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REVIEW ARTICLE OPEN ACCESS

Recent Advances in Detection and Control Strategies for Foodborne Bacteria in Raw and Ready-to-Eat Fruits and Vegetables

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ABSTRACT

The prevalence of foodborne outbreaks due to the consumption of uncooked and ready-to-eat fruits and vegetables has seen a noticeable increase, particularly in environments lacking sanitation. This article extensively explores recent advancements in the detection of foodborne pathogens in uncooked and ready-to-eat fruits and vegetables, alongside potential prevention strategies. Predominantly, pathogens like *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica* are the main culprits in outbreaks linked to these food items globally. Notably, contamination is more prevalent in fresh leafy greens than in fruit products. Various detection methods such as culturing, microscopy, immunological assays, polymerase chain reaction (PCR), biosensors, and hyperspectral imaging have proven effective in identifying pathogens in these foods. Nonetheless, these methods come with challenges, including time consumption, accuracy concerns, and high costs. Research is ongoing to refine these detection techniques, with efforts including combining methodologies like PCR–enzyme-linked immunosorbent assay and integrating culturing with PCR. Additionally, several interventions, including cold plasma treatment, ultraviolet irradiation, and the application of edible coatings, have shown promise in mitigating contamination risks, thereby enhancing the safety of these fresh produce items.

1 | Introduction

Consumption of fruits and vegetables has been increasing because of their nutritional value and health benefits; however, this increasing consumption is also associated with a signifi-

cant number of foodborne outbreaks, which pose significant challenges to public health and the economy on national and local scales (Aladhadh 2023; Nassarawa, Luo, and Lu 2022). Fruits and vegetables can be contaminated by foodborne bacterial pathogens during preharvest, harvest, and postharvest periods,

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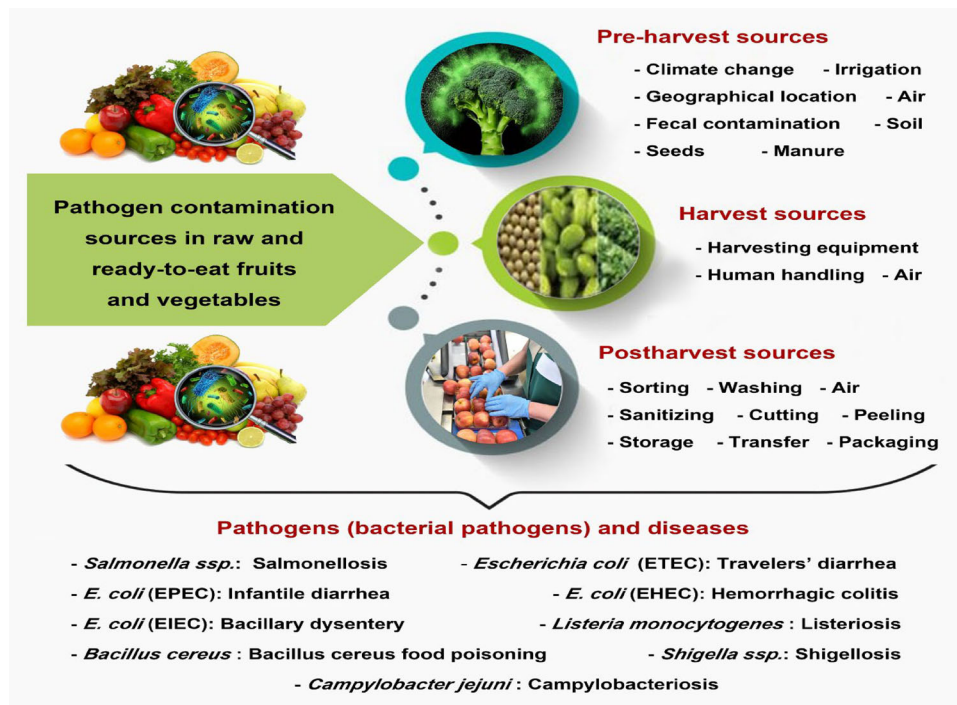


FIGURE 1 | Foodborne outbreaks associated with raw and ready-to-eat fruits and vegetables: sources and common bacterial pathogens.

and the main contamination sources of fruits and vegetables are fecal contamination, soil, manure, contaminated seeds, soil, irrigation water, geographical location, climate changes, packaging, preparation, and storage (Figure 1) (Nassarawa, Luo, and Lu 2022; Rashwan et al. 2020; Thomas et al. 2024). Infectious microbes, including bacteria, viruses, and fungi (or their toxins) are the main cause of contamination of fruits and vegetables. However, most bacteria outbreaks associated with fruits and vegetable crops were related to *Escherichia coli*, *Salmonella* spp., *Listeria* spp., *Aeromonas hydrophila*, *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Yersinia* spp., *Vibrio cholera*, *Campylobacter jejuni*, and *Shigella* spp. (Figure 1) (Azimirad et al. 2021; Lee et al. 2022). Many factors are responsible for the increase in reported outbreak numbers, including food industry globalization, recent changes in agricultural practice through various periods, and the developments in detection techniques of foodborne pathogens (Ölmez 2016; Rashwan et al. 2023).

Many detection methods, including culture methods, microscopic methods, immunological methods, polymerase chain reaction (PCR) techniques, biosensors, and hyperspectral imaging (HSI) technology can be applied for detection of foodborne bacterial pathogens in raw and ready-to-eat fruits and vegetables (Figure 2) (El-Moghazy et al. 2022; Fabiani et al. 2017; Fang et al. 2023; Johnson et al. 2023; Teixeira et al. 2020). Traditional methods include microbial cell culturing using a range of selective and nonselective enrichment methods, followed by biochemical confirmation. The key limitation of traditional methods is the time-to-detection when testing food, particularly those with short shelf-life, such as fresh fruits and vegetables. However, the rapid detection of foodborne bacteria in complex food materials is necessary to keep the quality and safety of foods (Ferone et al. 2020). Many rapid methods can be applied for the detection of foodborne pathogens in fruits and vegetables such as

immunological methods, biosensors, and HSI (El-Moghazy et al. 2022; Fabiani et al. 2017; Manthou et al. 2022).

Several strategies including physicochemical methods, and edible coating methods can be used for the protection of raw and ready-to-eat fruits and vegetables from contamination by foodborne bacteria (Kostić et al. 2023; Lee, Oh, and Min 2023). Studies focused on the detection methods of foodborne bacteria associated with raw and ready-to-eat fruits and vegetables and the potential strategies for preventing contamination by these pathogens are limited. This review comprehensively discusses the recent advances in the detection methods of foodborne pathogens associated with raw and ready-to-eat fruits and vegetables, including culture methods, microscopic methods, immunological methods, PCR techniques, biosensors, and HSI technology. Furthermore, associated bacterial outbreaks of raw and ready-to-eat fruits and vegetables as well as the recent advances in the control of contamination by these pathogens were reviewed. Therefore, this review could be a useful piece of work for detecting and controlling the contamination of raw and ready-to-eat fruits and vegetables by foodborne bacteria that may attract the attention of both food researchers and industrial individuals.

2 | Methodology of the Literature Review

This manuscript is based on a comprehensive review methodology, designed to provide a broad overview of the current advancements and challenges in the detection and control of foodborne pathogens in raw and ready-to-eat fruits and vegetables. The details of the methodology are as follows.

1. *Search platforms*: Searches were conducted across multiple reputable databases, including ScienceDirect, PubMed, and

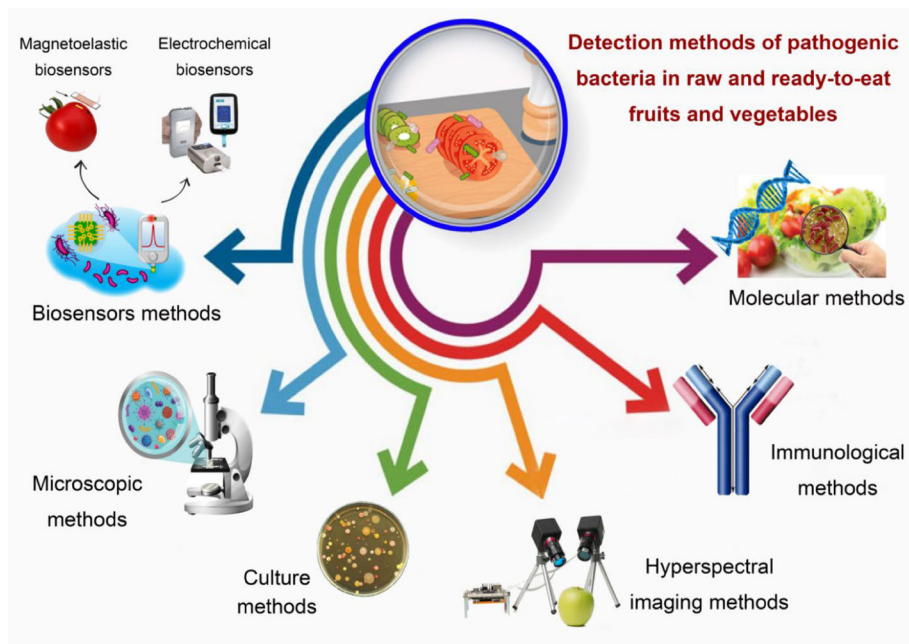


FIGURE 2 | Detection techniques of foodborne bacteria in raw and ready-to-eat fruits and vegetables.

Web of Science, to ensure the inclusion of high-quality and relevant studies.

2. **Keywords and search terms:** A combination of keywords and Boolean operators was employed, including “foodborne pathogens,” “detection techniques,” “control strategies,” “raw and ready-to-eat fruits and vegetables,” “bacterial contamination,” and “edible coatings.” These terms were selected to ensure a broad yet relevant coverage of the topic.
3. **Inclusion and exclusion criteria:**

Inclusion criteria: Articles were considered if they were published in peer-reviewed journals, discussed foodborne pathogens in raw and ready-to-eat fruits and vegetables, or covered advancements in detection and prevention strategies.

Exclusion criteria: Studies not published in English, lacking relevance to the topic, or unavailable in full text were excluded.

1. **Screening and selection process:** Articles were reviewed based on their titles and abstracts to assess relevance. Full-text reviews were then conducted to confirm the alignment of selected studies with the objectives of this review.
2. **Data extraction:** Relevant data, including study focus, detection methods, and control strategies, were extracted and synthesized to provide a comprehensive understanding of the topic.

This approach allows for a wide-ranging analysis, ensuring the inclusion of diverse perspectives and recent advancements in the field. The methodology emphasizes thoroughness and inclusivity rather than the strict procedural criteria of a comprehensive review.

3 | Bacterial Outbreaks in Produce: Prevention Measures and Associations

Fresh and ready-to-eat fruits and vegetables are recognized as important sources of foodborne outbreaks worldwide due to the contamination by different sources during various processes such as peeling, slicing, dicing, shredding, and a lack of active disinfection step through the production chain (Azimirad et al. 2021; Jeon et al. 2022). The increased consumption of fresh products such as fruits and vegetables is correlated with the increased outbreaks of microbial infection, vegetables are the most implicated food vehicle in fresh produce outbreaks (Aiyedun et al. 2021). Foodborne illness outbreaks can be caused by a range of microbial agents, including bacteria, viruses, parasites, fungi, and mycotoxins. Bacterial pathogens, including *Listeria monocytogenes*, *E. coli*, and *Salmonella enterica* are the second major contributor to outbreaks, representing 42 and 36% of outbreaks associated with consumption of fruits and vegetables in the European Union and United States, respectively, between 2004 and 2012. Furthermore, *Clostridium* spp., *Shigella* spp., *Campylobacter* spp., *V. cholerae*, and *B. cereus* were recognized as important causative of foodborne outbreaks (Havelaar et al. 2015; Thomas et al. 2024). Data from the Center for Disease Control showed the number of outbreaks, case numbers, and deaths associated with contaminated fruits and vegetables between 2006 and 2023 in the United States, *Salmonella* caused 34 outbreaks with 7256 cases and 10 deaths, *E. coli* caused 16 outbreaks with 998 cases and 10 deaths and *L. monocytogenes* caused 10 outbreaks with 303 cases and 54 deaths (Thomas et al. 2024).

The microbial safety of fresh-cut and ready-to-eat fruits and vegetables sold on retail markets in Canada was tested by culture-based isolation and identification method. (The markets located in 11 major cities, including Halifax, Saint John, Quebec, Montreal, Toronto, Ottawa, Vancouver, Kelowna, Calgary, Saskatoon, and Winnipeg.) The study indicated that *L. monocytogenes*

was detected in 0.51 and 0.24% of fresh-cut fruit and fresh-cut vegetable samples, respectively. Moreover, the detected *L. monocytogenes* was under 5 CFU/g in 67.6% of positive samples. *E. coli* O157:H7 and *Salmonella* were not detected in all tested samples (Zhang et al. 2020a). *Listeria* spp. was detected in 12.53% of samples of soft fruits (blackberries, raspberries, strawberries, and currants), vegetables (cucumbers, carrots, tomatoes), and ready-to-eat vegetables (machine-cut carrots, machine-cut salads, and machine-cut peppers) collected from Bavaria, Germany. *L. monocytogenes* was not detected in all samples, while it was identified in 1.72% of processed water and environmental samples. The study demonstrated that hygienic handling, cultivation, irrigation regime, and maintenance protocols are very significant in decreasing microbial contamination by *Listeria* in ready-to-eat fruits and vegetables (Wartha et al. 2023).

In a study that assessed the bacterial safety of fresh-cut fruits in urban markets across six south-western states of Nigeria, the enteric bacterial genera, including *Shigella*, *Pantoea*, *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* were identified in pineapple and watermelon using standard bacterial culturing methods, followed by analyses of partial 16S ribosomal RNA (rRNA) gene sequences. Eleven species were detected in these genera, including two potential pathogens, *Enterobacter sichuanensis* and *Enterobacter hormaechei*. Moreover, *Klebsiella pneumoniae* was identified in all samples, suggesting its prevalence in the fresh-cut fruit process chain. The hand swabs from the fruit wash water and fruit vendors showed phylotypes like those in the fresh-cut fruits, suggesting their involvement as potential sources of fresh-cut fruit contamination. The study recommended proper food safety measures for handlers and consumers of fresh-cut fruits to prevent bacterial contamination (Oyedele et al. 2020). Furthermore, *S. aureus* showed the highest prevalence (15.38%), followed by *E. coli* (9.23%) and *L. monocytogenes* (1.85%) in fresh-cut fruits and vegetables from Beijing, China, while no *Salmonella* was detected (Bai et al. 2024).

Contamination levels of total bacterial count and coliforms in meal kits vegetables from the Korean retail markets were recorded at 5.91 and 3.90 log CFU/g, respectively. A 3% of ready-to-eat fruits and vegetables, including purple cabbage, pineapple, and celery showed positive results for *S. aureus* (using PCR-based screening method). During the manufacturing process of ready-to-eat fruits and vegetables, contamination by *S. aureus* must be carefully prevented in the Korean retail markets (Jeon et al. 2022). The occurrence of bacterial foodborne pathogens in raw and ready-to-eat green leafy vegetables from markets located in Iran (Tehran) was investigated. The microbiological survey concluded that *C. perfringens*, *E. coli*, and *S. aureus* were the most frequent bacterial foodborne pathogens detected in fresh leafy and ready-to-eat vegetables. *V. cholerae* was not detected in any fresh leafy and ready-to-eat vegetables either by real-time PCR or by culture method. Furthermore, the microbiological survey showed that fresh leafy vegetables had higher levels of foodborne pathogens than ready-to-eat vegetables (Azimirad et al. 2021).

Focusing on the Brazilian findings, most of the published results indicated that the occurrence of *L. monocytogenes* and *Salmonella* spp. in ready-to-eat vegetables in the range from 0.6 to 3.1% and from 0.4 to 12.5%, respectively. The occurrence of these pathogenic bacteria in ready-to-eat vegetables is very

risky because consumers expect them to be safe for consumption without any additional care. The appropriate bacterial control during the production of ready-to-eat vegetables is required to produce foods with high safety and quality for consumers (Sant'Anna, de Melo Franco, and Maffei 2020). The presence of *Listeria* in frozen fruits (pineapple, papaya, melon, rhubarb, pomegranate, jackfruit, mango, grape, kiwi, coconut, cherry, avocado, banana, apple, and all types of berries) and vegetables (peas, peppers, beans, and broccoli) collected from retail and catering premises in England between 2018 and 2019 was studied. The results revealed that *Listeria* was detected in 24% vegetables and 2% fruit samples. The proper good manufacturing practice measures should be followed by all fruit and vegetable freezing plants to decrease *Listeria* contamination through processing (Willis et al. 2020).

The microbiological quality (aerobic colony count, *C. perfringens*, *L. monocytogenes*, *Salmonella* spp., and *S. aureus*) of ready-to-eat salad samples produced and commercialized in Italy, from January 2017 to January 2018 was assessed. *E. coli* was detected in 2.98% of tested samples, while other pathogenic foodborne bacteria were not detected. The authors stated that ready-to-eat salad samples from the industry were less contaminated than the supermarket ones. The washing of salads before consumption is beneficial to reduce the microbial load, especially *E. coli* number, but it is not active in eliminating pathogenic bacteria internalized within the plant's tissues (Calonico, Delfino, and Lo Nostro 2019). Two outbreaks of foodborne gastrointestinal infection linked to consumption of imported melons in the UK during 2021. The first outbreak was between March and July 2021, there was an outbreak of 17 cases of *Salmonella* (62% female, median age 61 years, 33% hospitalized). The second outbreak was between July and August 2021, there was an outbreak of 113 cases of Shiga toxin-producing *E. coli* O157:H7 (53% female, median age 21 years, 35% hospitalized) (Chan et al. 2023).

In summary, *L. monocytogenes*, *E. coli*, and *S. enterica* are the major bacterial pathogens associated with contaminated raw and ready-to-eat fruits and vegetables outbreaks around the world. The lack of hygiene conditions and decontamination steps are the major factors responsible for the increase in outbreaks associated with the consumption of raw and ready-to-eat fruits and vegetables. Good preparation practices as well as rapid and accurate detection methods of pathogens are required to control microbial contamination and improve the safety and quality of these fresh foods.

4 | Detection Techniques of Foodborne Bacteria in Raw and Ready-to-Eat Fruits and Vegetables

4.1 | Detection of Foodborne Bacteria Based on Culture Methods

Culture-based methods are generally regarded as the “gold standard” for food microbiological analysis (Foddai and Grant 2020). They are used for reliable and accurate detection of foodborne bacteria by bacteria growing on selective media to identify it after isolation of pure colonies with qualitative and quantitative data about growing bacteria. The count of alive bacterial cells in the tested samples is one of the most important advantages of the culture technique. Oxygen availability, selective media,

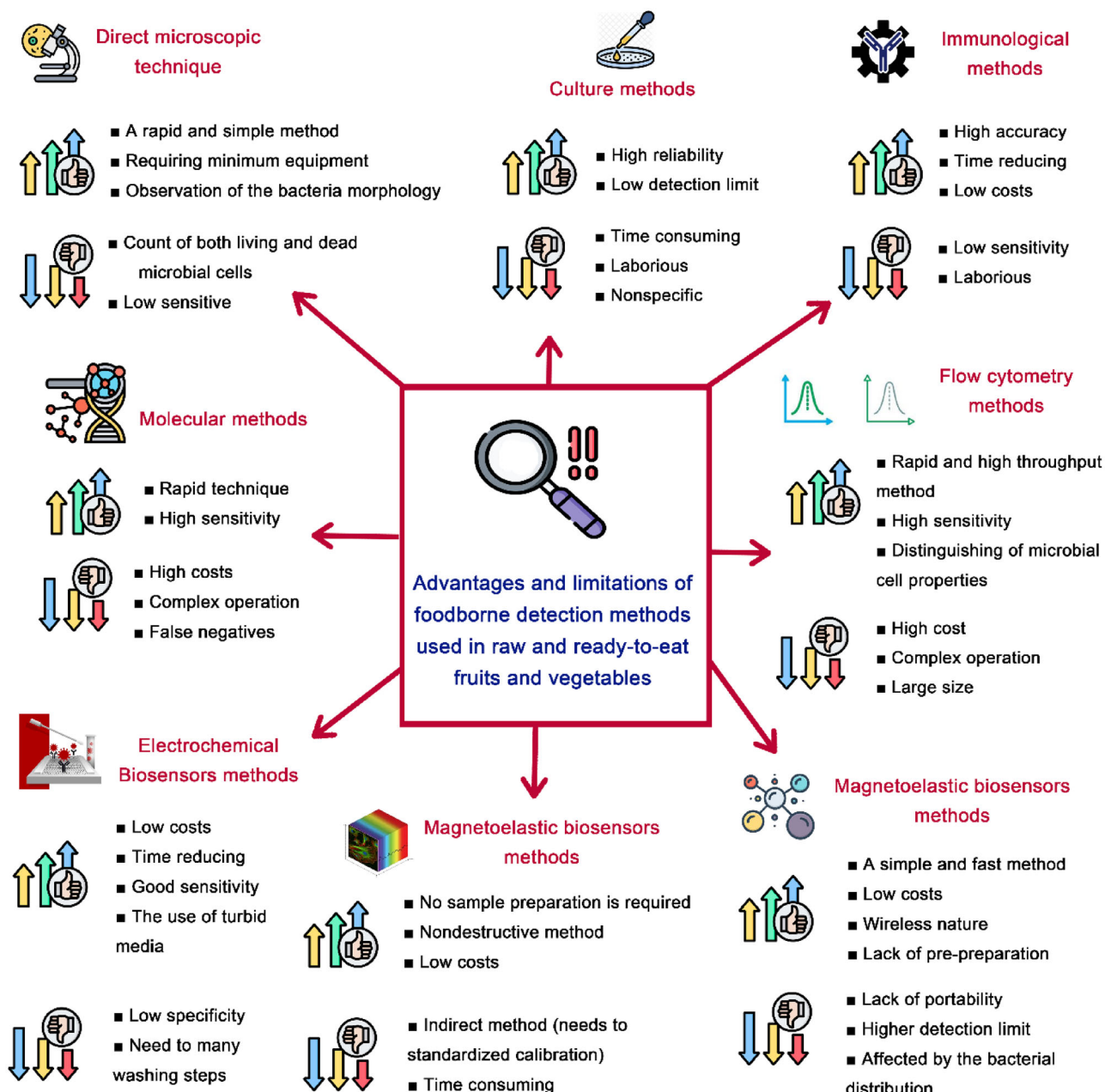


FIGURE 3 | Advantages and limitations of foodborne detection methods used in raw and ready-to-eat fruits and vegetables.

incubation temperature, and time are the most control conditions used to select the kind of bacterial growth (Ferone et al. 2020). Most foodborne bacterial pathogens need an incubation period ranging between 18 and 24 h and can increase to 72 h for some bacteria kinds. Every colony may have started from individual cells or a group of cells. Despite of many advantages of the culture technique, it is time consuming (requires 2–3 d for primary result and 7–10 d for verification) and labor-intensive method because of its many preparation steps (Figure 3) (Foddai and Grant 2020). Furthermore, aseptic methods should be thoroughly used to prevent probable contamination during the test (Ferone et al. 2020). Before isolation of target bacteria from raw food, many essential steps must be considered, such as homogenization, recovery of injured bacterial cells by pre-enrichment, nontarget bacteria inhibition, plating on selective, semi-selective, or nonselective agar, and confirming the colony purity. After the isolation step in

the culture method, biochemical identification tests are required to identify the bacterial pathogens (Lewin 2020). The culture method is used to detect many foodborne bacterial pathogens such as *E. coli*, *L. monocytogenes*, *C. jejuni*, *Yersinia enterocolitica*, and *S. aureus* in fruits and vegetables (Table 1). Furthermore, the culture method can be used in combination with other detection methods such as DNA techniques and biochemical-based techniques to obtain more rapid and accurate results.

4.2 | Detection of Foodborne Bacteria Based on Microscopic Methods

Total viable cell count can be determined in fruit and vegetable samples by direct microscopic technique. The stains were applied in the direct microscopic technique with fluorochromes based on

TABLE 1 | Various detection methods of foodborne bacterial pathogens associated with raw and ready to eat fruits and vegetables.

Detection technique	Detected pathogens	Limit of detection	Country	Associated horticulture crops	References
Microscopic techniques					
Flow cytometry	Dead and VBNC of <i>Listeria monocytogenes</i>	—	Spain	Wash water of fresh-cut lettuce	(Truchado Gambao et al. 2020)
Flow cytometry	<i>S. enterica</i> , <i>L. monocytogenes</i> , <i>E. coli</i>	—	Brazil	Mango-pineapple	(de Sousa Guedes and de Souza 2018)
Flow cytometry	<i>E. coli</i> O157: H7	—	Portugal	Lettuce	(Teixeira et al. 2020)
Immunological techniques					
ELISA with duplex PCR	<i>Salmonella</i> spp., <i>E. coli</i> O157: H7	10 CFU/mL	China	Cabbage	(Hu et al. 2018)
Magnetic bead-based immuno-detection using the Bio-Plex suspension array system	<i>L. monocytogenes</i> , <i>L. ivanovii</i>	10 CFU/g for <i>L. monocytogenes</i> , 100 CFU/g for <i>L. ivanovii</i>	USA	Lettuce, celery	(Day and Basavanna 2015)
Enzyme-linked-immuno-magnetic electrochemical (ELIME)	<i>S. napoli</i> , <i>S. thompson</i>	1–10 CFU/25 g sample	Italy	Lettuce, rucola, mixed salad	(Fabiani et al. 2017)
Molecular techniques					
Real-time PCR	<i>Pseudomonas aeruginosa</i>	10 ² CFU/mL for pure cultures	China	29 ready-to-eat vegetables	(Wang et al. 2022a)
Real-time PCR	<i>S. aureus</i> , <i>C. perfringens</i> and <i>E. coli</i>	—	Iran	Ready-to-eat and raw green leafy vegetables including radish, parsley, savory, basil, watercress, and leek	(Azimirad et al. 2021)
3-plex droplet digital PCR	<i>S. Typhimurium</i> , <i>S. enteritidis</i>	10–6 ng/μL	China	Spiked lettuce	(Fang et al. 2023)
Droplet digital PCR	<i>Campylobacter jejuni</i>	—	USA	Ready-to-eat coleslaw (made of sliced green cabbage), and ready-to-eat iceberg lettuce salad (composed of carrots, red cabbage and iceberg lettuce)	(Chon et al. 2021).

(Continues)

TABLE 1 | (Continued)

Detection technique	Detected pathogens	Limit of detection	Country	Associated horticulture crops	References
qPCR	Tetracycline-resistant <i>E. coli</i>	—	Indonesia	Ready to eat basil, cabbage, lettuce, long bean	(Mohamed, Muhaimin, and Ardiyati 2020)
qPCR	<i>S. enterica Typhimurium</i>	—	South Korea	Lettuce	(Kim et al. 2018)
Real-time PCR	Norovirus GI and GII, hepatitis A and E	—	Italy	Fresh and ready-to-eat lettuce, iceberg, carrot	(Terio et al. 2017)
Real-time PCR	Human adenoviruses, hepatitis A virus, norovirus GI, rotavirus group A	—	Egypt	Green onion, lettuce, leek, watercress	(Shaheen, Elmahdy, and Chawla-Sarkar 2019)
Reverse transcription qPCR	Hepatitis A virus, norovirus	—	Denmark	Lettuce, spinach, dates, figs, mango, blackberries, blueberries, strawberries, raspberries	(Rajjuddin et al. 2020)
Multiplex PCR	<i>Escherichia coli</i>	—	Indonesia	Eggplant, lettuce, lemon basil, cucumber, coriander, carrot, cabbage, apple, guava, tomato	(Waturangi, Hudiono, and Aliwarga 2019)
Multiplex PCR	Shiga toxin-producing <i>Escherichia coli</i> serogroups	—	Canada	Ready-to-eat strawberry, tomato, raspberry, spinach, broccoli, lettuce, apple, carrot	(Gao et al. 2018)
Multiplex PCR	<i>Y. enterocolitica</i> , <i>S. aureus</i> , <i>Shigella</i> spp., <i>Salmonella</i> spp., <i>L. monocytogenes</i> , <i>E. coli</i> O157:H7, <i>C. jejuni</i> and <i>B. cereus</i>	—	USA	Many fruits and vegetables	(Hodzic, Glavinic, and Wademan 2023)
Quadruplex PCR	<i>L. monocytogenes</i> , <i>L. ivanovi</i>	1–10 CFU/20 g	South Korea	Green romaine	(Rosimin et al. 2016)
PMA-qPCR	VBNC <i>E. coli</i> O157:H7	10 ³ CFU/g	Canada	Spinach lettuce	(Dinu and Bach 2013)

(Continues)

TABLE 1 | (Continued)

Detection technique	Detected pathogens	Limit of detection	Country	Associated horticulture crops	References
Real-time PCR with selective media	Enterotoxigenic <i>E. coli</i>	1.9–3.1 log CFU/mL	Japan	Leek, seaweed cherry tomato	(Ohtsuka et al. 2019)
Quadruplex PCR with PMA and IAC, with enrichment	<i>L. monocytogenes</i> , <i>E. coli</i> O157:H7, <i>S. enteritidis</i>	10 ¹ CFU/g	China	Lettuce, spinach Chinese cabbage	(Li et al. 2017)
Filtration, DNA concentration, and qPCR without enrichment	<i>E. coli</i> O157:H7, <i>S. Typhimurium</i>	7 CFU/25 g <i>E. coli</i> for cabbage and lettuce, 5 and 68 CFU/25 g <i>S. Typhimurium</i> , for cabbage and lettuce respectively	South Korea	Cabbage, lettuce	(Kim and Oh 2020)
Asymmetric PCR (aPCR) with fluorescent cascade amplification technique	<i>S. choleraesuis</i> , <i>S. enteritidis</i> , <i>S. Typhimurium</i>	6.9 × 10 ² CFU/g	China	Lettuce	(Yu et al. 2019)
Most probable number-multiplex polymerase chain reaction (MPN-mPCR)	<i>S. enterica serovar enteritidis</i> , <i>Salmonella spp.</i> , <i>S. enterica serovar Typhimurium</i>	—	Malaysia	Carrots, cucumbers, romaine lettuce, tomatoes, leafy lettuce, cabbages	(Saw et al. 2020)
Visual loop-mediated isothermal amplification	Salmonella strains	—	China	Ready-to-eat cucumber, lettuce	(Wan et al. 2020)
In situ-synthesized gene chip	<i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>S. aureus</i> , <i>V. parahaemolyticus</i> , <i>S. Typhimurium</i>	3 log CFU/g	China	Fresh-cut cantaloupe, lettuce	(Hu et al. 2020)
Biosensors					
Electrochemical DNA biosensor	<i>E. coli</i>	1–10 ⁴ CFU mL ⁻¹	USA	Fresh leafy vegetable	(El-Moghazy et al. 2022)
Electrochemical DNA biosensor	<i>Aeromonas hydrophila</i>	—	Poland	Carrots, lettuce	(Ligaj et al. 2014)
Wireless magnetoelastic (ME) biosensors and a surface-scanning detector	<i>S. Typhimurium</i>	10 ² to 10 ⁴ CFU/mm ²	USA	Tomatoes	(Horikawa et al. 2015)
Phage-based magnetoelastic biosensor	<i>S. Typhimurium</i>	10 ⁴ to 10 ⁵ cells/cm ²	USA	Fresh spinach leaves	(Horikawa et al. 2014)

(Continues)

TABLE 1 | (Continued)

Detection technique	Detected pathogens	Limit of detection	Country	Associated horticulture crops	References
Magnetoelastic (ME) biosensors	<i>S. Typhimurium</i>	1.5×10^3 CFU/mm ²	USA	Tomato	(Chai et al. 2013)
Lytic phage-based magnetoelastic Biosensors	Methicillin-resistant <i>S. aureus</i>	1.76 log CFU/25 mm ²	South Korea	Spinach leaves	(Byeon et al. 2015)
Microwire sensor-based immunofluorescence	<i>E. coli</i> K-12	10 ³ CFU/mL	USA	Baby spinach leaves	(Kim et al. 2011)
Hyperspectral imaging					
Hyperspectral imaging	<i>E. coli</i> K12	—	Japan	Spinach	(Siripatrawan et al. 2011)
Imaging technology and spectroscopy coupled with machine learning	Total plate count and <i>Pseudomonas</i> spp.	—	Greece	Baby spinach, rocket	(Manthou et al. 2022)
Hyperspectral imaging	<i>E. coli</i>	—	China	Fresh-cut potato slices	(Li et al. 2021a)
NIR hyperspectral imaging with (PLS-DA) technique	<i>Escherichia coli</i>	—	Iran	Lettuce	(Mobli et al. 2020)
Multispectral imaging with deep ultraviolet fluorescence sensing	<i>Salmonella</i> sp. and <i>Listeria</i> sp.	—	USA	Lettuce and spinach	(Johnson et al. 2023)

nonspecific stains (acridine orange, fluorescein isothiocyanate-FITC, and redox indicator), or specific stains combined with fluorescent antibody or oligonucleotide probes, which were advanced for *E. coli* O157:H7, *Salmonella*, and *Listeria* as well as total bacterial count. The direct microscopic technique was applied via electron microscopy, conventional microscopy, and confocal laser scanning microscopy. However, laser scanning microscopy can achieve the direct detection of thick samples inside a certain depth through interior construction without thin sectioning or processing complex samples (Takeuchi and Frank 2001). The direct epifluorescence filter technique needs some preparation steps, including sample filtration with a polycarbonate membrane, bacterial cell collection from the filter, and cell staining using fluorochrome. Direct epifluorescence filter technique can be used for bacterial cell detection with a detection limit of 10^3 cells/mL. Semi-automated and automated systems have been recognized to accelerate and enhance the epifluorescence microscopy technique because it is labor intensive (Bozal-Palabiyik et al. 2018). Direct epifluorescence filter technique can be combined with other detection methods such as aerobic plate count for the determination of mesophilic bacteria in fruits and vegetables (Araújo et al. 2009).

On the other hand, flow cytometry (FC) is an optical detection based on a laser technique. FC has hydrodynamically focused a narrow sample stream under a laminar flow of water, which has bacterial cells and moved it through a laser light beam that was emitted by fluorescent stains. This technique can be used to count and estimate the physical and chemical characteristics of microbial cells. The lenses collect the scattered light and point it to the sensitive detector that receives the data. The received data can be correlated with the physicochemical characteristics of bacterial cells. This technique has a detection limit of about 10^5 – 10^7 cells/g of food sample. FC equipment has major parts including the light source, flow cell, light detectors, optical filters, and data processing unit (Bozal-Palabiyik et al. 2018; López-Campos et al. 2012). FC is a promising method in food safety due to its ability to distinguish different physiological statuses of bacterial cells within a short time (Teixeira et al. 2020).

Using an Attune NxT Acoustic Focusing Cytometer (Thermo Fisher Scientific) equipped with a 488 nm excitation argon ion laser, Mok and coworkers detected *E. coli* K12 and *Listeria innocua* as nonpathogenic surrogates for pathogenic counterparts: *E. coli* O157:H7 and *L. monocytogenes*, respectively in the fresh apple. Double-staining of cells with propidium iodide and carboxyfluorescein diacetate were used to determine microbial cell injury viability (Mok et al. 2021). Additionally, FC (EC800 Sony Biotechnology Flow Cytometer) assay with LIVE/DEAD stains was used for *E. coli* determination in lettuce. The propidium iodide and SYTO-BC were used to determine the membrane integrity and were excited using a 488 nm laser of the flow cytometer. Results stated that FC can efficiently monitor the cell viability of pathogenic microorganisms (Teixeira et al. 2020).

Moreover, FC was applied with the fluorochromes including propidium iodide (for membrane integrity), bis-1,3-dibutylbarbituric acid (for membrane potential), ethidium bromide (for efflux activity), 5-cyano-2,3-ditoyl tetrazolium chloride (for respiratory activity), and thiazole orange (permeant dye enters all cells) for detection of *S. enterica*, *L. monocytogenes*, and *E. coli* in fresh

mango and pineapple. The author stated that multiparameter FC assay by the double-staining process was an effective method for quantitative and real-time detection of physiological functions of tested bacterial pathogens in mango and pineapple (de Sousa Guedes and de Souza 2018). FC can be successfully used for the detection of bacterial pathogens in raw and ready-to-eat fruits and vegetables with many advantages such as rapidity, high throughput, high sensitivity, and distinguishing microbial cell properties (Table 1). However, the high cost, large size, and complex operation are the main limitations recorded for this technique (Figure 3).

4.3 | Detection of Foodborne Bacteria Based on Immunological Methods

Many immunological methods were used for detection of foodborne pathogens such as ELISA (enzyme-linked immunosorbent assay), immunochromatography strip test, radio-immunoassays (RIA), immuno-precipitation assay, latex agglutination test, bioluminescent enzyme immunoassay, enzyme immunoassay, immuno-magnetic separation, and enzyme-linked fluorescent assay (Table 1) (Kim and Kim 2021; Mahari, Prakashan, and Gandhi 2023; Nassarawa, Luo, and Lu 2022). ELISA is the most multipurpose immunoassay methodology in foodborne diagnostic compared with immune-electrophoretic, immunofluorescence, and RIA assay (Välilmaa, Tilsala-Timisjärvi, and Virtanen 2015). ELISA technique has several advantages such as high levels of sensitivity, reproducibility, simplicity, and specificity in addition to the simple procedure because of its modifications to commercial kits that can be successfully used for the detection of many foodborne pathogens (Nagaraj et al. 2016).

The enzyme-linked-immuno-magnetic-electrochemical (ELIME) technique was used in comparison with real-time PCR to detect *Salmonella* Thompson and *Salmonella* Napoli in fresh raw leafy green vegetables, including canasta lettuce, romaine lettuce, cappuccina lettuce, and rucola as well as fresh ready-to-eat leafy green vegetables, including rucola, iceberg lettuce, and mixed salad. Both methods displayed a detection limit (1–10) CFU/25 g sample. The ELIME technique is exhibited to be a lower-cost and simpler technique compared with RTi-PCR, which makes it a proper method to monitor large numbers of samples without the need for advanced laboratories or skilled personnel. However, the RTi-PCR technique requires less time compared with the ELIME technique (Fabiani et al. 2017). The immunochromatographic strip test showed better capability for the detection of *S. Typhimurium* with lower detection limit, time, and costs than biochemical and morphological methods in many food materials including raw vegetables and fruits. Furthermore, this technique is suitable for use in developed countries and areas that lack advanced laboratories and it can be used by people with limited experience (Shukla et al. 2014). Additionally, many commercial test strips based on colloidal gold nanoparticles are available for the detection of *Salmonella* such as Singlepath *Salmonella* (Merck), Reveal *Salmonella* lateral flow (Neogen), VIP Gold (BioControl), RapidChek SELECT (SDIX), and DuPont Lateral Flow System *Salmonella* (Shukla et al. 2014).

Magnetic bead-based immuno-detection of *L. monocytogenes* and *Listeria ivanovii* in celery and lettuce was tested by a

combination of macrophage enrichment and Bio-Plex suspension array technique using antibody-coated MagPlex beads. The method of macrophage cell culture (RAW 264.7 macrophages) was used to isolate and enrich *L. monocytogenes* and *L. ivanovii* from selected vegetables. Antibody-conjugated MagPlex microspheres were recently applied for the isolation of some foodborne pathogens such as *L. monocytogenes* in food materials with subsequent detection by flow cytometric-based xMAP instrument. The occurrence of food components, such as proteins and carbohydrates in the tested samples may interfere with Magplex bead/analyte interactions resulting in a decrease of method sensitivity in the detection of *Campylobacter*, *Salmonella*, *Shigella*, *E. coli*, and *Listeria* in raw fruits and vegetables. To avoid this problem, these inhibitor particles must be removed from the food samples before exposure to antibody-conjugated microspheres. This technique displayed a detection limit of 100 CFU/g and decreased detection time (< 28 h for the presumptive result and < 48 h for the confirmation result). The author stated that the main restrictions of this technique were the high costs and the need for skilled personnel in the cell culture techniques (Day and Basavanna 2015; Kim et al. 2010).

The combination of PCR–ELISA detection technology has been developed to combine the advantages of both methods (high specificity of PCR and good sensitivity of ELISA). In this context, the duplex PCR–ELISA method was advanced for targeting the specific genes, *rfbE* of *E. coli* and *invA* of *Salmonella* spp. to detect these bacteria in some row vegetables such as cabbage. Compared with duplex PCR that had a detection limit of 10^3 CFU/mL, the results showed that the developed duplex PCR–ELISA displayed higher sensitivity with a detection limit of 1 CFU/mL. Moreover, duplex PCR–ELISA exhibited higher specificity than the duplex PCR technique when using 25 nontarget bacteria strains as controls. This technique has the potential to be used in the detection of many bacterial pathogens and to improve food safety (Hu et al. 2018). The combination of ELISA with PCR technique could produce a promising detection method of bacterial pathogens and needs further studies.

4.4 | Detection of Foodborne Bacteria Based on PCR Techniques

Many approaches have been developed to avoid the disadvantages of culture-based methods. In this context, the molecular technique relies on DNA amplification is one of the most important techniques for the accurate, fast, and reliable detection of bacterial pathogens in several food materials (Figure 3) (Altayb et al. 2023; Cao et al. 2024). PCR is the most molecular technique used for amplifying a few copies of DNA-specific regions. The primers of specific oligonucleotides that are generally about 20 base pairs long were used in the PCR technique. PCR is achieved by reduplicate cycle, containing double-stranded denaturation of DNA using heating, primer annealing using mixture cooling, DNA extension by Taq polymerase as well as amplification. The result of PCR is identified using electrophoresis of agarose gel for visual detection. Depending on the pathogen type, the results can be displayed after about 1 day, to rule out the negative samples (Ahmed and Karanis 2018; Bozal-Palabiyik et al. 2018; Sathyanarayana and Wainman 2024). PCR methods can be used for the detection of many foodborne bacterial pathogens such

as *E. coli* O157:H7 and *S. Typhimurium* in many food materials (Altayb et al. 2023; Cao et al. 2024). Besides the many advantages of molecular methods based on DNA, some limitations were recorded. Among them are the high costs, needing skilled workers, and the lack of capability to detect bacterial cells below 50 CFU/25 g without pre-enrichment. In addition, the dilution procedure resulted in reducing the final bacterial count of diluted samples (Aladhadh 2023; Bhunia 2014). Furthermore, PCR-based methods may have incorrect negative results because of the occurrence of PCR inhibitors in complex food samples, which may be prevented by the use of internal amplification control (IAC) (Aladhadh 2023; Li et al. 2017; Zhang et al. 2014).

An in situ synthesized gene chip was used for the detection of foodborne pathogens on fresh-cut cantaloupe and lettuce, including *Salmonella* Typhimurium, *Vibrio parahaemolyticus*, *S. aureus*, *L. monocytogenes*, and *E. coli* O157:H7. The optimized method exhibited high accuracy and strong amplification signals with a detection limit of 3 log CFU/g without culturing selected pathogens on the tested cantaloupe and lettuce samples (Sarengaowa et al. 2020). Many rapid and sensitive fluorescence detection methods were advanced for PCR with further signal amplification based on nucleic acids, such as loop-mediated isothermal amplification, hybridization chain reaction (HCR), helicase-dependent amplification, and rolling circle amplification (Li et al. 2018; Ma et al. 2017; Ma et al. 2018; Wan et al. 2020; Yu et al. 2019).

The fluorescent cascade amplification method was advanced using HCR with magnetic Fe_3O_4 nanoparticles to enhance the detection sensitivity of *Salmonella* in lettuce. The Fe_3O_4 nanoparticles separated the target ssDNA that hybridized with many fluorophores and led to reducing background signals. HCR amplification increased the sensitivity of the technique with a detection limit of 6.9×10^2 CFU/g. The fluorescence detection sensor coupled with the Fe_3O_4 nanoparticles capture displayed a promising method for the accurate detection of foodborne pathogens (Yu et al. 2019). Many PCR techniques can be applied for the detection of foodborne bacteria in raw and ready-to-eat fruits and vegetables, including real-time qPCR, droplet digital PCR, and multiplex PCR (Table 1).

4.4.1 | Real-Time qPCR

As an automated high-output technique, real-time PCR was presented to obtain a diagnostic test of pathogens in foods and reduce cross-contamination hazards (Aladhadh 2023; Fukushima et al. 2007). Microbeads, separation, filtration, and centrifugation were used as alternative methods to conventional enrichment methods in the detection of pathogens in food matrix (Choi et al. 2017; Kearns et al. 2019). DNA can be extracted from the pellet by centrifugation to separate pathogenic bacteria. However, some disadvantages were recorded in this method, including low efficiency in large-volume samples. The filtration process is an important step in the PCR technique for accurate detection of pathogenic bacteria to reduce food particles that may lead to interference and false results (Kim, Jung, and Oh 2020; Kim and Oh 2020). Quantitative real-time PCR based on species-specific novel gene targets identified by pangenome analysis was used

for the detection of *Pseudomonas aeruginosa*. The feasibility of this method was satisfactory in terms of efficiency, specificity, and sensitivity after the evaluation of artificially contaminated twenty-nine ready-to-eat vegetables. The developed method can be used for rapid detection of *P. aeruginosa*, providing accurate results to improve microbial safety of vegetables (Wang et al. 2022a). The real-time PCR assay was used to test the occurrence of foodborne bacterial pathogens in ready-to-eat and raw green leafy vegetables including radish, parsley, savory, basil, watercress, and leek. Generally, raw green leafy vegetables showed a higher bacterial contamination rate than ready-to-eat green leafy vegetables. The results indicated that *S. aureus*, followed by *C. perfringens* and *E. coli* were detected as the most prevalent pathogenic bacteria in the study. On the other hand, *V. cholerae* was not detected in any of the green leafy vegetable samples (Azimirad et al. 2021).

Using DNA concentration and filtration, a real-time qPCR technique was developed to decrease detection time and enhance the method accuracy in the detection of *Salmonella* Typhimurium and *E. coli* O157:H7 in cabbage and lettuce vegetables. Results indicated that the developed method detected *E. coli* O157:H7 with a detection limit of 7 CFU/25 g for both vegetables. On another hand, *S. Typhimurium* was detected in lettuce and cabbage with a detection limit of 68 and 5 CFU/25 g, respectively. DNA concentration and filtration increased the efficacy of recovery by 10–100 times and can be successfully applied as an alternative step to conventional enrichment with shorter time and better accuracy (Kim and Oh 2020).

Two real-time quantitative PCR detection techniques containing Ly Green saturated fluorescent dye for *Salmonella* spp. detection and DNA Green saturated fluorescent dye for *Salmonella* Enteritidis detection were operated. The results pointed out that the addition of graphene quantum dots increased the amplification system specificity. The R^2 values of standard curves were 0.998 and 0.999 for *Salmonella* spp. and *S. Enteritidis*, respectively. The sensitivity of two amplification types was estimated based on some characteristics, including bacterial cells without enrichment (2.49 CFU/mL, 2.49 CFU/mL); genomic DNA (1.36 fg/ μ L, 1.36 fg/ μ L); plasmid containing Gene ID:3335471 (1.08 copies/ μ L) and plasmid containing Gene ID:1828390769 (11.6 copies/ μ L). The molecular detection technique exhibited a higher detection rate than the traditional culture method in fresh ready-to-eat fruits and vegetables artificially contaminated with *Salmonella*, including strawberries, grapes, melon, watermelon, apple, tomato, carrot, cucumber, lettuce, and cabbage. The two detection techniques are appropriate for the detection of *Salmonella* in ready-to-eat fruits and vegetables with high specificity and sensitivity (Wan et al. 2021).

The 6S-23S ribosomal DNA region PCR analysis was used for detection of *Chryseobacterium indologenes*, *Gardmerella vaginalis*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *K. pneumoniae*, *Ochrobactrum anthropic*, and *Clostridium difficile* in fermented fruits, including banana, pineapple, and orange. The hybrid technique could introduce a high-resolution and active determination of bacteria kinds and confirm that no harmful bacterial cells are present in the final fruit product (Hussain et al. 2021). In a study aimed to the surveillance and tracking of pathogenic bacteria in the vegetable production

systems of India, the microbial analysis by PCR showed that the detection of *Pseudomonas aeruginosa* in tomato samples by 0.35 log₁₀ CFU/g, and *E. coli* in brinjal and lablab samples by 2.98–3.89 and 0.59–2.21 log₁₀ CFU/g, respectively. Also, the results indicated that *Salmonella* sp., *S. aureus*, *E. coli* O157:H7 were not detected in the tested vegetable samples. The study suggested that adequate knowledge of good agricultural practices for small-scale producers to cultivate safe vegetables is crucial to mitigating pathogenic outbreaks by fresh vegetables (Mohanapriya et al. 2024).

4.4.2 | Droplet Digital PCR

Recently, droplet digital PCR has been applied as a convenient technique for the detection and quantification of target DNA in complex food samples (Fernandez-Tejero et al. 2023; Persson et al. 2018). This method is based on sample portioning to many thousands of water-in-oil droplets before thermal cycling (Hindson et al. 2011). Moreover, this technique was effectively used for the accurate detection and quantification of many pathogens such as *Salmonella* Typhimurium and *B. cereus* in food samples (Persson et al. 2018; Peruziy et al. 2020). Although it is more expensive and time consuming than real-time PCR, droplet digital PCR is currently a more reliable method for the detection of pathogens in fruits and vegetables samples due to its higher sensitivity especially when zero-tolerance is required (Persson et al. 2018; Wang et al. 2018). In a recent study, the comparison between real-time PCR and droplet digital PCR for the detection of *Y. enterocolitica* in vegetables was carried out. The results indicate that droplet digital PCR may be introduced as a more reliable and alternative technique for the rapid detection of pathogens in vegetable samples (Cristiano et al. 2021). The higher sensitivity of droplet digital PCR may be attributed to its higher tolerance to inhibitors arising from food materials (Villamil et al. 2020).

Nanoplate digital PCR technique with bacteroides markers showed a sensitive and quick method to detect the fecal contamination of crops such as strawberries with a detection limit of 250 fg/ μ L (Fernandez-Tejero et al. 2023). A 3-plex droplet digital PCR assay displayed high specificity to detect and differentiate *Salmonella* and its two serovars Typhimurium and Enteritidis in spiked lettuce with a detection limit of 10⁻⁶ ng/ μ L. The developed assay showed satisfactory performance with high sensitivity and anti-interference ability (Fang et al. 2023). In another study, no significant differences ($p > 0.05$) were observed in the detection efficiency of positive *C. jejuni* samples from ready-to-eat coleslaw (made of sliced green cabbage), and ready-to-eat iceberg lettuce salad (composed of carrots, red cabbage, and iceberg lettuce) using real-time PCR and droplet digital PCR detection methods (Chon et al. 2021). In most reviewed studies, droplet digital PCR showed higher sensitivity than real-time PCR may be due to its higher tolerance to inhibitors arising from food materials.

4.4.3 | Multiplex PCR

Using multiple primers, multiplex PCR is used to simultaneously amplify multiple DNA sequences. Compared with other

PCR-based methods, this technique allows the detection of multiple target microbial cells in a single reaction and introduces many advantages such as reducing time and costs with higher accuracy (Ahari et al. 2020; Altayb et al. 2023; Awasthi et al. 2019). Multiplex PCR was widely used for the detection of foodborne bacteria such as *L. monocytogenes* and *E. coli* in fresh vegetables and fruits such as lettuce, sprouts, broccoli, parsley, spinach, tomato, apple, lemon, and guava. However, probable false positive results may be recorded by multiplex PCR due to the occurrence of DNA from dead or nonviable bacterial cells (Altayb et al. 2023; Moreno et al. 2012; Waturangi, Hudiono, and Aliwarga 2019).

In comparison with the conventional methods, the multiplex PCR can be considerably more reliable and rapid for detection of *Vibrio* spp. because *V. vulnificus* was detected in more samples by multiplex PCR in five groups of green vegetables, including spinach and onion, cress and radish, tarragon and basil, mint and dill, and parsley and coriander (Ahari et al. 2020). The combination of multiplex PCR with the most probable number method (mPCR–MPN) was successfully used for detection of *S. enterica* serovar Enteritidis, *S. enterica* serovar Typhimurium, and *Salmonella* spp. in fresh cucumbers, carrots, tomatoes, romaine lettuce, butterhead lettuce, leafy lettuce, iceberg lettuce, and cabbages (Saw et al. 2020). The detection of main foodborne bacteria, including *S. enterica*, *S. aureus*, *L. monocytogenes*, and *E. coli* in ready-to-eat organic lettuce was carried out using the combination of enrichment in a single culture broth and multiplex PCR assay. Buffered peptone water (BPW) was selected as the optimum enrichment medium among other tested enrichment media. BPW broth can successfully increase the simultaneous growth of selected pathogens and could be used before multiplex PCR assay in ready-to-eat vegetables, thus considerably decreasing the time, effort, and cost of the assay (Boukharouba et al. 2022).

A hydrolysis (TaqMan) probe-based system was developed and evaluated for simultaneous detection of 8 most common foodborne pathogens, including *Y. enterocolitica*, *S. aureus*, *Shigella* spp., *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7, *C. jejuni* and *B. cereus* in a single-step procedure by multiplex qPCR. The strategy of multicolor combinational probe coding (MCPC) was used to enable simultaneous detection, quantification, and identification of targeted genes. The effectiveness of individual qPCR reactions for each target gene had values comparable to those established for multiplex qPCR with a detection limit of less than 10 copies of DNA per reaction. The development showed a more rapid and sensitive system for the simultaneous detection of selected bacterial pathogens in fruits and vegetables (Hodzic, Glavinic, and Wademan 2023). Propidium monoazide (PMA) was used for quadruplex PCR to decrease the false positive results, which mostly resulted from PCR inhibitors. The IAC was used as an indicator for false negative results. The combination of PMA–mPCR–IAC system increased the accuracy and decreased the detection limit from 10^2 to 10^1 CFU/g for *L. monocytogenes*, *E. coli* O157:H7, and *S. Enteritidis* in spinach, lettuce, and Chinese cabbage (Li et al. 2017). To conclude, the multiplex PCR technique showed a prospective future for the detection of bacterial pathogens in fruit and vegetable samples due to its numerous advantages such as reducing time and costs with higher accuracy.

4.5 | Detection of Foodborne Bacteria Based on Biosensors

Biosensors are semiquantitative or quantitative analytical instrumental methods that contain sensing agents of biological origins that are combined within or in contact with a physiochemical transducer. Because of their rapidity, specificity, field applicability, ease of mass fabrication, and costs, biosensors have been introduced as important alternatives to conventional methods and successfully applied in many applications such as the detection of microbial and chemical contamination in many food materials (Figure 3) (Nnachi et al. 2022; Thakur and Ragavan 2013; Wang et al. 2023a). Generally, biosensors contain two elements, transducers and bioreceptors. Bioreceptors can distinguish and bind specific molecules and categorize them into five groups. Antibodies, nucleic acids, and enzymes are the main bioreceptor groups, in addition to bacteriophagic and cellular receptors. The transducer part is responsible for converting biological reactions into measurable signals. According to signal transducer types, biosensors are divided into numerous classes, including electrochemical and magnetoelastic (ME) biosensors (Table 1) (Sharma et al. 2013; Wang et al. 2023a).

4.5.1 | Electrochemical Biosensors

Electrochemical biosensors are advanced transduction-based techniques able to detect foodborne microorganisms with short detection time and high sensitivity. It measures the electrochemical responses and can directly transform the electrical signals to electronic fields. Compared with other analytical transduction approaches, electrochemical biosensors introduce several advantages, including the possibility to use in the turbid media, comparable instrumental sensitivity, and the possibility of miniaturization (analysis of small volume) (Wang et al. 2023a; Yahaya, Noordin, and Abdul Razak 2019; Zolti, Suganthan, and Ramasamy 2023). Electrochemical biosensors have main three parts, including an electrochemical signal output unit, a signal transduction part, and a target recognition part (Zhang et al. 2020b). A highly sensitive electrochemical biosensor was improved to detect *E. coli* on fresh leafy vegetables. The biosensor was assembled based on using genetically engineered bacteriophage T7 encoding with *phoA* gene as biorecognition elements, which can trigger the enzyme of alkaline phosphatase overexpression upon target bacterial infection. The alkaline phosphatase overexpression was electrochemically followed by a single-wall carbon nanotube-modified screen-printed electrode. The developed electrochemical biosensor can provide accurate and rapid detection of pathogenic *E. coli* on spinach leaves at a level of $1\text{--}10^4$ CFU mL⁻¹ within 1 h after pre-enrichment. Furthermore, it exhibited high specificity to *E. coli* in the presence of other food-bacterial contaminants (El-Moghazy et al. 2022). The development of new types of electrochemical biosensors with eco-friendly, biocompatible, and low-cost electrodes, advanced engineering of bioreceptors, and application of innovative nanomaterials for signals enhancement are the most important future trends and challenges concerning electrochemical biosensors (Rizzotto et al. 2023a). The voltammetric biosensor is another type of electrochemical biosensor that can detect the current change caused by oxidation or reduction reaction of active

electrochemical analysis (Chillawar, Tadi, and Motghare 2015). AuNPs-modified screen-printed carbon electrodes and polyaniline can develop the voltammetric biosensors leading to increasing surface area and biomolecule immobilization conductivity (Shoae, Forouzandeh, and Omidfar 2018).

Electroactive hybridization indicators including daunomycin (based on multiwalled carbon nanotubes modified carbon paste electrode and exploited daunomycin) and Hoechst 33258 (based on gold electrode modified with mixed SAM monolayer and exploited Hoechst 33258) were applied for detection of *A. hydrophila* bacteria in carrots and lettuce. The first biosensor displayed better reproducibility of its analytical outcome and shorter time than the second biosensor. Both biosensors are effective and reliable as detection tools for pathogens and can be applied in quality assurances of food production (Ligaj et al. 2014). The measurement of oxidation and reduction potential of the electrochemical reaction is the main principle of potentiometric biosensors. These biosensors use ion-selective electrodes to convert the biological reaction to an electrical signal. The binding of antigens-antibodies leads to small changes in protein charges that can be potentiometrically determined. The major drawback of potentiometric biosensors is the poor selectivity in some food samples (Chadha et al. 2022; Yahaya, Noordin, and Abdul Razak 2019). Impedimetric biosensors are active devices used for foodborne pathogens detection and based on conductivity change of environment through microbial metabolisms of electrically charged ionic compounds and inert substrate of acidic products including lactic acid, acetic acid, and amino acids (Yahaya, Noordin, and Abdul Razak 2019). Recently, the detection of pathogens by impedance linking of biological recognition technology has increased interest (Bhavadharini et al. 2022).

The use of nanomaterials in impedimetric biosensors achieved many advantages such as decreasing time and costs. The impedimetric biosensors with AuNPs-modified electrodes have higher effectiveness compared with impedimetric biosensors with planar electrodes, the self-assembled AuNPs and protein G could improve active surface areas by 2.2 times (Lin et al. 2019). Impedimetric immunosensors with AuNPs exhibited high specificity and sensitivity for *E. coli* O157:H7 detection by attaching with microbial cells through strong interaction and changed impedimetric signal (Wan et al. 2016). Si₃N₄ deposited nanogap electrode chip was developed for rapid detection of *S. aureus* DNA via measure of impedimetric signal (Lee et al. 2018). The impedimetric electrochemical biosensor based on the flower-like ZnO nanomaterials is another novel type of electrochemical biosensor. This innovative biosensor exhibited a lower detection limit, higher stability, and a broad response range (Tak, Gupta, and Tomar 2014). However, the applications of potentiometric and impedimetric biosensors in the detection of foodborne bacterial pathogens in fruits and vegetables are limited, this must be considered in future studies.

4.5.2 | ME Biosensors

ME biosensors are one of the most common biosensors that show promise for in situ, real-time detection of pathogens on fruit and vegetable surfaces due to their wireless, freestanding nature and

lack of pretest preparation (Chadha et al. 2022; Horikawa et al. 2014). The ME biosensor contains a transducer that was covered with a bio-molecular recognition element for specific capture and binding with target pathogens. The ME sensors (recognized by remote sensing) were produced using amorphous ferromagnetic alloys. When induced using magnetic fields, which regularly change, the materials display ME resonances that can be detected by noncontact signal collector coils (Senturk et al. 2018). Phage-based ME biosensors have been advanced for fast and direct pathogen detection such as *S. Typhimurium* in fresh fruits and vegetables such as tomato, spinach, and watermelon (Chai et al. 2013).

The phage-coated ME biosensor combined with a surface-scanning detector was introduced as a novel, revolutionary technique for the detection of *Salmonella* Typhimurium on the tomato surface. The developed method detected the target pathogen without sample preparation or enrichment through 2–10 min. This method can be applied for on-site and fast pathogens detection and investigation of outbreaks (Horikawa et al. 2015). The device of ME biosensor successfully detected *S. aureus* on spinach leaves surface at a level of 1.76 log CFU/25 mm² (Byeon et al. 2015). The application of chitosan-modified magnetic Fe₃O₄ nanoparticles in ME sensors led to amplifying its response to *E. coli* O157:H7 by improving the mass loading on the sensor surface (Lin et al. 2010). Moreover, gold-coated magnetic nanoparticles are applied as new amplification ways to enhance the sensor sensitivity (Campanile et al. 2020).

4.6 | Detection of Foodborne Bacteria Based on HSI Technology

HSI represents an advanced and nondestructive analytical method with a wide range of applications in evaluating the safety and quality of fruits and vegetables (Table 1) (Lu et al. 2020). Combining conventional imaging and spectroscopy, HSI furnishes a unique spectral signature for each pixel within a sample area, showcasing its effectiveness in scrutinizing fruits and vegetables for bacterial contamination and, thereby, playing a crucial role in ensuring the detailed analysis of hyperspectral images for the quality and safety assurance of these food products (Cen et al. 2022; Wang et al. 2023b). This method offers robust capabilities by scanning across multiple spectral ranges, most commonly within the visible (VIS) and near-infrared (NIR) bands. The architecture of a typical HSI system incorporates a light source, hyperspectral camera, and data acquisition and processing unit. The hyperspectral camera itself is often a sophisticated assembly of imaging optics, a narrow slit, a diffraction grating, and a 2D focal plane array (FPA) detector (Rodrigues et al. 2020). These elements work in tandem to capture light emanating from the illuminated sample, segregate it into discrete wavelengths via the diffraction grating, and project these segregated wavelengths onto the FPA. The data thus acquired are high-dimensional, offering both spatial (2D) and spectral (1D) information, and are commonly stored in the form of a hypercube (Ren et al. 2014). Figure 4 depicts the standard postprocessing sequence following the acquisition of a hypercube.

Although HSI provides substantial benefits over traditional methods for swift, noninvasive, and planar assays, it is not without its

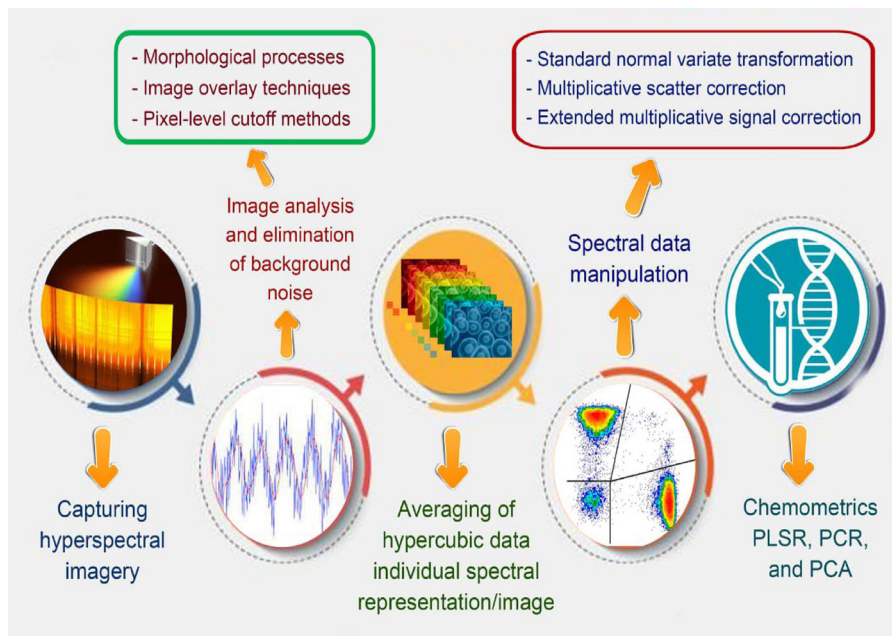


FIGURE 4 | Standard workflow for postprocessing following hypercube data acquisition.

limitations. These challenges include the complex composition of food matrices, variable bacterial population levels in samples, and inconsistent chemical attributes. Advanced chemometric methods and AI-based technologies like machine learning have been instrumental in mitigating these issues by efficiently modeling and extracting valuable data from HSI outputs. Machine learning methodologies can be categorized into three primary subtypes: supervised, unsupervised, and reinforcement learning (Misra et al. 2022; Ozdemir and Polat 2020).

HSI was applied as a rapid technique for the detection of *E. coli* K12 on inoculated fresh spinach. The hyperspectral camera (400–1000 nm), with spectral resolution (5 nm) was used to acquire a hyperspectral image of packaged spinach. The obtained reflectance spectra from different sites of the sample surface were pretreated using Sawitzky–Golay, then analyzed by chemometrics including an artificial neural network (to construct a prediction map of all pixel spectra of an image to display the number of *E. coli* in the sample) and principal component analysis (to remove redundant information of hyperspectral data). The study proposed that the application of HSI with chemometrics showed an innovative and rapid method for the detection of *E. coli* contamination in fresh packaged spinach (Siripatrawan et al. 2011).

Spectroscopy and imaging technology combined with machine learning can be successfully applied to detect bacteria associated with ready-to-eat leafy vegetables (Manthou et al. 2022). For this purpose, multispectral imaging (MSI), VIS spectroscopy, NIR, and Fourier-transform infrared (FTIR) were used for the assessment of total plate count and *Pseudomonas* spp. on the baby spinach and rocket. The results indicated that the models derived mainly from VIS sensors displayed better assessment of bacterial cells on baby spinach, while the models derived mainly from FTIR and MSI exhibited better assessment of bacterial cells on the rocket. The authors concluded that each vegetable type needs distinct sensors and computational analysis applications, and there

is no single combination of analytical approaches/algorithms that could be used for all fruits and vegetables (Manthou et al. 2022). HSI in VIS–NIR (400–1000 nm) could be successfully applied as a nondestructive and rapid detection of *E. coli* on the surfaces of fresh-cut potato slices. Four preprocessing approaches besides genetic algorithm were explored to handle spectral data and select characteristic wavelengths to establish a nonlinear and linear regression model. Based on full-spectrum, the performance of the back-propagation neural network model was satisfactory. (Overall accuracy was 97.6%, residual predictive deviation was 6.7.) Using this technique, the study effectively explored the optimum treatment time to inhibit *E. coli* on the surface of fresh-cut potato slices (Li et al. 2021a).

The NIR HSI with (PLS–DA) technique was used for rapid detection of contaminated lettuce by *E. coli*. The dimensionality reduction (spatial preprocessing) and spectral preprocessing were performed by principal component analysis and standard normal variate with the mean centering method. The wavelength of 1200 nm and spectral region of 1400–1500 nm were selected as the most important wavelength that provides the most information for group classification and target identification with high accuracy (90%) and class error (0.008). The NIR HSI with the PLS–DA method could be an accurate and fast technique for non-destructive and real-time detection of pathogens contamination in lettuce (Mobli et al. 2020). Deep ultraviolet (UV) fluorescence sensing with MSI was applied to monitor and detect foodborne pathogens, including *Salmonella* sp. and *Listeria* sp. on leafy green vegetables such as lettuce and spinach. This technique may result in the implementation of better strategies and technology to decrease the associated risks with contamination of foodborne bacterial pathogens (Johnson et al. 2023). HSI showed many advantages as a detection method of bacteria pathogens; however, further studies are required to overcome their limitations such as the complex composition of food matrices, variable bacterial population levels, and inconsistent chemical attributes.

5 | Advanced Techniques to Safeguard Raw and Ready-to-Eat Produce from Pathogens

Fruits and vegetables are particularly vulnerable to air, moisture, mechanical action, microbial spoilage, and endogenous enzymes during storage, transportation, and marketing. These factors lead to the deterioration of fruits and vegetables quality, particularly those that are fresh-cut and ready-to-eat (Adiani, Gupta, and Variyar 2021). Therefore, several strategies including physicochemical methods, and edible coating methods can be used for the protection of raw and ready-to-eat fruits and vegetables from contamination by foodborne bacteria (Table 2) (Kostić et al. 2023; Lee, Oh, and Min 2023; Rashwan et al. 2024).

5.1 | Control of Microbial Contamination by Physicochemical Methods

The antimicrobial activity of essential oils against many foodborne pathogens was reported by many previous studies (Adeyemi, Olajide, and Ogunlana 2024; Al-Geddawi et al. 2016). An environmentally friendly preservation method based on eucalyptus and rosemary essential oils (vapor and dipping) was tested to maintain the quality properties of cucumber during storage at 11°C for 2 weeks. The authors exhibited that the essential oils successfully preserved cucumber quality without any significant weight losses in the case of the vapor phase, while dipped cucumbers showed a greater weight loss (3.5%) compared with the control (3%). The results concluded that these essential oils (vapor or dipping) can be a potential natural alternative to be used to preserve fresh fruits and vegetables instead of the common sanitizer (chlorine) (Xylia et al. 2022). Furthermore, combining essential oils with nonthermal techniques such as ultrasound (US), cold plasma, and irradiation can prolong the shelf-life of fruits and vegetables and prevent spoilage by microorganisms without altering the organoleptic properties at low concentrations (Perumal et al. 2022). Cinnamon essential oil, TiO₂, and chitosan were combined to produce safe and renewable nanocomposites for the preservation of fruits such as strawberries. The results showed that cinnamon essential oil mainly destroyed the bacterial cell wall through penetration, while TiO₂ is through destruction. The strawberry coated with renewable nanocomposites showed lower weight loss, better hardness, and mildew rate. Additionally, the shelf-life of strawberries was extended for 4 days at 20°C compared with the control. All four nanocomposites were not cytotoxic and might be a good choice for fruit preservation (Yuan et al. 2023).

A variety of excited molecular, atomic, ionic, and radical species combine to form plasma, an electrically enhanced material with a multitude of uses. Fresh and fresh-cut vegetables and fruits can be decontaminated and preserved using cold plasma as a promising nonthermal technique. Plasma is a reactive oxygen and nitrogen species cocktail that acts quickly against a range of pathogenic and food spoilage organisms, making it an effective decontamination intervention. The plasma treatment of water known as plasma-activated water (PAW) has been widely employed to reduce the number of microorganisms in food products (Bezerra et al. 2023; Gao, Francis, and Zhang 2022). The authors examined the effects of washing fresh-cut pears with PAW treatment under three different conditions (peak voltage = 6, 8, and 10 kV) for

5 min and then stored at 4°C for 12 days on the native microflora survival. Results showed that all PAW treatments significantly inhibited the growth of aerobic bacteria, where the total counts of aerobic bacteria in fresh-cut pears were under the detection limit (1.0 log₁₀ CFU/g) at the beginning of the storage period (day 0), as well as total aerobic bacteria (log₁₀ CFU/g) in fresh-cut pears after 2 days of 8-kV PAW treatment was < 1 compared with 2.14 and 1.98 log₁₀ CFU/g for distilled water and sodium hypochlorite (200 µL/L), respectively (Chen et al. 2019).

Another study found that while there were very slight changes in the pH, color, texture, and total carotenoids of fresh-cut carrots treated with cold plasma, there was a 2 log₁₀ CFU/g drop in the population of total aerobic mesophiles in fresh-cut carrots treated at 100 kV for 5 min (~250 W power). Reactive oxygen and nitrogen species produced in plasma, which are derived from cellular disintegration, are the cause of the inactivation because of their antibacterial properties (Kumar Mahnot et al. 2020). Moreover, Song, Annous, and Fan (2020) investigated the effects of cold plasma-activated hydrogen peroxide (H₂O₂) aerosol (17.62 mL/m³) in a large chamber (4.27 × 2.44 × 2.14 m) on populations of *Listeria innocua* and *Salmonella* Typhimurium in apples, tomatoes, and cantaloupe during storage. The tested method achieved a significant reduction in the inoculated bacteria, down to a level below the detection limit (0.70 log CFU/piece) (Song, Annous, and Fan 2020). In a similar study, the fresh-cut apple treated with PAW showed a delay in bacterial growth (< 1 log CFU/g) compared with the control sample, which began to rise progressively on the second day (Perinban, Orsat, and Raghavan 2022).

The investigation of the possible application of PAW for the inactivation of blueberries inoculated with *S. aureus* and *E. coli* was studied. The authors discovered that PAW treatments showed noticeably larger decreases than water treatment, where the inactivation of *E. coli* and *S. aureus* by PAW was increased from 0.385 to 1.529 and 0.46 to 1.61 log, respectively, which were both significantly higher than that of the water treatment (from 0.01 to 0.12 and 0.048 to 0.254 log, respectively) at the corresponding time (Gan et al. 2022). The denaturation of DNA caused by reactive oxygen species generated by plasma and damage to the cell membrane were the main causes of the microbial inactivation of PAW, as demonstrated by electron microscopy, optical emission spectra of plasma, and propidium iodide and acridine orange staining (Gan et al. 2022). Based on the concept of hurdle technology, a combined nonthermal treatment approach for the decontamination of fresh-cut vegetables packaged in plastic containers has been created using cold plasma and H₂O₂ (HCP treatment). After 3 min of HCP treatment, the numbers of indigenous aerobic bacteria, *E. coli* O157:H7, and *L. monocytogenes* in mixed vegetables were reduced by 1.6, 1.2, and 1.3 log CFU/g, respectively. The numbers of indigenous aerobic bacteria in mixed vegetables were lower in the HCP group (by 1.6–2.4 log CFU/g during storage at 4°C, and by 0.4–1.6 log CFU/g during storage at 10°C) than in the control group. During 14 days of storage at 4°C, HCP treatment significantly prevented *E. coli* O157:H7 and *L. monocytogenes* growth in mixed vegetables (Lee, Oh, and Min 2023).

Food irradiation is a nonthermal, energy-efficient technology used for food preservation by food exposure to various ionizing

TABLE 2 | The recent advances in preventing pathogens contamination in raw and ready-to-eat fruits and vegetables.

Control methods	Fruits and vegetables	Method effectiveness or achievement	References
0.25% H ₂ O ₂ with peroxyacetic acid (40 ppm)	Tomatoes and cucumbers	Log reduction ranged between 2.65 and 3.35 log CFU/tomato and 1.28 to 2.66 log CFU/cucumber against <i>S. Tennessee</i> , <i>S. Typhimurium</i> , <i>L. monocytogenes</i>	(Li et al. 2020)
Plasma-activated water	Blueberries	Reduction of more than 1 log CFU against <i>S. aureus</i> , <i>E. coli</i>	(Gan et al. 2022)
1.22% H ₂ O ₂ with UV (68.4 kJ/m ²)	Apples	Reduction of more than 6.6 log CFU against <i>E. coli</i> O157:H7	(Ho et al. 2020)
7.8% H ₂ O ₂ and cold plasma	Cantaloupe, romaine lettuce, grape tomatoes, and Granny Smith apples	Reduction of 2.35–5.50 log CFU/piece against <i>L. innocua</i> , <i>S. Typhimurium</i>	(Song and Fan 2020)
Active edible packaging (pectin and oregano essential oil)	Fresh/fresh-cut fruit	Shelf-life extension	(Kostić et al. 2023)
Active edible packaging (algininate cross-linking enriched hexyl acetate)	Fresh-cut apples	Shelf-life extension	(Duong et al. 2023)
Renewable nanocomposites (cinnamon essential oil, TiO ₂ , and chitosan)	Strawberry	Shelf-life extension	(Yuan et al. 2023)
Aloe vera gel	Fruits and vegetables	Shelf-life extension	(Nicolau-Lapeña et al. 2021)
Edible coating based on carboxymethyl cellulose and pectin	Fruits and vegetables	Shelf-life extension	(Panahirad et al. 2021)

and nonionizing radiations. It extends the shelf-life of food products and reduces the microbial load and can be considered promising and well-established technology but still underutilized on a large scale. However, consumer buying behavior poses an important challenge with innovative food processing technologies such as food irradiation. The UV-C has the potential to be proved better than other methods at acceptable doses that can help maintain the quality of fruits and vegetables (Bisht et al. 2021). The synergistic effect between UV-C and MAP (modified atmosphere packaging) slowed the growth of bacteria in fresh-cut carrots. The microbial load on fresh-cut carrots was reduced to 1.02 log CFU/g by UV-C+MAP treatment and showed lower than that of the control sample (2.24 log CFU/g) (Li et al. 2021b). Besides, the preservation treatment of fresh raspberries using e-beam irradiation (3 kGy and during 7 days of refrigerated storage) showed a reduction of 2 log CFU/g of mesophilic bacteria (Elias et al. 2020). Furthermore, independent of the incubation temperature, a combination of UV-A light and low curcumin concentrations (1–10 mg/L) was effective in inactivating more than 5 log CFU/mL of *E. coli* O157:H7 and *L. innocua* in washing solution for fresh produce. In addition, lower pH improved curcumin's antibacterial action when combined with light against both *E. coli* O157:H7 and *L. innocua*. This increase in antimicrobial activity lowered the time required to inactivate 5 log CFU/mL of *E. coli* O157:H7 from 10 to 2 min (de Oliveira et al. 2018).

Moreover, the impact of low-concentration chlorine washing aided by the US on preventing cross-contamination in ready-to-eat winter jujube was investigated. The findings demonstrated that the cross-contamination incidence of *S. Typhimurium*, *E. coli* O157:H7, and non-O157 *E. coli* could not be reduced by US treatment (28 kHz) alone. Free chlorine treatment at 10 ppm reduced the incidence from 55.00 to 5.00% for *E. coli* O157:H7, 65.00 to 6.67% for non-157 *E. coli*, and 70.00 to 6.67% for *S. Typhimurium*. When the treatments were combined, the incidence of cross-contamination was fully eliminated (pathogens were not found in the sample). Besides, the numbers of aerobic mesophiles, aerobic psychrophiles, and three pathogens were significantly lower in the group that received combination therapy (28 kHz US + 10 ppm free chlorine) during storage (0–7 d at 4°C) than chlorine-treated, and US-treated sample (Wang et al. 2022b). H₂O₂ is a powerful oxidizing agent and causes cellular oxidative damage in microbial cells. Combining H₂O₂ with other sanitizers such as peroxyacetic acid and O₃ or physical disinfectants such as UV and cold plasma enhanced its antimicrobial activity and provides a promising disinfection strategy disinfection of raw and ready-to-eat fruits and vegetables (Abdelshafy et al. 2023; Abdelshafy, Neetoo, and Al-Asmari 2024). The sanitization step is one of the most important factors affecting the control of fruit and vegetable contamination. The combination of more than one control method produces a higher protection effect than the individual processes. However, the preparation of fruits and vegetables under hygienic conditions can effectively reduce the contamination risks by foodborne bacteria.

5.2 | Edible Coatings

Packaging materials (made from polysaccharides, proteins, polymers, and lipids) incorporated with bioactive compounds can

enhance the shelf-life of fruits and vegetables without affecting quality properties (Perumal et al. 2022; Rashwan et al. 2023b; Rashwan et al. 2024). Edible coatings or films can be also used for preventing and/or reducing foodborne pathogens contamination from the surface of fresh-cut and ready-to-eat fruits and vegetables, which are biopolymers that are being extensively researched for food packaging and preservation. These films have taken packaging to the next level, with shrinking boundaries discovered between packaging, preservation, and food. Concerns about the environment, the growing difficulty of recycling plastic waste and repurposing industrial food waste, and consumer aspirations for natural, wholesome foods are what gave rise to the concept of edible films (Al-Tayyar, Youssef, and Al-Hindi 2020; Rashwan et al. 2024; Youssef and El-Sayed 2018).

By lowering oxidation processes, gas exchange, solubility migration, moisture transfer, and preventing or lessening physiological diseases, edible films placed on food surfaces may extend their shelf life. Therefore, edible coatings that are typically made of food additives such as flavors, colorants, minerals, spices, and antibrowning agents or antimicrobial agents are becoming more and more relevant as crucial components to reduce the detrimental effects of processing vegetables and food (Duong et al. 2023; Kostić et al. 2023; Rashwan et al. 2023b). For instance, a biogenic ternary tannic acid/chitosan–citric acid (TA/CS–CA) preservation film was developed by Chang et al. (2023) to preserve strawberry fruit at room temperature. Their constituents contributed to their exceptional antibacterial activity (about 90% against *E. coli* and *S. aureus*) and remarkable oxidation resistance (the DPPH• scavenging activity of 90%). The freshness lifetime and edible rate of TA/CS–CA film-coated strawberries improved to 74% and 112 h, respectively, after being stored at 19–25°C for 7 days. These results were roughly twice that of uncoated strawberries. This film also showed good washability and natural biocompatibility, which gave it promise as a high-performing preservation medium (Chang et al. 2023).

Active edible packaging for fresh/fresh-cut fruit based on pectin and oregano essential oil (OEO) was developed by Kostić et al. (2023). The results revealed that the control pectin film did not show any inhibition against *E. coli*, *Bacillus subtilis*, and *Staphylococcus epidermidis*. In addition, films with 0.05 and 0.1% of OEO did not show an inhibitory effect on the growth of tested bacterial strains. However, the highest tested concentrations of OEO inhibition zones were VIS, where the diameters of inhibitory zones for 0.5% OEO against *E. coli*, *B. subtilis*, *S. epidermidis* were 15.44, 12.56, and 12.55 mm, while the diameters of inhibitory zones for 1% OEO were 15.89, 19.22, and 13.11 mm, respectively (Kostić et al. 2023). Furthermore, a distinct study showed that the function of film/coating was enhanced by alginate cross-linking enriched hexyl acetate (SAC–HA), whereby the various concentrations of HA added to SAC considerably reduced the microbiological growth (TVC) of coated fresh-cut rose apples. TVC was reduced more effectively by freshly cut rose apples covered with SAC containing 0.03 and 0.05% HA than by 0.01% (v/v). Following 10 days, the samples coated with SAC–HA–0.03% and SAC–HA–0.05% exhibited roughly 36 and 40% greater suppression of bacterial growth, respectively, in comparison with the control sample. Fresh-cut rose apples treated with 0.03 and 0.05% HA added to SAC showed a substantial reduction in TVC growth. Hexyl acetate as a volatile ester prevented microbial

spoiling by hydrolyzing to acid and alcohol, which in turn reduced microbial growth (Duong et al. 2023).

Carboxymethyl cellulose and pectin are two main polysaccharides with great potential in making edible coatings for the preservation of fruit and vegetable quality. They are commonly water-soluble, nonallergic, nontoxic, tasteless, odorless, transparent, and resistant to fat and oil, and could be good carriers for functional compounds such as antimicrobials, antioxidants, antibrowning agents, and nutraceuticals to avoid undesired changes such as microbial spoilage, oxidation, and enzymatic browning in fruits and vegetables (Panahirad et al. 2021). Gums are polysaccharides/carbohydrate polymers derived from renewable sources and have recently been considered promising biocontrol agents for the extension of fruits and vegetables' shelf-life and the use of petroleum-derived polymers. The plant extracts, phenolic compounds, essential oils, nanoparticles, and vitamins can be incorporated into these edible coatings to enhance their mechanical barrier, antimicrobial, and antioxidant characteristics (Tahir et al. 2019; Xing et al. 2019). In the last years, aloe vera gel has been used to preserve fruits and vegetables due to its antioxidant and antimicrobial properties. It is a natural hydrocolloid composed mainly of polysaccharides and can act as a semipermeable barrier for water vapor and gases, decreasing the respiration and ripening processes of fruits, maintaining the valuable compounds, firmness, and weight (Nicolau-Lapeña et al. 2021). Further studies are required on the new sources of edible coating for fruits and vegetables.

6 | Conclusion

Several species of foodborne bacterial pathogens such as nontyphoid *Salmonella*, and *E. coli* can contaminate the raw and ready-to-eat fruits and vegetables at various periods, including preharvest, harvest, and postharvest periods especially under poor hygiene practices, causing serious health problems. This review comprehensively discussed the recent advances in the detection methods of foodborne pathogens related to raw and ready-to-eat fruits and vegetables as well as the potential control strategies. Foodborne bacteria, including *L. monocytogenes*, *E. coli*, and *S. enterica* are the major bacterial pathogens associated with contaminated raw and ready-to-eat fruits and vegetables outbreaks worldwide. Moreover, fresh vegetable products, especially green leafy vegetables, showed higher contamination than fresh fruit products. Detection techniques, including culture methods, microscopic methods, immunological methods, PCR techniques, biosensors, and HSI technology can be successfully applied for the detection of foodborne bacteria related to raw and ready-to-eat fruits and vegetables. However, each detection method showed some limitations such as time consumption, low accuracy, and high costs. Several studies were investigated to develop these detection methods and decrease their limitations, including the combination of more than one detection method such as the combination of PCR-ELISA and the combination of culture method with PCR. Furthermore, many strategies can be effectively used to prevent the contamination of raw and ready-to-eat fruits and vegetables by foodborne bacteria such as cold plasma, UV, and edible coating methods, and promote the safety properties of these fresh foods. The design of innovative and in-site devices capable of monitoring the microbial properties of raw

and ready-to-eat fruits and vegetables during different periods needs further studies in the future. Moreover, finding low-cost methods that are suitable to use in developed countries needs further research.

Author contributions

Asem M. Abdelshafy: Conceptualization, software, methodology, writing—original draft, and writing—review and editing. **Hala A. Younis:** Conceptualization, methodology, writing—original draft, and writing—editing. **Ahmed I. Osman:** Resources, conceptualization, methodology, writing—original draft, and writing—editing. **Saleh M. Hussein:** Conceptualization, writing—review and editing. **Amr S. Abou El-Ela:** Writing—review and editing. **Elsayed A. Mahmoud:** Writing—review and editing. **Osama Elsherbiny:** Writing—original draft. **Ahmed K. Rashwan:** Conceptualization, software, methodology, supervision, resources, writing—original draft, and writing—review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Not applicable.

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