



Microbial Evaluation of Turkish Herbal Teas Before and After Infusion

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HIGHLIGHTS

- Three out of 20 herbal tea samples (15%) were contaminated with *Cronobacter sakazakii*; none of the samples were contaminated with *Salmonella* spp.
- *C. sakazakii* was not eliminated by the tea infusion with hot water.
- The results highlighted the need for quality control of tea during production to eliminate *C. sakazakii*.

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Acronyms and abbreviations

CFU=Colony Forming Unit

ABSTRACT

Background: Herbal teas are produced and sold in packaged or unpackaged forms all over the world. The aim of this study was microbial evaluation of Turkish herbal teas before and after infusion in boiled water.

Methods: A total of 20 packaged and unpackaged samples of Turkish herbal teas, including chamomile, salvia, green, mix, apple, mate, ginger, linden, fennel, and senna tea were collected. All of the samples were analyzed before and after infusion in hot water (~100 °C). Microbiological analyses were performed with tenfold serial dilution for yeast-mold, *Salmonella*, and *Cronobacter sakazakii* after enrichment by using the spread-on-plate method on selective agar. Data were statistically analyzed by using Microsoft Office Professional Plus 2013 Excel software.

Results: Three out of the 20 tea samples (15%) were contaminated with *C. sakazakii*. None of the samples were contaminated with *Salmonella* spp. No significant difference was found in occurrence of *C. sakazakii* before and after infusion of the samples ($p>0.05$). Mold and yeast contamination were found in 12 out of 20 teas samples (60%). No statistical significance ($p>0.05$) was found between the mold-yeast contents of the unpackaged and packaged herbal teas. After infusion, neither mold nor yeast was observed in any of the samples.

Conclusion: All of the herbal teas in this research were found to be within the microbiological limits for consumption according to Turkish Food Codex. However, the microbiological results highlighted the need for quality control of senna tea during production to eliminate the risk of *C. sakazakii* contamination.

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Introduction

Herbal teas are produced and sold in packaged or unpackaged forms all over the world. They are often con-

sumed against discomforts. They can be prepared by boiling, infusion, or maceration. The infusion process

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protects the beneficial effects more and is mostly preferred because of its simplicity (Jäger et al., 2011). However, during their growth, production, and storage, teas might be microbiologically contaminated through different routes, making them harmful rather than beneficial (Székács et al., 2018). Hence, heat treatment may not be sufficient to eliminate the microbial pathogens.

A variety of herbal teas are available on the market, providing various benefits for health, such as sedative and antioxidant effects in addition to supporting cardiovascular physiology, protecting against cold, strengthening the immune system, and providing defense against pathogens via their antimicrobial properties (Poswal et al., 2019). Phenolic compounds in herbal teas have been particularly investigated for their potentials against some gastrointestinal pathogens, including *Salmonella*, *Helicobacter pylori*, as well as *Campylobacter jejuni* (Jarriyawattanachaiikul et al., 2016). Despite the various health benefits of herbal teas, some microbiological assessments indicate high microbial contamination in numerous herbal tea products worldwide. Only some of these microorganisms can be inactivated with hot water, and importantly, most of the bacterial spores are not affected by hot water (Nas, 2004). Besides, antimicrobial efficacies of herbal phytochemicals might change with the pH level of tea (Oh et al., 2013).

Analysis of the consumer profile indicates that women prefer herbal teas more than men in Turkey, and this tendency decreases with age (Ulusoy and Seker, 2013). Even infants within their first years of life can encounter with herbal teas worldwide (Zhang et al., 2011). Relative to adults, infants are more vulnerable to pathogens due to their weak physiology and metabolism, and dose amounts per body weight they receive. Thus, although infants have a low risk of pathogenic exposure related to herbal tea consumption, they are more susceptible to develop serious infectious diseases (Bianco et al., 2008).

Another risk factor considered is that unpackaged herbal teas are assumed to be at higher risk of food-borne pathogens than packaged and controlled products. Nevertheless, *Salmonella* spp. contamination in packaged herbal tea products has previously caused multiple recalls (Gurtler et al., 2014). *Cronobacter sakazakii* is another pathogenic agent sometimes reported in herbal teas. It is a heat-resistant bacterium and presents a health risk particularly for infants (Stojanović et al., 2011). Manufacturers can reduce the microbial load in herbs by food processing practices, such as drying. However, several bacterial species such as *C. sakazakii* which are resistant to drying may endure the drying process (Beuchat, 2009). Besides, inappropriate drying or storage conditions can cause mold contamination and/or mycotoxin production, which can not be eliminated by boiling (Kosalec et al., 2009; Monbaliu et al., 2010).

In accordance with the above-mentioned reasons, the main aim of this study was microbial evaluation of Turkish herbal teas before and after infusion in boiled water.

Materials and methods

Sample collection

A total of 20 samples from 10 different types of herbal teas were collected as packaged (n=10) and unpackaged (n=10) during June-December 2017. The packaged and unpackaged herbal teas were obtained from markets and herbalists located in Istanbul, Turkey, respectively. The tea types included linden (*Tilia cordata*), sage (*Salvia officinalis*), green (*Camellia sinensis*), chamomile (*Matricaria chamomilla*), fennel (*Foeniculum vulgare*), senna (*Cassia angustifolia*), ginger (*Zingiber officinale*), mate (*Ilex paraguariensis*), apple (*Malus domestica*), and mixed tea consisting of cinnamon, raspberry, stinging nettle, and birch leaves in addition to fennel, gunpowder tree bark, rosehip, juniper fruit, calendula, elderberry fruit, and yarrow.

Infusion process

Infusion was performed by mixing 10 g of dry herb with 90 ml of boiling water (~100 °C). Samples with boiled water were left for 30 min to mimic tea-brewing. Microbiological analyses were conducted with pre-infusion (dry herbal tea) and post-infusion samples.

Microbiological analysis

Infused (10 ml) and dry herb (10 g) samples were separately mixed with 90 ml Enterobacteriaceae Enrichment Broth (Oxoid CM0317, ThermoFisher, United Kingdom) for the analyses of *C. sakazakii*, and with 90 ml Buffered Peptone Water (Oxoid CM0509, ThermoFisher, UK) for the analysis of *Salmonella* spp. (1/10 ratio). These bacteria are investigated regard to their presence on selective agar media, i.e. Brilliance *Enterobacter sakazakii* Agar [DFI] (Oxoid CM1055, ThermoFisher, UK) was used for the *C. sakazakii* analyses, whereas Modified Semi-Solid Rappaport Vassiliadis (Oxoid CM0910, ThermoFisher, UK) and Xylose Lysine Deoxycholate (XLD; Merck 105287, Germany) agar were used for the detection of *Salmonella* spp. after enrichment. For the analyses of yeast-mold contamination, tenfold serial dilution was prepared from 10⁻¹ to 10⁻⁵ with Peptone Water (Oxoid CM0009, ThermoFisher, UK). Appropriate dilutions were spread onto Dichloran Rose-Bengal Chloramphenicol (DRBC; Oxoid CM1148, ThermoFisher, UK) agar plates for enumeration of yeast-mold. The names of the microor-

ganisms investigated and media used in the isolation are given in Table 1.

The pH analysis

The pH of all infused samples were measured with a pH meter (MILWAUKEE MW 102, United States).

Data analysis

All data of this study were statistically analyzed using Microsoft Office Professional Plus 2013 Excel software.

Results

The results of the microbiological analyses are given in Table 2. Three out of 20 tea samples (15%) were contaminated with *C. sakazakii*. This bacterium survived after

infusion in both of the packaged and unpackaged senna tea samples. None of the samples were contaminated with *Salmonella* spp. No significant difference was found in occurrence of *C. sakazakii* before and after infusion of the samples ($p>0.05$).

Mold and yeast contaminations were found in 12 out of 20 teas samples (60%). No statistical significance ($p>0.05$) was found between the mold-yeast contents of the unpackaged and packaged herbal teas. After infusion, neither mold nor yeast was observed in any of the samples.

The pH values were measured after infusion. The lowest and highest pH values were found in the unpackaged apple and packaged linden tea samples as pH 3.17 and pH 6.63, respectively (Table 2). There was no statistical significance ($p>0.05$) between the pH values of the unpackaged and packaged teas. The overall median pH of the samples was 5.82.

Table 1: Methods used for microbiological analysis of Turkish herbal tea samples

Microorganism	Method	Reference
<i>Salmonella</i>	Pre-enrichment in Buffered Peptone Water (Oxoid CM0509, ThermoFisher, UK), incubation at 37 °C, 24 h.	Da Silva et al. (2013)
	Drop on MSR V motility agar (Oxoid CM0910, ThermoFisher, UK), incubation at 42 °C, 24 h.	
	Spreading on XLD agar (Merck 105287, Germany), incubation at 37 °C, 24 h. Isolation of typical (black) single colonies on XLD agar, and identification with API 20E (BioMérieux, France).	
<i>Cronobacter sakazakii</i>	Pre-enrichment in Enterobacteriaceae Enrichment broth (Oxoid CM0317, ThermoFisher, UK), incubation at 37 °C, 24 h.	Cetinkaya et al. (2013)
	Spreading on Brilliance <i>Enterobacter sakazakii</i> agar [DFI] (Oxoid CM1055, ThermoFisher, UK), incubation at 37 °C, 20 h.	
	Isolation of typical (green-turquoise) single colonies, and identification with API 20E (BioMérieux, France).	
Yeast-mold	Preparation of serial dilution in 0.1% Peptone Water (PW). Spreading on DRBC agar (Oxoid CM1148, ThermoFisher, UK), incubation at 25 °C, 5 days. Enumeration of the grown colonies.	Da Silva et al. (2013)

Table 2: Microbial counts (Colony Forming Unit/g) and pH levels of Turkish herbal tea samples

Samples	Unpackaged							Packaged						
	Before infusion			After infusion				Before infusion			After infusion			
	A	B	C	A	B	C	D	A	B	C	A	B	C	D
Chamomile tea (<i>Matricaria chamomilla</i>)	7.62×10 ³	-	+	<10	-	-	5.76	<10	-	-	<10	-	-	6.40
Sage tea (<i>Salvia officinalis</i> L.)	6.10×10 ³	-	-	<10	-	-	5.90	<10	-	-	<10	-	-	6.20
Green tea (<i>Camellia sinensis</i>)	<10	-	-	<10	-	-	5.43	5.0×10 ³	-	-	<10	-	-	5.94
Mixed tea	<10	-	-	<10	-	-	6.22	2.89×10 ³	-	-	<10	-	-	6.04
Apple tea	<10	-	-	<10	-	-	3.17	<10	-	-	<10	-	-	3.44
Mate tea (<i>Ilex paraguariensis</i>)	1×10 ²	-	-	<10	-	-	5.63	1×10 ²	-	-	<10	-	-	5.51
Ginger tea (<i>Zingiber officinale</i>)	<10	-	-	<10	-	-	3.92	<10	-	-	<10	-	-	5.69
Linden tea (<i>Tilia cordata</i> Miller)	2×10 ²	-	-	<10	-	-	5.66	7.6×10 ³	-	-	<10	-	-	6.63
Fennel tea (<i>Foeniculum vulgare</i>)	2.77×10 ⁴	-	-	<10	-	-	6.14	6×10 ²	-	-	<10	-	-	5.90
Senna tea (<i>Cassia angustifolia</i>)	3.76×10 ⁴	-	+	<10	-	+	5.89	1.1×10 ³	-	+	<10	-	+	5.66

A: Yeast-mold; B: *Salmonella*; C: *Cronobacter sakazakii*; D: pH

Discussion

In this research, *C. sakazakii* in the senna tea (*Cassia angustifolia*) still existed after infusion in boiled water. In the study of Stojanović et al. (2011), *C. sakazakii* was found in 32% of the 14 different types of tea from Belgrade, Serbia, including senna, sage, and several fruit teas. This bacterium can remain viable after infusion due to its resistance to drying and heating. Also, in our study, both of the packaged and unpackaged senna tea samples were contaminated with this bacterium, raising the possibility that senna plant may intrinsically have a high risk of contamination with this bacterium, and it needs to be investigated at what stage this contamination is likely to occur. In Pakistan, 27% of the herbal teas have been found contaminated with *Salmonella* within the range of 2×10^1 – 8.4×10^3 Colony Forming Unit (CFU)/g (Khattak, 2012). *Salmonella* was not found in any of the samples analyzed in the present study, complying with the microbiological criteria of Turkish Food Codex (TFC, 2011). However, for the presence of *C. sakazakii* in herbal teas, there is no criterion in the microbiological criteria of Turkish Food Codex (TFC, 2011).

In the United States, Tournas and Katsoudas (2008) detected mold and yeast contamination in 88% of the chamomile teas. European Herbal Infusions Association (EHIA, 2018) restricted the yeast and mold levels to 10^4 /g and 10^5 /g, respectively. According to the Directive on Microbiological Criteria of Turkish Food Codex, presence of yeast and mold in herbal teas is limited to 10^5 CFU/g-ml (TFC, 2011) and no sample in the present study exceeded these limits.

Generally, the antimicrobial properties of plant leaves are higher than that of roots (Oh et al., 2013). A protective effect of green tea against *Salmonella*, an inhibitory effect of mate tea against Gram-positive bacteria, and antimicrobial properties of sage tea were also investigated against many bacterial species, such as *Bacillus cereus*, *Klebsiella pneumoniae*, *Morganella morganii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Albayrak et al., 2012; Oh et al., 2013). Additionally, senna tea has been found to have antimicrobial activity against *B. cereus* after infusion process (Albayrak et al., 2012).

The pH levels of herbal teas may vary according to their growth and processing conditions. Consumption preferences may also vary among individuals in part due to differences in the pH values (Phelan and Rees, 2003). The mean pH of herbal teas are recorded to be 3.15 by Brunton and Hussain (2001), and ranges from 2.73 to 5.85 according to the study of Malik et al. (2013), which are slightly lower than the median result (5.82) of the current work. Phelan and Rees (2003) detected a higher pH value in chamomile tea from Thailand (7.08) than

that of the present study (6.40). The pH levels may affect the active ingredients of phytochemical compounds and antimicrobial activities of herbal teas. Allicin, a chemical metabolite extracted from sulphide/thiol plants, such as garlic, onion, and shallot, is most stable at the pH 5-6 (Jarriyawattanachaikul et al., 2016). In our study, 6 out of 10 unpackaged and 5 out of 10 packaged herbal teas were within this pH range. The apple tea had the lowest pH value (3.31 ± 0.19) among our samples.

Conclusion

All of the herbal teas in this research were found to be within the microbiological limits for consumption according to Turkish Food Codex. However, the microbiological results highlighted the need for quality control of senna tea during production to eliminate the risk of *C. sakazakii* contamination. Due to this sensitivity of contamination, necessary precautions should be taken for the storage conditions, such as temperature and humidity, as well as for the prevention of contamination by pests and microbes at every stage of production. Nevertheless, increasing the number of samples in future studies is expected to be useful in obtaining a wider data and making the necessary risk assessment.

Author contributions

Both authors participated equally in designing the research outline, analyzing the data, writing the manuscript, and conducting the experiment. Both authors read and approved the final revised manuscript.

Conflicts of interest

Both authors declare that there was no conflict of interest regarding the publication of this article.

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