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A Survey On The Microbial Spoilage Of Local Fruit Juices Available In Cuddalore District Of Tamil Nadu

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Abstract: Fruit juices are valuable from the nutritional point of view. They are rich in minerals, vitamins and other nutritive factors. In developed countries it is used a part of their diets. Researchers are going to produce safe high quality fresh juices which have good taste and longer shelf life through controlled minimal processing techniques. Microorganisms play a vital role in contamination and spoilage of fruit juices. The pathogens like Staphylococcus, Salmonella, Shigella, E.coli were transmitted through fruit juices. 12 different natural fruit juices are processed to isolate and identification of microorganisms present in it. It includes Staphyloccus aureus, Salmonella sp, Bacillus cerus, Streptococcus faecalis.

Key words:MPN Most Probable number method.NLF (Non Lactose Fermentor),LF (Lactose Fermentor)

INTRODUCTION:

Fresh juices have more "natural appeal, with flavor and taste closely resembling the original fruit and the potential for higher nutritional value. Fresh fruits are high in water, low in protein and low in fat. Water content is 85%, protein content is 3.5%, fat content is 0.5. Several fruits contain good amounts of Carotene which gets converted in to vitamin A in our body. Consuming fruits like orange, apple, lemon, and pomegranate can aid the proper functioning of the heart, fruits like mango, apple, date have a direct action on the central nervous system. Fruit juices are valuable from the nutritional point of view. They are rich in minerals, vitamins and other nutritive factors, fruit juices are delicious and have a universal appeal. In developed countries fruit juices commonly form part of the breakfast and are produced in very large quantities. Fruit juices are best in taste, aroma and colour when freshly expressed. The most important problem. Therefore in the fruit juice industry is to use such methods as would help retain these properties to the maximum extent various steps involved in the processing of juice are selection of fruit, extraction of juice, deacreation, staining and preservation. Hot research aims to produce safe, high quality fresh juices which taste good and have a longer shelflife through carefully controlled minimal processing techniques. The disease free fruits were used for juice extraction, using screw type juice extraction.the juice was preserved with 700 ppm SO2. The SO2 preserved juice ws clarified by pectinolytic enzyme. Improper handling and storage conditions after harvest can also facilitate mould contamination. In processing the juices or beverage from fruits, some isolates of fungi have the ability to tolerate this processing.

MATERIALS AND METHODS

SAMPLE COLLECTION

Fresh fruit juices were obtained from local shops. From the main sample one third was transferred to the 250ml sterile conical flask for further processing. The samples were processed to identify the type and number of organisms followed by two techniques.

STANDARD PLATE COUNT TECHNIQUE:

The collected fruit juices were serially diluted with Ringers solution. From each dilution 1 ml was transferred to sterile petriplates and to that sterile plate count agar, was poured and evenly distributed by rotating the plate clockwise and anticlockwise direction. Then the plates were incubated at 37°C for 24-48 hours. After the incubation period the plates were observed for the appearance of colonies and then colonies were counted.

MOST PROBABLE NUMBER TECHNIQUE:

The coliform group includes all of the aerobic and facultative anaerobic gram negative, non-sporing, rod shaped bacteria which ferment lactose with gas production within 48 hours at 37°C. The standard test for the estimation of number of coliform groups may be carried out by multiple tube dilution fermentation technique.

PRESUMPTIVE TEST:

Measured amount of single and double strength modified lactose broth were sterilized in test tubes containing Dhurham's tube to show the gas production. Totally 3 set of tubes were used each of which having five tubes. The first set of tubes were poured with 10 ml double strength lactose broth and the other two sets with 5ml single strength lactose broth. 10ml of sample was inoculated in first

set double strength lactose broth and 1.0 ml, 0.1 ml of sample were inoculated on second and third set respectively. Then all the tubes were incubated at 37°C for 24-48 hours. After the incubation period the tubes were analysed for gas production in the Dhurham's tube.

CONFIRMED TEST:

From the positive tubes of the presumptive test one or two loopful of culture were transferred to Brilliant green lactose bile broth (BGLB) with Dhurham's tubes. The tubes were gently rotated to mix the inoculum equally. Then the tubes were incubated at 37°C for 48 hours. After incubation, the formation of gas constitute the positive confirmed test.

COMPLETED TEST:

After incubation the tubes were examined for gas production which indicated the positive result. From such tubes one loopful of inoculum was transferred to Eosinmethylene blue agar (EMB agar) plate. After 24 hours of incubation the colonies were analysed for their characteristic morphology called metallic sheen indicating the growth of E.coli. Then the isolated colonies were confirmed by bio-chemical test.

MICROSCOPIC EXAMINATION:

GRAM'S STAINING:

The organisms grow on culture plates were stained using gram staining method. A thin smear was prepared on grease free slide. It was flooded with crystal violet solution and allowed to stand for one minute. Then it was washed with water and then flooded with grams iodine solution and the preparation was left for 30 minutes. It was drained and decolourised with 95% ethanol which was then washed gently in running water. Then the same was stained with counter stain called safranine for 1 minute. After drying the slide was observed under microscope to identify the organisms.

NEGATIVE STAINING:

Dilute suspension of organism was prepared. To that one loopful of culture was placed on to the slide. One drop of India ink or nigrosin was added to that culture and it spread evenly and allow the smear to dry in air. The slide should not heat fix. The organisms appear as hollow area and the background is black was the indication of positive.

BIO-CHEMICAL TESTS:

INDOLE TEST:

The colony from the slant was inoculated onto the peptone broth and it was then incubated at 37°C for 24 hours. The formation of red ring upon the addition of Kovac's reagent indicates the positive reaction.

METHYL RED TEST:

The colony from the slant was inoculated on to the MR-VP broth tubes and it was incubated at 37°C for 24 hours. The formation of red colour on the addition of methyl red indicates the positive reaction.

VOGES - PROSKAUER TEST:

A loopful of colony was inoculated in to vp broth and it incubated at 37°C for 24 hours. Development of pink to crimson red colour on the addition of barrit's reagent to the medium indicates positive reaction.

TRIPLE SUGAR IRON TEST:

TSI agar medium was prepared. The organisms taken from the slant was inoculated by stabbing down the centre of agar butt carefully withdraw the inoculating wire and then streak the surface of the slant and incubated at 37°C. The results reads only after 24-48 hours incubation. Appearance of yellow colour indicates acid formation, pink colour indicates alkali formation and blackening due to H2S production and gas formation.

UREASE TEST:

A loopful of culture was picked from the 4-6 hour growth of bacterium and it was streaked on to a christensen's urea Agar and incubate at 37°C for 24 hours. The positive reaction indicates the colour of the medium changes to pink.

COAGULASE TEST:

The test is performed specially to differentiate coagulase positive Staphylococcus aureus from Coagulase negative Staphylococus aureus. The tube coagulase test was done using 0.5 - 1.0 ml of plasma with an equal volume of overnight culture. After overnight incubation the presence of clumps indicates positive result and absence of this indicates negative result.

CATALASE TEST:

Few drops of 10% Hydrogen peroxide solution was added on the 24 hours colonies picked from the slant. A appearance of effervescence indicates catalase production.

OXIDASE TEST:

A solution of 1% Para Phenylene diamine dihydrochloride was prepared. Filter paper was taken and this paper was soaked on the reagent and kept it on a petridish with the help of a clean plastic loop, a colony from 24 hours growth of the test organisms were rubbed over the filter paper. The positive indicates the colour change to blue to purple within 10 seconds.

Table -1
Isolation and characterization of microorganisms Present in the available local fruit juice sample around of Cuddalore (Dt) of Tamil Nadu

S.No.	Fruit Juices	Isolate No. of colonies			Gram staining	Motility	Methyl red test	Voges proskauer test	Indole test	TSI
		Dilution		MPN/ 100 ml						
		10^{-5}	10^{-6}							
1	Mixed fruit juice	55	43	26	G +ve	Negative	Negative	Positive	Negative	Alk/Alk, H_2S
2	Pomegranate juice	32	16	7	G +ve	Negative	Negative	Positive	Negative	Alk/Alk, H_2S
3	Pine apple juice	42	26	6	G -ve	Positive	Positive	Negative	Negative	Alk/A, H_2S
4	Apple juice	-	-	>2	-	-	-	-	-	-
5	Grape juice	12	7	7	G +ve	Negative	Negative	Positive	Negative	Alk/Alk, H_2S
6	Sapota juice	45	39	14	G +ve	Negative	Negative	Positive	Negative	Alk/Alk, H_2S
7	Papaya juice	-	-	>2	-	-	-	-	-	-
8	Mango juice	41	30	9	G +ve	Negative	Negative	Positive	Negative	Alk/Alk, H_2S
9	Water melan	-	-	>2	-	-	-	-	-	-
10	Orange juice	36	18	14	G -ve	Positive	Positive	Negative	Negative	Alk/A H_2S
11	Sweet lime juice	32	16	7	G +ve	Negative	Positive	Positive	Negative	A/Alk Gas
12	Lemon juice	42	36	9	G +ve	Negative	Positive	Positive	Negative	A/Alk Gas

A – Acid, Alk - Alkaline

Table -2
Bio-chemical and sugar fermentation test of the isolated organisms from the fruit juice samples

S.No.	Fruit Juices	Bio-chemical test			Sugar fermentation test				Microorganism isolated
		Catalase test	Oxidase test	Coagulase test	Lactose	Glucose	Sucrose	Mannitol	
1	Mixed fruit juice	Negative	Negative	Negative	Positive	Negative	Positive	Positive	*Streptococcus faecalis*
2	Pomegranate juice	Negative	Negative	Negative	Positive	Negative	Positive	Positive	*Streptococcus faecalis*
3	Pine apple juice	Negative	Negative	Negative	Positive/Gas	Positive/Gas	Negative	Positive/Gas	*Salmonella species*
4	Grape juice	Negative	Negative	Negative	Positive	Negative	Positive	Positive	*Streptococcus faecalis*
5	Sapota juice	Negative	Negative	Negative	Positive	Negative	Positive	Positive	*Streptococcus faecalis*
6	Mango juice	Negative	Negative	Negative	Positive	Negative	Positive	Positive	*Streptococcus faecalis*
7	Orange juice	Negative	Negative	Negative	Positive/Gas	Positive/Gas	Negative	Positive/Gas	*Salmonella species*
8	Sweet lime juice	Positive	Positive	Positive	Positive	Positive	Positive	Positive	*Staphylococcus aureus*
9	Lemon juice	Positive	Positive	Positive	Positive	Positive	Positive	Positive	*Staphylococcus aureus*

RESULTS AND DISCUSSION

Twelve different local fruit juices were processed to standard plate count, MPN, and other biochemical tests. Out of 12 different local fruit juices 7 of them were positive for bacteria. In this streptococcus faecalis was isolated from mixed fruit juice, pomegranate juice, grape juice, mango juice, and sapota juice. This was also supported by Jedah. J.H. (2002). Salmonella sp were isolated from orange juice and pineapple juice. Salmonella species was isolated from orange juice, some strains of E.coli and shigella, Salmonella sp., can survive for several days in acidic food. Hatcher.W.S.S. et al., (1997). Out break of human diseases have been linked with the consumption of non pasteurized single strength orange juice. Preethi (1994). The organisms were characterized and identified as Salmonella sp, Staphylococcus aureus, Bacillus cereus, and streptococcus faecalis. Among the isolated were Streptococcus facalis were the predominant organism found in 5 different local fruit juice. Several workers have reported that contamination of salad, juices fruit and vegetables is due to unsanitary cultivations and marketing practices. Good rich (1998).

The main reason for contamination of fruit juices may be due to fruits, water which has to be taken for the preparation of fruit juice, handler's storage in retail shaps. Care should be taken to prevent the contamination of products. The workers of such fruit juice companies acts as a carries of disease and they may contaminate the fruit juices prepared by them. When consuming fruit juices are should be taken because disease causing microorganisms are present in the fruit juice. The microorganism mainly enters during improper handling. The result showed that the fruit juice preparations were carried out under unsterile condition. Fruit, water used for the preparation of fruit juice should be in first quality and also the handles hand equipment used in this process should be clean. Preparation of fruit juices were carried out in clean manner, the bacterial contamination is also minimized.

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