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
Muscat, Oman — Mohammad Shafiur Rahman

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We would like to thank the Almighty for giving us the opportunity to work on this interesting book project. We wish to express our sincere gratitude to the Sultan Qaboos University, Muscat, Oman, and Bangladesh University of Engineering and Technology, Dhaka, Bangladesh, for providing us the opportunity, facilities, and support to execute such an exciting book project.

We sincerely acknowledge our parents for their unconditional contribution in our educational progress and support in life. We acknowledge our spouses and children for their kind support and patience throughout the project. Special appreciations to our teachers and mentors in the progress of our careers. Special thanks to our colleagues and other research team members for their support and encouragement. We are pleased to find many dedicated contributors in this book. Special thanks to the editorial and technical staffs from Springer, New York, for providing continued cooperation in completing this book project.

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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 biosurface 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 postdoctoral 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.

Keywords Food safety · Food quality · Nutrition · Food contamination · Sensory property · Physical property · Chemical property · Microbial property · Assessment technique · ELISA · UV–Vis · GC-MS · Nuclear magnetic resonance · Electronic tongue · Point-of-care testing

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 nontargeted 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

Farrhin Nowshad, Nadira Mustari, and Mohidus Samad Khan

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 × 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

to be transported from the bulk liquid to the cells [7]. Microbial colonies that form in critical places contaminate the surfaces and consequently the products made in that particular process [8]. Once a biofilm has been formed, it can be a source of contamination for foods passing through the same processing line [28]. Harmful microbes may enter the manufacturing process and reach the end product in several ways, such as through raw materials, air in the manufacturing area, chemicals employed, process surfaces, or factory personnel. Any microbe can form biofilm under suitable conditions. However, some microbes naturally have a higher tendency to produce biofilm than others. Foodborne pathogens that readily form biofilms include *Bacillus cereus, S. aureus, M. paratuberculosis, C. perfringens, E. coli 0157:H7, S. typhimurium, C.jejunii, Yersinia enterocolitica, and L. monocytogenes* [29]. *L. monocytogenes* has been found to form biofilms on common equipment and food contact surfaces, for example, plastic, polypropylene, rubber, stainless steel, and glass, which are sometimes difficult to clean [8]. Therefore, the design of the equipment and process line in the food processing and packaging industry is important for preventing the formation of biofilms and so improving process and production hygiene.

3.4.4 Packaging, Distribution, Marketing, and Storage

Packaging is an important stage in providing quality foods to consumers, as it protects foods from microorganisms, prevents loss of moisture, protects from temperatures that cause deterioration during handling, storage, and transportation. However, the packaging material may transfer food spoilage or pathogenic organisms to the packed food [7]. The routes of contamination from the packaging material to food include the surface, cutting dust, or direct contact with the raw edge of the paperboard [8]. Microbial contaminations can be also spread through contaminated containers and possible contact with decaying products during storage and distribution. Even in the refrigerator, cross-contamination takes place from raw meat, poultry, or fish drips onto vegetables or other ready-to-eat foods on the shelf below. Contamination also occurs when foods are not kept at the right temperature, thus promoting the temperature danger zone (i.e., the temperature in which bacteria and such can be the most widely spread) [7]. In addition, the spraying of contaminated water to give a fresh appearance to the vegetables also adds microbial contamination.

4 Factors Influencing Microbial Growth in Foods

All microorganisms require a set of factors that allow them to grow/live in certain environments. The various factors that influence the growth of microorganisms in foods are generally designated as intrinsic and extrinsic factors [2, 4]. In addition, there are other factors related to the characteristics of microorganisms, which are

designated as implicit factors [4]. The manipulation of these factors allows to obtain products with a longer shelf-life and superior microbiological quality.

4.1 Intrinsic Factors

Intrinsic factors are those that are related to the physical–chemical characteristics of foods [4]. These factors have a preponderant action over microbial growth because almost all foods constitute a more or less favorable environment to the growth of most microorganisms. Like so, the type of nutrient contents [16, 30], pH [16], water activity [32–34], and oxygen [31, 35] are intrinsic factors that generally have a greater influence on microbial growth in foods.

Nutrient Contents: These nutrients required for microbial growth include carbohydrates, proteins, lipids, and small amounts of other materials such as phosphates, chlorides, and calcium [2, 16]. The growth of microorganisms can be controlled by controlling access and availability of corresponding nutrients [4, 31].

pH: Based on pH, foods can be grouped as high-acid foods (pH below 4.6) and low-acid foods (pH4.6 and above). Table 2.2 presents pH of some food items. The pH of a food has a profound effect on the growth and viability of microbial cells. Each species has an optimum and a range of pH for growth. In general, molds and yeasts are able to grow at lower pH than do bacteria: bacteria grow fastest in the pH range 6.0–8.0, yeasts 4.5–6.0, and filamentous fungi 3.5–4.0 [31]. The acidity of a product can have important implications for its microbial ecology and the rate and character of its spoilage.

Water Activity: For microbial growth, nutrients must enter the microbial cell through its cell wall. Therefore, nutrients must be soluble so that they can be carried into the cell by any free (or unbound) water that is available in the environment where the microbes are living (such as in food). The measure of this available free water is known as the water activity, or a_w, which ranges between 0 and 1. The a_w of food can be determined from its equilibrium relative humidity (ERH) by dividing

Table 2.2 pH of some common food commodities [40]

Item	Approx. pH
Apples	3.30–4.00
Bread, white	5.00–6.20
Cabbage	5.20–6.80
Egg white	7.96
Flour	6.00–6.30
Honey	3.70–4.20
Lime juice	2.00–2.35
Milk, cow	6.40–6.80
Oyster mushrooms	5.00–6.00
Sweet potatoes	5.30–5.60

Table 2.3 Minimum water activities at which active growth can occur [31–34]

Group of micro-organisms	Minimum a_w
Most gram-negative bacteria	0.97
Most gram-positive bacteria	0.90
Most yeasts	0.88
Most filamentous fungi	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.61

ERH by 100 (because ERH is expressed in percentage) [32–34]. Each microbial species (or group) has an optimum, maximum, and minimum a_w level for growth. Table 2.3 presents minimum water activities of different microorganisms at which active growth can occur. This information can be used to control spoilage and pathogenic microorganisms in food as well as enhance the growth of desirable types in food bioprocessing (such as adding salt in processing of cured ham) and in laboratory detection of microorganisms (adding salt to media to enumerate *Staphylococcus aureus*) [2].

Redox Potential: An oxidation–reduction (redox) reaction occurs as a result of the loss of electrons from a reduced substance (thus it is oxidized) and the gain of electrons by an oxidized substance (thus it is reduced). The tendency of a medium to accept or donate electrons, to oxidize or reduce, is termed as redox potential, E_h (measured in millivolts, mV) [31]. If free oxygen is present in a biological system, it can act as an electron acceptor [35]. Oxygen has a high redox potential and is a powerful oxidizing agent; thus, if sufficient air is present in a food, a high positive potential can result [31]. Increasing the access of air to a food material by chopping, grinding, or mincing can increase its redox potential. Similarly, exclusion of air as in vacuum packing or canning can reduce the redox potential [31]. The presence or absence of oxygen and the redox potential of food determine the growth capability of a particular microbial group in foods and the specific metabolic pathways used during growth to generate energy and metabolic by-products. This is important to understand and control microbial spoilage of foods (such as putrification of meat by *Clostridium* spp. under anaerobic conditions) and to produce desirable characteristics of fermented foods (such as growth of *Penicillium* species in blue cheese under aerobic conditions). Table 2.4 presents the redox potential of selected food materials [31].

4.2 Extrinsic Factors

Extrinsic factors are those related to food storage and environmental conditions [4], which include temperature, humidity, and gaseous environment. The relative humidity and gaseous condition of storage, respectively, influence the water activity and

Table 2.4 Redox potential of selected food materials [31]

	E (mV)	pH
Raw meat (post-rigor)	−200	5.7
Raw minced meat	+225	5.9
Cooked sausages and canned meats	−20 to −150	Ca. 6.5
Wheat (whole grain)	−320 to −360	6.0
Barley (ground grain)	+225	7.0
Potato tuber	Ca. −150	Ca. 6.0
Spinach	+74	6.2
Pear	+436	4.2
Grape	+409	3.9
Lemon	+383	2.2

redox potential of the food [2]. The influence of the storage temperature of food and the gaseous atmosphere on microbial growth is briefly discussed subsequently.

Temperature: Temperature is one of the most relevant factors in microbial growth. Microbial growth can occur over a temperature range from about −8 °C up to 61 °C at atmospheric pressure. The most important requirement is that water should be present in the liquid state and thus available to support growth [31]. Microorganisms in foods are mainly divided into three groups based on their temperature of growth, each group having an optimum temperature and a temperature range of growth [2]: (a) thermophiles (grow at relatively high temperature), with optimum at 55 °C and range 45–70 °C; (b) mesophiles (grow at ambient temperature), with optimum at 35 °C and range 10–45 °C; and (c) psychrophiles (grow at cold temperature), with optimum at 15 °C and range −5 to 20 °C. The correct use of temperatures during the maintenance of food and food products is fundamental for its preservation and longer shelf-life.

Gaseous Atmosphere: The presence and influence of oxygen on redox potential are important determinants of the microbial associations that develop and their rate of growth. Other than oxygen, there are other gases commonly encountered in food processing that also have microbiological effects. The inhibitory effect of carbon dioxide (CO_2) on microbial growth is applied in modified atmosphere packing of food and is an advantageous consequence of its use at elevated pressures (hyperbaric) in carbonated mineral waters and soft drinks [31]. Growth inhibition is usually greater under aerobic conditions than anaerobic and the inhibitory effect increases with decrease temperature, presumably due to the increased solubility of CO_2 at lower temperatures [31].

5 Health Impacts of Microbial Contamination of Food

The modality of foodborne microbial infection and toxication can be broadly classified into intoxication or poisoning, infection, and toxicoinfection.

Intoxication: Foodborne intoxication is caused by ingesting food containing bacterial toxins resulting from the bacterial growth in the food item [36]. For intoxication, a toxin has to be present in the contaminated food. Once the microorganisms have grown and produced toxin in food, consumption of viable cells cannot be required for illness to occur [2]. The foodborne bacteria that cause intoxication are *Clostridium botulinum, Staphylococcus aureus, Clostridium perfringens*, and *Bacillus cereus* [37].

Infection: Foodborne infection is caused by the ingestion of food and water contaminated with live enteropathogenic bacteria or viruses, which grow and establish themselves in the human intestinal tract [2, 36]. Viable cells, even if present in small numbers, have the potential to establish and multiply in the digestive tract to cause the illness. The foodborne bacteria that cause infection are *Salmonella* spp., *Listeria monocytogenes, Campylobacter jejuni, Vibrio parahaemolyticus, Vibrio vulnificus*, and *Yersinia enterocolitica*. The most common viral agents that cause foodborne disease are Hepatitis A, norovirus, and rotavirus [37].

Toxicoinfection: Illness occurs from ingesting a large number of viable cells of some pathogenic bacteria through contaminated food and water. Generally, the bacterial cells either sporulate or die and release toxins to produce the symptoms [2]. Some bacteria cause toxin-mediated infections while viruses and parasites do not. The foodborne bacteria that cause toxin-mediated infection are *Shigella* spp. and Shiga toxin-producing *Escherichia coli* [37].

The severity of the foodborne illness depends on the pathogenic microorganism or toxin ingested, the amount of food consumed (dose), and the health status of the individual. For individuals who have immunocompromised health conditions, or for the elderly population, children, or pregnant women, any foodborne illness may be life threatening [36]. The most common symptom associated with foodborne illnesses is diarrhea. Some other diseases caused by microbial contaminants are cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellosis, shigellosis, typhoid and paratyphoid fever, amoebiasis, and poliomyelitis [7]. Most foodborne illnesses are limited to brief episodes of diarrhea, nausea, or other acute gastrointestinal systems. Several other illnesses can result from the consumption of foods contaminated by microbial pathogens, which include fever, vomiting, weakness, chills and aches, headaches, abdominal pain, constipation, sore mouth, blurred vision, and muscle paralysis. In addition, septicemia, localized infection of other organs, and spontaneous abortion in pregnant women are the most severe acute illnesses associated with foodborne pathogen-contaminated food. Other complications associated with foodborne pathogens include reactive arthritis, hemolytic uremic syndrome (characterized by kidney failure), and Guillain-barre syndrome (characterized by neuromuscular paralysis) [7]. Table 2.5 shows some selected microbial contaminants, their commonly associated foods, and symptoms of illness.

Research showed that contaminated foods are alarmingly responsible for many more accidental fatalities than some products commonly perceived dangerous, including firearms, industrial machinery, and explosives [38]. Unsafe food is linked to the death of an estimated 2 million people annually including children [6]. Bacteria, parasites, and viruses, respectively, account for 72%, 21%, and 7% of

Table 2.5 Selected microbial contaminants, their commonly associated foods, and health impacts (symptoms) [7]

Microbial contaminant	Commonly associated foods	Health impacts (symptoms)
Bacillus cereus	Meat and vegetables dishes, cereals, spices, custards, puddings, and heat-treated desserts	Diarrhea and abdominal cramps; nausea and vomiting
Campylobacter species	Raw meats (beef and pork), water, unpasteurized milk, eggs, chicken, shellfish, and mushroom	Diarrhea (can be bloody), cramps, fever, and vomiting
Clostridium botulinum	Vegetables; improperly or home-canned or bottled foods, including canned meats, corn beef, canned fish, smoked fish and vegetables, honey, mushroom; improperly processed peppers, asparagus, soup, spinach	Vomiting, diarrhea, blurred vision, double vision, difficulty in swallowing, muscle weakness. Can result in respiratory failure and death
Clostridium perfringens	Raw meats, poultry, fish, stews, cooked turkey and beef, casseroles, gravy dressings, food that sits for extended periods, and dried foods such as spices and vegetables	Intense abdominal cramps and watery diarrhea
Cryptosporidium	Uncooked food or food contaminated by an ill food handler after cooking, contaminated drinking water	Diarrhea, stomach cramps, upset stomach, and slight fever
Escherichia coli OI57:H7	Ground beef, raw milk, chicken, vegetables and fruits, and any food exposed to raw fecal matter are at risk of being contaminated	Hemorrhagic colitis, severe (often bloody) diarrhea, abdominal pain and vomiting, little or no fever. Can lead to kidney failure.
Entamoeba hystolitica	Tap water, ice cream, ice cubes, shellfish, eggs, salads, raw or undercooked meat, peeled fruits, sauces	Amoebiasis; loose stool that may be bloody sometimes, stomach pain, fatigue, excessive gas, rectal pain, amoebic dysentery including high fever, severe abdominal pain
Hepatitis A	Raw produce, contaminated drinking water, uncooked foods, and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters	Hepatitis (diarrhea, dark urine, jaundice, and flu-like symptoms, i.e., fever, headache, nausea, and abdominal pain)
Listeria monocytogenes	Dairy (soft cheeses and coleslaw), meat products(pate, sausages, and gas-packed delicatessen goods), cold-smoked and gravid rainbow trout products, sliced cold cuts, soft cheese, butter, ice-cream, coleslaw, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish	Fever, muscle aches, and nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to premature delivery or stillbirth

(continued)

Table 2.5 (continued)

Microbial contaminant	Commonly associated foods	Health impacts (symptoms)
Noroviruses	Raw produce, contaminated drinking water, uncooked foods, and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters	Viral gastroenteritis, acute nonbacterial gastroenteritis, food poisoning, or food infection (nausea, vomiting, abdominal cramping, diarrhea, fever, headache).
Salmonella	Raw meats, eggs, fish shellfish, poultry, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces, salad dressing, cake mixes, cream-filled desserts and toppings, dried gelatine, peanut butter, cocoa, chocolate, pork. In general, beef is less often contaminated with salmonella than poultry and pork	Acute gastroenteritis, painful abdominal cramps, diarrhea that may be sometimes bloody, fever (100 °F to 102 °F), vomiting, headache, and body aches
Shigella	Salads of potato, chicken, seafood and vegetables, milk and other dairy products, and meat products, especially poultry	Abdominal cramps, fever, and diarrhea. Stools may contain blood and mucus
Staphylococcus aureus	The red meats, especially ham, poultry, potato, macaroni and tuna salads, custard and cream-filled bakery product, the sandwich sauces	Sudden onset of severe nausea and vomiting. Abdominal cramps. Diarrhea and fever may be present
Toxoplasma gondii	Raw or undercooked meat, especially pork, or wild game and water	Fever, swollen lymph nodes, especially in the neck, headache, muscle aches and pains, sore throat: People in high group may develop brain inflammations, seizures, mental issues such as confusion and psychosis
Vibrio vulnificus	Undercooked or raw seafood, such as shellfish (especially oysters)	Acute gastroenteritis; vomiting, diarrhea, abdominal pain, blood-borne infection. Fever, bleeding within the skin, ulcers requiring surgical removal
Yersinia enterocolitis	Raw vegetables, milk produces, tofu, minced meat, raw pork from where other foods may be cross-contaminated	Lymph node inflammation, appendicitis-like symptom

deaths associated with foodborne transmission. Five pathogens account for over 90% of estimated food-related deaths are Salmonella (31%), Listeria (28%), Toxoplasma (21%), Norwalk-like viruses (7%), Campylobacter (5%), and *E. coli* O157:H7 (3%) [7]. Some food-related illnesses and deaths also result from unknown pathogens. Infants, young children, pregnant women, the elderly, and those with an underlying illness are particularly vulnerable. Worldwide, diarrheal diseases are second to respiratory diseases as a cause of adult death and are the leading cause of childhood death. In some parts of the world, they are responsible for more years of

potential life lost than all other causes combined [5]. Each year, around 5 million children (more than 13,600 a day) die from diarrheal diseases in Asia, Africa, and South America. In the United States, estimates exceed 10,000 deaths per year from diarrhea and an average of 500 childhood deaths are reported [7]. A particular study showed that, in Nigeria, more than 200,000 people die annually from food poisoning caused by contaminated foods through improper processing, preservation, and service [7, 39].

6 Conclusion

Microbial contamination of food is a serious public health concern worldwide. To ensure safe and reliable supply of food, mitigation of microbial contamination and the consequential impacts is of paramount importance. Control of food microbial contamination and the consequent impacts must take place from the primary production to the dining table. 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, 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 [41]. Most importantly, food safety education for consumers and staff in the food industry is integral for the prevention of food contamination and its consequences and should be promoted widely. Proper measurement methods to measure microbial and chemical contaminants are required to produce safe and healthy foods.

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Chapter 3
Sources and Health Impacts of Chemical Contaminants in Foods

Shariful Kibria Nabil, Nadira Mustari, and Mohidus Samad Khan

Abstract Chemical contaminants are primarily responsible for major outbreaks of foodborne diseases. A wide range of organic and inorganic chemical contaminants can be naturally found in the environment or added in adulterated foods during their processing, packaging, and storage. 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. Chemical contamination can be food specific and can occur at any stage of food processing. Risk assessment and risk management need 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. This chapter presents different types and sources of chemical contaminants and their associated health risks. Furthermore, alternate options to minimize chemical contaminants in foods are addressed in this chapter.

Keywords Chemical contaminants · Origin and sources · Toxicity · Carcinogenicity · Pesticides · Growth regulators · Trace chemicals · Additives · Drug residues · Food processing · Health implication · Food safety · Alternative research · Integrated practices

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1 Introduction

Food contamination is a potential threat due to its adverse effect on the community and public health. According to World Health Organization (WHO), one in ten people fall ill due to eating contaminated food, and four hundred twenty thousand die every year resulting in the loss of 33 million (WHO, Food Safety, April 2020). The US Centre for Disease Control and Prevention confirmed more than 11,000 foodborne infections in 2013 [1], with several agents like viruses, bacteria, toxins, parasites, metals, and other chemicals [2].

Contamination of food can be originated due to chemical, microbiological, or physical causes. The chemical hazards are considered to be one of the main causes of food contamination that are associated with foodborne disease outbreaks [3]. Chemical contamination indicates the presence of undesired and harmful chemical substances in the food chain. These substances are termed as "contaminants" and can be naturally occurring or artificially introduced by humans. There is a wide range of organic and inorganic chemicals that can contaminate foods. Besides, implications at multiple phases of food cultivation, harvesting, processing, packaging, transport, and storage are also significant contributors to such contamination [4]. There are different analytical techniques to assess chemical contaminants in food which include mass spectrometry (MS), gas chromatography (GC), liquid chromatography (LC), GC-MS, LC-MS, high-performance liquid chromatography, polymerase chain reaction (PCR), ultraviolet–visible spectroscopy (UV–Vis), and electronic tongue.

Chemical contaminants of food are directly associated with health issues. The health risks of chemical contaminants are ranging from acute to chronic depending on the degree of exposure [5]. This chapter includes the classification of chemical contaminants and their sources including possible health impacts associated with specific contaminants of food. This book chapter will be useful for the professionals, policy makers, and researchers working on food processing and food safety to develop effective and integrated methods to reduce chemical contaminants in foods.

2 History and Background

Transition from the Paleolithic to the Neolithic age (hunting-gathering period to food-producing period) is believed to be the early inscription of food poisoning and contamination. [6]. The major incidents of food contamination have been occurring around the globe for centuries resulting from physical, chemical, biological, and environmental causes [7].

Such known history of mass contamination of food can be traced back to as early as the Roman Empire, where people suffered from lead poisoning due to the boiling of foodstuffs in lead-lined pots [8]. In the recent era, the "*Swill Milk Scandal*" of New York in 1850 is considered to be one of the earliest of adulterated food

contamination histories [9] along with "*1858 Bradford Sweets Poisoning*" and "*1900 English Beer Poisoning*" [10, 11]. The "*Minamata Disease*" in Japan caused by methylmercury poisoning in 1956 and 1965 is one of the deadliest incidents [12, 13]. In 1998, meat and milk were contaminated with dioxin in Germany and the Netherlands originating from the citrus pulp in Brazil [14]. In Belgium, during 1999, animal feed contamination by dioxin and PCB occurred at poultry and pig farms, and the severity led to the formation of the Belgium Federal Food Safety Agency [15]. In 2005, Worcester Sauce, United Kingdom, was found to contain banned Sudan I dye resulting in the recall of 576 food products [16]. Another infamous case is the contamination of infant formula with melamine in 2008 (affecting 300,000 infants and young children, 6 of whom died, in China alone) as reported by World Health Organization. In recent years, the use of artificial fruit-ripening agents, such as calcium carbide, ethephon, and ethylene, is becoming much prevalent; recent reports on fruit-ripening agents highlight health issues due to the use of artificial fruit-ripening agents.

Aside from these events, numerous other examples of chemical contaminations in food are present around the globe. All these events led to the importance of having regulatory bodies to develop specific regulations and monitor food contaminants to ensure health and safety. Most notables are the formation of the Food and Drug Administration (FDA) in 1930 in the United States, and the Food and Agricultural Organization (FAO) in 1945 by the United Nations [17]. There are other national and regional authorities in different parts of the world to formulate relevant laws to ensure safe food and safe health for the public [18].

3 Types of Chemical Contaminants

3.1 Pesticide Residues

Pesticides are the substances or mixture of substances used to prevent, destroy, or control pests that may cause harm during the production, processing, storage, transport, or marketing of foods and other agricultural commodities [19]. Globally, there is a growing concern about the improper use of pesticides in the agricultural sector. There have been frequent reports of occupational illnesses and injuries resulting from pesticide use, while some epidemiological studies have provided associations between occupational exposure to specific pesticides and the development of specific types of cancers [20]. The health effects of pesticides vary according to the exposure time, individual health condition, and the chemical toxicity of pesticides [21]. Pesticides can be classified according to their chemical structures, working principles, target molecules, and possible health effects. Considering the abovementioned factors, pesticides can be broadly classified as organochlorine pesticides, organophosphorus pesticides, carbamates, pyrethroids pesticides, biorational pesticides, and microbial pesticides [22]. These chemical substances are often persistent.

Because of the irrational use of pesticides during cultivation, pesticide residues can be found in crops, soils, and waterways [23]. Different analytical methods, such as various extraction, chromatographic, and spectrophotometric techniques, play an important role in the detection of pesticide residues in foods.

The most commonly identified pesticides include DDT, dieldrin, hexachlorobenzene, and toxaphene that are no longer allowed for use in different parts of the world but likely resulted in residues due to their significant environmental persistence, which makes them candidates to be taken up from the soil by food crops [24].

3.2 Plant-Derived Contaminants

Plant-derived contaminants are substances that are neither present naturally in the raw materials used for food production nor are added during the regular production processes [25]. Classes of plant-derived contaminants are of concern in the modern food chain because of their genotoxic carcinogenicity including pyrrolizidine alkaloids and ethyl carbamate. These compounds require bioactivation to a reactive proximate carcinogenic metabolite that forms DNA adducts and may result in tumor formation. Examples of foodborne phytotoxins include tropane alkaloids, opium alkaloids, grayanotoxin I, and delta-9-tetrahydrocannabinol.

3.3 Growth Hormones and Promoters

Growth promoters including hormonal substances and antibiotics are used both legally and illegally for the growth promotion of fruits, vegetables, and livestock animals. Hormonal substances are still under debate in terms of their human health impacts, and these are estradiol-17β, progesterone, testosterone, zeranol, trenbolone, and melengestrol acetate (MGA) [26]. Many of the risk assessments showed that natural steroid hormones caused negligible impacts when they are used under good veterinary practices. For synthetic hormone-like substances, acceptable daily intakes (ADIs) and maximum residue limits (MRLs) have been established for food safety along with the approval of animal treatment [27, 28]. Small amounts of antibiotics added to feedstuff present growth promotion effects via the prevention of infectious diseases, and these need to be lower than the therapeutic dose. The induction of antimicrobial-resistant bacteria and the disruption of normal human intestinal flora are major concerns in terms of human health impact. Regulatory guidance such as ADIs and MRLs fully reflect the impact on human gastrointestinal microflora [26, 29].

For the growth and development of plants, hormones specifically termed "phytohormones" are required which are organic substances naturally produced in minute concentration [30]. Aside from these, there are other natural or synthetic short-lived substances called plant growth regulators stimulating overall growth and

development (flowering time, differentiation, immune response, etc.) [31]. Major classes include auxins (i.e., IAA (indole-3-acetic acid)), gibberellins (i.e., terpenoids as a precursor), and cytokinins (i.e., adenosine-monophosphate as a precursor) as growth promoters and ethylene (i.e., methionine as a precursor) and abscisic acid (i.e., mevalonic acid as a precursor) as growth inhibitors [32]. The single purpose of these hormones and regulators is sometimes labeled as chemical transporter or messenger from the site of production/application to the site of action. Aside from growth, they play a predominant role in terms of signal propagation for interplant communication, resistance/tolerance level of plants, and responsive behavior/interaction against biotic (i.e. pathogens, pests) and abiotic (i.e. drought, low temperature) stresses [30, 33, 34].

3.4 Veterinary Drug Residues

Veterinary drug residues, as defined by the European Union (EU) and the Center for Veterinary Medicine, an agency under the Food and Drug Administration (FDA/CVM) in the United States, are pharmacologically active substances (whether active principles, recipients, or degradation products) and their metabolites remain in foodstuffs from animals. Under normal physiological conditions, following administration of a drug to an animal, most drugs are metabolized in order to facilitate elimination and to a large extent detoxification. In general, most of the parent product and its metabolites are excreted in urine and lesser extent via feces [35]. However, these substances may also be found in milk and eggs and in meat [36].

3.5 Additives and Color

Historical records show that injuries, even deaths, resulted from tainted colorants. In 1851, about 200 people were poisoned in England as a result of eating adulterated lozenges [37]. Since the nineteenth century, color additives have been widely used in all sorts of popular foods, including ketchup, mustard, jellies, and wine [38, 39]. Many synthesized dyes were easier and less costly to produce and were superior in coloring properties when compared to naturally derived alternatives [40]. Some synthetic food colorants are diazo dyes. Diazo dyes are prepared by coupling of diazonium compound with second aromatic hydrocarbons [41]. The attractiveness of the synthetic dyes is that their color, lipophilicity, and other attributes can be engineered and commercially produced in a large scale. The color of the dyes can be controlled by selecting the number of azo-groups and various substituents [42]. Synthetic food dyes of different types have long been controversial because of safety concerns, and many dyes have been banned because of their adverse effects on laboratory animals or inadequate testing [43].

3.6 Migrants from Processing and Packaging

Food packaging has been the subject of many debates concerning environmental and health issues. Due to the increasing awareness of consumers in terms of health matters, the importance of the migration of substances from food packaging materials to foods attracted the interest of the scientific and legislative communities [44–47]. In this case, the term "migration" describes a diffusion process that may be strongly influenced by an interaction of components of the food with the packaging material. This interaction may substantially affect the properties of the packaging material as well as food properties [48].

3.7 Trace Chemicals and Allergens

In the United States, food allergies in children under 18 years old increased by 18% between 1997 and 2008 [49]. There are eight common sources of food allergens popularly referred to as the "the big 8," which include cow's milk, hen's eggs, peanuts, tree nuts, soy, wheat, shellfish, and fish. In Europe, this list has been expanded to 14, with the inclusion of cereals containing gluten, celery, mustard, sesame, lupin, and sulfur oxide [50]. Allergenic foods contain proteins with special recognition sites known as epitopes, which can bind to immunoglobulin E (IgE), an antibody produced during the sensitization phase of allergic reactions [51]. These epitopes are referred to as IgE-binding epitopes. Food allergens present both mild and severe symptoms, including diarrhea, flushing, nausea/vomiting, urticaria, angioedema, laryngoedema, bronchospasm, and hypotension, dyspnea, abdominal pain, and anaphylaxis [52]. Various recommendations have been made by regulatory agencies for the management of food-induced allergic reactions which include identification of allergens, prevention of cross-contamination during freight, storage of raw materials and food additives during and after production, increase public awareness about food allergens, labeling of food allergens in food packages by manufacturers, and food processing [52–54].

3.8 Dioxins

Dioxins are lipophilic compounds that accumulate in the fat of animals. The types of foods that tend to have the highest dioxin concentrations are dairy products, meat and poultry, eggs, fish, and animal fats [55]. Green vegetables, fruits, and grains are the types of foods with the lowest dioxin concentrations. It was found that freshwater fish had the highest level of dioxins (1.43 Toxicity Equivalent Quantity), followed by butter (1.07 TEQ), hotdog/bologna (0.54 TEQ), ocean fish (0.47 TEQ), cheese (0.40 TEQ), beef (0.38 TEQ), eggs (0.34 TEQ), ice cream (0.33 TEQ),

chicken (0.32 TEQ), pork (0.32 TEQ), milk (0.12 TEQ), vegetables, fruits, grains, and legumes (0.07 TEQ) [56]. A person's intake of dioxins through the diet, therefore, depends on the relative intake of foods with high or low levels of contamination and the consumed quantity [57].

3.9 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are complex mixtures of chlorinated aromatic hydrocarbons that have relatively low water solubility levels and prolonged environmental and biological half-lives. PCBs comprise a family of 209 possible congeners ranging from three monochlorinated isomers to the fully chlorinated decachlorobiphenyl isomer. These organic chemicals are ubiquitous in the environment. They can be found in the adipose tissue, blood, and milk of the general population [58]. PCBs have entered, and continue to enter, the food chain through various means [59]. The single most important source is fish from contaminated aquatic environments, where PCBs have accumulated in the sediments [60–62] from nearby users of PCBs. In a recent study [63], residue profiles of a composite fish sample and a sediment sample from the Housatonic River in Massachusetts showed almost identical PCB patterns. Accidental spills and leakage from electrical transformers and capacitors in food and feed industries [64] and disposal of contaminated sewage sludge in estuaries or land [65] include some of the common contamination sources. Other sources of PCB contamination of food involve wrapping and packaging materials manufactured from recycled paper containing PCBs [66].

3.10 Emerging Environmental Organics

Industrial origin contaminants, such as perfluorinated compounds (PFCs), polybrominated biphenyls (PBBs), the new generation of pesticides, nanomaterials, and emerging groups of marine biotoxins (such as palytoxins and spirolides), are considered among the most prominent groups of emerging food contaminants. Many of them are of particular concern since they can be carcinogenic, may have endocrine disruptor effects, may cause severe damages to human health, and can be accumulated and biomagnified through the food chain [67].

3.11 Toxic Metals and Metalloids

Different toxic metals and metalloids can be transferred from the soil to the aboveground tissues of crops. Consumption of crops containing trace amounts of such toxic elements can be harmful to human health [68]. The residue of toxic metals and

metalloids in cereals, vegetables, and other plant-derived food is a good source of non-occupational cadmium exposure of the (nonsmoking) general population [69, 70]. Poisoning with arsenic residue is widespread in many countries, especially in South and Southeast Asia. In those countries, arsenic residue in drinking water and crops is a major source of arsenic exposure [71–73].

3.12 Heat-Generated Toxicants

Over the past decades, several foodborne toxicants, such as the heterocyclic aromatic amines (e.g., 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine, PhIP), polyaromatic hydrocarbons (e.g., benzo[*a*]pyrene), and chloropropanols (e.g., 3-monochloropropane-1-diol, 3-MCPD), have received attention as potential risk factors for human cancers [74]. Recently, a number of additional compounds have been added to the existing list of foodborne carcinogens. In April 2002, acrylamide was detected in cooked foods such as French fries, snacks, cereals and cereal-based products, biscuits, and coffee [75], and its formation was linked to the Maillard reaction [76, 77] In 2004, the US Food and Drug Administration (FDA) reported dietary exposure estimates based on the occurrence of furan in different foods, such as coffee, jarred, and canned foods [78, 79]. Recent attention has also been given to 3-MCPD based on the discovery that refined vegetable oils may harbor significant amounts of chloroesters (3-MCPD esters and 2-MCPD esters). Due to the widespread use of processed vegetable oils in many different foods, exposure to MCPD may be higher than previously assessed [80].

3.13 Nonthermally Generated Toxicants

A number of chemicals form during food processing, which include ethyl carbamate, 3-monochloropropane-1,2-diol, glycidol, biogenic amines, trans fatty acids, and fatty acid peroxides. These chemicals have been in the focus of safety concerns because of their possible adverse health effects. These chemicals can be formed during fermentation, acid hydrolysis, oil refining, hydrogenation, chlorination, and oxidation processes. These processes reflect the broad spectrum of pathways that may eventually lead to process-generated contaminants [81].

3.14 D-Amino Acids

Foods contain large quantities of non-natural substances of external origin, such as D-stereoisomer amino acids, which influence their digestibility to a considerable degree [82]. D-Stereoisomer amino acids are formed from common L-stereoisomer

amino acids either in the course of the production process or as a consequence of changes in the microbiological quality of the foodstuff. The presence of these D-stereoisomer amino acids results in a substantial reduction in the digestibility of dietary protein and the availability of the transformed amino acid [83].

3.15 Phycotoxins and Mycotoxins

Phycotoxins, more properly called "marine and freshwater toxins," belong to many different groups of small- or medium-sized compounds. Each group includes several compounds characterized by similar structures, which are either produced by algae or are secondary products of the primary algal toxin produced during metabolism by fish and shellfish [84].

On the other hand, a mycotoxin is a toxic secondary metabolite produced by organisms of the fungus kingdom and is capable of causing disease and death in both humans and other animals. Mycotoxins can contaminate human foods and animal feeds through the growth of molds before and during harvesting or because of improper storage after harvest. Postharvest contamination can be minimized when proper storage conditions are maintained. Preharvest contamination most often occurs when environmental conditions consistent with mold growth and mycotoxin formation occur [85].

4 Health Impacts

From the production process to commercial packaging, each and every food item is prone to chemical contamination from multiple sources. These contaminants can enter the human body through various possible routes. Figure 3.1 shows a simplified scheme of the routes by which chemicals in food may enter and have adverse effects on mammals (i.e., human). Most chemical contaminants proceed through a number of these routes, with the potential formation of many different metabolites and interaction with different tissues. As the exposure to a particular chemical increases, it becomes more likely that the detoxification and repair mechanisms become overwhelmed and the pathways leading to toxicity may be predominated [86].

Major chemical contaminants and potential sources in different food groups or items are presented in Tables 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, and 3.7 along with possible health impacts.

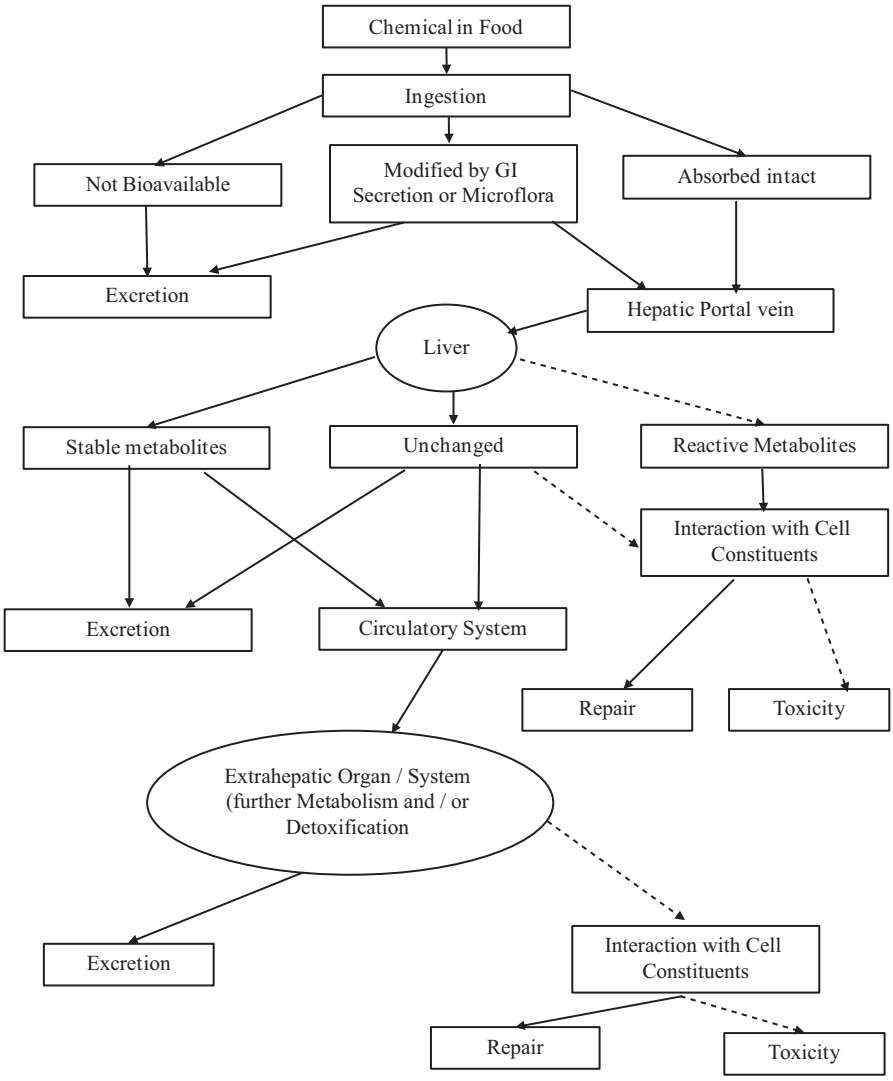

Fig. 3.1 Possible routes of chemicals to enter human body (David R. Tennant, Food Chemical Risk Analysis. 1997)

5 Minimizing Chemical Contamination: Recommendations and Alternatives

The level of chemical contaminants in food and feed can be minimized by implementing good agricultural practices (GAP), good manufacturing practices (GMP), and Hazard Analysis and Critical Control Point (HACCP) following an appropriate risk assessment. The following actions can be useful to reduce contamination of

Table 3.1 Sources and health impacts of chemical contamination in water and beverages

Items	Contaminants	Sources	Health impacts	Ref.
Water	Heavy metals • Arsenic • Aluminum • Lead • Nickel • Chromium • Mercury	• Industrial and municipal discharges • Natural geological formation	• Skin, bladder, and prostate cancers • Reproductive, cardiovascular, immunological, and neurological diseases	[4, 96–102]
	Disinfection by-products • Trihalomethanes • Dichloroacetic acid	• Water treatment process	• Leukemia, reproductive disease • Bladder and colon cancers	
	Pesticide residues • 2,4-D • Malathion • Diazinon • Fenpropimorph	• Use of pesticides • Urban and rural runoffs	• Leukemia, reproductive, immunological, and neurological cancers	
	Other inorganics • Flouride • Nitrate • Radon • Sulfate (gypsum, anhydrite, barite, celestine)	• Environmental pollution • Contamination of water distribution materials	• Diarrhea, laxative effect • Lung cancer • Osteosarcoma, skeletal fluorosis • Rectum, pancreas, ovarian, and kidney cancers • Adverse pregnancy outcomes, diabetes, and thyroid disorders	

(continued)

Table 3.1 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Beverages				
(a) Soft drinks	• Sodium benzoate	• Used as preservatives	• Forms benzene, a known carcinogen • Increases hyperactivity in children	[103–105]
	• High fructose corn syrup	• Used for sweetening process	• Obesity	
	Heavy metals • Cadmium • Lead • Mercury • Arsenic • Lead	• Contamination of water • Processing and packaging residues	• Cancer • Lead poisonings (central neurotoxicity anemia, peripheral motor neuropathy) • Interference of mercury with cellular processes	
	• Caffeine	• Added as a flavor additive	• Excessive consumption may lead to insomnia, nervousness, restlessness, irritability, muscle tremors, and upset stomach	
	Copper/zinc/ indigo-based dyes • Red#40 • Yellow#5 • Blue#1	• Used as artificial colorings	• Toxic effect and possibility of cancer • Hyperactivity of children • Double-blind challenges	
	• Bisophenol A (BPA)	• Component of polycarbonate beverage bottles	• Promote breast and prostate cancer • Obesity, diabetes, reproductive, and behavioral problems	
(b) Sport drinks	Artificial colorings • Dye yellow#5 & #6 • Blue#1 & #2 • Green#3 • Red#40	• Used as artificial colors	• Slower development of children • Adverse behavioral reactions • Thyroid, adrenal bladder, kidney, and brain cancer • Possibility of causing asthma	[106, 107]
(c) Energy drinks	Artificial colorings • Yellow#5 • Blue#1 • Red#40	• Used as additives	• Hyperactivity disorders • Behavioral problems of children • Double-blind challenges • Thyroid, adrenal bladder, kidney, and brain cancer • Possibility of causing asthma	[107]

(continued)

Table 3.1 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
(d) Flavored soda	• 4-Methylimidazole (MEI)	• Used as coloring agent	• Can have a carcinogenic impact	[108, 109]
	Preservatives • BHA, BHT • Benzoate • Calcium sorbate • Sodium erythorbate	• Used as antioxidants to reduce spoilage	• Dental decay and damage to the enamel • Mouth irritation • Digestive disorder • Allergic reaction, hypersensitivity • Childhood hyperactivity • Can provoke asthma attack	
	Sweeteners • High fructose corn syrup • Sorbitol • Saccharin • Mannitol	• Used to add sweetness without extra calories		
(e) Alcoholic drinks	Processing and storage components • Methanol • Pyridine • Copper sulfate • Acetaldehyde	• Used to denature alcohol (to reduce the effect of recreational use) • Can come from storing in glass/plastic bottles	• Blurred vision, blindness • Damage of immune system and internal organs (liver, kidney, etc.) • Carcinogenic components	[104, 110]

(continued)

Table 3.1 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
(f) Tea	Mycotoxins • Ochratoxin A • Aflatoxin • Fumonisin B1, B2	• Microbial contamination of leaves	• Can lead to immunosuppression, carcinogenesis, as well as genotoxic, hepatotoxic, and nephrotoxic effects • Esophageal cancer	[111, 112]
	Heavy metals • Cadmium • Lead • Arsenic • Mercury • Zinc • Chromium • Iron fillings	• Contamination of soil and water • Use of fertilizers • Irrigation and waste disposal	• Damage of digestive and central nervous system • Irritation of skin, damage of liver and kidney	
	Environmental contaminants • Polycyclic aromatic hydrocarbons (PAH) • Polychlorinated dibenzo-p-dioxins (PCDD) • Polychlorinated dibenzo furans (PCDF)	• Environmental pollution and contamination • Atmospheric deposition • Roasting and drying stages of tea	• Carcinogenesis and mutagenesis effect • Promote hepatoxicity and hormonal imbalance	
	Pesticides • Dicofol • Organophosphates • Organochlorines • Pyrethroids • Benzimidazoles • Nicotine	• Use of pesticides to reduce pest, insect infestation	• Can act as nerve poison • Cause allergic reaction • Damage of immune system	

(continued)

Table 3.1 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
(g) Coffee	Heavy metals • Cobalt • Tin • Nickel • Zinc • Aluminum	• Water contamination • Residues from packaging and storage	• Gastrointestinal problem and urine infection • Variable blood pressure • Long-term exposure can cause skin and lung cancer	[113]
	Mycotoxins • Ochratoxin A • Zearalenone	• Microbial (mold) contamination	• Hepatic, gastrointestinal, and carcinogenic diseases	
	Pesticide residues • Carbamates • Organophosphates • Organochlorine	• Use of pesticides at various stages—Primary production, primary processing, secondary processing and storage, transportation	• Affect nervous system • Irritation of eyes, skins • Adverse effects—Lung disorder, asthma, cancer, infertility	
	Organic compounds • Acrylamide • Bisphenol A • Furan • Phthalates	• Roasting and other processing stages of beans • Packaging and storage of products	• Can cause potential neurological damage • Muscle weakness, impaired muscle coordination • Carcinogenesis and mutagenesis • Can cause endocrine disruption, infertility, and precocious puberty	
	• Caffeine	• Added as additive	• Excessive consumption leads to insomnia, restlessness, and upset stomach	

(continued)

Table 3.2 Sources and health impacts of chemical contamination in fish and seafood

Items	Contaminants	Sources	Health impacts	Ref.
Fish	Preservatives • Formalin • Sodium nitrite (E250) • Potassium nitrite (E249)	• Used as preservatives	• Nitrites form carcinogenic nitrosamines during cooking • Can develop respiratory, digestive, cardiac, nephrological, and neurological problems along with cancer	[117–120]
	Organic contaminants • Tetrachlorodibenzo-p-dioxins • Polybrominated diphenyl ethers • Polychlorinated biphenyls • Polychlorinated diphenyl ethers • Organochlorine residues	• Water pollution • Environmental contaminants • Growth hormones	• Endocrine-disrupting chemicals • Development of tumors • Loss of fertility, estrogenic, antiandrogenic effects	
	Toxic metals • Arsenic • Mercury • Nickel • Copper • Zinc	• Water and environmental contamination • Processing and storage	• Carcinogenic at high consumption • Can lead to retarded growth and decreased fertility • Hamper brain development and cause neurological changes	
	• Gel	• Injected to increase weight during processing	• Highly carcinogenic substance	

(continued)

Table 3.2 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Seafood	Anthropogenic • Poly aromatic hydrocarbon (PAH) • Perflurooctane sulfonate (PFOS) • Perfluorooctanoic acid (PFOA) • Polychlorinated biphenyls • Dibenzofurans • Polybrominated diphenyl ethers	• Contamination during processing • Industrial pollutants and contaminants	• Hepatotoxicity, developmental toxicity, immunotoxicity, hormonal and carcinogenic effects • Cardiovascular diseases, neural damage, and reduced fertility	[121–123]
	Heavy metals • Lead • Cadmium • Mercury • Arsenic	• Water contamination • Environmental pollution	• Can lead to retarded growth and decreased fertility • Hamper brain development, cause neurological changes • Carcinogenic effect at long-term exposure	
	Additives • Benzoates • Phosphates • Ascorbates	• To preserve and prevent discoloration	• Can lead to digestive dysfunction, renal failure, and even cancer • Promote allergic reactions to specific people	
Processed fish	• Inorganic arsenic	• Environmental contamination	• Can cause skin, bladder, and lung cancer	[120, 124, 125]
	• Nitrite salts	• Used as antimicrobial agent against toxin-producing *Clostridium botulinum*	• Formation of carcinogenic nitrosamines with prolonged exposure	
	Dyes • Malachit green (MG) • Crystal violet (CV)	• Comes from veterinary drugs	• Highly carcinogenic in nature	

Table 3.3 Sources and health impacts of chemical contamination in poultry, red meat, and egg

Items	Contaminants	Sources	Health impacts	Ref.
Poultry meat and egg	Mycotoxins • Aflatoxins • Fumonisins • Zearalenone	• Contamination of crops and feedstuffs	• Induce genotoxic, carcinogenic, and immunotoxigenic problems	[126–129]
	Heavy metals • Cadmium • Lead • Mercury • Thallium • Arsenic	• Agricultural and industrial contamination of animal foods • Feed additives	• Disorders in the gastrointestinal, urinary, and nervous system • Damage of skin, muscle, liver, and kidney	
	Dioxins • Polychlorinated dibenzo-p-dioxins (PCDD) • Polychlorinated dibenzo furans (PCDF) • Polybrominated diphenyl ethers	• Contamination of food chain	• Health problems as carcinogenesis, teratogenesis, mutagenesis, immune deficiencies, and hormonal imbalances • Hormone, skin, immune, and neurological disruptors	
	Pesticides • DDT • Bipyridyliums • DDE	• On-farm use • Postharvest use • Use of pesticide at storage • Environmental metabolites of pesticides	• Allergic and toxic reactions • Chronic conditions such as mutagenesis or carcinogenesis	
	Veterinary medicines • Permethrins • Ionophoric compound (monensin, lasalocid, narasin) • Antibiotic residues (sulfonamides, β-lactams, cephalosporins	• Use of synthetic drugs and artificial hormones • Use of antiparasitics and anticoccidials • Use of unregistered antibiotics	• Mild neurotoxic effect • Responsible for acne, baldness, and precocious puberty • Can cause allergic reaction and gastrointestinal disorder	

(continued)

Table 3.3 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Red meat	Dioxins • Polychlorinated dibenzo-p-dioxins (PCDD) • Polychlorinated dibenzo furans (PCDF)	• Present as trace impurities • Forms during thermal processing • Air pollution • Contaminated livestock feeds	• Reproductive problem • Heart disease, diabetes • Increased risk of cancer	[130–133]
	Polychlorinated biphenyls (PCB) • Chlorinated naphthalenes • Chlorinated dibenzofurans	• Present as environmental contaminants • Contaminated livestock feeds	• Carcinogenic effect, skin disorder • Neurochemical behavioral change, immune dysfunction	
	• Poly brominated diphenyl ethers	• Environmental contamination	• Affect thyroid hormones and cause neurodevelopmental toxicity • Can act as endocrine disruptors	
	Pesticides • Organochlorine • Organophosphorus	• Use of illegal pesticides • Contaminated feedstuffs	• Reproductive problem, cancer, neurodevelopmental disorder • Abdominal pain, nausea, vomiting, skin and eye problems	
	Toxic metals • Lead • Cadmium • Arsenic • Mercury	• Fertilization, irrigation, and use of pesticides • Pollution at packaging and transportation	• Can cause cardiovascular, kidney, nervous, and bone diseases • Carcinogenesis and mutagenesis may also occur	
	Veterinary drugs • Antibiotics • Antimicrobials • Antiprotozoals • Hormones	• Used for therapy in animal rearing • Growth promoters	• Symptoms of tachycardia, nausea, headaches, and dizziness • May cause dementia, neurological problem, and allergic reactions in hypersensitive humans • May exert genotoxic, immunotoxic, carcinogenic, or endocrine effects in humans	

Table 3.4 Sources and health impacts of chemical contamination in rice, cereal, and spices

Items	Contaminants	Sources	Health impacts	Ref.
Rice	• Urea	• Used for whitening purpose	• Can cause kidney and liver damage	[114–116]
	Pesticide residues • Organophosphates • Carbamates • Organochlorines	• Use of pesticides against pests and insects	• Damage of nervous system • Can cause allergic reaction, asthma • Damage of lung, kidney, and reproductive system	
	Adulterant • Asbestos • Talc	• Comes from rice chiseling machine • Used as a preservative against moisture	• Carcinogenic and may lead to stomach cancer	
	Potentially toxic elements • Cadmium • Lead • Mercury • Arsenic	• Environmental contaminants • Comes from processing and packaging	• Carcinogenic elements • Can cause disruptive function of stomach, kidney, and neural damage • Potentially harmful for children	

(continued)

Table 3.4 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Cereals	Inherent toxicants • Phytate • Tannin	• Seed sowing and crop cultivation	• Anti-nutrients—ability to sequestrate metal ion inside the body • Inhibits digestive enzymes	[130, 143–148]
	Mycotoxins • Argot alkaloids • Aflatoxins		• Acute hepatotoxicity, hepatic cancer • Gangrenous necrosis, reproductive failure	
	Heavy metals • Arsenic • Cadmium • Lead		• Damage of nervous and cardiovascular systems, kidney • Carcinogenic compounds • Promote bone demineralization	
	Agrochemicals • Insecticides		• Neurological toxins	
	Mycotoxins • Zearalenone • Citrinin	• Harvesting period	• Oestrogenic effects • Yellow rice disease	
	Mycotoxins • Ochratoxin A	• Storage (post-harvest)	• Nephrotoxicity	
	Adulterants • Melamine	• Primary processing (mechanical reduction)	• Generation of toxic compounds inside the body	
	• Pesticides	• Storage (post-primary processing)	• Food poisoning	
	Processed food toxicants • Acrylamide • Chloro- propanols	• Secondary processing (flour treatment, dough conditioning, pH regulation)	• Can produce genotoxic and carcinogenic effects • Neurotoxins	
	Non permitted additives • Alum • Chlorine • Ascorbic acid		• Can be a source of genotoxic carcinogen during processing	
	• High fructose corn syrup	• Artificial sweetener	• Increases LDL cholesterol levels, contributes to diabetes development, tissue damage, and other effects	
	Artificial colors • Blue#1 • Blue#2 • Red#2 • Red#3 • Red#40 • Green#3 • Yellow#5 • Yellow36	• Used as artificial colorings	• Can debilitate the immune system • Chance of thyroid cancer, brain, and bladder tumors	

(continued)

Table 3.4 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Chili powder	Colorant • Sudan dyes • Allura red • Para red • Red oxide (iron III oxide) • Rhodamine B	• Used as colorings	• Carcinogenic, mutagenic, genotoxic, allergen, and gastrointestinal complexities • Hepatoxic, renal dysfunction, adversities on some neurotransmitter	[156–158]
	• Brick dust	• Used to increase product weight	• Gastrointestinal problems	
Turmeric powder	Colorants • Lead chromate • Tartrazine • Metanil yellow • Sudan dyes	• Used as colorings	• Renal and hematological dysfunction • Carcinogenic, mutagenic, and genotoxic substance • Intestine and liver damage, allergen, gastrointestinal complexities	[159, 160]
Coriander powder	Adulterants • Starch • Peanut powder	• Used to increase weight	• Can cause allergies to sensitive population	[158]

Table 3.5 Sources and health impacts of chemical contamination in fruits and vegetables

Items	Contaminants	Sources	Health impacts	Ref.
Fruits	Growth regulators • Calcium carbide • Ethrel (ethephon) • Ethion • Ethephon • Aldrin	• Used for artificial ripening, abscission, flower induction	• Causes dizziness, headache, memory loss, cerebral edema, and seizure • Can result in stomach upset, hypoxia, allergic reaction • Responsible for frequent thirst, mouth irritation, skin and lung damage	[151–155]
	Trace elements • Cadmium • Nickel • Lead • Arsenic	• Soil and environmental contamination • Secondary processing and packaging	• Damage of nervous and cardiovascular systems, kidney • Thyroid problems	
	Polycyclic aromatic hydrocarbons (PAH) • Benzo[*a*]pyrene (BaP), • Indeno(1,2,3-*cd*) pyrene (IcdP)		• Hormonal imbalance • Affect neurological development • Carcinogenesis, mutagenesis	
	Pesticide residues • Fluoride/arsenic • Organophosphates • Organochlorines • Organonitrogen	• Use of pesticides against infestation	• Damage of nervous system • Can cause allergic reaction, asthma • Damage of lung, kidney, reproductive system	
	• Formalin	• Used as a preservative	• Can develop respiratory, digestive, cardiac, nephrological, and neurological problems, along with cancer	
	Colorings • Sudan dye • Injectable dye	• Used as artificial colorings	• Allergens, sensitizers, and carcinogenic effect	

(continued)

Table 3.5 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Vegetables	Adulterant and dye • Copper salt (usually as copper sulfate) • Malachite green	• Used for a fresher and greener look	• Responsible for nephritis (i.e., disagreement still prevails on this among experts)	[151, 152, 161, 162]
	Trace elements • Cadmium • Nickel • Lead • Arsenic	• Soil and environmental contamination • Secondary processing and packaging	• Damage of nervous and cardiovascular systems and kidney • Thyroid problems	
	Polycyclic aromatic hydrocarbons (PAH) • Benzo[*a*]pyrene (BaP), • Indeno(1,2,3-*cd*) pyrene (IcdP)		• Hormonal imbalance • Affect neurological development • Carcinogenesis, mutagenesis	
	Pesticide residues • Fluoride/arsenic • Organophosphates • Organochlorines • Organonitrogen	• Use of pesticides against infestation	• Damage of nervous system • Can cause allergic reaction, asthma	
	Preservative • Formalin	• Used as a preservative	• Can develop digestive, cardiac, nephrological, and neurological problems, along with cancer	

Table 3.6 Sources and health impacts of chemical contamination in dairy products

Items	Contaminants	Sources	Health impacts	Ref.
MilkA.	Preservative • Formalin • Hydrogen per oxide	• Used as preservatives	• Causes kidney damage, ulcer and inflammatory diseases of gastrointestinal tract cause cancer in the long run	[163]
			• Causes gastric irritation and distension, emesis, and respiratory arrest	
	Adulterants • Melamine • Urea	• Used to increase whiteness and thickness	• Forms insoluble melamine cyanurate crystals in kidneys causing renal failure	
			• Can damage kidney severely	
	Hormone • Oxytocin	• Used to promote milk production in cows	• Responsible for early breast development in boys and girls and hormonal imbalance • Can develop various heart and kidney diseases	
Yogurt	Adulterants • High fructose corn syrup • Carrageenan	• Used as artificial sweetener	• Increases LDL cholesterol level, contributes to diabetes and tissue damage with other effects	[164]
		• Used as thickener and emulsifier	• Causes gastrointestinal inflammation and inflammatory bowel diseases, ulcers, and sometimes cancers	
Cheese	Dyes and colors • Yellow#6 (E110) • Yellow tartrazine (E102)	• Used as artificial colorings	• Increases the number of kidney and adrenal gland tumors, may cause chromosomal damage	[165, 166]
	Adulterant • Carrageenan	• Used as thickener and emulsifier	• Causes gastrointestinal inflammation and inflammatory bowel diseases, ulcers, and sometimes cancers	
Ice cream	Dyes and colors • Red #3 • Red #40 (E124)	• Used as artificial colorings	• Causes thyroid cancer, chromosomal damage, and brain–nerve transmission	[167, 168]
	Adulterants • High fructose corn syrup • Carrageenan	• Used as artificial sweetener • Used as thickener and emulsifier	• Increases LDL cholesterol level, contributes to diabetes and tissue damage with other effects • Gastrointestinal inflammation, inflammatory bowel disease, ulcers, and even cancers	

(continued)

Table 3.6 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Butter	Preservatives • BHA • BHT	• Used as preservatives	• Highly carcinogenic substances	[169, 170]
Sweetmeats	Heavy metals • Lead • Chromium • Mercury • Cadmium	• Raw materials contamination • Water contamination	• Behavioral abnormalities, decreased learning, and permanent neurological damage • Adverse effects on reproductive, hepatic, and renal systems • Cell damage and proliferation • May cause skin rashes, respiratory problems, ulcers, etc.	[171, 172]
	Artificial colors and toxic dyes • Rhodamine B • Orange 2 • Red 2 and 40 • Malachite green • Auramine • Metanil yellow	• Used for colorings	• Various digestive and neurological effects • Chronic effects like cancer upon long-term consumption	
	Adulterants • Vegetable oil	• Used in place of pure ghee	• Risk of cardiovascular diseases, can damage brain development • Risk of arthritis, inflammatory bowel diseases, allergic reactions	
	Toxins • Aflatoxin M1	• Contamination of feedstuffs	• Carcinogenesis • Reduced protein synthesis • Immunosuppressant effects	

Table 3.7 Sources and health impacts of chemical contamination in processed foods

Items	Contaminants	Sources	Health impacts	Ref.
Biscuit	Adulterants • Copper sulfate • Stannous chloride • Potassium carbonate • Potassium bromate	• Used to improve appearance	• Respiratory problems, stomach pain, cramping, weakness • Carcinogenic effects (lung cancer, endometrial cancer, ovarian cancer)	[134–136]
Bread	• Copper sulfate	• To improve appearance	• Risk of kidney cancer. • Large amounts can lead to nausea and vomiting. • Damage to body tissues, blood cells, and liver.	[137–139]
	• Stannous chloride	• To avoid differences between good grade and poor grade flour products	• Can be readily taken up by white blood cells and damage DNA	
	• Potassium carbonate	• Baking agent	• Uneven heartbeat, tiredness and weakness, severe stomach pain, cramping in legs, black/bloody/tarry stools	
	• Potassium bromate	• To increase white flour volume	• May cause cancer	
Jam/jelly	• Benzoic acid	• Used for preservation	• Damage to nervous systems • Linked to asthma and increased levels of hyperactivity in children	[140, 141]
	Artificial color • Red#2 • Red#3 • Red#40 Green#3 • Yellow#5 • Yellow#6	• Used for artificial coloring	• Can debilitate the immune system • Chance of thyroid cancer, brain, and bladder tumors	
	• Sulfur dioxide	• Fumes are used to maintain quality and nutrition values of raw fruits/pulps during drying and storage	• Destroys vitamin B1 content • Can cause asthmatic problems	
Honey	• Gypsum	• Used as binder	• Kidney stone, loss of appetite • Swelling of the abdomen, intense abdominal pain	[142]
	• High fructose corn syrup	• Artificial sweetener	• Increases LDL cholesterol levels, contributes to diabetes development, tissue damage, and other effects	

(continued)

Table 3.7 (continued)

Items	Contaminants	Sources	Health impacts	Ref.
Chips	• Butylated hydroxyanisole (BHA) • Butylated hydrozyttoluene (BHT)	• For preservation and avoiding oxidation	• Affects the neurological system of brain and alters behavior • Can form cancer-causing reactive components in the body	[149, 150]
	• Monosodium glutamate	• To enhance flavor	• Can cause headache and nausea	
	• Sodium chloride	• Used as flavoring	• Excessive amounts can affect cardiovascular function, leading to high blood pressure, heart attack, stroke, and kidney failure	
	• Olestra	• Used to make non-fat potato chips	• Can bind and eliminate fat-soluble vitamins A, E, D, K, carotenoids, and (substances thought to prevent cancer and maintain healthy immune system)	

feed and food: (i) preventing contamination at the source, for example, by reducing environmental pollution; (ii) applying appropriate technology control measure(s) in different food processing steps, which includes production, manufacture, processing, preparation, treatment, packing, packaging, transport or holding; (iii) applying measures to prevent contaminated feed or food to be marketed for consumption; and (iv) ensuring accurate detection and monitoring of chemical contaminants in different food processing steps.

Exploring alternative options and practices to reduce chemical contaminations can be a major scope of global food safety and regulation campaign. Soil remediation can greatly decrease environmental exposure and plant contamination [87]. Soil turnover and mixing process can result in lower level or dilution of contaminants like heavy metals (cadmium, mercury, and chromium) [88]. Long-term gardening can also have an impact to reduce the level of polycyclic aromatic hydrocarbon (PAH) due to microbial activity [89]. The slow release of nitrogen-based fertilizers can reduce cadmium levels in some plants like pak choi [90]. Water management during the initial growth stages of rice crops can reduce cadmium and arsenic levels [91]. Safer storage alternatives like edible film and coatings can decrease the probability and degree of exposure to contaminants [92]. Replacing smoking or open fire cooking with gas stoves can bring down the risk of PAH contamination of food [93].

Researchers have demonstrated that extracts of green tea, grape seed, and spices can be used as an alternative for chemical preservatives [94]. In addition, consumption of organic and locally produced foods, avoiding canned foods, and using containers made of glass, food-grade stainless steel, or polyethylene can reduce

exposure to chemical contaminants in foods. Alternative options in accordance with good agricultural and manufacturing practices along with integrated management during transportation and storage can act as the primary defense against this growing crisis [95].

6 Conclusion

In recent times, the risk of chemical contamination in food is much grave due to serious health implications in their wake. Although such contamination can occur naturally through toxins or environmental pollutants, the majority can be traced back to series of events including food processing, packaging, storage, and transportation. As technology advances, a wide range of contaminants are known and have been identified in terms of type, source, and potential health hazards for specific food items. The symptoms of the foodborne illness due to such contamination vary from mild gastrointestinal disorder to fatal incidents of hepatic, renal, and neurological syndromes. Keeping such scenarios in the mind and the possible health implications in the fore, this chapter reviews the types of chemical contaminants in specific food items along with daily human exposure to such contamination and further elaborates the health impacts of that food impurities. Rigorous measures and actions including good agricultural and manufacturing practices should be established to minimize individual exposure and health risks associated with chemical contamination of food at a global scale.

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Chapter 4
Conventional Microbial Counting and Identification Techniques

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Abstract The present chapter deals with conventional methods of detection and identification of microorganisms with a prime focus on food microbiology. It provides a brief overview of the different detection methods of microorganisms, such as standard plate count method, most probable number count and direct microscopic count. Similarly, the identification methods as mentioned in this chapter are a series of biochemical tests. These suggest the presence of certain enzyme systems in the microorganism or metabolic by-product in the culture medium and using this information, the target microorganism can be identified. The biochemical tests (i.e. IMViC test, catalase test, oxidase test, urease test and nitrate and nitrite reduction test), discussed in this chapter are widely used by the microbiological laboratory. Apart from the tests mentioned above, there are, however, different kinds of culture-dependent biochemical tests developed over the years to detect the presence of some specific enzyme system or metabolic by-products.

Keywords Microorganisms · Biochemical tests · Food pathogens · Colony formation unit (CFU) · Standard plate count · Membrane filtration · Pour plate method · Spread plate method · Drop plate method · Single dilution method · Direct microscopic count · Culture media

1 Introduction

Food microbiology deals with isolation, enumeration and characterization of foodborne or food spoilage microorganisms or their metabolic by-products. Their detection is essential to ensure safe food supply chain and reduced occurrence of foodborne diseases. Diseases like dysentery, diarrhoea and food poisoning are mainly caused by food pathogenic microorganisms, such as *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Shigella* spp. and *Salmonella* spp.

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[1]. Besides, microorganisms, such as *Pseudomonas* spp., *Aspergillus* spp., *Penicillium* spp. and *Botrytis* spp., are largely responsible for tremendous economic havoc associated with spoilage of food products [2, 3]. It is important to detect the pathogenic or spoilage microbes early at their growth phase to avoid the outbreak of foodborne diseases and spoilage of food products [4].

Conventionally, culture-dependent microbiological methods were used to observe and enumerate viable microorganisms in a given food sample, but none of the methods in practice could determine the exact number of microbes. Therefore, a single colony is considered to be 'colony-forming units' (CFU) that originated from a certain microorganism, and this shows specific characteristic features, namely size, shape and configuration. The groups, genera and species of microflora can be determined by qualitative and quantitative methods. The qualitative test involves the detection of the presence or absence of a specific microorganism, whereas the quantitative test deals with enumeration of the amount of the target microorganism. Based on these, three basic methods are traditionally used to estimate the total number of microbial cells [5–8]:

1. Standard plate count technique to determine colony-forming units.
2. Most probable number for statistical determination of viable cells.
3. Direct microscopic count.

These methods mentioned previously and their applicability in the determination of microorganisms from various sources are described in this chapter.

2 Standard Plate Count to Determine Colony-Forming Units

2.1 Procedures

Standard plate count term is interchangeably used as total aerobic mesophilic plate count or aerobic plate count. This technique involves homogenization or blending of the food samples followed by serial dilution in appropriate diluent and finally plating onto a suitable agar medium. It was then incubated for a predetermined time, and visible colonies were counted. It is the most commonly used method to enumerate microbial population in the food samples. Bacterial count is reported as colony-forming units (CFUs), which is presumed to have emerged from one cell or confluence of single cell type. The enumerated microbial population in any food samples using this method should be observed as a function of the selected food biota, initial microbial load, temperature and time of incubation, pH, water activity of the culture media, availability of oxygen, type of diluent (i.e. for serial dilution) and presence of synergistic or antagonistic microorganisms [8, 9]. Based on the process of bacterial inoculation, there are four different methods employed and they are: (i) pour plate method, (ii) spread plate method, (iii) drop plate method and (iv) membrane filtration technique.

2.1.1 Pour Plate Method

This method is used to determine the total aerobic mesophilic count, lactic acid bacteria count, *Enterobacteriaceae* and enterococci counts; and it has a detection level of 1 CFU/mL for liquid food samples and 10 CFU/g of solid food samples [10]. However, the limitation of this method relies on the usage of thermolabile components in the culture medium. Briefly, this method involves sample preparation, followed by inoculation with desired dilution. Inoculum volume of 1 mL is poured at the periphery of sterile Petri plates using a pipette, and molten agar medium is added at a temperature between 44 and 46 °C. The culture media must be added within 10 min of addition of inoculum in the plates to avoid the inoculum from drying out and to cohere to the plates [11]. Based on the estimated contamination level of the sample, the dilution is selected to obtain plates containing 10–300 colonies [12]. If the sample is supposed to have heavy contamination then higher dilution may be used as inoculum, and microbial load of less contaminated sample may be determined with lower dilution (say up to 10^{-3} dilution, which corresponds to 0.001 g or mL of sample). It is also possible to inoculate 1 mL of sample (liquid products) without any dilution if the expected contamination is very low. While enumerating bacterial load of particulate food samples, such as meals and flour, 0.005 to 0.05% of filter-sterilized 2,3,5-triphenyltetrazolium chloride (TTC) can be added to the sterile culture medium to produce red-coloured bacterial colonies [13]. This can avoid the interference of particles in food and the colonies grown in Petri plates from first dilution.

Microbial contamination on the surface of samples, such as carcasses of swine, poultry, bovine and fish, can be enumerated by surface washing and surface swabbing techniques. However, it is a sample preparation method, and the final CFU calculation based on pour plate technique is different from the homogenized food sample. The calculation for surface washing and surface swabbing techniques is mentioned in Sects. 2.3.1 and 2.3.2, respectively.

2.1.2 Spread Plate Method

Spread plating is performed by inoculating the sample or its dilution on the solidified culture medium using an L-shaped glass rod spreader. This technique offers faster growth of strict aerobes as opposed to microaerophilic organisms. In this method, the microbes are not exposed to the high temperature of molten culture medium; thus, characteristics and features of the grown colonies can easily be studied. On the contrary, the limitation of this technique lies in the inoculum volume, which is restricted to the maximum liquid absorption capacity of the selected culture medium, i.e. 0.5 mL per Petri plate [11]. However, crowding of colonies is a common problem associated with surface plating, which can be overcome by spreading the inoculum on the entire surface of the agar plate evenly. The standard procedure recommends using 0.1 mL of inoculum per plate of each dilution, and this provides a detection limit of 100 CFU/g of solid samples and 10 CFU/mL of

liquid samples. Pour plating is the commonly used procedure for standard plate count of a given sample. However, spread plating can also produce comparable results with an advantage in presumptive determination of aerobic psychrotrophs, yeast and mould, *Staphylococcus aureus* and *Bacillus cereus* count.

2.1.3 Drop Plate Method

This technique is similar to the spread plate method except for a point or drop of inoculum (10 μL or 0.01 mL) with selected dilution is deposited onto the surface of solidified culture medium. This method is useful when large number of dilutions are required to be inoculated, preferably three drops of same dilution in a same plate. The detection limit of this method is 1000 CFU/g of solid sample and 100 CFU/mL for liquid sample, which means the samples should not be contaminated. The dilution must be selected in a way that the point of the maximum inoculum yield is 30 CFUs. The calculation of CFU/mL of sample is done by dividing the product of average of the number of colonies in three drops and dilution selected for drop plating [10].

2.1.4 Membrane Filtration Technique

This technique is restricted to transparent liquid samples, as the presence of solid matter may clog the filtration membrane. The detection limit of this procedure is 1 CFU per inoculated volume, which means it can detect microorganisms in food samples having very low contamination. Briefly, the liquid sample is filtered through a membrane having pore size of 0.45 μm, and a large volume of the sample is filtered so as to concentrate the microorganisms on the membrane filter. This membrane filter containing microorganisms is placed onto the surface of solidified culture medium and incubated overnight to permit colonies to develop. This method is mainly applied to determine total aerobic mesophilic count, enterococci counts, total coliforms, faecal coliforms and *E. coli* in water samples. However, microbial load in solid samples, such as sugar and salts, can also be tested using this method provided they are transformed to clear solution prior to filtration. Rules regarding calculation of CFU/mL or CFU/g of sample filtered through this technique are mentioned in Table 4.1 [10]. For solid samples, counted CFU per filtered volume is converted to CFU/g with respect to the amount of solid sample present in the first dilution. The process of standard plate counting involves homogenization of the food samples, serial dilution of the sample with appropriate diluents, plating the inoculum on selected culture medium and counting the number of colonies upon incubation (Fig. 4.1).

Table 4.1 Rules to calculate CFU/mL of the sample using membrane filtration method

CFU/square of membrane	CFU/mL
Less than or equal to 2	∑(number of colonies) ÷ Filtered volume
Between 3 and 10	(Average of the number of colonies in 10 squares × 100) ÷ Filtered volume
Between 10 and 20	(Average of the number of colonies in 5 squares × 100) ÷ Filtered volume
More than 20	>2000 ÷ Filtered volume

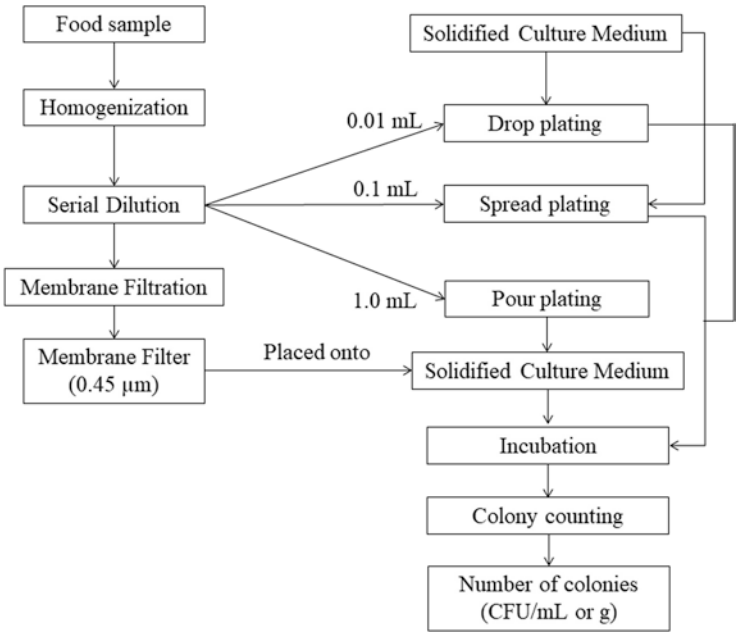

Fig. 4.1 Flow chart of the process of standard plate count method

2.2 Critical Control Points of Standard Plate Count Method

It is important to consider the flowing factors for the standard plate count method [8, 12]:

1. Microbial suspension must be mixed properly before pipetting the aliquot for subsequent serial dilution.
2. Microbial suspension should also be well mixed before pipetting an aliquot on the selected agar medium.
3. While spreading the inoculum on the surface of the agar plate, utmost care should be taken so as to avoid pooling around the periphery of the plates.

4. Inoculum spreader must be flame sterilized every time before using on a plate having different dilution.
5. Plates containing an estimated 10–300 should be used for calculating CFUs.
6. Plating of the dilution must be performed in less than an hour of sample re-suspension and thawing.
7. Plates must be kept in an inverted position during the incubation period. State reasons.

2.3 Calculations for Standard Plate Count Method

According to ISO 7218:2007 guidelines, Petri plates used for the SPC technique should be of 90 mm diameter and have count of 10–300 colonies in each plate for two consecutive dilutions. Set of rules to be followed to calculate CFU/mL or CFU/g of sample are mentioned in Table 4.2.

2.3.1 Calculation of CFUs for Surface Washed Samples

For food samples, washed suspension needs to be considered first for the undiluted sample, and CFUs are calculated as CFU/mL using the pour plate method. Initially, if washing of the sample is performed using an equal ratio of sample weight and diluent volume (1:1 w/v), then each mL of diluent corresponds to 1 g of sample, and CFU/mL of the suspension can be the same as CFU/g. However, if the ratio between the weight of the sample washed and volume of diluent used for washing is different, then CFU/g of sample can be the product of CFU/mL of the suspension and ratio of volume of diluent used for washing the sample (mL) to weight of sample washed (g) as shown in Eq. (4.1):

$$\text{CFU / g} = \text{CFU / mL of the washed suspension} \times \frac{\text{Volume of diluent used for washing}}{\text{Weight of the sample washed}} \quad (4.1)$$

2.3.2 Calculation of CFUs for Surface Swabbed Samples

In the swabbing technique, sample suspension is made by placing the swabs (15 cm × 0.3 cm long wooden stick having 2 cm × 0.5 cm absorbent cotton) in the diluent, and it is considered as undiluted, and CFU/mL of the sample suspension is determined according to pour plate method. The CFU per surface area (cm^2) of the swabbed sample is determined as a product of CFU/mL of the sample suspension and cm^2 swabbed per mL of diluent as shown in Eq. (4.2):

Table 4.2 Guidelines to calculate CFU/mL or g of the sample using the standard plate count method

Rule 1: The mean CFU in the selected sample can be calculated using equation:

$$CFU/\text{mL or } CFU/\text{g} = \frac{\text{Sum of colonies on two plates in two consecutive dilution}}{\text{Inoculum volume}(\text{mL}) \times 1.1 \times \text{First dilution retained}}$$

Inoculum for pour plating = 1 mL/dilution			Inoculum for spread plate = 0.1 mL/dilution		
10^{-1}	10^{-2}	CFU/mL or CFU/g	10^{-1}	10^{-2}	CFU/mL or CFU/g
171	30	(171 + 30)/ $(1 \times 1.1 \times 10^{-1}) = 1.7 \times 10^{3}$	135	22	(135 + 22)/ $(0.1 \times 1.1 \times 10^{-1}) = 1.4 \times 10^{4}$

Rule 2: If none of the inoculated Petri plates have produced 10 colonies then,

Scenario #1			**Number of colonies produced is greater than or equal to 4**		
Inoculum for pour plating = 1 mL/dilution			Inoculum for spread plate = 0.1 mL/dilution		
10^{-1}	10^{-2}	CFU/mL or CFU/g	10^{-1}	10^{-2}	CFU/mL or CFU/g
6	0	$6/(1 \times 10^{-1}) = 60$ (estimated)	5	0	$5/(0.1 \times 10^{-1}) = 5 \times 10^{2}$ (estimated)
Scenario #2			**Number of colonies produced is less than 4**		
10^{-1}	10^{-2}	CFU/mL or CFU/g	10^{-1}	10^{-2}	CFU/mL or CFU/g
2	0	Present $<4/(1 \times 10^{-1}) = <40$	3	0	Present $<4/(0.1 \times 10^{-1}) = 4 \times 10^{2}$
Scenario #3			**No colonies produced in any plates**		
10^{-1}	10^{-2}	CFU/mL or CFU/g	10^{-1}	10^{-2}	CFU/mL or CFU/g
0	0	$<1/(1 \times 10^{-1}) = <10$	0	0	$<1/(0.1 \times 10^{-1}) = <10^{2}$

Rule 3: If number of colonies produced are more than 300 in one dilution and less than 10 in successive dilutions then,

Scenario #1			**Number of colonies produced is less than 334 and equal to 8**		
Inoculum for pour plating = 1 mL/dilution			Inoculum for spread plate = 0.1 mL/dilution		
10^{-1}	10^{-2}	CFU/mL or CFU/g	10^{-1}	10^{-2}	CFU/mL or CFU/g
320	8	(320 + 8)/ $(1 \times 1.1 \times 10^{-1}) = 3 \times 10^{3}$	295	8	(295 + 8)/ $(0.1 \times 1.1 \times 10^{-1}) = 2.8 \times 10^{4}$
Scenario #2			**Number of colonies produced is less than 334 and less than 8**		
10^{-1}	10^{-2}	CFU/mL or CFU/g	10^{-1}	10^{-2}	CFU/mL or CFU/g
310	<8	$310/(1 \times 10^{-1}) = 3.1 \times 10^{3}$	328	<8	$328/(0.1 \times 10^{-1}) = 3.2 \times 10^{4}$
Scenario #3			**Number of colonies produced is greater than 334 and greater than 8**		
10^{-1}	10^{-2}	CFU/mL or CFU/g	10^{-1}	10^{-2}	CFU/mL or CFU/g
395	15	$15/(1 \times 10^{-2}) = 1.5 \times 10^{3}$	360	9	$9/(0.1 \times 10^{-2}) = 9 \times 10^{3}$
Scenario #4			**Number of colonies produced is greater than 334 and less than 8**		
Repeat the test					

Rule 4: If all the plates have too numerous colonies to count i.e. more than 300 then the calculation must be done based on 300 colonies and the result must be reported *as greater than the values obtained*.

$$\text{CFU / cm}^2 = \text{CFU / mL of the suspension} \times \frac{\text{Surface area used for sampling}}{\text{Volume of diluent used in sampling}} \quad (4.2)$$

As discussed above, the SPC method is a conventional method, and it has its own share of limitation. SPC method is unable to distinguish between pathogenic and non-pathogenic bacteria, yeast and mould, and over-crowding and clumping of small colonies can lead to estimation of a false-positive result, alongside SPC gives only a rough estimate of the microbial load in a given food sample. Finally, SPC is a basic and useful method to monitor and determine the contamination in food processing and product development, but it does not measure the presence of specific pathogens in a given food product.

2.4 Most Probable Number for Statistical Determination of Viable Cells

The most probable number (MPN) method is the quantitative method of determining target microorganisms mainly total coliform, faecal coliform and *E. coli* in water and food samples. The underlying principle of this method is based on probability statistics that estimate the frequency of occurrence of target microorganisms in a given sample. Theoretically, MPN considers the homogenous distribution of the microorganism throughout the sample. It is also assumed that all microorganisms present in the sample are in the same phase of growth. This is achieved by inoculating different volumes of sample in a series of tubes comprising culture broth suitable for its growth. The estimation of actual microbial load in the sample is made based on the number of tubes showing positive (microorganism present) and negative (microorganism absent) growth and standard MPN table. Briefly, homogenized samples are inoculated either according to multiple dilution method or single dilution method.

2.4.1 Multiple Dilution Method

Multiple dilution method is the most universal format of MPN technique because it encompasses a broad range of microbial concentration in the sample. The accuracy of the test depends on the number of dilution and the number of tubes used per dilution. The standard procedure involves diluting the homogenized sample into three consecutive decimal dilution and inoculation of respective aliquots in series of three tubes per dilution. The tube dilution method was introduced to determine the bacterial densities in a given sample and has been extensively used to perform sanitary analysis of coliforms in water, milk, other foods and public health specimen [14]. MPN estimates multiple tube method follows a logarithmic pattern that needs to be converted using standard MPN table [15]. The example of determination of total coliform count is shown in Fig. 4.2, where homogenized sample and three

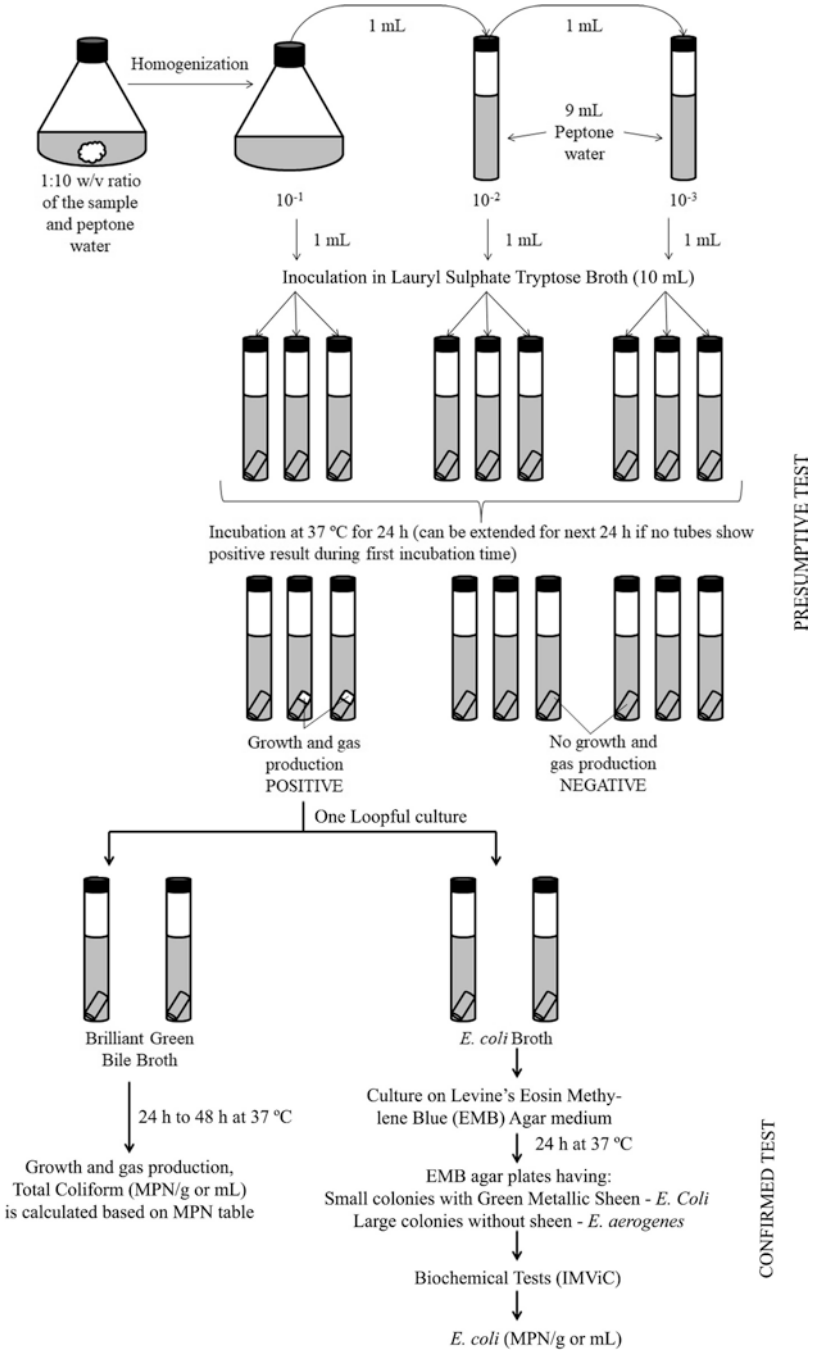

Fig. 4.2 Multiple dilution method for analysis of coliform in food sample

subsequent decimal dilutions, three tubes per dilution are inoculated in lauryl sulphate tryptose (LST) broth, which encourages the growth of coli-aerogenes bacteria and *E. coli*. The surface-active agent sodium lauryl sulphate restricts the growth of any microorganisms other than coli-aerogenes group [16]. LST broth also contributes to the recovery, rapid multiplication and abundant gas production of injured coliforms even with small inoculum volume. The cultures obtained from presumptive test in LST broth showing growth and gas production are selected, and a loop of the culture is transferred to brilliant green bile (BGB) broth using inoculation loop to calculate total coliform in the given sample. BGB broth is a selective medium comprising brilliant green and bile that promotes growth of coli-aerogenes group and inhibits Gram-positive bacteria [17]. Lactose present in BGB media is utilized as a carbon source through hetero-fermentative pathway leading to gas production.

2.4.2 Single Dilution Method

In a single dilution test, all five or ten aliquot of the sample (50 or 100 mL) or first dilution of solid sample is added to the equal volume of double-strength culture medium (LST broth), and the remaining process is similar to multiple dilution method (Fig. 4.3). Double-strength LST broth supposedly helps recover the damaged coliforms in the sample. This method is mainly applied when the level of coliform contamination in the sample is expected to be low, such as in potable water, fruit juices and carbonated soft drinks.

Presently, the MPN method is prominently being used to predict the presence of coliforms (gram-negative, rod-shaped, non-spore-forming bacteria such as *Escherichia coli* and *Enterobacter aerogenes*) in the given water sample. This method is relatively simple, reproducible and can determine the population load of diseases causing faecal coliforms. Alongside, this method is also helpful in determining specific microorganisms using selective and differential media. Eosin methylene blue (EMB) agar media used in this method is a selective as well as differential media. EMB agar is selective because it only allows gram-negative to grow as methylene blue dye restricts the growth of gram-positive bacteria. It is differential because it can differentiate two gram-negative bacteria grown on the medium based on its appearance. *Escherichia coli* can appear as green with metallic sheen, whereas *Enterobacter aerogenes* can produce colonies larger in size and are not green metallic sheen over the surface of the colonies. On the other hand, MPN method is time-consuming, laborious, requires more glassware and growth medium and also lacks precision.

2.4.3 Direct Microscopic Count

Direct microscopic count (DMC) is a quantitative method used to examine the total bacterial population (individual cells or clumps) as well as somatic cells in milk or milk products. It involves making a smear of 10 μL of a given sample over 1 cm^2 area of grease-free microscopic slide followed by immersion of the slide in

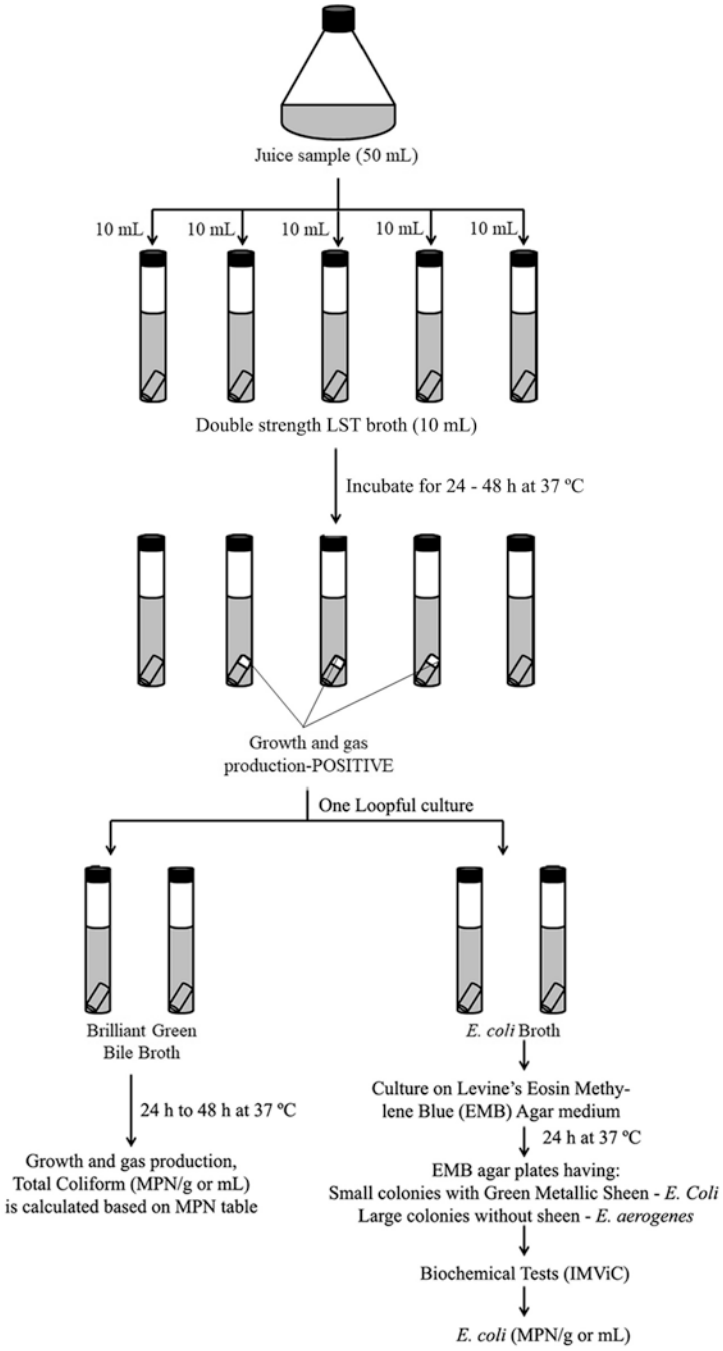

Fig. 4.3 Single dilution method for analysis of coliform in food sample

Newman's stain, drying and counting of cells under oil immersion objective of microscope. Newman's stain consists of tetrachloroethane, which defats the milk sample by dissolving the fat globules, ethyl alcohol fixes the smear onto the slide and methylene blue chloride stains the bacterial clumps. Average number of bacterial clumps in different microscopic field is calculated, and multiplied by the microscopic factor, and the outcome is represented as DMC per mL of sample. Microscopic factor is the ratio of smear area (cm^2) to the product of area of microscopic field (cm^2) and volume of sample (mL).

DMC is a rapid and simple method to estimate the bacterial load of a large volume of milk sample in less time. This method can also spot the presence of somatic cells in milk as a result of mastitis infection in cattle. Since it is a microscopic technique, it is useful to assess the cell morphology, and furthermore, fluorescence probe, such as acridine orange and aniline blue, can enhance the efficiency of the process as well. However, on the flip side, this is non-specific and provides low accuracy.

3 Identification Methods

The qualitative evaluation of a given sample suggests the presence or absence of any target bacteria. It can be achieved by culturing the sample in: (i) enrichment medium, (ii) isolation medium and (iii) confirming the identity of isolated culture based on morphology and biochemical features [18, 19].

3.1 Culturing the Sample in Enrichment Medium

The microbial flora in any given food can be either pathogenic or nonpathogenic. Based on the pretreatment or processing of the food, the viability of pathogenic microbes gets compromised, and microbial load goes down the detection limit of plate count and may take longer to flourish to eventually cause the food spoilage. To detect the presence or absence of such injured pathogens in the food sample, they are cultured in pre-enriched medium in order to resuscitate under favourable growth conditions, followed by culture in secondary selective medium to ensure complete retrieval of the pathogen [20]. Pre-enrichment medium are either moderately selective or non-selective, having reasonable buffering capacity in order to grow injured pathogens under optimal conditions [11]. Secondary selective enrichment medium also restricts the growth of competing microflora present in the sample and ensures a favourable growth environment for the target microorganism. This agent uses selective medium, which can be chemical compounds, namely brilliant green, bile salts, sodium lauryl sulphate, sodium tetrathionate, sodium selenite, potassium tellurite [21, 22] and antibiotics (vancomycin, polymyxin, streptomycin, tetracyclin etc.) isolated fungi [23].

For isolation of *Vibrio cholerae* from environmental water samples, overnight enrichment was performed in alkaline petone water (pH 8.6) followed by streaking of microaerophillic pellicle layer onto selective culture medium namely, thiosulphate citrate bile salt sucrose agar, tellurite taurocholate gelatin agar or Monsur medium and CHROMagar™ [24]. Similarly, for the isolation of *Salmonella* spp. from swine and poultry, it is shown better sensitivity (upto 95%) when neck skin or fecal samples are pre-enriched in universal pre-enrichment broth (UB), and these are followed by culturing on selective medium namely, brilliant green agar [25]. They also suggested that better buffering capacity and presence of sodium pyruvate in UB enhanced the repair mechanism of injured *Salmonella* spp. when compared to conventional buffered peptone water with pre-enrichment medium. Antibiotics are also used as selective enrichment agent that mostly targets the competing microflora growing alongside target microorganism. An improved recovery of cryo-injured *E. coli* O157:H7 cells from food samples with pre-enrichment (non-selective) can be observed within 2 h at 25 °C in modified *E. coli* broth without bile salt, while it can be observed within 18 h at 42 °C when enriched broth containing bile salt and novobiocin are used [26]. Among six selective agents (ampicillin, cephalotin, novobiocin, ethanol, bile salts and briiliant green) tested, cephalothin (at 10 ppm) can provide better efficacy for the isolation of *Aeromonas* spp. from chicken meat [27].

3.2 Isolation on Selective Differential Agar Medium

In the differential plating, a revived and multiplied target microorganism recovered from enrichment broth is performed on an agar medium to obtain a pure culture. This further ensures the elimination of competitive microflora and differentiates the target microorganism from the rest. pH indicators are the most commonly used differential agents because they can differentiate the microorganism based on the change in colour of the culture medium due to change in pH. Different pH indicators presently used as differential agents are phenol red, bromothymol blue, thymol blue, cresol blue, bromocresol purple and neutral red [28]. If the sample contains lactose or sucrose (carbohydrate) fermenting bacteria, then the presence of phenol red acid–base indicator in the medium can change the colour of the medium from brick red (at pH 8.5) to yellow (at pH 6.9) as a result of the reduction in pH due to acid production [11]. *Salmonella* spp. with better selectivity can be isolated when selective agent brilliant green, acid–base indicator phenol red and an organic sulphur compound sulfanilamide (up to 2 g/L) is used in the culture medium, which inhibits the growth of other competing microflora, namely *Proteus mirabilis, E. coli and Enterobacter* strains [29]. Similarly, hydrogen sulphide indicator has traditionally been used to isolate *Salmonella* spp. from a given food sample. Iron compounds, namely ferric citrate, ferric ammonium sulphate and ferric ammonium citrate, are used as H_2S indicators, which reacts with H_2S produced by bacteria to form black-coloured iron sulphide that diffuses through the culture medium to turn it into black

or black spots around the colonies [30]. Eosin methylene blue is a selective differential media that is commonly used to distinguish gram-positive and gram-negative bacteria. It contains two dyes, namely Eosin and methylene blue. Carbohydrate (lactose or sucrose) fermenters result in the reduction of pH of the culture medium, and under acidic condition, eosin change colourless (non-fermenters) bacterial colonies to black-coloured colonies (fermenter). On the other hand, methylene blue inhibits the growth of gram-positive bacteria [31].

3.3 Confirmation of the Isolated Cultures

The confirmation of the identity of isolated target microorganisms is based on its morphological and biochemical features. Morphological characteristic of the bacterial cells deals with staining properties, motility, shape (e.g. cocci, spiral shaped, curved rods, straight rods) and arrangement (tetrads, pairs, isolated, in cluster, in chains, in filaments), whereas fungal cells have conidia, hyphal arrangements [32]. Test carried out to study the biochemical properties of bacteria are commonly abbreviated as IMViC test, which includes a group of tests to differentiate coliform bacteria. Besides IMViC test, there are various tests that provide biochemical properties of bacteria, namely catalase test, oxidase test, urease test and nitrate reduction test.

3.3.1 IMViC Test

This conventional method is still under practice and has been very efficient in differentiating the Enterobacter (gram-negative, lactose fermenting and non-spore-forming bacilli) into species and varieties. This method has also seen several modifications over the years, and the most significant among them is the simplification of the method by using 48 h on agar plate [33, 34]. IMViC test that stands for indole test (suggest production of indole upon hydrolysis of an amino acid tryptophan), methyl red test (suggest change in pH of the culture medium due to bacterial fermentation of glucose to mixed acid), Voges-Proskauer test (suggest the ability of bacteria to ferment glucose into neutral end product acetoin) and citrate test (suggest the ability of the bacteria to grow in media containing citrate salts as only source of carbon).

Indole Test

Indole test has been used to distinguish indole-positive *E. coli* between indole-negative *E. aerogenes* and *Klebsiella* spp. [35]. It is performed to determine the ability of bacteria to produce indole upon deamination of tryptophan. The indole-positive bacteria use tryptophanase enzyme to produce indole, pyruvic acid and ammonia,

which further reacts with p-dimethylaminobenzaldehyde under acidic condition to form red-coloured dye rosindole [36]. On the other hand, tryptophanase enzyme system is absent in indole-negative bacteria.

Methyl Red Test

Fermentation of glucose by *E. aerogenes* and other members produces a higher ratio of CO_2 to H_2 gases that results in low acidification (less H^+ ions) of the culture medium (pH 6), whereas *E.coli* and other members upon undergoing similar fermentation produce less CO_2 to H_2 gas ratio under high acidification (more H^+ ions) of the culture medium having pH below or equal to 4.4 [37]. This is due to different pathways these bacteria choose to ferment the sugar source, *E. coli* uses mixed-acid pathway resulting in multiple 5 mol of low acid end-products (mainly lactic acid, acetic acid and formic acid) and 1 mol of ethanol alongside the gases per 1 mole of glucose. On the contrary, *E. aerogenes* uses butanediol fermentation pathway that produces 1 mole of acid and the gases per mole of glucose [38]. Addition of pH indicator methyl red to the culture medium results in change in colour to red (due to low pH 4.4) for MR-positive bacteria (*E. coli*) and MR-negative (*E. aerogenes*) results yellow colour (due to high pH 6) [39].

Voges-Proskauer Test

The principle of the Voges-Proskauer test is in continuation of methyl red test, where MR-negative bacteria reportedly produce acetoin (3-hydroxybutanone) as an intermediate product of glucose fermentation via butanediol pathway. The addition of Barritt's reagent A (5% w/v a-naphthol in absolute ethanol) to the culture medium catalyzes the oxidation of acetoin to diacetyl in presence of Barritt's reagent B (40% w/v potassium hydroxide in water). The formed diacetyl reacts with guanidine groups of peptone present in the MR-VP medium to produce pinkish-red colour [37, 39]. Therefore, VP-positive *E. aerogenes* can be pinkish-red colour, whereas culture medium appearing yellow colour is considered VP-negative (*E. coli*).

Citrate Test

Citrate test is performed to substantiate if the bacteria can utilized citrate as sole carbon for their growth. Citrate transport protein (permeases) present in citrate-positive bacteria (*Klebsiella pneumoniae, E. aerogenes*) enables the cell to uptake exogenous citrate [40], which is eventually cleaved by citrate lyase to form oxaloacetate and acetate. The oxaloacetate is further metabolized to pyruvate and CO_2 and based on the pH of the medium further catabolism of pyruvate takes place. If the medium has alkaline pH, pyruvate can be formed from acetate and formate, whereas under neutral pH (7.0) and below, lactate and acetoin are produced alongside CO_2,

respectively. The production of sodium carbonate is due to the reaction of CO_2 with sodium ions present in the culture medium, and the production of ammonium hydroxide due to utilization of ammonium salts increases the pH [41, 42]. This alteration in pH can be detected using bromothymol blue which changes its colour from deep green (at neutral pH) to blue (at alkaline pH 7.6 or more). However, visible growth can also be observed if the bacterium is citrate positive.

3.3.2 Catalase Test

Aerobic bacteria produce hydrogen peroxide during the metabolism of carbohydrates, and as a part of adaptive response to oxidative stress, it produces hydrogen peroxide reductase, also known as catalase [43]. While in the case of some obligate anaerobes, namely *Clostridium acetobutylicum* and *C. aminovalericum*, the presence of enzyme peroxidase attributes to its tolerance to less oxidative stress or microoxic condition [44]. The purpose of this test is to notice the presence of enzyme catalase in bacteria, which is responsible for neutralizing the bactericidal effects of hydrogen peroxide [45]. Anaerobic bacteria do not possess the enzyme and hence, this test could be used to differentiate between aerobes and obligate anaerobes [46]. The differentiation can be at the genus level (e.g. catalase-positive *Micrococcaceae* and catalase-negative *Streptrococcaceae*) or species level. For example, gram-negative *Campylobacter jejuni, C. fetus* and *C. coli* are catalase-positive as opposed to the remainder of *Campylobacter* species, which are catalase-negative [40].

3.3.3 Oxidase Test

The presence of cytochrome oxidase enzyme in bacteria is detected using this biochemical test. Oxidase-positive bacteria, namely *Neisseria* spp., *Pseudomonas* spp. And *Campylobacter* spp., possess the enzyme responsible for the reduction of molecular O_2 into water at end of electron transport system pathway [47, 48]. The test involves the reduction of cytochrome C by a colourless oxidase reagent tetramethyl-*p*-phenylenediamine-dihydrochloride (TMPD) to produce oxidized TMPD radical, which is blue in colour [49]. Based on variability in composition of cytochrome C, cytochrome oxidase and entire enzyme system of electron transport chain, a bacteria can also be classified as variable oxidase-positive [50].

3.3.4 Urease Test

This biochemical urease test involves the identification of bacteria that can utilize urea as a sole nitrogen source to produce ammonia and CO_2 in the presence of the enzyme urease [51]. Excessive ammonia produced raises the pH of the culture medium above 8.0, which eventually changes the colour from yellow to bright pink

[52]. The test was designed to distinguish urea hydrolyzing, i.e. urease-positive (*Proteus* spp.), urease-negative bacteria such as, *E. coli*, and delayed urease-positive such as, *Klebsiella* or *Enterobacter* [53].

3.3.5 Nitrate and Nitrite Reduction Test

This biochemical test intends to identify bacteria capable of reducing nitrate (NO_3) to nitrite (NO_2) or free nitrogen. The culture medium is supplemented with nitrate or nitrite so as to facilitate the target bacteria to utilize it as a nitrogen source. If the nitrate reductase enzyme system is present in the target organism, it can reduce NO_3 to NO_2, which may be further reduced to various nitrogen products such as, NO, N_2O, N_2 and NH_3 [32]. The produced NO_2 reacts with sulphanilic acid to form diazotize sulphanilic acid, which eventually reacts with α-naphthylamine to forms water-soluble red azo-dye [54, 55]. The nitrate-positive organism (e.g. *E. coli*) develops red colour, whereas organism like *Pseudomonas aeruginosa* reduces both NO_3 and NO_2 and results in no colour or delayed reduction of NO_3 and NO_2 results in no colour after 48 h of incubation [56].

4 Overview of Different Media

A suitable culture medium is necessary to obtain optimal growth, storage or transportation of the selected microorganism. The culture medium provides the source of energy in the form of carbon, nitrogen, inorganic phosphorus, sulphur and different minerals to carry out required metabolic activities by the microorganism to grow and flourish. The function of different constituents that are commonly used in the culture medium for growth, isolation and identification of microorganisms are mentioned in Table 4.3. Based on the nature or habitat of the selected microorganism, the nutrient requirement of the microorganism varies so does the ingredients of the culture media (Table 4.4).

5 Conclusion

This chapter presents the detection and identification of microorganisms in food microbiology. Presently, conventional methods of counting and identification of microorganisms have been taken over by more rapid culture-independent methods, such as polymerase chain reaction, enzyme-linked immunosorbent assay, immune-chromatographic test strips, nucleic acid sequence-based amplification, loop-mediated isothermal amplification etc. Although considerable advancement is progressed, there are evidence where molecular techniques could fail to come up with desired precision to identify certain bacteria. The advantage of the conventional or

Table 4.3 Commonly used ingredients in culture media and their basic function for proper growth, isolation and identification of microorganism

Commonly used ingredient	Function
Glucose and other carbohydrates	Carbon source
Peptone	Carbon, nitrogen source
Beef extract, yeast extract	Source of carbon, nitrogen, vitamins, nucleotides, organic acids, minerals
Acetate, citrate, phosphate buffers	Maintain pH
Blood, serum, vitamins, nicotinamide adenine dinucleotide	Growth factor
Bile salts, dyes (eosin red, methylene blue), desoxycholate	Selective agent
Phenol red, neutral red	Indicator
Agar, alginate, gelatin, silica gel	Solidifying agent
4-methylumbelliferyl-β-D-glucuronide (MUG)	Fluorogen
X-glucuronide, Salmon-GAL	Chromogen
Glycerol	Cryoprotectans

Table 4.4 Overview of different culture medium commonly used in isolation, identification and maintenance of microorganisms

Based on the state		Example
Liquid media	No solidifying agent is used	Nutrient broth
Solid media	Agar between 1% to 2%	Nutrient agar
Semi-solid media	0.5% of agar	Motility test medium
Based on ingredients		
Defined or synthetic media	All components are known in exact	BG-11 medium
Complex media	Contains certain ingredients of unknown composition	MacConkey agar, Tryptic soy broth, Nutrient broth
Enriched media	Fortification of media	Blood agar, Chocolate agar
Selective media	Favour growth of specific microorganism	Endo agar, EMB agar, MacConkey agar
Differential media	Allows differentiation and identification between different groups of bacteria	Blood agar, MacConkey agar

culture-dependent method is based on its routine analysis of bacteria on specific culture medium, whereas the results obtained using molecular methods need to be compared with the homology of identified strain. In general, the conventional method is tedious indeed, but they ascertain the specificity of identified bacteria at a genus level, and it should be combined with the outcome of culture-independent method for determining precise composition of the target microorganism.

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Chapter 5
Enzyme-Linked Immunosorbent Assay (ELISA) Technique for Food Analysis

Pallav Sengupta, Chee Woon Wang, and Zheng Feei Ma

Abstract Enzyme-linked immunosorbent assay (ELISA) techniques employ a highly sensitive and specific form of immunological reactions, and this technique shows wide applications in food analysis. The versatility functions of ELISA techniques render them suitable to detect specific constituents in food, including the natural components, pesticide, therapeutic agents, beneficial and spoilage microorganisms, and toxins. 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. The post-production of food products requires proper authenticity testing to ensure that their labeling does not falsify their adulterations. ELISA is also suitable to validate such food adulterations thereby providing the consumers to make diet choice. The most used ELISA techniques in the food industry include indirect, sandwich, and competitive ELISA that use both polyclonal and monoclonal antibodies as necessary. ELISA provides a suitable complementary approach in food analysis and minimizes the use of sophisticated, expensive, and time-consuming techniques by maintaining the sensitivity and reliability of this technique. Thus, the present chapter aims to present the basics of the ELISA technique and its application in food analysis. Its application can significantly contribute to the food industry in securing food quality control and safety.

Keywords ELISA · Food adulterants · Food analysis · Food technology · Food processing · Microorganisms · Food spoilage · Flavor constituents · Food additives · Plant growth substances · Pesticides

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1 Introduction

Food safety is one of the major global concerns. Altered food quality does not only falsify economically but also renders the consumers susceptible to health issues [1]. Food analysis deals to detect and characterize food constituents. These provide information that is vital for proper knowledge of the factors influencing food properties. Such factors include the presence of microorganisms (pathogens and spoilage microbes), food toxins, beneficial and undesirable food components, and adulterants [2]. Food quality testing assures the production of consistently safe and nutritious food products. It also enables the consumers to make choices regarding food intake, since these possess labeling information according to their measured quality [3].

Testing food products involved verification at various strata of product manufacturing, from raw material harvesting, processing, and storage until consumption [3]. Choice of food analytical technique relies upon the specificity, sensitivity reliability, simplicity, time, and finally cost effective. The conventional chromatographic methods require extensive procedural hassles, lacked enough sensitivity, and relatively time-consuming [4]. Genetic testing is the most specific food testing methods, but its limitations lie in the fact that these are expensive and need high expertise [5]. Immunoassays are the most convenient options for food testing. Among the immunoassays, enzyme-linked immunosorbent assay (ELISA) is the most widely accepted technique used for the detection of food components due to its simplistic operations, rapid detection, low-cost equipment, and high specificity [6–8]. The most applied ELISA variants in food testing are the indirect, competitive, and sandwich ELISA [8]. The indirect ELISA involves the use of two antibodies, one is antigen-specific while the other is enzyme coupled. The secondary antibody produces the detected signal following binding with fluorogenic or chromogenic substrate. The sandwich ELISA, as per its name, causes antigen to be bound between its two antibodies, namely, the capture antibody and the detection antibody. The latter is enzyme coupled that initiates biochemical reactions. Competitive ELISA is used to determine the concentration of an antibody or antigen in a test sample through detection level of interference in a framework of predetermined signal output. The sample antibody or antigen competes with a reference to bind with a limited quantity of their labeled specific antigen or antibody, respectively. Thus, the more the presence of sample antigen concentration, the lesser is the output signal, which signifies that the signal output is inversely related to the antigen in the food sample [8].

This chapter presents a concise overview of the principle of the ELISA technique and its applications in food testing, thereby including the use of ELISA in (a) detection of beneficial and harmful food ingredients, (b) verification of the quality of constituents used in food production and processing, (c) quantification of spoilage microorganisms during storage of food, and finally, and (d) testing the authenticity of food by proper detection of adulterants.

2 ELISA in the Detection of Food Constituents

2.1 General Technique of ELISA

2.1.1 Principle

ELISA presents antigen–antibody reaction. ELISA was introduced by Peter Perlmann and Eva Engvall in 1971 at the University of Stockholm, Sweden [9]. This is a popular laboratory technique used to test antibody or antigen concentrations in different samples (Fig. 5.1). ELISA is a plate-based method, where an antibody–enzyme conjugate interacts with a chromogenic substrate. It is used to identify and measure substances such as peptides, antibodies, enzymes, and hormones. Since various pathogens, flavors, and several other food constituents are identifiable via characteristic peptides, ELISA can be easily detected to determine the quality, safety, and authenticity of food products [10]. ELISA utilizes different enzymes including alkaline phosphatase (AP), β-galactosidase (BG), and horseradish peroxidase (HRP). Typically, 96-well polystyrene plates are used in ELISAs. Every well contains a specific test sample incubated into it. A positive control and a negative control sample would be included among the 96 samples [11]. A suitable antigen or antibody on the solid surface captures antibodies or antigens contained in the serum. After some time, serum and unbound antibodies or antigens are washed with consecutive use of wash buffers [11].

A secondary antibody attached to an enzyme-like alkaline phosphatase or peroxidase is added to every well in order to identify the bound antibodies or antigens. The secondary antibodies are washed off after an incubation period. In addition to an appropriate substrate, it reacts with the enzyme in the well, producing its

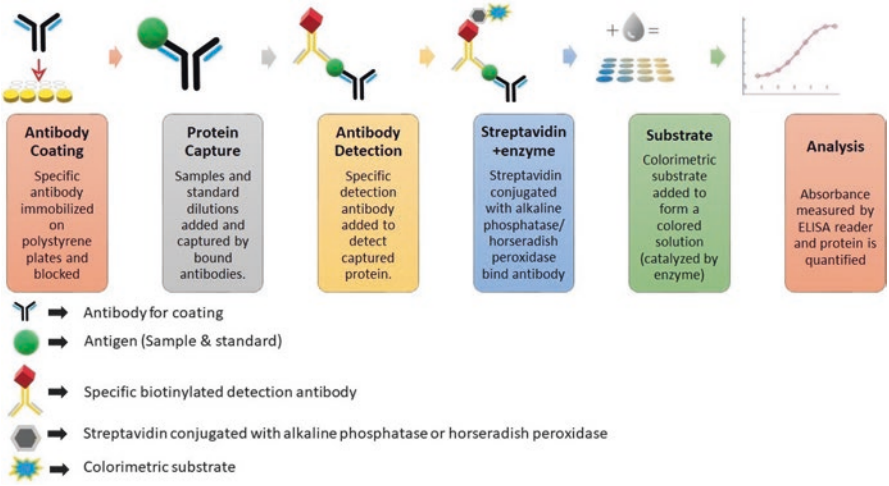

Fig. 5.1 General procedure of ELISA

characteristic color. This produced color can be measured as a function or amount of antigens or antibodies in the specified sample. The optical density corresponds to the intensity of the produced color in the wells, and it is measured spectrophotometrically at a wavelength of 450 nm. The color intensity shows how much antigen or antibody is present [11].

2.1.2 Main Types of ELISA

A number of changes to the basic protocol can be applied to ELISAs, and accordingly, they can be classified into direct, indirect, sandwich, or competitive ELISA. The key step is the immobilization of the antigen of interest by direct adsorption to the testing plate or by means of a capture antibody on the surface [12]. The antigen is then detected either directly (primary antibody labeled with enzyme) or indirectly (secondary antibody labeled with enzyme). The antibodies are generally labeled with AP or HRP. For performing the ELISA with HRP or AP conjugate, a wide range of substrates is available. Substrates are selected based upon the necessary assay sensitivity and the available signal detection equipment (i.e., spectrophotometer, luminometer, or fluorometer) [12].

Among the standards that have included variations in capture and detection, it is important to distinguish between the different available detection strategies. The detection step (as direct or indirect detection) is largely determined by an antigen captured to the plate (by direct adsorption to the surface and a precoated "capture" antibody as in the sandwich ELISA) (Table 5.1).

2.1.3 Analysis of Data

ELISA results can be categorized according to the data form obtained. Qualitative ELISA determines if the antigen is in the sample or not. It needs a blank well without antigen or a control antigen unrelated to it. Semi-quantitative ELISA allows the relative antigen levels to be compared between the samples [11]. Quantitative ELISA allows the quantity of antigen present in the specimen to be measured. It involves the comparison of the sample measured values with a standard curve provided by serial dilution in a known concentration of a purified antigen [13].

The norm or calibration curve for the measurement of antigen concentrations in the sample is an aspect of the quantitative ELISA. The standard curve is obtained from the plotting of known levels of the antigen reference against the read-out at each concentration (usually 450 nm optical) [10].

Table 5.1 Basic categorization of ELISA with their procedures, advantages, and disadvantages

ELISA	Procedure	Advantages	Disadvantages
Direct	Target antigen is coated to the ELISA plate and is directly detected by an antibody-enzyme conjugate	• Quick method as it uses only one antibody and very few steps • Secondary antibody cross-reactivity is eradicated	• Chemical bonds lead to adherence of non-adherent cells on coverslip • Immunoreactivity of primary antibody may be impaired by enzyme or tag labeling • Labeling of primary antibodies is both expensive and time-consuming • Lack of flexibility in primary antibody label selection for individual experiment • Amplification of signal is minimum
Indirect	• Target antigen is coated to the ELISA multi-well plate and needs two staged detection process. • Addition of an unlabeled primary antibody specific to the target antigen • Addition of an enzyme-labeled secondary (anti-species) antibody binds to the primary antibody	• High signal amplification as there are many secondary antibodies to bind to the primary ones • High flexibility in selection of secondary antibody since they can be used against variety of primary antibodies	• Chemical bonds lead to adherence of non-adherent cells on coverslip • There are chances of cross-reactivity with the secondary antibody, leading to nonspecific signal • Requirement of an extra incubation step
Sandwich	• Needs matched antibody pairs with each identical antibody for separate, nonoverlapping part of the antigen molecule (epitope) • ELISA plate is coated with the first antibody (capture) • Adding sample solution to the well • Adding secondary/ detection antibody to detect and quantify the antigen/protein in the sample	• High specificity in analyte protein capture and detection • High suitability complex samples as the method does not need analyte purification • Very flexible and sensitive experimental setup	The design is challenging since it is often difficult to obtain two antibodies for a single analyte that bind to different epitopes on the analyte as well as to be effective together

(continued)

Table 5.1 (continued)

ELISA	Procedure	Advantages	Disadvantages
Competitive	• ELISA plate is coated with capture antibodies reactive to the molecule of interest • Sample with target protein and enzyme conjugated recombinant protein (competing molecule) are added to the coated wells • Quantity of enzyme conjugated component is constant in each well • Thus, the quantity of target protein or the protein of interest in sample is the only determinant of binding ratio of enzyme conjugated protein vs target protein • After incubation and washing to remove unbound antibodies, the enzyme substrate is added. It transforms into a blue precipitate and its amount is directly proportional to the quantity of enzyme • The precipitate turns yellow with acid stop solution and the optical density is measured	• Sample processing is not needed • Dilution of sample and sample matrix have less impact on the result • High specificity and consistency • Highly flexible experimental setup as it can be based on sandwich or direct/ indirect ELISA	• Negative controls may produce positive results if blocking solution does not work. In this case, the secondary antibody or analyte can bind to any open sites • Dilution of primary antibody may highly strengthen or weaken signal

3 Food Constituents Measured by ELISA

3.1 Flavor Constituents in Food

A better perception of the origin and distribution of different flavor constituents in food, especially natural and developed components, provides a wider scope for the manipulation of its production and processing. For instance, the flavor responsible for bitterness in grapefruit is due to the presence of limonin-3, which can be detected by immunoassays [14]. Its concentration is higher in seeds when compared to the embryos of the plants/trees. Among the immunoassays, ELISA is used to rapidly screen for trees generating low levels of limonin and also for determining the quality of fruits and their processed products [15]. Similarly, the flavor that imparts

sweetness to the miracle fruit (*Richadella dulcifera)* is Miraculin that can also be effectively detected in the fruit extract by ELISA [16].

3.2 Naturally Occurring Undesirable Compounds

Apart from harmful adulterations of food substances and beneficial natural components, there are undesirable compounds that occur naturally in several food products. Almost 6000 plant species have been estimated to produce alkaloids [17]. There are several hundreds of naturally occurring individual pyrrolizidine compounds found in food, and these are reported to exert hepatotoxic, carcinogenic, mutagenic, and teratogenic effects [18]. Since these components are consumed by humans in varied forms, it is essential to perform quantitative assessment of these substances in foods.

Different varieties of potatoes contain diverse proportions of toxic glycoalkaloids [19]. Potatoes are one of the most consumed vegetables worldwide, and the glycoalkaloids, present in the potatoes, are suggested to be resistant to boiling, frying, baking, and even microwave heating [20, 21]. Therefore, it is important to quantify the glycoalkaloids present in the potatoes and select the varieties for consumption, with the least content of glycoalkaloids. For this purpose, the horseradish peroxidase ELISA proved to be the most effective method to estimate glycoalkaloid content in potatoes [22]. This method is widely used in the food industry for the rapid screening of huge quantity of potatoes with glycoalkaloids contents above its tolerance limit (i.e., 20 mg/100 g of potatoes). It also finds relevance in breeding new healthy potato varieties [19].

Certain carbohydrates like yams and other root crops also contain toxic alkaloids like saponins, retronecine, and monocrotaline, which can be quantified by ELISA [23]. ELISA is also a potential method in the detection of undesirable hormone content in food, such as steroids like androgen, progesterone, and estrogen [24].

3.3 Nutritionally Important Constituents

Immunoassays like ELISA are relatively inexpensive and a fast method to analyze tissue distribution of vital food constituents followed by ingestion [20]. ELISA is also widely used to estimate food pantothenic acid content and has been proved to be a better method than other standard microbiological methods (Table 5.2) [25].

Table 5.2 ELISA for the detection of spoilage microorganisms and viruses in food crops

Microorganism/virus	Crop/Food	References
Humicola lanuginosa	Rice	[44]
Ophiostoma ulmi	Cultures	[45]
Phytophthora megasperma	Soybean	[46]
Rhizoctonia solani	Soybean	[46]
Xanthomonas campestris	Citrus	[47]
Beet necrotic yellow vein virus	Beet	[48]

4 ELISA in Food Production and Processing

The major concerns regarding postharvest consequences of food involve senescence in vegetables and fruits, microbial contamination of foods, and chemical degradation of vegetables, fruits, animal products, fish, and meat products [26]. Improvement in food availability and its cost-effectiveness highly relies upon the extent to which such deleterious consequences of food damage are prevented. ELISA is a potential method to detect as well as quantify the constituents associated with food spoilage, such as enzymes, growth factors, and microorganisms, and contributes to the improvement of food quality (Fig. 5.2).

4.1 Detection of Plant Growth Substances

Perception about specific functions of plant growth substances is important in assessing quality management of plant-derived food products. In this regard, ELISA and other immunoassays find immense application as they are used to determine plant growth regulators [27]. Some examples of such regulators include cytokinins, isopentenyl adenosine, zeatin-riboside, 2-(methylthio)-isopentenyl adenosine, and related compounds in oranges and grapefruit [28], gibberellins in *Hyoscyamus niger* L. [29], and indole-3-acetic acid in various food crops [30]. ELISA is used in the quantification of indolic nitrogen [31] in crops like maize, oats, and avena coleoptiles as well as the growth inhibitor abscisic acid in citrus fruit [32]. This method is also suited to determine cis (+)-abscisic acid levels in plants [33].

4.2 Estimation of Food Constituents with Processing Characteristics

ELISA is a popular immunoassay for determining food constituents vital for maintaining food quality while processing. The sandwich inhibition ELISA has been used in the quantification of rye (water-soluble pentosans) which is important for

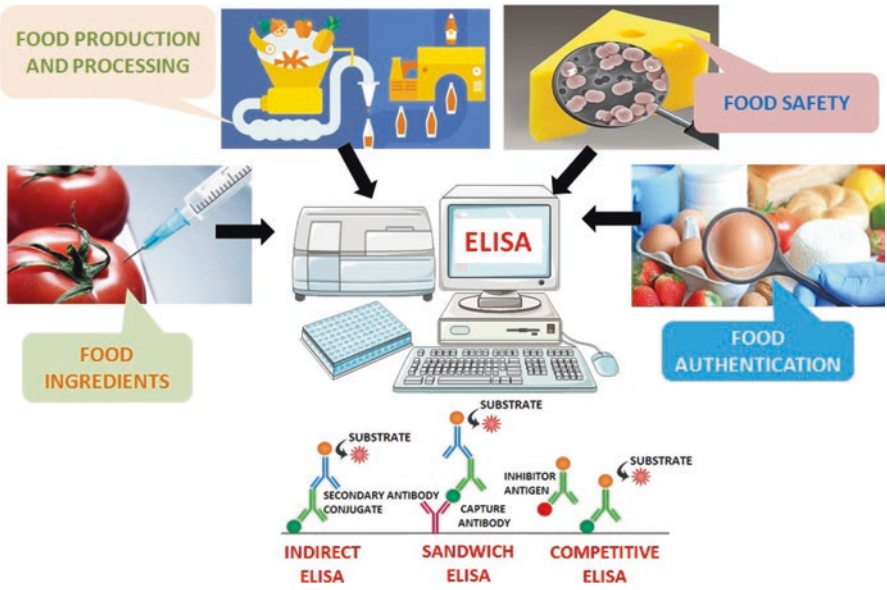

Fig. 5.2 Application of ELISA in food technology

desirable dough characteristics [34]. Moreover, food constituents such as globulin, gliadin, and glutenin polypeptides in wheat, protein content in barley, and soybean seeds are essentially determined by immunoassays to ensure such characteristics are restored after processing [35].

4.3 Spoilage of Foods and Food Crops

Microbial and parasitic contaminations are the common causes of food spoilage at various strata of food production, processing, and storage. A vast array of microbes have been identified in mediating food spoilage including molds, bacteria, and viruses [36]. ELISA is a useful method to be used in the early detection of such food-spoiling microbes so that food spoilage is controlled and/or prevented.

ELISA can also be used to determine mold counts in food products by raising antibodies against the antigenic factors in molds, estimating both viable and nonviable microbes [37]. This is employed in the detection of fungal and bacterial population and their virulence factors in food, such as *Pseudomonas* species in crops [38], *Phytophthora megasperma* f. sp. *glycinea* and *Rhizoctonia solani* in soybean plants, *Legionella pneumophila* detection, sulfate-reducing bacteria in canned vegetables, etc. [37]. Immunoassays, such as ELISA, thereby can provide needed insights into the pathogenic contaminants in food and aid initiation of timely management strategies against the diseases incurred by such microbes.

Unlike the presence of bacteria, fungi, and molds in foods, viral infections are far more deleterious with more severe consequences. Early and proper detection of viruses in crops is needed to avoid massive food losses. ELISA is a convenient immunoassay for the determination of viral contamination of food products, especially crops and seeds [39].

Meat and fish serve as potent sources of pathogenic microbes as well as parasites that may use human consumers as appropriate host for the completion of their developmental cycles. ELISA is useful in the detection of such pathogenic microorganisms in animal food before the slaughter process. Some of such pathogens include *Toxoplasma gondii* in sheep, rabbits, cattle, and pigs [40]; *Trichinella spiralis* in swine [41]; *Stephanuras dentatus* in pigs [42]; *Mycobacterium paratuberculosis* in goat [43]; and *Brucella* in cattle [44]. Timely diagnosis of pathogens in animal foods leads to the prevention of innumerable health hazards and economic loss to the food industry.

4.4 ELISA in Food Processing

ELISA is applicable in the identification of essential and helpful microorganisms required for food processing. Certain microorganisms are vital to obtain desirable nutritional traits in fermented foods. ELISA can be used for the assessment of slime-forming encapsulated *Streptococcus cremoris* for milk fermentation [45]. ELISA helps to detect specific immunoglobulins against pathogenic microorganisms which on introduction to processed foods to increase their resistance to those microorganisms [45, 46]. For example, human infant formulae food is enhanced by the introduction of anti-*E. coli* immunoglobulin (isolated from immunized chicken). Moreover, ELISA is employed in the determination of various enzyme activities needed in food production technology [47, 48].

5 ELISA in Food Safety

ELISA is extensively used in the detection and estimation of various food contaminants, additives, and adulterants that render food unsafe. The components that are vital to analyzing are the potential toxins from the pathogenic microbes, pesticides, anabolic agents, antibiotics, and other food adulterants.

5.1 Common Deleterious Food Components

The trend of consumption of preserved food and ready-to-eat items (i.e., heat treatments just prior to consumption) leads to higher toxic retention in food. Employing test animals to identify toxins and deleterious microorganisms produces non-uniform results. Moreover, such methods are expensive and involve ethical issues. On the contrary, ELISA overcomes these limitations and provides the needed sensitivity in the detection of the harmful food components (Table 5.2) [49–53].

5.1.1 *Clostridium perfringens* Enterotoxins

Highly specific and sensitive ELISA is employed for the detection of *C. perfringens* enterotoxins in human stools that is causative of gastroenteritis [54]. The same principle is used to detect the toxins in food [55] (Table 5.3).

5.1.2 *Clostridium Botulinum* Toxins

Given the high potency of botulinum toxin, immunoassays employed for its detection should bear high sensitivity. The amplified ELISA with monoclonal antibodies is suitable for its detection without any cross-reactivity with other potential bacterial toxins [56]. Thus, ELISA finds application in the food industry for detection of botulinum toxin in foods, more than any other immunoassays [57, 58] (Table 5.3).

Table 5.3 Application of ELISA in the assessment of pathogenic components, pesticides, anabolic and therapeutic agents, adulterants, and objectionable constituents in food

Agents	Specimen	Minimum detection level	References
C. Perfrigens enterotoxin A	Foods	5 ng/g	[50]
C. Botulinum toxin A	Salmon, beef	5–10 × mouse LD_{50}/mL	[53]
C. Botulinum toxin A	Pork	33 × mouse LD_{50}/mL	[51]
S. aureus enterotoxin A, B, C, D	Foods	0.1 ng/mL	[55]
Aflatoxin b1	Corn, wheat, peanut butter	3 ng/g	[73]
Atrazine	Water	50 pg/mL	[81]
Diflubenzuron	Milk	1 ng/mL	[82]
Iprodione	Foods	0.2 ng	[15]
Chicken adulterant	Beef, pig	1–30%	[92]
Impala adulterant	Goat	1–10%	[93]
Pig adulterant	Beef products	0.5%	[90]
Pork adulterant	Processed meat	1%	[94]
Quinine	Soft drinks	1 pg/mL	[95]

5.1.3 *Staphylococcus aureus* Enterotoxins

ELISA is the most commonly used immunoassay besides radioimmunoassay (RIA) to detect *Staphylococcus aureus* in food [59]. ELISA test has been used for the detection of *S. aureus* enterotoxins A, B, C, D, and E in meat samples [60]. The use of double-antibody solid-phase assay as well as enzyme amplification further improves the sensitivity of ELISA for this purpose [61]. Monoclonal antibodies are found to impart higher sensitivity and detectability of immunoassay, being capable to interact with every serotype of *S. aureus* enterotoxins. Thereby, indirect double sandwich ELISA with monoclonal antibodies rather than polyclonal antibodies has been reported to be the most sensitive in this case. It also highly enhances the range of toxin detection [62]. Thus, ELISA is reliable enough to help in the quality control of suspicious food products and raw food materials [63].

5.1.4 *Salmonella*

The development of immunoassays for *Salmonella* detection has received much attention in the last few decades [64–67]. *Salmonella,* fecal pathogenic microorganisms residing in the human and animal alimentary canal, is a potential food contaminant posing severe health problems [68]. Since its conventional culture methods are burdensome, their detection required extensive research. Given the fast growth of these bacterial species in perishable animal food and the high cost of storing suspicious food for clearance, a rapid cost-effective detection method for these bacteria is required. To meet such requirements of a fast, inexpensive yet reliable method, ELISA is used that detects *Salmonella* at 10^5 to 10^6 per milliliter of a one-day enrichment culture. It has been stipulated that horseradish peroxidase provides greater convenience than alkaline phosphatase as enzymes for color detection used in sandwich ELISA for the quantification of *Salmonella* in food [69].

5.1.5 *Listeria monocytogenes*

Detection methods of *Listeria monocytogenes* have been developed independently in the United States [70], United Kingdom [71], and Canada [72]. Among these, the ELISA developed in the United States is named Listeria-Tek and is used to detect these species in milk and meat [70]. With the advent of ELISA, the detection time of *Listeria monocytogenes* in animal products has narrowed down to 2 days from almost a month.

5.1.6 Other Microorganisms

Specialized immunoassays are applied for the detection of different other pathogenic microbes. ELISA is employed to detect *Yersinia enterocolitica* in milk, *Enterobacteriaceae* in drinking water [73], *E. coli* in oysters and chicken [74], and the biotype and serotype correlations of *Campylobacter jejuni* and *E. coli* in patient's stool samples [75].

5.1.7 Mycotoxins

Mycotoxins are small non-immunogenic molecules that elicit immunogenicity only by their conjugation with relevant proteins. Conventional detection methods of mycotoxins in food are tedious and prolonged chromatographic processes. Thereby immunoassays are adopted for their faster detection. ELISA reportedly portrays sixfold faster detection than high-performance liquid chromatography (HPLC) analysis, while also being more cost-effective [76]. While both RIA and ELISA are able to detect food mycotoxins at parts per billion levels, ELISA has been reported to bear higher accuracy and sensitivity. ELISA can estimate numerous mycotoxins in foods, such as aflatoxin BI (AFBI) in wheat, com, and peanut butter [77, 78]. The sensitivity of this ELISA procedure is further enhanced by the development of monoclonal antibodies against aflatoxins [79]. If the food extracts are cleaned, it imparts even higher sensitivity to ELISA detection. Hence, the application of ELISA in food mycotoxin analysis reduces the detection time to about 10 min along with the increase in sensitivity to parts per billion levels.

5.1.8 Mushroom Poisoning

Mushroom poisoning is becoming common worldwide due to the increased availability and intake of varied forms of mushrooms. The identification of ingested mushrooms via mere observation of clinical symptoms and classification of the samples are not adequate due to less specificity and more time factors. Such methods might delay the time of action and proper treatment leading to high risk of fatal outcome or chronic impairments to organs, especially to the liver and kidneys. For rapid, specific, and reliable detection of amanitin (a potent mushroom poison), ELISA has been introduced to determine mushroom poisoning in serum, urine, and fecal samples [80]. This method using ELISA provided greater specificity and sensitivity when compared to other immunoassays. Moreover, this method is less arduous as ELISA kits do not need the use of radioactivity or complex extraction procedures.

5.1.9 Algal and Seafood Toxins

Innumerable marine dinoflagellates species produce toxins that infect human via marine food products and severely affect human health. ELISA provides an efficient detection option of such toxins, such as neurotoxins, brevetoxin T17, and T34 (at 600 pg levels) using their specific antibodies [81]. Indirect ELISA is employed for the detection of saxitoxin [82]. Moreover, ELISA using polyclonal antibody is also available to evaluate saxitoxins and other gonyautoxins [83], while monoclonal antibody ELISA can be applied in the quantification of okadaic acid in fish. Consumption of various types of fish and seafood has been reported to cause poisoning in different geographical areas [84, 85]. ELISA is a best-suited detection method to rapidly screen marine food samples for toxin contents to prevent toxin ingestion by humans.

5.2 Pesticides

Pesticides, employed for crop improvement by prevention of pest-induced spoilage, have undesirable impact on human health. Assessment of food pesticides by conventional methods includes chemical analyses using HPLC, gas chromatography (GC), or gas chromatography-ion mobility spectrometry (GClMS). Immunoassays on the other hand provide more convenient and accurate assessments for pesticides in food products. ELISA and RIA are able to detect pesticide residues in food even at picograms or nanograms per gram of food (Table 5.3) [20, 86, 87] The problems involved in food pesticide analysis include environmental factors, processing hazards, and enzymatic actions upon pesticides that may lead to their degradation. Such transformation may take place during the extraction of pesticides from food products as observed with benomyl conversion to methyl-2-benzimidazolecarbamate [88]. The toxic derivatives of pesticides may not be detected via ELISA or other immunoassay if their transformations render them structurally different from the parent molecules. Moreover, cross-reactivity also may interfere with the results of ELISA for specific pesticides, but it is still a suitable method for estimation of the total food pesticide contents. Using a pesticide-specific antibody may be used be to estimate quantify pesticide in a number of food products, such as benomyl in lemon, grapes, orange, apple, tomato, peach, grapefruits, and cucumber; dichlorfop-methyl in sugar beet, soybean, wheat, and milk; metalaxyl in potato, avocado, squash, cucumber, and tomato; and triadimefon in apple, pineapple, pear, and grapes [88–90].

5.3 Anabolic Agents

Anabolic steroids (or anabolic–androgenic steroids) refer to steroidal androgens. These include synthetic androgens and natural androgens like testosterone. Anabolic agents in food products may severely disrupt human endocrine balance and can also be potentially carcinogenic [91]. Thus, it is essential to employ a highly specific and sensitive method to quantify food anabolic steroid content at levels as small nanogram or picogram. The difficulty in the analysis of food anabolic content is mainly the presence of similar endogenous hormones and rapid degradation or conversion of the anabolic agents after ingestion through food. The analysis should include the principal anabolic agent and its derivatives along with the metabolic products in the host animal tissues. When a second antibody is employed in ELISA, it doubles the assay sensitivity for the detection of anabolic agents in foodstuffs.

5.4 Therapeutic Agents

The presence of antibiotic residues in food products may result in the selection as well as the occurrence of antibiotic/drug-resistant pathogens paving the way to adverse health effects and even epidemic diseases. It is thereby important to monitor residual antibiotics in food, which can be mediated via various immunoassays, notably enzyme immunoassay (EIA), RIA, and ELISA.

5.5 Adulterants

The addition of inexpensive substitutes to expensive food products for financial profit, health issues, or religious factors often leads to adverse health consequences and inappropriate pricing of foodstuffs. The adulterants resemble the original product in terms of chemical composition, appearance, and organoleptic characteristics, hence their identification becomes difficult. The high specificity of immunoassays like ELISA and agar gel immunodiffusion renders them suitable for the identification of food adulterants. Such samples are undesirable or objectionable components in meat products, detection of adulterated meat with phylogenetically alike pairs of animal species like sheep and goat, horse and donkey, and buffalo and cattle [92]. Detection level in ELISA has been improved by the use of muscle proteins rather than sera as antigens to develop antibodies and employing monoclonal antibodies for the process [93]. Since muscle proteins are altered structurally after being heated, thermostable proteins have been used for the generation of antibodies for immunoassays [94]. Moreover, the presence of soya, barley, wheat, rye, or oat proteins or shrimp, and other seafood as adulterants in meat can also be detected using ELISA (Table 5.3) [95–100]. While applying immunoassays for adulteration

assessment of meat products, it is sufficient to qualitatively detect undeclared meat in the food products. The high sensitivity of ELISA does not pose any major issue, as meat is generally adulterated at large scales.

6 ELISA in Food Product Authenticity Testing

Authenticity testing of various food items like fish, milk, or meat is essential for proper assessment of their value, to determine the presence of any harmful constituents, and to ascertain customer safety against any fraudulence of the food industry. Conventional techniques for food authenticity include chromatographic and electrophoretic methods. These are inconvenient for routine sample analysis being relatively costly and complex methods requiring lots of time [101, 102]. Genetic techniques are the most sensitive and specific ones for authenticating foodstuffs. But, these techniques involve an expensive laboratory setup and a high level of expertise. Immunoassays seem to be the best alternative for food authentication testing. Among them, ELISA is the most common method in detecting food authenticity due to its simplicity, high sensitivity, specificity, and less testing time [103, 104].

6.1 Meat Products

Adulterations of meat and meat-based products have been a common concern in the retail market. The meat species of origin should be specifically identified to ensure consumer safety. ELISA is suited for the identification of meat-based products derived from different animal species, with the help of antibodies raised against animal serum, muscular proteins, and even thermostable proteins [105]. Both qualitative and quantitative analyses can be made using ELISA in meat species detection. For example, quantitative assessment of adulterated raw ground beef with pork and development of monoclonal antibodies for porcine thermostable muscle protein to quantify raw porcine meat as well as cooked or heated meat. ELISA is also used to detect the substitution of expensive proteins with cheap proteins like soya proteins or oat, barley, or wheat proteins.

The Indirect and Sandwich ELISA are mostly used in meat authenticity testing. Sandwich ELISA is widely employed in commercial ELISA kits since this allows direct addition of the dilute sample in the testing plate. More recently, a polyclonal antibody is added in Sandwich ELISA sample, and in the case of using two monoclonal antibodies, it is essential for the antibodies to possess affinity in addition to specificity. Commercially available Sandwich ELISA kits use the principle of recognition of cooked meats by the presence of specific glycoproteins [106]. The ELISA kits are found to be effective in detecting adulterations in beef, pork, mutton, poultry, and several other meat products as well as in canned meat-based items [107].

6.2 Fish and Fish-Based Food Products

It is difficult to identify fish species once the external characteristics are removed and processed following smoking, filleting, and canning. Thus, there is a high chance of fraudulent attempts of the food industry to mix low-priced fish with high-priced ones. Fish-based food authentication testing is usually mediated through genetic testing and ELISA [108]. The ELISA techniques being simpler and less expensive find advantage over PCR-based techniques. Indirect ELISA uses polyclonal antibodies against the muscular protein of various fish like flatfish species (European plaice, sole, flounder, Greenland halibut) [109], sardines [110], and clam fishes [111]. Moreover, monoclonal antibodies against fish muscle proteins are employed in the detection of red snapper [112] and raw and cooked adulterated fish food products [113].

6.3 Dairy Products

Fraudulent adulterations of inexpensive bovine milk in cheese have been associated with health issues, ethical, religious, financial, and cultural offenses. Therefore, proper evaluation of milk species in dairy product is essential. Many high-quality cheese brands prepared using goat's or sheep's milk have been registered by the European law with a Protected Designation of Origin (PDO) [114]. However, cheese can also be made using a mixture of high-quality milk with bovine milk, but they should be labeled as PDO quality. Such cases require accurate detection methods like ELISA for determining the pure quality of milk products [46]. For instance, Hurley et al. [46] put forth a sandwich ELISA and an indirect competitive ELISA to detect undeclared bovine milk in high-priced milk from sheep, goat, buffalo, and ewes. Indirect ELISA using monoclonal antibodies against bovine β-casein [45] imparted greater specificity in the detection of adulterated milk products [115]. There are plenty of ELISA kits available for species authentication in dairy products, which serve as a quick, reliable, and sensitive option for the authentication testing than any other techniques.

6.4 Fruits and Beverages

Falsification of fruit beverages is relatively easier than adulteration of other food products. Adulteration can range from water dilution of the main fruit extract to substitution with the juice of other cheap fruits or inexpensive ingredients such as acids, colorants, and sugars [116]. Thus, to ensure the safety of the consumers, appropriate and highly sensitive analytical tools like ELISA have been used to detect adulteration in juices. Sass-Kiss and Sass [117] prepared polyclonal

antibodies for peel and juice peptides of orange and grapefruit, which are employed in immunoassay allowing better evaluation of adulterated fruit products.

6.5 Genetically Modified Foods

Traceability systems record food product histories that are used both for product marketing and general health protection. Implementation of such a process requires highly effective techniques for every step of food harvest, processing, and storage [118]. Regulation (EC) 1830/2003 of the European Parliament and the Council suggests that all food products from genetically modified organisms (GMOs) should undergo proper tracing [119]. This enables accurate labeling of such food products. Thus, food products, which contain any DNA or protein product of genetic alterations should be specifically labeled. For this purpose, the presence of GMOs in foods, feeds, crops, and food ingredients are mediated via reliable, rapid, and sensitive techniques that include ELISA. This is mostly employed to quantify the proteins expressed by variety of processed fractions of modified crops such as dried soybean powder or soybean toasted meal [120]. The ELISA test kits for detecting GMOs are best suited for raw agricultural foodstuffs or food products that are slightly processed but they are not recommended to be applied for highly processed foods [121].

6.6 Irradiated Foods

Irradiation of food is the procedure where food products are subjected to treatment with X-rays or γ-rays and other sources of electron beams. It aids the reduction of a number of food pathogens, increases the time for food storage by inhibiting spoilage by microbes, delay ripening of fruits and sprouting of certain vegetables, and also protects food from insects [122]. With the enforcement of the treatment with ionizing radiation must be labeled accordingly, the need for proper detection of irradiated food surfaced. Besides other potent genetic methods, the less expensive ELISA can be applied for this purpose. Irradiated food often possesses dihydrothymidines (DiHT), which are transformed from DNA thymidines. ELISA can operate either by using antibodies against specific modified DNA bases in food products or competitive ELISA by using monoclonal antibodies against DiHT.

7 Conclusion

The present chapter leads to the consensus that ELISA has a broad spectrum of use in the food industry. Its application ranges from the detection of growth regulatory constituents of food crops, desirable and harmful components of raw materials, and

different ingredients of food processing to finally food authenticity testing. The development of more varied monoclonal and polyclonal antibodies for ELISA to target innumerable other food components will further elevate the scope of food testing with such a convenient, cost-friendly, rapid, and sensitive process like ELISA.

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Chapter 6
Vitek: A Platform for a Better Understanding of Microbes

Ismail M. Al Bulushi, Zahra S. Al Kharousi, and Mohammed Shafiur Rahman

Abstract Vitek systems are routinely used to identify clinical and environmental microorganisms and determine microbial susceptibility to antibiotics. The performance, accuracy, and reliability of these systems have been widely evaluated and presented in the literature. This chapter aims to introduce Vitek as a microbial identification and antibiotic susceptibility detection system and elucidate the principle of microbial identification. In addition, it presents conclusively the accuracy and reliability of Vitek systems to identify clinical and environmental microorganisms. It overviews the studies performed in the last 10 years. Vitek was launched in 1979 to identify pathogens, and later it was developed to different versions, such as Vitek 2, Vitek 2 compact, and Vitek MS. These systems identify microbe based on the developed color as a result of microbial utilization of the substrates in the cards and ionization of microbial proteins. Vitek systems provide good identification for wide ranges of clinical and environmental bacteria, yeasts, and molds such as staphylococci, enterococci, fermenting bacteria and Enterobacteriaceae, *Candida* sp., and *Aspergillus* sp. In addition, it detects microbial susceptibility and resistance to different antibiotics. In general, Vitek system's performances, accuracy, and reliability agreed with the other phenotypic, proteolytic, and genotypic techniques; however, in most cases, this concurrence has been found to be dependent on genus species. Some limitations in Vitek systems to identify certain microbes have been reported. Although these limitations have been mainly attributed to the capacity of the system's database and other factors, such as microbial age, purity, and load and user skills. It is established that the Vitek system could be used for routine identification of common microbes in food and clinical laboratories.

Keywords Microbes · Microbial identification · Rapid test · Gram-negative · Gram-positive · Clinical application · Environmental application · Phenotypic · Proteotypic · Genotypic · Accuracy · Reliability

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1 Introduction

New microbes are emerging in foods, and resistance to antibiotics is evidenced. There is a need for rapid identification of food poisoning outbreak causatives. Therefore, accurate, rapid, and simple microbial identification techniques are becoming an essential requirement to ensure food safety. Broadly, microbial identification techniques could be divided into genotypic, proteotypic, and phenotypic techniques. Different techniques, such as Vitek, API, biolog, ATP, and biotyper, are being developed within the phenotypic and proteotypic techniques for reliable microbial identification. In fact, these techniques differ in their principles, reliability, accuracy, simplicity, purpose, and cost. Vitek with its different versions is widely used in food and clinical laboratories for rapid and accurate microbial identification, such as Gram-positive bacteria, fermenting and non-fermenting Gram-negative bacilli, yeast and yeast-like organisms, and *Neisseria*, *Haemophilus*, and other fastidious Gram-negative bacteria. It uses different types of cards, which subject the microbes to different chemical substrates and evaluate their abilities to use such chemicals to identify them.

Vitek also has been used to determine microbial susceptibility and resistance to different antibiotics. This could be a useful guide to select a proper antibiotic for treatments. Despite its clearance by the FDA and high level of accuracy, the performance of Vitek was intensively evaluated by environmental and clinical studies. This chapter introduces Vitek as a microbial identification technique and elucidates the principle of microbial identification. It also evaluates the efficiency and limitations of Vitek in the identification of environmental and clinical microorganisms as well as elucidates the ability of Vitek to determine microbial susceptibility and antibiotic resistance. In general, this chapter provides useful information gained over the last 10 years by food and clinical microbiologists.

2 History and Development

The company bioMérieux (Marcy l'Etoile, France) has a long history of using innovative technology in the field of in vitro diagnostics and provides solutions for environmental and clinical microbial works. As one of its systems, Vitek has been developed continuously to enhance microbial identification and characterization. It was first launched in 1979, although it was initially developed to detect and identify pathogens from astronauts in spacecraft [1]. The Vitek system includes Vitek® 2 60, and Vitek 2 XL that can accommodate 60 and 120 cards, respectively. It also includes Vitek 2 compact 15, Vitek 2 compact 30, and Vitek 2 compact 60 (Fig. 6.1) that can accommodate 15, 30, or 60 cards, respectively (Fig. 6.2). Vitek 2 compact was launched in 2005 to be used by medium- to small-sized laboratories, while Vitek 2 60 and Vitek 2 XL systems are designed for large microbiology laboratories. Vitek 2 compact is also powered by the Advanced Expert System (AES™) software

Fig. 6.1 Vitek 2 compact equipment

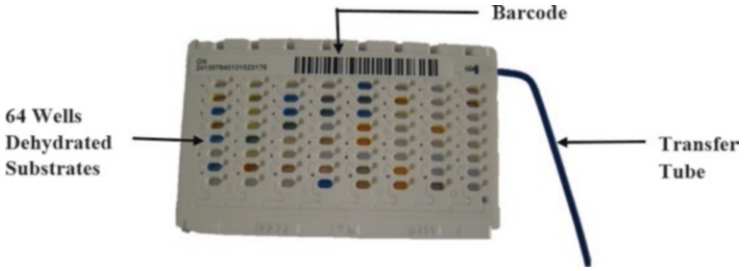

Fig. 6.2 Vitek 2 compact GN identification card

that detects mechanisms of antibiotic resistance after validating and interpreting the susceptibility test results [2].

Unlike the previous Vitek 2 system that relied on the fluorescent technique for microbial identification, the current Vitek 2 system uses a colorimetric technique. The colorimetric method widened the database and allowed better identification of microbes, especially nonfermenting bacteria and Gram-positive cocci [3]. For example, using the fluorometric method, the database for identification of Gram-negative bacteria included 101 taxa, whereas the database included 159 taxa using the colorimetric method [4]. The database for the identification of Gram-positive bacteria using the colorimetric method includes 115 instead of 51 taxa as included by the fluorometric method [5].

BioMérieux introduced Vitek MALDI-TOF MS that uses mass spectrometry for microbial identification in the US clinical laboratories in 2013, a half a century after the company was launched. Vitek MS is an automated mass spectrometry microbial identification system that can identify microbes in minutes. It uses matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology for the identification of microbes [6]. Vitek MS can be automatically connected and integrated with the Vitek 2 platform through Myla. This allows combined identification results as produced by Vitek MS with the antibiotic susceptibility test given by Vitek 2 [7]. Vitek system software is also continuously updated; for example, the database for

identification of *Bacillus* bacteria in the software version 9.01 includes 27 species when compared to 17 species in its previous version 7.01 [8].

3 Principle of Identification and Basic Steps

The identification principle of microbes and the basic steps depend on the type of Vitek. The manufacturer's instructions should be followed carefully to get the best results. These include the type of media, incubation conditions, age of culture, and turbidity of the inoculum. This section describes the principle and workflow with the most commonly used Vitek systems, Vitek 2, and Vitek MALDI-TOF MS.

3.1 Vitek 2

Vitek 2 system is available in three formats, Vitek 2 compact, Vitek 2, and Vitek 2 XL. These systems differ in the level of capacity and automation, while the same colorimetric reagent cards for microbial identification or antimicrobial susceptibility testing are used. The identification technology of the Vitek 2 depends on utilizing a growth-based automated microbiology system (Fig. 6.3). Specific microbial identification cards containing wells with various substrates are inoculated with the target test microorganism and after incubation, the system evaluates the optical signal. It is then compared to those found in the system database, and microbial identification is obtained [9].

There are different reagent cards used for the identification of microorganisms. The GN card is used for Gram-negative fermenting and non-fermenting bacilli, GP card for Gram-positive cocci and non-spore-forming bacilli, BCL card for Gram-positive spore-forming bacilli, NH card for the most clinically significant fastidious organisms, ANC card for anaerobic organisms and *Corynebacterium* species, CBC card for coryneform bacteria, and YST card for yeasts and yeast-like organisms. There are 64 wells in each reagent card, and each well has a specific substrate that measures microbial metabolic activity such as alkalization, acidification, susceptibility to growth inhibitors, and enzyme hydrolysis. Both sides of the card are sealed with an optically clear film that prevents contact with the substrate–microbe mixture while permitting transmission of an appropriate level of oxygen. Inoculation of the wells occurs through a preinserted transfer tube present in each card [10].

A Gram stain film is first prepared from fresh (18–24 h) pure colonies (Fig. 6.4). The appropriate card is selected according to the result of the Gram stain. A homogeneous organism suspension is prepared in 3 ml saline (0.45 to 0.5% NaCl) in plastic tubes using a sterile swab, loop, or applicator stick. A meter called DensiCheck™ is used to check the turbidity of the suspension, which is adjusted to be 0.5–0.63 McFarland (McF) for GN and GP cards, 1.8–2.2 McF for BCL and YST cards, and 2.7–3.3 McF for NH, ANC, and CBC cards. The suspension should be

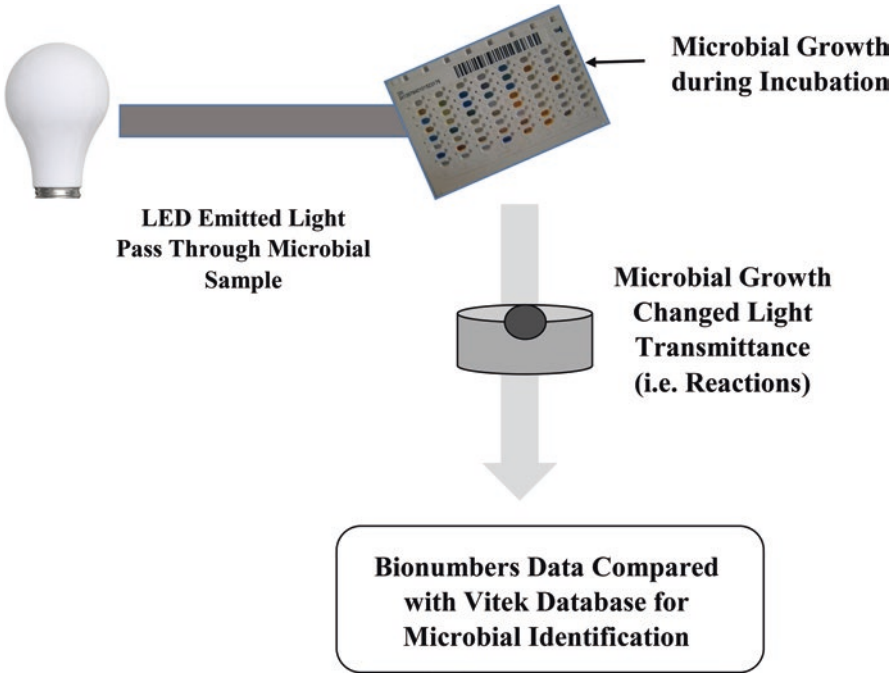

Fig. 6.3 The principle of work of the Vitek 2 system

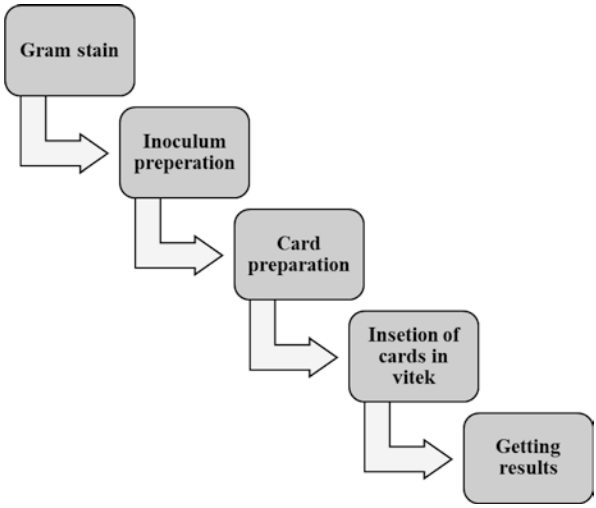

Fig. 6.4 Basic steps for the work in Vitek 2 compact

inoculated into cards within 30 min after preparation [11]. The tube containing microbial suspension is placed in a slot in a special rack known as a cassette, and the selected identification card is placed in the slot next to it, while the transfer tube of the card is inserted in the suspension tube. The cassette is placed in the fill station where the wells of the card are filled with microbial suspension from the suspension tube and through the transfer tube automatically by applying a vacuum and then air into the station. A machine then cuts off the transfer tubes from the inoculated cards and seals them. The cards are loaded into the carousel incubator and incubated at 35.5 ± 1.0 °C. Reactions in each card are read every 15 minutes by removing each card from the incubator, transferring it to the optical system, reading reactions, and then returning it to the incubator; thus, data are gathered every 15 minutes throughout the incubation period. The results of the test reactions are interpreted through a transmittance optical system using various wavelengths to determine changes in turbidity or color due to substrate microbial utilization [10]. The card is automatically ejected after analysis.

The results of the test reactions are compared to a threshold and presented as "+" or "−" if they are strong reactions but if they are weak reactions, they are presented in parentheses "(+)" or "(−)", in Vitek 2 compact or as "?" in Vitek 2 or Vitek 2 XL. The reaction biopattern of the test organism is compared to those found in the database to calculate a quantitative value for their closeness to each taxon in the database, which is given as a numerical probability that corresponds with a specific qualitative level of identification. The organism can be then identified. A list of possible microbes can also be given, or the organism cannot be identified because it is outside the database scope. Mixed taxa identification occurs if the biopattern represents a group-level, genus-level, or slashline identification. A species-level identification can also be mixed and composed of two subspecies. Supplemental tests can be used to discriminate between the reported species or subspecies. The organism may also be tagged as a low discrimination identification in which two or three choices are reported according to their probability calculation. Recommended supplemental tests are suggested and can be done to differentiate the low discrimination organisms. The result of "nonreactive biopattern" is reported if the biopattern is completely negative or contains both negative results and those are close to the test threshold [10].

3.2 Vitek MS

The Vitek MALDI-TOF MS works by ionizing the microbial sample with a precise laser, which allows a protein "cloud" to be released and accelerated by an electric charge in a ring electrode (Fig. 6.5). The generated proteins travel up a flight tube and then hit a detector [6]. A spectrum of the structural proteins that represent the protein makeup of each sample is created. This spectrum is digitized and compared to those found in the database for microbial identification [12].

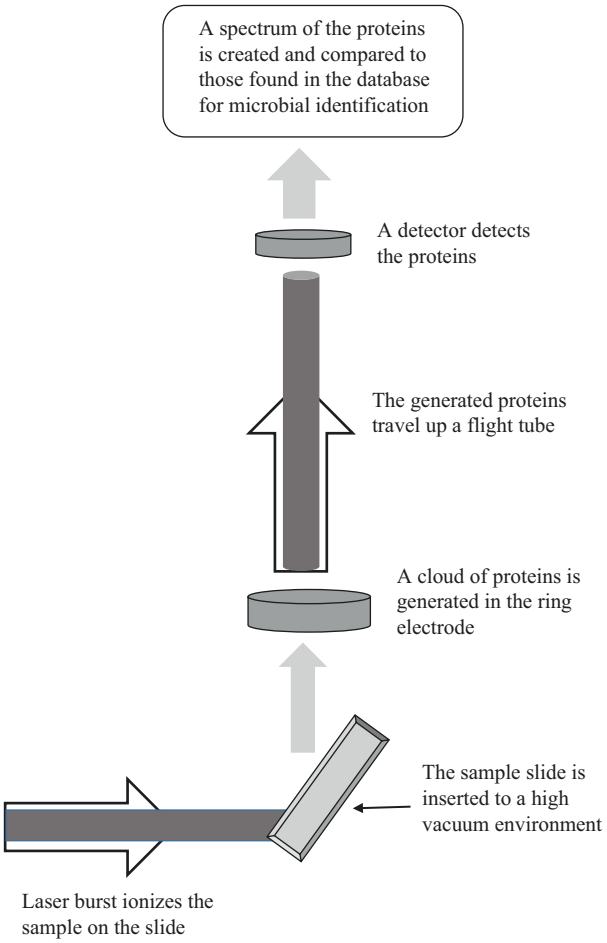

Fig. 6.5 The principle of work of Vitek MALDI-TOF MS

The basic steps of the workflow for bacterial identification in Vitek MS involve picking a pure colony, smearing it on the target slide, adding matrix, and drying and loading it into the Vitek MS. A similar procedure is done for yeast identification but with an additional step of adding formic acid to the colony on the slide before adding the matrix [13]. The generated protein spectra of test microbes are compared to reference spectra present in the system database. A confidence value from 60.0 to 99.9 is displayed for every single identification if one significant organism or organism group is matched. The test organism is tagged "Low-discrimination" if more than one but not more than four significant organisms or organism groups are matched. The organism can be considered "unidentified" if no match is found or more than four organisms or organism groups are matched [12].

4 Capacity and Microbial Database

The microorganisms that are used to construct and develop the Vitek systems databases are accurately characterized using various culture conditions. The used strains are derived from university culture collections, clinical, and environmental sources as well as from public sources such as ATCC [10]. Vitek systems have very flexible databases that can be customized and developed as required. Vitek 2 compact expanded database includes over 330 microbial species [14]. The database of GN card includes 159 taxa [4], GP card includes 115 taxa, YST card includes 49 taxa, BCL card includes 38 taxa [10], and ANC card includes 63 taxa [15], while the NH card includes 27 taxa [16]. The Vitek MS V3.2 database includes 1,316 taxa containing 207 molds and yeast, 16 *Nocardia*, and 39 Mycobacteria. The Vitek MS database is continually increasing to identify new emerging pathogens and other species. The enhanced database in Vitek MS V3.2 FDA 510(k) for bacteria and fungi contains *Mycobacteria tuberculosis*, non-tuberculous mycobacteria, dermatophytes, and dimorphic fungi, including the most prevalent human pathogens [6].

5 Vitek Applications in Microbial Works

Through its performance in microbial works, it could be found that Vitek has been more widely applied in the clinical areas than in environmental works. In fact, since its first version, Vitek JR to the latest version, Vitek MS MALDI-TOF, Vitek microbial database has been mainly built-up and revised with the clinical pathogenic bacteria and yeasts interests. Therefore, most of the studies cited in this chapter are based on clinical findings.

5.1 Clinical Applications

Clinically, Vitek and its latest versions have been applied to identify pathogenic bacterial and yeast isolates and to determine pathogens' susceptibility to different antibiotics. In its first reporting as a causative of the clinical outbreak in China, Vitek 2 compact was used to identify *Acinetobacter baumannii* as the causative of the outbreak [17]. In the investigation of the causatives of sepsis, Vitek 2 compact found 16 genera and 30 species among them were *Kocuria*, *Leuconostic*, *Cedecea*, *Pantoea*, and *Burkholderia* to cause this problem. Moreover, Vitek 2 compact was found to be the first modern automated identification system for these genera [18]. In analyzing the flora of the infectious wastes, Vitek 2 compact identified *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* among the flora of infectious wastes [19]. Also, Vitek has been used to diagnose the yeast-causing infectious diseases, and it is found to be a simple and useful identification system for clinical yeasts. In

this regard, different species of *Candida* sp. isolated from blood, wound, respiratory, and urine such as *C. albicans*, *C. tropicalis*, *C. dublininesis*, and *C. parapsilosis* were identified at a high level of confidence by Vitek 2 [20, 21]. In animal clinical studies, Vitek 2 identified *Aeromonas sobria* as an infection causative in fish sliver carp [22].

Vitek has been used to determine microbial susceptibility and resistance to different antibiotics, which has been considered a means to identify the microorganisms. For instance, Vitek 2 showed 100% concordance with the D-zone test for *Staphylococcus aureus* strains susceptibility to macrolides and lincosamides, in another study, Vitek 2 detected oxacillin-sensitive and cefoxitin-resistant certain strains of *Staphylococcus aureus* [23, 24]. In other studies, Vitek 2 detected the susceptibility of different pathogens, such as *Enterococcus* sp., *Staphylococcus* sp., and *Streptococcus* sp. to oxacillin and vancomycin, and the susceptibility of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* to amikacin [25, 26]. Moreover, Vitek 2 was found to be a rapid and accurate tool to evaluate the susceptibility of Enterobacteriaceae members, such as *Klebsiella pneumoniae*, *K. oxytoca*, *Escherichia coli*, *Proteus mirabilis*, and *Morganella morganii* against ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefazolin, cefoxitin, ceftazidime, ceftriaxone, cefepime, ertapenem, meropenem amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, and tetracycline [27]. In the identification of *Streptococcus uberis* by susceptibility to antibiotics, Vitek 2 was able to identify *Streptococcus uberis* susceptible against ampicillin, erythromycin, clindamycin, levofloxacin, linezolid, penicillin, cefotaxime, ceftriaxone, tetracycline, and vancomycin [28].

5.2 Environmental Applications

In environmental studies, Vitek has been widely used to identify bacterial isolates from food and food-processing environment. In aquatic studies, for instance, Vitek 2 was used to investigate the bacteria flora of Sapanca Lake, Turkey, in which Vitek 2 was capable to find a high diversity of bacterial species, such as *Aeromonas hydrophila*, *Citrobacter braakii*, *Enterobacter amnigenus*, *Enterobacter cloacae*, *E. sakazakii*, *Escherichia coli*, *E. hermanii*, *K. oxytoca*, *K. pneumonia*, *K. pneumonia*, *Pseudomonas aeruginosa*, *P. luteola*, *Bacillus lentus*, *B. licheniformis*, and *B. mycoides* [29]. Vitek 2 was also used to evaluate the microbial contamination level in water pipe components in which different contaminants such as *E. coli*, *Klebsiella. oxytoca*, *K. pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and Kocuria *rhizophila* were found in waterbowl and mouthpieces [30]. In other foods, Vitek has been used to know the composition of bacterial flora and confirm the incidence of certain bacteria. Investigating the microbial changes during poultry meat processing, Vitek 2 was capable to find common handling pathogens such as *Salmonella* sp., *Staphylococcus aureus*, *E. coli*, and *Listeria monocytogenes* in the poultry carcasses [31].

Moreover, Al Bulushi et al. [32] using Vitek JR found a high bacterial species diversity in some subtropical marine fish with the dominance of certain bacterial species such as *Micrococcus luteus* and *Streptococcus uberis* in fresh and ambient-stored fish. In a study aimed to know the distribution and existence of *Pseudomonas* sp. certain genes in camel meat, bacteria initially was identified by a Vitek system [33]. Vitek 2 also accurately identified *Cronobacter sakazakii* from retail foods and showed capabilities to differentiate this species from other *Cronobacter* species [34]. Moreover, a Vitek system has been used to evaluate the hygienic status of food handlers. In investigating the hygienic status of the food handlers' hands, noses, and wound sites, Khan [35] used Vitek 2 and found different types of Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterobacter agglomerans*, and *E. coli*.

6 Reliability and Accuracy

The performances of Vitek systems have been widely compared to that of different phenotypic and proteotypic systems to evaluate their efficiency, accuracy, and reliability. It is useful in the identification systems for clinical and environmental microorganisms. Despite the accuracy and reliability levels of Vitek systems in comparison with other phenotypic and proteotypic systems, these comparisons might lack precision and meaningful due to the limited database of microorganisms. In fact, the reality and acceptability of any comparison depend on the size of the database of the systems used to compare with it. Thus, meaningful and reality of comparison of any system can be achieved by comparing with genotypic techniques, since these techniques are built-up with a wide range of microbes from different sources. The performances of Vitek systems have been evaluated in comparisons with other systems using pure, clinical, and environmental isolates.

6.1 Comparison with Phenotypic and Proteotypic Techniques

6.1.1 Pure and Reference Microorganisms

Pure or reference microorganisms are usually used more frequently than isolates from other sources to compare the performance of the identification systems. This is because the identity of these microorganisms is already known, the effect of food systems on the identification performance is secured, and less work and practical skills are needed to perform the comparison. The accuracy and reliability of Vitek systems have been evaluated using pure or reference microorganisms (i.e., mainly from clinical sources). Using 160 clinical isolates and 50 reference strains, the performance of Vitek 2 was compared to that of microscan and phoenix systems to

identify these microbes at the genus and species levels. For identification of the clinical isolates to the species level, Vitek 2 correctly identified 93.7% of the isolates when compared to 82.4%, and 93.0% for Microscan and phoenix systems, respectively. However, for identification of the reference strains, Vitek 2 correctly identified only 55.3% of the strains when compared to 54.4%, and 78.0% for Microscan and phoenix identification systems, respectively. Examples of the misidentification of strains are *Streptococcus salivarius* (KCTC 3960T), *Vibrio proteolyticus* (KCCM 11992T), and *Aeromonas caviae* (KCTC 1653) [36].

In evaluating the performance of Vitek 2 to identify *Candida* sp. yeasts, Ochiuzzia et al. [21] mentioned that additional biochemical and molecular tests are needed to identify *C. albicans*, *C. dublininesis*, and *C. difficile*, whereas, at the strains level, Vitek 2 misidentified one strain of *C .tropicalis* and one strain of *C. krusei* as *C. parapsilosis*. Comparing Vitek 2 with the latest Vitek version, Vitek MS to identify anaerobic bacteria, Vitek 2 was able to identify correctly 86% of the strains when compared to 92% obtained by Vitek MS with the inability to differentiate among, *Clostridium difficile*, *C. bifermentans*, and *C. sporogenes* [37].

The accuracy and usefulness of Vitek 2 were also evaluated by comparing Microscan walkaway system for the identification of 20 Gram-positive cocci, 34 Gram-negative rods, and 13 reference strains of clinical sources. Vitek 2 accurately identified all bacteria when compared to about 90% accuracy for MicroScan walkaway. Moreover, faster and fewer difficulties were experienced in obtaining the results with Vitek 2 when compared to MicroScan walkaway [38].

The accuracy and reliability of Vitek MS MALDI-TOF were evaluated in some studies by comparison with other identification systems. For instance, Vitek MS correctly identified 92.7% of *Candida* species such as *C. tropicalis*, *C. albicans*, *C. parapsilosis*, and *C. lusitaniae* when compared to 94.6% correctly identified by Bruker Biotyper [39]. In another study, Vitek MS correctly identified 99% of 477 of the isolates to the genus level and 93.7% of the isolates to the species level. However, Vitek MS showed the inability to identify some of the isolates to the species level such as *Staphylococcus caprae*, *Streptococcus canis*, *Achromobacter denitrificans*, *Aeromonas hydrophila*, and *Proteus vulgaris* [40].

Vitek MS has the ability to identify 394 bacteria that belong to the genus and species of staphylococci, streptococci, and enterococci, and it is comparable with the results of Microflex LT. Both systems identified these bacteria correctly to the species level by 97.2% and 94.7%, respectively. Again Vitek MS showed the inability to identify species such as *Staphylococcus warneri* and *S. pettenkoferi* [41]. In a recent comparison study between Vitek MS and Microflex LT, Rocca et al. [42] evaluated the performance of both systems using 265 isolates of 77 Gram-negative bacilli, 33 Gram-positive cocci, 40 anaerobes, 35 actinomycetales, 19 fastidious microorganisms, and 61 Gram-positive bacilli. Although both systems showed 82% of accuracy in the identification of these microorganisms at the species level, non-fermenting bacilli such as *Bacillus amyloliquefaciens* and *B.cereusgroup* were identified by Vitek MS. Moreover, Vitek MS identified the following *Lactobacillus* species to the genus level only *L. gasseri*, *L. paracasei*, and *L. rhamnosus*.

6.1.2 Clinical and Environmental Microorganisms

In a small sample size, Vitek 2 was used to identify 70 microbial isolates responsible for bacteremia in comparison to a MALDI-TOF MS identification system, namely, biotyper. Vitek 2 identified 91.4% biotyper and enabled to identify different species of staphylococci, enterococci, and Enterobacteriaceae [43]. Vitek 2 showed the ability to identify and differentiate 31 isolates of Candida *haemulonii* complex, which includes *C. haemulonii*, *C. haemulonii* var. *vulnera*, and *C. duobushaemulonii*, and these were comparable with that of biotyper. Overall, Vitek 2 identified only 29% of the 31 isolates when compared with 77.4% and 100% achieved by biotyper. Some of the isolates were identified as *C. haemulonii*/*Kodamaea ohmeri* with a confidence level of 50%, whereas 4 of *C. duobushaemulonii* were misidentified as *C. haemulonii* with a probability level of 97–98% by Vitek 2 [44].

In another comparison with biotyper to evaluate the ability of Vitek 2 to speciate 183 *Candida* sp. of clinical sources. The Vitek 2 identified 92.9% of isolates as *C. glabrata* and provided low discrimination identification for 13 strains, while all isolates were identified as *C. glabrata* by biotyper [45]. Recently, Vitek MS was accurate to identify 138 isolates of *Bacteroides fragilis* of clinical sources when compared to Clin-ToF-II MS, Autof MS 1000, and VITEK 2 ANC identification systems. Vitek MS accurately identified 94.2% of the isolates as *Bacteroides fragilis* when compared to 94.2%, 98.6%, and 94.9% for Clin-ToF-II MS, Autof MS 1000, and Vitek 2 ANC, respectively. However, Vitek MS did not provide identification for other species of Bacteroides such as *B. ovatus* and B. *intestinalis* when compared to other systems [46].

Limited studies are presented to evaluate the reliability and accuracy of Vitek systems in order to identify environmental isolates. In a single study, the reliability and performance of Vitek MS and Vitek 2 were compared with biotyper and biolog to identify the industrial isolates, and performance of all systems was compared with standard genotypic identification. Approximately, 60% of the isolates were identified correctly to the genus level equally by Vitek MS and Biotyper. At the species level identification, Vitek MS showed a better performance than Biotyper; however, Vitek MS left more isolates unidentified when compared to biotyper [47].

6.2 Comparison With Genotypic Techniques

Genotypic techniques are precisely used 16S rRNA gene sequencing, and it is used as a golden standard reference and clear-cut identification. In fact, the accuracy and reliability of Vitek systems have been evaluated widely in comparison with 16S rRNA gene sequencing using pure, clinical, and environmental isolates. Nevertheless, most of these comparisons have been done using mainly the clinical isolates, and this could limit to draw general conclusion on the performance of the Vitek systems.

6.2.1 Pure and Reference Microorganisms

In evaluating the accuracy of Vitek 2 as a routine method for the identification of pathogenic *Roseomonas mucosa* in comparison with 16S rRNA gene sequencing, Vitek 2 incorrectly identified *R. mucosa* as *R. gilardii*, *Rhizobium radiobacter*, or *Sphingomonas paucimobilis* despite a high probability of identification level (i.e. 95% to 99%) [48]. However, Vitek 2 was found to be a reliable and better phenotypic identification system than other systems for identifying 171 coagulase-negative staphylococci isolates. It was a clinical source, and its accuracy was evaluated by 16S rRNA gene sequencing. In fact, Vitek 2 provided high level of accuracy for the identification of *Staphylococcus epidermidis*, *S. gallinarum*, *S. haemolyticus*, *S. sciuri*, *S. warneri*, while *S. nepalensis* and *S. pasteuri* were not identified by Vitek 2 [49].

Comparing with 16S rRNA gene sequencing to specify 495 isolates of clinical *Acinetobacter* sp., Vitek 2 identification agreed by 98–100% with that of 16S rRNA gene sequencing. In the identification of *A. baumannii* and *A. radioresistens*, Vitek 2 did not provide the identification for *A. nosocomialis*, *A. bereziniae*, *A. guillouiae*, *A. gyllenbergii*, *A. johnsonii*, and *A. ursingii*, whereas these species were identified by 100% accuracy with 16S rRNA gene sequencing [50]. Moreover, different disagreements were found between Vitek 2 and 16S rRNA gene sequencing in differentiating *Raoultella ornithinolytica* and *Klebsiella oxytoca*, where *Klebsiella* sp. were identified as *Raoultella ornithinolytica* by Vitek 2 [51]. Vitek MS performance was compared to that of 16S rRNA gene sequencing in some studies to evaluate its accuracy and reliability. For instance, Vitek MS showed high accuracy, reliability, and 100% agreement with 16S rRNA gene sequencing to identify 132 clinical isolates of *Enterococcus faecalis*, *E. faecium*, *E. casseliflavus*, and *E. gallinarum* [52]. While Vitek 2 showed some disagreements with 16S rRNA gene sequencing in the identification of *Raoultella* sp. [51], Vitek MS showed better performance and specificity to accurate identification of the species of this bacterium. For instance, Vitek MS identification accuracy level agreed with that of 16S rRNA gene sequencing in the identification of *Raoultella ornithinolytica* and *R. terrigena* [53].

6.2.2 Clinical and Environmental Microorganisms

The accuracy and reliability of Vitek 2 were evaluated with of 16S rRNA gene sequencing to identify *Myroides* sp. Vitek 2 accurately identified the isolates as *Myroides* sp. at a high confident level; however, it could not differentiate between the two species of *Myroides* sp., *M. odoratimimus* and *M. odoratus* [54]. In the identification of the clinical pathogens, *Chryseobacterium* and *Elizabethkingia* species, Vitek MS agreed by 42% with 16S rRNA gene sequencing in the identification of these pathogens showing inability to identify *C. gleum* despite the Vitek MS database coverage of this bacterium [55]. Moreover, considering 140 *Chryseobacterium* isolates, Vitek MS identified 98% (i.e., only one species isolates namely *C. indologenes*), whereas the rest of *Chryseobacterium* species isolates such

as *C. arthrosphaerae*, *C. culicis*, and *C. cucumeris* were incorrectly identified by Vitek MS [56].

The environmental isolates were evaluated by Vitek 2 in comparison with 16S rRNA gene sequencing. To identify pathogenic environmental *Burkholderia cepacia* complex from soil, Furlan et al. [57] found that Vitek 2 among other phenotypic techniques were not suitable for the identification of this pathogen. Considering pathogenic isolates from diseased fish, Vitek 2 correctly identified the isolates to the genus level as *Aeromonas* only, at the species level; however, Vitek 2 misidentified the isolates as *A. hydrophila* with a confidence level of 98% [58]. The Vitek systems were tested for its ability to identify molds. The Vitek MS was found as a useful system for the identification of clinical and environmental isolates of filamentous fungi such as *Aspergillus niger*, *A. flavus*, and *A. terreus* [59, 60]. However, it can be seen from these studies that Vitek MS did not provide identification for all isolates.

7 Efficiency and Limitations

Based on the purpose of use, identification systems could be divided into two main categories: techniques used for routine works and others used for investigation. Phenotypic and proteotypic systems can be used for routine works to confirm the expected microorganisms from certain genera and species. In fact, within this intention of use, Vitek systems showed good accuracy and reliability to identify wide ranges of microorganisms at genera and species levels [38, 39, 43, 49, 52, 53]. Another advantage of Vitek systems is its ability to determine microbial resistance and susceptibility to different antibiotics [23, 24, 27].

Moreover, Vitek systems provide the biochemical profiles of microorganisms, which can be used to determine microbial spoilage and the potential formation of biogenic amines. In fact, Vitek 2 identification cards include different chemicals and antibiotics. These detect protein degradation such as hydrogen sulfide and amino acid decarboxylation such as histidine, lysine, ornithine, and tyrosine [61, 62]. The former substrate determines microbial ability to spoil food by producing hydrogen sulfide, and the latter determines microbial ability to form biogenic amines, such as histamine, cadaverine, and putrescine.

Within these advantages, some limitations were found in Vitek system's reliability and accuracy to identify bacteria, yeasts, and molds [36, 37, 40, 42, 44, 46, 48, 49, 60]. Database limitation was mainly blamed and attributed for these limitations; however, updating the database is still identified as limitation [55, 63, 64]. In fact, the database is not the only limiting factor of Vitek's accuracy and reliability. Unlike genotypic systems (i.e., mainly rely on DNA from a microbe), any factor can influence the microbe and can consequently affect the performance of Vitek.

In this regard, many factors have been found to affect the identification accuracy and the performance of the techniques. Initially, to utilize the substrates in the card, the microbe is needed to be pure and young of 8–24 h old and fast growing with fast metabolism rate so that chemicals can be utilized by Vitek cards. The inoculum size was also found to be a determining factor in Vitek performance, for instance, a bacterial concentration of $>10^5$ CFU/mL enabled MALDI-TOF systems [65, 66]. The effect of the metabolism rate of a microbe on Vitek performance was explained by the fact that the slow rate of metabolism could cause weaker fluorescent biochemical reactions in the card components, which cause more difficulty for the Vitek system to recognize the microbe. Therefore, Vitek systems performed better with fast rate metabolism microbes such as Enterobacteriaceae and fermenters [67].

Vitek performance was also found to be affected by other factors such as colony morphology, sample pretreatment, microbial type, and user skills. In this regard, and in general, MALDI-TOF MS systems seemed to be more effective in the identification of Gram-negative bacteria and aerobic microbes than anaerobic and molds [13, 37, 68]. These observations, however, need more investigations to elucidate their possible influences on performances of Vitek systems. Finally, the user skills also could influence outcomes of Vitek systems, in simple means, the user must have enough skills to carefully select the purist colony and avoid any possible contamination from the closed colony [69].

8 Conclusion

Vitek systems were used for the routine identification of microbes in food and clinical laboratories. These systems identify different microbes at the genus and species levels and successfully detect antibiotics susceptibility for a wide range of microbes. Moreover, Vitek systems proved good tools in determining microbial biochemical profiles, which could be used to evaluate microbial spoilage and biogenic potentials. Vitek systems show good reliability and accuracy in the identification of certain microbes (i.e., pure, clinical, and environmental isolates) when compared to other identification methods. However, the performances of these systems are found to depend on genus species. For instance, Vitek systems show good performances in the identification of certain microbes, such as staphylococci, enterococci, fermenting bacteria, and Enterobacteriaceae at the genus and species levels. However, these encounter some difficulties and need additional tests in identifying other microbes, such as anaerobic bacteria and molds at their species level. Vitek systems are limited to identify different microbes due to a mainly limited database. However, other factors such as microbial age, load, purity, and user skills are also found to affect the performance of Vitek systems. Therefore, besides expanding the database size in these systems, further studies are needed to elucidate the role of these factors on Vitek systems.

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Chapter 7
Nuclear Magnetic Resonance Spectroscopy in Food Analysis

Ghulam Mustafa Kamal, Jalal Uddin, Muhammad Suleman Tahir, Muhammad Khalid, Sarfraz Ahmad, and Abdullah Ijaz Hussain

Abstract Nuclear magnetic resonance (NMR) spectroscopy is a powerful spectroscopic technique, conventionally used for structure verification, elucidation, and purity analysis in chemistry. NMR is a fast, reproducible, and nondestructive technique to provide detailed information of the compounds with little or no treatment required in the food samples. This makes NMR a suitable technique for the food analysis and is still a relatively underutilized methodology in the area of food science. It is due to its high cost, relatively low sensitivity, and required skills. The aim of this chapter is to explain NMR methodologies in the field of food analysis. This chapter covers the basic principles of NMR and its methodologies followed by their applications in food quality control and authentication (i.e., discrimination of foods based on different raw materials and its origin). In addition, a description of the chemometrics is provided considering combined NMR and multivariate statistical analysis. This combination is a powerful approach for addressing modern challenges in food quality control and authentication. In addition to standard NMR tech-

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niques, sophisticated NMR applications especially in hyphenation with other techniques are presented.

Keywords NMR spectroscopy · Hyphenated techniques · Metabolomics · Chemometrics · Data analysis · Statistical analysis · Food analysis · Food adulteration · Nutrition · Food fraud

1 Introduction

In 1946, two groups of scientists while working independently at Harvard University (Purcell, Pound, and Torrey) and Stanford University (Hansen, Bloch, and Packard) observed signals of proton resonance from paraffin water and wax, respectively [1, 2]. Bloch and Purcell were mutually awarded with the Noble Prize in Physics in 1952 for their invention. In 1950, when low-resolution NMR determined the moisture in food and milk analysis, it was the first time when NMR was used in the food science [3]. Due to inadequacy in instrumentation and convolution in food matrices, the application of NMR in food science began in the 1980s. Since then, it is being used effectively and systematically employed in food analysis and authentication. This usage has seen a dramatic increase in research and review articles involving NMR applications in food science. The popularity of NMR application in food science is evident from Fig. 7.1, where Pubmed results show data of last 5 years (2016 to 21 May 2020).

In domestic and international conferences, various oral and written communications have also been proposed. Every two years, in Europe, an International Conference on the applications of NMR in foodstuff is organized. The conference

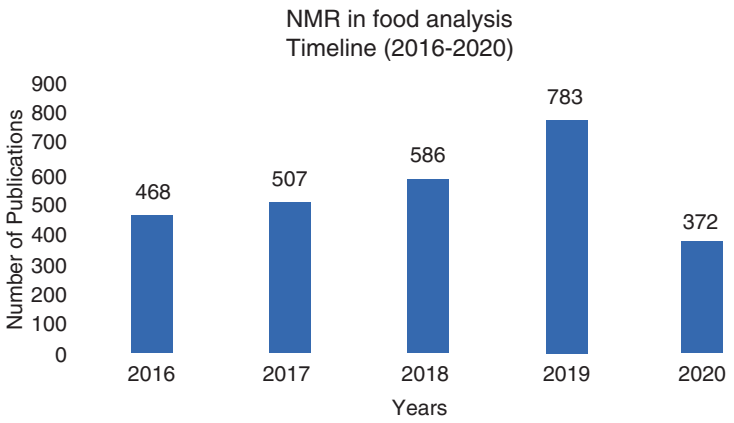

Fig. 7.1 PubMed search result of the number of publications with "NMR in food analysis" as a keyword

commenced in 1992 provided the opportunity for scientists from all over the world to propose new applications of NMR in food science and technology. European Union has approved NMR methods as official methods (e.g., determination of wine fraud) [4]. This development comprises of several reasons: (a) availability of the increased smoothness and user-friendly NMR instrumentation; (b) increased requirements of the industry to comprehend and formulate its products and processes; and (c) needs of efficient analytical techniques for the quality control and authentication of foods [5, 6] and thereby augmentation of the apposite constitution. Foods are very complicated and exceedingly heterogeneous matrix that contain a very great number of chemical compounds, whose constituents fluctuate significantly under specific conditions (e.g., slaughter or agronomical practices, industrial processes, storage, and maturation) [7]. Therefore, NMR could play a significant role in analyzing and characterizing foods.

2 Basic Principle and Theory of NMR

NMR spectroscopy is a technique based on the magnetic properties of certain nuclei. The ^{1}H-NMR and ^{13}C-NMR spectroscopy are important applications related to food analysis. The nuclei of certain atoms are considered to have a spin. These spinning nuclei possess a spin angular momentum (I) and a magnetic moment (μ). For each nucleus with spin, the number of allowed spins is quantized and can be determined by its I value by the formula "$2I + 1$" with integral differences ranges from $+I$ to $-I$. The nuclei possess an odd mass number, I becomes half-integer values (+1/2), whereas nuclei with an even mass number but odd atomic number I take integer values such as ^{13}C isotope. These spinning nuclei have $I \neq 0$, generate a magnetic moment called μ along the axis of spin, and act like a tiny magnetic bar. The nuclei have $I = 0$ are not NMR active nuclei, i.e., ^{12}C isotope. The magnitude of μ is directly proportional to (S) and gyromagnetic ratio (γ), which is a natural property of any nuclei and determines its magnetic strength ($\mu = \gamma S$). The nuclear magnetic moment, μ, interacts with the external magnetic field (B_0) generated by the magnets of NMR for understudied nuclei. For the nuclei like proton (^{1}H), magnetic moments show two alignments in the presence of applied B_0: either in the same direction (+1/2) or in opposite direction (−1/2) of the external magnetic field (Fig. 7.2). The aligned nuclei are more stable and of lower energy than nonaligned nuclei. In the presence of applied external magnetic field, there is a transfer of energy from original state to higher energy state. The wavelength at which it corresponds to the radio frequencies transfer of energy takes place and when the spin returns to its ground level, there is the emission of energy at the same frequency. This frequency is measured in many ways and processed to produce NMR spectra of corresponding nuclei [8].

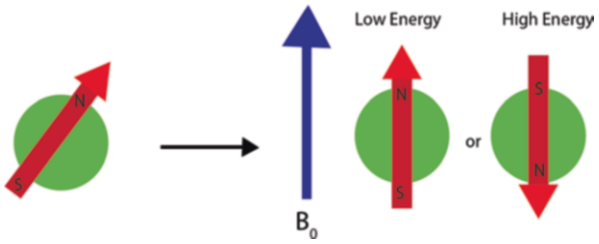

Fig. 7.2 Basic theory and principle of NMR [9]

2.1 Parameters of NMR

NMR gives valuable structural information of the compounds, which helps in quantitative and qualitative analysis. The valuable results are obtained from parameters like chemical shift, spin-spin coupling, and signal intensity.

2.1.1 Chemical Shift

Chemical shift (δ) is a plot of signal intensity (y-axis) versus frequency (x-axis) in one-dimensional (1D) NMR spectrum and expressed as ppm (part per millions). Universally, tetramethylsilane (TMS) or trimethylsilylpropanoic acid (TSP) is used as an internal standard because their protons are more shielded, and it is marked one end of the range to set the spectrum at 0 ppm. Chemical shift provides important information about the chemical environment of a nucleus in any molecule and assists in locating the signal in the NMR spectrum. Since in a compound not all nuclei may have the same chemical environment and therefore, they do not resonate at the same frequency. Moreover, chemical shift is proportional to the applied magnetic field, and nuclei absorb energy at various resonance frequencies due to the differences in the electron density among various nuclei. Nuclei with higher electron density around them have higher opposing magnetic field B_0 from electron and tend to resonate in lower chemical shifts (up field) and the nuclei are shielded when electron density around a nucleus decreases such as presence of electromagnetic element in the vicinity. The shielded proton experiences more B_0 and resonates at higher chemical shift (downfield). Chemical shift provides valuable information about the chemical environment and functional group of a molecule [9].

Figure 7.3 shows a schematic diagram identifying major components of an NMR system. Under the influence of external magnetic field, electrons around a proton produce their own magnetic field. This provides the shield to the proton. Thus, some protons are more shielded and some are less due to the different number of electrons around them. Due to this magnetic field of electrons, the influence on external magnetic field may increase or decrease on the nucleus. Thus, nuclei resonate at different frequencies due to the difference in their chemical environment. Shielding and de-shielding cause a shift to the frequency of the absorption of proton either left or

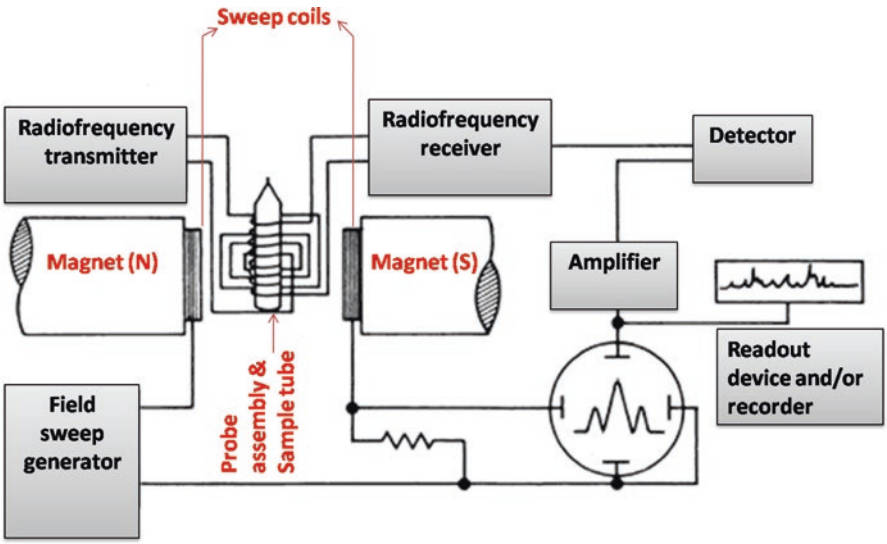

Fig. 7.3 Block diagram of NMR spectrometer [9]

right when compared to the position of the bare proton. This shift is called chemical shift. The resonance signals could overlap if two electrons present are in the same chemical environment and hence experience different chemical shift [10].

2.1.2 Spin–Spin Coupling

Spin–spin coupling is also called *J* coupling and responsible for the signal splitting in the NMR spectra. It is primarily arising from the interaction between neighboring spins mediated through the bonding electrons. It is very important for structural characterization, as its magnitude depends on the distance and relative orientation between the nuclei; however, *J* coupling further increases the complexity of the NMR spectra. The *J* coupling is measured in Hertz (Hz), and it can be either positive or negative and does not depend on the strength of the external magnetic field. The spin–spin splitting pattern can be explained empirically with $n + 1$ for spin ½ nuclei, whereas $2nI + 1$ rule is generally used for nuclei with integer values, where n represents the number of protons in neighbors and I is the nuclear spin quantum number [10, 13].

This splitting gives the information about the number of protons coupled, and its *J* value gives information about the relative orientation of adjacent C–H bonds. For example, ethyl iodide (CH_3–CH_2–I) is considered as a two spin system, where methylene proton after splitting appears as a quartet signal and methyl proton appears as a triplet in NMR spectrum. If a proton has no adjacent proton, then singlet appears in spectra that show no coupling [11].

2.1.3 Signal Intensity

The signal intensity is generally the area taken by peak in the NMR spectrum, and it is measured by digital integration. The intensity of an NMR signal is directly proportional to the number of equivalent nuclei in the sample, and it is used for spin multiplicity and quantitative analysis [12].

2.2 NMR Spectrometer

There are two basic types of NMR spectrometer currently in practice: the continuous waves (CW) and Fourier Transform (FT). In CW instruments, the magnetic field continuously varied from downfield (left) to the up field (right) end of the NMR spectrum for scanning. In this type of NMR spectrometer, one type of isotope nuclei under observation can be excited at a time. Another approach commonly used in modern sophisticated instruments is to use a short powerful burst of energy called a pulse that excites all the magnetic nuclei in the molecule at the same time. Since the molecule contains many different nuclei, many different frequencies of electromagnetic radiation are emitted simultaneously. This emission is called free-induction decay (FID)**.** FID is a time-domain signal of individual frequencies of different nuclei, which later converts into frequency domain signal using a computer and a mathematical method called as Fourier Transform (FT). For example, in an organic molecule, all the ^{1}H nuclei are induced to undergo resonance simultaneously, and at the same time, the pulsed FT instrument has several advantages compared to the CW instrument. For instance, it is more sensitive and can measure weaker signals. FT-NMR instrument are more sophisticated than CW instrument and require more complex program for signal detection. Before 1970, all experiments of NMR were taken by CW-NMR technique [13].

2.2.1 Magnet

The magnet of NMR is the most expensive part of the instrument, and it is considered as the main component. It is also called the heart of NMR instrument. In early spectrometers, permanent as well as electromagnets were used. They can produce a magnetic field of up to 2.3 T. Today, superconducting magnets are used which give high resolution in NMR experiments, and their field strength ranges from 6 to 23.5 T. In NMR spectrometer, active magnet shielding is present to avoid the effect of stray magnetic field surrounding the magnet [13, 14].

2.2.2 Shim Coils

In the high-resolution NMR spectroscopy, the homogeneity of magnetic field should be better than 1 ppb over the sample. Shims coils are used to narrow the spectral width and to remove the inhomogeneity, which is necessary for good quality spectral images. In most of the spectrometers, shim coil can be managed by computer. The computer finds out the best shim value using an appropriate algorithm.

2.2.3 Field Lock

After obtaining the required homogeneity using shim coils, stability of the homogeneous field is achieved by a field lock. The field strength varies over time due to temperature fluctuations and aging of the magnet. In a field lock deuterium resonance, the position is measured by spectrometer. Lock transmitter frequency is swept about 1 MHz as it searches the resonance frequency. When it detects resonance response, it stores these values and searches for another resonance response in other direction. When it detects response, it stores this frequency and jumps to midpoint of the stored values. It locks this frequency of midpoint as the resonance frequency of deuterium.

2.2.4 Probe

The probe is a core element in the NMR instrument, and it provides the interface for Radio frequency (RF), magnetic field, and samples. The sample is inserted into the probe and placed inside the magnet field, where it excites the nuclei and detects the NMR signal. RF coils are divided into three categories: (a) transmit and receive coils, (b) receive only coils, and (c) transmit only coils. In modern spectrometers, both transmit and receive coils are present. They act as transmitter of the magnetic field and receiver of RF energy from relaxed nuclei. Different types of probes like dual probe, broadband probe, triple resonance probe, inverse probe, cryogenic probe, micro coil probe, flow probe, solid probes, and MRI probes are used according to the type to experiment to be conducted [14].

2.2.5 Console

In spectrometer, a console is present next to a magnet, which supports the recording of the NMR spectrum. It provides three channels for radiofrequency that include observe, lock, and decoupling channels. These frequencies are controlled, amplified, pulsed, and transmitted to the probe head. In spectrometer, signals are amplified and then mixed, and NMR signal is attained using quadrupole phase detection. The two signal components are digitized in analog to digital converter (ADC) and fed into the computer memory [15].

3 1D NMR

1D NMR spectroscopy involves ordinary (^{1}H) Proton, ^{13}Carbon, and spectra of other cores. 1D NMR spectrum consists of two dimensions: The frequency axis corresponds to the x-axis (the chemical shifts) and the intensity corresponds to the y-axis (Fig. 7.4). Each one-dimensional NMR experiment consists of two portions: preparation and detection. While preparing, the spin system is fixed to a specific condition. The subsequent signal is recorded during detection. In the least complex case, the preparation is a 90° pulse (in our model connected along the x pivot) that turns the equilibrium charge Mz onto the y pivot (My). Each spin continues with its own Larmor frequency around the z-axis and generates a signal in the collector coil after this pulse. The signal decays because of T_2 relaxation and is named as free induction decay (FID).

As a rule, the analysis is directed ordinarily and the information is recorded to amplify the signal to noise ratio. After summation, the information is Fourier Transformed to give the last 1D spectrum. To terminate the signals of large molecules, one-dimensional methods including simple pulse-acquired 1-dimensional 1-Proton experiment and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence are employed. This method is also utilized to identify hetero-nuclei, like ^{13}C and ^{31}P [16].

A lot of structural and quantitative information in the form of parameters like signal intensities, coupling constants, and chemical shifts can be achieved by utilizing one-dimensional solid or liquid-state NMR spectroscopy of high resolution. Researchers can choose different nuclei, such as H^{1}, C^{13}, P^{31}, and F^{19},for the same sample to reveal the most important nuclei that can allow to study food samples under different prospects and gather the maximum information. Sample pretreatment and separation of many food components are not required [17].

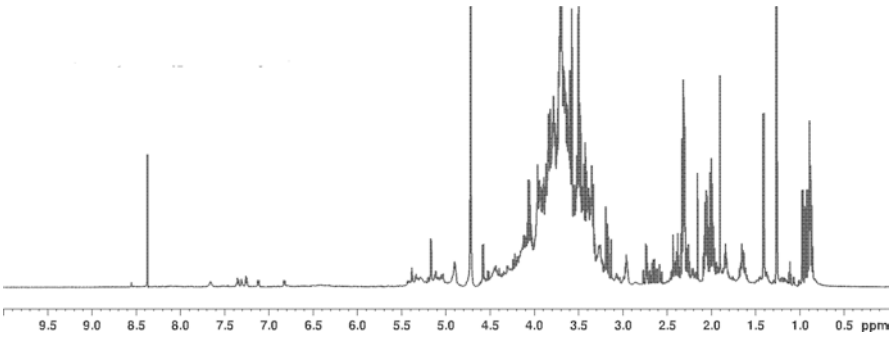

Fig. 7.4 A representative 1D NMR spectrum [9]

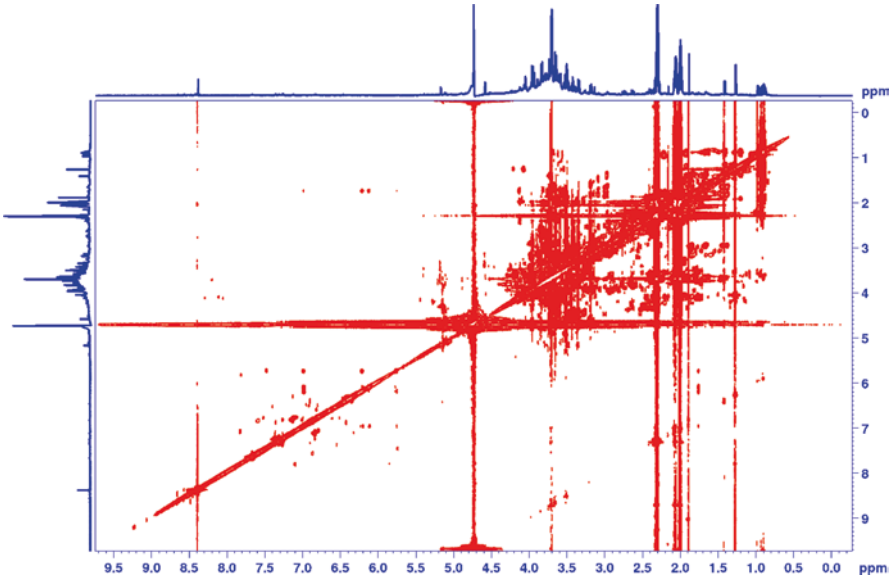

Fig. 7.5 A representative 2D ^{1}H–^{1}H TOCSY NMR spectrum [9]

4 2D NMR

A two-dimensional nuclear resonance spectrum consists of two frequency axes. Intensities represent the third axis and are commonly shown as contour plots (i.e., similar to the demonstration as utilized in geographical maps). The horizontal axis is termed as F2 (direct dimension) and the vertical axis as F1 (indirect dimension) as shown in the typical 2D NMR spectrum in Fig. 7.5. If both dimensions consist of chemical shifts, the experiment is termed as shift-correlated 2D-NMR, if one dimension indicates scalar couplings, the spectra are termed as *J* resolved.

With the assistance of 2D NMR spectroscopy, multipulse groupings are used to give extra data which are otherwise not achievable from 1D spectrum. The extensively employed types of 2D NMR spectroscopy give the relation between proton signals based on them, extensively indirect dipole-dipole coupling (C–C, H–H, or H–C), dipolar coupling like Nuclear Overhauser Effect (NOE interaction), and relative diffusion rates like diffusion ordered spectroscopy (DOSY).

4.1 COSY

COSY (correlation spectroscopy) is the first method of 2D NMR described by Jeener in 1971. It is two-pulse sequence that consists of two 90° pulses. Correlation spectroscopy enables association of all coupled protons (H^1–H^1 correlation) and

three bond H–H coupling that provides undeniable proof of proton assignments. COSY is helpful when peaks overlap in H^1 NMR and when many similar coupling constants are present. COSY also has a number of limitations. First due to polarization transfer between two spins, it cannot be used for all metabolites simultaneously. Second, it cannot provide 3D spatial localization. Third, it is not capable of identifying coupling constants [18].

4.2 HMBC

Heteronuclear multiple bond coherence (HMB) allows the correlation of protons and attached carbons over a long range. The spectra of HMBC can be used to determine the long-range H-C coupling constant. It determines association over 2 and 3 bonds. It is more sensitive than direct detection methods and provides information about the position of quaternary carbon. It gives useful information about the structure of molecule, but it is very complex [19].

4.3 NOESY

Nuclear Overhauser Effect Correlation Spectroscopy (NOESY) spectra provide us information about the protons that are close in space but not connected by chemical bonds. It uses the trough space interaction. It tells about the configuration of molecules. By NOESY, it is possible to find out the space relationship between all protons of a molecule. The disadvantage of this method is that it is time-consuming because all protons have to be radiated one by one. It also creates problems for protons which have very close chemical shifts [19].

4.4 TOCSY

Total correlation spectroscopy (TOCSY) yields homonuclear proton correlation and is based on scalar coupling between protons. It transfers magnetization of spin A on to spins B, C, etc. in a chain by relaying coherence from one proton to another. Principally, it can correlate all the protons present in a spin system. TOCSY spectrum shows all possible correlations even between spins that are not directly coupled. Another important experiment homonuclear Hartmann-Hahn (HOHAHA) spectroscopy (used in solid-state NMR spectroscopy) is identical to TOCSY because both have similarity in cross-polarization (transfer of polarization from proton to carbon nuclei). Therefore, TOCSY is also referred as HOHAHA [20].

4.5 HSQC

Heteronuclear single quantum coherence or heteronuclear single quantum correlation (HSQC) is the NMR experiment that generates a signal of NMR active nuclei that are bonded together [21]. It is employed for tallying chemical shift from proton (showed in F-2 pivot) to ^{13}C (in "roundabout," F-1 pivot) and about their straightforward connected carbons through $^{1}J_{CH}$ coupling. Multiplicity-edited HSQC usually used for the discrimination between carbons comprising of an odd (CH or CH_3) or even (CH_2) hydrogen's number [22].

5 NMR Hyphenated Techniques

The coupling of separation methods and NMR spectroscopy was proposed in the late 1970s when the implication of NMR in organic chemistry had already become extensive [23]. In the hyphenated technique, two different analytical techniques are combined by a proper interface. Mainly spectroscopic techniques are combined with chromatographic techniques. Hirsch Feld in 1980 precisely proposed the coupling of at least two instrumental analytical methods in a single output. For the coupling, the main reason is to attain data-rich and powerful information using a single analytical strategy [24]. The advantages of this system are: (a) it is rapid, (b) more prominent level of robotization, (c) larger sample throughput, (d) high reproducibility, and (e) it decreases contamination because of closed system [25].

5.1 LC-NMR

For coupling of NMR with liquid chromatography (LC) methods, optimization of chromatographic steps is needed to increase analyte concentration in the detection cell. High column loading and narrow elution are needed in order to maximize analyte concentration and tally the NMR detection [26]. One can perform liquid chromatography–nuclear magnetic resonance (LC-NMR) experiments in continuous-flow as well as in stop-flow manner. Other than NMR and HPLC instrumentation, the real need for online LC-NMR is constant-flow probe. To achieve NMR spectra of consistent or ceased-flow, a valve before the probe is inaugurated [27]. For liquid chromatography operation, UV-vis is also employed as a primary detector. In LC-NMR coupling, magnetic field strength of more than 9.4 T is suggested [28].

5.2 LC-NMR-MS

Liquid chromatography–nuclear magnetic resonance–mass spectroscopy (LC-NMR-MS) has been applied to detect the organic compounds in various fields, including natural products, drug metabolism, drug discovery and drug development, combinatorial chemistry, impurity profiling, metabolomics, and metabonomics [29]. The wide range of applications of LC-NMR-MS has been assigned as another optional technique used in academic and industrial laboratories, especially in the food analysis fields [30].

On-flow and stop-flow are the commonly available interfaces for LC-MS-NMR. With stop-flow, terminating the flow on the desired chromatographic peak being detected by NMR can be influenced by the MS instrument. In the loop collection mode, for conditions when the UV detector is not the most suitable. This is because of a deficiency of chromophores in the sample, and MS of the LC-MS-NMR system may also use to record the catching of the chromatographic peak inside the loop [31].

6 NMR Data Analyses of Foods

Foodstuff is considered as a complex mixture, comprising a wide range of metabolites produced by the metabolism of plants, bacteria, and animals. These metabolites have different chemical properties and concentrations. This complexity and the variability of food's metabolism, structure, and composition are evident in their NMR spectra. These NMR spectra comprise a vast amount of information that is not easy to extract and interpret by traditional univariate statistical methods, where the focus is on one variable at a time. Moreover, signal overlap often occurs in this type of NMR spectra, and it hindered the identification and/or quantification of the compounds of interest. It increases the uncertainty in the selection of molecules that are linked to certain food properties [13]. The combination of NMR spectroscopy with multivariate statistical methods is the most efficient way of analyzing the complex NMR spectral data of food samples. NMR spectra of a large number of samples could be used for statistical pattern recognition techniques (supervised or unsupervised) for determining spectral fingerprints. This method usually in combination with multivariate statistics and chemometrics approach to identify and quantify the metabolites in any system is termed as metabolomics [32]. Alternatively, this approach is also known as NMR-based food metabolomics (foodomics). Metabolomics does not require the determination of individual signals in the spectrum but follows to explore the precise spectral features that can distinguish and clearly indicate the existence of useful biomarkers or metabolites in the foodstuff [33].

6.1 NMR-Based Metabolomics

Every technique has its own advantage and disadvantage which results in different levels of authentication of metabolomics data. Therefore, the choice of well-suited instrumentation is very important to address a specific question in metabolomic analysis. Many factors are involved in the proper selection of instrumentation, including the availability of these analytical platforms and the possibility to combine different techniques in order to obtain a comprehensive metabolic profile. NMR is a powerful spectroscopic methodology, traditionally used for structure verification, elucidation, and purity analysis in chemistry [34]. NMR is a robust and reliable technique for metabolomic applications in which high reproducibility is paramount [35]. It offers rapid detection of structurally diverse metabolites at the same time and provides a metabolic "snapshot" of the sample [36]. There were some issues in the sensitivity of NMR which now have been efficiently overcome by advancements in NMR spectrometer hardware including the use of cryoprobes, cold-probes, or by using high field instruments, i.e., 800 MHz. Use of high-field instruments improves both the sensitivity and the resolution, but cost of the instrumentation is still an issue [37].

Depending on the aim and the information required to be generated from the experiment, we can use NMR-based metabolomics in two different ways. If the analyst is interested in a limited set of certain metabolites, the approach of choice is usually known as "targeted metabolomics," whereas in cases where the interest of analyst is to examine as many metabolites as possible, then it is known as "untargeted" approach [32]. Figure 7.6 shows a typical NMR-based metabolomics workflow in food analysis.

6.1.1 NMR-Based Targeted Metabolomics

NMR-based targeted metabolomics is used for the quantitative analysis of a few to several selected known metabolites. This involves the ability to differentiate the targeted metabolites from other intrusive metabolites, which may be achieved based on the chemical shift in an NMR spectrum [38]. Conventionally, quantitative analysis by NMR has been restricted to relatively simple mixtures with minimal peak overlap. This can be achieved by 1D ^{1}H-NMR because its peaks scale is linear with the concentration of the analytes in NMR spectrum. This makes NMR a natural choice for targeted metabolomics due to its analytical precision which usually does not depend on the chemical properties of target molecules [39, 40].

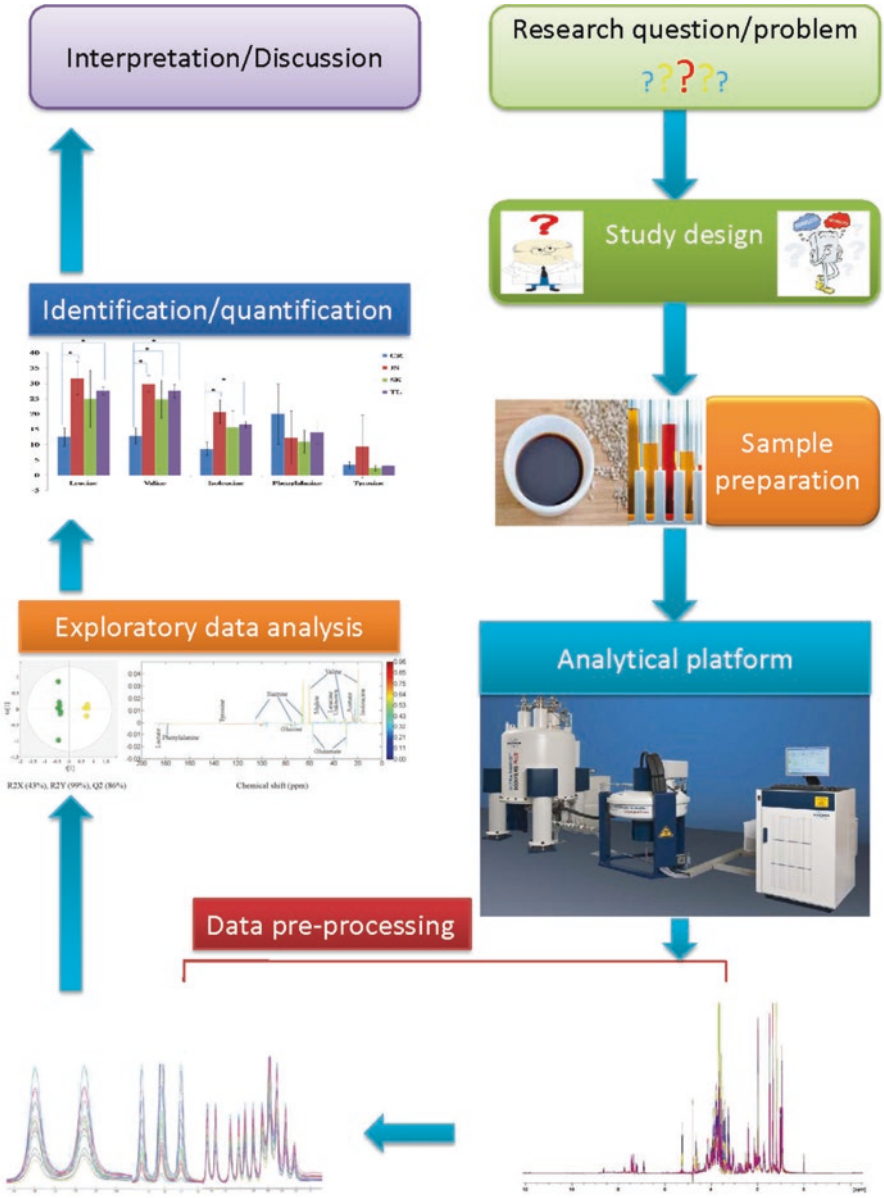

Fig. 7.6 NMR-based metabolomics workflow for application in food analysis [9]

6.1.2 NMR-Based Untargeted Metabolomics

The comprehensive analysis of all the quantifiable metabolites in a biological or food sample is known as the untargeted metabolomics. This approach is unbiased and thus makes it global in scope and sometimes is also termed as global metabolite profiling. Due to the comprehensive nature of untargeted metabolomics approach, it must be coupled to advanced chemometric techniques, such as multivariate statistical analysis which is useful in reducing the large datasets into a smaller set of manageable variables [40]. Several solvents and extraction methods should be applied and compared between the groups of samples because the nature of many compounds of interest in untargeted metabolomics is unknown [41].

6.2 Chemometrics in NMR

Svante Wold was the man who first introduced chemometrics in 1972. Isenhour and Jurs further contributed to its progress when they published the first pattern recognition article in 1972. However, the first real article on chemometrics was published by Kowalski, Massart, and Wold [42]. Chemometrics is a rather new technique, but it has a huge impact on the processing of spectroscopic data. An extended chemometric software is nowadays an essential part of a laboratory and process instrumentation. Chemometrics methods are still under development, and the definition may need to be modified from time to time in order to include all future developments [43]. In the 1980s, John explained the use of chemometrics (multivariate data analysis) coupled with NMR spectroscopy. Later on, further advancement was made by Nicholson and his group in the field of NMR-based metabolomics [44]. Chemometrics technique is commonly a complement to the analytical methods [45]. The simplest definition of chemometrics employs the use of mathematical and statistical methods to select optimal methodology and experiments to determine chemical information by scrutinizing the data [46]. The objectives of chemometrics at scrutinizing the NMR spectra in food matrix is threefold: (a) to classify and differentiate among groups of food samples, for example as a function of geographical origin or botanical origin (e.g., olive oil, honey, and wine); (b) to examine the relation between physiochemical properties and composition; and (c) to establish calibration prediction models to determine unfamiliar samples and additionally control the nourishment processes [47].

Chemometrics works with NMR data in two ways: (a) targeted or metabolic profiling and (b) chemometrics approach or metabolic fingerprinting [48]. Targeted analysis needs very little data for pretreatment, while metabolic fingerprinting needs a large amount of data, and chemometrics needs to be employed to draw useful information [49]. Figure 7.7 shows a workflow of chemometrics for NMR spectral data. One of the important approaches in chemometric-based NMR data analysis is targeted metabolic profiling, where all metabolites of interest in the NMR spectra are targeted and defined, followed by integrating the corresponding signals [40].

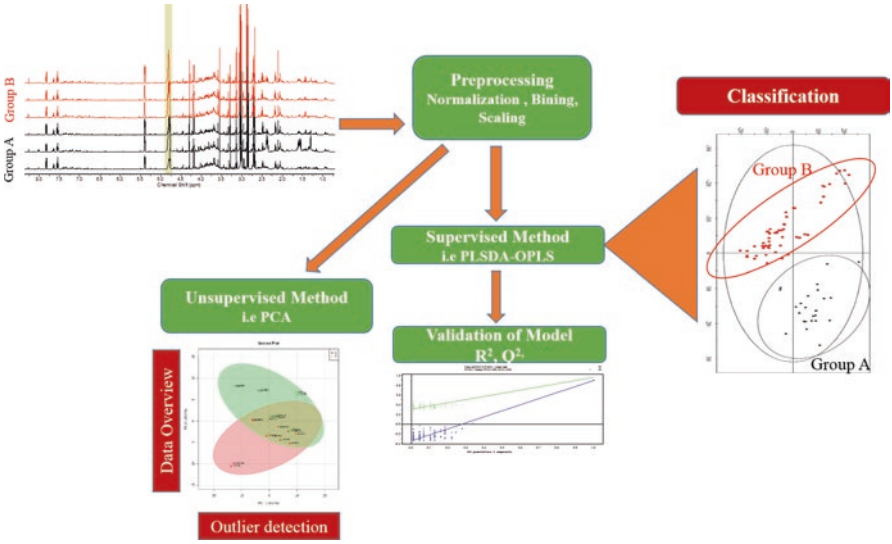

Fig. 7.7 A typical chemometric workflow in an NMR-based metabolomic approach [51]

The signal intensities or the calculated concentrations of a food's metabolites can then be employed as input variables into multivariate statistical methods. Not all the variables extracted from the spectra are used for statistical analysis. Variables with poor predictive performance and/or poor discriminatory power should be abolished. Data exploration is the first stage for data reduction, which provides information about the quality and suitability of each variable for succeeding statistical analysis [50].

6.2.1 NMR Data Processing in Chemometric

The chemometric approach does not require any chemical shift assignment. Upon applying the technique of buckets or bins, the spectra are prepared for chemometric analysis. Each spectrum can be pretreated and split into *(N)* number of buckets of 0.01 to 0.05 ppm width. Each bucket is integrated separately, and the number of buckets *N* and *S* values of integrals forms an $N \times S$ matrix. The total data matrix of (*n*) number of spectra, $n \times N \times S$, can go directly to chemometric analysis using a number of multivariate statistical techniques, and this could correlate the spectral data with constituents (variables) of a food system. The final step is the exploration of the ability of the statistical techniques to locate spectral regions (spectral fingerprints) that could be able to differentiate food samples with different physicochemical properties, for example, originated from different geographical areas or varieties. These spectral patterns are used subsequently to construct classification and prediction models for unknown samples. The main benefit of the chemometric approach

is its susceptibility to automation. However, it is unable to identify and/or quantify useful metabolites and biomarkers [48].

6.2.2 Univariate Statistical Analysis

Univariate statistical (UVS) analyses are used to test a hypothesis for individual variables to assess, whether a significant difference between groups exists. The most commonly used UVS parameter in chemometrics is analysis of variance (ANOVA). It is a statistical method in which the total variability present in a data set is divided into its individual components in order to highlight the contribution of specific variables [52]. ANOVA comprises a group of suitable statistical methods, such as one- and two-way ANOVA. One-way ANOVA checks the statistical importance of the mean parameter values of two or more groups of samples that are affected by a single independent variable. The statistical importance is assessed using the Fisher *F*-test, which investigates the null hypothesis of two or more specimens belonging to similar group. High value of *F*-test for each variable proposes the null hypothesis to be wrong, the samples belonging to different groups, and that specific variable may be exploited for classification. Two-way ANOVA ameliorates the statistical analytical ability of one-way ANOVA by considering the effect of two independent variables on the response, in addition to the possible interactions between these two variables [53].

It has been suggested that ANOVA could be replaced with multivariate analysis of variance (MANOVA) for large spectroscopic data. The ANOVA is used for the dataset where only one dependent variable is considered, while the MANOVA method includes multiple, dependent variables. For instance, ANOVA tests for the difference in means between two or more groups, while MANOVA tests for the difference in two or more vectors of the means [54]. MANOVA is superior to ANOVA because it discloses correlations between variables and reveals differences not shown by ANOVA.

6.2.3 Multivariate Statistical Analysis

Multivariate statistical analysis (MVA) is used for data reduction and visualization or for discrimination and classification of variables to be considered. In the first step of MVA, an unsupervised analysis is to be performed without prior information of neither the nature nor the group membership of the samples. This is often performed using principal component analysis (PCA) [55]. Exploratory treatment performed after data is cleaned or pre-processed. On the contrary, unsupervised methods such as partial least square (PLS) and orthogonal partial least square discriminant analysis (OPLSDA) are examples of exploratory analyses. It is used to identify the trends or clustering in data set, but does not attempt to relate observations to a class label or response. It is also of vital importance that models obtained from supervised

analysis must be validated [56]. Some of the multivariate statistical methods are described in the following sections.

Principle Component Analysis (PCA)

Principal component analysis (PCA) is the extensively employed multivariate analysis method for metabolomic and, in fact, chemometrics in general [57]. It was invented by Karl Pearson in 1901 [58], and later it was further developed and named by Harold Hoteling in the 1930s [59]. In the analysis of NMR data, PCA is the most common unsupervised method employed, particularly by the chemometric approach. It remains the principal statistical tool for inceptive analysis of big data sets to survey trends (similarities), classifications, and identification of outliers [48]. PCA is an analytical technique that employs an orthogonal transformation to change a set of observations of feasibly correlated variables into a set of linearly uncorrelated variables called principal components. If there are ṅ observations with variables, then the quantity of specific principle components can be determined. The modification is conveyed in such a manner that the primary principal component depicts the largest possible variance, and each following component has the exorbitant variance under the restrictions that it is orthogonal to anteceding components. The resulting vectors (each being a linear combination of the variables and containing *n* observations) are an uncorrelated orthogonal basis set. The relative scaling of the original variables makes PCA sensitive [60].

Partial Least Squares Discriminant Analysis (PLS-DA)

Unlike PCA, PLS-DA is a supervised method employed for optimizing segregation among different groups of samples that are achieved after joining two data matrices Y (i.e., groups, class membership, etc.) and X (i.e., raw data) [61, 62]. In these circumstances, a more focused evaluation and analysis of the data are possible. The basic principle is similar to PCA, but in PLS-DA, a second piece of information is used, namely, the labeled set of class identities. The major benefit of this technique is accessibility and managing extremely noisy and collinear information, which are extremely ordinary results from metabolomics experimental methods [63]. This technique gives an optical elucidation of complicated data sets via low-dimensional, feasibly explainable outcomes plot that demonstrates differentiation among various groups [64].

Orthogonal Partial Least Square Discriminant Analysis (OPLSDA)

OPLSDA was proposed as a refinement of the PLSDA method to differentiate two or more groups (classes) utilizing multivariate data [65, 66]**.** A regression model between the multivariate data and a response variable that only comprises class

information is calculated in OPLSDA. The benefit of OPLSDA compared to PLSDA is that a sole component is employed as a predictor for the class, while the other components explain the variation orthogonal to the first anticipating component [67].

7 NMR Applications in Food Analysis

Food adulteration and food fraud are common in foods. Due to the recent advancement of food fraud-related methods, it is gaining awareness in public, and problems are highlighted in public. It can involve public health risks due to the increasing ratio of economically motivated adulteration [68]. Adulterated food is an official term, which means that a food product fails to comply with the stated standards. Adulteration usually refers to fractious with health or safety standards. With the increasing number of food products, adulteration incidents are also increasing and result in serious economic and health issues. The food fraud issue has become a global threat for the food chain supply with its lethal destructive potential, particularly when many product ingredients are derived from different countries. Therefore, sometimes, it is difficult to detect and trace source of contaminants and related food safety concerns and even more difficult to detect and trace back cases of deliberate product fraud, especially in highly processed foods that are imported from multiple suppliers [69]. Food ingredients commonly associated with food frauds include oil, fish, honey, milk and dairy products, meat products, grain-based food products, wine and alcoholic beverages, and organic food. Many analytical techniques have been used to detect adulteration and fraud in food [68].

Present-day NMR instrumentation does not hold the disadvantages of the early days of NMR spectroscopy that include low sensitivity and high cost of analysis. Modern hardware consisting of a strong magnetic field of up to 23.5 T and cryogenic probes, which aid in the easy identification of components of food at microgram and nanogram levels. The screening of a large number of samples (overnight run) and decrease in experimental time to few minutes even for low sensitive nuclei (e.g. ^{13}C, ^{15}N). It is possible due to the development of sophisticated software and innovation in automation [70]. NMR has found applications in many fields of food science. Some of the important applications of NMR spectroscopy in the analysis of different foods are given in the following sections.

7.1 Fruits and Vegetables

Fruits and vegetables are complex, and their consumption is encouraged because they possess health benefits. They consist of a diverse range of compounds, which are responsible for several biological activities. NMR, being a nondestructive and powerful technique, can analyze this diversity in fruits and vegetables [17]. Among fruits, strawberry is an important fruit due to its lovely flavor and substance

properties including sugar, organic acids, amino acids, and essential metabolites. These metabolites are significant in strawberry during maturation [71], and these provide the essential support for its organoleptic properties, with sugar influencing the sweetness while amino acids and organic acids affecting the taste or aridity. A research has concentrated on disposing the impacts of PGRs (Plant Growth Regulator) on the yield of strawberry plant. A nuclear magnetic resonance-based methodology combined with multivariate analysis and pathway investigation has been utilized to assess the impact of Gibberellins, forchlorfenuron, and brassinolide associated with two diverse development stages [71]. A similar study demonstrated the application of NMR-based metabolomics with matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) and transcriptomics assistant [72].

Metabolomics is a powerful tool in the determination of changes in small molecules that emerge from the outer and internal environmental stimuli. A recent study shows the application of ^{1}H-NMR spectroscopy to investigate the metabolite profiling of Italian cherry tomatoes using multivariate analysis. In this study, cherry tomatoes were collected from different geographical origins and were analyzed by chemometrics [73]. NMR-based chemometrics approach was used successfully to characterize the cranberry supplements from whole cranberry powder as a reference standard. Compounds responsible for variation in metabolic profiling were ascertained using a chemometric approach. This includes citric acid and cranberry peel constituents ursolic acid, oleanolic acid, and hyperoside [74]. The classification of the Korean and Chines garlic and Chines and Korean onion was performed using ^{1}H-NMR spectroscopy approach with the conjunction of MVA. The results show that NMR can be used as a technique in tracking food origin and creates a possibility to tackle mislabeling of origin and thus providing a reliable technique in quality evaluation and fraud controls [75]. Allium genus (garlic and onion) is a profitable bioactive class of food crops with conceivably significant properties. Broad contrasts were seen between the sugar concentrations in onion species. Red onion contained a minimum measure of amino acids, and yellow onion contained the most elevated measure of amino acid. The level of flavanol was higher in yellow onions than in red onions, and garlic and leek contained a less measure of flavanols than the other allium species. ^{1}H-NMR together with HPLC-MS can be helpful in the identification and evaluation of the most metabolites, speaking to an effective way to pinpoint useful functional food ingredients from allium species [76]. NMR strategy is a quick and economical procedure and better than customary strategies, which are identified with variables, for example, taste, well-being, and security. ^{1}H-NMR together with HPLC-MS empowers the recognizable proof and measurement of an enormous number of metabolites from allium species [76].

7.2 Coffee and Tea

Coffee is the most consumed beverage in the world. The chemical composition of the coffee beans affects its flavor and quality. A high-resolution NMR is used to investigate espresso in aqueous solution and organic solvent. A detailed NMR investigation of fluid concentrates of green espresso bean was performed by Wei et al. [77]. Sixteen compounds were detected in the ^{1}H NMR spectrum [78]. Green coffee bean components were quantified using ^{13}C NMR. Coffee comprises of a complex blend of many different organic compounds ranging from traces up to 10% by weight [79]. A recent study of Brazil showed six different adulterants, namely, barley, corn, coffee husks, soybean, rice, and wheat in roasted coffee. They used ^{1}H-NMR with chemometrics as an assistant tool [80]. Okaru et al. [79] show the application of NMR spectroscopy for the routine screening of coffee for quality and authenticity. They investigated the influence of extraction time NMR device and nature of coffee. These parameters alter the level of caffeine, 16-O-methylcafestol (OMC), kahweol, furfuryl alcohol, and 5-hydroxymethylfurfural (HMF) [81]. Effect of roasting time on the structure of coffee melanoidins by NMR spectroscopy has been reported recently. This study shows that roasting affected the low molecular weight fractions of coffee melanoidins by incorporating with chlorogenic acid using 1D and 2D NMR [82].

Tea is also a well-known beverage consumed in the world. The quality of tea is evaluated through sensorial attributes, such as flavor and appearance. The tea is traditionally established using expert tea tasters. Numerous factors, for example, climatic conditions, soil, growth altitude and horticultural practices, plucking season, sorting of leaves, pressing, and storage, influence the flavor and chemical composition of the tea [83]. Le Gall et al. [86] have recognized 31 compounds in the ^{1}H-NMR spectrum of green tea extract. Metabolic profiles have been utilized to compare the high-quality Longjing teas and other Chinese teas. Longjing tea indicates a more elevated amount of theanine, gallic acid, caffeine, and other minor sugar compounds and lower levels of unsaturated fats and sucrose as compared to other teas. High-quality teas differ from low-quality ones considering the amount of caffeine, theanine, and catechins [83, 84]. NMR-based metabolomics approach showed the protective effect of tea polyphenols in sulfur mustard-induced injury in rats. In total, 13 biomarkers were identified related to sulfur mustard injury in rats [85].

7.3 Vinegar

Vinegar is a product utilized as a condiment for salad. It is produced by a variety of raw materials (e.g., grapes, apple, honey, orange, pineapple, and rice) by an alcoholic product pursued by acetic fermentation. The chemical composition of vinegar relies upon different factors, for example, manufacturing strategies and the

topographical environment of its production. Balsamic and traditional balsamic vinegar from Modena and Reggio Emilia are the most famous vinegar at the international level. ^{1}H-NMR spectrum of vinegar has been accounted for both using a D_2O buffered solution by Caligiani et al. [86, 87]. Wine vinegar is described by a high concentration of ethyl acetate, glycerol, methanol, and tartaric acid while ethyl acetate and glycerol signals are not perceptible in alcohol/agrin vinegars. Apple vinegar is rich in alanine although nectar vinegars and pineapple vinegar have highest amount of tartaric acid. Rice and orange vinegars are richer in lactic acid and contain less amount of methanol. Alanine signals are not noticeable in orange vinegar [88]. Recently, a comprehensive and nondestructive method based on ^{13}C isotopic evaluation was developed by Wang et al. [89] based on ^{1}H-NMR spectroscopy. The applied approach was successful to authenticate the quality of vinegar [89].

7.4 Oils

Olive oil is an integral part of the Mediterranean diet, and its fruit is used to extract natural juice. NMR spectroscopy has the potentials for controlling olive oil quality, authenticity, and geographical variations [90]. NMR spectroscopy coupled with multivariate analysis can differentiate between olive oils of different olive varieties as well as from different origins. ^{1}H-NMR has also been applied to the study of other vegetable oils (peanut, soybean, maize, and sunflower) and their mixture with olive oils [91]. Two main points are considered: olive oils are always classified by a high quantity of terpenes and a low quantity of saturated fatty acids. NMR metabolic profiling has been used to investigate the effects of different factors, such as altitude and irrigation [92]. A comparative study of oxidative products of commercially available refined and cold-pressed camellia oil, stored at room temperature for 1 year, was studied by ^{1}H and ^{31}P NMR spectroscopy [93]. Recently, solvent-based strategy was applied to improve the key parameter in edible fats and oils by ^{1}H-NMR spectroscopy. This methodology was successfully applied to the analysis of corn, sunflower, sesame, olive, and peanut oils. Moreover, the evolution of thermal oxidation and lipolysis of virgin olive oil and sunflower has been analyzed.

7.5 Fish

There is an increasing need for appropriate analytical techniques suitable for a complete snapshot of fish metabolome to assess the fish dietary quality. Fish is the main source of polyunsaturated fatty acids. A major application of NMR-based metabolic investigation of fish has been focused on the investigation of fish fatty acid composition mainly by using ^{13}C NMR spectroscopy. Various methods using ^{1}H NMR metabolomics are available to study fish-based foodstuffs [92]. Another study was conducted to assess the biomarker in biological fluid after fish consumption and to

examine the relationships with health parameters in a free-living population. The urine of the fish consumed population was compared to control and analyzed by NMR spectroscopy. This study determined trimethylamine-*N*-oxide (TMAO), dimethylamine, and dimethyl sulfone displayed a significant level in urine after fish consumption. Fish consumption yields a greater increase in urinary TMAO compared to red meat [94].

7.6 Juices and Beverages

NMR technique has been applied for quality control of different fruit juices, such as apple, black currant, grapefruit, lemon, orange, peach, and pineapple. NMR-targeted and non-targeted metabolomics approaches have been applied in food analysis [95]. The application of NMR metabolomics to examine orange juice adulteration. Microbial growth in fruits and their products is of great attention as it causes the decay process, loss of organoleptic properties, and generation of toxic substances. Due to the low pH of many fruit products, fungi are often predominant microorganisms while some bacteria are also responsible for food decay [96]. An example of such a study conducted recently by Cusano et al. [95] is based on NMR metabolomics of fermented juices. This study deals with metabolic changes in ciders fermented by six yeast species, and it was a valuable tool for the identification of metabolites from yeast. PCA was performed on NMR data from all spectral regions, and only the aromatic region revealed the potential to discriminate the yeast action [97]. Alcoholic and nonalcoholic beverages are a complicated mixture of different classes of compounds with varied concentrations. Alcoholic beverages consist of a varying quantity of ethanol in comparison to nonalcoholic beverages [98].

Fermented beverage, such as beer, is prepared from malted grains, hops, yeast, and water. Other specific fruits and herbs can also be added to give distinct characteristics to the product. NMR coupled with multivariate statistics has allowed beers to characterize considering the type, brewing site, production date, and malt type. Hyphenated NMR, namely, LC-NMR/MS, has allowed the description of some aromatic compounds, 2-phenylethanol, tyrosol, present in extremely low concentration [99].

Mixture of many compounds containing water, ethanol, glycerol, organic acid, and inorganic ions are utilized to manufacture wine. The chemical conformation of grapes and their wine is affected by the environmental situations of the vineyard. The ^{1}H-NMR metabolic approach has provided interesting information on the grape variety, geographical origin, and fermentation of wine acquired at each fermentation stage and aging period [100].

7.7 Soy Sauce

The ^{1}H-NMR has been applied on Zivania, the standard Cypriot alcoholic beverage from different countries. The evolution in metabolites in Korean standard soy sauces during 12-year maturing period has been examined by NMR combined with multivariate statistical analysis. The global metabolite profile of the soy sauce allowed a superior assessment of soy sauce quality [100, 101]. An improved strategy has also been developed and successfully applied for the differentiation of Chinese and Asian soy types by using ^{13}C NMR spectroscopy coupled with multivariate statistics [9, 102].

7.8 Milk and Dairy Products

Dairy milk is an important constituent of human food used in fresh as well as processed forms [103]. Two significant supplements, like lipids and lactose, are present in milk. Being an organic liquid, the composition of milk is influenced by different factors, such as breed, season, individual metabolism of animals, health state, diet, and milky protocols [104]. Milk is a critical dietary constituent being an extraordinarily complex and nutritionally complete biological fluid. Currently, it has been observed that the milk composition can be modified to upgrade its profitable attributes as well as to search for parameters that could identify the animal species from which the milk comes. Milk is an intricate emulsion and good ^{1}H-NMR spectra and ^{13}C provided a route of separation between the milk of various animal species according to fatty acid composition [105]. Coimbra et al. reported the detection of formaldehyde in raw milk by time domain-NMR and chemometrics approach. Different refrigeration storage time 0 and 48 h was assessed to check the growth of formaldehyde. The whiteness index of the milk is associated with the increased level of formaldehyde, while the lightness values indicate an increase in the yellowing index when compared to the control samples. This study shows a successful application of time domain-NMR in dairy products such as raw milk [106].

7.9 Butter and Margarine

NMR-based metabolic profiling has also been applied to analyze polar and apolar extracts from the spread and margarine [107]. The polar portion includes numerous acids including benzoic, sorbic, citrus, lactic, butyric, and formic acid, while a polar fraction contains rumenic and linoleic acid. The rumenic acid, trademark ruminant fat, has been found in all the margarine tests. The degree of natural acids, lactose, has been suggested as a significant marker of quality control and production process [107].

7.10 Cheese

Cheese is a fermented dairy product, harboring diverse microbial communities (microbiota) that change over time and vary depending on the type of cheese. The cheeses are varied as a function of starter and adjunct cultures. The final product of the cheese depends on the microorganisms, and they play an important role in their quality, flavor, and safety. Many studies have focused to explore the composition of cheese microbiota and the molecular mechanisms involved in cheese ripening [108]. The geographical origin of mozzarella buffalo cheese and graviera has been traced by using NMR [109, 110]. A good discrimination of mozzarella cheese samples from different geographical origins has been acquired utilizing a coupling of IRMs with NMR data [111].

7.11 Honey

Honey is considered a complex natural food product with various physiochemical properties. It is mainly composed of sugars and other constituents, such as amino acids, organic acids, carotenoids, vitamins, enzymes, minerals, and aromatic substances. Cheap sweeteners can be added to honey for adulteration or indirectly honeybees can be fed with sugar. Therefore, detecting and quantifying methods of adulteration are of prime importance. Due to its demand and interest of the consumer, honey authentication is increasing, and it is necessary to check the acceptability. Honey producers and quality control laboratories are now requiring increasingly sophisticated methods of analysis. One of the best techniques for honey characterization is NMR. NMR-based method for honey quality control and traceability are very well established [112]. NMR-based screening provides a cost-efficient, complete analysis that can be used reliably to ensure honey quality [113]. Song et al. [114] used 147 authentic monofloral honey from China to investigate NMR and chemometrics. NMR data from δ 0.00 to 6.00 ppm is the most suitable region to determine the adulteration of pure acacia honey [115]. A similar study was performed by Schievano et al. [116] on acacia honey from Italy, where carbohydrate profile can be traced to the authenticity of acacia honey when compared to commercially available acacia honey in the market. In this study, sugar profiles in honey were used as a fingerprint to confirm the authenticity or revealed the adulteration of the product by sweetener addition. NMR spectroscopy is used in the composition analysis and authentication of Chinese honey with a combination of chemometrics. A total of 65 major and minor components in honey were identified and quantified from their NMR spectra [117].

7.12 Rice

Rice is one of the most valuable cereal food crops in the world. H-NMR spectroscopy combined with multivariate analysis methods such as principal component analysis (PCA) and discriminate analysis (DA) has been applied to the profiling of metabolites in numerous rice. ^{1}H-NMR-based fingerprint combined with multivariate statistical analysis is the best tool for the classification of rice grains by geographical origin as well as for the discrimination among different pigmented rice grains [118].

7.13 Wheat

Proton NMR spectroscopy has been widely used to detect the metabolic profile of different samples of wheat [119]. A combined approach including HR-MAS to study the hydrated wheat flour and liquid state and ^{1}H-NMR to study the corresponding aqueous extracts have been employed for enzymatic degradation caused by endogenous hydrolases. The kinetic parameters resulting from the best fit have been extracted and subjected to multivariate statistical analysis resulting in good discrimination between the hard and the soft wheat. PCA showed that the environmental factors pose a great impact on metabolic profile than genotype.

8 Conclusion

Recently, demographic and commercial trends have shown an increase in the production of food of both plant and animal origin. Therefore, health risks are also intensifying in parallel. In recent years, food fraud has emerged as a potential threat to various countries. Various techniques are available for the quality control and authentication of foods. However, the majority of those techniques are targeted and provide information about a single aspect of foods. NMR spectroscopy combined with multivariate statistical methods is a powerful tool for the quality control and authentication of foods. This chapter also demonstrates that NMR is a valuable tool for the investigation and authentication of foods. NMR is a robust method to identify food adulteration and food fraud and is used to trace the geographical origin of food, evaluation of toxicants of food, and the raw material of different food. It has wide applications in food, including, fruits, coffee, tea, beverages, butter & margarine, cheese, vegetables, milk & dairy products, honey, rice, wheat, maize, juices, fish, meat, vinegar, etc. NMR spectroscopy could be preferred over most of the other techniques because of its nondestructive, robust, efficient, and nontargeted nature.

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Chapter 8
UV–Vis Spectroscopy for Food Analysis

Farah Haque, Saniya Yesmin Bubli, and Mohidus Samad Khan

Abstract Detection of food quality, authenticity, and adulteration is a great concern among consumers in the current market. Given the inherent complexity of food products, most instrumental techniques employed for quality and authenticity evaluation are time-consuming, expensive, and labor-intensive. Therefore, there has been an increasing interest in simple, fast, and reliable analytical techniques for assessing food quality attributes. One of these techniques is the absorption spectroscopy in the Ultraviolet and Visible (UV–Vis) region, which is used for qualitative and quantitative characterization of sample compounds. Due to simplicity and reliability, this technique has already been used in several research areas of food science and food processing industries. This chapter briefly discusses the effectiveness and relevance of using UV–Vis technique in food analysis. This chapter presents specific applications of UV–Vis technique to analyze different food matrices (e.g., meat, milk, coffee, wine, vegetables, fruits, drinks, and olive oil) with respect to food composition, authentication, adulteration, and quality.

Keywords UV–Vis spectroscopy · Absorption spectroscopy · Spectroscopy · Ultraviolet · Infrared · Visible region · Quantification · Food authentication · Polycyclic aromatic hydrocarbons (PAH) · Simplicity · Food composition · Sensory property

1 Introduction

Food is a complex, heterogonous system consisting of water, fat, proteins, and carbohydrates together with numerous minor components. Appearance, color, flavor, and texture are critical issues for the sensory quality of food. The quality of food includes different chemical, biological, and microbial factors [1]. Issues regarding food safety, integrity, and high quality in food production obviously call for high standards for quality and process control, which in turn requires appropriate

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analytical tools. The analysis of food composition has significantly evolved over the past 100 years and progressing from reliance on predominantly "wet chemistry" laboratory methods from the early to mid-twentieth century [2]. It was then developed gradually to modern instrumental techniques. The early focus of food analysis was to differentiate food components, assess purity, and detect economic fraud. Pioneering developments are in the pH measuring instruments, spectrophotometry, chromatography/separations, and spectrometry [3]. The growth and infrastructure of the modern global food distribution system heavily rely on food analysis [4], beyond simple characterization, as a tool for new product development, quality control, and regulatory enforcement.

Spectroscopic methods have been historically very successful at evaluating the quality of agricultural products, especially foods. They are highly desirable for the analysis of foods because they often require minimal or no sample preparation, provide rapid and online analysis, and ability to run multiple tests on a single sample. Spectroscopy has had an ever-increasing role in the determination of food composition and adulteration. It is important for determining food safety, accompanying food and beverage production, and for the control of food, beverages, and packaging in general. It is the most commonly used instrumental technique for both qualitative and quantitative food analysis because of its simplicity, accuracy, fast, and high precision. According to the region of electromagnetic spectrum employed in the food analysis, spectroscopy can be classified as ultraviolet (UV), visible (Vis), infrared (IR), and radio (nuclear magnetic resonance). Among these analytical techniques, 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 [5, 6]. 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 micro-volume spectrometric instrumentation has been developed to analyze samples with small volume or toxic solvent [7]. This chapter briefly discusses the UV–Vis spectroscopy technique for the quality and safety assessment of foods. The technical issues, key advantages, and limitations of UV–Vis spectroscopy for food analysis are also addressed in this chapter.

2 Basic Principles of UV–Vis Spectroscopy

Electromagnetic radiation consists of electromagnetic waves, which are synchronized oscillations of electric and magnetic fields that propagate at the speed of light through vacuum. Electromagnetic waves can be characterized by either the frequency or the wavelength of their oscillations, which determines their position in the electromagnetic spectrum through the following equation [7].

$$E = h\nu = hc / \lambda \tag{8.1}$$

Table 8.1 The electromagnetic spectrum [8]

Region	Wavelength range		Type of transition
ɣ ray	$<10^{-12}$ m	(<1 pm)	Nuclear
X-ray	1.0×10^{-12}–1.0×10^{-8} m	(0.001–10 nm)	Inner shell electron
Far-ultraviolet	1.0×10^{-8}–1.8×10^{-7} m	(10–180 nm)	Middle shell electron
Near-ultraviolet	1.8×10^{-7}–3.9×10^{-7} m	(180–390 nm)	Outer shell electron
Visible	3.9×10^{-7}–7.8×10^{-7} m	(390–780 nm)	Outer shell electron
Near-infrared	7.8×10^{-7}–2.5×10^{-6} m	(0.78–2.5 mm)	Molecular vibration
Mid- and far-infrared	2.5 × 10–6–1.0 × 10–3 m	(2.5–1000 mm)	Molecular vibration and rotation
Microwave	1.0 × 10–3–0.3 m	(0.1–30 cm)	Molecular rotation
Radio wave	>0.3 m	(>30 cm)	Electron and nuclear spin

where E is energy (J), h is Planck's constant (6.62×10^{-34} J s), ν is frequency (per seconds), c is the speed of light (3×10^{8} m s^{-1}), and λ is wavelength (m). The source of radiation defines the frequency of the electromagnetic wave. Sources that emit high-energy radiation produce electromagnetic waves with high frequencies but short wavelengths. The distribution of wavelengths and frequencies of electromagnetic waves is called the electromagnetic spectrum, which is shown in Table 8.1 and Fig. 8.1. Ultraviolet (UV) and visible radiation comprise only a small part of the electromagnetic spectrum, which includes other forms of radiation such as radio, infrared (IR), cosmic, and X-rays.

The electromagnetic spectrum refers to the known frequencies and their linked wavelengths of the known photons [5, 8]. Although all the wavelengths shown in Fig. 8.1 can be used for analysis, the range of wavelengths usually employed in spectroscopy is relatively narrow and includes mainly the UV, visible, infrared (IR), ultrasound, and FM radio (nuclear magnetic resonance [NMR]) regions.

2.1 UV–Vis Absorption Spectroscopy Mechanism

The absorption bands in the UV–Vis region are found in the range of 200–350 and 350–700 nm. The electronic state is made up of several vibrational energy sublevels. These different vibrational energy levels are composed of several rotational energy levels. The energy differences between the vibrational levels are much smaller than the differences between electronic energy states and the differences between rotational energy levels are even smaller. The electronically excited state also has several vibrational and rotational energy sublevels. Excitation of the electron involves a transition from any one of the vibrational and rotational levels in the ground state to any of the vibrational and rotational levels in the electronically excited state, resulting in several absorptions with small differences in wavelength that form very wide bands. Generally, species stay in the excited state for a short duration, then return to the ground state, or any other lower energy state in a process

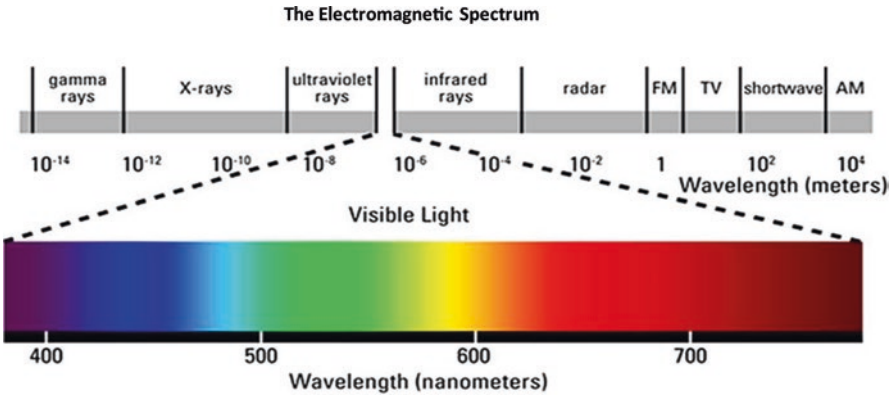

Fig. 8.1 Wavelength of electromagnetic spectrum (https://www.quora.com/Are-there-colors-that-exist-beyond-the-known-light-spectrum-that-have-not-yet-been-detected sited on 23/01/2019)

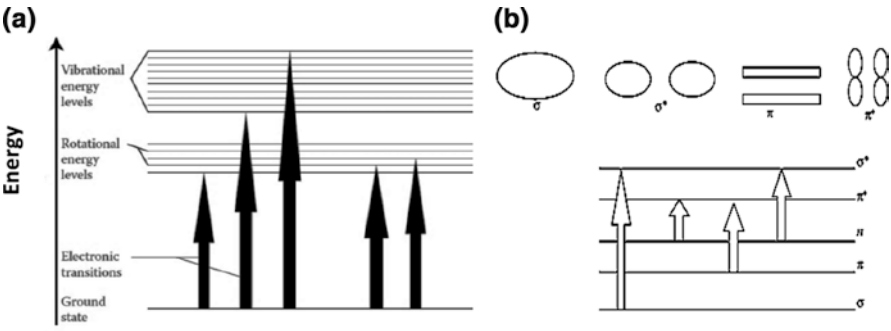

Fig. 8.2 (**a**) Electric, rotational and vibrational levels, (**b**) Molecular orbitals, bonding, antibonding and nonbonding orbitals (Munjanja, K. B. & Sanganyado, E. (2015). *UV-visible absorption, fluorescence, and chemiluminescence spectroscopy*. p. 572–583.)

called relaxation. During relaxation, the excited species release the energy they had absorbed through the emission of photons. The absorption and emission of photons, shown in Fig. 8.2a, are characteristics of the species and are thus utilized in order to determine the physical and chemical properties of samples.

The absorbance of light in the UV–Vis region occurs when an electron is excited and passes from a bonding (σ and π) or nonbonding orbital to an antibonding orbital (σ* and π*) (Fig. 8.2b). The amount of energy required to excite an electron depends on the difference in energy between the ground state and the excited state. Transitions of the σ–σ*, σ–π*, π–σ*, or simple π–π* type require light in the vacuum UV region. However, conjugated π systems exhibit π–π* transitions that absorb in the region between 200 and 800 nm. In conjugated systems, the excited state is more greatly stabilized by resonance than the ground state, so the difference in energy between the two states is smaller than in nonconjugated systems [9]. The smaller the difference in energy between the ground state and the excited state, the greater is the probability that a transition between the ground state and the excited state can occur.

The intensity of the absorbance is a function of this probability. For a given concentration, the intensity of absorbance will be greater when the difference in energy between the ground and the excited state is small. This difference can be smaller when there is a greater degree of conjugation in the molecule.

The quantification of analytes in UV–Vis technique is based on Beer's law, which states that the amount of light absorbed by an analyte in a homogeneous isotropic medium is proportional to the amount of analyte in the sample. Beer's law can be expressed as:

$$A = \varepsilon lc \tag{8.2}$$

where A is the absorbance, ε is absorptivity, l is path length through the solution, and c is the concentration of the species absorbing the light. However, it is important to note that Beer's law is only obeyed for very dilute solutions up to 10 mM in most cases. Furthermore, Beer's law is obeyed when the incident light is monochromatic and not polychromatic [10]. However, interferences from turbidity often occur and are usually eliminated or reduced by using derivative spectroscopy. The absorption of UV light is very accurate, and the linear relationship between absorbance and concentration has seen UV–Vis spectroscopy emerge as a workhorse technique in analytical chemistry.

2.2 Instrumentation

The main function of a spectrophotometer is to measure the absorbance or transmittance of a sample as a function of the wavelength of electromagnetic radiation [11]. In UV–Vis spectroscopy, light from a UV–Vis source is passed through a monochromator to isolate a specific group of wavelengths, and then passed through the analyte where a specific wavelength is absorbed, and finally, the light reaches the detector. The main components of the spectrophotometer are (1) source of energy, (2) monochromator (device for isolating a narrow range of wavelength), (3) sample-holding cell (cuvette), (4) detector, and (5) signal output to display the results conveniently for the analyst [12]. Single-beam and double-beam spectrophotometers are the types of settings marketed for conventional UV–Vis spectrophotometers. In Fig. 8.3, a single beam, widely used, is illustrated. In these conventional systems, polychromatic light from the source is focused on the entrance slit of a monochromator, which selectively transmits a narrow band of light that passes through the sample being detected.

The single-beam configuration was the earliest design and remains in common use, especially among low-end instruments. One beam of light is applied to make measurements in a simple configuration with a less expensive instrument (Fig. 8.3). In a double-beam configuration, the light beam is split into a reference and a sample beam and it measures the ratio of light intensities; therefore, the light beams are not as sensitive to fluctuations in the light source or detector.

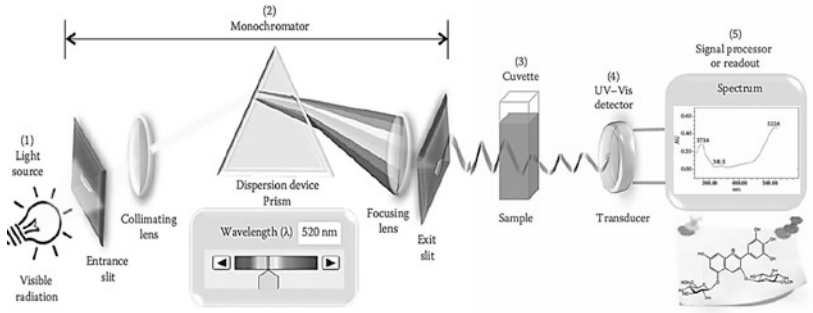

Fig. 8.3 Schematic view of a conventional UV–Vis spectrophotometer diagram (Franca, A. S., & Nollet, L. M. L. (2017). *Spectroscopic methods in food analysis*. Available from: http://www.crc-netbase.com/isbn/9781498754644.)

3 Applications of UV–Vis Spectroscopy for Foods Analysis

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. Sample preparation for UV–Vis spectroscopy is usually nondestructive, simple, quick, and relatively cheap. This technique can be applied for the analysis of food authentication, food adulteration, and determination of food quality. The quality of any food is intrinsically dependent upon the quality and composition of the raw materials and processes used to produce it. In many foods, the quality is also associated with other properties, such as geographic origin, botanical composition, or variety. Table 8.2 summarizes various applications of UV–Vis spectroscopy in different food categories.

3.1 UV–Vis Spectroscopy in Food Safety

According to Codex Alimentarius Commission/FAO, food safety refers to all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer [13]. Therefore, intrinsic characteristics of food should comply with specifications recommended or regulated by international bodies and authorities or even adopted by the industry itself for a product to be fit for use. UV–Vis absorption spectroscopy is often the technique of choice due to its high sensitivity to analyze food safety.

Table 8.2 Application of UV–vis spectroscopy based on food category

Serial no.	Basic food categories	Food types	Elements detected/adulteration	References
1	Dairy products	Milk	Melamine	[85]
			Lactose content	[82]
		Yogurt	Color, pH, reflecting index	[86]
		Cheese	Minerals, lactic acid	[82, 87]
2	Fish	Sea fish	*K* value	[69]
3	Egg	Egg yolk	Egg white contamination	[84]
4	Meat	Beef	CO concentration	[88]
5	Fruits	Cranberry, pomegranate juice	Anthocyanin	[89]
		Fruit juice	Indigotin	[25]
		Blueberry, cherry,	Phenolics	[32, 90]
		Packed fruit juice	Vitamin C	[91]
6	Vegetables		Vitamin C	[92]
7	Oil	Edible oil	PAH	[93]
			Tocopherol	[74]
		Extra virgin olive oil	Adultered with palm oil	[51]
8	Beverages	Drinks	Sulfite	[94]
		Wine	Phenolics	[32]
			Ethanol, glycerol, glucose, tartaric acid, malic acid, lactic acid, and acetic acid	[95]
		Drinks	Caffeine	[96]
9	Coffee	Roasted coffee	Unadultered coffee	[97]
			Methylxanthine	[59]

3.1.1 Heavy Metal Content in Water and Food Samples: Cadmium and Copper

UV–Vis spectroscopy can be used to assess heavy metal contents such as Cadmium and Copper in foods. Cadmium is classified as a prevalent toxic element with biological half-life in the range of 10–30 years and is known to damage organs such as kidneys, liver, and lungs, even at its very low concentration level [14]. Copper is an essential element whose role is complex in many body functions such as hemoglobin synthesis, connective tissue development, normal function of the central nervous system, and oxidative phosphorylation [15]. But excessive intake of copper would lead to accumulation of the metal in liver cells and hemolytic crisis, jaundice, and neurological disturbances [16]. Water and food are the potential sources that these metal elements enter human bodies; thus, the determination of cadmium and copper in these samples could afford some information of significant importance.

Because of the low concentration and the complexity of the environmental samples, an efficient preconcentration step is usually necessary prior to determination.

Researchers proposed a new method based on dispersive liquid–liquid microextraction (DLLME) using tetrachloromethane (CCl_4) as an extraction solvent for the spectrophotometric determination of cadmium and copper in water and food samples [14]. Under the optimal conditions, the limits of detection for cadmium and copper were 0.01 ng/L and 0.5 μg/L. The proposed method was applied to the determination of water and food samples with satisfactory analytical results. The LOD of Cd and Cu was found compatible with those obtained using FAAS and GF-AAS [17–20]. The main advantages of the proposed method were environmentally benign, fast, simple, and inexpensive. Using this technique, trace and ultra-trace Cd (II) and Cu (II) in food samples could be determined with good repeatability and spiked recoveries.

3.1.2 Indigotin (Color) in Food

Color is the first sensory parameter by which food quality and flavor are judged. The synthetic indigotin dyes, synthetic azo dye, and Ponceau-4R are among the colorants used in common foods, such as sweets, drinks, and ice cream. The analytical control of these dyes is of considerable importance in the food industry because of their toxic and carcinogenic potential [21, 22]. Derivative spectrophotometry has a great utility to resolve overlapping and evaluation of deformed spectra, particularly for binary [23] and ternary [24] mixtures.

Researchers have proposed spectroscopy-based sensitive and accurate method for the determination of Indigotin and Ponceau-4R [25]. Synthetic food samples and simulated food samples were prepared by adding known amounts of Ponceau-4R and Indigotin to a mixture of citric acid, vitamin C, sucrose, and three calcium phosphate. Commercial and simulated food samples with added Indigotin and Ponceau-4R dyes were dissolved in water and diluted with phosphate buffer at pH 7.0 to prepare samples for spectrophotometric analysis. The data obtained from the spectrophotometric method to determine Indigotin and Ponceau-4R dyes in food were found compatible with data from HPLC methods. Recovery data in spiked food samples were satisfactory, and the procedure was shown to be applicable to routine analysis of Indigotin and Ponceau-4R in food samples containing sucrose and citric acid.

3.1.3 Phenolic Compounds in Food

Phenolic compounds are ubiquitous compounds, which are diversified groups of phytochemicals derived from phenylalanine and tyrosine, found in all plants as their secondary metabolites [26–30]. Plant phenolics include simple phenols, phenolic acids (both benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins [31]. In food items,

Table 8.3 UV–vis used to detect phenolic compounds in different food samples

Sample	Individual phenolics[a]	Wavelength (nm)	References
Grape & wine	PCA, EC, PA, CA, GA, CAT, VA, SYA	280	[32]
Grape seeds	PCA, EC, GA, QC, CAT, RT, VA, SYA	280	[33]
Wine and tea	GA, PA, VA, CA, CAT, EC, SYA, QC, RT	280	[34]
Mushroom	RT	300	[98]
Guava leaf	MR, GA, QC, CAT	280	[35]
Plant material	PHA, VA, CA, SYA, PCA	254	[99]
Wine	RES	310	[100]
Wine	QC, RT, MR	360	[101]

[a]*CA* caffeic acid, *CAT* (+)-catechin, *EC* (−)-epicatechin, *GA* gallic acid, *MR* morin, *PA* protocatechuic acid, *PCA* *p*-coumaric acid, *QC* quercetin, *RES* *trans*-resveratrol, *RT* rutin, *SYA* syringic acid, *VA* vanillic acid

phenolics may contribute to the bitterness, astringency, color, flavor, odor, and oxidative stability of products. In addition, health-protecting capacity of some and antinutritional properties of other plant phenolics are of great importance to producers, processors, and consumers.

A number of approaches have been used to develop a simple and satisfactory UV spectrophotometric assay, which is shown in Table 8.3. These assays are based on different principles and are used to determine various structural groups present in phenolic compounds. Simple phenolics have absorption maxima of 280 nm [32–35], but their absorption is affected by the nature of the solvent employed and the pH of the solution.

3.1.4 Polycyclic Aromatic Hydrocarbons (PAHs) in Oil and Food

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings. They are primarily formed through incomplete combustion or pyrolysis of organic matter and during various industrial processes. At high temperatures, organic compounds are partially cracked to smaller unstable fragments (pyrolysis), mostly radicals, which recombine to give relatively stable PAHs [36]. PAHs have attracted the most attention because of their carcinogenic potential. Since edible oils and barbecued food are regularly consumed foodstuff, the level of PAHs should be determined regularly. Researchers have assessed different polycyclic aromatic hydrocarbons in corn, sunflower, olive oils, and barbecued meat and fish by HPLC/UV–Vis method [37]. The extraction procedure included saponification, liquid–liquid extraction, and finally purification of PAHs through silica–alumina column. The experimental analysis reported that oil samples contain different PAHs ranging from 0.44 to 98.92 μg L^{-1}. It was also found that barbecuing process increased the concentration (in the range of two- to eightfold) and caused the formation of PAHs in food samples.

3.2 UV–Vis Spectroscopy in Food Authentication

The term "authentication" used in food control refers to the confirmation of all requirements regarding the legal product description or the detection of fraudulent statements [38], especially in view of the following points: (a) substitution by cheaper but similar ingredients; (b) use of adulterants or undeclared processes; and (c) origin of the food (e.g., geographic, species, or method of production).

The analysis of specific marker compounds, which are indicative of certain properties of the product, is the most classical food authenticity assessment. Overall, the process through which food or beverages is verified as complying with its label description is called food authentication [39]. In recent years, food authenticity has become an issue of great concern due to unscrupulous practices by some manufacturers and food distributors of marketing fake and adulterated food products. Spectroscopic techniques such as UV–Vis absorption spectroscopy can be used for the identification of adulterated and fake food products, differentiation of food origins, and classification of food.

3.2.1 Beef Adulteration

Meat and meat products are closely associated with the daily eating habits of people around the world. Meat is also highly perishable due to its high water contents and good nutritional environment for microflora. Quality monitoring of meat and meat products is essential to ensure public health. In recent years, the meat industry has employed state-of-the-art, high-speed processing technology to analyze and ensure meat quality. Meat processors need rapid, nondestructive, and easy-to-use technology to monitor the safety and quality of meats and meat products for the economic benefit [40]. Instrumental methods and chemical analysis are being used for the detection of internal quality attributes of meat for decades.

Low-oxygen-modified atmosphere packages containing 60–70% CO_2, 30–40%N_2, and 0.4% of CO ensure prolonged shelf-life of fresh meat. In particular, CO is added because it binds to the myoglobin of the muscle tissue with a high affinity resulting in a bright, cherry-red colored Mb–CO complex. From 1985 to 2004, CO was used for modified atmosphere packaging (MAP) of fresh meat in Norway. In 2002, CO-MAP was approved in the United States, New Zealand, and Australia [41]. The European Community, however, pointed out that under inappropriate storage conditions, the presence of CO may mask visual evidence of spoilage [42]. Therefore, CO treatment of fish and meat at an industrial level is not admitted in the European Community, and CO is not included in the list of the allowed food additives. As a consequence, it is of interest to determine the amount of CO bound to the myoglobin of the muscle tissue, both to assess the effects of a CO-enriched atmosphere on the preservation and shelf-life extension of meat and fish and to prevent its fraudulent use in the European Community countries. Different research groups reported spectrophotometric method, based on the

electronic absorption and second-derivative spectra of beef drip, to detect the amount of CO bound to myoglobin in treated beef samples [43]. The accuracy of the method has been studied in terms of trueness and precision. The method is easy, rapid, and allows one to determine qualitatively and quantitatively the concentration of CO (as low as 0.14 μM) in the meat drip, by simply measuring in the UV–Vis spectrum (i.e., absorbance) at three specific wavelengths: 423, 429, and 434 nm. Any comparison with other methods???

3.2.2 Adulteration of Milk (Melamine Detection)

Milk quality can be compromised by adding a low-value component, such as water, fillers, preservatives, or melamine to maximize economic gain [44]. To detect adulteration in dairy products, determination of physical properties, such as specific gravity and freezing point, is widely used; however, these techniques are tedious and lack accuracy [45]. Therefore, spectroscopic techniques have emerged as suitable replacements because they are fast, cheap, and simple [46].

Melamine is a nitrogenous substance that is used by other food manufacturers to increase protein content, although it is not an approved food ingredient [47]. Ingestion of melamine at levels above the safety limit may result in adverse effects on people's health. For this reason, the analysis of melamine in milk products has been of great interest to scientists [48, 49]. Researchers have performed spectrophotometric study, using 0.1 M sodium hydroxide as a solvent to detect the melamine residue in raw milk and milk-related products [50]. In a study to detect melamine in milk samples, 31 milk samples (raw milk and milk-related product) were analyzed by the standard addition method [50]. The sample absorbance was carried out by the addition of a known quantity of standard, i.e., 3 ppm and 8 ppm at the same wavelength. The maximum absorbance was observed at 219 nm. Subsequently, the absorbance of varied concentrations of standard was measured at 219 nm, and a linearity curve was constructed. The linearity curve for the concentration of melamine ranged from 3 to 8 μg/mL. It was observed that all 31 samples contained melamine residue. This study confirmed the existence of melamine residue in commercially available pasteurized milk [50].

3.2.3 Adulteration of Olive Oil

Oil blends containing olive oil are commonly commercialized for economical and nutritional reasons. However, oil blends are adulterated with cheap oils or oil blends without extra virgin olive oil (EVOO). Palm oil (PO) is often used as an oil blend adulterant since it is relatively cheap compared to other vegetable oils. Edible oil adulteration is not only a major economic fraud but also exhibits major health implications for consumers. Therefore, detecting and quantifying EVOO and EVOO–vegetable oil blend adulterants are important. Hence, the UV spectroscopy technique was applied due to its simplicity and affordability as an analytical tool that also

requires less preparation time and process sample. When heated, the oil samples were monitored from ambient to high temperatures by UV–Vis spectroscopy. UV spectrometry coupled with partial least squares regression (PLSR) and principal component analysis (PCA) were used to detect the quality of EVOO–vegetable oil blends [51]. Furthermore, the method can be applied to distinguish among EVOO–vegetable oil blends, oil blends without EVOO, and oil blends with adulterant, combined with PCA models. This technique can be used to identify and quantify soybean oil (SO), sunflower oil (SFO), corn oil (CO) and palm oil (PO), and extra virgin olive oil (EVOO, Olivoila, Italy) from different oil blends.

3.2.4 Caffeine Content in Beverage

Nonalcoholic beverages, such as carbonated soft drinks, sports drinks, energy drinks, fortified beverages, bottled waters, tea, coffee, fruit juices, and vinegars, are an important component of the human diet [52, 53]. Because of the high consumption, there is a need to monitor the quality of nonalcoholic beverages. This involves determining and monitoring their composition, physicochemical properties, and sensory properties. Different research groups have demonstrated extensive use of UV–Vis spectroscopy to assess beverage consistency, quality, and authentication [54, 55].

Caffeine is a naturally occurring substance found in humans, and caffeine is a central nervous system (CNS) stimulant [56]. Beverages containing caffeine, such as coffee, tea, soft drinks, and energy drinks, enjoy great popularity. It is one of the most common ingredients of energy drinks which is added as a flavoring agent and to make the drinks addictive [57]. The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts, and tea leaves. Quantitative estimation of caffeine concentration in drinks was performed by UV spectrophotometric method using carbon tetrachloride as the extracting solvent at 270 nm wavelength [58, 59]. In the experimental study, researchers measured the caffeine concentration of a wide range of soft drinks and energy drinks. The pH of those soft drinks was ranging from 2.29 to 3.02, and in energy drinks, the pH varied from 2.85 to 3.28. The minimum caffeine level of soft drinks was observed to be 10.69 mg/serving, while the highest caffeine content was 42.17 mg/serving. On the other hand, the minimum caffeine level of energy drinks was observed at 32.04 mg/L, while the highest caffeine level in energy drinks was 101.70 mg/serving. The pH of these soft drinks was 2.29 to 3.02 and in energy drinks 2.85 to 3.28.

3.2.5 Identification of Adulteration in Roasted Coffee

The compositional analysis of coffee by UV–Vis spectroscopy is hindered by its inherent dark color and chemical complexity. Nevertheless, researchers have used UV–Vis spectroscopy coupled to successive projections algorithm for variable selection in association with linear discriminant analysis (SPA-LDA) for the

identification of adulterations in ground roasted coffees [60]. For this purpose, extracts of ground roasted coffees prepared in hot water alone (representing the final product as ingested by the consumers) were analyzed. The proposed methodology provided a simple and fast analysis of the aqueous extracts of ground roasted coffees. It can be used as a reliable tool to coffee consumers and regulatory agencies to prevent fraudulent labeling. In an experimental study, researchers successfully analyzed 45 unadulterated coffees and 57 adulterated coffees (adulterated with husks and sticks) [60]. Adulterations in the coffee samples were determined to be in the range of 1.04 to 7.63 g per 100 g coffee.

3.2.6 Adulteration of Pomegranate Juice

Among fruit juices, pomegranate juices (PGs) are considered a good source of phenolic compounds with strong antioxidant activity, whose consumption could improve cardiovascular health and inhibit the proliferation of many cancers [61]. PG contains a significantly high level of powerful antioxidants ellagitannins (e.g., ellagic acid, punicalagin, and punicalin) [62] and anthocyanins (i.e., delphinidin, cyanidin, and pelargonidin 3-glucosides and 3,5- diglucosides) which is responsible for its red–purple color [62, 63]. The most common way for adulteration of PG is either dilution or addition of another foreign juice, such as grape, apple, sour cherry, and strawberry to PG [64]. Undeclared added foreign juices could contain potential allergens [65]. For these reasons, analytical methods can verify the authenticity of pomegranate juices, and these have great importance.

Based on UV–Vis spectroscopy and chemometrics, a screening method was proposed for a quick screening of some common fillers of pomegranate juice that could decrease the antiradical scavenging capacity of pure products [63]. Fifty-nine different commercial fruit juices claiming to be authentic were analyzed which included 14 pomegranate juices (PG), 27 grape juices, 21 red grape juices, RG; two white grape juices, WG; four mix grape juices, XG, 11 apple juices (AP) and seven mix fruit juices containing pomegranate juice. UV–Vis spectroscopy was used to record the absorption spectra in the range 190–1100 nm. The outcomes were evaluated using multivariate exploratory analysis. The results indicate that the proposed strategy can be a useful screening tool to assess the addition of filler juices and water to pomegranate juices.

3.3 UV–Vis Spectroscopy in Food Composition (Food Quality)

Food composition may vary depending on soil, climate, macro, and micronutrients in fertilizers, genetic resources (varieties/cultivars, breeds), storage conditions, processing, and fortification. Accurate analysis of food composition is very important because of its relationship with both the quality and the specific characteristics of foods. UV–Vis spectroscopic method may provide quick and wide information on food composition.

3.3.1 Analyzing Fruit Composition

Parameters that are involved in analyzing fresh fruit quality include firmness, skin and flesh color, ethylene production, respiration rate, sugars, organic acids, pigments, phenolics, and volatile compounds. Although the quality is attributed to the balance of the above factors, the taste, such as sweetness, avidness, and bitterness, would be one of the most interesting factors for consumers. Especially, the acid and sugar content in fruit and their ratio are very important factors for the quality evaluation by consumers. The acidity and sugar content are conventionally evaluated with the acidimeter and Brix scale based on the titration and refractometry, respectively. Therefore, the predicted values indicate only the overall acidity and sugar content and do not have enough accuracy. Moreover, it is quite difficult to evaluate this kind of internal quality of fruit from the outside. UV–Vis spectroscopic technique has the potential to evaluate the quality of fruits by quantifying the important components. UV–Vis is applicable in measuring various parameters of different fruit juices that is shown in Table 8.4.

3.3.2 Freshness of Fish (K Value)

Freshness is the most important single criterion for judging the quality of fish products [66]. Currently, the K value of fish flesh is considered as a standard index for fish freshness in the scientific world [67]. The K value is defined as the ratio of inosine (HxR) plus hypoxanthine (Hx) to the total adenosine triphosphate (ATP) and related compounds (ADP, AMP, IMP, HxR, and Hx) in fish muscle extract [68].

Table 8.4 Summary of applications of UV–vis spectroscopy on various types of fruit

Fruits	Measured parameter	Spectrophotometer	Wavelength range	References
Apple	Sugar content	UV–vis spectrophotometer	300–900 nm	[102]
Mandarin	Total flavonoids, Individual flavanon Glycosides	Spectrophotometer UV UNICAM HELIOS β	420 nm	[103]
Peaches	Polyphenol oxidase and peroxidase	UV–Vis irradiation	250 and 740 nm	[104]
Kiwi, pomelo	Phenolic acids and flavonoids	UV-550 Jasco spectrophotometer	200–350 nm	[105]
Mango	Chrolofil, mangiferin	UV–Vis spectrophotometer	258.5 nm	[106]
Orange	Soluble solid content (SSC)	QE65000 VIS–SWNIR CCD spectrometer	200–1100 nm	[107]
Banana, Papaya, Mango, Avocado, Apple	Total polyphenols	SP65 UV–Vis spectrophotometer	511 nm	[108]

A method to predict K value of fish flesh using UV–Vis spectral properties (250–600 nm) of its eye fluid and a partial least squares (PLS) regression method was investigated [69]. In order to determine the K value of fish flesh, samples were taken from the dorsal part of the fish. For spectral measurement, about 0.5 mL of eye fluid was collected from both eyes of four fish (selection based on matching size and weight from fish population) using a syringe for each sample and centrifuged at 5000 rpm for 30 min to obtain a homogenous solution. An aliquot of the clear liquid fraction was then used for spectral measurement. Fisheye fluid spectra were acquired using a UV–Vis spectrometer. For each sample, the spectrum was recorded from 250 to 600 nm at a bandwidth of 0.5 nm and a scan speed of 400 nm per minute. The above technique successfully demonstrated that the use of UV–Vis spectroscopy combined with appropriate multivariate analysis has the potential to accurately predict K value of fish flesh.

3.3.3 Analyzing Milk Fat

The composition of dairy products is of high importance for quality purposes. The major components that are found in dairy products include fats, proteins, minerals, vitamins, enzymes, and carbohydrates [70]. Milk fat measurement is one of the key tasks performed in dairy industries for determining milk price and is necessary to know for casein–fat ratio normalization. UV spectrophotometry is a simple, fast, and cost-effective alternative screening method for milk fat measurement in fluid and powdered milk without prior extraction. This technique is based upon the property of fatty acids to absorb UV light proportional to their concentration. To analyze the fat content of milk samples, milk samples with different fat content mixing proper volumes of whole, skimmed, and defatted milk were prepared [70] Samples containing 3% of fat were prepared by adding milk cream to 3% fat milk. Milk (30 or 60 μL) was added to 3 mL of absolute ethanol at temperature −20 °C. All vials were hermetically capped and stored for 1 h at temperature −20 °C. This procedure allows the precipitation of proteins and hydrophobic peptides that interfere with UV measurement. Samples were centrifuged at 13,000 rpm for 15 min and allowed to reach room temperature. Aliquots of the supernatants were measured at UV wavelengths at spectral range of 200–300 nm. UV spectrophotometry technique was highly effective with compatible accuracy to measure milk fat.

3.3.4 Evaluation of Edible Oil

Edible oils provide essential fatty acids (FAs), which are building blocks required by hormones to regulate body systems. Edible oils are the most concentrated energy source [71]. Moreover, edible oils carry oil-soluble vitamins A, D, E, and K and enhance texture and mouthfeel, imparting flavor, and contributing to the feeling of satiety after eating. Edible oils are classified based on origin into three main groups: vegetable oils, marine oils, and animal fats. Edible oils are basically triglyceryl

esters of glycerol with three molecules of FAs. The physical and chemical properties of edible oils depend on the nature of FAs attached to glycerol molecule. FAs may be saturated fatty acids (SFAs) or unsaturated with short, medium, or long carbon chain length, which range from C12 to C24, of which C18 is often dominant [72]. Various edible oils are commercially available for human consumption in the form of cooking oils and food ingredients as well as raw materials for preparing cosmetics, soaps, surfactants, lubricants, plasticizers, paints, coatings, pharmaceuticals, and agricultural and industrial products [73].

UV–Vis technique can be useful to evaluate edible oils. Researchers have developed UV–vis spectroscopy coupled with chemometrics to authenticate edible oils and evaluate tocopherol in edible oils [51, 74]. To analyze the degradation of edible oils, commercially available edible soya, corn, sunflower, canola, and olive oils were analyzed in triplicate. The samples were heated from 30 °C until 170 °C, increasing by steps of 10 °C, the first spectrum being taken at the room temperature (25 °C). UV–Vis spectra were acquired in the range 300–540 nm. The results suggested that sunflower, colza, and olive oils offered more resistance with increasing temperatures, while soybean and corn oils were less resistant. This technique could be used to determine the contribution of chemical compounds to oxidative phenomena and the comparison of oils according to their stability.

3.3.5 Vitamin C in Fruits and Vegetables

Vitamin C (ascorbic acid) is an essential micronutrient required for the normal metabolic function of the body and plays an important role as a component of enzymes involved in the synthesis of collagen and carnitine. Ascorbic acid is reversibly oxidized to form L-dehydroascorbic acid (DHA) which also exhibits biological activity. Dehydroascorbic acid has been converted into acetic acid in the human body. For determining the activity of vitamin C, it is important to determine both acetic acid and dehydroascorbic acid in fruits and vegetables. Vitamin C is a water-soluble antioxidant within the body. It lowers blood pressure and levels cholesterol and is vital for the formation of bone and tissue repair. Citrus fruits, fruit juices, and vegetables are the best sources of vitamin C.

Ascorbic acid and dehydroascorbic acid have been investigated in fruits and vegetables using UV spectrophotometric method [75, 76]. In the reported experimental study, researchers homogenized a blended sample of various fruit and vegetable with 85% sulfuric acid and 10% acetic acid solution, and bromine water was added to oxidize ascorbic acid to dehydroascorbic acid in presence of acetic acid. After coupling with 2,4-dinitrophenyl hydrazine at 37 °C temperature for 2 h, the solution was cooled using an ice bath. After that, the solution was treated with dilute sulfuric acid to produce a red color complex, and the absorbance was measured by UV spectrophotometer (280–521 nm). It was observed that UV spectrophotometric method was a good agreement with the results obtained by titration method.

3.3.6 Lactic Acid Content in Cheddar Cheese

Cheese is prepared from milk having a dynamically balanced mixture of protein, fat, carbohydrates, vitamins, water, lactose, lactoglobulin, lactalbumin, and water-soluble minerals [77]. Cheddar is a hard type of cheese having high nutritional value owing to the concentration of caseins which contain various levels of all essential amino acids, fat, and small amounts of other nutrients like calcium, sodium, potassium, retinol, riboflavin, pyridoxine, and cyanocobalamin [78, 79]. The quality of cheddar cheese depends upon starter cultures, manufacturing technology, and composition of milk [77]. During ripening, Cheddar cheese experiences momentous biochemical modifications and transformations because of glycolysis, proteolysis, and lipolysis [78]. As a result, fresh curd, having a bland flavor and rubbery texture, is converted into a product with an attractive flavor and smooth texture. The flavors produced in cheese are very multifaceted and comprise substances including organic acids, which are the metabolites of bacterial cultures [79]. A primary function of bacterial culture is to convert the lactose into glucose or galactose, which are then converted into the end product of lactic acid. Lactic acid production has significantly influenced the quality of cheese. In addition, the acidification contributes a preservative effect with the result that many pathogenic and spoilage bacteria are inhibited [80]. Lactic acid is the most abundant organic acid present in cheese varieties. It is the metabolite of glycolysis reaction during cheese ripening [79]. Quantitative determination of lactic acid is important because it is an indicator of bacterial activity and contributes to cheese quality by imparting flavor [81]. Keeping in view the abovementioned facts, research groups performed to compare the lactic acid contents in Cheddar cheese prepared from buffalo and cow milk using UV–Vis spectroscopy [82].

Cow and buffalo milk samples were standardized at 4.0% fat level. Cheddar cheese samples were manufactured from cow and buffalo milk using direct in vat (DVS). Cheese samples were stored for ripening at 4 °C for a period of 120 days. During ripening, samples were evaluated for various quality parameters. Moisture, fat, protein, ash, pH, acidity, and salt were determined at 1-month intervals during ripening. Lactic acid concentration of cheese during ripening was determined after 2 and 4 months using UV–Vis spectroscopy [82]. During ripening, lactose and pH value are decreased, while the acidity and lactic acid contents are increased.

3.3.7 Contamination in Egg White

Eggs are a high-protein food and have the lowest price per unit weight when compared to other animal proteins. Because of their unique functionalities, egg yolk and egg white have different markets. Egg yolk, being an excellent emulsifier, is also used extensively in the food industry. Accidental contamination of egg white by yolk during egg-breaking operation often occurs, which decreases the functionalities and performances of egg white and results in economic loss. Yolk has a negative effect on the foaming properties of egg white. The presence of even small

quantities of yolk caused a significant reduction in egg white foaming capacity [83]. The problem of yolk contamination is becoming more critical with the high speed of egg breaking and high demand for product consistency. Because it is practically impossible to produce completely yolk-free white on a commercial production scale, it is important to develop a rapid and sensitive in-line detection method to provide timely feedback to the breaker operator. This can improve processing efficiency and product quality. A UV–Vis spectroscopy technique was developed as a rapid and noninvasive method for the identification and quantification of egg white by egg yolk [84]. The wavelength of 500 nm was used for the quantification of egg yolk in egg white at levels ranging from 0% to 0.5% (w/w). The optical absorbance of the "contaminated" egg white samples positively correlated with the yolk concentration, and its intensity was affected by the freshness of eggs, egg variety, and measuring temperature.

4 Limitations of UV–Vis Spectroscopy to Analyze Foods

UV–Vis spectroscopy is a widely used technique for the quantitative and qualitative analysis of analytes. UV–Vis spectroscopy follows Beers-Lambert law and can give high accuracy and precision. However, despite various advantages offered by UV–Vis spectroscopy, its application in food analysis can be limited due to low concentration target parameters in foods and high detection limits. Pesticides and metals in food are often lower than the detection limits of the spectroscopic technique. UV–Vis spectroscopy can analyze samples in liquid form. The results of the absorption can be affected by pH, temperature, contaminants, and impurities. Sample preparations for different parameters to be assessed using UV–Vis spectroscopy may take long time, and cuvette handling can affect the reading of the sample. Table 8.5 summarizes a few of the advantages and limitations of the use of UV–Vis spectroscopy to analyze foods.

5 Conclusion

Analyzing product quality and authenticity, the detection of adulteration are the major issues in the food industry. Given the inherent complexity of food products, most instrumental techniques (e.g., chromatographic methods) employed for quality and authenticity evaluation are time-consuming, expensive, and labor-intensive. Therefore, there has been an increasing interest in simpler, faster, and more reliable analytical methods for assessing food quality attributes. UV–Vis spectroscopy has developed in leaps and bounds over the past several decades. The UV–Vis analytical method has become of crucial importance and a ubiquitous technique in different scientific areas throughout the world due to its availability, simplicity, flexibility, and extensive uses in various areas, including biochemistry and analytical

Table 8.5 Advantages and limitations of UV–vis spectroscopy to analyze foods [109]

Serial no	Description	Advantages	Limitations
1	Sensitivity of application	High sensitivity	–
2	Cost of instruments	Relatively low cost	–
3	Remote sampling	Available using for fiber optics	–
4	Sample average	Good to very good depending on the optics	–
5	Solid samples	Analyzed using reflectance	–
6	Slurry samples	Analyzed using transflectance or fiber optics	Effect of scattering and path length
7	High chemical resolution	–	No
8	Effect of path length	–	Sensitive in changes of path length
9	Qualitative analysis(multiple wavelengths)	–	Need for chemometric Tools (principal components)
10	Quantitative analysis	Linear regression	Multivariable calibration needs of chemometric tools

chemistry. Hence, UV–Vis spectroscopy can be highly useful for food analysis. This chapter focused on UV–Vis techniques based on spectroscopy for the quality and safety assessment of foods and covers the major food items. This study reviewed the potential of UV–Vis spectroscopy for monitoring chemical composition, assessing physical and sensory parameters, ensuring authenticity and compliance with specifications, and detecting unintentional and economically motivated adulteration. The development of these methods and techniques provides the food industry a vast arsenal of tools that can be used to authenticate and trace foods as well as to assure consumers about the origin and safety of foods.

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Chapter 9
Gas Chromatography and Mass Spectroscopy (GC-MS) Technique for Food Analysis

Saniya Yesmin Bubli, Farah Haque, and Mohidus Samad Khan

Abstract Human health is strongly associated with food quality and safety. The presence of unwanted substances in foodstuffs makes them unqualified for human consumption. Therefore, the detection of inherent harmful ingredients and artificial hazardous compounds in food has become a serious concern to ensure the availability of safe food. Due to high accuracy, gas chromatography and mass spectroscopy (GC-MS) technique is widely accepted for the qualitative and quantitative analysis of food. This technique is also popular in forensic, energy, environmental, and pharmaceutical areas. Food quality can be assessed in terms of aroma, freshness, physical appearance, taste, and color. GC-MS technique can be used to detect and monitor different parameters of food quality as well as food adulteration. This chapter briefly discusses the mechanism of GC-MS, preparation of food samples prior to analysis, and detection of different food items.

Keywords Gas chromatography · Mass spectrometry · Mass-to-charge ratio · Ionization · Spectrum · Ion chromatogram · Quantification · Identification · Extraction · Volatile compounds · Organic compounds

1 Introduction

Food quality and safety are important for consumers, producers, food industries, policy makers, and researchers [1]. Apart from naturally occurring substances, food products may contain certain harmful compounds that can be contaminated during the cultivation, processing, or preservation of food. These toxic compounds can also be formed during processing, packaging, and storage. In addition, the nutritional and functional components of foods need to be analyzed. Therefore, valid methods

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of food analysis are always needed. Food analysis, a special branch of analytical chemistry, offers various techniques to identify the composition, structure, physicochemical, and sensory properties of food products [2]. With the advancement of analytical techniques, researchers and technicians can quantify the manifold unexpected natural and man-made substances found in food products [3]. With the increased demand for reducing detection limits, highly selective detection techniques are of critical importance for food analysis. For high selectivity and sensitivity, Gas Chromatography and Mass Spectroscopy (GC-MS) is considered a universal and versatile detection technique for food analysis [4, 5].

In the early 1980s, mass spectrometry (MS) was considered to be costly, intricate, and time-consuming. At the beginning of the 1990s, mass spectrometry became more widely recognized and also a vital detection method for gas chromatography (GC). The modern computer systems have made GC-MS more applicable alongside traditional spectroscopic methods in terms of its simple construction, comprehensible function, and operating system [5]. Conventionally, GC has been used to separate volatile and thermally stable compounds from a complex organic and biochemical mixture, whereas MS can provide the detailed structural identification of each component [6]. Therefore, the complete integration of gas chromatography and mass spectrometry into a single GC-MS method has been proved to be a synergistic combination in every aspect [5].

GC-MS has the capability to separate complex mixtures to quantify analytes even at sufficiently low concentrations and determine organic contaminants in trace levels. This technique has become the preferred analytical method due to its sensitivity, low detection limit, simultaneous identification, and quantification which solves the matrix interference problem [7]. The combination of MS with a fast GC instrument shortens the running time of sample analysis compared to conventional GC instruments [7]. On the other hand, liquid chromatography coupled to mass spectrometry is still difficult to control in comparison with GC-MS [2, 5].

GC-MS has a wide variety of application areas including environmental monitoring, food safety, packaging, flavor and fragrance analysis, biological and pesticides detections, forensic and criminal cases, drugs and pharmaceutical applications, energy and fuel applications, and also petrochemical and hydrocarbons analysis [4, 8, 9]. Especially, 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, and terpenes, in a wide range of food items [4]. In the domain of food quality assurance, this technique can be used for odor and freshness analysis, detection of contaminants, spoilage, and adulteration of food. In this chapter, the mechanism of GC-MS, sample preparation methods for various food items, and detection of different elements in a wide range of foodstuffs are briefly discussed. An overview of the application of GC-MS in food science is discussed in this chapter and it is summarized in Table 9.1.

Table 9.1 Application of GC-MS in food science

Serial no.	Basic food categories	Food types	Elements detected/ adulteration	References
1	Dairy products	Milk and milk products	Cyanuric acid (CYA) and melamine (MEL)	[26]
		Cheese	Major volatile-free fatty acids	[31]
2	Beverage and juice	Coffee	Polycyclic aromatic hydrocarbons (PAHs)	[35]
		Green coffee, tomato juice, and orange juice	Furan	[38]
		Coffee and barley	Adulteration of ground-roasted coffee with roasted barley	[40]
3	Meat and fish products	Meat	N-nitrosamines (NAs)	[42]
		Sea fish, freshwater fish, and crustaceans	Formaldehyde	[49]
4	Fruits and vegetables	Apple products and quince jam	Patulin	[51]
		Cranberry fruit	Benzoic and phenolic acids	[57]
		Berry fruits	Pesticides	[59]
		Tomato, pepper, and cucumber	Pesticides	[63]
5	Bakery products	Chocolate candies	Polycyclic aromatic hydrocarbons (PAHs)	[65]
		Honey	Volatile compounds	[68]
6	Fat and oil	Oil seeds	Pesticide residues	[71]
		Edible vegetable oil	Antioxidants	[73]

2 Mechanism of Operation

The GC component of GG-MS system separates chemical mixtures, and MS component identifies the constituents at a molecular level. A compound must be sufficiently volatile and thermally stable to meet the primary requirement of GC-MS analysis. Furthermore, chemical modification or derivatization may be required for the functionalized compounds to remove unexpected adsorption effects and to attain good quality data.

2.1 Principle of GC-MS

In GC, the elements of a vaporized sample are separated as a result of differential distribution between a mobile gaseous phase and a liquid or solid stationary phase. The mobile phase must contain a chemically inert carrier gas. The most commonly used carrier gas is helium; however, argon, nitrogen, and hydrogen can also be used as helium substitutes. The stationary phase, which is a column, and all the

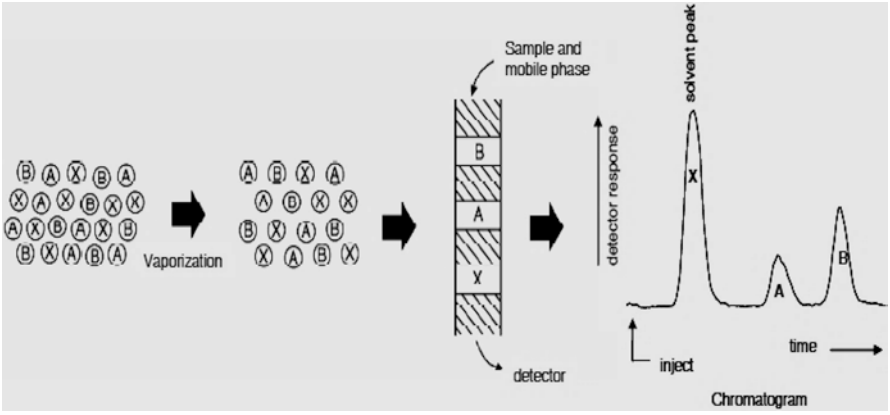

Fig. 9.1 Conceptual functioning aspects of a gas chromatograph (Mixture of A and B dissolved in solvent X and A has a lower solubility in the stationary phase than does B) [10]

separation mechanisms take place in the column. In the column, a microscopic layer of liquid or polymer on the inert solid support is placed inside glass or metal tubing [6]. The capillary column contains a stationary phase, a thin solid buttress surfaced with a nonvolatile liquid, where the solid itself can be the stationary phase. The specimen is swept through the stationary phase fixed in a column by a helium gas stream. Components are separated based on their retention time (RT) as the time varies to reach the detector [6]. A conceptual operational feature of a gas chromatograph is shown in Fig. 9.1.

The main criterion of mass spectroscopy is to deal with the mass of the isotopes of the elements, instead of atomic mass of the elements. Despite directly determining mass, MS determines the mass-to-charge ratio (m/z) of ions (mass-to-charge ratio is the ratio of the mass of the ions on atomic scale to the number of charges that the ions possess) [10]. Ions are charged particles, and in space, their position can be controlled with the use of electric and magnetic fields. When only individual ions are present, they can be grouped according to their unique properties (mass and the number of charges) and moved from one point to another. In order to attain individual ions free from any other forms of matter, it is necessary to analyze them in a vacuum. It is the fundamental requirement of mass spectrometry when the ions stay in the gas phase before they can be separated and detected according to their individual m/z values [10]. A conceptual picture of gas-phase ionization of analytes followed by ion separation is shown in Fig. 9.2.

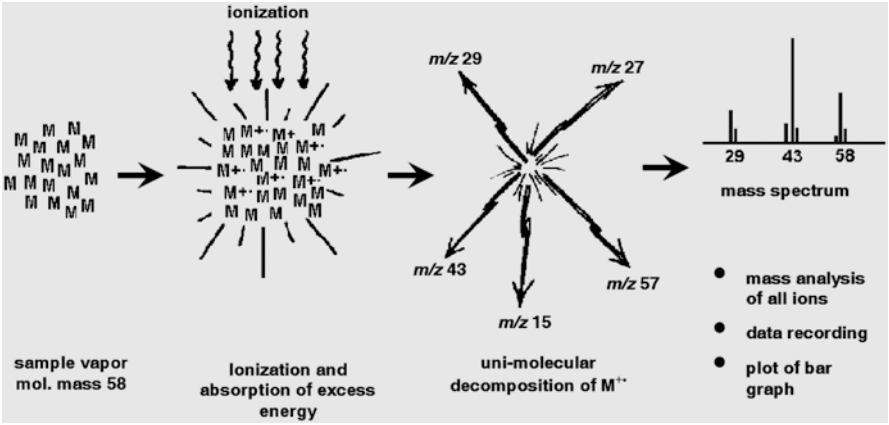

Fig. 9.2 Conceptual picture of gas-phase ionization of analytes according to the m/z value followed by ion separation [10]

2.2 Instrumentation and Working Steps of GC-MS

Gas is fed from a cylinder through a supply pipe to the instrument. It is important to ensure the purity of gases and appropriate gas supply pressure. In the injector part, the sample is vaporized, and the resulting gas is sent to the GC column through the carrier gas medium. In GC, retention of analyte molecules occurs due to strong interactions with the stationary phase than the mobile phase. The sample is separated into its constituent components in the column which is either packed or hollow capillary columns carrying the stationary phase coated onto the inner wall [6]. Based on applications, the length and internal diameter of columns can be changed [6].

The temperature in GC is controlled by a heated oven. The temperature of the column is an important variable; therefore, the oven is equipped with a thermostat that controls the temperature to a few tenths of a degree. The boiling point of the sample and the amount of separation are required to determine the temperature of operation [6].

After being separated in the column, the sample is passed through a detector. The phase ions are separated within the mass spectrometer by applying electrical or magnetic fields to differentiate ions [6]. Prior to the analysis in the mass spectrometer, the products are ionized in the ion source. The mass analyzer takes ionized masses and separates species on a mass-to-charge basis and passes them to the detector, where they are detected and later converted to a digital output [6]. The output signal is finally forwarded to a computer to be displayed and analyzed. The computer linked to the GC-MS system has a library of samples to assist in investigating the acquired data [6, 10, 11]. Data have been displayed in various ways: (i) the first one is the total ion chromatogram and (ii) the second one is the mass spectrum at a specific time in the chromatogram. A schematic diagram of GC-MS

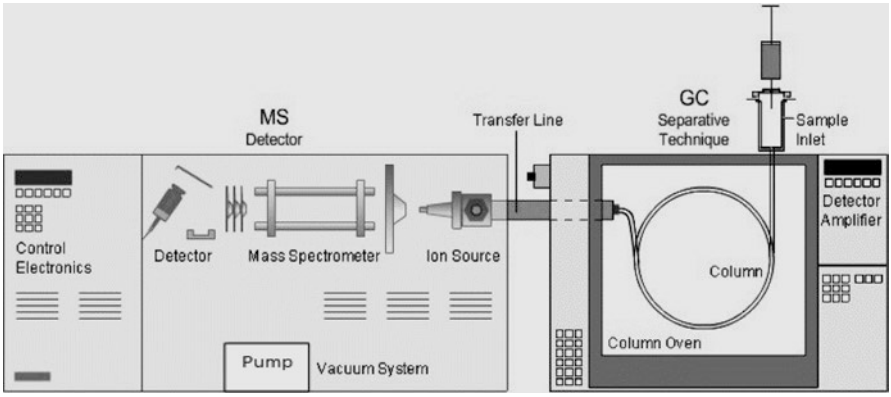

Fig. 9.3 Schematic diagram of gas chromatography and mass spectroscopy (GC-MS) technique [6]

method is presented in Fig. 9.3. The basic steps of GC-MS mechanism are shown in Fig. 9.4.

3 Sample Preparation Method

Effective sample preparation is highly required for receiving satisfactory analytical results. Hence, despite the vast development of analytical instrumentation during the last two decades, sample preparation remains a pivotal part of the complete analytical process. Therefore, over the years, researchers have been given considerable efforts to develop fast, accurate, and detailed sample preparation techniques to reduce the analysis time without compromising the integrity of the extraction process [12]. Conventionally, sample preparation is required to enhance the chromatographic behavior and detectability of analytes and to isolate analytes from the complex matrix [13].

Food samples cover a wide variety of physical types ranging from dry powders to biological matrices, like meat, fats, and liquids or solutions. Extensive sample extraction and preparation are required before instrumental analysis for determining trace residues and contaminants present in food. The amount of sample preparation depends on several factors such as the sample matrix and the characteristics and analyte level to be determined [14]. Methods to analyze different elements or quality of food involve a series of steps within sample preparation, which include sampling/homogenization, extraction, clean-up, derivatization, and concentration followed by the final analysis [14]. At present, a wide variety of modern techniques are available for extraction and clean-up of the target analytes from the food matrix such as solid-phase extraction (SPE), solid-phase microextraction (SPME), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), liquid–liquid extraction (LLE), and headspace techniques [5].

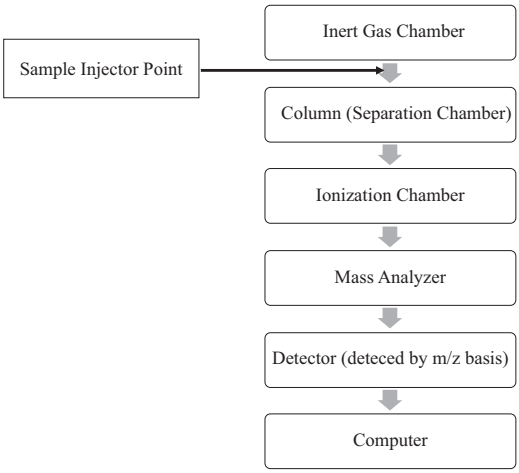

Fig. 9.4 A block diagram showing the basic steps of GC-MS instrumentation

3.1 Solid-Phase Extraction (SPE)

In the field of separation science, solid-phase extraction (SPE) is a fast, effective, and selective sample preparation technique. The major objectives of SPE include elimination of disturbing matrix elements and selective concentration and separation of target analytes. This ensures enhanced qualitative or quantitative analyses by GC, HPLC, GC-MS, or other chromatographic methods. SPE can either be performed off-line with the isolated sample preparation from the following chromatographic analysis or online by direct attachment to the chromatographic machine [15]. The equipment requires less space for SPE, and therefore, various parallel sample processing is thoroughly possible [5].

3.2 Solid-Phase Microextraction (SPME)

The solid-phase microextraction (SPME) device is constructed of a silica fiber coated with a fine layer (5–100 μm) of an appropriate polymeric sorbent or immobilized liquid [16]. SPME is a two-step process: (i) splitting of analytes between the sample matrix, which can be a headspace vapor or a liquid sample and (ii) the fiber coating followed by the desorption of the (concentrated) extract from the fiber into the analytical system, usually a GC, where the sample constituents are thermally desorbed [14]. The SPME methods coupled with GC or GC-MS system are widely used for volatile and semi-volatile compounds, while the SPME in combination with HPLC or LC–MS are carried out for the analysis of less volatile or thermally labile compounds [12].

3.3 Supercritical Fluid Extraction (SFE)

An environmentally friendly extraction technique is supercritical fluid extraction (SFE). It is an alternative to classical solvent extraction and has strong industrial and analytical advantages. It is mainly used off-line as a sample preparation step prior to the analytical determination, but it can be easily coupled online to chromatographic and other analytical instruments or directly to a detection system [12]. The extraction yields are largely influenced by the nature of the matrix and the analyte as well as the types of supercritical fluid. Due to the low toxicity, low cost, and convenient critical temperature and pressure, usually carbon dioxide is used as a supercritical fluid for providing faster reaction kinetics and more selective extraction than other liquids [14].

3.4 Pressurized Liquid Extraction (PLE)

Pressurized liquid extraction (PLE) is a green extraction approach that involves extraction using liquid solvents at elevated temperature and pressure to ensure quick and efficient extraction of the target analytes from the solid matrix [17]. The major advantage associated with this technique is the reduced extraction time and low consumption of extraction solvent which makes it widely used in biology, pharmaceutical, and food industries [18, 19].

3.5 Liquid–Liquid Extraction (LLE)

Liquid–liquid extraction (LLE) is also known as the partitioning and solvent extraction method [20]. It is used to segregate compounds according to their relative solubility in two different immiscible or slightly miscible liquids. This process is a fundamental technique of many analytical laboratories and is widely used in chemical, pharmaceutical, and food industries.

3.6 Headspace Technique

Headspace technique is considered as one of the most sophisticated and flexible methods for instrumental sample preparation and transfer for GC-MS techniques. This process is used to separate the volatile substances from the sample matrix [5]. The extraction of the analytes depends on the separation of the very and moderately volatile components between the matrix and the gas phase above the sample. After setting the partition equilibrium, the gas phase carries a qualitatively and

quantitatively characteristic cross section of the sample to analyze the components to be determined [5]. Liquid and solid sample matrices are mainly used for headspace techniques; however, gaseous samples can also be analyzed using this technique. Using the headspace technique, both qualitative and quantitative determinations are performed in combination with GC-MS systems. Generally, for the analysis of volatiles in a sample matrix, two methods are used—the static and dynamic (purge and trap) headspace techniques [5].

3.7 General Considerations

The type of GC column, flow rate, injection temperature, and oven temperatures are key parameters for sample analysis using GC-MS. [21]. To avoid any negative effect on the quality of chromatographic separation sample, injection needs to be carefully done in GC-MS sample inlet systems. A high-quality chromatographic analysis also amplifies the performance of mass spectrometer. The column diameter varies depending on application types, such as for complex mixtures, column with a smaller internal diameter is recommended to ensure better separation of critical compounds [5].

In the case of supercritical fluid extraction, the amount of sample has a considerable effect on the concentration of analytes and the length of the extraction. During coupling with GC-MS system, the possible water content of the extracts must be taken into inspection [5]. For solid-phase extraction, it is required to maintain strong retention of the analyte to prevent movement through the carrier bed at the time of sample application and washing [5].

4 Detection of Food Items

GC-MS has versatile applications in food science to detect natural constituents of food as well as different harmful compounds formed during processing or preservation of food [5]. Some of the appreciable utilization of GC-MS in food materials like dairy products, juice and beverage, fruits, vegetables, cereal-based food items, bakery products, fat and oil, meat, and fish are discussed in the following sections.

4.1 Dairy Products

4.1.1 Cyanuric Acid (CYA) and Melamine (MEL) in Dairy Products

Milk and milk products are good sources of high-quality protein, vitamins A, D, B-12, riboflavin, calcium, magnesium, potassium, phosphorus, and zinc. Therefore, milk products satisfy a large portion of the nutrient requirements of the human diet. Cyanuric acid (CYA) and Melamine (MEL) were found in dairy food items to boost apparent protein content [22]. Melamine (1,3,5-triazine-2,4,6-triamine, $C_3H_6N_6$) is a triazine-based industrial chemical that has the application of producing melamine–formaldehyde resin [23, 24]. In recent years, wheat flour, pet food, infant formula, and other protein-based food materials are discovered to be adulterated with melamine (MEL) [24, 25]. Melamine consumption may have low oral acute toxicity; however, excessive exposure in animals may result in renal stones. MEL and CYA have preferably low toxicities. However, they have a strong mutual affinity; therefore, when they are adulterated together in milk, toxicity will be increased [22]. When consumed by human beings, infants and children are affected the most as they have immature organ systems. GC-MS can be used for simultaneous determination of CYA and MEL in dairy products [26]. The detection limits of CYA and MEL were found to be 0.025 and 0.01 mg kg^{-1}, respectively [26].

4.1.2 Major Volatile Free Fatty Acids in Cheese

Cheese undergoes different biochemical changes during the ripening period which causes the formation of appropriate texture, flavor, and aroma [27, 28]. Different microorganisms used in the manufacture of cheese also actively contribute to the source of odorous compounds [27, 29]. The volatile fraction of food comprises many compounds, but only a limited number of aroma compounds are responsible for the flavor. Therefore, it is necessary to separate the key odor-active compounds from the less odorous or odorless components found in foods [30]. Ewe cheese made from raw ewe milk has a definite and heavily marked flavor. The major flavor of these cheeses originates from short- and medium-chain free fatty acids, which are copious components of the volatile fraction and each fatty acid consists of a characteristic odorous mark. Therefore, the presence of vast amounts of these fatty acids may contribute either to cheese aroma or to a rancidity defect [31]. Therefore, it is necessary for a low-priced, solvent-free, and reliable method providing outstanding sensitivity and better selectivity to evaluate short- and medium-chain free fatty acids at the time of cheese ripening. Researchers have developed a headspace SPME method coupled to GC-MS for the extraction, identification, and quantification of butanoic, hexanoic, octanoic, and decanoic acids in ewe cheese samples [31, 32]. From the experiments, a noted increase in the concentration of short-chain free fatty acids is considered during cheese ripening, the values ranging from 0.35 to

9.33 mg/100 g for butanoic acid, 0.363 to 4.34 mg/100 g for hexanoic acid, 0.343 to 2.0 mg/100 g for octanoic acid, and 1.291 to 3.85 mg/100 g for decanoic acid [31].

4.2 Beverage and Juice

4.2.1 Polycyclic Aromatic Hydrocarbons (PAHs) in Coffee

Roasting of coffee permits the development of color, aroma, and flavor, which are considered as primary factors to maintain the standard and quality of coffee. However, roasting may cause the formation of some unwanted compounds like polycyclic aromatic hydrocarbons (PAHs) [33]. Incomplete combustion or pyrolysis of carbonaceous materials at elevated temperatures can be responsible for the formation of polycyclic aromatic hydrocarbons that are known as universal pollutants. The presence of PAHs in food samples is of great concern, as they possess mutagenic and carcinogenic properties, mainly the strongest carcinogenic benzo[*a*]pyrene [34]. Food can be contaminated by PAHs in two ways: either food sources exposed to the environment where PAHs are present or formation of PAHs compounds during food processing stages because of the elevated temperatures [34]. The existence of polycyclic aromatic hydrocarbons in coffee samples has been discovered, and the source of contamination may be attributed to either adulteration of the initial green beans or formation of these compounds in the roasting process [33–35]. A more selective, rapid, and economical method for PAHs determination in coffee brew has been analyzed by GC-MS detectors in the single ion monitoring mode (SIM) [35].

The beverages were prepared using roasted coffee powder and water. To analyze possible PAHs traces in the coffee brew, some samples are extracted using a solid phase (SPE) using SPE cartridges holding a hydrophobic sorbent. In coffee brew, the total concentration of the 28 compounds investigated (expressed as the sum of concentration) varied from 0.52 to 1.8 μg/L. Carcinogenic PAHs, expressed as B[a]Peq, ranged from 0.008 to 0.060 μg/L. The results point out that coffee contributes low quantities to the daily human intake of carcinogenic PAHs [35].

4.2.2 Furan in Green Coffee, Tomato Juice, and Orange Juice

Furan (C_4H_4O) is an aromatic heterocyclic compound and a lipophilic contaminant having low molecular weight and high volatility. The flavor components of food are associated with furan and its derivatives. Furan is produced when food is passed through commercial or domestic thermal heat treatment [36]. It is found in a wide variety of foods, such as coffee, baked products, canned, or jarred foods, including baby foods. According to International Agency for Research on Cancer (IARC), furan is a possible human carcinogen (Group 2B) as it is a potentially hazardous chemical [37].

The formation of furan in various unprocessed food items, such as green coffee, tomato juice, and orange juice, can be determined using headspace GC-MS analysis under different equilibration temperatures and times [38]. In order to reduce furan loss during evaporation, samples are prepared in a cold room (4 °C). To make the food samples suitable for analysis, green coffee beans were prepared by grinding finely, while tomato and orange juices were freshly compressed.

Taking green coffee as an instance, a matrix-matched calibration curve is performed for furan. However, a green coffee sample is not blank, which is unanticipated. Furan concentration in green coffee can be found to be 4.2 ng/g during GC-MS analysis after equilibration for 30 min at 40 °C. The time-dependent manner needs to be utilized to examine green coffee at headspace equilibration temperatures of 40 °C and 70 °C so that furan existence could be discovered whether it is naturally present or developed during headspace sampling. Furan response can be continued to increase following first-order formation kinetics. In the case of freshly pressed tomato and orange juices, similar headspace equilibration kinetics can be observed [38].

4.2.3 Adulteration of Ground-Roasted Coffee with Roasted Barley

Adulteration of foods has become a very common practice all around the world, which has several harmful effects on human health. It is an age-old problem, especially where there is a challenge between the physical availability and the market demand for a food item [39]. This adulteration can be done intentionally or unintentionally, and an intentional one is a criminal act and a punishable offense. Adulteration is performed by mixing cheaper elements (which are usually tough to detect by consumers and by simple analytical procedures) or the removal of some vital ingredients.

Ground-roasted coffee is an easy target for adulteration, since few of its physical characteristics, such as particle size, texture, and color, can be easily replicated by a wide range of roasted biological materials (e.g., cereals, seeds, roots, and parchments). Among the commonly used contaminants, barley is particularly difficult to detect, especially at low concentrations [40].

Researchers have developed an efficient method to verify the feasibility of detecting coffee adulteration with roasted barley, based on GC-MS analysis of the headspace volatiles of several samples of ground roasted coffee and barley [40]. Both crude coffee and crude barley were roasted to three distinct degrees of roast in a laboratory oven. The degrees of the roast were characterized as light, medium, and dark according to industrial color standards. From the results, it was found that the highest the degree of roast, the more easily distinguished were the adulterated samples, allowing for detection of adulterations with as low as 1% (w/w) roasted barley in dark roasted coffee samples [40].

4.3 Meat and Fish Products

4.3.1 N-Nitrosamines (NAs) in Meat

Meat has high nutritional value and a large amount of free water. Therefore, meat is considered to be an ideal environment for microbial growth and spoilage. From ancient times, salt has been used in order to preserve meat, but it became evident only in the nineteenth century that the nitrate present as a contaminant in salt had a significant role to inhibit the growth of microorganisms [41, 42]. Nitrate and nitrite are used for the curing of meat products [43]. Unfortunately, the contamination of NAs in meat products occurs due to the use of nitrite and nitrate as they have a considerable role in the formation of the carcinogenic NAs in meat, especially under the specific conditions applied during the manufacturing process in the meat industry [44]. NAs are formed from the reaction between a nitrosating agent with primary and secondary amines and may exhibit potential genotoxicity and enhance the risk of gastric and colorectal cancer [42, 45, 46].

Researchers have developed a simple, accessible, and reproducible method for the determination of nine volatile N-nitrosamines (NAs) in meat products, using a low volume of organic solvent [42]. From the experimental analysis, the most abundant NAs found in the analyzed products are N-nitrosodipropylamine and N-nitrosopiperidine, and their values ranged from 1.75 to 34.75 μg kg^{-1} and from 1.50 to 4.26 μg kg^{-1} respectively [42].

4.3.2 Formaldehyde (FA) in Sea Fish, Freshwater Fish, and Crustaceans

Many species of fish are consumed as a good source of protein and other nutrients for humans. Preservation of fish is necessary to increase its shelf-life as well as maintain its nutritional value, texture, and flavor. However, freezing and frozen storage of fish products undergo physical and chemical changes which may affect the fish quality [47]. Protein aggregation in frozen fish depends on various factors like the fish species, storage temperature, storage time, variation of temperature, and enzymatic degradation. In frozen stored fish, formaldehyde (volatile toxic aldehyde) is produced, along with dimethylamine due to the enzymatic reduction of trimethylamine-N-oxide (TMAO) [48, 49]. The produced formaldehyde generates cross-links with proteins and thus resulting in aggregation, which causes protein denaturation and toughening of fish muscle [48, 49]. Moreover, formaldehyde has been recently categorized as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) [50].

The formaldehyde (FA) content in different fish products has been determined using a solid-phase microextraction (SPME) followed by GC-MS method based on fiber derivatization with pentafluorobenzyl-hydroxyl-amine hydrochloride [49]. In the experimental study, researchers have assessed fish quality by the analysis of 12 species (sea fish, freshwater fish, and crustaceans). In this study, higher FA levels

were found in species belonging to the Gadidae family (from 6.4 ± 1.2 mg kg^{-1} to 293 ± 26 mg kg^{-1}), whereas freshwater fish as well as crustaceans were generally characterized by lower values [49].

4.4 Fruits and Vegetables

4.4.1 Patulin in Apple Products and Quince Jam

Mold contaminate environmental air as well as food. When molds grow onto food, it causes the spoilage of food due to the production of enzymes that break down the food. Moreover, some molds produce mycotoxin onto the food, which causes a potential risk of health hazard to the consumers. Patulin is considered as one of the various secondary metabolites produced by the mold belonging to the genera *Penicillium, Aspergillus,* and *Byssochlamys* [51]. It has been revealed that the presence of patulin in foodstuffs, such as apples or processed apple products, may be a health concern, as this mycotoxin can be responsible for severe acute (e.g., convulsions, nausea, ulceration) and chronic (e.g., carcinogenic, genotoxic, and immunotoxic) hazardous effects in humans [52]. The quality of apples is generally measured by the amount of patulin present in those products. A reliable GC-MS method has been developed for the determination of patulin in trace levels (<10 μg L^{-1}) in apple products and quince jam [51].

The method was performed based on the extraction of patulin with ethyl acetate-hexane. The method was based on extraction of patulin with ethyl acetate-hexane, alkalinization, and silylation with N,O-bis-trimethylsilyltrifluoroacetamide with 1% of trimethylchlorosilane. The method was successfully applied to the determination of patulin in apple fruit and apple products including juice, cider, and baby food and also in quince fruit and quince jam [51].

4.4.2 Benzoic and Phenolic Acids in Cranberry Fruit

Many fruits and vegetables contain phenolic compounds that have attracted a large amount of public and scientific interest due to their potential health-stimulating effects as anticarcinogenic and antioxidants. Recent epidemiological studies have given strong evidence that intake of fruits and vegetables contributes lower risk of cancer, cardiovascular and neurological diseases [53–57]. The presence of phenolic and other antioxidant phytonutrients in fruits and vegetables protects against these diseases. Free radicals are responsible for oxidative destruction of nucleic acids, proteins, and lipids and may also play a role in cancer, heart problems, and other diseases. Phenolic and other plant antioxidants can scavenge these detrimental free radicals and thus prevent their oxidative reactions with vital biological molecules [57]. A GC-MS method has been reported for the separation and characterization of

widely different amounts of benzoic and phenolic acids as their trimethylsilyl derivatives simultaneously in cranberry [57].

Fifteen benzoic and phenolic acids were identified in cranberry fruit in their free and bound forms based on GC retention times and simultaneously recorded mass spectra. The experimental results indicated cranberry fruit contained a high content of benzoic and phenolic acids (5.7 g/kg fresh weight) with benzoic acid being the most abundant (4.7 g/kg fresh weight). Benzoic and phenolic acids occur mainly in bound forms and only about 10% occurs as free acid [57].

4.4.3 Pesticides in Berry Fruits

Dietary intake of berry fruits has a beneficial and intense impact on human health, performance, and disease. Many berry fruits contain micro- and macronutrients, including vitamins, minerals, folate, and fiber, but their various biological properties have been attributed to phenolic-type phytochemicals [58]. However, berries consumption can cause potential health hazards due to the use of pesticides and other hazardous chemicals including organophosphorus, organochlorine, carbamate, pyrethroid which are unavoidable or improperly used for protection against pests or spoilage during plant cultivation and food-manufacturing processes [59]. Pesticides are a numerous and diverse group of chemical compounds, which are used to remove pests in agriculture. However, the profound use of pesticides may lead to detrimental effects on humans and the environment. Moreover, their existence in food is remarkably dangerous. Fruits and vegetables are most likely to be contaminated by pesticides [60–62]. Therefore, it is important to pay more attention to ensure food safety using available analytical methods.

A method using solid-phase extraction (SPE) cleanup in combination with gas chromatography–mass spectrometry (GC-MS) has been developed for the quantitative determination of 88 pesticide residues in berry fruits, including raspberry, strawberry, blueberry, and grape [59]. Low limits of detection (0.006–0.05 mg kg^{-1}) and quantification (0.02–0.15 mg kg^{-1}) were readily achieved with this method for all tested pesticides [59].

4.4.4 Pesticides in Tomato, Pepper, and Cucumber

Pesticides are widely used during growing and post-harvest crop protection. Due to the possible risk of health hazards, there is a growing concern for monitoring pesticide residues in food [61, 63]. Nowadays, approximately 500 compounds are identified as pesticides or metabolites of pesticides, and among them 300 are responsive to gas chromatography [64]. For the determination of ten organophosphorus and organochlorine pesticides in vegetable samples (tomato, pepper, and cucumber), a simple, rapid, and sensitive multi-residue method has been developed [63]. A miniaturized ethyl acetate extraction was used in this system followed by large volume injection (10 μL) GC-EI-MS analysis in SIM (selective ion monitoring) mode.

Average recoveries were found between 63% and 99%, and good linearity was observed in the range from 0.01 to 1.00 mg kg^{-1} [63].

4.5 Bakery Products

4.5.1 Polycyclic Aromatic Hydrocarbons (PAHs) in Chocolate Candies

Chocolate is one of the most popular food items among all age-groups. It is the key ingredient of different food products, such as milkshakes, candies, bars, and cookies. A number of manufacturing steps are required for the production of homogenous chocolate of high quality in terms of flavor, consistency, and homogeneity. Therefore, the production of chocolates go through some complex procedures, including harvesting of coca, fermenting coca to cocoa beans, and shipping the cocoa beans to the manufacturing factory for cleaning, drying, roasting, winnowing, grinding, molding, blending, and conching, which may be responsible for the formation of polycyclic aromatic hydrocarbons (PAHs) [65, 66]. PAHs may cause potential health risk to people.

A sensitive, accurate, economic, and efficient extraction method has been established for simultaneous determination of 16 PAHs in chocolate candy samples by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GS-MS) as a confirmatory technique [65]. Limit of detection, limit of quantification, and correlation coefficients were found in the range of 0.3 to 4 ng g^{-1}, 0.9 to 12 ng g^{-1}, and 0.9109 to 0.9952, respectively [65].

4.5.2 Volatile Compounds in Honey

Honey is a naturally sweet, viscous substance produced by bees using flower nectar or honeydew from plants, combined with the bees' own secretions [67]. Usually, the determination of the floral and botanical origin of honey is carried out by melissopalynological analysis, which is based on the identification of pollen by microscopic examination [68, 69]. However, this method is very time-consuming and sometimes does not give reliable results. The aroma profile of honey is one of the most typical features and the selection of honey products is determined on the basis of aroma profile. The main factors responsible for aroma formation are the volatile substances which together with other factors such as taste and physical factors, contribute to the flavor [68]. The volatile compositions characterize the specific nature of honey samples and contribute to consumer's preferences among different types of honey. An analysis of volatiles present in honey could be a beneficial method for the identification of the botanical origin. The volatile profiles of various honey samples of different botanical and geographical origins have been determined by GC-MS [68]. The volatile profiles of 43 authentic honey samples have been determined by GC-MS. The results showed the presence of certain marker

compounds for the floral origins assessed (e.g., acacia, chestnut, eucalyptus, heather, lavender, lime, rape, rosemary, and sunflower) [68].

4.6 Fat and Oil

4.6.1 Pesticide Residues in Oil Seeds

With the advancement of cold pressing technology, cold-pressed vegetable oil has become more popular among consumers. The sensory features such as the typical, characteristic taste, specific aroma, and intensive color of cold-pressed oils make it more appreciated by the consumers [70]. On the other hand, food crops and products may contain a large number of contaminants, including pesticide residues or mycotoxins [61]. The pesticide residues (PRs) in vegetable oil sourced from oilseeds are considered as one of the major diseases of such cold-pressed vegetable oil [71]. An oil-absorbing matrix solid-phase dispersion extraction with GC-MS has been developed for screening pesticide residues in peanut, soybean, rapeseed, sesame, and sunflower seed [71]. A total of 68 pesticide residues have been determined by this method which include 27 kinds of organophosphorus, 23 organic chlorines, 11 synthetic pyrethroids, and 7 carbamates [71].

4.6.2 Antioxidants in Edible Vegetable Oil

Exposure of oily food materials to air for a long time makes them rancid. There are several factors on which oxidation depends like the material itself, exposure to air and light, storage conditions such as temperature and humidity, and the existence of catalysts such as enzymes and metals [72]. Synthetic antioxidants are ubiquitous in their presence as food additives to prevent oxidative damage of food products because of their high performance, low cost, and wide availability [73]. They particularly do not contribute to improving the quality of the food products; however, they maintain the quality by preventing oxidation of labile lipid components [74]. Due to the widespread use of antioxidants, it is necessary to evaluate safety factors, as some antioxidants have toxic effects at high doses [75]. Therefore, there is an increased demand for an effective method to monitor and quantify the prohibited and permitted antioxidants [73].

A simple, rapid, and nontoxic analytical method for the simultaneous determination of five synthetic antioxidants in edible vegetable oil (e.g., soybean oil, tea oil, edible blended oil, rap oil, peanut oil, peanut blended oil, and sesame oil) has been developed [73]. Using GC-MS technique, five synthetic antioxidants [*t*-butyl-4-hydroxyanisole (BHA), 2,6-di-*t*-butyl-hydroxytoluene (BHT), *t*-butyl hydroquinone (TBHQ), ethoxyquin (EQ) and 2,6-di-*tert*-butyl-4-hydroxymethyl phenol (Ionox 100)], in edible vegetable oil have been determined. After extraction by ethanol, the analytes were separated and detected by GC-MS. The limits of detection

(LOD) for BHA, BHT, TBHQ, EQ, and Ionox-100 were found 1.00, 0.92, 11.5, 0.83 and 1.39 μg/L, respectively [73].

5 Limitations of GC-MS

Gas chromatography integrated with mass spectrometry (GC-MS) is a robust, powerful, and widely used technique that combines high sensitivity and specificity for suitable analyte groups. GC-MS has been preferred by many analytical laboratories for sample analysis; however, GC-MS may be inappropriate in some cases due to some restrictions. The area of application of GC-MS is limited for volatile and thermally stable compounds. This method cannot directly analyze non-volatile, polar, and thermally labile components. In this case, to make the components volatile and compatible with the GC interface, they are often subjected to a chemical derivatization process [5, 76, 77]. Other purposes of derivatization may include achieving increased sensitivity, selectivity, or specificity for a given chromatographic separation [78].

Derivatization, large-scale sample preparation, and long chromatographic analysis make GC-MS a relatively low-throughput technique for some applications [76]. Moreover, there are challenges for the coupling of headspace analysis with GC-MS systems if moisture has to be driven out of the sample. Hence, water can weaken the focusing of volatile components at the initial stage of the GC column [5].

6 Conclusion

Quality and safety assurance have been considered as one of the most important issues in food industry with increasing market demands. It is necessary to detect unwanted compounds (such as toxins and hazardous elements) or unfavorable microorganisms (such as bacteria, viruses, yeast, and molds) at different stages of food production prior to its consumption. Therefore, different modern and powerful instrumental techniques have been discovered for detecting food materials. Among them, Gas Chromatography/Mass Spectrometry (GG-MS) is the unique analytical technique for the quantification and identification of organic compounds in complex mixtures. The application of GC-MS in the food sector is a noticeable one concerning other areas of science and technology. The food industry is leading toward the use of advanced and efficient instrumental techniques for detailed analytical assessment of food products. As food is a complex mixture to analyze, GC-MS has made it possible to accomplish this difficult task and brought new hope for the food industries as well as research sectors. It facilitates the use of multi-methods in parallel and multi-component analysis is an ideal way.

GC-MS is highly sensitive, effective, and rapid analytical technique, which can characterize food items at the molecular level. Desirable and undesirable

compounds in different foodstuffs can be detected and analyzed by GC-MS with great resolution. Its numerous application areas like environmental aspects, food safety, forensic and criminal cases, pharmaceutical areas, and energy and fuel applications have made this method the most ubiquitous analytical technique in the world. Moreover, there is an issue of ensuring human health (i.e., nutritional components) and safety (i.e., toxic components), GC-MS is at the forefront of this crucial challenge. Its exceptional working principle is a result of the combination of the high separation power of gas chromatography and subtle detection power of mass spectrometry to identify molecular structure in complex matrices like food.

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Chapter 10
Supercritical Fluid Extraction (SFE), Solid-Phase Micro Extraction (SPME), and Stir Bar Sorption Extraction (SBSE) Techniques

Mohammad Salauddin Kader and Md Ramim Tanver Rahman

Abstract Supercritical fluid extraction (SFE) is a sample preparation procedure according to the physicochemical characteristics of the analytes in a solid/liquid mixture. SFE technique is performed using different supercritical fluids (SFs) for the liquid/solid matrix to extract samples based on their interactions, including adsorption, hydrogen bonding, polar, and nonpolar interactions. SFE is routinely used to analyze various types of samples, including food components and contaminants. SFE is a rapid, no solvent consumption, and cheap technique compare to the other traditional extraction techniques. Solid-phase microextraction (SPME) can be used to prevent these common drawbacks from solid-phase extraction (SPE). SPME is a solvent-free microextraction technique, and it is a low-cost, highly sensitive, low detection limit and can be used for different types of analytes. Analytes can be extracted by headspace (HS), direct immersion (DI), or in tube method depending on the sample types. SPME technique can be used to detect quantitative analysis of food components and contaminates. Different SPME conditions are used for analysis, depending on the sample types. An open capillary tube is used in the SPME device, and SPME can be coupled with HPLC or LC/MS. Stir bar sorption extraction (SBSE) is another extraction technique that is similar to the SPME. SBSE is used for volatile or semi-volatile organic compounds in aqueous environmental samples. The stir bar is placed in the sample for 13–120 min. to perform the stir bar sorption extraction (SBSE). Then, the stir bar is set in a glass thermal desorption tube to be thermally desorbed or analyzed in a thermal or liquid desorption unit. In this chapter, the principle, application, and advancement of the extraction are discussed in relation to the analysis of food components and contaminants.

Keywords SFE · SPME · SBSE · Analytical instrument · Supercritical fluid · Sample preparation · Organic compounds · Aroma · Food product analysis

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1 Introduction

Sample preparation is performed for specific chemical analysis or for the particular purpose of modifying a sample to improve that analysis [1]. A method can be modified to evaluate a new analysis by changing specific parameters. Sample disintegration is a standard method when the sample is solid. A matrix is usually modified during sample washing, fractionation, and concentration. Chemical reactions may also be modified to achieve better efficiency in the chromatographic analysis [2]. In the case of any chemical analysis, the target component(s) needs to be extracted from the sample matrix, and it is a vital part of the analysis. There are several factors related to the sample preparation method, and these factors are interrelated with each other. Typically, sample preparation and component(s) extraction is the most time-consuming and difficult step of any chemical analysis [3].

Moreover, the use of a wide range of large-scale organic solvents and their disposal is a severe threat to the environment. Traditional sample preparation techniques require a large amount of organic solvents, and the steps are time-consuming, complicated, and expensive. The conventional methods of sample preparation lead to an increase in analytical errors and loss of analytes [4]. Fast, solvent-free, and environmentally friendly sample preparation or extraction techniques, such as Supercritical Fluid Extraction (SFE), Solid Phase Microextraction (SPME), and Stir Bar Sorption Extraction (SBSE), are becoming common in the food, pharmaceutical, and other chemical industries [5].

2 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is a powerful technique for the rapid sample preparation and enrichment of sample analytes, and it does not require any solvent; thus, it is environmentally friendly. The SFE technique is generally used to extract the target components from varied, complex solid mixtures [6]. However, it also applies to liquid complex mixtures. Different supercritical fluids can be used for the extraction process based on the analyte's properties. Supercritical fluids (SF) behave between liquid and gas. SFs can dissolve analyte components like liquid and can diffuse through solids like a gas, which makes penetration into the matrix easier. It supports diffusion and mass transfer from the matrix to the SFs. SFs perform faster extraction, low solvent consumption, and provide a higher yield when compared to the traditional extraction processes, such as solvent extraction, Soxhlet extraction, hydro-distillation, and ultrasound-assisted extraction [7].

The primary supercritical fluid used is carbon dioxide (CO_2). CO_2 has many advantages over other fluids. CO_2 has a low critical temperature (31 °C) and pressure (73.8 bar). The density of CO_2 is tunable according to the target analytes by adjusting suitable temperature and pressure to perform highly selective extraction.

Another advantage is that CO_2 is gas at room temperature, which gives solvent-free analyte components after extraction [8].

Furthermore, CO_2 is nontoxic, chemically inert, noncombustible, and cheap. CO_2 is a nonpolar solvent, which usually extracts nonpolar compounds from the mixture. But polar modifiers can be added with the CO_2 to increase polarity and solvating power toward the polar analytes. For example, co-solvent like methanol can be applied in a small amount with the CO_2 to improve the performance of the extraction toward polar analytes. Due to low viscosity and high diffusivity, CO_2 has strong solvating power [9]. Figure 10.1 shows the schematic diagram of the SFE technique.

SFE technique has similarities with other extraction techniques. It involves the partitioning mechanism between two phases for the separation. Liquid/liquid extraction involves partitioning between two immiscible liquid phases. On the other hand, SFE involves partitioning between SFs and a solid/liquid phase. In SFE, the sample can be in liquid/solid state, and the separation can occur due to the temperature, pressure, and density effects. SFE has many advantages over conventional liquid/liquid extraction methods. SFE is the more cost-effective technique compared to liquid/liquid extraction [10]. SFE does not require expensive glassware and a large amount of organic solvents. SFE does not have an incomplete phase separation problem. SFE technique can be automated with different analytical instruments, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), and mass spectrometry (GC-MS). SFE is a rapid and selective sample preparation method for liquid/solid samples [11].

SFE has a broad range of applications for the isolation of the organic and other interfering components from the different complex liquid mixtures to obtain a clean sample preceding chromatographic and other analyses. SFE can be used for fractionation, desalting, derivatization, trace enrichment, and purification. SFE is widely used for sample preparation in food analysis and the pharmaceutical industry. One drawback of CO_2-based SFE is the extraction of polar lipids from the complex biological matrix. In this case, dimethyl ether (DME) is an excellent alternative to CO_2, which is also nontoxic, nonreactive solvent, and provides solvent-free analytes at room temperature. Another advantage of CO_2 and DME is that they do not denature proteins during the extraction process [12]. Enzyme pretreatment is one of the strategies in the food industry to enhance the extraction efficiency of the food ingredients, which can effectively help to enrich target components before extraction. One

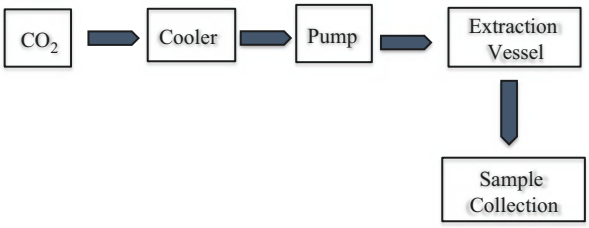

Fig. 10.1 Schematic diagram of the Supercritical fluid extraction (SFE) process

interesting recent application of CO_2-SFE in the food industry is to remove ethanol from alcoholic beverages. CO_2-SFE technique is very attractive for this dealcoholizing process because water, salts, carbohydrates, and proteins are not removed or denatured during the extraction process. SFE is a very effective extraction technique for product development in the food and pharmaceutical industries [13].

3 Solid-Phase Micro Extraction (SPME)

Solid-phase microextraction (SPME) is used as a new sample preparation technique since the early 1990s. SPME is a unique sample extraction process like SFE, which is also a solvent-free, simple, and rapid sample preparation process. Because of solvent-free sample preparation, SPME is also environmentally friendly [14]. SPME uses polymer-coated fiber to concentrate volatile/semi-volatile organic samples before any chromatographic analysis. SPME fiber extracts analytes from the sample using adsorption/absorption and desorption technique. The amount of sample extraction depends on the equilibrium time between different phases involved during extraction [15]. SPME fiber is the same type of fused silica used for the gas chromatography (GC) column, which is chemically inert and stable even at high temperatures. Different types of polymer coatings are used in the fiber, depending on the target analytes. SPME can be easily automated with different analytical instruments such as gas chromatography (GC), high-performance liquid chromatography (HPLC), and mass spectrometry (GC-MS). Different types of polymer coating used in SPME are polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate, carboxen/polydimethylsiloxane (CAR/PDMS), carbowax/divinylbenzene (CW/DVB), carbowax/templated resin (CW/TPR), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) [16].

PDMS is a nonpolar polymer coating, which adsorbs polar analytes from the sample. On the other hand, polyacrylate and carbowax are polar polymer coating, which tends to extract polar analytes from the sample [17, 18]. These polymeric coatings are desorbed inside the instruments (GC/HPLC/GC-MS) to introduce analytes for analysis. In GC/GC-MS, these polymeric coatings are desorbed thermally at high temperatures. In HPLC, different solvents (polar/nonpolar) are used for the desorption process, depending on the coating types. SPME syringe is portable, which is suitable for on-site sample preparation and analysis. SPME fiber can be analyzed directly using GC/HPLC/GC-MS by minimizing potential loss of sample analytes [19, 20].

SPME is widely used in the food industry, pharmaceutical industry, and environmental chemistry. In the food industry, aroma is a crucial factor for fruit and

vegetable products. To investigate volatile aroma compounds in various food products, SPME is an advantageous technique. Another traditional sample preparation technique is very time-consuming compared to the SPME. SPME provides excellent sensitivity and dynamic linear range for the analysis of aroma compounds. SPME required a very small amount of samples during the extraction process [21]. PDMS-coated fiber can be used to extract lipophilic compounds from fruit products. The polymer coating can be manipulated depending on the analysis. The equilibration time is an essential factor for both qualitative and quantitative analysis. Temperature also plays a vital role in the extraction process. Analyte concentration can be increased by increasing temperature, which also provides faster extraction during analysis [22]. PDMS-SPME can be coupled with time-compressed chromatography (TCC) and time-of-flight mass spectrometry (TOF-MS) for the rapid and efficient analysis of aroma compounds in the food industry. One major drawback of SPME is that sample recovery is very small, which is problematic for a large amount of sample analysis [23, 24].

In some cases, headspace is also used to avoid direct contact between SPME fiber and contaminated samples. In that case, the kinetics of the mass transport between the aqueous phase of the sample and headspace is very important. However, considering all aspects, SPME is an excellent solvent-free, rapid, and environmentally friendly sample preparation technique for the volatile and semi-volatile organic compounds in the food industry and pharmaceutical industry [25, 26]. Figure 10.2 shows the schematic diagram of the SPME technique.

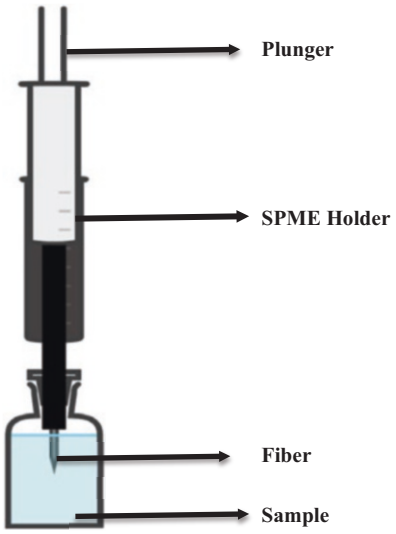

Fig. 10.2 Schematic diagram of the Solid-Phase Micro Extraction (SPME) device

4 Stir Bar Sorption Extraction (SBSE)

Stir Bar Sorption Extraction (SBSE) is another solvent-free and environmentally friendly sample preparation technique like SPME. SBSE was first introduced as a solvent-free sample preparation technique in 1999. SBSE is a newer extraction technique compared to the SPME. The sensitivity and recovery of SBSE are significantly higher than the SBSE. SBSE uses a magnetic stir bar coated with polydimethylsiloxane (PDMS) instead of fiber in the SPME. The magnetic stir bar absorbs/adsorbs target analytes from the sample. Finally, the stir bar is introduced in the desorption tube at high temperature (~250 °C) for 15–20 min. before any further chromatographic analysis. Volatile and semi-volatile organic analytes in an aqueous environment can be extracted using SBSE [27]. PDMS-coated SBSE is polar, which tends to extract mostly polar sorbent from the sample. Various methods have been applied to solve the problem, such as sol-gel technology and dual-phase stir bar to modify polymer coating based on the target analytes. With the PDMS polymer, different chemical groups can be added using sol-gel technology, including polyvinyl alcohol (PVA), divinylbenzene (DVB), and beta-cyclodextrin. These chemical modifications improved the extraction process for both the polar and the nonpolar analytes. In addition, specific sorbent materials may be used according to target analytes, such as polyacrylate (PA), poly (phthalazine ether sulfone ketone) (PPESK), and polypyrole (PPY), instead of PDMS [28]. Figure 10.3 shows the schematic diagram of the SBSE technique.

Extraction temperature, stirring rate, and mixing time are important factors to get maximum recovery and sensitivity in SBSE. Desorption time and the temperature is vital for the efficiency of the different chromatographic analysis. Headspace can also be used to avoid direct contact with the aqueous phase. In the food industry, different taint compounds can be analyzed more precisely using the SBSE technique when compared to the other sample preparation techniques. SBSE provides a wide linear dynamic range, high sensitivity, and high sample recovery for the analysis of taint compounds in the food industry [29]. SBSE, coupled with GC-MS/MS, can detect trace/ultra-trace level components in taint compound analysis. In aroma analysis, SBSE is more effective when compared to the SPME. SPME can extract a minimal amount of sample, which is a significant limitation of the SPME technique. But SBSE can do a large volume of sample extraction, which also provides high sample recovery and sensitivity. Despite all these outstanding advantages in sample

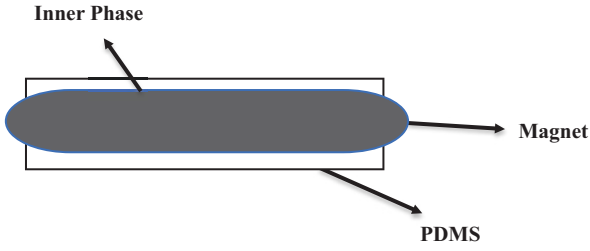

Fig. 10.3 Schematic diagram of the Stir Bar Sorption Extraction (SBSE) device

preparation, traditional PDMS-coated SBSE cannot extract abundant polar organic compounds [30–32]. Recently, solvent-assisted SBSE (SA-SBSE) was introduced in food product analysis, which provides enhanced efficiency in sample extraction. Different solvents, such as dichloromethane, di-isopropyl ether, and cyclohexane, are used to swallow PDMS coating on the SBSE stir-bar, which increases sensitivity in polar components analysis.

SA-SBSE is very useful in pesticide analysis of wine and beer. Food products consist of alcohol, fatty acids, and aromatic compounds. They can be overloaded in the GC column during analysis and reduce the efficiency of the column [33]. Traditional PDMS-coated SBSE is inefficient to extract abundant polar compounds from the sample. SA-SBSE can be used to overcome this problem, which can extract a large volume of these polar components from the sample matrix and increase the sensitivity of the chromatographic analysis [34, 35]. Another limitation of SPME is that the polymer coating on the stir bar can be easily damaged during stirring. Solid coating materials are critical to avoid cracking or damage to the polymer coating during the extraction process. Despite some limitations, SBSE is becoming a very much popular sample preparation technique in the food industry because of its solvent-free, high sensitivity, high recovery yield, environmentally friendly, and rapid sample preparation capabilities [36, 37].

5 Conclusion

Among these three sample preparation techniques, SFE is a highly selective sample preparation technique when compared to SPME and SBSE. In the case of sensitivity, SBSE and SPME are more sensitive than SFE. SBSE has a higher sample recovery capability than SPME. Both SBSE and SPME have limitations in extensive polar organic compounds extraction. Simple modifications can, however, overcome those limitations. Overall, all three are excellent sample preparation techniques compared to conventional solvent-based methods.

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Chapter 11
Electronic Tongue for Food Safety and Quality Assessment

Farrhin Nowshad and Mohidus Samad Khan

Abstract The sense of smell and taste play a fundamental role in human development and biosocial interactions. Taste is an important organoleptic property governing the assessment of food products. It is one of the prime factors determining the quality, market potential, and commercial success of foods. Thus, taste assessment is one of the most important quality control parameters for evaluating foods. The primary subjective method for taste measurement is generally by human panelists. However, the sensory method is very complicated to perform and analyze; it is variable depending on the panelists and conditions of assessment. In addition, it is time-consuming and thus difficult to include in the quality assessment in the food production line. Recruiting taste panelists and maintaining them can be highly difficult, especially when working with food products not preferred by the panelists. Furthermore, unsafe and toxic molecules are not allowed to be tested by the sensory method. Therefore, the analytical taste-sensing multichannel sensory system called as electronic tongue (also known as e-tongue or artificial tongue) could replace the sensory panelists. The concept of the electronic tongue is to measure a "fingerprint" of a sample allowing sensitive comparison in relation to the taste measurements. A sound basis for electronic tongues is provided by the extensive progress of developing well-known selective sensors, especially electrochemical and biological mimicking sensory systems of mammalians. The electronic tongue can be used for a wide range of food items to characterize, authenticate, quality evaluation, process monitoring, and quantitative analysis of foods. Thus, electronic tongue includes benefits like reducing reliance on human panel, and it could be rapid and used in the quality control in the food production line. This article briefly discusses the mechanism and application of electronic tongue with respect to food safety and quality assessment, and the contents would be highly useful to the food professionals working in the academic, research, and industry.

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Keywords Electronic tongue · Biomimetic sensor · Sensory panel · Taste sensation · Taste-sensing technology · Sensor array · Multisensory system · Pattern recognition · Taste assessment · Food-process monitoring · Authenticity assessment

1 Introduction

Determining the quality of foods is critical to the consumers. 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. However, we are inherently varied by our inability to assess food quality considering smell, sight, sound, touch, and taste [1]. There are various types of analytical methods that can provide information about physical and chemical characteristics of foods. However, these methods are often complicated and time-consuming. For processing and quality control, the food industry generally requires methods that are simple, rapid, and able to provide reliable data about foods. Therefore, there is a high need to develop rapid and low-cost methods to measure attributes related to the sensory taste of different foods.

Taste sensation in humans arises through physicochemical interactions of selected molecules present in food and complex system of cell buds located on the tongue [1]. The sense of taste includes sourness, saltiness, bitterness, sweetness, and umami [2]. We perceive each type of taste attribute during food consumption. Taste buds are composed of approximately 50–100 taste cells [2]. Each taste cell has receptors, which bind to the molecules and ions and the result is the taste sensations [3]. Taste information perceived by taste buds is transmitted to taste nerves through neurotransmitters, and finally, the signal reaches the gustatory area in the brain [2]. In brief, taste formation is related to chemical transduction in the papillae of the human tongue with activation of intrinsic and extrinsic neuronal circuits. It is mediated by regulatory membrane receptors through a complex network [4–7]. According to the Classical Threshold Theory, taste sensations depend on the intensity of the attributes of the stimulus. Stimulus is perceived only when its intensity is above a specific level or threshold [4]. Scale descriptors and/or geometric mean values are normally used in human tasting panels [6], and it is not easy to assign a scale or unit for measuring taste. Therefore, it can be said that taste is identified through a combination of nonspecific responses obtained by a series of nonspecific molecular recognition events [8]. The pattern created by the simultaneous response of these receptors is specific for a particular set of stimuli [1]. Unfortunately, the inherent variability of the receptor density over the tongue and palate causes the differences between human to human perceptions of taste. Therefore, even if members of a taste panel are well trained and calibrated, the evaluation still remains subjective. Therefore, it is still troublesome in some industrial applications. For this reason, researchers and management associated with food, beverage, and pharmaceutical industries have been seeking a reliable, low-cost, reproducible analytical tool to analyze the taste of foods.

The term E-tongue was coined owing to the similarity with the human gustatory system, which is based on the concept of global selectivity [9–11]. The electronic tongue systems are designed as an array of non-specific and low selective chemical sensors with partial specificity (cross-sensitivity) to different components present in the sample under investigation [1]. The electronic tongue consists of an array of liquid sensors with a different selectivity, a signal collecting unit, and a pattern recognition software [12]. When exposed to a sample containing different compounds, it 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 [13]. The sensors of electronic tongue "taste" raw substances, semi-products, and finished products in a fast and nondestructive method; hence, the 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 [1]. E-tongue has thus emerged as a very capable, rapid, and easy-to-use tool for evaluation of food quality [14]. In addition, they have also been widely used in the monitoring of environmental conditions, medical diagnostics, detection of herbal products, and detection of endotoxins and pesticides in different products [13].

In this chapter, a brief portrayal of the development of the electronic tongue is included along with different types of sensors used in electronic tongue systems. Applications described in this chapter include process monitoring, freshness evaluation, authenticity assessment, foodstuff recognition, quantitative analysis of foods, and food safety assessment. Finally, future directions, strengths, and weaknesses of electronic tongues are also mentioned.

2 Electronic Tongue in Food Analysis and Its Other Applications

Food analysis has become a very important area of research due to the fast growth of the food trade and increasing awareness of food safety [15]. Food analysis provides information about different characteristics of foods, including their composition, structure, physicochemical properties, and sensory attributes [15]. This information is critical to our rational understanding of the factors that determine the properties of foods as well as to our ability to economically produce foods that are consistently safe, nutritious, and desirable. One of the most important reasons for analyzing foods is to ensure that they are safe. In food analysis, a wide range of traditional methodologies such as liquid chromatography, IR, and UV spectroscopy are used to determine or detect characteristics compounds in foods [14, 16]. These methodologies show good precision, accuracy, and reliability [14]. However, they are destructive, time-consuming, complex, require specialized personnel and extensive instrumentations, and inconvenient for in situ or at site monitoring [12, 14]. Electronic tongues can be used to overcome these drawbacks, and it could be very promising and easy-to-use tools for the evaluation of food quality [14]. Electronic

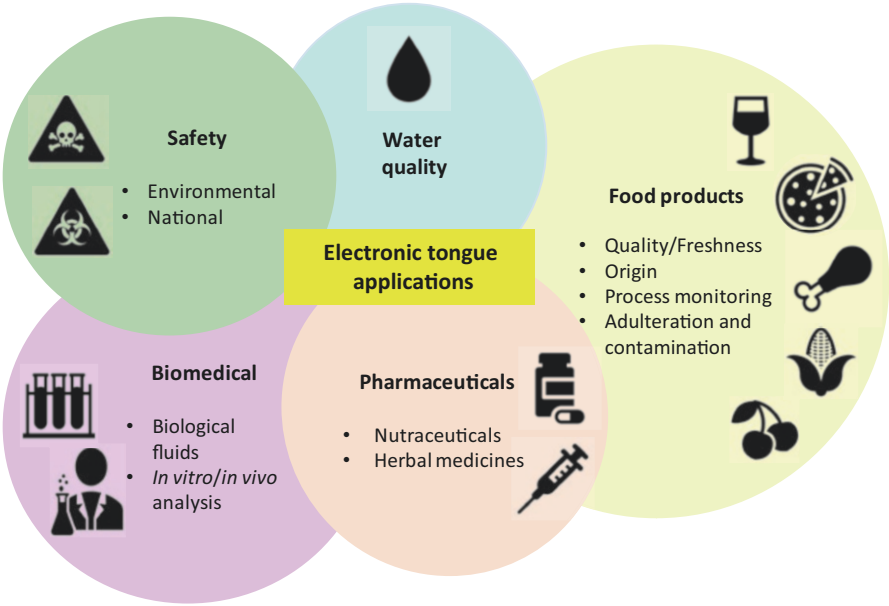

Fig. 11.1 Fields of application for electronic tongue systems. E-tongues are widely used for identifying hazardous and toxic chemicals, assessing water quality, ensuring food safety and in biomedical research and pharmaceutical industry

tongues can be highly useful for their rapid analysis and online capabilities to meet the trends of automation and continuous processing in the food industry [12]. This device is frequently used for the recognition, classification, and quantitative determination of multiple component concentrations [14].

Electronic tongues can be used for the detection of all types of dissolved compounds, including volatile compounds (i.e., odors) if dissolved in the solution [16]. Electronic tongues have already been used for the determination of fruit juice, onions, soft drinks, tea and herbal products, beverages, apples, milk, tomatoes, alcohol, coffee, sake, olive oil, beer, rice, cork, meat, and soya paste. However, electronic tongues are now also being used for environmental monitoring, water quality analysis, pesticide detection, medical diagnostics, and analyzing the fermentation process [17]. Figure 11.1 summarizes the versatile fields where E-tongue system is being used extensively.

3 Development of Electronic Tongue System

Electronic tongues have been developed based on the human taste mechanism. The human tongue is a mass of interlacing skeletal muscle, connective tissue with some mucous and serous glands, and pockets of adipose tissue, covered in oral mucosa

[18]. The mucosa covering the upper surface of the tongue is thrown into numerous tiny projections called the *papillae* [18]. Several thousands of taste buds are present on the surfaces of the papillae which are the collections of taste cells that connect to nerves running into the brain. In human gustatory system, the taste-producing substances are received by the biological membrane of gustatory cells in non-specific taste buds on the tongue [19–21]. The taste buds are chemoreceptors; they transduce or translate chemical signals in food into electrical signals in the body called action potentials, which travel to the brain through the nervous system allowing the sensation of taste [22]. In an E-tongue, the output of the nonspecific sensor array shows different patterns for the different taste-causing chemical substances, and such data are statistically processed [21].

3.1 General Mechanism of Electronic Tongue

The electronic tongue can be defined as "a multisensory system" for liquid analysis based on the combination of signals from non-specific and overlapping chemical sensors with suitable pattern recognition routines [1, 21]. The whole system of an e-tongue imitates what is happening when molecules with a 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. These signals are compared to physiological action potentials that are recorded by computer, which correspond to the neural network at the physiological level. Depending on the objective, the data obtained can further be evaluated on the basis of an already existing matrix of sensor responses, and these can be compared to human memory or association to existing taste patterns [14, 22]. Figure 11.2 presents a simplified flowchart of the electronic tongue system.

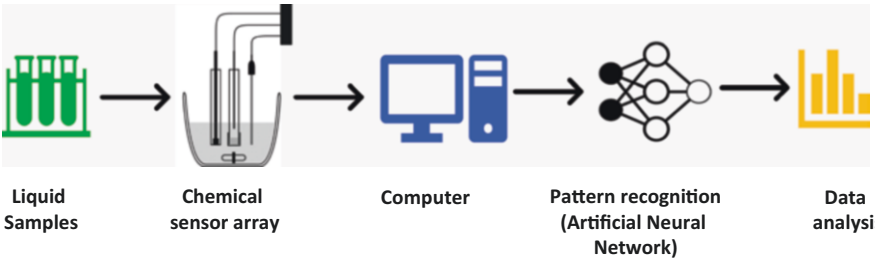

Fig. 11.2 Simplified flow chart of electronic tongue system. This system imitates what is happening when molecules with specific taste nature interact with taste buds on the human tongue. The taste buds are represented by sensors which interact with these molecules at the surface initiating changes in potential. These signals are compared with physiological action potentials which are recorded by computer (pattern recognition), which correspond to the neural network at the physiological level. The data obtained can further be evaluated using statistical methods based on already existing matrix of sensor responses

Currently, two major electronic tongue systems are commercially available: the taste sensing system SA402B (Insent Inc., Atsugi-chi, Japan) and the ASTREE e-tongue (Alpha M.O.S, Toulouse, France) [23, 24]. Both of these measures change in electronic potential while investigating liquid samples but the underlying sensor technologies are different. The taste-sensing system SA402B is equipped with lipid membrane sensors, whereas the ASTREE uses chemical field-effect transistor technology [24]. In addition, other taste-sensing systems are under development. An electronic tongue system is generally made of the following parts:

3.2 Different Sensor Arrays

In an e-tongue, the output of the nonspecific sensor arrays shows different patterns for different taste-causing chemical substances, which is statistically analyzed. Thus the e-tongue can be defined as "a multisensory system for liquid analysis based on chemical sensor arrays and a suitable pattern recognition method" [14]. Regarding the sensor arrays used in the design of e-tongues, a wide variety of chemical sensors have been employed, which include electrochemical (potentiometric, voltammetric, amperometric, impedimetric, conductimetric), optical, mass, and enzymatic sensors (biosensors) [1, 25–27]. The most applied operational principle of the e-tongue is typically voltammetry or potentiometry [28]. A potentiometric E-tongue system generally consists of the following parts:

Working Electrode: The working electrode is an inert material such as Gold, Platinum, Glassy Carbon, iridium, and rhodium [24]. The working electrode serves as a surface on which redox reactions occur. The surface area should be very less (few mm^2) to limit the current flow [24].

Reference Electrode: An Ag/AgCl reference electrode is used in measuring the working electrode potential. A reference electrode should have a constant electrochemical potential as long as no current flows through it [23, 24].

Auxiliary electrode: A stainless steel counter electrode is a conductor that completes the cell circuit. It is generally an inert conductor. The current flow into the solution via the working electrode leaves the solution via the counter electrode [23]. A relay box is used, enabling the working electrodes to be connected consecutively to form four standard three-electrode configurations [24]. The potential pulses/steps are applied by a potentiostat which is controlled by a PC. The PC is used to set and control the pulses, measure and store current responses, and operate the relay box [23, 24]. Figure 11.3 represents different components of a potentiometric E-tongue system.

Potentiometric sensors are the most widely used type in e-tongue systems, especially ion-selective electrodes (ISEs) [14]. The main disadvantages of potentiometric sensors are their temperature dependence and the adsorption of solution components that affect the membrane potential [14]. These factors can be minimized by controlling the temperature and washing the electrodes. On the other hand, the advantages of potentiometric sensors include their well-known operation

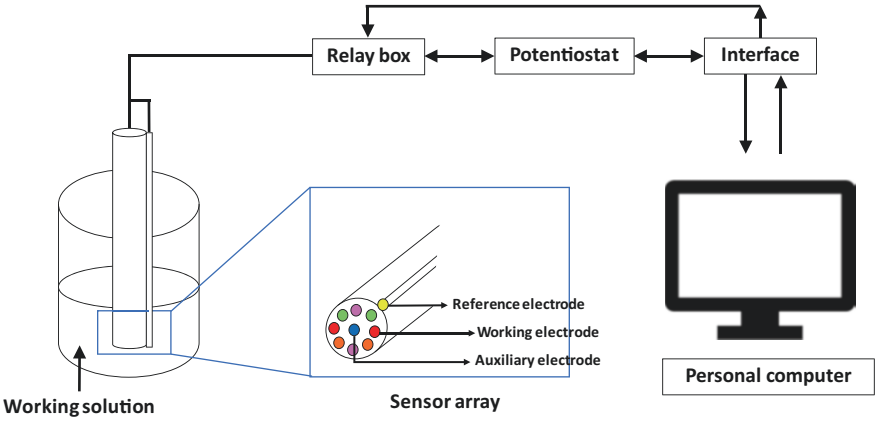

Fig. 11.3 Schematic diagram of a potentiometric type E-tongue. Sensor array is comprised of reference, auxiliary and working electrodes. These sensors measure dissolved organic compounds in liquids including taste and flavor compounds. A relay box is used to enable the working electrodes to be connected consecutively. The potential pulses/steps are applied by a potentiostat which is controlled by a personal computer. This computer is used to set and control the pulses, measure and store current responses and to operate the relay box

principle, low cost, simple set-up, easy fabrication, and the possibility of obtaining sensors selective to many various species [29].

Voltammetric sensors are also extensively used in e-tongue systems. These devices are advantageous for multicomponent measurements because of their high selectivity, high signal-to-noise ratio, low detection limits, and various modes of measurement [14]. Furthermore, the surface of the electrodes can be modified with various chemosensitive materials obtaining sensors of various sensitivity and selectivity toward a variety of species. However, their applicability is limited to redox-active substances [25].

Impedimetric e-tongues are based on the measurement of impedance either at one fixed frequency or a broader spectrum using impedance spectroscopy. They have been applied to the recognition of basic taste substances, beverages, and mineral waters, and these have been shown to have excellent sensitivity [30]. Moreover, there is no requirement of active species in the measuring system, and unlike other electrochemical methods, when they do not require a standard reference electrode (which might be troublesome in many practical applications because a reliable reference is a critical issue in miniaturized sensor arrays) [14].

In an amperometric e-tongue, an electrochemical conversion occurs (potentiostatically) at an electrode, and the resulting current due to this electrochemical reaction is measured. With the amperometric method, only partial electrolysis takes place. To be detected by amperometric e-tongue, the compound of interest has to be electroactive (at the applied potential, in the solution used, and at the prevailing pH) [1]. This is both a limitation and an advantage. This electrode can only be used to detect electroactive species, which is a major limitation. On the other hand, the

selectivity of the electrode can be considered as a key advantage of amperometric e-tongue. Thus, it is possible to detect electroactive components without the interference of the non-electroactive compounds. Using an array of sensors working at different potentials, it is possible to resolve between several electroactive compounds, since at low positive/negative potential, only strong reducing/oxidizing compound may be detected. On the contrary, at large detection potentials, the total electroactive compounds are detected [1].

Optical sensors are also used in e-tongues technology. These devices offer several modes of operation, such as fluorescence, absorbance, and reflectance. Additionally, analytes are difficult to detect electrochemically (e.g., uncharged and/or non-electroactive), and it can often be studied with optical sensors. However, some drawbacks, such as sensor preparation, durability, and signal interferences, limit their applications [31]. Optical e-tongues have been mainly used in biomedical analysis and food analysis [14].

3.3 Data Processing

Apart from the sensor arrays, signal processing is an important aspect of multisensory analysis. In a multicomponent environment, the sensor array produces complex signals (patterns) that contain information about different compounds and other features. These signals should be analyzed together to extract valuable analytical information. Various methods of multivariate calibration and pattern recognition are now available and can be used for sensor array data processing. The electronic tongue may be applied in principle to two main tasks: (i) quantitative determination of the content of components and (ii) classification (recognition, identification, and discrimination). The choice of the data processing technique for a particular case depends on the task to be solved and the structure of the data (nonlinearity, correlations, etc.). A brief overview of some data processing methods, together with their main features, is presented in Table 11.1 [32].

4 Applications of Electronic Tongue in Food Safety and Quality Assessment

To ensure food quality and to comply with the safety requirements, food products need to be monitored and controlled consistently during the entire food supply chain. For this purpose, the electronic tongue system appeared to be an ideal device, as it enables fast, precise, and direct analysis [33]. Moreover, an electronic tongue can be applied for automatic online monitoring during food processing. The following sections briefly describe the most relevant applications of taste sensors, such as sample recognition/origin tracing, freshness evaluation, process monitoring,

Table 11.1 Selected methods of multivariate calibration and pattern recognition used for electronic tongue data processing

Method	Linear	Supervised	Advantage	Drawback
Principal component analysis (PCA)	Yes	No	Easy to interpret	Sensitive to the drift in the data
Partial least squares (PLS)	Yes	Yes	Statistical description of the results Small calibration data set	
Self-organizing map (SOM)	No	No	2D representation of data of any dimensionality	Works as black box
Back propagation neural network (BPNN)	No	Yes	Easily deal with nonlinear data	

authenticity assessment, quantitative analysis, and quality control. Applications of the electronic tongue in relation to different categories of foods are presented in Table 11.2.

4.1 Foodstuff Recognition and Characterization

An electronic tongue comprising 17 ion-selective electrodes can be applied to discriminate between different brands of mineral waters and apple juices [14, 16]. Since the main components of mineral water are ionic, high-selective sensors were able to easily differentiate the water samples. However, in the case of juice samples, a lower recognition accuracy was obtained due to ionic inorganic but also organic species characterizing juice taste [14]. To solve this problem, a system based on selective and partially selective sensors was developed to discriminate between different brands of orange juice, tonic, and milk with an accuracy of 90–100% [14]. In this way, the combination of two types of sensors provides a versatile device for qualitative analysis of various types of beverages.

Researchers have proposed a hybrid electronic tongue based on a combination of potentiometry, voltammetry, and conductivity measurements for the classification of six different types of fermented milk [34]. Using data from the voltammetric, potentiometric, and conductivity measurements, independently, a partial overlapping between sample classes was observed. However, it was reported that the combination from all information sources could separate all six samples [14].

4.2 Authenticity Assessment

Food authentication is one of the greatest concerns for researchers, consumers, industries, and policymakers. An authentic raw material or finished product has to comply with labeling in terms of ingredients, brand, origin as well as production

Table 11.2 Applications of the E-tongue in relation to different categories of foods

Sample	Type of study	Principle of detection	References
Milk	Detection of adulteration of samples with cow milk	Potentiometry	[54]
	Freshness monitoring	Voltammetry	[55, 56]
	Quality and storage time monitoring	Voltammetry	[57]
	Classification of milk samples	Potentiometry	[58]
	Detection of antibiotic residues	Potentiometry	[59]
	Detection of clinical mastitis	Voltammetry	[60]
Bacteria cultures used in cheese production	Fermentation process monitoring	Potentiometry	[61]
Red wines	Discrimination among 12 Spanish red wines	Voltammetry	[62]
Wines	Monitoring of wine aging	Potentiometry	[63]
Beer	Correlation with human taste panel scores	Potentiometry	[64]
	Variability monitoring of brewing process	Impedance	[65]
	Monitoring of fermentation process	Impedance	[65]
Tea	Determination of caffeine and catechins in green tea	Voltammetry	[66]
Coffee	Tastes of different kinds of coffee	Potentiometry	[4]
Fruit juice	Semi-quantitative and quantitative analysis of non-alcoholic beverages	Potentiometry	[67]
Tomato	Analysis of tomato taste	Potentiometry	[68]
Apricot	Discrimination between apricot varieties	Potentiometry	[69]
Apples	Discrimination of varieties and determination of organic acids	Potentiometry	[70]
Pear	Determination of sugar content and firmness of non-climacteric pear	Voltammetry	[71]
Olive oil	Detection of olive oil adulteration	Voltammetry	[48]
Meat	Prediction of NaCl, nitrate, and nitrite contents in minced meat	Impedance, voltammetry	[72]
Fish	Discrimination between storage times, prediction of chemical and biochemical degradation parameters	Potentiometry	[73]
	Prediction of fish degradation degree	Voltammetry	[74]
Rice	Sensory evaluation of milling	Voltammetry	[75]

technology. Information about the geographical origin is also crucial since it may affect the price [35]. E-tongues have proven their potential in the authenticity assessment of different kinds of foods due to their simplicity, efficiency, speed, and low cost of the determinations [14].

Frequently studied food products in terms of label authentication are oils, dairy products, alcoholic and non-alcoholic beverages, honey, tea, and coffee. An electronic tongue constructed with an array of 20 all-solid-state potentiometric electrodes with polymeric membranes can be used to differentiate. One of such studies demonstrated that electronic tongue can be used to classify honey samples according to the most predominant pollen type with reasonable efficiency (84% and 72% of success in calibration and cross-validation, respectively) [36]. The same tool also presented a promising behavior for monofloral honey assortment. This type of electronic tongue can also be used to detect raw goat milk adulterations with raw cow milk [37]. However, to use this electronic tongue as a routine methodology, it is needed to improve the multisensor system by testing and including more sensible sensors to milk composition variations [14].

Another important application area of electronic tongues is the authenticity assessment of vegetable oils. A potentiometric electronic tongue containing cross-sensitive lipid membranes was applied to discriminate monovarietal extra virgin olive oils according to olive cultivar and geographic origins. Recorded signals were analyzed by meta-heuristic simulated annealing algorithm together with the LDA resulting in sensitivity above 97% [38].

Studies on the fraudulent performances are increasingly carried out in different alcoholic beverages, and among them, wines are the important targets. Electronic tongues have been used for the identification or authentication of wines according to grape variety or their geographical origins, brands and for the detection of fraudulent samples derived from deliberated addition of chemicals to wine to correct or enhance its organoleptic properties [14]. In this context, a hybrid array of voltammetric sensors formed by different families of sensitive materials (phthalocyanines and conducting polypyrrole polymers) can be used to detect various chemical adulterations in wines and other beverages [14]. Another study presented a portable electronic tongue system based on disposable screen-printed electrodes capable of distinguishing between different types of Lager beer, predicting its color and alcoholic strength (accuracy of 76% and 86%, respectively) [39]. A very recent study of a novel paper-based potentiometric electronic tongue with an integrated reference electrode demonstrated that it can be served as a tool for discriminating beers from 19 brands and 12 different types [35]. Moreover, it allowed the detection of stabilizers, antioxidants, dyes, and substances added during the fermentation process [35]. In the case of wine, samples could be classified according to the grape variety. A significant advantage of the system is its low cost, adaptability, and ability to work with microliter sample volume [40].

4.3 Freshness and Food Quality Evaluation

The quality of food products is the ever-growing interest of the consumers. Electronic tongues have the potential to predict the freshness or spoilage of different foods like meat, fish, seafoods, raw materials, and other food products. In case of meat and fish products, freshness evaluation is highly important because rotten or spoiled products may negatively affect the health of consumers. Therefore, a multisensor system based on modified screen-printed electrodes has been tested as a possible tool for the detection of ammonia and putrescine (toxic diamine produced during decomposition of amino acids) in a powdered beef extract [35, 41]. The proposed sensor matrix showed an excellent sensitivity toward amine compounds (LOD of 1.85 mol/L for ammonia and 0.34 mol/L for putrescine) [41].

The application of electronic tongue to evaluate fish freshness has also been reported [14, 42]. Experimental results demonstrated the usefulness of electronic tongues for in situ and at site freshness evaluation, and it could be used as an alternative to other destructive, high-cost, and time-consuming methodologies [14]. In an experiment, the evolution with time on fillets of cultured sea bream (*Sparus auratus*) using a potentiometric electronic tongue containing 16 electrodes was used [42]. Fish freshness indicators, such as texture, pH, color, microbial analysis, total volatile basic nitrogen, and biogenic amines, were also determined with respect to time. Multivariate analysis of electronic tongue data allowed the assessment of the storage time of fish fillets with a rate of success of 100% [42]. On the other hand, good correlations were found between potentiometric data and fish freshness indicators with correlation coefficients higher than 0.90 [42]. In another study, two voltammetric e-tongues based on carbon paste or screen-printed electrodes modified with phthalocyanines were developed and applied to monitor fish spoilage. The screen-printing technology allowed for the preparation of miniaturized electrodes, which are promising for the fabrication of low-cost sensors with higher sensibility compared to the corresponding modified carbon paste electrodes [43].

4.4 Process Monitoring

Electronic tongues can also be successfully applied for the monitoring of changes occurring in the composition of foodstuff during its production. One of the most significant applications is the fermentation process. Continuous control of the fermentation helps to avoid unfavorable deviations, detect microbiological contamination, and ensure the feasibility of the processes [44]. In many cases, mixed cultures of microorganisms are used, which trigger themselves at specific time points of the process. In addition, due to the variations in the composition of major substance used in the process, it is difficult to find any other analytical technique that could be reliably applied to analyze fermentation samples. Electronic tongue systems are able to deal with such samples that are extremely complex, present huge variations

in the background composition, and have to be studied in detail as in some cases lack of certain nutrient can stop the process or lead to unwanted by-products [44, 45]. A study showed that an electronic tongue based on potentiometric sensors can quantify organic acids (such as citric, lactic, and orotic) in the fermentation media with average prediction errors in the 5–13% range while conducting in both "normal" and "abnormal" operating conditions. Electronic tongue has thus become a promising tool for fermentation process monitoring [46].

In the dairy industry, the demands of sensors are gradually increasing as they are able to withstand extreme conditions (hot base and acid solutions), hygienic, and can be sterilized easily. Also, no reference electrode is allowed [14]. Researchers have demonstrated that specially designed voltammetric electronic tongues were inserted in the process line of a dairy industry for direct inline measurements, and they were in operation for 8 months without any malfunction and maintenance [34]. The electronic tongues were used to follow different sources of raw milk coming into the process and to monitor the cleaning process of the pasteurization unit.

An electronic tongue based on voltammetric electrodes chemically modified with different sensitive materials (polypyrrole, metallophthalocyanine derivatives, and perylene derivatives) can be used to monitor the aging of red wines and to discriminate wine samples aged in oak barrels of different characteristics [47]. The diversity of the sensing materials in the electronic tongue allowed obtaining a high cross-selectivity in the responses of the sensors forming the array. Multivariate inspection of voltammetric data as used in e-tongue showed a high capability of discrimination and classification.

4.5 Quantitative Analysis

An electronic tongue composed of a polymer membrane sensor array and data processing routines can be used as an analytical tool for quantification of saltiness (NaCl) and sourness (citric acid) in synthetic taste solutions as well as for discrimination between wines from different grapes [14]. A study showed that electronic tongues, based on 23 potentiometric cross-sensitive chemical sensors, can measure several quantitative parameters of the wines (total and volatile acidity, pH, and contents of several wine components) with precision within 5–12% [16]. In addition, the system is capable to predict human sensory scores with precision errors within the 4–27% range [14].

Apart from wine and grape juice samples, electronic tongues can be used for the determination of taste compounds in other kinds of foodstuffs. An electronic tongue comprising of voltammetric electrodes as modified by different sensitive materials was proposed to evaluate the phenolic content of extra virgin olive oils [43]. Brazilian researchers have recently shown that an artificial hand-held tongue of four sensors made from ultrathin films deposited on gold interdigitated electrodes was able to distinguish the four basic tastes [46]. Some of the samples, e.g., 5 mm NaCl and sucrose, were detected below the human threshold level, and suppression of

quinine by sucrose was also observed. The high sensitivity was attributed to the ultrathin nature of the films. By using four sensors for the different tastes, an electronic fingerprint of the taste was obtained. These responses were combined into a single data point which allows predicting the taste of a particular solution [46].

4.6 Food Safety Assurance

The intentional addition of inferior quality material as well as biological and chemical contamination during the period of growth, storage, processing, transport, and distribution of the food products pose a serious risk to the consumer's health. Thus, developing quick and precise methods for the detection of contaminations in food products is of the highest importance. Electronic tongues can play a very important role to detect adulterants. One of the examples includes the examination of virgin olive oil adulteration [48]. An array of modified carbon paste electrodes was employed for the evaluation of the percentage content of edible oils (sunflower, soybean, and corn oils) in olive oil samples. Data obtained after processing of voltammetric signals using PLS (partial least squares) discriminant analysis and regression demonstrated the ability of the sensor to classify precisely the aforementioned adulterant oils with a concentration level below 10% [48].

Recently, an automated hydrodynamic bioelectronic tongue based on genetically modified acetylcholinesterase was applied for the quantification of pesticide (chlorpyriphos-oxon and malaoxon) mixtures in milk [49]. In another study, a voltammetric electronic tongue enabled quantification of formaldehyde, urea, and melamine in milk with limits of detection (10.0, 4.16, 0.95 mmol/L, respectively) below the limit of the recommended tolerable intake dose [50]. A different study described a voltammetric electrode array which showed that it was able to predict levels of the most widely used curing agents, nitrate, nitrite, and chloride in minced meat and saline solution [51].

Finally, another recent, important application of electronic tongues is the monitoring of organophosphate pesticides at nanomolar levels in food items [35]. The presence of pesticides in foods can not only cause a number of health effects but is also linked to a range of serious illnesses and diseases in humans, from respiratory problems to cancer. The potential presence of pesticides forces additional quality control in the case of vegetables and fruits as they are toxic. Before the development of electronic tongue, many analytical methods have been developed to detect these compounds such as chromatographic and spectroscopic techniques.

5 Future Directions

Being an advancing biomimetic measurement technology, electronic tongue makes the use of sensor arrays that combine both hardware and software to exhibit high performance in analyzing the product and process quality. However, the concept of

biomimetics should not be exaggerated, as they cannot be as effective as the human sensation, which is strongly linked to the signals from the brain and memories about previous taste experiences [52]. Artificially designed sensor arrays give results based only on smell, and they do not follow the same mechanism as the human senses. Also, there are several difficulties associated with sensors manufacturing, such as reproducibility, drift, and the transfer of properties from one lot of sensors to another. This learning and understanding are important, as we continue to use man-made sensor arrays in biomimetics for a wide range of applications [52]. Regardless of these concerns, the future for the electronic tongue appears to be promising as it can fulfill niche analyses. Research and algorithm development activities for data analysis in an efficient manner are continuing apace in different laboratories around the world. Since the early instruments have performed well for some applications, it is believed that the newer prototypes could be advanced in the field further. Researchers are investigating the application of electronic tongues as a detection scheme in flow-based analytical systems. It is remarkable that flow-based electronic tongues are currently experimenting, which confirms them as a trend in modern analytical chemistry. There is no doubt that these systems may be used for the resolution of more complex analytical problems, increasing the number of analytes to be determined, and also the identification/classification of even more similar samples [14]. The other key areas for future development can be envisioned inline monitoring of food production process, especially alcoholic fermentations.

6 Conclusion

The unique capabilities of electronic tongue systems, such as the ability to deal with complex and changing background and diminish the impact of interferents are the reasons of their paramount importance. Electronic tongues have become a very promising and prospective field of chemical sensor science. In addition to being a multicomponent quantitative analysis instrument, different sensing techniques, possible use of unconventional fabrication methods and numerous data treatment procedures indicate that electronic tongue systems can be tailored to various application areas. The strengths of the electronic tongue include the fact that they are easy to build, cost-effective, and provide a short time of analysis [46]. Also, they have greatly reduced the exposure and risk of using human panel to test food products, permitting better analytical results to quickly define the best formulation and get the product to the market. Therefore, these devices are becoming more and more popular to monitor food safety and quality assessment of foods. Although some electronic tongues have already been offered commercially, this is still an emerging scientific direction and more research has to be carried out in order to implement them in the process production widely. Major concerns include the lack of intermediate precision studies (i.e., using different operators or instruments) and long-term studies, more validation studies, and higher number of analyzed samples being required in some cases to extract more reliable conclusions [53]. To expect new

achievements in the upcoming future, extensive efforts are needed in sensing mechanism studies, in the development of new sensor compositions, and in the procedures for the application of electronic tongues to practical tasks.

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Chapter 12
Paper-Based Kits for Food Analysis and Authentication

Md Mursalin Rahman Khandaker and Mohidus Samad Khan

Abstract Food safety and security have emerged as an issue of global public concern and trade implications. Food contamination and adulteration are major threats toward public health. Traditional and conventional benchtop technologies are being utilized all over the world for the identification and quantification of food contaminants and adulterants. But these technologies are expensive, labor-intensive, require highly skilled manpower, and resources for proper operation. There is an urgent need for developing point-of-care devices for food analysis and authentication, which can be cost-effective, reliable, simple to operate, and analyze. Bioactive paper 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 point of need. In recent years, bioactive paper research has been a topic of great interest, as it is robust, simple, and affordable, and it can be an alternative to the traditional expensive instruments for detections, such as gas chromatography, mass spectroscopy, or high-performance liquid chromatography. This article focuses on paper-based point-of-care (POC) devices that are being developed for food analysis and authentication. It discusses paper-based devices as developed for the detection of foodborne and waterborne pathogens, organic and inorganic toxins, pesticides, and illegal food additives. Finally, this study also sheds light on the future aspect of bioactive papers in the food industry, limitations, and way forward.

Keywords Bioactive paper · Point of care devices · Food analysis · Food authentication · Food safety · Foodborne pathogens · Toxins · Pesticides · Adulterants · Robustness · Smartphone application

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1 Introduction

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. Food authentication is another analytical process to verify that a food product complies with its label description [1]. Food analysis and authentication are integral parts of food safety and security. Food safety is a global public health issue, and it has been aggravated in recent times. According to World Health Organization, almost 1 in 10 people in the world, or in other words 600 million people around the world fall ill after eating contaminated food and 420 thousands die every year, resulting in the loss of 33 million healthy life-years [2]. Children under the age of 5 are one of the most common victims of such untimely deaths. Foodborne illnesses are mainly caused by foodborne contaminants, which comprise biological and chemical species. Chemical toxicants basically include mycotoxin, marine biotoxin, pesticide residue, persistent organic pollutants, heavy metals, and cyanogenic glycosides. Such contaminants present in foods cause serious fatalities, even cancer. Biological contaminants are mainly foodborne and waterborne pathogens. Diseases such as cholera, diarrhea, typhoid, and meningitis are caused by such pathogens, which are life-threatening. *Salmonella* sp., *Escherichia coli*, *Cronobacter* sp., *Campylobacter* sp., and *Vibrio cholerae* are basically involved in food and water contamination. Infection by *Listeria monocytogenes*. causes miscarriage in pregnant women or death to newborn babies [3]. Besides bacteria, viruses like Norovirus, Hepatitis A virus, and Novel Coronavirus (SARS-CoV2) also contaminate foods causing severe illnesses. These are spread from uncooked or raw meat, fish, poultry, eggs, milk, fruits, and vegetables, or in some cases, from infected food handlers. Hence, it is necessary to analyze foods before consumption. There are many ways to identify contaminants in foods or relate to the authenticity of a food product, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), simplex or multiplex quantitative real-time polymerase chain reaction (qPCR), and enzyme-linked immunosorbent assay (ELISA) [4]. However, these techniques are expensive, time-consuming, and labor-intensive, which make them less applicable in resource-limited settings, importantly in developing countries, where foodborne illnesses are prevalent. To this end, there is a high demand to develop cost-effective and robust analytical devices for food safety monitoring to create effective prevention and control strategies.

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 the use in detecting food contaminants [5, 6]. A bioactive paper device is a specific, accurate, low-cost, time-, and labor-saving tool for food analysis and authentication. It has gained much attraction in food analysis research due to its capacity to replace conventional, expensive, and cost-intensive technologies such as gas chromatography, mass spectrometry, real-time or multiplex

polymerase chain reaction, or high-performance liquid chromatography [7] The detection technique using bioactive paper devices relies on colorimetric, fluorescent, and electrochemical signals developed in the presence of specific organic and/or inorganic substances, which can be observed and recorded in a rapid and simple manner [8]. This article reviews bioactive papers and paper technologies that have been used worldwide for food analysis and authentication. It enlists detection of foodborne and waterborne pathogens, toxins, pesticides, and illegal food additives and discusses the mechanisms, assay times, and sensitivity of the analysis. Besides, this article also sheds light on the future aspects of bioactive papers in food safety and security.

2 History of Bioactive Papers

Biosurface engineering consists of optimizing the biomolecular activity on a surface in terms of biofunctionality and biocompatibility. Biomolecule immobilization on the porous surface is of special interest to manufacture bioassays and functional surfaces. Paper, a nonwoven material made of cellulosic fibers, is highly wettable when untreated, easy to functionalize, biodegradable, sterilizable, biocompatible, and cheap. It is also chemically stable, porous, lightweight, and strong [9]. It is an ideal material for functional materials for functional bioactive surfaces [10]. The main advantages of paper biosensors are (i) high surface to volume ratio, (ii) adsorption properties, (iii) capillary action, (iv) compatibility with biological samples, (v) chemical functional groups for immobilization of proteins and antibodies, and (vi) straightforward sterilization [11]. Paper is biodegradable and easily disposable. Paper can transport reagents in its matrix which eliminates handling of the chemical solution. The currently available paper-based diagnostic devices are dipstick assays, lateral flow assays (LFAs), and microfluidic paper-based analytical devices (μPADs). The major substrates of dipstick and μPADs are cellulose fiber-based material and that for LFAs are nitrocellulose membranes [12]. The concept of bioactive paper is about 200 years old. However, low-cost paper-based tests, like pregnancy, blood glucose tests, and blood typing, are commercially available since the last century. Since the early 2010s, bioactive paper research and innovation have been receiving strong global attention and momentum. Figure 12.1 shows a timeline of bioactive paper research diagnosis and product development.

The major bioactive paper research initiative is carried out by SENTINEL Bioactive Paper Network (Canada), Harvard University (USA), University of Washington (USA), Monash University (AUS), and VTT Technical Research Centre of Finland. Bioactive paper research is also gaining momentum in other parts of the world including Brazil, China, Scandinavia, Japan, India, and Bangladesh [13]. Bioactive papers and diagnostics could be the future means to ensure better health care, safer food, and environment both in developed and in developing nations at an affordable cost.

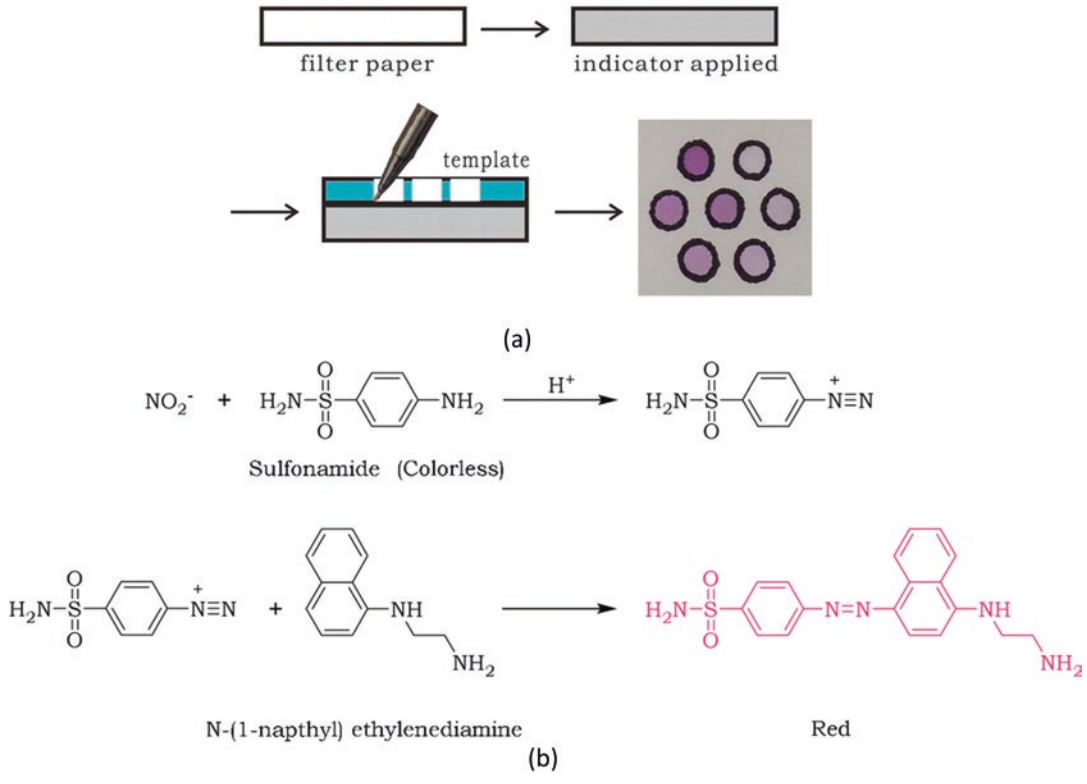

Fig. 12.1 Detection of nitrite ion by a microfluidic device in a colorimetric assay (**a**) loading sample in the indicator device, (**b**) reaction mechanism of colorimetric detection [20]

3 Paper-Based Diagnostic Devices for Food Analysis

Conventionally, additives and chemicals present in food are mostly detected and quantified by benchtop equipment like HPLC, GC, and GC-MS [14]. These methods are typically expensive, and these procedures are time-consuming, labor-intensive, and usually require highly trained workers [15]. Culturing and plating assays are known as the gold standard to detect foodborne pathogens [16]. ELISA (enzyme-linked immunosorbent assay) can fast detect targets, but it requires multiple processing steps including rinsing and reagent addition steps, which is time-consuming [17]. Unlike ELISA, qPCR (quantitative polymerase chain reaction) technique has high accuracy, but it requires numerous operation steps (i.e., nucleic acid extraction, amplification, and detection) [18]. Collectively, the above-mentioned conventional detection methods are costly and laborious, which make them less applicable in resource-limited settings and limited accessibility to well-established laboratories. To overcome the problem, different POC devices, such as lateral flow assays and microfluidic paper-based analytical devices (μPAD), have been developed to detect food contaminants rapidly, sensitively, and specifically for food safety monitoring [19]. Lateral flow immune-chromatographic assays are paper-based simple devices that identify the presence of target substances without the need for any specialized equipment. A microfluidic paper-based analytical device (μPAD) is typically a small piece of patterned paper with 2D or 3D structure that can rapidly test liquid samples of desired substances in a smaller volume. Figures 12.1, 12.2, 12.3, 12.4, and 12.5 present few examples of paper-based devices to detect different chemical compounds and biomolecules in food items [20–24]. These emerging devices come with several advantages, including: (i) being cost-effective, (ii) high portability for onsite analysis, (iii) high throughput, (iv) requiring

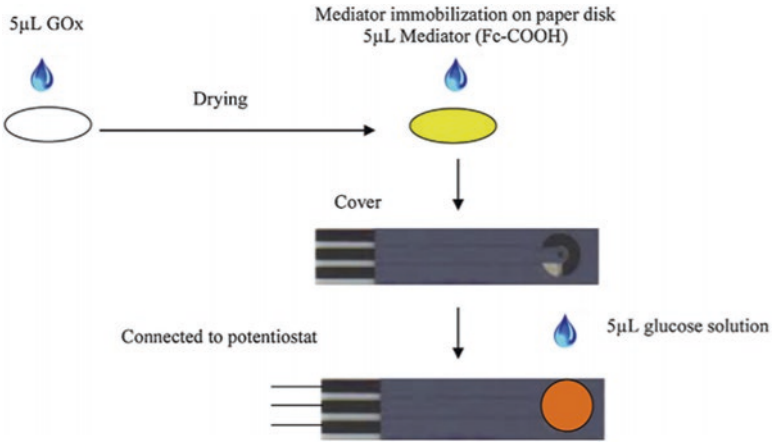

Fig. 12.2 Preparation of paper disk for electrochemical detection of glucose from beverages [24]

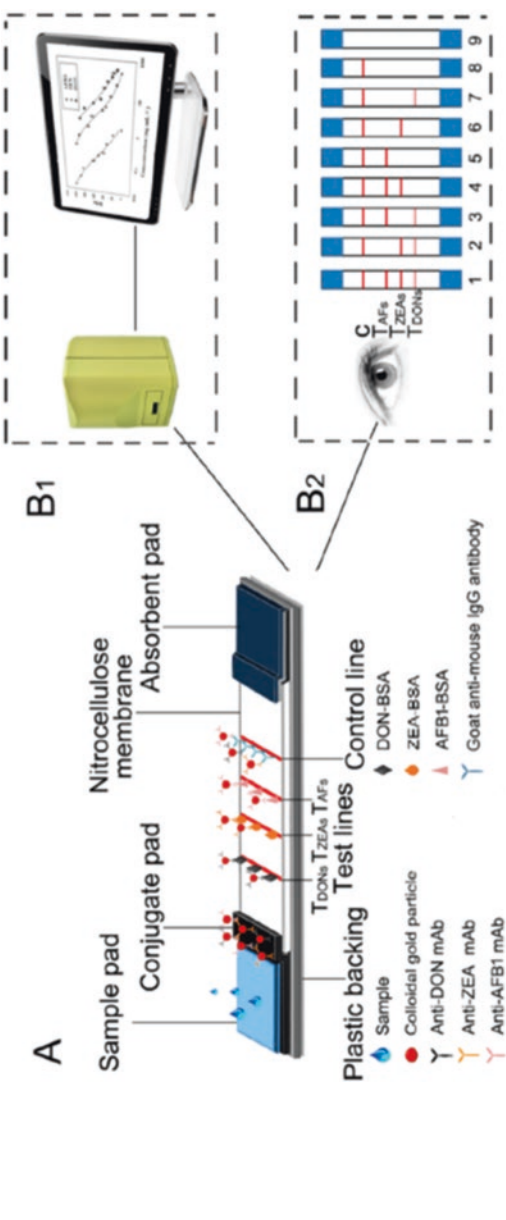

Fig. 12.3 Detection mechanisms of paper-based devices: scheme of the multiplex lateral flow immunoassay (LFA) for multiplex mycotoxins. B1: semiquantitative analysis platform for LFA. B2: qualitative analysis platform for LFA. Strips 1 to 9 are the schematic illustrations of detection results [21]

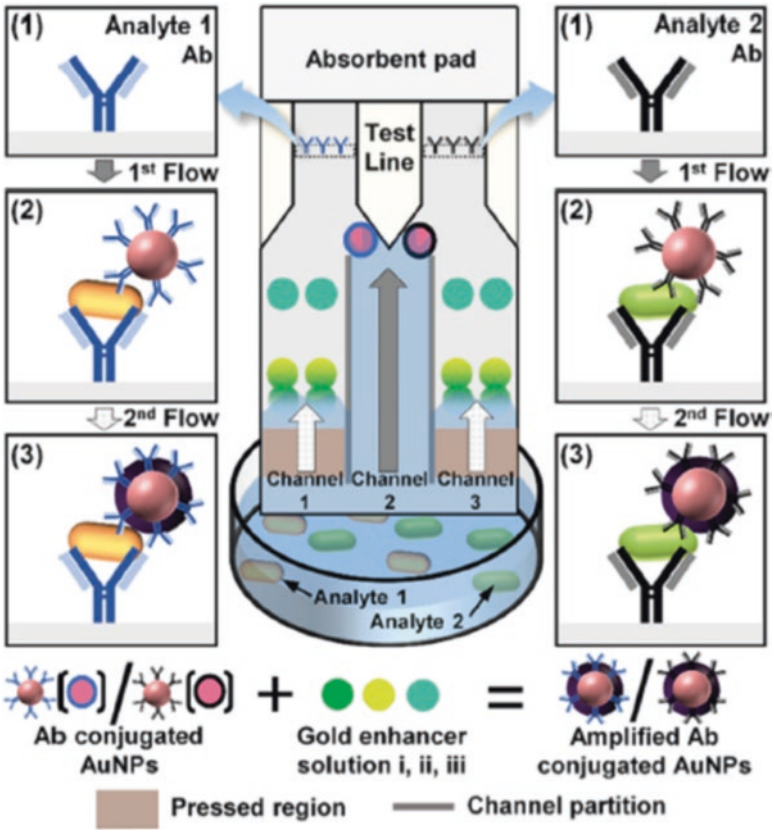

Fig. 12.4 Schematic of operation principle of pressed paper-based dipstick to detect foodborne pathogens. (*1*) Capture antibodies are fixed at the test lines before device is dipped into a sample solution. (*2*) Development of colorimetric signal by rehydration of Ab conjugated AuNPs with pathogens in the first flow through channel 2. (*3*) Colorimetric signals are amplified by rehydration of three components of gold enhancer with the second flows through channels 1 and 3 [22]

a low volume of reagents and samples, (v) having simple operation steps, and (vi) short analysis time [25].

These characteristics offer enormous potential for improving food safety issues, especially in developing and underdeveloped countries, where foodborne illnesses are on the rise. The details of emerging paper-based POC devices for food analysis are briefly discussed in the following sections and are summarized in Tables 12.1, 12.2, 12.3, 12.4, and 12.5.

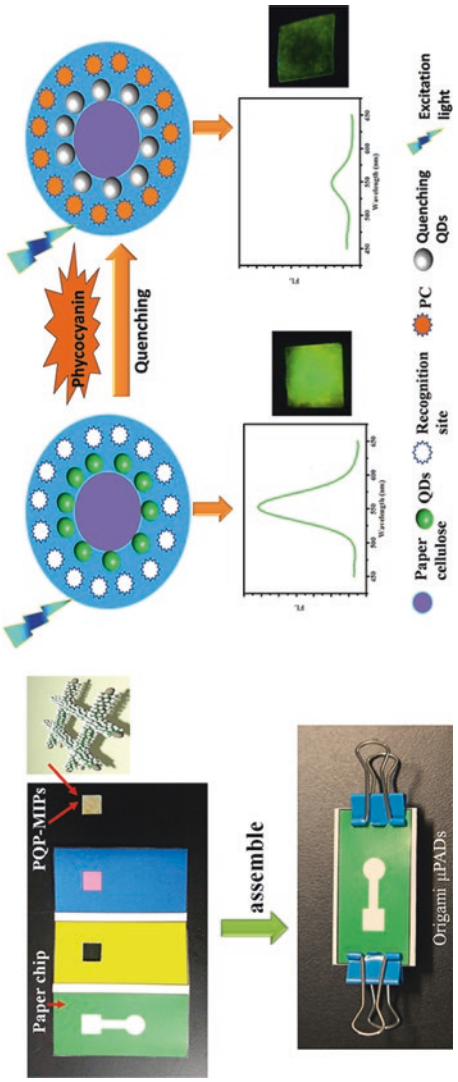

Fig. 12.5 Fluorescence detection of Phycocyanin using paper based device [23]

Table 12.1 Paper-based devices for detecting foodborne pathogens

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
1	*Cronobacter* sp.	Different kinds of food	Colorimetric	External step of sample enrichment	10 CFU/cm^2	8–12 h	[26]
2	*E. coli* O157:H7, *S. paratyphi A, S. Paratyphi B, S. paratyphi C, S. Typhi, S. enteritidis, S. choleraesuis, Vibrio cholera* O1, *V. cholera*e O139, and *V. parahaemolyticus*	Dairy products, marine food products, beverages, snacks, and meats	Fluorescence	External step of homogenization	10^4–10^5 CFU mL^{-1}	20 min	[27]
3	*Shigella boydii* and *Escherichia coli* O157:H7 (*E. coli* O157:H7)	Bread, milk, and jelly	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	Upto 4 CFU mL^{-1}	5–10 min	[28, 29]
4	Shiga toxin-producing *E. coli*	Undercooked meat, unpasteurized dairy products, vegetables, and water			vt1, vt2, eae, ehxA and 16S: 10^4–10^5 CFU mL^{-1} or 0.1–0.9 ng/µL DNA	<1 h	
5	*S. Typhimurium, S. Enteritidis*	Chicken feed and other veterinary products			10^4–10^6 CFU mL^{-1}	5–15 min	
6	*Salmonella typhimurium*	Solid/liquid food items	Mie scattering	Immunoagglutination of antibody conjugated particles	Single-cell level	<1 min	[30]

(continued)

Table 12.1 (continued)

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
7	(a) *E. coli* O157:H7 (b) *S. typhimurium*	Phosphate-buffered saline	Colorimetric		(a) 10^5 CFU mL^{-1} (b) 10^6 CFU mL^{-1}	10 min	[22]
8	*Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella typhimurium*	Ready-to-eat meat	Colorimetric	Microspot assay	10^1 colony-forming units/cm^2	12 h or less	[31, 32]

Table 12.2 Paper-based devices for detecting waterborne pathogens

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
1	*P. aeruginosa*	Water	Colorimetric		20 CFU mL^{-1}	50 min	[33]
2	*E. coli* O157:H7	Phosphate-buffered saline, milk, water, and apple juice			10 CFU mL^{-1}	35 min	[34]
3	*E coli*	Water	Fluorescence	On board DNA extraction and amplification	5 cells	60 min	[35]
4	*E. coli*	Water, milk	Colorimetric		10–1000 CFU mL^{-1}	1 h	[36]
5	*E. coli* O157:H7	Milk	Fluorescence		10–1000 CFU mL^{-1}	1.5 h	[37]
6	*E coli*	Water, milk	Fluorescence		Single cell level	<1 min	[38]
7	*E. coli* and *Bacillus sp*	Water	Fluorescence		1.9×10^3 CFU mL^{-1}	45 min	[39]
8	*Escherichia coli*	Water	Colorimetric	Phage-mediated cell lysis	<10 CFU mL^{-1}	~6 h	[40]
9	*Escherichia coli*	Water	Chemiluminescent	Paper chip immunoassay coupled with silver enhancement	1 CFU mL^{-1}	1 h	[41, 42]
10	*Salmonella typhimurium*	Water, poultry packing liquid	Fluorescence		100–1000 CFU mL^{-1}	5 min	[43]
11	*Salmonella typhimurium*	Milk, juice, egg	Colorimetric	DNA extraction and amplification	100–1000 CFU mL^{-1}	1 h	[44]
12	*Salmonella sp*	Water	Colorimetric and electrochemical		2.6×10^7 CFU mL^{-1}	35 min	[45]
13	*V. cholerae* O1 and O139 serogroups	Water	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	10^2 CFU mL^{-1}	~6 h	[46]

Table 12.3 Paper-based devices for detecting toxins

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
1	Nitrite ion	Water	Colorimetric	External step of filtration	0.5 nmol/L 73 ng mL^{-1}	5 min, 15 min	[20, 47].
2			Colorimetric	UV lithography on OTS coating	~6 µM	12 h	[48]
3			Colorimetric	Reaction in which first nitrite reacts with sulfanilamide and the formed diazonium salt reacts with naphthylethylenediamine dihydrochloride	5.6 µM	12 h	[49]
4	Clenbuterol, a veterinary drug	Milk	Colorimetric	Enzyme-linked immunosorbent assay (ELISA)	0.02 ppb	~1 h	[50]
5	Iodine	Fortified salt	Colorimetric	Iodometric titration	0.8–15 ppm	1.5 h	[51]
6	Benzoic acid	Water, fruit juice	Colorimetric	Janovsky reaction	500 ppm	20 min	[52]
7	Copper ions	Water, tomato juice, Rice	Colorimetric	Catalytic etching of silver nanoplates (AgNPls) by thiosulfate ($S_2O_3^{2-}$)	0.3 ng mL^{-1}	2 min	[53]
8	Glucose	Drinks	Surface-enhanced Raman spectroscopy (SERS)	Formation of complexes	0.18 mM	~1 h	[24, 54]
9	Alkaline Phophatase	Milk	Colorimetric	External step of target separation	0.1 U/L	10 min	[55]
10	Phycocyanine	Water	Fluorescence	External step of filtration	2 mg/L	30 min	[23]

11	Mercury (II) ion, silver (I) ion, aminoglycoside antibiotics	Water	Fluorescence		(a) 121 nM (b) 47 nM (c) 153 nM	10 min	[56]
12	Ethanol	Beer	Electrochemical		0.52 mM	1 h	[57]
13	Lead (II) ion, cadmium (II) ion, copper (II) ion	Water, rice, and fish Samples	Electrochemical	External step of filtration for water samples external steps of grinding, extraction and filtration for rice and fish samples	(a) 0.1 ng mL^{-1} (b) 0.1 ng mL^{-1} (c) 5 ng mL^{-1}	~10 min	[58]
14	Heavy-metal ions and glucose	Aqueous solutions	Electrochemical	Photolithography or wax printing and electrodes screen-printed from conducting inks (e.g., carbon or Ag/AgCl)		~10 min	[59].
15	Pb (II) and Cd (II)	Aqueous solutions	Electrochemical	Square wave anodic stripping voltammetric (SWASV)	Pb(II) 2.0 and Cd(II) 2.3 ppb	~6 min	[60]
16	Cu (II)	Water	Colorimetric	Color intensity of polymer inclusion membrane (PIM)	0.06 mg L − 1	15 min	[58, 61]
17	Hg (II); Ag(I); Cu(II); Cd(II); Pb(II) Cr(VI); Ni(II)	Water	Colorimetric	Colorimetric visualization of the enzymatic activity of β-galactosidase (B-GAL)	Hg(II) = 0.001 ppm; Ag(I) = 0.002 ppm, Cu(II) = 0.020 ppm; Cd(II) = 0.020 ppm; Pb(II) = 0.140 ppm; Cr(VI) = 0.150 ppm; Ni(II) = 0.230 ppm	10 min	[62]

(continued)

Table 12.3 (continued)

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
18	Phenol, bisphenol A, catechol and cresols	Water and liquid foods	Colorimetric	Specific binding of the enzymatically generated quinone to the multilayers of immobilized chitosan	0.86 (±0.1) μg/L each	6–17 min	[63]
19	Food supplement and water	Halide ions	Colorimetric		$10^{-4.8}$ to 0.1 M for bromide and iodide and from $10^{-4.5}$ to 0.6 M for chloride	~20 min	[64]

Table 12.4 Paper-based devices for detecting pesticides

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
1	Paraoxon, an organophosphate pesticide	Liquid (i.e., milk and apple juice), solid (apple and head lettuce	Colorimetric	Acetylcholine esterase inhibition (AChE)	0.5 ppm	1–2 h	[66]
2	Organophosphate and carbamate pesticide	Food items (solid/liquid)	Colorimetric	Acetylcholine esterase inhibition (AChE)	Carbofuran 0.003 ppm, dichlorvos, carbaryl 0.3, ppm,	2–2.5 h	[67]
3	Pirimicarb, a carbamate pesticide	Lettuce and brown rice	Colorimetric	Acetylcholine esterase inhibition (AChE)	0.6 ppm	~1–2 h	[68]
4	Organophosphate (methomyl) and carbamate (profenos) pesticides	Food items (solid/liquid)	Colorimetric	Acetylcholine esterase inhibition (AChE)	Methomyl 6.16×10^{-4} mM and profenofos 0.27 mM	~5 min	[69]
5	Methyl-paraoxon, chlorpyrifos-oxon, malathion	Food items (solid/liquid)	Colorimetric	Acetylcholine esterase inhibition (AChE)	18 and 5.3 ng $mL^{-1}$for methyl-paraoxon and chlorpyrifos-oxon, respectively	2–3 h	[70]
6	Pentacholorphenol (PCP)	Food items (solid/liquid)	Colorimetric	MIP technique	0.01–100 ng mL^{-1}	20–30 min	[71]
7	Imidacloprid, chlorpyrifos-methyl, isocarbophos	Rice, lettuce, water, Chinese cabbage, milk, muscle and liver of swine, fruit juice	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	(a) 50 μg L^{-1} (b) 100 μg L^{-1} (c) 100 μg L^{-1}	~7 min	[72]
8	Thiram	Water, apple juice	Colorimetric	Luminescence of NaYF4:Yb/tm	0.1 μM	~15 min	[73]

(continued)

Table 12.4 (continued)

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
9	Thiram, Thiabendazole, methyl parathion	Apples, oranges, tomatoes, and green vegetables	Chemiluminescence	External step of fruit cutting	0.1 U/L	10 min	[74].
10	Thiram, ferbam	Water	Surface-enhanced Raman scattering	Conjugation of monoclonal nanoparticles with antigen	(a) 0.46 nM (b) 0.49 nM	~5 min	[75]
11	Aflatoxin B1, Ochratoxin A Zearalenone	Peanuts, maize, and rice sample	Surface-enhanced Raman scattering	Conjugation of monoclonal nanoparticles with antigen	(a) 0.25 ng mL^{-1} (b) 0.5 ng mL^{-1} (c) 1 ng mL^{-1}	20 min	[76]

12	Deoxynivalenol (DON), Zearalenone (ZEA)	Grains (rice, wheat, and maize)	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	(a) 50 ng mL^{-1} (b) 1 ng mL^{-1}	5–15 min	[21, 77–82]
13	Fumonisin B1, Zearalenone	Corn and wheat	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	(a) 6 ng mL^{-1} (b) 50 ng mL^{-1}	<15 min	
14	Fusarium group mycotoxins	Wheat, oats, and maize	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	80, 400, 1400 and 3200 μg kg^{-1}, respectively, for zearalenone, T-2/HT-2 toxins, deoxynivalenol and fumonisins in maize, and 80, 400 and 1400 μg kg^{-1}, respectively, for zearalenone, T-2/HT-2 toxins and deoxynivalenol	30 min	
15	Aflatoxin, Deoxynivalenol (DON), zearalenone (ZEA)	Cereal	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	0.05 μg/kg, 1 μg/kg, and 3 μg/kg, respectively	15 min	
16	AFB1, AFB2, AFG1, AFG2	Agro products	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	(a) 0.06 ng mL^{-1} (b) 0.25 ng mL^{-1} (c) 0.125 ng mL^{-1} (d) 0.25 ng mL^{-1}	15 min	

Table 12.5 Paper-based devices for detecting illegal food additives

Sl. no	Target analyte	Food sample	Detection mode	Detection mechanism	Detection limit	Assay time	References
1	Gliadin	Wheat, barley, oat, rice, foxtail millet, corn, buckwheat, soybean, and rye)	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	10 μg mL^{-1}	30 min	[85]
2	Formaldehyde	Fish, dried food	Colorimetric	Acetylcholine esterase inhibition (AChE)	10 ppm	>1 min	[87–89]
3	Horse meat residue	Meat and processed meat	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	Positive/negative test with around 0.01% precision	35 min	[90]
4	17 β estradiol	Milk	Colorimetric	External step of target separation	0.25 μg/L	10 min	[91]
5	Glucose, sucrose and fructose	Beverage	Electrochemical	Cu electrode signal	Glucose, fructose, and sucrose are 270 nM, 340 nM, and 430 nM, respectively	~10 min	[92]
6	Cyromazine, Melamine	Foods of animal origin	Multicomponent immunochromatographic assay (ICA)	Conjugation of monoclonal nanoparticles with antigen	(a) 0.22 ng mL^{-1} (b) 0.26 ng mL^{-1}	20–30 min	[94]

3.1 Foodborne Pathogens

Cronobacter sp. is an opportunistic foodborne pathogen, which is mainly found in foods and food-producing environments persisting for a long time. A micro spot paper-based analytical device (μPAD) was developed to detect *Cronobacter* in food within 8–12 h of assay time and has a detection limit of 10 CFU/cm^2 [26]. It has a sensitivity of about 80–100%, and it is a low-cost instrument, with a price lower than 15 cents per test.

A 10-channel up-converting phosphor technology-based lateral flow (TC-UPT-LF) assay was established by researchers for the rapid and simultaneous detection of 10 epidemic foodborne pathogens. Food samples of 279 were tested, including dairy products, marine food products, beverages, snacks, and meats. Without enrichment, the TC-UPT-LF assay had a detection sensitivity of 10^4–10^5 CFU mL^{-1} for each pathogen, and after sample enrichment, it was 10 CFU/0.6 mg. The TC-UPT-LF assay allows the rapid, quantitative, and simultaneous detection of 10 kinds of foodborne pathogens within 20 min [27].

A colloidal gold immunochromatographic strip with double monoclonal antibodies was presented for rapid and simultaneous detection of *Shigella boydii* and *Escherichia coli* O157:H7 (*E. coli* O157:H7). Parallel analysis of pathogen detection from bread, milk, and jelly showed consistent results between the strip test and enzyme-linked immunosorbent assay (ELISA). The detection limit was substantially improved to 4 CFU/mL of the original bacterial content after preincubation of the bread, milk, and jelly samples in broth for 10 h, 10 h, and 8 h, respectively. This ICS was able to finish the test within 5–10 min and has advantages in high throughput and easy operation. Researchers from different parts of the world also worked on developing immunochromatographic test kits to detect shiga toxin-producing *E.coli* [28, 29].

An immunochromatographic assay was developed to detect *Salmonella enterica* serovars *typhimurium* and *enteritidis* in a single strip. The test strips can immediately detect *S. Typhimurium* and *S. Enteritidis* specifically in a culture medium at levels as low as 10^4 and 10^6 CFU/mL, respectively. The contamination of *S. typhimurium* and *S. enteritidis* at 1 CFU/mL or greater can be detected by the test strips after 6–24 h. incubation. This test strip also provides advantages of simplicity and very rapid detection of these specific bacterial contaminants in chicken and can be useful for mass detection on chicken farms and in other veterinary products.

Another optical-based technique was developed in which *Salmonella* samples were premixed with anti-*Salmonella* conjugated particles to allow immune agglutination before loading into the μPAD. A pressed paper-based dipstick was presented that enables detection of foodborne pathogens with multistep reactions by exploiting the delayed fluid flow and channel partition formation on nitrocellulose (NC) membrane. The paper can detect *Escherichia coli* O157:H7 and *Salmonella typhimurium* in phosphate-buffered saline within 10 min by signal enhancement [30].

In another study, a μPAD was developed for the micro spot assay of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* in ready-to-eat meat samples. The pathogens were collected from foods by a swab sampling technique and then cultured in media before adding to a chromogen-impregnated paper-based device. A color change was observed indicating the presence of an enzyme associated with the pathogen of interest, and detection was achieved [31, 32].

3.2 Waterborne Pathogens

A simple and sensitive approach for visual and point-of-care detection of *P.aeruginosa* and its toxin genes was presented based on multiple loop-mediated isothermal amplification (mLAMP) and lateral flow nucleic acid biosensor (LFNAB). The device is capable of detecting concentrations of *P. aeruginosa* as low as 20 CFU/mL in water sample within 50 min without complicated instrument, which is more sensitive than traditional PCR [33].

A simple and sensitive aptamer-based biosensor was developed for rapid detection of *Escherichia coli* O157:H7 (*E. coli* O157:H7). The colorimetric change on the device can be seen by naked eyes, and it can detect *E. coli* upto 10 CFU/mL from phosphate-buffered saline, milk, water, and apple juice samples in 35 min [34]. Another device that integrates sample preparation and loop-mediated isothermal amplification (LAMP) with end point detection using a hand-held UV source and camera phone was established. This device detects *Escherichia coli* malB gene to proceed with an analytical sensitivity of one double-stranded DNA target copy. It can detect *E.coli* with a limit of detection of five cells. The assay time is 60 min [35].

Researchers have also demonstrated an integrated paper-based biosensor based on nucleic acid extraction, amplification, detection, and quantification using a smartphone. The biosensor can successfully detect *Escherichia coli* (as a model analyte) in spiked drinking water, milk, and spinach with a detection limit of as low as 10–1000 CFU mL^{-1} [36]. The process takes up to 1 h. Another group of scientists have developed an immunochromatographic assay kit using Eu (III)-doped polystyrene nanoparticle for detection of *Escherichia coli* in the milk sample. This device can detect *E. coli* as low as 100 CFU mL^{-1} in 30 min Among other immunochromatographic assays used, EuNP-SA-ICA exhibited the highest sensitivity and the widest linear range with good specificity, accuracy, and precision [37].

A paper-based lateral flow immunoassay was reported for detection of *Escherichia coli* that avoids the use of secondary antibodies and is revealed by the photoluminescence quenching ability of graphene oxide. The proposed device can display a highly specific and sensitive performance with a limit of detection of 10 CFU mL^{-1} in standard buffer and 100 CFU mL^{-1} in bottled water and milk within 60–90 min [38]. Researchers have reported an innovative, simple, and low-cost, paper-based probe for detection of *E. coli* and *Bacillus sp.* in water, fabricated

by screen printing carbon electrodes onto hydrophobic paper. The device can detect bacterial concentrations ranging from 10^3 to 10^6 CFU mL^{-1}, with an estimated lower detection limit of 1.9×10^3 CFU mL^{-1} within 45 min [39]. A one-step multiplexed fluorescence (FL) strategy for detecting pathogens was also developed by [40] using a μPAD that was a hybrid of PDMS and glass. Other works on *E. coli* detection in water by μPADs were reported by Burnham et al. [41] and Ma et al. [42].

A rapid, paper microfluidic-, and smartphone-based protocol was developed for the extraction and direct fluorescent identification of the nucleic acids of *Salmonella typhimurium* from field and clinical samples. The limit of detection of *Salmonella Typhimurium* in 10% poultry packaging liquid with cellulose paper was 10^3 CFU mL^{-1} while that extracted with nitrocellulose paper was 10^4 CFU mL^{-1} (as determined by both PCR and fluorescence reflectance) [43]; the proposed assay time was 5 min

A fully disposable and integrated paper-based sample-in-answer-out device was developed for nucleic acid testing (NAT) by integrating nucleic acid extraction, helicase-dependent isothermal amplification, and lateral flow assay detection into one paper device. It can sensitively detect *Salmonella typhimurium*, as a model target, with a detection limit of as low as 10^2 CFU mL^{-1} in wastewater and egg, and 10^3 CFU mL^{-1} in milk and juice in about an hour [44]. A cost-effective microfluidic paper analysis device (μPAD) with a special Z-folding design was developed for controlling the fluidic flowing and substrate transportation for detecting pathogens in water. The limit of detection (LOD) was 1 μM for ATP detection and 2.6×10^7 CFU mL^{-1} for *Salmonella* live cell detection in 35 min [45]. A method based on CL detection of *Salmonella* via adenosine triphosphate (ATP) quantification on μPAD was also presented [45]. *Salmonella* was cultured and then lysed after harvesting by boiling method. Color change was observed in the μPAD only when ATP is present as an indication of the presence of Salmonella in the sample.

Epidemic and pandemic cholera are exclusively associated with Vibrio cholerae serogroups O1 and O139. In light of the need for rapid diagnosis of cholera and to prevent the spread of outbreaks, scientists developed and evaluated a direct one-step lateral flow biosensor for the simultaneous detection of both *V. cholerae* O1 and O139 serogroups using alkaline peptone water culture. The assay is based on the immunochromatographic principle, where the antigen–antibody reaction would result in the accumulation of gold nanoparticles, thus the appearance of a red line on the strip. The limit of detection by this device is 10^8 and 10^7CFU mL^{-1}, respectively, for the serogroups [46].

3.3 Organic and Inorganic Toxins

A simple μPAD was developed by using a permanent marker pen to identify nitrite ions in water in about 5 min time. The detection limit is 0.5 mmol/l, and it is coupled with a camera phone which detects the colorimetric change of the assay. A

colorimetric paper-based platform coupled with a smartphone was also proposed for quantification of nitrite ion in water in about 15 min [20, 47].

A μPAD was developed by coupling hydrophobic octadecyl-trichlorosilane (OTS) to the fibers of Whatman No. 1 filter paper, followed by UV-lithography of the OTS coating [48]. In another study, researchers used a preheated metal stamp to transfer paraffin wax from a waxed filter paper to another stacked native filter paper. Both studies used Griess reaction—a two-step diazotization reaction in which nitrite reacts with sulfanilamide first and the formed diazonium salt reacts with naphthyl-ethylenediamine dihydrochloride to produce strong pink-colored azo dye that detects the presence of nitrite ions [49]. Detection of clenbuterol, a veterinary drug, in milk was diagnosed using a paper-based enzyme-linked immunosorbent assay (ELISA) [50].

A multiplexed μPAD (called a saltPAD) was developed which is capable of making an iodometric titration in a single printed card [51]. Multiple reagents were stored on every compartment of each detection zone of the saltPAD. The reagents could recombine and undergo surface-tension-enabled mixing upon introduction of the iodized salt sample solution for determination.

An integrated microfluidic platform comprising a μPAD and a portable detection system was proposed for detecting benzoic acid in water and fruit juice having a minimum concentration of 500 ppm in 20 minutes via Janovsky reaction theory [52]. After colorimetric change in the device, it is detected by a CMOS camera, and results are interpreted by an RGB software.

A novel, highly sensitive, and selective paper-based colorimetric sensor was developed for detecting trace amounts of copper ions from water and food. This device based on the catalytic etching of silver nanoplates (AgNPls) by thiosulfate ($S_2O_3^{2-}$) can detect copper ions up to 0.3 ng mL^{-1}, and it takes only 2 min to obtain the colorimetric change [53]. The data obtained from this device is in good agreement with data derived from inductively coupled plasma-optical emission spectrometry.

Amperometric detection of glucose on a screen-printed electrode μPAD was fabricated. One μPAD designed for detecting colorants was presented, where a poly (sodium 4-styrenesulfonate)-functionalized paper substrate was used for the rapid separation, preconcentration, and detection of colorants in drinks by the formation of complexes via a surface-enhanced Raman spectroscopy (SERS) method [24, 54].

Researchers developed a disposable lateral flow-through strip for the smartphone to fast one-step quantitatively detect alkaline phosphatase (ALP) activity in raw milk. A trace amount of ALP as low as 0.1 U L^{-1} with a linear dynamic range of 0.1–150 U L^{-1} (R2 = 0.999) in pasteurized milk and raw milk can be one-step detected by the developed flow-through strip within 10 min, demonstrating the potential of smartphone-based portable sensing device for pathogen detection [55]. This device offers a rapid, sensitive, and inexpensive platform in food inspection and monitoring.

A novel strategy was reported using fluorescent quantum dots (QDs) combined with molecularly imprinted polymers (MIPs) on three-dimensional (3D) origami paper-based microfluidic devices for specific recognition and sensitive detection of

phycocyanin. Results revealed that the method exhibited a dynamic response to phycocyanin in the range of 10–50 mg/L with a limit of detection of 2 mg/L in 30 min [23].

A low-cost and simple paper-based microfluidic device integrated with fluorescence-labeled single-stranded DNA (ssDNA) functionalized graphene oxide sensor was fabricated for the multiplex determination of different types of chemical contaminants in food. This device has been successfully applied in multiplex detection of heavy metal mercury (II) ion (Hg^{2+}) and silver (I) ion (Ag^{+}) and aminoglycoside antibiotics residues in food within 10 min [56].

The first example of a paper-based, screen-printed biosensor was reported for the detection of ethanol in beer samples. The developed biosensor allowed a facile quantification of ethanol up to 10 mM (0.058%vol), with a sensitivity of 9.13 μA/mM cm^2 (1574 μA/%vol cm^2) and a detection limit equal to 0.52 mM (0.003% vol) [57].

A microfluidic paper-based analytical device (μPAD) coupled with dual electrochemical and colorimetric detection was developed to obtain high sensitivity and specificity for the simultaneous determination of lead, cadmium, and copper in food samples like rice, fish, and water. The μPAD is divided into two parts. The first part is electrochemical detection for the determination of lead and cadmium using a bismuth-modified, boron-doped diamond electrode (Bi-BDDE). The limit of detection is 0.1 ng mL^{-1} (for both metals). The second part is colorimetric detection for the determination of copper based on the catalytic etching of silver nanoplates (AgNPls) by thiosulfate ($S_2O_3^{2-}$). In the second phase, the detection limit is 5 ng mL^{-1} and the assay time is nearly 10 min [58].

A μPAD was developed for the versatile and quantitative electrochemical detection of biological and inorganic analytes in aqueous solutions as well as water [59]. An electrochemical μPAD was also developed for Pb(II) and Cd(II) detection in water based on square wave anodic stripping voltammetry, (SWASV) relying on in situ plating of bismuth film [60]. Two other applications of μPAD with colorimetric detection for Cu(II) were also reported by researchers [58, 61]. A multiplexed μPAD was reported that is capable of detecting heavy metals simultaneously in single μPAD [62]. Two μPADs were developed in 2015 and 2016 for detecting Cu(II), Pb(II), and Cd(II) in different food commodities based on calorimetry and electrochemistry [53, 58].

For the detection of phenolic compounds, researchers [63] developed a paper sensor that produces different color responses for phenol (reddish-brown), bisphenol A (blue-green), dopamine (dark-brown), cathecol (orange), and m-cresol (orange), and p-cresol (orange), resulting from the specific binding of enzymatically generated quinone to chitosan immobilized in multiple layers on the paper. An electrochemical μPAD for halide detection in food supplement and water samples via cyclic voltammetry was also developed which utilized silver elements as working and counter/reference electrodes [64].

3.4 Pesticide

Lateral flow assays (LFA) and microfluidic paper-based analytical devices (μPADs) can test potentially hazardous pesticides and relevant residues in food and water samples. One common technique of pesticide detection by μPADs is acetylcholinesterase inhibition (AChE) [65]. A bidirectional lateral flow bioactive paper sensor was fabricated to detect paraoxon, an organophosphate pesticide in liquid (i.e., milk and apple juice) and on the surface of solid foods (i.e., apple and head lettuce) [66].

A μPAD was developed to detect pirimicarb, a carbamate pesticide, in lettuce and brown rice with the same principle but a different design [67]. A method was developed based on the inhibition of acetylcholinesterase (AChE) on the degradation of acetylcholine molecules into choline and acetic acid by organophosphate (methomyl) and carbamate (profenos) pesticides [68]. A μPAD sensor and a novel smartphone application were developed for colorimetric detection of organophosphates (paraoxon, malathion) based on inhibition of AChE by pesticides [69].

A nanoceria-coated μPAD was fabricated by a colorimetric approach to detect methyl-paraoxon, an organophosphate pesticide, in cabbage and green mussel [70]. The μPAD was mounted on Whatman No. 4 filter paper and the 5-mm diameter circle pattern was generated by screen-printing of polystyrene (25% w/v in toluene). Pentachlorophenol (PCP) is carcinogenic and has severe health effects [71]. Researchers developed a photoelectrochemical (PEC) sensor that utilized the MIP technique on a μPAD to detect PCP [72]. A novel lateral flow immunoassay (LFIA) was presented for the simultaneous detection of the pesticides imidacloprid, chlorpyrifos-methyl, and isocarbophos based on three competitive immunoreactions. The detection time is within 7 min, and the detection limits are 50, 100, and 100 μg L^{-1}, respectively [73].

An integrating upconversion nanosensor with smartphone digital imaging was demonstrated for point-of-care analysis on a paper-based platform. The blue luminescence of NaYF4:Yb/Tm upconversion nanoprobes is quenched after additions of pesticide "thiram" through resonance energy transfer mechanism. The device detects thiram concentration as low as 0.1 μM in about 15 min [74].

Researchers screen printed with silver nanoparticles and graphene oxide on the paper surface and fabricated a paper device as controllable Surface-enhanced Raman scattering (SERS) substrates for the detection of pesticide residues in apples, oranges, tomatoes, and green vegetables. The device was capable to detect of thiram, thiabendazole, and methyl parathion in complex surfaces with a low limit of detection of 0.26, 28, and 7.4 ng cm^{-2}, which are much lower than the maximal residue limits in fruit prescribed by the U.S. Environmental Protection Agency [75].

A hydrophobic paper-based surface was developed for the fabrication of SERS sensors to detect pesticide residues in water. The limits of detection of thiram and ferbam as test pesticides are measured to be 0.46 and 0.49 nM, respectively, within an assay time of 5 min [76]. Mycotoxins are highly toxic contaminants and have induced health threat to human and animals. Researchers have developed multiplex lateral flow immunochromatographic assays (LFICS) to simultaneously detect

different mycotoxins which commonly occur in agro-based food and feed. Such mycotoxins are AflatoxinB1 (AFB1), ochratoxin A (OTA), Deoxynivalenol (DON), Fusarium, Fumonisin B1, and zearalenone (ZEA) [21, 77–82].

3.5 Illegal Food Additives

Food additives are used worldwide due to consumer demand. Additives are generally used to enhance flavors, make the food more palatable, improve shelf-lives of raw and uncooked foods, maintain nutritional value, improve overall taste, texture, and appearance. In its broadest sense, a food additive is any substance used in the production, processing, treatment, packaging, transportation, or storage of food [83]. Additives come in different forms, namely, food colors, preservatives, and chemical compounds. Illegal or misuse of additives might lead to adulteration, causing food fraud. Food may also get contaminated naturally. Adulteration or illegal use of additives are difficult to detect at the point of need, since it requires conventional benchtop technologies and tedious sample preparation procedures. Hence, scientists and researchers have explored point-of-care devices to detect additives or adulterants used on food [84].

Gliadin in wheat is an alcohol-soluble compound that contains approximately 50% of the wheat gluten protein and it is also a food allergen. A barcode style lateral flow immunoassay has been developed using two cutoff values (10 and 50 mg/kg gliadin) to provide a semi-quantification for identifying "gluten-free" and "very low gluten" foods, based on the international Codex Alimentarius Standard. This device is tested on 48 food samples and results of this assay closely agreed with those obtained using AOAC-approved ELISA or strip kits. The assay time is about 30 min [85].

Formaldehyde is widely used in the preservation of fish, but excess use of formaldehyde illegally to preserve fishes is a major threat to public health [86]. Researchers developed a paper-based kit to identify formaldehyde in fishes. The device offers a low-cost colorimetric detection of formaldehyde concentrations as low as 10 ppm in 2–3 min [87]. Besides, the scientists have also developed fluorescence μPADs for detecting formaldehyde in dried goods (e.g., ginseng) [88, 89].

The issue of fraudulent incorporation of horse meat in foods intended for human consumption gained major public attention in 2013, following a meat adulteration scandal in Europe wherein ~5.0–7.5% of beef-based products in Europe were documented to contain undeclared horse meat residues at contamination levels exceeding 1.0% [90]. Hence, for meat authentication, a highly sensitive Lateral Flow Device (LFD) has been developed for horse meat detection in beef and pork intended for deployment outside the laboratory setting. It has got 0.01% precision and the assay time is 35 min.

17β-estradiol (17β-E2) is an environmental estrogen, which endangers food security by causing endocrinal problems to humans. This is mostly used in the dairy and meat industries. A molecularly imprinted polymer (MIP) grafted paper-based

method has been reported for the detection of 17β-estradiol in milk. The limit of detection (LOD) of detection method by the paper device for milk and human urine samples could reach 0.25 μg L^{-1} within 10 min [91].

The toxicity level of additives like color, glucose, fructose, and sucrose used in food and beverage industries depends on intake concentration. Thus, there is a point of need for μPAD technology for testing the samples of food and beverage industries. A μPAD was presented that utilized microwire electrodes as an alternative to screen-printed electrodes for the nonenzymatic electrochemical detection of glucose, fructose, and sucrose in beverage samples [92].

Melamine (MA), with an IUPAC name of 1,3,5-triazine-2,4,6-triamine, is an industrial chemical and has been used for decades in the manufacturing of dishes, plastic resins, ingredients of paper and paperboard, flame retardant fibers, and industrial coatings. It has been found that many dairy industries use melamine with powdered milk to increase the crude protein content. In 2008, infants in Gansu province in China were found infected with kidney stones, 6 babies died around, and 300,000 children were found sick due to the use of melamine in powdered milk [93]. Cyromazine (CA) is a triazine pesticide used for fly control in cattle manure, field crops, vegetables, and fruit. In order to provide rapid testing facilities, scientists developed multiplex paper-based lateral flow immunochromatographic assay kits for simultaneous detection of cyromazine (CA) and melamine (MA) in animal origin foods [94].

4 Future Aspect of Paper-Based Kits for Food Analysis

The simplicity, cost-effectiveness, user-friendliness, and portability of paper-based POC devices play a key role in a broad range of applications. Recent advances in the field have made it possible to achieve sensitive and specific detection of food contaminants. Furthermore, quantification could also be achieved by using a simple smartphone application, enabling signal analysis to be performed by users without the requirement of benchtop equipment. More recently, several other materials coupled with paper such as textile, nanomaterials, or carbons were explored in POC applications to improve device sensitivity, simplicity, and functionality [95, 96]. To further improve simplicity and functionality, some studies have also attempted to integrate sample-to-answer process into a single device. These paper-based devices show great promise for possible development into a stand-alone device for the detection of food contaminants outside the laboratory, thus enhancing food safety and security especially in developing countries [8].

In order to mitigate the challenges faced in this field, future goals should focus on further simplifying the user steps by creating an automated fluidic delivery as well as incorporating multiple steps into a single device (e.g., nucleic acid extraction, amplification, and detection) in a simple and cost-effective manner [8]. The ability of preserving all reagents on board can eradicate the need for the laboratory

storage unit and the capability of multiplexing could improve the assay productivity [19]. Furthermore, studies should focus on developing advance and robust smartphone applications that allow onsite analysis while providing swift transfer and data storage to keep track of records. Given the fact that wireless network supply is limited in most resource-limited settings, the device should also be supported by asynchronous data transmission [97].

Novel nanomaterials have been widely explored recently in the field of sensing and food safety applications. The benefits of using carbon-based nanomaterials (e.g., graphene and GO), noble metal nanoparticles (e.g., AuNP and AgNP), and MIPs have also been frequently reported [56, 95], especially the capabilities of producing enormous signal enhancement and amplification with high selectivity. In fact, a better understanding of the fundamentals of nanomaterials in terms of chemical, structural, and physical properties would allow the engineering of these materials to produce more biocompatible substrates. The stability of these materials should also be tested to ensure its robustness and reliability for real-world applications.

5 Conclusion

Food analysis and authentication are the inseparable parts of food safety and security. Recent advances in point-of-care testing have shown great promise in food analysis. Novel approaches toward high-end, instrument-free detection of amplicons along with the development of PCR-on-paper or nitrocellulose, whole-cell detection on paper/nitrocellulose, tissue-on-paper, and all-in-one assays will be important contributions to the field. This area is likely to grow with many devices being developed and likely to reach the commercial market in the next few years. The global paper diagnostics market size was estimated at USD 5.69 billion in 2017. It is poised to witness a CAGR (compound annual growth rate) of 8.0% during the forecast period [98]. The increasing need for cost-effective, point-of-need testing in remote areas is leading to the rising popularity of point-of-care diagnostic methods. The involvement of smartphones and other devices can help amplifying and recording signals in a faster and simpler way.

Emerging bioactive paper technologies will be capable of offering robust, portable, easy-to-use, cost-effective, sensitive, and specific sample-to-answer devices with multiplexing capabilities. With the help of these devices, food contaminants and adulterants such as toxic chemicals, pathogens, pesticides, and infectious agents could be swiftly identified and quantified, mitigating foodborne illnesses and fraudulence.

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Chapter 13
Differential Scanning Calorimetry (DSC) for the Measurement of Food Thermal Characteristics and Its Relation to Composition and Structure

Preetinder Kaur, Manpreet Singh, and Preeti Birwal

Abstract Food, a complex biological system incorporating commonly three states of matter, is constituted mainly of carbohydrates, lipids, vitamins, and proteins apart from moisture. These food components are affected by various heat and mass transfer operations during processing, packaging, and storage that lead to changes in their structure and function, hence causing a physical, sensory, and nutritional influence on the food properties. This chapter discusses the Differential Scanning Calorimetry (DSC) measurement techniques adopted by researchers for thermal characterization of food and its components. It also elucidates how the changes in thermal properties of food components, i.e., carbohydrates, proteins, oils/fats, vitamins, and moisture can be used as a measure to describe the changes in food during processing, preservation, and storage.

Keywords DSC · Thermal characterization · Thermo-optical analysis · Thermal treatment · Thermophysical-properties · Food components · Heat capacity · Enthalpy · Crystallization temperature · Thermal conductivity · Fusion · Crystallization · Food authenticity

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1 Introduction

Food is a complex biological system containing a heterogeneous mixture of high- and low-molecular-weight components. Processing of foods is accomplished largely by various thermal and non-thermal techniques involving different biophysical processes [1]. The heat and mass transfer operations cause a variety of changes in food components, namely, carbohydrates, lipids, vitamins, and proteins, which further caused alteration in food structure and function, thus affecting the physicochemical, sensory, and nutritive characteristics of food.

Thermal treatment during food processing, i.e., heating, cooling, freezing, vaporization, and melting, causes conformational and phase or state transitions in food components. These bring changes in the thermophysical properties of food products during processing and/or storage. Thermal characterization of food systems generates data that can be related directly to the processing protocols. Many food materials exist in an amorphous state in disordered form, making it highly imperative for food researchers to understand the concept of amorphous state, and crystalline state, such as freezing, solids melting, solids decomposition glass transition, and related phenomena, including enthalpy relaxation [2]. Characterization of structure and properties of food materials before and after processing is important in gaining an insight into the impact of processing and storage conditions and serves as a prerequisite for optimization of food processes.

DSC, a widely used thermal analysis technique, enables us to determine a number of parameters connected with the physical or chemical processes in a condensed phase. A sample with a known mass is heated or cooled, and the changes in its heat capacity are tracked as changes in the heat flow. It measures the amount of heat required to raise the temperature of a sample with a specific heating rate by maintaining zero temperature difference between a sample and a reference (i.e., a blank cell). The particular temperature at which any kind of severe thermal or mass changes occur is noted, as this gives a complete idea regarding the nature of the nanoparticle, its melting range, purity, homogeneity [3]. It also enables measurement of heat capacities, temperatures of first- and second-order phase transitions, enthalpies of phase transitions, polymorphism in food and pharmaceuticals, liquid crystalline transitions, phase diagrams, thermoplastic polymer phase changes, glass temperatures, purity measurements, and kinetic studies. DSC basically helps to ascertain the phase transitions involved when a colloidal system is heated over a span of temperature. It helps to ascertain the crystalline nature of the fats or emulsions that affect their stability.

DSC has been used conventionally for measuring the thermophysical properties of foods [4]. It has attracted the interest of the scientific community because it requires only a small amount of sample for the analysis without any specific sample preparation, and is a repeatable and reliable method. In addition, DSC methodology needs a short time of experiments when compared to other techniques used for the same purpose. This chapter discusses the measurement techniques adopted by researchers for thermal characterization of food and its components and how the changes in thermal properties of food components (i.e., carbohydrates, proteins, oils/fats, vitamins, and moisture) can be used as a measure to describe the changes in food occurring during processing, preservation, and storage. The applications of DSC in food industry are also highlighted.

2 Principle of DSC

DSC functions to measure heat flow to maintain zero temperature difference between sample and reference. Sample needs heat if endothermic process is involved, while sample releases heat if exothermic process is involved. In this technology, the system measures the amount of heat a sample requires for raising the temperature with respect to a reference. The system tries to maintain the temperature of both sample and reference at the same level in an ongoing experiment. A number of temperatures are noted throughout the experiment [5]. When a sudden increase in temperature is observed, a complete idea of melting temperature, composition, heterogeneity, and homogeneity is known [3]. DSC, in short, estimates the quantity of energy either released or absorbed by the sample upon heating and cooling. DSC is suitable only for those materials that do not contain F, Cl, Br, and I, as these can corrode the equipment [6].

In DSC, a sample is placed in the sample pan and the reference is kept blank; both are subjected to the heating scan (i.e., heating rate), and the analysis is performed based on the temperature or time and enthalpy (Fig. 13.1). The sample holder is basically an aluminium pan, and the reference is aluminium pan without the sample. An aluminium sample is preferred instead of keeping reference empty, as this leads to very precise control and accurate measurement of heat capacity and enthalpy. The heat is produced through the furnace that is placed in a cell, and the temperature profile is analyzed through the sensors that are positioned in both the sample holder and reference pan. Basically, the temperature difference of sample holder and reference along with the absolute temperature of both is recorded periodically. With time, the temperature of the sample keeps on increasing in the linear manner, and this gives the heat capacity of the sample. With a linear increase in temperature, the sample undergoes physical transformation and required heat might

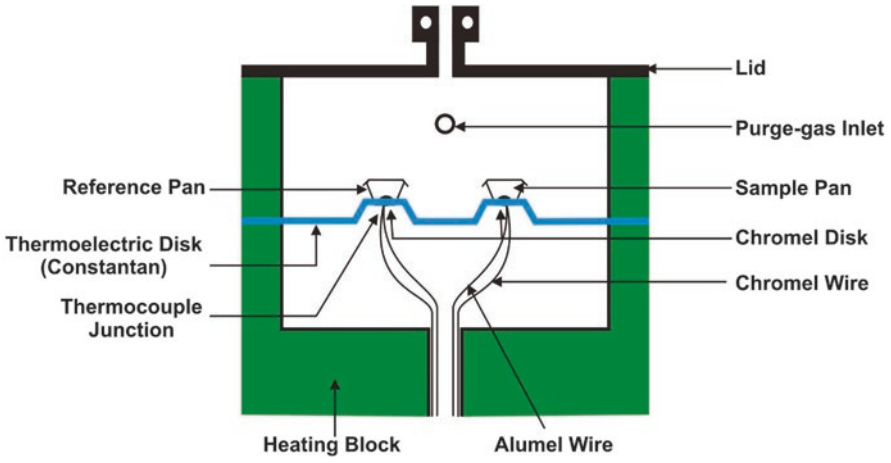


Fig. 13.1 Schematic diagram of DSC

be less or more depends upon the type of transformation, which could be exothermic, endothermic, or only specific heat change. DSC measures the heat of exothermic or endothermic reaction based on the transitional changes of a sample.

3 DSC Curve

The output of a DSC is a curve where the y-axis depicts heat flow, which is generally measured in mW, and x-axis is the temperature (°C) or time (s). The curve shows different peaks and troughs, peaks resulting from an endothermic transition and trough due to an exothermic transition. This curve is used to calculate the enthalpy of the sample with the following formula:

$$\Delta H = \mathrm{c}\, A \tag{13.1}$$

where, ΔH is the enthalpy of sample heat transition (J/g), c is the calorimetric constant, and A is the area of the curve (mJ). This calorimetric constant (c) is different for different types of DSC system. The heat flow in the sample can be calculated using following formula:

$$\left(\mathrm{d}T / \mathrm{d}t\right)\mathrm{p} = \mathrm{d}H / \mathrm{d}t \tag{13.2}$$

where $\mathrm{d}T/\mathrm{d}t$ is the sample heating rate (°C/min), $\mathrm{d}H/\mathrm{d}t$ is the heat flow (W), and p is a constant. Heat flow can be calculated using the following formula:

$$\Delta\left(\mathrm{d}H / \mathrm{d}t\right) = \left(\mathrm{d}H / \mathrm{d}t\right)_{\text{sample}} - \left(\mathrm{d}H / \mathrm{d}t\right)_{\text{reference}} \tag{13.3}$$

If there is no exothermic or endothermic process, the reference (usually an empty pan) generally is heated faster than the sample side during heating of the DSC measuring cell (i.e., reference temperature (T_{R}, green) increases a bit faster than the sample temperature (T_{P}, red)) (Fig. 13.2). The two curves exhibit parallel behavior during heating at a constant heating rate - until a reaction occurs in the sample. In the case shown, the sample starts to melt at t_1. The temperature of the sample does not change during melting; the temperature of the reference side, however, remains unaffected and continues exhibiting a linear increase. When melting is completed, the sample temperature also begins to increase again and beginning with the point in time t_2, again exhibits a linear increase.

Parameters measured on the graphs are shown in Fig. 13.3. The glass transition temperature (T_g) is an additional significant attribute of noncrystalline and semicrystalline materials; however, T_g is an especially critical property of many polymers. Generally, at a temperature below T_g, amorphous and semicrystalline polymers are hard and weak. This is because at this temperature the polymer chains are in coiled position and in tangled position. Above T_g, the polymers become more ductile and softer because at this temperature the chain of polymers is able to rotate

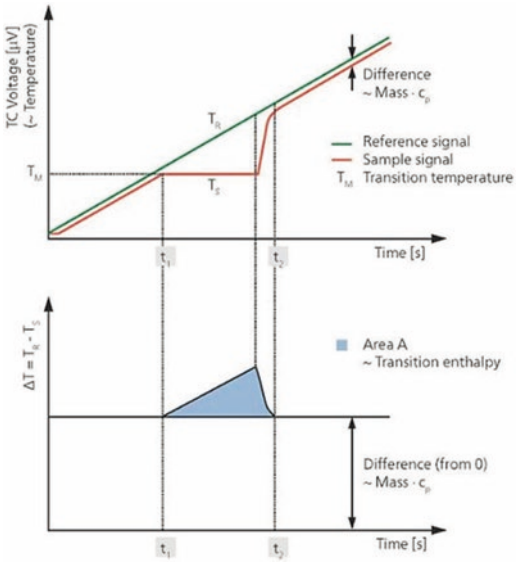


Fig. 13.2 Illustration of an endothermic melting process during specific heat measurement of sample wrt reference

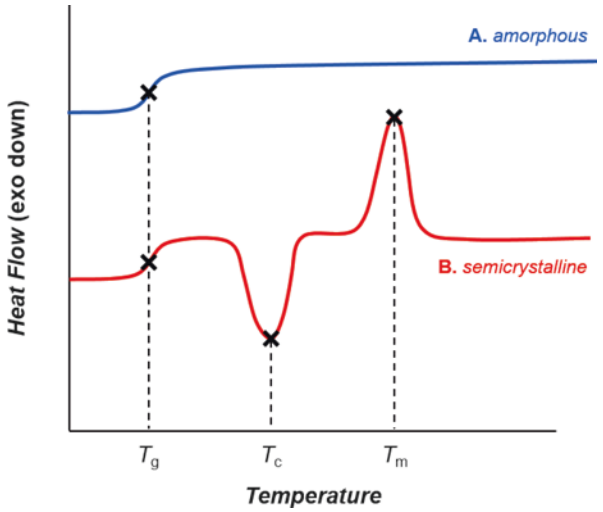


Fig. 13.3 Glass transition temperature (T_g), the crystallization temperature (T_c), the melting temperature (T_m), and the curing temperature

more easily. Glass transition affects the material's properties, such as structure, bonding, and molecular weight. These bonds require energy to break, and due to this, the glass transition appears on DSC curve as endothermic process. The glass transition temperature can be found as a decrease in baseline heat flow in DSC curve, and generally T_g is the onset, inflection, and end points on the curve.

The crystallization temperature (T_c) is another significant change that happens in crystalline materials. The arbitrary chain arrangement of polymer is not lost at crystallization temperature, whereas intermolecular bond and the polymer molecules become more ordered. The formation of bonds during crystallization is an exothermic process, so an increase in heat flow (a peak on the DSC curve) accompanies the crystallization process. Generally, the T_c is found as the onset point of the crystallization curve. However, many amorphous polymers never undergo crystallization.

When material changes its phase from a solid to liquid, it melts. During this time, the intermolecular bonds absorbed energy, and it started to break and the process is endothermic, which appears as a large temporary decrease in heat flow. When material melting is complete, the DSC plot of heat flow returns to its original baseline value. Generally, melting temperature is measured at onset point of the melting curve. Crosslinking happens when individual chains form strong bonds to other releasing energy making the process exothermic.

4 Characteristic Terms of the DSC Curve

A few characteristic terms are necessary to understand the DSC curve shown in Fig. 13.4. The first one is zero line, which is measured using empty instrument where even crucible is not present. Any standard deviation can be added as a variation in the instrument. Also, the (interpolated) baseline connects all the peaks. Peak is recorded on sudden disturbance in the steady state either by exothermic or endothermic reactions. Endothermic curves are upward, i.e., positive direction. The first

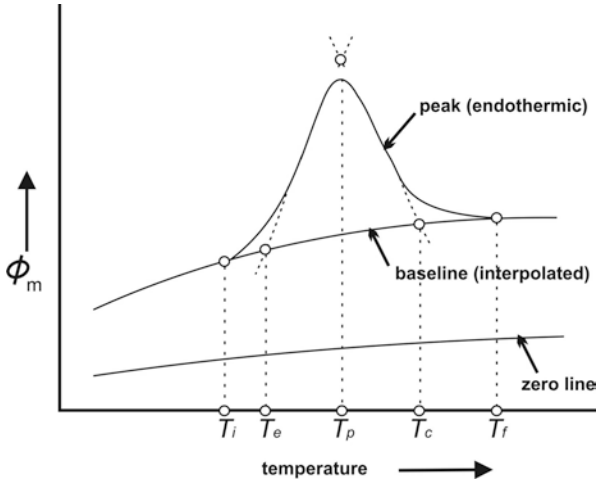


Fig. 13.4 A characteristic DSC Curve illustrating different temperature representations

deviation is reported at T_f, maximum/minimum by T_p, and the point where the curve merges the baseline is represented by T_f [7].

5 Parameters Influencing DSC Curve

The parameters affecting the DSC curve are (i) heating rate, flow velocity, and surrounding surface temperature; (ii) crucible unequal masses and material, and their emissivity; (iii) properties of reference material, i.e., heat capacity and glass transition; (iv) thermal conductivity of the sample; (v) mass and heat capacity of the sample; (vi) structure and composition of the sample; (vii) thermal resistance between sample and temperature sensor; (viii) location of the sample in the crucible; and (ix) sample purity.

6 Types of DSC

6.1 Heat Flux DSC

This type of DSC measures the temperature or heat flux change between a test material and a reference material and calculates the amount of heat flow with respect to calibrated data. In this measurement, the temperature of the material remains unchanged, whereas the temperature of the reference material increases linearly upon melting. The samples remain unchanged until it reaches melting temperature. The sensor areas can be integrated with a thermocouple. This process is less sensitive to small changes and gives less accurate results.

6.2 High-Pressure DSC (HP-DSC)

High-pressure DSC is more precise and gives more accurate result than heat flux DSC, especially under the following four situations: (i) if a sample is to be tested for oxidative stability under atmospheric conditions, it takes more time causing sample deterioration during measurement. High pressure reduces measurement time with improved results; (ii) if any particular material undergoes temperature changes and produces methanol or water as a by-product, foams are created inside the measuring chamber that damage the probe and sensor of DSC. High pressure removes the foams for proper measurement; (iii) heat flux DSC creates much fluctuation that can disturb the final results. This can be decreased by applying pressure so that the samples are measured under a controlled environment; and (iv) some samples are sensitive to pressure. So, the HP-DSC helps in measuring the heat flow with a change in pressure.

6.3 Fast Scan DSC

As the name indicates, its heating rate is very fast and accurate which increases its sensitivity. The heating rate in this type of DSC varies between 100 and 300 °C/min The fast increase in temperature allows the enhancement of weak transition of the product. It is also possible to analyze the low levels of amorphous material in this type of DSC.

6.4 Ultraviolet DSC (UV-DSC)

In this DSC, samples are exposed to UV light. Different types of UV emitters like light-emitting diodes (LEDs) or mercury vapor lamps can be used. These UV light sources can perform over various ranges of intensities and frequencies. This DSC also provides the kinetic properties and mechanical strength of a sample. This DSC is suitable for material that requires a UV-initiated curing process, e.g., bone cement, dental resins, coatings, orthopedic hydrogels, paints, adhesives, or for materials that decompose under UV radiation, e.g., drugs, food products, and packaging materials.

6.5 Modulated Temperature DSC (MT-DSC)

MT-DSC is used to separate the kinetic and thermodynamic data when a nonlinear heating or cooling rate is applied to the sample. This can be achieved by applying a series of heating and cooling rate followed by isothermal hold. An equilibrated heat capacity curve is obtained which shows the thermodynamic response and isothermal kinetic baseline. The kinetic noise from the sample is also removed in this type of DSC.

6.6 DSC in Conjunction with Other Techniques

Differential Thermal Analysis (DTA): Differential thermal analysis is a technique in which temperature difference is recorded with respect to reference material, against temperature or time, as the two samples are exposed to an identical temperature in an environment of heating or cooling at a controlled rate. This instrument consists of two measurement cells situated in a temperature-controlled environment with temperature varied at a controlled rate. The samples are placed in the sample cell with reference material having known thermal properties (often distilled water) kept inside the reference cell. These cells are heated and cooled simultaneously at a controlled rate. The small difference in temperature between these two samples is

measured using thermocouples placed within the cells. The temperature of the external environment is measured and varied in a controlled manner. The output of the measurement is plotted against the temperature difference. A temperature of the sample cell greater than the reference cell suggests that the sample has experienced an exothermic reaction as indicated by the release of heat. A lower temperature of the sample cell with the reference cell demonstrates that the sample experienced an endothermic reaction indicating absorption of heat. The nature of peaks in the thermogram indicates the type of transition, and the area under the peak gives an idea about the sample enthalpy change per unit amount of the material.

DSC with Infra-Red Spectroscopy (DSC-IR): DSC-IR is used to perceive and quantify different types of adulterants in many products like food and pharmaceuticals. In this equipment, first, the emission spectrum of a broadband lamp is analyzed and then the same lamp shining through the sample is analyzed. The first one is termed as background spectrum, whereas the second one is termed as sample spectrum and its ratio is directly related to the absorption spectrum of the sample. The normal equipment consists of (i) source: infrared radiation is emitted through the source, generally a black body. Aperture controls the amount of energy to which a sample is exposed, (ii) interferometer: spectral encoding happens in the interferometer, and the resulting signal is termed as interferogram; (iii) sample: beam then enters the sample section and is transmitted through the surface of the specimen. The amount of energy stored by the sample can be detected with the help of detector; (iv) detector: it measures the amount of energy stored by the sample and is particularly designed to quantity the special interferogram signal; and (v) computer: this helps to digitalize the signal where the Fourier transformation takes place, and it presents the final data to the users for further interpretation.

DSC with Raman Spectroscopy (DSC-RS): Laser Raman spectroscopy is based on the principle of Raman scattering of monochromatic light, which is generated in infrared, ultraviolet, or visible light. When there are excitations in the system the laser light interacts with those phonons, which results in the energy shifting either up or down. This up and down shift in the energy predicts the vibrational modes of the system. The Raman scattering occurs due to a change in the polarization of a molecule. In Raman spectroscopy, the light is illuminated by the sample subjected to a laser beam. This illumination is collected with the lens and sent to the monochromator. The common problem is the separation of the intense Rayleigh scattered light from the weak inelastic scattered light. However, the modern instrument is very precise and has edge filters for rejection purpose [8].

DSC with Fourier Transform Infra-Red Spectroscopy (DSC-FTIR): DSC-FTIR procedure gives spectroscopic and thermodynamic data about a solid or fluid specimen experiencing thermal adjustment. DSC measures the exothermic and endothermic reactions of the specimen, while the FTIR analysis observes their arrangements in terms of chemical and physical composition. An infrared spectrum denotes a fingerprint of a sample with absorption peaks, which resemble the frequencies of vibrations among the bonds of the atoms making up the material. Since each dissimilar material is an exclusive combination of atoms, two compounds can produce the same infrared spectrum. Consequently, infrared spectroscopy is highly suitable

for subjective examination of any particular material. The size of the peak in the range estimates the amount of material present. With present-day programming calculations, infrared is a powerful tool for the quantitative study of any material [9].

DSC Equipped with Microscopy: In this equipment, a microscope is positioned directly above the sample crucible. Images of the sample are captured during heating or cooling in the DSC using a CCD camera mounted on the microscope. The images are useful in understanding the transformations taking place during the analysis and enable appropriate interpretation of the effects observed on the DSC curve.

DSC Equipped with Chemiluminescence: This equipment combines a DSC with an optical attachment for recording chemiluminescence emitted by a sample during analysis in the DSC through a highly sensitive CCD camera. Chemiluminescence mainly investigates the oxidation behavior of polymers and other materials.

DSC Equipped with Photo-calorimetry: In this type of DSC technique, it is mainly used to study light-induced curing reactions, the sample is exposed to light of a particular wavelength range and intensity for a defined time, and the heat flow from the sample is recorded.

DSC with Thermo-optical Analysis using Hot-stage Microscopy (DSC-TOA): TOA is a method for the study of thermally induced phase transitions. Thermo-optical analysis (TOA) with hot-stage systems is a widely used technique that can be combined with DSC. TOA permits visual observation of the physical changes produced in a sample as a function of temperature or time. Combined with DSC, heat flow can be simultaneously recorded for a more comprehensive analysis.

7 Thermal Characteristics of Food

Thermophysical properties of foods have immense importance with regard to the design of processes and equipment to carry out innumerable food engineering operations in food industries. These properties are required to perform the various heat transfer calculations that are not only a part of the design of equipment for storage, refrigeration, and processing, estimation of the process time and energy for cooling, heating, drying, or freezing of foods in solid, semisolid, or liquid form. It is also needed for mathematical modeling and simulation of heat and moisture transfer.

7.1 Specific Heat (C_p)

Specific heat is the amount of heat required to raise the temperature of a unit mass of a material by 1 degree and the unit is kJ/kg K. The DSC method measures very small thermal effects occurring during thermal processes. When the sample is analyzed at a particular rate of temperature increase over a selected time interval, a thermogram is produced. The area under the curve is a measure of the heat energy absorbed or released by the sample during the heating or cooling process. Several

factors especially the structural state of molecules, macromolecular transitions, and physical environment can affect the measurement of specific heat while using DSC, thereby influencing the position, sharpness, and shape of transition(s) in DSC scans [10]. The native stable protein structure is disrupted when heat is applied, leading to changes in C_p.

The composition of food, i.e., moisture content and fat content, has also been reported to have a considerable effect on specific heat of various foods, namely, cereals (Emami et al. 2007), milk [11], coconut milk [12], tofu [13], cheese [14], meat products [15], fish [16], oysters [17], fruits, and vegetables [18–22]. The water activity of foods at temperatures above zero is a much-researched topic because of the significant effect of the presence of water and its availability on the specific heat of foods. The stability of foods in unfrozen state is more closely associated with water activity than with total moisture content. Models for predicting specific heat of different categories of food have been developed over the years by various researchers and can be helpful in reducing the experimentation time (Table 13.1).

7.2 Enthalpy, H

Enthalpy consists of sensible energy when a food material is above the freezing point, but below the freezing point, enthalpy consists of both sensible and latent energy. The enthalpy of phase transition can be determined using DSC and DTA to detect endothermal and exothermal changes taking place either as a function of temperature or time under isothermal conditions. The DSC thermogram shows the heat flow versus temperature, and the area under the curve gives an estimate of the energy used in phase transition. Peaks are obtained in the thermograms for first-order phase transitions and a step change in heat flow occurs during second-order transitions. The transition temperatures can be obtained from first-order transition thermograms and the latent heat of the transition through peak integration. As the changes in the specific heat are low compared to heat of the phase transition during the first-order transition, i.e., melting [7], the enthalpy of phase transition ΔH can be approximated from the area under the DSC peak [23]. In this case, the absolute value of ΔH is determined using reference substances. Thermograms displaying the second-order transitions can help in the derivation of transition temperatures and changes in heat capacity as shown for glass transition.

7.3 Thermal Conductivity (K) and Diffusivity (α)

Thermal conductivity is an intrinsic property that measures the ability of a food to conduct heat, while thermal diffusivity is defined as the ability to conduct thermal energy relative to its ability to store energy. The thermal properties, geometry, and thermal processing condition are used as the major parameters for calculating heat transfer in a food. The equation connecting the primary thermal properties is:

Table 13.1 Models for prediction of specific heat of various foods considering the effect of different factors

Food	Factors	C_p and models	References
Cereals			
Chickpea flour, isolated starch, and isolated protein	Different temperatures and bulk densities	• C_p showed a linear relationship with the temperature and moisture content • Models were developed to predict the thermal properties of samples	Emami et al. (2007)
Corn	Moisture Content: 8–12% Temperature: 323–353 K	• C_p: 1.839–2.976 kJ/kg·K • Effect of temperature and moisture content on C_p was described by second-order polynomial relationships	Kang et al. (2012)
Milk and milk products			
Milk	Fat content: 0.1–35% Temperature: 274–332 K	• Empirical equation was developed to determine C_p as a function of fat mass content and temperature	Hu et al. (2009)
Coconut milk	Fat content: 20–35% of Temperature: 333–353 K	• C*p*: 3.277–3.711 J/g·K • Empirical model for dependence of specific heat on fat content and temperature	[12]
Cheese		• Composition of the cheeses was analyzed • Equations from the literature were used to calculate the heat capacity • Differences between calculated and measured values increased with a decrease in moisture content of the cheese	[14]
Tofu	Moisture Content: 30–70% Temperature: 283–378 K	• Empirical models were developed as a function of moisture content and temperature of the tofu • Perpendicular model and the thermal conductivity data with DSC showed good agreement	[13]
Meat and fish			
Meat products	Moisture Content: 30–75% Temperature: 233–313 K	• Correlated C_p as a function of moisture content • C_p increased significantly with increasing moisture content than the temperature	[15]
Raw skipjack tuna		• C_p of loin meat: 3.536 kJ/kg·K • C_p red meat: 3.505 kJ/kg·K • C_p viscera: 2.263 kJ/kg·K • Significant differences in C_p values between the backbone and loin meat, backbone and red meat, and backbone and viscera	[16]

(continued)

Table 13.1 (continued)

Food	Factors	C_p and models	References
Shucked oysters	Temperature 283–323 K	• C_p increased from 3.795 to 4.047 kJ/kg·K when temperature was increased from 283 to 323 K	[17]
Fruits and vegetables			
Apples	W = 0–0.9 Temperature: 283–363 K	• The excess contribution of bound water to the specific heat was defined in terms of C_p of water plasticized apple and C_p of dry apple	[19]
Cassava root, yam tuber and plantain fruit	Moisture Content: 10–68% Temperature: 309–324 K	• Heat capacity varied from 1.64 to 3.26 kJ/kg·K	[20]
Pomegranate (*Punica granatum* L.) juice	SSC: 12, 40 and 65 °Brix Temperature: 298–243 K	• C_p decreased linearly with increasing soluble solid content and decreasing temperature	[21]
Fresh and osmotically dehydrated kiwifruit	Temperature: 233–313 K	• C_p of kiwifruit decreased with increasing osmotic dehydration at a fixed temperature	[22]
Alginate-restructured sweet potato puree		• C_p increase during freezing (or melting): 1.9 to 90 kJ/kg K • C_p of restructured and non-restructured sweet potato puree: 3.695 and 3.404 kJ/kg·K after freezing	[18]

$$\propto = \frac{k}{\rho C_p} \tag{13.4}$$

where α is the thermal diffusivity (m^2/s); k is the thermal conductivity (W/m^2 K); ρ is the density (kg/m^3); and C_p is the specific heat capacity (kJ/kg K). Buhri and Singh [24] developed an attachment to be used with a conventional DSC for the measurement of thermal conductivity. Based on a steady-state method, the thermal conductivity, K, could be calculated using the Fourier's steady-state solution of heat conduction equation:

$$k = L\frac{\Delta Q}{A}\left(\Delta T_2 - \Delta T_1\right) \tag{13.5}$$

where L is the sample length (m); ΔQ is the difference of energy required to maintain pan temperature (J); A is the sample area perpendicular to heat flow (m^2); ΔT_2 is the final temperature difference between DSC heating pan and sample (K); and ΔT_1 is the initial temperature difference between DSC heating pan and sample (K). The developed DSC method resulted in an average coefficient of variation of 2.88%. The authors suggested that the use of the DSC method was appropriate for small-size homogeneous samples.

7.4 Fusion and Crystallization

Glass transition is a second-order phase transition occurring in food products having high moisture content at low and ultralow temperatures. The material exists in solid phase, but the matter is not structured as in the case of crystalline state. Glass transition occurs at conditions when viscosity is high (more than 10^{12} Pa s) [25]. This state is characterized by low molecular mobility enabling the product to be stable for long durations at these conditions. The mechanical structure is characterized by brittleness and high Young's modulus.

The state diagram is commonly used to identify different state boundaries in a material. The DSC method is used to detect the change in heat capacity occurring over the transition temperature range. Rahman [26] developed the state diagram as shown in Fig. 13.5 and hypothesized 13 microregions having the highest to the lowest stability based on the location from the glass and BET–monolayer lines. For example, region 1 (relatively nonreacting zone, below the BET–monolayer line and glass line) is the most stable and region 13 (highly reacting zone, far from BET–monolayer line and glass line) is the least stable. The stability decreased as the zone number increased. The most unstable microregion is the region 13 since it is the most reactive mobile region.

Yakimets et al. (2005) described the process of hydration of gelatin in three stages (i) water bound by high-energy sorption centers, (ii) structural water, and (iii) polymolecular layer water. Upon addition to gelatin, the water molecules form hydrogen bonds with hydrophilic groups inside the helical structure of gelatine gel. An outward expansion of structure took place resulting in increased pore size. An increase in food moisture content increased the amount of freezing water. Below a moisture content of 40% (w/w), all moisture existed as nonfreezing or bound water but above that, it was constituted of both bound and free water. The fusion enthalpy of the DSC curve gives an idea about the state of the water as well as the amount of free and bound water in gelatine gels [27].

8 Food Compositional Analysis Using DSC

8.1 Carbohydrates

Carbohydrates include low-molecular-weight sugars, such as glucose, fructose, and sucrose, and also amylopectin and cellulose. The amylopectin fraction of starch is made up essentially of glucose. Whereas, some complex carbohydrates may be composed of numerous sugars and noncarbohydrates, like proteins, lipids, and phenolics. Thermal treatment during any process changes the structure of food, e.g., bread/flatbread is different from the dough, and baked/boiled/fried potato is different from raw potato.

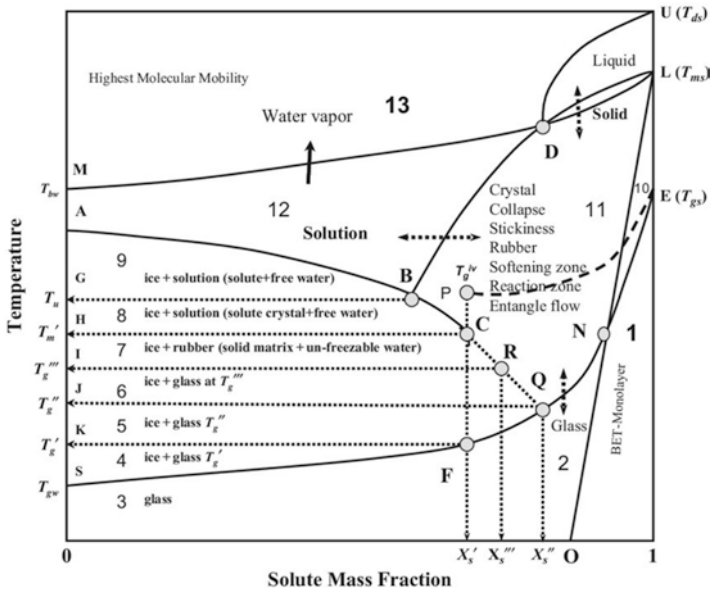


Fig. 13.5 State diagram showing different regions and state of foods T_{ds}: solids-decomposition temperature, T_{ms}: solids melting temperature, T_{gs}: solids–glass transition temperature, T_g^{iv}: end of solids-plasticization temperature, T_{gw}: glass transition of water, Tu (solute crystallization temperature during freeze-concentration), T_m' (maximal-freeze-concentration condition, i.e. end point of freezing), T_g'' (glass transition of the solids matrix in the frozen sample as determined by Differential Scanning Calorimetry (DSC)), T_g'' (intersection of the freezing curve to the glass line by maintaining the similar curvature of the freezing curve), and T_g' (glass transition at maximal-freeze-concentration, i.e. at the end point of freezing), T_{bw}: boiling temperature of water. (Source: Rahman [26])

8.2 Starch Gelatinization

Starch, a major structure-forming food hydrocolloid, comprises essentially amylose and amylopectin along with small amounts of noncarbohydrate constituents, such as lipids, proteins, and phosphorus. When starch is heated with excess water, molecules in starch granules experience a transition from ordered to disordered, which can be observed using a DSC. Owing to the hydrogen bond arrangement of amylopectin and amylose, penetration of water into intact starch granules is difficult, but swelling of granules and gelatinization occurs when the water is heated. The temperature when the granule undergoes irreversible changes can be recorded through the DSC. The DSC thermograms can show different endothermic peaks for native or modified starch used in various food products. Under the influence of heat and moisture, starch undergoes physicochemical transformation referred to as gelatinization. The use of the term *gelatinization* should be limited to the loss of *birefringence*. Subsequent to the loss of birefringence, further heating of starch to a higher temperature in the presence of water brings diverse changes that can be described as *solubilization*.

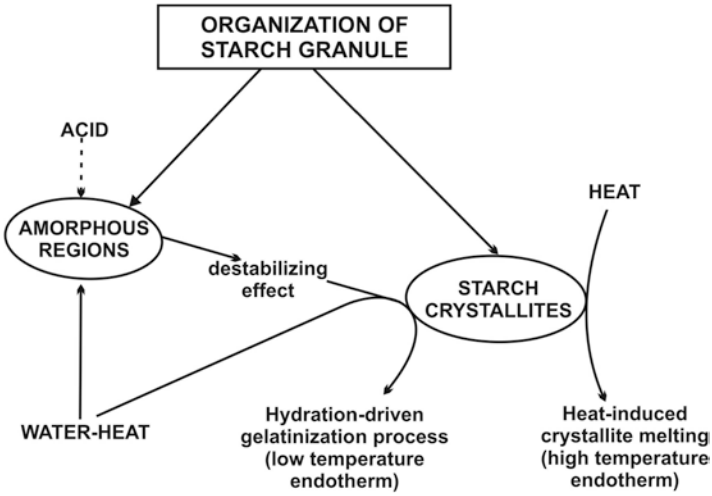


Fig. 13.6 An illustration of phase transition mechanisms of granular starch

Cooked foods are usually a mixture of solubilized and insoluble starch, as most foods are heated with limited water to less than 100 °C. An illustration of the phase transition mechanisms of granular starch is shown in Fig. 13.6. Swelling and water absorption are limited due to the crystalline nature of the starch, and loss of crystallinity due to gelatinization allows the granules to swell freely enabling them to absorb more water. In this process, the starch becomes smooth or cooked and loses its raw starchy taste. Beyond its gelatinization temperature, starch forms a paste that becomes a gel on cooling as a result of the hydrogen bonding between water and soluble starch molecules. Freezing and thawing can cause expression of water from gel known as *syneresis* over time because of hydrogen bonding of starch with concomitant elimination of water. The starch retrogradation process is represented by the appearance of cloudiness as a consequence of recrystallization of the starch when starch gels are stored, especially at low temperatures.

DSC has been used for the study of starch gelatinization at different temperatures and moisture levels with and without additives (sugar, salt, honey, glycerol, etc.). The gelatinization temperature is influenced by the source of starch as well as the type and number of molecules that are dissolved in the available water. Dissolution of small molecules in water or addition of sugars lowers the water activity thereby increasing the gelatinization temperature. The shape of the DSC curves (Fig. 13.7) and the content of bound water in corn starch suspension with different water contents were significantly different (Grabovska and Mykhailyk 2008). The starch thermal treatment at different temperatures from 293 to 343 K influenced the bound water content in different starch–water systems as shown by clear changes in the melting peak when treatment temperature was close to gelatinization temperature (Grabovska et al. 2011). Below the starch gelatinization temperature, bound water

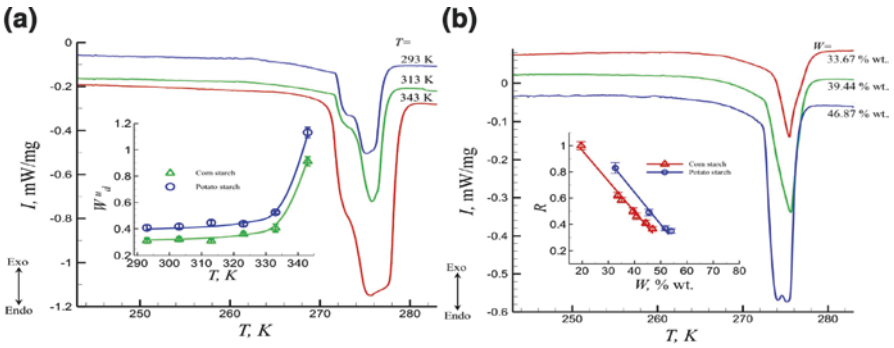


Fig. 13.7 DSC thermograms for (**a**) melting of free water in potato starch suspensions treated thermally for 15 min at different temperatures (293–343 K) Inset figure shows unfreezable moisture contents (DB) versus temperature of thermal treatment for potato and corn starches Source: Grabvoska et al. 2011; (**b**) heating of corn starch suspensions with different moisture content. Inset figure shows fraction of unfreezable water versus moisture content in corn and potato starch suspensions. (Source: Grabvoska and Mykhailyk 2008)

linearly increased with an increase in temperature, but beyond 330 K, a sharp increase was observed.

Figure 13.8 shows a large gelatinization endotherm for both waxy and normal wheat starch at about 70°C, but the peak temperatures of gelatinization and gelatinization enthalpy for waxy wheat starch were found to be higher than those of normal wheat starch. Higher gelatinization enthalpy for waxy wheat starch suggests its higher crystallinity in comparison to normal wheat starch. Phase transition of wheat starch during gelatinization is affected by the heterogeneity and complexity of starch granular structure. Two endotherms were detected for normal wheat starch, first endothermic transition depicting the melting of the crystalline lamellae and the second one representing the melting of incompletely solvated starch crystallites or the dissociation of the amylose–lipid complexes. The absence of second transition indicates the low amylose content in waxy wheat starch.

An increase in water content made the gelatinization transitions more noticeable and symmetrical [28]; however, not much variation was observed in the thermal transition temperatures (T_c) of rice starch when compared to maize starch. The addition of electrolytes, concentration, and type of anions and cations have also been known to affect starch gelatinization. Relatively low concentrations of solutes, i.e., sucrose or NaCl in 40% wheat starch suspension in water, increased the gelatinization temperature of the starch as observed by DSC [29]. Figure 13.9 shows that application of high hydrostatic pressure (HHP) affected the glassy and crystalline transitions of wheat starch in aqueous solutions with sodium chloride concentrations ranging from 0 to near saturation using DSC [30].

Starch retrogradation, first discovered in 1852 by Boussingault (1852), occurs upon aging of starch and involves reassociation and recrystallization of the polysaccharide chains. This exothermic process also accounts for the firming, shrinkage, and syneresis of aged starch gels [31]. Amylose retrogradation is quite faster than

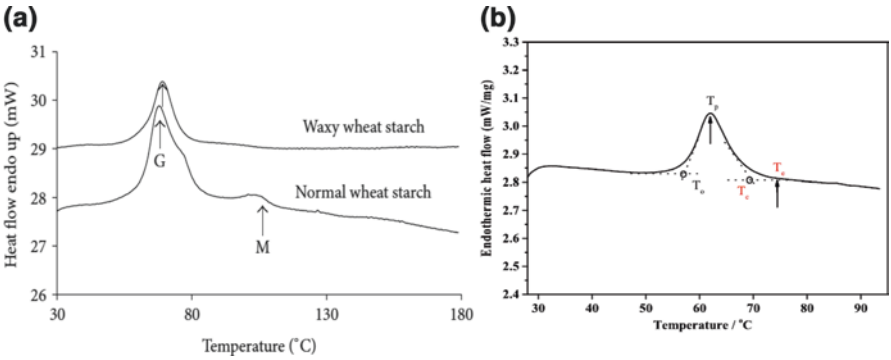


Fig. 13.8 (**a**) DSC gelatinization endotherms for waxy and normal wheat starches with excess water (75%) (Source: Ref. [73]) (**b**) DSC thermogram of wheat starch at a water: starch ratio of 3.0:1 showing *Tc* and *Te*. (Source: Wang et al. [28])

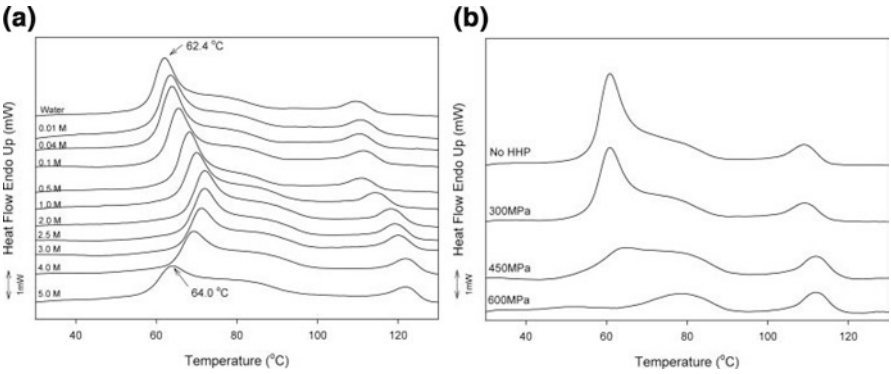


Fig. 13.9 DSC thermograms for wheat starch (**a**) suspended in solutions (1:1, w/w) of various NaCl concentrations at atmospheric pressure (**b**) after HHP treatment in water (1:1, w/w) for 15 min at 257 °C (**c**) after HHP treatment in 2M NaCl (1:1, w/w) for 15 min at 257 °C (**d**) after HHP treatment in 5M NaCl (1:1, w/w) for 15 min at 257 °C. (Source: Kweon et al. [30])

amylopectin retrogradation. The amylose fraction of the starch retrogrades very rapidly in dilute systems [32], but in food systems with limited water, the amylopectin retrogradation seems to occur slowly. This phenomenon of bread staling as well as firming visualized in many starchy foods can be attributed to starch retrogradation.

The major factors affecting starch retrogradation are source, temperature, and moisture content. Faster retrogradation of potato starch, when compared to waxy maize, is due to the presence of amylose in potato starch that could form an ordered matrix and act as seed nuclei for the amylopectin (van Soest et al. 1994). Several researchers (Orford et al. 1989; Kalichevsky and Blanshard 1993; Farhat et al. 2000b, Levine and Slade 1988) showed that water can act as an effective plasticizer for starchy materials, lowering T_g and T_m of the system to which it is added. Rate of retrogradation increases when the moisture content of the system increases.

8.3 Proteins

Protein, with a highly complex structure, is comprised of 20 amino acids as building blocks regardless of their origin, e.g., animal, plant, or microbial (Well 2004). The proteins usually exist in a certain preferred folded conformation, and its native conformation is achieved through optimization of various inter- and intramolecular interactions that represents a thermodynamic equilibrium state signified by the least free energy of the molecule.

Proteins are essential food ingredients derived from different plant and animal protein sources, such as milk, eggs, fish, soy, and wheat and used to improve the flavor, textural, and organoleptic properties of the processed foods. Many proteins, such as soy protein (Campbell et al. 2009; Chen et al. 2012a, 2012b), whey protein (Briviba et al. 2016), lentil protein (Avramenko et al. 2013), mushroom protein (Jalali et al. 2015), and hazelnut meal protein (Gul et al. 2018), are multifunctional ingredients important for their functional attributes, like solubility, water and oil holding capacities, gelation, emulsification, and foaming. Processing conditions, namely, temperature, pH, ionic strength, and water content influence the conformational state (i.e. degree of denaturation) of protein isolates, thereby affecting the functional properties by altering the structure of the protein. Therefore, many scientists (Kinsella 1982; Damodaran 1994; Aluko and Yada 1995, Srinivasan 2005) have extensively studied the structure–function–process relationships of food proteins and modification of protein properties through conventional processing or biological treatments [33].

Protein denaturation is the change in the structural configuration of proteins and is affected to a large extent by heat, salt, pH, and mechanical shear, etc. A higher denaturation temperature (T_d) usually suggests higher thermal stability for a globular protein, and hence, lower disruption of hydrogen bonds is responsible for maintaining tertiary and quaternary structures of proteins, particularly the tertiary ones indicating that the tertiary structure of polypeptides is more compact.

In the DSC thermograms, denaturation is indicated to start from the point where the curve deviates from the baseline. The peak temperature is defined by extrapolated slope and the onset temperature of denaturation by the baseline due to difficulty in identification of the exact temperature of denaturation. Proteins show a wide range of thermal transition temperatures, and the peak temperature is commonly regarded as the temperature of denaturation [34]. The DSC thermograms of the extracted protein isolate samples showed the endothermic peaks in the range of 75.1–89.1 °C, which is the characteristic denaturation temperature of the isolates (Fig. 13.10).

Pressure-induced denaturation is a complex phenomenon that depends primarily upon the structure of protein and range of pressure application along with other external factors temperature, pH, and solvent composition like the presence of sugars, salts, and other additives [35]. The three-dimensional native structure of proteins results from the compact folding of the polypeptide chain that can be disrupted by pressure application. In general, reversible effects are observed below 1–2 kbar

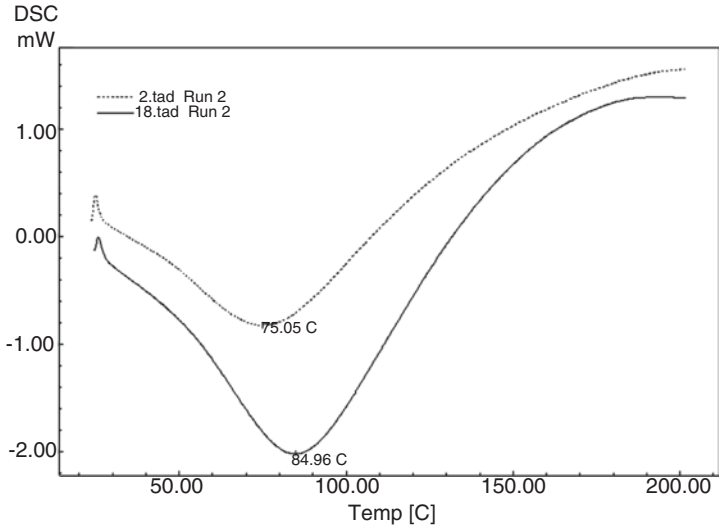


Fig. 13.10 DSC thermograms showing the effect of temperature and time of defatting and amount of activated carbon on the temperature and enthalpy of denaturation for two protein isolate samples (Source: Mehryar et al. [34])

whereas nonreversible effects occur above 2 kbar. Dissociation of oligomers at pressures <1.5–2 kbar can be followed by subunit aggregation or by precipitation. At pressures higher than 2 kbar, significant tertiary structure changes, namely, unfolding of proteins and reassociation of subunits from dissociated oligomers are observed. Reversible unfolding of small proteins occurs at higher pressures (4 to 8 kbar). Nonreversible denaturation is observed when changes in secondary structure take place at very high pressures above 7 kbar. A typical example of such hysteresis is shown in Fig. 13.11. High pressure can modify the structure and function of meat proteins (Cheftel and Culioli 1997; Jimenez Colmenero, 2002), but the mechanism of protein denaturation may differ based on the pressure/temperature combination (Messens et al. 1997).

Heat denaturation of 10% soy proteins in distilled water and 0.2 M salt solutions was studied, and greater thermostability was observed in the isoelectric region (pH 4–5) confirming the stabilizing role of salt in maintaining the tertiary and quaternary structure of soy proteins at any given pH value outside the isoelectric region (Fig. 13.12). Salt has little influence in the maintenance of conformational stability of whey proteins at low concentrations, but it significantly affected the aggregation of unfolded protein molecules at higher concentrations observed as an exothermic peak due to the formation of a new intermolecular bond. DSC thermograms of 10% whey protein dispersions with 0.2 M NaCl showed only a slight increase in the denaturation temperatures when compared to those without salt (Fig. 13.13a), but when NaCl concentration reached at 100 mM NaCl, an exothermic peak (Fig. 13.13b) was detected. Sucrose addition increases protein denaturation temperature, gelation temperature, and the final rigidity of the cooled gels [36]. The use of stabilizing

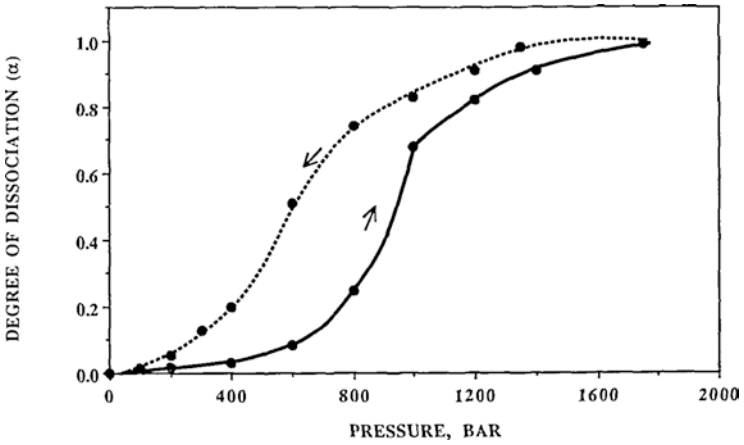


Fig. 13.11 Hysteresis effects as a result of pressure-induced dissociation: increasing pressure-continuous curve, decreasing pressure-dashed curve (Source: Balny and Masson [35])

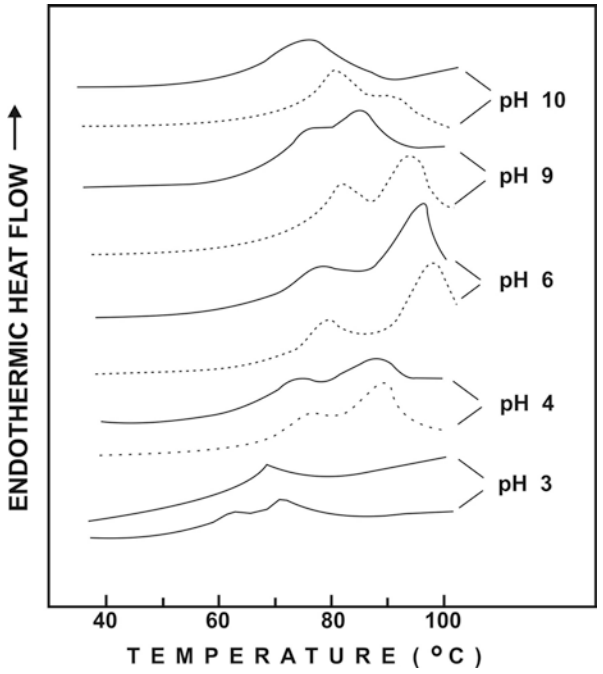


Fig. 13.12 Effect of pH on denaturation of soy protein dispersions in distilled water (continuous curve) and 0.2M NaCl (Hermansson 1978)

agents such as low-molecular-weight disaccharides with higher T_g (e.g. sucrose and trehalose) can help in preserving the protein structure during the freezing and drying process [37], while polysaccharides exhibit the ability to stabilize the emulsions

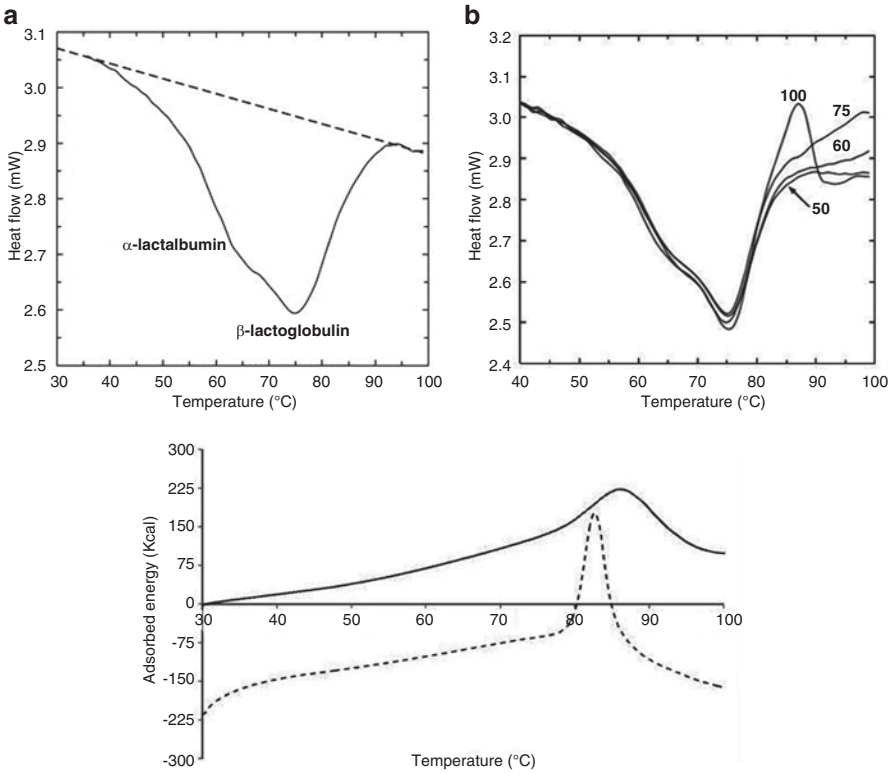


Fig. 13.13 DSC Thermograms showing the denaturation of α-lactabumin and β-lactoglobulin in WPI in (**a**) water (**b**) 50, 60, 75 and 100 mM NaCl solutions (Source: Fitzsimons et al. 2007) (**c**) β-lactoglobulin without (solid line) and with pectin (dashed line) (Benjamin et al. 2012)

by increasing the system viscosity (Dickinson 2001; Benjamin et al. 2012). The presence of pectin modified the onset temperature of the exothermic peak and enthalpy (Fig. 13.13c). The interactions during protein gel formation are influenced by intrinsic factors classified as, electrostatic interactions, hydrophobicity, disulfide bonds, molecular weight, and amino acid composition, or extrinsic factors, namely, protein concentration temperature, pressure, pH, ionic strength, and ion type (Phillips et al. 1994). The gelation process of proteins can be induced physically by heat and high pressure or chemically by the use of ions, urea, acid, or enzymes.

8.4 Lipids

Vegetable oils mainly consist of triacylglycerols (TAGs), the molecules formed by three fatty acids esterified to a glycerol structure (Fig. 13.14). Due to their high molecular weight, these tend to crystallize in three main polymorphic forms, namely, α (alpha), β′ (beta-prime), and β (beta) from the liquid phase (Sato 2001).

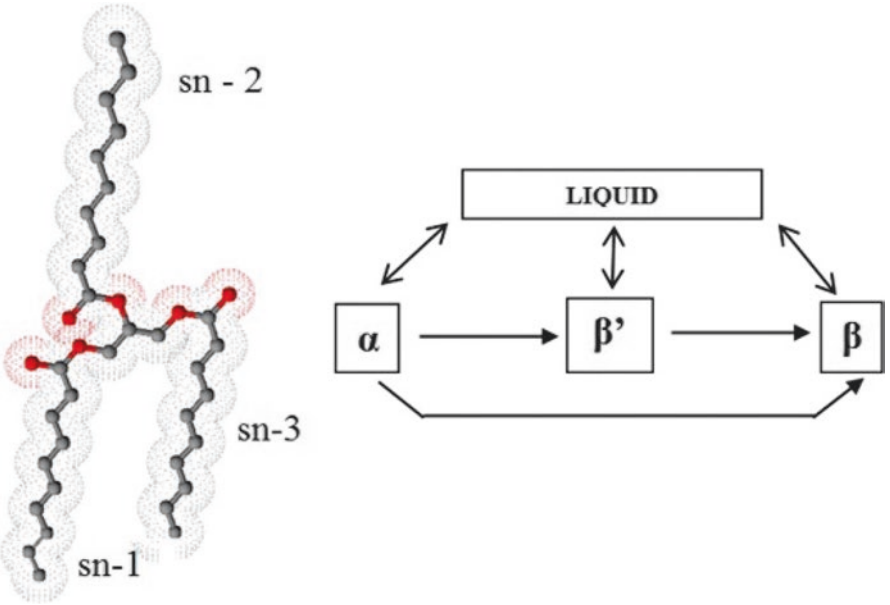


Fig. 13.14 Solid-liquid transitions in triacylglycerols molecules

The triacylglycerols are accountable for the various physical characteristics, e.g., melting points. Differences in properties may arise on the account of different positions of the same fatty acids or different fatty acids profiles in blends with similar bulk fractions of saturated/unsaturated fatty acids. Various characteristics of fatty foods, including organoleptic properties and flavor release, are influenced by the solid–liquid phase behavior of vegetable oils [38]. The temperatures where the main phase transitions occur are observed by differential scanning calorimetry (DSC).

The onset temperature, the heat of fusion (ΔH), the identification of polymorphic behavior, and crystallization of oils and fats are the main characteristics assessed using a heat–cool–heat DSC program. An isothermal method or scanning method with an oxygen atmosphere is used to determine the oxidation induction time (OIT) in the case of hydrogenated vegetable oils. The oxidative stability or the thermal-oxidative resistance of greasy materials can be assessed from the enthalpic changes by calorimetry or by thermal analysis using DSC and pressurized DSC owing to the fact that oxidation and thermal-oxidative decomposition of oils and fats are exothermal reactions (Kowalski et al. 1997).

8.5 Thermal and Oxidative Stability

Thermogravimetric analysis shows three steps for thermal decomposition of lipids. It shows first corresponding to the polyunsaturated fatty acids occurs between 230 and 380 °C, the second corresponding to the monounsaturated fatty acids, such as

Table 13.2 Estimating quality by thermal analysis of food components-starches, proteins, and lipids

Food component and/or source	Salient findings	References
Starches		
Dry native starch, amylose and amylopectin Wheat and maize	• Amorphous phase swollen by bound water (temperature: 323–428 K) • Swelling was linearly dependent on the volume fraction of bound water and bound water caused melting points depression of crystallites • Degree of perfection of crystallites in the starch granules could be related to gelatinization temperatures, whereas the degree of crystallinity could be related to gelatinization enthalpies	[39]
Starch Green Banana flour (*Musa cavendishii* L.)	• A single endothermic transition and a flow of maximum heating at highest temperatures from 340.95 to 351.63 K • Drying temperature significantly affected the gelatinization peak temperature (*Tp*) • Gelatinization enthalpy (ΔH_{gel}): 9.04–11.63 J/g	[40]
Polysaccharides *Musa sapientum* L.	• Glass transition observed at 313.8 K without finding a melting peak. The lower value may be due to plasticization due to residual water molecules remaining unmoved during fast scanning • The continuous endothermic transition indicates moisture loss in the sample. The weight loss onset at 513 K indicates good thermal stability of polysaccharides. The onset peak and conclusion temperature of phase transition were observed to be 384.84 K • Thermal behavior was influenced by structural and functional group differences in polysaccharides and affected the transition temperature	[41]
Gum arabic and cashew gum, the exudate polysaccharides *Acacia* and *Anacardium occidentale L*	• Gum arabic showed an endothermic event at about 363 K (*To*) with low water content (0–40%) and multiple melting endotherms and associated enthalpies were observed with increasing water content (50–80%) • Cashew gum showed a similar trend to arabic gum but with *To* ≈370 K	[42]
Xanthan gums	• Enthalpy value was observed to be 1924 ± 2.01 J/g for commercial Xanthan • From the DSC curves the onset (*To*) and peak (*Tp*) determined were found to be 379 ± 1 K and 427 ± 1 K, respectively	[43]
Natural gums from seeds of Diospyros melonoxylon Roxb, Buchanania lanzan spreng, Manilkara zapota	• Major intense exothermic transition (around 473 K) followed by weaker exotherm(s)	[44]

(continued)

Table 13.2 (continued)

Food component and/or source	Salient findings	References
Proteins		
Whey protein	• Denaturation of whey proteins by the disruption of hydrophobic interactions • pH affected the formation of protein aggregates during heating • Whey Protein denaturation affected by pH and protein concentration during heating (413 K) was studied. DSC data revealed that whey protein denaturation depended upon concentration	[45]
Mixtures of soybean protein isolates and corn starch in water	• Presence of soybean protein isolates restricted the corn starch gelatinization and the presence of corn starch protected soybean protein isolates from denaturation	[46]
Mixtures of whey protein concentrate, starch, gelatin, and sucrose in water	• The starch gelatinization temperature was less than the whey protein denaturation temperature. In the presence of sucrose, whey proteins got denatured before the gelatinization of starch	[47]
High protein systems on the base of whey protein isolate (WPI)	• DSC data showed a presence of protein hydration transition for rehumidified WPI, WPI–oil, WPI–sugar, and WPI–oil–sugar mixtures • Changes in protein conformation, denaturation, and glass transitions have been discussed • Stability of these systems depended upon hydration and reactions in both hydrophilic and hydrophobic phases	[48]
Chickpea protein concentrates (CPCs)	• Effects of freeze-drying and convective drying on denaturation were studied • Significant differences were observed in peak denaturation temperature and enthalpy of transition for different drying methods	[49]
Mushroom (*Agaricus bisporus*) slices	• Protein denaturation under the electrohydrodynamically (EHD) assisted hot air drying was studied • Enthalpy ΔH and transition temperatures of endothermic peaks were measured. The correlations between ΔH and parameters of EHD treatment (electrode gap and voltage) were discussed • Significant effect of EHD-treatment on thermodynamic responses related to protein denaturation was observed • Changes in state of water in DSC cooling thermograms of dried mushroom slices • Mushroom slices dried by all the combined hot air–EHD drying treatments have no freezable water	[50]

(continued)

Table 13.2 (continued)

Food component and/or source	Salient findings	References
Jumbo squid muscle	• DSC studies for freezing storage of jumbo squid muscle at 253 K for up to 30 days did not reveal any denaturation of proteins	[51]
Meat proteins	• Impact of biopolymers (starch, nonmeat protein, and hydrocolloids (carrageenan, flaxseed gum, curdlan, and barley β-glucan)) on thermal behavior and denaturation of meat proteins using DSC were reviewed • Three denaturation steps were observed in transition temperatures ranges: 216–340 K for myosin subunits, 340–342 K for sarcoplasmic proteins and collagen, and 344–356 K for actin	[52]
Lipids		
Sunflower oil	• Oxidative stability was studied • Phase transition behavior of sunflower oil was studied as influenced by the oxidation level • Upon oxidation, the crystalline structure changes and its crystallization and melting enthalpy significantly decrease with the increase in oxidation level	[53]
Vegetable oil in salad dressing	• Photo-oxidation of vegetable oil in salad dressing as affected by riboflavin was evaluated • With the increase in storage time, the crystallization peaks of the oil samples shifted to lower temperatures, and a decrease in enthalpies was observed • Endo- or exo-therm peaks of canola oil which became sharp on the addition of palm olein to canola oil. This may be attributed to the modifications in fatty acid composition	[54]
Palm oil, canola oil and its blends	• Oxidative stability during the frying process was studied • Comparatively lower amounts of oxidation products were formed in the blends of palm and canola oils compared to canola oil during the frying process	[55]
P. serotina seed oil	• Oxidative stability during toasting was assessed • Physicochemical characteristics of oils extracted from *Prunus serotina* raw and toasted seeds with hexane and supercritical CO_2 were evaluated • Three-step oxidation of oil was observed with the mean onset and oxidation temperatures of 394 and 403–546 K, respectively, depending upon processing • Oil extracted using supercritical CO_2 had the lowest oxidation temperatures with the absence of the third exothermic peak. However, the oil extracted using hexane showed the highest oxidation temperature	[56]

(continued)

Table 13.2 (continued)

Food component and/or source	Salient findings	References
Bauhinia purpurea, rice bran and cotton seed oil	• *Bauhinia purpurea* oil was found to be a very stable oil as compared to rice bran and cotton seed oil	[57]
Soybean oil	• Heat-bodied soybean oil has higher oxidative stability with even higher stability for microwave irradiated soybean oil • onset temperature of the exothermic thermal transition of heat-bodied soybean oil is considerably high (*To* = 428.7 K) compared to that for untreated soybean oil (*To* = 405.7 K) • Microwave-irradiated soybean oil (473 K for 20 min) had higher *To* = 433 K as compared to untreated soybean oil, and even higher than the heat-bodied soybean oil • High oxidative stability attributed to decrease in double bonds and formation of cyclic triacylglyceride ring structures	[58]
Moringa oleifera seed oil	• Degradation kinetics was studied • Activation energy of *Moringa oleifera* seed oil, *Ea* = 1.593 kJ/mol	[59]
Roselle seed oil	• Studied degradation kinetics during heating • Thermal oxidation of the double bonds of the oil showed a first-order thermal oxidation kinetics • Arrhenius plot yielded a straight line (activation energy, *Ea* = 9.041 kJ/mol)	[60]
Carica papaya seed oil	• Degradation kinetics was studied • Activation energy, *Ea* = 7.752 kJ/mol	[61]
Gumbo seed oil	• Degradation kinetics was studied • Activation energy of gumbo seed oil, *Ea* = 8.646 kJ/mol	[62]
Robusta coffee bean	• Optimal roasting conditions for robusta coffee bean for the best nutritive value are *T* = 483 K and humidity content in roasting air = 1% and flow velocity = 1 m/s • Roasted beans obtained a very high quality aroma and oil under such conditions	[63]
Sesame oil	• Oxidative stability of the oils extracted using SC-CO_2 was not significantly affected by temperature and pressure levels • Oil extracted using *n*-hexane had lower oxidative stability, compared to the oils extracted with SC-CO_2 due to higher temperature and extraction time	[64]
Sesame oil	• Thermal oxidation of the double bonds of the sesame oil showed a first-order thermal oxidation kinetic • Activation energy, *Ea* = 12.428 kJ/mol	[65]
Flaxseed oils	• Thermal oxidation behaviour of flaxseed oils obtained from different varieties of seeds was studied • Not significant differences in the thermal and oxidative stabilities of oils obtained from brown and golden flaxseeds were observed	[66]

(continued)

Table 13.2 (continued)

Food component and/or source	Salient findings	References
Balanites aegyptiaca beans	• Characterization of oil extracted from the *Balanites aegyptiaca* bean • Presence of natural antioxidants in the bean protected the triglycerides present in its oil are well against aging	[67]
Rambutan seed (*Nephelium lappaceum* L.) fat	• Thermal stability of *Nephelium lappaceum* L. fat analyzed in an inert N_2 atmosphere and in a normal oxidizing atmosphere • With three stages of decomposition, fat decomposition begins at 500 K and concludes at 802 K, in a normal oxidizing atmosphere	[68]
Edible oil	• 17 edible oil samples were characterized • DSC melting and crystallization profiles were observed at higher temperature regions for oil samples with a high degree of saturation (IV < 65) than the oil samples with a high degree of unsaturation (IV > 65) • Significant difference between T_o of the crystallization curve and T_f of the melting curve for all the oil samples	[69]
Vegetable oils	• 11 edible oil samples were characterized • Vegetable oil samples that were cooled at a constant rate (5 °C/min) from the melt showed between one and seven melting endotherms upon heating at four different heating rates (1, 5, 10, and 20 °C/min) • With increased heating rates, the melting transition temperature shifted to higher values, the breadth of the melting endotherm and the area under the melting peak also increased	[70, 71]

oleic acid, occurs between 380 and 480 °C, and the third corresponding to saturated fatty acids, such as palmitic acid, occurs between 480 and 550 °C. The decomposition of the unsaturated fatty acids begins with the decomposition of the polyunsaturated fatty acids, thus making it most important for the thermal stability of edible oils. The oxidation in edible vegetable oils starts with the absorption of oxygen through the fatty acid chain resulting in the formation of peroxides as the product oxidation. The second step involves the breaking of double bonds leading to saturation of the triglyceride molecules in the edible vegetable oils. The third step includes the thermal decomposition of saturated fatty acids.

Most of the studies are related to thermal and oxidative stability of lipids and a few relate to development of new products (Table 13.2). Thermal degradation of edible vegetable oils occurs due to prolonged heating. The deterioration in quality is usually indicated by a change in color, increase in viscosity, and occurrence of unpleasant odor. The thermal stability of the vegetable oils is lowered as a consequence of auto-oxidation of unsaturated fatty acids causing a decrease in the oxidative induction time. An increase in frying time enhances the degradation of these oils caused by a decrease in unsaturation due to prior heat treatment. The oxidative rancidity is observed in oils on repeated frying due to the formation of

hydroperoxides and release of volatile degradative compounds, such as hydrocarbons, aldehydes, ketones, furans, and carboxylic acids owing to degradation. The onset temperature of thermal decomposition (T_{onset}) is also lowered, and this loss of stability is accelerated by an increase in process time. The degradative effect of frying time can also be noted from a decrease in the onset temperature of decomposition obtained from TG/DTG curves for the process of degradation of sunflower oils (Fig. 13.15), with higher onset temperature for oils with antioxidant at frying times of more than 4 h.

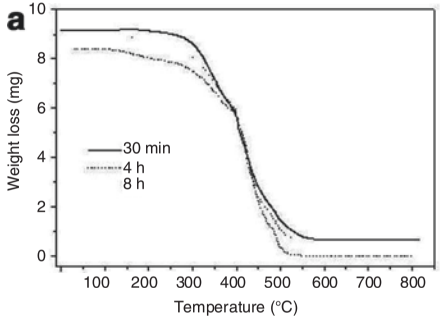
The thermal decomposition profiles for the sunflower oils presented in Fig. 13.16a, b also show three thermal decomposition steps ranged between 230 and 550 °C, and it is related to the decomposition of polyunsaturated, monounsaturated, and saturated fatty acids, respectively, with no residue remaining at 800 °C [72]. Addition of antioxidant to oils help in increasing the stability indicated by smoother curve along with narrow and sharp peaks in the thermogram (Fig. 13.16c, d). The specific heat curve also smoothes for oil with antioxidants.

9 Applications of DSC in Food Industry

DSC may serve as a quality control technique to ensure that excessive processing conditions are not incurred. The most important applications of DSC in the examination of food components, foods, and food ingredients include studies on protein stability and denaturation, phase transition of aqueous starch systems, thermal gelation of some other polysaccharides, phase behavior of frozen carbohydrate systems, characterization of glass transition and cryo-stabilization of food, melting points and degree of crystallinity of lipids, and their testing for oxidative stability.

9.1 Estimation of Food Quality and Nutritional Characteristics

Starch has wide application in the food industry. It finds use as a thickening agent in dry sauce bases, instant soups, mayonnaise, spreads, or as stabilizing agent for oil emulsions, e.g., in instant dressings. Starch can exhibit two different thermal properties known as gelatinization and retrogradation [2]. The process of starch gelatinization is important in the food industry, as its influences the structure of starch-based products (Lelievre and Liu 1994). Gelatinization is considered a combination of glass transition of amorphous regions and the melting of crystalline regions [74, 75]. The use of additives and modification of starches have been suggested for avoiding retrogradation of starch. Starch–lipid complex formation is a frequently observed phenomenon in the food industry. The addition of surfactants/emulsifiers can form an amylopectin–lipid complex that would obstruct the growth of the amylopectin crystal (Eliasson and Larsson 1993) resulting in a decrease in retrogradation indicated by a decrease in the melting enthalpy measured by DSC (Gudmundsson



b

Time (h)	T_{onset} (°C)	
	Sunflower Oil A	Sunflower Oil B
0	246.67	218.78
0.5	192.80	207.78
4	186.51	177.95
8	184.66	169.60

Fig. 13.15 Effect of frying time on onset temperature of decomposition shown in (**a**) TG curves (sunflower oil with antioxidant) (**b**) Variation in onset temperature for sunflower oil with and without antioxidant

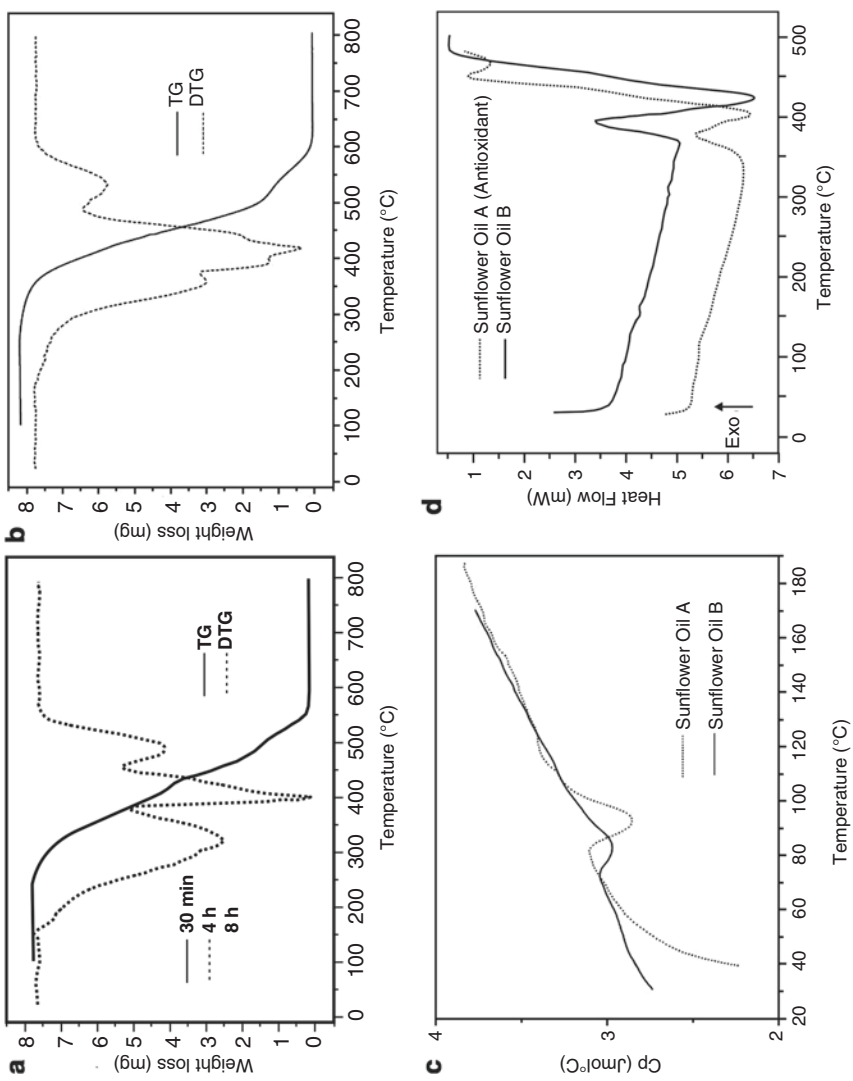


Fig. 13.16 TG/DTG curves for sunflower oil (**a**) without artificial antioxidants and (**b**) with artificial antioxidants (**c**) Thermogram and (**d**) specific heat curves for sunflower oil with and without antioxidant (Source: Souza et al. [72])

and Eliasson 1990). The use of "anti-staling" enzymes to avoid retrogradation in bread and chapati at low temperatures has been recommended as a useful approach. These exo-alpha amylases generate maltose and other malto-oligosaccharides from the nonreducing ends of amylopectin resulting in shorter amylopectin side chains in the wheat flour making it less prone to retrogradation. Modified starches are primary crosslinked starches that produce gels having good freeze–thaw stability. The use of modified starches instead of pure starches in emulsions tends to reduce syneresis and retrogradation enhancing the stability of the preparations during congelation and decongelation. Pregelatinized starches can be used as thickeners in products that do not undergo severe heat treatment.

Proteins, the most studied food components ([76, 77]; Hettiarachchy, [78]), consist of 20 different L-α-amino acids and may be in fibrous (water insoluble) or globular (water soluble) forms. The DSC application studies are presented in Table 13.2, and it includes the confirmation of changes caused by various factors, thermal denaturation, conformational transitions, thermodynamics involved with proteins; food enzymes and enzyme preparations for the food industry; and the effects of various additives on their thermal properties. The denaturation temperature is a measure for the thermal stability of proteins, essential in food science. Thermal denaturation of several proteins (bovine serum albumin, lysozyme, and whey protein isolate) in the presence of hydrocolloids [79], thermal behavior of herring muscle, skin and pyloric caeca during salting and ripening (Schubring 1999), gelation mechanism of globular proteins upon heating [80], and the structural change, equilibrium thermodynamic stability, and folding mechanism of the proteins as well as the interactions between the proteins and other food ingredients (Johnson 2013). DSC is particularly well suited to the characterization of protein stability and provides an insight into enthalpy changes associated with the unfolding transition, essential in food science. It a widely used technique for this purpose because monitoring the unfolding of a macromolecule induced by exposure to elevated temperature is essential for evaluating stability. The food industry has started introducing enzymes and structural proteins into modern food materials and their processing, making it pertinent to understand the chemical and physical properties of proteins [1]. Protein precipitation is a common problem in many industrial applications of WPC (Theologou et al. 2005; Dickson and Parkison 2004). It is known that maintaining the pH of the food system at or below 3.7 prior to thermal processing can prove useful in checking this not-so-desirable phenomenon. Under these conditions, precipitation of most whey proteins did not occur after heating at 95 °C for 5 min (Sudhir et al. 2005; [81]).

DSC technique has been extensively used by researchers to study thermal properties of food lipids having a different composition, including phase transition temperatures and the associated enthalpies (Table 13.2). The reviews on DSC studies of phospholipid membranes [82] and phospholipids in vegetable oils and fats in relation to lipid modification processes [83] have been reported. Thermal oxidative decomposition of edible oils can be used for predicting oil stability. The oxidation induction time (OIT) and oxidation onset temperature (OOT), measured using DSC, are the main parameters evaluated by the food industry as a measure of oil stability during heat treatment and storage. It was established that the bigger the size of the

hydrocarbon chain, the higher the enthalpy of activation [84]. The DSC was used as a rapid and effective method to characterize the olive oil at different levels of oxidation [85, 86]; to determine both oxidative stability and antioxidant capacity of grape seed oils [87], buriti pulp oil (*Mauritia flexuosa Mart*), rubber seed oil (*Hevea brasiliensis*), and passion fruit oil (*Passiflora edulis); and the* efficacy of δ-tocopherol was the most effective antioxidant for sunflower oil and propyl gallate for the less unsaturated oils [88, 89].

9.2 Food Authenticity and Adulteration Testing

Food authenticity testing is one of the prominent applications of DSC with a major focus on oil- and fat-containing samples. Scientists in the food industry used DSC as a quality control tool to visualize how the crystallization and melting properties were affected by the cooling and heating rates. Recent investigations conducted to detect food adulteration and check food authenticity in oils, fats, butter, meat, milk, and honey have been tabulated (Table 13.3). This approach was based on the fact that unique compositions of TAG and fatty acid together with minor components (i.e., diacylglycerols and lipid oxidation products) of a particular fat or oil enabled identification by their unique crystallization or melting curve properties. This information can be further utilized for determining the cultivar, geographic origin, commercial categories, and also adulteration. In the context of oil authenticity, most of the documented work reports about olive oil. Due to its high-economic value, it has always been exposed to potential adulteration. Several chromatography-based methods and alternatives have been proposed for determining the authenticity of olive oil [103]. Cost, time, effort, and expertise are required for such analysis, and it justifies the development and use of rapid and simple methods such as DSC for this purpose. Bodurov et al. [104] and Jafari et al. [105] also applied DSC to detect the presence of soybean, sunflower, or canola oils in olive oil. Laddomada et al. [106] endorsed the application of a modulated adiabatic scanning calorimeter in temperature-scanning mode for analysis at various points along the olive oil supply chain using a tailor-made time–temperature protocol that was simpler, compact, and portable when compared to the conventional DSC. The fast DSC was evaluated to replace the conventional DSC for adulteration detection with comparable yet not always distinguishable results [107].

9.3 Design of Novel Food Products

The use of DSC measurements for optimizing the production process and quality control is highly effective during the development of new products. Polymorphism, the ability of a chemical compound to crystallize into different states/modifications, has immense importance in food science. Chocolate products with cacao butter

Table 13.3 Recent investigations related to the detection of food adulteration using DSC

Investigation	Salient findings	References
Pure ghee adulteration with caprine body fat and groundnut oil	• Adulteration of pure ghee with caprine body fat when added singly (5, 10, and 15%) and in combination with groundnut oil (GNO) (5, 10, and 15%) was detected using DSC • Transition behaviour in terms of crystallizing and melting curves was studied • Adulterated samples showed a shift in the midrange temperature of thermal curves, indicating the presence of foreign fats	[90]
Extra virgin oil adulteration with hazelnut oil	• Hazelnut oil having similar chemical composition is often used as an adulterant in extra virgin olive oil. Hazelnut oil adulteration is difficult to detect, especially when its concentration is below 20% • On the basis of alterations of both heating and cooling thermogram profiles of pure extra virgin olive oil detection of at least 5% of refined hazelnut oil in extra virgin olive oil was done using DSC • Exact percentage of adulteration of hazelnut oil could not be done as thermal properties did not exhibit a significant difference in the range of 5–20% and 30–40%, respectively	Chiavaro et al. (2008)
Virgin coconut oil adulteration with lard	• Physical properties of Virgin Coconut Oil (VCO) and lard are almost similar but they contain different amounts of saturated and unsaturated TAG and FA. Lard adulteration in VCO was determined using cooling and melting profiles • Lard heating thermograms exhibit two major endothermic peaks with lower melting points at 3.93 and 18.83 °C as lard has more unsaturated FA and TAG than VCO • lard adulteration could be successfully detected using DSC when VCO is successively adulterated with lard from 1 to 30%, on the basis of the endothermic max T peak A, which increased in magnitude as lard percentage increased	[91]
Canola oil adulteration with lard stearin	• Cooling and heating profiles were analyzed for pure canola oil samples spiked with lard stearin ranging from 5 to 15% (w/w) • The canola oil cooling curve at 19.5 °C was strongly influenced by lard stearin and displayed significant variations when adulteration levels reached 5% (w/w)	[92]

(continued)

Table 13.3 (continued)

Investigation	Salient findings	References
Avocado oil adulteration with refined bleached deodorized palm Superolein	• Adulteration of avocado oil with Refined Bleached Deodorized Palm Superolein (RBDPSO) was successfully detected using DSC combined with multivariate calibration • Characterization of avocado oil and adulterant was done by significantly different cooling and heating DSC thermal profiles • Adulteration with RBDPSO shifts the overall transition temperature towards lower temperature, enhancing crystallization, melting enthalpy, and developing both processes over a narrower temperature range • Change of characteristic exothermic and endothermic event in avocado oil with increasing adulterant may be due to the increase in oleic and stearic acids and the decrease of palmitoleic acid	[93]
Detection of lard in cocoa butter	• Changes in fatty acids composition, triacylglycerols profile, and thermal characteristics were studied for detection of lard in cocoa butter. Cocoa butter was mixed with 1-30% (v/v) of lard and analyzed • Heating thermogram showed that as the concentration of lard increased from 3 to 30%, two minor peaks at −26 °C and 34.5 °C started to appear and a minor peak at 34.5 °C gradually overlapped with the neighboring major peak • Cooling thermogram showed a minor peak shift to a lower temperature of −36 °C to −41.5 °C	[94]
Detection of refined olive oil as adulterant in extra virgin olive oil	• Admixtures of EVOO: ROO were prepared in different ratios (50:50, 60:40, 70:30, 80:20, 90:10 and 95:5 w/w) • Exothermic and endothermic thermograms were investigated which indicated that by increasing refined olive oil, onset temperature (T_{on}) was increased from −61.08 to −56.16 °C and offset temperature (T_{off}) decreased from 19.09 to 12.57 °C • With the increase in ROO ratio the average enthalpy values of both exothermic and endothermic curves decreased	[95]

(continued)

Table 13.3 (continued)

Investigation	Salient findings	References
Detection of adulteration in honey	• Thermal behavior of authentic honey and sugar syrups (industrial and homemade) were investigated by DSC • 30 honey samples (Robinia, lavender, chestnut, and fir) were analyzed and their T_g was also measured • DSC showed a detection level of 5–10% depending on the type of syrup for honey samples artificially adulterated with different industrial syrups • An endothermic phenomenon occurring between 40 and 90 °C during the heating was studied by TMDSC and a new thermal transition similar to a glass transition was highlighted	[96]
Adulteration of wild boar meat in meatball formulation	• Characterization of oil extracted from WBM was done using different cooling and heating DSC thermal profiles • The change of characteristic exothermic and endothermic event in oil with increasing crystallization, melting enthalpy, and developing both processes over a narrower temperature range was studied	[97]
Adulteration of virgin coconut oil with palm kernel oil and soyabean oil	• Virgin coconut oil (VCO) was spiked separately with PKO and SBO from 2 to 40% (w/w) of adulterant oils • PKO-adulterated oils did not show any adulteration peak but demonstrated a gradual decrease in the peak height of the major exothermic peak • The heating curve of SBO-adulterated oils showed the adulteration peak appearing at the lower temperature region at 10% adulteration level	[98]
Detection of butter adulteration with water	• To calculate the water content for seven tested kinds of butter correlation equations were adopted. High correlation coefficients were found between the water content and the enthalpies of the ice melting/water crystallization • Water content determined by the reference method and by DSC had a difference in the range of 0.2–2.6% for the measured enthalpy of ice melting, and between 1.0 and 5.6% for the enthalpy of water crystallization	[99]

(continued)

Table 13.3 (continued)

Investigation	Salient findings	References
Detection of animal fat contaminations in sunflower oil	• Sunflower oil samples were spiked separately with lard, beef tallow, and chicken fat in the range 1–20% (w/w) and analyzed using differential scanning calorimetry to obtain their heating profiles • Both lard and beef tallow below 20% (w/w) in sunflower oil can be detected using characteristic contaminant peaks appearing in the higher temperature region (0–50 °C) • Chicken fat contamination in sunflower oil did not show any characteristic peaks in this region but caused changes only in the exiting thermal transitions in the low-temperature region	[100]
Detection of butter adulteration with lard	• Heating thermogram of the mixture shows that there was one major endothermic peak (peak A) with a smaller shoulder peak embedded in the major peak that gradually smoothed out to the major peak with the increase in the percentage of lard • Cooling thermogram of the mixture shows that there was one minor peak (peak B) and two major exothermic peaks, peak C which increased with the increase in lard percentage, and peak D which decreased with the increase in lard percentage • The stepwise multiple linear regression (SMLR) equation of lard percent adulteration in butter is 293.1–11.36 (Te A) − 2.17 (Tr D); where Te A is the endset of peak A and Tr D is the range of thermal transition for peak D	[101]
Detection of dilution of milk	• The glass transition temperature (*T*g) of pure milk varied between −114 and −118 °C. There is a linear relationship between glass transition temperature and composition of the milk • The addition of sugars (glucose, fructose, and sucrose) also led to a significant change in glass transition temperature. Adulterations in milk by dilution with water only and water and sugars were detectable even at 0.5 and 5%, respectively	[102]

have desirable consumer properties only if the p-form with a melting point at 35 °C is applied. A large number of studies related to polymorphism in crystallization (Wille and Lutton 1966; Bricknell and Hartel 1998; Loisel et al. 1998; Le Révérend et al. 2010; Miyasaki et al. 2015) and process kinetics (Los et al. 2002; Foubert et al. 2002) have been conducted to check the suitability of natural as well as modified fats and oils for close imitation of physical properties, such as cloud point, solid fat content, brittleness, and hardness of cocoa butter (Gregersen et al. 2015). DSC can help improve the cold consistency (spreading property) of butter and the concurrent reduction of softening with an increase in temperature. DSC has been used to differentiate between natural and imitation Mozzarella cheese made from calcium caseinate, using a proposed mechanism of the alteration of crystallization properties of milk fat in cheese [108], detect changes in fat crystallization that impact the fat globule structure and functional properties, such as free oil formation, to examine

the differences in cheese melting profile [109], to determine the aging behavior of the nonequilibrium glassy state, assess for final quality and the storage stability of isomalt [110, 111], a frequently used sugar substitute.

The difference in physical properties of various oils, e.g., the occurrence of final solid–liquid transition, melting points, and recrystallization, offers a huge industrial potential for a large diversity of formulations using these fats. The final melting point of cocoa butter (CB) occurs above typical room temperature but below mouth temperature, making it solid under ambient conditions but melting in the mouth, releasing desired sensory properties, such as cooling sensation (Torbica et al. 2006). Teles dos Santos et al. (2013) showed the efficacy of computational tools and modeling approach for prediction of SFC of binary blends of vegetable oils. Computation of physical properties in solid–liquid mixtures helps in using this approach in identifying new mixtures using vegetable oils for industrial food applications.

Phase transitions and melting range of fats studied using DSC describes the physical and textural properties of fats. The melting and crystallization behavior of different types of oils and fats are helpful when replacing them in a product. Different ingredients are added at different stages during production, and improper temperature of an ingredient at the time of addition can cause encapsulation of other ingredients or the presence of that ingredient as a particle in the product. The temperature of a product at the time of filling is important to obtain the desired firmness of a product and to prevent graininess. DSC is widely used to compare batches of a product by studying differences in crystallinity of the fat or composition of the end product. Different scanning rates are used to investigate the cooling effect on the crystallization of a specific fat. The solid fat content (SFC) of a system can be determined over a given melting range. The aging behavior of a fat or end product is assessed by exposing the sample at an isothermal temperature to imitate refrigerated conditions. Information on phase transitions during these storage conditions can be obtained by comparing the DSC thermograms of a fresh sample and after a known storage time.

9.4 Food Microbiology

DSC techniques are finding increasing applications in food microbiology by measuring heat production of microbial cultures in various substrates. The bacterial contamination in food and spoilage in canned foods can be rapidly detected using microcalorimetry with the thermograms obtained by modern thermal bioactivity monitors allowing calculation of various parameters like growth and activation energy. DSC thermograms of a range of bacteria having different thermal resistances were obtained by Miles et al. [112]. Equations developed to estimate the rate of decline of viable organisms in a calorimeter with an increase in temperature showed a direct relationship between the onset of heat denaturation and the heat resistance of different organisms. Differences in heat sensitivity could be judged from the features in DSC thermograms. It is possible to understand the thermoadaptive mechanisms of important microorganisms by DSC if membrane fluidity of thermophilic bacteria is studied. Several studies demonstrate the applicability of

DSC for resolving transitions that represent lipid melting, protein unfolding, and melting of nucleic acids in relation to the thermostability of cellular constituents in whole cells. Such application is exceedingly important for heat preservation of food, as it helps in detecting the critical target of thermal deactivation of bacteria and also comprehending the heat destruction mechanism of microorganisms. The exothermic heat flow observed probably represented the oxidative metabolism of the cells in the vital temperature range while the appearance of series of endothermic transitions indicated a loss of viability. The "melting" process of the intracellular DNA was depicted by a reversible transition near to 90 °C.

9.5 Nano Emulsions

Nano emulsions consist of emulsified oil and water systems. An average droplet size between 100 and 500 nm can exist as oil-in-water (o/w) or water-in-oil (w/o) form, where the core of the particle is either oil or water. Nano emulsions are made from surfactants that are generally regarded as safe (GRAS). Characterization of nano emulsions involves the physical and chemical tests that include compatibility of the nano emulsion components, isotropicity of the formulation, uniformity of content, appearance, pH, viscosity, density, conductivity, surface tension, size and zeta potential of the dispersed phase, etc., with respect to the effect of the composition on physical parameters. The application of DSC can generate information related to the stability of emulsions, thermal transitions, and interactions of different components of the formulation.

10 Conclusion

This chapter presents the differential scanning calorimetry technique that is often used for thermal analysis in foods because of its speed, simplicity, and availability. The basic principle of DSC, its types, and DSC curves have been discussed. A main advantage of this technique is its ability to study the changes occurring during processing under dynamic temperature conditions as well as to provide both thermodynamic and kinetic data. Detailed protocols for the determination of thermal properties like specific heat, enthalpy, thermal conductivity, thermal diffusivity, and glass transition using DSC have been summarized. Various applications of DSC in the food industry like studying the food composition, thermal and oxidative stability, estimation of food quality and nutritional characteristics, food authenticity testing, design of food products, and food microbiology have been discussed in detail compiling reviews by different researchers on various important food materials. DSC is a useful investigative tool for studying various heat-related phenomena, like denaturation, gelatinization, retrogradation, in food components. Indicative changes in DSC thermograms due to admixtures in the product also make its application in

adulteration detection. Besides this, DSC can significantly contribute to the development of novel food products. In the food industry, DSC has numerous applications that have been discussed, and the researchers can effectively use this technique for need-based or commodity-specific research.

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Chapter 14
Quality Assessment of Frying Oil Degradation

Mohammed Al-Khusaibi and Mohammad Shafiur Rahman

Abstract Deep-fat frying is a quick method of cooking that produces a product with attractive organoleptic properties. However, the degradation of frying oil that occurs thr ough series of reactions and the transfer of a substantial amount of oil to the product raises health concerns. The main oil degradation reactions are oxidation, hydrolysis, and polymerization. Each reaction produces specific compounds that negatively affect health, reduce the foods, and oil quality. Hence, frying oil must be discarded when it reaches a certain level of degradation. There are several methods to detect the degradation of oil, such as the peroxide value, free fatty acids, *p*-anisidine value, and total polar compounds. The fatty acid profile (FAP) of frying oils is a good indicator of their stability. FAP changes with the degradation of oil by the breakdown of unsaturated fatty acids because of their susceptibility to oxidation. According to the American Oil Chemists Society, official methods, rapid methods, and test kits are being developed to reduce time, chemicals, and/or subjectivity of the test methods.

Keywords Fried food · Frying oil · Oil degradation · Fatty acid · Fatty acid profile (FAP) · Free fatty acid · Oxidation · Hydrolysis · Polymerization · Peroxide value · *Para*-Anisidine value · Iodine value · Oil stability index

1 Introduction

Frying is a very popular and rapid cooking method. Frying can be classified into shallow frying and deep-fat frying. Shallow frying is also called pan frying or stir frying. In deep-fat frying, a food sample is completely immersed in an oil bath at a preheated temperature between 160 and 200 °C. The process is commercially well established, and it is used to produce a wide range of products, such as potato chips,

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Table 14.1 Oil content and moisture content of some common fried food products (USDA National Nutrient Database for Standard Reference)

Product	Oil content (g/100 g)	Water (g/100 g)
Fried potato chips	34.6–38.4	1–2.5
Corn-based chips	28.4–32.7	0.3–1.2
Tortilla chips	21.6–26.1	1.5–2.3
French fries	14.5–17.4	36.7–41.1
Doughnut	19.3–25.3	16.30–29.8
Fried chicken,		
Breast, with skin/without skin	4.37/14.8–15.54	64.2/50.6–53.1
Drumstick, with skin/without skin	7.1/16.2–9.1	64.2/48.1–0.9
Fish fillets—Battered or breaded	13.3–13.8	53.6–60.1
Chicken nuggets	19.8–23.2	43.1–48.6
Onion rings, breaded	18.69–6.7	28.50–37.09

Data obtained from Ref. [4]

French fries, chicken products (e.g., breaded nuggets, fillets, breasts, and drumsticks), fish fillets, onion rings, and doughnuts). Table 14.1 shows the examples of some common fried foods along with their oil and water content. The process is characterized by a simultaneous heat and mass transfer. Heat is transferred from the oil to the food, and oil is also transferred to the food, while water is transferred from the food to the oil [1, 2]. This phenomenon negatively affects the nutritional and physicochemical quality of fried food and oil. The oil absorbed by the fried food increases oil content, which varied from <1% (i.e., potato tubers) up to 35% (i.e., potato chips). This may also have a negative health impact, since the health recommendation is to reduce the consumption of fat. On the other hand, several factors cause the degradation of oil during frying, which results in the production of different compounds that are believed to pose health risks and affect the quality of fried food [3].

This chapter discusses the mechanisms of oil degradation to understand the formed compounds, different methods used to assess oil quality, and their principles and procedures. This chapter discusses mainly the official methods published by the American's Oil Chemistry Society (AOCS) and the International Union of Pure and Applied Chemistry (IUPAC). Other methods that can be used to assess the same parameters are also briefly discussed. It also presents some of the commonly used rapid methods or kits to evaluate the quality of frying oil.

2 Mechanism of Oil Degradation

Vegetable oils used for frying are made up triglycerides (i.e. approximately 98%), and these are composed of glycerol linked to three fatty acids. During frying, the oil is exposed to different factors (moisture, oxygen, and temperature) that contribute

to its degradation by hydrolysis, oxidation, and polymerization. The extent of those reactions depends on different factors, such as frying temperature ([5, 6]), degree of saturation [7, 8], type and proportion of fatty acids [9, 10], presence of antioxidants [11–14], presence of air (i.e., atmospheric vs. vacuum frying) [15–17], types of fried foods, minor compounds, such as metals, pigments, and phospholipids [18].

2.1 Oxidation

Oxidation process is well-known for its initiation, propagation, and termination stages. Thermal oxidation is believed to be similar to autoxidation with the exception at higher temperatures [19]. This process forms both volatile and nonvolatile compounds. The primary compounds formed due to oxidation at low or moderate temperatures are hydroperoxides. These are called primary oxidation products, and they are unstable under high frying temperatures ([20, 21]). For this reason, these are absent above 150 °C [22]. At high temperatures, hydroperoxides breakdown into secondary oxidation products, and these include oxidized monomers (e.g. alcohols and ketones), short-chain monomers (e.g., aldehydes), oxidized dimers and oligomers, and volatile compounds (e.g., aldehydes and ketones) [19].

2.2 Hydrolysis

Hydrolysis of triglycerides during frying is enhanced by the presence of water escaped from foods when fried in an oil bath at high temperature. This reaction breaks down the triglyceride molecule into diglyceride and free fatty acid. It has been reported that the content of free fatty acids increases with the continued use of oil during frying [23–25].

2.3 Polymerization

Polymerization reactions are complex due to the different types of natural unsaturated fatty acids present in the frying oil. These are responsible for the majority of oil degradation products [22], such as high molecular weight nonvolatile polar compounds [19, 26]. Several other compounds are also formed, such as the nonvolatile polar compounds, dimers, and polymers. These compounds result in a change in several properties of the oil including its viscosity and interfacial tension, color, and smoke point. The total polar compounds are reported to increase with the continuous use of frying oil [27, 28]. A summary of the reactions and typical degradation products is shown in Table 14.2.

Table 14.2 Typical oil breakdown during frying

Reaction	Description	Main constituents
Hydrolysis	Interaction between oil and water, which leads to cleavage of TAG bonds between glycerol and fatty acids	MAG, DAG, glycerols, FFA
Oxidation	Interaction between oil and oxygen (air) at high temperature that promotes the formation of hydroperoxides (primary oxidation) and fission of hydroperoxides (secondary oxidation), which produce secondary decomposition products	Hydroperoxides, carbonyl constituents (ketones, aldehydes), alcohols, FFA
Polymerization	Oxidation of the secondary oxidation constituents at high temperature; and alteration of oil molecules or fatty acids by heat, which leads to the formation of large molecules	PTAG, OTAG, dimers, cyclic fatty acids monomers

3 Assessment of Frying Oil Quality

Compounds formed in oil by oxidation, hydrolysis, and polymerization reactions reduce the nutritional quality of the fried foods [29–31]. It is very crucial to monitor the quality of oil and to determine when to discard considering the concentration limits of these compounds. In the catering services, the point of discard is usually considered based on visual assessment of color, viscosity, and foaming or smoking. Such methods are subjective and not reliable. Different methods have been developed to evaluate the quality of frying oil. The assessment methods rely on the measurement of physical changes and chemical compounds, which are produced as a consequence of the deteriorative reactions. Some methods approved by the American Oil Chemists' Society (AOCS) and the International Union of Pure and Applied Chemistry (IUPAC) are listed in Table 14.3.

3.1 Peroxide Value

The peroxide value is an indicator for the concentration of hydroperoxides (also called peroxides) that are formed as a result of primary oxidation [32]. Peroxide value is a measure of the amount of chemically bound oxygen to an oil or fat, and it is reported as milliequivalents of active oxygen per kilogram of oil (mEq/kg) [33]. The codex standards for fat and oils from vegetable sources have set a maximum of 10 mEq/kg for refined oils [34]. Virgin oils are allowed to have higher values, 15 mEq/kg for cold-pressed and virgin oils, and 20 mEq/kg for virgin olive oil [35]. Peroxide value can be evaluated according to AOCS Cd 3d-63, IUPAC 2.201, or ISO 660:1996 methods [36]. The principle is to determine the quantity of active oxygen (i.e., representing peroxides) that oxidizes potassium iodide to form iodine under recommended controlled conditions. The iodine formed needs to titrate with thiosulfate, and the endpoint is visually determined. This method is considered as accurate and has a linear response, but its sensitivity is limited due to the difficulty

Table 14.3 The Association of Oils' Chemist Society (AOCS) and the International Union of Pure and Applied Chemistry (IUPAC) official methods to measure oil degradation compounds

Parameter	AOCS	IUPAC
Peroxide value		
Acetic acid–chloroform method	Cd8-53	2.501
Acetic acid–isooctane method	Cd8b-90	NA
Iodine value		
Wijs method	Cd1-25	2.205
Calculated method	Cd1c-85	NA
Free fatty acid/acid value	Ca5a-40	2.201
Total polar compounds	Cd20-91	2.507
p-Anisidine value	Cd18-90	NA
Fatty acid composition by GLC	Ce1-62	2.302
Fat stability		
Active oxygen method	Cd12-57	NA
Oil stability index method	Cd12b-92	NA
Stability test by Swift method	NA	2.506

NA Method is not available

in determining exact titration endpoint. Crowe and White [37] developed an AOCS-modified version that uses a small sample size and 10% less chemicals while keeping good sensitivity and precision.

The concentration of hydroperoxides can be determined by other methods such as Ferric Thiocyanate (FeSCN) Assay and Xylenol Orange (XO). In the FeSCN assay, ferrous iron (Fe^{2+}) is used to oxidize hydroperoxides under acidic medium. The reaction resulted in Fe^{3+} complex that reacts with thiocyanate to form a red-violet complex (FeSCN) and it absorbs at 500–510 nm [38, 39]. Xylenol Orange Assay depends on the oxidation of ferrous ions in an acidic medium containing xylenol orange (XO) dye. The resulting Fe^{3+} complex reacts with the XO dye and formed a blue–purple complex, which absorbs at 550–600 nm. [21, 40]. Saad et al. [41] developed a new procedure to determine peroxide value by tri-iodine detector. In this method, oil is emulsified through a reaction with potassium iodide (KI), and the aqueous phase iodide reacts with hydroperoxides. The method showed a linearity in the range 0.35–28.0 (mequiv.O_2/kg) and a detection limit of 0.32.

It must be noted that hydroperoxides are generally unstable under high temperatures and decompose rapidly into secondary oxidation products [42]. It has been noted by several authors that during repeated frying, the value increases during the induction period and then tends to decrease as the frying process progresses [24, 43].

3.2 Para-*Anisidine Value* (p-*AnV)*

As mentioned earlier, hydroperoxides breakdown at high frying temperatures and form secondary oxidation products [44]. The *p*-AnV is a measure of the amount of aldehydes present in the oil. The oil/fat sample is dissolved in *iso*-octane, and it is allowed to react with *p*-anisidine in glacial acetic acid. The reaction results in yellowish compounds with maximum absorbance at 350 nm. The *p*-AnV is determined by measuring the absorbance before and after the reaction. For long-term storage and deep-fat frying, *p*-AnV is more reliable than peroxide value. *Para*-anisidine value is not one of the quality characteristics indicators of the codex standard for vegetable oils [34]. However, Rossell [33] reported that the value in fresh oil should not exceed five units [33]. During frying, *p*-AnV increases with the increased use of oil [6, 45, 46]. The value is used (along with the peroxide value) to calculate "total oxidation value," also called "ToTox":

$$\text{Total oxidation value}\left(\text{ToTox}\right) = 2 \times \text{Peroxide value} + \text{Anisidine value} \tag{14.1}$$

Saad et al. (2007) developed a flow injection method to determine *p*-anisidine value similar to the method as developed to determine peroxide value. It was similar to Saad et al. [41] as they used a triiodide detector. In addition to *p*-Anv, nonvolatile compounds can be measured by thiobarbituric acid (TBA) assay. The reaction between 2-thiobarbituric acid and 2-alkenals and 2,4-alkadienals results in red-colored compounds that can be measured at 532 nm. This test is more suitable for fish and meat products, while *p*-Anv is more suitable for bulk oils and fats [47].

3.3 *Free Fatty Acids*

Free fatty acids are formed as a result of hydrolysis reaction through a breakdown of triglycerides to free fatty acids and diglyceride. Hence, free fatty acids are a good measure for hydrolytic degradation of oil, and it is used very widely in frying industry to monitor the degradation of fried oil. It is given as a percentage of free fatty acids calculated as oleic acid for most of the oils. The only exception is the palm oil and its derivatives when the value of percent of FFA is calculated as palmitic acid [48]. FFA concentration can be expressed as acid value. It is defined as the number of milligrams of KOH required to neutralize the free acids in 1 g of oil.

Free fatty acids (FFA) are present naturally in crude vegetable oils, and they are removed by the refining process [49]. Table 14.4 shows the maximum values for acid values in different types of oils according to codex standards [34, 35]. Several authors reported a steady increase in free fatty acids expressed as % or acid value in oil used for repeated frying [42, 50]. A value of 2% FFA is an indicator of abused frying oil that needs to be discarded [51]. Satyarthi et al. [52] reported the use of ^{1}H NMR to measure the concentration of free fatty acids. The method relies on the

Table 14.4 Codex Alimentarius maximum limits of acid value in vegetable oils

Oil	Maximum acid value KOH/g oil
Refined oils (all types)	0.6
Cold-pressed and virgin oils	4.0
Virgin olive oil	6.6
Virgin palm oils	10.0

presence of a quartet-like spectral pattern in the R-CH_2 region of the NMR spectrum in oils containing free fatty acids and esters. The intensity of the peaks depends on the concentration of FFA in esters, and the unmerged peak of the FFA triplet can be used to determine the FFA according to the following equation:

$$\%\text{FFA} = 4\text{X area of unmerged peak of } \alpha - \text{CH}_2 \text{ of FFA} / \text{total area of } \alpha - \text{CH}_2 \text{ of both FFA and ester} \quad (14.2)$$

Skiera et al. [53] also reported an NMR method with higher sensitivity. Oil sample is dissolved in a mixture of deuterated chloroform and dimethyl sulfoxide-d. It was found that the COOH protons of all FFAs resonate as a singlet in the downfield region at about δ = 11.6 ppm. FFA are quantified by integrating the COOH and a carbonyl CH_2 signals at d = 2.2–2.4 ppm. While the titration method measures FFA in addition to the acidic impurities, the ^{1}H NMR measures only the free fatty acids.

3.4 Total Polar Compounds

The polymerization process during frying results in the formation of high polar compounds [54]. The accumulation of polar compounds is steady during frying and these compounds are low volatility; hence, these are a reliable indicator of the degree of abused oil [55]. The concentration of polar compounds expressed as total polar compounds (TPC) or total polar material (TPM) in frying oil has been used to monitor the change in the quality and several countries have set TPC of 25–27% as a discard point [56, 57]. A recommendation in the Third International Symposium on Deep-fat Frying (2000) was made that two tests on frying fats and oils need to be used to confirm abuse; a TPC concentration of 24% and a Polymeric Triglycerides concentration of 12% [58]. The increase in the concentration of polar compounds causes an increase in the oil viscosity, foaming, and color darkening [59]. Total polar compounds can be measured by AOCS method Ca 20–91 [48] and IUPAC method 2.507 [60]. The column chromatography methods need reasonable experience and consume large quantities of chemicals. Xu [61] developed a spectrophotometric method to assess the concentration of TPC. The oil is scanned at 490 nm and using a quadratic equation:

$$Y = -2.7865x^2 + 23.78x + 1.0309 \tag{14.3}$$

where Y is the % polar compounds and X is the absorbance at 490 nm. The method is quick, consumes less chemicals and time, and showed good correlation with the AOCS method.

3.5 *Fatty Acid Profile and Iodine Value*

Fatty acid profile of oils has been used as an indicator of their stability. Vegetable oils differ in their fatty acid profile and hence in their stability. Oils with a high ratio of saturated to unsaturated fatty acids have been reported to be more stable. Table 14.5 shows the typical fatty acid profile of some vegetable oils. It has been reported that the different unsaturated fatty acids present in oil differ in their susceptibility to oxidation. Table 14.6 shows the oxidative rate of unsaturated fatty acids compared to stearic acid, a saturated fatty acid [26]. Several researchers concluded that modifying oils by the increased proportion of oleic acid (C18:1) can increase their stability [13, 62–64].

One of the consequences of oil oxidation is the change in their degree of saturation which increases due to the decrease in unsaturation of fatty acids. The fatty acid profile can be obtained by gas chromatoraphy (GC) analysis. Fatty acids present in the oil are esterified and converted to fatty acid methyl ester (FAME) that can be

Table 14.5 Typical fatty acids compositions of unmodified oils [65]

Fatty acid	PO	POo	SBO	CnO	CO	SFO	CoO	SaFO	SeO	OO
C6:0	–	–	–	–	–	–	0.5	–	–	–
C8:0	–	–	–	–	–	–	7.8	–	–	–
C10:0	–	–	–	–	–	–	6.7	–	–	–
C12:0	0.2	0.2	–	–	–	–	47.5	–	–	–
C14:0	1.1	1.0	0.1	0.1	–	–	18.1	–	–	–
C16:0	44.0	39.8	10.8	3.5	11.4	6.2	8.8	5.3	9.5	8.4
C16:1	0.1	0.2	0.2	0.2	0.1	0.2	–	–	–	0.7
C18:0	4.5	4.4	4.0	1.5	1.9	4.7	2.6	1.5	4.8	2.5
C18:1	39.2	42.5	23.8	60.1	25.3	20.4	6.2	15.0	42.5	78.0
C18:2	10.1	11.2	53.3	20.1	60.7	68.5	1.6	77.0	42.5	8.3
C18:3	0.4	0.4	7.1	9.6	–	–	0.1	–	–	0.8
C20:0	0.4	0.3	–	0.6	–	–	–	–	–	0.5
Others	–	–	0.7	4.3	0.6	–	0.1	1.2	0.7	0.8
Saturated	50.2	45.7	14.9	5.7	13.3	10.9	92.0	6.8	14.3	11.4
Monounsaturated	39.3	42.7	24.0	60.3	25.4	20.6	6.2	15.0	42.5	78.7
Polyunsaturated	10.5	11.6	60.4	29.7	60.7	68.5	1.7	77.0	42.5	9.1

CnO Canola oil, *CoO* Coconut oil, *CO* Corn oil, *PO* Palm oil, *POo* Palm olein, *SaFO* Safflower oil, *SeO* Sesame oil, *SBO* Soybean oil, *SFO* Sunflower oil, *OO* Olive oil

Table 14.6 Relative oxidation rate of fatty acids [26]

Fatty acid	Relative oxidation rate
Stearic	1
Oleic	10
Linoleic	100
Linolenic	150

injected to GC using nonpolar columns and FID detector. Iodine value is highly related to the degree of unsaturation of oils. This degree of unsaturation changes during frying due to the cleavage of double bonds due to oxidation and polymerization of oil at high temperatures ([32, 66, 67]). Iodine value (IV) is a test that indicates the degree of unsaturation of oil. IV can be defined as the number of grams of iodine that is added to 100 g of fat. The test is based on the ability of unsaturated carbon–carbon bonds to react with halogens (e.g., iodine, bromine, or chlorine) [68]. The AOCS and IUPAC methods depend on Wijs method that uses iodine trichloride (Wijs reagent). Additionally, AOCS has approved what is called "calculated iodine value" which calculates the value based on the fatty acid composition. The degradation of oil can be positively correlated with the decrease in iodine value [69–71]). Recently, two methods using cyclohexane (Cd 1b-87) and cyclohexane-acetic acid (Cd 1D-92) were reapproved by AOCS [72]. Haryati et al. [73] developed a method to determine the IV through the determination of oil triglycerides (TG) by high-performance liquid chromatography (HPLC). IV was determined by regression analysis of the disaturated, trisaturated, and triunsaturated TG. The coefficient of determination of the method was 0.990. The use of Fourier Transform Infrared Spectroscopy (FTIR) [74] and Fourier Transform Near-Infrared (FT-NIR) [75] was reported as rapid tests when compared to conventional methods.

One of the challenges in FTIR is that the near-infraRed (NIR) spectral profile contains less spectral detail and consists of overlapping and poorly defined overtone. Li et al. [75] recommend the use of more sophisticated multivariate analysis technique such as PSL. As frying oil degrades, there is a possibility of overlapping between spectra of oil sample functional groups and different oxidation products functional groups. Zahir et al. [76] observed overlap between a saturated aldehyde functional group or other secondary oxidation products (at 1728 cm^{-1}) and the stretching vibrations of the ester carbonyl functional group of the triglycerides at 1746 cm^{-1}. In FTIR, calibrating the background spectrum with relevant triglycerides is essential.

3.6 Oil Stability Index (OSI)

The Oil Stability Index (OSI) measures the relative resistance of oils or fats to oxidation. The principle of the test depends on passing a flow of air through the sample at temperatures between 70 and 110 °C, which accelerates the sample oxidation.

The oxidation of volatile organic acids is carried by the air stream to a conductivity cell containing water in which they dissolve. The water conductivity changes according to the concentration of acids. Resistance of the oils and fats to oxidation depends on several factors, such as the degree of saturation and the presence of antioxidants or pro-oxidants. Due to the experimental parameters (i.e., air and temperature), the oil resistance to oxidation fails at a specific point depending on the factors mentioned above. The induction period is the point in which a rapid increase in conductivity is detected. The OSI is measured as time (hours) to the induction point. The OSI can be measured according to the AOCS method, Cd 12b-92 [77].

4 Rapid Methods to Evaluate Oil Quality

The AOCS official conventional methods are accurate and reliable. However, these methods are subjective to some extent, require skillful personnel, are time-consuming, use a high amount of solvents, and are costly [54, 78]. In order to overcome these challenges, several rapid tests have been developed. These tests either depend on the change in the dielectric constant or colorimetric reactions. It has been shown by several researchers that dielectric constant of frying oils increases proportionally with the increase in polar compounds [79–82]. A very good correlation was found between several rapid tests and total polar compounds [79, 82–84]. Other methods are based on colorimetric reactions and detect the amount of carbonyl or oxidized fatty acids [84–86]. Table 14.7 shows some of the available kits/instruments and some selected specifications.

In addition, electronic nose (a chemosensory system) has also been investigated as a rapid method to monitor the oil quality. E-nose systems are equipped with metal oxide semiconductor (MOS) systems that can detect the headspace volatiles of frying oil. A multivariate statistical classification method such as principal components analysis (PCA), Linear discriminant analysis (LDA), or discriminant function analysis (DFA) is used to group the detected signals and help in discriminating oils with different degrees of degradation. Innawong et al. [80] found a good correlation between physicochemical parameters and sensor signals. They reported that raising the temperature of the oil to 80 ° C while keeping the sensor at room temperature resulted in better discrimination.

According to Chatterjee et al. [87], the presence of different MOS sensors results in a wide variety of volatile organic compounds (VOCs) that can be detected. Chatterjee et al. [87] reported that the multiple number of sensors results in poor sample discrimination, and there is a need to identify the most relevant sensors. In order to improve the discrimination ability of the system, Upadhyay et al. [88] used chemometrics and fuzzy logic analyses to identify the most effective MOS sensors against headspace volatiles of fried oils. A good correlation was found between e-nose odor index and conventional chemical methods.

Table 14.7 Rapid methods available to evaluate the quality of frying oil

Kit/instrument	Compound tested	Range	Cooking oil operating temperature	Manufacturer
Testo 270	Total polar compounds	0–40.0% TPC	40–200 °C	Testo SE & Co.
Food oil monitor—FOM 330	Total polar compounds	0–40% TPC	50–200 °C	Ebro (Germany)
CapSens 5000	Total polar compounds	0–35%	50 °C	CCIT Sensors AG (Switzerland)
OleoTest®	Total polar compounds	5-level color scale (<5– > 24%)	60–80 °C	Castro, Pinto and Costa, Lda
VITO® FT 440	TPC	0–40%	50–200 °C	VITO AG (Germany)
1.3.4Frying oil monitor (DOM-24 and DOM-24 Ti)	Total polar materials	0.5–40.0%	0–225 °C	ATAGO CO., LTD.
	Acid value(AV)	0.00–9.99		
CDR FoodLab®	Free fatty acids	0.01–26% acidity (oleic acid)	Room temperature	CDR s.r.l. (ITALY)
	p-Anisidine value	0.5–100		
	Peroxide value (PV)	0.3–50 ($mEqO_2$/kg)		
Oxifrit®	Oxidized fatty acids	4-level scale color comparison	Not available	Merk (Germany)
Low range shortening monitor (test strips)	Free fatty acids	4-level scale color comparison (1–2.5%)	Not available	3 M™ (USA)

5 Conclusion

Several chemical reactions take place during frying, and the result is the formation of oxidized, polymerized, and hydrolytic compounds. These are called oil degradation compounds and food absorbs these compounds during frying. The consequences of these compounds are the decrease in fried product's quality and cause of negative health issue. Legislations/recommendations have been made by several countries to set the maximum limits of degradation compounds. Researchers have proposed different analytical methods that are used to evaluate the quality of frying oil and measured these compounds. In addition, rapid test kits/instruments have been commercialized to reduce the time and chemicals needed for the evaluation.

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Chapter 15
Sensory Properties of Foods and Their Measurement Methods

Subhajit Ray

Abstract Consumers' demand toward the consumption of various foods is growing rapidly, and sensory or organoleptic properties in this regard play a significant role to maintain the quality standards apart from nutritional, physicochemical, and microbiological properties. Evaluation of sensory properties, e.g., appearance, color, mouthfeel, taste, body and texture, odor, flavor, aroma, and overall acceptability are the important determinants to assess the food quality. The sensory experiences evoked by various foods and processed food products are the key to deliver pleasure, and these are crucial to commercial success. It is very much essential to measure, understand, and optimize consumers' sensory experiences for designing and marketing food products. The science of sensory evaluation requires a suitable understanding of different sensory methods, and their applicability to solve sensory enquiries and sensory parameters, and their efficient use in describing the quality characteristics of foods and their products. Sensory attributes of food products can be evaluated using two methods, namely, subjective or qualitative and objective or quantitative techniques. Subjective measurement of sensory attributes deals with organoleptic responses to food qualities and is conventionally measured by a hedonic rating test with the active role of trained panelists. An objective or discriminative analysis is essential for routine quality control and usually, the application of instrument or equipment is necessary for testing purposes. The most effective use of sensory evaluation is the establishment of data relationships between consumer responses, instrumental assessment, and descriptive sensory measurements. Both the subjective and the objective methods of sensory evaluation are important in the measurement of food quality attributes and their control in processing and preparation. Therefore, sensory analysis is an essential tool to judge the quality characteristics of the newly developed product and consumers' acceptability. In this chapter, an overview on the sensory attributes and their measurement methods including facilities and panels are presented.

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1 Introduction

It is important that foods meet the expectations of the consumers, and it should be commercially viable. Food acceptance studies thus form an important aspect of commercial food production. Acceptability of foods always depends upon food quality, including sensory or organoleptic quality, physicochemical properties, nutritional quality, and microbiological quality. Food acceptance involves human sense organs, which measures and records the flavor, touch or feel, taste, and aftertaste of foods. These are known as sensory or organoleptic attributes of food acceptance0. has been defined as a scientific method used to evoke, measure, analyze, and interpret responses of products as perceived via the senses of sight, smell, touch, taste, and hearing [1]. A sensory property can be defined as the human physiological–psychological perception of a number of physical and other properties of food and their interactions. The physiological apparatus examines the food and reacts to the food's properties. Signals are sent to the brain, which interprets the signals and comes to a decision about the food's sensory quality; this is the psychological bit [2]. The methods generally used to measure these quality parameters are known as sensory or organoleptic tests. Therefore, sensory analysis plays a major role in defining food quality. Sensory evaluation of food products is carried out for the following reasons: (a) to study sensory characteristics of foods in relation to the consumer preferences, (b) to investigate the impact of food processing on the final products, and (c) to monitor and check the quality assurance of the food products based on quality standard.

The aim of the sensory evaluation is to describe the food quality in terms of magnitude and direction. The performance of the product is characterized by the sum of the organoleptic characteristics. Sensory evaluation has evolved into a complex, multidisciplinary field that requires a high degree of scientific knowledge and skill to carry out successfully [3]. The efficacy of the evaluation of sensory characteristics is basically based upon the successful empirical and theoretical contributions of different professionals, such as physiologists, psychologists, philosophers, anthropologists, marketing scientists, chemists, technologists, and data analysts [4]. Evaluation of sensory or organoleptic qualities of a food product is very much essential in the area of sensory science as well as to judge the particular product for consumers' acceptance. For effective evaluation of sensory data, many facilities must be considered, which include, for example, sensory evaluation facilities, trained panelists, sufficient area for food sample preparations, and necessary utensils. This chapter discusses the various sensory attributes of food, an overview of different sensory evaluation methods, role of sensory panels and sensory facilities in the evaluation process, and the applicability of sensory evaluation techniques.

2 Sensory Attributes of Food

Sensory attributes of food are characterized by appearance, color, flavor, texture, and taste, and these are the five key elements of sensory perception measurement. Now suitable description and understanding of all attributes are very much important in the sensory evaluation process.

2.1 Appearance

Appearance or sight of a food product gives information, such as the size, color, shape, and surface texture. The human visual system detects the light of the wavelengths from about 400 nm (violet) to about 700 nm (red). The color mixing is created in the eye, and it depends on the stimulation of the incoming light where the receptors are stimulated. Moreover, it is generally designated by the characteristic property of the surface. Surface properties of food materials can be determined by some desirable characteristics, e.g., brightness or shiny appearance in fish scale, lightness in bakery and confectionery products, transparency, translucency, opaqueness, turbidity, dullness, and glossiness in processed fruits and vegetable products. Similarly, some undesirable characteristics can also be measured, e.g., sandiness in ice cream, lumpiness in pudding, or liquid milk.

2.2 Color

Color is considered to be an important quality attribute of food. It generally influences the attraction and acceptance of foods. Most of our acceptance or rejection of foods depends on its visual observation. The color of a food material can be used to judge its quality. Different foods appear in different colors, and it creates attraction to the consumers, e.g., orange–yellow color of ripe mango, yellow color of ripe banana, red color of ripe tomato, green color of leafy vegetables, unripe mango, and unripe banana. Moreover, the pale or dark color sometimes indicates the immaturity or staleness of fruit.

Color compounds are generally classified into two categories, e.g., natural colors and synthetic colors. Both colors can be added for the preparation of food to enhance the attractivity for consumers' acceptance. Saffron, betalain, carotene, chlorophyll, lutein, and lycopene are some examples of common natural colorants for food preparations, e.g., rasgulla, sandesh, and mihidana-type Bengali sweets. However, synthetic coal-tar dyes, e.g., sunset yellow FCF, brilliant blue FCF, tartrazine, carmoisine, and amaranth, can be used in preserved fruit and carbonated beverages.

2.3 Flavor

Flavor comprises three basic components, e.g., odor, taste, and a composite sensory impression of mouth feel. This is the most important attribute of food, which generally contributes to choosing a food. Food's flavor or aroma is closely associated with the preparation or manufacturing of food. Therefore, the acceptability of food, as well as processed food, is directly related to its flavor.

2.4 Odor

The odor or smell of a food also pays a significant impact on acceptability. Generally, odor-developing components are volatiles, and during consumption, these are carried by air into the nose and are finally transmitted to olfactory nerves followed by neural signals to our brain. The diagrammatic representation of human olfactory system and transmission of odor signals to brain is given in Figs. 15.1 and 15.2 [5, 6]. The olfactory system, or sense of smell, is the part of the sensory system used to smell a food (i.e., olfaction). Most mammals and reptiles have a main olfactory system and an accessory olfactory system. The main olfactory system detects airborne substances, while the accessory system senses fluid-phase stimuli. The senses

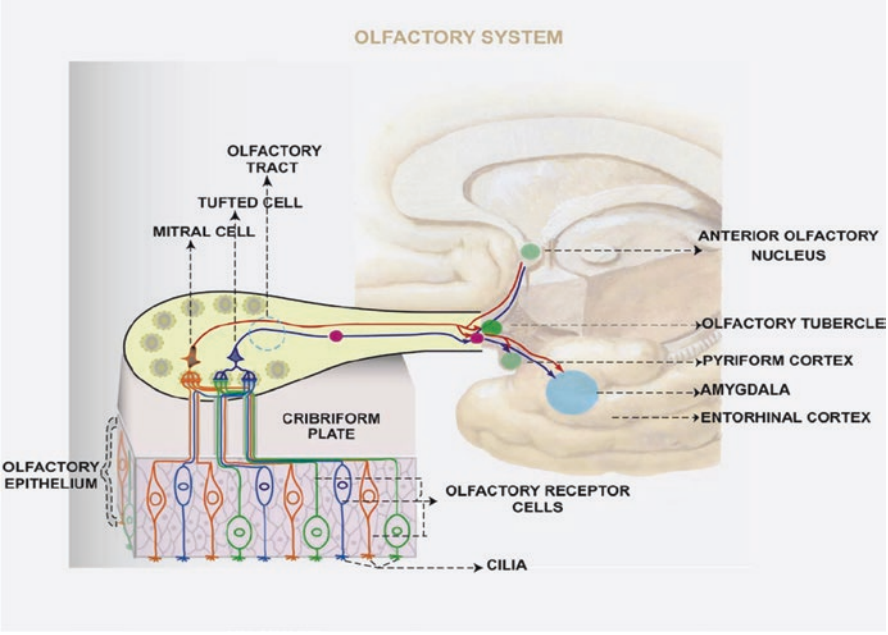

Fig. 15.1 Human olfactory system [5]

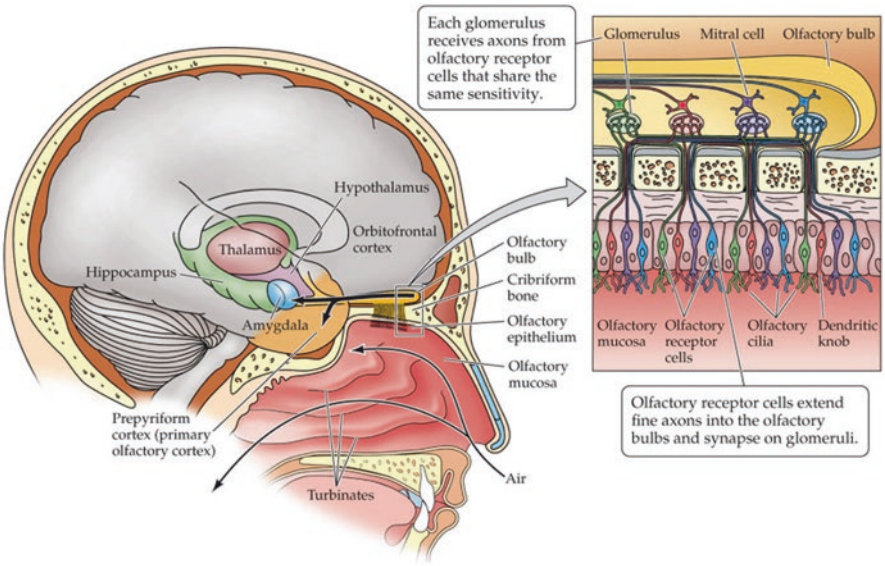

Fig. 15.2 Transmission of odors to human brain [6]

of smell and taste (gustatory system) are often referred to the chemosensory system because they both give information to the brain on the chemical composition and it is considered a process called transduction. The process by which olfactory information is coded in the brain to allow proper perception is still being researched and is not completely understood. When an odorant is detected by receptors, they break the odorant signals, and then the brain puts the odorant back together for identification and perception [7]. The odorant binds to receptors that recognize only a specific functional group or feature of the odorant; therefore, the chemical nature of the odorant is important [8]. After binding the odorant, the receptor is activated and sends a signal to the glomeruli [8]. Each glomerulus receives signals from multiple receptors that detect similar odorant features. Because several receptor types are activated due to the different chemical features of the odorant, several glomeruli are activated as well. All of the signals from the glomeruli are then sent to the brain, where the combination of glomeruli activation encodes the different chemical features of the odorant. The brain then essentially puts the pieces of the activation pattern back together in order to identify and perceive the odorant [8]. This distributed code allows the brain to detect specific odors in mixtures of many background odors [9]. The volatility of odor or aroma is temperature-dependent. High temperature always prefers volatility, whereas low temperature or cold temperature inhibits volatility. The odor sensation can be characterized by various parameters, e.g., pungency, earthy, rancid, leathery, oily, rotten, creamy, and woody, and these are found in different food products.

2.5 *Taste*

Taste sensations are the aggregate of the sensations created by food when it is inserted into the mouth. The sensation of taste is perceived when the taste receptors or taste buds are represented by Fig. 15.3 [10] and shows how taste buds in the tongue are stimulated. The taste buds are generally located on the surface of the tongue. Tongue contains approximately 10,000 taste buds. The tongue is covered with thousands of small bumps called papillae, which are visible to the naked eye. Within each papilla, there are hundreds of taste buds [11]. The exception to this is the filiform papillae that do not contain taste buds. There are between 2000 and 5000 [12] taste buds that are located on the back and front of the tongue. Others are located on the roof, sides and back of the mouth, and in the throat. Each taste bud contains 50 to 100 taste receptor cells. The taste receptor cells within a bud are arranged with their tips, which form a small taste pore and through this pore is extended microvilli from the taste cells. The microvilli of the taste cells bear taste receptors. Interwoven among the taste cells in a taste bud is a network of dendrites of sensory nerves called "taste nerves." When taste cells are stimulated by binding of chemicals to their receptors, they are depolarized, and this depolarization is transmitted to the taste nerve fibers resulting in an action potential that is ultimately transmitted to the brain. One interesting aspect of this nerve transmission is that it rapidly adapts—after the initial stimulus, a strong discharge is seen in the taste nerve fibers, but within a few seconds, that response diminishes to a steady-state level of much lower amplitude. Once taste signals are transmitted to the brain, several efferent neural pathways are activated that are important to digestive function.

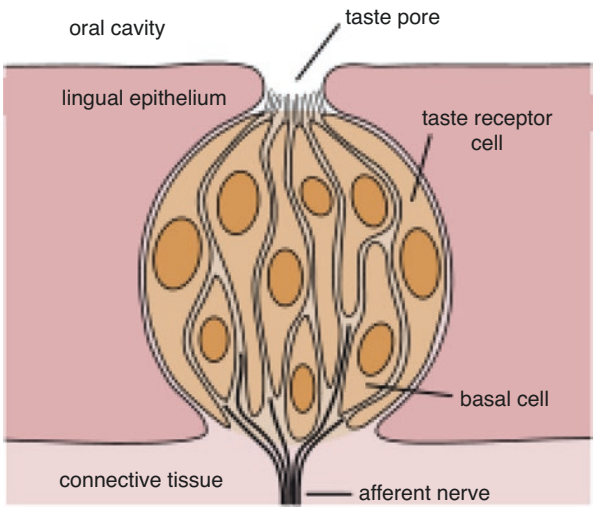

Fig. 15.3 Diagram of taste bud [10]

For example, tasting food is followed rapidly by increased salivation and by low-level secretory activity in the stomach.

Among humans, there is a substantial difference in taste sensitivity. Roughly, one in four people is a "supertaster" that is several times more sensitive to bitter and other tastes than those that taste poorly. Therefore, non-tasters need more salt to have the same level of sensation as supertaster. Such differences are heritable and reflect differences in the number of fungiform papillae and hence taste buds on the tongue. In addition to signal transduction by taste receptor cells, it is also clear that the sense of smell profoundly affects the sensation of taste. Fungiform papillae (FP) are mushroomed-shaped pink structures located on the anterior two-thirds of the tongue with a higher density being present on the tongue tip compared to other areas of the tongue [13]. Fungiform papillae (FP) density is frequently reported to be higher in individuals who are classified as supertasters of the bitter substance 6-n-propylthiouracil (PROP) compared to PROP medium tasters (PMTs) and PROP non-tasters (PNTs) [14–17]. Supertasters generally avoid sugary as well as fatty foods often packed with high calorific values that cause less weight than average and even non-tasters. Super taster also avoids alcohol consumption and smoking due to the fact that the bitter flavors picked up by the tongue's taste buds are far too overpowering to enjoy. Non-tasters like hot spicy foods and usually require more seasoning to make it taste good. This is true except for salt. Because salt masks bitterness, super-tasters tend to consume more sodium than non-tasters [18]. Non-tasters have a clear preference for high-fat and sweeter foods. They also show the greatest alcohol intake and a higher rate of alcoholism [19]. The sensation of taste includes five established basic tastes: sweetness, sourness, saltiness, bitterness, and umami [20, 21] as represented in Fig. 15.4 [22]. Scientific experiments have demonstrated that these five tastes exist and are distinct from one another. Taste buds are able to distinguish between different tastes through detecting interaction with different molecules or ions. Sweet, savory, and bitter tastes are triggered by the binding of molecules to G protein-coupled receptors on the cell membranes of taste buds. Saltiness and sourness are perceived when alkali metal or hydrogen ions enter taste

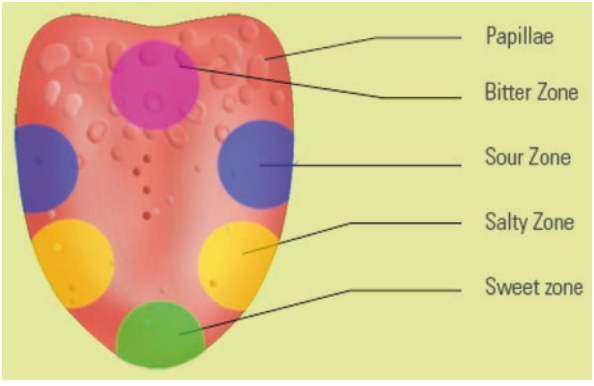

Fig. 15.4 Different taste sensations in taste bud located in tongue [22]

buds, respectively [23]. The basic tastes contribute only partially to the sensation and flavor of food in the mouth—other factors include smell, detected by the olfactory epithelium of the nose [24]; texture [25], detected through a variety of mechanoreceptors and muscle nerves [26]; temperature, detected by thermoreceptors; and "coolness" (such as of menthol) and "hotness" (pungency), through chemesthesis. As taste senses possess both harmful and beneficial attributes, all basic tastes are classified as either aversive or appetitive, depending upon the effect of the attributes they sense [27]. Sweetness helps to identify energy-rich foods, while bitterness serves as a warning sign of poisons [28].

The concentration required for identification is known as the threshold for that particular food material. Generally, the four primary taste sensations, namely, bitter, sour, salty, and sweet, differ in their sensitivity, and the threshold of each taste is conventionally not at the same level with respect to the individual. There is a possible interaction of different tastes when food materials are composed of mixtures of substances. Some threshold concentrations of salt can increase the apparent sweetness of sucrose. Sugar in subthreshold concentration reduces the saltiness of sodium chloride. Therefore, a pinch of sugar may improve the taste characteristics of over-salted vegetable soup. Acid can also increase the sensation of saltiness. There are three main approaches to taste masking [29]: (a) peripheral interactions, where a compound antagonizes a particular taste receptor; (b) central cognitive interactions, where one strong taste or aroma reduces perception of the other in the brain; and (c) encapsulation, where the compound is physically prevented from interaction with the active sites either by modifying solubility or by introducing a barrier [30, 31]. The bitterness of drugs and foods is readily masked by cyclodextrins, e.g., the addition of about 0.5% of cyclodextrin halved the bitterness of naringin and limonene [32]. Milk fat content in an emulsion had a significant effect on suppressing the bitterness of only one of two polyphenolic plant extracts considered [33]. It has been reported [34] that the bitter taste threshold of caffeine was increased in the presence of 1% linoleic acid sonicated with 5% acacia gum solution (pH 4.57). It was found [35] the opposite trend and caffeine in full fat milk (4% milkfat) was more bitter than in reduced-fat or skim milk (2% or 0% fat, respectively).

2.6 Texture

The texture is the tactile sensory and functional manifestation of the structural, namely, molecular, microscopic or macroscopic, mechanical, and surface properties of foods detected through the senses of vision, hearing, touch, and kinesthetics, and odors and tastes are not included. Therefore, texture is a multiparameter attribute. The characteristic textural property is detected by the senses of touch and pressure [36]. Textural characteristics can be designated by the following parameters as discussed in the following sections.

2.6.1 Consistency

All the sensations resulting from stimulation of the mechanical receptors and tactile receptors, especially in the region of the mouth, and varying with the texture of the product.

2.6.2 Hardness and Softness

As a texture characteristic, describes a product that displays substantial resistance (i.e. force) to deformation or breaking. A texture characteristic describes a product that displays slight resistance to deformation.

2.6.3 Tenderness and Firmness

A texture characteristic describes a product that, during mastication, displays little resistance to breaking. A texture characteristic describes a product that during mastication displays moderate resistance to breaking.

2.6.4 Crunchiness

It is the perceived cumulative intensity of force required by repeated incremental failures of the product by chewing up to five times with the molars [37].

3 Overview of Sensory Evaluation

Sensory or organoleptic evaluation of a food product is usually assessed human sensory organs, i.e., subjective. Sensory quality is a combination of different senses of perception during the consumption of food. Sensory evaluation is a process that scientifically elicits, measures, analyses, and interprets psychological and/or physiological responses to physical stimuli produced by a food product. A sensory property can be defined as physiological–psychological perception of a number of physical and other properties of food, including tactile properties, textural properties, color, appearance, taste, odor, and sound [2]. Although many of the instrumental sensory properties can be measured objectively with instruments, these are not sensory properties as psychological components are missing. However, these instrumental measurements could be correlated with sensory attributes and could also be used in the quality evaluation of foods. Rheological behavior, an important instrumental textural characteristic of semisolid foods, namely, jam, jelly, marmalade, tomato sauce, basically depends upon shear stress and shear rate relationship, and these are related to the sensory texture.

4 Sensory Evaluation Methods

Various methods may be used to measure the sensory attributes of foods [38]. Sensory evaluation can be divided into two areas, like analytic and hedonic. In analytic testing, the sensory attributes of a product are evaluated by a selected and trained panel. In hedonic, the reactions of consumers to the sensory properties of products are measured. The full potential of sensory evaluation is realized when sensory, consumer, and/or instrumental analyses are combined. Properly conducted sensory evaluations can lead to improved decision-making with the involvement of less risk while targeting and achieving the goals and a way of categorizing attributes.

The selection of a sensory evaluation method is determined based on the type of information that is needed. The discrimination and description of both qualitative and quantitative sensory parameters of food products assessed by trained panels involve the sensory descriptive analysis [39, 40]. In this regard, the sensory descriptive analysis method can be applied in various ways, e.g., Flavor Profile [41], Texture Profile [42], Spectrum™ method [39], and Quantitative Descriptive Analysis (QDA) [43]. The scientific background of sensory evaluation requires an understanding of sensory test methods, their proper application to sensory questions and sensory attributes, and their proper use in describing the appearance, flavor, and texture properties of samples and products [44]. Therefore, the chief objective of the sensory analysis is to determine the attributes and their relationship with consumer acceptance.

The sensory evaluation process is very much useful to fulfill various purposes with regard to consumers' acceptability. It can identify the similarities/differences in a group of food products and able to evaluate an existing food product against benchmark sample. The improvements in the food products could be performed based on market feedback. Specific response to a food sample whether acceptable or not by consumers will be able to be elicited. It is also helpful to study a particular property in an ingredient or a food product and can be able to evaluate if a ready food product meets its original specification/standard sample. Product information interacts in complex ways with consumer attitudes and expectancies [45–54]. Expectations can cause assimilation of sensory reactions toward what is expected under some conditions and under other conditions will show contrast effects, enhancing differences when expectations are not met [55–58]. Packaging and brand information will also affect sensory judgments [59–61]. The method is very much effective to obtain feedback data for decision-making and to carry out suitable modifications in food as well as processed food products. Different types of sensory tests have been devised to fulfill a number of specific objectives. These tests are grouped into mainly four categories, e.g., (a) difference or discrimination tests, (b) preference tests or rating tests, (c) sensitivity tests, and (d) descriptive tests.

5 Difference or Discrimination Tests

The difference test is one of the most useful sensory tests and is sometimes called the discrimination test. It is generally used for the selection and training of sensory panelists. A difference test is designed or used to detect small discrimination or difference between two or more samples. This test consists of a variety of specific methods, namely, paired comparison, duo-trio, triangle, and multiple sample tests [62].

5.1 Paired Comparison Test

This test is useful when two types of the same food, e.g., baked beans, yogurt, and juice are compared. The test is called as directional difference test, where different attributes, such as sweetness, acidity, hardness, and color, are responsible for this difference. The paired comparison test is relatively easy to organize and implement. There are two different types of paired comparison tests, e.g., simple difference paired comparison test and directional paired comparison test.

5.1.1 Simple Difference Paired Comparison Test

In this test, the tester or panelist is presented with two coded samples, and the panelist has to decide if there is any difference or not. The two coded samples are generally represented by AA, BB, AB, and BA, where A &B are two products and are served simultaneously (Tables 15.1 and 15.2).

Table 15.1 Scorecard for simple difference paired comparison test

Product…… Name ……..……
Date..... Time.....
You are presented with two coded samples. Please taste the samples in the order given. Can you detect a difference between the samples?
Yes_____No_____

Table 15.2 Record sheet for simple difference paired comparison test

Food Product A: _____

Food Product B: _____

When recording results, transfer responses from the scorecards by indicating whether testers answered Yes or No. Tick ✓ those that are correct.

			Response	**✓ If Correct**
Tester 1				
Food product	A	B		
Code	□	○		
Tester 2				
Food product	B	A		
Code	□	○		
Tester 3				
Food product	A	B		
Code	□	○		
Tester 4				
Food product	B	A		
Code	□	○		
Tester 5				
Food sample	A	B		
Code	□	○		
Tester 6				
Food product	B	A		
Code	□	○		
Total number of correct responses				

5.1.2 Directional Paired Comparison Test

In this test, the tester or panelist is presented with different samples in each pair, and the panelist or tester is asked to determine which of the samples has a greater degree of intensity in terms of a particular characteristic, e.g., sweetness, bitterness, saltiness, or rancidity [63]. The two odd samples are generally represented by AA, BB, AB, and BA, where A & B are two products and are served simultaneously (Tables 15.3 and 15.4).

5.2 Duo-Trio Test

The duo-trio test is an alternative to the triangle test. This test is also considered a modified paired comparison test. This test employs three samples where two are identical and one is different. One sample was identified as the reference (R), which is the first one given to the panelists for evaluation [64]. Subsequently, two coded samples, one of which is identical to reference, are presented. The panelist is asked to match which of the two samples is the same as "R." The test is suitable for products that have a relatively intense odor, taste, and/or kinesthetic effects such that the sensitivity of the evaluator is significantly reduced. In industry, the reference is normally the product currently being manufactured (Tables 15.5 and 15.6).

5.3 Triangle Test

The Triangle test is the most well-known and more frequently used out of the three different tests. The triangle test is used to see if there is a detectable difference between two similar products [65]. This test employs three samples where two are identical and one is different or odd. Here, the panelist's task to determine which two are most similar or which one is most different from the other two. Two food

Table 15.3 Scorecard for directional paired comparison test

Product…… Name ……..……
Date..... Time.....
In front of you are two coded samples.
Starting with the sample on the left, taste each sample and circle the sample that is _____
You must make a choice. You may re-taste as often as you wish.
□○

Table 15.4 Record sheet for directional paired comparison test

Food Product A: _____

Food Product B: _____

When recording results, circle the letter that corresponds with the symbol selected on the scorecard. Tick ✓ the correct responses.

			✓ **If Correct**
Tester 1			
Food product	A	B	
Code	□	○	
Tester 2			
Food product	B	A	
Code	□	○	
Tester 3			
Food product	A	B	
Code	□	○	
Tester 4			
Food product	B	A	
Code	□	○	
Tester 5			
Food product	A	B	
Code	□	○	
Tester 6			
Food product	B	A	
Code	□	○	
Total number of correct responses			

products A and B can be represented in two combinations AAB and BBA and can be arranged in six different combinations, namely, AAB, ABA, BAA, BAB, ABB, and BBA. The Triangle test is more difficult because the panelist must recall the sensory characteristics of two products before evaluating the third and then make a decision (Tables 15.7 and 15.8).

Table 15.5 Scorecard for duo-trio test

Product…… Name ……..……
Date..... Time.....
You are presented with three samples, one marked R and two other coded samples. Starting from the left, taste the R sample followed by the two coded samples in the order given. Circle the sample that is different from R. You may retaste the samples. You must make a choice.
R ○∗

5.4 Multiple Sample Test

Test involving more than three stimuli are classified as multiple sample tests. They may have equal (symmetrical) or unequal (asymmetrical) numbers of each stimulus. When they are applied as true difference tests, the judge is required to separate the sample into two groups of like samples. When they are applied as directional tests, the judge is asked to identify the groups of higher or lower intensity of a given criterion.

6 Preference/Acceptance Tests/Rating Tests

Affective or acceptance testing is a sensory technique usually performed at consumer's levels. It refers to measuring liking or preference for a product. Preference can be measured directly by the comparison of two or more products with each other. Indirect measurement of preference is achieved by determining which product has scored significantly higher rating than another product in a multiproduct test. This method can be classified into six methods, namely, (a) Paired preference test, (b) Hedonic rating test, (c) Ranking test, (d) Food action rating test, (e) Numerical scoring test, and (f) Composite scoring test [66–74].

Table 15.6 Record sheet for duo-trio test

Food Product A: _____

Food Product B: _____

When recording the results circle the letter that corresponds with the symbol selected on each scorecard.

Tick ✓the appropriate column if the tester correctly identified the sample that was *different* from R.

				✓**If Correct**
Tester 1				
Food product	A	A	B	
Code	R	○	✳	
Tester 2				
Food product	A	B	A	
Code	R	○	✳	
Tester 3				
Food product	A	A	B	
Code	R	○	✳	
Tester 4				
Food product	A	B	A	
Code	R	○	✳	
Tester 5				
Food product	A	A	B	
Code	R	○	✳	
Tester 6				
Food product	A	B	A	
Code	R	○	✳	
Total number of correct responses				

6.1 Paired Preference Test

A paired preference test is generally used to express a preference between two products. Two coded samples or food products, e.g., A and B are presented with a tester or panel expert. Finally, the tester decides about the preference level. The score card and record sheets are indicated below (Tables 15.9 and 15.10).

Table 15.7 Scorecard for triangle test

Product…… Name ……..……
Date..... Time.....
In front of you are three coded samples, two are the same and one is different.
Starting from the left, taste the samples and circle the one that is different from the other two.
You may re-taste the samples. You must make a choice.
□ ○ ✳

6.2 Hedonic Rating Test

Hedonic rating tests can be used to measure consumer acceptability in terms of the extent of liking or disliking food products. The term hedonic means having to do with pleasurable or unpleasurable experiences. This test can rate the acceptability of a food product on a scale usually of nine points ranging from like extremely to dislike extremely although five points ranging from like extremely to dislike extremely can also be considered. The score card and record sheets are indicated below (Tables 15.11 and 15.12).

6.3 Ranking Test

Ranking tests or preference ranking tests are used to rank foods in order of preference based on a single characteristic. They are used when two or more samples are being tested simultaneously with code numbers. The number of samples used is dependent on the tester's attention span and memory. The score card and record sheets are presented below (Tables 15.13 and 15.14).

6.4 Food Action Rating Test

The food action rating test is one of the important tests for sensory evaluation. In a food action rating test, a scale is used to determine the attitudes of testers or panelists to a food product. It is often referred to as a "FACT Scale." The test can be carried out on one or multiple sample of food products. The score card and record sheets are presented below (Tables 15.15 and 15.16).

Table 15.8 Record sheet for triangle test

Food Product A:_____

Food Product B:_____

When recording the results circle the letter that corresponds with the symbol selected on each scorecard.

Tick ✓ the appropriate column if the tester correctly identified the sample that was different.

				✓ If
Tester 1				
Food product	A	B	A	
Code	□	○	∗	
Tester 2				
Food product	A	A	B	
Code	□	○	∗	
Tester 3				
Food product	B	B	A	
Code	□	○	∗	
Tester 4				
Food product	B	A	B	
Code	□	○	∗	
Tester 5				
Food product	A	B	B	
Code	□	○	∗	
Tester 6				
Food product	B	A	A	
Code	□	○	∗	
Total number of correct responses				

6.5 Numerical Scoring Test

The numerical scoring test is another potential technique of judging consumers' acceptability. In this test, one or more samples are presented to each tester in random order. The rating of the sample is usually carried out based on particular characteristics on a specific scale. A suitable understanding of sensory characteristics to the tester always results in the success of the test (Table 15.17).

Table 15.9 Scorecard for paired preference test

Product…… Name …… Date..... Time..... In front of you are two coded samples. Taste each sample and tick ✓ the sample that you prefer. □○

Table 15.10 Record sheet for paired preference test

Food Product	Ticks	Total Number of Ticks
□		
○		

6.6 Composite Scoring Test

In the composite scoring test, the specific characteristic of a product is separately judged. The resultant scores are compounded for any one panelist to achieve a composite score. This method is very much useful in judging product gradation and comparison of food quality factors based upon the indicative characteristics in a poor-grade product. The method is so informative and a good alternative to the numerical scoring technique. Here, the tester or panelist is capable to judge the individual quality characteristics (Table 15.18).

7 Sensitivity Tests

Sensitivity tests are based upon the perception of the individual to identify different sensory attributes, e.g., color, flavor, taste, and mouthfeelness. These tests are basically used to select and train panelists for evaluating the quality of food products, e.g., spices, pickles, and tomato ketchup. In this test, the panel members/panelists/testers must have the ability to sense very accurately the sensory parameters in evaluating food quality, and it is classified into two categories: (a) Sensitivity-threshold test and (b) Dilution test.

Table 15.11 Scorecard for hedonic rating scale

Product …… Name…….
Date..... Time.....

In front of you are three coded samples. Taste each sample and tick ✓ how much you like or dislike it.

	□	○	∗
Like a lot	_____	_____	_____
Like very much	_____	_____	_____
Like moderately	_____	_____	_____
Like slightly	_____	_____	_____
Neither like neither dislike	_____	_____	_____
Dislike slightly	_____	_____	_____
Dislike moderately	_____	_____	_____
Dislike very much	_____	_____	_____
Dislike extremely	_____	_____	_____

Table 15.12 Record sheet for hedonic rating scale

Food Product □_____

Food Product ○_____

Food Product ∗_____

Score Value Assigned:

Like extremely=9 Like very much=8 Like moderately=7 Like slightly=6 Neither like nor dislike = 5 Dislike slightly=4 Dislike moderately=3 Dislike very much dislike=2 Dislike extremely=1

Food Product	**No. of Testers/Panelists**					**Total Score**	**Average Score** (total score ÷ number of testers/panelists)
	1	**2**	**3**	**4**	**5**		
□							
○							
∗							

Table 15.13 Scorecard for ranking test

Product…… Name ……..……

Date..... Time.....

In front of you are three coded samples. Taste each sample.

Please indicate your preference by placing:

1st choice beside the sample that you prefer most

2nd choice beside your next preference

3rd choice beside the one you least prefer.

□ _____ ○ _____ ∗ _____

Table 15.14 Record sheet for ranking test

Food Product □_____

Food Product ○_____

Food Product ∗_____

For each tester place a tick ✓ in the box that corresponds to their choice for that product.

Score Value Assigned:

1st choice give 3 points

2nd choice give 2 points

3rd choice give 1 point

Food Product	1st choice	2nd choice	3rd choice	Score	Rank Order
□					
○					
∗					

7.1 Sensitivity Threshold Test

Sensitivity tests are used to measure the ability of an individual to smell, taste, or realize the specific characteristics of food products for evaluation. Defining and measuring sensory thresholds requires setting the sensitivity limit such that the perception observations lead to the absolute threshold. The threshold is defined as a statistically determined point on the stimulus scale at which a transition in a series

Table 15.15 Scorecard for food action rating test

Product……	Name ……
Date.....	Time.....

You are presented with a food sample.

Please taste the sample and tick ✓ the box that best describes how you feel about it.

- ☐ I would eat this every opportunity that I had
- ☐ I would eat this very often
- ☐ I like this and would eat it now and then
- ☐ I would eat this if available but would not go out of my way
- ☐ I don't like this but would eat it on occasion
- ☐ I would hardly ever eat this
- ☐ I would eat this only if forced to

Table 15.16 Record sheet for food action rating test

Action	**Total Ticks**
I would eat this every opportunity that I had	
I would eat this very often	
I like this and would eat it now and then	
I would eat this if available but would not go out of my way	
I don't like this but would eat it on occasion	
I would hardly ever eat this	
I would eat this only if forced to	

of sensations or judgments occurs [75]. The level of sensitivity is usually assumed to be constant in determining the threshold limit. There are three common methods used to determine sensory thresholds [76].

7.1.1 Method of Limits

In the first step, the subject is stimulated by strong, easily detectable stimuli that are decreased stepwise (descending sequence) until panel members cannot detect the stimulus. Then, another stimulation sequence is applied called ascending sequence. In this sequence, stimulus intensity increases from subthreshold to easily detectable. Both sequences are repeated several times. This yields several momentary threshold values. In the following step, mean values are calculated for ascending and

Table 15.17 Scorecard numerical scoring test

Product…….....Name ……

Date..... Time.....

Rate these samples according to the following descriptions

Score	Quality description
90	Excellent
80	Good
70	Fair
60	Poor

Sample	Score	Comments

Table 15.18 Scorecard for composite scoring test

Product …… Name…….

Date..... Time.....

Quality	Possible score	□	○	∗
		Sample scores		
Colour	20	_____	_____	_____
Consistency	20	_____	_____	_____
Flavour	40	_____	_____	_____
Absence of defects	20	_____	_____	_____
Total scores	100	_____	_____	_____

Comments: Signature

descending sequences separately. The mean value will be lower for descending sequences. In the case of audiometry, the difference of the means in the case of ascending vs. descending sequences has diagnostic importance. In the final step, the average of the previously calculated means will result in the absolute threshold.

7.1.2 Method of Constant Stimuli

Stimuli of varying intensities are presented in random order to a subject. Intensities involve stimuli that are surely subthreshold and stimuli that are surely supra-threshold. For the creation of the series, the approximate threshold is judged by a simpler method (i.e., by the method of limits). The random sequences are presented

to the subject several times. The strength of the stimulus, perceived in more than half of the presentations, is taken as the threshold.

7.1.3 Adaptive Method

Stimulation starts with a surely supra-threshold stimulus; then further stimuli are given with an intensity decreased in previously defined steps. The series is stopped when the stimulus strength becomes subthreshold (this is called the turn phenomena). Then, the step is halved and the stimulation is repeated, but now with increasing intensities, until the subject perceives the sound again. This process is repeated several times until the step size reaches the preset minimum value. With this method, the threshold value can be delineated very accurately. The initial size of the step can be selected depending on the expected accuracy.

The recognition threshold tests with basic tastes, odors are most frequently employed for the selection of panel. A universal absolute threshold is difficult to define a standard because of the variability in the measurements [77]. While sensation occurs at the physical nerves, there can be reasons why it is not consistent. Age [78] or nerve damage can affect sensation. Similarly, psychological factors can affect the perception of physical sensation. Mental state, memory, mental illness [79], fatigue [80], and other factors can alter perception. The specimen evaluation card for the sensitivity threshold test will be represented below (Table 15.19).

7.2 Dilution Test

The dilution test is generally designated to determine the smallest amount of unknown test material that can be detected when it is mixed with standard food products, e.g., spray dried milk powder in liquid toned milk, synthetic flavor, or color in natural jelly, jam, and marmalade. The quality of the test material is designated by the dilution number which is none, but the percentage of test material in the mixture of the standard food product in such a way that a fine difference in odor and taste exists. The data are evaluated using the evaluation card by the judgment of the tester or panelist. The specimen evaluation card for the dilution test is represented below (Table 15.20).

8 Descriptive Tests

Descriptive analysis is a sensory methodology that provides quantitative descriptions of products based on the perceptions of a group of qualified subjects. It is a total sensory description taking into account all sensations that are perceived (e.g., visual, auditory, olfactory, and kinaesthetic) when the product is evaluated. The

Table 15.19 Scorecard for sensitivity threshold test

Product …… Name……
Date…..... Time…....

You receive a series of samples with increasing concentrations of one of the 4 taste qualities (sweet, salty, sour, bitter)*.
Start with Samples No. 1 and continue with Samples No. 2, No. 3, etc.
Retasting of already tested solutions is not allowed.
Describes the taste* and the feeling factors and give intensity scores.

Use the following intensity scale:

0 = None or pure water taste
? = Different from water, but taste quality not identifiable
X = Threshold very weak (identify the taste)
1 = Weak
2 = Medium
3 = Strong
4 = Very strong
5 = Extremely strong

Sample No.	**Description of Taste and Feeling Factors**
1.	_____
2.	_____
3.	_____
4.	_____
5.	_____
6.	_____
7.	_____
8.	_____
9.	_____
10.	_____

Comments Signature

Table 15.20 Scorecard for dilution test

Product …… Name……
Date..... Time.....

Quality factors	Possible score	□	○	∗
		Sample scores		
Colour	20	_____	_____	_____
Consistency	20	_____	_____	_____
Flavour	40	_____	_____	_____
Absence of defects	20	_____	_____	_____
Total scores	100	_____	_____	_____

Comments: Signature

descriptive analysis provides complete sensory descriptions of an array of products and provides a basis for determining those sensory attributes that are important to acceptance. The results enable one to relate specific process variables to specific changes in some of the sensory attributes of a product. From the product development viewpoint, descriptive information is essential in finding out those product variables that are different and from which one can establish cause and effect relationships. Screening should be product category-specific as is the subsequent training effort. Training is primarily focused on the development of descriptive language which is used as a basic form of scoring the product. Apart from this, the other important activities that are part of training include the grouping of attributes by modality (i.e., appearance attributes, aroma attributes, and so on), them by occurrence, developing a definition for each attribute, identifying helpful references for use during training, and familiarizing the subjects with the scoring procedure. There are numerous applications for descriptive analysis, including monitoring competitions, storage stability/shelf life, product development, quality control, physical/chemical, and sensory correlation. Depending upon the test methods used, the training can be quite different.

8.1 Flavor Profile Method

The flavor profile method is the only formal qualitative descriptive procedure and is probably the most well-known of sensory test methods. This method utilizes a panel of four to six screened and selected subjects who first examine and then discuss the product in an open session. Once agreement is reached on the description of the product, the panel leader summarizes the results in report form. The method has considerable appeal because results could be obtained rapidly and would obviate the need for statistics.

8.2 Texture Profile Method

This method represents an advancement in descriptive analysis with respect to the development of descriptive terminology, the scales for recording intensities, and the word/product anchors for each scale category. In developing the method, the objective is to eliminate problems of subject variability and to allow direct comparison of results with known materials, and provide a relationship with instrument measures. There is considerable appeal to the direct link between specific instrumental measures of these rheological properties of a product and the responses of a panel of specific sensory attributes, for example, texturometer units and hardness sensory rating. However, separation of texture from other sensory properties of a product such as color, aroma, tastes, and so forth limits the total perception of the product's sensory properties.

8.3 Quantitative Descriptive Analysis

The quantitative descriptive analysis (QDA) method was developed with an approach that is primarily behavioral in orientation with a consensus approach to language development, use of replication for assessing subject and attribute sensitivity, and for identifying specific product differences and defined statistical analysis. The method possesses the following criteria: (a) it should be responsive to all sensory qualities of food products, (b) analytical process should rely on a limited number of subjects for each test, (c) it utilizes the parameters which will qualify before participation, (d) it evaluates multiple product individual booths, (e) it should be quantitative and use a repeated trials design, and (f) method should have a useful data analysis system. In a QDA test, the subjects evaluate all the products on an attribute-by-attribute basis on more than a single occasion.

9 Other Methods

The spectrum descriptive analysis involves extensive training activities, reflecting the basic Flavour and Texture Profile procedures, with particular reliance on training the subjects with specific standards of specified intensities. Free choice profiling is another approach in which no subject screening or training is required, and the subject can use any words they want to describe the products being evaluated. The time advantage may, however, require the experimenter to spend time explaining the testing procedures to the subject.

10 Sensory Panels

Sensory tests are well integrated with the overall plan of development of the product. It produces reliable results only when good experimental control is exercised at each step of the testing process with the active assistance of sensory panels. Careful planning and thorough standardization of all procedures should be done before the actual testing begins. Particular attention should be given to the selection of panelists, suitable design of testing laboratory, sampling food materials, preparing and presenting samples to the panel for assessing sensory attributes, testing time and experimental design, and using reference and control samples and sensory evaluation card. The performance of the panel is monitored in terms of discrimination power, the agreement between panelists, and reproducibility during training to achieve the most accurate, reliable, and consistent results possible. However, training panelists are quite costly, and some new methods could be used by utilizing untrained panelists [81].

10.1 Selection of Panel Members/Panelists

The panelists are people who test the food and judge it. A panelist can be one person or several hundred. It depends on the type of sensory method. The panel members analyze food products through properly planned experiments and their opinions are expressed by appropriate analysis. The panelists can be classified into three groups, namely, trained panelists, semi-trained panelists, and untrained panelists. Careful training for specific products is a must for the trained panelist. Periodically, the panel undergoes refresher training and tests. The number of members for trained panelists generally vary from 5 to 10. Discriminative, communicative, or semi-trained panelists are normally technical people familiar with the qualities of different types of foods. Conventionally, 15 to 20 people are preferred for testing purposes. Untrained panelists are also known as consumer panelists. A group of a minimum of 100 people is considered for assessing the food quality as a consumer preference test. The more the number, the more will be the reliability as well as dependability of the result [81–83].

10.2 Quality of the Pannel Members

The ideal panel member should have the following criteria and they should have (a) good health; (b) no smoking habits; (c) able to discriminate easily between samples and distinguish between taste and smell; (d) good sensory memory, sound mental condition, and motivational power; (e) positive attitude and lack of prejudice; (f) thoroughness and good analyzing and synthesizing ability; (g) conscientiousness and willingness to spend time for sensory analysis; (h) solid character and prudence; (i) disciplined, able to concentrate, and derive an appropriate conclusion; (j) ability to suppress emotional factors; (k) availability and willingness to carry out the periodic test for a consistent result; (l) associative and have good selection skills; (m) quality of perseverance to tolerate criticism; and (n) critical and self-critical approach ability [84–88].

11 Sensory Facilities

11.1 Sensory Analysis Booth/Testing Laboratory

A sensory analysis booth or testing laboratory requires some basic facilities if reliable and efficient tests need to be achieved. The basic requirements for all sensory testing facilities consist of five separate units: (a) a food preparation area, (b) a separate panel discussion area, (c) a panel booth area, (d) a desk or office area for the panel leader, and (e) supplies for preparing and serving samples.

11.2 Food/Sample Preparation Area

The area for sample or food preparation needs some basic requirements includes adequate space for counters, sinks, cooking equipment and utensils, refrigerator, storage, and ventilation. The sample preparation area should be clean enough to conduct the sensory test.

11.3 Panel Discussion Area

Food product testing needs a room where the panelists can meet with the panel head for necessary instruction, training, and discussion. This discussion area should be completely separate from the food or sample preparation area so that noise and cooking odors do not interfere with the panelists' works. It should be located such that there are no interruptions from other laboratory personnel. A comfortable well-lit area, with a large table and chairs or stools to seat at least ten people, is ideal. A large chalkboard, flipchart, or whiteboard should be located where it can be easily seen by the panelists around the table. A bulletin board located close to the entrance allows the posting of notices and information about panelists' performance [44, 84].

11.4 Panel Booth Area

The booth area should be completely separate from the food or sample preparation area. It is always suggested to have a self-contained panel booth room, areas can be combined by having the booths constructed along one wall of the group discussion room, with no dividing wall between the booth and discussion areas. The panel booth area should contain individual compartments, where panelists can assess samples without influence by other panel members. Generally, five or ten individual sections are most common. Each booth should be equipped with a counter, a tool or chair, a pass-through opening to the food preparation area, and individual lighting and electrical outlets. It is useful to have the entrance to the panel area within a partial view of the food preparation facilities.

11.5 Office Area

A separate space is required for panel leaders or experts to prepare ballots and reports, data analysis, and storage of data. This area should be equipped with a desk, a filing cabinet, and desktop computer equipped with a statistical program for data analysis.

11.6 Supplies for Preparing and Serving Samples for Sensory Testing

There should be good and adequate supply facilities of necessary utensils and glass vessels for preparing and serving samples for sensory evaluation to be carried out by panelists. All utensils should be made of materials not be able to transfer odors or flavors to the prepared foods or samples. Food preparation and serving equipment, utensils, and glassware for the sensory testing area should be purchased new, sufficient in quantities, and used exclusively for sensory testing.

11.7 Sampling Food Materials for Sensory Evaluation

All food materials presented to the panelists for testing must be safe for consumption. Panelists should not be instructed to taste or eat moldy food or microbiologically as well as chemically contaminated food. Now, only the odor and appearance attributes of the food can be evaluated if a food, or an ingredient of the food, has been treated or stored in a way that may make it unsafe for consumption.

11.8 Preparation of Samples for Assessing Sensory Attributes

Samples for sensory comparison should all be prepared by a standardized method to eliminate the possibility of preparation effects. A careful sampling of the food is necessary for sensory evaluation. Preparation steps should be standardized during preliminary testing and documented before the beginning of sensory testing, to ensure uniformity during each testing period. Now samples to be tested should be at the same specified temperature and kept constant.

11.9 Testing Time and Design of Experiment

Testing time is an important factor in determining the sensory quality of food. Generally, testing time should be considered when panelists are fresh and cool in mind. A suitable and ambient environment always influences the evaluation of sensory characteristics. Morning time of around 9 to 10 is generally preferred. At a time, a maximum of 4 to 5 samples can be allowed; otherwise, testing of so many samples may lead to an erroneous result. Experimental design or presentation of samples are carried out with 3 to 5 digit code marking to secure the identity of the samples. The presentation sequence of samples for assessing sensory attributes

should be randomized within the interval of each session to minimize the experimental error. Statistical design is usually preferred to determine the variables and to achieve significant results.

11.10 Use of Reference Samples

Reference samples are often used in sensory testing. These designated samples, against which all other samples are to be compared; or they can be identified samples used to mark the points on a measurement scale; or they can be hidden references, coded and served to panelists with the experimental samples in order to check panelist performance. Reference samples are often called standards. These references may be of food similar to that being tested or maybe totally different. A reference will improve panel consistency only if the reference itself is consistent.

11.11 Testing Procedures

Samples to be tested should be homogeneous in nature. Food materials are presented with 3 to 5 digit code marking to obscure the identity of the sample. Panel members finally judge the sensory attributes, e.g., odor, taste, texture, etc., by means of subjective evaluation or assessment in the sensory chamber. A special technique is used to perceive the aroma for the testing of the odor of food products. Smelling is done with a short interval and rapid sequence of sniffs. Tasting of the food product, e.g., coffee or tea or fruit juice, is done generally by slurping. One teaspoon of the liquid is rolled on the tongue so that the liquid reaches all parts of the tongue where the taste buds are located. Conventionally, rinsing of mouth is required between every testing operation while evaluating the taste and flavor of food items, e.g., dahi, fruit juice concentrate, squash, jam, jelly, marmalade, tomato sauce, etc. Therefore, the panel members contribute a significant role to assess the sensory quality of food products in terms of sensitivity and consistency during testing operations.

11.12 Sensory Evaluation Card

The questionnaire or scorecard should be prepared carefully for each test. The card should be clearly typed or printed. It should be simple and use unambiguous terms and directions in the desired sequence of action as a guide to the evaluation. The design of scorecards for sensory evaluation is challenging and difficult because the key characteristics of the product need to be evaluated on

paper in a way that permits the judges to transmit their assessments of the samples accurately to the researcher. A scorecard with too much detail and clutter may discourage careful judgment and too brief a form may fail to obtain some important information. A scorecard may be as simple as indicating which sample is different as is done when duo-trio or triangle testing is the mode being used. A sheet for indicating rank order for a single characteristic also is extremely simple. It is in the descriptive tests that the scorecard becomes a critical part of the planning for an experiment. A table utilizing the hedonic ratings ranging from unacceptable to very acceptable is relatively easy to construct. No single scorecard fits all experiments. Instead, the scorecard needs to be developed for the specific experiment. All scorecards should contain the date and name of the judge [89].

12 Applicability of Sensory Evaluation

Sensory evaluation techniques can be suitably applied for the commercial development of processed food products as well as for branding new food products. Monitoring of sensory evaluation during processing stages can give important information to the process industry. Moreover, the process of sensory evaluation can suitably assess the product preparation strategy based upon either batch or continuous mode. Consumers' acceptability of a new food product can always be determined by sensory evaluation techniques. Statistical quality control (SQC) of sensory data is very much useful in sensory science, and it can be suitably employed to judge the quality of a newly developed food product.

13 Conclusion

The need for sensory science in food quality evaluation is growing rapidly. Human sensory organs, e.g., nose, eye, ear, and tongue, are very much sensitive for qualitative evaluation of food as well as processed food products. Subjective or organoleptic evaluation of food quality plays an important role in the food processing industry. The sensory department apart from the quality assurance division is an integral part of every food industry. In this regard, the trained panelist or tester has a vital role to determine the overall acceptability of the product with respect to sensory quality attributes, namely, color, flavor, taste, and touch for consumers' acceptance. Sensory research is delivering increasingly sophisticated techniques and a better understanding of consumer perception and behavior using a multidisciplinary approach by linking with fields such as physicochemistry, psychophysics, psychology, physiology, neuroscience, and genomics [90]. It is interesting that the ability of organoleptic sensations for evaluation of sensory attributes of food has a marked difference

with instrumental assessment, although the necessity of both these two methods is equally important given the growing need for product development by the food manufacturing organizations. Although objective evaluation is essential for routine quality control, subjective or sensory evaluation is essential for product development and good marketing of new food products [91]. Therefore, the requirement of evaluation of food quality attributes in terms of overall acceptability, consumers' satisfaction, and market demand proves the importance of sensory studies in the current socioeconomic scenario.

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Chapter 16
Quality Assessment of Milk by Sensory and Instrument Methods

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Abstract Heat and other processing treatments can adversely affect the sensory characteristic of milk and dairy products. Taste and aroma are the most affected and studied attributes in milk. This work reviewed the human sensory tests as well as the instrumental development in this field and their application in milk. For many years, human sensory-like discrimination (e.g., triangle and Duo-trio) and descriptive methods (e.g., quantitative descriptive analysis) have been used for milk assessment. The discrimination tests give general differences between samples, whereas descriptive methods give a more detailed description of the attributes and their intensity which is mostly measured by a 15-line scale. Affective methods were also applied to assess consumer acceptability using the hedonic scale (e.g., 9-point scale). Special facial scale successfully used to assess children acceptability instead of verbal scale. Application of GC/O, e-nose, and e-tongue in fluid milk assessment gives a more clear explanation of the flavor cause, development, and its impact character compounds. The e-nose and e-tongue showed comparable results with human sensory tests. Future enhancement and development in sensors can improve the response to cover a wide range of chemical compounds.

Keywords Fluid milk · Sensory · E-nose · E-tongue · Milk oxidation · Sensory evaluation · Chemical analysis · Gas chromatography · GC olfactory · Artificial sensors · Electronic nose · Electronic tongue

1 Introduction

Milk is highly perishable, and it needs further processing, such as pasteurization. In addition, milk is preserved by processing it to other food products, such as milk powder and fermented products (e.g., cheese, yogurt, buttermilk, sour cream, kefir,

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koumiss, and acidophilus milk). In addition, many by-products could be developed, such as whey powder and lactose from the waste of cheese-processing plants. These by-products are being used in the products, such as infant formula, bakery items, chocolate, and confectionary.

Around 42.9% of milk is used as fresh milk, 23.1% is used for butter and ghee production, 25.2% is used for cheese production, and 5.1% and 3.7% are used for skim and whole milk powder [1]. The production of butter, skim milk powder, whole milk powder, and cheese was expected to grow at 1.9, 1.3, 1.2, and 1.2% per capita, respectively, until 2028 [2]. The FAO expected that the consumption of dairy products would be increased (2018–2028). Improving the current commercial products or developing new products in the dairy sector requires maintaining their sensory quality. The sensory attributes are commonly measured by a sensory panel, and often these are related to instrumental techniques such as sensors. These easy and fast instrumental methods could be easily used in quality control in production line.

Sensory analyses are used to evaluate the freshness and off-flavors of foods as well as to determine the acceptability. In milk, smell/aroma is an initial attribute to evaluate the initial spoilage. However, safety assessment is not recommended to assess by sensory. Off-flavors, such as unclean, putrid, and fruity, in milk could be developed because of its microbial activity. This includes thermal-resistant lipases or proteases from psychotropic bacteria, and this can cause bitterness of dairy products and physical changes, like coagulation. Thermal treatment can adversely affect the odor and taste of milk and consequently can affect the consumer acceptability (e.g., development of cooked flavor in UHT milk).

The sensory test can be used for many purposes, including quality assessment, determination of acceptability, and failure during milk processing. The sensory test can be applied in many methodologies ranging from simple tests by untrained or trained panelists to complex consumer preference by survey methods [3]. Instrumental methods (such as electronic nose and tongue) are being developed to selected limitations of the sensory methods. Furthermore, gas chromatography (GC) and GC/olfactometry (GC/O) are also essential to identify the volatile organic compounds (VOCs) in milk to determine the active key compounds, which can be correlated with the human sensory test. This chapter presents different human sensory analysis methods as well as different interments used for quality evaluation of milk, especially flavor and odors.

2 Raw Milk Quality

Good-quality raw milk is required for good quality milk products. Therefore, milk used for pasteurization should pass the quality control tests (i.e., microbial, chemical, and sensory analysis). Raw milk with visible changes in color, odor, and/or texture should be rejected, as it cannot be improved by the pasteurization process.

Several factors need to be considered for determining the sensory quality of milk. These include the type of feed, milking environment (i.e., milk absorb aroma), microbiological quality, light exposure, processing methods (i.e., raw, thermal, and nonthermal treatments), and storage conditions. The milk color is described as white or yellow-white, opaque liquid. Milk flavor is described as bland, pleasantly sweet, and free of defects [4]. Additionally, Clark [5] described fresh milk could have umami and a fatty taste. Milk with less fat content can have more umami (i.e., linked with protein) taste. The deviation from these flavors is an indication of poor quality, which can lead to the rejection of milk. The sensory evaluator should use a warm milk sample placed in closed containers to allow the volatiles to escape to the headspace before sniffing. Milk with full fat should have a clean aroma of milk fat [5]**.** VOCs, such as acetone, acetaldehyde, 2-pentanone, toluene, limonene, and heptanol, can be used as freshness indicators for raw milk [6]. The low quality of raw milk is detrimental to the processed milk. Therefore, milk should be chilled at 4 °C directly after milking to avoid bacterial growth.

The growth leads to acid production and consequently coagulation (visual detection) of the milk and changes its odor (e.g., malty and fruity). In addition, animal feeds can affect the chemical and physical properties, which indeed affect the milk aroma and flavor (e.g., cowy and feed). Animal feed studies indicated the effect of feed on VOCs in milk such as terpenes and free fatty acids (FFAs) [7–11], and these are considered as feed or milk origin tracers [12]. In general, butane-2-one, dimethyl sulfide, and secondarily ethanol and propane-2-one are considered as indicators of off-flavors resulting from the feed [13].

Clark [5] classified the milk off-flavors into four classes as absorb (e.g., barny, cowy, and feed), bacterial (e.g., acid, fruity, and malty), chemical (e.g., cooked and oxidized), and delinquency (flat and forging). These off-flavors are described and their causes are discussed [14]. A summary of some reported off-flavor in different milk samples is presented in Table 16.1. More details are discussed in the following sections.

The heat-stable enzymes (i.e., lipase and protease produced by psychotropic bacteria) of raw milk and the activity of native plasmin and lipoprotein lipase can survive during the heat treatment, and these can affect the milk quality [15–18], for example, bitterness and age gelation UHT milk. Rancid off-flavor in pasteurized milk is attributed to the activity of lipase on milk lipids (lipolysis) [19], and it can form a high concentration of FFAs. However, the presence of FFAs at low concentrations can cause desired flavors in milk.

3 Milk Oxidation

Both milk fat and protein are susceptible to oxidation [20]. Although lipids in food give desirable flavors, they are also responsible for the formation of undesirable flavors via lipid oxidation or lipolysis. Auto-oxidation affects mainly the unsaturated fatty acids, which are present adequately in milk, leading to the formation of

Table 16.1 Aroma defects in milk

Off-flavor	Compounds	Sample	Cause	References
Fruity	Ethyl butyrate and ethyl hexanoate	Pasteurized homogenized milk	Post pasteurization contamination with *Pseudomonas fragi*	Reddy et al. [135]
Fishy	Tri methylamine	Milk	Presence of high concentrations of trimethylamine	Lunden et al. [136]
Cowy or feed-like flavor	Butane-2-one, dimethyl sulfide, ethanol, and propane-2-one	Cow milk	Feeding lactating cows fresh silage before 3 h of milking	Mounchili et al. [13]
Cooked flavor	Hydrogen sulfide (H2S), methanethiol (MeSH), dimethyl sulfide (DMS), dimethyl trisulfide (DMTS)	UHT milk	H2S: (a) Strecker degradation of cysteine (b) thermal degradation of thiamine MeSH: Strecker degradation of methionine and also from riboflavin DMS and DMST: Strecker degradation of methionine	Al-Attabi et al. [40]
Stale (oxidized)	2-Butanone, 2-pentanone, 2-heptanone, 2-nonanone, heptanal, octanal, nonanal	Lactose-hydrolyzed UHT processed milk	Maillard reaction and lipid oxidation during the heat processing of milk	Jensen et al. [39]
Rancid off-flavor	FFAs	Pasteurized milk	Hydrolysis of milk lipids by lipase	Meunier-Goddik and Sandra [19]
Barnyard	P-cresol	Milk	*P*-cresol results from deamination and decarboxylation of tryptophan and tyrosine which are present in the protein of grass and grass/clover feed	Faulkner et al. [137]

many carbonyl compounds [21]. These compounds resulted from the autoxidation of oleic acid, linoleic acid, and linolenic acid [22]. The short-chain carbonyl compounds (i.e., aldehydes and ketones) are considered the most important, especially hexanal, which is reported as a shelf-life indicator or biomarker because of its high volatility [23]. On the other hand, light exposure is a real obstacle facing the dairy industry. It causes many problems such as the formation of off-flavors, like sunlight or burnt-feather [24], discoloration, and lipid oxidation [25]. The degree of oxidation is affected by milk composition, packaging material, and light intensity [24].

The oxidation by light was linked to riboflavin, where reactive oxygen species (ROS) formed [26]. They may interact with amino acids (e.g., methionine) and caused the formation VOCs (e.g., dimethyl disulfide) and resulting off-flavors [27,

28]. On the other hand, when ROS attacks unsaturated fatty acids, hydroperoxides and carbonyl compounds, such as hexanal and thiobarbituric acid, were formed [29]. In addition, pentanal was also formed and caused off-flavor [30]. Furthermore, different types of light (e.g., LED and fluorescent) can cause different flavor changes based on the intensity "threshold" and exposure time [31, 32].

4 Processing of Dairy Products

Commercial milk normally undergoes different processing methods, such as pasteurization (e.g., 72 °C, 15 s), extended shelf-life (ESL) (125 °C, 2 s), ultra-heat treatment (UHT) (e.g., 145 °C, 2–4 s), and sterilization (120 °C, 16 min). Such processes lead to the formation of new compounds and the disappearance of some native compounds [33], which in turn affect the flavor and aroma of milk. The aroma compounds of raw milk are different from those of heat-treated ones. Fresh or lightly pasteurized milk has a mild with characteristic taste [34]. The ESL should have a similar flavor to pasteurized milk with extended shelf-life [4]. Its flavor was found to be close to the pasteurized milk flavor at the end of the shelf-life, while at the beginning, these could be different [35]. In contrast, ELS flavor was found to be like UHT flavor at the beginning of the shelf-life.

The degradation (e.g., thermal processing) of milk components, such as protein, fat, and lactose, can lead to the generation of VOCs [22]. These VOCs belong to different classes such as ketones, aldehydes, sulfur, and alcohol. However, their contribution to the overall or specific milk aroma/flavor depends on the perception threshold value of each compound. For instance, hydrocarbons have a very high perception level, and thus its influence on food flavor is minimum. In contrast, aldehydes have a low perception level, so they affect food flavor significantly [36]. Free fatty acids also have a low flavor threshold; therefore, they are considered as the main cause of milk flavor [7]. The VOCs that are present in levels lower than the perception threshold could also contribute to the overall flavor of milk due to their combined additive levels.

Heating milk above 100 °C caused hydrolysis and volatilization of ethyl esters compounds and formation of methyl ketones. The presence and absence combination of methyl ketones, ethyl butanoate, and ethyl hexanoate could be used as the indicator for mild or severe heat treatment [37]. The fat content of raw and UHT milk had a significant effect on methyl ketones concentrations (i.e., higher fat content milk had higher methyl ketones content) [38].

Stale off-flavor was formed in lactose-hydrolyzed UHT-processed milk as a result of butane-2-one, dimethyl sulfide, ethanol, and propane-2-one formation [39]. In addition, VSCs were responsible for cooked off-flavor in the UHT milk. Al-Attabi et al. [40] studied the development of sulfur VOCs in raw, pasteurized, and UHT milk. Raw milk contained four VSCs: dimethyl sulfide (DMS), dimethyl sulfone (Me2So2), carbon disulfide (CS2), and dimethyl disulfide (DMDS). On the other hand, CS2, DMS, DMDS, and dimethyl sulfoxide (DMSO) were detected in

pasteurized milk. With increasing severity of heat treatment (i.e., UHT), the concentrations were increased with more additional volatiles detected: hydrogen sulfide (H2S), carbonyl sulfide (COS), methanethiol (MeSH), and dimethyl trisulfide (DMTS). The H_2S, MeSH, DMS, and DMTS were present initially in concentrations higher than their reported threshold values, indicating that they contribute to cooked flavor in UHT milk. Further processing of milk to a higher temperature than UHT such as sterilization led to the Millard reaction that resulted in the formation of several compounds, e.g., methylpropanal, 2- and 3-methyl butanal, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone [34].

Nonthermal (e.g., high hydrostatic pressure (HHP), Ultraviolet Light-C (UV-C), pulsed electric field (PEF)) treated foods are microbiologically safe if properly used, and these cases have less effect on their nutritional value and sensory properties [41]. Many studies investigated the effect of nonthermal treatments on milk volatile compounds. For instance, Hu et al. [36] studied the influence of two nonthermal treatments (i.e., UV-C and multiple-cycle HHP) on the composition of volatile compounds of treated cow milk. Both HHP and UV-C treatments caused the increase in the concentrations of volatile compounds, thus accelerating flavor formation when compared to non-treated milk. Aldehydes, alcohols, aliphatic hydrocarbons, esters, and ketones were higher in UV-C than in HHP samples. The UV-C milk samples were characterized by five VOCs (1-hexanol, 1-tetradecanol, 1-heptene, 3-methylphenol, and 2-Methylcyclopentanone).

Compared to the common high-temperature short time (HTST) pasteurization (i.e., 75 °C, 15 s) and ultra-high pressure homogenized (UHPH) (300 MPa), UHPH-treated milk with 200 MPa had fewer carbonyl compounds but a higher level of hexanoic acid indicating lipolysis of milk fatty acids. Conversely, 300-MPa UHPH treatment produced higher concentrations of aldehydes than common pasteurized milk, but these contained less concentration when compared to UHT and in-bottle pasteurized and sterilized milk [42]. In addition, negligible changes in dimethyl sulfide were noticed between UHPH-treated milk and heat-treated milk. The increased trend was obtained for both aldehydes and methyl ketones in the heat-treated milk when increasing the intensity of heat treatment, while nonthermal-treated milk showed an increased trend just for aldehydes. Liepa et al. [43] found no significant differences in aroma between high-pressure (HP)-treated milk (400 MPa) and pasteurized milk (78 °C for 15–20 s) when compared to color, taste, and aftertaste preferences. These properties scored high acceptance in HP-treated milk compared to pasteurized milk.

Zhang et al. [44] found that HTST pasteurized milk showed an increase in the aldehydes and methyl ketones when compared to raw milk, while only aldehydes increased with PEF-treated milk. Treatment of milk with PEF of 30 kV/cm led to the highest concentrations of pentanal, hexanal, and nonanal. On the other hand, PEF-treated milk showed less concentrations of heptanal and decanal compared to pasteurized milk but higher than those of raw milk. There was no significant change in acids (such as acetic acid, butanoic acid), lactones, and alcohols (e.g., ethanol, 3-methyl-butanol) between raw, pasteurized, and PEF-treated milk. In addition, 2(5H)-furanone was detected in PEF-treated milk only.

Bandla et al. [45] found no significant differences among the odor of UV-light-treated, untreated (lamp off), and control (fresh raw milk), right after the UV treatment. On the other hand, a notifiable difference was found on the third and seventh days after storage at 4 °C. Additionally, the smell of the UV-C-treated sample was significantly different from the untreated one after 1 day of storage. This was related to the higher lipid oxidation in UV-treated sample.

5 Sensory Evaluation Methods

5.1 Human Sensory Methods

Sensory methods are categorized into descriptive (e.g., profile attribute analysis) and discrimination methods (e.g., triangle test and paired test). Project and test objectives should be considered while selecting the proper method. Selecting the proper test, test conditions, and data analysis can lead to reproducible, powerful, and beneficial results [46]. Moreover, ethical approval is vital to insure the safety of panelists. Additionally, the consent form must be signed before conducting the test. Panelists' motivation and rewarding are necessary to be considered, especially with volunteer panelists. Furthermore, the preparation and presentation of samples plus the environmental conditions (e.g., lightening, temperature, and humidity) in the sensory room should be controlled from one session to another. Palate cleaners (e.g., water, crackers, and plain bread) must be provided for assessing the samples. Panelists with lactose intolerance should be excluded from the test. The sensory tests in foods [47–49] and dairy [3, 46, 50] are discussed in the literature.

5.2 Discrimination Methods

Discrimination methods determine whether there is a significant difference between two products or more. The difference can be either general or based on a specific attribute. Therefore, these methods are called difference tests. They are less often used in research when compared to the acceptability or descriptive tests [50], and these are widely used in fluid milk. Paired test, duo-trio, and triangle testing are the most used tests for fluid milk sensory analysis [3].

Triangle and Duo-Trio test represent general difference, whereas paired directional test represents difference with respect to a single attribute. Paired comparison test (same or different test) was also used to determine the basic differences of liquid milk [51]. These tests can be easily implemented, and the data can be simply analyzed using published statistical tables. The number of panelists depends on the degree of the difference, for example, in triangle test, 12 panelists are suggested when the difference can be easily identified compared to 20–40 panelists when the

difference is small [49]. A minimum of 16 panelists is required for Duo-Trio test. The paired test requires a minimum of 30 panelists ([49]: [35, 45]).

In the triangle test, the panelists are presented with three coded samples in which two are the same and one is odd. The panelists are asked to identify the odd sample or can be asked to identify the similar ones. On the other hand, in Duo-Trio test, panelists are presented with two coded samples and one reference (i.e., one of the presented samples). They should try the reference and then test the sample and identify which sample is like the reference. These tests can be either constant reference or balanced reference depending on certain factors. The triangle test (1/3) is more efficient than Duo-Trio test and the directional-paired test (i.e., 1/2).

Bandla et al. [45] used the triangle test to study the effect of UV-C on cow milk odor using nontrained panelists. The milk samples (>15 mL) were presented at 4 °C in semi-opaque covered plastic cups. Furthermore, the triangle test was used by Grabowski et al. [35] to study the sensory difference between pasteurized (P), ESL, and UHT processed milk using inexperienced panelists. They were provided with a combination of three milk samples (i.e., each 100 mL, presented ambient temperature) (i.e., P/ESL, P/UHT, and UHT/ESL). Recently, Abdulghani [52] showed an insignificant sensory difference between control and pasteurized milk processed from raw milk treated with CO_2 using a triangle test with10 panelists.

Significant differences between specific attributes of multiple samples can be analyzed by ranking test. Villegas et al. [53] used the ranking test to assess the perception of yellow color, brightness, vanilla flavor intensity, sweetness, and thickness of milk and soymilk beverages. Thirty-six assessors with experience were asked to rank the samples (presented at 10 °C) from the least to the most intense attribute. The data were analyzed using Friedman's analysis of variance. The data showed that milk samples were significantly more acceptable (i.e., using a hedonic scale) with stronger yellow and less light in color, more intense sweetness, stronger vanilla flavor, and thicker consistency as compared to soya milk.

5.3 Descriptive Methods

Descriptive sensory analysis (e.g., quantitative descriptive analysis method, QDA) can be employed to identify and quantify food attributes using a well-trained sensory panel to produce results as precise and reproducible. This is achieved by training a panel on the selected and defined sensory attributes of the sample studied and the used scale [46]. Standard definition milk sensory attributes from the literature can be used or can be developed by the panelist through discussion [54] (Table 16.2). References should be provided to reduce the panelists' variability and calibrating the panelists to use the scale [49]. Different sensory attributes are used to describe different types of fluid milk (i.e., raw/fresh, processed, and stored), and their references/recipes are compiled in Table 16.3. The results can be presented using a spider plot, where the center represents zero intensity. The attribute intensity mean is plotted on the lines extending from zero, and these are joined with the line (Fig. 16.1).

Table 16.2 Sensory attributes used for fluid milk analysis treated at different temperatures [54]

Descriptive term	Definition[a]	Direction
Odor	Odor associated with	Positive
O-boiled milk[b]	Boiled milk	Positive
O-maize	Brine from canned maize in milk	Negative
O-cardboard/sour	Cardboard soaked in milk	
Appearance	Sensation associated with the appearance	Neutral
A-Gray color	Gray color	Neutral
A-yellow color	Yellow color	
Flavor/taste	Sensation associated with flavor/taste of	Positive
F-boiled milk	Boiled milk	Negative
F-watery	Diluted milk	Positive
T-sugar sweet	Sugar in milk	Positive
F-toffee	Sweet toffee in milk	Positive
F-maize sweet	Brine from canned maize in milk	Negative
F-cardboard/sour	Cardboard soaked in milk	Negative
F-metallic	Fe2(SO4)3, 7 H2O in milk	Negative
F-plastic/chemical	Odourized plastic	Negative
T-bitter	Quinine in milk	
Aftertaste	Sensation associated with aftertaste/mouthfeel of	Neutral
AT-astringent	Astringency 0 s after mouth emptying	Negative
ATMF-cardboard/sour	Cardboard soaked in milk	Neutral
AT-astringent 20	Astringency 20 s after mouth emptying	

O odor, *A* appearance, *F* flavor, *T* taste, *ATMF* aftertaste/mouthfeel

[a]Definition of the sensory terms as derived during vocabulary development using reference samples

[b]Suffix to sensory term indicates method of assessment by panelists

During the recruitment process, the questionnaire can be used to assess the potential panelists on their ability to conduct the test, preference, health issues, availability, willingness to participate, and prior experience on trained panels [55, 56]. Additionally, their ability to conduct the experiment can be tested using several sensory tests, such as taste and odor recognition, differences between samples using triangle test, and difference from control test [55, 57]. A small number (i.e., 5–12) of well-trained panelists can be used [46, 49], but extensive training is required. The training sessions depend on the tested attributes, panelists experience, and availability. Different training sessions are reported (i.e., 35, 9, 7, 18, and 40 h) for different number of sensory attributes (i.e., 11, 12,17, 17, and 7) in fluid milk [54, 56–59], respectively. Furthermore, Li et al. [60] spend 50 h to train the panelists on assessing viscosity and astringency in milk samples.

The quantification of intensity measurement is normally done using certain scales, such as line marking scale (i.e., 15 cm, 9 cm) and category scale, where the panelists are trained on how to use this scale. Line scale can be labeled with anchors at the end and 1 or 1.25 or 2 cm from the end [49, 57, 58]. The intensity of some references can be added on the line scale (i.e., structured and unstructured) for easy comparison and measurement (Fig. 16.2). The 15-cm scale has been used to assess fluid milk aroma and taste of different heated treated milk with standardized fat content [59], flavored milk attributes [61], fluid milk with different processing

Table 16.3 Sensory attributes of different types of fluid milk (i.e., raw/fresh, processed, and stored) and their corresponding reference/recipe[a]

	Attribute	Definition	Reference/recipe
Texture	Viscosity	Amount of force required to slurp 4.93 mL (1tsp) of liquid from a spoon over lips	Water = 1, heavy cream = 32
Appearance	Opacity	Visual term denoting the degree of opacity	Water = 0.0, whole fat milk = 12
	Yellow color	Degree of yellow color visible to the human eye	[b]Behr paint chips: "Ültra-pure White" (PPU18-06_ = 0.0, "glass of Milk" (P260-1u) = 3.5
Flavor/aroma	Sweet aromatics	Sweet aromatics generally associated with materials also having a sweet taste	Vanilla, caramelized sugar
	Cheese aroma	NL	Colby cheese
	Sweet taste	• fundamental taste sensation elicited by sugars • as in the sweetness associated with sucrose (not vanilla, malt, nor biscuit)	• Sucrose (5% in water) • 1:1100% lactose free Lactaid and 2% fat milk
	Aroma intensity In-mouth	The overall orthonasal effect of the sample	NL
	Feed	NL	Add 5 mL "alfalfa tea" (steep alfalfa or Timothy hay 5 min in boiling water) to 1 L of milk within 6 h of tasting
	Creamy	As in the flavor of 100% cream	100% cream (meadow fresh cream, Goodman fielder, Auckland, New Zealand)
	Cheesy	As in the flavor of parmesan, cheddar cheese	–
	Cultured/fermented	As in the flavor of sour cream, yogurt	• A 75% (w/v) mixture of sour cream and trim milk • Sauerkraut
	Malty/biscuity/cereal	As in the flavor of malt (but not the sweetness)	A (50% w/v) mixture of cornflakes and trim milk soaked for 30 min and strained
	Barny/cowy	As in the flavor associated with barny/cowy odor	–
	Metal oxidized	NL	• Add a cleaned and sanitized copper penny to 1 L milk; allow oxidation for at least 4 h; filter out the penny before serving • Add 1 mL 0.25% copper sulfate to 1 L of milk at least 4 h before serving • Add 1.8 mL 1% copper sulfate to 600 mL whole milk

	Light oxidized	NL	• Expose milk in glass or plastic containers to fluorescent or UV light for 15–30 min • Expose 600 mL whole milk to bright direct sunlight for 12–15 min
	Rancid	NL	• Strong provolone cheese • Mix 100 mL raw milk with 100 mL pasteurized milk and agitate in a blender for 2 min. Add 400 mL to obtain 600 mL total (note: Pasteurize after smelling the defect by heating to 70 °C for 10 min)
	Fruity/fermented	As in the fruity, tropical fruit, perfumy flavor	• 1.25 mL of 1% ethyl hexanoate (food grade) per 600 mL • Add up to 3 mL pineapple juice, apple juice, or V8 splash tropical blend juice to 1 l of milk
	Milk fat/lactone	Aromatics characteristic of milk fat, lactones, and coconut	Fresh coconut meat, heavy cream, δ-dodecalactone (40 mg/kg)
	Cooked	Aromatics associated with heated/cooked milk	• UHT milk 2% fat microwaved for 30 s • Freshly pasteurized milk (79 °C/18 s) • Skim milk heated to 85 °C for 30 min • Heat 600 mL milk to 80 °C for 1 min and cool • Boil 500 mL of milk and bring to 1 L with "no defect" milk
	Bitter	NL	Add 1–2 mL of a 0.25% quinine sulfate solution or a 0.25% caffeine solution to 1 L of milk
	Astringency	Chemical feeling factor on the tongue or oral cavity described as puckering or dry	Alum (1% in water)
Mouthfeel	Thickness	As in the mouthfeel caused by the viscosity of the matrix (i.e., milk)	Cream/trim milk
After swallow	Mouth coating	Referring to the coating film formed on the tongue/mouth after swallowing milk.	NL
	Sweet aftertaste	As in the sweetness of sucrose (not vanilla, malt, nor biscuit)	1:1100% lactose-free Lactaid and 2% fat milk
	Creamy	As in the after swallow flavor associated with cream.	100% cream
	Sour	NL	Cultured buttermilk
	Metallic	NL	$FeSO_4$ (0.445 g/L)
	Cardboard	NL	Cardboard box

[a]References: [5, 47, 58, 64, 66, 133]
[b]Behr: Santa Ana, CA; NL: not listed

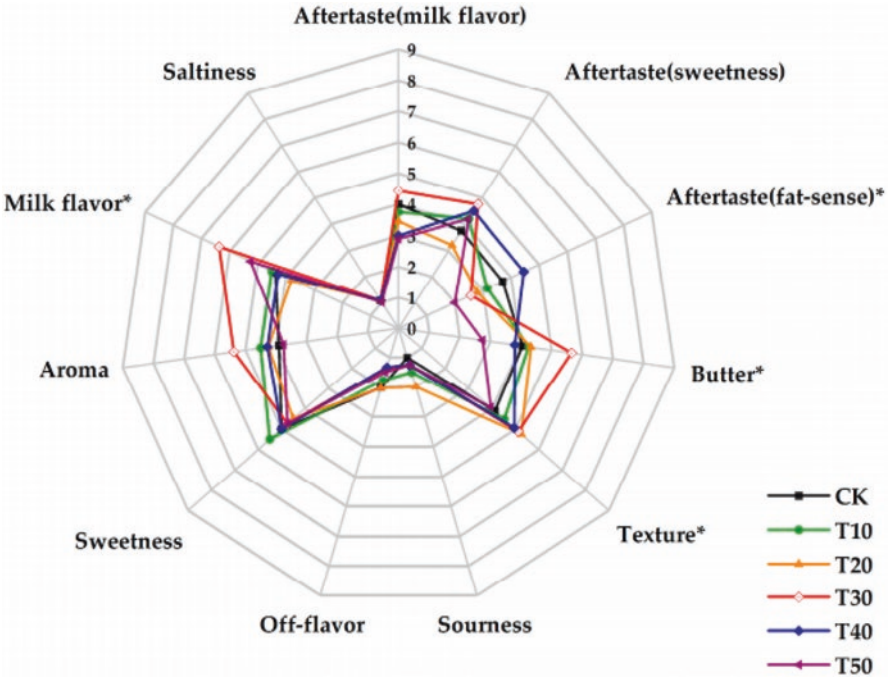

Fig. 16.1 Spider plot for sensory responses of skim milk preheated at different time. Reprinted with permission from Pan et al. [62] under Creative Commons CC BY 4.0 license

Fig. 16.2 15-cm line scale of cooked milk flavor with reference (i.e., reference with intensity 4 represent UHT milk 2% fat parmalat whereas 10 represent UHT milk 2% fat parmalat microwaved for 30 s) [133]

conditions [54], and commercial milk samples [57] using 6, 20, 3, and 12 panelists, respectively. On the other hand, 9 cm unstructured line scale was used to assess the sensory attributes of commercial probiotics fermented milk and skim milk processed using 5 panelists at different preheating treatments [62]. A 10-cm line scale with anchors "none" and "extreme" was also used to evaluate the sensory attributes (i.e., milkfat, grassy/vegetable oil, sweet taste, and astringency) fortified milk with conjugated linoleic acid [63].

The milk sample presentation (i.e., quantity and temperature) should be considered and controlled during and between the sessions. The reported amount of milk presented in descriptive method ranged between 25 and 80 mL, while different

temperatures were used including 5, 7, 10, 12, and 16 °C [54, 55, 57–60, 63, 64]. Additionally, covering the used cup is very important for the sensory analysis of aroma in fluid milk. This allows the volatiles to be collected in the headspace [64] as well as turning off the overhead lights to prevent light oxidation [59, 63].

The flavor or aroma profile method is one of the descriptive methods, and it is used for the profile attribute analysis including appearance, tactile, and auditory attributes [49]. The aroma profile method was used to identify the aroma profile of goat milk by orthonasal (o) and retronasal (r) means [65] (Fig. 16.3). Caramel-like, cooked milk-like, fatty, goat-like, hay-like, metallic, nutty, stable-like, sweaty, and vanilla-like attributes were evaluated. The odor profiles of pasteurized and UHT-treated milk were similar. Goat-like (r) and caramel-like (o, r) rated high in pasteurized/UHT-treated and sterilized milk, respectively. Additionally, cooked milk-like, fatty, stable-like, and sweaty attributes (o/r) were also characterized pasteurized and UHT-treated milk when compared to vanilla-like odors (o), cooked milk-like, fatty, goat-like, and sweaty odors (r) for sterilized milk.

Alothman et al. [58] studied the sensory attributes (i.e., odor, flavor, mouth feel, and after swallow) of stored (4.5 °C) whole fresh chilled pasteurized milk using nine trained panelists. These attributes were evaluated against a fresh sample as a reference. The researcher used 15-cm unstructured line scale (0 is none, 15 is extreme) with middle reference and anchors at 1 and 14 cm (Fig. 16.4). In contrast, Gandy et al. [56] used the 15-cm unstructured scale but without reference labeling to evaluate the aroma and flavor (using 8 panelists) of 2% pasteurized milk at different temperatures.

Recently, Li et al. [60] studied the effect of processing methods (HTST, Ultra-pasteurized (UP), fat content, homogenization pressure, and storage time on the sensory attributes (i.e., viscosity and astringency) during storage (1, 4, and weeks). The test protocol requested the panelist (i.e., 7) to use nose clips to mask any other attributes while doing the test. They have been requested to place around ½ of the presented sample in their mouth and hold for 10 s before expectorating and then evaluate the intensity using 15-cm line scale (0 is not detected, 15 is extremely strong). In this test, 10-min break was given between samples plus rinsing with water and followed by 0.1% (w/w) solution of carboxymethylcellulose. The viscosity of UP milk was increased as compared with HTST pasteurization. Fat content increased sensory viscosity and decreased astringency in both processing methods. Furthermore, astringency is increased with increasing storage time compared to no effect on viscosity.

Furthermore, McCarthy et al. [64] described the sensory attributes (i.e., opacity, color, viscosity, and flavor) of different milk samples with different fat concentrations using eight trained panelists. The panelists were standardized using warm-up samples. The intensity was measured using 15-cm line scale. Likewise, QDA/principal component analysis (PCA) was used by Piotrowska et al. [4] to study the sensory quality (i.e., 18 defined attributes) of commercial milk samples subjected to different preservation methods (i.e., microfiltration and low pasteurization,

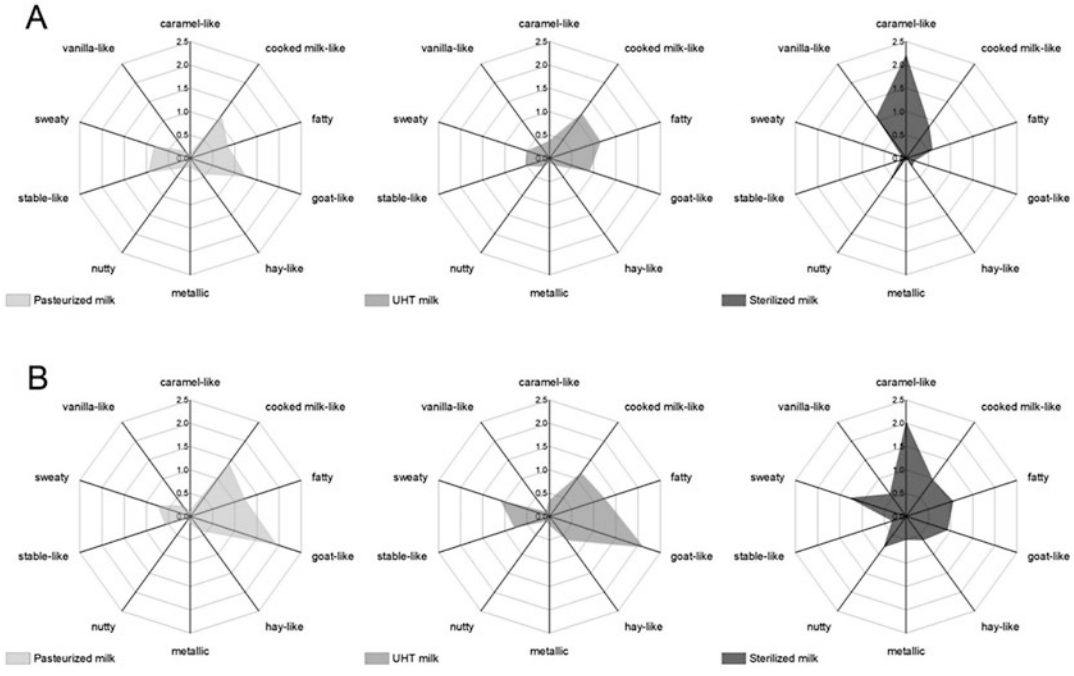

Fig. 16.3 Orthonasal (**a**) and retronasal (**b**) odor profiles of goat milk: heated milk profiles after pasteurization, UHT-treatment, and sterilization. Reprinted with permission from Siefarth and Buettner [65]. Copyright (2020) American Chemical Society

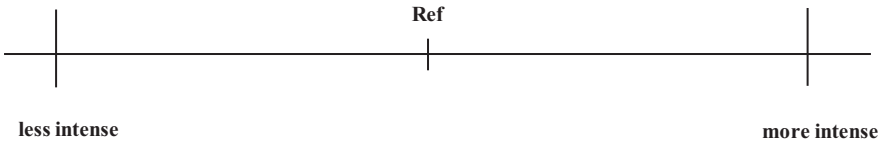

Fig. 16.4 Unstructured line scale

pasteurized, and UHT). These attributes were tested by 10 trained and expert panelists. Three clusters formed (i.e., represent the samples with the same heated treatment) with the varied sensory profile. Microfiltered and pasteurized milk had a lower intensity of milk flavor.

The QDA/PCA was also used to evaluate HTST pasteurized 2% fluid milk samples throughout shelf-life (i.e., 7, 14, and 17 days of storage at 6 °C) using 13 panelists [66]. These panelists had developed the descriptors/terminologies to describe the aroma, taste, and aftertaste through discussion. References were provided during training. A 15-cm line scale anchored (none: 0 and strong: 15) was used. A 50 mL of the milk sample in plastic-covered cup was stored in a cardboard box at 6 °C. The sample was presented at 15 °C by heating the cardboard box in the microwave for 22 s. In this work, the panelist was provided with a list of the attributes, their definitions, and references during evaluation.

A 5-point scale (0 is no difference, 1 is very slight difference, 2 is slight difference, 3 is moderate difference, 4 is large difference, or 5 is extremely large difference) was also reported to be used to evaluate the flavor of spontaneously oxidized flavor bovine milk (SOF) [67]. The panelists (i.e., 11) were presented with 30 mL whole milk sample as reference. During the test, they were given one control (same as the reference) and two recombinant SOF (i.e., milk spiked with terpenoid compounds). They were asked to describe the perceived flavor differences.

5.4 Affective Analysis

This kind of test is used to measure the consumer acceptably of the products by measuring their preferences of liking and dislike. Hedonic scale is one of the most widely used scales. It is a "balanced bipolar scale centered around neutral, with categories labeled with phrases representing various degrees of liking" [61]. This scale can be numerical, verbal, category, and facial scale ([49, 50, 68]) (Fig. 16.5). It can be 5, 7, or 9 points hedonic scale. This kind of test requires a high number of nontrained panelists (i.e., consumers) than discrimination and descriptive methods. The number of panelists and sample presentation information of the hedonic scale used in some related studies is summaries in Table 16.4.

A hedonic scale with 9 points is widely used for milk assessment. It has been used by Gandy et al. [56] and Conti-Silva and Souza-Borges [55] to evaluate the

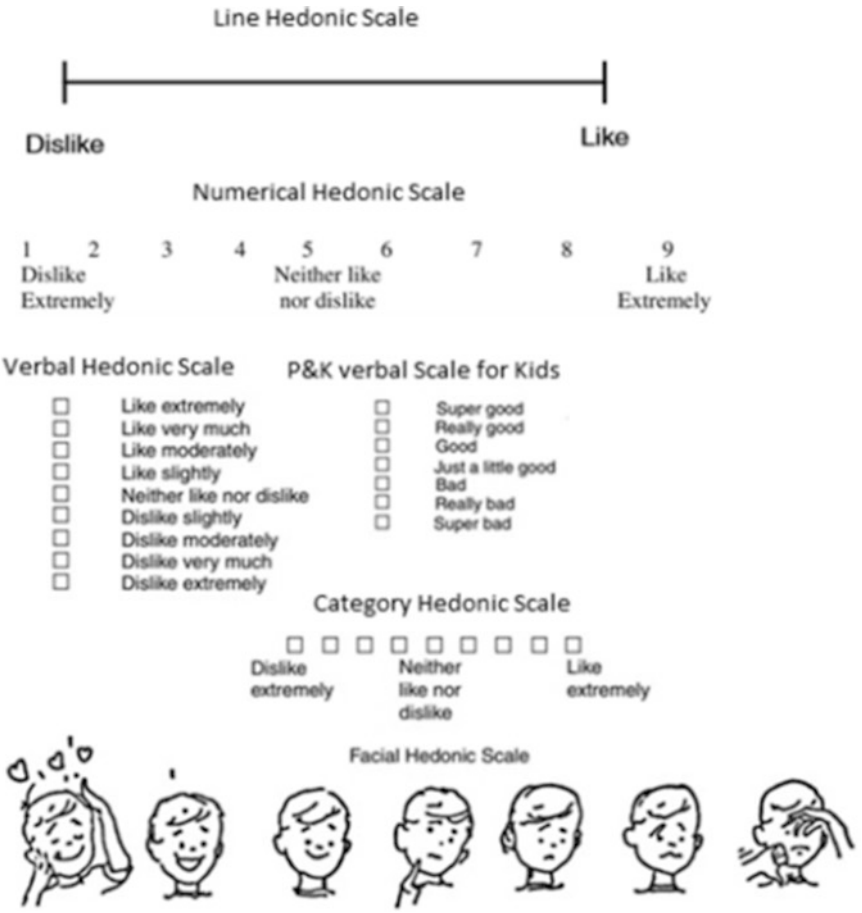

Fig. 16.5 Types of hedonic scale [49, 50, 68]

Table 16.4 Panelists and sample presentation information of hedonic scale used in some milk sensory study

Sample type	Number of panelists	Sample size (mL)	Temperature (°C)	References
Milk fortified with conjugated linoleic acid	101	–	5	Campbell et al. [63]
2% pasteurized	50	28.4	–	Gandy et al. [56]
Flavored milk	656	–	18	Zhi et al. [61]
High pressure treated milk	55	30	16	Liepa et al. [43]
Pasteurized and UHT milk	240	40	Room temperature	Phu and Hang [71]
Probiotic fermented milks	85	30	10	Conti-Silva et al. [55]
Commercial vanilla-flavored milk and soymilk beverages	142	30	10	Villegas et al. [53]

flavor and overall acceptability of 2% pasteurized at different temperatures (i.e., 77, 79, 82, and 85 °C for 15 s) during storage and liking of the commercial probiotic fermented milks, respectively. In addition, hedonic scale of 5 points (1 is dislike very much, 2 is dislike slightly, 3 is neither like nor dislike, 4 is like slightly, 5 is like very much) was also used to study milk acceptance (i.e., color, aroma, taste, and aftertaste) [43]. Similarly, flavored milk acceptance (i.e., sourness, sweetness, fresh milk flavor, thickness, and smoothness) was also measured [61].

Selecting the target consumers is very important when studying any product using affecting methods. Campbell et al. [63] used a short questionnaire for selecting and recruiting the panelists to evaluate the overall acceptability, overall flavor, and freshness of fluid milk fortified with conjugated linoleic acid. Demographic information, milk consumption habits, and interest in nutraceutical/functional foods are collected for the questionnaire. Similarly, Villegas et al. [53] used knowledge and consumer habits with respect to milk and vegetable beverages to recruit the panelists to evaluate the differences in acceptability between commercial vanilla-flavored milk and soymilk beverages. The questionnaire was delivered by e-mail.

The facial hedonic scale is more appropriate for children, which makes their assessment easy. The facial hedonic scale of 3, 5, and 7 points with Peryam and Kroll (P&K) verbal scale (super good/super bad) were used to evaluate 2% commercial UHT milk and 2% commercial pasteurized milk preference for taste, color, and mouthfeel among the children (i.e., 79) [69] (Fig. 16.6).

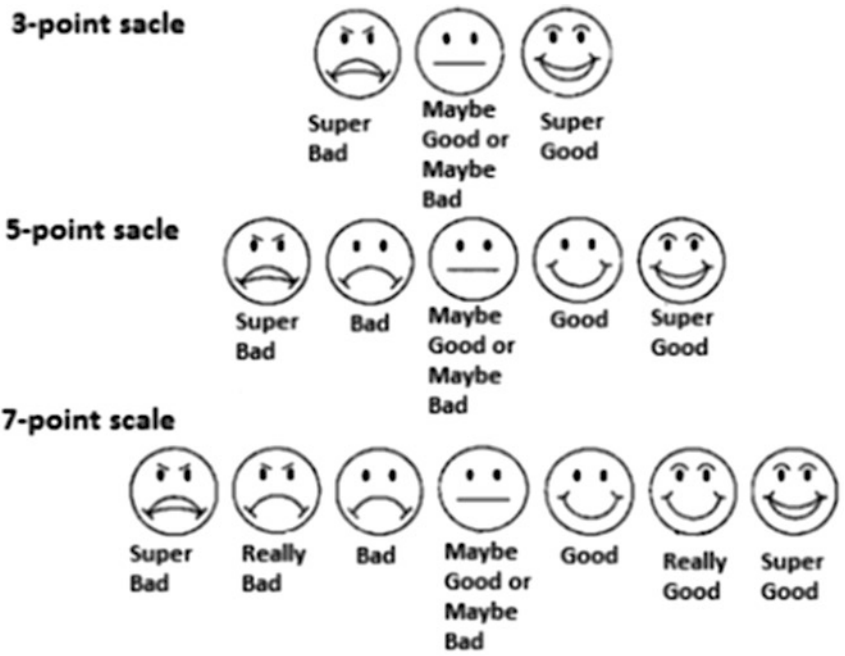

Fig. 16.6 Facial hedonic scale with 3, 5, and 7 points used with children aged between 3 and 6 years old to assess their preference of pasteurized and UHT milk [69]

The 7-point hedonic facial scale was used to assess the acceptability of camel milk among children (i.e., 173), with 1 is dislike very much and 7 is like very [70]. Recently, Phu and Hang [71] also used 7-point facial hedonic scale using emoji faces to measure Vietnamese children's preference/liking of pasteurized and UHT milk (Fig. 16.7). Parent approval is granted which is always required whenever children are involved in sensory evaluation. The emoji facial scale can be used as an alternative to P&K verbal scale (super good/super bad) to measure food liking among children (i.e., 8–11 years old) [72].

5.5 Threshold Test

The threshold value can be defined as the lowest concentration at which a sensory response is detectable [49]. This can be defined as an absolute threshold. Other types of thresholds are also used such as recognition (the concentration at which a stimulus can be recognized and identified), difference (changes in the stimulus to product noticeable difference), and terminal thresholds (concentration above which there is no perceived increase in the sensory stimulus). Just noticeable difference (JND) terminology is used to describe the difference threshold. It requires changing the stimulus concentration higher and lower the standard until the difference is noticed by panelists. The threshold determination can be either orthonasal or retronasal. Thus, it requires knowing the threshold value of the volatile compounds such as for flavor. These can be either determined by the researcher using the appropriate method. However, some threshold values in different food samples were compiled, and these were published [73, 74]. In the dairy industry, sensory threshed is very important to identify the minimum concentration of an off-flavor compound in pasteurized milk that can be detected by the consumers [75]. It also is used to identify the desirable flavor.

Three alternative force choice method (AFC) is the most commonly used [46]. This method requires giving several concentrations in ascending way to 25–40 panelists or more [46, 49], and it requires some warm-up examples giving before

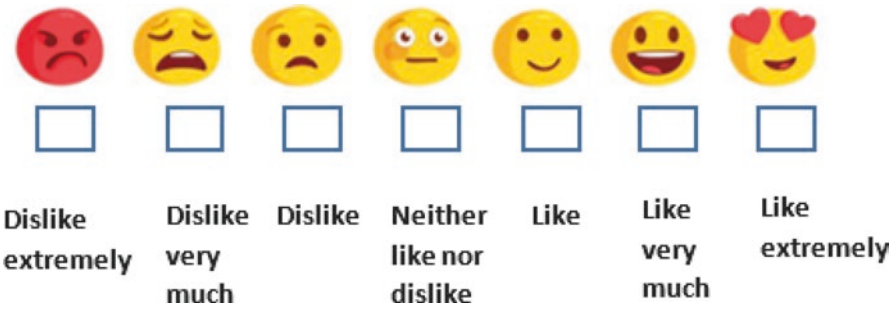

Fig. 16.7 Emoji Facial hedonic scale with 7 points [71]

running the experiment. The panelists are provided with three coded samples of which two control and one contain the stimulus. They are asked to select the odd sample. The geometric mean is used to measure the individual and group threshold values. This method was applied by Aardt et al. [76] to study the acetaldehyde threshold value in milk, milk chocolate, and spring water. The results clearly showed that the carrier or food matrix plays a major role in threshold determination. Different milk fat contents showed no significant difference in the acetaldehyde threshold ranging from 3939 to 4040 ppb. In contrast, chocolate-flavored milk and spring water showed thresholds of 10,048 and 167 ppb, respectively. Furthermore, Santos et al. [75] studied the threshold value of off-flavor in 2% fat milk caused by lipolysis (free fatty acids) using 25 untrained panelists. The off-flavor (rancid) induced by native milk lipases was in the range of 0.32–0.351 meq of FFA/kg of milk.

Chapman et al. [77] studied the light-oxidized flavor of milk (2% fat) samples subjected to different light intensities and different times using the semi-ascending paired difference method. Although the time of exposure was set ascending, but the samples within the set are randomized. Each panelist is given three samples of which one is control (unexposed to light) and two samples of adjacent exposure time (Fig. 16.8). Each time, the panelists (i.e., 10 trained) were required to compare each sample with the given reference (unexposed) and rate the intensity of difference. The research used untrained panelists (consumers). The trained panelists detected off-flavor after 15–30 min of light exposure compared to the untrained ones (i.e., between 54 min and 2 h).

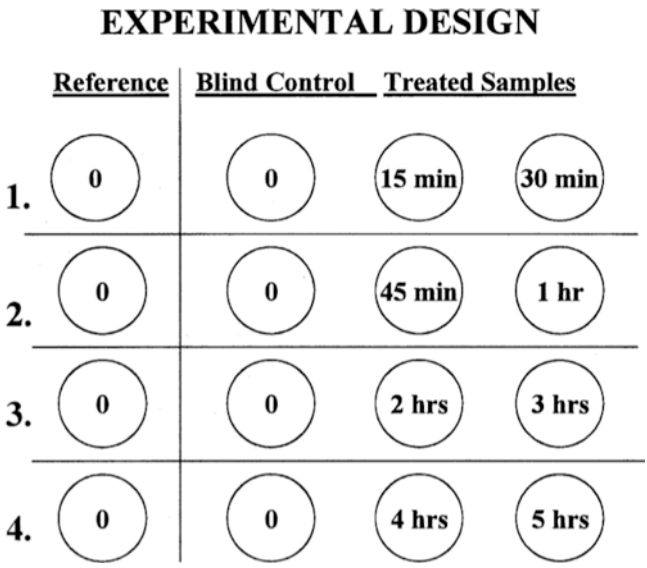

Fig. 16.8 Experimental design of the semi-ascending paired difference method [77]

It is well known that milk fat contributes to the desirable milk flavor. Furthermore, it contributes to different milk characteristics such as color and texture (e.g., viscosity). McCarthy et al. [64] studied the milk fat threshold (i.e., JND) using forced-choice method. Milk with 0.1, 1, 2, and 3.5% fat was used as standards. The researchers tasted the milk with masking visual. To do that, milk samples (i.e., 59 mL) were presented at 4 °C under controlled light (i.e., red). Panelists (i.e., 60 consumers) were presented with coded randomized samples, two standards, and one tested sample (with increased fat concentrations). The concentrations were increased by different step factors of 2.2, 1.7, 1.5, and 1.4 for skim milk, 1%, 2%, whole milk samples, respectively. They have been asked to determine the odd sample. Furthermore, the JND for milk by visual-only (i.e., without tasting) was determined. In this test, the panelists present the milk samples under the fluorescent light. The concentrations increased by 1.5 for skim milk and 1.3 for 1%, 2%, and whole milk. The results showed that the JND is higher for tasting only compared to visual-only within each standard. For skim milk (0.1%), the JND for visual-only was 0.4% compared to 4.4% for tasting only. On the other hand, for whole milk (3.25%), the visual only was 4.3% compared to 6.0% for tasting only.

6 Chemical Analysis/Gas Chromatography

Several VOCs were reported in milk that contribute individually or combined to the milk flavor or aroma. Therefore, GC is used to identify and quantify these compounds over the headspace (HS) of the milk package. Therefore, many headspace extraction techniques could be applied to characterize VOCs of milk. These include static headspace (SH), dynamic headspace (DH), solid-phase extraction, and solid-phase microextraction (SPME). After the extraction, the VOCs are injected into the GC-heated injector. These volatiles are then transferred through the GC-system using carrier gas (i.e., helium). They are separated in the column based on their polarity and volatility [78]. The oven temperature is usually programed to start at a low temperature (i.e., 35–40 °C). Appropriate column selection (i.e., polar or nonpolar) can enhance separation and identification [78]. Thereafter, the eluted VOCs are analyzed by detector (e.g., mass spectrometry, MS; flame ionization detector, FID; pulsed flame photometric detector, PFPD), and these are evidenced on the chromatogram as peaks. Standards are used to identify and quantify the VOCs. The application of these extraction techniques in milk and dairy product has been reviewed [78, 79].

In the SH extraction method, the milk sample was placed in a closed vail and left to equilibrate at a certain time and temperature. Thereafter, the headspace sample withdraws using a gas-tight syringe or it can be directly linked to the GC injector. On contrary, DH does not require equilibration. The milk sample in the closed system is purged with inert gas (i.e., nitrogen and helium). The VOCs are then adsorbed or trapped in a sorbent before transferred to the GC injector. The sorbet works as a pre-concentration step, which improves its sensitivity and selectivity compared to SH.

SPME can be described as fast, sensitive, and solvent less that can be used to extract and pre-concentrate volatile and semi-volatile compounds from gas, liquid,

and solid samples. It can detect VOCs at very low concentrations when compared to static headspace. It has better precision and accuracy when compared to dynamic headspace. The SPME extraction should be optimized to obtain the right fiber selection and best extraction time and temperature. Sample size, addition of salt, and agitation could enhance the extraction. Different milk studies used different extraction conditions, which can be related to the research objective (e.g., extraction of sulfur compounds). SPME has been wildly used for VOCs extraction over milk samples in recent years [38, 40, 56, 62, 80–82]. Examples of some SPME and GC conditions used for VOCs analysis in milk are summarized in Table 16.5.

Al-Attabi et al. [40] were able to identify and quantify nine sulfur compounds in raw, pasteurized, and UHT milk using GC/SPME/PFPD. The PFPD is a very selective detector, especially to sulfur compounds compared to the FID, which is the most common detectors used. CAR/PDMS and extraction conditions of 30 °C for 15 min were a good combination for sulfur extractions [40, 80]. The sulfur compounds are responsible for cooked or sulfurous aroma in milk.

Gandy et al. [56] used SPME/GC/MS to detected VOCs in pasteurized milk samples heated at different temperatures (i.e., 77, 79, 82, and 85 °C). This technique helped to characterize each treated milk sample based on the volatiles after treatment and during storage (i.e., product shelf-life). For example, at day 0, 77 °C treatment (hydroxylamine, phenol, and butanoic acid), 79 °C treatment (hydroxylamine, phenol, butanoic acid, hexanoic acid, and hexanol), 79 °C treatment (benzoic acid, p-xylene, nonanone, and eugenol), and 82 °C treatment (benzoic acid, p-xylene, nonanone, and eugenol). On the day 10 and 13, all treatments were closely characterized by acetic acid, heptanone, and hexanone, which were linked to sour or acid flavor detected by the panelists at the end of the shelf-life. The GC output (i.e., VOCs) and consumer acceptability were not able to find the difference between heat-treated milk. The SPM/GC method allowed the detection of highly volatile compounds (e.g., sulfur compounds) compared to stir-bar sorptive extraction in milk [59]. Fifty-five compounds were detected in the tested milk (HTST, ultra-pasteurized by direct steam injection, and ultra-pasteurization by indirect heating). Pan et al. [62] correlated the preferred preheating condition before defatting for skim milk (50 °C, 30 min) to the high concentration of acids (e.g., fatty acids). Recently, SPME was also used to identify and quantify the VOCs of UHT hydrolyzed lactose milk (HLM) with GC/MS [82]. Seventeen VOCs were detected. The post-UHT samples were separated from the rest of the samples, which is related to the methyl ketones consecrations (Fig. 16.9).

For a better assessment of milk flavor quality, multivariate analysis (MVA) is used to correlate the chromatographic profile of milk with the human sensory scores [83, 84]. For example, Marsili [84] correlated the SPME/MS/MVA data with the sensory to predict the shelf-life of pasteurized milk and homogenized reduced-fat milk and whole-fat chocolate milk over a 7-month period. On the other hand, Vallejo-Cordoba and Nakai (1994) used dynamic headspace correlation to predict the shelf-life of milk. Volatile free fatty acids, which are significant contributors to milk off-flavors and hard to be detected at low levels using static and dynamic headspace techniques, were extracted and accurately detected by Marsili [84] using SPME-GC.

Table 16.5 SPME/GC extraction and detection conditions

Sample	SPME fiber	Sample size (mL)/vial size (mL)	Conditions (temperature, (C)/time (min)	Agitation/ salting	Column	Detector	References
Pasteurized and homogenized reduced-fat milk and whole-fat chocolate milk	CAR/PDMS	3/6	50/20	Agitation	DB-5 fused-silica capillary column (30 m × 0.25 mm i.d., 1 μm film thickness)	MS	Marsili [84]
Raw, pasteurized, and UHT milk samples with various fat contents	DVB/CAR/PDMS	20/40	35/60	Agitation	HP-5 capillary column (50 m × 0.32 mm i.d., 0.52-m film thickness)	FID	Vazquez-Landaverde et al. [38]
Raw, pasteurized, UHT milk	CAR/PDMS	–	30/5	Agitation	Fused silica capillary column (DB-FFAP, 30 m × 0.32 mm i.d., 1.0-m film thickness)	PFPD	Vazquez-Landaverde et al. [80]
Pasteurized Milk	DVB/CAR/PDMS	10/40	50/30	Agitation	Rtx-5 capillary column (30 m × 0.25 mm i.d., 0.25 m film thickness)	MS	Gandy et al. [56]
Raw, pasteurized, UHT milk	CAR/PDMS	5/10	30/15	–	CP-SIL 5 CB column fused silica, (30 m × 0.32 mm i.d., 4 μm film thickness)	PFPD	Al-Attabi et al. [40]

(continued)

Table 16.5 (continued)

Sample	SPME fiber	Sample size (mL)/vial size (mL)	Conditions (temperature, (C)/time (min)	Agitation/ salting	Column	Detector	References
Pasteurized, ultra-pasteurized using direct steam injection or indirect heat	DVB/CAR/PDMS	5/20	35/40	Agitation	ZB-5 ms (30 m × 0.25 mm i.d., 0.25 μm film thickness)	MS	Jo et al., [59]
Skim Milk	PDMS/DVB	8/20	50/30	Agitation	DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness)	MS	Pan et al. [62]
Whole pasteurized milk	CAR/PDMS	2/4	–/60	Agitation/ salting	DB-1 60-m column	MS	Ziyaina et al. [95]
UHT hydrolysed-lactose milk	DVB/CAR/PDMS	5/20	40/60	–	HP-Innowax Fused-silica capillary column (30 m, 0.32 mm i.d., 0.5 mm film thickness)	MS	Bottiroli et al. [82]

DVP divinylbenzene, *CAR* carboxen, *PDMS* polydimethylsiloxane

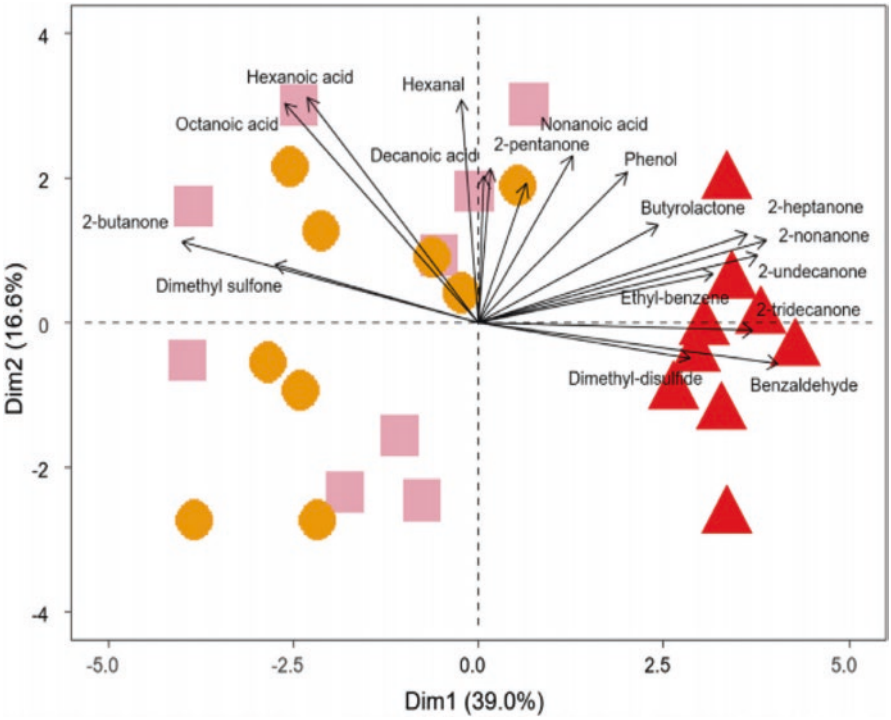

Fig. 16.9 Principal component analysis (PCA) bi-plot for the 1st and 2nd component (Dim1 and Dim 2), which describe respectively the 39.0% and 16.6% of the total variance. The displayed points represent the three production replicates of HLM samples taken during "in batch" production (square, before lactase addition; circle, after lactose hydrolysis; triangle, after the UHT treatment) [82]

7 Sensory and Instrument Method (GC Olfactory)

Gas chromatography has the ability to detect and identify many VOCs in food samples but cannot identify the individual ones that contribute to the aroma. Smelling GC effluent, called GC-olfactometry (GC-O), permits to determine the relative importance of specific VOCs according to their concentration divided by the odor threshold of that compounds [85]. Thus, odor active compounds can be discriminated from those nonactive ones in a specific food sample. The GC-O principle is discussed and presented its application to dairy products [86–90]. At the end of the column, the VOC are analyzed simultaneously by GC column and human olfactory system using sniffing port (olfactometer), where an expert and trained panelist (i.e., 1–3) sniff the outcome VOC and describe its smell nature using software (Fig. 16.10). Several odor descriptors are reported in dairy products and summarized in Table 16.6.

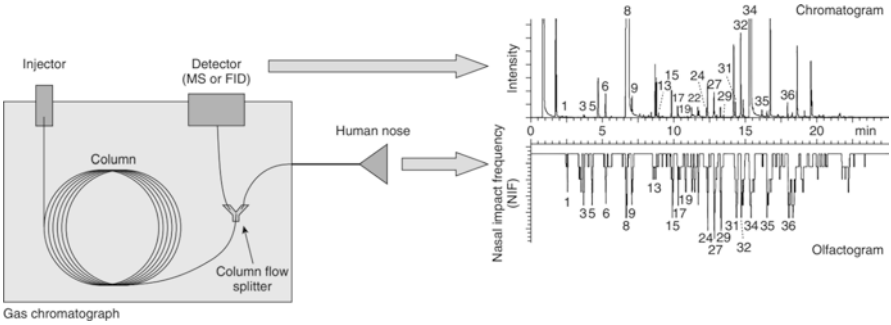

Fig. 16.10 GC/O system and data output [89]

Table 16.6 Odor descriptors in dairy products [87]

Compound	Odor descriptors
Ethyl butanoate	Fruity, sweet
Ethyl hexanoate	Fruity, pineapple
Heptanal	Green, sweet
Indole	Fecal, putrid, musty, floral in high dilution
Nonanal	Sweet, floral
1-Octen-3-ol	Mushroom-like
Dimethylsulfone	Sulfurous
2-Hetanone	Fruity, spicy, cinnamon
2-Undecanone	Floral, rose-like
Hexanal	Green, cut-grass
2-Nonanone	Grassy-herbal, green-fruity
Benzothiazole	Quinolone, rubbery
d-Decalactone	Coconut
Diacetyl	Buttery
HDF	Cotton candy
EHMF	Sweet, maple, caramel
1-Octen-3-one	Mushroom-like
Methional	Boiled potato-like
3-Methylbutana	Green, malty
Butyric acid	Sharp, cheesy, rancid, sweaty, sour, putrid
Isovaleric acid	Rancid, cheesy, sweaty, fecal, putrid
Ethyl butyrate	Ethereal-fruity, banana, pineapple
Ethyl caproate	Fruity-winey, apple, banana
Acetic acid	Vinegar-like
2,3-Butandione	Buttery, creamy
Acetaldehyde	Ethereal, pungent
Dimethyl sulfide	Intense, boiled cabbage, sulfurous

Different olfactometric methods are used to collect and analyze the GC-O data to determine the active odor compound. These are flavor dilution methods, detection frequency methods, and time-intensity methods. The most dilution method used is dilution analysis or Charm analysis in which panelists are presented with several dilutions in random order until no odor is detected. The data are demonstrated by a Charm chromatogram. In aroma extract, dilution analysis (AEDA) is presented in an increased order and flavor dilution value (DF) is calculated and presented by aromagram. Time-intensity methods such as OSME method are implemented by measuring the intensity of the compound as a function of time using a computerized system. The higher the intensity recorded the high the effect on food aroma.

In the detection frequency method, the eluted time of the aroma concentrate, and its aroma quality is recorded. The detection frequency is then summed based on the aroma detected at the same time by each panelist [91]. The more panelists respond simultaneously to the same aroma, the higher the estimated odor impact. Trained (e.g., 13) and untrained (e.g., 8–10) panelists were reported to be used with this method [90, 91].

The aroma active compounds in different types of milk were determined by GC-O-Charm analysis [37, 92, 93]. In bovine milk (i.e., raw, pasteurized, and UHT), 15 key aroma impact characters were identified. Among these, dimethyl sulfone and indole were active compounds in all three types of milk. Additionally, ethyl butanoate and ethyl hexanoate were reported for raw milk and 2-hexanone and 2-nonanone for UHT milk [92]. Moio et al. [93] found 14 odor active compounds in ovine milk in which 9 (i.e., ethyl butanoate, heptanal, dimethyl sulfone, 1-octen-3-01, ethyl hexanoate, octanal, 4-methylphenol, nonanal, and indole) compounds were identified as shown in Fig. 16.11.

Forty-one aroma active compounds were detected from seven whole milk powder samples using GC-O by detection frequency method [91]. The aroma volatiles were first extracted by solvent-assisted flavor evaporation method. Thereafter, 2.0 μL injected into the GC injector. Thirteen trained (i.e., 60 h) panelists were used with three-port sniffing GC-O system used simultaneously. On the other hand, Siefarth and Buettner [65] used GC-O-AEDA and human sensory method (i.e., aroma profile analysis) to study the odor profile of pasteurized, UHT-treated, and sterilized goat milk. Two panelists were used to sniff the aroma and to determine the active compounds in which 66 aroma-active compounds were detected when heated.

Recent work by Potts and Peterson [67] was able to identify the active compounds responsible for the claimed spontaneous oxidized flavor (SOF) in milk (i.e., pasteurized at 74 °C for 20 s) using GC-M-O-OSME and sensory test. These are endo-borneol, 2-methylisoborneol, and α-terpineol. The authors pointed that these were the microbial origin, which in turn highlight the importance of determining the active compounds of any reported flavor. A sensory test with spiked milk samples with terpenoid compounds (endo-borneol, 2-methylisoborneol, and α-terpineol) confirmed the finding. In GC-O, three trained panelists were requested to record the elution time of the compound, perceived odor descriptor, and intensity on a scale from 1 to 5: 1 is very weak; 2 is weak; 3 is moderate; 4 is strong; and 5 is very strong. The identified compounds with their odor descriptors are presented in Table 16.7.

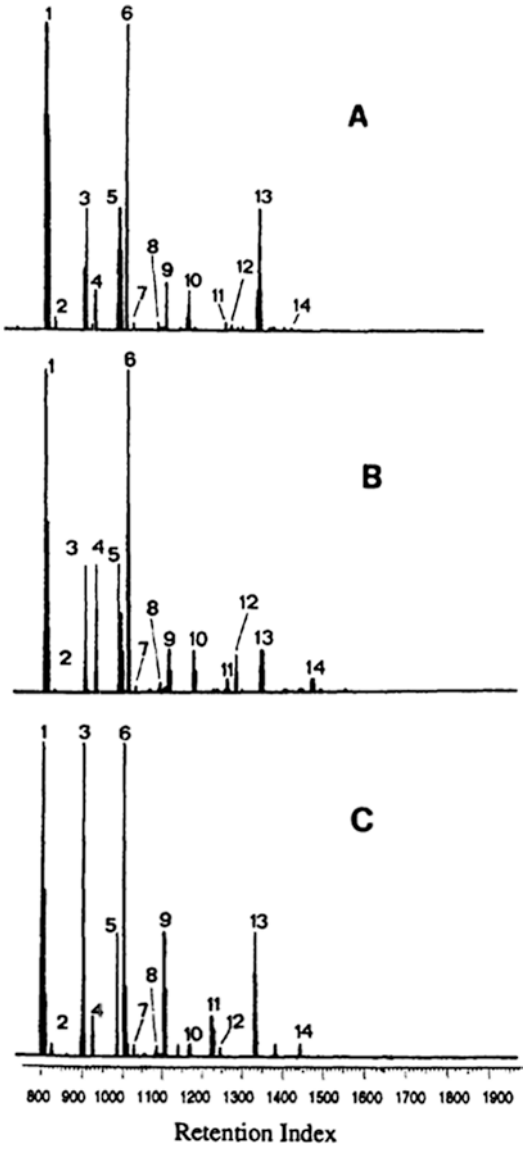

Fig. 16.11 Aromagrams of the neutral extracts of ovine milk from ewes fed on natural pasture (**a**), grass meadow (**b**), and mixed grain ration (**c**) [93]

The SPME-GC-O was used to evaluate the aroma profile of pasteurized milk (HTST), UP by direct steam injection (DSI-UP), and ultra-pasteurization by indirect heating (IND-UP) and to determine the active ones [59]. Two trained panelists were requested to record aroma character, perceived intensity, and retention time. This method allowed the detection of 55 active compounds in which H_2S, DMTS, and methional in DSI-UP and 2 and 3-methylbutanal, furfural, 2-heptanone, 2-acetyl-1-pyrroline, 2-aminoacetophenone, benzaldehyde, and dimethyl sulfide in

Table 16.7 Aroma compounds identified in the neutral/basic fraction of spontaneous oxidized flavor labeled milk [67]

Identified compounds	Odor descriptor	Average OSME intensity[a]	
		d 0	d 14
Propanoic acid	Pungent, rancid	–	1.8
Pentanal	Pungent	2.2	2.0
Hexanal	Oxidized, fat	1.7	1.0
Butyric acid	Cabbage, onion	–	1.7
Hexanol	Hay, grass	1.7	1.8
2-Heptanone	Fatty	1.7	2.3
3-Octanol	Mushroom, earthy	2.0	2.0
Benzyl alcohol	Roasted, meaty	2.2	1.8
1-Octanol	Chemical, metal	2.3	1.7
2-Nonanone	Green	2.3	2.0
Nonanal	Fat, cream	2.0	2.3
Endo-borneol	Paper, musty	2.2	2.7
2-Methylisoborneol	Musty	2.3	3.7
Octanoic acid	Sweat, cheese	2.3	2.5
Naphthalene	Musty	–	2.5
α-Terpineol	Musty	2.3	2.3
Decanal	Green, fat	2.3	2.2
E-2-Decenal	Cheese, fat	1.8	2.0
E,*E*-2,4-decadienal	Paper, cardboard, bean	2.0	2.0
δ-Octalactone	Peach, coconut	2.2	2.3
Decanol	Fat	2.0	2.7
δ-Nonalactone	Coconut	2.3	2.7
p-Vinyl guaiacol	Malty, mushroom	2.2	2.8
γ-Nonalactone	Coconut, peach	1.8	2.3
Decanoic acid	Rancid, fat	2.5	2.2
δ-Decalactone	Lactone, cream	2.5	2.7
Dodecanoic acid	Oil, soap	–	1.8

[a]Averaged among three panelists

IND-UP were considered high-impact aroma active compounds. The sulfur compounds were associated with sulfur or cooked flavor in milk in DSI-UP, whereas the compounds in IND-UP give milk sweet aromatic as it was linked to sugar degradation and Maillard reactions.

8 Artificial Sensors

Chemical sensors are developed as rapid methods, which permit in-site detection of VOCs without complex sample preparation. Some chemical sensors and commercial systems (e.g., electronic nose and tongue) can be used in dairy for spoilage

detection, shelf-life prediction, and quality monitoring. In addition, smart packaging in which sensors were incorporated in milk packaging was developed [94]. Recently, colorimetric nano-sensors were incorporated in pasteurized whole milk packaging material and showed success in the detection of VOCs and these emitted as a result of microbial activity during storage at 7, 13, 15, and 19 °C. It has been noticed a good correlation between signals from nano-sensors and aerobic plate counts [95].

8.1 Electronic Nose

Electronic nose (e-nose) was invented to solve many problems, which are normally faced when using human panels such as individual variability, adaptation, fatigue, infections, mental state, subjectivity, and difficulty to expose to hazardous compounds. The extra feature of e-nose is its capability of giving the aroma profile of samples [96, 97]. When studying complex mixtures like foods, e-nose is better than GC when it shows the differences between samples in terms of the emitted VOCs without a need to know the composition or concentration [98].

E-nose is an instrument that consists of chemical array sensors, signal conditioning system, and pattern recognition system to recognize smell print of odors. The sample placed in a closed container like vail, and it is left for certain time at a specific temperature to allow VOCs to accumulate over the sample headspace. Later, the VOCs transferred (e.g., syringe or pump) to the electronic nose sensors and then signals are sent to the recognition system (Fig. 16.12).

There are different available brands of e-nose that were reviewed by Wilson and Baietto [99] such as PEN2 (metal oxide sensors), Aromascan A32S (conducting polymers sensors), and Cryanose 320 (Carbon black polymers) (Fig. 16.13). The commercials are handy and compact but cannot analyze all volatiles [100]. The

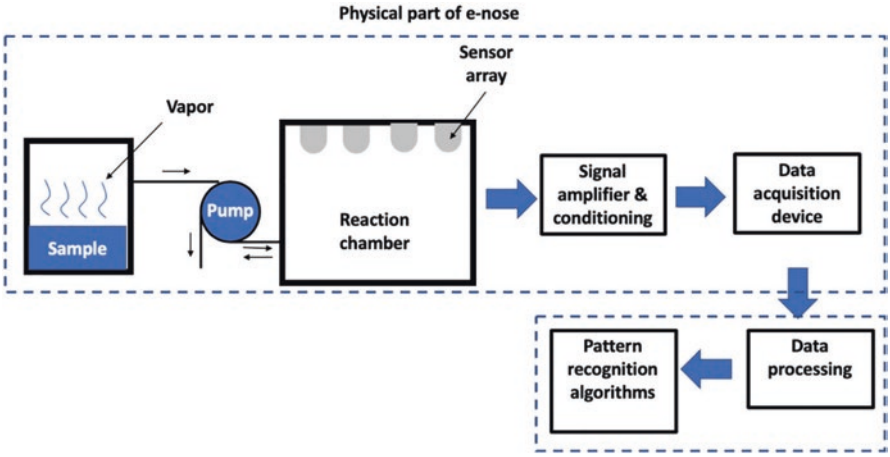

Fig. 16.12 E-nose system [100]

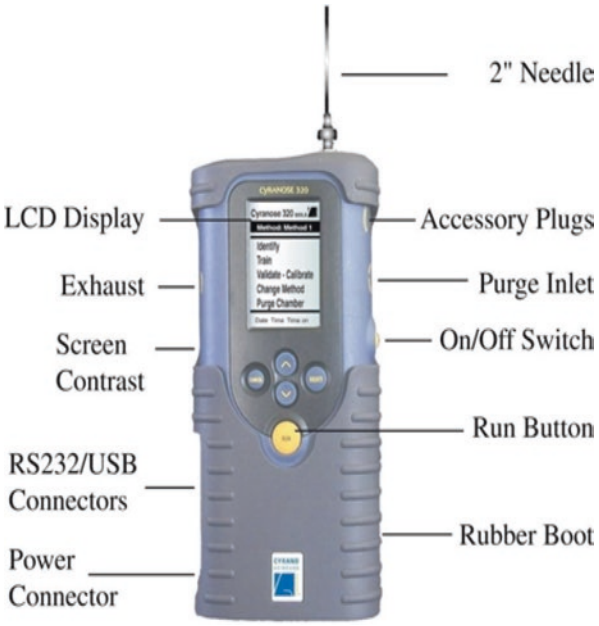

Fig. 16.13 Commercial Cyranose 320 (http://sensigent.com/instrument.html)

e-nose principle and application in dairy products were discussed [97, 101, 102]. There are a couple of sensors that had been reviewed [89, 101, 103, 104]. For instance, conducting organic polymer sensors (CP), mass spectrometry-based sensors, thickness-shear mode (TSM) sensors, piezoelectric sensors, metal oxide sensors (MOS), and metal-oxide semiconductor field-effect transistors (MOSFET).

The resulted raw data from e-nose sensors need to be processed, analyzed, and classified in order to get meaningful information [105]. The pattern recognition techniques are characterized by their ability to distinguish complex mixtures without dealing with individual components [98]. Several pattern recognition methods can be used such as PCA, cluster analysis (CA), discriminant function analysis (DFA), and Artificial neural network (ANN). More detailed discussion on the pattern recognition algorithms and classification methods can be found in recent publications [100, 104].

The portability of some commercial electronic nose devices makes them convenient, reliable, and simple preparations for use in the production line [106–108]. E-nose is described as easy to use [98], and it does not need highly trained people to use [101]. Besides, e-nose data have a good correlation with sensory and microbiological tests. In addition, simplicity, low cost, and low time for measurement make e-nose a convenient option [109]. Although many types of e-noses were developed, their practical use is still limited mainly to laboratories [110]. Sensor drift or noise is the main defect of e-noses. Different factors could affect sensor drift such as temperature, humidity, and sensor aging [108].

Measurements by e-nose are highly affected by water vapor because gas sensors are very sensitive to humidity [98]. The water that appears in liquid samples affects the sensor coatings and can disturb the effect of other compounds, which e-nose need to be detected [110]. For example, Ali et al. [111] suggested that an electronic nose with six-element array-coated Quartz Crystal Microbalance could not distinguish between uncontaminated UHT milk and contaminated with *Pseudomonas fragi* because of the affectivity of sensors to water vapor, where milk contains about 87% of water, which prevented sensors from detecting the volatile compounds emitted by bacteria.

On the other hand, it is difficult to detect VOCs when they are produced by bacteria in low concentrations. In such cases, sensitive sensors are needed [134]. Lack of selectivity and difficulty to replace the sensor with the same characteristics are reported [107, 109]. E-nose defects can be mitigated sometimes by the combination of e-nose with other detection approaches such as microbial or sensory tests [102].

Application of e-nose in fluid milk was reported. Classification of different heat-treated milk (i.e., pasteurized and UHT) and milk with different fat content has efficiently used an e-nose with seven tin oxide-based gas sensors and support vector machine (SVM) neural approach [112]. E-nose with 32 sensors, when used with 2% fat pasteurized milk subjected to different intensities of LED light for 4 h, was able to show the difference between milk packaged with glass and translucent high-density polyethylene (HDPE) bottles with and without light protective material [113]. In addition, it was possible to discriminate between three natural milk flavorings, two synthetic flavorings, and one self-made enzyme-induced milk flavoring when an electronic nose with 18 metal MOS sensors and PCA as a method of data analysis. E-nose could also show better than sensory tests when different natural milk flavorings were detected [114].

A study of goaty flavors in goat milk was conducted using e-nose with 10 different MOS. The PCA and LDA graphs of e-nose results were correlated well with sensory scores and GC data showed specifically differences in C6:0, C8:0, and C10:0 fatty acids in goat milk samples. Four intensities of goaty flavor (none, low, mild, and strong) were well-distinguished using e-nose with Fisher Discriminant Analysis (FDA) and back-propagation neural network (BP-NN) [115]. Furthermore, combined with CO_2 sensor, a gas MOSFET-sensor array technology was able to classify milk from mastitic cows with that from healthy cows both between and within cows. The results of gas sensor array were correlated well with GC results. Besides, good discrimination was clear along with the second principle of PCA graph between the gram-negative bacteria in mastitis milk (i.e., negative values), and gram positive showed positive values [116]. Following up the evolution of rancidity during the aging of pasteurized and UHT milk was performed using an electronic nose with SnO_2-based semiconductor thin films sensors (Fig. 16.14).

On a three-dimension PCA graph, clear clusters were shown a good classification of milk with different degrees of rancidity [117]. Rancidity of raw milk during 4 days of storage at 4 °C was tracked using a simple and home-developed e-nose with six MOS. On a 3D PCA graphs, three distinguished clusters appeared to be correlated with three different levels of rancidity (Fig. 16.15). Similar clusters were

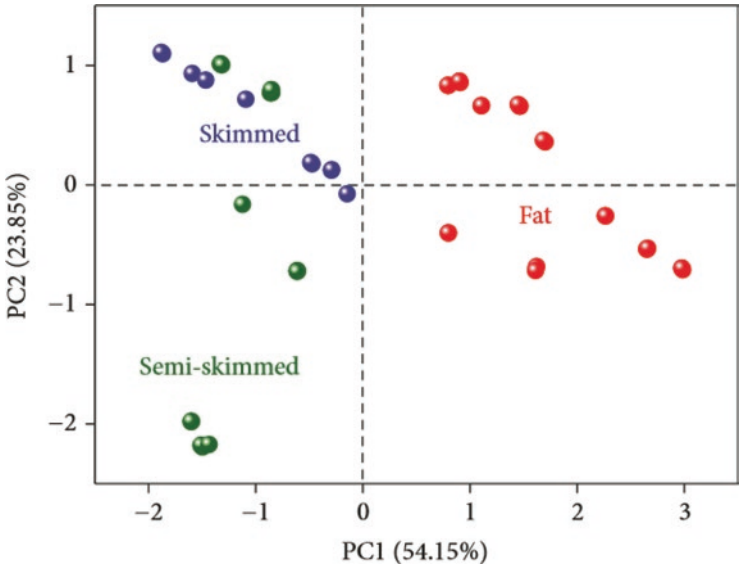

Fig. 16.14 Electronic nose set-up used in the measurements of milk rancidity [117]

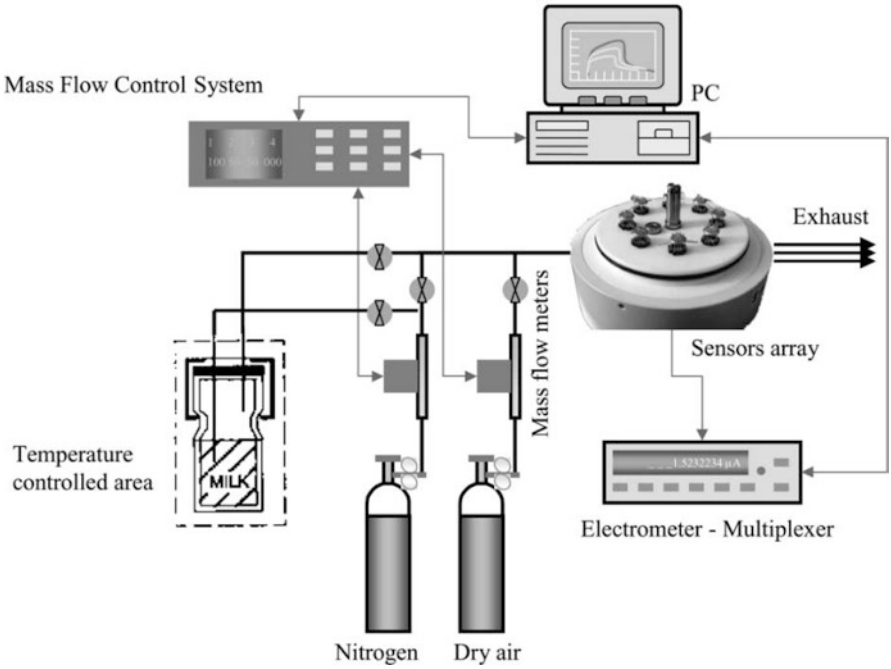

Fig. 16.15 PCA plot for UHT (left) and pasteurized milk collected during 8 and 3 days respectively [117]

obtained when a 2D graph of PCA was used. Another 3D graph of PCA showed distinguishable clusters of three types of milk from three dairy farms [118].

Five brands of pasteurized milk were discriminated on three-dimensional PCA by a hybrid e-nose with four micro-machined gas sensors and four commercial tin-dioxide gas sensors on the first day of storage. Three of the five brands had similar characteristics according to the PCA plot. When studying the storage of one of the five brands, by increasing of storage period, microorganisms increased and produced volatile compounds caused different e-nose responses and showed clear clusters on a PCA graph. Furthermore, more accurate discrimination between pasteurized milk samples during storage using a combination of hybrid e-nose with a voltammetric electronic tongue (e-tongue) and a data fusion approach was achieved [106].

8.2 Electronic Tongue

E-tongue (Fig. 16.16) is a mimic of the human tongue, where chemical sensors react with the liquid sample and then signal to send to the data processor and these can be analyzed with different pattern recognition algorithms. The common pattern recognitions used are PCA, LDA, hierarchical cluster analysis (HCA), DFA, SVM, convolutional neural network (CNN), and ANN. Chemical sensors (i.e., potentiometric, voltammetric, and impedimetric), biosensors, and optical mass sensors are being developed. Sensors and pattern recognition algorithms for e-tongue are thoroughly discussed in the literature [89, 100, 101, 119]. The e-tongue is a qualitative analysis that has the ability to classify the samples based on their taste. Different applications of e-tongue have been reported for the food and dairy industry [101, 119, 120]. The e-tongue is descried as label-free detection, low cost, rapid and accurate, sensitive;

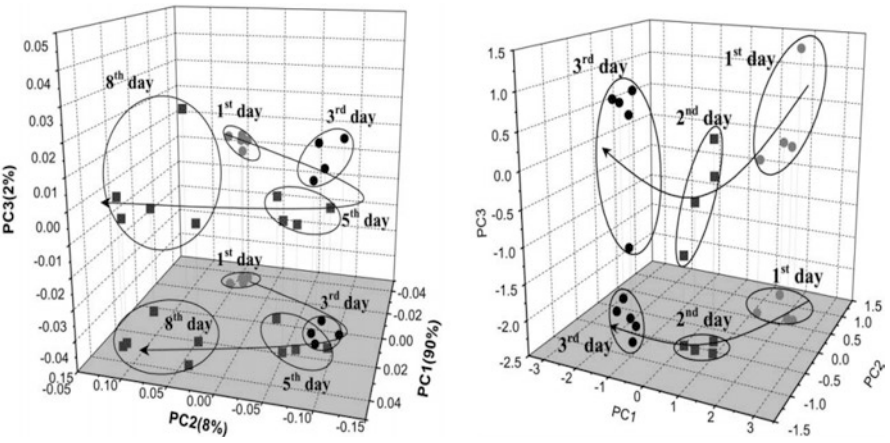

Fig. 16.16 Electronic nose system [100]

however, different limitations are reported including pretreatment time required for multiple analytes analysis and short life of the sensors [100, 120].

Electronic tongue was successfully used to classify milk based on sources/brands [121–123] as well as unsealed pasteurized milk stored at different times [124]. Furthermore, it has been used to assess the safety of milk including antibiotics [125, 126], adulteration [127–130], and milk spoilage [94]. Figure 16.17 shows an example of milk adulteration analyzed by e-tongue. The discrimination analysis successfully separated the three types of sample (i.e., cow, goat, and goat and cow mixture). Pan et al. [62] used e-tongue with PCA and CA to assess the best preheating conditions before defatting milk and compared it with the sensory evaluation. Food and beverage sensors called chemically modified field-effect transistors (CHEMFETs) used 20 mL of milk sample at room temperature by immersing the sensors array for 120 s. Each sample was analyzed 10 times, and the first 3 readings were excluded due to varied and unstable mV reading. The authors, Pan et al. [62], found that the results of the sensory and e-tongue are in line to determine preheating milk before defatting, and the best treatment conditions were observed as 50 °C for 30 min. These conditions were classified as the same group with raw milk indicating taste similarity. The pasteurized milk taste profile was studied at different storage temperatures [131]. The e-tongue data indicated that the milk taste is unchanged within 3 days of storage at 0, 4, or 10 °C, while its sweetness significantly dropped when stored at 15 or 25 °C due to the milk spoilage. Furthermore, the e-tongue had classified milk samples successfully based on fat content (Fig. 16.18) [132]. The two samples of semi-skimmed milk are located close to skimmed milk in Fig. 16.18 may be related to the lower fat content in these samples, since semi-skimmed samples can contain a range of fat (1–2%).

Humans normally evaluate flavor using combined senses (nose and tongue) simultaneously. Therefore, using both e-nose and e-tongue with human sensory can provide a clear explanation of milk flavor. Hu et al. [36] used both instruments to study the high hydrostatic pressure cow milk flavor exposed to ultraviolet light C. Six metal sensors were used for e-tongue analysis. They were immersed in 15 mL

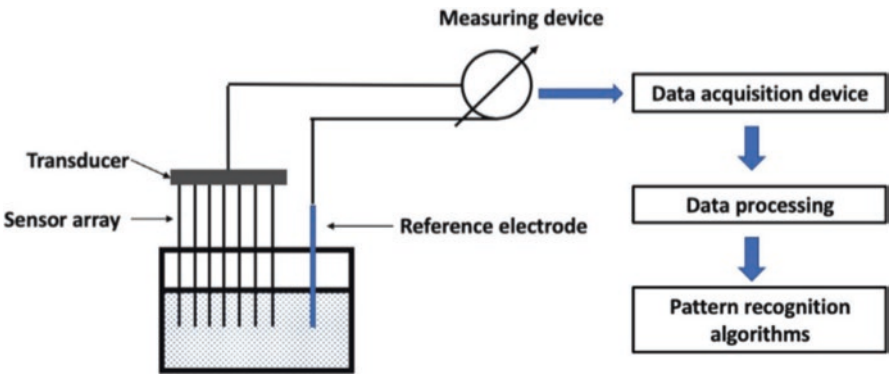

Fig. 16.17 Discriminant analysis of adulterated skim raw milk samples [129]

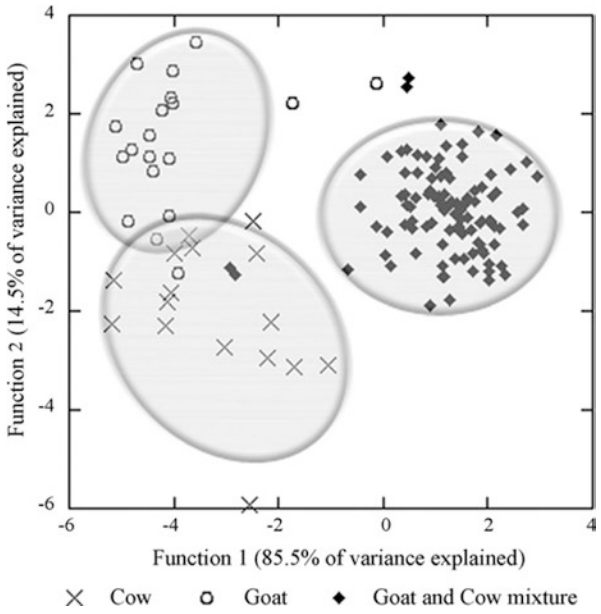

Fig. 16.18 PCA plots for milk samples with different fat content with using E-tongue [132]

of sample (25 ± 1 °C) and 180 s of acquisition time and 1 s of acquisition period were used. It was observed that insignificant differences between the control and UV-C sample. Furthermore, simultaneous application of e-nose and e-tongue gives an acceptable classification of milk.

9 Conclusion

Sensory analysis is a very important field of food science. Its application covers food quality, food safety, and product development. For many years, the sensory tests are evolved to include sensor detection technology like e-nose and e-tongue. These technologies developed to cover some of the human sensory drawbacks such as individual variability, adaptation, fatigue, and mental state. The sensors can be described as convenient, reliable, simple to use, and cost-effective; moreover, it can be installed over the production line for quick assessment of any sensory deviation, which could minimize loss. They are capable of classifying samples with the same characteristic, source, treatment, and processing with help of the pattern recognition systems. However, identifications of VOCs of the odor attribute would not be possible without GC, which maps the odor profile (i.e., VOCs) with varying concentrations. Further development (GC-O) allowed identification of the key impact characters (i.e., VOCSs) that contributed significantly to the odor. When the flavor is evaluated by humans; chemical compounds react by the nose, tongue receptors, and signals analyzed by the brain. Similarly, using e-nose, e-tongue, and GC-O

simultaneously is a powerful technique that could elicit more details and explanation of the milk flavor.

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Chapter 17
Measurement of Instrumental Texture Profile Analysis (TPA) of Foods

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Abstract Texture is one of the sensory attributes that consumers use to evaluate food acceptability and purchasing decision. It is a very complex attribute where different senses (e.g., vision, hearing, and touch) are used simultaneously to assess different textural properties, such as hardness, cohesiveness, adhesiveness, gumminess, and springiness. These and others are evaluated using different sensory methods including the descriptive method. However, the subjective sensory measurements have limitations. Instrumental Texture Analysis Profile (TPA) is a very important breakthrough in the field of sensory analysis. It is now widely used by the food industry and scientific community. TPA is an objective method, and the parameters defined by this method can be correlated with the sensory textural attributes. Therefore, this method could be fast and needs easy sample preparation and could be used in quality control in the processing line. This chapter addresses the theoretical development of the TPA and its applications in different food products.

Keywords Texture profile analysis · Texturometer · Fracturability · Texture · Sensory properties · Mechanical · Composition · Cohesiveness · Adhesiveness · Chewiness · Juiciness · Modulus · Electromyography · Poisson's ratio · Young's modulus · Empirical tests · Imitative tests

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1 Introduction

Food sensory describes the interaction between human senses and food attributes (i.e., appearance, color, aroma, taste, and texture), and it provides a critical decision on the sensory attributes of food as well as preference/acceptability of certain food. Food texture is defined as "the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics" [1]. The flavor and taste are excluded from the texture. The complexity of texture assessment by human sensory is raised from its multi-mechanical (e.g., hardness, cohesiveness, and adhesiveness), geometrical (i.e., particle size, shape, and orientation), and compositional attributes (i.e., moisture and fat). These attributes are initially evaluated by hand and later by mouth (i.e., from the first bite followed by mastication, swallowing, and residual feeling in the mouth and throat). The evaluation covers both kinesthesis (i.e., exerting pressure) and somesthesis properties (i.e., tactile/surface structure). The textural attributes and assessment method are described by Szczesniak [1].

Texture is used as a quality indicator for natural and formulated foods. It is a useful indicator for the harvesting stage and assessing the effect of handling/transportation of postharvest products (e.g., tomato). Consumers, in many cases, used the appearance to make an initial decision on food purchasing based on visual texture (i.e., surface smoothness or roughness). Visual texture (i.e., surface) could also provide the healthiness perception of biscuits [2]. It was found that "visual texture, independent of color, affects the taste and flavor as well as the appearance of foods" [3]. Furthermore, texture perception could also affect the aroma and taste perception [4]. Therefore, in many instances, sensory studies on texture and flavor are combined [5]. Additionally, food texture has a very important role in developing food satiety (e.g., solid vs. liquid) [6]. Therefore, the texture of new products must be carefully optimized and developed. Thus, knowledge of food chemistry, physics (i.e., acoustics), physiology (i.e., oral processing), and psychology (i.e., perception of texture) is required [7].

Moreover, the mastication (i.e., chewing/breakdown of food) process may differ among individuals [5]. Hence, understanding mouth behavior among targeted consumers is important in product development [5], and this can increase the chance of product success (i.e., re-purchase). The mouth behavior theory was developed by Melissa Jeltema and Jacqueline Beckley who developed JBMB™ typing tool (stand for Jeltema/Beckley Mouth Behavior) [8]. Four mouth behavior groups were identified by Jeltema et al. [8]: crunchers, chewers, suckers, and smoothers. This could explain the individual difference in texture perception in sensory analysis. However, in the descriptive method, trained panelists perceived attributes similarly [5]. As fast-eating rates were linked with obesity and higher energy intake, modifying food texture can slow down the eating rate [9]. Wee et al. [9] highlighted the effects of eating behavior (i.e., number of bites, average bite size, total chews, chews per bite, oro-sensory exposure time, and eating rate) and macronutrients on instrumental textural properties (i.e., hardness, adhesiveness, springiness, cohesiveness, chewiness,

resilience, and modulus). They observed correlations between oral processing parameters and textural properties (i.e., springiness, cohesiveness, chewiness, and resilience). For example, the protein content of the food was associated with springiness and chewiness, and this may help to reduce eating rates due to the enhanced eating process. Therefore, instrumental texture attributes can guide the slow eating rate by smaller average bite-size pieces and need more chews per bite. The numbers of chews are related to springiness, cohesiveness, chewiness, and resilience.

Quantitative descriptive analysis (QDA), texture profile method, spectrum method, and time-intensity method (TI) are used to evaluate texture. A hedonic scale can be used to assess the consumer acceptability of food texture. The sensory and instrumental methods are reviewed by Lawless and Heymann [10] and Chen and Opara [11]. Several sensory limitations are compiled and reported by Chen and Opara [11]. These include (a) fatigue, adaption, and level of training can affect the response, (b) panelists calibration, and (c) time consuming and expensive compared with the instrumental method. To overcome these limitations, instrumental analysis such as Texture Profile Analysis (TPA) was introduced. However, the instrumental and oral assessment of texture may be different; however, in many instances, these can be correlated and can be used further [12]. Limited numbers of sensory texture attributes can be evaluated in a single sitting by panelists (i.e., due to consumer fatigue) when compared to TPA (i.e., numbers of parameters/properties can be evaluated in one run). Therefore, combining both sensory and instrumental methods gain more interest, and coloration is established [11]. Peleg [13] has raised some concerns regarding TPA validity in which he stated some faults with attributes measurement, which could be measured differently by incorporating fundamental material science. This could be "more problematic" [14]. Peleg [13] also suggested harmonizing the nomenclature and definitions with other industries; however, this could be very challenging [14]. Some technical improvement was suggested by Johnson [14], including the development of guidelines for foods that are suitable for TPA analysis and "set guidelines regarding the range of speeds and other common settings that might be appropriate for different food subindustries (e.g., dairy, processed meats, baked goods)." Other important suggestions are also listed in this chapter. This chapter discusses the basic principle of TPA measurement, definitions of the attributes, and the factors affecting the TPA measurement. Finally, examples of TPA in evaluating selected foods are presented.

2 Instrumental Methods

The physical measures of instrumental texture do not completely mimic the sensory experience [15]; however, these methods are quick and easy to use. In addition, these are in great demand especially in industrial environments, and economically more profitable [16]. No single measurement and no single instrument can quantify or relate the complete texture due to its multidimensional nature of texture. Forces greater than gravity are applied to cutting, squeezing, compression, shearing,

crushing, and pulling. It is important to know the types of applied force and their direction and rate. In addition, temperature, moisture, composition, and structure also need to be considered for the instrument. Instrumental methods related to texture are divided into six classes: fundamental, empirical, imitative tests, TPA analysis, acoustic method, and electromyography.

3 Fundamental Tests

Fundamental tests in foods measure properties that are familiar to engineers, such as toughness, ultimate strength, Poisson's ratio, yield point, and various moduli (i.e., Young's modulus, shear modulus, bulk modulus, storage modulus, and viscosities). When engineers handle the mechanical properties of solids, they are more concerned to know the failure point, whereas, in the case of texture, food scientists and engineers need to go further more (grinding, crushing, mixing, and flow) beyond the crack or breakage and the progress of cracking, crushing, and mixing. Fundamental tests usually correlate poorly with the sensory textural properties of foods [17]. Measures of fundamental properties do not encompass either singly or in combination all measures that embody multidimensional food texture. These tests describe only a portion of the property or attribute sensed in the mouth during mastication [18]. Young's modulus of elasticity is rarely applicable to food systems because of the assumptions associated [19]. These assumptions are: (a) test material must be continuous, isotropic, homogeneous, and of uniform shape and size (e.g., cylinder); (b) materials must be purely elastic, but foods generally exhibit viscoelastic properties rather than pure elastic behavior, and (c) the strain used in measurement is small. The fundamental tests are time-consuming and require detailed technical calculations. However, these properties can provide a much better insight into the chemistry and physics of the foods, and sometimes they are the only meaningful measurements that can be made [20].

Considering the perception of crispness upon eating, large deformation and fracture tests seem to be the most suitable instrumental tests [16]. Small deformation data through dynamic rheology may provide information not directly related to crispness but to the molecular basis of this attribute. The most commonly used tests can be categorized into three groups: flexure, shear, and compression. The latter is probably the most commonly employed because of their similarities with the process of mastication. Puncture test is also used extensively as well since they can simulate the incisors impact at biting. The force-deformation pattern is characterized by a series of sharp force peaks corresponding to the rupture of the individual structure. The force deformation plots are analyzed either by extracting some parameters or by considering the signal as a whole. The first approach uses only the linear region of the force-deformation plot, and it reflects the mechanical properties. Another approach or option is to collect information from the jagged part of the force-deformation curves.

4 Empirical Tests

Empirical tests are developed from practical experience, which is correlated with textural quality. These are puncture, shear, cutting, and extrusion. A number of tests in this category have been developed that give a good correlation with a sensory evaluation of texture on a limited number of foods [17]. The empirical methods can usually be carried out more quickly, and in many instances, it correlates with texture as perceived by a taste panel. This empirical approach could be easily used in food quality control.

5 Imitative Tests

Imitative tests are developed to imitate with instruments the conditions that the food is subjected in the mouth or on the plate. Imitative tests started with the work of Volodkevich [21], Proctor et al. [22], Szczesniak et al. [23], and Bourne [24]. The instrumental measurement of food texture requires the active manipulation or deformation of food. The imitative test could be performed by compressing either by deforming the sample to a fixed degree or by compressing the sample at a predetermined set force. In both cases, compression is exerted for a given time to reach a predetermined force or deformation [25]. A number of parameters are measured in one-cycle compression analysis. These are maximum force or firmness (i.e., force necessary to produce a given deformation), modulus of deformability (i.e., slope of the force–deformation curve), the degree of deformation produced by a given compression force, energy, and elasticity, or the recovered height by the sample in a given time following compression. In addition, the relaxation time or the time taken for the compression force to reduce a predetermined percentage is also considered [26]. There exist correlations between one-cycle compression measurements and sensory parameters, such as elasticity in mackerel and white hake, or firmness in various species [27, 28]. Single compression test was correlated well with seven sensory attributes, including cohesion of bolus, adhesion to lips, hardness, cohesiveness of mass, roughness of mass, toothpull, and toothpack of cooked rice [29]. Furthermore, single and double compression cycles were found to give satisfactory prediction with sensory attributes for adhesion to lips as well as toothpull, and it is the moderate prediction for manual stickiness, initial cohesion, and hardness of cooked rice [30]. The single cycle would be more appropriate additionally due to its less time consumption. Good correlation with a tactile sensory evaluation of trout was observed by pressing the muscle with a finger [31]. While firmness generally tends to increase with time in frozen storage, no clear trend was observed for elasticity [26]. Sesmat and Meullent [29] defined 14 instrumental parameters from a compression test on five kernels of cooked rice in one layer and attempted to develop predictive models for 11 sensory textural attributes. Kasapis et al. [32] identified

four instrumental parameters from one cycle compression test of fish burger and correlated with overall sensory desirability.

6 Instrumental Texture Profile Analysis (TPA)

Instrumental Texture Profile Analysis (TPA) is the extension of the one-cycle imitative tests. Instrumental TPA is one of the major breakthroughs in the imitative test [17]. In the literature, it is reported as Texture Profile Analysis (TPA). However, it is scientifically sound to use instrumental TPA, since human senses are not incorporated in these tests.

6.1 Background and Procedure

Instrumental TPA involves two or more cycles of compression of a sample between two parallel surfaces. The first attempt was to imitate mastication by instrumental means was the denture tenderometer [22]. The difficulty with this instrument was not to have more information than peak force, which could be obtained from the resulting force–time curve [33, 34]. The major breakthrough in instrumental TPA came with the development of the General Foods Texturometer, and this gave a foundation of the current TPA [17]. In this attempt to simulate mastication, a group at General Foods, led by Dr. A. S. Szczesniak, developed the Texturometer [35]. The equipment was modified from the original MIT Denture Tenderometer and was designed to simulate the masticating action of the human mouth. It was a unit composed of a flat plate supported by a flexible arm, which was attached to a strain gauge, and a plunger, which was acted on a food sample. The sample is usually a bite-size piece, and it is compressed twice in a reciprocating motion that imitated the biting action of the jaw. The strain gauge detected the force generated, and it was recorded on a strip chart recorder. The plunger was originally constructed with three materials and considered five sizes. The sizes usually varied from 2 to 5 cm in diameter. All the plungers were 2.5 cm in height. The materials used were lucite, aluminum, and brass [36]. This device usually compressed a bite-size piece (i.e., a cube approximately 1.3 cm) of food twice and generated a force–time curve, which was then used to define the textural characteristics (Fig. 17.1). This device was then followed by Instron, which produced similar output. The Texturometer uses a small flat-faced cylinder to compress to 25% of its original height (i.e., 75% compression) two times in a reciprocating motion imitating the biting action of the jaws by back and forth movement. A force–time curve portrayed the entire force history of the simulated mastication action and was plotted by strain gauges and a strip-chart recorder. Original analyses of the force–time curve led to the extraction of seven textural parameters or attributes: five measured and two calculated from the measured parameters [35]. Cohesiveness and elasticity values cannot be obtained

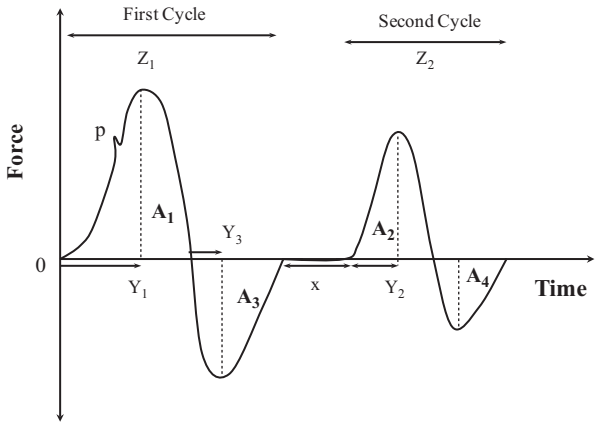

Fig. 17.1 A typical graph of Instrumental Texture Profile Analysis (TPA), p: Fracture point, Z_1: Time of first compression and decompression cycles, Z_2: Time of second compression and decompression cycles, A_1: Area of first compression cycle, A_2: area of first decompression cycle, A_3: Area of first decompression cycle, A_4: Area of second decompression cycle, Y_1: Time to reach the peak of the first compression cycle, Y_2: Time to reach the peak of the second compression cycle, Y_3: Time to reach the peak of the first decompression cycle, x: time lag between first and second compression-decompression cycle

from Texturometer's curves because they were very difficult to perceive from this test.

6.2 Limitations of GF (General Food) Texturometer

The limitations of (General Food) GF Texturomter compressing device and its concepts are identified as [36–39]: (a) the plunger was driven by an electric device; therefore, the compression rate was essentially a sinusoidal function of time (i.e., cycles of increasing and decreasing), whereas the paper chart moved linearly with time; (b) human mastication showed approximately follow a sine-wave pattern, and it decelerated as it approached at the end of the compression stroke and slowly accelerated again as it began its upward motion, and speed was reached at a maximum at the middle position of each stroke; (c) strip chart moved linearly with time, and thus the plot was force-time (i.e. not force-distance relationships); (d) contact area between the sample and the plunger was not constant during the test, since the plunger moved through the arc of a circle (i.e., one edge of the plunger containing the food first and, as the down stroke continued, the area of plunger pressing on the food increased until the entire plunger area was in contact with the food at the end of the down stroke) [24], and (e) there was a deflection of the sample supporting plate [40, 41].

6.3 Instron Adaptation

Pons and Fiszman [36] pointed that a very important milestone was the adaptation of the Instron Universal Testing Machine to food studies as described by Bourne et al. [42]. The Instron was a better tool for determining instrumental TPA parameters than the GF Texturometer for the following reasons as identified in the literature: (a) speed of the Instron compression is constant during the down stroke and during the immediate reversal of the compression stroke at the end of the first bite can produce a sharp peak in the TPA curves; (b) plunger moves in a strictly rectilinear manner, therefore the plunger area in contact with the food is constant throughout the compression, assuming flat sample surface with no slippage at the interface; and (c) Instron chart gives force–distance curve, and therefore the area is a true measurement of the work performed.

6.4 Computer-Assisted TPA

Modern computer-assisted TPA (such as TA-XT2, Stable Micro System, Texture Technologies Corporation) provides great versatility. It is possible to perform TPA tests and to obtain all TPA parameters directly by means of its software, without any previous selection of curve values for calculations. One of the most recent instruments from this group is the TA-XT Plus Texture Analyzer. It is improved with the ability to plug-in peripheral instruments, which can provide multichannel data acquisition. This allows relevant test equipment can be used in conjunction with other units, such as temperature monitoring systems (e.g., cheese) and humidity monitors to test dry products (i.e., crackers and biscuits). These can give additional advantages to the system. In this equipment, speed can be changed in proportion to the changing height of the compressing sample, and this allows to test the product at a constant strain rate. Instron Corporation introduced a hand-held mechanical tester, called the In-Spec 2200. Food Technology Corporation manufactured food texture gauges based on Kramer Shear Press. It is offering the newly redesigned TP-6 shear press. The press offers four programmable testing modes: Manual, One-Bite, TPA, and Multi-Step. Additionally, it offers automatic calculations and estimations of integrals, slopes, curvatures, statistics, and TPA parameters [43].

6.5 Instrumental TPA Attributes

A typical curve is shown in Fig. 17.1. The original parameters are defined from the curve given by Friedman et al. [35], and a discussion on their improvements is given below.

Fracturability: Fracturability (*FR*) is defined as the force at the first significant break in the curve shown as a small peak in Fig. 17.1 as marked at p. This is originally called *brittleness*. The unit is Newton. This appears as a sudden change in direction due to the sample's first point fracture or cracking or crumbling or fragmenting of structure into pieces. In brittle materials, cohesiveness is low whereas hardness can vary. In many cases, it is referred to as the yield point or bio-yield point [44].

Hardness: Hardness (*HA*) is defined as the peak force (i.e., force necessary to attain a given deformation) of area A_1 during the first compression cycle visualized as the first bite. The unit is Newton. Many authors termed it as firmness, which already exists in Szczniak's textural classification [39, 45, 46]. This peak force could be vague when the sample is broken into pieces by the plunger, and in this case, measurement conditions, such as sample size or compression, could be reduced [47].

Cohesiveness: Cohesiveness (*CO*) (i.e., strength of the internal bonds making up the body) is defined as the ratio of positive force area during the second compression to the first compression ($CO1 = A_2/A_1$). The unit is dimensionless. It is one of the complex attributes since it does not correspond to a single sensation, such as hardness and brittleness. The area of integration is a measure of the power needed to impose that force, assuming the head moves at a constant speed. The ratio does imply some measure of the ability of the food to recover and the process to be repeated with a different resistance or reaction. Samples that are very cohesive (high values) are perceived as tough and difficult to break up in the mouth. It was modified as the positive areas of compression and excluded the negative areas of the decompression for each cycle and calculated as $CO_2 = (A_2 - A_4)/(A_1 - A_3)$ [17, 38]. This procedure was also followed by Olkku and Rha [48] and Peleg [49]. Sherman [39] presented in his critical review on the instrumental TPA and mentioned that hardness and cohesiveness are dependent on elasticity and therefore should not be treated separately, and definition of elasticity, gumminess, and chewiness have very little practical significance.

Adhesiveness: Adhesiveness (*AD*) is defined as the negative force area of A_3 to the first bite, representing the work necessary to pull the compressing plunger away from the sample. If the material is at all sticky or adhesive, the force becomes negatives. The unit is N m. Actual adhesiveness is usually measured by touching the plunger without compression of the sample and then withdrawing it. In the case of TPA, the negative area is not actual adhesiveness since additional compression is applied, and a pull or push action from the sample like spring could affect the area A_3 [50]. Thus, adhesiveness is a function of surface adhesion and elastic–plastic nature of the sample.

Springiness: Springiness (*SP*) (originally called elasticity) is a measure of the recovery after the first compression. It is an indication of the recovery during the time that elapses between the end of the first bite and the start of the second bite. The unit is second or m. It is a measure of how much the original structure of the sample was broken down by the initial compression. The spring back is measured at the downstroke of the second penetration, and this can consider the wait time

between two strokes and make it more relatively important. In many instances, an excessively long waiting time allows a product to spring back more considering the conditions being researched in human chews (e.g., human may not be waiting 60 s between chews) [51]. The original definition of springiness (Type 1) used the length 2 ($SP1 = Y_2$) only and units are m or s. Springiness was modified typically as the distance of the detected height of the product on the second penetration as divided by the original penetration ($SP2 = Y_2/Y_1$) (Type 2). By expressing springiness as a ratio of its original length, comparisons can be made between a more broad set of samples and products [51]. Pons and Fiszman [36] defined two different parameters in gels. These are *instantaneous recoverable springiness* and *retarded recoverable springiness*. However, minimal experimental results are presented to check their validity and applications. The *instantaneous recoverable springiness* ($SPI=Y_3/Y_1$) is defined from the first bite. It is the ratio of the distance (or time) during the first decompression cycle of the sample to the first sample compression cycle. The *retarded recoverable springiness* ($SP2 = SPR = Y_2/Y_1$) is defined as the ratio of the distance (or time) recorded during the second compression of the sample to the first compression cycle. This considers the height recovered during the time elapsed between bites as shown in Fig. 17.1 (i.e., first and second compression). It can be considered that the first parameter would be an index of the ideal elastic characteristics (i.e., instantaneous recovery for elastic materials), whereas the second would reflect the characteristics due to the viscous behavior (i.e., retarded recovery for viscoelastic materials). Therefore, this could provide the dominating behavior of the sample in relation to its elastic and viscous behaviors. An SPI value near or equal to 1 would indicate a high elastic component in a sample, while SPR > SPI indicates high viscous component (i.e., time elapsed between bites becomes longer).

Resilience: Resilience (*RE*) is the instant springiness since it is measured on the withdrawal of the first penetration before the waiting period. It is the area during the withdrawal of the first penetration (i.e. decompression) divided by the area of the first penetration (A_3/A_1). Resilience can be measured with a single penetration by maintaining the withdrawal speed as the same as the penetration speed [51].

Gumminess: Gumminess (*GU*) is defined as the product of hardness and cohesiveness. The unit is Newton. Gumminess only applies to semi-solid products. Gumminess is a measure of the force to disintegrate the particles ready for swallowing, thus the higher the hardness the higher the gumminess.

Chewiness: Chewiness (*CH*) is related to the primary parameters of hardness, cohesiveness, and elasticity. It is defined as the product of gumminess and springiness (=hardness × cohesiveness × springiness). The unit is N s or N m (i.e., when Type 1 springiness used), and N (when Type 2 springiness used). Chewiness is the energy for masticating the food until it is ready for swallowing. It is incorrect to quantify and report chewiness and gumminess in TPA of solid or semisolid products. Gumminess refers to semisolid foods and chewiness to solid foods, and therefore chewiness and gumminess should not be reported both for the same product [52]. However, many products may transform from solid to semi-solid state in the course processing, storage, and consumption.

Juiciness: Juiciness (*JU*) is defined as the juice released during the first compression. The level of compression should be chosen to have sufficient tissue failure, and the consequence is a high juice expression. In this case, two filter papers are placed one above and one below the specimen. After compression, for example, 80%, the wetted areas are determined for two papers and expressed as m^2/kg sample. The filter papers could be pretreated by dipping in a 50 g/kg copper sulfate solution and dried [53]. For fruits, more juice was released indicating higher juiciness, whereas in the case of meat, types of bound water are more related to the feeling of juiciness.

Modulus: Although modulus (*E*) could not be included by Szczesniak's research team for TPA, in many instances, a parameter called *modulus* (i.e. initial slope of the force-deformation curve before failure point in the first bite) is measured. It is a measure of the sample's behavior when it is compressed to a small degree of deformation [37, 54]. It was indicated that it is better to derive the true stress-strain data from the force–deformation curve during the initial compression cycle considering true or Hencky's strain and stress [55, 56].

7 Factors Affecting Instrumental TPA Measurement

Undoubtedly, the instrumental TPA approach provides more in-depth information on the structure of foods although in many cases the parameters correlate with sensory. Mochizuki [57] outlined three protocols for instrumental TPA: protocol 1 for vegetables, protocol 2 for cooked beef, and protocol 3 for gels. Halmos [58] pointed that more analysis is needed before all the parameters, which are generated from the TPA graph, can be accepted as a valid or representing, reproducible, and meaningful measure.

7.1 Sample Preparation

In the case of TPA, consistent preparation of samples is probably the most critical step in obtaining good data (i.e., steps in the cutting and slicing of samples). Preparing the same size for each test sample is very critical. Generally, it is also important to avoid edge effects in a test sample and the aging effect of prepared samples [57]. The degree of compression, plunger size, and cross-head speed need to be kept the same among tests. Adjustment in the operating characteristics of the machine is needed when testing different types of samples. For example, if the samples are harder than those used in one protocol, equipment settings need to be reduced as the degree of compression and/or the cross-head speed [57].

7.2 Cross-Head Speed

The original proposed standard speed employed was 42 bites per min, which is equivalent to approximately 80 cm/min (13.33 mm/s) [35]. The panelists observed that the chewing force was applied at a slower rate for harder foods than soft foods. Large forces are required to chew hard foods than to chew soft foods. The applied force in chewing and also rate of force application needs to be rational to relate with sensory texture. In the case of gels, it varied from 0.17 to 4.17 mm/s. In recent years, Instron was performed at a speed of 0.83 mm/s [36]. Typical rate varied from 0.033 to 0.833 mm/s [46]. As the rate of loading is increased, the force required to achieve a particular compression is also increased. Alternatively, a constant force achieves a lower compression with the increased rate of loading [46]. This is due to the fact that slower rate allowed greater relaxation of the gel. A speed value of 3.33 mm/s was recommended for gels based on the correlation between sensory and TPA hardness [59, 60]. A circular plate of 15 cm in diameter was used to compress the specimens so that samples do not experience shearing [24]. Meullenet et al. [61] determined the first bite velocities of ten cheeses for seven panel members (subjects) trained in descriptive analysis using an electrognathograph. Three subjects showed average biting velocities of 19.8, 19.9, and 20.2 mm/s, respectively. The other four subjects showed significantly higher biting velocities as 26.0, 27.8, 30.8, and 35.1 mm/s, respectively. The effectiveness of instrumental TPA as performed at predefined and individual crosshead velocities to predict hardness perception of cheese were assessed by Meullenet et al. [61]. Considering five of the seven subjects, the perception of hardness was adequately described by imitative instrumental tests as performed with upper and lower teeth impressions cast attached at the upper load cell and lower platform. Four of seven subjects showed improvement when Instrumental TPA was performed at the matching individual's biting velocity. Overall, peak load and energy to peak seemed to be the best predictors of hardness while other factors (i.e., initial slope within 2% strains, average slope between 0% and failure, and maximum slope) indicated a lower effect.

7.3 Sample Size and Shape

In general, samples used are prepared with cutting or molding standard-sized cubes or cylinders. The sample size is generally well specified within 1–3 cm [36]. Single grain cooked rice kernel was used for 50% compression with plunger of 4 cm diameter and crosshead speed of 0.17 mm/s [62]. Few kernels are also used for a single compression cycle. However, reproducibility of a few kernels test is poor when compared to the bulk test [29].

7.4 Compressing Unit and Sample Size

The size of the compressing unit (i.e., sometimes called punch, plunger, or probe) is very important, especially in relation to the sample size [36]. When the compressing unit is larger than the sample, the forces registered are largely due to uniaxial compression, whereas when the puncture is used then a combination of compression and shear is experienced by the sample. In the case of first paper on instrumental TPA, all samples are made up with an area at least that of the plunger base, and thus it exerts combined compression and shear forces [35]. Gels with plungers smaller than the samples are used in GF Texturometer for plant protein pastes [47], soybean [63], puddings, and whipped toppings [60]. Instron punch was used in the case of cheese (plunger and sample diameters: 0.64 and 2.3 cm) [64], gels of carrageenan/gelatin, and agar/gelatin [65]. However, it is interesting to mention that a larger brass plunger (i.e., 5 cm diameter) could simulate the squeezing action of the tongue against the palate [60]. Henry et al. [45] found that Instron TPA parameters for a series of commercial desserts (puddings, custards, and gelatin) and whipped toppings and marshmallow cream correlated with sensory attributes in a plunger 5.7 cm in diameter with samples of 2.54 cm in diameter. Others also used 1.5 cm cubes with compression between flat parallel plates with much larger cross-sectional areas [46], and a flat plate 5 cm in diameter with cylinder sample with cross-sectional areas about 1.0 cm^2 [48]. Kadan et al. [66] used a rectangular flat probe of 3.3 mm wide. In general, most of the recent work uses a compression unit larger than the sample size, and thus it applies mainly uniaxial compression force [36]. Upper and lower molar teeth impression cast is also used for compression [61]. Abbas et al. [67] used whole palm fruit for 10% penetration in the case of abscission and mesocarp layer, while 7% compression was used for palm kernel.

7.5 Degree of Compression

Compressions could be 10–90%, whereas the original protocol used 75% compression. In the case of many attributes, such as hardness, compressions selected should be such that the samples had undoubtedly suffered more than surface deformation and undergone significant structural breakdown. In general, 75–80% deformation was the choice of most authors who worked on fruits, vegetables, and meats [36]. In the case of gels, 20–50% are commonly applied since compressions greater than 70–80% completely break the gels. At these levels, samples do not break (i.e., brittleness peak does not appear in the curve), but it is still possible to obtain valuable information in the instrumental TPA parameters. At 3.33 mm/s, all compression forces measured beyond rupture point correlated better with sensory measurements than force values registered before or at rupture point, whereas at 0.83 mm/s forces values at smallest deformation (i.e. 40%) were more highly correlated with the sensory TPA [59]. Later, the authors used 20% deformation value (i.e., very far away

from the break point) for hardness, springiness, and cohesiveness [68]. Pons and Fiszman [36] recommended two levels of deformation to consider all possible information about different aspects of mechanical behavior of a given sample. One problem could exist when the compression device has a larger diameter than the sample causing the sample to adhere to the plunger during upward movement, thus making it impossible to measure adhesiveness at the end of the first compression cycle [36].

7.6 Time Lag Between Two Compressions

It was difficult to program zero time lag either with the GF Texturometer or with the Instron. Current machines, such as the TA.XT2 (Stable Micro Systems), give the option of selecting a variable period to elapse between first and second bites. If a period is selected, it should be specified in the testing conditions because the amount of time between bites clearly affects instrumental TPA parameters [36].

7.7 Effect of Lubrication

The use of unlubricated plates in compression tests can produce frictional phenomena at the sample–plate interface and can result in non-reproducible data. In addition, compression data with unlubricated plates can yield parameters that depend on the material's bulk properties and frictional characteristics of the sample–plate surface interface [36]. Lubrication can be performed using mineral or vegetable oil between the sample and the plates. The sample without lubrication usually provides much higher magnitudes of hardness than expected [69, 70]. In the case of Gouda cheese, the compression forces observed with lubricated plates could be only half as the forces observed when plates possessed rough (i.e., high friction surfaces) [71]. Lubrication in real situations inside mouth could be counteracting influences since it is likely to act as a lubricant, thus reducing the frictional effects while the cusps in the teeth would tend to anchor the samples. Brennan and Bourne [72] tried to simulate this situation using an opposing pair of artificial molar teeth mounted on a TA.XT2 texture analyzer. They compressed the samples to a 50% deformation with and without lubrication (i.e., mineral oil). The frictional effect of the cusps on the molar teeth can cause the samples a barrel shape when deformed, this shape effect can be greater than the effect of lubrication. Therefore, lubrication is called into question if the objective of the testing is to simulate what happens in the mouth [36].

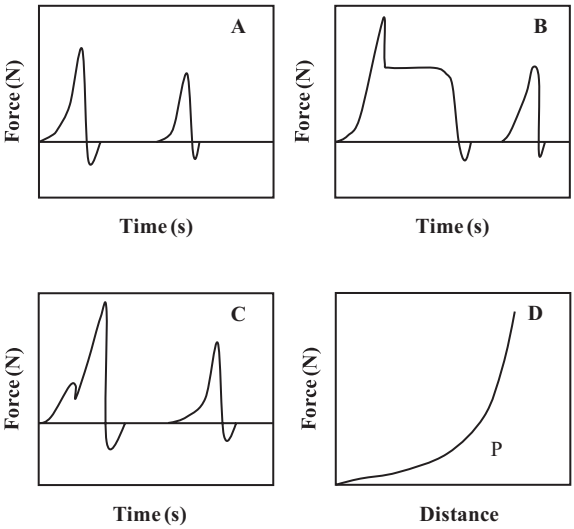

Fig. 17.2 Types of TPA graphs for different food products, (**a**) two positive peaks (i.e. compression) and two negative peaks (i.e. decompression) without fracture, (**b**) First compression peak showed hardening of the sample before fracture or changing shape, (**c**) First compression peak shows fracture before break or changing shape, (**d**) Very hard sample without any fracture or break

8 Instrumental TPA of Food Products

Types of TPA curves are shown in Fig. 17.2. In this section, TPA attributes of different food products are presented. These attributes are grouped into different classes.

8.1 Fresh and Dried Fruits

Instrumental TPA of pears during storage is presented in Table 17.1. In the case of fresh fruits, such as pears, peaches, apple, plantain, and dried apple, the curves A_3 and A_4 as shown in Fig. 17.1 could not be observed [24, 73–75]. This indicated that during decompression there was no sticking or pull-off force from the material. However, curves A_3 and A_4 were observed in the case of fresh banana [75] and palm fruit [67]. In the case of fresh pears, a fracture was identified, and tissues continue to crack open and squash flat as the downstroke continues. When the compressing plate contacts the fruit for a second time, the force again rises in a smooth curve with no fracture point. In the case of palm fruit fracture was also observed [67]. A *rubbery-leathery* transformation was observed by Seow and Thevamalar [76] in rice-based products when hardness versus water content was plotted. The transition was considered when a sharp rise in hardness was observed and at this point rubbery (i.e., more easy to deform) dried flesh transformed into leathery (tough to deform) behavior. They identified the point of rubbery-leathery transformation when the product was humidified at different atmospheres. Firmness and stickiness are also interrelated [77]. Bourne [74] presented the instrumental TPA attributes of fresh and dried apples (Table 17.2). The hardness of apple within water activity from 0 to 1

Table 17.1 Instrumental TPA attribute values of fresh pears as a function of storage time

Days	*FR* (N)	*SP1* (mm)	*CH* (mJ)	*HA* (N)	*MT* (N)	*GU* (N)	*CO1*
4	119.7	0.80	1.28	–	133.9	7.01	0.055
7	92.2	0.81	1.00	132.4	80.4	6.28	0.046
9	78.5	1.00	0.82	102.5	66.7	5.49	0.053
11	58.9	0.85	0.60	84.9	46.1	4.32	0.048
14	43.2	1.10	0.20	54.9	35.3	1.96	0.033
16	32.9	1.32	0.10	36.3	29.4	1.18	0.031
18	25.5	1.58	0.12	23.5	28.4	0.98	0.038
21	19.6	1.58	0.10	22.1	24.5	0.59	0.037
23	19.6	1.75	0.08	22.1	24.5	0.30	0.034

Instrument: *G.F.* Texture Profile, *SP1* springiness type 1, *MT* Magness-Taylor instrument, *CO1* cohesiveness type 1
A_3 is zero
Size: Disk of 2 cm diameter and 1 cm thickness
Source: Bourne [24]

Table 17.2 Instrumental TPA for apple as a function of moisture content

a_w	X_w	*HA* (N)	*FR* (N)	*CO1*	*SP1* (mm)	*GU* (N)	*CH* (N m)	*DF* (mm)
0.01	0.01	414	90	0.23	1.52	100	2.0	0.02
0.12	0.04	6046	2	0.25	0.57	1550	12.0	0.02
0.23	0.05	709	0	0.53	2.58	400	11.2	0.20
0.35	0.07	600	0	0.53	3.60	320	10.2	0.46
0.45	0.10	500	0	0.53	2.50	220	6.0	0.46
0.55	0.15	500	0	0.53	2.20	200	5.6	0.84
0.65	0.20	300	0	0.53	2.40	200	4.0	0.84
0.75	0.25	200	0	0.42	1.63	100	1.8	0.80
0.85	0.35	104	0	0.24	1.63	10	0.6	1.88
0.98	0.85	114	40	0.10	1.85	10	0.1	0.20

a_w water activity, X_w Moisture content (kg water/kg sample), *CO1* cohesiveness type 1, *SP1* springiness type 1, *DF* deformation
Instrument: Instron
Size: 1.0 cm cube, Compression: 90%, Plate
Crosshead speed: 0.83 mm/s, Chart speed: 8.33 mm/s
DF Deformability (mm)
Source: Bourne [74]

showed a sharp rubbery-leathery transition. In addition, at very low moisture content below 5%, the water content of the material transformed from leathery to brittle and thus hardness again dropped to a very low value. The leathery and chewy characteristic is important for dried fruits when it is used as an intermediate moisture snack. Table 17.3 shows the TPA attributes of fresh apples as a function of variety and tissue structure. *Cohesiveness* is the extent to which a material can be deformed before it ruptures. Brittle materials possess low cohesiveness but have high fracturability values. This could be explained by the fact that an increase in hardness can reduce the cohesiveness (material fights back less).

Table 17.3 Instrumental TPA of fresh apple as a function of inner and outer tissue structure

Attributes	Variety	Orientation			
		Inner structure		Outer structure	
		Tangential	Radial	Tangential	Radial
Hardness (N)	Kim	50.7	59.7	39.1	48.8
	Mutsu	75.2	77.2	62.6	60.7
	Jonagold	62.1	60.4	55.9	51.7
Cohesiveness	Kim	0.098	0.075	0.105	0.082
	Mutsu	0.081	0.069	0.072	0.059
	Jonagold	0.078	0.068	0.075	0.069
Springiness [SP1] (mm)	Kim	1.49	1.77	1.62	1.66
	Mutsu	1.36	1.57	1.28	1.57
	Jonagold	1.34	1.29	1.34	1.38
Chewiness (mJ)	Kim	7.48	8.07	6.67	7.02
	Mutsu	8.35	8.46	5.80	5.62
	Jonagold	6.02	5.34	5.25	4.88
		First 6 mm	6–12 mm	12–18 mm	
Density (kg/m^3)	Kim	805	767	727	
	Mutsu	841	811	765	
	Jonagold	830	799	753	
Porosity	Kim	0.144	0.179	0.214	
	Mutsu	0.114	0.143	0.186	
	Jonagold	0.122	0.155	0.198	

Porosity and density were measured by vacuum infiltration method
Conditions for instrumental TPA
Cylinder (height: 5.98 mm, diameter: 15.79)
60% Compression
Speed: 50 mm/min
Source: Mavroudis et al. (2004) [103]

Cohesiveness is a measure of the strength of the internal bonds of food material and thus more energy would be required for de-bonding the material [75]. Cohesiveness was found constant for fresh clingstone peaches for the two ratios of the areas (i.e., the cohesiveness parameter decreased at the same rate as the puncture test) [73], whereas cohesiveness of pears decreased with the ripening [24]. In the case of dried apple, cohesiveness increased with the decrease in water content and showed a plateau and then decreased further at low moisture content [74]. The plateau region was within 20 to 5% water content. The adhesiveness of fresh pears was found to be nearly zero during ripening [24]. In the case of different soy protein isolates, few isolates did not show adhesiveness, and the lack of adhesiveness in these gels seemed to be related to the highest values of hardness [78].

The chewiness of fresh and dried apple was a function of water activity and found chewiness increased with the decrease in water content and reached to a peak and further decrease in water content caused the chewiness values to decrease [74]. In the case of pears, instrumental TPA (i.e., hardness, cohesiveness, springiness,

elasticity, brittleness, chewiness, and gumminess) decreased during ripening at approximately the same rate as the Magness-Taylor pressure test. There is an initial rapid decrease in value of each parameter, which lasts for about 16 days; thereafter, a slow decrease in each parameter was observed. Cohesiveness does not decrease as much proportionately as the other parameters and thus much greater scatter in the experimental points [24]. These trends make complex texture simple because all the parameters run parallel as the pears ripen. Similarly, the texture of peaches is complex, but it appears to be simple because all the textural parameters change in the same direction and at approximately the same rate as the peaches ripen over time [73]. Paoletti et al. [53] studied the instrumental and sensory TPA (failure stress, failure strain, modulus, fracturability, firmness, cohesiveness, mealiness, and juiciness) for different cultivars of apple (Table 17.4). Highly significant correlations

Table 17.4 Instrumental and sensory TPA of different cultivars of apple

Cultivars	D (m)	ρ_a (kg/m^3)	Instrumental TPA						Sensory TPA				
			σ_m (kPa)	ε_m	*FI* (N)	*E* (MPa)	*CO1*	*JU* (m^2/kg)	*FI*	*FA*	*CO*[a]	*JU*	*ME*
Golden delicious extra	0.083	740	196.7	0.191	6.8	1.4	0.0135	1.221	3.9	3.6	95	6.0	3.3
Golden delicious I	0.073	680	164.4	0.185	6.8	1.3	0.0299	1.203	3.2	2.8	81	5.6	4.1
Stark delicious extra	0.090	750	129.9	0.145	9.2	1.2	0.0156	0.723	1.7	1.5	74	3.6	6.5
Stark delicious I	0.081	670	257.4	0.173	8.2	1.9	0.0205	1.377	2.1	2.0	97	3.9	6.0
Renetta	0.080	820	177.8	0.154	9.2	1.6	0.0090	0.386	2.6	2.1	85	4.1	5.6
Annurca	0.074	740	299.7	0.181	12.7	2.5	0.0192	1.230	4.0	3.6	100	5.1	3.5
Granny Smith	0.109	770	309.2	0.173	14.2	2.3	0.0520	2.595	7.3	6.9	114	7.2	1.6
Emperor	0.083	740	216.1	0.166	8.4	1.7	0.0171	0.951	2.9	2.4	110	4.2	5.0

D diameter, ρ_a apparent density, σ_m maximum stress, *E* Modulus, *CO1* cohesiveness type 1, *ME* Mealiness
Instrument: Instron UTM model 4501
FI Firmness by penetration force by 6.5 mm probe
ε_m: Hanky's strain
Sample size and shape: Cylinder (Diameter: 0.015 m, height: 0.010 m)
Compression: 80%, Crosshead speed: 0.83 mm/s
Sensory scale: 1–9 hedonic
Train panelists: 10
FL1 Factor Loading 1 (failure stress, firmness, modulus, cohesiveness, juiciness)
FL2 Factor Loading 2 (failure strain)
[a]Paiwise rank test value
Source: Paoletti et al. [53]

were observed when sensory fracturability compared with failure stress, sensory firmness with TPA firmness, sensory mealiness with TPA cohesiveness, sensory juiciness with TPA juiciness. No significant correlation was found between TPA and sensory cohesiveness, showing an incorrect understanding of how cohesiveness was measured by the panel. This may be difficult in perceiving cohesiveness because of the kind of product tested (too easy to break). The PCA and factorial analysis were conducted for the attributes for its grouping. The first factor includes failure stress, firmness, modulus, cohesiveness, and juiciness, while the second factor includes only failure stain. Myhara et al. [79] studied the firmness of dates as a function of maturity days. Rahman and Al-Farsi [50] measured the instrumental TPA attributes of dates as a function of water content (Table 17.5). Date flesh did not show any fracture point in instrumental TPA curves. Hardness increased sharply below 21.5% moisture content due to rubbery–leathery transition. Adhesive or cohesive failure was observed depending on the moisture content. In case of adhesive failure, samples stuck either with base or with top plate, whereas in case of cohesive failure, the sample separated into halves and stuck on both base table and plate. Since fracture was not observed in the sample during compression, the cohesiveness measured is the elastic–plastic nature of the sample, which is affected by a combination of cohesiveness and adhesiveness. Hardness, resilience, and chewiness increased exponentially with the decrease in moisture content, whereas cohesiveness, adhesiveness, and springiness showed a peak at around 21.5% moisture content. The trends or curvature of the curves above and below the peaks were different. Principal components and factorial analysis showed that two factors, elastic nature

Table 17.5 Instrumental TPA attributes of Khalas variety of date flesh at *tamr* stage of maturity

Sample	X_w	a_w	*HA* (N)	*CO2*	*AD* (J)	*SP2*	*RE*	*CH* (N)
Commercial product	0.171	0.300	98	0.20	4.60×10^{-3}	0.55	0.034	10.15
Equilibrated sample	0.172	0.113	171	0.20	8.42×10^{-3}	0.50	0.025	19.07
Equilibrated sample	0.177	0.231	118	0.19	7.18×10^{-3}	0.43	0.038	11.80
Equilibrated sample	0.186	0.331	119	0.24	9.50×10^{-3}	0.61	0.044	16.59
Equilibrated sample	0.215	0.432	60	0.40	13.93×10^{-3}	0.82	0.175	15.76
Equilibrated sample	0.246	0.544	36	0.28	4.76×10^{-3}	0.71	0.127	5.62
Equilibrated sample	0.257	0.570	27	0.25	3.26×10^{-3}	0.71	0.121	4.87
Equilibrated sample	0.345	0.755	6	0.13	0.79×10^{-3}	0.59	0.134	0.43
Equilibrated sample	0.453	0.851	4	0.20	0.68×10^{-3}	0.54	0.164	0.47
Equilibrated sample	0.582	0.976	3	0.20	0.55×10^{-3}	0.34	0.145	0.25

a_w: water activity, X_w: Moisture content (wet basis, kg water/kg sample), *CO2:*cohesiveness type 2, *SP2:* Springiness type 2
Instrument: TA-XT2
Force applied: Compression
CP commercial product, *ES* equilibrated sample in the lab
Composition at *tamr* stage (weight basis): X_w: 0.284, *protein*: 1.81, *fat*: 0.0023, *ash*: 1.76, *total fiber*: 1.76, *fructose*: 21.9, *glucose*: 29.4, sucrose: 0.0
Size: 1.0 × 1.0 × 0.4 cm (with skin), Compression: 75%, *t*: 20 °C, Compression speed: 1 mm/s, Plate diameter: 7.5 cm
Source: Rahman and Al-Farsi [50]

(hardness, adhesiveness, and chewiness) and plastic nature (cohesiveness, resilience, and springiness), can explain the TPA attributes of dried dates. Abbas et al. [67] measured TPA attributes of fresh and sterilized palm oil fruit in order to assess their structure after sterilization.

The effectiveness of using texture as a ripening indicator was tested using three red grape varieties (i.e., Mencía, Brancellao, and Merenzao) [80]. A clear texture difference between varieties and ripening stages was observed, and ripeness grade was assessed using cohesiveness. The ripening grade increased with the berry cohesiveness for all the varieties studied. Singh et al. [81] studied instrumental TPA of date fruits as a function of its physicochemical properties. They observed a significant linear correlation of hardness with moisture content, crude fiber and pectin content, adhesiveness with color, springiness 1 with color, chewiness 1 with moisture content, length of whole date fruit, crude fiber, and pectin content. In addition, gumminess 1 with pectin and crude fiber, elasticity 1 with pectin, crude fiber and with color, and cohesiveness1 with the mass of whole date fruit. Date was classified into three groups: hard-resilient, soft-springy, and firm-adhesive. The developed date-tamarind fruit leathers containing hydrocolloid (i.e., starch, pectin, dextrin, or guar gum) and water showed different textural attributes [82]. Hardness and gumminess increased when hydrocolloids were added compared to the blank, whereas cohesiveness, resilience, and springiness decreased. All hydrocolloids' addition increased chewiness except for dextrin. The product was classified into hard-chewy, soft-springy, hard-fragile, and soft-resilient leathers. The flesh of different peach and nectarine cultivars was successfully grouped according to their textural parameters using TPA [83].

8.2 Cooked Vegetables

Mittal [84] studied instrumental TPA of potatoes and carrots as a function of cooking temperature. They found hardness, cohesiveness, gumminess, and chewiness decreased with the increase in cooking temperature, whereas springiness did not follow any pattern (Table 17.6). There was a sharp decrease after 60 °C. The major changes in texture during thermal processing were due to the breakdown of cellular material, and these TPA parameters and penetration force are suitable to monitor the cooking process. The stress relaxation test was not appropriate to measure property changes during the cooking of potato, as the behavior of many test parameters was random with respect to the temperature. However, for carrots, stress relaxation and penetration tests proved to be better tests to monitor the cooking process [84]. Recently, De Castro et al. [85] evaluated different cooking methods (i.e., boiling, steaming, cooking in the combined oven, microwave, and steaming on the microwave) for different vegetables (i.e., broccoli, carrot, and Brazilian zucchini) using sensory and instrumental analysis of texture and color. Boiling mothed was appropriate for broccoli, steaming in the microwave for zucchini, and steaming or steaming in the microwave for carrots. It has been found that firmness showed no

Table 17.6 Changes in the texture of potatoes and carrots cooked to various center temperatures

t (°C)	Potatoes								Carrots				
	Instrumental TPA						Puncture test		Instrumental TPA				
	CH (mJ)	*CO1*	*FR* (N)	*GU* (N)	*HA* (N)	*SP1* (mm)	HA[a] (N)	*HA*[b] (N)	*CH* (mJ)	*CO*	*GU* (N)	*HA* (N)	*SP1* (mm)
20	38.5	0.087	107	12.6	145	3.05	232	209	73.7	0.104	22.3	215	3.30
30	–	–	–	–	–	–	–	–	48.3	0.080	17.0	213	2.84
40	29.4	0.071	104	10.5	148	2.81	220	151	39.6	0.067	15.3	228	2.59
50	26.6	0.061	122	9.3	153	2.85	239	132	52.9	0.087	17.6	202	3.01
60	16.2	0.049	121	6.6	135	2.45	145	73	22.0	0.048	8.9	186	2.46
70	7.7	0.047	85	3.9	84	1.95	111	52	15.9	0.044	8.0	182	1.99
80	2.8	0.036	26	1.4	37	2.10	[c]	[c]	16.0	0.065	5.9	91	2.69
90	0.4	–	–	0.3	3	1.39	[c]	[c]	7.2	0.065	2.8	44	2.51

CO1 cohesiveness type 1, *SP1* springiness type 1
Instrument: Instron Model 4204
Force applied: Compression, Compression: 50%
Crosshead speed: 0.18 mm/s, Chart speed: 1.67 mm/s
Sample size: 10 mm height and 11.4 mm diameter
Puncture with 10 mm diameter probe up to 15 mm depth
Crosshead speed: 0.083 mm/s, Chart speed: 0.33 mm/s
Source: Mittal [84]
[a]First cycle
[b]Second cycle
[c]Sample cracked

difference among cooking methods for carrots, whereas, for zucchini, microwaving showed higher firmness and lesser acceptance.

8.3 Gels

Henry et al. [45] studied the TPA analysis of a series of commercial desserts, whipped toppings, and marshmallow crème. Products were evaluated by taste panel considering 15 sensory attributes related to texture. Multivariate analysis was conducted, and four factors (accounted for 90% variation) were derived from the sensory ratings. Although 15 TPA attributes were available, only 8 were needed for the prediction of the four sensory factors. They also emphasized that some of the additional physical test values obtained under tension were needed for these predictions as well as the standard physical test values obtained under compression. Edwards et al. [86] studied the texture of jet-cooked high amylose corn starch–sucrose gels by instrumental TPA. In general, values of attributes increased with cooking temperature and storage time. These were inversely related to sucrose–starch ratio and water added–solids ratio. In addition, the attributes hardness, fracturability, gumminess, and chewiness were exponentially related to quadratic functions of the

variables, while resilience, cohesiveness, and springiness were observed linearly related. It was recognized that adhesiveness values were low and independent of the variables [86]. In the case of gelatin gels, hardness and fracturability increased as the concentration increased, and these were dependent on the crosshead speed. Cohesiveness was not significantly correlated with concentration [59]. Cohesiveness increased with the increase in gelatin concentration followed by a maximum value at 24 g solid/m^3 solution and then decreased with a further increase in gelatin concentration [59]. This indicated that at low and high moisture, the bonding of the matrix is low and the maximum bonding was achieved at the critical moisture content. Munoz et al. [68] studied the sensory textural attributes firmness (i.e., manual, oral, shear, and compression), elasticity, nature of breakdown, and sourness of three types of gels at two concentrations. It was observed that gelatin was the firmest and elastic compared to alginate and κ-carrageenan gels. It broke down into few large pieces in the process of mastication, while carrageenan produced the least firm and elastic, which failed into large number of small pieces. Time-intensity recordings demonstrated that more oral manipulation time was required to break down carrageenan and alginate gels in contrast to thermally degradable gelatin gel. The perceived oral firmness reached a maximum in less than 5 s after placement of a gel sample in the mouth, and maximum sourness was not perceived until 13–18 s. In addition, greater discrimination between gel concentrations was obtained by sensory when compared to the mechanical measures (i.e., beyond yield point rather than below). Sensory firmness by shear was highly correlated with mechanical shear and compression. Manual compression correlated only with surface rupture force, while oral compression correlated only with shear maximum force. For cooked rice instrumental texture, one of the most popular and reliable instrumental methods involves the use of an Ottawa extrusion cell [87, 88]. The dimensions of the traditional Ottawa cell require rather large quantities, around 100 g milled rice. Juliano et al. [87] demonstrated that an instrumental method of compression utilizing small sample sizes (i.e., a few kernels) is less reliable than a test performed on bulk samples. Sesmat and Meullenet [29] developed a single compression method on few kernels relating to sensory which could be invaluable to rice-breeding programs to quickly and inexpensively assessing the texture of cooked rice. Eleven sensory textural attributes were evaluated via a trained descriptive panel and 14 instrumental parameters from a single compression test. They used multivariate analysis techniques and stepwise optimization method, and it allowed the satisfactory prediction of seven main attributes (cohesion of bolus, adhesion to lips, hardness, cohesiveness of mass, roughness of mass, toothpull, and toothpack). They concluded that the compression test has some limitations because it uses few kernels that may not be representative of the distribution of kernel properties, although it gives advantages of the requirement of small sample size. Gujral and Kumar [62] and Sesmat and Meullenet [29] found curve A_3 in case of single grain cooked rice when cooking times varied with the variety of rice. The instrumental TPA attributes are presented in Tables 17.7 and 17.8. Perez and Juliano [89] reported increased hardness of cooked aged rice. They observed the accelerated aging lead to an increase in the hardness of raw rice, and this may be attributed to the filling up of air

Table 17.7 Instrumental TPA of single (one grain) cooked rice

Rice	X_w^o	X_w	*HA*[a] (N)	*HA* (N)	*CO1*	*SP1* (mm)	*AD* (mJ)
IR-8[b]	0.14[d]	0.534	82.9	6.99	0.133	0.383	0.0232
	0.14[e]	0.582	112.0	7.44	0.188	0.450	0.0092
	0.18[e]	0.611	167.3	8.47	0.206	0.540	0.0005
	0.22[e]	0.657	317.0	10.94	0.250	1.033	0.0001
IR-8[c]	0.14[d]	0.588	61.6	5.38	0.206	0.483	0.0551
	0.14[e]	0.624	105.8	5.50	0.229	0.491	0.0151
	0.18[e]	0.652	158.7	5.75	0.248	0.899	0.0024
	0.22[e]	0.675	257.7	6.07	0.273	1.140	0.0007

X_w^o: moisture before processing
Instrument: Instron Testing Machine
Plunger: 4 cm diameter, Compression: 50%, crosshead speed: 0.17 mm/s
[a]Raw rice
[b]Brown
[c]Milled
[d]Controlled
[e]Accelerated aged
Source: Gujral and Kumar [62]

spaces and fissures in the rice due to starch gelatinization. However, brown rice showed harder than the milled rice because of the presence of the fibrous bran layer around the kernel. Cooked brown rice showed lower cohesiveness compared to cooked-milled rice. This could be due to the presence of outer fibrous branny layers, the structural collapses after the first compression, and prevents the rice from retaining its shape after compression. In addition, gelatinization introduced an additional effect. Milled rice has more springiness compared to brown rice. The increase in conditioning moisture content increased the springiness of both brown and milled rice. Montejano et al. [90] measured textural attributes of eight different heat-induced protein gels by torsion failure testing, instrumental, and sensory TPA (Table 17.9). They developed correlation and regression equations with instrumental and sensory TPA attributes. Initial shear modulus and 50% compression force had the poorest correlation with sensory. True shear strain at torsion failure was the most frequent and significant predictor of sensory. Toda et al. [47] studied the sensory and instrumental (i.e., texturometer, *TM*, gelometer, *GM*, and curd meter, *CM*) TPA of protein-based hard gels, soft gels, and pastes. The gels were first classified by the sensory method. The principal component and factorial analysis indicate the factors as factor 1 (*TM* hardness, *TM* gumminess, *CM* breaking stress, *GM* breaking stress, *GM* breaking work), factor 2 (*TM* cohesiveness, *TM* springiness), and factor 3 (*TM* adhesiveness, *GM* penetration rate). Other attributes measured are *CM* hardness, *GM* breaking strength, and *GM* softness. They termed first component as hardness or breaking strength, the second component as springiness, and the third component as adhesion. Sensory ratings for both the gels and the pastes correlated highly with instrumental measurements contributing to the first component. From the bi-plot of first versus second components, hard gels can be easily distinguished from the other two, but soft gels and pastes could not be differentiated. From the

Table 17.8 Instrumental TPA of single (one grain) cooked rice

Rice type 1								Rice type 2							
Rice	X_w^o	X_w	*HA*[a] (N)	*HA* (N)	*CO1*	*SP1* (mm)	*AD* (mJ)	Rice	X_w^o	X_w	*HA*[a] (N)	*HA* (N)	*CO1*	*SP1* (mm)	*AD* (mJ)
GOV[b]	0.14[d]	0.544	72.0	1.96	0.139	0.249	0.0462	SHA[b]	0.14[d]	0.525	93.8	1.97	0.108	0.199	0.0243
	0.14[e]	0.555	124.8	2.35	0.144	0.261	0.0162		0.14[e]	0.537	120.2	1.99	0.111	0.257	0.0056
	0.18[e]	0.611	217.3	2.86	0.207	0.433	0.0008		0.18[e]	0.619	217.4	2.75	0.115	0.332	0.0018
	0.22[e]	0.657	233.7	3.10	0.301	0.816	0.0004		0.22[e]	0.642	312.8	3.35	0.172	0.333	0.0004
GOV[c]	0.14[d]	0.549	42.1	1.45	0.155	0.253	0.2403	SHA[c]	0.14[d]	0.628	64.3	1.57	0.177	0.293	0.0356
	0.14[e]	0.649	96.7	2.43	0.175	0.424	0.0126		0.14[e]	0.674	108.3	1.61	0.220	0.368	0.0082
	0.18[e]	0.675	125.0	2.45	0.248	0.666	0.0046		0.18[e]	0.707	184.4	1.66	0.226	0.586	0.0029
	0.22[e]	0.692	178.7	2.54	0.371	0.911	0.0042		0.22[e]	0.726	291.8	2.30	0.242	0.691	0.0021

X_w^o*:* moisture before processing, *GOV* Govinda, *SHA* Sharbati
Instrument: Instron Testing Machine
Plunger: 4 cm diameter, Compression: 50%, crosshead speed: 0.17 mm/s
[a]Raw rice
[b]Brown
[c]Milled
[d]Controlled
[e]Accelerated aged
Source: Gujral and Kumar [62]

Table 17.9 Sensory and instrumental TPA attributes of heat-induced protein gels

Gel	Sensory TPA													Instrumental TPA					
	SP	*FI*	*CO*	*DE*	*CH*	*GP*	*GU*	*AD*	*MR*	*CR*	*GR*	*COM*	*PA*	*HA*[1]	*HA*[2]	*CO1*	*SP2*	*GU*	*CH*
EW	5.6	3.3	3.4	7.9	18.1	6.9	2.4	1.2	5.1	4.4	7.9	3.6	5.0	17.3	6.1	0.26	0.57	4.8	2.7
EW (OAT)	10.6	8.9	9.1	11.6	27.9	12.2	1.0	1.0	4.7	11.5	2.3	1.4	3.6	30.6	6.4	0.51	0.87	15.9	13.9
EW (S)	11.6	10.6	11.7	12.2	31.6	12.7	1.0	1.0	5.6	12.2	1.3	1.1	3.0	30.6	3.6	0.76	0.93	23.4	21.7
Beef	6.4	6.1	5.6	5.8	17.4	5.4	4.1	2.1	10.0	6.2	7.9	4.2	5.7	40.7	14.4	0.31	0.66	12.5	8.3
Pork	5.6	5.8	4.6	5.8	16.8	4.8	4.2	1.7	9.2	5.1	8.4	4.1	5.7	38.2	11.3	0.37	0.70	14.1	9.9
Turkey	9.4	9.7	7.8	8.8	30.9	10.7	1.7	1.2	9.6	9.7	4.9	4.1	5.7	68.9	21.3	0.42	0.69	28.6	19.7
Surimi (A)	11.6	11.1	11.1	11.3	33.3	12.2	1.0	1.0	7.6	12.3	1.1	1.2	6.2	120.6	24.7	0.56	0.71	67.2	47.7
Surimi (B)	13.2	13.5	12.4	12.2	39.7	13.0	1.0	1.0	7.6	13.1	1.0	1.2	6.0	179.1	32.2	0.64	0.81	114.9	93.7

GP Gel persistence, *MR* Moisture release, *CR* Coarseness, *GR* Graininess, *COM* Cohesiveness mass, *PA* Particles perceived, *CO1* cohesiveness type 1, *SP2* springiness type 2
Instrumental TPA: 75% compression, 1.67 mm/s, cylinder: 1.25 cm diameter and 2.54 cm length
HA[1]: 75% compression, HA[2]: 50% compression
OAT Oleic acid treated, *S* Succinylated treated, *EW* Egg white
Source: Montejano et al. [90]

bi-plot of second and third components, soft gels and pastes can be distinguished by excluding the points of hard gels. Kadan et al. [66] measured the instrumental TPA of rice fry as affected by gum and mechanical perforation (Table 17.10). Increasing gum lowered hardness and fracturability values, decreased fat absorption, and retained more moisture. The incorporation of a pinhole decreased moisture and increased fat contents. Therefore, gum application improved the overall appearance as well as decreased popping and blistering of fries during the final frying step.

One cycle and two compression–decompression cycles of instrumental TPA were used to assess the textural or gelling characteristics of fish gelatin compared to commercial gelatin (bovine and porcine) gels [91]. The hardness of fish and commercial gelatin was found to be increased significantly as the concentrations of gels increased. At 10% gel, the hardness of bovine gelatin was higher than porcine and fish skin gelatin gels, respectively. TPA attributes of 10% fish skin gel showed significant differences from those obtained from 20% and 30% gels. Mechanical characteristics of 10% gels of gelatin from fish skin, determined from one cycle compression, were significantly lower than other sources of gelatin gels. Gelatin texture properties from freshwater fish skin were also assessed [92].

Rosenthal, [93] studied starch-glycerol gel using texture analyzer under different analysis conditions (i.e., two sample-instrument geometries, various speeds of compression and strain levels, both with lubrication or not) using two-bite compression test. This study aimed to highlight how changing the parameters in the test protocol can affect the texture properties of the sample. The compression speeds from 0.1 to 10 mm/s showed a logarithmic relationship with hardness, cohesiveness, corrected cohesiveness, and adhesiveness. A minimum crosshead speed of 2 mm/s was recommended with 75% deformation with parallel plates. Strain level has an effect on studied parameters, whereas lubrication or no-lubrication has no effect.

8.4 Cooked Patties

In the case of cooked ground beef patties, hardness, cohesiveness, and springiness decreased with the increase in fat level from 5 to 30% as shown in Table 17.11 [94]. This shows that fat reduced the internal bonding of the protein matrix. In the case of different soy protein isolates, cohesiveness and springiness varied from 0.18 to 0.56 and 0.24 to 0.68, respectively, when the moisture content was 85% (wet basis) [78]. If springiness was high, it required more mastication energy in the mouth. Bernal et al. [95] studied the sensory with trained panel and instrumental TPA, Warner-Bratzler shear, and three tensile tests of restructured beef with two levels of connective tissue (high and low), three flake size (3, 6, 13 mm), and three levels of sodium chloride as 0.25, 0.50, and 0.75% (Table 17.12). Most sensory texture attributes could be predicted accurately using instrumental methods. Reduction of the sensory attributes by PCA revealed two underlying dimensions as connective tissue and binding strength. A consumer panel rated the product with 6-mm flakes and 0.75% sodium chloride at either connective tissue level to be the most acceptable of the 18

Table 17.10 Instrumental TPA of rice-fry as affected by gum and mechanical perforation

			Composition					Instrumental TPA						
θ (min)	WR: R (%)	Pin Hole (mm)	Gum (%)	X_w		X_F		*HA* (N)	*FR* (N)	*SP2*	*CO1*	*GU* (N)	*CH*	*RE*
				BF	FF	PF	FF							
5.0	60:40	0.0	0.00	0.290	0.196	0.106	0.123	6.08	5.59	0.83	0.60	3.63	2.94	0.22
	60:40	0.0	0.25	0.311	0.192	0.101	0.123	3.83	3.43	0.85	0.54	2.06	1.67	0.26
	60:40	0.0	0.50	0.316	0.199	0.106	0.110	4.22	4.02	0.87	0.46	1.77	1.67	0.25
	60:40	2.6	0.00	0.292	0.164	0.101	0.143	5.89	5.69	0.84	0.48	2.65	2.16	0.23
	60:40	2.6	0.25	0.309	0.185	0.111	0.129	6.47	6.28	0.88	0.59	3.83	3.43	0.27
	60:40	2.6	0.50	0.308	0.189	0.116	0.132	4.51	4.32	0.83	0.49	2.26	1.96	0.35
	80:20	0.0	0.00	0.292	0.192	0.099	0.174	4.12	3.53	0.83	0.56	2.35	1.86	0.20
	80:20	0.0	0.25	0.284	0.201	0.115	0.160	4.22	3.83	0.90	0.59	2.45	2.26	0.24
	80:20	0.0	0.50	0.297	0.212	0.102	0.127	4.22	3.73	0.82	0.55	2.26	1.86	0.20
	80:20	2.6	0.00	0.283	0.180	0.113	0.158	4.41	4.22	0.83	0.51	2.26	1.96	0.18
	80:20	2.6	0.25	0.311	0.189	0.118	0.166	4.12	3.63	0.81	0.55	2.26	1.86	0.19
	80:20	2.6	0.50	0.281	0.198	0.112	0.135	3.73	3.43	0.81	0.54	1.96	1.77	0.19
10	60:40	0.0	0.00	0.290	0.196	0.106	0.123	12.07	8.73	0.89	0.66	7.95	7.16	0.24
	60:40	0.0	0.25	0.311	0.192	0.101	0.123	7.85	5.89	0.90	0.55	4.32	4.02	0.31
	60:40	0.0	0.50	0.316	0.199	0.106	0.110	8.04	6.77	0.88	0.48	3.92	3.43	0.31
	60:40	2.6	0.00	0.292	0.164	0.101	0.143	17.95	17.85	1.12	0.53	9.61	10.69	0.26
	60:40	2.6	0.25	0.309	0.185	0.111	0.129	10.60	10.30	1.26	0.52	5.49	6.87	0.32
	60:40	2.6	0.50	0.308	0.189	0.116	0.132	11.58	11.38	1.33	0.46	5.40	7.26	0.21
	80:20	0.0	0.00	0.292	0.192	0.099	0.174	6.97	6.97	0.86	0.62	4.32	3.73	0.22
	80:20	0.0	0.25	0.284	0.201	0.115	0.160	6.18	5.49	0.82	0.50	3.14	2.55	0.20

(continued)

Table 17.10 (continued)

			Composition					Instrumental TPA						
θ (min)	WR: R (%)	Pin Hole (mm)	Gum (%)	X_w		X_F		*HA* (N)	*FR* (N)	*SP2*	*CO1*	*GU* (N)	*CH*	*RE*
				BF	FF	PF	FF							
	80:20	0.0	0.50	0.297	0.212	0.102	0.127	5.79	5.30	0.79	0.54	3.14	2.55	0.27
	80:20	2.6	0.00	0.283	0.180	0.113	0.158	6.57	5.10	0.80	0.55	3.63	2.94	0.21
	80:20	2.6	0.25	0.311	0.189	0.118	0.166	6.57	5.00	0.85	0.60	3.92	3.34	0.31
	80:20	2.6	0.50	0.281	0.198	0.112	0.135	6.18	4.81	0.94	0.64	4.02	3.63	0.26

θ: Time (s), X_w: Moisture content (wet basis, kg water/kg sample), *WR* Waxy rice, *LR* Long grain, *PF* Pre-fry, *FF* Final fry, X_F Lipid, *CO1* cohesiveness type 1, *SP2* springiness type 2

3.3 mm wide rectangular flat probe, 30% strain, 1 mm/s, and contact area: 33 mm^2

Source: Kadan et al. [66]

Table 17.11 Instrumental TPA values of cooked ground-beef patties with different fat levels

Parameter	Fat level (%)					
	5	10	15	20	25	30
CH	0.475	0.410	0.378	0.380	0.345	0.360
HA (N)	0.96	0.84	0.69	0.59	0.59	0.52
SP2	86.2	85.8	82.9	82.2	77.9	78.1

SP2 springiness type 2
Source: Troutt et al. [94]

products manufactured. Table 17.13 presents instrumental TPA attributes for frankfurters.

8.5 Cheese

Cheese texture is widely recognized as an important quality attribute affecting consumer perception. It can only be measured directly by sensory evaluation due to its complexity. A clear fracture is usually found in the case of instrumental TPA. Truong et al. [96] studied the texture of cheddar cheese by sensory, instrumental TPA, vane method, and uniaxial compression. Vane parameters were significantly correlated with compression, instrumental TPA, and sensory tests. Multivariate analysis indicated that seven sensory attributes (out of 10) of 10 commercial cheddar cheeses were satisfactorily predicted by vane, uniaxial compression, and instrumental TPA methods. In particular, cheese firmness and cohesiveness evaluated by the sensory panel were described satisfactorily by vane stress and apparent strain. It was observed that the low-fat cheeses had higher fracture stress and strain than their counterparts. The cheeses became *tougher* with fat reduction, while the full-fat cheeses were more brittle. Instrumental TPA curves showed distinct fracturability peaks, and this indicated that the materials had moderate brittleness and low cohesiveness (0.12–0.27). These cheeses had detectable adhesiveness (4.34–7.63 N s), which was negatively correlated with cohesiveness. Instrumental TPA adhesiveness and cohesiveness of cheeses were independent of other TPA parameters. Correlations of vane and compression parameters and univariate correlation coefficients between instrumental parameters and nine sensory attributes are shown in Table 17.14. The principal component analysis biplot showed the relationships of the types of cheeses and their sensory attributes, and the PCA score plot indicated that the cheeses were mapped, and these were visualized more distinctly by the instrumental techniques than the sensory attributes. It was suggested that instrumental methods were more sensitive in distinguishing the mechanical properties of the tested cheeses.

Significant correlations were found between human sensory attributes with instrumental TPA springiness, gumminess, chewiness, and resilience of ezine cheese that is made of a mixture of cow, sheep, and goat milk [97]. The texture was studied through 12 months (analyzed every 3 months) of aging, at 2–4 °C, which

Table 17.12 Sensory and instrumental Texture Profile Analysis (TPA) of restructured beef product

	Sensory Texture Profile Analysis (TPA)								Instrumental Texture Profile Analysis (TPA)								
	CT		Flake size (mm)			Salt level (%)				CT		Flake size (mm)			Salt level (%)		
Attribute	Low	High	3	6	13	0.25	0.50	0.75	Attribute	Low	High	3	6	13	0.25	0.50	0.75
SP	5.7	5.7	5.9	5.1	6.0	3.9	6.1	7.0	HA (N)	120.3	114.1	115.2	107.5	128.8	101.5	119.2	130.9
HA	4.9	4.3	3.8	3.8	6.1	4.2	4.4	5.1	CO	0.539	0.551	0.567	0.519	0.547	0.523	0.545	0.563
CO	5.7	5.1	5.1	5.0	6.1	4.0	5.6	6.6	GU (N)	80.2	80.7	82.7	70.5	88.0	69.9	79.0	92.4
MOR	5.2	5.3	5.5	5.1	5.1	3.6	5.5	6.6	CH (J)	872.3	921.8	960.3	791.0	939.8	740.5	878.3	1072.3
SF	4.1	4.2	4.5	4.2	3.8	2.2	4.2	6.2	WB (N)	141.4	120.9	104.6	123.7	165.1	136.7	129.8	126.9
MF	5.0	5.4	5.2	5.4	5.1	4.9	5.4	5.4	GLT (N)	5.6	4.8	6.5	5.3	3.8	3.7	5.2	6.9
COM	4.7	4.2	3.3	3.7	6.2	4.1	4.5	4.7	GIT (N)	15.7	15.4	16.6	14.5	15.4	11.5	17.0	18.2
SCP	4.6	4.3	3.8	3.5	5.9	4.2	4.1	5.0	EL (g)	23.6	24.8	23.6	24.4	24.5	25.8	23.9	22.9
CH	5.1	4.4	3.6	3.9	6.9	4.6	4.8	4.9	DL (g)	4.5	5.0	3.8	4.2	6.2	7.2	4.1	2.9
ACT	4.0	3.5	2.3	2.9	6.0	3.9	3.7	3.6	TCL (g)	27.7	29.3	27.0	28.3	30.1	32.3	27.8	25.4
FL	6.3	6.2	6.2	6.4	6.1	5.8	6.3	6.6									
TEX	6.0	5.9	6.0	6.1	5.7	5.8	6.3	6.6									
OA	6.2	6.1	6.2	6.3	6.0	5.6	6.2	6.6									

MOR moisture/oil release, *SF* salt flavor, *MF* meat flavor, *COM* cohesiveness mass, *SCP* size of chewed pieces, *CH* chewiness, *ACT* amount of connective tissue, *FL* flavor, *TEX* texture, *OA* overall acceptability, *CT* connective tissue, *WB* Warner-Bratzler, *GLT* glue tensile, *GIT* Gillet tensile, *EL* evaporative loss, *DL* drip loss, *TCL* total cook loss

On average the composition (wet basis), X_w: 0.75, *protein*: 0.75, *fat*: 0.15, *ash*: 0.05

Soluble collagen: for high CT (8.2 kg/100 kg total collagen), for low CT (10.1 kg/100 kg total collagen)

Insoluble collagen: for high CT (91.8 kg/100 kg total collagen), for low CT (89.9 kg/100 kg total collagen)

Source: Bernal et al. [95]

Table 17.13 Chemical composition and instrumental TPA of the commercial and experimental frankfurters

Type	X_w	X_F	X_p	DF (cm)	CO1	AD (mJ)	HA (N)
G1	0.579	0.225	0.131	0.881	0.618	0.0162	8.29
G2	0.613	0.177	0.144	0.865	0.588	0.0310	12.86
G3	0.617	0.166	0.111	0.821	0.534	0.0204	5.88
CM	0.689	0.103	0.129	0.830	0.542	0.0420	10.27
S	0.628	0.205	0.123	0.890	0.622	0.0241	7.77
C	0.670	0.157	0.127	0.853	0.544	0.0241	5.38
CP	0.689	0.102	0.128	0.911	0.657	0.0122	8.16
CC	0.681	0.106	0.124	0.876	0.621	0.0137	7.27

X_F: fat, X_p: protein, DF: deformation, CO1: cohesiveness type 1
1 mm/s, 70% compression
Source: Ordonez et al. [104]

was found to be more adhesive and less cohesive. Instrumental TPA was able to identify a textural difference between camembert cheese and Chinese sufu that have a similar processing technology [98]. Texture profile analysis indicators (i.e., harnesses, chewiness, adhesiveness, cohesiveness, springiness, and resilience) of cheese were higher than sufu. The effect of chemical composition and storage temperature on texture attributes of different sliced cheese types was evaluated [99]. Firmness, cohesiveness, adhesiveness, springiness, chewiness, and resilience were analyzed after storage at 4 and 25 °C for 4 h. Protein, fat, moisture, and sodium chloride contents as well as storage temperature significantly affected the texture of sliced cheeses. Fat in the dry matter and moisture in the nonfat substances were negatively correlated with the firmness of sliced cheeses. Increasing temperature from 4 to 25 °C significantly reduced the average values of firmness, chewiness, and resilience.

8.6 Snacks and Sweets

Product optimization is very important during the formulation process in product development. The texture of shelled sunflower seed caramel snacks was studied using different proportions of sugar and sunflower kernels using TPA [100]. Both sugar and shelled seed affected the textural characteristics significantly. Hardness, cohesiveness, and chewiness showed an increasing trend compared to springiness and resilience (i.e., decreased) with increase in sugar proportion. Best proportion that can give acceptable texture was not indicated in his study. Extruded snacks with different shapes (i.e., cylindrical, pelleted, shell-shaped, and ring-shaped) were evaluated using descriptive tests and texturometer [101]. Cylindrical snake was characterized by crispness and fracturability, pelleted and shell-shaped by chewiness, and ring-shaped by adhesiveness and hardness. Rahman et al. [102] evaluated the textural properties of commercial Omani halwa. The instrumental TPA

Table 17.14 Correlations instrumental and sensory TPA of different types of cheeses

Type	TC	X_F	X_p	X_c	Attribute	VAN-σ_p	VAN-σ_e	Δ	VAN-$\varepsilon_{a,ma}$
Cracker sharp	Firm	0.36	0.21	0.0	TPA-HA	0.67*** (0.46)	0.90*** (0.63**)	0.56** (0.39)	–
Cracker extra	Firm, crumble	0.36	0.21	0.0	TPA-FR	0.61** (0.45)	0.83*** (0.53)	–	0.74*** (0.71**)
Cracker S-light	Firm, elastic	0.21	0.25	<0.04	TPA-AD	–	–	–	–
Cabot sharp	Firm, brittle	0.32	0.25	0.04	TPA-CO	–	–	–	0.91*** (0.91***)
Cabot extra	Firm, crumble	0.32	0.25	0.04	TPA-SP	–	–	–	–
Cabot light	Firm, elastic	0.16	0.29	0.04	TPA-GU	–	0.80*** (0.40)	–	0.80*** (0.88***)
K-sharp	Firm	0.36	0.21	0.0	COM-$\varepsilon_{a,m}$	–	–	–	0.85*** (0.89***)
K-mild	Firm, elastic	0.36	0.21	0.0	COM-τ_m	0.70*** (0.40)	0.71*** (0.48)	0.66*** (0.38)	–
K-medium	Firm, elastic	0.36	0.21	0.0					
K-mild light	Firm, tough	0.21	0.25	<0.04					
Sensory and Instrumental Texture									
Attribute	**h-elasticity**	**m-firmness**	**m-coh**	**m-elasticity**	**m-adhe-teeth**	**c-coh-mass**	**c-adhe-mass**	**s-mass**	**s-film**
VAN-σ_p	0.64**	0.54*	–		–	–	–	−0.56*	–
VAN-σ_e	0.64**	0.55*	–		−0.60*	–	−0.55*	–	–
Δ	–	–	–		–	–	–	−0.65**	−0.63**
VAN-$\varepsilon_{a,m}$	–	–	0.62**	0.66**	−0.58*	0.69**	−0.86***	0.68**	0.71**
TPA-HA	–	0.75***	–	0.70**	–	–	−0.70**		–
TPA-FR	–	–	–	0.68**	–	–	−0.69**		–
TPA-AD	–	–	–	0.66**	–	–	−0.63**		0.54*

Type	TC	X_F	X_p	X_c	Attribute	VAN-σ_p	VAN-σ_e	Δ	VAN-$\varepsilon_{a,ma}$
TPA-CO	–	–	0.55*	0.72***	–	0.58*	−0.88***		0.57*
TPA-SP	–	−0.64	–	0.55*	–	–	–		–
TPA-GU	–	–	–	0.72***	−0.54*	–	−0.84***		–
COMP-$\varepsilon_{a,m}$	–	0.63**	–	–	–	–	–		–
COMP-τ_m	–	–	0.67**	0.56*	–	0.65**	−0.84***		0.71**

TC Textural Characteristics, *COMP* Compression test, *VAN* Vane method, σ_p: normal stress (Pa), σ_e equilibrium stress (Pa), $\varepsilon_{a,m}$ maximum strain, τ_m maximum shear stress (Pa)
TPA: TA.XT2, sample size: 15 mm cube, plate 5 cm, Compression: 80%, time pause: 5 s
Vane speed = 1.65 rpm, values for vane speed of 0.38 rpm are in the parentheses
Δ: difference between VAN-σ_p and VAN-σ_e
Sensory attributes
Handheld elasticity (h-elasticity)
Biting: firmness (m-firmness), elasticity (m-elasticity), adhesive between teeth (m-adhe-bt), adhesiveness to teeth (m-adhe-teeth)
Chewing: cohesiveness of mass (c-cohe-mass), adhesiveness of the mass (c-adhe-mass), smoothness of mass (s-mass)
After swallow: smoothness of film (s-film)
*Denotes $p < 0.10$, **Denotes $p < 0.05$, ***Denotes $p < 0.01$
Source: Truong et al. [96]

attributes were correlated with the moisture, fat, fatty acid, and sugar contents. Furthermore, halwa was classified into four groups based on the physicochemical properties and instrumental TPA attributes: hard-chewy (fatty and medium-sugar), soft-resilient (low-fat and high sugar), soft-springy (fatty and medium-sugar), and soft-cohesive-springy (high-fatty and low sugar).

9 Conclusion

The sensory test is considered as time-consuming and expensive when compared to the instrumental method. In addition, panelist's fatigue, adaption, and level of training adversely affect the sensory responses. Therefore, instrumental TPA is one of the important developments in instrumental texture measurements, which mimics the human oral processing of food. In many instances, TPA attributes are correlated with the sensory texture. It involves two or more cycles of compression of a sample between two parallel surfaces. Thereafter, the generated TPA curve is used to calculate different instrumental textural attributes, including fracturability, hardens, cohesiveness, adhesiveness, springiness, resilience, gumminess, and chewiness. Thus, TPA can be used in routine quality control and product development due to its ability to correlate with sensory texture. However, combining both tools (i.e., human sensory and TPA) can give more understanding of textural behavior in the mouth and the human perceptions. Different factors must be considered when using TPA that can affect the output. These include sample size and shape, sample preparation, compression unit, degree of compression, time lag between two compressions, and lubrication. Currently, the applications cover a wide range of fresh and processed food including fruits, vegetables, meat, fish, dairy (e.g., cheese and cream) jelly, and snacks. Studies on the relationship between texture and food satiety as well as texture and mouth behavior contributed significantly to the understanding of texture behavior in the mouth.

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