

Enzymes in Food Processing

Edited by
Tilak Nagodawithana
Gerald Reed

ENZYMES IN FOOD PROCESSING

Third Edition

EDITED BY

Tilak Nagodawithana

Research and Development
Universal Foods Corporation
Red Star Specialty Division
Milwaukee, Wisconsin

Gerald Reed

Milwaukee, Wisconsin



ACADEMIC PRESS, INC.

Harcourt Brace & Company

San Diego New York Boston

London Sydney Tokyo Toronto

This book is printed on acid-free paper.



Copyright © 1993, 1975, 1966 by ACADEMIC PRESS, INC.

All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Academic Press, Inc.

1250 Sixth Avenue, San Diego, California 92101-4311

United Kingdom Edition published by Academic Press Limited 24-28 Oval Road, London NW1 7DX

Library of Congress Cataloging-in-Publication Data

Enzymes in food processing / edited by Tilak Nagodawithana, Gerald Reed. -- 3rd ed.

p. cm. -- (Food science and technology) Includes bibliographical references and index. ISBN 0-12-513630-7

1. Enzymes-Industrial applications. 2. Food industry and trade.

I. Nagodawithana, Tilak W. II. Reed, Gerald. III. Series.

TP456.E58E6 1993 664'.024-dc20

92-41628

CIP

PRINTED IN THE UNITED STATES OF AMERICA

93 94 95 96 97 98 MM

ENZYMES IN FOOD PROCESSING

Third Edition



FOOD SCIENCE AND TECHNOLOGY

International Series

SERIES EDITOR

Steve L. Taylor University of Nebraska

ADVISORY BOARD

John E. Kinsella University of California, Davis

Douglas Archer FDA, Washington, D.C.

Jesse F. Gregory, III University of Florida

Susan K. Harlander University of Minnesota

Daryl B. Lund Rutgers, The State University of New Jersey

Barbara O. Schneeman University of California, Davis

Robert Macrae University of Hull, United Kingdom

A complete list of all books in this series appears at the end of the volume.

FOOD SCIENCE AND TECHNOLOGY

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Jens Adler-Nissen (159), Department of Biotechnology, Technical University of Denmark, DK 2800 Lyngby, Denmark

Patrick Adlercreutz (103), Department of Biotechnology, Chemical Center, Lund University, S-221 00 Lund, Sweden

Ramunas Bigelis (121), Biotechnology Division, Amoco Technology Company, Naperville, Illinois 60566

Rodney J. Brown (347), Department of Nutrition and Food Sciences, College of Agriculture, Utah State University, Logan, Utah 84322

Sven Erik Godtfredsen (205), Novo Nordisk, DK 2880 Bagsvaerd, Denmark

Frank E. Hammer (221), Biotechnovation, Inc., Oak Forest, Illinois 60452

Ronald E. Hebeda¹ (321), Enzyme Bio-Systems, Ltd., Arlington Heights, Illinois 60005

Henry Heinsohn (71), Genencor, Inc., South San Francisco, California 94080

Tilak Nagodawithana (401), Red Star Specialty Products, Universal Foods Corporation, Milwaukee, Wisconsin 53218

Kirk L. Parkin (7, 39), Department of Food Science, University of Wisconsin-Madison, Madison, Wisconsin 53706

Walter Pilnik (363), Agricultural University, Department of Food Science, 6700 EV Wageningen, The Netherlands

Joseph Power (439), Siebel Institute of Technology, Chicago, Illinois 60645

Gerald Reed (1), 2131 North Summit Avenue, Apartment #304, Milwaukee, Wisconsin 53202

Bruno G. Sproessler (293), Kirschenallee, 6100 Darmstadt, Germany

Gudmundur Stefansson (459), Department of Food Technology, Icelandic Fisheries Laboratories, 121 Reykjavik, Iceland

Thomas Szalkucki (279), Center for Dairy Research, University of Wisconsin-Madison, Madison, Wisconsin 53706

Jean-Claude Villettaz (423), Ecole d'Ingènieurs du Valais, Food and Biotechnology Department, 1950 Sion, Switzerland

Alphons G. J. Voragen (363), Agricultural University, Department of Food Science, 6703 WD Wageningen, The Netherlands

Jo Wegstein² (71), Genencor, Inc., South San Francisco, California 94080

¹ Present address: Corn Products, Moffett Technical Center, 6500 South Archer Road, Summit-Argo, Illinois 60501-0345.

² Present address: 6346 Escallonia Drive, Newark, California 94560.

Preface to the Third Edition

The 1967 monograph, Enzymes in Food Processing, and the 1975 edited second edition covered the uses of enzymes in the food industry in a comprehensive manner. The present and third edited edition has been completely rewritten because of the extensive changes in the way enzymes are used and the availability of new enzymes.

We believe that the third edition will be more useful to readers because it emphasizes basic information on enzymes, newly discovered uses, and uses that have not been adequately described in the literature. Thus, chapters on enzyme functionality and the effect of environmental parameters have been expanded, a chapter on the genetic modifications of enzymes has been added, and a chapter on the use of enzymes in fish processing has been included.

Therefore, less emphasis is devoted to the routine uses of enzymes, and subjects in which relatively few changes have occurred were omitted. The chapter on the production of microbial enzymes has been replaced by an extended introductory chapter that deals with practical aspects of the formulation, standardization, and assay of microbial enzymes as they are sold to the food industry.

We express our gratitude to Academic Press for their continued support of *Enzymes in Food Processing* and to Red Star Specialty Products Division, Universal Foods Corporation, Milwaukee, Wisconsin for their encouragement, and, most of all, to the authors who have so competently and patiently contributed to the book.

We hope that the third edition will be as well received and as widely used as the two earlier editions.

Tilak Nagodawithana Gerald Reed

Preface to the Second Edition

The purpose and scope of *Enzymes in Food Processing* have been adequately described in the Preface to the first edition, which follows. A deeper understanding of the action of enzymes, some changes in enzyme technology, and the introduction of new enzyme processes into the food industry have made it desirable to publish a second edition.

This edition, unlike the first one, is an edited work. The authors of individual chapters have contributed a deeper knowledge of their field and a greater expertise than the editor could muster for the writing of the first edition. Hence, the chapters dealing with the properties of specific enzymes and the chapters dealing with enzyme applications should be authoritative and up-to-date.

I am greatly indebted to the contributors who have given freely of their time to share their expert knowledge with their colleagues. I am equally indebted to readers of the first edition who have contributed encouragement and criticism and who have made publication of a second edition worthwhile. I am grateful to Academic Press and to the Board of Editors of the "Food Science and Technology" series and, in particular, to Dr. George F. Stewart for assistance in editing. Finally, I wish to thank Universal Foods for permission to undertake this work.

Gerald Reed

Preface to the First Edition

The manufacture of foods has rapidly changed from an art to a highly specialized technology based on discoveries in the natural sciences. However, the translation of scientific knowledge from the fields of microbiology and biochemistry into useful food technology has been rather slow. Art and tradition still play an important part in the fermentation industries and in various uses of enzymes in food processing. It is, therefore, important to bridge the gap between available scientific knowledge and food technology in these particular areas.

During the past 25 years the use of commercial enzymes has grown from an insignificant role to an important aspect of food processing. However, no comprehensive treatise on the use of enzymes in food processing has been published in the past 15 years; this monograph was written to fill that gap. Primarily, it is directed to food technologists. They will find in it a description of the properties of those enzymes which are important in food processing as well as a description of the many practical applications of enzymes in their industry. It will also be of value to the microbiologist and enzyme chemist who may wish to acquire some knowledge of the fields in which their discoveries are put to practical use. This volume will acquaint them with present applications of enzymes in the food industry and will perhaps suggest new uses for enzymes.

The subject is treated in two parts. Part I describes the properties of enzymes in general and the properties of enzymes used specifically in food processing. Part II describes the practical application of these enzymes to various phases of the food industry with cross references to the basic properties of the enzymes described in Part I. It is hoped that this method will foster a clearer understanding of the relationship between the basic properties of enzymes and their application.

I am greatly indebted to the following people who have reviewed one or several of the chapters and who have provided extensive assistance: Dr. M. L. Anson, Mr. W. G. Bechtel, Dr. T. Cayle, Dr. S. L. Chen, Dr. G. I. de Becze, Mr. F. Hammer, Dr. K. Konigsbacher, Dr. E. R. Kooi, Dr. J. H. Nelson, Mr. M. C. Reed, Dr. D. Scott, Dr. E. Segel, Dr. C. V. Smythe, Dr. G. F. Stewart, and Dr. L. A. Underkofler. Dr. Underkofler has written Chapter 10, "Pro-

XXIV Preface to First Edition

duction of Commercial Enzymes." Without his help this subject could not have been treated adequately. He also reviewed a considerable portion of the manuscript and made many helpful suggestions. I am deeply grateful to Dr. M. L. Anson, one of the editors of the Food Science and Technology series. Without his advice, counsel, and criticism it would have been difficult to write this book.

I want to thank Mrs. F. W. Chen and Mr. R. Liu for the illustrations, and Mrs. M. Ziesch for typing the manuscript. My wife has encouraged me and helped me with the preparation of the manuscript.

April, 1966 Gerald Reed

| Preface to the Third Edition xix Preface to the Second Edition xxi Preface to the First Edition xxiii CHAPTER I |
|--|
| Preface to the First Edition xxiii CHAPTER IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII |
| CHAPTER I Introduction I GERALD REED CHAPTER 2 General Characteristics of Enzymes KIRK L. PARKIN I. Introduction 7 II. Primary Features of Enzymes and Enzyme Reactions 7 A. Enzymes as Polypeptides 7 |
| Introduction I GERALD REED CHAPTER 2 General Characteristics of Enzymes KIRK L. PARKIN I. Introduction 7 II. Primary Features of Enzymes and Enzyme Reactions 7 A. Enzymes as Polypeptides 7 |
| GERALD REED CHAPTER 2 General Characteristics of Enzymes KIRK L. PARKIN I. Introduction 7 II. Primary Features of Enzymes and Enzyme Reactions 7 A. Enzymes as Polypeptides 7 |
| General Characteristics of Enzymes KIRK L. PARKIN I. Introduction 7 II. Primary Features of Enzymes and Enzyme Reactions 7 A. Enzymes as Polypeptides 7 |
| I. Introduction 7 II. Primary Features of Enzymes and Enzyme Reactions 7 A. Enzymes as Polypeptides 7 |
| II. Primary Features of Enzymes and Enzyme ReactionsA. Enzymes as Polypeptides7 |
| C. Specificity 12 III. Nomenclature 18 IV. Enzyme Kinetics 20 A. Basic Considerations 20 B. Estimating Kinetic Constants 23 C. Use of Linear Plots 25 |
| C. Use of Linear Plots 25 D. Allosteric Kinetics 27 |

| V. Inhibition 29 | |
|---|-------|
| VI. Cofactors 32 | |
| VII. Enzyme Assays and Units 34 | |
| A. Assays 34 | |
| B. Units 34 | |
| References 36 | |
| | |
| | |
| CHAPTER 3 | |
| Environmental Effects on Enzyme Activity | |
| KIRK L. PARKIN | |
| I. Introduction 39 | |
| II. Effect of pH 39 | |
| A. General Considerations 39 | |
| B. Mechanistic Considerations 40 | |
| III. Effect of the Aqueous Environment 46 | |
| A. General Considerations 46 | |
| B. Water Activity 47 | |
| C. Ionic Strength 52 | |
| D. Freezing 54 | |
| IV. Effect of Temperature 57 | |
| A. General Considerations 57 | |
| B. Effect on Enzyme Activity and Stability | 58 |
| C. Other Effects 63 | |
| V. Effect of Other Environmental Conditions | 64 |
| VI. Summary 66 | |
| References 67 | |
| | |
| | |
| CHAPTER 4 | |
| Modern Methods of Enzyme Expression and D | esign |

JO WEGSTEIN and HENRY HEINSOHN

| ١. | Summary | 71 | | |
|-----|----------------|---------------------|----|----|
| II. | Background | 72 | | |
| | A. Historical | Perspective | | 72 |
| | B. Simplified | Model | 73 | |
| | C. Classical M | 1 utagenesis | | 75 |

| III. Genetic Engineering 76 | |
|--|----|
| A. Recombinant DNA Techniques 76 | |
| B. Enzyme Design 83 | |
| C. Metabolic Pathway Modification 85 | |
| D. Engineering for Production Improvement 87 | |
| IV. Commercialization of Genetically Engineered Products | 88 |
| A. Example 88 | |
| B. Regulatory Considerations 95 | |
| C. Ownership Protection 95 | |
| D. Consumer Attitudes 97 | |
| V. Implications for the Future 98 | |
| Supplemental Reading 100 | |
| References 100 | |
| | |
| | |
| CHAPTER 5 | |
| Immobilized Enzymes | |
| PATRICK ADLERCREUTZ | |
| I. Introduction 103 | |
| II. Immobilization Methods 104 | |
| A. Covalent Immobilization on a Solid Support 105 | |
| B. Cross-linking 106 | |
| C. Adsorption 107 | |
| D. Enzymes in Organic Media 107 | |
| E. Entrapment 108 | |
| F. Immobilization by Membranes 109 | |
| G. Immobilization in Two-Phase Systems 109 | |
| III. Effects of Immobilization 110 | |
| A. Inactivation during Immobilization | |
| B. Mass Transfer Effects | |
| C. pH Shift 113 | |
| D. Substrate and Product Partitioning 113 | |
| E. Stabilization by Immobilization | |
| F. Catalytic Properties of Immobilized Enzymes in | |
| Organic Media 115 | |
| IV. Applications 115 | |
| V. Free or Immobilized Enzyme? 117 | |
| References II8 | |

viii Contents

| | | | | _ | _ | R | • |
|---|---|-----|---|---|---|---|---|
| | н | А | ۲ | | - | ĸ | 6 |
| • | | , , | | | _ | | • |

| ~ | | | |
|-----|-----|-----|------|
| Car | boh | var | ases |

RAMUNAS BIGELIS

| Intrac | luction | 121 |
|--------------|---------|-----|
| IIILI OC | luction | 121 |

II. Food-Processing Carbohydrases 121

A. Amylases 121

B. Pectic Enzymes 134

C. Lactases 139

D. Invertases 142

E. α -Galactosidases 143

F. Cellulases 144

G. Hemicellulases 14

H. Dextranases 147

References

147

CHAPTER 7

Proteases

JENS ADLER-NISSEN

I. Introduction 159

II. Substrates 161

A. Protein Chemistry in Brief 161

B. Food Proteins as Substrates 165

III. Enzymes

ymes 107

A. Classification of Proteases 167

B. Peptide Bond Cleavage and Protease Specificity 168

C. Peptide Bond Cleavage and Kinetics 172

D. Activity and Stability Profiles of Proteases 178

E. Protease Preparations in Practice 180

IV. General Issues in Protein Hydrolysis Processes 182

A. Protein Hydrolysis Indices 182

B. Application Screening of Proteases 184

C. Peptide Bitterness 186

D. Plastein Formation 188

V. Individual Proteases 188

A. Serine Proteases 191

B. Cysteine Proteases 193

C. Aspartic Proteases 193

| D. Metalloproteases 195 | |
|--|-----|
| E. Naturally Occurring Protease Inhibitors | 196 |
| VI. Summary of the Food Uses of Proteases | 196 |
| A. Proteases as Processing Aids 196 | |
| B. New Functional Protein Ingredients | 197 |
| References 199 | |
| | |
| CHAPTER 8 | |
| Lipases 205 | |
| SVEN ERIK GODTFREDSEN | |
| References 214 | |
| | |
| CHAPTER 9 | |
| CHAFTER 7 | |
| Oxidoreductases | |
| FRANK E. HAMMER | |
| I. Polyphenol Oxidase 221 | |
| A. General Characteristics 221 | |
| B. Of Mushroom 223 | |
| C. Of Grape 224 | |
| D. Of Pear 225 | |
| E. Of Kiwi Fruit 227 | |
| F. Of Sago Palm 227 | 1 |
| G. Of Tea 228 | |
| H. In Cocoa Processing 229 | |
| I. Of Green Pepper 230 | |
| J. Of Strawberry 230 | |
| K. Of Potato 231 | |
| L. Of Pomegranate 231 | |
| M. Of Persimmon 232 | |
| N. Of Olive 232 O. Of Beet 232 | |
| II. Peroxidase 233 | |
| A. General Characteristics 233 | |
| | 233 |
| C. Other Analytical Peroxidases 234 | |
| D. Blanching of Foods 235 | |
| | |

X Contents

| | E. Reduction of P | eroxidase | Activity b | by Other Method | is 236 |
|---------|---|---------------------|------------|-----------------|---------------|
| | F. Of Apple | 237 | | | |
| | G. Of Orange | 238 | | | |
| | H. Of Bean | 238 | | | |
| | I. Of Grape | 238 | | | |
| | J. Of Brussels Spr | routs | 239 | | |
| | K. Of Barley | 239 | | | |
| | L. In Spices | 239 | | | |
| | M. Of Potato | 240 | | | |
| | N. Of Cucumber | 240 | | | |
| | O. Of Pea 24 | Н | | | |
| | P. Of Tomato | 242 | | | |
| | Q. Of Paprika | 242 | | | |
| | R. Of Peanut | 243 | | | |
| | S. In Canola | 243 | | | |
| III. | Lactoperoxidase | 244 | | | |
| | A. General Chara | cteristics | 244 | | |
| | | 44 | 5 | <i>y</i> - | |
| | C. In Milk Produc | ets 2 4 | 45 | | |
| IV. | Catalase 246 | | | | |
| | A. General Chara | | 246 | | |
| | B. In Various Fru | | egetables | 247 | |
| | | 47 | | | |
| ٧. | Sulfhydryl Oxidase | 248 | - 40 | | |
| | A. General Chara | | 248 | | |
| | B. Mammalian | 248 | | | |
| 371 | C. Microbial | 250 | | | |
| VI. | Glucose Oxidase | 25 I | | 251 | |
| | A. Failure in the | | | 251 | |
| | B. Of Aspergillus C. Of Penicillium | niger 253 | 252 | | |
| | | | 254 | | |
| VII | D. Of <i>Talaromyces</i> Pyranose Oxidase | 2.1 | 234 | | |
| | Xanthine Oxidase | 255 | | | |
| ¥ 1111. | A. General Chara | | 255 | | |
| | | 55 | 233 | | |
| IX | Lipoxygenase | 257 | | | |
| ., | A. General Chara | | 257 | | |
| | B. Of Soybean | 258 | | | |
| | C. Of Potato | 260 | | ii ii | |

Contents xi

| D. Of Tomato 261 |
|---|
| E. Of Maize 261 |
| F. Of Strawberry 262 |
| G. Of Canola Seed 262 |
| H. Of Bean 262 |
| I. Of Other Plants 263 |
| J. Of Fish 264 |
| K. Of Chicken Meat 265 |
| X. Dehydrogenases 265 |
| A. General Characteristics 265 |
| B. In Various Plants 265 |
| C. Meat Lactate Dehydrogenase 267 |
| References 267 |
| CHAPTER 10 |
| Applications of Oxidoreductases |
| THOMAS SZALKUCKI |
| I. Alcohol Oxidase 279 |
| II. Catalase 280 |
| A. H ₂ O ₂ /Catalase in Cheese Milk 282 |
| B. H ₂ O ₂ /Catalase in Eggs 282 |
| C. H ₂ O ₂ /Catalase in Whey and Other Products 283 |
| III. Glucose Oxidase / Catalase 284 |
| A. Desugaring of Egg Components 284 |
| B. Deoxygenation of Beverages and Other Applications 285 |
| IV. Lactoperoxidase 287 |
| A. Preservation of Raw Milk 287 |
| B. Preservation of Other Products 288 |
| V. Sulfhydryl Oxidase 289 |
| A. UHT Milk Treatment 289 |
| B. Dough Strengthening 289 |
| References 289 |
| CHAPTER II |
| Milling and Baking BRUNO G. SPROESSLER |
| l. Introduction 293 |
| II. Enzymes in Flour 294 |
| A. Amylases 295 |