e-ISSN 2581-0405
Asian Journal of Applied Research for Community Development and Empowerment
Journal home page: http://ajarcde-safe-network.org ISSN 2581-0405

Evaluation of Artificial Sweeteners, Microbiological Contamination, and Hygiene and Sanitation of Tea Beverages

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ARTICLE INFO

Article History:
Received: 30 June 2026
Final Revision: 07 July 2026
Accepted: 09 July 2026
Online Publication: 11 July 2026

KEYWORDS

Coliform, Escherichia coli, hygiene sanitation, saccharin and cyclamate, tea beverages

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A B S T R A C T

The high incidence of diarrhoea in Rungkut District, Surabaya, which reached 3,341 cases, highlights critical concerns regarding the safety of beverages sold at container-based outlets. This study aimed to evaluate the food safety of iced tea beverages sold by container-based vendors in Rungkut District by analyzing the presence of artificial sweeteners (saccharin and cyclamate), assessing microbiological contamination (TPC, Coliform bacteria, and *Escherichia coli*), and examining their relationship with vendors' hygiene and sanitation practices. A descriptive survey with a cross-sectional approach was conducted, involving interviews, observation, and laboratory analysis for 20 container-based tea vendors selected through purposive sampling. Data were statistically analyzed using Fisher's exact tests at a 95% confidence level ($p<0,05$). Laboratory results showed that 100% of the tea samples were negative for saccharin and cyclamate. However, microbiological evaluation revealed that 85% of the samples failed to meet the national safety standard (PerBPOM No. 13 Year 2019) with critically high TPC values ranging from 47×10^3 to 134×10^5 CFU/mL. Furthermore, 80% of the samples exceeded the Coliform safety limit (<1.8 MPN/mL), although 100% of the samples were negative for *Escherichia coli*. Statistical analysis confirmed there was no significant relationship ($p>0.05$) between hygiene sanitation factors (personal hygiene, equipment sanitation, serving sanitation, and environmental sanitation) and the microbiological contamination levels, suggesting external risk factors such as raw water or contaminated ice blocks as the primary sources of contamination.

Contribution to Sustainable Development Goals (SDGs):
SDG 3: Good Health and Well-being
SDG 6: Clean Water and Sanitation
SDG 12: Food Safety

1. INTRODUCTION

1.1. Research Background

Food safety defined as critical effort to mitigate biological, chemical, and other hazardous contaminants that may risk human health [1]. According to government regulation, the realization of food safety is systematically established through seven main implementations, including food sanitation and the regulation of Food Additives [2]. Food sanitation serves as a preventive measure against the growth of spoilage and pathogenic microorganisms within foods, beverages, utensils, and processing environments [3].

A field study by Yulistiani et al.[4], highlighted that while some vendors demonstrated satisfactory personal hygiene, a significant proportion still neglected standard sanitation practices: approximately 56% of vendors worked without aprons or hairnets, 11% failed to wash their hands prior to food handling, and 22% operated without gloves or suitable utensils. According to Hadi et al., these sanitary deficiencies among street vendors are

primarily driven by restricted technical knowledge and a distinct lack of access to formal food safety training [5].

Along with sanitation, food safety requires strict monitoring of food additives. These substances are intentionally added to alter product characteristics or appearance, including artificial sweeteners [2], [6]. Despite regulatory limits which specifies an Acceptable Daily Intake (ADI) of 0–5 mg/kg body weight for saccharin and 0–11 mg/kg body weight for cyclamate—compliance issues remain frequent [2]. The BPOM annual report [7] documented extensive violations, identifying 498 samples exceeding permitted limits for cyclamate, 138 for saccharin, 15 for acesulfame-K, and 11 for aspartame. This excessive consumption poses severe physiological risks, where cyclamate is associated with metabolic disorders, hyperglycemia, and hyperinsulinemia, while saccharin is linked to hepatic and renal dysfunctions [8], [9].

Monitoring data compiled by the BPOM (2017) highlights that colored beverages, syrups, and ice products consistently demonstrate a high status of not meeting requirements, particularly regarding yeast-mold counts, Total Plate Count, Coliform contamination, and cyclamate levels. Epidemiological trends indicate that beverages have become a predominant vector for foodborne illnesses; poisoning cases attributed to beverages rose from 903 cases in 2016 to 890 cases in 2017, outnumbering solid food-related incidents within the same period [7], [10].

These persistent violations in the beverage sector present a major public health challenge, notably in high-density commercial zones like Rungkut District, Surabaya, where 20 container-based vendors sell tea beverages. Critically, Rungkut District was designated as a public health red zone due to severe gastrointestinal outbreaks, recording 3,341 diarrhea cases within the 2023–2024 period [11].

Recent local research by Wahyuni [12] detecting Coliform and *Escherichia coli* presence alongside positive saccharin and cyclamate in tea products. Driven by these immediate risks, this study was initiated to comprehensively evaluate the chemical safety and microbiological contamination profiles of street-vended tea beverages across Rungkut District.

1.2. Literature Review

Food safety serves as a critical pillar in supply chains, requiring rigorous standards to ensure products are free from biological, chemical, and physical hazards [13]. Within urban food safety, this quality assurance becomes highly critical for tea beverages sold by container-based vendors due to their vulnerability to environmental and chemical contamination during open processing. Recent study conducted by Wahyuni [12] confirmed widespread non-compliance within this sector, detecting notable Coliform and *Escherichia coli* contamination alongside positive qualitative traces of artificial sweeteners, specifically saccharin and cyclamate, in street-vended tea products.

Tea is categorized as a beverage produced by steeping *Camellia sinensis* leaves or tea extracts [14]. While tea contains beneficial bioactive compounds like polyphenols and catechins that act as antioxidants [15], its safety can be compromised by external contaminants. Factors influencing the bacterial profile of iced tea include the quality of brewing water, the hygiene of ice cubes, and the personal hygiene of the food handler [16]. Food sanitation specifically aims to prevent the growth of spoilage and pathogenic bacteria in food, beverages, equipment, and the processing environment [3].

Alongside sanitation, food additives are substances added into food to influence its characteristics or appearance [6]. Among the most commonly used additives in the beverage industry are artificial sweeteners like saccharin and cyclamate, often favored for their economic value despite being chemically synthesized [17]. Saccharin is a non-nutritive sweetener with a sweetness intensity 300-700 times greater than sucrose [18]. Meanwhile, cyclamate provides a sweetness level approximately 30 times higher than sugar but is often associated with a bitter aftertaste [19].

The use of these sweeteners is strictly regulated by BPOM Regulation No. 11 of 2019, which sets the Acceptable Daily Intake (ADI) for saccharin at 0-5 mg/kg body weight and cyclamate at 0-11 mg/kg body weight [20]. Excessive long-term consumption of saccharin has been linked to potential renal and hepatic dysfunction [8], while overexposure to cyclamate may lead to metabolic disturbances such as hyperglycemia [9].

Microbial contamination in beverages, particularly those sold in open distribution chains, remains a significant public health challenge. Common indicators used to evaluate the sanitary quality of food are Coliform bacteria and *Escherichia coli* [21]. Coliforms are Gram-negative, rod-shaped bacteria that ferment lactose to produce gas and acid [22]. Their presence in beverages indicates potential fecal contamination and correlates with risks of gastrointestinal diseases such as diarrhea and typhoid [23]. Specific to *Escherichia coli*, this bacterium is a primary inhabitant of the human intestinal tract and serves as a direct indicator of hygiene failure [24]. Furthermore, the overall microbial load is measured through the Total Plate Count (TPC), which quantifies the total viable microorganisms in a sample [25]. High TPC levels in street-vended beverages are often attributed to poor raw material quality, such as contaminated ice or unsanitary water sources [26].

1.3. Research Objective

This study aims to evaluate the food safety profile of iced tea beverages sold by container-based vendors in Rungkut District, Surabaya. Specifically, it identifies the presence of the artificial sweetener saccharin and cyclamate, evaluates their compliance with personal, equipment, serving, and environmental hygiene sanitation protocols, and quantifies target microbiological contamination levels involving Total Plate Count, Coliform bacteria, and *Escherichia coli*. Furthermore, this research analyzes the statistical relationships between the container-based vendors' hygiene sanitation practices (personal hygiene, equipment, serving, and environmental sanitation) and the resulting microbial counts of the tea products.

2. MATERIALS AND METHODS

The research was carried out from August 2025 to February 2026 at the Microbiology Laboratory and Food Analysis Laboratory, Department of Food Technology, Universitas Pembangunan Nasional "Veteran" Jawa Timur. The sampling was localized within Rungkut District, Surabaya. The study employed an exploratory and descriptive survey design using a cross-sectional approach. Utilizing a non-probability purposive sampling method based on specific inclusion criteria—namely active container-based vendors operating from morning to evening under a

https://doi.org/10.29165/ajarcde.v10i3.1197

recognized trade brand—a total of 20 eligible tea vendors were identified and designated as respondents.

Chemical analysis for the presence of saccharin and cyclamate in iced tea samples was conducted qualitatively using commercial rapid test kits, with a provision for further quantification through alkalimetric titration for saccharin and gravimetric methods for cyclamate upon any positive indication. At the same time, microbiological evaluation was performed through colony density estimation; total viable bacterial counts were quantified using the Total Plate Count (TPC) method, while Coliform bacteria levels were monitored using Most Probable Number (MPN), and *Escherichia coli* presence confirmed through selective isolation on *Eosin Methylene Blue Agar*. At the same time, the vendors' hygiene sanitation compliance was evaluated using structured observation checklists and questionnaires, consisting of personal hygiene (physical health, handwashing habits, and use of protective gear), equipment sanitation (cleanliness, use of running water, and proper storage), serving sanitation (boiled water/ice safety, material quality, and processing limits), and environmental sanitation (waste disposal and pollutant protection). Statistical dependencies between vendors' hygiene sanitation practices, and the laboratory outcomes were computed through Fisher's exact tests at a 95% confidence interval ($p < 0.05$).

3. RESULT AND DISCUSSION

3.1. Identification artificial sweeteners

The qualitative analysis of 20 iced tea samples from Rungkut District yielded negative results for both saccharin and cyclamate, indicating full compliance with the safety standards set by BPOM Regulation No. 11 of 2019. These negative findings may be attributed to growing awareness among container-based vendors of the long-term health risks posed by these additives. However, this does not exclude the potential utilization of other permitted artificial sweeteners; for instance, Abeiasa et al. [27] previously detected excessive aspartame levels (35.15–49.47 mg) that surpassed the limits Acceptable Daily Intake (ADI) of 40 mg/kg body weight in commercial beverages.

Although the use of saccharin and cyclamate is formally permitted within specific thresholds 0-5 mg/kg for saccharin and 0-11 mg/kg for cyclamate per day—the negative results in this study ensure that the iced tea products sold by container-based vendor in Rungkut District comply with safety regulations [20].

3.2. Container-based vendor's hygiene and sanitation

Hygiene is defined as a health initiative focused on maintaining and protecting personal cleanliness, such as through handwashing and dishwashing, while, food sanitation is an effort to establish and sustain healthy and hygienic food conditions that are devoid of biological, chemical, and physical contamination risks. By integrating these hygiene and sanitation measures, vendors can effectively reduce the presence of pathogenic microorganisms during the preparation and serving processes.

Referring to the Decree of the Minister of Health of the Republic of Indonesia Number 942/MENKES/SK/VII/2003 [28], food handlers are required to meet physical health requirements, utilize personal hygiene equipment such as aprons and masks, and implement hygienic behaviors. This protocol includes the obligation to be in good health and free from infectious diseases, maintain personal cleanliness (such as handwashing and keeping clean nails), and avoid activities that risk triggering product contamination, such as smoking or wearing accessories during food processing.

Table 1. Observational results of vendors' hygiene and sanitation practices in Rungkut District

Category	Hygiene Personal	
	n	%
Bad	4	20
Fairly Good	16	80
Good	0	0
Total	20	100
Category	Equipment Sanitation	
	n	%
Bad	8	40
Fairly Good	5	25
Good	7	35
Total	20	100
Category	Serving Sanitation	
	n	%
Bad	5	25
Fairly Good	6	30
Good	9	45
Total	20	100
Category	Environmental Sanitation	
	n	%
Bad	7	35
Fairly Good	2	10
Good	11	55
Total	20	100
Category	Overall Hygiene and Sanitation	
	n	%
Bad	2	10
Fairly Good	13	65
Good	5	25
Total	20	100

Based on table 1, observational data in Lampiran 9 showed that 80% of vendors maintained a fairly good personal hygiene, whereas 20% were classified as bad. Direct on-site monitoring revealed that 100% of the vendors neglected to wash their hands immediately prior to beverage preparation, and 95% failed to utilize basic Personal Protective Equipment (PPE) like aprons, head covers, or face masks. According to Almasari & Prasasti [29], a lack of protective gear and the presence of hand jewelry during food handling significantly escalate the risk of microbial shed and cross-contamination into the products.

Regarding equipment sanitation, 40% of the container-based vendors exhibited poor practices, 25% were fairly good, and 35% were good. Notably, 55% of the operators failed to wash utensils under running water, and 25% did not dry them thoroughly using a dedicated clean cloth. Some vendors merely placed the wet equipment upside down on open and non-sterile shelves after a rudimentary rinse. This behavior aligns with the warnings of Fadhila et al. [30], who stated that a damp drying phase heavily accelerates bacterial colonization, especially when the same wiping cloth is used repeatedly for multiple utility cycles.

For serving sanitation, 45% of the vendors were good, 30% were fairly good, and 25% were classified as bad. This poor

compliance was primarily triggered by the fact that 80% of the operators failed to re-boil or thermally re-process the tea bases that had been stored at ambient temperatures for more than six consecutive hours. Reheating beverages before serving is an essential step to eliminate vegetative bacterial cells and prevent rapid bacterial growth, as strictly mandated by the Decree of the Minister of Health of the Republic of Indonesia [28].

Lastly, the environmental sanitation assessment showed that 55% of the operations were in good condition, 10% were fairly good, and 35% were in poor condition. The primary structural vulnerability identified was that 70% of these container structures were located directly on roadsides. According to Decree of the Minister of Health of the Republic of Indonesia [28], street food facilities must be located far from open pollution sources. Operating in a high-mobility urban area directly exposes preparation surfaces and ice storage units to airborne dust particles and heavy-vehicle emissions, creating a continuous recontamination pathway that compromises the overall safety of the beverages.

3.3. Microbiological quality

The microbiological quality of tea beverages is strictly regulated by Indonesian safety standards to ensure public health. According to SNI 3143:2011 (Indonesian National Standard for Packaged Tea Beverages) [31] regarding packaged tea beverages and BPOM Regulation [32] concerning maximum limits for microbial contamination in processed foods, specific limits are defined for various pathogens. For tea-based drinks, the maximum allowable limit for the Total Plate Count (TPC) is typically set at 1×10^3 CFU/mL, while Coliform bacteria must not exceed 1.8 MPN/mL. Furthermore, the presence of *Escherichia coli* must be non-detectable or negative per sample to comply with safety requirements. These regulations serve as a critical benchmark for evaluating whether container-vended beverages in Rungkut District meet the necessary hygiene standards and are safe for human consumption.

Table 2. Analysis results of Total Plate Count, Coliform bacteria and *Escherichia coli* in tea beverages

Sample Code	TPC (CFU/mL)	Coliform	*Escherichia coli*
P1	130×10^5	>1100	negative
P2	45×10^4	35	negative
P3	43×10^5	240	negative
P4	132×10^5	>1100	negative
P5	21×10^2	0	negative
P6	40×10^5	290	negative
P7	47×10^2	0	negative
P8	47×10^3	7.2	negative
P9	134×10^5	>1100	negative
P10	$38\text{x}10^2$	6.2	negative
P11	96×10^5	1100	negative
P12	67×10^5	9.2	negative
P13	155×10^4	210	negative
P14	109×10^4	43	negative
P15	35×10^4	28	negative
P16	50×10^3	11	negative
P17	107×10^3	21	negative
P18	73×10^5	460	negative
P19	89×10^3	27	negative
P20	65×10^3	11	negative

The microbiological quality of iced tea beverages sold by container-based vendors in Rungkut District was evaluated using TPC, Coliform bacteria, and Escherichia coli counts. Laboratory analysis revealed that 85% of the samples failed to meet the safety standards defined by BPOM Regulation No. 13 of 2019 [32]. The highest TPC level was recorded in sample P9 at 134×10^5 CFU/mL. High TPC levels generally indicate poor hygiene during preparation or the use of contaminated raw materials. This finding is supported by Suryani and Astuti [33], who state that poor sanitation facilities and limited hygiene resources at vending locations significantly increase the risk of cross-contamination, which in turn triggers high microbial counts in the products, as 70% of vendors operate roadside locations, increasing the risk of exposure to airborne contaminants and dust.

The study further identified that 80% of the samples were contaminated with Coliform bacteria, exceeding the maximum limit of <1.8 MPN/mL mandated by SNI 3143:2011 [31]. Rahmayani dan Simatupang [34], stated that high Coliform prevalence serves as a primary indicator of poor quality sanitation and potential environmental contamination during the production or handling process. Several factors contribute to this contamination, including the practice of not washing hands before preparation (observed in 100% of vendors) and the use of unboiled water or block ice distributed in open conditions. Additionally, the repeated use of drying cloths for equipment, as noted by Fadhila et al. [30], significantly increases the risk of bacterial transfer to the serving utensils.

In contrast to the high Coliform counts, all examined samples (100%) tested negative for *Escherichia coli*, thereby complying with SNI 3143:2011 [31] standards. The absence of *Escherichia coli* suggests that there is no direct evidence of recent contamination in the water sources or ice cubes used by the vendors [26]. However, despite the negative *Escherichia coli* results, the overall high microbial load and Coliform presence indicate that the hygiene and sanitation protocols required by Decree of the Minister of Health of the Republic of Indonesia [28] are not being adequately implemented. Continuous monitoring and improved educational interventions regarding food safety are essential to protect consumers from potential foodborne illnesses.

3.4. Correlation between hygiene and sanitation and microbiological quality

The statistical analysis of the relationship between hygiene sanitation and Total Plate Count (TPC) in Table 3 indicates that container-based vendors' hygiene sanitation does not have a significant relationship with TPC quality, with a p-value of 1,000 > 0,05. High TPC levels can be triggered by several factors, one of which is the poor implementation of hygiene practices by food handlers during processing. According to observational results on personal hygiene by Almasari and Indria [29], it was found that some food handlers did not utilize standard Personal Protective Equipment (PPE), such as masks, gloves, and aprons. Furthermore, some food handlers were found wearing hand jewelry, such as rings and bracelets, while interacting directly with food ingredients, which technically has the potential to serve as a medium for both physical and biological contaminants to the product. This behavior technically poses a severe threat to food safety, as the microscopic in jewelry can accumulate organic debris and moisture, effectively serving as a continuous medium for transferring both physical and biological contaminants into the final product.

https://doi.org/10.29165/ajarcde.v10i3.1197

Table 3. Correlation between hygiene and sanitation with TPC

Hygiene Sanitation	Unacceptable		Acceptable		*p-value*
	n	%	n	%	
Bad	2	10	0	0	
Fairly Good	13	65	0	0	1,000
Good	2	10	3	15	
Total	17	85	3	15	

Based on Table 4, there was no significant relationship between Coliform contamination and hygiene and sanitation, with p-values> 0.05. The findings of this study contrast with the research by Yulistiani et al. [4], which concluded that hygiene sanitation practices have a significant impact on Coliform contamination. The primary contamination pathways identified include non-aseptic behavior of food handlers (such as failure to use gloves or perform handwashing), poor equipment sanitation, and low microbiological water quality resulting from incomplete boiling processes.

Table 4. Correlation between hygiene and sanitation with Coliform

Hygiene Sanitation	Unacceptable		Acceptable		*p-value*
	n	%	n	%	
Bad	2	10	0	0	
Fairly Good	13	65	0	0	1,000
Good	2	10	3	15	
Total	18	90	2	10	

4. CONCLUSION

This study concludes that while all iced tea samples in Rungkut District are free from saccharin and cyclamate, they exhibit high microbiological contamination in the Total Plate Count and Coliform parameters. Despite good personal hygiene and serving sanitation, equipment and environmental conditions remain critical points of contamination. The lack of a significant statistical relationship implies that microbial loads likely originate from external factors beyond the observed and documented hygiene practices, such as the initial quality of raw materials (water and ice cubes) or constant exposure to roadside pollutants.

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