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Studies on the Microbial Spoilage in Fruit Juice and Enhancing the Shelf Life using Bio-preservatives

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A B S T R A C T

A fruit juice contains essential nutrient, mineral, antioxidant and vitamins for overall health. However, food borne illness related to fruit and fruit product is increasing. So, the main objective of this study was to assess the bacteriological quality of fresh fruit juices available for the consumers in Thuraiyur town. The study was conducted from May 2024 to September 2024. A total of 20 samples was purchased from cafeteria, restaurants and supermarkets which consisted of 20 fresh juice samples 5 each of Apple, Pomegranate, Mango, and pineapple whereas from the total samples. Also, detection of pathogens and antimicrobial susceptibility testing was conducted. All fresh fruit juice samples were found to harbor TVC, TCC, and TSC within the range between 2.95 ± 0.52-5.91 ± 0.52, 0.30 ± 0.10-4.65 ± 0.44 and 1.00 ± 0.15-2.86 ± 0.33 $\log_{10}$ cfu/ml, respectively. In this study the prevalence of *E. coli*, *Salmonella* and *Staphylococcus aureus* was detected for all fresh fruit juices samples of this Pomegranate was more dominated. Antibiotic susceptibility test for *E. coli*, *Salmonella* isolates and *Staphylococcus aureus* revealed completely resistant (100%) to a Biopreservatives (Ginger, Honey and Lactic acid bacteria (LAB). In general the study, especially exhibits the level of bacterial load found in fresh juice samples was unsatisfactory compared to gulf standards. This cause health problems and possible vehicle of foodborne outbreaks to the community. Therefore, good quality of water used; hygienic conditions related to washing of utensils, good personal and domestic hygiene during fresh fruit juice preparation can improve the bacterial quality and safety of the finished product.

Introduction

Fruit juices are very common among the people of all ages everywhere in the world. Fruit juices are flesh flavor which provides health benefit and are well consumed for their nutritive value, mineral and vitamin content. However, fruit juices by their nature contain various organisms and many of these microorganisms will be harmless bacteria such as saprophytic Rahman *et al.,* (2011). One possible source can be damaged surfaces, such as perforations, wounds, cuts and splits that occur during growing or harvesting through which pathogenic

organisms can enter fruits Tambekar *et al.,* (2009). The disease agents spread by juice like drink not only harm large groups of people but also sometimes result in serious disability and death Rahman *et al.,* (2011).

The practice of consuming fruit and vegetable juices cannot be stopped on unhygienic grounds or prohibited from selling such items, since it is a source of their livelihood Tambekar *et al.,* (2009) and Olaniyi *et al.,* (2013). The total viable bacterial count in most of the fresh juice samples was higher than the commercially packed juice Rahman *et al.,* (2011). Many researchers believe that consumption of commercially packed juice is safe than the locally produced fresh fruit juice Rashed *et al.,* (2013) and Kader *et al.,* (2014). This might be the reason of using mechanized machine and also some preservatives during fruit juice processing. The juices comprise primarily water, sugar, preservatives, color, fruit pulps and other additives. But some preservatives of higher concentrations can be dangerous for our health. In spite of all these problems, enormous number of coliforms and *staphylococcus* count were identified from commercially packed fruit juices Kader *et al.,* (2014). Food-borne disease is usually caused by certain bacteria or their toxins, which are poisonous proteins produced by these bacteria. Contamination of juices with pathogenic microorganisms has caused various illness and even some fatalities Cody (1999).

There are generalized opinions among investigators that, the most common food borne pathogenic bacteria are Bacillus cereus, Clostridium botulinum, *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Streptococcus pyogenes*, *Mycobacterium bovis*, *Listeria monocytogenes*, *Klebsiella* spp., *Enterobacter* spp., *Staphylococcus* spp. Tambekar *et al.,* (2009); Rashed *et al.,* (2013) and Reddy *et al.,* (2009). In Thuraiyur town, there is always a great demand for both fresh and packed fruit juices as the climate remains hot and humid for most part of the year. So, the main objective of this study was designed to evaluate the bacteriological load of fresh fruit juices existing in Thuraiyur town for the customers.

Materials and Methods

Sampling technique and source of sample

A random sampling technique was used to take the representative of fruit juice. Locally prepared fresh fruit juices of sample were purchased from Restaurants and cafeteria from Thuraiyur. Commercially prepared packed fruit juice of sample where purchased from super market.

Collection of Samples

The study was conducted from May 2024 to September 2024. A total of 20 samples was purchased from cafeteria, restaurants and supermarkets which consisted of 20 fresh juice samples 5 each of Apple, Pomegranate, Mango, and pineapple whereas from the total samples, another 20 commercially packed juices viz., Apple, Pomegranate, Mango, and pineapple were collected from supermarkets. Also, detection of pathogens and antimicrobial susceptibility testing was conducted

Sample Processing Method

Thirty milliliter (30 ml) of fruit juice were separately drawn and blended in 200 ml of physiological saline solutions (0.85% NaCl). The serial dilution was done for fresh fruit juice (Apple, Pomegranate, Mango, and pineapple) and packed fruit juice (Apple, Pomegranate, Mango, and pineapple).

The samples were homogenized and appropriate dilutions (10^{-1} up to 10^{-6}) were performed for each fresh fruit and packed juice samples. Appropriate dilutions of the sample were plated in triplicate on the solid media for bacteriological count.

The actual numbers of bacteriological colony count were estimated as colony forming unit per ml (cfu/ml) by using the following formula.

Number of bacteria colony in original sample = Number of colony counted x 1/*df* x V (ml)

Where: df, Dilution factor; V, Volume in ml

Bacteriological analysis

Total viable count (TVC)

Triplicate plate of nutrient agar was inoculated with 1 ml of diluted solution by using spread technique. All plates were incubated at temperature of 37ºC for 24 hrs. The temperature was chosen to differentiate the mesophylls which constitute most medically important pathogenic bacteria. The colony developed on the plate was counted from incubated plate.

Total coliform count (TCC)

From each samples of previously serial dilution, 1 ml was transferred on MacConkey agar (MCA). Then the plate was incubated at 37°C for 24-48 hrs. Purple red colonies surrounded by zone of precipitated bile were counted by using digital colony counter.

Total staphylococcus count (SCC)

From each samples of previously serial dilution, 1 ml was transferred in to Mannitol salt agar (MSA) and it was incubated at 37ºC for 24-48 hrs, yellow and orange colonies surrounded by yellow zone due to mannitol fermentation was counted.

Estimation of bacterial load was performed by Gulf standard method known as the recommended microbiological standard for fruit juices for all bacteriological analysis Cheung *et al.,* (2007) and Gulf Standards (2000).

Microbial Characterization

For microbial characterization, 10-15 colonies with different morphology and color were picked randomly from countable plate and were purified by repeated plating and characterized to the genus and species level using the following tests like Gram's reaction, urease test, catalase test, oxidase test, indole test, nitrate reduction, citrate test, H_2S test and VP test.

Detection of microbial pathogens

Salmonella

For detection of *Salmonella*, 25 ml juice samples were added to 200 ml buffered peptone water, vigorously shaken and the suspension was incubated at 37ºC for 24 hrs for metabolic recovery and proliferation of cells. From this, 1 ml of culture was transferred into separate tubes each containing 10 ml of Selenite Cystein Broth.

The broth was incubated at 37 ºC for 24 hrs. After secondary enrichment, culture from enrichment broth was separately streaked on plates of Xylose Lysine Desoxycholate (XLD) (Oxoid) medium. Pink colonies with or without black centers from selective medium was picked, purified and tested biochemically Cheung *et al.,* (2007).

Escherichia coli

Some pathogenic bacteria such as *E. coli* were detected according to the procedures outlined by Food and Drug Administration (FDA, 2001).

Staphylococcus aureus

For detection of *S. aureus*, golden yellow colonies from Mannitol Salt Agar (MSA) during staphylococci count were picked, purified and preserved. Coagulase test was done by two ways: slide coagulase test and tube coagulase test, Cheesbrough (2006).

Antimicrobial Susceptibility testing

From the total of 20 fresh fruit juice samples 5 of each Apple, Pomegranate, Mango, and pineapple, around 60 bacteria isolates of *Escherichia coli*, *Salmonella* isolates and *Staphylococcus aureus* were isolated. Out of the total isolates, 12 *Escherichia coli*, 10 *Salmonella* isolates and 15 *Staphylococcus aureus* isolates were subjected to antibacterial sensitivity testing. In vitro test was used to confirm susceptibility of isolates to chosen antimicrobial agents by means of a disc diffusion method on Mueller-Hinton Agar (Difco) Bauer *et al.,* (1966).

Briefly, a single colony of each isolate was introduced into 2 ml of Mueller-Hinton broth, incubated for 4 hours, and the culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and were spread evenly over the entire agar surface.

The suitable seeded medium was prepared, and poured in sterile petriplates and then cork borer is used to form the well (1 cm diameter). The extract is prepared in different concentration ginger, honey and LAB.

The extract is poured into the well. Plates were incubated for 16-24 hour at 35ºC. The diameters of zone of inhibition were measured to the nearest whole millimeter using the transparent rule interpreted as susceptible, intermediate and resistant.

Results and Discussion

Fresh fruit juices are well consumed by consumer for their fresh flavor, vitamins content and nutritive value. Also in Thuraiyur town, prepared fresh fruit juice is

becoming more and more popular as they are usually delicious, flavor, cheapest and easily available than packed fruit juice in the study area. But many fresh fruit juice are easily contaminated during the preparation and has many hazardous effects on health of human being. Several bacteria, for example coliform, *salmonella*, *shigella* and *staphylococcus* species are representing the pathogenicity of fruit juice.

After overnight incubation, distinctive morphological characteristics like pink, circular, convex colonies on MacConkey Agar and yellow colonies on Mannitol Salt Agar and biochemical tests were recorded which indicates the presence of coliforms, *Salmonella* and *Staphylococcus* species.

The fruit Juices may be contaminated with microbes from raw material, juice machince, handler and unhygienic conditions. Pathogenic microbes may enter in fruits during their growth and harvesting through damaged surfaces and punctures.

The main microbial contamination in fruit juices are bacteria like *E.coli, Staphylococcus aureus, Salmonella, Bacillus cereus* and molds like *Aspergillus flavus, Aspergillus niger*

Examination of (TVC, TCC, TSC) Microbial Load in Fruit Juice Sample (log10cfu/ml)

Total viable count (TVC) is a measure of microbial quality of fruit juices. Presence of microbes in high numbers (TVC>4 log 10 CFU/ml) is responsible for fruit juices (Gulf standard, 2000) and Codex standards (2005).

In the current study, all the Four Fresh Fruit juice Samples (Apple, Pomegranate, Mango, Pineapple) are collected and enumerated for their Total Viable Count (TVC), Total Coliform Count (TCC) and Total Staphylococcus Count (TSC) was summarized in Table 2. Accordingly, the Total Viable Count (TVC) of pomegranate juice was highest 5.91 ± 0.52(log10 cfu/ml). Whereas the Total Viable Count of Apple, pineapple was 5.32±0.49 and 3.08±0.65 (log10 cfu/ml) respectively.

The lowest 2.95±0.52 (log10 cfu/ml) TVC was observed in Mango juice Sample. Likewise the Total Coliform Count (TCC) of pomegranate juice was highest 4.65 ±0.44(log10 cfu/ml) Whereas the Total Coliform Count (TCC) of Apple and Mango were 2.59±0.42 and 0.60±0.35 (log10 cfu/ml) respectively. The lowest 0.30±0.10 (log10 cfu/ml) TCC was observed in pineapple juice. Regarding the Total Staphylococcus Count (TSC) the highest 2.86±0.29(log10 cfu/ml) bacterial load was counter in pomegranate juice Sample. On the other hand, the lowest TSC was recorded in the case of pineapple juice 1.00 ±0.15 (log10 cfu/ml) fruit juice.

Morphological character and biochemical test of detected pathogens from fruit juice samples

The Morphological, Microscopic and Biochemical Characters of *E.coli, Salmonella* and *Staphylococcus aureus* strains were Studied based on the particulars of Bergey's Manual of Determinative Bacteriology and the results are presented in Table 3. *E.coli* were Characterized as Mucoid as the Colour of the colony, Gram negative, Rod shape, Circular Configuration with Entire Margin and Slightly Raised Elevation. *Salmonella* were Characterized as pale yellow colour colony, gram negative, rod shape, Circular Configuration with Entire Margin and convex Elevation. *Staphylococcus aureus* were Characterized as yellow colour colony, gram positive, cocci in bunch of colony, circular configuration with Entire Margin and convex Elevation. Biochemical Test such as Nitrate Reduction test, Urease Test, Catalase Test, Oxidase Test, Indole Test, Citrate Test, H2s Test and Voges-Proskauer (VP) Test were carried out and confirmed as *E.coli, Salmonella* and *Staphylococcus aureus.*

Effect of *Lactobacillus acidophillus* against food borne pathogens

According to Mohammed *et al.,* (2007), The Anti microbial compounds (lactic acid, diacetyl and hydrogen peroxide) produced by lactic acid bacteria (LAB) have been proven to have great anti microbial effect against fruit juice spoilage organisms without any adverse on the consumers. In the current study the effect of *Lactobacillus acidophillus* on the inhibition of growth of *E.coli*, *Salmonella* and *Staphylococcus aureus* was studied in agar well diffusion method and results are presented on Table 04. The inhibition zone of the food borne bacterial strains increases with increase in the quantity of culture filtrate from 0.2 to 1ml/well. The higher inhibition zone (8.95, 8.20, 8.00 mm) at 1.00 ml and lower inhibition zone (2.80, 2.66, 2.00 mm) at 0.2 ml were recorded from *S.aureus*, *E.coli* and *Salmonella* respectively. No inhibition zone was observed in control.

Table.1 List of Microorganisms Responsible For Spoilage of Fruit Juice

S.No	Bacteria	Mold
1	*E. coli*	*Aspergillus flavus*
2	*Staphylococcus aureus*	*Aspergillus niger*
3	Salmonella	
4	*Bacillus cereus*	

Table.2 Examination of (TVC, TCC, TSC) Microbial Load in Fruit Juice Sample (Log10cfu/ml)

S.No	Types of fruit juice (sample)	TVC (log10cfu/ml) mean ±SD	TCC (log10 cfu/ml) mean ±SD	TSC (log10 cfu/ml) mean ±SD
1	Apple	5.32 ± 0.49	2.59 ± 0.42	2.08 ± 0.29
2	Pomegranate	5.91 ± 0.52	4.65 ± 0.44	2.86 ± 0.33
3	Mango	2.95 ± 0.52	0.60 ± 0.35	1.30 ± 0.56
4	Pineapple	3.08 ± 0.65	0.30 ± 0.10	1.00 ± 0.15

TVC - Total Viable Count, TCC -Total Coliform Count and TSS - Total Staphylococcus Count

Table.3 Morphological character and biochemical test of detected pathogens from fruit juice samples

S.No	Morphological character & Biochemical test	*E. coli*	*Salmonella*	*S.aureus*
1	Colony of the colony	Mucoid	Pale yellow	Yellow
2	Gram's reaction	**-**	**-**	**+**
3	Shape of the isolate	Rod	Rod	Cocci in bunch
4	Configuration	Circular	Circular	Circular
5	Margin	Entire	Entire	Entire
6	Elevation	Slightly raised	Convex	Convex
7	Nitrate reduction test	+	+	-
8	Urease test	+	+	+
9	Catalase test	+	+	+
10	Oxidase test	-	+	-
11	Indole test	+	-	-
12	Citrate test	-	-	-
13	H2S test	-	+	-
14	VP test	-	-	-

+, positive; -, Negative

Table.4 Effect of *Lactobacillus acidophillus* against food borne pathogens

Lactobacillus acidophillus culture filtrate (ml/well)	Inhibition zone mm in (well diffusion assay method)		
	E. coli	*Salmonella*	*S.aureus*
0(control)	-	-	-
0.1	2.66	2.00	2.80
0.5	4.10	4.00	4.50
1.0	8.20	8.00	8.95

Table.5 Effect of Honey against Food Borne Pathogens

Honey (ml/well)	Inhibition zone mm in(well diffusion assay method)		
	E. coli	***Salmonella***	***S.aureus***
0(control)	-	-	-
0.1	1.15	1.00	1.56
0.5	3.16	3.00	3.96
1.0	6.00	6.10	6.98

Table.6 Effect of Ginger Extract against Food Borne Pathogens

Ginger Extract (ml/well)	Inhibition zone mm in(Well diffusion assay method)		
	E. coli	***Salmonella***	***S.aureus***
0(control)	-	-	-
0.1	5.10	4.30	5.65
0.5	8.50	8.00	9.96
1.0	12.10	10.10	13.50

Effect of Honey against Food Borne Pathogens

Honey has very good inhibitory potential against *E.coli, Staphylococcus aureus, Salmonella*. Various factors are responsible for variation of antimicrobial potency among the different honeys that include its seasonal, geographical and botanical source as well as harvesting, processing and storage conditions.

In our study the effect of bio preservative honey on the inhibition of growth of *E.coli, Salmonella,* and *Staphylococcus aureus* was studied in agar well diffusion method and result was presented in Table 5. The inhibition zone of the food borne bacterial strains increase with increase in the quantity from 0.2 to 1 ml/well. The higher inhibition zone (6.98, 6.10, 6.00 mm) at 1ml and lower inhibition zone (1.56, 1.15, 1.00 mm) at 0.2ml were recorded for *Staphylococcus aureus, E.coli* and *Salmonella* respectively. No inhibition zone was observed in control.

Effect of ginger extract against food borne pathogens

Ginger can preserve foods up to 4 months at room temperature (25℃), also discovered that aqueous extract of ginger caused an increase in the protein and mineral content thereby reducing the microbial growth on juice samples. In our study the effect of plant extract (Ginger) on the inhibition of growth of *E.coli, Salmonella* and *Staphylococcus aureus* was studied in agar well diffusion method and result are presented in Table 6. The inhibition zone of the food bore bacterial strains increases with increase in the quantity from 0.2 to 1 ml/well. The higher inhibition zone (13.50, 12.10, 10.10 mm) at 1 ml and lower inhibition zone (5.65, 5.10, 4.30 mm) at 0.2 ml were recorded for *Staphylococcus aureus, E.coli* and *Salmonella* respectively. No inhibition zone was observed in control.

The overall assessment of the fresh fruit juice samples analyzed bacteriologically indicated high count and highly contaminated as showed in all analysis, total viable count, total coliform count and total *Staphylococcus* count.

These high counts, however, may pose hazard to the health of consumers especially in the current study pathogenic species like *E. coli*, *Salmonella* isolates and *Staphylococcus aureus* are present in the fresh fruit juices to be consumed by the community of the study area. This contamination is due to poor quality of water used; unhygienic conditions related to washing of utensils, poor personal and domestic hygiene during fresh fruit juice preparation.

In this study has shown that Ginger (*Zingiba officinale*) has some anti-oxidative and anti-microbial effects which can extend the shelf life of fruit juice samples. Samples treated with higher concentrations of ginger inhibited the growth of bacteria and also increased the shelf-life of the fruit juice. Ginger has shown excellent antibacterial

properties and effective in controlling virus, bacterial and fungal diseases. In many countries, gingers have been used to preserve foods. In addition, 2% ginger and honey was most effective in the preservation of fruit juice and taste enhancement and no side effects to human health.

Author Contributions

P. Elamparithi: Investigation, formal analysis, writing—original draft. A. Hemapriya: Validation, methodology, writing—reviewing. G. Hemasruthi:—Formal analysis, writing—review and editing. G. Pooja: Investigation, writing—reviewing. V. Priyadharshini: Resources, investigation writing—reviewing. K. Sundarasan: Validation, formal analysis, writing—reviewing. S. Sakthi Pooja: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. V. Poovizhi: Investigation, formal analysis, writing—original draft. K. Prabhavadhani: Validation, methodology, writing—reviewing. M. Ilakkiya:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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