysis. The sample was tested on day1, day 8 and day 15.

Identification of the Microbial Strains

Identification of the microbial strains was done on the basis of Gram's staining and Lacto phenol cotton blue staining technique.

Results

Microbiological Content of Fresh and Preserved Fruits

Table 1 represents the bacterial count of fresh, preserved and unpreserved fruits. In apple the bacterial count increased from 3.9x10^3 to 6.3x10^{14} in 15 days in preserved apple, while in unpreserved the count was 4.4x10^{18}. In mausami the bacterial count increased from 1.1 x 10^2 to 1.9 x 10^{14} in 15 days in preserved mausami, while in unpreserved the count was 5.6 x 10$^{18.}$ In guava the bacterial count increased from 3.7 x 10^3 to 4.8 x 10^{12} in 15 days in preserved guava, while in unpreserved the count was 5.2 x 10^{18}. In pineapple the bacterial count increased from 3.9 x 10^3 to 4.9 x 10^{10} in 15 days in preserved fruit, while in unpreserved the count was 5.0 x 10$^{18.}$ In pomegranate the bacterial count increased from 2.4 x 10^3 to 3.5 x 10^8 in 15 days in preserved pomegranate, while in unpreserved the count was 4.9 x 10$^{18.}$

Table 1: Bacterial count of fruits

SAMPLE	Day 1	Day 8	Day 15
Apple			
Fresh Fruit	3.9 x 10^{3}	-	-
Fruit Preserved		5.4 x 10^{3}	.3 x 10^{14}
Fruit Unpreserved		4.4 x 10^{5}	4.4 x 10^{18}
Mausami			
Fresh Fruit	1.1 x 10^{2}	-	-
Fruit Preserved		5.3 x 10^{2}	1.9 x 10^{14}
Fruit Unpreserved		4.6 x 10^{4}	5.6 x 10^{18}
Guava			
Fresh Fruit	3.7 x 10^{3}	-	-
Fruit Preserved		1.1 x 10^{5}	4.8 x 10^{12}
Fruit Unpreserved		4.9 x 10^{8}	5.2 x 10^{18}
Pineapple			
Fresh Fruit	3.9 x 10^{3}	-	-
Fruit Preserved		6.3 x 10^{5}	4.9 x 10^{10}
Fruit Unpreserved		4.1 x 10^{8}	5.0 x 10^{18}
Pomegranate			
Fresh Fruit	2.4 x 10^{3}	-	-
Fruit Preserved		5.6 x 10^{4}	3.5 x 10^{8}
Fruit Unpreserved		3.2 x 10^{5}	4.9 x 10^{18}

Microbiological Analysis of Fresh and Preserved Juices.

Table 2 represents the bacterial count of fresh fruit juice and preserved fruit juice. In apple fresh juice, the bacterial count increased from 1.3 x 10^{4} to 6.5 x 10^{4} in 7 days, while in preserved juice the count increased from 1.1 x 10^{2} to 4.2 x 10^{6} in 15 days. In mausami fresh juice the bacterial count increased from 5.1 x 10^{2} to 5.6 x 10^{3} in 7 days, while in preserved juice the count increased from NIL to 3.2 x 10^{5} in 15 days. In guava fresh juice the bacterial count increased from 5.5 x 10^{2} to 6.1 x 10^{6} in 7 days, while in preserved juice the count increased from 25 to 2.2 x 10^{4} in 15 days. In pineapple fresh juice the bacterial count increased from 2.8 x 10^{4} to 7.9 x 10^{4} in 7 days, while in preserved juice the count increased from 4.5 x 10^{1} to 2 x 10^{4} in 15 days. In pomegranate fresh juice the bacterial count decreased from 3.1 x 10^{3} to 1.3 x 10^{4} in 7 days, while in preserved juice the count increased from 1.5 x 10^{1} to 4.7 x 10^{2} in 15 days.

Table 2: Bacterial count of fruit juices

Sample	Day 1	Day 7	Day 15
Apple			
Fresh Juice	1.3 x 10^{4}	6.5 x 10^{4}	TNTC
Preserved Juice	1.1 x 10^{2}	-	4.2 x 10^{6}
Mausami			
Fresh Juice	5.1 x 10^{2}	5.6 x 10^{3}	TNTC
Preserved Juice	NIL	-	3.2 x 10^{5}
Guava			
Fresh Juice	5.5 x 10^{2}	6.1 x 10^{6}	TNTC
Preserved Juice	25	-	2.2 x 10^{4}
Pineapple			
Fresh Juice	2.8 x 10^{4}	7.9 x 10^{4}	TNTC
Preserved Juice	4.5 x 10^{1}	-	2 x 10^{4}
Pomegranate			
Fresh Juice	3.1 x 10^{3}	1.3 x 10^{4}	TNTC
Preserved Juice	1.5 x 10	-	4.7 x 10^{2}

Gram characterization of the strains found in the microbiological analysis of various samples showed the dominance of gram positive rod shaped bacteria (Table 3).

In apple only the gram positive rod shaped cells were observed.

In guava, gram positive rods and gram positive cocci were observed.

In the mausami, pineapple and pomegranate fruit samples, both gram positive rods and cocci were seen but rods were dominating in number.

Table 3: Colony and Gram characteristics of the microbial colonies

SAMPLE	NO OF STRAINS OBTAINED	GRAM CHARACTERISTICS
Apple Fruit	12	Gram postive rods
Mausmi Fruit	14	Gram postive rods and cocci
Guava fruit	5	Gram postive rods and cocci
Pine Apple Fruit	9	Gram postive rods and cocci
Pomegranate fruit	8	Gram postive rods and cocci

In fungal analysis of the samples various fungal strains were observed (Table 4):

In Apple, colonies of *Aspergillus* sp. were observed.

In Mausami, the colonies of *Penicilium* sp and *Mucor* sp were seen.

In Guava, the fungal colonies observed were of *Penicillium* sp, *Helminthosporium* and *Mucor* sp.

In Pineapple, *Aspergillus* sp. and *Rhizopus* sp. were observed.

In Pomegranate, the colonies of *Penicillium* sp and *Mucor* sp were observed.

Table 4: Characteristics of the Fungal Colonies found in the Fresh and Preserved Fruits and Juices

Fruit	Fruit/Fruit juice	Fungal species found
Apple	Fresh Fruit	No colonies
	Unpreserved Fruit	*Aspergillus* sp

	Preserved Fruit	No colonies
	Preserved Juice	No colonies
Mausami	Fresh Fruit	No colonies
	Unpreserved Fruit	No colonies
	Preserved Fruit	*Penicillium* sp
	Preserved Juice	*Penicillium* sp *Mucor* sp
Guava	Fresh Fruit	No colonies
	Unpreserved Fruit	*Penicillium* sp *Helminthosporium* sp *Mucor* sp
	Preserved Fruit	*Penicillium* sp
	Preserved Juice	No colonies
Pineapple	Fresh Fruit	No colonies
	Unpreserved Fruit	*Aspergillus* sp
	Preserved Fruit	*Rhizopus* sp
	Preserved Juice	*Aspergillus* sp
Pomegranate	Fresh Fruit	No colonies
	Unpreserved Fruit	*Penicillium* sp
	Preserved Fruit	No colonies
	Preserved Juice	*Mucor* sp

The fungal analysis of the samples also revealed the following results

Apple: Fungal growth was seen only in unpreserved fruit, no colonies were seen in other samples of fruits & fruit juices.

Mausami: Fungal contamination was seen in preserved fruit & preserved fruit juice, no colonies were seen in other samples of fruits and fruit juices.

Guava: Fungus had grown only in preserved and unpreserved fruit, no colonies were seen in other samples of fruits and fruit juices.

Pineapple: Fungi was observed in preserved fruit, unpreserved fruit and preserved juice, no colonies were seen in other samples of fruits & fruit juices.

Pomegranate: Fungi were seen in unpreserved fruit and preserved juice, no colonies were seen in other samples of fruits & fruit juices.

3. DISCUSSION

In spite of the potential benefits offered by fruit juices, concerns over their safety and quality have been raised. Freshly squeezed fruit and vegetable juices have little or no process that reduces pathogen levels, if contaminated (Mahale et.al, 2008).

In the present investigation it was found that in the fresh fruits, highest contamination was seen in Apple (3.9x10^{3}**)** and pineapple and lowest in Mausami (1.1x10^{2}) on day1. This shows that among all fresh fruits taken for the study, Mausami shows least growth of microorganisms when it is freshly consumed.

Contamination in Fresh fruits can be ranked as:

Mausami < Pomegranate < Guava < Apple < Pineapple

In preserved fruits highest contamination was seen in Apple (6.3x10^{14}) & lowest in Pomegranate (3.5x10^{8}) on day15. So we can conclude that among all fruits taken for the study, Pomegranate can be preserved for longer time span without the chances of greater microbial growth.

Contamination in Preserved fruits can be ranked as:

Pomegranate < Pineapple < Guava < Mausami < Apple

In unpreserved fruits highest contamination was seen in Mausami (5.6x10^{18}) & lowest in Apple (4.4x10^{18}) on day15. So we see when the fruits are kept unpreserved, the mausami catches more contamination than others.

Contamination in Unpreserved fruits can be ranked as:

Apple < Pomegranate < Pineapple < Guava < Mausami

It was observed that bacterial contamination on day1 was seen only in fresh fruits. In all other fruit samples (preserved and unpreserved) used, there was no growth seen on the 1st day. When results of preserved and unpreserved fruits were analyzed it was seen that preservation reduces the bacterial growth to a great extent. On 15th day, it was found that in all the fruits samples, bacterial growth was much more in unpreserved as compared to the preserved ones.

When the results of fruit juices was analyzed it was observed that in Fresh fruit juices, highest contamination was seen in Guava (6.1x 10^{6}) & lowest in Mausami (5.6x10^{3}) on day7.

Contamination in Fresh fruit juices can be ranked as:

Mausami <Pomegranate < Apple < Pineapple < Guava

In Preserved fruit juices highest contamination was seen in Apple (4.2x10^{6}) and lowest in Pomegranate (4.7x10^{2}) on day15.

Contamination in preserved fruit juices can be ranked as:

Pomegranate < Pineapple < Guava < Mausami < Apple

It was observed that among fresh and preserved juices, as predicted, preserved ones gave better results i.e. fresh juices got contaminated earlier than preserved juices.

When the colony characteristics of the microbial stains were studied, all the fruits and their juices chosen for the study, showed the occurrence of different colonies of rod shaped (Bacilli) and cocci bacteria. Both the two types of bacteria namely Bacilli and Cocci were seen in all the fruits (Mausami, Guava, Pineapple, and Pomegranate) except apple in which only rods were seen.

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