PUBLIC HEALTH RESEARCH

Assessment of Microbial Load in Made Tea and Antimicrobial Property of Made Tea Infusion

Md Monir Hossain[1], Rezaul Karim[1], Shamima Begum[2], GM Rabiul Islam[1] and Md Mozammel Hoque[1]*

[1]Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Bangladesh.
[2]Department of Microbiology and Biotechnology, Jagannath University, Bangladesh.

[*]For reprint and all correspondence: Assoc. Prof. Dr. Md. Mozammel Hoque, Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh.
E-mail: hoque-fet@sust.edu

ABSTRACT

Accepted 5 June 2013

Introduction This study aimed to find out that a cup of tea is or is not safe for human health from microbial contamination and to point out the antimicrobial property of made tea liquor.

Methods Different made tea brands were collected randomly from different super shop of Dhaka city. The Association of Official Agricultural Chemists (AOAC), 2005 was used as official methods of analysis. The Standard Plate Count (SPC) technique was used for total microbial load, yeast and fungal count. Most Probable Number (MPN) technique was used for the enumeration of coliform in tea samples.

Results Bacteria, yeast, mould and coliform were observed before and after boiling in all studied the samples. Before boiling, total microbial load and coliform were found at significantly higher of its' acceptable limit ($p<0.05$) whereas yeast and fungus were found of its' acceptable limit ($p>0.05$). After boiling, only coliform was observed significantly higher in all except Tetley tea at its' non-acceptable limit ($p<0.05$). Fecal coliform was not present at every stage of this study. Made tea liquor has shown to have antimicrobial property.

Conclusions Boiling in tea preparation and its' liquor antimicrobial property considerably reduced the level of microbial load to safe level for public consumption.

Keywords Antimicrobial activity - Microbial load - Peptone solution - Tea liquor - Tea.

INTRODUCTION

Tea (*Camellia sinensis*) is one of the most widely consumed beverages in the world and its health effects have been widely explored[1,2]. The leaves or buds of tea plants are collected from the tea garden and subjected to processing without cleaning or washing. The important parameters in tea preservations are dry, cool, dark, and inert storage[3]. Though the final drying step at a temperature of approximately 80°C is sufficient to reduce the high bacterial and fungal load, the storage, handling, and packing after drying may result in microbial contamination of the processed tea leaves[4,5]. However, use of water at sub-boiling temperature may not eliminate all pathogens, including spores of bacteria. Green and white tea is often prepared with sub-boiling water to keep tea flavor intact. Instant tea infusion is commonly prepared these days by dipping commercially sold tea bags in hot water. If tea leaves are contaminated with pathogenic microorganisms during processing and storage, the infusion may pose a potential health risk. There are reports of the presence of pathogens in tea leaves[5,6]. The presence of fungal strains in tea leaves and the presence of aflatoxin have also been reported[7,8,9,10]. The tea phenolics are known to inhibit xanthine oxidase and are beneficial to health[11] with the prevention of chronic conditions such as cancer, atherosclerosis and neurological diseases[12]. Antibacterial activity of tea against selected bacteria has also been reported[11,13]. The antimicrobial activities and bactericidal effects of tea compounds of tea catechin-3-gallates, theaflavins, and tea infusions complement have been reported against pathogens[14]. Though tea liquor has a medicinal value but sporadically[15] it may contaminates with some hazardous bacteria, yeast and moulds and tea dust or made tea undergoes spoilage by microorganisms. Fundamentally, the drinking tea must be safe, visually attractive, organoleptically acceptable, nutritious and convenient to prepare and serve with minimal use of preservatives. In nature most of the microorganisms are beneficial to human welfare and very little are predominantly detrimental causing damage, spoilage and disease. The pivotal sources of microorganisms are food and food stuff. Made tea could also be a source of microorganisms, but every day millions people drink at least one cup of tea. Hence, this study is intended to assess the level of microbial load and antimicrobial property of collected local tea brands of Bangladesh.

METHODS

Collection and preparation of samples

To evaluate the microbiological status of different local made tea brands were collected randomly from different super shop of Dhaka city, Bangladesh. Each brand of made tea were collected for six times from different super shop. The Association of Official Agricultural Chemists (AOAC), 2005 was used as official methods of analysis between April to August, 2012.

Preparation of tea sample before boiling

Using sterile instrument and aseptic technique, 25 g of tea sample were taken in a conical flask containing 225 ml 0.1 % peptone solution and kept in room temperature for 1 hour. Then 1.0 ml suspension from initial suspension (10^{-1}) was transferred to 9 ml of 0.1% peptone solution in a screw cap bottle to give a 10^{-2} dilution. In this way 10 fold serial dilutions of samples were prepared. Sample for each dilution was thoroughly mixed using the vortex mixer.

Preparation of tea sample after boiling

Three grams of tea samples were taken into a beaker containing 100 ml boiling water and allowed to boil for 2 minute on a burner. Then the beaker was removed from the burner and kept for 30 minutes at room temperature for settle down the tea dust under aseptic conditions. Tea extraction was decanted into a 100 ml beaker with aseptic condition in a laminar air flow. Then 1.0 ml suspension from initial suspension (10^{-1}) was transferred to 9 ml of 0.1% peptone solution in a screw cap bottle to give a 10^{-2} dilution. In this way 10- fold serial dilution of samples were prepared. Sample for each dilution was thoroughly mixed using the vortex mixer.

Preparation of tea sample with tea bag

Before boiling 100 ml 0.1% peptone solution was taken into a beaker. One tea bag of each sample was submerged into this beaker for one hour. After boiling, one tea bag of each sample was taken into a beaker containing 100 ml boiled water for two minutes. In both cases before and after boiling 1.0 ml suspension from initial suspension (10^{-1}) was transferred to 9 ml of 0.1% peptone solution in a screw cap bottle to give a 10^{-2} dilution. In this way 10 fold serial dilutions of samples were prepared. Sample for each dilution was thoroughly mixed using the vortex mixer.

Preparation of nutrient media by using made tea liquor

Nutrient culture media was prepared by made tea liquor (at 100°C) instead of 0.1% peptone solution for aerobic, fungal, yeast and coliform count. This media was used for the test of antimicrobial property of made tea.

Standard Plate Count
The Standard Plate Count (SPC) is an indicator of overall degree of microbial contamination of any food sample. One ml of 10^{-1} and dilution up to 10 folds of the tea samples were inoculated using pour plate method. The plates were then incubated at 37°C for 24 hours. After 24 hours of incubation in nutrient agar media visible bacterial colonies were counted. All bacterial plate counts were expressed as the number of colony forming units (cfu) per gram (g). A dilution was selected which yields less than 300 colonies per plate due to accurate count. When replicate plates were prepared at each dilution, the arithmetical mean of the colony counts at the chosen dilution was used to calculate the microbial concentration of original sample.

Total Fungal Count
Rose Bengal Agar media was used for total fungal count. Tea samples were placed on this selective fungal media that encourages the growth of yeast and fungus while preventing the growth of others. The pH of the media was adjusted to 4.5 ± 0.1. One ml of 10^{-1} and dilution up to 10 folds of the tea samples were inoculated using pour plate method. The plates were then incubated at 30°C for 72 hours. After 72 hours of incubation visible fungal colonies were counted (cfu g^{-1}).

Total Yeast Count
Rose Bengal Agar media was used for total yeast count. The pH of the media was adjusted to 4.5 ± 0.1. One ml of 10^{-1} and dilution up to 10 folds of the tea samples were inoculated using pour plate method. The plates were then incubated at 30°C for 48 hours. After 48 hours of incubation visible yeast colonies were counted (cfu g^{-1}) and yeast colonies were confirmed by microscopic examination.

Total Coliform Count
Most Probable Number (MPN) technique (American Public Association, 1971, Standard methods for the examination of water and waste water, 13th ed., New York, USA) was used for the enumeration of coliform in tea samples. Lauryl Sulfate Tryptose (LST) broth was used for the presumptive test of coliform. Inoculation of three tube MPN series into LST broth using 1 ml inoculation of 1:10, 1:100, and 1:1000 dilutions, with triplicate tubes at each dilution. Each tube contains a durhum tube. Tubes were then incubated at 37° for 24-48 hours. After 24 or 48 hours incubation the gas forming tubes were observed and considered as coliform positive. For coliform confirmation gently agitate LST tubes and transfer a loopful of suspension to selective enrichment Brilliant Green Bile Broth (BGLB) broth. BGLB tubes were incubated at 35-37°C and examined for gas production for 24 and 48 hours. All gas positive tubes were considered to be presence of coliform. Calculation of most probable number of coliform per gram of each tea samples were done from the number of tubes showing gas formation, using a MPN index for the determination of most probable number. Positive data were recorded by matching with MPN index.

Fecal Confirmation Test
In the confirming test procedure for fecal coliform bacteria, the positive presumptive cultures are transferred to EC broth, which is specific for fecal coliform bacteria. Any presumptive tube transfer which shows gas production after 24 (+/-2) hours incubation at 44.5°C (+/-0.2°C) confirms the presence of fecal coliform bacteria in that tube and is recorded as a positive confirmed tube.

Statistical analysis
The observations were analyzed and expressed in terms of mean and standard error of mean of all studied samples by using SPSS, version 17. In order to identify whether the microbial load in acceptable limit as well as tea liquor's antimicrobial property student t test were used.

RESULTS

The assessment of microbial load of different made tea brands were done before and after boiling of made tea dust and antimicrobial property of made tea was assessed by using made tea liquor as a media solvent instead of 0.1% peptone solution in the preparation of nutrient agar media.

Microbial load before boiling
In this study, before boiling the total microbial load in all studied samples were found greater than 10^6 cfu g^{-1} and coliform load was also found greater than 10 cfu g^{-1} ($p<0.05$). The load of yeast and fungus were found less than 10^4 cfu g^{-1} ($p>0.05$). After the fecal confirmation test fecal coliform was not found. These findings are shown in Table 1.

Table 1 Microbial load in all studied samples at different conditions

Sample name	SPC (cfu/g)			Yeast (cfu/g)			Fungus (cfu/g)			Coliform (cfu/g)			Fecal coliform (cfu/g)		
	Before boiling in 0.1% peptone solution	After boiling in 0.1% peptone solution	After boiling in boiled made tea liquor solution	Before boiling in 0.1% peptone solution	After boiling in 0.1% peptone solution	After boiling in boiled made tea liquor solution	Before boiling in 0.1% peptone solution	After boiling in 0.1% peptone solution	After boiling in boiled made tea liquor solution	Before boiling in 0.1% peptone solution	After boiling in 0.1% peptone solution	After boiling in boiled made tea liquor solution	Before boiling in 0.1% peptone solution	After boiling in 0.1% peptone solution	After boiling in boiled made tea liquor solution
Tetley	1.00×10^{8} ±$3.88\times10^{2*}$	10030.75 ±3.63	149.75 ±2.17	2.00 ±0.41	2.00 ±0.41	2.00 ±0.41	10.75 ±0.47	5.00 ±0.41	3.00 ±0.41	14.00 ±0.41*	7.00 ±0.81	3.00 ±0.40	Nil	Nil	Nil
Lipton Taza	1.49×10^{10} ±$2.75\times10^{4*}$	76031.00 ±25.53	550.75 ±2.78	58.75 ±0.75	14.00 ±0.41	8.00 ±0.41	40.00 ±0.81	15.00 ±0.81	10.50 ±0.65	39.00 ±0.40*	10.25 ±0.47*	7.00 ±0.41	Nil	Nil	Nil
Ispahani Mirzapore	2.19×10^{10} ±$2.89\times10^{4*}$	84038.25 ±38.33	603.25 ±3.79	55.75 ±0.62	18.00 ±0.82	10.50 ±0.62	200.00 ±2.04	17.00 ±0.81	10.50 ±0.64	15.00 ±0.82*	10.25 ±0.47*	4.25 ±0.43	Nil	Nil	Nil
Finley	1.60×10^{10} ±$3.02\times10^{4*}$	92003.00 ±39.19	750.50 ±1.55	49.00 ±0.41	12.00 ±0.41	9.00 ±0.41	250.00 ±2.04	24.00 ±1.22	13.00 ±0.41	23.00 ±0.82*	14.00 ±0.82*	11.00 ±0.52*	Nil	Nil	Nil
Finley Tea Bag	1.63×10^{10} ±$1.24\times10^{5*}$	150008.5 0 ±80.86	1401.25 ±2.05	1199.50 ±2.10	45.25 ±1.03	26.50 ±0.95	1400.00 ±2.04	55.00 ±1.63	24.75 ±1.03	93.00 ±1.22*	28.00 ±1.22*	14.50 ±0.62*	Nil	Nil	Nil
Mirzapore Tea Bag	1.36×10^{10} ±$3.88\times10^{2*}$	120011.7 5 ±122.88	1604.00 ±1.22	1298.75 ±4.27	53.75 ±1.63	33.75 ±0.83	1600.00 ±2.04	65.00 ±2.04	30.25 ±1.25	64.00 ±1.23*	23.00 ±0.82*	19.75 ±0.85*	Nil	Nil	Nil

The data present the mean ± standard error of mean, * indicates excess to the acceptable limit significantly ($p<0.05$)

Microbial load after boiling

After boiling the findings of microbial load in nutrient media prepared by 0.1% peptone solution are shown in Table 1. In this stage the total microbial load were found to vary in the range of 10^4 to 10^5 cfu g^{-1} in all studied samples ($p>0.05$). Coliform load was found in Tetly tea less than 10 cfu g^{-1} ($p>0.05$), but in Lipton Taza, Finley, Ispahani Mirzapore, Finley Tea Bag and Mirzapore Tea Bag the coliform load was found in greater than 10 cfu g^{-1} ($p<0.05$). Fecal confirmation test was done and fecal coliform was not found. In different studied samples yeast and fungus were found to vary in the range of 2 to 65 cfu g^{-1} ($p>0.05$).

Antimicrobial property of tea

The result of microbial load of all studied samples in nutrient agar media prepared by boiled made tea liquor instead of 0.1% peptone solution which are shown in Table 1 along with the microbial load of all studied samples after boiling in the nutrient agar media prepared by 0.1% peptone solution. When the nutrient agar media was prepared by boiled made tea liquor instead of 0.1% peptone solution after boiling Mirzapore Tea Bag contained the highest microbial load 1604 cfu g^{-1} ($p>0.05$, Table 1) but in the case of nutrient agar media prepared by 0.1% peptone solution instead of boiled made tea liquor after boiling Mirzapore Tea Bag contained 120011 cfu g^{-1} ($p>0.05$, Table 1). This kind of reduction in microbial load was also found for each sample for yeast, fungus and coliform (Table 1) which is the indication that made tea liquor has the antimicrobial property. In the study of antimicrobial property of made tea liquor fecal coliform confirmation test was done and it was not found.

DISCUSSION

According to Tijburg[16], the microbial load in made tea was not safe to consume when it exceed the total microbial load 10^6 cfu g^{-1}, coliform 10 cfu g^{-1}, yeast & fungus 10^4 cfu g^{-1}. In this study before boiling the total microbial load in all studied samples were found (>10^6 cfu g^{-1}) in excess of its acceptable limit significantly ($p < 0.05$, Table 1). Bianco[17] and Street[18] also reported the high levels of microbial load were found in herbal tea. The coliform load was found (>10 cfu g^{-1}) that exceed its acceptable limit significantly ($p<0.05$) whereas the load of yeast and fungus were found (<10^4 cfu g^{-1}) in its acceptable limit ($p>0.05$, Table 1). These finding are also coincided with the findings of Tijburg[16].

After boiling the total microbial load were found to vary in the range of 10^4 to 10^5 cfu g^{-1} in all studied samples which belongs to its acceptable limit[16] ($p>0.05$, Table 1) . Coliform load was found in Tetly tea (<10 cfu g^{-1}) at acceptable limit ($p>0.05$), but in Lipton Taza, Finley, Ispahani Mirzapore, Finley Tea Bag and Mirzapore Tea Bag the Coliform load (>10 cfu g^{-1}) was found in significantly ($p<0.05$) higher from its acceptable limit and similar finding was also pointed by Tijburg[16]. After boiling yeast and fungus were found to vary in the range of 2 to 65 cfu g^{-1} in different studied samples which were in their acceptable limit($p>0.05$) and similar results were also disclosed by Tijburg[16], Romagnoli[19] and Donia[20]. According to Donia[20], fecal coliform is very harmful to human health and in this study fecal confirmation test was done and fecal coliform was not observed. So, it can postulate that properly boiled tea is safe to drink for human health.

In order to investigate the antimicrobial property of made tea, boiled made tea liquor which was extracted from the studied samples were used in nutrient agar media preparation as a solvent instead of 0.1% peptone solution and after boiling the resulted total microbial load in the nutrient agar media showed that Mirzapore Tea Bag contained the highest microbial load 1604 cfu g^{-1}. On the other hand after boiling Tetley tea showed the lowest microbial load 10030 cfu g^{-1} ($p>0.05$, Table 1) in the nutrient media which was prepared by 0.1% peptone solution. It is seen that after boiling the highest microbial load in the nutrient media prepared by made tea liquor instead of 0.1% peptone solution is lower than the lowest microbial load after boiling when the nutrient media was prepared by 0.1% peptone solution instead of made tea liquor. Consequently it was inferred the significant ($p<0.05$) difference of microbial load at each studied sample in both cases. It was seem that the fundamental factor for the different findings was the solvent of nutrient agar media preparation. Because of for the lowest microbial findings nutrient agar media was prepared by using the solvent of boiled made tea liquor whether for the highest findings the nutrient agar media was prepared by using 0.1% peptone solution. As a result the difference of microbial load is pointed that made tea liquor has antimicrobial property.

CONCLUSIONS

Throughout this study, it is revealed that made tea liquor is not safe to human health without boiling, because of excess microbial loads were found on the various local made tea brands, particularly before boiling which could not be acceptable. But after boiling microbial load were found in acceptable limit, therefore, properly boiled tea drinking is safe to human health from microbial hazards.

REFERENCES

1. Namiki M. Antioxidant/antimutagens in food. Critical Reviews in Food Science and Nutrition. 1990; 39: 273–300.

2. Wiseman S, Waterhouse A, Korver O. The health effects of tea and tea components: opportunities for standardizing research methods: report of an international workshop organized by the ILSI international subcommittee on the health effects of tea components. Critical Reviews in Food Science and Nutrition. 2001; 41: 387–412.
3. Bokudava MA, Skobeleva NI. The biochemistry and technology of tea manufacture. Critical Reviews in Food Science and Nutrition. 1980; 12: 303–370.
4. Bouakline A, Lacroix C, Roux N, Gangneux JP, Derouin F. Fungal contamination of food in hematology units. Clinical Microbiology. 2000; 38: 4272–4273.
5. Wilson C, Dettenkofer M, Jonas D, Daschner FD. Pathogen growth in herbal teas used in clinical settings a possible source of nosocomial infection. Journal of Infection Control. 2004; 32: 117–119.
6. Hauer T, Jonas D, Dettenkofer M, Daschner FD. Tea as a source of Acenetobacter baumanniiventilator-associated pneumonia. Infection Control Hospital Epidemiology. 1999; Vol. 20, pp. 592-594.
7. Abdel-Hafe AI, El-Maghaby OMO. Fungal flora and aflatoxin associated with commonly used coffee and tea powders in Egypt. Cryptogamie Mycologie. 1992; 13: 31–45.
8. Elshafie AE, Allawatia T, Albahy S. Fungi associated with black tea and tea quality in sultanate of Oman. Mycopathologia. 1999; 145: 89–93.
9. Hasan HAH, Abdel-sater MA. Studies on mycoflora and aflatoxin in regular and decaffeinated black tea. Journal of Islamic Academy of Science. 1993; 6(2): 1–6.
10. Yde M, Rillaer WV, Maeyer-Cleempoel SD. Microbiological study of tea and herb tea. Archeves Belges Medicine Sociale. 1981; 39: 488–497.
11. An BJ, Kwak JH, Son JH, Park JM, Lee JY, Jo C, Byun MW. Biological and antimicrobial activity of irradiated green tea polyphenols. Food Chemistry. 2004; 88: 447–451.
12. Sumary PD, Joseph, WMP, Kennedy E J, Gomezulu E. Indicative investigation of amount caffeine in some commercial teas and coffees found in Dodoma markets. 2011; 2(1): 323-327.
13. Tiwari RP, Bharati SK, Kaur HD, Dikshit RP, Hoondal GS. Synergistic antimicrobial activity of tea and antibiotics. Indian Journal of Medical Research. 2005; 122: 80–84.
14. Hu ZQ, Zhao WH, Yoda Y, Asano N, Hara Y, Shimamura T. Additive, indifferent and antagonistic effects in combinations of epigallocatechin. Journal of Antimicrobial Chemotherapy. 2002; 46(2): 558-60.
15. Tournas VH, Katsoudas EJ. Microbiological Quality of Various Medicinal Herbal Teas and Coffee Substitutes. Microbiology Insights. 2008; 1: 47–55.
16. Tijburg LBM. Tea flavonoids and cardiovascular disease a review. Critical Reviews in Food Science and Nutrition. 1997; 37: 771-785.
17. Bianco MI, Lúquez C, De Jong LIT, Fernández RA. Presence of Clostridium botulinum spores in Matricaria chamomilla (chamomile) and its relationship with infant botulism. International Journal of Food Microbiology. 2008; 121: 357–360.
18. Street RA, Stirk WA, Van Staden J. South African traditional medicinal plant trade - Challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology. 2008; 119: 705–710.
19. Romagnoli B, Menna V, Gruppioni N, Bergamini C. Aflatoxins in spices, aromatic herbs, herb-teas and medicinal plants marketed in Italy. Food Control. 2007; 18: 697–701.
20. Donia MAA. Microbiological quality and aflatoxinogenesis of Egyptian spices and medicinal plants. Global Veterinaria. 2008; 2: 175–181.