Chapter 3
Microbial Adulterants in Food: Challenges to Overcome

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1 Introduction

Microbiological contamination of food refers to the inadvertent presence of pathogenic organisms such as bacteria, fungus, yeasts, protozoa, and viruses in food because of their exposure during manufacturing and processing, rendering it unfit for human consumption (Bintsis 2017). Microbial contamination happens most frequently during the voyage from the farm to the processing facility, during processing, storage, transportation, distribution, and before consumption. Microbial poisons are also potential dietary toxins; nevertheless, microbes and their products can also be used to combat pathogenic organisms. Food spoilage is a complex process, and even with contemporary preservation techniques, microbial spoilage causes enormous volumes of food to be wasted. (Alum et al. 2016; Amit et al. 2017). The widespread contamination of food by pathogenic microorganisms, their capacity to grow and survive in low-oxygen environments, and the low microbial dose required for food poisoning epidemics point to a significant public health issue. Ionizing radiation and heating are two well-known and widely utilized microbial control strategies. On the other hand, novel nonthermal and modified thermal technologies have become effective methods for killing pathogenic microbes in food.

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The food processing industry's main challenge is to keep up with innovative microbial decontamination technologies that will allow it to provide safe, high-quality food items with a long shelf life. In fact, during the next 50 years, the development or re-emergence of the novel or unanticipated microbial contaminants will impact the food sector (Tauxe et al. 2010). With the globalization of the food trade, the risk of foodborne illness has increased, leading to import bans, which have hampered the economies of many developing countries worldwide (Bhat 2004). The World Health Organization (WHO), a component of primary health care, has advocated food safety. Keeping this in mind, the present chapter endorses the causes, routes, and mechanisms of microbial food spoilage and foodborne infections, novel technologies for controlling foodborne pathogens, the need to monitor food contamination, and the effect on the economy due to microbial food spoilage.

2 Microbes and Food

2.1 Microbe Assisting Food Production

Microorganisms associated with foods can be categorized as "spoilage," "pathogenic," or "useful." Nature uses microorganisms to carry out fermentation processes. Yeasts, molds, and bacteria have been exploited for thousands of years to make bread, beer, wine, vinegar, yogurt, cheese, fermented fish, meat, and vegetables. Fermentation is one of the oldest methods of food transformation and preservation. This biological process increases food's nutritional and organoleptic characteristics while preserving it (relating to the senses, taste, sight, smell, and touch). A well-run fermentation will favor beneficial organisms over undesired flora to minimize spoilage and increase taste and texture.

Functional microorganisms during fermentation of food transform its chemical constituents resulting in enhancement of nutrients bio-availability and sensory quality of food, imparts bio-preservative effects and improve food safety, degrading toxic/anti-nutritive factors, producing antioxidant and antimicrobial compounds, stimulating probiotic functions along with fortification of health-promoting bioactive compounds (Tamang et al. 2016).

In the food processing industry, microorganisms play a significant role. They are utilized in developing a wide range of food products and are also responsible for food spoiling, resulting in poisoning and illness. Although the microflora of raw materials is typically varied, food preparation frequently imposes a distinct and very particular microbial flora. The natural flora of low-acid canned goods that have been severely heat processed but not sterilized is made up of thermophilic spore-forming bacteria, which are the raw materials' most heat-resistant microbial components. The significant flora of shelf-stable canned cured meats is made up of mesophilic aerobic and anaerobic spore-forming bacteria, which are resistant to the heat treatment used to preserve these items. Small populations of spore-forming bacteria, yeasts, and lactic acid bacteria compromise the natural flora of mayonnaise and salad dressing.

Aerobic spore-forming bacteria predominate in dry spices and in several dry vegetable products. Molds and yeasts are the most common microorganisms found in dried fruits. Yeasts make up the typical flora in carbonated beverages. The nature of the raw materials, production conditions, packaging, and storage of the shelf-stable product are all reflected in the surviving and predominating microflora in each of the examples above. The microflora that survives processing in perishable products may be diverse, but the percentage that develops during storage and causes deterioration is usually rather particular. As a result of contamination from the animal and the processing environment, a heterogeneous flora can be found on raw red meats, poultry, and fish. However, spoiling is caused mainly by a highly specialized group of microbes, particularly *Pseudomona*s and closely related aerobic, psychrotrophic Gram-negative bacteria, during refrigerated storage of such products. Changes in perishable food processing must consider the impact these changes may have on the spoilage flora and the product's usual rotting pattern.

2.2 Food Spoilage

Food spoilage caused by microorganisms is known as microbial spoilage. It's also the leading cause of food poisoning. Microorganisms are ubiquitous and have the potential to spoil food and induce foodborne illness. Microbial contamination of food products occurs most commonly on the journey from the farm to the processing facility, during processing, storage, transportation, and distribution, and prior to consumption. Food spoiling is a complicated process, and even with modern preservation procedures, large amounts of food are lost owing to microbial spoilage. The microbiota that develops during storage and in deteriorating foods can be anticipated, despite the heterogeneity in raw ingredients and processing environments.

Both intrinsic and extrinsic causes can influence microbial spoilage in foodstuffs. The inherent qualities of foods affect the type and rate of microbial decomposition and their projected shelf life or perishability. The essential intrinsic qualities of food spoilage include endogenous enzymes, substrates, light sensitivity, and oxygen. The pH, water activity, nutrient content, and oxidation-reduction potential are all intrinsic factors in food deterioration. Food deterioration is caused by extrinsic variables such as relative humidity, temperature, and the presence and activities of other microorganisms (Amit et al. 2017).

Changes in appearance (color, pockets of gas/swelling), texture (soft and mushy), color, aroma, flavor, or slime development are all signs of microbial deterioration of food. It covers Gram-positive, Gram-negative aerobic bacteria, yeasts, molds, and fungal pathogens, among other spoilage species. Food spoilage has an enormous economic impact, and microbial food spoilage plays a significant role in food waste and loss.

2.2.1 Microbial Spoilage of Raw Foodstuff

Microbiological contamination can be found everywhere in the biosphere, in plants, animals, soil, and water. Many bacteria, such as pseudomonads, lactic, micrococci, and coliforms, grow readily on agricultural and horticultural plants. For example, Raw/fresh produce and highly perishable products like fluid milk account for a disproportionately high percentage of annual losses compared to other commodity groupings. Liquid milk, although pasteurized, is an excellent growing medium for spore formers that have survived the heat process. Produce is frequently harvested before it has fully ripened and continues to breathe throughout transportation. Still, it is not until senescence, when the native protections have been compromised, that produce becomes most susceptible to deterioration.

2.2.2 Microbial Spoilage in Processed Foodstuff

All of the preventative measures and sanitation tactics still apply to processed products, but there is new potential for spoiling mitigation in processing and packaging. Economically, further spoilage complications in food processing have arisen due to shifting consumer preferences. Demands include minimizing antimicrobial compounds and heat processing and changing compositions to incorporate more contamination components.

Groups of Foods Spoilers

Ubiquitous microorganisms found in soil, water, and air, as well as specific sources of contamination such as spoiled raw materials, food waste, biofilm on equipment surfaces, and personal hygiene from food workers or consumers, depending on the ecological microbial niche, cause microbial spoilage. Microorganisms involved in food spoilage can be divided into three major categories: molds, yeasts, and bacteria. Table 3.1. presents the operational conditions of different microorganisms that affect foods.

Yeasts are generally single-celled organisms adapted for life in specialized, usually liquid, environments and do not produce toxic secondary metabolites. They often colonize foods with high sugar or salt content and contribute to maple syrup, pickles, and sauerkraut spoilage. Fruits and juices with a low pH are other targets, and some yeasts grow on the surfaces of meat and cheese.

Molds are essential for recycling dead plant and animal remains in nature but also attack various foods and other materials helpful to humans. They are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes. Most molds grow at a pH range of 3 to 8; some can grow at deficient water activity levels (0.7–0.8) on dried foods.

Table 3.1 List of food spoilage microbes

Group of microorganism	Common microbes	Types of foods	Activities/ mechanism	References
Lactic acid bacteria	*Lactobacillus, Weisella, Leuconostoc, Lactococcus, Pediococcus, Streptococcus, Enterococcus.*	Dairy products sourdough beverages (fruit juices, beer)	Acetic acid, gas blowing, post acidification	Sakamoto and Konings (2003)
Acetic acid bacteria	*Acetobacter, Gluconobacter, Acidomonas, Gluconacetobacter, Asaia, Kozakia, Swamina – thania, Saccharibacter, Neoasaia, Granulibacter, Tanticharoenia and Ameyamaea.*	Wine	Strictly aerobic	Bartowsky and Henschke (2008)
Filamentous fungi, Moulds	Zygomycetes	Rot in stored fruits and vegetables	Formation of spores	Rawat (2015)
	Penicillium spp.	Citrus, pear, and apple fruits, refrigerated and processed foods such as jams and margarine	Produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem)	
	Aspergillus spp.	Grains, dried beans, peanuts, tree nuts, and some spices.	Produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid	
	Fusarium spp.	Harvested grains	Mycotoxins	
Yeast	Zygosaccharomyces	Honey, dried fruit, jams and soy sauce	Producing off-odors and flavors and carbon dioxide	Rawat (2015)
	Saccharomyces spp.	Alcoholic beverages	Producing gassiness, turbidity, and off-flavors associated with hydrogen sulfide and acetic acid.	
	Candida	Fruits, some vegetables, and dairy products		
	Dekkera/ Brettanomyces	Fermented foods, including alcoholic beverages and some dairy products.	Produce volatile phenolic compounds responsible for off-flavors	

Bacteria: Lactic acid bacteria are a group of Gram-positive bacteria, including species of *Lactobacillus*, *Pediococcus*, *Leuconostoc,* and *Oenococcus*; under low oxygen, low temperature, and acidic conditions, these bacteria become the predominant spoilage organisms on a variety of foods. Undesirable changes caused by LAB include greening of meat and gas formation in cheeses (blowing), pickles (bloater damage), and canned or packaged meat and vegetables. Off-flavors described as mousy, cheesy, malty, acidic, buttery, or liver-like may be detected in wine, meats, milk, or juices spoiled by these bacteria.

3 Foodborne Diseases

Hippocrates (460 B.C.) highlighted the correlation between human food consumption and diseases. The ingestion of food contaminated with microbial pathogens and toxins results in foodborne illness (Bintsis 2017). Microbiological contamination of food refers to the unintentional presence of pathogenic microorganisms such as bacteria, fungi, yeasts, protozoa, and viruses owing to its exposure during food production and processing, making it unfit for human consumption. The appearance or re-emergence of the novel or unexpected microbial contaminants will affect the food industry within the next 50 years (Tauxe et al. 2010). According to The Centre for Disease Control and Prevention (CDC), an estimated 76 million people get affected by foodborne diseases, with yearly medical expenses up to 5–6 billion dollars (Kirch 2008). Sudershan et al. (2010) reported that a foodborne illness outbreak affecting 60 people in the Indian city of Hyderabad led to an economic burden of U.S. $ 2070. European Union (E.U.) in 2015 reported 4362 foodborne outbreaks in 26 member states which accounted for 45,874 cases of illness, 3892 hospitalizations, and 17 deaths (EFSA 2015). With the globalization of food trade, the probability of the spread of foodborne illness leads to the ban of imported food, which undoubtedly hampers the economy of many developing nations worldwide (Bhat 2004).

3.1 Microbial Contamination in the Food Industry: Routes and Mechanism

The first significant route for food contamination in the food-processing sector is due to the failure of industry personnel to follow the standard hygiene rules, which include hand-washing and gloves during food manufacture or packaging (Green and Selman 2005). Secondly, using water contaminated with pathogens results in microbial contamination of food (Ashbolt 2004). Along with the above factors, the microbial contamination of instruments/equipment routinely used for food processing is a significant route for microbial contamination of processed food, stressing the need for food industries to follow proper protocols for sterilization of the equipment (Alum et al. 2016). Bacterial, fungal, and viral pathogens frequently contaminate processed food in the industry due to improper handling. Foods like red meats, poultry, and seafood products are more prone to microbial contamination than fruits or vegetables. As per EFSA (European Food Safety Authority), the toxin from bacterial pathogens are ranked second among the microbial pathogens responsible for food- and waterborne outbreaks, followed by viruses responsible for 19.5% and 9.2% of the total episodes in 2015. On the other hand, parasites and other causative agents resulted in less than 3% of the outbreaks, while for 34% of attacks, the causative agent could not be determined. Studies have shown that most foodborne diseases were related to *Norovirus*, nontyphoidal *Salmonella* spp., *Clostridium perfringens,* and *Campylobacter* spp. Foodborne microbial illnesses are categorized into:

(a) **Foodborne infections:** With the advent of next-generation sequencing technology, it has been possible to carry out sequencing of the foodborne pathogens' whole genome, which provides insights into their pathogenicity. Foodborne pathogens such as *Salmonella* spp., *Campylobacter* spp., *E. coli* O157:H7, etc., are ingested via food, followed by colonization of intestinal epithelial cell lining, which spreads further to the lower intestinal tract and liver coupled to release of toxin (Fig. 3.1).
(b) **Foodborne intoxications:** Foodborne intoxications involve ingesting toxins into the human body instead of bacterial or fungal cells. e.g., fungal toxins like fumonisins, aflatoxin B, ochratoxin A, bacterial toxins such as botulinum neurotoxin, cholera toxins Shiga toxin, and Staphylococcus enterotoxin.
(c) **Foodborne toxico-infections:** The toxic foodborne infections involve dormant cells (spores) of *C. perfringens*, *Bacillus anthracis*, *B. cereus*, before, etc., ingested, which, after death, release toxins (Fig. 3.2).

4 Antibiotic Resistance Pathogens (ARGs): An Emerging Challenge for the Food Industry

The application of antibiotics to livestock has resulted in the emergence of antibiotic-resistant foodborne pathogens and livestock bacteria. Foodborne pathogens and other opportunistic pathogens such as *Salmonella*, *E. coli*, and *Campylobacter* exhibiting multidrug resistance have been found in fresh produce and food-producing animals. The pathogens have been resistant to various antibiotics such as azithromycin, tetracycline, nalidixic acid, amikacin, ciprofloxacin, trimethoprim-sulfamethoxazole, and cephalosporin. Antibiotic resistance in foodborne microbes has resulted from the inappropriate use of antibiotics to promote growth and fight infections. One of the commonly used growth promoters in Belgium, India, China, and Brazil is colistin, and as a consequence, colistin-resistance genes have been

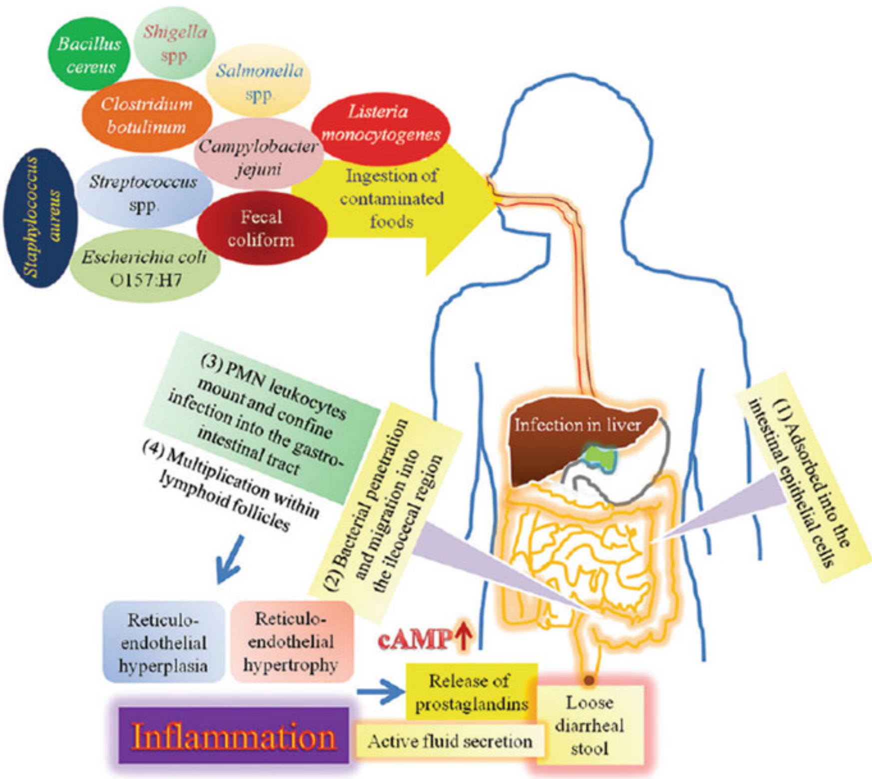

Fig. 3.1 Schematic representation of the mode of infections by foodborne pathogens. The ingestion of pathogenic microbes at a dose equal to/above the infectious amount adheres to the intestine's epithelial cells, followed by dissemination to the liver and lower intestinal tract. This results in the weakening of the immune system or diarrheal syndromes. (Reproduced from Noor 2019)

predominantly found in the environment. Mcr-1 and mcr-2 are in pork carcasses, chicken meat, and mutton in Belgium, Brazil, and India, respectively. The contaminated surfaces during food processing serve as a source of antibiotic resistance gene transfer. The bacteria on contaminated surfaces exhibit antibiotic resistance by taking up antibiotic resistance genes indicating how resistance is spread in the inter-connected environment. In addition, food processing techniques routinely applied to control foodborne pathogens create microbial stress resulting in their inactivation. However, the processing techniques can also serve as a route for transferring antibiotic resistance genes in pathogens with prolonged exposure to such stress (Thakali and MacRae 2021). Low and middle-income countries apply animal waste as agricultural fertilizers or as fish feed without necessary treatment, resulting in contamination of food products such as vegetables, fish, shellfish, and

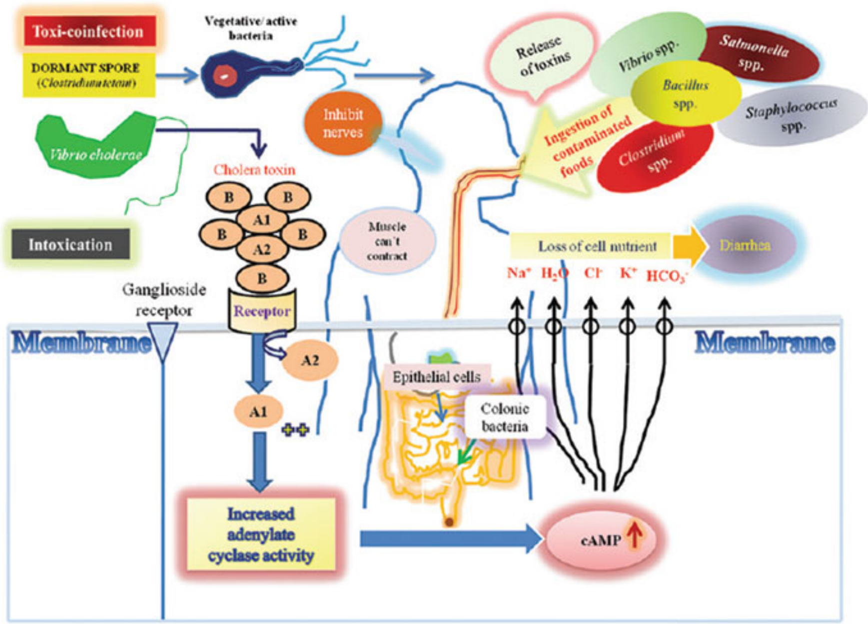

Fig. 3.2 The mode of foodborne microbial intoxication and toxico-infection in host cells. Food contaminated with pathogens such as *Clostridium* spp., *Bacillus* spp., *Vibrio cholera,* and *Staphylococcus* spp. Results in foodborne intoxication and toxico-infection using toxins apart from foodborne infections are described in Fig. 3.1. This diagram shows the route of transmission and mode of toxin action in cholera (intoxication) and botulism (toxico-infection) caused by *Vibrio cholerae* and *Clostridium botulinum*, respectively. (Reproduced from Noor 2019)

water with antibiotic-resistant bacteria. This entry of antibiotic-resistant genes into the food chain ultimately enhances the risk to public health. Tao et al. (2022) reported the prevalence of foodborne pathogens resistant to antibiotics in different food types such as aquatic products, meat, milk, and dairy products. Multidrug-resistant (MDR) pathogens comprise ≥36% of foodstuffs, with the highest rate of 52% found in meat. The pathogens resistant to β-lactams at the rate of ≥57% were most common among all the food products. Among aquatic food products, fluoroquinolones and sulphonamides-resistant microbes were around 13% while isolates resistant to β-lactams were over six times higher.

5 Strategies to Prevent Microbial Contamination of Food

The ubiquitous contamination of food by pathogenic microbes, their ability to grow and survive under refrigeration/ reduced oxygen conditions, and the low microbial dose required for food poisoning outbreaks indicate a potential risk to public health. The matter has been further complicated by documentation of food poisoning by microbes previously unknown to cause foodborne disease. Conventional and established microbial control techniques like ionizing radiation and heating are widely used. On the other hand, novel nonthermal and modified thermal technologies have gained attention as efficient techniques to kill pathogenic microbes in food. The various conventional and novel technologies for controlling foodborne pathogens have been described in the following sections.

5.1 Thermal Treatment

Heat application to kill pathogenic microbes is the most widely used foodborne pathogen control strategy. The thermal treatment is designed for specific lethality of foodborne pathogens to ensure the target food's shelf life and microbiological safety. D- and Z-values are two factors that govern the heat resistance of foodborne pathogenic bacteria. D-value denotes the heating time required to kill 90% of viable cells or spores at a specific temperature. Z-value is the change in heating temperature required to change D-value by 90% (1 log cycle). Based on the D- and Z-values, thermal processing strategies are designed to control foodborne pathogens in specific food products. The sterilization of food products is being carried out using the following methods:

(a) **Direct heating:** This is carried out either by steam injection or steam infusion.
(b) **Indirect heating:** The food product is subjected to indirect heating by using steam or hot water as the medium of heat in the plate, tubular, or scraped surface heat exchanger.
(c) **Ohmic heating:** This novel sterilization technique utilizes electric current to generate heat in the food products to be sterilized. This technology works on the principle that it is possible to generate heat in the material by passing electric current, using the inherent electrical resistance of the material. Ohmic heating results in rapid and uniform heating of the material. Figure 3.3 shows the essential part of ohmic heating equipment, which comprises a power supply, heating chamber, electrodes, thermocouple, current sensor, and data acquisition system. Ohmic heating results in heating the food rapidly and uniformly by Joule's law of heating. This makes it possible to heat the food material volumetrically and efficiently. Ohmic heating inactivates undesirable enzymes and pathogenic microbes in food in a comparatively shorter time than conventional heating. Thermal effects of ohmic heating results in enzyme inactivation. Apart from this, nonthermal effects such as chemical changes and cell membrane electroporation result in microbial killings during ohmic heating. High microbial pathogens could be treated at lower pH by ohmic heating. However, fat contents compromise the activity of ohmic heating, resulting in low microbial inactivation efficiency. Thus, owing to the advantages of ohmic heating, such as quick and volumetric heating, high energy efficiency, rapid microbial/enzyme inactivation, etc., ohmic heating is an alternative to conventional thermal strategies (Makroo et al. 2020).

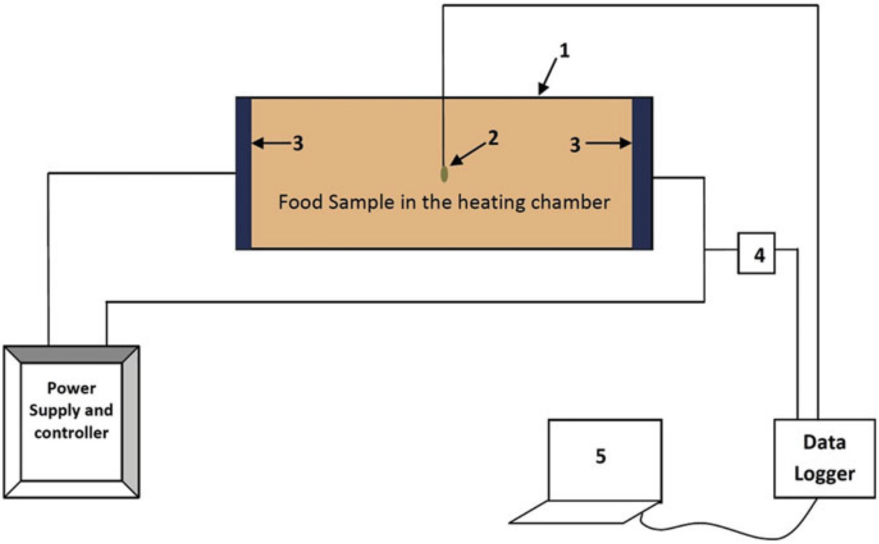

Fig. 3.3 Schematic representation of ohmic heating setup; (1) O.H. chamber (2) Thermocouple (3) Electrode (4) Current sensor (5) Personal computer. (Reproduced from Makroo et al. 2020)

(d) **Microwave heating:** For food sterilization, microwave heating encompasses several advantages over conventional thermal treatment as heating speed, better process control with rapid start and shut down times, and better taste, texture, and nutritional content of the treated food product. Microwave heating of food products occurs by the interaction of microwaves with the ionic or dipolar content of the food. Food's dipolar content comprises water, proteins, and carbohydrates, which are volumetrically distributed in food. Thus, the microwave could cause volumetric heating, which is governed by the material's dielectric properties and the microwave's frequency. The food product dielectric property is responsible for the number of incident microwaves reflected, transmitted, or absorbed by the material (Juneja et al. 2007). Osaili et al. (2021) evaluated the effect of microwave heating on treating pathogenic microbes in tahini, a traditional food associated with multiple foodborne-related outbreaks due to *Salmonella*, *L. monocytogenes,* and *E. coli* contamination. They reported microwave heating as a potential method for lowering the risk of Salmonella spp., E. coli O157:H7, and L. monocytogenes in tahini with no fadverse effect on quality. The treatment showed no effect on acid, peroxide, p-anisidine, or color values of tahini up to 90 °C. Hashemi et al. (2019) reported that microwave treatment showed significant decrease in pathogens as *Escherichia coli, Salmonella Typhimurium, S. Enteritidis and Staphylococcus aureus* and content of vitamin C, β-carotene, phenolic compounds whereas the pH of samples did not show significant changes in cantaloupe juice.

5.2 Pulsed Electric Fields

Pulsed electric fields (PEF) are a nonthermal technique dependent on short electrical pulses to inactivate foodborne pathogens. This technology is preferred over thermal processing, which causes detrimental sensory and physical changes in foods. PEF is considered over thermal treatment since it destroys pathogens while keeping the nutritional quality, flavor, texture and colour of food intact. PEF technology delivers pulsing power to the food product placed between electrodes. The high voltage pulse generator, treatment chamber having a system for fluid handling, and necessary devices required to monitor and control the system are important sections of the equipment (Fig. 3.4). The food to be treated is placed in a treatment chamber containing two electrodes to which high voltage electrical pulses are applied which conducts high-intensity electrical pulse to a food product. The electric field to which food is exposed causes the breakdown of membranes in foodborne pathogens. This technology is preferred for pasteurizing juices, milk, yogurt, etc. (Mohamed and Eissa 2012). Bulut et al. (2020) developed a pilot-scale pulsed electric fields system to treat sesame seeds by analyzing their physicochemical properties and *Aspergillus parasiticus* inactivation. The pulsed electric field energy in the range of 0.97 to 17.28 J resulted in maximum reductions of peroxide value (67.4%) and acidity number (85.7%) with no change in color. Applying maximum pulsed electric field energy led to a 60% reduction of *A. parasiticus* counts. The study proved that a pulsed electric field could be used to treat sesame seeds by preserving physicochemical properties and inactivating *A. parasiticus*. Mendes-

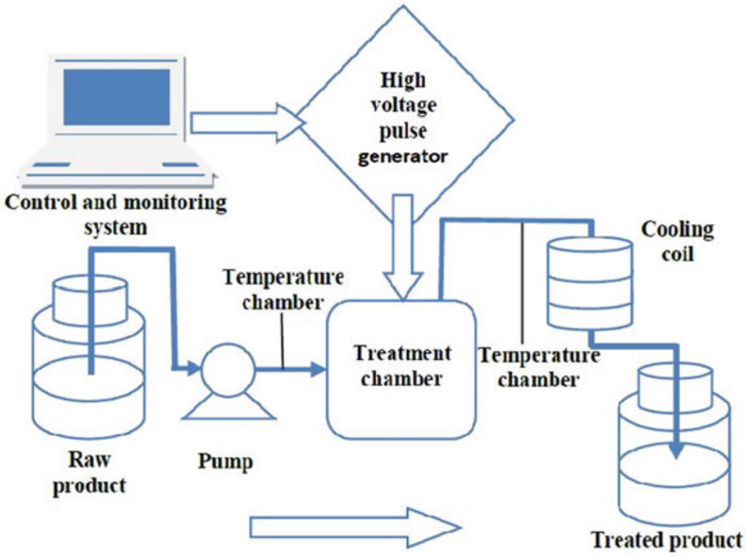

Fig. 3.4 Schematic diagram of a pulsed electric field food processing system. (Reproduced from Mohamed and Eissa 2012)

Oliveira et al. (2021) processed juice inoculated with *Escherichia coli* O157:H7 and *Salmonella Typhimurium* in a continuous PEF process system. They reported an over 5 log reduction of pathogens with no regrowth during storage at 4, 10, and 22 °C. The native microbes population in treated juices after 24 week-storage was found to be same or lower compared to control samples. PEF showed no significant negative effect against pH, color, and vitamin C retention.

5.3 Electrolyzed Water

Electrolyzed water has been used in Japan for several years due to its antimicrobial properties. The application of electrolyzed water to destroy foodborne pathogens is preferred since it has no hazardous effects on the human body. Electrolyzed water has strong bactericidal effects on foodborne pathogenic bacteria. It is produced by passing the diluted salt solution through an electrolytic cell containing an anode and cathode separated by a membrane. The electrodes are exposed to direct current voltages. Negative ions like chloride and hydroxide move in salt solution towards the anode to release electrons and produce oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid, and hydrochloric acid. On the other hand, positively charged ions like hydrogen and sodium migrate towards the cathode and accept electrons to produce hydrogen gas and sodium hydroxide. This results in the production of electrolyzed water having properties of low pH (2.3–2.7) and high oxidation-reduction potential (ORP, >1000 mV) at the anode. Liang et al. (2019) analyzed the application of slightly acidic electrolyzed water (SAEW) for microbial reduction in buckwheat. This treatment resulted in molds, yeasts, and total bacteria count reduction in buckwheat seeds and harvested sprouts. SAEW treatment showed strong efficiency for removing *E. coli* and *L. monocytogenes* inoculated on harvested shoots. Medina-Gudiño et al. (2020) analyzed the activity of Neutral Electrolyzed Water (NEW) was tested in vitro and on artificially contaminated eggs against *Salmonella enterica* subsp. *enterica* or *Escherichia coli*. Neutral electrolyzed solution do not damage egg cuticle and causes pore formation in *Salmonella enterica* and *E. coli* surfaces after 30 s treatment.

5.4 High-Pressure Processing Technology

High-Pressure Processing (HPP) is a nonthermal method for processing food where food is exposed to high pressure in the range of 100–800 MPa. It is also considered a cold processing technique since the temperature is in the ambient field. This technique kills foodborne pathogens but the food possesses better texture and color than heat-treated foods. This technique does not result in a change of molecules conferring flavor and nutritional content to the food. This technique can treat liquid and solid food materials except for foods with air pockets. For HPP to be applicable in the control of food pathogens, the pressure should exceed 400 MPa. The microbial resistance to the treatment depends on several factors, such as pressure, temperature, duration of therapy, and the microbe type. The resistance of gram-positive bacteria to HPP is higher than Gram-negative bacteria due to teichoic acid (a bacterial polysaccharide). Spores are highly resistant to vegetative cells owing to the presence of dipicolinic acid. It has been found that heat-resistant microbes are generally resistant to pressure, while cells in the exponential phase are more sensitive to stress than cells in the stationary phase. The control of viral pathogens in food is related to capsid protein denaturation required for attachment to the host cell. The cell membrane damage at HPP is related to compression of the cell membrane on applying pressure with lipid bi-layer expansion following the release of tension. This leads to loss of integrity of cell membrane, making microbes unable to reproduce. The damaged cells cannot control water and ions transport across membranes resulting in cell death (Naik et al. 2013). Stratakos et al. (2019) attempted to study the effect of high-pressure processing on microbiological safety/shelf life and raw milk quality compared to that of conventional heat pasteurization and untreated milk. It was observed that high-pressure processing could achieve 5 log reductions for pathogenic *E. coli*, *Salmonella,* and *L. monocytogenes,* respectively. High-pressure processing prolongs the shelf life of raw milk by decreasing the levels of Total mesophilic aerobic bacteria, Enterobacteriaceae, lactic acid bacteria, and *Pseudomonas* spp. Levels as compared to those in pasteurized and raw milk. The milk processed using high-pressure exhibited that the treated milk did not affect the quality attributes characterizing raw milk, such as color and mouth sensation due to particle size. Considering the increasing demand for raw milk, high pressure processing can be an alternative for microbiologically safe milk while retaining fresh-like characteristics.

5.5 Ultrasonication

Ultrasonication is a rapid and non-destructive technology based on mechanical waves applied for the microbial safety of food products. Ultrasound in a liquid medium results in acoustic cavitation, i.e., phenomena of the bubbles' production, growth, and collapse. The propagation of ultrasound waves causes bubbles to oscillate and collapse, leading to thermal, mechanical (collapse pressure, turbulence, and shear stress), and chemical effects (production of free radicals). The results cause significantly high temperatures (5000 K) and pressures (1000 atm). The microbial control by ultrasonication accounts for the production of acoustic cavitations, leading to an increase in membrane permeability, thinning of the cell membrane, confined heating, and production of hydroxyl radicals (Fig. 3.5). FDA recommends ultrasonication for a 5-log reduction of the microbial population. Ultrasonic 100 W power is considered optimum for microbial control, demonstrating its efficiency against Escherichia coli, Listeria monocytogenes, and other pathogens. The effectiveness of this treatment depends on several microbial properties such as size, hydrophobicity, Gram status, and phase of growth. Microbes with soft and thick capsules are resistant to ultrasonication (Majid et al. 2015). Zhang

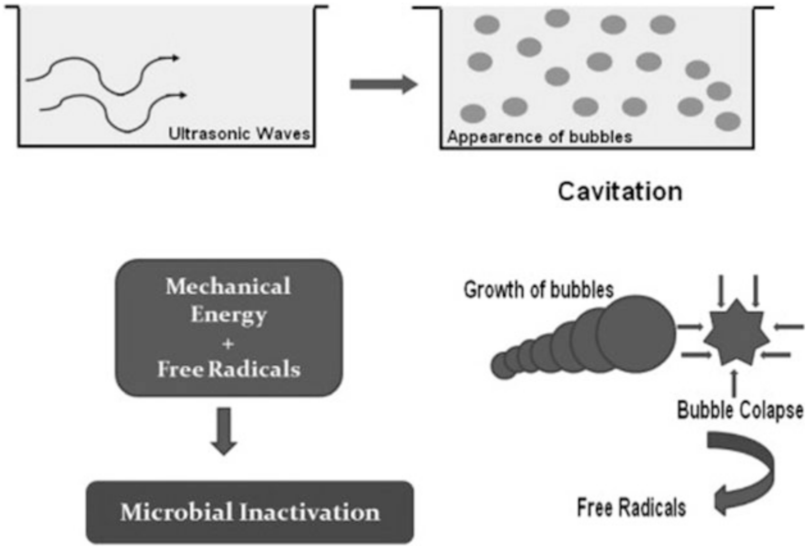

Fig. 3.5 Cavitation and inactivation of microbes by ultrasonication. (Reproduced from de Sao Jose et al. 2014)

et al. (2020) showed the antimicrobial activity of natural compounds in combination with low/high-frequency ultrasound against *E. coli* K12 or *L. innocua*. The synergistic bacterial inactivation was achieved with 0.5–2.0 log reduction on the simultaneous application of U.S. 1 MHz / 20 kHz and citral, carvacrol, or geraniol compared to the sum of individual treatments. Bacterial inactivation was based on ultrasound frequency, bacteria species along with the presence of antimicrobial compounds. The physical impact and dispersion of citral droplets determine the effectiveness of low-frequency ultrasound processes. On the other hand, high frequency causes inactivation by oxidative stress production inside the bacterial cells. The combined treatment showed better bacterial treatment on surfaces of blueberry and washed water used for washing. Tavsanli et al. (2021) showed that ultrasound treatment could serve as an alternative to pasteurization of raw goat milk with 99% inactivation of food pathogens *Brucella melitensis* type 3, *Salmonella Typhimirium*, *Escherichia coli*, *Listeria monocytogenes* and methicilin resistant *Staphylococcus aureus* in goat milk.

5.6 Pulsed-Light System

The pulsed-light system is a nonthermal technology for microbial control in food using short-duration and intense pulses on the food surface or its packaging. The efficiency of microbial decontamination is based on light intensity and the number of pulses the food is exposed to. The high U.V. content in pulsed light is crucial for microbial decontamination. Nucleic acids are the cell target of pulsed light.

U.V. light is related to the photochemical transformation of pyrimidines in microbial DNA forming dimers. This prevents the unzipping of DNA for replication leading to the incapability of the microbe to reproduce. Without sufficient DNA repair mechanisms, such damage leads to mutation, impairment of reproduction, and gene transcription, finally leading to cell death. The decontamination procedure's efficacy depends on the food's composition. This technology is unsuitable for treating food with high oil and protein content since a part of radiation will be absorbed by proteins/oils. Pulsed-light sterilization of packaged products is possible if the packaging is U.V. transparent (Elmnasser et al. 2007). Tao et al. (2019) analyzed the pulsed-light system's efficiency for controlling foodborne pathogens inoculated on the lettuce surface and the treatment impact on lettuce quality through 8 days of storage at 4 °C. They reported that pulsed-light treatment could effectively decrease pathogens' load on leaves based on the fluence applied. However, the pathogens tested showed varied susceptibility to the treatment where *Staphylococcus aureus* was the most sensitive, *Escherichia coli* and *Salmonella enteritidis* being moderately susceptible, while *Listeria monocytogenes* were comparatively resistant. After the refrigeration period, the pulsed-light treated lettuce had low bacterial/yeast/mold count while retaining the food quality by minimizing weight loss and color and preserving the loss of total soluble solids, chlorophyll, and ascorbic acid. Chen et al. (2019) attempted to determine the effect of an intense pulsed light on inactivation of *Cronobacter sakazakii* and *Enterococcus faecium* in powdered food including non-fat dry milk, wheat flour and egg white. They reported up to 5 $\log_{10}$ CFU/g and 2.7 $\log_{10}$ CFU/g reductions for *C. sakazakii* and *E. faecuium*, respectively with no undesirable agglomeration.

6 The Scope and Need for Monitoring Food Contamination

Unsafe food is a global concern to human health and economics, with an estimated 600 million cases of foodborne illness every year. As a result, ensuring food safety is a top priority for public health and a critical step toward achieving food security. Food safety and quality control systems are essential for protecting people's health and well-being, encouraging economic development, and improving livelihoods by facilitating access to domestic, regional, and international markets. Protecting the health of a country's population is one of a government's most important responsibilities, and it's directly tied to the achievement of several Sustainable Development Goals (SDGs). Food safety regulations or monitoring, on the other hand, is essential for ensuring fair practices in the food trade and fostering economic opportunities for all stakeholders involved in the food chain. Controlling foodborne dangers across the entire food chain has become increasingly important in an age of fast-evolving food technologies and ever-increasing global food trade. Food control/monitoring systems must be up to date with the newest advances, function based on risk analysis, and be harmonized with international standards and best practices to meet the complex developing challenges of the twenty-first century.

Due to the water, energy, and material consumption required for the production, processing, storage, and transportation of food that is not being used effectively, this loss of food due to microbial contamination is fundamentally unsustainable (Pleissner 2018). Foodborne illness can be contracted by ingesting microorganisms that cause food poisoning or by eating toxins produced by toxigenic pathogens in food (Bintsis 2017). Livestock and human mobility, land application of raw manure, polluted irrigation water, immature compost application, contaminated soil, and runoff from compost and manure stockpiles on the farm are all sources of pathogen contamination of fresh produce at the farm level (Maurice Bilung et al. 2018; Ssemanda et al. 2018). Environmental samples (soil, feces, water), poorly sanitized food contact surfaces (conveyor belts, knives, slices, etc.) and poorly sanitized non-food contact surfaces (walls, drains, floors, etc.), unhygienic plant design, unregulated traffic patterns, non-sanitized worker's hands, transport trailers, and crates are some of the contamination sources (Perez-Arnedo and Gonzalez-Fandos 2019).

Monitoring food contamination provides information and evidence on the types of contaminants in food. It gives an insight into the increasing trends or drifts in food contamination—this aid in initiating proactive precautionary measures before any severe or threatening health hazard occurs and becomes widespread. Moreover, monitoring programs also help evaluate the feasibility and success of any activity or initiative to minimize contamination. However, monitoring itself is insufficient to solve the problem of contamination. The information obtained must be followed up, like identification of the source of contamination, its control, and elimination. Simultaneously, any food, if found to be contaminated, must immediately be banned by taking appropriate measures. The need/requirement of monitoring food contamination is crucial to ensure public safety and health and proper management of food and agricultural resources to stop or prevent any economic or financial loss. It provides a timely investigation of the changes in the level of contaminants and corroborates that their amount does not exceed any standards. The lack of a proper food contamination monitoring system, therefore, not only poses the risk of severe health complications but also affects the finance and economy. The scope of any monitoring program depends on the availability of resources, its significance concerning health and economy, and any technical limitations like lack of proper analytical tools. Environmental monitoring systems (EMP) are a hands-on method developed to ensure and check food safety by monitoring sanitation and hygiene processes. It is an indicator for preliminary and final product testing since the entire production process must be thoroughly scrutinized to warrant product quality. Environmental sampling is a rational means to check the various contamination sources, maintain cleanliness, and highlight any issues that might require counteractive action.

Commercial technology for the efficient and low-cost detection of microbial contamination in food, industrial wastewater, and clinical samples is in high demand. Microorganisms' optical, electrochemical, metabolic, and physical capabilities have been used in various detection approaches. The necessity for a technology that can generate a quick, reliable, accurate, critical analysis for clinical, industrial, and environmental applications has resulted in significant progress in developing biosensors for microbial detection in recent years. Several instruments have been commercialized as a result of this comprehensive investigation.

Rapid detection and monitoring technologies will be classified as either non-biochemical or bioelectrochemical. Non-biochemical approaches can be split into two categories: standard (dry weight, viable count, and turbidity), specialized (Dry weight, viable count, and turbidity), and sophisticated (Microcalorimetry, Epifluorescence filter technique, Fluorescent-antibody technique, Radiometry, Bio-luminescence, Coulter counter, Electronic particle analysis, Micro-ELISA, Electron microscopy, spectroscopy, etc.). For the assessment of microbial biomass, a variety of electrochemical detection/monitoring technologies (impedimetry and conductivity, fuel cell technology, cyclic and square wave voltammetry) have been presented. Like the preceding strategies, their goal is to lower the time it takes to detect microorganisms, eventually developing a quick, accurate, economic, and repeatable biomass probe (Hobson et al. 1996).

Microbial food contamination is a crucial area of food safety where hazard analysis and critical control points (HACCP) will significantly impact preventing bacteria from being transmitted to humans through food. When foodborne bacteria cause illnesses, one of the main concerns is that the germs may become invasive and necessitate medical intervention, such as using antibiotics. In the context of food microbiological contamination, antibiotic-resistant microorganisms are a subpopulation of organisms that, when present, can be carried inside the food product and offer a significant obstacle to illness treatment and remediation in humans. The hazard of disease and illness caused by food contamination with microbes is far greater than the threat of resistance transfer from animals to people, based on the number of recorded cases. More explicitly, the relationship between the actual ailment caused by an antibiotic-resistant organism and the incidence of a genetic component of resistance being passed to microbes and causing disease must be tracked. Furthermore, because of the potential introduction of antibiotic residues and microbiological infections from countries with less rigorous agricultural production practices and quality-control systems, imported foods may need to be strictly regulated (The Use of Drugs in Food Animals: Benefits and Risks 1999. National Academies Press (U.S.)).

To ensure human health and safety, it is necessary to systematically detect, assess, and regulate the harmful consequences and related probability resulting from microbiological pathogen-contaminated foods. Food safety, on the other hand, is difficult to pin down and quantify. Factors like microbiological safety, chemical safety, and personal and environmental hygiene are crucial to ensure the overall well-being of the food. It necessitates consistent and reasonable efforts by competent authorities, corporate operators, scientists, consumers, and monitoring body/organizations representatives like the Food Safety and Standard Authority of India, 2011. Globally, proposed measures include (i) the implementation of standards and guidelines such as ISO 22000, the Hazard Analysis Critical Control Point (HACCP) scheme, the Good Manufacturing or Management Practices (GMP) scheme, and (ii) the scientific basis for risk management related to food consumption, such as the Food Safety Barometer developed by the Belgian Food Safety Scientific Committee (Saad et al. 2013).

7 Effect of Microbes on the Economy – Effect on the Import and Export Sector

Food spoilage poses a severe challenge to food security, i.e., our ability to provide a sufficient food supply to the world's growing population. In the opinion of a former World Health Organization official, "*This large increasing world population needs food, and we have a moral obligation to utilize all our skills and technologies to increase food production and limit food spoilage*."

In recent years, an increase in the international food trade, extensive production typically involving multiple sites, and a complex supply chain have contributed to microbial food deterioration. Food hygiene is critical for people's general well-being and daily lives, as well as for economic development, social stability, and the reputation of the government and country (Hussain and Dawson 2013).

Food processing sectors have a disproportionately large share of the global economy. The processed food business is constantly expanding due to technical advancements, rising demand, and changing customer preferences. As a result of this improvement, both developed and developing countries are adopting innovative food processing and delivery technologies. (Amit et al. 2017). The food processing industry loses millions of dollars yearly owing to microbiological contamination and substantial reductions in products that do not fulfill consumer expectations. Food is vulnerable to deterioration and pathogenic microbial activity. Some microorganisms generally attach to solid surfaces with sufficient nutritional content for nourishment and growth in natural habitats. Reduced crop yield and quality, as well as considerable economic losses owing to buyer rejection and mycotoxin contamination of grains, are all consequences of microbial infection of food crops (e.g., poisonous fungal secondary metabolites).

Spoilage is a business concern in these countries, affecting producers' and manufacturers' profits and losses. Spoilage continues to be a severe worry in less developed countries. It is impossible to calculate the actual economic cost of food deterioration. Approximately 30% of manufactured food products are damaged, with microbial food spoilage being the most common cause (Gram et al. 2002).

Food waste is inefficient and expensive, and it has the potential to harm the economy and diminish customer confidence. According to the E.U. 2020 Resource Efficiency Flagship, which presents a strategic framework for more sustainable and efficient use of natural resources, each person wastes approximately 179 kg of food annually. This amounts to around 89 million tonnes per year in total. In Europe, food waste is anticipated to increase to almost 126 million tonnes.

An increase in the globalized food trade in recent years, extensive production often involving many sites, and a complex supply chain all contribute toward an increased number of microbiological food. The presence of foodborne pathogens in a country's food supply impact not only the health of the local population but also poses a risk of pathogen transmission to travelers and customers in other nations where food is exported. Awareness of these issues has prompted international initiatives to harmonize food safety standards, which has complicated international food commerce.

More than 200 ailments, ranging from diarrhea to cancer, are caused by contaminated food carrying pathogenic bacteria, viruses, parasites, or inorganic chemicals. Every year, an estimated 600 million people worldwide become infected after eating tainted food, with 420,000 deaths resulting in the loss of 33 million disability-adjusted life years. With 125,000 deaths annually, children under five account for 40% of the foodborne disease burden. Diarrheal infections are the most frequent illnesses caused by contaminated food, affecting 550 million people each year and resulting in 230,000 fatalities. Different parties involved in the national or international food trade may be defrauded, such as manufacturers, co-packers, distributors, and others along the distribution chain. Because it impacts individuals of all ages, races, genders, and income levels worldwide, food safety in the food market is one of the most critical areas of concern in public health. Food marketing on a local and international scale continues to impact public food safety and health substantially. Food supply systems now span many national borders, putting health concerns on a global scale (Gizaw 2019).

Food loss is significant in the efforts to combat hunger, raise income and improve food security in the world's poorest countries. Food loss due to spoilage or contaminated food hurts the food industry and customers, resulting in financial losses and higher hospitalization costs. Infectious diseases associated with food intake are one of the impediments to economic growth, and they have an impact on a country's productivity as well as medical costs. Apart from the sudden drop in market transactions that commonly precede such occurrences, which can take years to stabilize, the result of such incidences is significant economic consequences as a result of taking products off shelves, reporting consumers, and the cost of lawsuit reparations. Food processing, packaging, and formulation procedures are frequently designed to suppress or control microbial development to prevent spoilage. The most restricted techniques are pH reduction, preservatives, water activity limiting, oxygen tension management, thermal processing, and hermetic packing. These procedures are used in tandem to inactivate or prevent the growth of possible spoiling microorganisms. On the other hand, the lack of competition from different background microbiota enhances the propagation of certain microorganisms that can resist these controls.

8 Conclusion

Food rotting caused by bacteria or microbiological food contamination threatens the global system. Furthermore, the emergence of antibiotic-resistant pathogens has aggravated food safety risks. To keep a circular food supply chain viable, extremely cautious skills and expansion are required to reduce the level and recurrence of food contamination, as well as scientific studies into the eventual demise of contaminants during treatment, techniques for simplified, economic, and reliable monitoring, and policy options to safeguard the framework. Therefore, from the conventional thermal and chemical strategies for food decontamination, the food processing industry implements novel and innovative techniques like pulsed electric fields, nonthermal plasma, electrolyzed water, etc., which will ensure the safety and improved shelf-life of the food. In addition, various food contamination monitoring technologies have also emerged, which detect specific physical and chemical characteristics of the microorganisms leading to their rapid identification and development of control mechanisms. These technologies will make the food industry aware of the various food-related diseases, identify and combat them, and strengthen their manufacturing and packaging processes to deliver the products safely.

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