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DETERMINATION OF TOTAL MICROBIAL BIOLOAD AND PATHOGEN TESTING FROM PACKED AND FRESH FRUIT JUICE

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Abstract: Fruit juices are popular because they are high in vitamins, minerals, and fibre, all of which are essential for human health. In this study we have conducted a total microbial bio load and pathogen testing of packed (Mango, Guava, Apple, Grape, Lychee) and fresh (Pomegranate, Banana, Papaya, Lemon) fruit juices. For the pathogen testing, a total of nine juice samples were used. Here we prospect the isolation of bacteria, total colony count and pathogen testing. Isolation of bacteria was done using nutrient agar medium, total colony count was prosecute by colony counting method, pathogen testing were carried out using specific media like Mannitol salt agar(MSA), Eosin methylene blue agar(EMB), Cetrimide agar and Bismuth sulphite agar(BSA).After completion of pathogen testing we concluded that there was no growth of pathogen in the packed and fresh fruit juices.

Keywords-Packed fruit juice, Fresh fruit juice, Isolation, Specific media, Pathogen testing.

I. INTRODUCTION

Bioactive substances can be found in abundance in fruit juices. These are nutrient-dense beverages with a delicious taste and a long list of health advantages (Jay *et al.*, 2010). When compared to entire fruits, pure fruit juice has less dietary fibre and vitamin C. The majority of studies on pure fruit juice focuses on CVD risk variables like blood pressure and serum cholesterol. Preservatives are often added to packaged juices in order to extend their shelf life. As a result of their high sugar content, they make great food for bacteria (Romika Dhiman*et al.*, 2014).

Without a doubt, mango juice is the world's most popular non-alcoholic beverage, especially amongst people of all ages. Mango juice includes roughly 30 grams of sweet carbs per cup and adds vitamin C to the diet (Dietary fibres, sugars - glucose, fructose, maltose, sucrose) (Ruhul Amin *et al.*, 2018). Guava is a tropical fruit that belongs to the *Myrtaceae* family and is known as the "apple of the tropics". Guava has outstanding medicinal, pharmacological, digestive, and nutritional properties, as well as a nice flavour, good palatability, and abundant availability at a reasonable price (Md.Atiqur Rahman Sarker*etal.*,2018).

Antibacterial properties of pomegranates (*Punica granatum L.)* date back to biblical times. Pomegranates were utilised by Egyptians to treat a variety of diseases. It has been used as a traditional cure in the Ayurvedic school of medicine for thousands of years, with extracts from the fruit's rind and the tree's bark being beneficial against diarrhea and dysentery. It has been shown that eating grapes and grape products reduces the risk of cardiovascular disease and thrombosis control of hypertension and dyslipidemia in addition to antioxidant, anti-inflammatory, anti-aging and diabetic properties that are directly linked to polyphenol profile, including flavonoids (anthocyanins, flavan-3-ols, flavonols, and flavanones) and non-flavonoids, grapes (phenolic acids, stilbenes and lignans).

Banana is one of the world's most popular fruits and a significant food crop in tropical and subtropical regions. You may use it in a broad range of goods such as banana puree and figs. When it comes to food preparation, lemon juice is a staple in both commercial and household kitchens. It is also included in many processed foods and beverages. A wide range of phyto nutrients and antioxidants may be found in lemon (Citrus limon) juice. Lychee was a South-east Asian tropical to subtropical crop. They're highly sought after on the international market for their taste, as well as their beautiful red skin and semi-translucent white arid. It's based on the work of Susan Patricia Miranda-Castro and colleagues (2016).

The bacteria that cause illness in humans via virulence machinery are known as food borne pathogens. This is the case even when the infectious dosage is very low (Yousef and Abdelhami et al., 2019). Extending the shelf life of food while also producing healthier and more nutritious foods has been possible because to advances in microbe control throughout the course of human history.

II. MATERIALS AND PROCEDURE

2.1 Collecting Samples

The fresh and packed sample of fruit juice were collected from the local market in Hosur. The packed fruit juice samples were Mango (Maaza), Guava, Apple, Lychee (Paper boat swing), Grape (Real fruit power grape) and the fresh fruit juice samples were Pomegranate, Banana, Papaya, Lemon.

2.2 Sample Preparation

99 ml of distilled water was kept in autoclave for sterilization and then 1 ml of sample was added

2.3 Isolation and estimation of microorganism

Prepared 200 ml of nutrient agar (HiMedia, chemtech laboratories) medium containing 1g of peptone and 1g of sodium chloride (fisher scientific), 3g of agar, 0.4g of yeast extract. The pH of the solution was maintained at 7.

300µl of sample was poured into petri plate and then freshly prepared autoclaved nutrient agar (HiMedia, chemtech laboratories) is added and incubated at 37ºC for 24 hours. After the incubation time, the plates were examined for the existence of colonies, and the actual number of bacteria was calculated as colony forming unit per ml (cfu ml). The formula below is used to calculate the total number of microorganisms present in 1 ml.

$$\textit{Total number of microbe per ml} = \textit{Total number of colonies} \times \textit{Dilution Of Sample} \quad \ldots\ldots\ldots\ldots 3.1$$

Colony morphology was done by observing the size, form, elevation, margin, opacity, surface, color, type and Gram's characteristics for every colonies that grown on the petri plate.

2.4 Isolation of pure culture

Prepared 200 ml of nutrient agar (HiMedia, chemtech laboratories) medium containing 1g of peptone and 1g of sodium chloride (fisher scientific), 3g of agar, 0.4g of yeast extract. The pH of the solution was maintained at 7 and poured into petriplate after solidify we taken a loop of culture from mother culture and streaked the plate and kept it in incubator at 37ºC for 24 hours.

2.5 Specific media

Pathogen testing were carried out by using specific media like Manittol salt agar (MSA),Bismuth sulphite agar(BSA), Cetrimide agar and Eosin methylene agar(EMB).Mannitol salt agar (MSA) includes 1% mannitol and 7.5% sodium chloride, as well as phenol red indicator and peptone in 40ml.The pH of the solution was maintained at 7.4 and mannitol salt agar media was kept in autoclave for 30 minutes and poured into petriplate after solidify we taken a loop of culture in pure culture and streaked the plate and kept it in incubator

at 37ºC for 24 hours. It was shown that *Staphylococcus aureus* could be isolated and identified using Mannitol Salt Agar (MSA) as a selective and differential medium from clinical and non-clinical materials.

40ml of cetrimide agar contains 0.3g of cetyltrimethylammonium bromide and 13.5g of agar, 10ml of glycerine, and magnesium chloride. The pH of the solution was maintained at 7.1. Cetrimide agar media was kept in autoclave for 30 minutes and poured into petriplate after solidify we taken a loop of culture from pure culture and streaked the plate and kept it in incubator at 37ºC for 24 hours. Cetrimide Agar, commonly known as Pseudomonas Cetrimide Agar or Pseudosel Agar, is a selective and differentiating medium used to isolate and identify *Pseudomonas aeruginosa* from clinical and non-clinical materials.

40ml of Bismuth sulphite agar(BSA) contains 0.41% of brilliant green and 13.5% of agar, Bismuth sulphite indicator, and ferrous sulphite. The pH of the solution was maintained at7.6 and Bismuth sulphite agar media was heated gently with frequent until the medium just begins to boils and simmer for 30 seconds to dissolved the agar do not overheated and do not autoclave. Bismuth sulphite agar was cooled to 50-55ºC and mixed well to disperse suspension and it was poured into periplate after solidify, a loop of pure culture was taken and streaked the plate and kept it in incubator at 37ºC for 24 hours. According to the available data, the only medium that can identify biochemically unusual salmonellae, particularly lactose fermenting bacteria, is Bismuth sulphite agar (BSA).

40ml of Eosin methylene blue agar(EMB) contains 0.41% of eosin and 13.5% of agar, methybule ,and lactose. The pH of the solution was maintained at 7.1 and Eosin methylene agar media was kept in autoclave for 30 minutes and poured into petriplate after solidify we taken a loop of culture from pure culture and streaked the plate and kept it in incubator at 37ºC for 24 hours. In order to differentiate *E.coli* from other gram-negative bacteria, Eosin methylene blue agar may be used.

RESULTS AND DISCUSSION

3.1 Isolation of bacteria

Isolation of microorganisms from packed and fresh fruit juice samples was carried out initially by serial dilution procedure then the isolated samples were grown in the pour plate(subculture). The following table 1 contains the total microbial count present in each samples. There is no growth in the packed grape and fresh lemon juice because of the presence of acids like tartaric acid and citric acid. Other samples like pomegranate, lychee, banana, apple, mango, guava in nutrient agar plate have growth of colony.

Table 1: Total microbial count of packed and fresh fruit juice

S.No	Sample	Dilution rate	Colony	Total count	Mean value	Standard deviation
1	Packed Mango Juice	10^{-3}	1 1	1×10^{-3} 1×10^{-3}	1×10^{-3}	0.707×10^{-3}
2	Packed Grape Juice	10^{-3}	0	0	0	0
3	Packed Guava Juice	10^{-3}	1 1	1×10^{-3} 1×10^{-3}	1×10^{-3}	0.707×10^{-3}
4	Packed Apple Juice	10^{-3}	1	1×10^{-3}	1×10^{-3}	0
5	Packed Lychee Juice	10^{-3}	1 2 3	1×10^{-3} 2×10^{-3} 3×10^{-3}	2×10^{-3}	2.303×10^{-3}
6	Fresh Pomegranate Juice	10^{-3}	6 4 7	6×10^{-3} 4×10^{-3} 7×10^{-3}	5.6×10^{-3}	6.58×10^{-3}
7	Fresh Papaya Juice	10^{-3}	4 3 1	4×10^{-3} 3×10^{-3} 1×10^{-3}	2.6×10^{-3}	3.117×10^{-3}
8	Fresh Banana Juice	$10-3$	1	$1\times10-3$	$1\times10-3$	0
9	Fresh Lemon Juice	$10-3$	0	0	0	0

3.2 Colony Morphology

Colony morphology is the visual colony characteristics of a bacterial colony on an agar plate. The table here shows the morphology of colonies like shape, margin, elevation, size, surface and colour.

Table 2: Colony characteristics observed on Nutrient agar plate

Sample	No of Colony (cfu)	Shape	Margin	Elevation	Size	Surface	Colour
Fresh Papaya Juice	4	Rhizoid	Filiform	Flat	Moderate	Wrinkled	White
	3	Round	Entire	Convex	Small	Smooth	White
	1	Irregular	Undulate	Raised	Moderate	Smooth	White
Packed Mango juice	1	Circular	Entire	Convex	Moderate	Smooth	White
	1	Irregular	Undulate	Convex	Moderate	Smooth	White
Packed Guava Juice	1	Circular	Entire	Convex	Moderate	Smooth	White
	1	Irregular	Entire	Convex	Moderate	Smooth	White
Fresh Banana Juice	1	Circular	Entire	Convex	Small	Smooth	White
Packed Apple Juice	1	Circular	Entire	Convex	Large	Smooth	White
Packed Lychee Juice	1	Irregular	Entire	Crateriform	Large	Smooth	White
	2	Irregular	Undulate	Umbonate	Small	Smooth	White
	3	Rhizoid	Undulate	Convex	Large	Smooth	White
Fresh Pomegranate Juice	6	Irregular	Undulate	Crateriform	Large, moderate	Smooth	White
	4	Filamentous	Lobate	Umbonate	Moderate	Smooth	White
	7	Irregular	Undulate	Crateriform	Morderate	Smooth	White
Packed Guava juice	-	-	-		-	-	-
Fresh Lemon Juice	-	-	-		-	-	-

3.3 Gram staining

Gram staining was used to identify the gram negative and gram positive bacteria. Table 3 shows the gram reaction for the packed and fresh fruit juice sample.

Table 3: The morphology character from gram's staining

Sample	Gram Reaction	Shape	Type
Fresh Papaya Colony1	Gram negative	Spherical shaped	*cocci*
Fresh Papaya Colony2	Gram negative	Spherical shaped	*cocci*
Fresh Papaya Colony3	Gram positive	Spherical shaped	*cocci*
Fresh Pomegranate Colony1	Gram negative	Rod shaped	*cocci*
Fresh Pomegranate Colony2	Gram positive	Spherical shaped	*cocci*
Fresh Pomegranate Colony3	Gram negative	Spherical shaped	*cocci*
Packed Mango Colony1	Gram negative	Rod shaped	*Bacilli*
Packed Mango Colony2	Gram positive	Rod shaped	*Bacilli*
Packed Apple Colony1	Gram positive	Spherical shaped	*Cocci*
Packed Lychee Colony1	Gram negative	Rod shaped	*Bacilli*
Packed Lychee Colony2	Gram positive	Spherical shaped	*Cocci*
Packed Lychee Colony3	Gram negative	Rod shaped	*Bacilli*
Packed Banana Colony1	Gram negative	Spherical shape	*cocci*
Packed Guava Colony1	Gram negative	Rod shaped	*Bacilli*
Packed Guava Colony2	Gram positive	Rod shaped	*Bacilli*

3.4 Pathogen testing

Pathogen testing was done for all the colonies that were obtained from the streak plate(pure culture). It was carried out using specific media namely Mannitol salt agar, EMB agar, Centrimide Agar, Bismuth Sulphite Agar. A common selective and differential growth medium is Mannitol Salt Agar (MSA), which is a salt-based solution. EMB agar is a selective and differential culture media. Bismuth sulphite agar (BSA) is the only recommended medium for detecting biochemically unusual *salmonellae*, particularly lactose fermenting bacteria. There are many distinct names for Cetrimide Agar, but they all refer to the same thing: A selective and differentiating medium used for the isolation and identification of the bacterium *Pseudomonasaeruginosa* from both clinical and non-clinical samples.

Table4: Pathogen testing in specific media

SAMPLE	DILUTION RATE	COLONY	MSA	EMB	BSA	CETRIMIDE AGAR
Packed Mango juice	10^{-3}	1,2	-	-	-	-
Fresh Pomegranate juice	10^{-3}	1,2,3	-	-	-	-
Packed Guava juice	10^{-3}	1,2	-	-	-	-
Fresh Papaya juice	10^{-3}	1,2,3	-	-	-	-
Packed Banana juice	10^{-3}	1	-	-	-	-
Packed Lychee juice	10^{-3}	1,2,3	-	-	-	-
Packed Apple juice	10^{-3}	1	-	-	-	-

The table 4 shows that (-) denotes the absence of pathogen and (+) denotes the presence of pathogens, MSA- Mannitol salt agar, EMB- Eosin methylene blue, BSA- Bismuth sulphite agar. Packed (Mango, Guava, Apple, Grape, Lychee) and fresh (Pomegranate, Banana, Papaya, Lemon) fruit juice samples in Bismuth sulphite agar (BSA), Mannitol salt agar, Eosin methylene blue agar and Cetrimide agar after incubation for 24 hours at 37ºC was observed that there was no growth of pathogen, because of pasteurization and addition of preservatives in packed fruit juice and the presence of acidic nature in the fresh fruit juice.

III. CONCLUSION

The present study shows the pathogen testing for packed Juices like Mango, Guava, Apple, Grape, Lychee and fresh juices like Pomegranate, Banana, Papaya, Lemon. Due to the juices' low pH, they were contaminated with significant levels of moulds and yeasts, and had to be thrown out. Indications of food-borne epidemics include the presence of pathogenic bacteria in juices. Therefore good handling and contact surface as well as proper hand hygiene are crucial to the production of safe juices products as they are part of the food and beverage industry. Fruit juices also contains acids like critic acid, tartaric acid, malic acid, ascorbic acid etc... To ensure that their products are pathogen-, yeast-, and mould-free, juice producers use a fast, high-heat pasteurisation process, which gives their products a shelf life of 9 to 12 months, depending on the packaging. It is concluded from the pathogen testing that their was no growth of pathogens, because of pasteurization, addition of preservatives and their acidic nature.

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