ASSESSMENT OF MICROBIAL QUALITY ALONG WITH ISOLATION, IDENTIFICATION OF BACTERIAL STRAIN AND EFFECT OF HEAT TREATMENT ON STREET HERBAL JUICE SAMPLE IN DHAKA CITY

Mehrin Mamun Mourin, Nawsheen Islam, Nurul Abadin Chowdhury and Nayan Chakrabarty

Department of Food Engineering and Technology, School of Science and Technology, State University of Bangladesh, Dhaka-1205, Bangladesh

ABSTRACT

The aim of this research was to measure the microbial load and evaluate the effects of heat treatment on microbial quality of herbal street juice, which was collected from Malibagh (M), Farmgate (F) and Kawranbazar (K) in Dhaka city. Plate count, MacConkey and PDA were used to determine the Standard Plate Count (SPC), Total Coliform Count (TCC) and Total Fungal Count (TFC), respectively. SPC showed that microbial load in all juice samples exceeded the acceptable range when E. coli, Klebsiella pneumoniae, Bacillus sp., Salmonella, Proteus sp., Enterobacter sp. and Shigella sp. were identified by morphological and biochemical characteristics. TCC and TFC in juices were not in acceptable range. After heating juices at 55ºC for 30 min, the count of Coliform was massive but after heating at 63ºC for 30 min, TCC was NIL in the M, F sample and small amount of TCC was found in the K sample.

KEYWORDS

Herbal juice, Microbial quality, Biochemical, Morphological

1. INTRODUCTION

In Bangladesh, street juices are very common and popular because of cheapest cost and unique flavour. It is sold as a main beverage item in many tropical countries. In developing countries food borne illnesses are caused by the foods and juices which are sold by street vendors. Various types of juices or drinks can be found in the streets of Dhaka City i.e. Fruit Juices, Lemon drink, Aloe Vera juice, mixed fruit and plant herbal juice, Blond Plantain and Tank mixed juice, Rooh afza juices.

Bacterial pathogens like E. coli O157:H7, species of Salmonella, Shigella and Staphylococcus aureus are found in the juice [Ball, C.O.1943]. According to the standards provided for drinking water showed that the infectious that create for these contaminating bacteria in fruit juices is not yet well established [Buchanan et al., 1999]. The level of microbiological quality of street-food and juice samples was found unsatisfactory for the presence of high levels of coliforms, E. coli and S. aureus [Liu, R.H., 2003]. Sierra Leone has a significant risk factor for cholera infection by the street-sold water in Cholera epidemic regions [Muinde et al., 2005]. E. coli (37.5%), Salmonella (5.36%), Shigella (19.64%) and various microbial species from 37 samples of street- sold foods are found in a study included Silchar city, Assam in India [Lewis et al., 2006]. A cross sectional study has been shown that the health risk posed from street-sold food in densely populated cities

of a developing country like Bangladesh, food vendors in these countries have ample knowledge and awareness of food safety issues [Cappuccino et al., 2008].

Ingredients that are used for the preparation of these street juices are not washed properly which add bacteria that causes contamination. The water used by the street juice shops is unhygienic. The other ingredients such as ice are unhygienic. Air flies, refrigeration technique, airborne dust are also the sources of contamination. The main reasons for this microbial contamination of such foods are preparation and storage, infrastructure, cleaning and serving utensils, cooking, quality of water and personal hygiene maintained by food handlers [Kuldiloke at al., 2008]. Street Juices are very popular drinks due to the presence of various antioxidants, vitamins, minerals, and naturally occurring phytonutrients which play a major role for preventing different types of diseases such as Cataracts, Alzheimer disease, and some of the aging related functional disorders [Bhattacherjee et al., 2011].

We collected the herbal juice samples from different places of Dhaka city for our study. The juice contains Ocimum basilicum seeds, Plantago ispaghula, Phyllanthus emblica, Terminalia bellirica, Aegle marmelos, Terminalia chebula, Aloe vera, Azadirachta indica root, Terminalia arjuna, Cassia Angustifolia, Mangifera indica root, Nelumbo nucifera root and 51 other ingredients. For enhancing consumer's health these ingredients could play many important roles.

Ocimum basilicum seed helps controlling blood sugar of those people who have type-2 diabetes, lowering the cholesterol level, bladder infection and treats colds, flu, cough and asthma. Plantago Ispaghula is a source of dietary fiber. It helps to prevent irritable bowel syndrome, constipation, and diarrhoea. Phyllanthus emblica contains antioxidant, Gallic acid and anti- inflammatory chemicals which prevent free radicals, fight cancer and reduce inflammation. Terminalia bellirica is used to protect liver and treat respiratory conditions, cough and sore throat. It helps to prevent heart disease, HIV infection, severe diarrhoea, urinary problems and lung conditions. It also helps to lower the blood cholesterol level. Aegle marmelos is used to treat tuberculosis, prevent gynaecological problems, diabetes, reduce irritable bowel syndrome. Terminalia chebula is used to prevent constipation by speed of the intestinal tract. It reduces irritable bowel syndrome, Alzheimer's and Parkinson diseases. Terminalia arjuna fights against the damage from free radicals, inflammation, lipid disorder. It tackles the high blood pressure, boosts energy and exercise performance. It boosts heart function and heals cardiac injury. Azadirachta indica root is used as a detoxifying agent. It reduces acne and fever, prevents dandruff, and treats skin disorder. Cassia Angustifolia is effective against viral infection, helps to cure diarrhoea. It is good for antidepressant, provides relief from joint pain and constipation. Aloe vera contains antioxidants which prevent the growth of harmful bacteria. It reduces the dental plaque, constipation, blood sugar level, helps to treat mouth ulcers, improves skin and prevents wrinkles. Mangifera indica root regulates diabetes, dissolves gall and kidney stones. Nelumbo nucifera root is used to improve heart health and helps to reduce weight.

This herbal juice is very nutritious for human. Quality of this herbal juice should be maintained properly. Developing countries have rules and regulations for maintaining a hygienic condition. In Bangladesh the vendors of the shops are not concerned with the cleanliness and hygiene because of the absences of proper law against these conditions. If it is processed properly it could enhance consumer's health through preventing many diseases. The research work was designed with aims to evaluate physico-chemical properties, microbial quality parameters, identification of pathogens, and effect of heat treatment on microbial quality parameters of herbal street juice in Dhaka City.

2. MATERIALS AND METHODS

2.1. Site Selection and Samples Collection

Street herbal juices were collected from Malibagh, Farmgate and Kawranbazar; mostly popular and crowded areas of Dhaka city. People of these places drink the street herbal juice most. There are also lots of street herbal juice shops which are presented into these areas. The samples were collected from the different street shops of Dhaka City. Samples were collected into sterilized bottles and immediately transferred into the laboratory for the microbial test. Every sample was coded differently. Malibagh sample was coded M, Farmgate sample was coded F and Kawranbazar sample was coded K. Sampling of the street herbal juice for estimation of total microbial count and identification of the bacterial strain were carried out using the sterile equipment and environment. The date of the sampling and manufacturing were recorded.

2.2. Preparation of Dilution

Saline diluent was prepared using 0.90% Sodium chloride (NaCl) and distilled water. Then 1ml of each sample weighted and poured into 9ml of diluents. For the juice sample it was selected for the preparation of initial dilution 10-1. The diluents then vortexed at 1400 rpm for mixing properly. When the juice sample dissolved properly into the diluents then it became ready for serial dilution. For the assessment of microbial quality 10-1 to 10-10 dilutions were made.

2.3. Media Preparation

Different types of media are prepared for the total microbial count and identification of bacterial strain i.e. Plate Count Agar (PCA), MacConkey (MAC), Motility Indole Urease (MIU), Mannitol Salt Agar, Triple Sugar Iron (TSI), Potato Dextrose Agar (PDA), Simmons Citrate, Carbohydrate Fermentation media, Citrate, MR and VP.

2.4. Thermal Processing

Different thermal processing methods were applied for inactivation of the enzyme activity, to destroy microbial growth and increase the shelf life of the street herbal juice. Consume fruit juices, blends and fortified beverages are a popular way to maintain a healthy diet [Manguiat, L.S. et al., 2013]. For this reason, thermal processing is done to analysis whether it is manufactured commercially or not. It is also applied for an attractive appearance of the juice. We compared three different temperatures such as ambient, 55ºC and 63ºC after thermal processing of the street juice sample. We used to heat the juice at 50ºC for 15 min because to inactive the yeast activity and increasing the temperature decreased the growth of microorganisms rapidly [Al Mamun et al., 2013]. Pasteurization temperature of 63°C for 30 minutes which was applied, were considered as the complete thermal death time for a range of pathogenic bacteria [Lee, H., Kim et al., 2013]. Coliforms are sensitive to heat treatment. Heating is the most important process to destroy the E. coli 0157:H7 and other pathogenic E. coli. To control the food borne illness juice processors needed to reduce the pathogen by 5 log units which is required by the U.S. Food and Drug Administration (FDA) [Nguyen et al., 2014]. In juice, Salmonella Listeria or E. coli O157:H7 can be present and survive for a certain amount of time [Sharma et al., 2014]. By applying heat, they can easily be destroyed. Extreme heat treatment over 80ºC causes various changes for the juices such as unwanted flavour and colour [Sharma et al., 2014]. Loss of nutrients is negligible both in 55°C and 63°C temperature. At 55°C number of microorganisms slightly eliminate but at 63°C for 30 min heat destroy the pathogenic bacteria like E. coli and Salmonella. At this condition microorganisms are removed from juice. Heat treatment was also applied to increase the quality, prevent from

chemical activity, for improvement in availability of some nutrients and destruction of anti-nutritional components.

3. RESULTS AND DISCUSSION

3.1. Total Microbial Count

The Standard Plate Count (SPC) was recorded in the sample K (2.87×10^6cfu/ml), F (3.5×10^6cfu/ml) and in the sample M (4.2×10^5cfu/ml). Total Coliform Count was found more in sample K (1.81×10^6cfu/ml), F (1.95×10^6cfu/ml) and lowest recorded in the sample M (2.22×10^5cfu/ml). The Total Fungal Count was found in the sample K (1.85×10^6cfu/ml), F sample (1.90×10^6cfu/ml) and in the sample M (3.6×10^5cfu/ml). Count of the samples are not in acceptable range and thus these street juices are not considered as safe for consumption [14]. In Figure 1, light blue colour columns indicate the growth of microbial count in M juice sample, orange columns indicate the growth of microorganisms in F and blue columns indicate the microorganisms in K juice sample. Standard Plate Count is higher in F sample than in K, M samples because the water was contaminated more than other two places and the vendors were not maintaining the personal hygiene at all. Total Coliform Count is lower in M sample than F, K samples. Total Fungal Count is higher in F sample than in K, M samples. All-over Total Microbial Count is too high for human consumption. For this the ranges of microorganisms in both samples are not acceptable.

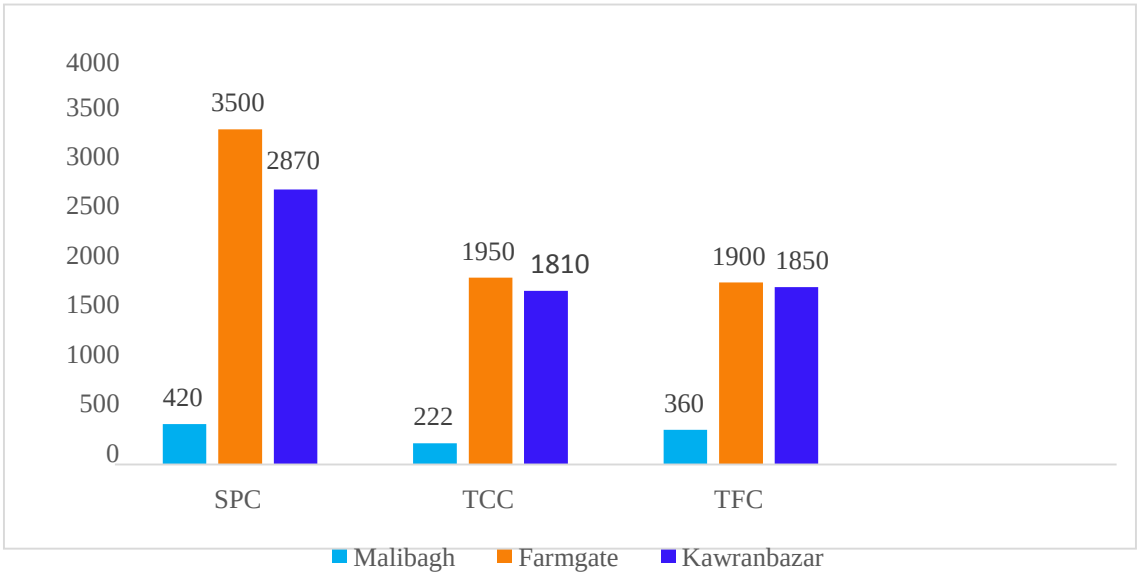

Figure 1: Total Microbial Count (in Thousands). Graph showing No. of SPC in different samples (102).

3.2. Total Coliform Count (TCC) after Thermal Processing

The total coliform count (TCC) of the street herbal juice samples gave an indication of the total number of coliform bacteria present. In the ambient temperature highest TCC was found in the sample K (1.81×10^6cfu/ml), F (1.95×10^6cfu/ml) and lowest in sample M (2.22×10^5cfu/ml). After heating the sample at 55ºC highest TCC was found in the sample K (1.06×10^6cfu/ml), F (1.80×10^6cfu/ml) and lowest in M (1.16×10^5cfu/ml). After heating at pasteurization temperature (63ºC for 30 minutes); it shows that TCC was NIL in the M, F samples and small amount of coliform found in the sample K.

Table 1: TCC in three different temperatures are given below.

Serial Number	Sample Code	Growth in ambient temperature	Growth after heating at 55°C temperature	Growth after heating at 63°C temperature
01	M	2.22×10^5cfu/ml	1.16×10^5cfu/ml	NIL
02	F	1.95×10^6cfu/ml	1.80×10^6cfu/ml	NIL
03	K	1.81×10^6cfu/ml	1.06×10^6cfu/ml	1.2×10^2cfu/ml

In Figure 2, the X-axis indicates different temperature and the Y-axis shows the number of microorganisms. White columns indicate the growth of microorganisms at ambient temperature, grey columns indicate the growth of microorganisms at 55°C temperature and green colour columns indicate the growth of microorganisms at 63°C temperature. Highest growth has seen in the ambient temperature and lowest growth in 63°C.

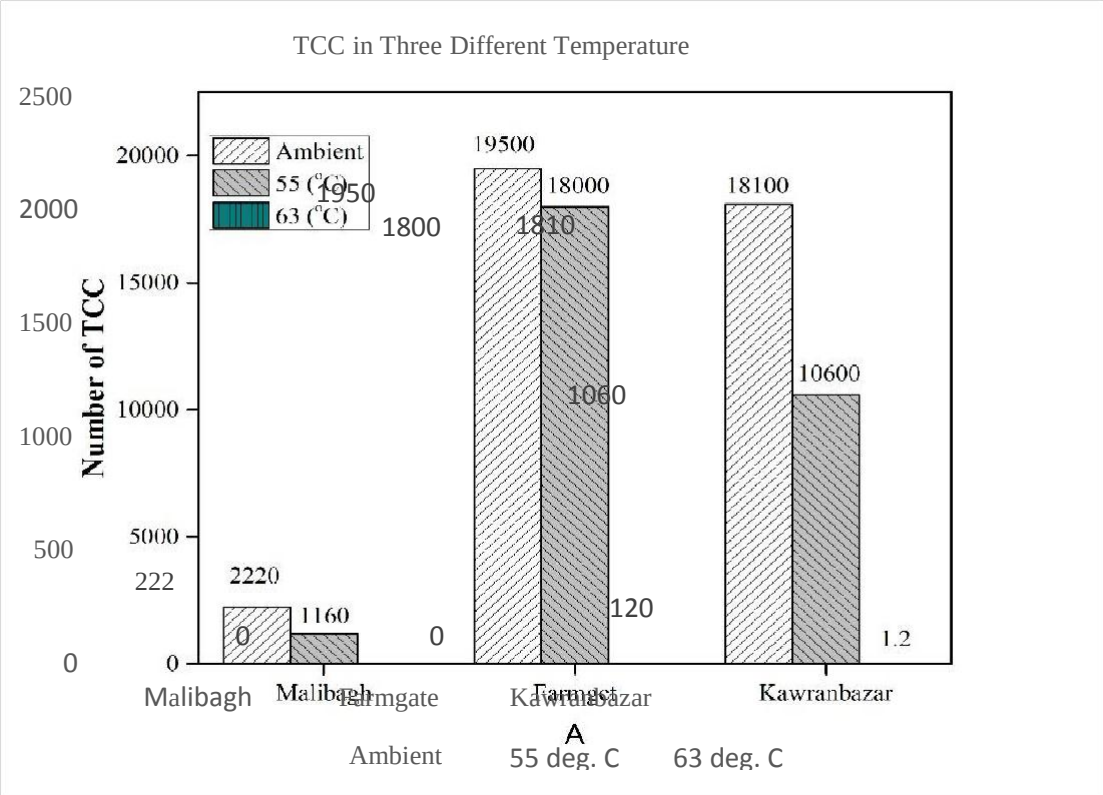

Figure 2: A bar chart showing the TCC found (in thousands) in three different samples at three different temperatures. Graph showing No. of TCC in different samples (102).

3.3. Gram Staining

For differentiating the Gram-positive and Gram-negative bacteria Gram Staining technique is used. Plate Count Agar media was used for the growth of microorganisms and then gram staining was used for differentiating the microorganisms whether it is gram positive or gram negative. Microscopic observation showed both gram-positive (Figure 3) and gram-negative (Figure 4) bacteria were found in the street juice samples.

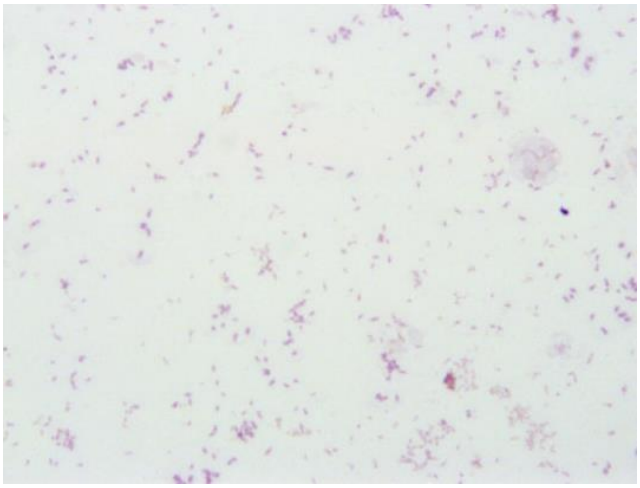

Figure 3: Microscopic observation of Gram-positive bacteria from isolated colonies (Gram- stained).\

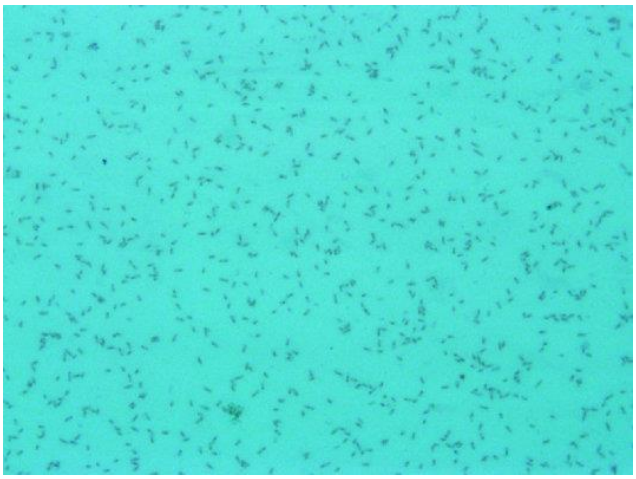

Figure 4: Microscopic observation of Gram-negative bacteria from isolated colonies (Gram- stained).

3.4. Morphological characters

Isolates were Gram stained and then observed under bright field microscope for the identification of the morphological characteristics. Morphological characteristics can be clearly visible when difference between refractive index was shown. Structural details of live bacteria are not understandable under the light of microscope due to lack of contrast. For this reason, staining technique is useful to detect the morphological characteristics of microorganisms. Bacterial colony was also observed for morphological study. The microscopic observations of eight strains from the sample M are described in the Table 2. There were three size of strain found and maximum of them are circular. Maximum isolates have grey and opaque pigments.

Table 2: Morphological characters for sample M

SL. No.	Strain Code	Colony Size	Form	Margin	Pigments	Growth on Agar Slant
01	M11	Large	Irregular	Entire- Sharply Defined	Grey	Filiform
02	M12	Moderate	Irregular	Entire- Sharply Defined	Opaque	Echinulate
03	M13	Small	Circular	Entire- Sharply Defined	Opaque	Beaded
04	M14	Large	Circular	Entire- Sharply Defined	Grey	Beaded
05	M15	Pin Point	Circular	Entire- Sharply Defined	Opaque	Effuse
06	M16	Pin Point	Circular	Not clearly defined	Grey	Effuse
07	M17	Pin Point	Circular	Entire- Sharply Defined	Grey	Beaded
08	M18	Small	Circular	Entire- Sharply Defined	Grey	Beaded

The microscopic observations of seven strains from the sample F are described in the Table 3. There were four size of strain found and maximum of them are circular and irregular. Maximum isolates have opaque pigment and only two of them have transparent pigment.

Table 3: Morphological characters for sample F

SL. No.	Strain Code	Colony Size	Form	Margin	Pigments	Growth on Agar Slant
01	F11	Large	Irregular	Entire- Sharply Defined	Opaque	Echinulate
02	F12	Small	Irregular	Entire- Sharply Defined	Opaque	Echinulate
03	F13	Pin Point	Circular	Entire- Sharply Defined	Transparent	Filiform
04	F14	Pin Point	Circular	Entire- Sharply Defined	Opaque	Filiform
05	F15	Moderate	Irregular	Entire- Sharply Defined	Opaque	Beaded
06	F16	Pin Point	Irregular	Entire- Sharply Defined	Opaque	Beaded
07	F17	Pin Point	Circular	Entire- Sharply Defined	Transparent	Beaded

Seven strains were observed in microscope from the sample K, described in table-4. There were two size of strain found and maximum of them were small and only two of them were pin point. Maximum isolates have opaque pigment and only three of them have transparent pigment.

Table 4: Morphological characters for sample K

SL. No.	Strain Code	Colony Size	Form	Margin	Pigments	Growth on Agar Slant
01	K11	Small	Circular	Entire- Sharply Defined	Opaque	Beaded
02	K12	Pin Point	Circular	Entire- Sharply Defined	Opaque	Beaded
03	K13	Small	Circular	Entire- Sharply Defined	Opaque	Beaded
04	K14	Small	Circular	Filamentous	Transparent	Effuse
05	K15	Small	Irregular	Filamentous	Opaque	Effuse
06	K16	Pin Point	Circular	Filamentous	Transparent	Effuse
07	K17	Small	Irregular	Filamentous	Transparent	Beaded

3.5. Biochemical Tests

Microorganisms were picked depending on their morphological characteristics, growth pattern from different culture media. Microorganisms then compared with targeted microorganisms

[Siddiqua, S., 2016; Uddin et al., 2017]. Biochemical test results from the Table 5 shows that the sample M contained several species of microorganisms such as E. coli, Klebsiella pneumoniae, Bacillus species.

Table 5: Biochemical characteristics of isolates from M

Media	**M11**	**M12**	**M13**	**M14**	**M15**	**M16**	**M17**	**M18**
Indole	+	+	-	+	-	-	-	-
Motility	+	+	+	+	+	+	+	+
MR	+	+	-	+	-	-	+	+
VP	-	-	+	-	+	+	-	-
Citrate	-	-	+	-	+	+	-	+
TSI	sugar ferment G+, H2S-	sugar ferment G-, H2S-	sugar ferment G+, H2S-	sugar ferment G+, H2S-	sugar ferment G+, H2S-	sugar ferment G+, H2S-	sugar ferment G+, H2S-	Glucose G-, H2S-
Urease	-	-	+	+	+	+	+	-
Catalase	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	+/-	+	-	-
Glucose	G+	G+	G+	G+	G+	G+	G+	G+
Dextrose	G+	G+	G+	G+	G+	G+	G+	G+
Sucrose	G+	G+	G+	G+	G+	G+	G+	G+
Lactose	G+	A+	A+. G-	G+	A+	G+	Partially ferment A+,G-	G+

For identification of isolates the various biochemical tests were performed and results are shown in the Table 6 for the sample of F. The results showed that the sample from F contained a lot of microorganisms like Klebsiella pneumoniae, Salmonella paratyphi, Bacillus species, Proteus species compared with targeted microorganisms [Uddin et al., 2017; Ahmed et al., 2018].

Table 6: Biochemical characteristics of isolates from F

Media	**F11**	**F12**	**F13**	**F14**	**F15**	**F16**	**F17**
Indole	-	-	-	-	-	-	-
Motility	+	+	+	+	-	+	+
MR Test	+	+	-	+	+	-	+
VP Test	-	-	+	-	-	+	-
Citrate	+	-	+	-	-	+	+
TSI Test	Sugar ferment G+, H_2S-	Glucose G+, H_2S-	Glucose G+, H_2S-	Sugar ferment G+, H_2S-	Sugar ferment G+, H_2S-	Sugar ferment G+, H_2S-	Sugar ferment G+, H_2S-
Urease	-	-	+	+	-	+	-
Catalase	+	+	+	+	+	+	+

Oxidase	+	-	+	-	-	-	+
Glucose	G+	G+	G+	G+	G+	G+	G+
Dextrose	G+	G+	A+	A+	G+	G+	G+
Sucrose	G+	G+	G+	G+	G+	G+	G+
Lactose	Partially ferment A+, G-	Partially ferment A+, G-	Partially ferment A+, G-	G+	Partially ferment G-, A+	A+	A+

Biochemical tests of the sample from K are shown in Table 7. The results shows that E. coli, Klebsiella pneumoniae, Enterobacter species, Salmonella microorganisms presented into the juice compared with the targeted microorganisms [Siddiqua, S., 2016; Uddin et al., 2017].

Table 7: Biochemical characteristics of isolates from K

Media	**K11**	**K12**	**K13**	**K14**	**K15**	**K16**	**K17**
Indole	+	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+
MR Test	+	-	-	-	+	+	-
VP Test	-	+	+	+	-	-	+
Citrate	-	+	+	+	+	+	+
TSI Test	Sugar ferment G-, H2S-	Sugar ferment G+, H2S-	Sugar ferments G+, H2S-	Sugar ferment G-, H2S-	Sugar ferment G+, H2S-	Sugar ferment G-, H2S-	Sugar ferment G+, H2S-
Urease	-	+	+	-	-	-	-
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Glucose	G+	G+	G+	G+	G+	G+	G+
Dextrose	G+	G+	G+	G+	G+	G+	G+
Sucrose	G+	G+	G+	G+	G+	G+	G+
Lactose	G+	G+	A+	G+	A+	G+	G+

Biochemical test results of the herbal juice samples were compared to standard references mentioned in microbiology Laboratory Manual [Siddiqua, S., 2016] and presumptive bacteria are enlisted in the Table 8.

Table 8: List of presumptive bacteria from samples on the basis of biochemical test for sample M, F and K

Strain Code	Identified Bacteria	Strain Code	Identified Bacteria	Strain Code	Identified Bacteria
M11	E. coli	F11	Klebsiella pneumoniae	K11	E. coli
M12	E. coli	F12	Salmonella paratyphi a	K12	Klebsiella pneumoniae
M13	Klebsiella pneumoniae	F13	Bacillus species	K13	Klebsiella pneumoniae
M14	E. coli	F14	Proteus species	K14	Enterobacter species
M15	Bacillus species	F15	Klebsiella pneumoniae	K15	Salmonella
M16	Bacillus species	F16	Klebsiella pneumonia	K16	Salmonella
M17	Klebsiella species				
		F17	Klebsiella	K17	Enterobacter
M18	Salmonella		pneumonia		species

E coli, Klebsiella spp, Enterobacter spp, Enterococci spp. were found in the street vented juice [Padamadan et al., 2016]. Fecal contamination could be caused by the presence of Coliforms on the surface [Reddy et al., 2000]. Total fungal count in street herbal juice is alarming and similar with the previous result of Uddin et al. [Uddin et al., 2017]. Inadequate cleaning of fruits, proper preservation, temperature of cooked foods and the personal hygiene of vendors and the surrounding unhygienic environment of the street vendor could cause a high microbial load described by Uddin et al. [Uddin et al., 2017]. Presence of Klebsiella spp. in street herbal juice is minacious and similar with the previous study of Haryani et al. [Haryani et al. 2007]. Load of E. coli found in this study is quite similar with the study of Subbannayya et al. [Subbannayya et al. 2007]. Pasteurization at 60 ℃ to 100 ℃ of fruit juices destroy pathogenic microorganism and also inactivate enzymes [Ağçam et al., 2018]. Pasteurization in this temperature has no effect on the spores of fruit juices [Ramesh, 2007]. Bioactive compounds like phenolic compounds, flavonoids, and anthocyanins are affected by the heat treatment process [Ağçam et

al., 2018]. High product quality can be developed by pasteurizing at 63°C for 30 minutes which also reduce the severity of heat treatment [Ağçam et al., 2018].

4. CONCLUSION

The herbal street juice is affluent in minerals, vitamins, antioxidants and polynutrients. Herbal Juice is particularly healthy for human consumption. It also has many therapeutic benefits which help to prevent many kinds of diseases. Presences of bacteria, yeast, mold found in the study made the juice harmful for human consumption. In the study we found that highest microorganisms (2.87×10^6cfu/ml), coliform (1.81×10^6cfu/ml), fungal (2.87×10^6cfu/ml) in Kawranbazar sample. There are several reasons for too much microbial load such as the ingredients are in liquid form and the water is also contaminated. On the other hand, in the Farmgate sample we found microorganisms (3.5×10^6cfu/ml), coliform (1.95×10^6cfu/ml), fungal (1.90×10^6cfu/ml) and in Malibagh sample we found microorganisms (4.20×10^5cfu/ml), coliform (2.22×10^5cfu/ml), fungal (3.60×10^5cfu/ml) which are not also in acceptable range. In Farmgate and Malibagh samples ingredients are in powder form which is the reason for lowering the microbial load.

The juices need thermal processing for human consumption. In the study we use two temperatures (55 ºC and 63ºC for 30 minutes) for the thermal processing. Loss of nutrients is negligible in 55°C temperature but the number of microorganisms eliminated slightly. Heating at 63°C for 30 min eliminated all the pathogenic bacteria like E. coli and Salmonella. At this condition, microorganisms are removed from juice and also the loss of nutrients are negligible. Coliform spp., Klebsiella pneumoniae, Bacillus species and Salmonella spp. are harmful for human. It causes serious health problems for human. For this reason, they need to be reduced completely for human consumption and the study shows that pasteurization temperature (63ºC for 30minutes) is good for reducing the microorganisms. The limitation of the study includes the partial loss of vitamin C and bioactive compounds due to heat treatment which should be considered in the future research.

ACKNOWLEDGEMENT

We are grateful to Md. Nurul Abadin Chowdhury of Food Engineering and Technology in State University of Bangladesh, for his helps and inspiration.

REFERENCES

[1] Ball, C.O.1943. Short-time pasteurization of milk. Industrial & Engineering Chemistry, 35(1), pp.71-84.

[2] Buchanan, R.L., Edelson, S.G., Miller, R.L. and Sapers, G.M., 1999. Contamination of intact apples after immersion in an aqueous environment containing Escherichia coli O157: H7. Journal of food protection, 62(5), pp.444-450.Manguiat, L.S. and Fang, T.J., 2013.Microbiological quality of chicken-and pork-based street-vended foods from Taichung, Taiwan, and Laguna, Philippines.Food microbiology, 36(1), pp.57-62.

[3] Liu, R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The American journal of clinical nutrition, 78(3), pp.517S-520S.

[4] Muinde, O.K. and Kuria, E., 2005. Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. African Journal of Food, Agriculture, Nutrition and Development, 5(1).

[5] Lewis, J.E., Thompson, P., Rao, B.V.V.B.N., Kalavati, C. and Rajanna, B., 2006. Human bacteria in street vended fruit juices: A case study of Visakhapatnam city, India. Internet Journal of Food Safety, 8(1), pp.35- 38.

[6] Cappuccino, J.G. and Sherman, N., 2008. Microbiology: a laboratory manual. Pearson.

[7] Kuldiloke, J. and Eshtiaghi, M.N., 2008. Application of non-thermal processing for preservation of orange juice. KMITL Science and Technology Journal, 8, pp.64-74.

[8] Bhattacherjee, A.K., Tandon, D.K., Dikshit, A. and Kumar, S., 2011. Effect of pasteurization temperature on quality of aonla juice during storage. Journal of food science and technology, 48(3), pp.269-273.

[9] Manguiat, L.S. and Fang, T.J., 2013. Microbiological quality of chicken-and pork-based street-vended foods from Taichung, Taiwan, and Laguna, Philippines.Food microbiology, 36(1), pp.57-62.

[10] Al Mamun, M., Rahman, S.M.M. and Turin, T.C., 2013.Microbiological quality of selected street food items vended by school-based street food vendors in Dhaka, Bangladesh. International Journal of Food Microbiology, 166(3), pp.413-418.

[11] Lee, H., Kim, H., Cadwallader, K.R., Feng, H. and Martin, S.E., 2013. Sonication in combination with heat and low pressure as an alternative pasteurization treatment–Effect on Escherichia coli K12 inactivation and quality of apple cider. Ultrasonicssono chemistry, 20(4), pp.1131-1138.

[12] Nguyen, V.D., Sreenivasan, N., Lam, E., Ayers, T., Kargbo, D., Dafae, F., Jambai, A., Alemu, W., Kamara, A., Islam, M.S. and Stroika, S., 2014. Cholera epidemic associated with consumption of unsafe drinking water and street-vended water—eastern Freetown, Sierra Leone, 2012. The American journal of tropical medicine and hygiene, 90(3), pp.518-523.

[13] Sharma, I. and Mazumdar, J.A., 2014. Assessment of bacteriological quality of ready to eat food vended in streets of Silchar city, Assam, India. Indian journal of medical microbiology, 32(2), p.169.

[14] Worametrachanon, S., Techarang, J., Chaikham, P. and Chunthanom, P., 2014. Evaluation of quality changes of pressurized and pasteurized herbal-plant beverages during chilled storage. International Food Research Journal, 21(5).
[15] Siddiqua, S., 2016. Microbial profile testing of ready-to-eat street vended foods collected from different university premises (Doctoral dissertation, East West University).
[16] Uddin, M.E., Akter, T., Parvez, M.A.K., Nahar, S., Pervin, S., Debnath, B. and Datta, S., 2017. Microbial safety of street vended fruit juices in Dhaka City of Bangladesh. Journal of Advances in Microbiology, 3(2), pp.1-7.
[17] Ahmed, T., Das, K.K. and Uddin, M.A., 2018. The Microbiological Quality of Commercial Fruit Juices- Current perspectives. Bangladesh Journal of Microbiology, 35(2), pp.128-133.
[18] Padamadan, C.J., Raju, S. and Shenpagam, N.H., 2016. Isolation and identification of microorganism from street vented fruit juices and packed juice and its antibiogram. IJAR, 2(5), pp.1099-1105.
[19] Reddy, S.M. and Ram Reddy, S., 2000. Microbiology A Laboratory Manual. Revised edition BSC. Publishers and Distributors, Hyderabad, 105.
[20] Haryani, Y., Noorzaleha, A.S., Fatimah, A.B., Noorjahan, B.A., Patrick, G.B., Shamsinar, A.T., Laila, R.A.S. and Son, R., 2007. Incidence of Klebsiella pneumonia in street foods sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD–PCR analysis. Food Control, 18(7), pp.847-853.
[21] Subbannayya, K., Bhat, G.K., Shetty, S. and Junu, V.G., 2007. How safe is sugarcane juice? Indian journal of medical microbiology, 25(1), pp.73-74.
[22] Ağçam, E., Akyıldız, A. and Dündar, B., 2018. Thermal pasteurization and microbial inactivation of fruit juices. In Fruit juices (pp. 309-339). Academic Press.
[23] Rahman, M.S. ed., 2007. Handbook of food preservation. CRC press.