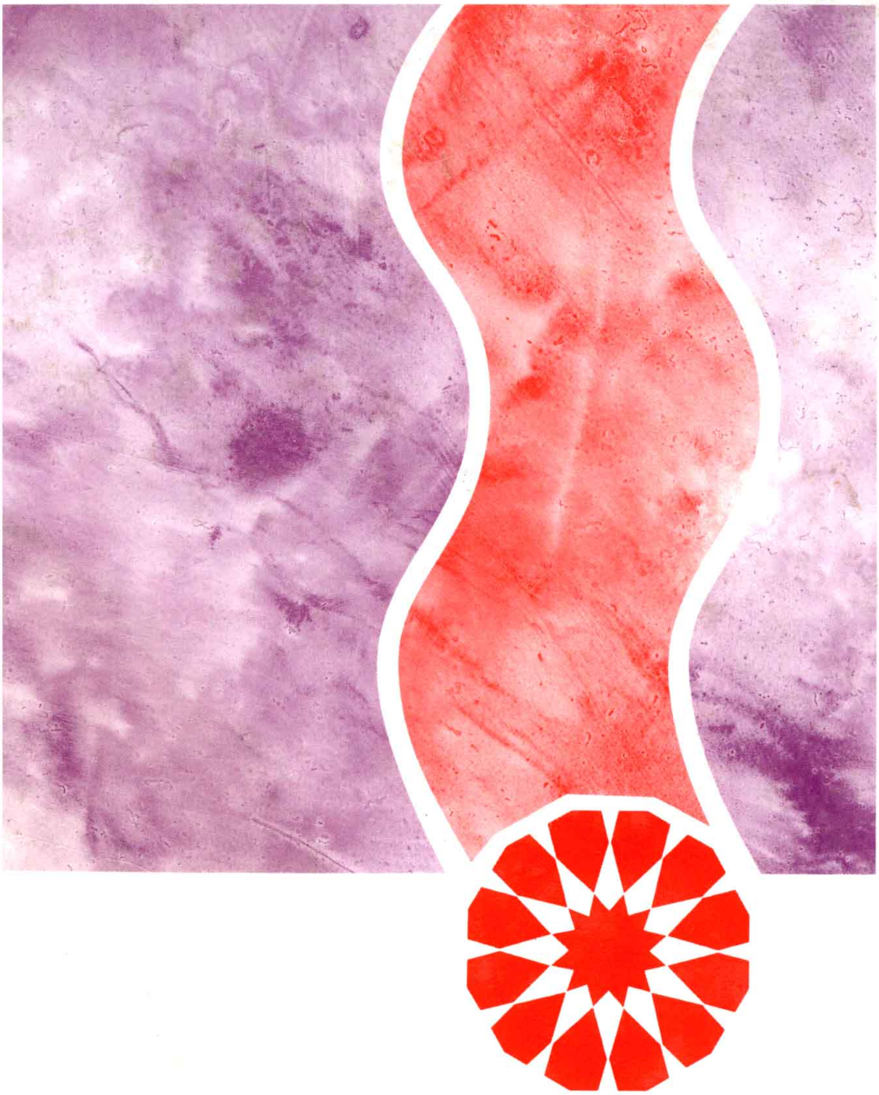
VOLUME 2



FOOD ENZYMOLOGY

EDITED BY
P. F. FOX

ELSEVIER
APPLIED
SCIENCE

FOOD ENZYMOLOGY

Volume 2

Edited by

P. F. Fox

University College Cork
Cork, Ireland

ELSEVIER APPLIED SCIENCE
LONDON and NEW YORK

FOOD ENZYMOLOGY

Volume 2

ELSEVIER SCIENCE PUBLISHERS LTD
Crown House, Linton Road, Barking, Essex IG11 8JU, England

Sole distributor in the USA and Canada
ELSEVIER SCIENCE PUBLISHING CO., INC.
655 Avenue of the Americas, New York, NY 10010, USA

WITH 45 TABLES AND 58 ILLUSTRATIONS

British Library Cataloguing in Publication Data
Food enzymology.
1. Food. Enzymes
I. Fox P. F.
664.06

ISBN 1-85166-615-X V.1
ISBN 1-85166-616-8 V.2

Library of Congress Cataloging-in-Publication Data
Food enzymology.
p. cm.
Includes bibliographical references and indexes.
ISBN 1-85166-615-X (v. 1). — ISBN 1-85166-616-8 (v. 2)
1. Enzymes. 2. Food—Composition. I. Fox, P. F.
TX553.E6F66 1991
664—dc20

Typeset and printed by The Universities Press (Belfast) Ltd.

Preface

Throughout its history, the development of enzymology has been very closely associated with food science, or, perhaps, vice versa. In many respects, food science might be regarded as applied enzymology. With a few minor exceptions, our food is derived from animal or plant tissues or excretions and, consequently, its constituents are synthesized via enzyme-catalyzed reactions. These reactions do not cease after the animal has been slaughtered, or the plant harvested, but continue more or less actively to cause changes in foods which may be desirable or detrimental to quality, depending on the circumstances. Food constituents are susceptible to alteration by enzymes produced by contaminating microorganisms, which may lead to food spoilage. On the other hand, enzymes produced by certain microorganisms may cause desirable changes in foods and such organisms are widely exploited in the production of a very wide range of fermented foods. Many food processing operations are concerned with controlling or preventing the activity of indigenous or microbial enzymes, e.g. heat treatment, dehydration and chilling/freezing. Food constituents are amenable to modification by exogenous (added) enzymes which may be used to induce specific changes, perhaps resulting in entirely new food products; the food industry is probably the principal industrial user of enzymes.

Not surprisingly, various aspects of the importance of enzymes in foods and food processing have been the subjects of an extensive scientific and technical literature, including several textbooks, for example, Whitaker (1972, 1974), Reed (1975), Schwimmer (1981), Birch *et al.* (1981), Kruger *et al.* (1987) and Stauffer (1989) and chapters in many others. However, most of these books focus on the enzymes rather than on the foods containing them. This book is an attempt to review food enzymology more from the viewpoint of the food scientist or technologist rather than that of the enzymologist. The book, consisting of thirty chapters, in two volumes, attempts to assess the importance of indigenous, microbial and exogenous enzymes in a wide range of foods based on milk, meat, cereals, fruits and vegetables including beverages and food ingredients.

A chapter is devoted to the importance of enzymes in food analysis, both as indicators of quality and as analytical reagents. Two major new aspects of enzymology, immobilized enzymes and genetic engineering, are also considered.

Certain gaps in the coverage will be apparent due to the failure of some chapters to materialise—most noticeably those on enzymes in baking, enzymes in the starch and sugar industries and the enzymatic modification of lipids. It is hoped to include these in a later volume.

P. F. Fox
University College, Cork

List of Contributors

VOLUME 1

J. B. ADAMS
Campden Food & Drink Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK

J. M. ALONSO
Instituto de Agroquímica y Technología de Alimentos (CSIC), Jaime Roig 11, 46010 Valencia, Spain

A. T. ANDREWS
AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT, UK

R. B. ARIE
Agricultural Research Organization, The Volcani Center, Institute for Technology and Storage of Agricultural Products, PO Box 6, Bet Dagan 50250, Israel

G. BENGTSSON-OLIVECRONA
Department of Medical Biochemistry and Biophysics, University of Umea, S-90187 Umea, Sweden

L. BJÖRCK
Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Box 7024 Ultuna, S-75007 Uppsala, Sweden

G. CAMPBELL-PLATT
Department of Food Science and Technology, University of Reading, PO Box 226, Whiteknights, Reading RG6 2AP, UK

J. CHAMARRO
Instituto de Agroquímica y Technología de Alimentos (CSIC), Jaime Roig 11, 46010 Valencia, Spain

P. E. COOK
Department of Food Science and Technology, University of Reading, PO Box 226, Whiteknights, Reading RG6 2AP, UK

J. F. D. DEAN
Plant Hormone Laboratory and Plant Molecular Biology Laboratory, Beltsville Agricultural Research Center (W), ARS, US Department of Agriculture, Beltsville, Maryland 20705, USA

M. J. Desmazeaud
INRA, Centre de Recherches de Jouy-en-Josas, Station de Recherches Laitières, Domaine de Vilvert-78350, Jouy-en-Josas, France

D. Dubourdieu
Université de Bordeaux, Institut d'Oenologie, Bordeaux, France

N. Y. Farkye
Food Chemistry Department, University College, Cork, Republic of Ireland

P. F. Fox
Department of Food Chemistry, University College, Cork, Republic of Ireland

J. L. Garcia-Martinez
Instituto de Agroquímica y Technología de Alimentos (CSIC), Jaime Roig 11, 46010 Valencia, Spain

J.-C. Gripon
INRA, Centre de Recherches de Jouy-en-Josas, Station de Recherches Laitières, Domaine de Vilvert-78350, Jouy-en-Josas, France

M. B. Grufferty
Department of Food Chemistry, University College, Cork, Republic of Ireland

E. Harel
Department of Botany, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

J. S. Hawker
Department of Botany, The University of Adelaide, GPO Box 498, Adelaide, South Australia 5001, Australia

M. Knee
Department of Horticulture, The Ohio State University, 2001 Fyffe Court, Columbus, Ohio 43210, USA

G. Lamberet
INRA, Centre de Recherches de Jouy-en-Josas, Station de Recherches Laitières, Domaine de Vilvert-78350, Jouy-en-Josas, France

A. K. Mattoo
Plant Hormone Laboratory and Plant Molecular Biology Laboratory, Beltsville Agricultural Research Center (W), ARS, US Department of Agriculture, Beltsville, Maryland 20705, USA

A. M. Mayer
Department of Botany, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

M. Mazelis
Department of Food Science and Technology, University of California, Davis, California 95616, USA

V. Monnet
INRA, Centre de Recherches de Jouy-en-Josas, Station de Recherches Laitières, Domaine de Vilvert-78350, Jouy-en-Josas, France

N. M. O'Brien
Department of Nutrition, University College, Cork, Republic of Ireland

T. P. O'Connor
Department of Food Chemistry, University College, Cork, Republic of Ireland

T. Olivecrona
Department of Medical Biochemistry and Biophysics, University of Umea, S-90187 Umea, Sweden

R. E. Paull
Department of Plant Molecular Physiology, University of Hawaii at Manoa, 3190 Maile Way, Honolulu, Hawaii 96822, USA

W. Pilnik
Department of Food Science, Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands

N. C. Price
Department of Molecular and Biological Sciences, University of Stirling, Stirling FK9 4LA, UK

D. S. Robinson
Procter Department of Food Science, University of Leeds, Leeds LS2 9JT, UK

T. Sørhaug
Department of Dairy and Food Industries, Agricultural University of Norway, 1432 Ås-NLH, Norway

L. Stepaniak
Department of Dairy and Food Industries, Agricultural University of Norway, 1432 Ås-NLH, Norway

L. Stevens
Department of Molecular and Biological Sciences, University of Stirling, Stirling FK9 4LA, UK

J. C. Villettaz
Ecole d'Ingénieurs du Valais, Food Technology Department, Rte du Rawyl 47, 1950 Sion, Switzerland

A. G. J. Voragen
Department of Food Science, Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands

J. R. Whitaker
Department of Food Science and Technology, University of California, Davis, California 95616, USA

VOLUME 2

H. V. Amorim
Fermentec S/C Ltda, Rua 13 de Maio 768, 13400 Piracicaba SP, Brazil

S. Arai
Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-Ku, Tokyo 113, Japan

M. E. Bailey
Department of Food Science and Nutrition, University of Missouri, 21 Agriculture Building, Columbia, Missouri 65211, USA

P. S. Dimick
Department of Food Science, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

L. W. Doner
Eastern Regional Research Center, US Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pennsylvania, USA

S. J. B. Duff
Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6

R. E. Feeney
Department of Food Science and Technology, 1480 Chemistry Annex, University of California, Davis, CA 95616, USA

R. FitzGerald
Microbiology Department and National Food Biotechnology Centre, University College, Cork, Republic of Ireland

M. Fujimaki
Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-Ku, Tokyo 113, Japan

J. E. Kruger
Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, Manitoba, Canada R3C 3G8

A. S. Lopez
CEPLAC/CEPEC, CxP 07, 45600 Itabuna BA, Brazil

A. W. MacGregor
Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, Manitoba, Canada R3C 3G8

B. A. Marchylo
Canadian Grain Commission, Grain Research Laboratory, 1404–303 Main Street, Winnipeg, Manitoba, Canada R3C 3G8

M. Melo
Fermentec S/C Ltda, Rua 13 de Maio 768, 13400 Piracicaba SP, Brazil

P. Monsan
BioEurope, 4 Impasse Didier Daurat, Z. I. de Montaudran, 31400 Toulouse, France

F. A. Murdock Jr.
Department of Food Science and Nutrition, University of Missouri, 21 Agriculture Building, Columbia, Missouri 65211, USA. Present address: Department of Animal and Food Science, River Falls, Wisconsin, USA

W. D. Murray
Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6

M. Nuti
Dipartimento di Biotecnologie Agrarie, Università degli Studi di Padova, Padova, Italy

F. O'Gara
Microbiology Department and National Food Biotechnology Centre, University College, Cork, Republic of Ireland

T. Osuga
Department of Food Science and Technology, 1480 Chemistry Annex, University of California, Davis, California 95616, USA

F. Paul
BioEurope, 4 Impasse Didier Daurat, Z. I. de Montaudran, 31400 Toulouse, France

F. G. Priest
International Centre for Brewing and Distilling, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK

J. C. Slaughter
International Centre for Brewing and Distilling, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK

H. E. Swaisgood
Department of Food Science, University Biotechnology Program, North Carolina State University, Raleigh, North Carolina 27695–7624, USA

M. R. Ullah
Tocklai Experimental Station, Jorhat, Assam, India. Present address: BSB College, Jorhat-15, Assam, India

J. R. Whitaker
Department of Food Science and Technology, University of California, Davis, California 95616, USA

Contents

VOLUME 1

VOLUME 2

17

Endogenous Cereal Enzymes

J. E. KRUGER, A. W. MACGREGOR & B. A. MARCHYLO
Canadian Grain Commission, Grain Research Laboratory, 1404-303 *Main Street, Winnipeg, Manitoba, Canada R*3*C* 3*G*8

INTRODUCTION

It is impossible in one chapter to describe in detail all aspects of the numerous enzymes that exist in cereals, both from the standpoint of their role in the physiology of the plant and their effects on secondary processing. For excellent in-depth treatises, the reader is referred to reviews by Fox and Mulvihill (1982), Kruger *et al.* (1987), Hill and MacGregor (1988), Kruger and Reed (1988) and Muthukrishnan and Chandra (1988). This chapter focuses on those enzymes which are of most interest to food scientists, i.e. those which influence the quality of various food products. Enzymes from different cereals quite often have similar modes of action, and so similar assays can be used to determine them. Also, the occurrence and location of these enzymes in the cereal seed must be understood, in order to fully appreciate their potential effects on various food end-products. This is examined with particular emphasis on the hydrolases, which are perhaps the most important quality-determining enzymes in cereals. Finally, the most technologically important enzymes in a wide range of cereals, including wheat, barley, rice, rye, triticale, oats, maize, sorghum and millet, are discussed, with reference to their specific influence on the quality of their characteristic end-products.

MODE OF ACTION OF ENDOGENOUS CEREAL ENZYMES

In order to fully appreciate the means by which endogenous cereal enzymes can influence food quality, it is necessary to have some concept of how these enzymes carry out their catalytic action. In this section, the basic mode of action of some of the most important endogenous cereal enzymes is presented.

Further discussion of the role played by these enzymes in food processing is presented later.

Hydrolytic enzymes

Various endogenous hydrolytic enzymes are present in cereals, for the purpose of degrading storage constituents such as starch, proteins and lipids. The storage components of the grain, such as starch and protein, as well as cell-wall β-glucan, are degraded by the combined action of exo- and endo-enzymes. Exo-enzymes attack polymers from one end of the molecule, sequentially removing one or two of the monomeric units. Endo-enzymes, on the other hand, attack interior portions of the polymers, releasing smaller, but still relatively large, molecular fragments. Broadly speaking, endo-enzymes serve to solubilize macromolecules by reducing their size, while exo-enzymes produce lower molecular weight species from products of endo-enzyme hydrolysis (Bamforth, 1986).

Hydrolysis of starch

Starch is degraded by a combination of enzymes including α- and β-amylases and limit dextrinase (Fig. 1), ultimately resulting in the production of sugars.

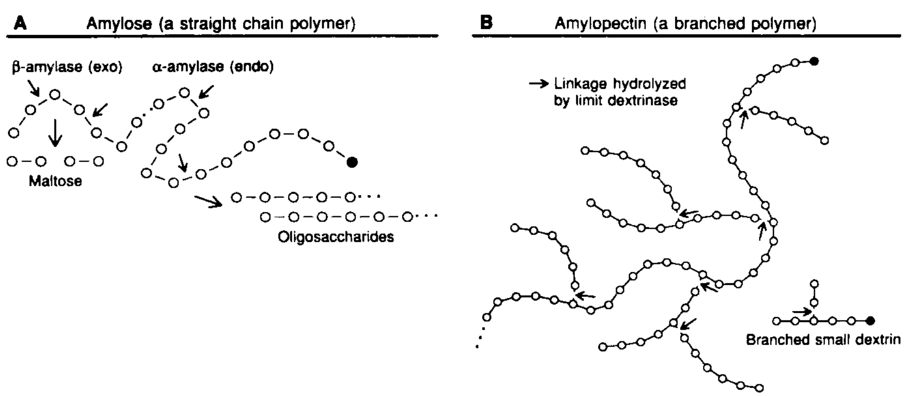


FIG. 1. Hydrolysis of starch amylose (A) and amylopectin (B) by the combined action of α- and β-amylase and limit dextrinase (O, glucose residue; ●, reducing-glucose residue; — α-(1-4) bond and ·· α-(1-6) bond).

Hydrolysis of barley β-glucan (mixed-linkage (1,3)-(1,4)-β-D-glucan)

Endo-(1,3)-(1,4)-β-glucanase from barley malt, which is also known as endo-barley-glucanase or lichenase, hydrolyzes β-(1,4) bonds, regardless of the number of adjacent (1,3) or (1,4) linkages, only when the glucosyl residue is substituted at the C-3 position (Fig. 2).

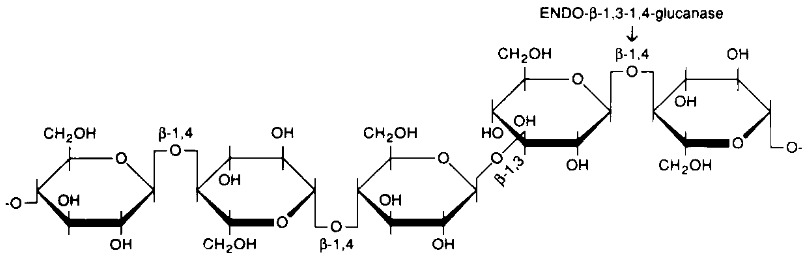


FIG. 2. Mixed-linkage, barley β-glucan bond cleaved by endo-β-(1-3)-(1-4)-glucanase.

Protease

There is much confusion in the scientific literature on the nomenclature of cereal enzymes that hydrolyze proteins. In general, 'proteases' or 'proteolytic enzymes' are commonly used as a general name for enzymes that hydrolyze peptide bonds (Storey & Wagner, 1986). Endopeptidases hydrolyze internal bonds in proteins, while aminopeptidases and carboxypeptidases are exopeptidases that liberate single amino acids from the amino end and carboxyl end of peptide chains, respectively (Fig. 3).

Endopeptidase ↓

$$^{+}H_3N - AA_1 - AA_2 - AA_3 - [AA_{4 \rightarrow N}] - AA_{N+1} - AA_{N+2} - COO^-$$

↑ ↑ ↑ ↑

(aminopeptidases) Exopeptidases (carboxypeptidases)

FIG. 3. Proteolysis of peptide linkages in a cereal protein.

Lipases

True lipases are enzymes that attack triacylglycerols, as shown in the following example, and only act at an oil–water interface (Galliard, 1980):

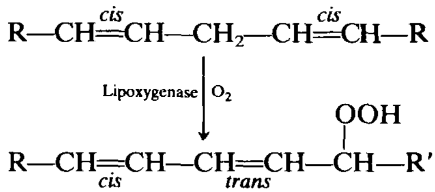
$$\begin{array}{c} CH_2OOCR \\ | \\ HCOOCR' \\ | \\ CH_2OOCR'' \end{array} \xrightarrow[\text{Lipase}]{} R''COOH + \begin{array}{c} CH_2OOCR \\ | \\ HCOOCR' \\ | \\ CH_2OH \end{array}$$

R, R′ and R″ represent fatty-acid chains

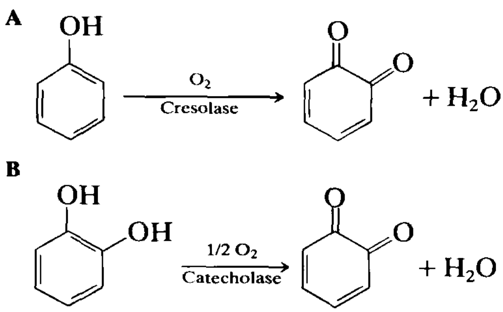
Oxidases

Lipoxygenase

Lipoxygenase (linoleate:oxygen oxido-reductase) refers to a group of enzymes that catalyze the oxygenation, by molecular oxygen, of fatty acids containing a *cis,cis*-(1-4)-pentadiene system to produce conjugated hydroperoxydiene derivatives (Galliard & Chan, 1980), as follows:



Phenol oxidase
There is a range of enzymes in cereals that catalyze the oxidation of mono- and diphenols to quinones. These are often referred to as polyphenol oxidase, phenolase, tyrosinase, catecholase and cresolase. Two distinct reactions are catalyzed (Mayer & Harel, 1979; Stauffer, 1987), as shown below:



These enzymes are involved in the formation of coloured polymeric materials. This reaction may be called 'enzymatic browning' or 'melaninization'.

Peroxidase
Peroxidase is a member of a large family of enzymes called the oxido-reductases (Burnette, 1977) and is a haemoprotein that catalyzes the oxidation of a number of aromatic amines and phenols by hydrogen peroxide (Kruger & Reed, 1988). The enzyme catalyzes the general reaction (Scott, 1975):

$$ROOH + AH_2 \rightarrow H_2O + ROH + A$$

ROOH can be HOOH or another organic peroxide, such as ether peroxide, ethyl hydrogen peroxide or butyl peroxide (Sumner & Somers, 1947). The enzyme reaction proceeds via a number of intermediate complexes, as shown in the following equation, where AH_2 stands for the hydrogen donor and A for the oxidized donor (Burnette, 1977):

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{Complex I}$$

$$\text{Complex I} + AH_2 \rightarrow \text{Complex II} + AH$$

$$\text{Complex II} + AH_2 \rightarrow \text{Peroxidase} + A$$