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Peeping into The Kettle: A Review on the Microbiology of 'Made Tea'

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Abstract

Tea constitutes one of the most widely consumed aromatic beverages of the world, with a multitude of health benefits attributed to it. Post-collection, the tea leaves are subjected to various grades of processing and fermentation to derive its aroma and impart characteristic flavor. Normally, the processed tea is boiled in water, with or without other condiments, milk, sugar, etc to make 'made tea'. However, use of water at a sub-boiling temperature might not eliminate all microbes, including spores of certain bacteria. Although tea has medicinal and antimicrobial principles, yet it may occasionally be laden with microbes and contaminated with pathogenic bacteria, yeast and molds during processing and storage, which may pose a potential health risk. As every day millions of people drink at least one cup of tea, an overall analysis of the contamination profile of different kinds of made tea is of utmost importance and an absolute mandate these days. Hence, this article intends to review the level of microbial load of made tea based on available literature. Over the past few years, reports have also been accumulated indicative of the presence of different bacterial pathogens in tea leaves, besides its contamination by pathogenic fungal strains and mycotoxins. The potential of a number of other herbal condiments and organic spices that could be supplemented with made tea to impart additional antimicrobial properties to this highly sought-after drink has also been discussed here.

Keywords
Contamination profile, health risk, made tea, microbial load, pathogens.

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INTRODUCTION

A Tea Tale

Drinking has been a large part of socializing throughout the centuries. Beverages (from Latin *'bever'* meaning to '*rest from work'*) are drinks intended for human consumption. They not only satiate thirst, but also play important roles in human culture [1]. There are various kinds of beverages, including tea, coffee, hot chocolate, carbonated soft drinks and alcoholic drinks.

Tea is one of the most ancient and popular beverages consumed across the world. Drinking of tea is a long-

established practice, which is firmly rooted in the heritage of almost every global civilization. Tea is the second most-consumed drink, only after water [http://www.teausa.com/14655/tea-fact-sheet]. It is an aromatic beverage commonly prepared by pouring hot or boiling water over the processed leaves of tea plant (*Camellia sinensis* L.) [https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=506801#null] The black tea is almost a part of daily diet in the Indian subcontinent and is widely considered to be a boon for healthy living [2].

The primary center of origin of tea was South-East Asia [3]. Today India and China are the largest producers of tea globally [https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN & search_value=506801#null].

In India, Assam is the largest tea producer [4] (Figure 1).

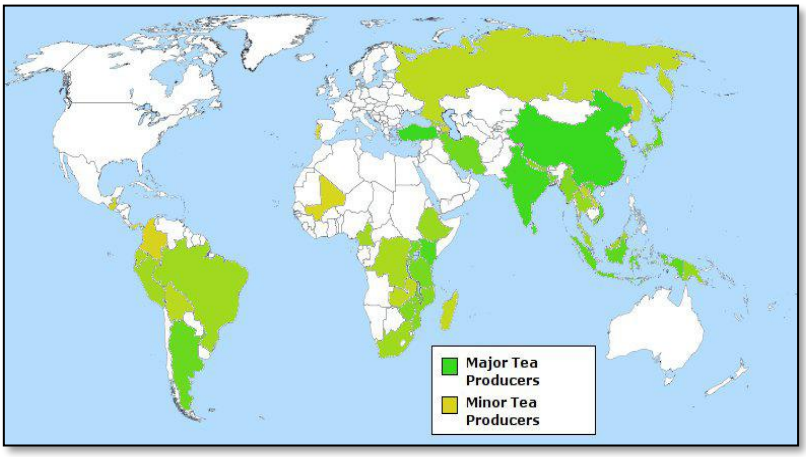

Figure 1. World Map of Tea Producing Regions
[Picture Courtesy: FAOSTAT, Food and Agriculture Organization]

Tea shoots harvested for processing generally comprises of the apical bud and the youngest two leaves including the stem. Young green tea shoots are extremely rich in polyphenolic compounds which are responsible for the unique character of processed tea [5]. Different varieties of tea are processed by different ways and means, and almost invariably involve a few common steps [6]. The leaves or buds of tea plants are collected from the tea garden and subjected to processing without cleaning or washing, and thereafter stored and packaged for sale.

The evidences emerging from scientific research on health benefits of various kinds of tea is very encouraging indeed. Tea makes a remarkable contribution to a healthy lifestyle, and in particular, in areas of chronic diseases including cancer and cardiovascular infirmities. However, for all the beneficial effects of tea to healthy life, there are substantial risks of contamination of tea leaves by both normal microflora and harmful pathogens. There are no regulations or load limits concerning microbial contamination of teas and infusions. Even though tea phyllosphere contains a natural microflora, because of its low water activity (a_w) and inherent antibacterial activity against selected bacteria, the risk of microbial proliferation is not very significant [7, 8]. However, any excessive moisture could lead to profuse growth of microbes in tea. Prior reports have already pointed to the fact that different tea samples analyzed have various kinds of bacterial and fungal contamination. Tea infusions or 'made tea' prepared at a sub-boiling temperature to preserve its aroma, do not eliminate these microbes to safe limits [9]. If tea leaves are contaminated with pathogenic microorganisms during processing and storage, the drink may pose a potential health risk culminating in disease manifestations [10]. This could be alarming, given that tea is 'generally recognized as safe' (GRAS) and almost 2.16 billion cups of tea are consumed worldwide, per day [https://www.fda.gov/, https://worldteanews.com/tea-industry-news-and-features/tea-consumption-second-only-to-packaged-water]! Because of these reasons, it is important to check whether tea prepared from different varieties of branded packed and loose tea have any such precarious situation. So, this review is intended to present an overview of the research work that has been carried out to assess the status

of microbial load and the potential risk of a number of made tea varieties.

In contemporary times, people are making a shift to using more natural ingredients for the foods they eat, and often choose tea mixed with healthy dried herbs [11]. Thus, this review also assesses the potential of a number of herbal condiments and organic spices that could be supplemented with tea to impart added antimicrobial properties to it, in addition to the natural active principles of tea itself.

Tea Processing

The method by which the leaves from the tea plant are transformed into dried leaves for brewing is called tea processing (Figure 2).

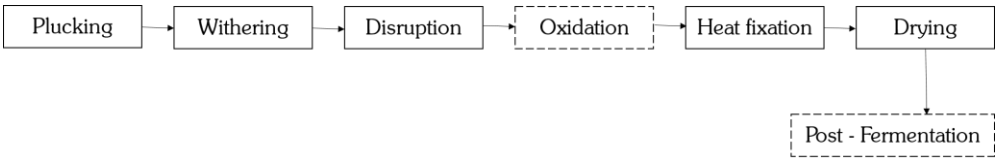

Figure 2. Basic steps of tea processing
[Flowsheet Courtesy: Tan S. Master Thesis, Lund University, Lund, Sweden, (2014)]

Plucking of leaves is the first step, done either manually or mechanically. Plucking requires a defined bud leaf configuration called a 'plucking standard', viz., two leaves and a bud [12]. Thereafter, the fresh tea leaves are withered or dehumidified of redundant water, either by sun exposure or by storage in a cool breezy place. For tea leaves requiring oxidation, this is followed by mechanical disruption of the leaves by bruising, rolling and crushing. When a leaf's cells are damaged as a result, their insides are exposed to oxygen. When polyphenols in the cell's vacuoles, and the peroxidase in the cell's peroxisomes mix with polyphenol oxidase in the cell's cytoplasm, an oxidation reaction begins [13]. The degree of oxidation is further enhanced by adapting a suitable temperature and moisture to the disrupted tea leaves. The polyphenols known as catechins are oxidized to complex tannins and theaflavins, thus contributing to the dark color, as well as the unique taste and aroma of tea [14]. Thus, the degree of oxidation is in direct relation to the sensory quality of tea. For tea leaves which do not require oxidation, heat fixing is done to limit the oxidation process. Frying the tea leaves reduces their moisture level and inactivates their oxidative enzymes [6, 15]. For the fermented tea varieties, selected microorganisms such as molds (*Aspergillus* spp.) are artificially introduced post-heat fixation [6, 15]. These fungi catalyze exo-oxidation in tea leaves, which contributes to the deep color and distinctive taste of fermented tea. Then, all types of tea leaves are sun-dried or baked, before being packaged for sale in the market. Finally, tea infusion is brewed properly by consumers in gentle boiling or sub-boiling water (80–100°C) for 3-5 minutes, depending on the tea type. The tea leaves are allowed to steep for the proper length of time, decanted and served after cooling to room temperature. For making a decoction of tea, the leaves are boiled in the water continuously, and then served hot.

Different Kinds of Tea

All types of tea come from the same basic plant, *Camellia sinensis* L. Differences between types of teas arise predominantly from processing (depending on different degrees of oxidation), growth conditions and geographical parameters. Two principal varieties of the tea are *C. sinensis* var. *assamica* and *C. sinensis* var. *sinensis*. The *assamica* variety of tea grows large leaves and is endemic to the Assamese belt. This has been traditionally used for black tea, although in recent years some green and white teas have been produced. The *sinensis* variety of tea grows short leaves and is grown in the Sino-Tibetan region. This is used for most other types of teas [https://tandemtea.com/blogs/tea-master-class/meet-the-tea-family-camellia-sinensis-sinensis-and-camellia-sinensis-assamica].

Teas can be divided into six basic kinds (Figure 3):

Figure 3. Six various kinds of teas: (a) Black Tea, (b) Pu'er Tea, (c) Oolong Tea, (d) Green Tea, (e) White Tea, (f) Yellow Tea.
[Picture Courtesy: Google Image]

(i) Black Tea

Black tea is allowed to wither, during which moisture evaporates out of the leaf and the leaf imbibes more oxygen from the air. They usually undergo 100% oxidation, resulting in their characteristic dark brown and black leaves. Typically, they have more robust and pronounced flavors, and higher caffeine content than less oxidized teas [16].

(ii) Dark Tea (including Pu'er Tea)

Dark tea is a flavorful aged probiotic tea that steeps up very smooth with a natural slightly sweet note. Pu'er tea is a type of Chinese dark tea known for its medicinal properties. It is very strong, with an incredibly deep and rich flavor, and no bitterness, and peaty flavor [17].

(iii) Oolong Tea (Tieguanyin Tea)

Oolong tea is wilted, bruised, rolled, followed by partial oxidation (5-40% oxidized). It is then pan-fried or steamed to kill oxidative enzymes. These teas have intermediate caffeine content. Oolong teas have their own extremely fragrant and intriguing tones [18].

(iv) Green Tea

Green tea is allowed to wither only slightly, after being plucked. Then the oxidation process is stopped very quickly by firing the leaves. Therefore, when brewed at lower temperatures and for less time, green teas tend to have less caffeine. They also produce more subtle flavors with many undertones and accents [19].

(v) White Tea

White tea is the most delicate of all teas. This tea is slightly passively oxidized by being allowed to wither for 12-36 hrs. Post withering, its oxidative enzymes are destroyed. They are hand-processed using the youngest shoots of the tea plant. They have their own subtlety, complexity, low caffeine content and natural sweetness. They are brewed at a very low temperature, and a short steeping time [20].

(vi) Yellow Tea

Yellow teas typically go through more oxidation than green teas, and a longer and slower drying period. It is a rare category of tea, without the usual grassiness of the green teas [https://sevencups.com/learn-about-tea/yellow-tea/].

MICROBIAL ANALYSES OF DIFFERENT TEA SAMPLES

Most people consider that tea is safe to drink after being brewed with boiling water. However, scientists around the world have reported quite a few studies that indicated that tea can harbor potentially harmful bacteria, including pathogens. It has been reported that excessive microbes in cut tea (*dhool*) can interfere with the quality of the final product. Higher microbial counts are also found if the tea is not handled carefully during packaging and sorting [http://www.teaboard.gov.in/pdf/Quality_Control_laboratory_pdf4416.pdf].

The Tea Board of India in Siliguri of Darjeeling district, West Bengal, maintains a Quality Control Laboratory which is in charge of maintaining proper factory hygiene. According to their report, they regularly study the microflora to maintain the quality of tea for internal consumption and export purpose [http://www.teaboard.gov.in/pdf/Quality_Control_laboratory_pdf4416.pdf]. According to Tijburg, made tea is not safe to consume when it harbors more than a total microbial load of 10^6 cfu g^{-1} (colony forming units per gram), coliform 10 cfu g^{-1}, yeast & fungus 10^4 cfu g^{-1} [21]. According to Donia, fecal coliforms

are very harmful to human health, and there should be zero tolerance for it [22]. The upper limit prescribed for microbial count in tea is variously followed by different countries as per their regulatory norms.

Studies have majorly delved into the analysis for total viable count (TVC), coliform bacteria, molds and yeasts, and mycotoxins from cultured or non-cultured samples of made teas or tea infusions. Some reports have also made use of recent techniques like DNA barcoding and Random Amplified Polymorphic DNA (RAPD) analyses to conclude over the microflora distribution in teas and tea infusions, before and after brewing. The characterization of mycotoxins like aflatoxin, ochratoxin, and others are done mainly using liquid chromatography techniques, namely High Performance Liquid Chromatography (HPLC) (Figure 4).

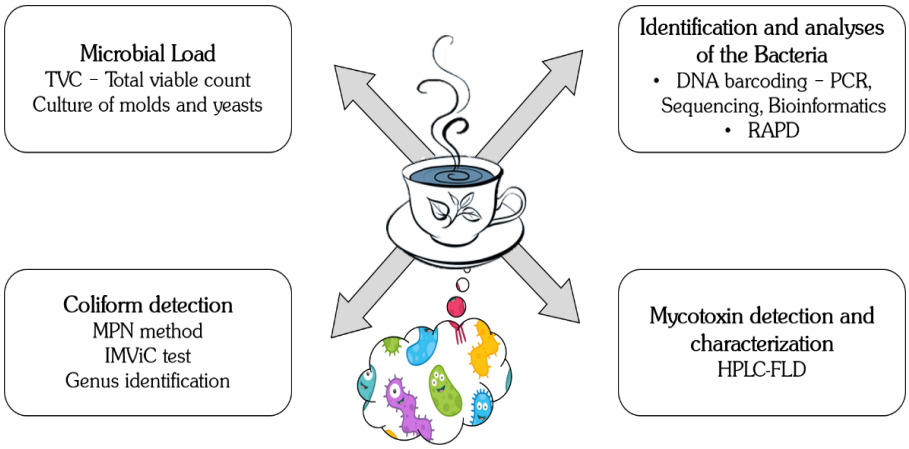

Figure 4. Scheme of Microbial analyses of made teas or tea infusions

Below is discussed a few reports which have found higher than safe limits of microbial loads and pathogens in some unique made teas (Figure 5):

Figure 5. Some unique made teas that have been analyzed microbiologically: (a) Sun Tea, (b) Fu Brick Tea, (c) Miang (pickled) Tea, (d) Iced Tea.
[Picture Courtsey: Google Image]

(i) Sun Tea can harbor dangerous bacteria:

Sun tea (Figure 5a) is made by placing loose or bagged tea leaves in glass jars of water which are then left in direct sunlight. But sun tea can be a dangerous breeding ground for bacteria. According to the Centers for Disease Control (CDC), using the sun's rays to make tea can facilitate the growth of deadly bacteria. Tea steeped in a jar on the porch does not get any hotter than 130°F, which is not enough to kill germs lurking in the tea. The sugar in the solution also promotes the microbial contamination. The bacteria *Alcaligenes viscolactis* consequently grows in sun tea [https://www.snopes.com/fact-check/steep-risk/].

(ii) Microorganisms present in commercial Black and Green Tea:

Carraturo *et al*. evaluated the microbial quality of tea bags of black and green tea, and characterized pathogens isolated from there [7]. They also reported the presence of ochratoxin A in those samples tested. Microbial loads, for over 80% samples, ranged from 1.0×10^2 to 2.8×10^5 CFU g^{-1} tea: most of identified microorganisms were classified as belonging to Bacillaceae family. Using DNA barcoding technique, they characterized the bacterial strains of *Pseudomonas psychrotolerans*, *Staphylococcus warneri, Pantoea gaviniae* and *Clostridium perfringens*, most of which are pathogens. The most prevalent molds characterized were *Aspergillus niger* and *A. tubingensis* [7].

(iii) Fungal and Bacterial contamination in Ceylon Tea:

Ceylon tea is well known throughout the globe because of its natural flavour. It is produced by subjecting the green tea to several production steps. Dayananda *et al*. reported the microbial contaminations in the tea brew samples of Ceylon tea [23]. The tea brew samples made from packed tea had fungal contaminations as high as 13.6 cfu mL^{-1}, whereas the tea brew samples made form loose tea brands had fungal contaminations ranging from 3.0 - 9.1 cfu mL^{-1}. Brewing reduced fungal contaminations by more than 97%. Nonetheless, there was about 3% of possibility to have heat-resistant fungal spores which can withstand the elevated temperature of the boiling water. *Aspergillus niger* was also detected from there. The samples also had bacterial contaminations which were viable even at 100°C [23].

(iv) Fungal and Bacterial communities in Fu Brick Tea:

(v) Fu brick tea (Figure 5b) is a unique post-fermented tea product, which is fermented with microorganisms during the manufacturing process. Li et al. determined the microbial community shift and succession in the various stages of pile-fermentation of Fu brick tea [24]. The fungal genera of Cyberlindnera, Aspergillus, Uwebraunia and Pleosporales and bacterial genera of Klebsiella and Lactobacillus were predominant in the early stages of the process, but only Cyberlindnera and Klebsiella dominated the later stages also [24].

(vi) Pu'er Tea microbes:

Tan's report analyzes the colony count and microbial composition of Pu'er tea using pre- and post-brewing culturing methods, RAPD, and 16S rRNA analyses [6]. Bacterial composition and counts were found to be much more robust than other less oxidized varieties of tea. Bacteria like *Pseudomonas fluorescens* and *Paenibacillus pueri* were isolated. More surprisingly, a potential probiotic *Bacillus coagulans* was also found as the major bacteria in the brewed Pu'er tea [6].

(vii) Microbes in Miang (pickled) Tea:

Miang (Figure 5c) is a microbial fermented Thai tea made from fresh tea leaves of *C. sinensis* var. *assamica*. Miang is produced by lactic acid bacterial fermentation. Ketwal *et al.* detected microorganisms in samples of miang, and showed the high number of TVC ranging from $10^6 – 10^{10}$ cfu g^{-1} [25]. Yeasts and molds were also detected in all samples, ranging from $10^6 – 10^{10}$ cfu g^{-1}. Some samples even showed the results as too numerous to count (TNTC) [25].

(viii) Bacterial contamination of Iced tea:

Consumption of iced tea (Figure 5d) has also raised certain health concerns. High coliform counts were detected (upto 2×10^5 cfu mL^{-1}) in a few restaurant samples. The CDC in 1996 released a memo that stated that since iced tea was prepared at inadequate temperature, it might not be safe and could have coliform contaminations, most frequently *Klebsiella* and *Enterobacter* [26].

(ix) *Salmonella* can grow in Chamomile, Peppermint, and Green Tea:

Salmonella is a ubiquitous foodborne pathogen that is especially hazardous for those with impaired immune systems. The thermal treatments provided during tea brewing are not sufficient to destroy the pathogen. Keller *et al.* simulated home brewing process and inoculated *S. enterica* in brewed tea that did not change after desiccation, and 28 days of storage [27]. Moreover, all brewed teas tested supported the growth of the bacteria. Thus, there is every possibility of survival and growth of *Salmonella* after a home brewing process [27].

HERBAL TEAS: NOT THE MICROBES' CUP OF TEA

It is important that what we eat and drink be satisfying in all senses. The organoleptic acceptability of various types of tea depends on the blending of certain supplements to augment its flavor and aroma. It is a common practice to add some condiments, herbs and spices with teas, especially black and green tea. Typical condiments for black tea include milk, sugar, cardamom, cloves, pepper, honey and lemon. [https://www.pinterest.com/pin/433682639089552645/]. Recently, there is a growing trend among people to shift towards the various types of the so-called 'herbal teas' or 'tisanes', which are essentially an herbal mixture made from leaves, seeds and/or roots of various plants [2]. Some of them are being consumed for their 'adaptogenic' properties to help induce rejuvenation, and also strengthen the immune system. Popular amongst herbal teas are green tea, chamomile tea, ginger tea, turmeric tea, ginseng tea, cinnamon tea, basil tea, and others. Thus, not only is there an enhancement in taste, but also there are overarching health benefits of herbal teas.

Food contamination of different teas could be controlled by natural means, such as through incorporation of herbal condiments and spices in them. Many of these herbal teas have proven antibacterial properties, and their active principles have been isolated and extensively studied in the recent years [28]. Therefore, it is logical to hypothesize that the supplementation of tea with various herbs and condiments will enhance its antimicrobial properties, and the brewed tea will be free from pathogenic microbes and high microbial loads. Therefore, research in this area should be focused on the optimization of tea – herb combinations, and their applications to obtain effective antimicrobial activity at sufficiently low concentrations.

CONCLUSION

Tea is a beverage with little history of disease transmission, and many people opine that '*a cup of tea makes everything better*'. Given its unique and rich composition and flavor, it is popularly, as well as scientifically, considered a healthy drink. Even though there had been multiple studies with respect to the chemical composition and health benefits of tea, the microbiological aspects of tea have been relatively neglected. Notwithstanding the paucity of data relating to the quantitative aspects of tea microbiology, this review tries to give a picture of the ongoing research in this field. Given the popular consumption of tea worldwide, it is essential that stringent regulations be put into place for public benefit. In order to guarantee true tea pleasure, a set of universal quality control measures should be in place. Further studies in this field are warranted, and it will be interesting to assess the microbial profiles of the different varieties and flavors of tea, grown particularly in India.

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