Isolation and Identification of possible Microbes Associated with Black Tea in a Commercial Tea Blending Process Factory

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Abstract: Exportation of tea in Sri Lanka, formerly called Ceylon Tea, is one of the main income source. Quality deterioration of blended tea during storage and post-process handling are significant concern which makes tea unfit for exporting and human consumption. Present study was undertaken to investigate the microbes associated with blended black tea. Samples of tea from 3 collection points as raw material feeding point, conveyor belt, and bag feeding point have been examined for the enumeration of total aerobic bacteria, Coliforms, *Escherichia coli*, *Salmonella*, yeast and mould. Microbial count of raw black tea and blended black tea for total aerobic bacteria and yeast and mould were $\log 12.21 \pm 0.62$ cfu/g, $\log 13.36 \pm 0.87$ cfu/g and $\log 11.99 \pm 0.74$ cfu/g, $\log 12.08 \pm 0.87$ cfu/g respectively and exceeded the acceptable level according to the Sri Lanka tea Board. Major species isolated were *Aspergillus niger*, *Aspergillus flavus*, *Mucor* sp. and *Klebsiella* sp. However with boiling water these microbes were destroyed. Findings revealed that, raw material receiving to the factory was the main contamination which can be minimize with initiating Good Manufacturing Practices (GMP) in the primary processing factories.

Keywords: Blended tea, raw black tea, *Aspergillus niger*, Good Manufacturing Practices

Introduction

Tea, which is the main exporting agricultural commodity in Sri Lanka has recorded a positive growth in tea production in 2017, reversing the declining trend of past four years .Sri Lanka produced 307.7 million kilograms of made tea in 2017 with a growth of 5.2 per cent compared to 292.6 million kilograms in 2016 (Oktaviana, Masyhuri, & Hartono, 2016). Tea manufacturing process is conducted in more than 700 factories all over Sri Lanka under different climatic conditions, different elevations and using slightly different manufacturing procedures. Manufactured tea is then sold in Colombo tea auction and afterwards buyers blend the bought tea lots prior to export or sell in the local market. Tea blending is the last process step of manufacturing tea, before exporting tea as bulk (Wijayasiri & Jayaratne, 2011). In recent years, food poisoning has become one of the major health problems locally and internationally (McMeekin et al., 1997). Among them microbial foodborne diseases may occur due to presence of microbial pathogens or microbial toxins (Lammerding & Fazil, 2000). Other than processing conditions worker hygiene and equipment cleanliness are important sources among various sources of contaminations in food processing (Heaton & Jones, 2008). Based on the findings of study on *Salmonella* Agona outbreak from aniseed-containing herbal tea in Germany 2003, Koch et al. (2005) 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" (p. 1127).

Quality of the product plays a vital role in the bilateral trade (Hallak, 2006). It can be degraded due to unfavorable temperature, atmosphere, relative humidity and microbiological activities in food products. Hence, it is essential to maintain the quality of "Ceylon tea" also to regain the loss market and to compete with rising

competitors (Hicks, 2009). Processed dry tea leaves are disposed to microbial contamination during post-processing handling and storage. Also, during storage under high humidity, absorption of moisture may boost fungal growth and result in caking of the product (Mishra, Gautam, & Sharma, 2006). Hence, there are several possibilities in microbial contaminations in tea blending process. These microbial contaminations during the tea blending process may lead to physical damage, chemical alterations (oxidation, colour changes) or off-flavours and off-odours and product can affect hazardously on consumer health (Gram et al., 2002).

Microbial contamination in food raises questions not only on consumer health but also on economic consequences (Smith, Van Ravenswasy, & Thompson, 1988). Microbial contamination of tea may lead to reject at point of export and foodborne diseases. Rejecting tea at point of export will cause a loss of international tea market. Exporting quality deteriorated tea will degrade the brand reputation of “Ceylon tea” (Selnes, 1993). Negative impression regarding food safety might reflect a main effect on purchases than positive impression. Restoring customer confidence regarding food safety is harder than initiating (Smith et al., 1988). Hence, exporting firms should concern about the mandatory SLS standards regarding microbial contaminations to identify possible microbial contamination sources in tea blending process to eliminate microbial contaminations in tea. Therefore, the objective of this study was to isolate and identify possible microbes associated with black tea in a commercial tea blending process factory.

Materials and methods

Sampling procedure

Tea samples were collected from the following predetermined places: conveyer feeding point (starting point), residual removing process (mid-point) and bag feeding hopper (end point). In addition swab samples were collected from equipment use to tear tea bags in the conveyor feeding point, equipment use to collect tea from blending floor, equipment use to fill tea bags in end point, tea blending floor and employee's hand. This sample collection procedure was repeated until contamination points in tea blending process were identified with considerable replicate samples and the hygienic conditions practicing by the relevant factory was recorded. All samples were kept in pre-chilled containers with chiller packs and were processed within 24 hours of collection. 2.5g of tea sample was sampled for the determination of microorganisms indicated in microbiological analyses section.

Microbiological Analyses

Enumeration of total aerobic bacteria

Plating was performed into plate count agar /PCA (AOAC, 2016) from the prepared dilutions by spread plate method. Colonies formed after 48 h incubation at 30 °C under aerobic conditions were counted.

Enumeration of yeast and mould

Plating was performed into potato dextrose agar /PDA (AOAC, 2016) from the prepared dilutions by spread plate method. Colonies formed after 48 h incubation at 30 °C under aerobic conditions were counted.

Enumeration of coliform

MacConkey agar (AOAC, 2016) was used for presumptive and confirmed tests for coliforms. Colonies formed after 48 h incubation at 30 °C under aerobic conditions were counted.

Enumeration of Escherichia coli (E.coli)

Plating was performed into eosin methylene blue /EMB (AOAC, 2016) from the prepared dilutions by spread plate method. Colonies formed after 48 h incubation at 30 °C under aerobic conditions were counted. Enumeration of Salmonella.

From the prepared dilutions of the samples, plating was performed by spread plate method onto Xylose Lysine Deoxycholate agar/XLD (AOAC,2016). Colonies formed after 48 h incubation at 30 °C under aerobic conditions were counted.

Isolation and Identification Procedure for Bacteria

Dilutions of all the samples were prepared by using tryptone water and incubated for 1 h at 37 °C. Then prepared dilutions of samples were cultured on Blood Agar and MacConkey agar for the isolation of bacteria and for the isolation of Salmonella spp. All the samples were enriched in Selenite broth and cultured on Xylose Lysine Deoxycholate (XLD) agar. Gram staining was then performed for the positive cultures. After that the biochemical tests were performed including Triple Sugar Iron Agar (TSI) test, Urease test, Citrate test and Indole test as the confirmation tests.

Isolation and Identification Procedure for Fungi

Dilutions of all the samples were prepared by using tryptone water and incubated for 1 h at 37 °C. All samples were cultured on Sabouraud Dextrose Agar (SDA) for the isolation of fungi. Isolated fungal species were identified according to the morphology and Lactophenol Cotton Blue staining.

Statistical analysis

Resulted numerical data were analyzed through mean value comparison (mean $\pm$ standard deviation) using Minitab 17 package. Mean values of tea samples according to collecting points were compared using one way ANOVA. Colony forming unit values of each test were benchmarked with SLS mandatory standards using hypothesis testing with sample-t test.

Results and discussion

The average total aerobic bacterial count and yeast - mould count in made black tea were exceeds the acceptable limits of Sri Lanka Tea board: SLS 516 part 1 and SLS 516 part 2, respectively (Figure 1). Temelli, Dokuzlu, & Sen, (2006) has explained that total aerobic microbial count as an important indicator for both determine the hygiene of food and microbiological contamination sources during food processing. According to the Sri Lanka warehouse tea blending reports in 2017, yeast and mould outbreaks have occurred in four times in 2017.

Even though Salmonella should absence in 25g of Black tea according to SLS threshold limit followed by Sri Lanka Tea Board (SLS 516: Part 5: 1992/ISO 6579:2002), 10% samples of black tea samples, which were checked for the presence of Salmonella, were positive. Low-water-activity foods did not support growth of vegetative pathogens such as Salmonella (Podolak, Stone, Black & Elliott, 2010). Therefore the risk of multiplying these in black tea is minimum. However in order to overcome the risk of presence of Salmonella, moisture content should be kept in minimum level in black tea.

On the other hand, 12% black tea samples were positive for the E. coli test. However the acceptable threshold limit in Black tea should be absent (SLS 516: Part 3: 1982/ISO 7251:2005). Contamination of fecal matter and manure may be the reason for presence of E. coli in Black Tea (Solomon, Yaron, & Matthews, 2002). Temelli et

al. (2006) in his research revealed that it is also as an indication of poor sanitation conditions, insufficient heat application or recontamination.

From analyzed black tea samples 91.67% samples were negative for Coliform test. Bacteria such as fecal coliforms are used as indicators of fecal pollution (Whitlock, Jones, & Harwood, 2002). The results show that samples had less contamination chances through fecal Coliforms. This gives a better condition than the previous two where Salmonella and E. coli were positive.

From the initial sample collecting point, microbial count was higher than the acceptable limits. It indicates that raw materials were already contaminated in the primary manufacturing process (Figure 1). Statistically proved that there is no significant difference between sample collecting places in blending process ($p>0.05$). Use of quality deteriorated raw materials cause to contamination of blending process.

Figure 1 Aerobic microbial count and yeast-mould count in blending process

When comparing swab samples from brand new packing bags and used tea bags: brand new packing bags were negative for Salmonella, Coliforms and only 8.33% samples were positive for E. coli test. Swab samples from used tea bags show 25%, 16.67%, 8.33% positive for E.coli, Salmonella and Coliforms tests respectively. Remaining tea particles from previous packing may be the reason to show positive results. Used tea bags should undergo a proper cleaning and sanitation process prior to use.

Swab samples from blending floor shows highest risk of contamination comparing the rest. Swab samples were positive for E. coli, Salmonella and Coliforms in 41.67%, 41.67% and 50% respectively (Figure 2). Walking across the blending floor and remaining particles from previous blending may be the reason for risk of contamination. Strict cleaning process should apply to the blending floor and walking across the blending floor should be avoided.

Figure 2 Swab samples from blending floor (% values)

Employees' hand is another point which has high risk of contamination. Employees engage with blending process without using gloves creates high risk of contamination results show 25%, 25% and 33.33% positive percentages for *E. coli*, *Salmonella* and *Coliforms* tests respectively (Figure 3). Presence of *Coliforms* indicates risk of contamination from fecal matter (Whitlock et al, 2002). Therefore use a sanitizer to maintain hand hygiene and gloves usage when involving with blending process should be recommended to minimize this risk of contamination.

Figure 3 Swab samples from employee's hand (% values)

Supporting equipment were positive for *E. coli*, *Coliform* and *Salmonella* tests but all percentage values are equal or below 25%. Equipment surfaces contaminated from previous blending and particles remaining even after cleaning. Although microbes have higher viability on stainless steel surfaces proper cleaning and removing of remaining particles from previous blending can minimize cross contamination (Noyce, Michels, & Keevil, 2006).

Enumeration of Specific moulds in the made tea samples

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 organisms were identified morphologically and microscopically. Among them *Aspergillus niger* was the most frequent isolation and *Aspergillus flavus* were found to be in occasional occurrence (Plate 1).

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

Summary

These results conclude that contaminated raw materials can be identified as the major source of contamination in tea blending process while labour hygiene and microbial quality of blending floor were identified as possible contamination sources in tea blending process. It indicated a contamination before the initiate tea blending process. Hence attention should be focus on primary tea processing process in factories to minimize contaminations and contamination points in order to export quality tea. As consequences, there is a requirement to initiate GMP for primary tea processing to select tea with low initial microbial counts to the plant. More emphasis should be focused on processing in proper hygiene conditions with sufficient sanitary applications to eliminate remaining particles from initial blending to minimize cross contaminations and reduce the risk of contamination from blending floor and employees' hands. Further studies are needed to confirm the existing microorganisms in tea and recommended changes should be verify after implementing them in the black tea blending process.

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