Isolation and Enumeration of Bacteria from Packed Mango Juice

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Abstract: - The present study was isolated the bacteria from packed mango juice. Mango juice has always been considered as a delicious, nutritious popular drink, but processed juice may not always be safe due to chemical and microbial risks. Determination of physicochemical and microbiological qualities of some packed mango juices of Coimbatore will help consumers to know the present scenario. Material and Methods of commercially available Mango juice sample were collected from the market. Carbohydrate profiles were determined using HPLC, crude protein content was calculated using the Kjeldahl method and other parameters were determined by standard AOAC methods. Standard culture techniques were followed to assess the total viable count (TVC), E. coli and other fecal coliforms. Results the highest quantity of monosaccharide (9.867) was recorded. Conclusion that the locally available mango juices contain a safe level of nutritional and microbial elements for human consumption, but not highly satisfactory. In this study, antimicrobial susceptibility test was done from collected isolates from packed Mango juice sample .this study specially highlights the level of microbial loads found in packed mango juice sample.

Key Words: — *Fruit juices, packaged drinks, Isolation and enumeration, antimicrobial susceptibility.*

I. Introduction

A Structural part of a plant is Fruit. Fruits contain various photochemical compounds and high percentage of water averaging 80% also in small amount of fat; protein and carbohydrate are present. Fruit juice are part of our daily consumption. All over the world in everyone's diet chart it is always included as a variety of any fruit juices, beverages, energy drinks etc., Fruit juices are well recognized for their nutritive value, mineral, vitamins and antioxidant contents that are essential for human beings[1]. They contain large amounts of antioxidants, vitamins C and E, and possess pleasant taste and aroma.

Most fruits contain bacterial counts up to 1.0 × 10^5 cm^2 on their surface.

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1.1 Mango

Mango and its juice, commonly known as "Aam Ras" contain many vital vitamins and minerals that are essential for a healthy human body. Mangoes are a powerhouse of vitamin C, beta carotene, potassium, iron and many other nutrients which help in fighting infections, as well as maintaining overall well-being of the body.[2]

Mango juice is high in antioxidants. It is packed with polyphenols. It has over a dozen of different mangiferin, catechins,anthocyanine,quercetin,kaempferol,rhamnetin,benzoic acid and many others. It is a good source of immune-boosting nutrients. It have several qualities that make it excellent for

digestive health. For one, it contains a group of digestive enzymes called amylase.

II. REVIEW OF LITERATURE

2.1 Sample Collection

Sample, which are most popular and available all over the country, of commercially packed mango juice, were collected from the cafeteria (kalapatti area) in 17 March 2021. The most common food-borne pathogenic bacteria, associated with mango juice, are Bacillus cereus, Escherichia coli, Salmonella spp., Staphylococcus aureus etc.

Penicillium, Byssochlamys, Aspergillus, Paecilomyces, Mucor, Cladosporium, Fusarium, Botrytis, Talaromyces, and Neosartorya are filamentous fungi most frequently isolated from fresh fruits and juices. Among bacteria, lactic acid bacteria and acetic acid bacteria have been isolated from fruit juices'[3]

2.2 Aim of the Study

The aim of the project is to check microbial quality in commercially packed juice. It from the sample growth of different microorganisms have been found following steps will be done;

- Isolation of microorganisms from juice sample available in Kalapatti area.
- Isolates will be identified with different Biochemical test

To detect the antibiotic resistance Pattern of the bacterial isolates found in juice.

III. MATERIALS AND METHODS

MATERIALS	MEDIA
Petri dishes	Nutrient agar
Microscope	SS Agar
Colony counter	Urea Agar
Inoculating loop	Triple sugar iron Agar
Test tubes	Simmons citrate Agar
Bunsen burner	Baird Parker Agar
Measuring cylinder	Bacillus cereus Agar
Autoclave	
Flasks	
Water bath	

Physiochemical test method (PTH):

Sample were collected in sterile containers, kept in the icebox, maintained at 4ºC during transportation to the microbiological laboratory and analyzed within 1 to 2 hours. The pH value was measured with a pH meter.

Total Soluble solids:

Total soluble solids, primarily sucrose, fructose and glucose, were measured minerals in the juice also contributed to the soluble solids. Brix is reported as "degrees Brix" and is equivalent to a percentage. For example, a juice measured as 12 degrees Brix has 12% total soluble solids.

Determining solubility:

Citric acid and a small amount of malic and tartaric acid were added in juice for its tartness and unique taste. The amount of acid present in the juice was reported as the percent citric acid. A titration with sodium hydroxide was used to calculate the value.

Kjeldahl Method to Determine protein:

Juice was digested with a strong acid (H2SO4) so that it released nitrogen, which could be determined by a suitable titration technique. The amount of protein present was calculated from the nitrogen concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used for applications. This was only an average value and each protein has a different conversion factor depending on its amino-acid composition. The Kjeldahl method can conveniently be divided into three steps: digestion,

neutralization and titration. Anhydrous sodium sulfate and a catalyst (copper) were introduced to alleviate the boiling point of the medium (from 337°C to 373°C). The initially very dark-colored medium had become clear and colorless to indicate that the chemical degradation of the sample was completed.

Fat content determination:

The fat content of the sample was determined as free and total fat. Free fat was extracted from the lyophilized sample by Soxhlet using ether as a solvent. The total fat content was determined by the acid-hydrolysis method [27]. Samples (1.5 g) were digested with dilute hydrochloric acid (5 ml) for about 45 minutes in a water bath. The mixture obtained was then extracted with a combination of solvents comprising of methanol (2.5 ml), diethyl ether (7.5 ml) and petroleum ether (7.5 ml). Thereafter, the mixture was centrifuged, ether–fat layer was decanted and evaporated and the fat content was measured.

Total Reducing Sugar:

A number of chemical methods were used to determine monosaccharides and oligosaccharides which are based on the fact that many of these substances are reducing agents. Those reducing agents can react with other components to yield precipitates or colored complexes, which could be quantified. Acidity was measured according to the AOAC method and expressed in g/L as citric acid.

Carbohydrates Profile chromatographic Methods:

HPLC is commonly used to separate and identify carbohydrates because of rapid, specific, sensitive and precise measurements. Sugar content tests were performed using high-pressure liquid chromatography [HPLC Model# CTO20A, Shimadzu, Japan]. The solution (2% w/v) was prepared by the doubled refined distilled and sonicated (10 minutes) water. The sample was injected into the 20 µl column (solid phase). The separation was conducted at 80°C with the mobile phase water at 1.3 ml/min flow rate. The identification of monosaccharide was done by comparing the retention times of individual sugars in the reference vs. standard solution. The quantitative assays were made of the carbohydrates as fructose, glucose, sucrose, maltose, malt triose and maltotetraose. The contents of those compounds were assayed based on the comparing peak areas obtained in the examined samples with those from the standard. To make the presentation of the obtained results more comprehensive, the following was calculated: total sugars, fructose to glucose ratio and total monosaccharides. The calculation was done by the Lab Solution.[4]

Bacterial Analysis of Fruit Juice:

Microbiological analysis was done using appropriate media designed enumeration and identification of different microbial groups following standard procedures. The total colony count was done by spread plate method using plate count agar for bacteria. Fecal coliform Counts (FCC) were determined using the most probable number (MPN) method.

3.1 Enumeration of bacterial Isolates

Staphylococci count:

Enumeration of Staphylococci was done using Mannitol Salt Agar (MSA) in four replicates following standard methods and procedures. From appropriate dilutions 0.1 mL of sample fruit juices were spread plated on MSA and then incubated at 30°C for 24–36 h. Then, each plate was observed after 24–30 h of growth and presumptive colonies were counted for confirmation of S. aureus, coagulase test was performed. To do this, inoculum from each presumptive colony of MSA plate was transferred to a separate tube of Brain Heart Infusion (BHI) broth and incubated at 35°C for 18–24 h under aerobic condition. Then, 0.2 mL of BHI broth culture was transferred into sterile 13 × 100 mm tubes containing 0.5 mL certified coagulase plasma and mixed thoroughly. The mixture was incubated at 35°C and examined after 1 h and 4 h. A firm clot, which did not move when the tube was tipped on its side (coagulase reaction), was considered a positive test for S. aureus. If no clot was observed, it was considered as a negative test for S. aureus. Gram staining was also done for confirmation of S. aureus by preparing smears from the deep yellow opaque colonies.

Total Coliform Count:

The three-tube procedure using lactose broth in three replicates was used to detect the coliform and determine the MPN of coliform. The MPN method was used as a combination of presumptive, confirmed, and complete incubated for 48 ± 3 h at 35 ± 0.5°C and examined for gas formation. Formation of gas in an amount in the inverted vial at any time within 48 ± 3 h was recorded as a positive confirmed test. Negative test tubes were re-incubated for additional 24 hour Number of positive tubes for each dilution was recorded. Tubes showing positive results were streak plated on eosin tests.1 mL of each of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions was inoculated into three test tubes of LB each containing Durham's tube. After incubating

for 24 h, the number of tubes in each set of three that showed positive for acid and gas production was recorded. Negative test tubes were re-incubated for additional 24 hour. Each presumptive positive tube of lactose broth was gently swirled and a loopful of each positive culture was transferred to tubes of brilliant green lactose bile 2% broth (BGLBB) using a sterile inoculating loop. Inoculated BGLBB tubes were eosin methylene blue (EMB) agar and incubated at 37°C for 48 h. From each EMB agar plate a typical coliform colony Pink to dark red with a green metallic surface sheet) was transferred to a tube containing lactose broth and nutrient Agar slant, incubated at 35 ± 0.5°C for 48 ± 3 hour. Calculation of MPN was done from the completed test results using the formula employed by Thomas.

MPN = P/TN

Where: P = the number of positive tubes

T = Total quantity of sample in all tubes in mL

N = Total quantity of sample in negative tubes in ml.

Fecal Coliform Count:

Fecal coliform were obtained by MPN Technique.1 ml of each of the 10-3,10-4, and 10-5 dilution was inoculated into three test tubes of LB with Durham's tube and incubated at 37°C for 48 hr. .Each presumptive positive tube of lactose broth was gently swirled and a loopful of each positive culture was transferred to tubes of EC broth using a sterile inoculating loop. Inoculated EC broth tubes were incubated at 48 hour at 45+/- 0.5°C in water bath. Gas production in an EC broth culture was considered as a positive fecal coliform reaction .Only tubes which were positive in the EC medium within 24 hr. , were used in the calculation of fecal coliform. The presence of fecal coliform was confirmed by streaking from positive EC broth cultures on EMB Agar plates. Bacterial colonies developed were considered as fecal coliform[5].

Antibacterial Susceptibility Test:

All isolates of pathogenic bacteria were tested for their sensitivity to antibiotics by means of the dis diffusion method on Muller-Hinton Agar (Disco, Detroit, MI) as described previously by Bauer et al. All disks used in the disk diffusion test were obtained from BECTON, USA in the following concentrations : Ciprofloxacin (5 mu g), Ceftriaxone (30 mu g), Gentamicin (10 mu g), Cotrimoxazole (25 mu g), Erythromycin (15 mu g), moxclavul acid (30 mu g),Ampicillin (10 mu g), Chloramphenicol (30 mu g), and Tetracycline (25 mu g).Briefly ,five colonies of each isolate were introduced into 5 ml of Nutrient broth, incubated for 4 hr., and the culture turbidity was adjusted to a 0.5 Mc Farland standard sterile cotton swab was dipped into the suspension and spread evenly over the entire Muller-Hinton Agar surface The antibiotics impregnated disc's were then placed onto the surface of the inoculated plates and incubated at 37°C for 16-18 hour. After incubation, diameters of the zones of inhibition were measured in mm and interpreted as susceptible, intermediate and resistant.[6],[15]

3.2 Biochemical analysis

Biochemical characterization of the bacteria:

Several biochemical tests were carried out in order to have a presumptive identification of the potential bacteria chosen before. Most of the methods were done according to the microbiology laboratory manual. The biochemical tests performed were Triple sugar iron agar test, IMViC test (Indole production test, Methyl red test, Voges- Proskauer test, Citrate utilization test), MIU test (Motility test, Indole test and Urease test), Nitrate reduction test, Catalase test, Oxidase test, Casein hydrolysis test, Gelatin hydrolysis test, Starch hydrolysis, Blood agar, Eosin methylene blue agar, and Cetrimide agar.

Antibiotic resistance and susceptibility analysis:

In clinical microbiology laboratory it is an important task to check the performance of antimicrobial susceptibility testing of significant bacterial isolates.[7] The aim of this test is to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. Manual methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods.

IV. RESULTS AND DISCUSSION

4.1 Physiochemical Test Method

Total Soluble Solids:

The TSS content is significantly influenced by the percentage of solid materials (mango pulp, sugar, glucose and other ingredients) dissolved in water in the juice. Sometimes, the

producer adds a sweetening agent instead of sugar and glucose. On the other side, some producers add other ingredients as sodium CMC to increase TSS artificially. TSS value of the juice was 13.5%[8].

Protein Content in Collected Juice Sample:

Most of the fruit juices were low in protein content. Protein is insoluble in fruit juice so a considerable proportion of the protein content is present in the fruit juices. Our sample is 0% protein as displayed in Table

Fat Content in Collected Juice Samples:

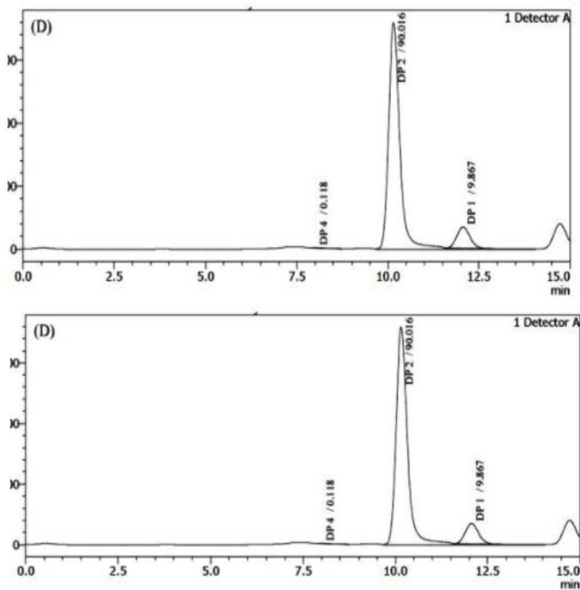
Fruit juice does not contain fat; somehow a little amount could be present in raw ingredients. Fat was detected as 0.13% in juice sample.

Total Reducing Sugar of Collected Juice Sample:

It is estimated that reducing sugar and total sugar content increased with the advanced ripening of fruits. The combined effect of the stages of maturity and ripening conditions significantly affected the reducing sugar and total sugar content of the fruit pulp and juices. 3.6% TRS in sample.

Carbohydrate Profile of Collected Juice Sample:

In this study, Carbohydrate Profile was analyzed by the HPLC and dextrose profile was segregated from other oligosaccharides. 9.867% monosaccharides in a taken sample.[9]



S.no	Dextrose (%)	Sucrose (%)	Triose (%)	Textrose (%)
1.	9.867	90.01 6	0	0.118

4.2 Total bacterial count of collected juices

S. No	Different counts	Mango juice
1	Total Viable count (TVC) (CFU/ ml)	1.25×10^6
2	Total Coliform Count (TCC) (CFU/ ml)	1.5x10^2
3	Fecal Coliform Count (FCC) (CFU/ ml)	-Nil
4	Total Staphylococcal Count (TSC) (CFU/ ml)	2.5x10^3
5	Total Salmonella count (CFU/ ml)	Nil

4.3 Antimicrobial susceptibility patterns of isolated bacteria from mango juice

Antibiotics	Bacterial isolates											
	S.aureus N = 6			*Klebsiella spp* N = 3			*E. coli* N = 4			*B. cereus* N = 2		
	R	I	S	R	I	S	R	I	S	R	I	S
Er	100	0	0	100	0	0	100	0	0	100	0	0
Amx	100	0	0	0	33.3	66.7	25	25	50	100	0	0
S	33.3	16.7	50	33.3	66.7	0	50	25	25	100	0	0
Te	16.7	0	83.3	66.7	33.3	0	75	25	0	50	50	0
C	66.7	16.7	0	0	0	100	25	0	75	50	0	50
Gm	33.3	33.3	33.3	0	0	100	25	0	75	50	0	50
P	0	0	100	66.7	33.3	0	0	0	100	0	0	100
Amp	0	16.7	83.3	66.7	0	33.3	50	25	25	50	0	50

N= number of isolates, Er = Erythromycin, Amx = Amoxicillin, S= Streptomycin, Te = Tetracycline, C = Chloramphenicol, Gm = Gentamicin, P = Penicillin, Amp = Ampicillin.[10]

4.4 Biochemical Test Result

Organism	Gram stain									
		IN	MR	VP	Cit	H2S	N03	CAT	Coag	sta
E.coli	-	+	-	-	-	ND	ND	ND	ND	ND
Salmonella	-	-	+	-	+	+	+	+	-	-
Shigella	-	+	+	-	-	-	+	+		-
S .aureus	+	-	+	+	-	-	+	+	+	-
Klebsella.sps	-	-	-	+	+	ND	ND	ND	ND	ND
Psuedomonas.sps	-	-	-	-	+	ND	ND	ND	ND	ND

4.5 Discussion

From this study, there was more contamination in the juice dilution of 10-1 Mango samples, respectively. Whereas the juicing dilution 10-7 was the least contaminated than the others. By this result clearly indicated that the water used in the preparation of fruit juices was highly contaminated with many entro bacterial spices[11].In addition to this, the contamination of juices was also due to the use of unhygienic conditions of water storage and use of unclean utensils and unhygienic physical and biological contaminants. [12]

V. Conclusion

The present study was isolated the bacteria from packed mango juice The different bacteria identified and isolated from these samples were E.coli, Bacillus cereus, Salmonella typhimrium, Listeria monocytogenes and Staphylococcus aureus. [13]'[14]

The testing of fruit juices helps to aware people of unhygienic conditions in the freshly prepared juices and helps to analyze the presence of pathogens to prevent various health problems caused due to contamination.

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