Mohidus Samad Khan
Mohammad Shafiur Rahman *Editors*

Techniques to Measure Food Safety and Quality

Microbial, Chemical, and Sensory

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Preface

Food safety is a growing concern due to the increase in food-borne illnesses caused by food adulteration, excessive use of pesticides, chemical preservatives, chemicals used for artificial fruit ripening, microbial contaminations, and improper food handling. Chemical contaminants in food could be transferred from environmental or agrochemical sources, which include heavy metals, pesticide, herbicide, pharmaceutical and personal care products, and other by-products of water disinfection. On the other hand, microbial food safety can be threatened due to the presence of many pathogens, such as *Salmonella*, *Escherichia coli*, *Clostridium botulinum*, *Staphylococcus aureus*, and *Listeria monocytogenes* in foods. Strict regulations are imposed worldwide to limit the potential contaminates in foods. In order to control all contaminants, it is important to have a reliable method(s) to measure these microbial and chemical contaminants. Therefore, accurate, rapid, portable, and inexpensive approaches to test food contamination and adulteration are a major challenge to ensure global food safety.

This book addresses the basic understanding of the food contaminants and their sources followed by selected measurement techniques for microbial, chemical, and sensory attributes. After ensuring the microbial safety, it is important to address the sensory properties since it is the ultimate determinant for consumer acceptability of any food product. The first part of this book presents the sources of contaminants in foods, their associated health risks, and integrated management and alternative options to minimize contaminants. The second part discusses the conventional methods and selected advanced methods for the detection, identification, and enumeration of microbial contaminants. This part presents conventional microbial counting and identification techniques, such as standard plate count technique, most probable number for statistical determination of viable cells, and direct microscopic count. This part also discusses enzyme-linked immunosorbent assay (ELISA) and Vitek techniques for food analysis. The third part included different chemical measurement techniques, such as nuclear magnetic resonance (NMR), UV–vis spectroscopy, gas chromatography and mass spectroscopy (GC-MS), supercritical fluid extraction (SFE), solid-phase microextraction (SPME), and stir bar sorption extraction (SBSE), electronic tongue, paper-based detection, and differential scanning

calorimetry techniques. Sensory properties, such as flavours, aroma, taste, texture, and mouthfeel, are among the most important attributes of food products in ensuring their acceptability to the consumers. The responses are perceived by all five senses: smell, taste, sight, touch, and hearing. The approach used in sensory evaluation varies depending on the types of foods and the ultimate goal of the testing. Two approaches are available to evaluate sensory properties of food product, namely subjective and objective analyses. The subjective approach is also known as sensory evaluation, while the objective approach is known as instrumental evaluation. Subjective analysis or sensory evaluations determine human responses to food properties. The fourth part includes selected subjective (i.e. sensory) and objective (i.e. instrumental) methods. The sensory attributes of food include appearance, colour, flavour, odour, taste, texture, consistency, hardness, softness, tenderness, firmness, and crunchiness. The fourth part briefly discusses different instrumental techniques for sensory and chemical analysis of milk, and instrumental texture profile analysis (TPA) of various foods.

This book will be an important resource for food scientists, technologists, and engineers for analysing foods for their microbial and chemical contents in relation to compositions (i.e. protein, carbohydrate, fats, minerals, nutrients, and active components) and contaminants (i.e. undesired components). In addition, it will be a source of sensory measurement techniques as well as instrumental methods in relation to the sensory analysis. We are confident that this book will definitely be informative and enlightening to the readers.

Dhaka, Bangladesh   Mohidus Samad Khan
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Acknowledgement

Contents

About the Editors

Mohidus Samad Khan is an aspiring researcher and innovator, currently working as an associate professor at the Department of Chemical Engineering, Bangladesh University of Engineering and Technology (BUET). Dr. Khan aims to address different socio-economic issues through his research. His research interests encompass Biotechnology, Food and Environmental Engineering. Till date, Dr. Khan has authored and co-authored over 90 technical articles, which include peer-reviewed journal and conference articles, international patents, industrial reports, books, and book chapters. He also serves as a reviewer of several reputed international journals. Dr. Khan completed his BSc in chemical engineering (2004) from Bangladesh University of Engineering and Technology (BUET). He began his career as a research engineer in an international research project on managing and monitoring of industrial pollution, led by DFID, USAid, and the Government of Bangladesh (GoB) (2004–2006). Starting in 2006, Dr. Khan completed his PhD in 2010 in bio-surface and biotechnology from Monash University, Australia. His PhD research work won several major awards, including **University Medals and Vice-Chancellor's Commendation** for Best PhD Thesis. Dr. Khan worked as a post-doctoral fellow at the Department of Chemistry, McGill University, Canada, to continue his research work on biotechnology (2010–2013). Since 2013, Dr. Khan is working as a full-time faculty member in the Department of Chemical Engineering, BUET. He also served as a visiting professor (2015) at McGill University, Canada, and as a visiting research scholar (2016–2017) at Texas A&M University, USA.Dr. Khan has been involved in food, health, and environment projects led by the World Health Organization (WHO), the United Nations Development Programme (UNDP), the Government of Bangladesh (GoB), international donor agencies, and local Industries. As a researcher and innovator, Dr. Khan has won several prestigious awards, which include the "**Young Innovator Award**" entitled "**TR35@Singapore Awards** for 2012" organized by "*MIT Technology Review Inc*", and "**iCFP2016 Young Scientist Award**". His research work has been highlighted across several continents and different journals/organizations, including the Royal Society of Chemistry Journal "Chemistry World", MIT Technology Review, American Chemical Society, NY Times, India Times, and Science Daily.

Mohammad Shafiur Rahman is a professor at the Sultan Qaboos University, Oman, and the author or co-author of 400 technical articles including 136 refereed journal papers, 129 conference papers, 73 book chapters, 36 reports, 17 popular articles, and 12 books. He is the author of the internationally acclaimed and award-wining Food Properties Handbook, published by CRC Press, Boca Raton, Florida, which was one of the bestsellers from CRC Press in 2002. The second edition is now released with his editorship. He is the editor of the popular book Handbook of Food Preservation published by CRC Press, Boca Raton, Florida. The first edition received one of the bestsellers from CRC press in 2003, and the third edition is now in the market. The first edition was translated into Spanish. He is one of the editors of Handbook of Food Process Design (two volumes) published by Wiley-Blackwell, Oxford, England, in 2012. He was invited to serve as one of the associate editors for the Handbook of Food Science, Engineering and Technology, and one of the editors for the Handbook of Food and Bioprocess Modeling Techniques published by CRC Press, FL. Recently, he has released a coedited book Minimally Processed Foods: Technologies for Safety, Quality and Convenience published by Springer, New York (2016), and Glass Transition and Phase Transitions in Food and Biological Materials published by Wiley-Blackwell, England (UK) (2017).Professor Rahman has initiated the International Journal of Food Properties (Marcel Dekker, Inc.) and serving as the founding editor for more than 20 years. In addition, he is serving in the editorial boards of 10 international journals. He is a member in the Food Engineering Series Editorial Board of Springer Science, New York. He is serving as an editor-in-chief for the Sultan Qaboos University journal, Journal of Agricultural and Marine Sciences (JAMS). In 1998, he has been invited and continued to serve as a Food Science Adviser for the International Foundation for Science (IFS) in Sweden. Professor Rahman is a professional member of the New Zealand Institute of Food Science and Technology (NZ) and the Institute of Food Technologists (USA) and Member of Executive Committee for International Society of Food Engineering, ISFE. He was involved in many professional activities, such as organizing international conferences, training workshops, and other extension activities. He was invited as key note speaker and plenary speaker in 10 international conferences in the food science and engineering area. He received the B.Sc. Eng. (Chemical) (1983) and M.Sc. Eng. (Chemical) (1984) degrees from Bangladesh University of Engineering and Technology, Dhaka, the M.Sc. degree (1985) in food engineering from Leeds University, England, and the Ph.D. degree (1992) in food engineering from the University of New South Wales, Sydney, Australia.Professor Rahman is an eminent scientist and academic in the area of food processing. He is recognized for his significant contribution to the basic and applied knowledge of food properties related to food structure, engineering properties, and food stability. Professor Rahman has received numerous awards and fellowships in recognition of research/teaching achievements, including the HortResearch Chairman's Award, the Bilateral Research Activities Program (BRAP) Award, CAMS Outstanding Researcher Award 2003, SQU Distinction in Research Award 2008, and the British Council Fellowship. In 2008, Professor Rahman ranked among the top five Leading Scientists and Engineers of 57 OIC Member States in the Agroscience Discipline.

Chapter 1
Introduction on Techniques to Measure Food Safety and Quality

Mohidus Samad Khan and Mohammad Shafiur Rahman

Abstract Food safety is always a concern due to the increasing trends of foodborne illnesses caused mainly by microbial and chemical contaminants. Chemical contamination indicates the presence of undesired and harmful chemical substances in the food chain, which is caused by excessive use of pesticides, chemical preservatives, chemicals used for artificial fruit ripening, and other processing aids. On the other hand, microorganisms like molds, yeasts, bacteria, and viruses can cause food spoilage and foodborne diseases. There are different ways of contaminating foods during preproduction, production, and post-production stages. Hence, it is important to understand the sources of contaminations and their measurement techniques to determine contaminated microbial and chemical levels. In addition to microbial and chemical safety, it is important to measure the sensory attributes to achieve consumer satisfaction.

1   Introduction

Food is defined as edible substances, usually of plant or animal origin, consisting of nourishing and nutritive components, such as carbohydrates, fats, proteins, essential minerals, and vitamins. Quality of foods considers two aspects: the first one is the safety (i.e. physical, chemical, and microbial) and the second one is the sensory and

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nutrition. Appearance, color, flavor, and texture are critical factors for the sensory quality of food. Detection of food quality, authenticity, and adulteration is a great concern among consumers and authorities. Quality of food can often be associated with taste since undesired taste often indicates degradation of foods. The relationship among taste, flavor, and consumer preference is the key to retain and increase customer satisfaction. Food safety is a growing concern due to the increase in food-borne illnesses caused by food adulteration, excessive use of pesticides, chemical preservation, artificial fruit ripening, microbial contamination, and improper food handling. Food safety is a global public health issue. According to World Health Organization, almost 1 in 10 people in the world, or in other words 600 million, people fall ill after eating contaminated food and 420 thousands die every year, resulting in the loss of 33 million healthy life years [1].

Contamination of food can be due to chemical, microbiological, or physical causes. Chemical contaminants in food could be transferred from environmental or agrochemical sources, which include heavy metals, pesticides, herbicides, pharmaceutical, and personal care products, and other by-products of water disinfection. On the other hand, microbial food safety can be threatened due to the presence of many pathogens, such as *Salmonella*, *Escherichia coli*, *Clostridium botulinum*, *Staphylococcus aureus,* and *Listeria monocytogenes* in foods. Food analysis and authentication are integral parts of food safety and security.

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret responses to products as perceived via the senses of sight, smell, touch, taste, and hearing [2]. It is a subjective method and it is generally used to measure these quality parameters, which are known as sensory or organoleptic tests. The physical and chemical properties of food interact with the human sense organs and the signals are then transferred to the brain for a response. Therefore, sensory analysis plays a major role in defining food quality.

Strict regulations are imposed worldwide to limit the potential contaminants in foods. The development of accurate, rapid, and inexpensive approaches to test food contamination and adulteration is a major challenge to ensure global food safety. The analysis of food composition has significantly evolved over the past 100 years [6, 7]. The early focus of food analysis was to differentiate the levels of food components, assess purity, and expose economic fraud. Pioneering developments in pH instruments, spectrophotometry, chromatography/separations, and spectrometry often had immediate applications to food analysis [8]. The growth and infrastructure of the modern global food distribution system heavily relies on food analysis [9], beyond simple characterization, as a tool for new product development, quality control, product automation, regulatory enforcement, and problem-solving.

The development of accurate, rapid, and inexpensive approaches to measure food quality is the major challenge to ensure global food quality. There are existing processes to ensure the safety of food products from chemical and microbial contaminants. Apart from the existing measurement technologies, varieties of new techniques are also emerging, and these could be potential to ensure effective food safety and quality.

2 Overview of Food Safety and Sensory Quality

2.1 *Microbial Contaminations*

Microbial contamination of food is a serious public health concern worldwide. Microorganisms like molds, yeasts, bacteria, and viruses can cause food spoilage and foodborne diseases. Research also showed that contaminated foods are alarmingly responsible for many accidental fatalities [7]. Pathogenic microbes can potentially be found more or less everywhere. Some persist in the environment or multiple hosts and contaminate foods via pathways that reflect the variety of ecosystems that make up our food supply [7]. Microbial contaminations can be introduced at preharvesting, harvesting, post-harvesting, preslaughtering, slaughtering, and post-slaughtering stages. There are different intrinsic factors (e.g., nutrient, pH, water activity, and redox potential) and extrinsic factors (temperature, pressure, and gaseous atmosphere) influencing microbial growth in foods [3]. To ensure safe and reliable supply of food, mitigation of microbial contamination and the consequential impact is of paramount importance. The best practice is to prevent microbial contamination through good agricultural practices. Different preventive measures such as good agricultural practices, good manufacturing practices, good hygienic practices, good transportation practices, hazard analysis and critical control point, and good storage practices can be implemented to minimize microbial food safety hazards [7]. The exclusion and control of the well-estimated risk factors through the above-mentioned practices can help to develop safe food all over the world and reduce the socioeconomic burden of foodborne diseases [8].

2.2 *Chemical Contaminations*

Chemical contaminations are considered to be one of the main causes of food contamination that are associated with foodborne disease outbreaks [9]. Chemical contamination indicates the presence of undesired and harmful chemical substances in the food chain. A wide range of organic and inorganic chemical contaminants can be naturally found in the environment or added to adulterated foods during their processing, packaging, and storage [10]. Potential sources of chemical contaminants include pesticide residues, plant derived, growth hormones, artificial fruit ripening, additives, and colors. The presence of these unwanted chemicals in foods at a higher concentration above the allowable limit could be fatal to human health. Potential health implications can be mild gastrointestinal symptoms to severe hepatic, neurological, and other disorders [11]. Chemical contamination can be food specific and can occur at any stage of food processing. Risk assessment as well as risk management needs to be applied at different levels of food processing and handling to limit contamination below the permitted threshold. Analytical detection of food contaminants and successive control and monitoring should be done at all

phases of food production, processing, and distribution. Alternative options in accordance with good agricultural and manufacturing practices along with integrated management in the supply chain can help to minimize chemical contaminations in foods [12].

2.3   Overview of the Sensory

Food must meet the expectations of the consumers, which depends upon food quality including sensory or organoleptic quality, physicochemical properties, nutritional quality, and microbiological quality. Sensory properties, such as texture, mouthfeel, flavor, and taste, are among the most important attributes of food products in ensuring their acceptability to the consumers [2]. Two approaches are available to evaluate sensory properties of the food product, namely, subjective and objective analyses. The subjective approach is also known as sensory evaluation, while the objective approach is known as instrumental evaluation. Subjective analysis or sensory evaluations determine human responses to food properties and materials. The responses are perceived by all five senses: smell, taste, sight, touch, and hearing. The approach used in sensory evaluation varies depending on the types of foods and the ultimate goal for desired attributes' testing.

3   Technological Assessment of Food Safety and Quality

To ensure food quality and safety, it is important to analyze physical, chemical, and sensory properties, authenticity, microbial and chemical contaminants, and toxicity of foods [13]. Therefore, effective and valid methods of food analysis are always needed. Food analysis, a special branch of analytical chemistry, offers various techniques to identify the composition, structure, physicochemical, nutritional, and sensory properties of food products [14]. With the advancement of analytical techniques, researchers and technicians can quantify the manifold unexpected natural and man-made substances found in food products [15]. With the increased demand for reducing detection limits, highly selective detection techniques are of critical importance for food analysis [16, 17]. There are different techniques available to assess microbial and chemical contaminants and food sensory quality:

- High-performance liquid chromatography (HPLC) technique
- Fourier-transform infrared spectroscopy (FTIR) technique
- Portable sensors technique
- Paper-based detection kits
- Biosensors
- Ultraviole–visible spectroscopy (UV–vis) technique
- Gas chromatography (GC) technique
- Mass spectroscopy (MS) technique
- GC-MS technique

- LC-MS technique
- Image analysis technique
- Texture profile analysis (TPA)
- Bioelectrochemical systems (BES)
- NMR spectroscopy
- Supercritical fluid extraction (SFE) technique
- Solid-phase micro extraction (SPME) technique
- Stir bar sorption extraction (SBSE) technique
- Microwave-assisted extraction (MAE) technique
- Laser-induced breakdown spectroscopy (LIBS) technique
- Surface-enhanced laser desorption ionization (SELDI) technique
- Surface plasmon resonance (SPR) technique
- Polymerase chain reaction (PCR): (q-PCR and immuno-PCR) technique
- Reverse transcription PCR (RT-PCR) technique
- Rapid validity PCR (RV-PCR) technique
- Enzyme-linked immunosobent assay (ELISA) technique
- DNA sequencing technique
- Electronic tongue (E-Tongue) technique
- Electronic nose (E-Nose) technique
- Microarrays technique
- Differential scanning calorimetry (DSC) technique
- QuEChERS (quick, easy, cheap, effective, rugged, and safe) technique
- VITEC-automated instrument for fast, accurate microbial identification, and antibiotic susceptibility testing
- Computer-guided AI

Principles, methodology, sample preparation, and applications of the few selected key techniques are briefly discussed in the following sections.

3.1 Enzyme-Linked Immunosorbent Assay (ELISA) Technique

Enzyme-linked immunosorbent assay (ELISA) is a plate-based assay technique that employs a highly sensitive and specific form of immunological reactions. The sensitivity, specificity, and versatility functions of the ELISA technique allow it to detect food adulterants and specific constituents in food, including the natural components, pesticides, organic and inorganic toxins, and spoilage microorganisms [18]. It is a convenient and reliable analysis tool for the detection and quantification of constituents related to food production and processing as well as food safety. In the food sector, ELISA can be used for a wide range of applications including discrimination of meat, fish, and milk species; fruit juice labeling authentication; genetically modified and irradiated food detection; feedstuffs origin; and allergen ingredients identification [19]. ELISA is also suitable to validate food adulterations and provides a suitable complementary approach in food analysis and minimizes the use of sophisticated, expensive, and time-consuming techniques.

3.2 Ultraviolet–Visible Spectroscopy (UV–Vis) Technique

Spectroscopic methods have been very successful at evaluating the quality of agricultural products, especially food. It is the most commonly used instrumental technique for both qualitative and quantitative food analysis because of the simplicity, accuracy, fast, and highly precise result. According to the region of electromagnetic spectrum employed in the analysis, light absorption spectroscopy in the Ultraviolet and Visible region (UV-Vis) (200-800 nm) is one of the most used techniques for the characterization and determination of several organic and inorganic substances [20, 21]. Due to the simplicity and reliability, this technique has already been used in several research areas of food science and food processing industries. UV–Vis absorption spectroscopy is extensively used to analyze a wide range of food samples, such as meat, dairy products, processed foods, oils, beverages, wine, spices, flavors, and fresh and processed fruits and vegetables. Currently, UV-vis microvolume spectrometric instrumentation has been developed to analyze samples with small volume or toxic solvent [22].

3.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy combined with multivariate statistical methods is a powerful tool for the quality control and authentication of foods [23]. NMR technique is a fast, reproducible, and non-destructive technique highly suitable for food analysis [24]. NMR can be used to identify food constituents, food adulterants, and food authenticity. It has wide applications in food including cereals, fish, red meat, poultry, milk and dairy products, fruits and vegetables, and beverages. High cost and relatively low sensitivity are limiting its wide applications in the area of food science. However, NMR spectroscopy could be preferred over most of the other techniques because of nondestructive, robust, efficient, and non-targeted nature.

3.4 Gas Chromatography and Mass Spectroscopy (GC-MS) Technique

Gas Chromatography and Mass Spectroscopy (GC-MS) technique has a wide variety of application areas including environmental monitoring, food safety and quality control, biological and pesticides detections, forensic and criminal cases, drugs and pharmaceutical applications, energy and fuel applications, and also petrochemical and hydrocarbons analysis [16, 25, 26]. Due to high accuracy, high sensitivity, and low detection limit, GC-MS is highly accepted for the qualitative and quantitative analysis of food [27]. GC-MS can separate complex mixtures to quantify

analytes even at sufficiently low concentrations and to determine organic contaminants in trace levels. The combination of MS with a fast GC instrument shortens the running time of sample analysis compared to conventional GC instruments [27]. In the field of food analysis, GC-MS is considered one of the most powerful tools because of the cooperative interaction between GC and MS techniques. GC-MS can detect various organic compounds, such as esters, fatty acids, alcohols, aldehydes, terpenes in a wide range of food items [16]. This technique can also be used for the detection of contaminants, spoilage, and adulteration of food.

3.5 Electronic Tongue for Food Safety

Electronic tongue is an analytical taste-sensing multichannel sensory system that can be used for a wide range of food items to characterize, authenticate, quality evaluation, process monitoring, and quantitative analysis of foods [28–30]. The electronic tongue systems are designed as an array of nonspecific and low selective chemical sensors with partial specificity (cross-sensitivity) to different components present in the sample under investigation [31]. The electronic tongue consists of an array of liquid sensors with a different selectivity, a signal collecting unit, and a pattern recognition software [32]. The whole system of an e-tongue imitates what is happening when molecules with specific taste nature interact with taste buds on the human tongue. The taste buds are represented by sensors that interact with these molecules at the surface initiating changes in potential. When exposed to a sample containing different compounds, electronic tongue generates an output pattern representing a combination of all the components in the sample. The output pattern is given by different selectivity and sensitivity of individual sensing units and is correlated with a specific taste or quality aspect [33]. In an electronic tongue, the output of the nonspecific sensor array shows different patterns for the different taste-causing chemical substances and such data are statistically processed [34]. The sensors of electronic tongue "taste" raw substances, semi-products, and finished products in a fast and nondestructive method; hence, electronic tongue could contribute to improve automation of food processes. Electronic tongue techniques can also be used in food classification, freshness evaluation, authenticity assessment, and quality control [31].

3.6 Paper-Based Kits for Food Analysis

With the advances in point-of-care testing (POCT), researchers have sought to develop microfluidic chip-based devices (e.g., poly(methyl methacrylate) (PMMA), polydimethylsiloxane (PDMS)-based chips), and paper-based devices (e.g., lateral flow test strips and three-dimensional paper-based microfluidic devices), which are fast gaining popularity for use in detecting food contaminants [35, 36]. Paper-based

detection device is a promising tool that can be used over a wide range of applications involving food safety and security. Paper-based devices are typically rapid, cost-effective, and user-friendly, offering a high potential for rapid food safety analysis at the point of need. In the recent years, bioactive paper research has been a topic of great interest as it is robust, simple, affordable, and user-friendly [37]. The bioactive paper gained much attraction in food analysis research due to its capacity to replace conventional expensive and cost-intensive technologies like Gas Chromatography, Mass Spectrometry, Real-time or multiplex Polymerase Chain Reaction, or High-Performance Liquid Chromatography [38]. The detection approaches are usually based upon colorimetric, fluorescent, and electrochemical detection, and the signals can be observed rapidly and simply, providing an effective platform for food safety monitoring [39]. Paper-based detection devices are in use to detect foodborne and waterborne pathogens, organic and inorganic toxins, pesticides, illegal food additives, and discuss the mechanisms, assay times, and sensitivity of the analysis.

3.7   Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a widely used thermal analysis technique that can be used for measuring thermophysical properties of foods [40]. DSC is becoming very popular in thermal studies of foods, since it is rapid, facile, and capable of supplying both thermodynamic and kinetic data on protein denaturation [41]. This technique requires a small amount of samples for analysis without any specific sample preparation [41]. It requires less experimental time when compared to other techniques used for the same purpose. DSC is also preferred because it allows the detection of transitions in a wide range of temperatures (−90 to 550 C) and ease in the quantitative and qualitative analysis of the transitions [42]. Thermal transitions are used to determine the stability of foods as well as the characteristics changes that occur in the foods during processing and storage.

4   Conclusion

Food safety is one of the major global concerns. Food contamination is a potential threat due to its adverse effect on the community and public health. Therefore, it is important to properly analyze food composition and monitor the physical, chemical, biological, and sensory properties of food to ensure food safety and quality. Food analysis is a branch of analytical chemistry that deals with the information related to food composition, processing, quality control, and compliance with trade laws. The purpose of food analysis is to confirm the safety and quality of food and helps differentiate levels of food components, assess purity, and expose economic fraud. Therefore, it is important to ensure accurate, rapid, and robust technologies for food

analysis. This chapter gives a brief overview of food safety and sensory quality with respect to microbial contaminations, chemical contaminations, and sensory properties. This chapter also identifies different techniques available to assess microbial contaminants, chemical contaminants, and food sensory quality and briefly discusses principles and applications of key technologies. The following chapters of this book entitled, 'Technologies to Measure Food Safety and Sensory Quality' will address new measurement techniques into three parts: (i) understanding of food safety and sensory quality, (ii) technological assessment of microbial and chemical contaminants, and (iii) technological assessment of sensory quality. This book could be helpful for the students, researchers, academics, and engineers working in different aspects of the food safety in relation to their contaminants.

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Chapter 2
Overview of Microbial Contamination of Foods and Associated Risk Factors

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Abstract Microorganisms like molds, yeasts, bacteria, and viruses can cause food spoilage and foodborne diseases. For the past decade, the increase in foodborne infections has become an important public health concern worldwide. According to a report of the World Health Organization, hundreds of millions of people worldwide suffer from diseases caused by contaminated food. In order to ensure the protection of consumers from detrimental impacts of food microbiological contamination, it is important to improve the understanding and awareness of the sources and to identify the routes of transmission of pathogens into foods. This chapter thus addresses the microbiological contamination of foods including the mechanisms of microbiological contamination, microbial contaminants, and their commonly associated foods. In addition, it discusses the impacts of microbial contaminations and their risk factors associated with foodborne diseases.

Keywords Food microbiology · Microorganism · Microbial contamination · Foodborne disease · Food pathogen · Microbial toxin · Microbial growth factor · Food spoilage · Toxicoinfection · Food safety hazard · Risk factor · Food security · Food hygiene · Safe food processing

1 Introduction

Food is defined as edible substances, usually of plant or animal origin, consisting of nourishing and nutritive components, such as carbohydrates, fats, proteins, essential minerals, and vitamins. When ingested and assimilated through digestion, they sustain life, generate energy, and provide growth, maintenance, and health [1]. Food also meets emotional, social, and psychological needs. Despite their beneficial roles in the body, foods can also serve as vehicles for disease transmission and cause of death if contaminated with harmful microorganisms, microbial toxins, or

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environmental contaminants, such as chemical allergens and microphysical particles [2]. For the past decade, the increase in foodborne infections has become a global public health concern [3]. Microorganisms play an important role in food poisoning. There are many types of microorganisms of different forms and complex structures. Bacteria, molds, yeasts, and viruses have a greater impact on food deterioration [4].

Food contaminated by pathogens is a serious issue, as it can lead to a wide range of health problems. Foodborne illnesses can be fatal and may cause suffering, discomfort, and debilitation among the survivors. Pathogens are responsible for more than 200 diseases, including typhoid fever, diarrhea, and cancer [5] and can lead to the death of unsuspecting consumers in both developing and developed countries [6]. In the United States, between 1972 and 1978, among the total number of cases reported of foodborne disease outbreaks, pathogenic microorganisms caused 94.4%, and only 1.1% and 4.3% were caused by parasites and chemicals, respectively. At present, the occurrence of foodborne diseases caused by pathogenic microorganisms far exceeded compared to all other contamination combined. The consumption of pathogen-contaminated foods also creates an economic impact that can be devastating to the consumers, the nation, food dealers, and food companies.

The microbiological aspect of food safety involves exclusion of pathogenic microorganisms or microbial toxins from food, which can pose potential health hazards by their presence in food items. This implies the prevention and management of microbiological contamination of foods [7]. There are different ways foods can be contaminated in preproduction, production, and post-production stages. Hence, reliable and safe food supply needs to be free from harmful contaminants, and it is highly essential for the general health and daily life, economic development, and social stability [2, 8]. This chapter addresses different aspects of microbial contamination of foods including sources and health impacts of microbial contaminants. The information can help researchers, policy makers, industries, and consumers to take measures to early detect and eliminate sources of microbial contaminations and to prevent health issues and economic losses caused by microbial food contamination.

2 Types of Microbial Contaminants in Foods

The microbial groups responsible for causing foodborne diseases and food spoilage consist of several types of bacteria, yeasts, molds, and viruses. Among them, bacteria constitute the largest group because of their prevalent presence and rapid growth rate when all nutrients are available in the foods; they can grow even under conditions where yeasts and molds cannot grow. As a result, they are considered the most detrimental in food contaminations [2].

2.1 Bacteria

Among all types of microorganisms that contaminate foods, bacteria make up the most important group due to their diversity and actions of greater frequency. In the presence of favorable conditions, bacteria are capable of quickly multiplying on the foods, by causing alterations in foods and sometimes cause intoxications [4]. Bacteria are unicellular organisms that exist in various forms. The most common forms are spherical (cocci), rod-shaped (bacilli), and curved (spirilla) [9]. Regarding reproduction, bacteria reproduce by binary fission, which is the division of the cell into two equal parts. In certain situations, some bacteria (*Clostridium, Bacillus*) can form highly resistant structures to temperatures. These structures are called endospores, which are formed when the cells are in the presence of unfavorable growing conditions (i.e., lack of nutrients). Once the endospores encounter favorable growing conditions, they can germinate and form cells identical to the originated ones [4].

A wide range of bacterial genera are accountable for food spoilage and foodborne diseases, which include *Citrobacter*, *Escherichia*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Aeromonas*, *Plesiomonas*, *Pseudomonas*, *Acetobacter, Acinetobacter, Morexella, Flavobacterium, Alcaligenes, Psychrobacter, Micrococcus, Staphylococcus, Streptococcus, Enterococcus, Bacillus, Sporolactobacillus, Clostridium,* and *Listeria*. On the contrary, there are good bacterial genrea that are used in food industries as biopreservatives (*Lactococcus*, *Lactobacillus*), probiotics (*Lactobacillus*), and food bioprocessing (*Lactococcus*, *Lactobacillus*). A brief description of few important bacterial genera that are responsible for most of the foodborne diseases is given in Table 2.1.

2.2 Yeast and Mold

Although they present very different features, molds and yeasts belong to the same taxonomical group—fungi [4]. The large and diverse group of microscopic foodborne yeasts and molds includes several hundreds of species. The ability of these organisms to attack many foods is due to their relatively versatile environmental requirements. Although the majority of yeasts and molds are obligate aerobes (require free oxygen for growth), their acid/alkaline requirements (pH2–9) and temperature requirements (−8 to 45 °C) for growth are quite broad, with a few species capable to grow below or above this range. Moisture requirements of foodborne molds are relatively low; most species can grow at a water activity (a_w) of 0.85 or less, although yeasts generally require a higher water activity [10].

Both yeasts and molds cause various degrees of deterioration and decomposition of foods. Their detectability in or on foods depends on food type, organisms involved, and degree of invasion. The contaminated food may be slightly blemished, severely blemished, or completely decomposed. Abnormal flavors and odors may also be produced. Several foodborne molds, and possibly yeasts, may also be

Table 2.1 Important bacterial genera responsible for food spoilage and foodborne diseases [2, 4, 9]

Name of the genus	Shape	Size (μm)	Important species	Possible source/location	Relation with temperature
Citrobacter.	Straight rod	1 × 4	*Citrobacter freundii*	Found in the intestinal contents of humans, animals, birds, and in the environment	Mesophiles
Escherichia.	Straight rod	1 × 4	*Escherichia coli*	Found in the intestinal contents of humans, warm-blooded animals, and birds	Mesophiles
Enterobacter.	Straight rod	1 × 2	*Enterobacter aerogenes*	Found in the intestinal contents of humans, animals, birds, and in the environment	Mesophiles
Proteus.	Straight rod	0.5 × 1.5	*Proteus vulgaris*	Found in the intestinal contents of humans and animals and the environment.	–
Salmonella.	Medium rod	1 × 4	*Salmonella enterica* ssp. *enterica*	Found in the intestinal contents of humans, animals, birds, and insects	Mesophiles
Shigella.	Medium rod	–	*Shigella dysenteriae*	Found in the intestine of humans and primates	Mesophiles
Yersinia.	Small rod	0.5 × 1	*Yersinia enterocolitica*	Present in the intestinal contents of animals	–
Vibrio.	Curved rod	0.5 × 1	*Vibrio cholerae, Vib. parahaemolyticus, Vib. vulnificus, Vib. alginolyticus*	Found in freshwater and marine environments	Mesophiles
Aeromonas.	Small rod	0.5 × 1	*Aeromonas hydrophila*	Found in water environment	Psychrotrophs
Plesiomonas.	Small rod	0.5 × 1	*Plesiomonas shigelloides*	Found in fish and aquatic animals	–
Pseudomonas.	Straight/curved rod	0.5 × 5	*Pseudomonas fluorescens, Pse. Aeruginosa, Pse. Putida.*	Found widely in the environment	Psychrotrophs
Acetobacter.	Ellipsoid to rod	0.6 × 4	*Acetobacter aceti*	Widely distributed in plants and in places where alcohol fermentation occurs	Mesophiles
Acinetobacter.	Rod	1 × 2	*Acinetobacter calcoaceticus*	Found in soil, water, and sewage	Mesophiles

Name of the genus	Shape	Size (μm)	Important species	Possible source/location	Relation with temperature
Morexella.	Short rod	1×1.5	*Morexella lacunata*	Found in the mucous membrane of animals and humans	Mesophiles
Flavobacterium.	Rod	0.5×3	*Flavobacterium aquatile*	Found in soil and fresh water in a variety of environments-	Psychrotrophs
Alcaligenes.	Rods/coccobacilli	0.5×1	*Alcaligenes faecalis*	Present in water, soil, or fecal material	Mesophiles
Psychrobacter.	Coccobacilli	1×1.5	*Psychrobacter immobilis*	Found in fish, meat, and poultry products	Psychrotrophs
Micrococcus.	Spherical	$0.2-2$	*Micrococcus luteus*	Found in mammalian skin	Mesophiles
Staphylococcus.	Spherical	$0.5-1$	*Staphylococcus aureus*	Present in the skin of humans, animals, and birds	Mesophiles
Streptococcus.	Spherical/ovoid	1	*Streptococcus pyogenes*	Present as commensals in the human respiratory tract	Mesophiles
Enterococcus.	Spherical	1	*Enterococcus faecalis*	Found in intestinal contents of humans, animals, and birds, and the environment	Mesophiles
Bacillus.	Rod	$0.5-1 \times 2-10$	*Bacillus cereus, bac. Coagulans, bac. Stearothermophilus*	Present in soil, dust, and plant products (especially spices)	Mesophiles
Sporolactobacillus.	Medium rod	1×4	*Sporolactobacillus inulinus*	Found in chicken feed and soil	–
Clostridium.	Rod	–	*Clo. Tyrobutyricum, Clo. Saccharolyticum, Clo. Laramie*	Found in soil, marine sediments, sewage, decaying vegetation, and animal and plant products	Mesophiles / Psychrotrophs
Listeria	Short rod	0.5×1	*Listeria monocytogenes*	Widely distributed in the environment and have been isolated from different types of foods	Psychrotrophs

hazardous to human or animal health because of their ability to produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking. Although the generating organisms may not survive food preparation, the preformed toxin may still be present [10].

There are wide ranges of molds that are associated with spoiled fruits and vegetables (e.g., *Aspergillus*, *Alternaria*, and *Rhizopus*), dairy products (e.g., *Alternaria*), grains (e.g., *Penicillium*), meats (e.g., *Penicillium*), and bread (e.g., *Penicillium*). Few molds are also used as food additives (e.g., *Asp. Oryzae., Asp. Niger.*) and food-processing aid (e.g., *Mucor rouxii*) [2].

Yeasts are important in food because of their ability to cause spoilage. Many are also used in food bioprocessing. Some are used to produce food additives [2, 11]. *Saccharomyces* form pellicles in beer, wine, and brine to cause spoilage. *Saccharomyces cerevisiae* variants are also used in baking for leavening bread and in alcoholic fermentation. *Rhodotorula* are pigment-forming yeasts and can cause discoloration of foods such as meat, fish, and sauerkraut (e.g. *Rhodotorula glutinis*). *Torulopsis* cause spoilage of milk because they can ferment lactose (e.g., *Torulopsis versatilis*). They also spoil fruit juice concentrates and acid foods. *Candida* spoils foods with high acid, salt, and sugar and forms pellicles on the surface of liquids (e.g., *Candida lipolyticum*). *Zygosaccharomyces* cause spoilage of high-acid foods, such as sauces, ketchups, pickles, mustards, mayonnaise, and salad dressings, especially those with less acid and less salt and sugar (e.g., *Zygosaccharomyces bailii*).

2.3   *Virus*

Viruses are regarded as noncellular entities. Bacterial viruses (bacteriophages) are important in food microbiology and are widely available in nature [12, 13]. Unlike bacteria, yeasts, and molds, viruses are incapable of reproducing independently. Instead, they must first invade the cells of another living organism called the host before they can be multiplied [14]. Viruses are composed of nucleic acids (DNA or RNA) and several proteins. A bacteriophage attaches itself to the surface of a host bacterial cell and inoculates its nucleic acid into the host cell. Subsequently, many phages form inside a host cell and are released outside followed by lysis of a cell [2]. Bacteriophage infections of starter cultures of lactic or other fermentative bacteria can interfere seriously with the manufacture of cheese, buttermilk, sauerkraut, pickles, wine, beer, and other desirable fermentative products [15]. On the other hand, virus-borne diseases can be transmitted by food to human beings. Although viruses require a live host cell and cannot multiply in foods, they can remain viable and infectious for long periods, even under highly adverse conditions, such as drying, freezing, and pasteurization.

Several pathogenic viruses have been identified as causing foodborne diseases in humans. Hepatitis A and Norwalk-like viruses have been implicated in foodborne outbreaks. Several other enteric viruses, such as poliovirus, echo-virus, and

coxsackievirus, can cause foodborne diseases [2]. Heating a product to an internal temperature of at least 72°C is considered adequate to inactivate both these viruses [16].

3 Sources of Microbial Contamination

Alarmingly, pathogenic microbes can potentially be found more or less everywhere. Some of them are sustained in humans and contaminate food supply via the excreta of infected humans, while many others are sustained in animals. Some persist in the environment or multiple hosts and contaminate foods via pathways that reflect the variety of ecosystems that make up our food supply [7]. Food safety depends on understanding these pathways or mechanisms well enough to prevent them. The different mechanisms or pathways through which pathogenic microorganisms or microbial toxins get into foods are briefly discussed subsequently.

3.1 Preharvest Factors

While being grown in the field, plant foods can be contaminated with microorganisms through water used for irrigation, application of pesticides, manure applied as fertilizer, migratory or wild animals, and practices of workers in the field [7].

3.1.1 Irrigation Water

The inside tissue of foods from plant sources is essentially sterile, except for a few porous vegetables (e.g., radishes and onions) and leafy vegetables (e.g., cabbage and Brussels sprouts) [2]. Contaminated water used for irrigation has been suspected as the likely cause of contamination of lettuce and tomatoes with *E. coli* 0157:H7 and mangoes with *Salmonella* [7]. A study conducted on crops (lettuce, carrots, and amaranthus) demonstrated that the crops got contaminated with *Salmonella*, *Vibrio* spp. and *E. coli* due to the presence of these pathogens in irrigation water [17]. Researchers have also demonstrated that the method of application of irrigation water directly influences food contamination with microorganisms during harvest [18].

3.1.2 Manure Applied as Fertilizer

Many types of molds, yeasts, and bacterial genera (e.g., *Enterobacter, Pseudomonas, Proteus, Micrococcus, Enterococcus, Bacillus,* and *Clostridium*) can enter into foods from the soil [2]. This microbial contamination can be amplified when

untreated sewage and manure are applied as plant fertilizers with improper application time or improper composting [19]. Soil contaminated with fecal materials can be a source of enteric pathogenic bacteria and viruses in food [2]. A number of studies have demonstrated the long-term survival of *E. coli* 0157:H7 and *Salmonella* in manure [17, 20].

3.2 Harvest Factors

A wide variety of equipment used in harvesting can be easily contaminated by many types of pathogenic microorganisms from air, raw foods, water, and personnel getting into the equipment. Microorganisms including pathogens can be built up in improperly washed and sanitized harvesting equipment, such as knives, chippers, and containers including trailers, boxes, bins, and truck beds. Depending on moisture, nutrients, temperature, and exposure time, microorganisms can be multiplied in harvesting equipment from a low initial population to a high level and contaminate large volumes of foods [2]. Microbial contamination of plant products can also occur through the contaminated unwashed hands of the field workers at the time of harvesting [7].

3.3 Preslaughter and Slaughter Contamination Factors

Livestock may be colonized by potentially pathogenic microorganisms prior to slaughter, and if care is not taken during the procedures of handling, transporting, slaughtering, and dressing livestock, the edible portions of the meat carcass surface can be contaminated with organisms (e.g., *Salmonella spp*. and *E.coli* (EHEC)) capable of causing foodborne illness in humans [21]. For meat and poultry products, studies showed that many potential human pathogens colonize their animal hosts without causing clinical signs, making it difficult to detect carriers [22]. In apparently healthy livestock going to slaughter, pathogenic microorganisms are confined primarily to the gastrointestinal tract and exterior surfaces, such as the hooves, hide and skin, hair, or fleece [21, 23], while internal organs and intact internal muscle are free of microorganisms. Poultry farms with a large population of birds are a potential source, where microbial pathogenic contaminants can be spread rapidly [24]. In the case of poultry, the feeding of infected foodstuffs can result in large numbers of chickens and their eggs carrying food-poisoning bacteria [19]. Drinking water, rodents, insects, dogs, cats, human stools, farm equipment, footwear, and work clothes of handlers are other potential sources to infect poultry flocks [7]. When care is not taken in removing animal skin or the hide, such pathogens find their way into meat or poultry during slaughtering and may cross-contaminate other foods that come in contact with the raw contaminated meat or poultry [7]. Meat and poultry can be contaminated during evisceration (the removal of the internal organs),

where the stomach or intestines may burst and release bacteria that can contaminate the flesh (the raw meat or poultry prepared for human consumption) [25]. This evisceration of chickens or turkeys by the same equipment or knife can lead to the transfer of pathogenic *Campylobacter* and *Salmonella* from one infected bird to several others.

3.4 Post-harvest/Slaughter Contamination Factors

3.4.1 Food Processing/Preparation and Serving

Food processing is the transformation of agricultural products into foods or food ingredients. Food processing involves applying scientific and technological principles to preserve foods [26, 27]. Food preparation or processing can introduce pathogens into a product if not done properly [7]. One important factor of microbial contamination during food processing and preparation is the use of shared equipment, which may introduce pathogens from one food to another. Studies showed that *Listeria monocytogenes* has been found on food processing equipment and process surfaces, which are difficult to clean [25].

3.4.2 Unhygienic Practices of Food Handlers

Between production and consumption, foods can be in contact with different people handling the foods. Humans (their skin, mucous membranes, and cuts, open sores, or a skin infection) can serve as potential sources of pathogens from where foods can be contaminated if handled under unhygienic conditions, especially through unwashed hands [5]. Improperly cleaned hands, lack of aesthetic sense and personal hygiene, dirty clothes, and hair can be major sources of microbial contamination in foods [2, 8]. The presence of blemishes, pimples, boils, open wounds and soiled tissues, minor cuts and infection in hands, and mild generalized diseases such as flu, strep throat, or hepatitis A in an early stage can amplify the situation [2, 7]. Pathogens such as *Staphylococcus aureus, Salmonella serovars*, *Shigella spp.*, pathogenic *E. coli*, and hepatitis A can be introduced into foods from human sources [2]. Handlers who do not wear gloves commonly spread Staphylococcus bacteria to meat, cream-filled desserts, potato salads, and egg products [7].

3.4.3 Biofilm Formation in Food Processes

During food processing, microbial colonies can inhabit or accumulate on critical places such as food contact and environmental sites on equipment to form biofilms. Biofilms are microbial cell clusters with a network of internal channels in the extracellular polysaccharide and glycoprotein matrix, which allows nutrients and oxygen