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Research Article

Microbiological Quality Assessment of Handmade Juice in Street of The Dhaka City

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Abstract

The present study was conducted for bacteriological study of handmade juice in street of Dhaka city. For this total viable bacterial count (TVBC) Isolation, purification, Gram staining, selective isolation, result interpretation were determined in Mango juice (Mangifera indica), Apple juice (Malus domestica), Orange juice (Citrus sinensis), Malta juice (Helichrysum melitense) and Lacchi. In such investigation highest TVBC ($1.4x10^6$) and ($1.2x10^6$) was observed in Mango juice and Alovera juice which is form Khilkhet (street) and Sadarghat (street) and the lowest TVBC ($9.0x10^5$) was observed in Malta juice which is collect form banani($1.2x10^6$) and TVBC ($9.0x10^5$) was observed in Papaya which is collect form Banani.

In conclusion 10 types of selected isolate were selected depending on their Growth, colony color, Morphology for final study. Enterobacter aerogenes was present in Mango juice sample, Pseudomonas aeroginosa was present in Apple juice sample, Salmonella typhimurium was present in Malta juice sample, Bacillus cereus was present in Orange juice sample and Klebshilla pneumoniae was present in Lacchi sample. When prepare of these juices of different street area of Dhaka city it was not properly stored and handling condition of those street shop. As a result we can say that these street juice product can cause serious health effect even death of human being due to presence of harmful pathogen.

Introduction

Juice is the liquid that is naturally contained in fruit or vegetable tissue. It is commonly consumed as a beverage or used as an ingredient or flavoring in foods. Juice is prepared by mechanically squeezing fruit or vegetable flesh without the application of heat or solvents. For example, grape juice is the liquid extract of the fruit of the grape tree.

Juice may be prepared in the home from fresh fruit and vegetable using a verity of hand or electric juicer. Many commercial juices are filtered to remove fiber or pulp.

Food juice consumption overall Bangladesh has increased in recent years probably due to public perception of juice as a healthy natural source of nutrients and increase public interest in health issues. Indeed fruit juice intake has been consistently associated with reduced risk of many cancer types, might be protective against stroke.

Therapeutic benefits of some juices: Alovera juice can help lower blood sugar level in people with type 2 diabetes. Alovera juice can reduce cholesterol. Alovera juice can cure ulcer or reduce inflammation or pain.

Grape juice can be used for cure of asthma. Grapes increase the nitric oxide levels in the blood, which prevent blood clots thereby reducing the chances of heart attack. Ripe grape are important home remedy for curing migraine. It should be taken early in the morning without adding water.

Papaya is good to cure the skin infections and wounds that don't heal quickly. It is low in cholesterol and high in nutritional values. For pregnant women, regular consumption of a small slice of papaya helps to cure nausea and morning sickness. Papaya is rich in vitamin A and vitamin C that helps in boosting the body's immunity.

Street foods are described as wide range of ready to eat foods and beverages prepared and/or sold by mobile or stationary vendors and hawkers specially on a street and around public institution such as schools, hospitals, railway stations and bus terminals. Street foods like different types of juices feed millions of people daily with a wide verity that are relatively cheap and easily accessible. Street food offers significant amount of

employment, often to persons with the little education and training (FAO, 1997) street foods play an important role in developing countries in meeting the food demands of the urban dwellers. Food security in terms of adequate quantity and quality of food to lead an active and healthy life must be considered as the prime function of a food system. FAO reports that street foods have significant nutritional implication. For consumers particularly middle and low income sector of the population who depend heavily on them.

Selling foods in the streets is a widespread phenomenon in the city of Dhaka, the capital of Bangladesh. About 15 million people live in this city. Among various types of informal sectors of activities, street food vending is distinctive in the sense that it provides a basic need to the urban inhabitants and involves issues of hygiene and food safety. A large number of city dwellers from different spheres of life such as students, tourists, rickshaw pullers, cart pullers and other such workers relay on street food vendors for their daily meals. According to the report there are as many as 200000 street food vendors in Dhaka city who are increasing mainly due to the demand of an urban population growing at a rate of 5% a year.

The rising cost of food in recent years provides an important dimension to the existence of street food vendors. They can provide

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food and services at relatively low prices since they do not incur overhead expenditure to the extent of their counterpart. Within this context, street as an informal food supply system provided opportunities for resource poor groups in urban and pre-urban environments, not only as a means of employments but also as an effective way of providing low cost nutrition to the people.

Objectives of this study

Identification of selected isolates.

Measurement of the total viable count.

Methods and Materials

Table 1 Types of samples and their collection area and date

Collection of samples

Sample of different types of handmade juice were collected from different street area in Dhaka city. The samples were collected from (Sadarghat, Rampura ,Khilkhet, Uttara, Tongi,Gulshan , Banani etc).

Selection of samples

Sample of different street juice were taken from different from area. All collection samples were labeled immediately and transfer to the laboratory as quickly as possible.

Culture media

Different types of culture media were used in this experiment (Appendix). Figure 1

Methods

Sterilization: All equipment's and glass ware sterilized by autoclaving at 15psi for 15 minutes. All culture media and solutions were sterilized by autoclaving at 15 psi for 15 minutes in the autoclave.

Sample preparation:1ml sample was transferred to a test tube containing 9ml of sterile distilled water to make 10^{-1} dilution and shaken with vortex mixture. A serial dilution up to 10^{-5} was also made in same procedure.

Pour plate method: For the pour plate technique 0.1ml after serial dilution was pipette in to sterilized Petri plate. Sterilized nutrient agar medium was cooled to about 45^0 C and was poured onto the plate. The media was mixed well by a gentle swirling motion. The Petri plates were then allowed to solidify. The plates were incubated at the required temperature for 24 hour to 72 hour.

Table 1: Types of samples and their collection area and date.

Name of sample (code)	Collection area	Date
Mango juice (S1)	Khilkhet	15-09-12
Mango juice (S2)	Uttara	17-09-12
Malta juice (S3)	Banani	20-09-12
Malta Juice (S4)	Banani	24-09-12
Orange Juice (S5)	Airport	26-09-12
Orange Juice (S6)	Tongi	30-09-12
Apple Juice (S7)	Gulshan	30-09-12
Apple Juice (S8)	Banani	08-10-12
Lacchi (S9)	Banani	08-10-12
Alovera juice[S10]	Sadarghat	09-11-12
Alovera juice[S11]	Rampura	17-11-12
Alovera juice[S12]	Banani	20-11-12
Grape Juice[S14]	Banani	24-09-12
Papaya Juice[S15]	Sadarghats	26-09-12

Figure 1: MAC, XLD, NA agar media.

Growth of microorganisms: All culture were inoculated and incubated under aseptic condition. The samples were inoculated in a laminar airflow cabinet. The inoculation media for static growth condition were incubated in the incubator. A colony counter was used to count the microbial colonies on solid agar plates. A compound microscope was used to observe the microscopic characteristics of bacteria.

Preservation of pure culture: Transfer the purified isolates on nutrient agar slant in test tube an incubated at 37^0C overnight. After incubation, the tubes were tightly cotton plugged properly marked and preserved at 4^0C in a refrigerator as stock for further study.

Microscopic study

Gram staining

This is the most extensively used differential stain the divides bacteria in to two major groups. Those which retain crystal violate dye after treatment with iodine and alcohol appears purple or bluish purple are designated as gram positive. On the other hand those bacteria which loss the crystal violate show the color of the counter stain employed. The commonly used counter stain is safranin which gives pink red color to bacteria and these organisms are leveled as gram negative.

Small drop of distilled water or normal saline was placed on a slide. Then loopful isolated colony was taken and smeared over the surface of the slide. The smeared was allowed to dry thoroughly. The smeared was fixed quickly through the burner flame three times. After cooling the smeared was stained. Between each staining reagent the smeared was washed under genteelly running tap water.

Staining and reagent were applied as per following sequence:

1. Ammonium Oxalate. (Crystal violate) (60 sec)

2. Grams Iodine. (60 sec)

3. 95 % Ethanol (30 sec)

4. Safranin (45 sec)

Then it was air dried and observed under × 40 and × 100 objective lance in a compound microscope.

Screening

Isolated colonies were transferred to the fresh nutrient agar to purify them and finally 5 pure colonies were selected, based on their morphology, color and growth on selective media for further study.

Result

Table 2

Biochemical study

Conventional biochemical test used for the identification of the isolates mention below:

Imvic test

The imvic series of test indole, methyl red, and voges-proskauer and citrate utilization can be used for this purpose.

Indole test

Incase of positive reaction tryptophan is oxidize by the microbial enzyme tryptophanase, yielding indole and pyruvic acid. Indole reacts

Table 2: Total viable count in studied samples.

Sample no.	TVBC on Nutrient Agar NA(cfu/ml)	TVBC on MCA (cfu/ml)	TVBC on XLD (cfu/ml)
S10M5	1.2×10^6	1.80×10^6	7.0×10^5
S6M1	1.4×10^5	4.1×10^5	2.1×10^5
S9N4	7.2×10^5	4.0×10^5	2×10^4
S10X8	5.5×10^5	9×10^4	7×10^4
S7X2	9.0×10^5	1.2×10^5	1.4×10^5
SA_1	1.4×10^6	1.80×10^6	7.0×10^5
SA_2	1.6×10^5	4.0×10^5	2.2×10^5
SG_1	7.2×10^5	4.0×10^5	2×10^4
SA_3	5.5×10^5	9×10^4	7×10^4
SP_1	9.0×10^5	1.2×10^5	1.4×10^5

with paradimethylminobenzaldehyde producing a visible colorful dye. The tryptophan broth medium was prepared the P^H was adjusted. Dispensed in to the test tube at the rate of 10 ml per tubes. The medium was then sterilized at 121^0C temperatures and 15 lb pressure for 30 minutes in the autoclave and cooled. The tubes of tryptophan broth in duplicate or inoculated with 48 hours old nutrient broth culture. The tubes were then incubated at 37^0C for 3 days. After incubation few drops of kavac's solution or were added to the tubes. The tubes were shaken vigorously for 1 minute and observed for the oink color formation in the tubes. Figure 2

MR test

This test was used for determined the ability of microorganisms to oxidized glucose with the production and stabilization of high concentration of acid end products. MR-VP broth was used for this purpose. The broth was then sterilized at 121^0C temperature and 15 lb pressure for 30 minutes in the autoclaved and cooled. Inoculated the test culture and incubate at 37^0C for 48 hours. Add five drops of MR reagent red color as the surface indicates positive result and absence of negative results. Figure 3

VP test

MR-VP broth was used for this purpose. Inoculate the test culture on VP broth and incubated at 37^0C for 48 hours after incubation add 15 drops of VPA reagent (O_2 is need to reaction). After few times add five drops of VPB reagent and mixed well. Pink red color indicates positive and absence the red color shows negative results. Figure 4

Citrate utilization

The test is used to differentiate among enteric organism on the basis of there ability to ferment citrate as a soul source of carbon. Figure 5

Catalase test

This test was used to differentiate those bacteria that produce the enzyme catalese from non catalase producing one. Aerobic, facultative aerobes, microaerophiles can produce catalase while the anaerobes unable to produce this enzyme. The production of bubble indicates the positive catalase test results and the absence of bubble production indicate negative results. Figure 6

Oxidase test

Place a piece of filter paper. Then add 3 drops of Oxidase reagent. After that organism transfer on filter paper. Blue color indicates positive result and no color indicates negative results. Figure 7

NO_3- reaction

Nitrate broth was used for this purpose. Inoculation of test culture on the nitrate broth solution. Incubate 37^OC for 48 hours. After incubation add sulfanilic acid and 1- napthal amin. Red color indicates positive result. After some time add small amount of zinc. Red color production indicates positive result and colorless indicate negative result.

Lactose fermentation

Weight and dissolve triptycase nutrient broth and phenol red in 100 ml distilled water at 0.5 ml lactose insert Durham's tube in to all test tubes should be fully filled with lactose broth. Sterilizing at 121^OC for 15 psi. Inoculation culture organism by loop. Incubation for 18-24 hours at 37^OC. Blanks Durham's tubes indicates gas production and color change indicate produce acid. Not color change indicates alkaline. Figure 8

Glucose fermentation

Weight and dissolve triptycase nutrient broth and phenol red in 100 ml distilled water at 0.5 ml Glucose insert Durham's tube in to all test tubes should be fully filled with Glucose broth. Sterilizing at 121^OC for 15 psi. Inoculation culture organism by loop. Incubation for 18-24 hours at37^OC. Blanks Durham's tubes indicates gas production and color Change indicate produce acid. Not color change indicates it alkaline. Figure 9

Sucrose fermentation

Weight and dissolve triptycase nutrient broth and phenol red in 100 ml distilled water at 0.5 ml Sucrose insert Durham's tube in to all test tubes should be fully filled with Sucrose broth. Sterilizing at 121^OC for 15 psi. Inoculation culture organism by loop. Incubation for 18-24 hours at 37^OC. Blanks Durham's tubes indicates gas production and color change indicate produce acid. Not color change indicates it alkaline. Figure 10

Gelatin hydrolysis

The medium used is nutrient gelatin. An inoculum from a pure culture to transferred aseptically to sterile tube of nutrient gelatin. The inoculated tube is incubated at 35-37^0C for 24 hour. Gelatin becomes liquid at temperature modestly above the incubation temperature. If the gelatin has been digested by gelatinase, the will fail to solidify after refrigeration. Figure 11

Starch hydrolysis

Starch is polysaccharide made of 2 components amylose and amylopectin. Amylose is truly soluble in water, which produce blue color when combined With iodine. Amylopectin produce a violet color when mixed with iodine. Figure 12, Table 3

Discussion

Juice is popular among the people throughout of the year particularly in the summer. In Dhaka city, we frequently find juice vendors in the streets. The make fresh juices for the thirsty peoples. People drink these types of juices overlooking the microbiological as well as hygienic standard. For that people often get sick due to drinking of such unhygienic juices. In this study mainly use three type of sample at different area in Dhaka city. All of the juices samples had very high level of microbial contamination. The sources of contamination may be the polluted water or ice use to dilute the juices. It also contaminated for the unsterile container, place, air, naked hand etc. The samples are allovers, grapes and papaya.

The highest number TVBC (1.4×10^6) To (1.2×10^6) were present in alovera and Mango juice sample and lowest number of TVBC were present in papaya (9.0×10^5) and Malta (5.5×10^5)

A total number of 14 samples were studied and total viable bacterial count was found in the range between 2×10^4 and 8×10^6.

Ten isolates were finally selected for identification and was identified by considering the colony morphology, gram straining & biochemical test result. Ten types of bacteria finally isolated from my Project work. They are *Escherichia coli, Streptococcus lactis, Pseudomonas aeruginosa, Proteus vulgaris, Micrococcus luteus. Enterobacter aerogenes, Bacillus cereus, Klebshiella pneumoniae, Salmonella typhimurium..*

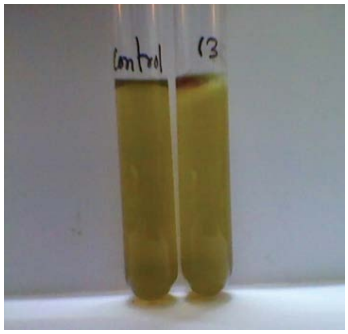
Above all organisms are harmful/pathogenic for human being. We should not overlook the hygienic standard of street juice in Dhaka city. Steps must be taken to reduce the spread of multi drug resistance bacteria/microorganism for healthy and clean environment.

Conclusion

The practice of consuming fresh juices from street cannot be stopped on either nutritional ground or hygienic standard nor the street vendor prohibited from selling such items. Present study exhibited the microbiological status of available local street juices to ensure the exact public health risk. The microbial loads in the most street juice sample were still above the standard limit for consumption. These juice samples are collected from different area of Dhaka city. This is not satisfactory as *E.coli* , *Streptococcus lactis* , *Micrococuss luteus* , *Pseudomonas aeruginosa*, and *Proteus vulgaries* were deceted in those juice. Lack of knowledge of safe fruit juice preparation as well as the contamination sources.

Table 3: Biochemical test result of isolate colony form juice sample.

Sample	**S10M5**	**S6M1**	**S9N4**	**S10X8**	**S7X2**
Gram stain + shape	G(-) Rod	G(+) Rod	G(+) Rod	G(-) Rod	G(-) Rod
Color	Creamy	White green pigment	White	White	Off White
Lactose	++	Alkaline	Alkaline	Acid+Gas	Alkaline
Glucose	Acid + Gas	Alkaline	Acid	Acid+Gas	Acid+Gas
Sucrose	Acid + Gas	Alkaline	Acid	Acid	Acid+Gas
H_2S	-	-	-	-	+/-
N_03-	+	-	+	+ (Zn add)	+
Indole	-	-	-	-	-
MR	-	-	-	+	+/-
VP	+	+	+	-	+/-
Citrate utilization	+	+	+/-	+	+
Urease	-	-	-	+/-	-
Catalase	+	+	+	+	+
Oxidase	-	+	-	-	-
Gelatin	-	+/-	+	-	-
Starch	-	-	+	-	-
Organism presunptive	*Enterobacter aerogenes*	*Pseudomonas aeroginosa*	*Bacillus cereus*	*Klebshiella pneumoniae*	*Salmonella typhimurium*



A **B**

Figure 2: Indole test: positive (A) and negative (B).

A **B**

Figure 4: VP Test: Positive (A), Negative (B).

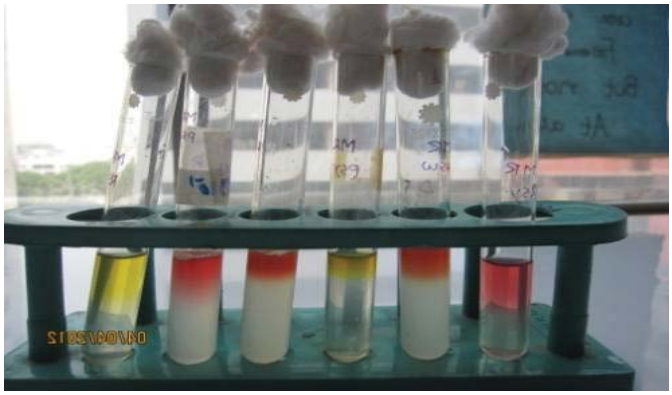


Figure 3: Methyl red test.

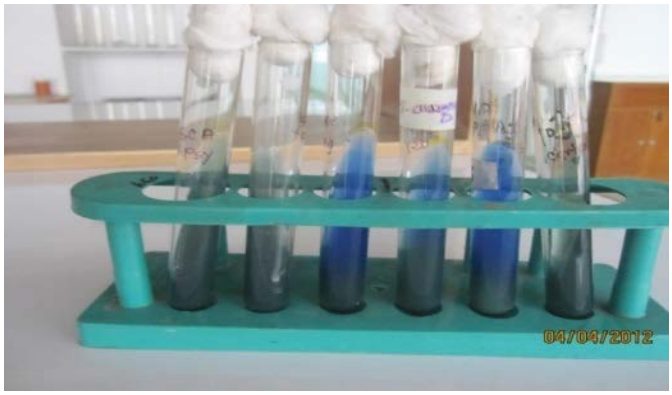


Figure 5: Citrate Utilization test: Positive test (Blue color), Negative test (No change).

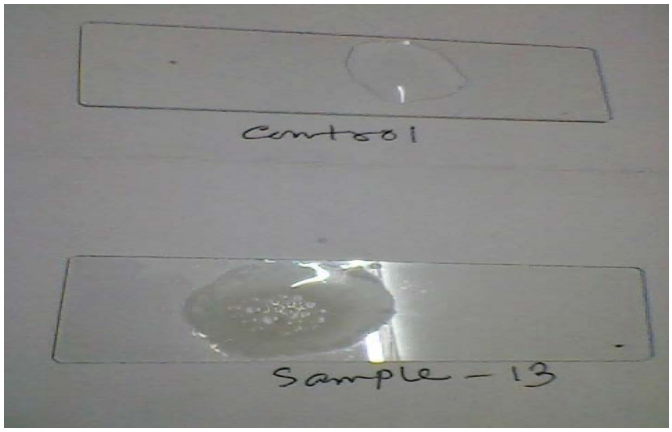


Figure 6: Catalase Test of different juice sample.

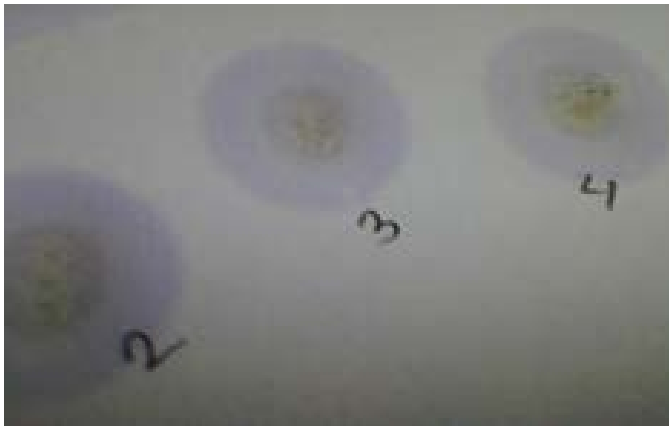


Figure 7: Oxidase test of different juice samples.

Figure 8: Lactose fermentation.

Figure 9: Glucose fermentation.

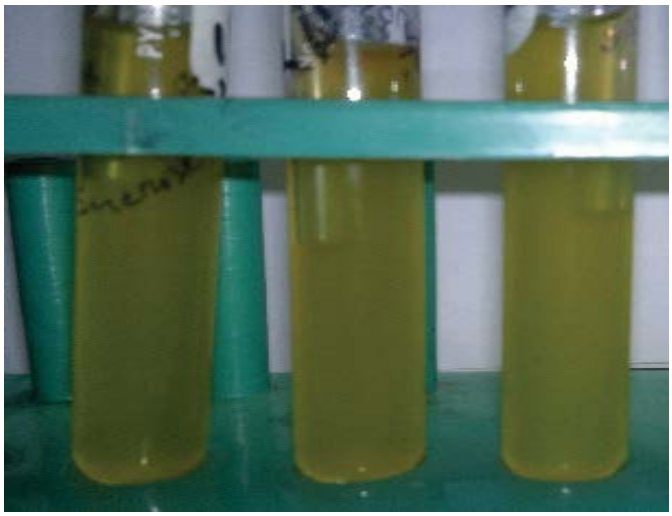


Figure 10: Sucrose fermentation.

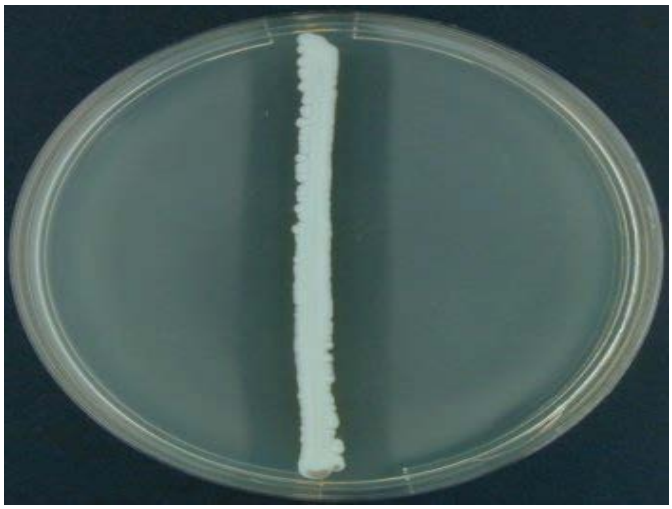


Figure 11: Gelatin hydrolysis..

Figure 12: Starch hydrolysis.

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