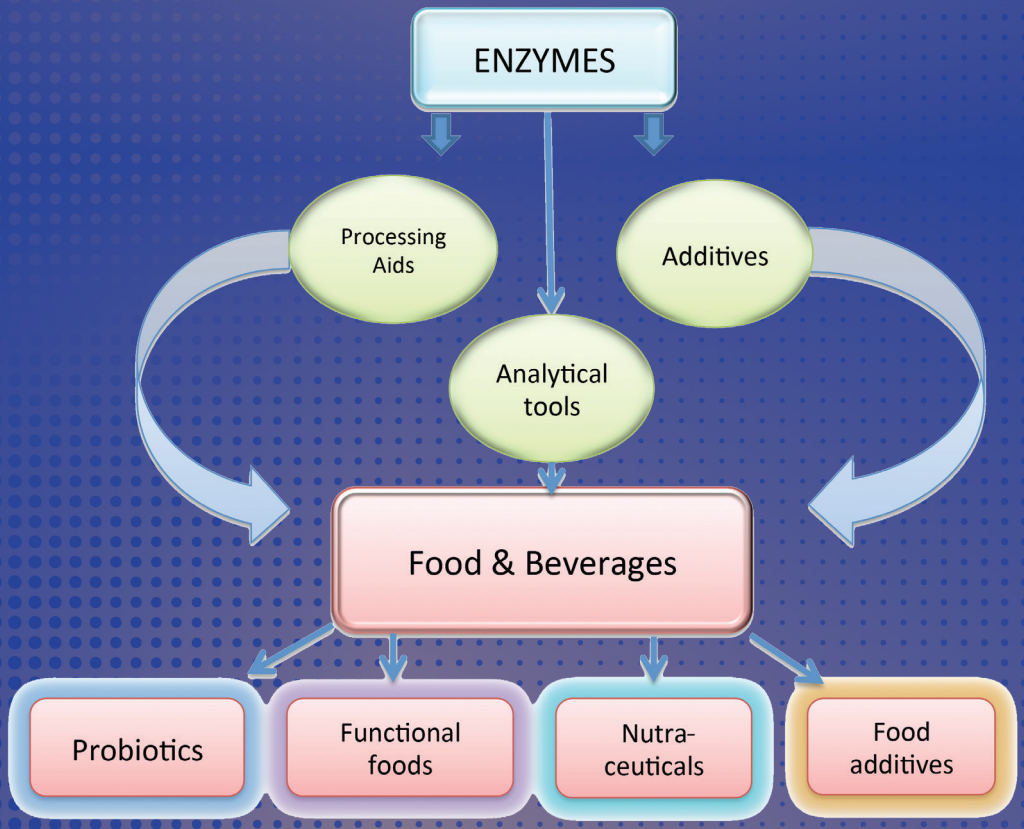
Enzymes in Food and Beverage Processing



Edited by
Muthusamy Chandrasekaran

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Preface

Food and beverage production has registered tremendous growth worldwide, concomitant with population growth and demand for processed food and beverages that are nutritive, delicious, and aesthetic in addition to having an extended shelf life. Further advancements in food science and technology have significantly contributed to a better understanding of food and its importance in sustenance of life, to the scope and need for augmentation of a diverse range of quality food and beverages for consumption, and to meeting ever-increasing food demand.

Toward meeting consumer demands, the food and beverage industries have adopted the latest developments in modern food technologies and biotechnologies. Among the biotechnologies, enzyme technology holds the key for implementation of green technologies that are ecofriendly and an ideal alternative technology to conventional chemical technologies, which use chemicals that may cause pollution on disposal into the environment. Thus, recently, there have been large-scale applications of enzymes in selected food processing industries. This is mainly due to the fact that enzymes have become an essential component of food processing as well as part of the foods that are consumed. Enzymes are principally used as additives in food or as processing aids in the production of food and beverages to improve texture, flavor, quality, appeal, and to extend shelf life. Moreover, recent developments in enzyme biotechnology, nanobiotechnology, metagenomics, and proteomics have contributed to the design and development of tailor-made enzymes with novel properties, and catalytic activities have played a significant role in the discovery and development of new enzymes and newer applications in food and beverage processing and valorization of food processing by-products and wastes. Enzymes have also found applications as analytical tools in the food industry.

A huge amount of scientific literature is available in the public domain on rapid developments in enzyme structure, enzyme kinetics, and characteristics; application of a range of food-grade enzymes derived from diverse groups of microorganisms, including extremophiles and plant resources; the scope for effecting desirable modification in the enzyme structures favoring activity under harsh conditions through enzyme engineering and protein modification; enzyme-mediated novel biotransformations of naturally available biologicals; and development of new ranges of functional foods, nutraceuticals, probiotics, and enzyme inhibitors that regulate enzyme activity. These advances and developments in food enzyme research have to be adopted and exploited in food and beverage processing production. This book is an attempt to document such developments for the benefit of and use by the food research fraternity and the food and beverage industries.

Unlike other books that deal with the application of specific enzymes in production of a specific product of interest, this book presents the application of different enzymes in various food and beverage industries in addition to presenting information on basic concepts and fundamental principles of enzymes, emerging enzyme technologies, and enzyme engineering. Further, this book also deals extensively with the latest advances in food science technology with respect to emerging food industries, such as functional foods, nutraceuticals, probiotics, the use of enzyme inhibitors, and enzyme biotransformations in addition to the use of enzymes in valorization of food and beverage processing by-products and waste. In addition, emerging trends and future prospects in the use of enzymes by food industries are also discussed.

The book is presented in 22 chapters grouped under four sections. Section I deals with enzyme basics and development of novel biocatalysts covered in Chapters 1 through 4. Chapter 1 presents comprehensive information on enzymes: their nomenclature, mechanism of action and kinetics, characteristics, and sources of food-grade enzymes. Chapter 2 deals extensively with enzyme technologies: current and emerging technologies for development of novel enzyme catalysts. This chapter includes a discussion on the scope for utilization of new technologies, such as directed evolution for designing new enzymes and use of nanomaterials for applications in food processing. Chapter 3 includes a detailed discussion on the

use of enzymes as analytical tools for the assessment of food quality, food safety, and monitoring of food processing with reference to the use of modern developments in techniques such as ELISA and biosensors. Chapter 4 deals with the latest developments and scope for application of enzyme engineering and protein modifications in food processing.

Section II covers various applications of enzymes in food and beverage industries and is presented in Chapters 5 through 15. Chapter 5 includes an overview of market trends in various food and beverage industries in the world and enzyme-producing companies that cater to the needs of the food and beverage industries. Chapter 6 deals with the latest advancements in the application of various enzymes in the starch processing industries and the products derived. Chapter 7 presents an illustrative account of applications of various enzymes in bakery industries that manufacture bakery products. Chapter 8 includes a detailed discussion on the various applications of enzymes in confectionery industries that produce various confections based on sweeteners and different categories of chocolates. Chapter 9 includes an account of the role of enzymes in oil and lipid processing. Chapter 10 describes the importance of enzymes in the processing of fruit juices and vegetables in light of the latest developments in the field. Chapter 11 deals with applications of enzymes in the processing of alcoholic and nonalcoholic beverages. Chapter 12 presents an account of the prospects of enzyme applications in the production of flavors and food additives. Chapter 13 includes advancements in enzyme applications in the production of milk, cheese, and associated dairy products. Chapter 14 includes a discussion on the role of enzymes in meat tenderization and prospects of applications of enzymes for other applications in meat processing industries. Chapter 15 deals with the utilization of current developments in enzyme technologies for the processing of different species of seafood.

Section III exclusively deals with recent advances in food-grade enzyme biotechnology covered under Chapters 16 through 21. Chapter 16 presents advancements in enzyme applications for the synthesis of novel functional food ingredients. Chapter 17 essentially deals with current developments in enzyme-assisted extraction technology for processing of nutraceuticals from plant resources. Chapter 18 presents an in-depth account of the scope of enzyme-mediated novel biotransformations for deriving a new range of biomolecules and products of value in the food and beverage industries. Chapter 19 deals with the prospects of applications of enzymes in the development of probiotics, prebiotics, synbiotics, and cobiotics. Chapter 20 includes a discussion on the recent developments and prospects of using enzyme inhibitors in regulating enzyme processing of food and beverages. Chapter 21 includes a comprehensive account of the use of enzymes in the valorization of food and beverage by-products and waste toward deriving value-added products of commercial importance. Section IV comprises Chapter 22, which presents the various emerging trends and prospects of enzyme applications in food processing industries in the future.

The contents of this book will definitely cater to the needs of food science researchers, enzyme biotechnologists, and food and beverage industries as a reference book for guidance and a roadmap for taking up research toward harnessing the diverse range of enzymes for applications in food and beverage processing, as well as the design and development of novel enzymes utilizing emerging technologies. Moreover, this book holds the potential to serve as a useful text cum reference for advanced courses in food science technology, food biotechnology, food engineering, enzyme biotechnology, enzyme technology, and food waste management. Nevertheless, there may be overlapping content presented under different chapters, by different authors, which could not be avoided. Readers of this book will derive maximal benefits in terms of knowledge on the use of enzymes in food processing.

Acknowledgments

This book could not have been accomplished but for the grace and blessings of the Almighty, who infused me with the physical and mental strength to take up such a challenging task of developing a book on enzymes in the processing of food and beverages, a topic of contemporary interest and significance and for which huge volumes of literature are available in the public domain. I am grateful to the Almighty for the wisdom and intuitive guidance to perform the duties of editor. I am thankful also to all the contributing authors who extended kind cooperation and support in addition to making significant contributions in their own field of specialization. But for their contributions, this book would not have become a reality.

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I record my sincere gratitude to all the authorities of King Saud University, College of Science, Department of Botany and Microbiology, Riyadh, Saudi Arabia, for extending moral and physical support for completing this book. I am very grateful to Prof. Dr. Ali H. A. Bahkali and Prof. Dr. Fahad M. A. Al-Hemaid, former chairmen of the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, for their constant encouragement and moral support extended during the course of preparation of this book.

The moral and physical support I received from many people who extended valuable assistance directly or indirectly in collecting materials, and extending healthy and useful discussions are also gratefully acknowledged here.

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Editor

Professor Muthusamy Chandrasekaran is a distinguished scientist and a teacher, who has made significant contributions in the fields of marine microbiology and biotechnology. Professor Chandrasekaran earned his BSc degree in zoology from the University of Madras, India; MSc degree in marine biology from Annamalai University, India; and PhD degree in microbiology (food microbiology) from Cochin University of Science and Technology, India. He did his postdoctoral research in genetic engineering of bacteria for wastewater treatment at Hiroshima University, Japan. His major areas of research interest are harnessing marine microorganisms for novel enzymes, bioactive molecules, and microbial and enzyme technologies for enzyme production and waste management.

He began his career as a lecturer in the Department of Applied Chemistry in the Cochin University of Science and Technology in 1983 and later in 1991, as founder head, organized the Department of Biotechnology in Cochin University of Science and Technology, India. After more than 31 years of service, he retired from Cochin University of Science and technology in December 2014, and currently serves as a professor of biotechnology in the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

Professor Chandrasekaran has made significant contributions to the growth of marine microbiology through publications in peer-reviewed international journals. He demonstrated for the first time that marine bacteria and fungi could be harnessed efficiently for the production of industrial enzymes, such as L-glutaminase, chitinase, alkaline protease, lipase, beta glucosidase, and tannase. He showed for the first time in the world that the L-glutaminase enzyme from marine bacteria is a good antileukemic agent. He also did pioneering work in developing fermentation processes for the large-scale production of these marine microbial enzymes in addition to characterizing the enzymes, finding industrial applications for them, and isolating the full gene coding for an alkaline protease from marine fungi and characterizing it. His studies have significantly advanced the existing knowledge on marine microbial enzymes, which were never studied before for their possible application. He has also worked on value addition, employing microbial enzymes to shrimp processing waste and banana and cabbage waste using solid-state fermentation in addition to environmental solid waste management, among other subjects.

Professor Chandrasekaran has earned recognition from the University Grants Commission, India, as a career awardee for his contribution in microbiology. He was a recipient of the Indian National Science Academy Visiting Fellowship and Overseas Associateship of the Department of Biotechnology, Ministry of Science and Technology, Government of India.

Professor Chandrasekaran edited the book *Valorization of Food Processing By-Products* in 2012, published under the Fermented Foods and Beverages series, CRC Press, Taylor & Francis Group, Boca Raton, Florida, U.S.A.

In 2012 he also coauthored the book *L-Glutaminase Production by Marine Fungi* with Sabu Abdulhameed, Lap Lambert Academic Publishing, Germany.

Professor Chandrasekaran has guided 27 PhD candidates and has several publications in peer-reviewed ISI-listed journals and a number of presentations in international and national symposia, seminars, and conferences. He has completed several sponsored research projects funded by UGC, CSIR, and Department of Biotechnology (DBT), Government of India and has organized many national symposia and popular lecture programs in biotechnology.

Professor Chandrasekaran has served as a member of the editorial board and a reviewer for several international research journals in the fields of food science, microbiology, and biotechnology. He has also served as a subject expert on the boards of studies in microbiology and biotechnology of several universities in India and contributed to the development of the curriculum at the UG and PG levels.

Professor Chandrasekaran founded the Society for Biotechnologists of India in 1995 as founder president. He is also a life member in several professional societies, including the Association of Food Science Technologists of India, the Association of Microbiologists India, the Marine Biological Association of India, the Society of Fishery Scientists and Technologists of India, the Indian Biophysical Society (1996), and the Mycological Society of India.

Professor Chandrasekaran has served as a member of the formulation group for the establishment of the Marine Biotechnology Application Centre, Department of Biotechnology, Ministry of Science and Technology, Government of India, 2000–2001; member, steering committee, National Bioresource Development Board, Department of Biotechnology, Ministry of Science and Technology, Government of India, 2002–2007; nominee of the University Grants Commission (UGC) India, to the Advisory Committee of DRS Programme of Department of Botany, University of North Bengal, India (April, 2002–March 2007); member, Task Force on Biotechnology, ICAR, Government of India (July 2003–2006); member, subcommittee of the Earth Sciences Research Committee on Disaster Preparedness, Council of Scientific & Industrial Research, HRD Group, New Delhi (May 2006 to March 2009); member, Task Force on Aquaculture and Marine Biotechnology, Department of Biotechnology, Ministry of Science and Technology, Government of India, 2006–2009; and member, Research Advisory Committee (RAC) of the Central Institute of Fisheries Education (CIFE) Mumbai, Indian Council of Agricultural Research ICAR, Government of India for the period 2010–2013.

Contributors

Fahad M. A. Al-Hemaid
Department of Botany and Microbiology
College of Science
King Saud University
Riyadh, Saudi Arabia

Caio C. Aragon
Departamento de Biocatálisis
Instituto de Catálisis (CSIC)
Campus UAM
Madrid, Spain

Ali H. A. Bahkali
Department of Botany and Microbiology
College of Science
King Saud University
Riyadh, Saudi Arabia

Soorej M. Basheer
Molecular Biophysics Unit
Indian Institute of Science
Bangalore, India

P. S. Beena
SciGenom Labs Pvt. Ltd.
Cochin, India

Ummalyama Sabeela Beevi
Centre for Biofuels
Biotechnology Division
CSIR-National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram, India

Sarita G. Bhat
Department of Biotechnology
Cochin University of Science and Technology
Cochin, India

Cinthia Baú Betim Cazarin
Department of Food and Nutrition
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

Honey Chandran Chundakattumalayil
School of Biosciences
Mahatma Gandhi University
Kottayam, India

Muthusamy Chandrasekaran
Department of Botany and Microbiology
College of Science
King Saud University
Riyadh, Saudi Arabia

Sreeja Chellappan
Molecular Biophysics Unit
Indian Institute of Science
Bangalore, India

Fabiano Jares Contesini
Laboratory of Food Biochemistry
Department of Food Science
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

Juliana Kelly da Silva
Department of Food and Nutrition
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

Clarissa Hamaio Okino Delgado
São Paulo State University "Júlio de Mesquita Filho"—UNESP
IBB/DQB
Botucatu, São Paulo, Brazil

Débora Zanoni do Prado
São Paulo State University "Júlio de Mesquita Filho"—UNESP
IBB/DQB
Botucatu, São Paulo, Brazil

Angela Dura
Department of Food Science
Institute of Agrochemistry and Food Technology (IATA-CSIC)
Spanish Research Council
Paterna, Spain

Pedro Fernandes
Institute for Biotechnology and Bioengineering
Centre for Biological and Chemical Engineering
Department of Bioengineering
Instituto Superior Técnico
Universidade de Lisboa
and
Faculdade de Engenharia
Universidade Lusófona de Humanidades e Tecnologias
Lisbon, Portugal

Marco Filice
Departamento de Biocatálisis
Instituto de Catálisis (CSIC)
Campus UAM
Madrid, Spain

Luciana Francisco Fleuri
São Paulo State University "Júlio de Mesquita Filho"—UNESP
IBB/DQB
Botucatu, São Paulo, Brazil

Xiao Hua
School of Food Science and Technology
Jiangnan University
Jiangsu, China

Ayman Salih Omar Idris
Centre for Biofuels
Biotechnology Division
CSIR-National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram, India

Amit Kumar Jaiswal
School of Food Science and Environmental Health
College of Sciences and Health
Dublin Institute of Technology
Dublin, Republic of Ireland

T. R. Keerthi
School of Biosciences
Mahatma Gandhi University
Kottayam, India

Sapna Kesav
Department of Biotechnology
Cochin University of Science and Technology
Cochin, India

Jissa G. Krishna
National Centre for Biological Sciences
University of Agricultural Sciences (UAS)
Gandhi Krishi Vignana Kendra (GKVK)
Bangalore, India

Ravinder Kumar
Animal Biotechnology Centre
ICAR-National Dairy Research Institute
Karnal, India

Sanjeev Kumar
Department of Life Science
Assam University
Silchar, India

Tsz Him Kwan
School of Energy and Environment
City University of Hong Kong
Hong Kong, People's Republic of China

Wan Chi Lam
School of Energy and Environment
City University of Hong Kong
Hong Kong, People's Republic of China

Pengfei Li
School of Food Science and Technology
Jiangnan University
Jiangsu, China

Gláucia Carielo Lima
Department of Food and Nutrition
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

Carol Sze Ki Lin
School of Energy and Environment
City University of Hong Kong
Hong Kong, People's Republic of China

Aravind Madhavan
Centre for Biofuels
Biotechnology Division
CSIR-National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram, India

Manzur Ali Pannippara
Department of Biotechnology
MES College
Aluva, India

Mário Roberto Maróstica Jr.
Department of Food and Nutrition
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

Gustavo Molina
Laboratory of Bioflavors
Department of Food Science
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

and

Institute of Science and Technology
Food Engineering
UFVJM
Diamantina, Minas Gerais, Brazil

Renu Nandakumar
Department of Medicine
Columbia University Medical Center
New York, New York

T. Nidheesh
Department of Meat and Marine Sciences
Academy of Scientific and Innovative Research
CSIR-Central Food Technological Research Institute
Mysore, India

Paula Kern Novelli
São Paulo State University "Júlio de Mesquita Filho"—UNESP
IBB/DQB
Botucatu, São Paulo, Brazil

Gaurav Kumar Pal
Department of Meat and Marine Sciences
Academy of Scientific and Innovative Research
CSIR-Central Food Technological Research Institute
Mysore, India

Jose M. Palomo
Departamento de Biocatálisis
Instituto de Catálisis (CSIC)
Campus UAM
Madrid, Spain

Gláucia Maria Pastore
Laboratory of Bioflavors
Department of Food Science
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

Mayara Rodrigues Pivetta
São Paulo State University "Júlio de Mesquita Filho"—UNESP
IBB/DQB
Botucatu, São Paulo, Brazil

Anil K. Puniya
College of Dairy Science and Technology
Guru Angad Dev Veterinary and Animal Sciences University
Ludhiana, India

Monica Puniya
Dairy Microbiology Division
ICAR-National Dairy Research Institute
Karnal, India

Cristina M. Rosell
Department of Food Science
Institute of Agrochemistry and Food Technology (IATA-CSIC)
Spanish Research Council
Paterna, Spain

Utpal Roy
Department of Biological Sciences
Birla Institute of Technology and Science, Pilani
K. K. Birla Goa Campus
Goa, India

Meena Sankar
Centre for Biofuels
Biotechnology Division
CSIR-National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram, India

Vani Sankar
Centre for Biofuels
Biotechnology Division
CSIR-National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram, India

Vaisakhi Satheesh
Centre for Biofuels
Biotechnology Division
CSIR-National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram, India

Hélia Harumi Sato
Laboratory of Food Biochemistry
Department of Food Science
Faculty of Food Engineering
UNICAMP
Campinas, São Paulo, Brazil

Samriti Sharma
School of Biomolecular and Biomedical Science
University College Dublin
Dublin, Republic of Ireland

Juliana Wagner Simon
São Paulo State University "Júlio de Mesquita Filho"—UNESP
IBB/DQB
Botucatu, São Paulo, Brazil

S. Raghul Subin
Department of Biotechnology
Cochin University of Science and Technology
Cochin, India

Rajeev K. Sukumaran
Centre for Biofuels
Biotechnology Division
CSIR-National Institute for Interdisciplinary Science and Technology
Thiruvananthapuram, India

P. V. Suresh
Department of Meat and Marine Sciences
Academy of Scientific and Innovative Research
CSIR-Central Food Technological Research Institute
Mysore, India

Mamoru Wakayama
Department of Biotechnology
College of Life Sciences
Ritsumeikan University
Kusatsu, Japan

Ruijin Yang
School of Food Science and Technology
Jiangnan University
Jiangsu, China

Wenbin Zhang
School of Food Science and Technology
Jiangnan University
Jiangsu, China

Section I

Enzymes: Basics and Development of Novel Biocatalysts

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Enzymes: Concepts, Nomenclature, Mechanism of Action and Kinetics, Characteristics and Sources of Food-Grade Enzymes

S. Raghul Subin and Sarita G. Bhat

CONTENTS

1.1 Introduction

The use of enzymes in the food processing industry dates back to 6000 BC or earlier with the brewing of beer, bread baking, cheese and wine making, and vinegar production (Poulsen and Buchholz 2003). Enzymatic degradation in meat was first observed by Spallanzani (1729–1799), and a substance in barley was found by Kirchhoff (1815) to be capable of starch paste liquefaction into sugar (Roberts 1995). The term *diastase* was coined by Payen and Persoz for this saccarification process (Payen and Persoz 1833), and this is still in use for amylases in the brewing industry. Now the enzymes find applications in a wide range of food industries including dairy, baking, brewing, food processing (vegetables, fruits, and egg), sweetener production, protein hydrolysis, distilling, and fruit juice and wine production and in lipid modification. In all of these industries, enzymes are meant mainly for the production of fermentation substrates, flavor development and enhancement, or for product production.

The history of the food industry reveals the efficient use of enzymes as they were exploited for their specificity and catalytic activity in food processing. The first immobilized enzyme used in the food industry was invertase in the production of invert sugar syrup. Even as early as the 1960s, the large-scale application of enzymes in the food industry was established with the acid hydrolysis of starch replaced by the application of amylases and amyloglucosidases.

Enzymes are biological catalysts and are natural in origin unlike chemical catalysts. They are polymers catalyzing chemical reactions that are fundamental to life, comprising rapid synthesis of complex compounds, degradation of high-molecular-weight structures and processing of biomolecules into their active state. Enzymes are superior to any other chemical catalysts, with much greater catalytic power, and stereo specific with the ability to convert nonchiral substrates to chiral products, and these properties are exploited in the food industry. Further, they are highly specific, and even absolute specificity is seen in others. With the exception of a few catalytic RNA molecules, the ribozymes, most enzymes are proteins. All the enzymes used in the food industry are protein in nature, and food technologists normally use them in the manufacture, processing, preparation, and treatment of food. In this chapter, the basic enzymology, including the concepts of nomenclature, mechanism of action, enzyme kinetics, their characteristics, and source of food enzymes, is discussed to present the reader with an instant reference and emphasize the significance of enzymes in food and beverage processing.

1.2 Enzyme Nomenclature and Classification

Traditionally, enzyme names end in "-ase." Some exceptions to this rule are the proteolytic enzymes, such as trypsin and chymotrypsin, which end in "-in." Names of some other enzymes involve the use of the substrate names, such as lactase, which hydrolyze the disaccharide lactose into glucose and galactose. Transaminases indicate the nature of the reaction catalyzed, that is, the transfer of amino groups without specifying the names of the substrate, whereas the name catalase, which neither indicates the name of the substrate nor the reaction catalyzed, catalyzes the degradation of hydrogen peroxide into water and oxygen.

This lack of consistency in the nomenclature of the enzymes was apparent when the list of enzymes grew longer, thereby necessitating the requirement of a systematic method of naming and classifying enzymes.

Reports of the commission appointed by the International Union for Biochemistry and Molecular Biology (IUBMB, formerly the International Union for Biochemistry) were published in 1964, and they were updated from time to time (in 1972, 1978, 1984, and 1992). The additions made to the enzyme database are available at the website dedicated to enzyme nomenclature: http://www.enzyme-database.org/.

Enzymes are classified according to the IUBMB report in consultation with the IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (JCBN). Each enzyme is assigned a recommended name and a four-part distinguishing number by the enzyme commission. Nevertheless, it is clear that some alternative names still remain in such common usage that they will be used, where appropriate, in the text.

The specific property that differentiates one enzyme from another is the chemical reaction that is catalyzed, and it is reasonable to utilize this as the basis for the classification and naming of enzymes. The trivial system is another type of naming of enzymes, not recognizing the rules of any formal system of nomenclature. Recommended names or trivial names are original names or named by appending "-ase" to either the name of the substrate or to the type of catalytic reaction. Some common examples of trivial names are lipase (acts on lipids), protease (acts on proteins), and cellulase (acts on cellulose). The IUPAC name is vital for unambiguous communication between biochemists and product chemists. However, the trivial name of the enzyme, derived from the truncated substrate name with "-ase" added, identifies the substrate or substrate range better for food technologists than does its systematic name or its International Union of Biochemistry Enzyme Commission (IUB or EC) number (Dixon and Webb 1999). In certain cases, food technologists use traditional names for enzymes, such as malt, pepsin, and rennet. The nomenclature of major enzymes used in the food industry is presented in Table 1.1.

The enzyme commission (EC) has divided enzymes into six main groups according to the type of reaction catalyzed (http://www.chem.qmul.ac.uk/iubmb/enzyme/rules.html). Each enzyme was assigned a code number, consisting of four digits, separated by dots. The first digit shows the main class to which the enzymes belong, which is as follows.

Class 1: Oxidoreductases catalyze redox reactions in which hydrogen or oxygen atoms or electrons are transferred between molecules. This extensive class includes the dehydrogenases (hydride transfer), oxidases (electron transfer to molecular oxygen), oxygenases (oxygen transfer from molecular oxygen), and peroxidases (electron transfer to peroxide). The second digit in the code indicates the donor of the reducing equivalents involved in the reaction. For example, glucose oxidase (EC. 1.1.3.4, systematic name, β-D-glucose: oxygen 1-oxidoreductase) is used in dough strengthening in the food processing industry. Laccase (EC. 1.14.18.1) and lipoxygenase (EC. 1.13.11.12) are other major enzymes of this class used in the food industry.

Class 2: Transferases catalyze the transfer of an atom or group of atoms between two molecules except those enzymes included in other groups (e.g., oxidoreductases and hydrolases). EC recommends that the names of the transferases should end as X-transferase, with which X is the group transferred. For example, glucanotransferase (EC. 2.4.1.19) is used for the modification of starch into cyclodextrins. The second digit describes the group that is transferred.

Class 3: Hydrolases include enzymes catalyzing the hydrolytic cleavage of bonds such as C–O, C–N, C–C, and some other bonds, including phosphoric anhydride bonds. They are classified according to the type of bond hydrolyzed. This is presently the most commonly encountered class of enzymes in the field of enzyme technology. In the food industry, a majority of the enzymes categorized as such includes α-amylase (EC. 3.2.1.1), β-amylase (EC. 3.2.1.2), lactase (EC. 3.2.1.23), lipase (EC. 3.1.1.3), and proteases, which include aminopeptidase (EC. 3.4.11), trypsin (EC. 3.4.21.4), subtilisin (EC. 3.4.21.62), papain (EC. 3.4.22.2), ficin (EC. 3.4.22.3), pepsin (EC 3.4.23.1), and chymosin (EC. 3.4.23.4).

Class 4: Lyases are involved in nonhydrolytic removal of groups from substrates. These are elimination reactions in which a group of atoms are removed from the substrate, often leaving double bonds. The second digit in the classification indicates the broken bond. This includes the aldolases, decarboxylases, and dehydratases. For example, acetolactate decarboxylase (EC. 4.1.1.5) is used in the beer industry.

Class 5: Isomerases are those enzymes that can catalyze different molecular isomerization reactions. These enzymes catalyze geometric or structural changes within one molecule. According to the type of isomerism involved, they may be called racemases, epimerases, cis-trans-isomerases, isomerases, tautomerases, mutases, or cyclo-isomerases, for example, phospho glucose isomerase (EC. 5.3.1.9).

Class 6: Ligases, also known as synthetases, catalyze the synthesis of new bonds between two molecules. They join molecules together with covalent bonds in biosynthetic reactions. These reactions require the input of energy by the hydrolysis of a diphosphate bond in ATP or a similar triphosphate, and this property adds to the difficulty in their commercial application. The second digit indicates the type of bond synthesized.

In the code, the second and third digits describe the kind of reaction being catalyzed. There is no general rule here as the meaning of these digits is specified separately for each class of enzymes.

TABLE 1.1

Nomenclature of Major Enzymes Used in the Food Industry

EC No.	Systemic Name (Other Names)	Application in Food Industry	References
	Oxidoreductase		
1.1.3.4	β-D-glucose: O_2 1-oxidoreductase (notalin; glucose oxidase)	Oxygen removal from food packaging and as food preservative, also has been used in baking, dry egg powder production, and wine production	Wong et al. 2008
1.8.3.2	Thiol: O_2 oxidoreductase (sulphydryl oxidase)	Application in dairy and baking industry; flavor enhancement	Faccio et al. 2011
1.11.1.6	H_2O_2: H_2O_2 oxidoreductase (catalase)	Prevent food spoilage, soft drink manufacturing	Whitehurst and Law 2002
1.11.1.7	Donor: H_2O_2 oxidoreductase (lactoperoxidase)	Cold sterilization of milk, prevents spoilage of food	Whitehurst and Law 2002
1.13.11.12	Linoate: O_2 13-oxidoreductase (lipoxygenase)	Dough strengthening, bread whitening	Kirk et al. 2002
1.14.18.1	*o*-Diphenol: O_2 oxidoreductase (laccases)	The production and treatment of beverages, including wine, fruit juice, and beer; baking industry, flavor enhancer	Gianfreda et al. 1999; Kirk et al. 2002
1.1.3.5	D-hexose: oxygen 1-oxidoreductase (hexose oxidase)	Acts as an oxygen scavenger in food products, bread and wheat flour industry, bakery product preparation, preparation of milk products	Smith and Olempska-Beer 2004
	Transferase		
2.3.2.13	Protein-glutamine: γ-glutamyltransferase (transglutaminase)	Manufacture of cheese and other dairy products, in meat processing, to produce edible films, and to manufacture bakery products	Kieliszek and Misiewicz 2014
2.4.1.19	1,4-α-D-glucan: amine 4-α-D(1,4-α-D-glucano)-transferase (cyclodextrin, glucanotransferase)	Widely used in cyclodextrin production, which is used as food-grade micro-encapsulants for colors, flavors, and vitamins	Van Der Maarel et al. 2002
	Hydrolase		
3.1.1.3	Triacyl glycerol acylhydrolase (lipase, tributyrase)	Widely used in food processing, which includes oil and fat modification, flavor development, and improving quality	Hasan et al. 2006
3.1.1.4	Phosphatidylcholine 2-acylhydrolase (phospholipase A, lechithinase A)	Dairy, baking products, emulsifying agents, manufacture of edible oils	Casado et al. 2012
3.1.1.11	Pectin pectylhydrolase (pectinesterase, pectase)	Fruit and vegetable juice preparations	Jayani et al. 2005
3.1.1.20	Tannin acyl hydrolase (tannase)	Tea and cold drink manufacturing; gallic acid and propylgallate preparation, used as food preservative and antioxidant	Chae et al. 1983
3.2.1.1	1,4-α-D-glucan glucanohydrolase (α-amylase, diastase, ptyalin)	Liquefaction in starch industry	Van Der Maarel et al. 2002
3.2.1.2	1,4-α-D-glucan maltohydrolase (β-amylase)	Production of high malt syrups	Van Der Maarel et al. 2002

(*Continued*)

TABLE 1.1 (CONTINUED)

Nomenclature of Major Enzymes Used in the Food Industry

EC No.	Systemic Name (Other Names)	Application in Food Industry	References
3.2.1.3	1,4-α-D-glucan glucohydrolase (amyloglucosidase, glucoamylase)	Conversion of dextrins to glucose, corn syrup production, beer and malt liquor preparation, fruit juice preparation	Van Der Maarel et al. 2002
3.2.1.4	1,4-(1,3;1,4)-β-D-glucan 4-glycanohydrolase (cellulase)	Fruit juice production, solubilization of pentosan in baking, natural flavors and color extracts, used in tea industry, additive in detergents	Uhlig 1998
3.2.1.7	2,1-β-D-fructan fructanohydrolase (inulinase)	Inulin hydrolysis	Fernandes and Jiang 2013
3.2.1.8	1,4-β-D-xylan xylanohydrolase (xylanase)	Separation and isolation of starch and gluten from wheat flour	Heldt-Hansen 1997
3.2.1.15	Poly-(1,4-α-D-galacturonide) glycanohydrolase (pectinase, endopolygalacturonase)	Production of high-quality tomato ketchup and fruit pulps, production of cloudy vegetable juice of low viscosity, fruit and vegetable juice preparations, coffee and tea manufacturing industry, retting and degumming of fiber crops	Bhat 2000; Grassin and Auquembergue 1996; Heldt-Hansen 1997; Jayani et al. 2005; Kashyap et al. 2001; Uhlig 1998
3.2.1.17	Peptidoglycan N-acetylmuramoylhydrolase (lysozyme, muramidase)	Prevention of late blowing defects in cheese by spore-forming bacteria	Whitehurst and Law 2002
3.2.1.23	β-D-galactoside galactohydrolase (β-galactosidase, lactase)	Lactase-reducing enzyme, ice cream preparation	Uhlig 1998
3.2.1.26	β-D-fructofuranoside fructohydrolase (invertase, saccharase, glucosucrase)	Hydrolysis of sucrose in confectionery, flavor development in fruit juices	Uhlig 1998
3.2.1.37	4-β-D-xylan xylohydrolase (pentosanase)	Beer manufacturing, degerming, distillery mesh, vineger mesh, and production of nonstandardized bake goods	Uhlig 1998
3.2.1.40	α-L-rhamnoside rhamnohydrolase (naringinase)	Processing of citrus fruit juice	Whitehurst and Law 2002
3.2.1.41	Pullulan 6-α-glucanohydrolase (pullulanase)	Starch saccharification (improves efficiency)	Van Der Maarel et al. 2002
3.4.11	Aminopeptidases	Cheese manufacturing	Whitehurst and Law 2002
3.4.21.4	Trypsin	Production of hydrolysates for food flavoring	Whitehurst and Law 2002
3.4.21.62	Subtilisin (alkaline proteinase)	Milk coagulation for cheese making, hydrolysate production for soups and savory foods, beer manufacturing, bread and flour; meat tenderizer	Uhlig 1998; Whitehurst and Law 2002
3.4.22.2	Papain	Meat tenderization, chill haze prevention in brewing industry	Uhlig 1998; Whitehurst and Law 2002
3.4.22.3	Ficain (ficin)	Hydrolyzation of animal, milk, and plant proteins; meat tenderization	Uhlig 1998; Whitehurst and Law 2002
3.4.22.32	Stem bromelain (bromelain)	Meat tenderization, beer and malt liquor preparation	Uhlig 1998; Whitehurst and Law 2002
3.4.22.33	Fruit bromelain (juice bromelain)	Meat tenderization, beer and malt liquor preparation	Uhlig 1998; Whitehurst and Law 2002

(*Continued*)

TABLE 1.1 (CONTINUED)

Nomenclature of Major Enzymes Used in the Food Industry

EC No.	Systemic Name (Other Names)	Application in Food Industry	References
3.4.23.1	Pepsin A (pepsin)	Manufacture of fat-free soy flour, precooked instant cereals, beer and cheese production	Uhlig 1998
3.4.23.4	Chymosin (renin)	Cheese production	Uhlig 1998
3.5.1.1	L-asparagine amidohydrolase (L-asparaginase)	Degradation of asparagine in food industry, especially baked and fried foods	Yadav et al. 2014
3.5.1.2	L-glutamine amidohydrolase (L-glutaminase)	Flavor-enhancing enzyme in food and tea industry	Nandakumar et al. 2003
3.5.1.5	Urea amidohydrolase (urease)	Reduction of ethyl carbomate in wine manufacturing industry	Sujoy and Aparna 2013
	Lyases		
4.1.1.5	(2S)-2-hydroxy-2-methyl-3-oxobutanoate carboxy-lyase (acetolactate decarboxylase)	Beer maturation, reduction of wine maturation time	Godfredsen and Ottesen 1982
4.2.2.10	(1→4)-6-O-methyl-α-D-galacturonan lyase (pectin lyase)	Fruit and vegetable juice preparations, coffee and tea manufacturing industry, retting and degumming of fiber crops	Jayani et al. 2005
	Isomerase		
5.3.1.9	D-glucose-6-phosphate ketolisomerase (phospho glucose isomerase)	Conversion of high glucose syrup into fructose syrup	Van Der Maarel et al. 2002

Source: *Enzyme Nomenclature* (recommendations of the Nomenclature Committee of the International Union Biochem. & Mol. Biol. On the Nomenclature and Classification of Enzymes) Academic Press, San Diego, New York, London, 1992.

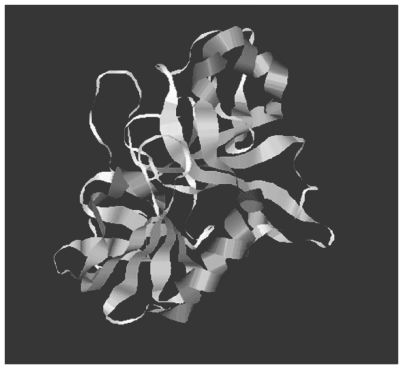
Apart from this technical classification, enzymes may be differentiated in terms of their location and mode of synthesis:

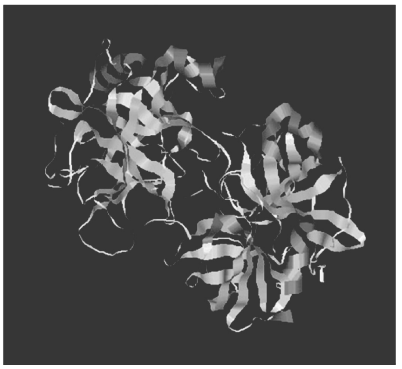
- Intracellular enzymes: Synthesized and stored inside the cell. They are responsible for cellular metabolism.
- Extracellular enzymes: Synthesized and transported outside the cell into the environment. They may be either released directly into the environment or may be located in the periplasmic space of the cell. They are responsible for the breaking down of complex polymeric substances into monomeric forms before uptake into cells.
- Constitutive enzymes: Enzymes routinely synthesized and stored in the cell for various functions.
- Induced enzymes: Enzymes that are synthesized on induction by respective substrates or biosynthetic signaling molecules.

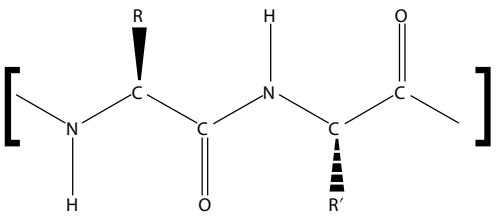
1.3 Enzyme Structure and Chemistry

Enzymes may be differentiated into *monomeric enzymes* and *oligomeric enzymes* based on their chemical structure.

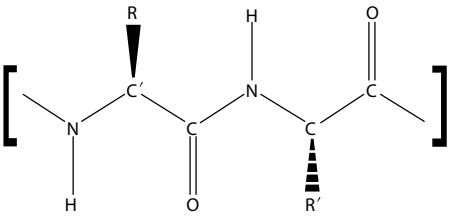
Monomeric enzymes are those that consist of a single polypeptide chain so that they cannot be dissociated further. Several proteases are monomeric in nature. In order to prevent hydrolytic cleavage and therefore cellular damage due to generalized cleavage of proteins, several of these enzymes are produced







Structure of polypeptides where trypsin can act
R = Arg and Lys



Structure of polypeptides where chymotrypsin can act
R = Phe, Trp, and Tyr

FIGURE 1.1 Tertiary structures of trypsin (PDB ID 1PTN) and chymotrypsin (PDB ID 1YPH).

as zymogens, or proenzymes, which require activation. Serine proteases, so called due to the presence of serine in their active site, include trypsin and chymotrypsin produced by the mammalian pancreas. The tertiary structures of these endopeptidases are similar, and their catalytically important residues match exactly although they show only 40% similarity in their primary structure (Figure 1.1).

Oligomeric enzymes consist of two or more polypeptide chains termed as subunits, usually linked by noncovalent interactions and never by peptide linkages. These have large molecular weights, in excess of 35,000. Lactate dehydrogenase is an oligomeric enzyme, which has five isoenzyme forms. Pyruvate dehydrogenases in bacteria and in eukaryotes are multienzyme complexes that catalyze the conversion of pyruvate to acetyl-CoA.

1.4 Solubility of Enzymes

Enzymes are globular proteins and are soluble in aqueous solvents or dilute salt solutions. Their solubility is enhanced by weak ionic interactions, such as hydrogen bonds between the solute and water. All factors that influence or interfere with this process have an effect on solubility. The four factors that influence solubility of enzymes are salt concentration, pH, temperature, and organic matter of the solvent. Solubility of enzyme proteins depends on the concentration of dissolved salt. It can be increased by the addition of neutral salt in low concentrations. The added ions can interfere with the ionization of the side chains of amino acids, which, in turn, can interfere with interactions within the protein molecule but increase interactions with the solute and solvent. This process of increasing solubility by the addition of salt in low concentrations is known as "salting in." When higher salt concentrations are used, there is greater interaction between the ions and water. This leads to reduced protein–water interactions, often causing precipitation from the solution. This process is known as "salting out." Divalent ions are more effective than monovalent ions. When salts such as ammonium sulfate that have higher solubility are

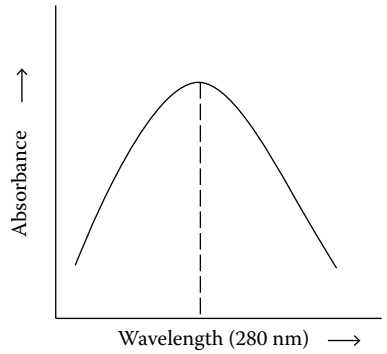


FIGURE 1.2 Absorption maximum of proteins (at 280 nm).

used, some proteins will precipitate at particular salt concentrations, and others will not. Most proteins will precipitate out in more than 80% $(NH_4)_2SO_4$ saturation.

Cations such as Zn^{2+} and Pb^{2+} decrease solubility by forming insoluble complexes with the enzyme protein. Proteins are also precipitated by the addition of acids such as trichloroacetic acid or picric acid due to the formation of acid-insoluble salts, a property used in analytical techniques to separate proteins from the solution before estimation of other substances.

When ethanol, a water-miscible molecule, is added into the solvent, its dielectric point is altered. This causes an increased attraction between the oppositely charged groups within the protein, further reducing its interaction with water molecules and consequently decreasing protein solubility.

Enzymes are charged proteins due to the presence of amino acids with the charge depending on the pH of the solution. At low pH, the amino acids are fully protonated, and there is a positive charge on the protein. As pH is increased, the protein loses a proton to neutralize the OH^- ions and becomes a zwitter ion. As more alkali is added, the NH_3^+ gives its H^+ ions, and the protein becomes positively charged. Under extremes of pH, the ionizable side chains of the protein have charges that are very different from those under normal physiological conditions. This change in the charge pattern of the enzyme protein causes a disruption of the tertiary structure of the protein, a process termed "denaturation." The tertiary structure of the protein has hydrophilic amino acids on the outside, shielding the hydrophobic groups well hidden within the molecule.

The disruption of the tertiary structure brings these hydrophobic groups in close proximity to the aqueous solvent, thereby decreasing the solubility. Restoring the normal conditions may sometimes cause the refolding of the protein into its original tertiary structure required for function and activity. Solubility of enzyme protein will decrease over a narrow pH range called its "isoelectric point" when there is no net charge on the protein; that is, they are electrically neutral. When the temperature is between 40°C and 50°C, solubility of these enzymes increases. At temperatures above this, the tertiary structure is disrupted; the protein is denatured and loses its activity. Rates of enzyme-catalyzed reactions increase with an increase in temperature as the frequency of collisions between molecules increases until the enzyme is denatured and loses its catalytic activity.

Enzymes are proteins and therefore give maximum absorption at 280 nm due to their content of aromatic amino acids (trp, tyr, phe) (Figure 1.2).

1.5 Mechanism of Enzyme Action

The reactant in an enzyme-catalyzed reaction is called "substrate," and the *active site* of an enzyme is responsible for the catalytic action of the enzyme. An enzyme usually contains one or more active sites, which may comprise only a few amino acid residues; the rest of the protein is required for maintaining the three-dimensional structure. The mechanism of enzyme action is well explained by several researchers. The *three point combination* concept by Ogston (1948) pointed out that there were at least

three points of interaction between the enzyme and substrate (Figure 1.3), which can explain the stereospecificity of enzymes. These interactions have either a binding or a catalytic function.

The binding sites link to specific groups in the substrate, ensuring that the enzyme and the substrate molecules are held in a set orientation with respect to each other with the reacting group in the vicinity of the catalytic sites. Regions with binding and catalytic sites are termed "active sites" or the "active center of an enzyme." All enzymes contain an active site (Figure 1.4), which is responsible for the catalytic action of the enzyme, and this is the region where substrates bind. These active sites consist of different regions of the protein, brought together by folding and bending of the protein chain by secondary and tertiary structure formation. This indicates the importance of structural arrangement of enzymes and the destructive effect caused by the denaturation of the protein by heating, which changes the three-dimensional configuration.

There are some amino acid residues in active sites that do not have a binding or catalytic action and therefore may interfere with the binding of chemically similar substances. The three-point interaction theory, for that reason, cannot explain the enzyme action and specificity in detail.

There are several other hypotheses that detail the mechanism of enzyme action. It is believed that enzyme action occurs in two steps. First, the active site of the enzyme combines with the substrate to form an *enzyme–substrate complex*. This enzyme–substrate complex then breaks up to form the products and the free enzyme, which can react again.

According to the hypothesis of the *lock-and-key model* by Fischer (1980), the substrate must fit into the active site of the enzyme; it is very specific, and all structures remain fixed throughout the binding process (Figure 1.5a). The lock-and-key model failed to explain the flexibility of enzymes as it did not successfully distinguish between free and substrate-bound enzymes.

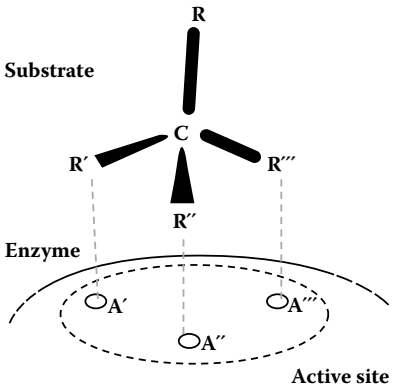


FIGURE 1.3 Three-point interactions between enzyme and substrate. A′, A″, and A‴ are sites on the enzyme that interacts with the groups R′, R″, and R‴, respectively, of the substrate.

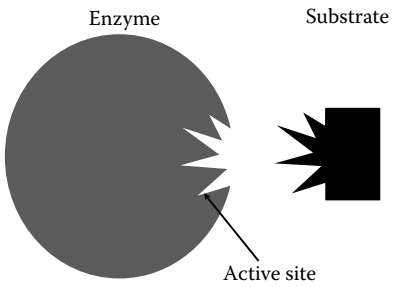


FIGURE 1.4 Active site of enzyme where substrate binds.

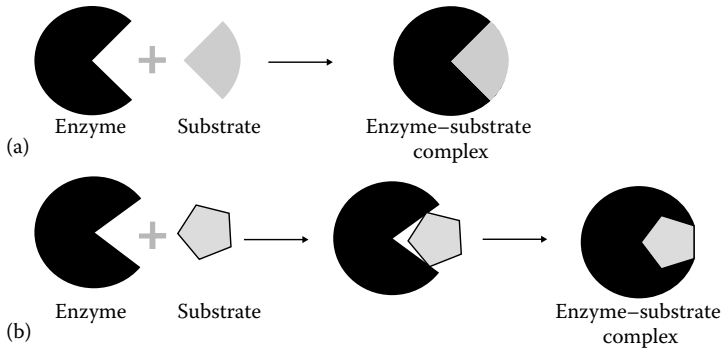


FIGURE 1.5 Models of enzyme action. (a) Lock-and-key hypothesis and (b) induced fit hypothesis.

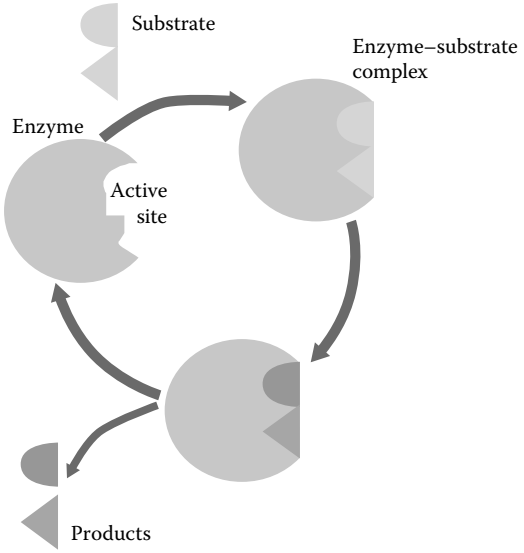


FIGURE 1.6 Mechanism of enzyme action.

The conformational changes of an enzyme, that is, the changes in the three-dimensional structure of an enzyme, resulting from the binding of a substrate to an enzyme, was explained by Koshland (1958) in his *induced fit hypothesis*. He proposed that the structure of a substrate may be complementary to the active site of an enzyme in the enzyme–substrate complex but not in the free enzyme state, and this was due to the conformational change that takes place in the enzyme upon binding with the substrate (Figure 1.5b). This mechanism could explain the flexibility of the enzyme and its high degree of specificity in action.

The mechanism of enzyme action depicted in Figure 1.6 explains the conformational change that happens in the active site of an enzyme that allows substrate to bind to the modified active site and carry out the reaction process.

1.5.1 Mechanism of Catalysis by Enzymes

There are four general mechanisms underlying enzyme catalysis. First, is *catalysis by proximity*; that is, in order to carry out chemical reactions, the substrate and enzymes must come within a bond-forming

distance of each other. Thus, as the concentration of substrate increases, the possibility of chemical reactions occurring is greater. When an enzyme binds a substrate at its active site, a zone of higher substrate concentration is developed, causing the substrate molecule to orient spatially in a position ideal for chemical reaction to occur, resulting in enhanced reaction rates. Certain enzymes catalyze reactions by acting as acids and bases due to the presence of ionizable functional groups of aminoacyl side chains and prosthetic groups.

Enzyme-catalyzed lytic reactions are achieved due to straining of the covalent bonds of substrates. When the enzymes bind with substrates, conformational changes unfavorable for the bond that undergoes cleavage is generated, resulting in strain stretches, or distorts the targeted bond, weakening it and making it more susceptible for cleavage.

Covalent catalysis involves the formation of a covalent bond between the enzyme and one or more substrates. The modified enzyme then becomes a reactant and the chemical modification of the enzyme is transient. On completion of the reaction, the enzyme returns to its original state. The proteolysis by trypsin is an example for covalent reactions.

1.6 Specificity of Enzymes

The specificity of enzymes is one of their unique properties. They are specific not only to the reactions they catalyze, but also to the substrates they utilize and can therefore be categorized based on their different exhibited specificities, as *absolute specificity*, *group specificity*, *relative specificity*, *stereospecificity*, and *dual specificity.*

- Enzymes that exhibit *absolute specificity* act only on one substrate, for example, glucokinase, which acts only on glucose, and lactase, which acts only on lactose.
- Enzymes with *group specificity* are specific not only to the type of bond, but also to the structure around it, for example, aminopeptidases and carboxypeptidases are exopeptidases that hydrolyze the peripheral peptide bonds at the amino terminal and carboxy terminal, respectively, and chymotrypsin is an endopeptidase that hydrolyzes the central peptide bonds in which the carboxyl groups belong to aromatic amino acids.
- Enzymes showing *relative specificity* act on substrates that are similar in structure and show the same type of bonds, for example, amylases act on α-1-4 glycosidic linkages of starch, dextrin, and glycogen, and lipases act on ester linkages in triglycerides.
- In *optical specificity*, the enzyme is specific not only to the substrate but also to its optical configuration, for example, L-amino acid oxidases act on L-amino acids, and β-glycosidase acts on β-glycosidic bonds in cellulose.
- Enzymes with *dual specificity* have the ability to act on two substrates, for example, xanthine oxidase converts hypoxanthine to xanthine and xanthine to uric acid.

1.7 Bioenergetics and Enzyme Catalysis

1.7.1 Concepts of Bioenergetics

Bioenergetics deals with changes in energy and in comparable factors, even as a biochemical process takes place, but not with the mechanism of the reactions or the speed thereof.

The *first law of thermodynamics* states that energy can neither be created nor destroyed but can be converted into many other forms of energy or be used to do work.

The *second law of thermodynamics* deals with entropy or degree of disorder. It states that entropy in the universe is perpetually increasing. It does not distinguish between different systems,

be it any living cell, a locomotive engine, or any chemical reaction. However, from a state of low entropy maintained by the consumption of chemical energy (in the form of food) in other organisms to light energy by photosynthesis in plants, life ultimately approaches thermodynamic equilibrium via death and decay.

Living systems operating at constant temperatures and pressures cannot use heat energy to perform work. Under these circumstances, the concept of two energy forms has been put forth: one that can be used to perform work, also called *free energy*, and another that cannot.

1.7.2 Enthalpy, Entropy, and Free Energy

Under thermodynamic considerations, a system that allows exchange of energy with its surroundings but not with matter is a *closed system*. In a closed system, if a process that takes place involves transfer of heat to or from the surrounding environment and causes a change in the volume of the system, then, according to the first law of thermodynamics,

$$\Delta E = \Delta H - P\Delta V \tag{1.1}$$

where ΔE is the increase in the intrinsic energy of the system, ΔH is the increase in enthalpy and $P\Delta V$ is the work done on the surrounding environment by increasing the volume of the system by ΔV at constant pressure P and temperature T. Change in enthalpy is defined as the quantity of heat absorbed by the system under the above conditions (at constant pressure P and temperature T) and determined calorimetrically.

The increase in entropy of the surroundings is $-\Delta H/T$ under the above conditions. If the process conditions are thermodynamically reversible (but take place infinitely slowly), then the increase in entropy of the system, ΔS, would be $\Delta H/T$. If the process has to occur spontaneously under thermodynamically irreversible conditions, then ΔS must be greater than $\Delta H/T$. This, as required by the second law of thermodynamics, gives an overall increase in entropy of the system including the surroundings.

Thus,

$$S - \frac{\Delta H}{T} > 0 \tag{1.2}$$

and

$$\Delta H - T\Delta S < 0 \tag{1.3}$$

In 1878, Gibbs defined the increase in free energy of the system ΔG as

$$\Delta G = \Delta H - T\Delta S \tag{1.4}$$

Therefore, at constant pressure and temperature, $\Delta G < 0$ for a spontaneous process.

1.7.3 Standard Free Energy

Standard free energy change ΔG° can be calculated if the equilibrium constant of a reaction is known.

$$\Delta G^\circ = -hRT \log_e K_{eq} = -2.303\, RT \log_{10} K_{eq} \tag{1.5}$$

It is also the difference in the standard free energy of formation of the reactants and the standard free energy of formation of the products with each term adjusted according to the stoichiometry of the reaction equation.

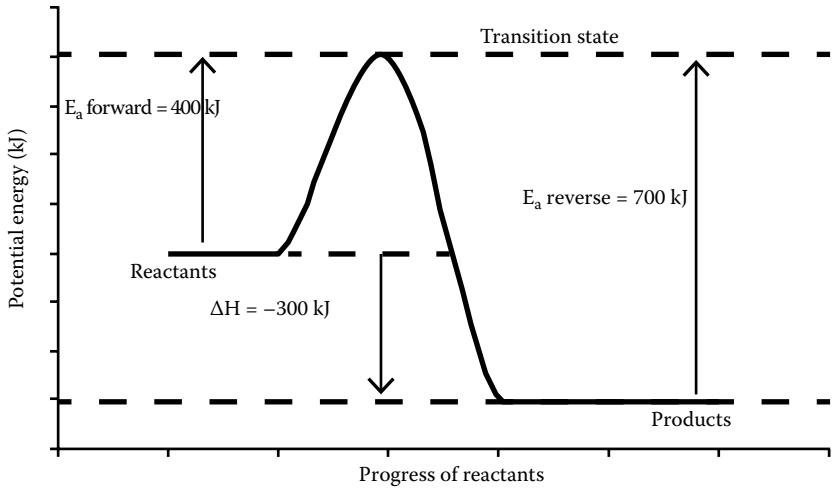


FIGURE 1.7 Reaction profile for hypothetical reaction.

1.7.4 Factors Affecting Rates of Chemical Reactions

Chemical reactions can take place only if the molecules can interact with each other. Molecules can interact only if they are in contact with each other and collide with each other. According to the principles put forth by Arrhenius and van't Hoff (Atkins and DePaula 2006), factors that can increase the rate of collision of molecules will also increase reaction rates, that is, concentration of reactants or increase in temperature. However, it is also important to know that not all colliding molecules will react due to steric hindrance. Further, these colliding molecules may not possess between them sufficient energy for a reaction to take place.

Molecules that are alike may not necessarily possess the same amount of energy even considering the different forms of energy. For example, the energy possessed by individual molecules will depend on the type of collisions they were recently involved in. The energy levels of the colliding molecules must be sufficiently large to overcome a potential barrier known as the "energy of activation."

The *transition state theory* developed by Eyring (1935) explains the requirement for activation energy in a chemical reaction. This postulates that every chemical reaction proceeds via the formation of an unstable intermediate between the reactants and products. The activation energy is required for the formation of the transition state complex from the reactants.

The theory suggests that three major factors determine if a reaction will occur: concentration of transition state complex, rate of breakup of complex, and the way the complex breaks, that is, to reform the reactants or to form the products.

Using a *reaction profile*, the energy necessary to complete a reaction can be determined by plotting energy values on the y-axis of a Cartesian plane. On the x-axis, the reaction progress is plotted.

Figure 1.7 illustrates where the molecules exist as reactants, products, or in the transition state. The relationship between potential energy and reaction rate is clear. Also illustrated is the amount of energy required to initiate a reaction—the activation energy (E_a). A reaction profile can also be used to find the enthalpy (ΔH) of the reaction by subtracting the energy of the products from the energy of the reactants. This example reaction is exothermic. The Gibbs energy change of the reaction (ΔG) is equal to the activation energy of the forward reaction.

1.7.5 Reaction Catalysis

A catalyst increases the rate of a chemical reaction without changing itself and can be separated unchanged or unmodified from the end product of a reaction. This indicates that it has no overall thermodynamic effect, that is, the presence of the catalyst does not change the amount of free energy liberated or utilized (Figure 1.8) when the reaction reaches completion. Catalysts act frequently by reducing the energy of activation for a chemical reaction, wherein a part or the whole of the catalyst interacts with

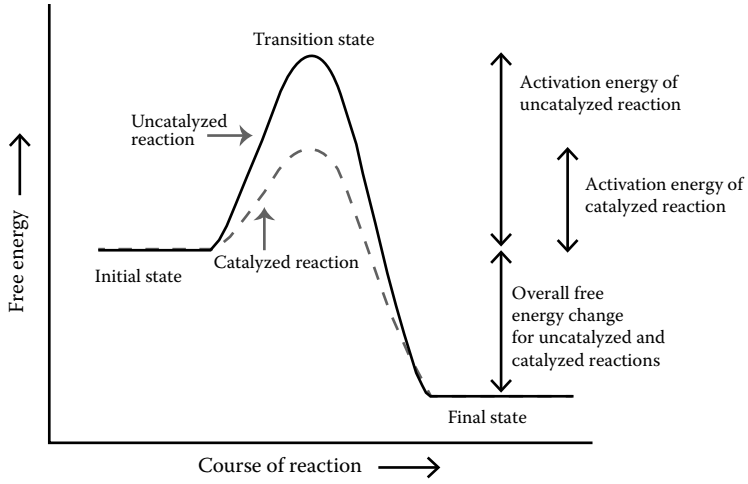


FIGURE 1.8 Effect of catalyst on free energy changes in a chemical reaction.

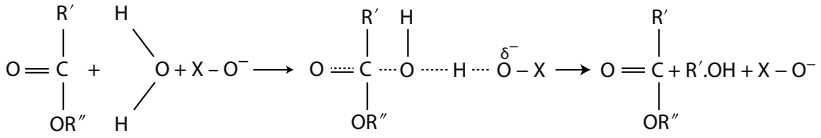


FIGURE 1.9 Hydrolysis of ester by a base.

the reactants to form a transition state complex that is not only very different from that produced by an uncatalyzed reaction, but also more stable and hence with lower energy.

Catalysts are often acids or bases. The transition state is stabilized by donation of a proton by acids and acceptance of proton by bases, for example, hydrolysis of an ester by a base (Figure 1.9).

In covalent catalysis, on the other hand, the transition state is stabilized by changes in covalent bonds whereas, in metal ion catalysis, it is stabilized by electrostatic interactions with a metal ion.

1.7.6 Kinetics of Uncatalyzed Reactions

The *law of mass action* proposed by Guldberg and Waage (1867) forms the basis for all kinetic work. This states that the rate of reaction is proportional to the product of the activities of each reactant (each activity) raised to the power of the number of molecules of that reactant participating. For example, for a reaction **xA + yB → products**, the reaction rate is proportional to (activity of A)^x × (activity of b)^y.

However, for all practical purposes, concentration is used instead of activity although this holds good only for ideal gases and in very dilute solutions.

1.7.7 Order of Reactions

The law of mass action helped to develop the concept of the *order of reactions*.

A *first-order reaction* is one in which a reaction rate is dependent on the concentration of a single reactant (Figure 1.10).

Thus, for a first-order reaction **A → P** occurring at constant temperature and pressure in a dilute solution the reaction rate ν at any time t is given by

$$\nu = -\frac{d[\mathrm{P}]}{dt} = +\frac{d[\mathrm{P}]}{dt} = k[\mathrm{A}] \tag{1.6}$$

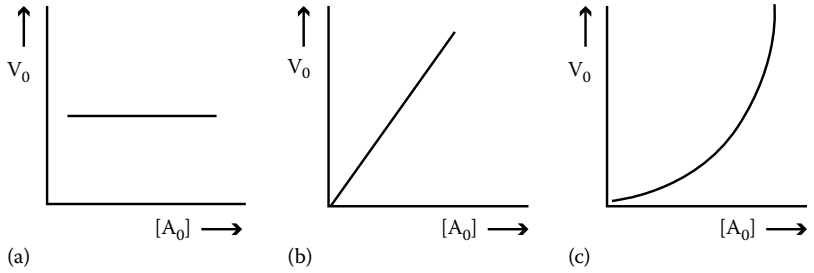


FIGURE 1.10 Graphs representing initial velocity against initial reactant concentration for single-reactant reactions (order of reactions). (a) Zero order, (b) first order, and (c) second order.

where ν is the reaction rate at time t, k is the rate constant, [A] is the concentration of reactant A at time t, [P] is the concentration of product P at time t, $-\frac{d[P]}{dt}$ is the rate of decrease in [A], and $+\frac{d[P]}{dt}$ is the rate of increase in [P].

A second-order reaction involves two reactants and proceeds at a rate proportional to the concentration of the two reactants or the second power of a single reactant (Figure 1.10). Thus, for a second-order reaction A + B → P occurring at constant temperature and pressure in a dilute solution, the reaction rate ν at any time t is given by

$$\nu = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = +\frac{d[P]}{dt} = k[A][B] \tag{1.7}$$

For a second-order reaction $2A \rightarrow P$ the above equation may be rewritten as

$$\nu = -\frac{d[A]}{dt} = +\frac{d[P]}{dt} = k[A][A] = k[A]^2 \tag{1.8}$$

However, if one of the reactants is in far greater excess than the other, a two-reactant reaction may be considered a *pseudo single-order reaction*.

Zero-order reactions are also possible. These are reactions in which the reaction rates are independent of the concentrations of any of the reactants involved.

1.7.8 Initial Velocity

The appearance of the product with time can be graphically represented as given in Figure 1.11. The reaction rate at any time is the slope of the curve at that time on the graph, which may be constant for a short while initially; this will later decrease with a decrease in concentration of the reactant(s) as the reaction proceeds, finally reducing to zero. At this point, all reactants have either been converted to products or a reaction equilibrium has been reached, wherein the rate of forward and backward reaction are equal.

Initial velocity (ν_0) is the reaction rate when $t = 0$ and can be determined from the graph by drawing a tangent as shown in Figure 1.11. The units for ν_0 are those used for product concentration divided by those used for time:

$$\nu_0 = \frac{[P]_2 - [P]_1}{t_2 - t_1} \tag{1.9}$$

Initial velocity is a kinetic parameter determined for a reaction, especially when the conditions can be easily specified. Thus, it is evident that initial velocity is dependent on initial concentration of the

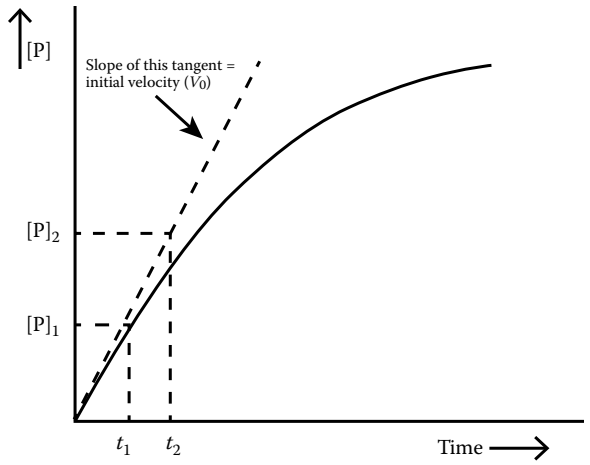


FIGURE 1.11 Graph of product concentration against time for a chemical reaction.

reactants. For a first-order reaction, that is, a single reactant reaction, $\nu = k$ [A]. Consequently, at time t = 0, $\nu_0 = k$ [A_0], where [A_0] is the initial concentration of A.

Accordingly, for a single-reactant second-order reaction, $\nu_0 = k$ [A_0]^2, and for a single-reactant zero-order reaction, $\nu_0 = k$ [A_0]^0.

This approach can be applied to reactions with more than one reactant. The overall order of the reactions is the sum total of the orders of each of the individual reactants in a reaction.

1.7.9 Catalysis Causes Reduction of Energy Barriers

Catalysis causes a change in the stable condition, which can be achieved by supplying energy. Catalysis occurs at active sites, during which process an energy barrier must be surmounted. To surmount the energy barrier to carry out a chemical reaction, enzymes that cause reduction of energy barriers may be used. Lowering of energy can be done with the help of an enzyme–substrate interaction that takes place at the active sites (Uhlig 1998). The active sites of multimeric enzymes are often located at the interface between the subunits and recruit residues from more than one monomer. The three-dimensional active site can shield substrate from solvent and facilitate catalysis.

1.7.9.1 Nature of Enzyme Catalysis

Enzymes behave like other catalysts and combine with reactants to form a transition state with lower free energy than that formed by an uncatalyzed reaction. Formation of enzyme–substrate complexes are not synonymous with transition states. In the case of a single-reactant reaction, the substrate binds with the specific substrate-binding site to form an enzyme–substrate complex; this process is preceded by the formation of the unstable transition state (transition state 1). The groups that react are held in close proximity with each other and with the catalytic site in the enzyme–substrate complex. The reaction proceeds further to form the enzyme–product complex via transition state 2. However, in circumstances in which the product may still be enzyme-bound, there would exist another transition state (3) before the product is released. The free energy profile of this type is depicted in Figure 1.12.

1.8 Kinetics of Enzyme-Catalyzed Reactions

Enzymes can increase the rate of a given reaction. Enzyme kinetics is primarily concerned with the measurement and mathematical description of reaction rates and their associated constants. It is primarily

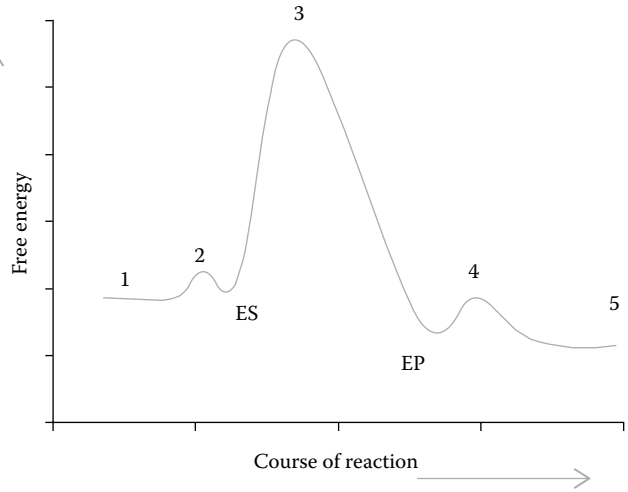


FIGURE 1.12 Free energy profile of an enzyme-catalyzed reaction involving the formation of an enzyme–substrate and enzyme–product complex. (1) Initial state, (2) transition state 1, (3) transition state 2, (4) transition state 3, and (5) final state.

applied to analyze data obtained from an enzymatic reaction and to use the data to optimize the reaction, and these data can be used for detailed characterization of the enzyme.

Both quantitative and qualitative enzyme kinetics are important to determine the minimum level of enzyme needed to carry out a chemical reaction efficiently in particular conditions in order to carry out economical and efficient conversion of food products. The enzyme kinetics of most industrial enzymes have been determined and are available online (www.brenda.uni-koeln.de).

Wilhelmy (1850) demonstrated that the rate of sucrose hydrolysis was proportional to its concentration. Brown (1902) demonstrated that, at low sucrose concentrations, the invertase catalyzed reaction was a first-order reaction, but at higher concentrations, it became zero order. This holds true for all single-substrate reactions. In the case of multisubstrate reactions, this applies only when all but one of the substrate concentrations is kept constant.

A graph of initial velocity, ν_0, against initial substrate concentration ($[S_0]$) at constant enzyme concentration ($[E_0]$) was shown to be a rectangular hyperbola (Figure 1.13).

This was first explained by Brown (1902) pertaining to sucrose hydrolysis.

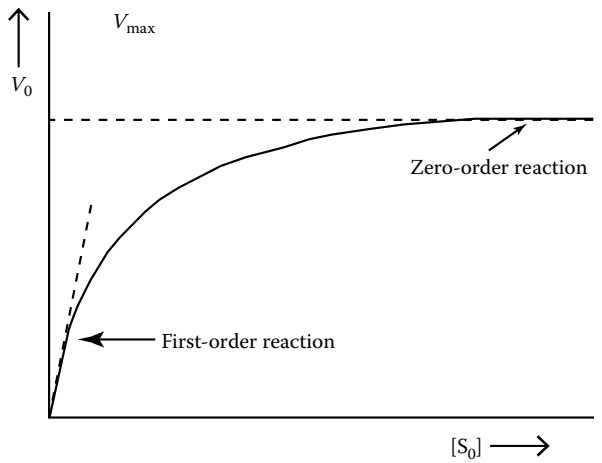


FIGURE 1.13 Graph of initial velocity against initial substrate concentration.

The general equation to explain the graph is

$$v_0 = \frac{V_{max}[S_0]}{[S_0] + b} \tag{1.10}$$

where V_{max} is maximum value of ν_0, and b is the value of $[S_0]$ when $\nu_0 = 1/2\ V_{max}$.

Kinetic models to explain this were studied by both Henri (1903) and Michaelis-Menten (1913). Not all enzymes give hyperbolic curves. However, it is characteristic of all enzymes to attain maximum initial velocity with increasing substrate concentration when the enzyme concentration is kept constant. Sufficient increase in substrate concentration will cause enzyme saturation. When this happens, all enzymes will be substrate bound, and there will not be any free enzyme.

Then, $[ES] = [E_0]$, and therefore, the reaction rate will be $k_2\ [E_0]$.

This is independent of substrate concentration and will therefore not increase with further increase in substrate concentration. Hence, $V_{max} = k_2\ [E_0]$.

However, in low concentrations of substrate, the enzyme will not be saturated. Therefore, the overall reaction rate will be limited by the rate of formation of the enzyme–substrate complex. When enzyme concentration is constant, the reaction rate is proportional to $[S_0]$ and therefore results in the first-order reaction. Rates of reactions are affected by factors such as enzymes/catalysts, substrates, effectors, temperature, and pH.

1.8.1 Michaelis-Menten Equation

First, the enzyme (E) and the substrate (S) react reversibly and quickly form a noncovalent ES complex:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \tag{1.11}$$

Second, the ES complex undergoes a chemical transformation and dissociates to give product (P) and enzyme (E).

$$ES \xrightarrow{k_2} E + P \tag{1.12}$$

Many enzymatic reactions follow Michaelis-Menten kinetics. The Michaelis-Menten model is a simple model to explain single-substrate enzyme-catalyzed reactions involving the formation of a single intermediate. However, not all enzyme mechanisms are as simple and are always more complicated and can involve more than one substrate or more than one substrate-binding site to name a few variations.

The Michaelis-Menten model is based on several assumptions. It is assumed that the first step is fast and is always at equilibrium.

The catalytic step (ES → E + P, *or* ES → EP) is the rate-limiting step, and therefore, k_1 and k_{-1} are much greater than k_2 (i.e., $k_1, k_{-1} >> k_2$).

Under these circumstances, Michaelis-Menten equilibrium assumption is valid, and $K_m \cong K_s$.

K_s gives the affinity of the enzyme for the substrate; a low K_s indicates high affinity, and a high K_s indicates a low affinity of the enzyme for the substrate.

Second, the system is assumed to be in a steady state, that is, $d[ES]/dt \approx 0$. It is also assumed that there is only a single reaction or dissociation step (i.e., $k_2 = k_{cat}$). The other assumption of the Michaelis-Menten model is that the initial substrate concentration $[S_0]$ is much greater than the initial enzyme concentration $[E_0]$; hence, the formation of the enzyme–substrate complex will not result in a significant change in the free substrate concentration. Thus, $S_{Tot} = [S_0] + [ES]$, $[S_0] \approx [S]$. Another assumption is that there is no back reaction of P to ES, and initial velocities are measured when $[P] \approx 0$.

1.8.1.1 Briggs-Haldane Modification of Michaelis-Menten Equation

Briggs-Haldane (1925) introduced a more general assumption, such as *steady state*, to the Michaelis-Menten equation. According to them, initially, because the concentration of the enzyme and, therefore, that of the enzyme–substrate complex, was very small compared to the substrate concentration, the rate of change of [ES] would be much less than the rate of change of [P].

Thus, for the reaction,

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P \tag{1.13}$$

The rate of formation of ES at time t (initially, product concentration is negligible) = k_1[E][S].

The rate of breakdown of ES at this time = k_{-1}[ES] + k_2[ES] (ES can either form products or reform the reactants).

Using the steady-state assumption,

$$k_1\,[\mathrm{E}][\mathrm{S}] = k_{-1}\,[\mathrm{ES}] + k_2\,[\mathrm{ES}] = [\mathrm{ES}]\,(k_{-1} + k_2) \tag{1.14}$$

Separating constants from variables, we get

$$\frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} = \frac{k_{-1} + k_2}{k_1} = K_\mathrm{m} \tag{1.15}$$

where K_m is another constant.

Substituting [E] = [E_0] − [ES], we get

$$\frac{([\mathrm{E}_0] - [\mathrm{ES}])[\mathrm{S}]}{[\mathrm{ES}]} = K_\mathrm{m} \tag{1.16}$$

From which we get

$$[\mathrm{ES}] = \frac{[\mathrm{E}_0][\mathrm{ES}]}{[\mathrm{S}] + K_\mathrm{m}} \tag{1.17}$$

Because $\nu_0 = k_2$[ES],

$$\nu_0 = \frac{k_2[\mathrm{E}_0][\mathrm{ES}]}{[\mathrm{S}] + K_\mathrm{m}} \tag{1.18}$$

Because $V_{max} = k_2$[E_0] and [S] > [E], [S] ≈ [S_0], so

$$\nu_0 = \frac{k_2[\mathrm{E}_0][\mathrm{S}_0]}{[\mathrm{S}_0] + K_\mathrm{m}} \text{ at constant } [\mathrm{E}_0] \tag{1.19}$$

and hence,

$$\nu_0 = \frac{V_{max}[\mathrm{S}_0]}{[\mathrm{S}_0] + K_\mathrm{m}} \text{ at constant } [\mathrm{E}_0] \tag{1.20}$$

This equation has retained the same form as that of the Michaelis-Menten equation as well as the name, and K_m is called the Michaelis-Menten constant.

A graph of ν_0 against $[S_0]$ at constant $[E_0]$ is shown in Figure 1.12.

K_m can be obtained from the graph. When $\nu_0 = 1/2\ V_{max}$, then

$$\frac{V_{max}}{2} = \frac{V_{max}[S_0]}{[S_0] + K_m} \tag{1.21}$$

Therefore,

$$V_{max}\ ([S_0] + K_m) = 2\ (V_{max})\ [S_0] \tag{1.22}$$

So $K_m = [S_0]$.

1.8.1.2 Significance of Michaelis-Menten Equation

This equation and its Briggs-Haldane modification regard single-substrate enzyme-catalyzed reactions with a single substrate-binding site.

1.8.1.3 Michaelis-Menten Constant (K_m)

K_m is also called the Michaelis-Menten constant. It is the apparent dissociation constant of the enzyme–substrate complex. K_m is the substrate concentration $[S_0]$ required to reach half maximum velocity (i.e., when $\nu_0 = 1/2\ V_{max}$). Under circumstances in which k_1 and k_{-1} are much greater than k_2 (i.e., k_1, $k_{-1} >> k_2$), the Michaelis-Menten equilibrium assumption is valid, and $K_m \cong K_s$, is used to describe the enzyme affinity for the substrate.

1.8.1.4 Turnover Number (k_{cat})

The constant k_{cat} is called the turnover number. This is applied to simple single-substrate enzyme-catalyzed reactions. It can be obtained from the expression $V_{max} = k_2[E_0]$. The turnover number indicates the maximum number of substrate molecules that can be converted to product per molecule of enzyme per unit of time. The turnover number ranges from 1 to 10^4 per second for most enzymes.

1.8.1.5 Catalytic Efficiency (k_{cat}/K_m)

The term k_{cat}/K_m is used to denote the catalytic efficiency of an enzyme and is used to rank them. A high value of k_{cat}/K_m (close to that of k_1) indicates that the substrate is bound tightly by an enzyme and the frequency of collisions between the enzyme and substrate is the limiting factor. A low value supports the equilibrium assumption; k_{cat}/K_m is a measure of the enzyme specificity.

1.8.2 Lineweaver-Burk Plot

The Michaelis-Menten graph of ν_0 against $[S_0]$ is not able to determine K_m and V_{max} satisfactorily as ν_0 becomes V_{max} only at infinite substrate concentrations. Hence, from the Michaelis-Menten plot, the determination of an accurate value for V_{max}, and hence K_m, is difficult. Besides, as the plot is a curve, extrapolation from the values of ν_0 at nonsaturating concentrations is inaccurate. This problem was circumvented by Linweaver and Burk (1934) by simply inverting the Michaelis-Menten equation:

$$\frac{1}{\nu_0} = \frac{[S_0] + K_m}{V_{max}[S_0]} = \frac{[S_0]}{V_{max}[S_0]} + \frac{K_m}{V_{max}[S_0]} \tag{1.23}$$

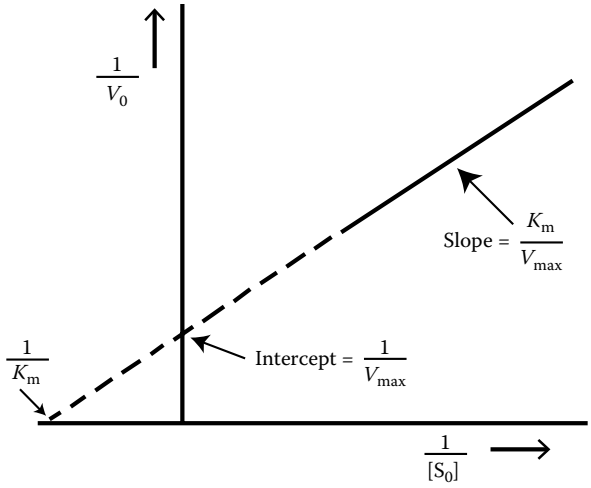


FIGURE 1.14 Lineweaver-Burk plot.

Therefore,

$$\frac{1}{v_0} = \frac{K_m}{V_{max}} \frac{1}{[S_0]} + \frac{1}{V_{max}} \tag{1.24}$$

(Lineweaver-Bulk equation).

This is in the form of $y = mx + c$, which is an equation for a straight-line graph on which the plot of y against x gives a slope m with an intercept c on the y-axis.

Because the graph (Figure 1.14) of $1/v_0$ against $1/[S_0]$ is linear for systems obeying the Michaelis-Menten equation, it can be extrapolated to determine the values of V_{max} and K_m.

1.8.3 Eadie-Hoftree and Hanes Plots

In the Eadie-Hoftree plot (Figure 1.15a), based on the Lineweaver-Burk equation, (which, in turn, is based on the Michaelis-Menten equation), both sides of the equation are multiplied by the factor

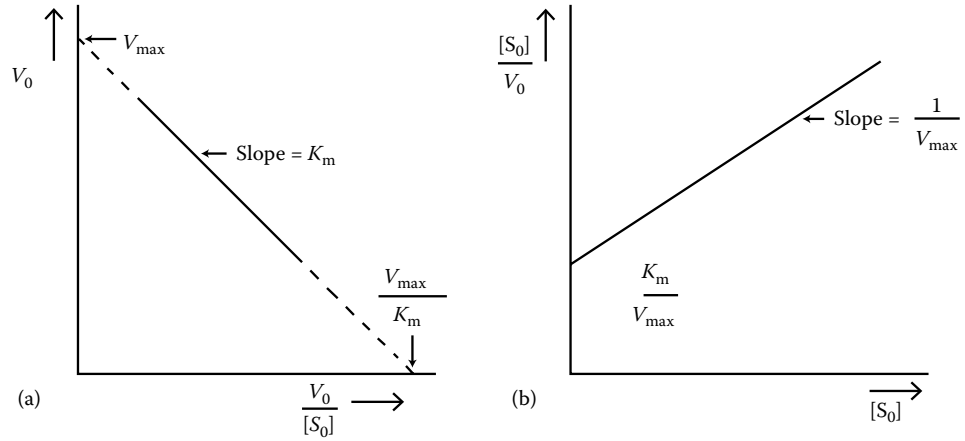


FIGURE 1.15 (a) Eadie-Hoftree plot, (b) Hanes plots.

v_0 V_{max} to give the Eadie-Hoftree equation, which is a straight line from which V_{max} and K_m can be determined.

$$v_0 = -K_m \frac{v_0}{[S_0]} + V_{max} \tag{1.25}$$

The Hanes plot (Figure 1.15b) also starts with the Lineweaver-Burk equation, but here it is multiplied by $[S_0]$ to obtain a linear plot from which both V_{max} and K_m can be determined.

$$\frac{[S_0]}{v_0} = \frac{1}{V_{max}}[S_0] + \frac{K_m}{V_{max}} \tag{1.26}$$

1.9 Enzyme Inhibition

Enzyme activity can be inhibited by several substances, small molecules, or ions that bind with the enzyme. Inhibitors are substances that decrease the rate of enzyme-catalyzed reactions. These can act either on the substrate or the coenzyme or combine directly with the enzyme. The inhibitors that directly interact with the enzyme are of two types: reversible inhibitors that bind enzymes reversibly and can be removed by dialysis to restore enzyme action and irreversible inhibitors that cannot be removed by dialysis.

In reversible inhibition, the dissociation of the enzyme–inhibitor (EI) complex is much faster than in irreversible inhibition as there are no covalent interactions between inhibitor and enzyme. When the inhibitors bind the enzyme, due to the close resemblance to the substrate, and competes for the same binding site as that of the substrate, it is called competitive inhibition. The inhibitor prevents the substrate from binding with the active site. A competitive inhibitor competes with the substrate for the binding site, whereby enzyme activity is reduced due to decreased enzyme-bound substrate. The competitive inhibition can be overcome by increasing the concentration of the substrate. An example of a competitive inhibitor is malonate, which is a structural analogue for succinate and inhibits succinate dehydrogenase activity.

Competitive inhibition is dependent not only upon inhibitor and substrate concentration, but also on their relative affinities for the binding site. Consequently, the degree of inhibition is greater when the inhibitor concentration is greater than that of the substrate. Alternatively, when the substrate concentration is high, the inhibitor competes with the substrate for available binding sites, thereby reducing the extent of inhibition. When substrate concentrations are much higher, the inhibitor molecules are outnumbered by the substrate molecules, leading to insignificant inhibition. Therefore, V_{max} remains unchanged. However, the apparent K_m clearly increases due to the inhibition and is termed K'_m (Figure 1.16).

In the presence of competitive inhibitor I, the Michaelis-Menten equation is

$$v_0 = \frac{V_{max}[S_0]}{[S_0] + K_{m\left(1+\frac{[I_0]}{K_i}\right)}} \tag{1.27}$$

where K_i is the inhibitor constant.

Here, V_{max} remains unaltered, but K_m is altered and becomes K'_m

$$K'_m = K_{m\left(1+\frac{[I_0]}{K_i}\right)} \tag{1.28}$$

where K'_m is the apparent K_m in the presence of initial concentration of competitive inhibitor $[I_0]$. For competitive inhibition, the Lineweaver-Burk equation is given as

$$\frac{1}{v_0} = \frac{K'_m}{V_{max}}\frac{1}{[S_0]} + \frac{1}{V_{max}} \tag{1.29}$$

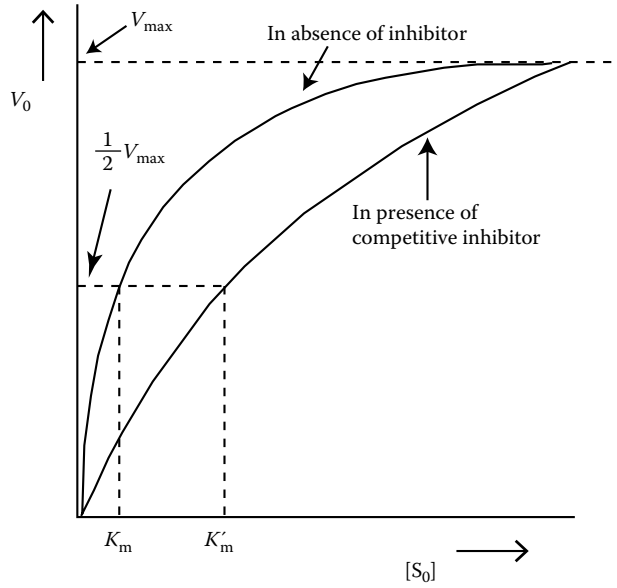


FIGURE 1.16 Michaelis-Menten plot in the presence of a competitive inhibitor.

The Lineweaver-Burk plot showing the effect of competitive inhibitor on enzyme action is given in Figure 1.17.

Although competitive inhibitors bind free enzymes and form the enzyme–inhibitor complex, uncompetitive inhibitors bind only to the enzyme–substrate complex. Here, the inhibitor does not compete for the substrate-binding site but binds at a completely different site. Hence, the inhibition cannot be overcome by increasing the substrate concentration. Both V_{max} and K_m are altered. The Michaelis-Menten form of the equation for uncompetitive inhibition is

$$v_0 = \frac{\dfrac{V_{max}}{\left(1+\dfrac{[I_0]}{K_i}\right)}[S_0]}{[S_0]+\dfrac{K_m}{\left(1+\dfrac{[I_0]}{K_i}\right)}} \tag{1.30}$$

where

$$V'_{max} = \frac{V_{max}}{\left(1+\dfrac{[I_0]}{K_i}\right)} \tag{1.31}$$

and

$$K'_m = \frac{K_m}{\left(1+\dfrac{[I_0]}{K_i}\right)} \tag{1.32}$$

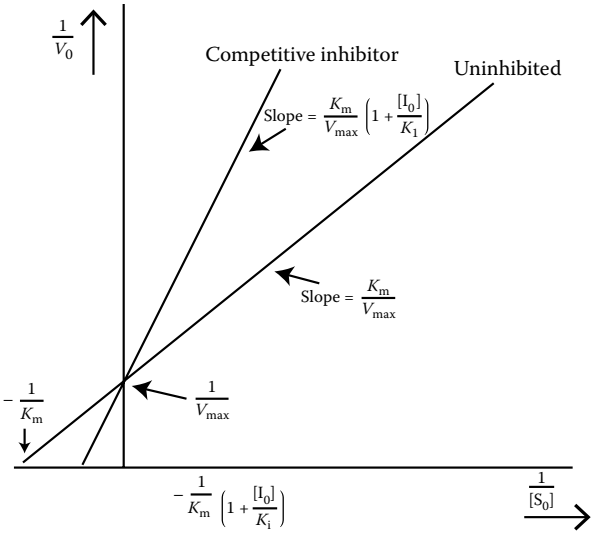


FIGURE 1.17 Lineweaver-Burk plot in the presence of a competitive inhibitor.

where V'_{max} is the value of V_{max} and K'_m is the apparent K_m in the presence of $[I_0]$ concentration of the uncompetitive inhibitor.

The Lineweaver-Burk equation for uncompetitive inhibition is

$$\frac{1}{v_0} = \frac{K'_m}{V'_{max}}\frac{1}{[S_0]} + \frac{1}{V_{max}} \tag{1.33}$$

The Lineweaver-Burk plot showing the effect of the uncompetitive inhibitor on enzyme action is given in Figure 1.18.

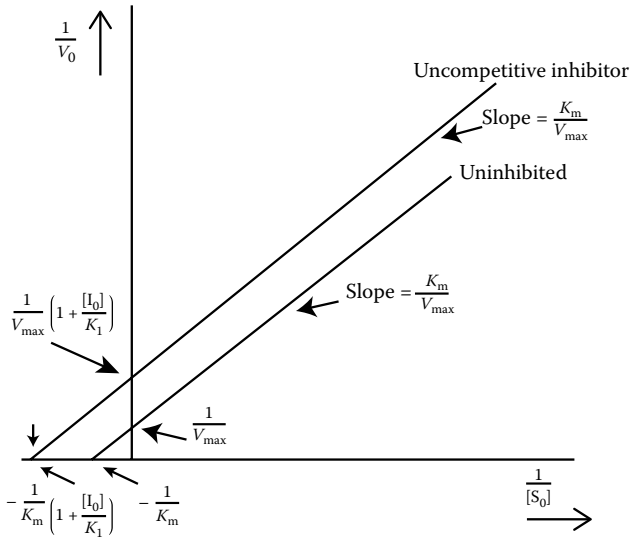


FIGURE 1.18 Lineweaver-Burk plot showing the effect of uncompetitive inhibitor on enzyme action.

In noncompetitive inhibition, there is no competition for the substrate-binding site as the inhibitor molecule binds to the enzyme regardless of the binding of the substrate; that is, the binding sites for the substrate and inhibitor molecules are different. The enzyme turnover number is decreased in this case, and increasing the concentration of substrate molecules can reverse inhibition.

1.10 Factors Affecting Enzyme Activity

Enzyme stability is an important factor to be considered in the application of enzymes in the food industry. The stability and activity of enzymes is influenced by their inherent physical stability, presence of inhibitors/poisons/antagonists in the food/reacting mixture and physicochemical factors such as pH and temperature. Therefore, most enzymes exhibit maximal activity at their optimal conditions that influence enzyme activity. Optimum conditions of enzymes are those favorable conditions that allow them to perform efficiently. Most influencing physicochemical conditions for enzymes are pH, temperature, substrate concentration, and enzyme concentration.

All enzymes exhibit an optimum pH, temperature, and substrate concentration at which they demonstrate maximum activity (Figure 1.19), and they behave according to well-established rules. All enzymes have a specific pH value or pH range for optimal activity (Table 1.2). A change in pH can affect enzymatic activities, and extremely low or high pH values can cause deformity in the structure of enzymes, which, in turn, result in loss of action for most enzymes. The pH value at which an enzyme shows its maximum activity is called its optimum pH, which is one of the important criteria in its application in the food industry. This can be exemplified by the enzymes involved in the brewing industry as a majority of these enzymes have an optimum pH in the range of 3.0–7.0 (Whitehurst and Law 2002).

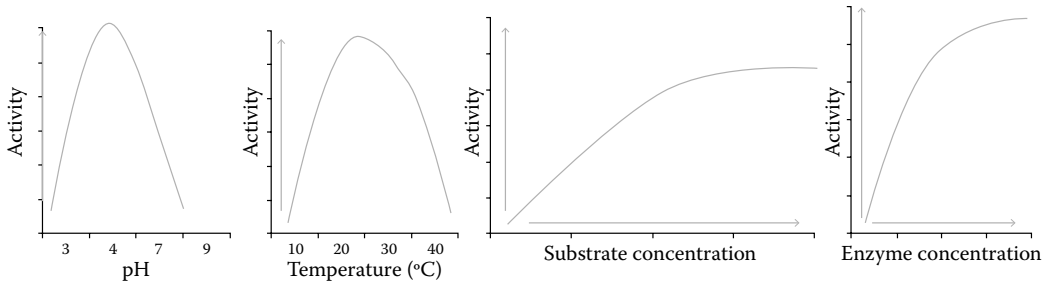


FIGURE 1.19 Effect of temperature, pH, enzyme concentration, and substrate concentration on the initial rate of reactions.

TABLE 1.2

Optimum pH and Temperature Range of Microbial-Derived Enzymes Widely Used in the Food Industry

Sl. No.	Enzyme	Optimum pH Range	Optimum Temperature (°C)	References
1	α-Amylase from *B. licheniformis*	6.0–11.0	76	Saito 1973
2	α-Amylase from *B. stearothermophilus*	4.0–5.2	80	Srivastava and Baruah 1986
3	α-Amylase from *A. oryzae*	5.0	50	Patel et al. 2005
4	Lipase from *Aspergillus nidulans* WG312 strain	6.5	40	Mayordomo et al. 2000
6	*Substilisin* Carlsberg from *B. licheniformis*	8–9		Aunstrup 1980
7	Alkaline protrease from *Aspergillus oryzae*	7.4		Bergkvist 1963
9	Trypsin	5–11	30–60	Kristjansson 1991
10	β-Galactosidase from yeast	7.0	30	Itoh et al. 1982

As with pH, every enzyme has its optimum range of temperature, and enzyme performance usually improves with increasing temperature. Enzymatic reactions increase with every 10°C temperature increase, and this may be applicable up to 10°C–40°C. Some enzymes may be active at low temperatures. Beyond the optimum temperature, enzymes may be denatured, and optimal temperature is therefore another important parameter in the food industry. A wide range of enzymes with various temperature ranges are needed for various purposes in the food industry.

Carrying out enzymatic reactions efficiently requires an optimum level of substrate concentration. The substrate should saturate the enzyme. At a constant enzyme concentration, enzyme activity increases with substrate concentration, and the substrate concentration value at which the activity of the enzyme is found to be maximum is called the optimum substrate concentration, and a further increase in substrate concentration will not cause any elevation in the enzymatic activity. Likewise, each enzyme has its own optimum value for enzyme concentration. In order to carry out reproducible and profitable usage of an enzyme in the food industry, all these physicochemical parameters should be optimized. Performance of enzymes is optimum in aqueous solutions as they must have constant and unimpaired contact for maximal enzymatic activity.

Inhibitors are substances that decrease the rate of enzyme-catalyzed reactions. These can act on either the substrate or the coenzyme or combine directly with the enzyme. Enzyme inhibitors are of two types: (i) reversible inhibitors that bind enzymes reversibly and can be removed by dialysis to restore enzyme action, and (ii) irreversible inhibitors that cannot be removed by dialysis.

1.10.1 Role of Prosthetic Groups, Cofactors, and Coenzymes

Catalysis can be promoted by small nonprotein molecules and metal ions that participate directly in substrate binding, termed "prosthetic groups," "cofactors," and "coenzymes." They are distinguished by their mode of action and binding strength (Murray et al. 2009).

Prosthetic groups are tightly integrated with enzyme structure by covalent or noncovalent forces. Metals, including Co, Cu, Mg, Mn, Se, and Zn, are the most common prosthetic groups, and enzymes containing tightly bound metal ions are called "metalloenzymes." The biotin and flavin dinucleotide are considered to be prosthetic groups other than metal ions. They facilitate the binding and orientation of substrates or the formation of covalent bonds with reaction intermediates, or they interact with substrates to make them more susceptible to chemical reaction. *Cofactors* serve the same function as that of prosthetic groups, but the binding is not as tight as in prosthetic groups. Unlike metalloenzymes, enzymes requiring a metal cofactor are termed "metal-activated enzymes." In the case of cofactors, they should be in the medium surrounding the substrate and enzyme in order to carry out catalytic reactions.

Coenzymes are mainly meant for transfer of substrates from the point of origin to the point of utilization, for example, coenzyme A, tetrahydrofolate.

1.11 Sources of Enzymes in the Food Industry

Enzyme preparations used in food industries are obtained from animal, plant, or microbial sources and may consist of whole cells, parts of cells, or cell-free extracts of the source used. They may contain one or more active components as well as carriers, solvents, preservatives, antioxidants, and other substances consistent with good manufacturing practice. They may be available in liquid, semiliquid, dry, or an immobilized form (www.fao.org). Various enzymes, their sources, and their applications in the food industry are detailed in Table 1.3.

Because enzymes are present in almost all raw food material, their high substrate specificity, rapid reaction rates, and ability to work effectively at relatively low temperatures make them ideal for food processing. Plants, animals, and microorganisms are the primary sources of enzymes in the food industry. For instance, intrinsic enzymes found in plant materials such as amylases and pectinases have a significant role in bread making, beer brewing, and fruit juice production. Plant-based proteases such as papain, bromelain, and ficin find wider application in meat tenderization and hydrolysis of animal, milk, and plant proteins. These proteases are characterized by the sulfhydryl group in the active site, responsible

TABLE 1.3

Sources of Major Food Enzymes

Enzymes	Source	References
Glucose oxidase	**Fungi**	
	Aspergillus niger	Kona et al. 2001
	Penicillium notatum	Keilin and Hartree 1948
Sulphydryl oxidase	**Fungi**	
	Aspergillus niger	De la Motte and Wagner 1987
Catalase	**Animal**	
	Liver of *Bos taurus*	Uhlig 1998
	Fungi	
	Aspergillus niger	Fiedurek and Gromada 2000
Lactoperoxidase	**Animal**	
	Cheese whey: bovine colostrums	Kussendrager and Hooijdonk et al. 2000
Lipoxygenase	**Plant**	
	Soy bean	Brash 1999
Laccases	**Plant**	
	Rhus vernifera	Yoshida 1883
	Fungi	
	Agaricus brunnescens	Fagan and Fergus 1984
	Aspergillus nidulans	Aramayo and Timberlake 1990
	Bacteria	
	Azospirillum lipoferum	Givaudan et al. 1993
Hexose oxidase	**Red algae**	
	Chondrus crispus	Sullivan and Ikawa 1973
Cyclodextrin	**Bacteria**	
	Bacillus sp.	Tonkova 1998
Transglutaminase	**Bacteria**	
	Streptoverticillium sp.	Ando et al. 1989
	Animal	
	Muscles of atka mackerel, botan shrimp, carps, rainbow trout, and scallop	Fernandes 2010
Lipase	**Animal**	
	Pig pancreas, gullet of goat and lamb, calf abomasums	Uhlig 1988
	Fungi	
	Penicillium roqueforti	
	Rhizomucor miehei	Herrgard et al. 2000
	Bacteria	Macedo et al. 2003
	Bacillus subtilis	
		Ruiz et al. 2005
Phospholipase A, lechithinase A	**Animal**	
	Pancreatic glands of porcine	Uhlig 1988
	Bacteria	
	Bacillus mycoides strain 970	Chang et al. 2010
Pectinesterase	**Plants**	
	Carica papaya, *Lycopersicum esculentum*	Fayyaz et al. 1993;
	Fungi	Warrilow et al. 1994
	Aspergillus niger	Maldonaldo and de Saad 1998
Tannase	**Bacteria**	
	Bacillus sphaericus	Raghuwanshi et al. 2011

(*Continued*)

TABLE 1.3 (CONTINUED)

Sources of Major Food Enzymes

Enzymes	Source	References
	Bacillus cereus	Mondal et al. 2001
	Lactobacillus plantarum	Ayed and Hamdi 2002
	Fungi	
	Aspergillus niger	Barthomeuf et al. 1994
	Aspergillus awamori	Beena et al. 2010, 2011
α-Amylase, diastase	**Animal**	
	Hog or pig pancreas	Uhlig 1998
	Fungi	
	Aspergillus sp.	Kvesitadze et al. 1978
	Bacteria	
	Bacillus stearothemophilus	Srivastava and Baruah 1986
	Bacillus licheniformis	Madsen et al. 1973
	Bacillus amyloliquefaciens	Pazur and Okada 1966
β-Amylase	**Plant**	
	Sweet potato	Laurière et al. 1992
	Cereal seeds	
	Fungi	
	Aspergillus sp.	Abe et al. 1988
Amyloglucosidase	**Fungi**	
	Aspergillus niger *Aspergillus awamori*	Fogarty and Benson 1983; Bertolin et al. 2003
Cellulase	**Fungi**	
	Aspergillus niger	Kang et al. 2004
	Trichoderma sp.	Cheol et al. 2003
Inulinase	**Fungi**	
	Aspergillur sp.	Gupta et al. 1994; Viswanathan and Kulkarni 1996
	Penicillium sp.	Viswanathan and Kulkarni 1996
	Cladosporium sp.	Viswanathan and Kulkarni 1996
Xylanase	**Fungi**	
	Aspergillus sp.	Gawande and Kamat 1998
	Trichoderma sp.	Cheol et al. 2003; Royer and Nakas 1989
	Bacteria	
	Bacillus thermophilus	Khasin et al. 1993
Pectinase, endopolygalacturonase	**Fungi** *Penicillium frequentans*	Said et al. 1991
	Saccharomyces cerevesia	Blanco et al. 1994
	Bacteria	
	Bacillus sp.	Kelly and Fogarty 1978
Lysozyme, muramidase	**Animal**	
	Hen egg white	Whitehurst and Law 2002
β-Galactosidase, lactase	**Bacteria**	
	Escherichia coli	Hall and Hartl 1974
	Fungi	
	Aspergillus sp.	Park et al. 1979
Invertase, saccharase, glucosucrase	**Fungi** *Neurospora crassa*	Braymer et al. 1971

(*Continued*)

TABLE 1.3 (CONTINUED)

Sources of Major Food Enzymes

Enzymes	Source	References
Pentosanase	**Fungi**	
	Alternaria sp.	Simpson 1954
	Aspergillus sp.	Simpson 1954
	Fusarium sp.	Simpson 1954
	Trichothecium sp.	Simpson 1954
	Trichoderma sp.	Simpson 1954
	Bacteria	
	Bacillus sp.	Simpson 1954
Naringinase	**Fungi**	
	Aspergillus niger	Puri and Kalra 2005
Pullulanase	**Bacteria**	
	Bacillus acidopollulyticus	Martin and Birgitte 1984
Aminopeptidases	**Animal**	
	Pig kidney	Delange and Smith 1971
Trypsin	**Animal**	
	Bovine/porcine pancreas	Keil 1971
Subtilisin	**Bacteria**	
	Bacillus subtilis	Ottesen and Spector 1960
	Bacillus amyloliquefaciens	Peng et al. 2003
Papain	**Plant**	
	Latex of *Carica papaya*	Uhlig 1998
Ficin	**Plant**	
	Latex of *Ficus carica*	Whitaker 1957
	Latex of *Ficus glabrata*	
Bromelain	**Plant**	
	Pineapples such as *Ananas comosus* and *Ananus bracteatus*	Uhlig 1998
Pepsin A	**Animal**	
	Bovine abomasums	Fruton 1971
	Arctic capelin	Fernandes 2010
	Atlantic cod	Fernandes 2010
	Spheciospongia vesperiam	
Chymosin, rennin	**Animal**	
	Calf abomasums	Cheeseman 1981
Asparaginase	**Plants**	
	Sphagnum fallax	Yadav et al. 2014
	Lupine araboreuse	Borek et al. 2004
	Lupin amgustplius	Borek et al. 2004
	Bacteria	
	Pseudomonas flourescence	Mardashev et al. 1975
	Fungi	
	Aspergillus oryzae	Hendriksen et al. 2009
L-Glutaminase	**Bacteria**	
	Bacillus circulans	Kikuchi et al. 1971
	Fungi	
	Saccharomyces cerevisiae	Soberon and Gonzalez 1987

(*Continued*)

TABLE 1.3 (CONTINUED)

Sources of Major Food Enzymes

Enzymes	Source	References
Urease	**Bacteria**	
	Lactobacillus reureri	Kakimoto et al. 1989
	Klebsiella aerogenes	Mulrooney et al. 2005
	Fungi	
	Rhizopus oryzae	Geweely 2006
Acetolactate decarboxylase	**Bacteria**	
	Lactobacillus casei DSM 2547	Godtfredsen et al. 1984
Pectin lyase	**Bacteria**	
	Penicillium italicum	Alaña et al. 1990
	Bacillus sp. *DT 7*	Kashyap et al. 2000
Glucose isomerase	**Bacteria**	
	Pseudomonas sp.	Marshall and Kooi 1957
	Lactobacillus sp.	Kent and Emery 1974
	Actinomycetes	
	Streptomyces sp.	Tsumura and Sato 1965

for their catalytic activity. Similarly, animal-based proteases such as chymosin from calf abomasums, trypsin from bovine and porcine pancreas, pepsin from bovine abomasums, and lysozyme from hen egg white have a role in the food manufacturing industry. Animal-based lipases extracted from the gullet of goat and lamb, calf abomasums, and pig pancreas were mainly used in the past for flavor enhancement in the cheese-making industry. Catalases and lactoperoxidases derived from animal sources are known to prevent food spoilage as they have antimicrobial activity. However, it is important that animal tissues used for the preparation of enzymes comply with meat inspection requirements and good hygienic practice. Similarly, plant materials used in the production of enzyme preparations must not contribute to health problems in the processed finished food under normal conditions of use. However, a majority of animal- and plant-derived enzymes cannot survive the extreme conditions of food processing. In spite of the fact that the animal- and plant-based enzymes are widely acceptable in the food industry, the use of microbial enzymes is also desired and preferred because they have more advantages due to the higher specificity and stability of the enzymes produced. For example, rennet of microbial origin replaced the use of animal rennet to curdle milk in the cheese-manufacturing industry with one third of rennet used in cheese manufacturing being of microbial origin in recent years.

Sources of microbial enzyme preparation may vary from native strains or variants of microorganisms or be derived by genetic modification. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is an international expert scientific committee that is administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). It gives general specifications and considerations for enzyme preparations used in food processing, which are available at http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm. According to them, the production strains for food enzyme preparations should be nonpathogenic and nontoxigenic. Care should be taken in the case of fungal enzyme preparation to avoid mycotoxin contamination. Enzyme preparations by commercial agencies are produced in accordance with good food manufacturing practice. Care must be taken to ensure that the carriers, diluents, supports, and other additives and ingredients (including processing aids) used in the production, distribution, and application of enzyme preparations are acceptable for the relevant food uses of the enzyme preparations concerned, or that they are insoluble in food and removed from the food material after processing (www.fao.org).

Among the commercially available microbial enzymes, fungus-derived enzymes are significant. More than 240 enzymes are listed as commercialized by the Association of Manufacturers and Formulators of Enzyme Products (AMFEP; http://www.amfep.org/). Approximately 55% of the commercial enzymes are of fungal origin. More than 25% of all industrial enzymes are from *Aspergillus*, followed by *Penicillium*

and *Rhizopus*. Fungi are heterotrophic organisms, they absorb only smaller molecules through their cell wall, and extracellular digestion of complex organic matters is required. They secrete a complex battery of extracellular enzymes for extracellular digestion, and these exo-enzymes have evolved naturally under harsh conditions, making them ideal candidates for industrial biocatalysts. The solid-state fermentation is more suitable for fungal production of enzymes, making downstream processing easy and more economical. As with fungal enzymes, both natural and recombinant bacterial enzymes are widely used in the food industry. The genus *Bacillus* is dominant among the bacterial enzyme producers in the list of enzyme producers for commercialized enzymes by AMFEP. Nevertheless, scientists worldwide continue to search for novel potential biocatalysts especially from extreme environments. There are reports of enzymes from extremophiles, which are often difficult to grow under typical laboratory conditions. The advancement of recombinant DNA technology aids in raising recombinant microorganisms using mesophiles as hosts with which the genes of interest from extremophiles have been expressed (Fujiwara 2002). There are several strategies available for screening novel genes of potential biocatalysts, which include sequence-based and activity-based metagenomic and metatranscriptomics approaches. There has been remarkable progress in this field of research due to the recent advances of next generation sequencing (NGS) technologies (Schuster 2007).

Plants, animals, and microorganisms from terrestrial environments have been the routine source for potent industrial and food enzymes over the years. However, extreme environments and marine environments hold promise as potential sources of new enzymes with unique and desirable properties for application in food industries. In this context, it must be noted that the marine environment constituting 75% of the earth is the least explored environment for novel biocatalysts. In marine environments, including hydrothermal vents, deep sea sediments, salt marshes, coral reefs, giant kelps, and estuaries, as all life forms are subject to perpetual competition and stress, it is not surprising that the organisms living in these environments produce an enormous range of biocatalysts. Thermo-stable proteases, lipases, esterases, amylases, and xylanases have been actively sought and, in many cases, were found in bacterial and archaeal hyperthermophilic marine microorganisms. The higher marine organisms are also known as potent food enzyme producers (Fernandes 2010). Muscles of atka mackerel, botan shrimp, carp (*Cyprinus carpio*), rainbow trout, and scallop can be exploited for the production of trans-glutaminase. From higher marine organisms, enzymes such as proteases from mud crab (*Scylla serrata*) and sardine orange roughy (*Hoplostethus atlanticus*); amylases from gilt-head (sea) bream, turbot, and deepwater redfish; chymotrypsin from Atlantic cod, crayfish, and white shrimp; and pepsin from Arctic capelin, Atlantic cod, and marine sponges (*Spheciospongia vesperiam*) were reported (Fernandes 2010).

1.12 Conclusion

In the food industry, enzyme substitutes are not as successful as enzymes, which show unique properties such as supreme specificity; the ability to operate under mild conditions of pH, temperature, and pressure while displaying high activity and turnover numbers; and the property of biodegradability, as enzymes are biostatic and more or less biological in origin, which makes them ideal candidates for application in the food industry (Bommarius and Riebel-Bommarius 2004; Illanes 2008). A further significant property of enzymes that makes them suitable for industry is that very small amounts are needed for the bioconversion of a large amount of substrates. They have been used to modify flavor, texture, appearance, and storage stability of foods in the food industry. With the development of enzyme technology, specific enzymes that mediate such effects have been identified, isolated, and characterized. Apart from that, a large number of intrinsic enzymes in plants and the genes coding them, mediating flavor generation, tissue softening, color production, and other qualities that makes food acceptability, have been identified. Thus, enhancement of desirable properties or delay in the development of undesirable properties in foods is available for food processors. The advancement in enzyme protein engineering, recombinant enzyme production, and site-directed mutagenesis enable the introduction of potential biocatalysts in the food industry. Although a search for novel enzymes with unique properties are being pursued through intensive screening of natural sources, biotechnology and enzyme engineering hold immense promise for tailor-making potential enzymes for use in food and beverage processing.

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