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Assessment of Microbial Contaminations inDried Tea And Tea Brew.

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Abstract: *During the dried tea production process, green tea leaves are undergone several steps at which microbial contaminations in tea would be possible. This study aimed to enumerate fungal and bacterial contaminations in different dried tea brands available in the local market and to enumerate microbial contaminations in the respective tea brew samples. Ten packed tea samples and five loose tea samples were purchased randomly from the local super markets and vendors under three different batches per each brand. Three different culture media; DG 18, DRBC and SPCA were used for the enumeration. Tea brew samples were prepared as per the ISO standards. Results showed that the dried tea and tea brew samples had fungal and bacterial contaminations. A significant reduction of microbial contaminations was observed in the tea brew samples which was more than 94%. Heat resistant spore forming fungal and bacterial colonies found in tea brew samples. Two packed tea brands and four loose tea brands exceeded the accepted levels for fungal contaminations. None of the packed tea brands exceeded the acceptable levels for bacterial contaminations. However, two loose tea brands exceeded that level. Such microbial contaminations may affect the human health while reducing quality and demand for tea brands.*

Keywords: *Bacterial contaminations, Fungal contaminations,Heat resistant spores, Dried Tea, Tea brew*

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I. Introduction

Sri Lanka is a well-known country for growing tea (*Camellia sinensis*) and exporting tea under a unique name called "Ceylon Tea"around the globe. Ceylon tea is well known throughout the globe because of its natural flavour. According to Sri Lanka Central Bank Annual Report, 2015[1], production of tea during that period was 329 million kilograms. History of Ceylon tea goes back to the ancient time where the British governors governed the country. Ceylon tea has been ranked number one tea for several years. During the dried tea production processes, green tea leaves are undergone several production steps such as plucking of fresh tea leaves, withering, rolling, fermentation, firing, grading and bulk packing. The possibility of having microbial contaminationswould increase during the tea production and the possible fungal contaminations may cause, due to the processing steps conducted with fresh tea leaves without cleaning or washingthem.Water, air and soil act as the primary sources for fungal contaminations while poor farming practices act as secondary sources for such contaminations [2]. Further, the improper storage conditions, improper handling andpacking conditions of tea, increased the possibility of having fungal contaminations. Soilsamples taken from the plantation area showed the aflatoxigenic fungal species whenanalyzed. Moreover, the study found that the working environment of the tea factories were also contaminated with these aflatoxigenic fungal species and such conditions may increase the health hazardous to the humans[3].

Previous studies reported that the tea samples (under weight loss tea category) taken from super markets had different types of fungal colonies and found that black tea was one of the tea types which had significantly higher number of fungal colonies when analyzed [2]. Fungal species which belong to the genus Aspergillus were also found by the previous studies [2],[3]. Because of these reasons, it is important to check whether the tea brands (packed tea and loose tea brands) available in the local Sri Lankan market are having the same situation. Since Sri Lanka has several tea manufacturing industries, they may face risks if they produce tea with microbial contaminations even at the time of consumption of tea brew. This may lead to break the competition with other competitors in the tea manufacturing field while reducing the quality of Sri Lankan tea.

This study aimed to assess fungal and bacterial contaminations found in dried tea which are available in the local market and also to determine whether there are any possible microbial contaminations even at the time of consumption of tea brew. The significance of this research project was to improve the guidelines for tea

processing, since the acceptable fungal and bacterial contaminations in tea brew have not been given by the Sri Lanka Tea Board Guidelines.

II. Materials And Methods

2.1. Sample collection

Ten different packed tea brands and five different loose tea brands were purchased randomly under three different batches per each brand. Packed tea samples were purchased from the local super markets and the loose tea samples were purchased from the local boutiques, none of which was labeled as export quality.

2.2. Isolation and enumeration

Ten grams of dried tea was put into a sterile stomacher and mixed with 90 mL of 0.1% peptone water to prepare 10^{-1} dilution. Up to 10^{-3} dilutions were respectively prepared. Two grams of dried tea was weighed into a flask and mixed with 100 mL of boiling sterile distilled water to prepare the tea brew 10^{0} dilution and up to 10^{-2} dilutions were prepared.Tea brew samples were prepared as per the specifications given by ISO 3103 [4].Isolation of common fungal contaminations in dried tea was performed with dichloran glycerol (DG 18) agar medium as per the guidelines given by ISO 21527-02 [5]. Fungal contaminations in the respective tea brew sample were isolated with dichloran rose Bengal chloramphenicol (DRBC) agar medium according to the ISO 21527-01 standards [6]. Bacterial contaminations in both dried tea and tea brew samples were isolated using standard plate count agar (SPCA) medium as per ISO 4833-01 [7].Spread plate technique was performed on DG 18 and DRBC agar media while pour plate technique was performed on SPCA medium. The DG 18 plates and DRBC plates were incubated at 25 ^{0}C for 5 days and the SPCA plates were incubated at 30 ^{0}C for 3 days.

After the incubation period, colonies found in each plate werecounted and converted to CFU / g [Colony Forming Unites per gram]for respective dried tea sample and CFU / mL [Colony Forming Unites per milliliter] for each tea brew sampleusing an equation proposed by the ISO 7218 [8].

III. Results

Figure 1. Different types of fungal contaminations found in a dried tea sample.

Almost all dried tea samples were contaminated with different fungal species with different colors and textures. Aspergillus species were abundant on the culture medium.

Figure 2. Commonly found blackish fungal colonies in dried tea.

Different fungal species were observed on DG 18 medium cultured with a dried tea sample.*Aspergillusniger*was found as dominant species most samples tested.

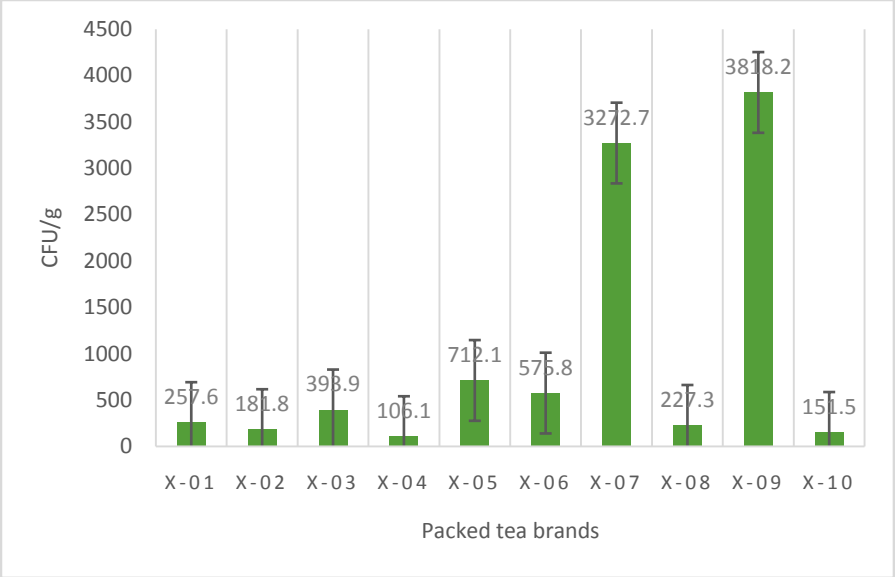

Figure 3. Fungal contaminations found in packed tea brands. (X-01 to X-10 refer to packed tea brand codes.)
Packed tea brand X-09 showed the maximum values whereas packed tea brand X-04 showed the minimum. Both packed tea brands X-07 and X-09 had fungal contaminations more than 30 times compare to the tea brand X-04.

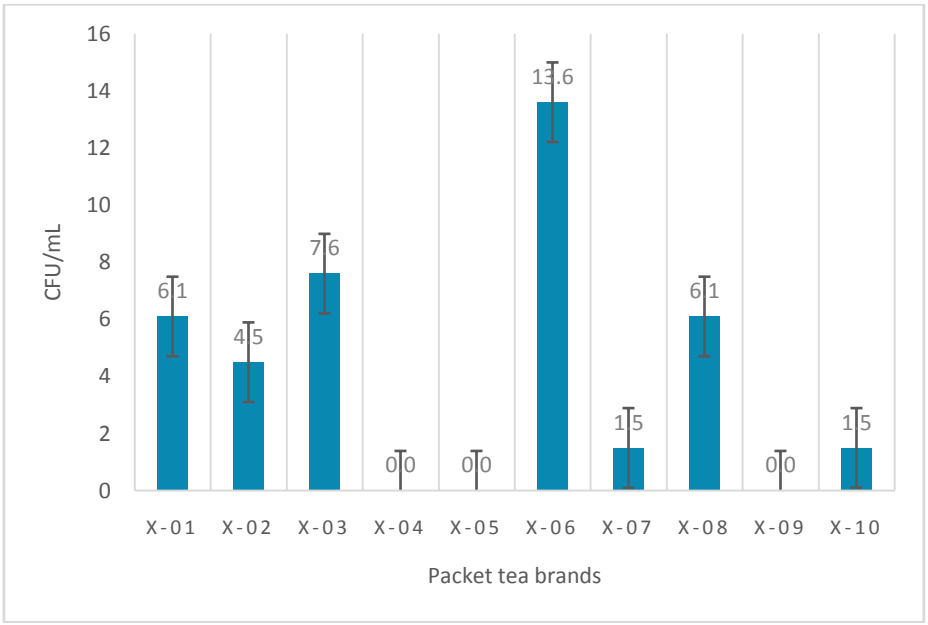

Figure 4. Fungal contaminations found in tea brew samples made from respective packed tea brands. (X-01 to X-10 refer to packed tea brand codes.)

Tea brew samples made from packed tea brands X-04, X-05 and X-09 showed zero level fungal contaminations while the tea brew sample made from packed tea brand X-06 showed the maximum value, 13.6 CFU/mL.

Table 1. Reduction percentages for fungal contaminations in tea brew samples made from respective packed tea brands. (X-01 to X-10 refer to packed tea brand codes.)

Packed tea brand	Reduction percentage for fungal contaminations
X-01	97.60%
X-02	97.50%
X-03	98.10%
X-04	100.00%

X-05	100.00%
X-06	97.60%
X-07	99.90%
X-08	97.30%
X-09	100.00%
X-10	99.00%

Tea brew samples made from packed tea brands X-04, X-05 and X-09 showed 100% reduction of fungal contaminations. Other brands showed reduction more than 97%.

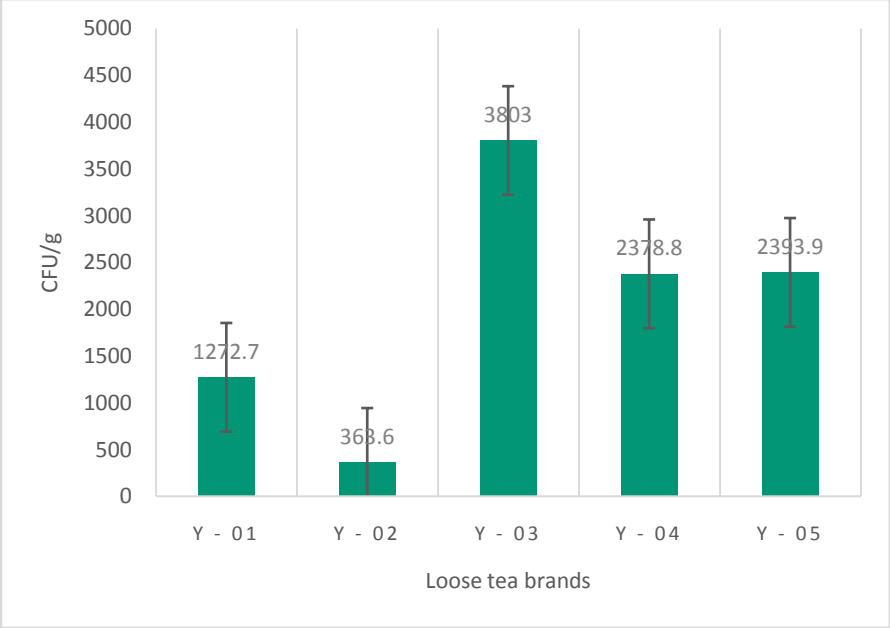

Figure 5. Fungal contaminations found in loose tea brands. (Y-01 to Y-05 refer to loose tea brand codes.)
Except loose tea brand Y-02, other four loose tea brands had high values of CFU/g.The highest CFU/g was recorded in Y-03 which was more than ten times of fungal contaminations compared to Y-02.

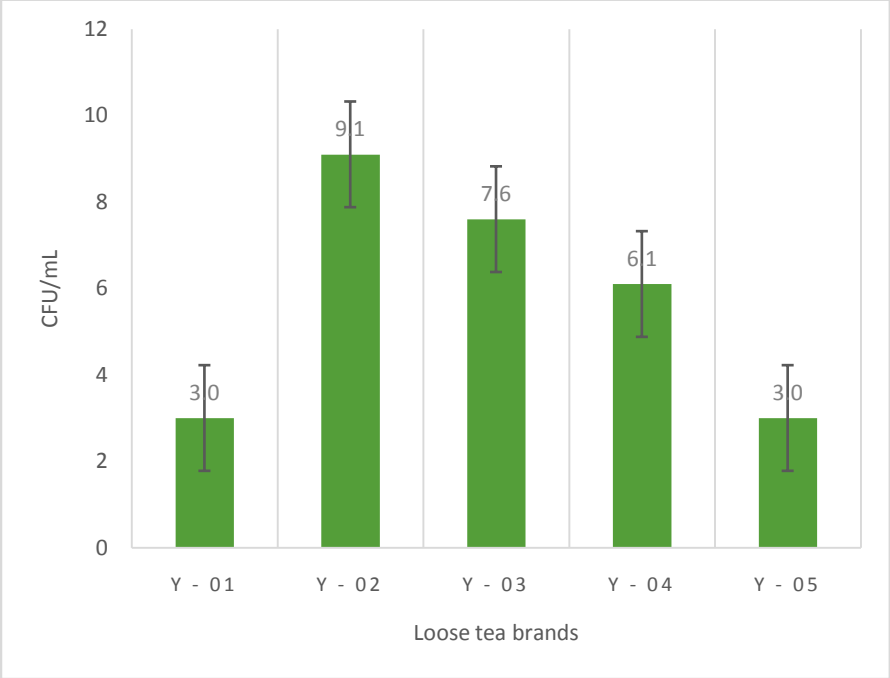

Figure 6. Fungal contaminations found in tea brew samples made from respective loose tea brands. (Y-01 to Y-05 refer to loose tea brand codes.)

Fungal contaminations were observed in tea brew samples made from the respective tea brands. Among them both tea brew samples made from loose tea brand Y-01 and Y-05 showed the minimum value, 3.0 CFU/mL while Y-02 showed the maximum, 9.1 CFU/mL.

Table 2. Reduction percentages for fungal contaminations in tea brew samples made from respective loose tea brands. (Y-01 to Y-05 refer to loose tea brand codes.)

Loose tea brand	Reduction percentage for fungal contaminations
Y-01	99.76%
Y-02	97.49%
Y-03	99.80%
Y-04	99.74%
Y-05	99.87%

Except Y-02, other loose tea brands showed more than 99% reduction of fungal contaminations.

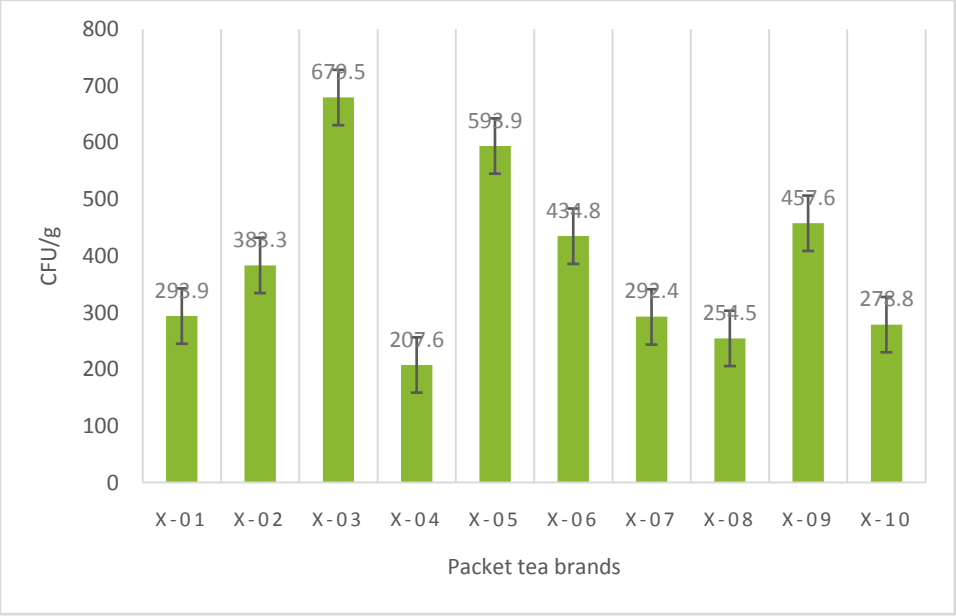

Figure 7. Bacterial contaminations found in packed tea brands. (X-01 to X-10 refer to packed tea brand codes.) Maximum bacterial contamination was given by the packed tea brand X-03 while the minimum was showed by the packed tea brand X-04.

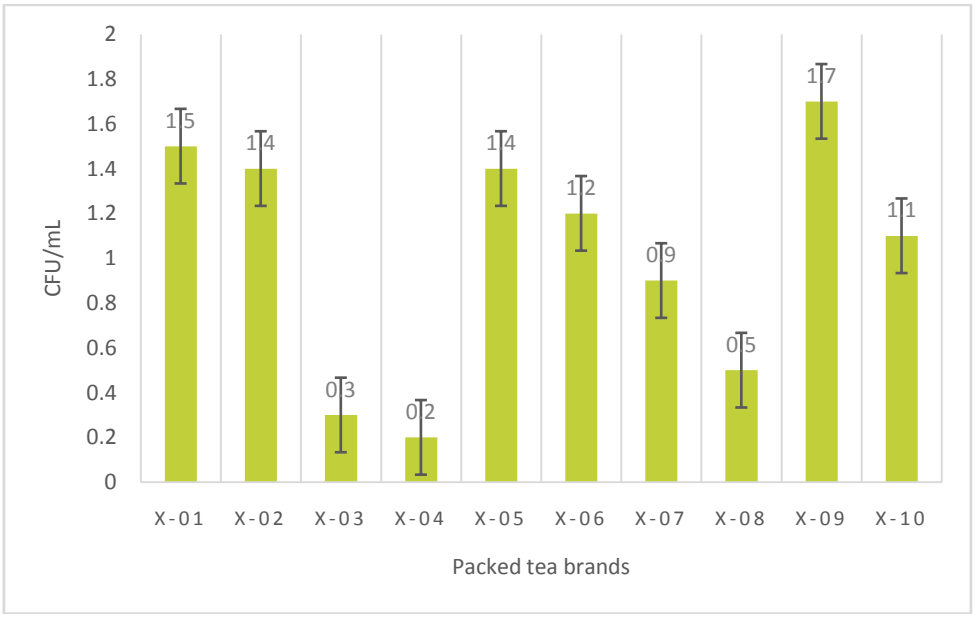

Figure 8. Bacterial contaminations found in tea brew samples made from respective packed tea brands. (X-01 to X-10 refer to packed tea brand codes.)

Maximum value was observed in X-09 and the minimum was given by X-04. All tested tea ten tea brew samples showed less than 2.0 CFU/mL.

Table 3. Reduction percentages for bacterial contaminations found in tea brew samples made from the respective packed tea brands. (X-01 to X-10 refer to packed tea brand codes.)

Packed tea brand	Reduction percentage for bacterial contaminations
X-01	99.50%
X-02	99.60%
X-03	99.90%
X-04	99.90%
X-05	99.80%
X-06	99.70%
X-07	99.70%
X-08	99.80%
X-09	99.60%
X-10	99.60%

More than 99% reduction was showed by all the tea brew samples made from the respective packed tea brands.

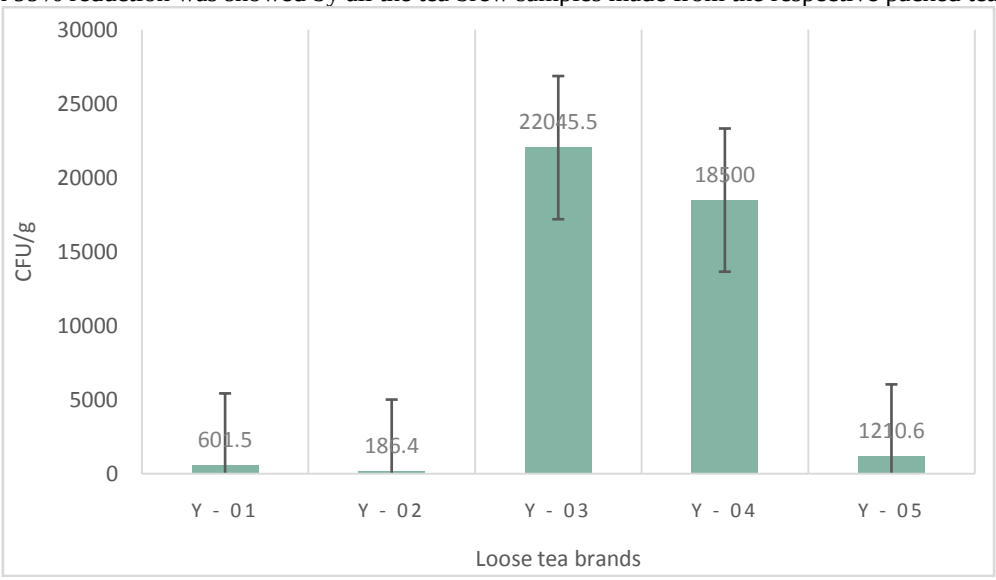

Figure9. Bacterial contaminations found in loose tea brands. (Y-01 to Y-05 refer to loose tea brand codes.)
Loose tea brand Y-03 had more than 118 times of bacterial contaminations when compared to Y-02 brand which showed the minimum.

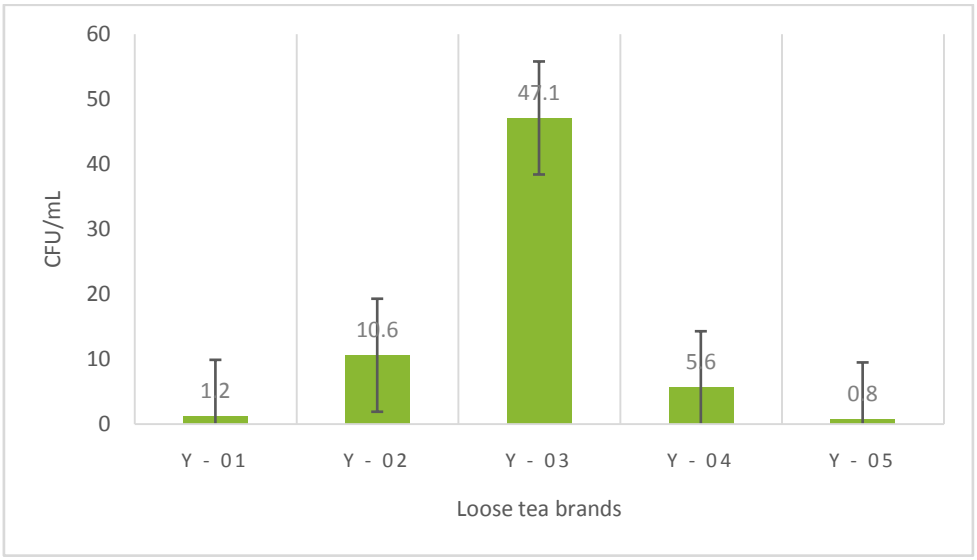

Figure 10. Bacterial contaminations found in tea brew samples made from respective loose tea brands. (Y-01 to Y-05 refer to loose tea brand codes.)

Y-03 had more than 58 times of bacterial contaminations in the tea brew sample compared to Y-05 which showed the minimum value.

Table 4. Reduction percentages for bacterial contaminations found in tea brew samples made from the respective loose tea brands. (Y-01 to Y-05 refer to loose tea brand codes.)

Loose tea brand	Reduction percentage for bacterial contaminations
Y-01	99.80%
Y-02	94.31%
Y-03	99.78%
Y-04	99.96%
Y-05	99.93%

Except tea brew sample made from Y-02, others showed a reduction which was more than 99% .

IV. Discussion

Results revealed that the dried tea samples were contaminated with different fungal species which exhibited different colors and textures.The morphological appearances proved that the dried tea had been contaminated with fungal species which belong to the genus Aspergillus [9], [10] which were common in almost all the dried tea samples. *Aspergillusniger* seemed to be dominant in the culture medium.

Fungal contaminations in dried tea was ranging from 1.06×10^2 to 3.81×10^3 CFU / g in packed dried tea brands.It was reported that the fungal contaminations on the DG 18 agar medium was ranging from 1×10^2 to 2.6×10^6 CFU / g in Pu-erhtea [11]. Fungal contaminations in dried tea, ranging from 4.1×10^3 to 4.5×10^3 CFU / g was found by a study which was performed for the weight loss tea [2].Loose tea brands showed fungal contaminations which were ranging from 3.63×10^2 to 3.80×10^3 CFU / g (Fig.5).This indicates that there were heat stable fungal species in the original dried tea samples. The tea brew samples made from packed tea had fungal contaminations ranging from 0.0 CFU / mL to 13.6 CFU / mL whereas the tea brew samples made form loose tea brands had fungal contaminations ranging from 3.0 CFU / mL to 9.1 CFU / mL.Though the tea brew was made from boiling water less fungal contaminations were observed on DRBC agar medium. However, a significant reduction of fungal contaminations was observed when it came to the time of consumption of tea brew. The reduction percentage of fungal contaminations was between 97.3% to 100.0% for the packed tea brands (Table 1),while reduction percentage ranging from97.49% and 99.87% for the loose tea brands (Table 2).

Though there was more than 97% reduction of fungal contaminations in tea brew samples, there is about 3% of possibility to have heat resistant fungal spores which can withstand the elevated temperature of the boiling water. Results revealed that the packed tea are much more suitable for human consumption as they showed 100% reduction of fungal contaminations in tea brew (Table 1 and 2) when compared with the tea brew samples made from loose tea brands.

The Sri Lanka Tea Board Guidelines [12], have given accepted levels for fungal contaminations in dried tea. The acceptable fungal contaminations have to be between 100 CFU / g and 1000 CFU / g. However, the results showed two packed tea brands exceeded this acceptable levels (Fig. 3).Though the packed tea brand X-09 showed the maximum fungal contaminations in dried tea, there was no fungal contaminations in the respective tea brew sample (Fig. 4). This indicates the idea that packed tea brand X-09, did not have any heat stable fungal spores. Among the loose tea brands, except one brand (Y-02), others exceeded the accepted levels for fungal contaminations in dried tea (Fig.5). The minimum fungal contaminations wereshown by the loose tea brand Y-02 and the same brand had the maximum heat stable fungal spores in the respective tea brew sample (Fig.6).

In the present study, it was observed that the dried packed and loose tea brands had been contaminated with bacteria.The bacterial contaminations found in packed tea were ranging from 2.07×10^2 to 6.79×10^2 CFU / g (Fig.7) whereas loose tea had bacterial contaminations ranging from 1.86×10^2 to 2.20×10^4 CFU / g (Fig.9). Tea brew samples made from the respective tea brand also had bacterial contaminations which were viable even at the boiling temperature of water.

Results revealed that there is a possibility of having heat stable bacterial spores even at the time of consumption of tea brew. Bacterial contaminations in tea brew samples made from the respective packed tea brands and loose tea were ranging from 0.2 CFU / mL to 1.7 CFU / mL and 0.8 CFU / mL to 47.1 CFU / mL respectively. (Fig. 8 and 10). However, though the packed tea and loose tea brands had bacterial contaminations in their dried form, there was a significant reduction of bacterial contaminations when it came to the time of consumption of tea brew (Table 3 and 4). There was a reduction of bacterial contaminations ranging from 99.5% to 99.9% in tea brew samples made from packed tea brands (Table 3). This indicates that there could be heat stable bacteria in tea brew ranging from 0.1% to 0.5% in the respective tea brew sample. The tea brew samples

made from the loose tea brands also showed a significant reduction in bacterial contaminations (Table 4). The reduction percentages were ranging from 94.3% to 99.9%.

According to the Sri Lankan Tea Board Guidelines, maximum bacterial contaminations found in dried tea has to be 10,000 CFU / g [12]. None of the packed tea brands had exceeded this recommended level. However, two loose tea brands (Y-03 and Y-04) had exceeded this maximum levels for bacterial contaminations. Further, loose tea brand Y-03 had the maximum heat stable bacterial contaminations in tea brew and the maximum levels of bacterial contaminations in its dried form.

Studies onweight loss tea and Pu-erh tea confirmed that the tea samples used for the studies were contaminated with *Aspergillusniger*. [2],[11]. The same fungal species was identified by this study while stating it as the most common fungal species found in almost all dried tea brands (Fig. 2).

V. Conclusions

Both packed tea brands and loose tea brands had fungal and bacterial contaminations in their dried form. Even after making the tea brew from the respective tea brands, it was possible to observe that the tea brew samples also had heat stable fungal and bacterial contaminations which were viable even at the boiling temperature of water. Results showed that the dried tea had fungal contaminations belong to the genus Aspergillus. This may indicate the possibility of havingmycotoxinsin dried tea.

Among 10 packed tea brands, 2 brands exceeded the accepted levels for fungal contaminations. Except one loose tea brand, other four loose tea brands exceeded this accepted level for fungal contaminations. None of the packed tea brands had exceeded the accepted levels for bacterial contaminations. Among five loose tea brands, two had exceeded this accepted levels for bacterial contaminations. However, there was a significant reduction of microbial contaminations at the time of consumption of tea brew.

The present study therefore would help to act as an eye-opener for the relevant authorities to pay their attention to consider the level of microbial contaminations found in the tea brew at the time of consumption.

Potential Conflicts of Interest

The authors declare that there is no conflict of interestsregarding the publication of this paper.

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