

# MICROBIOLOGICAL ASSESSMENT OF *ESCHERICHIA COLI* IN COMMERCIALLY AVAILABLE FROZEN FOODS FROM JABALPUR, MADHYA PRADESH, INDIA

DIVYA VERMA\* AND MONICA AGRAWAL

Department of Microbiology-Swami Vivekanand University, Sagar, M.P., India

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**Abstract**—This study was conducted to investigate the occurrence of *Escherichia coli* in frozen food stuffs sold in market of Jabalpur, Madhya Pradesh. Total 133 samples of frozen foods *i.e.*, frozen peas, beans frozen cut vegetables, frozen snacks, frozen corn and frozen cottage cheese etc. were collected. After pre-enrichment, the selective isolation for *E. coli* was performed on Eosin Methylene Blue (EMB) agar plates. In total, 17% frozen samples were found contaminated with *E. coli*. The presence of *E. coli* was confirmed by presence of green metallic sheen on EMB agar and presence of Gram-negative bacilli under microscope and other biochemical tests. Our study shows that frozen foods may predominantly harbor *E. coli* as pathogenic bacteria, although other pathogenic bacteria may also be present.

## INTRODUCTION

Frozen foods are increasingly consumed in India due to urbanization, changing lifestyles, and the expansion of cold-chain infrastructure. Freezing is an effective preservation method that retards microbial growth and extends shelf life; however, it does not eliminate microorganisms present at the time of processing (Jay *et al.*, 2008; Willis *et al.*, 2020). Consequently, frozen foods may still pose microbiological risks if adequate hygiene and temperature control are not maintained throughout the food supply chain.

*Escherichia coli* is widely recognized as an indicator organism for fecal contamination and hygienic quality of foods. Under the Food Safety and Standards Act, 2006, and the Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011, the presence of *E. coli* in specified food categories is considered unacceptable and indicative of non-compliance with sanitary practices. Although most *E. coli* strains are non-pathogenic, certain pathogenic variants are capable of causing serious foodborne illnesses, posing a significant public health concern (Doyle and Erickson, 2006).

In the Indian context, challenges such as inconsistent cold-chain maintenance, variable

implementation of Good Manufacturing Practices (GMP), and limited adherence to Hazard Analysis and Critical Control Point (HACCP) systems contribute to microbiological contamination of frozen foods (Tambekar *et al.*, 2008). Several studies conducted in India have reported the presence of *E. coli* in commercially available frozen foods, including meat and ready-to-cook products, suggesting contamination during processing, storage, or distribution (Kumar *et al.*, 2019), but limited information is available on frozen vegetables and other frozen foodstuffs specially produced by local manufacturers.

Therefore, microbiological assessment of *Escherichia coli* in commercially available frozen foods is essential to evaluate compliance with FSSAI standards, identify potential hygiene lapses, and ensure consumer safety. Such studies support regulatory surveillance and aid in strengthening food safety management systems in India.

## MATERIALS AND METHODS

The present study was undertaken to characterize *Escherichia coli* in frozen foodstuffs. The study was conducted in the Microbiology laboratory of Excellent Bio Research Solutions Pvt. Ltd., Jabalpur, a FSSAI notified food testing laboratory.

### Sample Collection

Frozen food samples were collected from various retail sources in Jabalpur, Madhya Pradesh, including supermarkets, Kirana (grocery) shops, and local markets. The samples were transported to the laboratory under cold chain conditions in thermostable containers containing ice packs, in order to maintain their frozen state and prevent microbial changes during transit.

### Storage and Handling of Samples

Upon arrival at the laboratory, all samples were stored in a deep freezer at 0°C until further analysis. Prior to microbiological testing, samples were thawed at room temperature under aseptic conditions to minimize the risk of cross-contamination.

Isolation of *Escherichia coli* from Frozen Foodstuffs (Food Safety and Standards Authority of India (FSSAI, 2024).

### Sample Preparation

A 25 g portion of each frozen food sample was aseptically weighed and transferred into a sterile stomacher bag. To this, 225 ml of sterile buffered peptone water was added. The sample was then homogenized using a stomacher machine for 30 to 60 seconds, or until a uniform suspension was achieved.

### Pre-Enrichment of *E. coli*

The homogenized sample was transferred to a Class II Biosafety Cabinet for further processing. A 1 ml aliquot of the homogenate was aseptically drawn using a sterile 1 ml micropipette, ensuring that no solid particles or debris entered the pipette tip. The aliquot was then inoculated into a sterile test tube containing 10 ml of MacConkey broth, which serves as a pre-enrichment medium for *Escherichia coli*. The inoculated tubes were incubated at 37 °C for 24 hours.

### Selective Isolation of *E. coli*

Eosin Methylene Blue (EMB) agar plates were prepared by pouring 15–20 ml of sterilized EMB agar medium into sterile petri plates. Once the medium solidified, the plates were used for the selective isolation of *E. coli*. After incubation, the MacConkey broth tubes were removed and brought into the biosafety cabinet. A sterile inoculating loop was used to streak a loopful of the enriched culture onto the surface of the EMB agar plates. The

inoculated plates were then incubated at 37 °C for 24–48 hours.

### Observation and Identification

Following incubation, the EMB agar plates were examined for the presence of typical *Escherichia coli* colonies. Colonies of *E. coli* are generally identified by their characteristic green metallic sheen on EMB agar, indicating lactose fermentation and acid production. Further Gram staining, biochemical tests for indole, MR-VP, citrate, urease, TSI, oxidase tests were performed as per standard guidelines for identification of *E. coli*.

## RESULTS

For the present study, commercially available frozen foodstuff samples were collected during different time interval for their microbiological assessment with reference to the presence of *E. coli*. Among these samples were frozen vegetables (peas, beans, carrots and corns, as well as potato fries (Fig 1). All the samples were assessed for presence of *E. coli*, and the results showed that in total, 23 frozen food samples were found contaminated with *E. coli* out of 133 frozen samples collected during the study (Fig. 2).

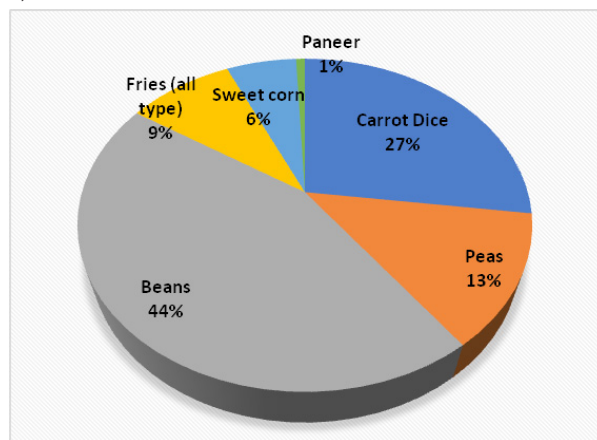


Fig. 1. Distribution of different frozen food stuffs collected during the study

The presence of *E. coli* was established by presence of green metallic sheen on EMB agar (Fig. 3) and presence of Gram-negative bacilli under microscope. For further confirmation, the biochemical tests were performed with isolated strains of *E. coli* from EMB agar. The strains showing positive indole and methyl red reactions, production of acid and gas on triple sugar iron agar

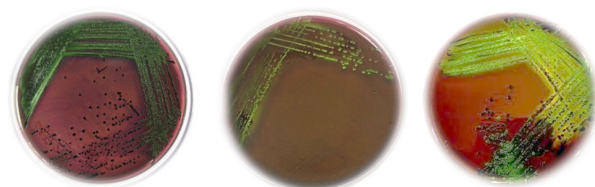


Fig. 2. The growth of presumed *E. coli* on EMB agar showing green sheen

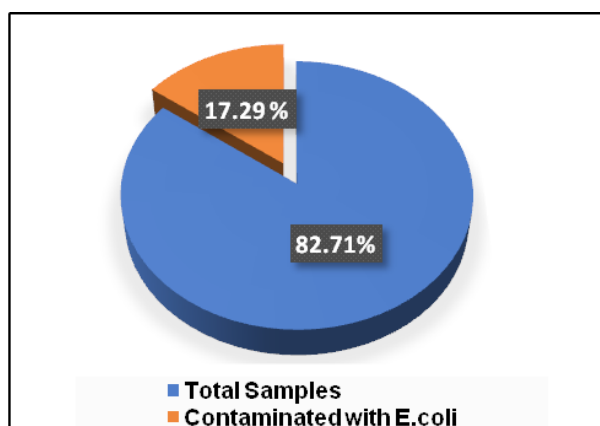


Fig. 3. Distribution of *E. coli* in frozen foodstuffs collected from Jabalpur (MP)

without  $H_2S$ , negative reactions with Voges Proskauer test, citrate utilization test, urease production and oxidase test were identified as *E. coli* strains.

## DISCUSSION

The microbiological assessment of *Escherichia coli* in commercially available frozen foods highlights important concerns regarding food safety and hygienic quality. Although freezing is widely employed to inhibit microbial growth and extend shelf life, it does not eliminate microorganisms already present in food products. *E. coli* is commonly used as an indicator organism for fecal contamination and overall sanitary conditions during food production and handling (Jay *et al.*, 2008; ICMSF (International Commission on Microbiological Specifications for Foods), 2005).

Several studies have reported the presence of *E. coli* in frozen food commodities, including meat, poultry, seafood, and vegetables. Kumar *et al.* (2019) reported detectable levels of *E. coli* in frozen meat and ready-to-cook products marketed in India, suggesting contamination during slaughtering, processing, or post-processing handling. Similarly, Rahman *et al.* (2021) observed *E. coli* contamination

in frozen foods sold in retail markets, emphasizing inadequate hygiene practices during processing and storage. These findings align with the current assessment, indicating that freezing alone is insufficient to ensure microbiological safety.

The persistence of *E. coli* in frozen foods can be attributed to its ability to survive low temperatures by entering a dormant physiological state. Previous studies have demonstrated that freezing causes minimal reduction in viable *E. coli* counts, and bacteria may regain metabolic activity upon thawing (Doyle and Erickson, 2006). Moreover, repeated freeze-thaw cycles may increase cellular injury but do not necessarily eliminate pathogenic strains, posing risks when frozen foods are improperly handled, stored or inadequately cooked (Beuchat *et al.*, 2001).

The detection of *E. coli* in frozen foods also reflects lapses in good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) implementation. Cross-contamination from raw materials, contaminated equipment, water, or food handlers has been identified as a major source of contamination in frozen food processing units (FAO/WHO, 2008). In developing countries, fragmented cold-chain infrastructure and poor retail hygiene further exacerbate microbial risks (Tambekar *et al.*, 2008; Kumar *et al.*, 2019).

In conclusion, the occurrence of *E. coli* in commercially available frozen foods underscores the need for strict hygiene control throughout the food supply chain. While freezing is effective in delaying microbial proliferation, it should not be considered a lethal process. Strengthening sanitation practices, enforcing regulatory standards, ensuring proper cold-chain management, and educating consumers on safe handling and thorough cooking are essential measures to minimize foodborne risks associated with frozen foods.

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**Conflict of Interest-** None

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