# QUALITY ASSESSMENT OF NON-ALCOHOLIC BEVERAGES SOLD IN UTTARAKHAND MARKETPLACES

Sakshi Gupta[1], Deepti Gulati[2]
[1] Student , [2] Associate Professor
Department of Biotechnology, Dolphin (PG) Institute of Biomedical & Natural Sciences,

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## ABSTRACT

Non-alcoholic beverages produced and distributed for commercial purposes are susceptible to microbial contamination. This study aimed to assess microbial loads and identify specific microbes in these beverages. Through a cross-sectional study, 467 samples were randomly selected and tested for aerobic plate count (APC), Enterobacteriaceae count (EBC), total coliform count (TCC), total yeast and mold counts (TYMC), and specific contaminating microbial genera. The average mean ± SD microbial counts were 3.62 ± 0.29 log CFU/ml for APC, 3.47 ± 0.27 log CFU/ml for EBC, 3.36 ± 0.08 log CFU/ml for TCC, and 3.39 ± 0.17 log CFU/ml for TYMC across the sampled products. Variations in APC and EBC levels were observed based on product type, packaging formats, points of purchase, and countries where the products were manufactured. TCC remained relatively consistent across all variables, with a count of 3.37 log CFU/ml overall. Products obtained from street vendors showed a relatively high TYMC count of 4.28 ± 0.91 log CFU/ml. Microbial analysis revealed the presence of various genera, including *Bacillus* (5.2%), *Streptococcus* (2.8%), *Enterobacter* (2.1%), *Clostridia* (1.9%), *Actinomycetes* (1.6%), Yeasts (1.2%), *Corynebacterium* (0.8%), *Neisseria* (0.6%), *Micrococcus* (0.7%), Molds (0.1%), and unidentified microbes (1.9%). Certain levels and types of microbes may present health hazards and could reduce the

shelf life of these beverages. For this reason, it is crucial to maintain strict quality control throughout production, transportation, handling, and distribution processes. Additionally, raising public awareness is vital to effectively reducing these risks.

**Keywords**: Microbial contamination, Non-alcoholic beverages, Quality control.

## INTRODUCTION

Non-alcoholic beverages such as juices, sports drinks, and health drinks have become increasingly popular in recent years due to their ready availability, flavour variety, marketing, and advertised health claims[15] . The processing and transportation of these packaged beverages to distribution centres and retail stores require careful measures to ensure that the beverages are of high quality, safe, and ready for consumption. Although processing methods are designed to control spoilage microorganisms [1] , the beverages produced are not fully sterile and may still contain low levels of surviving microorganisms [9] . The nutrient-rich composition and high-water content of juices and health drinks make them fertile ground for microbial growth.

Proper storage and transportation at appropriate temperatures is critical for preserving the safety and overall quality of these beverages. Exposure to extreme temperatures, whether hot or freezing, can significantly affect their taste, texture, and nutritional integrity [16] . Microorganisms such as yeasts, molds, and certain acid-tolerant bacteria present in such beverages pose significant risks to public health [10] .

Bacteria are a common concern in juices and health drinks and can include pathogenic species such as Escherichia coli, Salmonella, Bacillus, and Clostridia. These bacteria can cause foodborne illnesses, leading to symptoms such as diarrhoea, vomiting, and fever. Yeasts can also grow in juices and health drinks, causing spoilage or fermentation. While most yeasts are not harmful to humans, some can produce toxins that may cause illness. Similarly, molds can grow in juices and health drinks, causing spoilage or discoloration. Some molds can produce toxins that pose serious health risks [14] .

The presence and number of potentially harmful microorganisms in non-alcoholic beverages can be estimated using analytical methods such as aerobic plate count (APC), Enterobacteriaceae count (EBC), total coliform count (TCC), and yeast and mold count (TYMC) [2] . Previous reports indicate multiple documented outbreaks associated with contaminated fruit juices [10] . Despite scattered reports, comprehensive assessments of microbial contamination in commercially produced non-alcoholic beverages sold in India remain limited, underscoring the need for systematic evaluation.

Given the year-round demand for non-alcoholic beverages and the potential risk of emerging foodborne outbreaks linked to their consumption, this study assessed the extent of microbial contamination in non-alcoholic beverages collected from two urban locations in northern India. The findings of this study can serve as foundational data for the development of new technologies aimed at improving microbial quality and ensuring product safety.

# METHODOLOGY

### Study design

A cross-sectional survey was carried out from January to June 2023 to isolate microorganisms and examine the extent of microbial contamination in non-alcoholic beverages sold in the markets of Dehradun (Dehradun district) and Haridwar (Haridwar district) in Uttarakhand, North India.

### Study area

The study was conducted in Dehradun (30.3165° N, 78.0322° E) and Haridwar (29.9457° N, 78.1642° E), located approximately 240.2 km and 189.5 km from New Delhi, the capital of India. Dehradun and Haridwar have populations of 16.96 and 18.91 lakh, respectively, with average annual temperatures of 25°C and 27°C. The laboratory where the experiments were conducted is located 15 km and 71.5 km from Dehradun and Haridwar, respectively.

### Sample size calculation

Sample size estimation was performed with a 95% confidence interval (CI), 5% desired absolute precision, and an anticipated 50% prevalence of microbial contamination in non-alcoholic beverages at each site (570 samples per location). A minimum of 230 samples from each city yielded a total of 460; however, 467 samples were ultimately analyzed.

### Sampling and description of juices and health drinks produced

Samples were collected using a simple random sampling method. The study targeted specific cities, identifying public supply points for juices and health drinks. The target population included various brands, flavors, and packaging types. Samples were categorized based on product type, packaging material (tetra packs, metal cans, glass, and plastic bottles), expiry date, and location (*Table 1*). Each sample was assigned a

unique identification number and transported in ice boxes to the laboratory, after which they were stored at 4°C until further examination.

| Factors studied | Description of item | Total no. of samples | Frequency |
|---|---|---|---|
| Date of expiry | Within shelf life | 467 | 100 |
| | Expired | 0 | 0 |
| Source cities | Dehradun | 235 | 50.6 |
| | Haridwar | 232 | 49.4 |
| Category of product | Juice | 322 | 67.9 |
| | Energy drink | 145 | 32.1 |
| Material of packaging | Glass bottles | 154 | 38.1 |
| | Cans | 138 | 28.8 |
| | Plastic bottles | 99 | 21.7 |
| | Tetra pack | 76 | 11.4 |
| Public supply location | Restaurants/Café | 120 | 25.8 |
| | Hotels | 102 | 19.9 |
| | Street | 113 | 24.2 |
| | Supermarkets | 132 | 30.1 |
| Country of origin | Bangladesh | 38 | 8.1 |
| | India | 260 | 55.7 |
| | Thailand | 86 | 18.4 |
| | Indonesia | 83 | 17.8 |
| Total | | 467 | 100 |

*Table 1 : Factors studied and description of items for juice and energy drink samples from both cities*

**Physicochemical analysis**

Physical and chemical properties of the samples, including pH, density, moisture, titratable acidity, sugar content, and total soluble solids, were analyzed to provide insights into the sensory and nutritional quality of the products and to detect any deviations from expected values. The acidity of each beverage sample was determined using a benchtop pH meter (Model pH 700, Oakton/Eutech Instruments). Sample density was determined by measuring the mass of a 10 mL portion at ambient laboratory temperature (23 ± 2 °C) and expressing it as mass per unit volume. For moisture content and total solids, a juice or health drink sample (30 mL) was poured into a pre-weighed, clean, and dry crucible. The mass of the crucible containing the sample was noted, after which it was dried in a hot-air oven at 105 °C until all moisture had evaporated. Once the crucible had returned to room temperature, its mass together with the dried residue was measured. Moisture content was determined by subtracting the weight of the dry residue from the initial weight of the crucible and liquid, dividing the difference by the combined weight of the crucible

and liquid, and multiplying the result by 100. Total solids were determined by deducting the measured moisture percentage from 100%.

To measure titratable acidity, a 10 mL sample of juice or health drink was transferred to a 250 mL beaker and mixed with 50 mL of distilled water. Three drops of phenolphthalein indicator were introduced into the diluted sample, followed by gradual addition of 0.1 M NaOH solution while continuously stirring. The NaOH solution neutralized the acids in the juice or health drink, causing a color change from colorless to pink or red. The addition of NaOH was stopped once the color stabilized, indicating complete neutralization of the acid. The volume of NaOH solution used was recorded, and titratable acidity was calculated. Reducing sugars in the juices were measured using Benedict's test. Benedict's reagent was prepared by dissolving 17.3 g of copper sulfate pentahydrate, 100 g of sodium carbonate, and 173 g of sodium citrate in distilled water. Glucose (2-10 g/L) served as the standard. Benedict's reagent (1 mL) was combined with 5 mL of the sample, a positive control (glucose), or a negative control (distilled water). The reaction mixture was subsequently heated in a water bath at 60 °C for approximately five minutes to allow colour development. After cooling, the samples were centrifuged, and the clear filtrate was subjected to spectrophotometric analysis at 735 nm.

**Estimation of microbial load**

For microbial enumeration, 10 mL of each beverage was homogenized on a vortex mixer and subsequently diluted in 90 mL of buffered peptone water (BPW) to prepare the initial suspension. Microbial load was assessed under aseptic conditions through serial dilution and surface plating techniques.

For determining the aerobic plate count (APC), successive ten-fold dilutions were prepared using sterile 0.9% saline as the diluent. From the second dilution, 0.1 mL was dispensed onto plate count agar, and the inoculum was spread uniformly across the surface using a sterile spreader. The inoculated plates were incubated at 35 ± 2 °C for approximately 48 hours. After incubation, colonies were enumerated using an automated counter, and CFU/mL values were derived based on standard dilution factors. To determine Enterobacteriaceae count (EBC), 0.1 mL from the primary dilution was transferred onto Violet Red Bile Glucose Agar (VRBGA) and spread evenly. The VRBGA plates were incubated at 35 ± 2 °C for about 48 hours, after which colonies showing the characteristic pink coloration were enumerated with an automated colony counter to determine CFUs per milliliter. For assessing total coliforms, 0.1 mL of the primary dilution was inoculated onto MacConkey agar, and the sample was distributed uniformly over the plate surface using a sterile spreader. Following incubation at 35 ± 2 °C for roughly 48

hours, the resulting colonies on MacConkey agar were enumerated to determine the coliform load. To enumerate yeasts and molds, 0.1 mL from the primary dilution was inoculated onto Sabouraud Dextrose Agar (SDA) containing chloramphenicol (100 mg/L), and the inoculum was spread uniformly across the agar surface. The SDA plates were incubated at 25 ± 2 °C for approximately five days, after which fungal colonies were enumerated with an automated counter, and CFU/mL values were computed based on dilution factors [7] [1] .

**Isolation of microbes**

For isolating microbial contaminants, the primary 1:10 BPW dilution was incubated at 35 °C for roughly 20 hours to allow pre-enrichment, after which it was held at 4 °C until further processing. For primary bacterial isolation, a loopful of the enriched broth was streaked onto blood agar containing 5% sheep blood, followed by incubation at 37 °C for approximately 18 hours. Colonies showing distinct morphological features were transferred to fresh nutrient agar plates for purification and subsequently examined using standard staining methods and basic biochemical assays.

Gram staining was performed to differentiate isolates into Gram-positive and Gram-negative categories. Within the Gram-positive group, organisms exhibiting rod-shaped, coccoid, or filamentous morphologies, some resembling Actinomycetes, were noted during microscopic examination. Bacilli were incubated at room temperature for 3 days before re-staining. Non-spore-forming bacilli were designated as *Bacillus*, while spore-forming bacilli were categorized under *Clostridium*. Catalase-positive bacteria with irregular shapes and swollen ends were classified as *Corynebacterium*. Cocci were further classified as *Streptococcus* or *Micrococcus* based on their arrangement under the microscope.

Gram-negative bacteria were categorized as coccobacilli, cocci, or bacilli. Cocci were grouped as *Neisseria*. Coccobacilli and bacilli were differentiated using biochemical tests, such as lactose utilization and oxidase activity, and classified under Enterobacteriaceae. Colonies grown on Sabouraud Dextrose Agar supplemented with chloramphenicol were also subjected to Gram staining to distinguish between filamentous fungi/molds and yeast, the latter exhibiting characteristic yeast morphology [3].

**Heavy metal analysis**

Analysis for heavy metals (Pb, As, Zn, Cd, Cu, Cr) was performed by AAnalyst400, Perkin Elmer flame atomic absorption spectrophotometer. Juices were homogenised and filtered to remove contaminants before being used as samples. To determine the correlation

between metal ion concentrations and absorbance data, the equipment was calibrated using standard solutions. The spectrophotometer was then used to detect the absorbance of particular wavelengths that were associated with the heavy metal ions in the prepared samples. By comparing the absorbance results with the calibration curves, the concentrations of the heavy metals were calculated. For accuracy and precision, quality control procedures involving blank samples and replicate measurements were used.

### Statistical Analysis

Data were analyzed using Microsoft Excel 2016 and IBM SPSS Statistics 20.0. Microbial counts were calculated in logarithmic form (CFU/mL) with dilution factors. Data are expressed as mean ± standard deviation, and statistical comparisons were carried out using a two-tailed unpaired t-test with a significance threshold of $P < 0.05$.

## RESULTS AND DISCUSSION

### Physicochemical analysis

*Table 2* and *Table 3* summarize the physical and chemical data of juice and health drink samples, including pH, density, moisture content, titratable acidity, total solids, and reducing sugars. Sample pH values spanned from 3.1 to 4.9, with variations observed based on the source city, product category, packaging format, point of sale, and country of origin. Density values were between 0.92 and 1.06 g/cm³, titratable acidity ranged from 0.11 to 0.89 g/100 mL, total solids ranged from 5.2% to 18.8%, moisture content ranged from 75.1% to 95.1%, and reducing sugars were between 4.09 and 9.42 g/L.

Notably, the average pH of all juices studied by Vantarakis et al. [16] was reported as 3.47. Oranusi et al. [10] found titratable acidity levels ranging from 0.19 to 0.48 g/100 mL and sugar content (% sucrose) from 0.30% to 13.00%. In an analysis by Zahoor et al. [19], the density of juice samples ranged from 0.98 to 1.01 g/cm³, moisture content ranged from 84.86% to 90.62%, and total solids content varied from 9.39% to 20.49%.

The acidity of beverages can negatively affect dental and digestive health. Over time, acidic drinks may erode tooth enamel, leading to cavities and tooth sensitivity. Additionally, the acidic nature of these beverages can cause heartburn and acid reflux, especially when consumed in substantial amounts or without accompanying food. In contrast, alkaline beverages may help neutralize acidity in the body, potentially reducing the risk of health issues such as osteoporosis and muscle wasting.

Beverage density has little direct relevance to its health effects, as density does not directly influence the nutritional value or chemical composition of the beverage. However, density can affect calorie content, as denser beverages generally contain more calories per unit volume than less dense ones. Commercially available juices and health drinks often have added sugars and other additives, which can increase their density and calorie content.

Moisture content strongly influences the storage stability and overall microbiological safety of beverages. High moisture content can create an environment conducive to the growth of bacteria and other microorganisms, potentially leading to spoilage and foodborne illnesses. Conversely, low moisture content can result in dehydration and deterioration of the beverage, reducing its shelf life.

Total solids represent the quantity of dissolved and suspended constituents in the beverage, including sugars, minerals, and other compounds. Regular consumption of beverages with added sugars can contribute to weight gain, obesity, and has been linked to several chronic health conditions, including type II diabetes, cardiovascular disorders, and some forms of cancer. However, beverages with high total solids may also provide beneficial components, such as vitamins, minerals, and dietary fiber, that contribute to general health and nutrition.

Accurate estimation of reducing sugars in juices and health drinks is crucial, as excessive sugar intake is associated with conditions like obesity, type II diabetes, and tooth decay. It is important to note that some health drinks may contain added sugars not always listed on the label, emphasizing the need for precise sugar content estimation.

These findings highlight the diverse characteristics of beverages influenced by factors such as geography, product type, and packaging. Further analysis could provide valuable insights for improving quality control and understanding consumer preferences within the beverage industry.

| Study variables | | Samples studied | pH Range | Mean ± SD | Density (g/cm$^3$) | Mean ± SD | Moisture (%) | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| Source cities | Dehradun | 235 | 3.1-3.9 | 3.6±0.45 | 1.02-1.06 | 1.05±0.09 | 78.1-94.9 | 91.7±0.77 |
| | Haridwar | 232 | 3.4-4.1 | 3.7±0.86 | 1.01-1.05 | 1.03±0.17 | 76.9-95.1 | 89.2±0.18 |
| Category of product | Juice | 322 | 3.6-4.3 | 3.9±1.12 | 0.92-0.98 | 0.96±0.62 | 79.2-93.1 | 89.3±0.04 |
| | Energy drink | 145 | 3.4-4.0 | 3.7±0.78 | 0.96-0.99 | 0.98±0.73 | 74.9-88.2 | 83.2±0.26 |
| Material of packaging | Glass bottles | 154 | 3.5-3.8 | 3.7±0.21 | 0.94-0.97 | 0.95±0.12 | 75.1-89.0 | 83.7±0.54 |

| Study variables | | Samples studied | pH Range | Mean ± SD | Density (g/cm$^3$) | Mean ± SD | Moisture (%) | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| | Cans | 138 | 3.6-3.9 | 3.8±0.33 | 0.93-0.98 | 0.96±0.08 | 79.9-87.6 | 85.6±0.33 |
| | Plastic bottles | 99 | 3.3-4.1 | 3.9±0.84 | 0.93-1.00 | 0.97±0.65 | 76.2-83.7 | 81.9±0.08 |
| | Tetra pack | 76 | 3.2-3.9 | 3.6±0.99 | 0.96-0.99 | 0.98±0.19 | 80.1-92.3 | 89.6±0.11 |
| Public supply location | Restaurants/ Café | 120 | 3.7-4.8 | 4.3±0.32 | 0.98-1.01 | 1.00±0.92 | 82.3-93.6 | 88.1±0.79 |
| | Hotels | 102 | 3.4-4.5 | 3.9±0.56 | 0.99-1.01 | 0.99±0.68 | 76.6-92.4 | 89.6±0.09 |
| | Street | 113 | 3.6-4.9 | 4.4±0.18 | 0.95-0.99 | 0.97±0.14 | 75.2-87.9 | 84.2±0.81 |
| | Supermarkets | 132 | 3.5-4.3 | 3.8±0.27 | 0.98-1.01 | 1.00±0.05 | 79.3-91.1 | 87.0±0.15 |
| Country of origin | Bangladesh | 38 | 3.8-4.5 | 4.3±0.65 | 0.99-1.02 | 1.01±0.86 | 80.2-92.9 | 86.7±0.09 |
| | India | 260 | 3.6-4.0 | 3.8±0.12 | 1.00-1.05 | 1.03±0.43 | 83.7-95.0 | 90.2±0.08 |
| | Thailand | 86 | 3.4-4.2 | 3.9±0.74 | 1.01-1.03 | 1.02±0.08 | 81.1-92.4 | 87.9±0.17 |
| | Indonesia | 83 | 3.5-4.4 | 4.1±0.32 | 0.98-1.02 | 1.01±0.61 | 79.7-90.8 | 87.1±0.08 |

*Table 2 : Physicochemical analysis of juices and health drinks*

| Study variables | | Samples studied | TSS (%) | Mean ± SD | TA (g/100 ml) | Mean ± SD | RS (g/L) | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| Source cities | Dehradun | 235 | 12.2-18.8 | 16.2±0.25 | 0.14-0.57 | 0.39±0.71 | 4.09-7.29 | 5.21±0.05 |
| | Haridwar | 232 | 12.4-18.2 | 15.4±0.36 | 0.13-0.62 | 0.41±0.16 | 4.12-7.32 | 5.32±0.13 |
| Category of product | Juice | 322 | 8.4-16.1 | 12.0±0.11 | 0.17-0.61 | 0.52±0.09 | 5.22-8.04 | 6.14±0.25 |
| | Energy drink | 145 | 7.9-13.7 | 11.8±0.07 | 0.11-0.54 | 0.37±0.12 | 4.19-7.34 | 5.63±0.27 |
| Material of packaging | Glass bottles | 154 | 7.6-14.1 | 11.1±0.42 | 0.23-0.72 | 0.56±0.06 | 5.03-8.16 | 6.81±0.09 |
| | Cans | 138 | 9.1-14.4 | 12.2±0.15 | 0.42-0.85 | 0.64±0.19 | 5.12-8.91 | 6.79±0.42 |
| | Plastic bottles | 99 | 6.4-13.9 | 10.4±0.51 | 0.16-0.53 | 0.42±0.41 | 5.36-8.89 | 6.51±0.38 |
| | Tetra pack | 76 | 7.5-14.4 | 12.7±0.71 | 0.35-0.88 | 0.58±0.34 | 4.42-8.63 | 6.65±0.11 |
| | Restaurants/Café | 120 | 5.7-13.7 | 9.6±0.27 | 0.54-0.87 | 0.72±0.11 | 4.17-8.83 | 7.12±0.04 |

| Study variables | | Samples studied | TSS (%) | Mean ± SD | TA (g/100 ml) | Mean ± SD | RS (g/L) | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| Public supply location | Hotels | 102 | 7.6-13.5 | 10.3±0.05 | 0.53-0.89 | 0.65±0.31 | 4.83-8.81 | 7.07±0.16 |
| | Street | 113 | 5.2-11.1 | 9.7±0.88 | 0.49-0.76 | 0.64±0.15 | 5.65-9.42 | 7.98±0.43 |
| | Supermarkets | 132 | 8.5-14.3 | 12.6±0.11 | 0.37-0.79 | 0.59±0.06 | 4.28-8.04 | 6.98±0.52 |
| Country of origin | Bangladesh | 38 | 7.9-14.6 | 11.2±0.31 | 0.29-0.54 | 0.45±0.13 | 5.97-7.91 | 6.04±0.21 |
| | India | 260 | 10.2-17.2 | 15.1±0.02 | 0.23-0.61 | 0.52±0.11 | 4.14-7.64 | 5.34±0.07 |
| | Thailand | 86 | 11.1-15.8 | 13.4±0.56 | 0.27-0.67 | 0.51±0.22 | 4.98-7.98 | 5.62±0.35 |
| | Indonesia | 83 | 10.9-15.9 | 13.1±0.24 | 0.34-0.72 | 0.58±0.35 | 4.67-7.93 | 5.72±0.41 |

*Table 3 : Physicochemical analysis of juices and health drinks*

**Estimation of microbial load**

The study examined various microbial parameters across different variables, including source cities, product categories, packaging materials, point of purchase, and country of manufacture. The results are summarized in *Table 4*.

The Aerobic Plate Count (APC) in juices and health drinks serves as a general indicator of spoilage bacteria, which can cause products to spoil or develop off-flavors or unpleasant odors. An overall mean of 3.62 ± 0.29 log CFU/mL was observed. The APC ranged from 3.47 to 3.51 log CFU/mL among different product categories, 3.69 to 4.08 log CFU/mL among various packaging materials, 3.43 to 4.32 log CFU/mL among points of purchase, and 3.53 to 3.84 log CFU/mL based on the country of origin. These observations are consistent with the patterns documented in the study by Hiko and Muktar [3] for commercial juices in Ethiopian town markets, which recorded a total viable bacterial count of 3.26 log CFU/mL.

Detection of *Enterobacter* in juice or health drink samples is commonly interpreted as evidence of lapses in hygiene or sanitation during processing or handling. An overall mean of 3.47 ± 0.27 log CFU/mL was observed. The Enterobacter count ranged from 3.41 to 3.42 log CFU/mL across product categories, 3.40 to 3.58 log CFU/mL across packaging materials, 3.47 to 3.82 log CFU/mL among points of purchase, and 3.49 to 3.64 log CFU/mL based on the country of manufacture. These counts are lower than the 4 log CFU/mL reported in soft drinks by Park and Chen [10] .

The Total Coliform Count (TCC) in juices and health drinks are often interpreted as markers of possible fecal-associated contamination. The presence of coliforms in these products may signal inadequate hygiene practices during production, processing, or storage. The overall TCC averaged 3.36 ± 0.08 log CFU/mL. Across different product categories, values varied between 3.43 and 3.45 log CFU/mL. When examined by packaging type, TCC ranged from 3.39 to 3.46 log CFU/mL, while points of purchase showed values from 3.37 to 3.46 log CFU/mL. Differences by country of origin were minimal, with counts spanning 3.38 to 3.41 log CFU/mL. Akond et al. [2] reported that Total Coliforms (TC) and Fecal Coliforms (FC) were present in 68%–100% of the examined brands, with TC counts between 5 and 213 CFU/100 mL and FC levels ranging from 3 to 276 CFU/100 mL.

The Total Yeast and Mold Count (TYMC) is a crucial metric of microbial quality, product safety, and the shelf-life stability of juice beverages. By monitoring and controlling yeast and mold levels, manufacturers can uphold product standards, meet regulatory requirements, and maintain consumer satisfaction. An overall mean of 3.39 ± 0.17 log CFU/mL was observed. The TYMC ranged from 3.41 to 3.46 log CFU/mL across product categories, 3.32 to 3.57 log CFU/mL across packaging materials, 3.39 to 3.62 log CFU/mL among points of purchase, and 3.39 to 3.44 log CFU/mL based on the country of manufacture. High levels of yeast and mold (over 4 log CFU/100 mL) have been recorded in soft drinks in Georgia by Park and Chen [10] .

The current investigation reveals that the levels of APC, EBC, TCC, and TYMC exceeded the acceptable CFU/mL thresholds for total count, coliform count, and yeast count as prescribed by the FSSAI (2011) [3] guidelines. According to these guidelines, the total microbial count should be below 25 CFU/mL, coliforms must be undetectable in 100 mL of sample, and yeast levels should remain under 2 CFU/mL.

The high microbial counts noted in this study are likely linked to the growth of microorganisms introduced during processing. These findings underscore the importance of monitoring and controlling microbial contamination at various stages of production, packaging, and distribution to maintain product safety and quality. Further analysis and interpretation of these results could provide valuable insights for enhancing food safety regulations and practices.

| Study variables | | Total Samples studied | APC log CFU/ml (Mean ± SD ) | t | EBC log CFU/ml (Mean± SD ) | t | TCC log CFU/ml (Mean± SD ) | t | TYMC log CFU/ml (Mean± SD ) | t |
|---|---|---|---|---|---|---|---|---|---|---|
| Source cities | Dehradun | 235 | 3.36±0.14 | 102.5 | 3.38 ±0.17 | 7.1 | 3.37±0.01 | 1.5 | 3.39±0.07 | 2.8 |
| | Haridwar | 232 | 3.41±0.26 | 148.5 | 3.34±0.21 | 20.6 | 3.38 ±0.04 | 1.09 | 3.39±0.01 | 245.7 |
| Category of product | Juice | 322 | 3.51±0.32 | 112.3 | 3.42 ±0.11 | 102.0 | 3.45 ±0.02 | 145.7 | 3.41±0.13 | 36.4 |
| | Energy drink | 145 | 3.47±0.11 | 92.6 | 3.41 ±0.32 | 38.3 | 3.43 ±0.01 | 5.7 | 3.46±0.09 | 1.7 |
| Material of packaging | Glass bottles | 154 | 3.82±0.37 | 88.1 | 3.45 ±0.09 | 187.6 | 3.41 ±0.06 | 227.8 | 3.32±0.07 | 347.2 |
| | Cans | 138 | 3.76±0.25 | 100.9 | 3.40 ±0.11 | 123.7 | 3.39 ±0.04 | 3.2 | 3.43±0.24 | 78.9 |
| | Plastic bottles | 99 | 4.08±0.34 | 78.2 | 3.58 ±0.19 | 79.2 | 3.42 ±0.09 | 9.7 | 3.57±0.17 | 66.4 |
| | Tetra pack | 76 | 3.69±0.24 | 352.8 | 3.49 ±0.41 | 213.6 | 3.46 ±0.03 | 16.5 | 3.48±0.21 | 237.1 |
| Public supply location | Restaurants/ Café | 120 | 3.71±0.33 | 156.4 | 3.61 ±0.56 | 47.8 | 3.37 ±0.07 | 694.1 | 3.41±0.14 | 323.9 |
| | Hotels | 102 | 3.57±0.20 | 173.2 | 3.57 ±0.23 | 228.9 | 3.40 ±0.03 | 79.3 | 3.39±0.07 | 782.1 |
| | Street | 113 | 4.32±0.41 | 328.7 | 3.82 ±0.34 | 116.4 | 3.46 ±0.07 | 1.8 | 3.62±0.34 | 376.4 |
| | Supermarkets | 132 | 3.43±0.38 | 113.7 | 3.47 ±0.22 | 192.0 | 3.40 ±0.08 | 77.6 | 3.47±0.19 | 613.0 |
| Country of origin | Bangladesh | 38 | 3.84±0.24 | 67.1 | 3.64 ±0.16 | 87.6 | 3.41 ±0.02 | 236.4 | 3.39±0.18 | 2,8 |
| | India | 260 | 3.53±0.13 | 176.0 | 3.49 ±0.07 | 119.2 | 3.39 ±0.02 | 119.3 | 3.40±0.05 | 112.6 |
| | Thailand | 86 | 3.68±0.19 | 154.3 | 3.58 ±0.17 | 598.0 | 3.38 ±0.03 | 397.5 | 3.40±0.11 | 256.2 |
| | Indonesia | 83 | 3.72±0.21 | 560.3 | 3.61 ±0.29 | 457.4 | 3.39 ±0.01 | 654.2 | 3.44±0.06 | 201.3 |

*Table 4 : Microbial load in juices and health drinks determined by Mean±SD of APC, EBC, TCC, and TYMC using two-tailed unpaired t test (t value)*

**Prevalence of bacterial genera, yeast and mold in samples**

The investigation examined the microbial profiles of samples collected from multiple sources, grouped according to city of collection, beverage category, type of packaging, point of purchase, and country of manufacture. The microbial analysis revealed the presence of several genera, including *Bacillus* (5.2%), *Streptococcus* (2.8%), *Enterobacter* (2.1%), *Clostridia* (1.9%), *Actinomycetes* (1.6%), yeast (1.2%), *Corynebacterium* (0.8%), *Neisseria* (0.6%), *Micrococcus* (0.7%), and molds (0.1%).

In samples sourced from Dehradun and Haridwar, *Bacillus* and *Enterobacter* were the most prevalent, with minor occurrences of other microbial species. *Bacillus* was prominent in both cities, followed by *Enterobacter*, indicating a consistent microbial profile across the region.

Regarding product types, juices exhibited a diverse microbial composition, with *Bacillus* being the predominant genus, followed by *Streptococcus*. Energy drinks, in contrast, showed relatively lower microbial diversity, with *Bacillus* and yeast being the most prevalent.

The type of packaging material also influenced microbial presence. Glass bottles harbored a wide variety of microbes, with *Bacillus* being the most common, followed by *Micrococcus* and yeast. Cans exhibited a higher prevalence of *Bacillus* and *Enterobacter*, while plastic bottles and tetra packs showed a more diverse microbial profile, with *Bacillus* and *Corynebacterium* being predominant.

Public supply locations demonstrated varying microbial compositions as well. Samples from restaurants/cafés and streets showed higher microbial diversity compared to those from hotels and supermarkets. *Bacillus* and *Streptococcus* were prevalent in restaurants/cafés and street-sourced samples, whereas supermarkets exhibited a lower microbial load.

The country of origin further influenced microbial diversity. Products from Bangladesh and Indonesia exhibited higher microbial diversity compared to those from India and Thailand. *Bacillus* and *Enterobacter* were predominant in products from Bangladesh, while *Bacillus* and *Corynebacterium* were common in products from Indonesia.

Additionally, 1.9% of microbial isolates remained uncharacterized due to the lack of adequate laboratory facilities. These isolates were classified as "unidentified" microbial strains.

The results highlight the diverse microbial compositions present in different samples based on various parameters. The prevalence of *Bacillus* across most samples suggests its ubiquitous nature and adaptability to various environments and conditions. *Enterobacter*, another common finding, is known for its presence in food and water, indicating potential sources of contamination.

Differences in microbial profiles among cities, beverage categories, packaging formats, purchase locations, and countries of origin highlight the roles of environmental conditions, handling procedures, and local microbial communities in shaping the microbial diversity of these products. The higher microbial diversity observed in juices compared to energy drinks may be attributed to differences in processing, ingredients, and storage conditions. Similarly, the microbial profiles associated with different packaging materials reflect their susceptibility to microbial colonization and survival.

The significant microbial diversity observed in restaurants/cafés and street-sourced samples highlights the importance of hygiene and sanitation practices in food handling and distribution. Products sourced from different countries showed varying microbial compositions, likely reflecting differences in agricultural practices, manufacturing standards, and regulatory frameworks.

Taken together, the results enhance our understanding of the microbial communities associated with these beverage products and underscore the need for stringent quality control measures and monitoring protocols to ensure food safety and public health. Further research is warranted to elucidate the specific factors influencing microbial dynamics in food products and to develop targeted interventions for microbial control and mitigation.

| **Study variables** | | **Samples studied** | ***Actinomycetes* No. (%)** | ***Bacillus* No. (%)** | ***Clostridia* No. (%)** | ***Enterobacteriaceae* No. (%)** | ***Corynebacterium* No. (%)** | ***Micrococcus* No. (%)** | ***Streptococcus* No. (%)** | ***Neisseria* (%)** | **Fungi/Mold (%)** | **Yeast (%)** | **Unindentified (%)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source cities | Dehradun | 235 | 3 (1.28) | 9 (3.83) | ND | 5 (2.13) | 2 (0.85) | 1 (0.43) | 1 (0.43) | ND | ND | 1 (0.43) | ND |
| | Haridwar | 232 | 7 (3.02) | 11 (4.74) | ND | 4 (1.72) | 2 (0.86) | 1 (0.43) | 2 (0.86) | ND | ND | 1 (0.43) | 2 (0.86) |

| **Study variables** | | **Samples studied** | ***Actinomycetes* No. (%)** | ***Bacillus* No. (%)** | ***Clostridia* No. (%)** | ***Enterobacteriaceae* No. (%)** | ***Corynebacterium* No. (%)** | ***Micrococcus* No. (%)** | ***Streptococcus* No. (%)** | ***Neisseria* (%)** | **Fungi/Mold (%)** | **Yeast (%)** | **Unindentified (%)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Category of product | Juice | 322 | 6 (1.86) | 39 (12.11) | 2 (0.62) | 9 (2.80) | 1 (0.31) | 3 (0.93) | 5 (1.55) | 4 (1.24) | 1 (0.31) | 2 (0.62) | 7 (2.17) |
| | Energy drink | 145 | ND | 2 (1.38) | ND | ND | ND | ND | 1 (0.69) | 1 (0.69) | 1 (0.69) | 2 (1.38) | 3 (2.07) |
| Material of packaging | Glass bottles | 154 | 1 (0.65) | 6 (3.90) | ND | 2 (1.30) | ND | 3 (1.95) | 2 (1.30) | 2 (1.30) | ND | 1 (0.65) | 4 (2.60) |
| | Cans | 138 | 2 (1.45) | 15 (10.87) | ND | 1 (0.72) | ND | ND | 2 (1.45) | ND | 1 (0.72) | 2 (1.45) | 3 (2.17) |
| | Plastic bottles | 99 | ND | 5 (5.05) | 1 (1.01) | ND | ND | ND | 1 (1.01) | 1 (1.01) | 2 (2.02) | 3 (3.03) | 2 (2.02) |
| | Tetra pack | 76 | ND | 2 (2.63) | ND | 2 (2.63) | ND | ND | 1 (1.32) | ND | ND | 1 (1.32) | 3 (3.95) |
| Public supply location | Restaurants/Café | 120 | 3 (2.5) | 11 (9.17) | ND | 8 (6.67) | 1 (0.83) | 4 (3.33) | 3 (2.5) | 1 (0.83) | 1 (0.83) | 2 (1.67) | 5 (4.17) |
| | Hotels | 102 | 1 (0.98) | ND | ND | ND | ND | ND | ND | 1 (0.98) | ND | 1 (0.98) | 2 (1.96) |
| | Street | 113 | 9 (7.96) | 21 (18.58) | 2 (1.77) | 19 (16.81) | 5 (4.42) | 3 (2.65) | 12 (10.62) | 6 (5.31) | 1 (0.88) | 5 (4.42) | 7 (6.19) |
| | Supermarket | 132 | ND | 10 (7.58) | ND | ND | ND | ND | 2 (1.52) | 3 (2.27) | ND | 3 (2.27) | 3 (2.27) |
| Country of origin | Bangladesh | 38 | 3 (7.89) | 5 (13.16) | 1 (2.63) | 2 (5.26) | ND | ND | 1 (2.63) | 1 (2.63) | ND | 1 (2.63) | 2 (5.26) |
| | India | 260 | ND | 2 (0.77) | ND | 3 (1.15) | ND | ND | ND | ND | ND | ND | 2 (0.77) |

| Study variables | | Samples studied | *Actinomycetes* No. (%) | *Bacillus* No. (%) | *Clostridia* No. (%) | *Enterobacteriaceae* No. (%) | *Corynebacterium* No. (%) | *Micrococcus* No. (%) | *Streptococcus* No. (%) | *Neisseria* (%) | Fungi/Mold (%) | Yeast (%) | Unindentified (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Thailand | 86 | 2 (2.33) | 3 (3.49) | ND | 1 (1.16) | ND | ND | ND | ND | 1 (1.16) | 1 (1.16) | 3 (3.49) |
| | Indonesia | 83 | 5 (6.02) | 4 (4.82) | 1 (1.20) | 2 (2.41) | 1 (1.20) | ND | 1 (1.20) | ND | ND | 1 (1.20) | 1 (1.20) |

*ND=Not Detected*

*Table 5 : Prevalence of bacterial genera, yeast and mold in juices and health drinks by study variables*

## Heavy metal analysis

The heavy metal analysis conducted on the samples yielded reassuring findings regarding consumer safety. Using a flame atomic absorption spectrophotometer, the analysis assessed the presence of various heavy metals, including lead (Pb), arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), and zinc (Zn), in juices and health drinks. The results were compared against the permissible limits set by the World Health Organization (WHO) [18] , which specify limits (in mg/L) for chromium (0.05), copper (2.0), cadmium (0.003), and lead (0.01), while limits for zinc and arsenic were not provided.

Encouragingly, cadmium and arsenic were either absent or present below the detection limit in all fruit juice samples, indicating negligible levels of these potentially harmful heavy metals. Moreover, the mean concentrations of heavy metals in the juice samples generally fell within the permissible limits or were undetectable, highlighting the safety of these beverages.

Across various study variables, such as source cities, product categories, packaging materials, public supply locations, and countries of origin, heavy metal concentrations remained within acceptable ranges or were non-detectable. Exceptions were rare, with sporadic instances of certain metals being detected, though still within permissible limits.

These findings confirm the safety and quality of the analyzed juices and health drinks, affirming their compliance with established regulatory standards and their suitability for consumption. However, continuous monitoring and strict adherence to food safety practices remain essential to maintain compliance and safeguard consumer health.

| Study variables | | Total Samples studied | Pb (mg/L) | As(mg/L) | Cd(mg/L) | Cu(mg/L) | Cr(mg/L) | Zn(mg/L) |
|---|---|---|---|---|---|---|---|---|
| Source cities | Dehradun | 235 | ND | ND | ND | ND | ND | ND |
| | Haridwar | 232 | ND | ND | ND | ND | ND | 0.002±0.06 |
| Category of product | Juice | 322 | ND | ND | ND | BDL | 0.007±0.006 | 0.096±0.062 |
| | Energy drink | 145 | ND | BDL | ND | ND | 0.034±0.001 | 0.019±0.005 |
| Material of packaging | Glass bottles | 154 | ND | ND | ND | 0.08±0.096 | 0.007±0.001 | 0.061±0.084 |
| | Cans | 138 | 0.001±0.0015 | ND | ND | ND | 0.009±0.004 | 0.042±0.006 |
| | Plastic bottles | 99 | BDL | ND | BDL | BDL | 0.041±0.002 | 0.082±0.017 |
| | Tetra pack | 76 | ND | ND | ND | 0.07±0.041 | 0.008±0.001 | 0.011±0.045 |
| Public supply location | Restaurants/Café | 120 | ND | ND | ND | ND | 0.03±0.003 | 0.062±0.08 |
| | Hotels | 102 | BDL | ND | BDL | ND | BDL | 0.097±0.015 |
| | Street | 113 | 0.005±0.005 | BDL | BDL | 0.074±0.085 | 0.042±0.006 | 0.08±0.006 |
| | Supermarket | 132 | BDL | BDL | BDL | ND | ND | 0.027±0.041 |
| Country of origin | Bangladesh | 38 | ND | ND | BDL | ND | 0.05±0.002 | 0.091±0.008 |
| | India | 260 | ND | BDL | ND | BDL | 0.01±0.003 | 0.07±0.062 |
| | Thailand | 86 | ND | ND | ND | 0.004±0.07 | BDL | 0.093±0.034 |
| | Indonesia | 83 | ND | ND | ND | ND | 0.026±0.012 | ND |

*ND=Not detected; BDL=below detection level (<0.0001 mg/L)*

*Table 6 : Heavy metal concentration (Mean ± SD) in juices and health drinks by study variables*

## CONCLUSION

The results demonstrate that all analysed non-alcoholic beverages exhibited notable levels of microbial contamination (APC, EBC, TCC, and TYMC), independent of beverage type, packaging format, point of purchase, or manufacturing origin. Interestingly, microbial

profiles remained mostly consistent across products from both surveyed towns. These results underscore the critical importance of implementing rigorous quality control measures throughout the production, transportation, and distribution processes of non-alcoholic beverages. Additionally, there is a pressing need for public awareness campaigns regarding beverage safety.

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# APPENDIX

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The authors declare that they have no competing interests.

**Authors' contributions**

1st author planned the experiments, analyzed the data, and drafted the manuscript. 2nd author carried out the experiments. All authors read and approved the final manuscript.

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**End**