

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.sp13.050>

Microbiological Evaluation of Fresh and Commercially Packed Mango Juice

M. Pooja¹, D. Rithiga² and Sriram Chandrasekaran^{3*}

¹Department of Microbiology Auxilium College (Autonomous), Vellore-632006, India

²Department of Microbiology Valliammal College for Women, Chennai-600102, India

³Bionyme Laboratories Chennai-600044, Tamil Nadu, India

*Corresponding author

Keywords

Antibiotic Sensitivity, Gram's Stain, Microorganism, positive, amoxicillin-clavulanate, FSSAI.

Article Info

Received:

20 August 2026

Accepted:

28 September 2026

Available Online:

10 October 2026

ABSTRACT

Mango juice is a widely consumed fruit beverage that may contain microorganisms due to raw material quality, handling, processing and storage conditions. The present study aimed to compare the microbiological quality of fresh and packed mango juice. The samples were analysed using the spread plate technique for colony count and colony morphology, followed by Gram staining, catalase, biochemical and antibiotic sensitivity tests. Both samples showed microbial growth with white to cream-coloured colonies of varying sizes and characteristics. While the packed mango juice isolate had Gram-negative rods with a positive catalase reaction, the fresh mango juice isolate was seen as Gram-positive cocci with a negative catalase reaction. Antibiotic sensitivity testing indicated different susceptibility patterns, whereas biochemical assays demonstrated variations in the isolates' traits.

Introduction

Microbiology is the branch of biological science devoted to the study of microorganisms, including bacteria, archaea, fungi, viruses, and protozoa. These microscopic organisms play essential roles in human health, agriculture, industry, and the environment (15, 16) Food microbiology is a vital field of food science that focuses on the study of microorganisms present in food and their impact on food quality, safety, and human health. Understanding microbial behavior in food systems is essential for controlling contamination, extending shelf life, and ensuring consumer safety (5, 15, 16).

This highlights the importance of studying microorganisms in food systems to ensure food safety and quality in daily consumption (15, 17).

Among various food products, fruit juices are highly susceptible to microbial contamination due to their nutrient-rich composition and high water activity (1, 5, 7). Fruit juices are considered as one of the important sources of carbohydrates, minerals, vitamins, and several proteins and therefore included as a part of diet due to their nutritional value. There is a need to evaluate the microbiological load in fruit juice samples due to the increased consumption of such juices (2, 7).

For the processed juices, containers are used for packing to keep liquid food materials safe, but due to packaging materials and human handling contamination can occur (4, 17, 18). Therefore, it is critical to minimize microbial contamination in packaged juice products (5, 15, 17).

The constituents of processed juices are mainly water, sugar, preservatives, colour, and fruit pulp. The most commonly used preservatives are benzoic acid, sorbic acid, or sulphur dioxide (1, 3).

Based on these considerations, a comparative analysis between fresh and packaged fruit juices becomes essential. The objective of the study is to determine microbial contamination in fresh and packaged commercial fruit juices.

Materials and Methods

Collection of samples

Two juice samples were collected from Chennai City, Tamil Nadu, for the present study. Sample A considered as freshly prepared fruit juice made at home, while Sample B considered as commercially processed fruit juice purchased from a retail outlet. Both samples were collected under aseptic conditions and transported to the laboratory for further analysis. The samples were properly labeled as Sample A (fresh juice) and Sample B (processed juice) and stored under appropriate conditions until analysis.

Measurement of pH

The pH of juice samples was measured using a pH meter. The pH of sample A is 6.2 and for sample B is 3.

Isolation of organisms

Dilution is a standard fluid operation widely employed in the sample preparation process of many bio (chemical) assays. The collected samples were subjected to serial dilution for microbial isolation. Each sample was serially diluted in a 1:10 ratio by transferring 1 mL of the sample into 9 mL of sterile distilled water. Successive dilutions were prepared up to 10^{-3} , resulting in dilution levels of 10^{-1} , 10^{-2} , and 10^{-3} . These dilutions were then used for the isolation and enumeration of microorganisms. Following serial dilution, nutrient agar was prepared, sterilized and poured into sterile petri plates. The plates

were allowed to solidify at room temperature. A 0.1 mL volume from each dilution was aseptically spread onto the surface of nutrient agar plates using the spread plate technique. The inoculated plates were incubated at 36 °C for 24–48 hours.

Identification of organisms

Gram Staining

The gram staining is the most crucial staining in microbiology and is the first step in differentiating bacteria. The staining technique also gives a picture about morphological forms and arrangements of bacteria. Gram staining was performed using four main steps. Initially, the bacterial smear was stained with crystal violet, a primary water-soluble stain. Subsequently, Gram's iodine was applied as a mordant to enhance the binding of the dye to the bacterial cells. The smear was then decolorized using acetone or 95% ethanol, which helps differentiate Gram-positive and Gram-negative bacteria. Thereafter, a counterstain, either safranin or diluted carbol fuchsin (1:10), was applied to visualize the decolorized cells. Finally, the stained slides were examined under a 45× objective lens.

Catalase test

The catalase test facilitates the detection of the enzyme catalase in bacteria. Catalase expedites the breakdown of hydrogen peroxide into water and oxygen.



Place a microscope slide inside a petri dish. Keep the petri dish cover available. Using a sterile inoculating loop, collect a small amount of organism from a well isolated 18-24 hour colony and place it onto the microscope slide. Using a dropper, place 1 drop of 3% H_2O_2 onto the organism on the slide. Do not mix, immediately cover the petri dish with a lid to limit aerosols and observe for immediate bubble formation.

Biochemical test

The IMViC test is based on the variations in the metabolic requirements and properties of different genera and species of bacteria. It begins with the organism inoculated into the peptone water and incubated for 24 hours at 37°C.

1. Indole test – Prepare 1% tryptophane broth and introduce a loopful of culture into it. Following incubation, Kovac's reagent are added and shake gently.

2. Methyl red test - The bacterium is need to be tested, inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37°C for 48 hours. After incubation few drops of MR reagent is added.

3. Voges Proskauer – The same procedure is repeated for VP test, instead of MR reagent Barritt's solution 1 and Barritt's solution 2 are added.

4. Citrate utilization test – The culture is inoculated into the Simmon's citrate agar and incubated overnight at 37°C.

Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method, helps to identify the effectiveness of different antibiotics by measuring the zone around the disc and classified the organism under susceptible, intermediate, or resistant. One of the most important step in this test is the inoculum preparation. This test involves by selecting the suitable bacterial colonies to suspend them into broth to ensure accurate results.

The direct colony method technique will provide the reliable results. The turbidity of the broth must be standardized with the 0.5 McFarland standard. The Muller Hinton agar is prepared and poured into the sterile petriplates and allow them to solidify at room temperature. By using a cotton swab make lawn culture and place antibiotics firmly on the plate. Finally, the plates are incubated at 37°C for 24 hours, after which the zones of inhibition are observed and measured.

Results and Discussion

Colony Count and Morphology

The colony count and morphology analysis showed microbial growth in both fresh and packed mango juice samples (FIG.1, FIG.2). White to cream, circular colonies of varying sizes were observed. The fresh mango juice showed higher visible colony growth, while the packed mango juice showed comparatively lower

microbial growth.

Gram Staining

Gram staining showed Gram-positive cocci in the fresh mango juice and Gram-negative rods in the packed mango juice (FIG.3, FIG.4). This indicates the presence of different bacterial types in the two samples based on their Gram reaction and cell morphology.

Catalase Test

The catalase test showed that the fresh mango juice isolate was catalase negative, while the packed mango juice isolate was catalase positive, indicating differences in their enzymatic characteristics.

Antibiotic Sensitivity

The fresh mango juice isolate was sensitive to ampicillin, clindamycin and penicillin, but resistant to tetracycline and vancomycin. The packed mango juice isolate was sensitive to amoxicillin-clavulanate, cefotaxime and gentamicin, while showing resistance to azithromycin and ceftazidime.

The present study was conducted to determine the microbiological quality and safety of a commercially packed fruit juice. The sample was collected, serially diluted, and inoculated on nutrient agar. The total viable count in the packed fruit juice was 3.5×10^5 CFU/mL, determined by the spread plate technique, indicating the presence of viable microorganisms. The Food Safety and Standards Authority of India (FSSAI) is a statutory body established under the Food Safety and Standards Act, 2006, under the Ministry of Health and Family Welfare, Government of India. According to the guidelines of FSSAI, the acceptable aerobic plate count using the pour plate technique is around 1×10^6 CFU/mL (14).

The elevated bacterial count obtained in the present study denotes poor microbiological quality and suggests possible contamination (14, 15, 17).

The microbial contamination can occur from various sources such as the food processing chain, food transport, cleaning processes, food preparation, food storage, food packaging, and food handling. The contamination observed in the present study may have occurred from one of these sources (15, 18).

Fig.1 Colony Morphology on Nutrient Agar (Fresh Juice Sample)



Fig.2 Colony Morphology on Nutrient Agar (Packed Juice Sample)



Fig.3 Gram's Stain Image of Isolates from Fresh Juice Sample

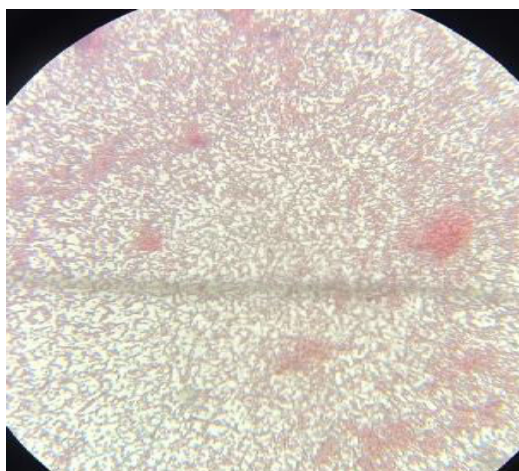


Fig.4 Gram's Stain Image of Isolates from Packed Juice Sample

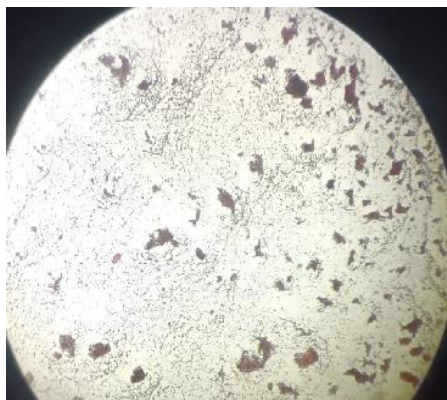


Fig.5 Biochemical Analysis of Isolates from Fresh Juice Sample

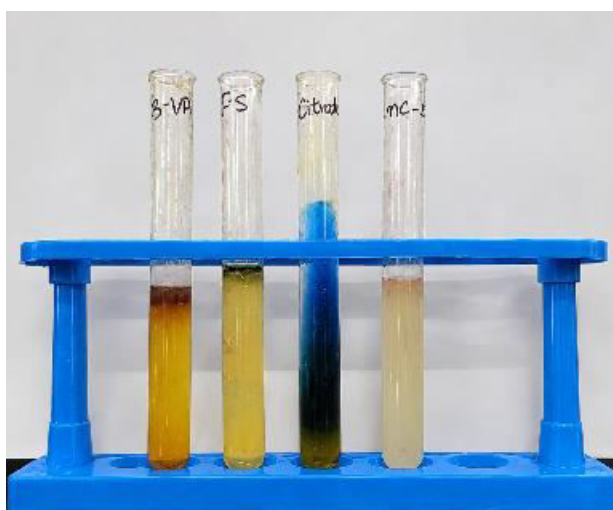


Fig.6 Biochemical Analysis of Isolates from Packed Juice Sample

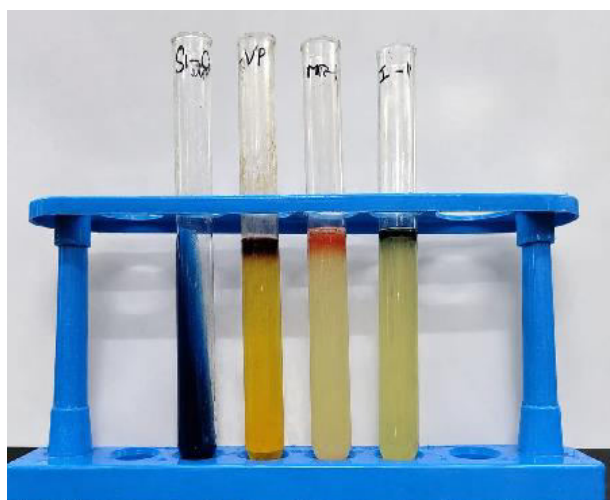


Table.1 Details of Biochemical analysis

TEST	FRESH JUICE (Lactobacillus sp.)	PACKED JUICE (Citrobacter koseri)
Indole	Negative	Negative
Methyl red	Negative	Positive
Voges proskauer	Negative	Negative
Citrate utilization	Negative	Positive

Ingredients of samples

Sample A	Mango pulp, sugar, milk
Sample B	Tested RO water, mango pulp, sugar, acidity regulators (INS 330, INS 296), contains permitted class II preservatives (INS 224), food colour (110), added mango flavours (nature identical and artificial flavouring substances)

A previous study reported standard plate counts ranging from 2×10^3 to 4×10^3 CFU/mL in industrially processed fruit juices available in Dhaka City, Bangladesh (6). Similarly, another study reported microbial counts ranging from 10^2 to 10^5 CFU/mL in packed fruit juices collected from Owerri, Nigeria (19). Another investigation detected coliform bacteria in packed fruit juices and reported that the microbial counts remained below 10^6 CFU/mL (3). Compared with these studies, the bacterial count observed in the present study was higher than that reported in study (1) but was within the range reported in study (2) and below the permissible limit specified by the FSSAI (3, 6, 14, 19).

Further identification of the bacterial isolate was carried out using Gram staining, biochemical tests, the catalase test, and antimicrobial susceptibility testing. Based on these observations, the bacterial isolate was identified as *Citrobacter koseri* (10). The isolate identified in the present study belongs to the genus *Citrobacter*, a member of the family Enterobacteriaceae (10, 11). Members of this genus are Gram-negative, non-spore-forming, facultatively anaerobic, rod-shaped bacteria that utilize citrate as a primary carbon source, characteristics consistent with the biochemical profile observed in this study (9, 10). They are commonly found in water, soil, and food, and occasionally colonize the gastrointestinal tract of animals and humans (10, 11).

Citrobacter species are recognized as opportunistic pathogens that can cause a range of infections, including septicemia, respiratory tract infections, urinary tract infections, keratitis, and meningitis, particularly in infants and immunocompromised individuals (11, 12, 20,

21). Since *Citrobacter* species are commonly found in water, the sample used in the present study may have been contaminated with the organism through contaminated water during processing or from other sources such as raw materials, equipment, or handling (15-18). Therefore, the presence of *Citrobacter koseri* in the packed fruit juice indicates microbial contamination particularly in susceptible individuals (10, 15-18).

In conclusion, the study demonstrated the presence of microorganisms in both fresh and packed mango juice samples. The fresh mango juice showed Gram-positive cocci with catalase-negative characteristics, whereas the packed mango juice showed Gram-negative rods with catalase-positive characteristics. The biochemical and antibiotic sensitivity tests further showed differences between the isolates. Overall, the results indicate that both fresh and packed mango juices can harbour microorganisms, highlighting the importance of proper hygiene, processing and storage conditions to maintain the microbiological quality and safety of fruit juices.

Author Contributions

Pooja M: Equal contribution of collecting data, analysis, writing. Rithiga. D: Equal contribution of collecting data, analysis, writing. Sriram Chandrasekaran: Work Design, Review of Methodology.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

1. Aneja KR, Dhiman R, Aggarwal NK, Aneja A. Emerging preservation techniques for controlling spoilage and pathogenic microorganisms in fruit juices. *Int J Microbiol.* 2014; 2014: 758942. <https://doi.org/10.1155/2014/758942>.
2. James V, Panchal H. Estimation of microbial contamination in fresh and commercial fruit juices: a comparative study. *J Pharm Res Int.* 2021; 33(50A): 145-153.
3. Ogodo AC, Ugbogu OC, Ekeleme UG, Nwachukwu NS. Microbial quality of commercially packed fruit juices in South-East Nigeria. *J Basic Appl Res Biomed.* 2016; 2(3): 240-245.
4. Jadhav MP, Waghmare VN. Isolation of microorganisms from soft drinks and fruit juices and their impacts on human health. *Int J Curr Res Technol.* 2021; 9(7): (article). <https://doi.org/10.6084/m9.figshare.15097317.v1>.
5. Tribst AAL, Sant'Ana AS, de Massaguer PR. Review: microbiological quality and safety of fruit juices—past, present and future perspectives. *Crit Rev Microbiol.* 2009; 35(4): 310-339. <https://doi.org/10.3109/10408410903241428>.
6. Tasnim F, Hossain MA, Hossain MK, Lopa D, Haque KMF. Quality assessment of industrially processed fruit juices available in Dhaka City, Bangladesh. *Malays J Nutr.* 2010; 16(3): 431-438.
7. Aneja KR, Dhiman R, Aggarwal NK, Kumar V, Kaur M. Microbes associated with freshly prepared juices of citrus and carrots. *Int J Food Sci.* 2014; 2014: 408085. <https://doi.org/10.1155/2014/408085>.
8. Cappuccino JG, Welsh C. *Microbiology: A Laboratory Manual.* 12th ed. New York: Pearson; 2020.
9. Tille PM. *Bailey & Scott's Diagnostic Microbiology.* 15th ed. St Louis: Elsevier; 2021.
10. Janda JM, Abbott SL, Cheung WKW, Hanson DF. Biochemical identification of *Citrobacter* in the clinical laboratory. *J Clin Microbiol.* 1994; 32(8): 1850-1854. <https://doi.org/10.1128/JCM.32.8.1850-1854.1994>.
11. Gross RJ, Rowe B. *Citrobacter koseri* (syn. *C. diversus*): biotype, serogroup and drug resistance patterns of 517 strains. *J Hyg (Lond).* 1983; 90(2): 233-239. <https://doi.org/10.1017/S0022172400028904>.
12. Martínez-Lage JF, Martínez-Lage Azorín L, Almagro MJ, Bastida ME, Reyes S, Tellez C. *Citrobacter koseri* meningitis: a neurosurgical condition? *Eur J Paediatr Neurol.* 2010; 14(4): 360-363. <https://doi.org/10.1016/j.ejpn.2009.08.002>.
13. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966; 45(4): 493-496. <https://doi.org/10.1093/ajcp/45.4.493>.
14. Food Safety and Standards Authority of India. Food Safety and Standards (Food Products Standards and Food Additives) Regulations: Microbiological standards for fruits and vegetables and their products—process hygiene criteria. New Delhi: FSSAI; 2021.
15. World Health Organization. Food safety. Geneva: World Health Organization; 2026.
16. World Health Organization. Assessing microbiological risks in food. Geneva: World Health Organization.
17. World Health Organization. Safe food handling. Geneva: World Health Organization.
18. World Health Organization, Food and Agriculture Organization of the United Nations. Prevention and control of microbiological hazards in fresh fruits and vegetables: parts 1 & 2—general principles. Microbiological Risk Assessment Series 42. Geneva: WHO; 2024.
19. Onuoha CO, Braide W, Orji JO, Uzoh CV, Adeleye SA, Okoronkwo C, *et al.*, The microbiological quality assessment of commercially available packaged fruit juices sold in Owerri, Imo State, Nigeria. *Biotechnol Ind J.* 2018; 14(2): 160.
20. Xian M, Ji X, Zhong M, Su D, Guan J, Chen R, *et al.*, Severe asthma patient with secondary *Citrobacter koseri* abdominal infection: first case report and review of the literature. *Gut Pathog.* 2023; 15: 49. <https://doi.org/10.1186/s13099-023-00574-9>.
21. Ribeiro CD, Davis P, Jones DM. *Citrobacter koseri* meningitis in a special care baby unit. *J Clin Pathol.* 1976; 29(12): 1094-1096. <https://doi.org/10.1136/jcp.29.12.1094>.

22. Sharma S, *et al.*, Antibiotic resistance in microbes from street fruit drinks and hygiene behavior of the vendors in Delhi, India. (Journal article). 2020.
23. Petruzzi L, Campaniello D, Speranza B, Corbo MR, Sinigaglia M, Bevilacqua A. Thermal treatments for fruit and vegetable juices and their effects on nutritional and sensory properties. (Review/article). 2017.
24. Putnik P, Barba FJ, Lorenzo JM, *et al.*, Novel food processing and preservation technologies for improving the quality and safety of fruit juices. (Review). 2020.
25. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

How to cite this article:

Pooja M., Rithiga D. and Sriram Chandrasekaran. 2026. Microbiological Evaluation of Fresh and Commercially Packed Mango Juice. *Int.J.Curr.Microbiol.App.Sci*. Special Issue-13: 488-495.

doi: <https://doi.org/10.20546/ijcmas.2026.sp13.050>