

Lutz Popper, Klaus Lösche

# Understanding Baking Enzymes



# Basics and Application



**Enzyme and substrate fit together like lock and key**  
is a thesis from the early days of enzyme research. It is still valid in a slightly modified form.

Today, it is added that the enzyme adapts to the substrate, i.e. is not rigid – comparable to a modern lock that scans the complicated key with movable pins.

# Contents

## Understanding Baking Enzymes

Basics & Application

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<b>1</b>	<b>The discovery and use of enzymes</b>	<b>22</b>
1.1	The long road to baking enzymes	22
1.2	From yeast to the Nobel Prize: a chronology of enzymes	25
1.3	References	29
<b>2</b>	<b>The mode of action of enzymes – nature’s energy savers</b>	<b>30</b>
2.1	Origins and kinetics	30
2.1.1	The nature of enzymes	30
2.1.2	Enzyme subunits	34
2.1.3	Enzyme cofactors	34
2.1.4	Enzyme effectors	36
2.1.5	Specificity	36
2.1.6	Enzyme kinetics	39
2.1.7	Induced fit model	41
2.1.8	Reasons for the specificity and effectiveness of enzymatic catalysis	41
2.1.9	Classification of enzymes (nomenclature)	42
2.1.10	Units or katal	42
2.1.11	Limits to comparability	43
2.2	Factors affecting enzyme action	46
2.3	Effect of water activity	46
2.3.1	Definition of water activity	46
2.3.2	Practical examples of the effect of water activity	47
2.4	Availability of substrate (reactants) for the enzymatic reaction	50
2.5	Effect of temperature on enzymatic activity	52
2.5.1	Principles of the effect of temperature	52
2.5.2	Practical examples of the effect of temperature on enzymatic activity	54
2.6	Effect of the pH on enzymatic activity	57
2.6.1	Principles of the effect of the pH	57
2.6.2	Practical examples of the effect of the pH	57
2.7	Enzyme inhibition	59

2.8	Cereal enzymes.....	59	2.18	Transglutaminase.....	103
2.8.1	Distribution of enzymes in cereal grains.....	59	2.19	Asparaginase.....	105
2.8.2	Technical measures to take account of enzymes in cereals.....	62	2.20	Phytase.....	106
2.8.3	Germinating grain as a source of enzymes.....	62	2.21	Other Enzymes.....	108
2.8.4	Controlling flour quality by managing enzymatic activity.....	64	2.21.1	Invertase and inulinase.....	108
2.9	Starch – the substrate of the amylases.....	64	2.21.2	Cellulase and $\beta$ -glucanase.....	108
2.10	The “large family” of the glycosidases.....	66	2.21.3	Arabinofuranosidase and feruloyl esterase.....	109
2.11	Amylolytic enzymes.....	67	2.21.4	Chitinase.....	109
2.11.1	$\alpha$ -Amylase.....	68	2.21.5	Polymer-producing enzymes.....	109
2.11.2	The mechanism of enzymatic catalysis, taking $\alpha$ -amylase as an example.....	69	2.22	Summary of the effects of enzymes in baking.....	110
2.11.3	The $\alpha$ -amylolysis of amylose.....	70	2.23	Enzymes – Nature’s energy savers.....	112
2.11.4	The $\alpha$ -amylolysis of amylopectin.....	71	2.24	References.....	115
2.11.5	Fungal $\alpha$ -amylase.....	71			
2.11.6	Bacterial $\alpha$ -amylase.....	73	<b>3</b>	<b>The effect of enzymes on dough stability and rheology.....</b>	<b>120</b>
2.11.7	Maltogenic amylases.....	73	3.1	Dough stability.....	120
2.11.8	$\beta$ -Amylase.....	74	3.1.1	Amylases.....	120
2.11.9	Glucoamylase.....	75	3.1.2	Xylanases.....	120
2.11.10	$\alpha$ -Glucosidase (maltase).....	76	3.1.3	Proteases.....	121
2.11.11	Debranching enzymes.....	76	3.1.4	Glucose oxidase.....	121
2.12	Malt flours.....	77	3.1.5	Carboxylester hydrolases.....	121
2.13	Flour and dough improvement with amylases.....	78	3.1.6	Other enzymes with the effect of increasing dough stability.....	121
2.13.1	Promoting fermentation.....	78	3.2	Stickiness of dough.....	122
2.13.2	Effects on flaking of the crust of fully baked frozen goods.....	79	3.3	Dough extensibility.....	122
2.13.3	Limits to the use of malt products.....	79	3.4	Dough elasticity.....	122
2.13.4	Effects on dough and baked products.....	80	3.5	Dough plasticity.....	122
2.13.5	Effect of the puroindolines.....	80	3.6	Relevance of rheological data to baking.....	122
2.14	Protease.....	81	3.7	Falling Number.....	123
2.15	Hemicellulases.....	83	3.8	Farinograph.....	124
2.16	Carboxylester hydrolases.....	87	3.9	Mixolab.....	125
2.16.1	Wheat lipids and enzymes hydrolyzing wheat lipids.....	87	3.10	Extensograph and Alveograph.....	126
2.16.2	Effect of carboxylester hydrolases on dough and baking properties.....	89	3.11	Kieffer rig.....	128
2.16.3	Effect on starch retrogradation.....	91	3.12	Sedimentation.....	128
2.16.4	Use of lipolytic enzymes in cake.....	91	3.13	References.....	130
2.17	Oxidative enzymes, oxidoreductases.....	91	<b>4</b>	<b>Controlling the effects of enzymes.....</b>	<b>131</b>
2.17.1	The fate of oxygen in dough preparation.....	92	4.1	Climate change and grain quality.....	133
2.17.2	Glucose oxidase, hexose oxidase, pyranose oxidase and carbohydrate oxidase.....	92	4.2	Enzyme applications in baking.....	134
2.17.3	Dehydrogenase.....	96	4.3	Enzyme combinations.....	136
2.17.4	Oxygenase.....	98	4.4	Interaction between sourdoughs / sponges and enzymes.....	136
2.17.5	Sulfhydryl oxidase.....	100	4.4.1	Effect of carbohydrases.....	137
2.17.6	Peroxidase, catalase.....	101	4.4.2	Proteolytic processes in sourdough.....	139
2.17.7	Laccase.....	102	4.4.3	Controlling dough consistency.....	140
2.17.8	Polyphenol oxidase.....	102	4.4.4	Different interactions of sourdoughs and sponges with enzymes.....	140
2.17.9	Protein disulfide isomerase.....	103	4.4.5	Bioavailability of minerals and trace elements.....	141

4.5	Controlling the effect of enzymes with raw materials, formulas and processes.....	141
4.5.1	Controlling enzymatic activity with available water .....	141
4.5.2	Ionic reactions and enzymatic effects.....	143
4.5.3	Non-ionic, polar solutes (e.g. sucrose) and enzymatic effects.....	145
4.5.4	Reactions at interfaces in doughs and batters .....	146
4.5.5	Changes resulting from freezing of dough and baked goods.....	147
4.5.6	pH and the effect of enzymes as a condition for aroma and flavor.....	148
4.5.7	Fermentation control: a means of enzyme control.....	149
4.6	References .....	155
<b>5</b>	<b>Synergisms and interactions between enzymes .....</b>	<b>158</b>
5.1	Introduction.....	158
5.2	$\alpha$ -Amylase, $\beta$ -amylase, $\alpha$ -glucosidase and glucoamylase.....	158
5.3	Glucose oxidase and glucoamylase .....	159
5.4	Lipase and lipoxygenase .....	159
5.5	Peroxidase and glucose oxidase .....	160
5.6	Xylanase, ferulic acid esterase and arabinofuranosidase .....	160
5.7	Endo- and exo-peptidases .....	161
5.8	Oxidases and glutathione dehydrogenase.....	161
5.9	Synergistic combinations of different enzymes.....	162
5.10	References .....	168
<b>6</b>	<b>Enzyme inhibition.....</b>	<b>170</b>
6.1	The significance of enzyme inhibition in baking .....	173
6.2	Significance and effect of enzyme inhibitors of biological origin.....	174
6.3	Inhibition of protein-degrading enzymes .....	178
6.4	Inhibition of starch-degrading enzymes .....	178
6.5	Inhibitors of non-starch-polysaccharide-degrading enzymes .....	179
6.6	Nutritional aspects.....	179
6.7	Relevance for milling and baking .....	180
6.8	References .....	180
<b>7</b>	<b>Enzyme inactivation.....</b>	<b>184</b>
7.1	Thermal inactivation .....	184
7.2	Inactivation through a shift in the pH.....	190
7.3	$a_w$ value .....	191
7.4	Electrolytes and ionic strength .....	192
7.5	Pressure .....	192
7.6	Detergents.....	193
7.7	Miscellaneous effects .....	193
7.8	References .....	195
<b>8</b>	<b>Interactions with other constituents of the recipe.....</b>	<b>197</b>
8.1	Water.....	197
8.2	Yeast.....	197
8.3	Ascorbic acid .....	198
8.4	Salts .....	198
8.5	Sugar .....	199
8.6	Sourdough and dough acidifiers.....	199
8.7	Baking powder and its constituents .....	199
8.8	Fats and emulsifiers.....	200
8.9	References .....	200
<b>9</b>	<b>Optimizing the enzyme addition.....</b>	<b>201</b>
9.1	Controlled baking trials .....	202
9.2	Design of experiments for process optimization .....	203
9.3	Choice of boundaries of the experimental space .....	206
9.4	References .....	207
<b>10</b>	<b>Specific applications.....</b>	<b>208</b>
10.1	Differences between artisanal and industrial processing .....	208
10.1.1	Straight-dough processes.....	209
10.1.2	Retarded fermentation and freezing.....	211
10.2	Flour treatment for standardizing flour properties.....	212
10.3	Influence of the size of products .....	214
10.4	Hamburger buns.....	215
10.5	Baguettes and similar products.....	215
10.6	Ciabatta and similar products .....	216
10.7	Pizza bases.....	216
10.8	Arabic flat bread, baladi, Lebanese bread, kubus, khubz .....	216
10.9	Roti, chapati, poori, naan and many other types of flat bread .....	217
10.10	Paratha (parotta) .....	219
10.11	Wheat tortillas .....	219
10.12	Turkish yufka.....	220
10.13	Ancient cereals.....	221
10.14	Gluten-free .....	221
10.15	Products made from fine laminated yeast doughs (e.g. croissants and Danish pastries).....	223
10.16	Puff pastry .....	224
10.17	Sponge cake bases.....	225
10.18	Biscuits, hard biscuits, crackers, wafers.....	226
10.19	Saccharification of leftover bread.....	226
10.20	Enzymatic modification of the by-products of milling .....	226
10.21	Modification of proteins.....	227
10.22	References .....	228

<b>11 Puroindolines – companions of enzymes or alternatives to them?.....</b>	<b>230</b>		
11.1 Introduction.....	230		
11.2 Occurrence.....	230		
11.3 Properties.....	231		
11.4 Technical relevance.....	232		
11.5 Effects similar to those of enzymes.....	232		
11.6 Interaction with enzymes and other components.....	233		
11.7 Conclusions.....	236		
11.8 References.....	236		
<b>12 Improvement of crumb softness and shelf life.....</b>	<b>238</b>		
12.1 $\alpha$ -Amylase.....	239		
12.1.1 Fungal $\alpha$ -amylase.....	240		
12.1.2 Cereal $\alpha$ -amylase.....	241		
12.1.3 Cereal $\beta$ -amylase.....	241		
12.1.4 Bacterial $\alpha$ -amylase.....	241		
12.1.5 Maltogenic amylase.....	241		
12.2 Hemicellulases.....	243		
12.3 Carboxylic ester hydrolases (triacyl-, phospho- and glycolipases).....	244		
12.4 Lipoxigenases.....	244		
12.5 Other enzymes.....	244		
12.6 References.....	245		
<b>13 Steamed bread.....</b>	<b>246</b>		
13.1 Introduction.....	246		
13.2 Amylases.....	247		
13.3 Hemicellulases.....	248		
13.4 Glucose oxidase.....	249		
13.5 Sulfhydryl oxidase.....	250		
13.6 Carboxylic ester hydrolases (triacylglycerol lipase, phospholipase, glycolipase).....	250		
13.7 References.....	252		
<b>14 Rye and high-extraction wheat flour.....</b>	<b>253</b>		
14.1 Healthy nutrition with wholemeal products.....	254		
14.2 Functions specific to baking.....	255		
14.3 Enzymes for wheat and rye “volume” bread.....	256		
14.3.1 Amylases.....	256		
14.3.2 Hemicellulases.....	257		
14.3.3 Non-specific pentosanase.....	257		
14.3.4 Dough “drying” with specific pentosanases.....	257		
14.3.5 Proteases.....	257		
14.3.6 Asparaginase.....	257		
14.3.7 Phytase.....	258		
14.3.8 Invertase and inulinase.....	258		
14.4 Crispbread.....	258		
14.5 Biscuits and crackers.....	259		
14.6 References.....	259		
<b>15 Enzymes for biscuits, crackers and wafers.....</b>	<b>260</b>		
15.1 Biscuit and cracker applications.....	261		
15.1.1 Use of proteases.....	261		
15.1.2 Amylases for biscuits and crackers.....	262		
15.1.3 The problem of checking.....	263		
15.1.4 Gluten-strengthening enzymes.....	264		
15.2 Wafer applications.....	264		
15.3 Control of acrylamide formation.....	267		
15.4 References.....	268		
<b>16 Enzyme-assisted wheat tempering.....</b>	<b>269</b>		
16.1 Introduction.....	269		
16.2 Enzymatic support.....	270		
16.3 Obstacles and challenges.....	270		
16.3.1 Water migration.....	270		
16.3.2 Protein migration.....	270		
16.3.3 Water availability.....	270		
16.3.4 Accessibility of the substrate.....	271		
16.3.5 Carryover.....	271		
16.4 Suggestions for wheat tempering.....	271		
16.5 Combined processes.....	272		
16.6 Summary.....	274		
16.7 References.....	274		
<b>17 Retarded fermentation and freezing.....</b>	<b>275</b>		
<b>18 Legal framework for the safe use of enzymes.....</b>	<b>276</b>		
18.1 Background.....	276		
18.2 Safety in modern biotechnology.....	277		
18.3 The FIAP initiative.....	277		
18.4 Approval of enzymes according to FIAP.....	278		
18.5 Label declaration of enzymes.....	278		
18.6 Future labelling requirements.....	279		
18.7 Enzyme regulations in Canada, Mexico and the U.S.....	279		
18.8 References.....	281		

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# Forewords

## 1<sup>st</sup> Preface

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## 1<sup>st</sup> Preface

“... so that any new scheduled study  
 must almost certainly lead to the discovery  
 of physiologically important facts.”

*Wilhelm F. Kühne, 1876*

Despite their extraordinary importance as one of the key principles enabling life on earth, the story of scheduled studies on enzymes is comparatively short. After the first discovery of an enzyme, diastase, in 1833 by the French chemist Anselme Payen, the German physiologist Wilhelm F. Kühne coined the term enzyme, from the Greek words *en* and *zymi*, meaning *in yeast*. He reported before the Heidelberg Association for Natural History and Medicine on the isolation of a ferment that unfolds its effectiveness outside of living organisms or organized structures; the specific digestive enzyme he had isolated in 1876 is the one we now know as trypsin. In subsequent years, numerous discoveries revealing crystalline structures and catalytic modes of action were made, and the first enzyme-based baking improver was patented in 1902. From this date, countless industrial applications of enzymes have been developed, including a wide variety of applications in the feed and food industries.

To help users keep track of the enormous and rapid developments in this field, this book aims to provide an overview of the activity, functionality and applications of enzymes in cereal-based products in an easily comprehensible and practice-oriented way.

Written by the acknowledged experts Dr Lutz Popper (Head of Research & Development, Mühlenchemie) and Prof. Dr Klaus Lösche (Managing Director, Northern Food Tec), the

book provides a short history of enzymes and explains their mode of action before going into the subject of cereal-based applications by describing the effect of enzymes on dough stability and rheology, control of enzymatic effects, synergisms and interactions between different enzymes, mechanisms of inhibition and inactivation, interaction with other constituents of the recipe and possibilities of optimizing enzyme addition. One major asset of the book is its in-depth focus on specific applications for a wide variety of cereal products and processes, making it extremely valuable for everyone involved in the cereal value chain. With its clear structure of 18 chapters, attractive layout designed to highlight the most important aspects at a glance, descriptive figures and comprehensive tables, this book will be a useful resource for cereal breeders, handlers, millers, bakers, food scientists and also undergraduate and graduate students seeking to understand the use of enzymes in cereal-based products. I am convinced that this book will serve as a valuable, comprehensive and practice-oriented reference that I recommend highly to anyone seeking to explore the diverse and exciting success story of enzymes.

*Prof. Dr Katharina Scherf*  
 April 2021

## 2nd Preface

Food technology is one of the key technologies of the 21st century, and food production will rank among the most significant industries of the future. The sustainable provision of safe, affordable and appetizing food for a growing world population is one of the greatest challenges of our time. It is no exaggeration to say that an adequate supply of food on the global and also the regional level is a matter of war or peace.

A vital contribution to meeting this challenge will be made by the “specialty food ingredients” industry. It will be functional food ingredients like enzymes that make it possible to transform basic constituents of food into attractive consumer products under sustainable conditions. This applies especially to the baking industry that uses enzymes to control the baking properties of flours. Its basic raw material – grain – is a natural product with properties that fluctuate under a diversity of influences. On the one hand, baking is an ancient cultural technique, but it is also a complex technological process. So it is chiefly practical considerations that speak in favor of using enzymes in baking.

Only the products and innovations brought about by bio and enzyme technology can close the widening gap between a growing world population, with its increasing needs in respect of the availability and quality of food products, and the still limited supply basis. We are faced with the task of feeding as many as ten billion people in the foreseeable future, and we will not succeed without resource-saving processing of cereals as basic raw materials. In this context we also measure sustainability in terms of avoiding waste and reducing energy

and water consumption in food production. Enzymes are magnificent process optimizers, and technologists the world over appreciate them as such.

With “Understanding Baking Enzymes” we are presenting a book that can make a substantial contribution to the beneficial use of enzymes in baking applications by making their effect comprehensible and setting it in a context of practical applications.

*Dr Matthias Moser,  
Peter Steiner  
April 2021*

## 3rd Preface

This compendium of baking enzymes owes its origin to the awareness that without the complex effects of enzymes we would be unable to satisfy our culinary ambitions, and even life itself would be impossible.

The control and intelligent use of enzymatic reactions already offer an outstanding basis for innovation and progress and will doubtless acquire even more significance in the future – not only for diagnostics in medicine and pharmacy (CRISPR-Cas, ELISA, PCR etc.) and for technical applications (detergents, textiles, paper, bioethanol etc.) but also for food processing.

The field of baking enzymes was chosen specifically for this compendium because the resulting bakery products have positive connotations for nearly all of us. The reason is presumably that with their enormous diversity they offer something for everyone. But very few people realize that many of the positive attributes of baked goods are directly attributable to the effects of enzymes.

With the aid of countless enzymes, a cereal grain grows and develops and strives towards reproduction. But when it comes to processing ground cereals, the natural properties of the grain are by no means ideal for achieving high-quality bakery products. Specific modifications frequently have to be made in order to generate milled products and baked goods with consistently good processing properties and quality attributes. It is here that enzymes offer a host of possibilities for specific optimization.

In spite of the many obvious advantages of enzymatic processes, some consumer circles and

even certain professional users view enzymes with skepticism where food applications are concerned. This was the authors’ main motivation for creating a compendium which they hope will give users an understanding of enzymes and their applications.

Moreover, by explaining important biochemical principles, backed up with numerous practical examples from milling and baking, they wish to help professional users with the optimization of their processes and products with which they are often faced. For this reason even Chapter 2, which is highly theoretical, contains numerous cross-references to baking applications in order to show the practical relevance of the scientific findings. Besides this, it is hoped that the book will encourage readers to study enzymatic reactions and applications in order to use them as outstanding and highly specific instruments for innovation and progress.

While we were writing this book we constantly discovered new sources that had so far been unknown to us, and many of which we were able to include. But one or the other piece of information has doubtless been overlooked, not least because more and more findings relevant to the use of baking enzymes were published while the compendium was being compiled. We ask you to excuse these gaps, and hope that a new book to be published in the future will fill them.

*Dr Lutz Popper,  
Prof. Dr Klaus Lösche  
April 2021*



## TEXT IN BLUE CHARACTERS

Text passages with special reference to practical baking applications are highlighted in blue font, unless the context is exclusively about baking applications.

## ABBREVIATIONS

<b>AA</b>	L-Ascorbic Acid	<b>DNSA</b>	DiNitroSalicylic Acid, used to detect reducing sugars	<b>IUBMB</b>	International Union of Biochemistry and Molecular Biology	<b>SI</b>	Système International
<b>AACC</b>	American Association of Cereal Chemists (now Cereal & Grains Association)	<b>DOE</b>	Design Of Experiments	<b>IUPAC</b>	International Union of Pure and Applied Chemistry	<b>SKB</b>	Amylase activity units as determined by a method established by Sandstedt, Kneen and Blish
<b>ADH</b>	Alcohol DeHydrogenase	<b>DP</b>	Degree of Polymerization			<b>SOX</b>	Sulfhydryl OXidase
<b>ALARA</b>	As Low As Reasonably Achievable	<b>DP</b>	Diastatic Power	<b>JECFA</b>	Joint FAO/WHO Expert Committee on Food Additives	<b>SSL</b>	Sodium Stearoyl-2-Lactylate
<b>AS</b>	Amino Acid					<b>T550, T1150 etc.</b>	T stands for Type and the number for the amount of minerals (determined as ash) in the flour, in mg per 100 g
<b>ATI</b>	Amylase Trypsin Inhibitor	<b>EC</b>	Enzyme Commission	<b>LAB</b>	Lactic Acid Bacteria	<b>TAG</b>	TriAcylGlycerol
<b>ATP</b>	Adenosine TriPhosphate	<b>EDTA</b>	EthyleneDiamineTetraAcetic acid	<b>LIP</b>	Lipase-Inhibiting Proteins	<b>TAXI</b>	Triticum Aestivum Xylanase Inhibitor
<b>AU</b>	Amylogram Units	<b>EFSA</b>	European Food Safety Authority	<b>LOX</b>	LipOXygenase	<b>TG</b>	TransGlutaminase
<b>a<sub>w</sub></b>	Water Activity	<b>EPS</b>	Extracellular PolySaccharide, exopolysaccharide	<b>LTP</b>	Lipid Transfer Protein	<b>TLXI</b>	Thaumatococcus-like Xylanase-Inhibitor
<b>AX</b>	ArabinoXylan	<b>ES-AX</b>	Enzyme-Solubilized water-unextractable ArabinoXylans	<b>MAG</b>	MonoAcylGlycerol	<b>TTG</b>	Tissue TransGlutaminase
<b>BASI</b>	BArley Subtilisin Inhibitor	<b>ESL</b>	Extended Shelf Life	<b>MAP</b>	Modified Atmosphere Packaging	<b>US FDA</b>	U.S. Food and Drug Administration
<b>BIPEA</b>	Bureau InterProfessionnel d'Études Analytiques (Interprofessional Bureau of Analytical Studies)	<b>FAA</b>	Fungal Alpha-Amylase	<b>MDD</b>	Minimum Durability Date	<b>USDA</b>	U.S. Department of Agriculture
<b>BRENDA</b>	BRaunschweig ENzyme DAtabase	<b>FAD</b>	Flavine Adenine Dinucleotide	<b>MGDG</b>	MonoGalactosyl DiGlyceride	<b>USMCA</b>	United States-Mexico-Canada Agreement
<b>BU</b>	Brabender Unit	<b>FADH</b>	Flavin Adenine Dinucleotide, reduced (Hydrogenated)	<b>MGMG</b>	MonoGalactosyl MonoGlyceride		
<b>CAZy</b>	database of CArbohydrate-active enZymes	<b>FADH<sub>2</sub></b>	Flavin Adenine Dinucleotide, reduced (Hydrogenated) twice	<b>mTG</b>	Microbial TransGlutaminase	<b>WASI</b>	Wheat α-Amylase/Subtilisin Inhibitor
<b>CCD</b>	Central Composite Design	<b>FAE</b>	Ferulic Acid Esterase (feruloyl esterase)	<b>NAD<sup>+</sup></b>	Nicotinamide Adenine Dinucleotide, oxidized	<b>WE-AX</b>	Water-Extractable ArabinoXylan
<b>CD</b>	Coeliac Disease	<b>FALCPA</b>	Food Allergen Labelling and Consumer Protection Act (U.S.)	<b>NADH</b>	Nicotinamide Adenine Dinucleotide, reduced (Hydrogenated)	<b>WF</b>	Wheat Flour
<b>CFIA</b>	Canadian Food Inspection Agency	<b>FAU</b>	Fungal Amylase Unit	<b>NADP<sup>+</sup></b>	Nicotinamide Adenine Dinucleotide Phosphate, oxidized	<b>WFLI</b>	Wheat Flour Lipase Inhibitor
<b>CFR</b>	Code of Federal Regulation (U.S.)	<b>FIAP</b>	Food Improvement Agent Package (E.U.)	<b>NADPH</b>	Nicotinamide Adenine Dinucleotide Phosphate, reduced (Hydrogenated)	<b>WK</b>	Degree Windisch-Kolbach
<b>COFEPRIS</b>	Comisión FEderal para la Protección contra Riesgos Sanitarios (Federal Commission for the Protection Against Sanitary Risks, Mexico)	<b>FODMAP</b>	Fermentable Oligo-, Di-, and Monosaccharides And Polyols	<b>NAFTA</b>	North American Free Trade Agreement	<b>WU-AX</b>	Water-Unextractable ArabinoXylan
<b>CRC</b>	Consolidated Regulations of Canada	<b>GAA</b>	Cereal (Grain) Amylase Analyzer	<b>NAPE</b>	N-Acyl PhosphatidylEthanolamine	<b>XIP</b>	Xylanase Inhibition-Protein
<b>CRISPR-Cas</b>	Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-associated (genes)	<b>GdL</b>	Glucono delta-Lactone	<b>NCWS</b>	Non-Coeliac Wheat Sensitivity		
<b>CWRS</b>	Canadian Western Red Spring (hard wheat variety)	<b>GH</b>	Glycoside Hydrolase	<b>NOM</b>	Norma Oficial Mexicana (Mexican Official Norm)		
		<b>GI</b>	Glycemic Index	<b>NSP</b>	Non-Starch Polysaccharide		
<b>DAG</b>	DiAcylGlycerol	<b>GM</b>	Genetically Modified				
<b>DATEM</b>	DiAcetyl Tartaric acid Ester of Mono- and diglycerides	<b>GMO</b>	Genetically Modified Organism	<b>PCR</b>	Polymerase Chain Reaction		
<b>DE</b>	Dextrose Equivalents, used to express the reducing power	<b>GMP</b>	Gluten MacroPolymer	<b>PDI</b>	Protein Disulfide-Isomerase		
<b>DGDG</b>	DiGalactosyl DiGlyceride	<b>GOX</b>	Glucose OXidase	<b>PEF</b>	Pulsed Electric Field		
<b>DGMG</b>	DiGalactosyl MonoGlyceride	<b>GRAS</b>	Generally Recognized As Safe (U.S.)	<b>PEF</b>	Pulsed Electric Field		
<b>DH</b>	Degree of Hydrolysis	<b>GSH</b>	Glutathione	<b>PIN</b>	PuroINDoline		
<b>DHAA</b>	DeHydroAscorbic Acid	<b>GSH-DH</b>	Glutathione DeHydrogenase	<b>PUFA</b>	PolyUnsaturated Fatty Acid		
<b>DL</b>	Dough Liquor	<b>GSSG</b>	Glutathione disulfide				
<b>D.M.</b>	Dry Matter	<b>GSP</b>	Grain Softness Protein	<b>QPS</b>	Qualified Presumption of Safety (E.U.)		
<b>DNA</b>	DeoxyriboNucleic Acid			<b>RF</b>	Retarded Fermentation		
<b>DNS</b>	Dark Northern Spring (U.S. hard wheat variety)	<b>HFCS</b>	High Fructose Corn Syrup	<b>RF</b>	Rye Flour (Tab. 5 only)		
		<b>HPMC</b>	HydroxyPropyl MethylCellulose	<b>SBD</b>	Starch Binding Domain (of an amylolytic enzyme)		
		<b>ICC</b>	International Association for Cereal Chemistry (now International Association for Cereal Science and Technology)	<b>SDS</b>	Sodium Dodecyl Sulfate		



# 2. The Mode of Action of Enzymes

## NATURE'S ENERGY SAVERS

### 2.1 ORIGINS AND KINETICS

#### 2.1.1 The nature of enzymes

Enzymes have been in common use in the food industry for years. Unlike most other applications in foods, the **enzymes used for flour treatment do not react at the place where they are added, namely at the flour mill; they do not take effect until the baker adds water.** This difference in time and place is a great challenge to the flour treatment sector in general, but in the case of enzymes, it is an especially complex matter.

In their uses, enzymes are highly specific. If they are pure enough, they act on selected targets and only have to be added in small quantities. In their sources, they are entirely

natural. They can only be obtained from microorganisms by way of fermentation or from vegetable or animal tissue and fluids by means of extraction (Fig. 1).

Enzymes are proteins with catalytic properties. They are biocatalysts, capable of speeding up chemical reactions under comparatively mild conditions (temperature, pH etc.) by reducing the activation energy of a reaction, and they emerge from this reaction unchanged.

Fig. 2 diagrams shows this catalytic effect. Only "Gibbs free energy of activation"  $\Delta G$  is reduced; thus, the forward and reverse reaction speeds up by the same factor. In other words, a state of equilibrium is achieved faster. The bonding that forms the temporary enzyme-substrate complex activates the substrate, thus

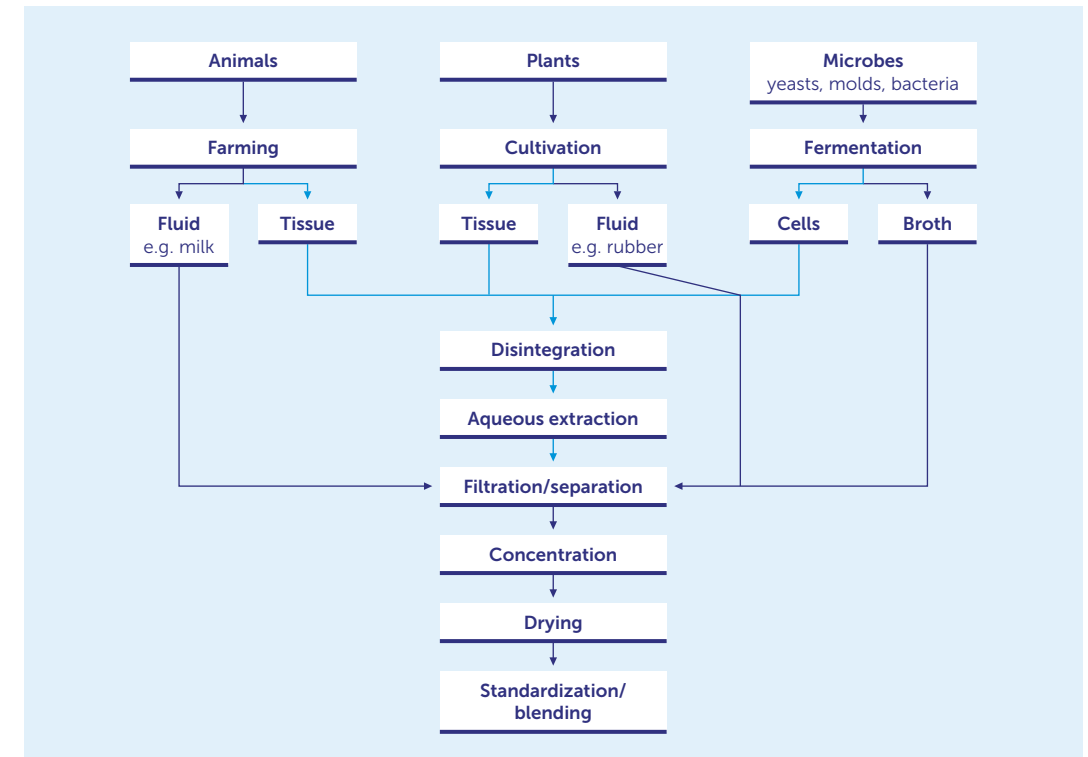


FIG. 1 Natural sources only: enzyme recovery

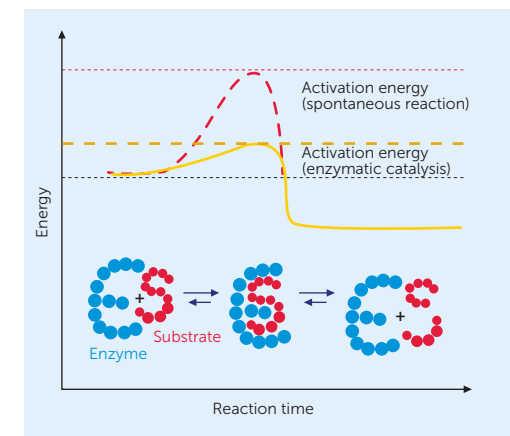


FIG. 2 As biocatalysts, enzymes reduce the activation energy  $\Delta G$  of a reaction

reducing the activation energy for the entire reaction. This ultimately results in an increase of the reaction rate. The enzyme comes out of the reaction cycle unchanged, whereas the substrate undergoes a change.

The attributes (Fig. 3) and properties that characterize individual enzymes differ according to their origin (different enzyme sources or organisms).

Very high catalytic efficacy and specificity characterize enzymes. By reducing the activation energy required for a chemical reaction, enzymes achieve a thousandfold or even millionfold increase in the speed of the reaction.

### 2.1.2 Enzyme subunits

Enzymes do not always consist of a single molecule; they are often globular structures made up of several subunits (often called isoenzymes<sup>2</sup>). The subunits may be identical, or they may differ.

Most of the enzymes suitable for baking do not form subunits (these include cereal amylases, proteases and xylanases), but other enzymes do. Glucoamylase from *Aspergillus niger*, however, is a glycoprotein with a carbohydrate content of about 13% that can be split by electrophoresis into two isoenzymes (and is therefore a *dimer*). Typical xylanases used for optimizing flour and dough each consist of only a single polypeptide chain. *Trichoderma lignorum*, a source of xylanases, also forms a  $\beta$ -xylosidase with a molar mass<sup>3</sup> of about 100,000 Da<sup>4</sup> that consists of two subunits (and is also a dimer). This enzyme is a glycoprotein with a carbohydrate content of 25%, which contains the monosaccharides mannose, glucose and xylose in the ratio of 9:3:1 (Schmidt *et al.* 1979).

Similarly, baker's yeast forms alcohol dehydrogenase (ADH), an enzyme with two subunits. Among other things, these isoenzymes differ in kinetic data and electrophoretic mobility. Whereas fermentation ADH is thermolabile, the second – more thermostable – isoenzyme is above all responsible for introducing ethanol into the metabolic system (Dellweg and John 1974). Many enzymes do not exist only in the form of a defined protein; instead, they exist in several molecular forms that catalyze the same

reaction. According to the recommendations of IUPAC/IUBMB<sup>5</sup>, these multiple enzyme forms are termed *isoenzymes* if the difference between them is genetic in origin and not due to modifications after biosynthesis.

Fig. 4 shows catalase, which can consist of as many as four isoenzymes, each of which exercises catalytic activity of its own. Further properties of enzymes made up of subunits are described by Ebner (1975).

### 2.1.3 Enzyme cofactors

Enzyme proteins can be bound to organic or inorganic molecules. Although necessary for the catalytic function, these *cofactor* molecules are not proteins.

In a complex of an enzyme protein and a cofactor, the actual enzyme, i.e. the protein component, is called the *apoenzyme*. Together, the cofactor and the apoenzyme make up the *holoenzyme*.

#### Prosthetic groups

An organic molecule that is covalent or bound to an enzyme with high affinity and therefore not dissociable is called a *prosthetic group*. This active group is not itself a protein, but it is firmly bound to the protein component and influences control of the enzyme. The groups include the *hemes* (porphyrin rings) that contain iron as their central atom. They form the prosthetic group of peroxidase, catalase and the cytochromes, among others (Karlson 1972).

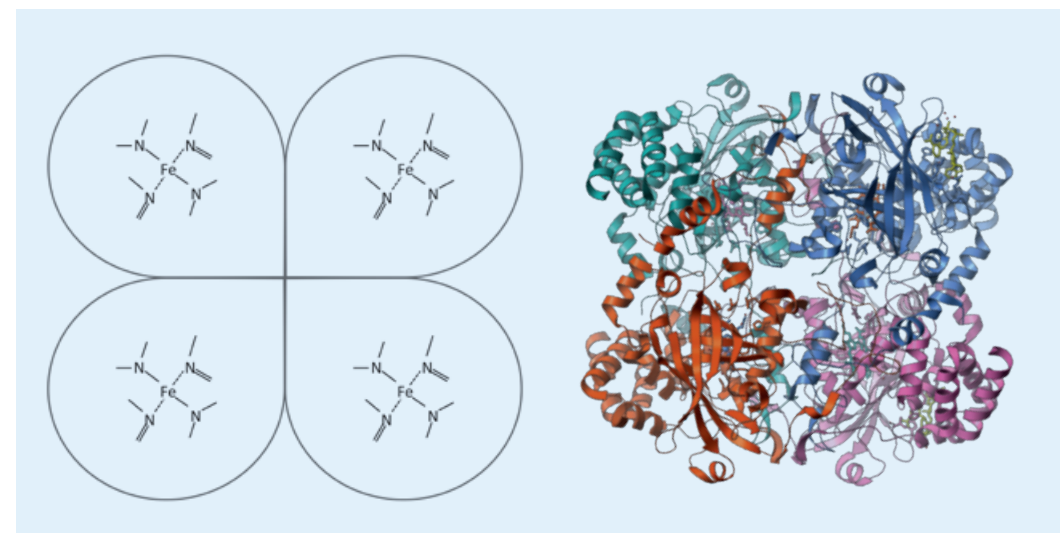
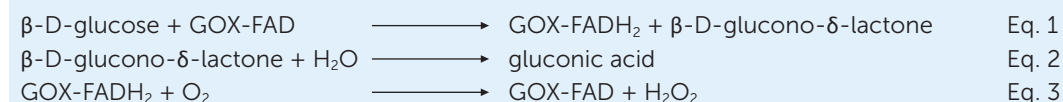


FIG. 4 Structure of the catalase tetramer. Catalase consists of four identical subunits with heme prosthetic groups. Left: simplistic schematic. Right: ribbon diagram (Vossman 2009)

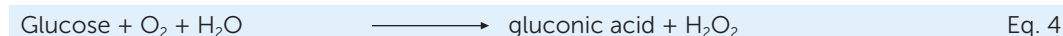
#### Coenzymes

Smaller organic molecules that are not covalent, i.e. that are less firmly bound to the enzyme protein and participate in the reaction, are called *coenzymes* or *co-substrates*. During catalysis, they take up electrons or protons, for example, and subsequently have to be “regenerated”.

One example is FAD (flavine adenine dinucleotide), a coenzyme of some oxidative enzymes such as glucose oxidase (GOX). During the reaction, FAD takes up protons ( $H^+$ ) and has to be regenerated with oxygen before it can take part in the reaction again (Eq. 1 - Eq. 3).



In the literature, the simplified equation Eq. 4 is often found,



which does not indicate the participation of the coenzyme; this is the case in Fig. 6 in Chapter 2.1.5.

<sup>2</sup> Isoenzymes have the same, or almost the same, effect, but they differ in respect of their primary structure (sequence of the amino acids).

<sup>3</sup> The term molar mass (or mole) describes the weight of a molecule. The carbon atom with a mass of 12 g/mol serves as a reference.

<sup>4</sup> Molar mass is measured in Dalton units (Da) instead of g/mol. By definition, one mole of a substance contains  $6.022 \times 10^{23}$  (ca. 602 trillion – so-called the Avogadro constant) particles, i.e. atoms, of an element or molecules of a chemical compound. Amino acids have molar masses of 75 Da (glycine) to 204 Da (tryptophan). Their presence in a protein indicates the latter's molar mass. The weighted average of the amino acids of many proteins is about 130 Da per amino acid. The smallest enzyme molecule discovered so far contains 62 amino acids, the largest more than 2,500.

<sup>5</sup> Enzyme nomenclature was created by a joint committee of the International Union of Biochemistry (IUB; now the International Union of Biochemistry and Molecular Biology, IUBMB) and the International Union of Pure and Applied Chemistry (IUPAC).

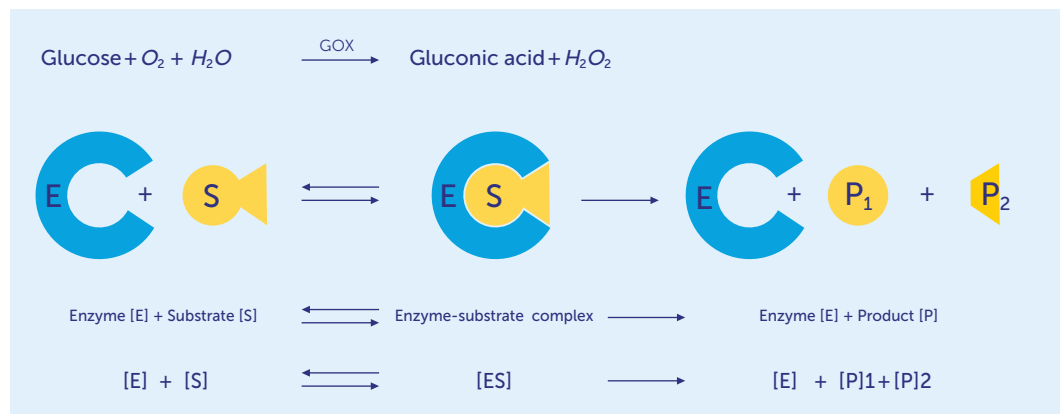


FIG. 6 Enzymatic conversion of a substrate (glucose) to a product (gluconic acid) according to the lock-and-key principle

Fig. 6 illustrates substrate specificity, taking the example of the conversion of glucose with glucose oxidase.

In this example, glucose (the substrate) is the “key” to the “lock” glucose oxidase (the enzyme).

Other sugars, such as maltose and sucrose, and glucose polymers like dextrin or starch are unsuitable as keys. Although fructose fits into the enzyme lock it is unable to close the lock because it does not have the exact shape. For that reason, it is not converted by the enzyme (Renneberg 1990).

Although enzymes must have high substrate specificity for regulating metabolism in the cell, the reverse is true of digestive enzymes. They act outside the cell (extracellular action) in the stomach or intestine, and it would be uneconomical of the organism to produce a specific enzyme for every protein that finds its way into the stomach. So a digestive protease like pepsin must have low substrate specificity but high reaction specificity.

Pepsin hydrolyzes all proteins at specific peptide bonds, with a preference for the hydrophobic amino acids phenylalanine, tryptophan and tyrosine.

It may be said, with certain reservations, that the enzymes of cell metabolism and synthesis show stricter specificity than those of digestion, which act outside the cell in any case. It is assumed that where a high degree of specificity is meaningless, e.g. with the enzymes of the digestive tract, there was no evolutionary selection pressure to create them<sup>9</sup>.

In contrast to *chemical* reactions in industry, in which unwanted side reactions and by-products are a great problem, in the case of *biochemical* reactions, only one of several potentially possible reactions is catalyzed. The desired end product is accessible at a high yield under mild conditions (e.g. at room temperature, normal pressure, neutral pH) (Fig. 7).

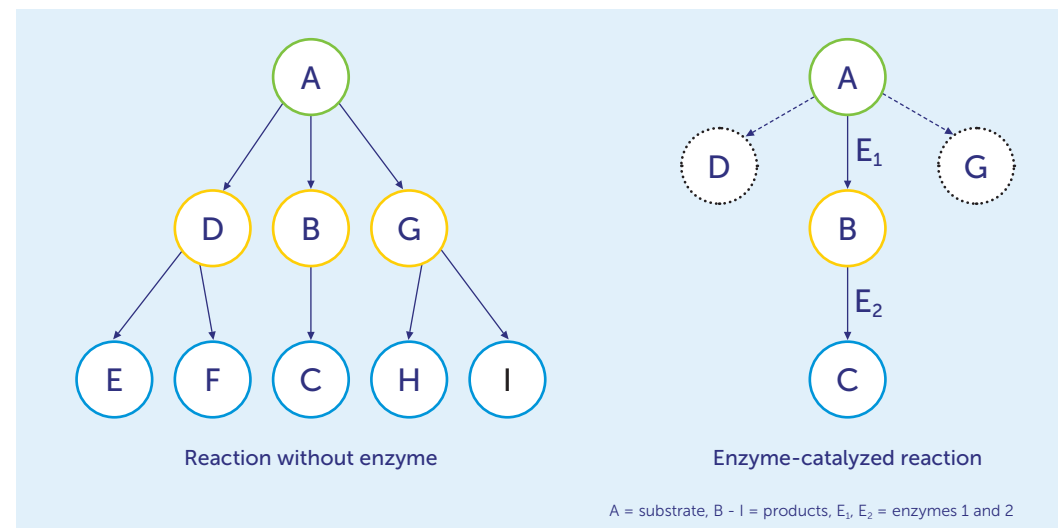


FIG. 7 Comparison of the reaction specificity of chemical and biochemical reactions (dotted line = conversion to the reaction products D and G is only marginal)

### 2.1.6 Enzyme kinetics

#### Reaction rate and Michaelis-Menten constant

According to the model concepts of Leonor Michaelis and Maud Menten (1913), a free-substrate binds to the active site of an enzyme. The enzyme-substrate complex passes through an activated transition state in which the conversion of the substrate takes place. The reaction products are released, and the enzyme can bind a substrate again. According to this lock-and-key principle (see also Chapter 2.1.5), the enzyme has a shape complementary to the substrate. The active site is understood to be a rigid matrix to which a substrate only attaches itself if it fits like a key in a lock (Fig. 8).

The course of an enzyme-catalyzed reaction (kinetics) can be described with the equations Eq. 5 through Eq. 8 as follows.

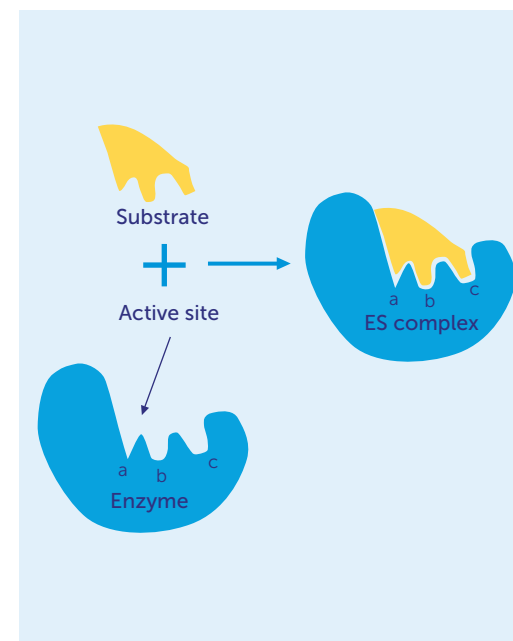


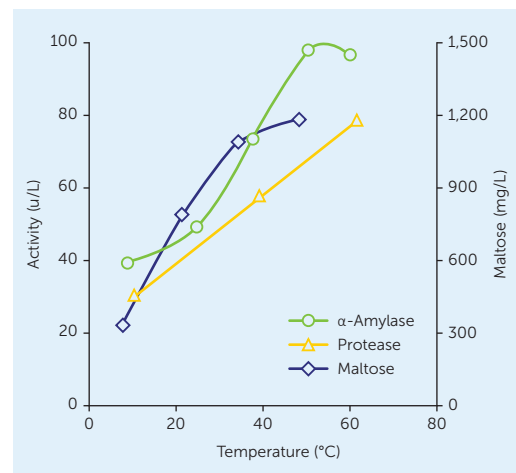
FIG. 8 Model concept of enzyme-substrate interaction after Michaelis-Menten

<sup>9</sup> Interestingly, enzymes that catalyze a bimolecular reaction are not necessarily specific towards both substrates. Alcohol dehydrogenase, for example, is strictly bound to NAD<sup>+</sup> as a hydrogen acceptor and NADH as a hydrogen donor and cannot react with NADP<sup>+</sup> and NADPH. But the enzyme converts quite a number of alcohols and aldehydes. This is possibly an expression of the necessity to achieve a clear distinction between different areas of the metabolism, which is possible with the alternate use of two hydrogen conversion systems, whereas the low specificity towards alcohol is meaningless when practically only one such area exists in the cell.

However, this also leads to the conclusion that residual enzymatic activity is to be expected in dough even at temperatures below  $-5^{\circ}\text{C}$  ( $23^{\circ}\text{F}$ )<sup>18</sup>.

Retarded fermentation and freezing serve to interrupt the baking process, but they are also an instrument of enzyme management and control of enzyme activity. At refrigerator and freezer temperatures, these actions are more important, in principle, than microbial activities (yeasts, lactic acid bacteria) and are therefore technological variables and an important basis for premium qualities in the baking industry.

Whereas storage-stable grain and mill products have an  $a_w$  value of ca. 0.64, frozen dough portions have  $a_w$  values of ca. 0.75, depending on the recipe (high ratio<sup>19</sup> or lean doughs) and the temperature. At room temperature, bread roll doughs and the *crumb* of the baked products have an  $a_w$  value of ca. 0.96, whereas the *crust* has only ca. 0.5. Products with a high percentage of free (available, freezable) water include



**FIG. 14** Effect of dough temperature on the activity of selected enzymes (wheat flour Type 550 (ca. 0.6% ash), Falling Number 380 s, dough yield = 200,  $t = 150$  min)

meat and fish and also milk and cream, fruit and vegetables (Tab. 7).

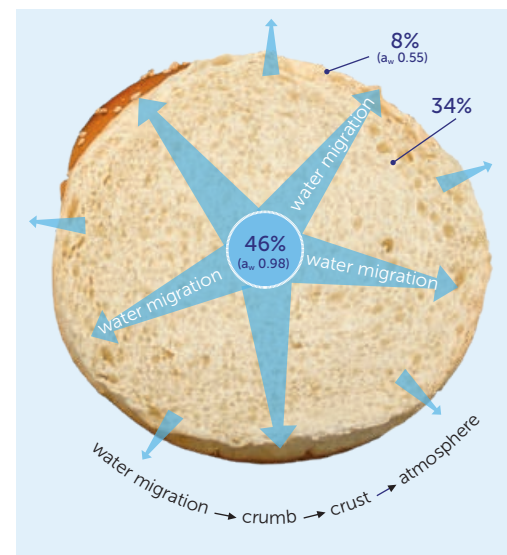
#### Water migration

Two ingredients may have the same moisture content but very different  $a_w$  values. The free water migrates from regions with a high  $a_w$  value to regions with a low  $a_w$  value and *not* between regions with a different moisture content (Fig. 15).

Water migrates between the different layers of a food and causes unwanted changes in texture that are a major cause of staling in baked goods (Chapter 12).

**TAB. 7** Typical  $a_w$  values of selected foods

$a_w$	Examples of foods
0.99	Fresh fish, fresh meat, quark
0.98	Milk, cream, juices, fruit, vegetables, soft cheeses
0.95 - 0.97	Water doughs (bread rolls, baguettes etc.), semi-hard and hard cheese, sausage, crumb of bread
0.86 - 0.92	Light yeast pastry, Parmesan cheese, raw ham, raw sausage, salami
0.80 - 0.90	Sauces like ketchup, jam, cake, light yeast pastry, ready-made doughs, syrup, sugared evaporated (condensed) milk
0.70 - 0.80	Frozen doughs, heavy yeast pastry, sponge and Madeira cake batters, soup seasonings, marzipan, dry fruit cake, prunes, jam with higher concentrations
0.75 - 0.77	Fondant (10.5 - 11.5% water)
0.60 - 0.70	Cereals, honey, nougat, raisins, muesli, nuts, confectionery fillings, dried fruits, flour, rice, brown gingerbread
0.50 - 0.55	Raisins (14.5 - 15.5% water)
0.5	Pasta (12% water), spices (10% water), crust of bread, hard and soft gingerbread "printen"
0.40 - 0.50	Chocolate (0.1 - 0.5% water)
0.4	Egg powder (5% water)
0.3	Biscuits, crackers, crispbread (4% water)
0.2	Milk powder (3% water), meringue, flat wafers



**FIG. 15** Water distribution and migration along a cross-section of a baked product

Fig. 15 shows a cross section of a small baked product (consisting of about 55% water and some 45% solids) which loses its characteristic quality attributes primarily through water migration.

#### Enzymatic reactions

Most enzymatic reactions require only a minimal water content. The function of this is chiefly:

- To solubilize the substrates,
- To increase substrate mobility,
- To provide water as a reactant.

Enzyme stability is also influenced by water. Denaturation takes place through:

- Hydrolysis,
- Deamination (loss of ammonia groups,  $\text{NH}_3$ ),
- Oxidation.

Other mechanisms can also be involved for each of which sufficient water must be

available. As a rule, dry enzyme preparations are highly stable and resist even temperatures above the denaturation temperature for a certain time.

#### Technological relevance

If a piece of dough develops a "skin" in the course of processing, the reason is desorption (drying) caused by ambient air with a relative humidity below the  $a_w$  value of the dough. Accordingly, the available (free) water in this skin is reduced and so also is the activity of the enzymes and yeast and the thermal conductivity of the dough piece. Consequently, heat and mass transfer processes are reduced during the baking process. The browning reaction (Maillard reaction) is delayed and minimized; the crispness, aroma and flavor of the products are retarded or come about only sluggishly. The result is a thin, fine-pored crust with a rather weak, brittle material response (reduced splintering, limited crispness, reduced browning reaction, reduced flavor etc.).

To minimize undesirable "flaking" (detachment of the crust from the crumb in semi-baked products) enzymes (e.g. glucoamylase) with sugar-producing and water-binding effects can reduce formation of ice crystals.

Enzymatic activity therefore depends on the  $a_w$  value of a particular food:

- Below an  $a_w$  value of 0.8, hardly any activity is to be expected,
- The activity increases with the water content,
- Lipases, phospholipases, lipoxygenases and some peroxidases are active down to  $a_w = 0.20$ . (Lipases are used industrially even in water-free systems, for example, to achieve interesterification of edible fats.)

<sup>18</sup> The freezing range of the free water in doughs is ca.  $-1$  to  $-5^{\circ}\text{C}$  ( $30$  to  $23^{\circ}\text{F}$ ). But even below these temperatures, some of the water is still present in liquid form. Not until temperatures fall below ca.  $-70^{\circ}\text{C}$  ( $-94^{\circ}\text{F}$ ) is all water frozen, which means that at typical freezer temperatures of  $-18$  to  $-24^{\circ}\text{C}$  ( $0$  to  $-11^{\circ}\text{F}$ ), a certain amount of enzymatic activity must still be expected.

<sup>19</sup> "Heavy" (or "high-ratio") yeast doughs, e.g. brioche, contain ca. 25 to 50% fat (flour basis). They also often contain 10% or more sugar.

Lipid oxidation is one of the most common reasons for food spoilage and may be caused by metal ions (so-called prooxidants) but also by enzymes. Reaction rates of this type decrease down to a minimum as the  $a_w$  value falls and then increase again.

The minimum is around  $a_w = 0.3$ . Reasons for the increase of the prooxidative effect in the  $a_w$  range 0.3 to 1.0 are the increase in the solubility of the catalysts, including the enzymes, the increased mobility of the reaction partners and the swelling of the food accompanied by enlargement of the surface.

## 2.4 AVAILABILITY OF SUBSTRATE (REACTANTS) FOR THE ENZYMATIC REACTION

Wheat flour dough contains various sources of fermentable sugars. Free saccharides are naturally present in wheat flour in small amounts, ranging from approximately 0.05% (for glucose, fructose and maltose) to 0.2 or 0.3% (for sucrose and raffinose). In wheat bran, sucrose concentrations are higher (1.75 to 3.0%). Wholemeal therefore contains higher concentrations of free saccharides than flour. For wholemeal dough, sucrose concentrations of about 1% have been reported.

The majority of fermentable sugars in dough are generated by amyolytic degradation of damaged<sup>20</sup> starch. Recent studies have shown that fructan is also an important source of fermentable sugars in dough. The amount of fructan<sup>21</sup> in wheat grains ranges from 0.7 to 2.9%. The levels in flour are considerably lower than in bran (1.4 to 1.7% as against 3.4

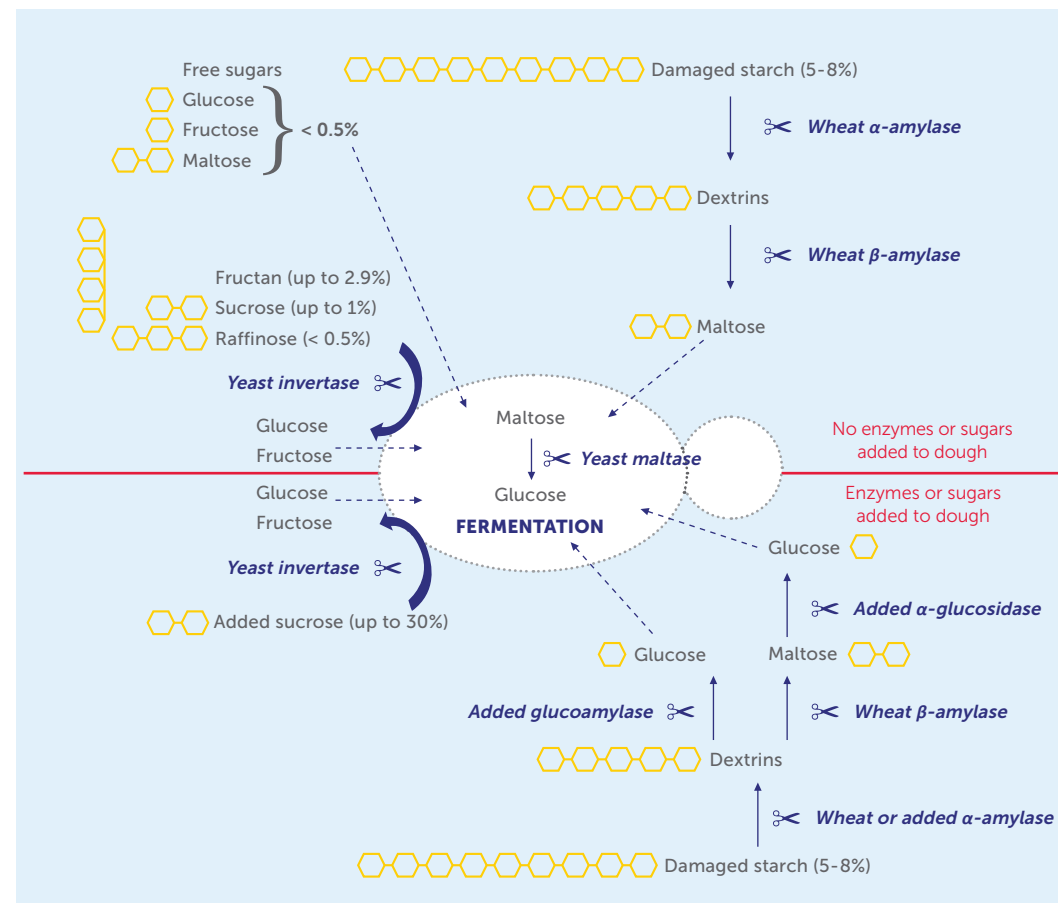
to 4.0%; Haska *et al.* 2008). In wholemeal, fructan levels range from 0.6 to 2.9% (e.g. Struyf *et al.* 2017).

Fig. 16 gives an overview of the different endogenous and added sources of fermentable sugars in dough and their degradation by endogenous (cereal) enzymes and added or yeast enzymes.

Damaged starch is degraded to the fermentable sugar maltose by amylases that act on the  $\alpha$ -1,4 and/or  $\alpha$ -1,6 linkages of the starch polymers (Van der Maarel *et al.* 2002). Two types of amylases are present in wheat flour:  $\alpha$ -amylases (Chapter 2.11.1) and  $\beta$ -amylases (Chapter 2.11.8). The ability of a flour-water suspension to produce maltose is known as the amyolytic activity of the flour (Dodić *et al.* 2005). In dough without yeast, the maltose concentration increased from 0.1% in flour to 1% after mixing and 2% after 180 min of incubation (Struyf *et al.* 2016).

Maltose levels in dough depend on the total damaged starch content and the  $\alpha$ -amylase activity of the flour (Struyf *et al.* 2016). Doughs prepared from flours with higher  $\alpha$ -amylase activities or higher damaged starch content therefore contain higher maltose levels. Since  $\alpha$ -amylase activity is often limited in wheat flour, fungal or malt  $\alpha$ -amylase is often added to wheat flour to increase the level of fermentable sugars in dough (Hebeda and Zobel 1996, Hruskova *et al.* 2003, Cauvain and Young 2007).

A noticeable drop in the fermentation rate is observed when glucose, fructose, sucrose and fructan are nearly depleted, and yeast cells need to adapt to the consumption of malt-



**FIG. 16** Generation of fermentable sugars by endogenous wheat, yeast and added enzymes. Grey: sources of fermentable sugars; yellow: structures of fermentable sugars; black (and scissors): enzymes (Struyf *et al.* 2017)

ose (Struyf *et al.* 2017a). The maltose content of dough mainly influences the production of  $\text{CO}_2$  after the lag phase. Consequently, it determines the total productive fermentation time rather than the fermentation rate in the initial stages of fermentation (Chapter 4, Fig. 4).

Determination of the maltose value is an attempt to describe the fermentative power of

a flour. It serves as a measure of the ability to form maltose. A flour-water suspension is left to incubate for an hour at 27°C (80°F). Maltose is formed by enzymatic activity. The maltose value is the difference between the total amount of maltose measured after 1 hour and the maltose already present in the flour. Typical maltose values are 2 to 3% in wheat and 2.5 to 5% in rye (T550 or T1150).

<sup>20</sup> Damaged starch refers to starch granules broken mechanically during milling. Its amount ranges from 5 to 8% (flour basis) in hard wheat flours obtained by roller milling.

<sup>21</sup> Fructans are polymers of fructose molecules. The term fructooligosaccharide is used for fructans with a short chain length (up to 7 fructosyl subunits). In most fructans, a sucrose unit is located at the otherwise reducing end of the polymer. Fructans occur in wheat, artichokes, asparagus, leeks, garlic and onions, for example. The type of linkage between the fructose molecules determines the type of the fructan.  $\beta$ -2,1-linked fructose is found in inulin,  $\beta$ -2,6-linkages in levan.

“One major asset of the book is its in-depth focus on specific applications for a wide variety of cereal products and processes, making it extremely valuable for everyone involved in the cereal value chain.

... I am convinced that this book will serve as a valuable, comprehensive and practice-oriented reference that I recommend highly to anyone seeking to explore the diverse and exciting success story of enzymes.”

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