RESEARCH ARTICLE

A study on level of microbiological contamination in made tea as a raw material in commercial tea bagging factory and its workers' hand hygiene

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ABSTRACT

Hand hygiene prevents contamination in food processing; however, workers' adherence is poor. Made tea used in tea bagging were investigated with respect to microbial contamination to evaluate efficacy of hand hygiene practices. Investigation was based on workers with hand washing using selected sanitiser against wearing gloves. Samples were analysed according to Latin Square Design with replicates (n=3) and Standard microorganisms' detection methods. Study showed that no significant differences (P>0.05) were in the aerobic plate count (APC), yeast and mould between hand hygiene treatments, indicating log 11.08±0.17 cfu g^{-1} and log 10.74±0.18 cfu g^{-1}, respectively. Most frequent isolates were Aspergillus niger and, Aspergillus flavus in made tea samples. Findings of the study revealed that not only the substantial aspects of proper hand hygiene practices in workers associated with tea bagging but also using quality raw materials act as the one of fundamental importance to avoid the microbial contamination in made tea.

***Keywords**: Microbial contamination, made tea, hand hygiene, yeast and mould*

INTRODUCTION

Tea is considered to be one of the major and most popular beverages in the world and consumed by a large number of people (Oktaviana *et al*., 2016). More than 75% of the global exports of tea is from countries like China, India, Sri Lanka, Kenya and Indonesia (Basu *et al*., 2010). Among them the Sri Lankan tea industry contributes more than 13% of the total export earnings of the country (Central Bank of Sri Lanka, 2016). Bulk tea is the major form of exporting tea from Sri Lanka and recently “Value added” tea such as packaged tea and tea bags increase the relative industry capacity as another determinants of export market (Herath and De Silva, 2011). Although "Tea export Industry" makes a much more contribution to the foreign exchange earnings and employment generation, it increasingly facing with some challenges in meeting different standards when accessing the markets aboard such as industrialised countries (Wijayasiri and Jayaratne, 2011). Moreover, to protect the image of “Ceylon tea” established globally, Sri Lanka Tea Board (SLTB) has introduced and mandated a set of standards/guidelines for tea exports. All registered manufacturers and exporters of Sri Lankan origin tea must comply with these SLS standards and accepted limits for microbial contaminants in order to ensure that products meet certain desired quality characteristics (SLTB, 2005). Meanwhile it may help to regain the loss market and to compete with rising competitors by concerning more about the public health.

With regard to food, it provides ideal conditions such as nutrition source and pH value in the range for micro-organisms proliferation (McMeekin *et al.*, 1997). For instance, during post processing handling, storage and packing dry tea also prone to microbial contamination (Koch *et al.*, 2005). Based on the findings of study on *Salmonella* Agona outbreak from aniseed-containing herbal tea in Germany 2003, it stated that in dried food products such as aniseed herbal tea, *Salmonella* can adapt to the dry state and may become resistant to environmental stress (e.g., heat, lack of nutrients) (Koch *et al.*, 2005). Therefore, exporting and tea producing companies also need to develop procedures to ensure micro-biological safety of their products. The workers who engage in food manufacturing can spread foodborne illness in the food service environment through hand contact acting as a potential important mechanism which pathogens may enter the food supply (Todd *et al.*, 2007). However, published data on the effectiveness of worker's hand washing and gloves wearing practices in food service setting are limited (Montville *et al.*, 2001). With that matter when evaluating hand hygiene products for potential use in tea bagging sections, they must be aware about the relative efficacy of antiseptic agents against various sources of food contaminants and acceptance of hand hygiene products by workers (Todd *et al.*, 2010).

Wearing of gloves had become much more popular in food service establishments due to an assumption that it prevents the food contaminations from food handlers by acting as a good physical barrier (Montville *et al.*, 2001). However, the biggest issue aroused, recently is many workers feel and claim that their gloves simply make their hands too hot and sweaty, encumbering their work (Green *et al.*, 2006). Concerning the above fact makes the reason for workers to reject wearing the gloves while they are engaging in production (Green *et al.*, 2007). Hence, above reason makes a turning point in many tea export factories to engage in different hand hygiene practices from no washing to washing with soap and water also followed by sanitizers.

As hand hygiene is recognised as a key element in helping prevent the spread of foodborne illness, or aim should be to evaluate the efficacy of different hand hygiene practices such as disinfectant hand sanitisers and wearing gloves (Taylor *et al.*, 1999). Also there is a growing awareness of its relevance to food handlers or operators from different food preparation areas of tea exporting companies not only for health of consumer but also on positive motivation of workers to full fill their duties (Greig *et al.*, 2007). In the same way satisfying workers with acceptable hand hygiene practices may also plays a vital role to enhance their productive working capacity. With that recent concerns, assessment of microbial load of tea bag samples under four hand treatments were undertaken for the aerobic plate count (APC), yeast and mould, *E. coli, Salmonella* and *Coliform* to identify contaminations during tea bag handling by workers.

Therefore, the objective of this study was to evaluate the effect of wearing gloves in the tea bagging section to minimise the contamination from the foodborne pathogen.

MATERIALS AND METHODS

Sampling frame for sample collection procedure

Samples were collected from two different locations of the whole production line where manual bagging was involved. Each of locations can be categorised as follows: With gloves application (WG), Powder gloves (PG), Powder free gloves (PF) and without gloves application (WOG) as sanitiser (WS) and bare hand treatments (WOS). The above samples were then taken from workers who engage in tea bagging, after applying above four different hand treatments. All participated workers were required to present with two type of gloves wearing practices and hand washing practice with and without applying sanitizer. A total number of 24 samples was taken from the same worker, representing his/her right and left hands under each category level for a working day. Number of replications was 3.

Microbiological sample processing

All tea bag samples, which were collected into sterilised bags, were kept on ice immediately after collection and transported to the Microbiology Laboratory for further analysis following the method described in Association of Official Agricultural Chemists (AOAC), 2016. Microbial culturing was carried out to detect the level of aerobic plate count, yeast and mould count and presence of *Escherichia coli*, *Salmonella* and total *coliform* after a series of dilution was undertaken. Spread plate technique was followed inside the Bio Safety Cabinet (AC2-4E8) with sterilised glassware and culture media by autoclaving at 121 °C, 15 atm for 15 min using autoclave (LS-B100L-1). Working top was sterilised with 70% ethanol prior to culturing.

Inoculation of collected samples for bacteria

Tea samples were dissolved in buffered peptone water. Plate Count Agar, Potato Dextrose Agar, Eosin Methylene Blue (EMB) Agar, Xylose-Lysine Deoxycholate (XLD) Agar and MacConkey Agar were prepared according to the guidelines. Then, each sample was diluted up to 10^{-10} and cultured. After the inoculation samples were incubated in an incubator (FOC 2151) maintained at 37 +/- 1 °C for 48 hours (AOAC, 2016).

Total colony count and yeast and mould count

The petri plates, which afford the counting, were kept on the slanting platform of the colony counter (Galaxy 230). The number of colonies was counted with the help of digital reading meter and multiplied the count per plate by the reciprocal of the dilution factor, with which the plate was prepared (AOAC, 2016).

Hand swab sample testing

Hand swabs were carried out on bare hands of workers', prior to entering the processing. Twisting technique was used and the swab was moved across the palm covering as much as possible on the palm, and also in between 1 or 2 of the fingers were swabbed. All the Swab samples were returned into separate containers and sealed. Results were obtained after the microbiological processing (AOAC, 2016).

Enumeration of specific moulds in the made tea samples

The samples, which afford the enumeration, were mixed with Tryptone water and kept inside the incubator (FOC 2151) maintained at 37 +/- 1 °C for 24 h and then cultured on the appropriate media. Then, they were again subcultured based on colony morphology and biochemical tests were performed as required for identifications (AOAC, 2016).

Statistical analyses

As this research was conducted to investigate the effect of different hand hygiene practices on tea bag contamination, each working day and working session were considered as separate blocks and different hand hygiene practices as the treatments and accordingly, the data were subjected to analysis of variance (ANOVA) under Latin Square Design (LSD) using Minitab Version 16. Means were compared according to the Turkey test.

RESULTS AND DISCUSSION

Microbial status of tea bag samples

Research on foodborne illness risk factors has indicated that most outbreaks associated with food service establishments can be attributed to food worker's improper food preparation practices (Bryan, 1988). Todd *et al*., (2007) reported that workers who engage in food manufacturing can spread foodborne illness in the food service environment through hand contact acting as a potentially important mechanism, which pathogens may enter the food supply.

Tea bag sample analysis for aerobic plate count and yeast and mould count

The findings of microbial load in tea bag samples were not significantly ($P>0.05$) different in all four hand treatments applied. However, these values were higher than the accepted limits reported in the Sri Lankan Tea Board Standard values under SLS 516: Part 1 and Part 2 for Aerobic Plate Count (10,000 cfu g^{-1}) and, yeast and mould counts (1000 cfu g^{-1}) as microbiological requirements for made black tea. In general, bacteria require higher values of water activity (a_w) for growth than fungi where gram- negative bacteria having higher requirements than gram-positives, as yeast and moulds grow over a wider pH range than bacteria. Certain relationships have shown that optimum temperature and the presence of nutrients increases the range of a_w over which the organisms can survive (George, 2016).

Tea bag sample analysis for presence of *E. coli, Salmonella* and *Coliform*

The percentages of available *E. coli, Salmonella* and *Coliform,* were compared for the different four hand treatments applied. Accordingly, *Salmonella* test was obtained the highest percentage value for the positive results for PG treatment (Figure 1). Similarly, PF treatment and WS treatment separately indicated the highest percentage for *Salmonella* test positive results (Figure 2 and 3), except WOS treatment. The WOS treatment indicated the highest percentage for the *coliform* test.

The presence of *Salmonella* in low-moisture products is a concern because low numbers of *Salmonella* in food can cause illness. According to Figures 1, 2 and 3; *Salmonella* tests showed the high number of presence percentages for PG treatment, PF treatment and WS treatment, except WOS treatment. Even though, *Salmonella* outbreaks from low moisture products are relatively rare it often impacts large numbers of people. Similar results were obtained for spices and herbs as source of *Salmonella*-related foodborne diseases (Zweifel and Stephan, 2012). It revealed that if spices or herbs are contaminated with bacterial pathogens like *Salmonella* such pathogens, might enter the food chain (Michels, 2000). This observation is in agreement with the fact that, due to high tolerance to desiccation stress, *Salmonella* spp. can survive for an extended period of time in dried products (Hiramatsu *et al.*, 2005; Ristori *et al.*, 2007). In some instances, infection has occurred from consuming low-moisture products contaminated with less than 1 cfu g^{-1} depending on the host, the product, and the *Salmonella* strain. Food contamination caused by fecal contamination is a serious problem due to the potential for contracting diseases from pathogens (disease-causing organisms). Coliforms are often referred to as "indicator organisms" because they indicate the potential presence of disease-causing bacteria (Halkman *et al.*, 2003). According to the findings presented in Figure 4 for coliform bacteria, high counts generally indicate that unsanitary conditions or poor hygienic practices during or after the production.

Microbial status of made tea samples used as raw material

In the food supply chains, incoming raw materials include as a potential source of bacterial contamination (Simonne and Treadwell 2008). A study of microorganisms in made tea, and their activity under conditions of storage, indicated that the drying of dhools did not sterilise the made tea but did give a reduction in bacterial count (Ekanayake, 1987). So, the values, which exceed the accepted limits, can take as another agreement with the findings of Ekanayake (1987).

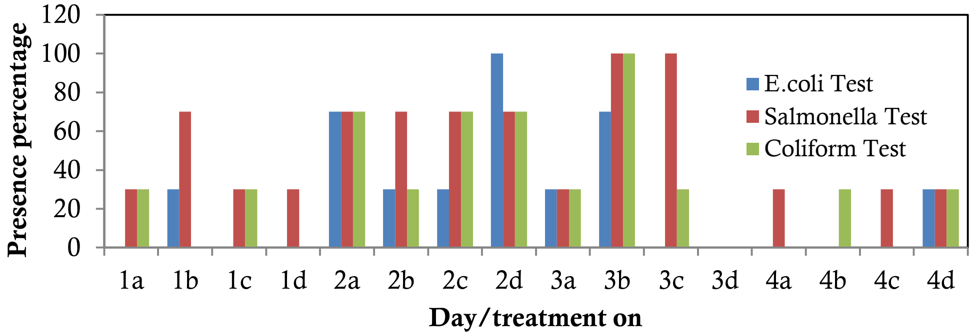


Figure 1: Percentages of *E. coli, Salmonella* and *Coliform* tests in powder gloves treatment. a = Female worker (right hand), b = Female worker (left hand), c = Male worker (right hand) and d = Male worker (left hand).

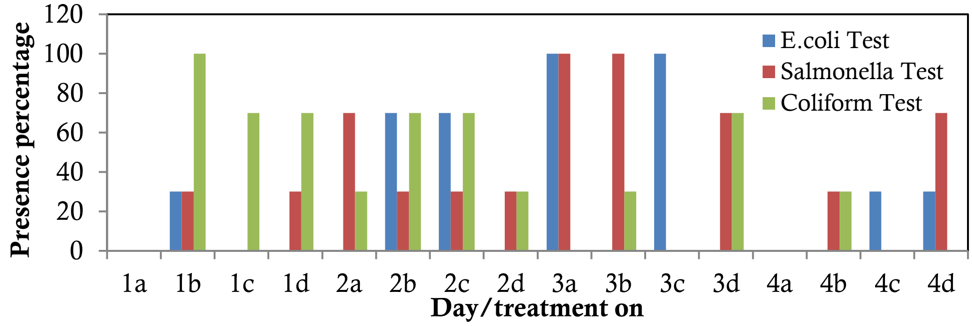


Figure 2: Percentages for *E. coli, Salmonella* and *Coliform* tests in powder free gloves treatment. a = Female worker (right hand), b = Female worker (left hand), c = Male worker (right hand) and d = Male worker (left hand).

Made tea analysis for aerobic plate counts and yeast and mould counts

Made Tea as a raw material is a leading factor of microbial contaminations in addressing the final quality of "Ceylon Tea" (Anon, 2006). It revealed that, the count values obtained were higher than the hypothesised mean (Sri Lankan Tea Board Standards for Microbiological requirements for Black tea SLS 516: Part 1 and SLS 516: Part 2). These findings are shown in Table 1. Since this study was basically focused about the employee hand hygiene practices, who engaged in tea bagging by directly contact their hands with tea bags, it indicates that it is necessary to concern more about the raw materials that they are used in this manufacturing process as they were already contaminated beforehand-contacting.

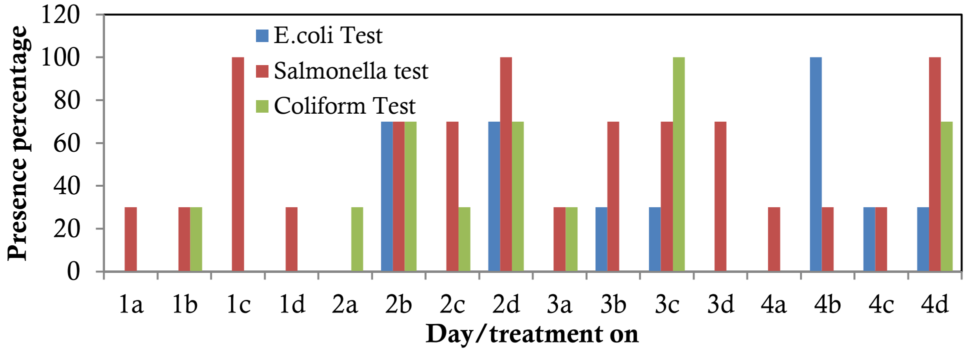


Figure 3: Presence percentages for *E. coli, Salmonella* and *Coliform* tests in Sanitizer treatment. a = Female worker (right hand), b = Female worker (left hand), c = Male worker (right hand) and d = Male worker (left hand).

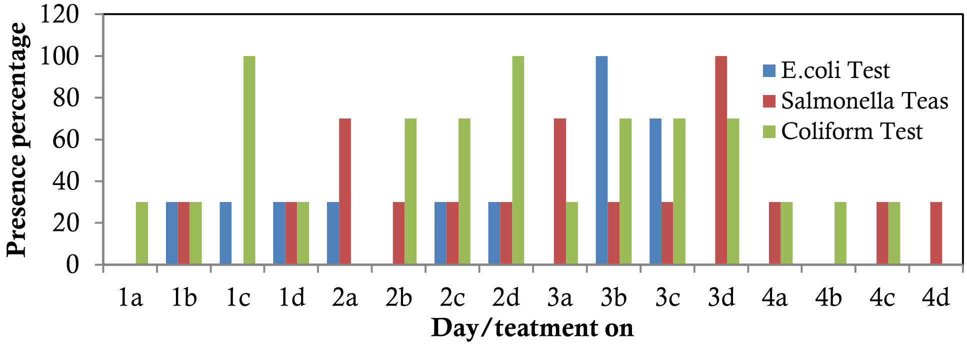


Figure 4: Percentages for *E. coli, Salmonella* and *Coliform* tests in bare hand treatment. a = Female worker (right hand), b = Female worker (left hand), c = Male worker (right hand) and d = Male worker (left hand).

Table 1: Aerobic plate count and yeast and mould count values for made tea.

	Day 01	Day 02	Day 03	Day 04
	(log CFU g^{-1})	(log CFU g^{-1})	(log CFU g^{-1})	(log CFU g^{-1})
Aerobic plate count	11.17 $\pm$ 0.11	10.50 $\pm$ 0.60	11.26 $\pm$ 0.16	10.93 $\pm$ 0.04
Yeast and mould count	10.535 $\pm$ 0.18	10.72 $\pm$ 0.03	10.66 $\pm$ 0.12	10.95 $\pm$ 0.15

Made tea analysis for presence of *E. coli, Salmonella* and *Coliform*

Microbial contamination or microbial spoilage is one of the major reasons, which basically affect the quality of any food in which made black tea is not exceptional (Stagg, 1974). As recorded results indicated that out of 96 made tea samples, as the raw materials examined, 72 (75%) samples, 56 (58%) samples and 64 (67%) samples were positive for *E.coli*, *Salmonella* and *Coliform* tests, respectively.

Worker's hand swab samples

The results between the standard of tea bag handlers with hands swab samples demonstrate that out of 72 samples examined, number of positive samples for *E. coli* and *Salmonella* tests on female worker's hands were within satisfactory limits for both right and left hand (Figure 5 and 6). In the same manner for male worker's hands the *E. coli* and *Salmonella* tests showed satisfactory limits for both right and left hands: 11 & 22% and 0 & 22%, respectively (Figure 5 and 6). *Coliform* was found to be the highest among the other two indicators as all of the samples (100%) were positive. Food handlers should improve on good hand hygiene practices relevant to washroom use as the incidence of *Coliform* on food handlers hands were extremely high.

Generally, Good Manufacturing Practices (GMP) can help diminish potential microbial contamination in made tea, as that employee hand hygiene practices and raw material quality came out as the major contributing in this study (Wijayasiri and Jayaratne, 2011). Since this study confined only to tea bagging in commercial tea export company it is necessary to extend this to primary black tea manufacturing process in factories that cover the different manufacturing practices after drying of black tea such as grading, tasting and auction process before engage in packing, in addressing the final quality of "Made Ceylon Tea" in general.

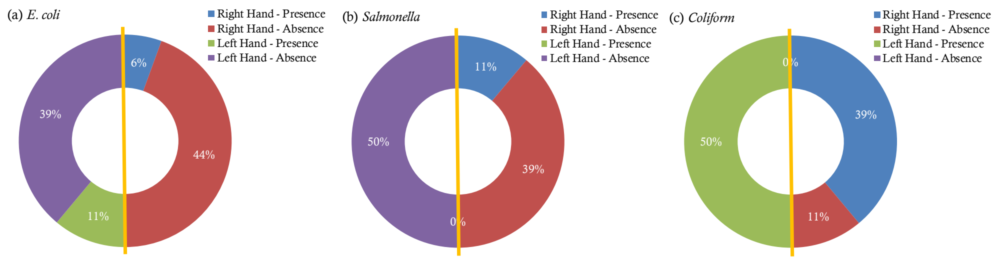


Figure 5: Presence and absence percentages for (a) *E. coli,* (b) *Salmonella* and (c) *Coliform* tests in swab sample from female worker's hand.

Enumeration of specific moulds in the made tea samples used in tea bagging

Made tea can contain a range of main moulds species as storage with free access to air and moisture increased the fungal count in made tea. In meeting the specific objective, special attention was paid to the *Aspergillus* spp. and the organisms were identified morphologically and microscopically. Among them, *Aspergillus niger* was the most frequent isolation and *Aspergillus flavus* was found to be an occasional occurrence (Plate 1).

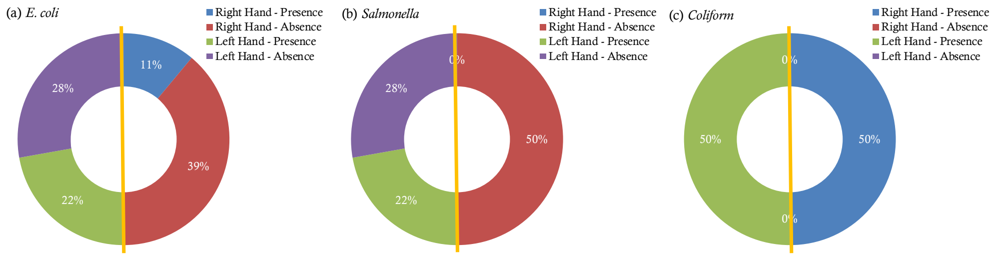
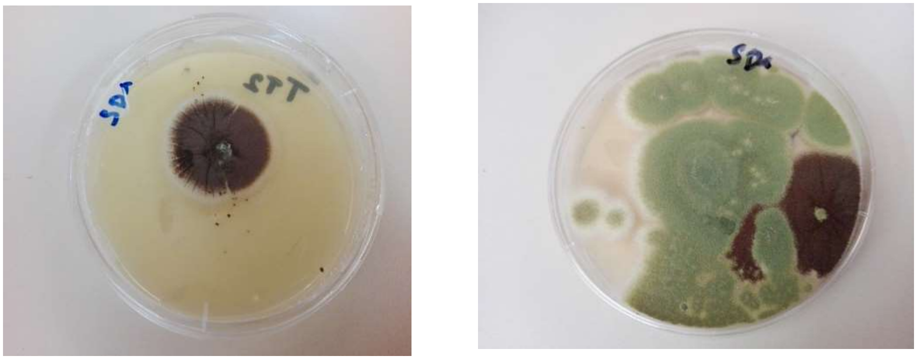


Figure 6: Presence and absence percentages for (a) *E. coli,* (b) *Salmonella* and (c) *Coliform* tests in swab sample from male worker's hands.



(a) (b)

Plate 1: Observed samples of different *Aspergillus* species (a) *Aspergillus niger,* (b) *Aspergillus niger* and *Aspergillus flavus.*

CONCLUTIONS

Major challenges for microbial control in made tea include sourcing of low-risk raw materials and ingredients, controlling cross-contamination from storage to post-process handling, and implementing and monitoring validated employee personal hygiene practices. The ability of microbes to survive for long periods of time in dry foods, ingredients, and processing environments greatly increases the challenge of controlling microbes in made tea. In this study, microbial counts were found to have no significant difference for the all four hand treatments applied, as the values are considerably higher than the accepted limits reported in the Sri Lankan Tea Board Standard values for microbiological requirements in made black tea. It indicates raw material as the primary contamination source in tea bagging. Hence, the efficacy of glove application and sanitiser application found no significant difference as hand hygiene practices, which food handlers engaged. Effective control for incoming raw materials should be in place as well the monitoring programmes should address the external environment, employee hand sanitation, operational and primary manufacturing practices to address the final quality of the tea bags.

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