Enzymes for the Milling and Baking Industry

Lutz Popper, Ph.D., Head R & D
Mühlenchemie GmbH & Co. KG
Ahrensburg, Germany

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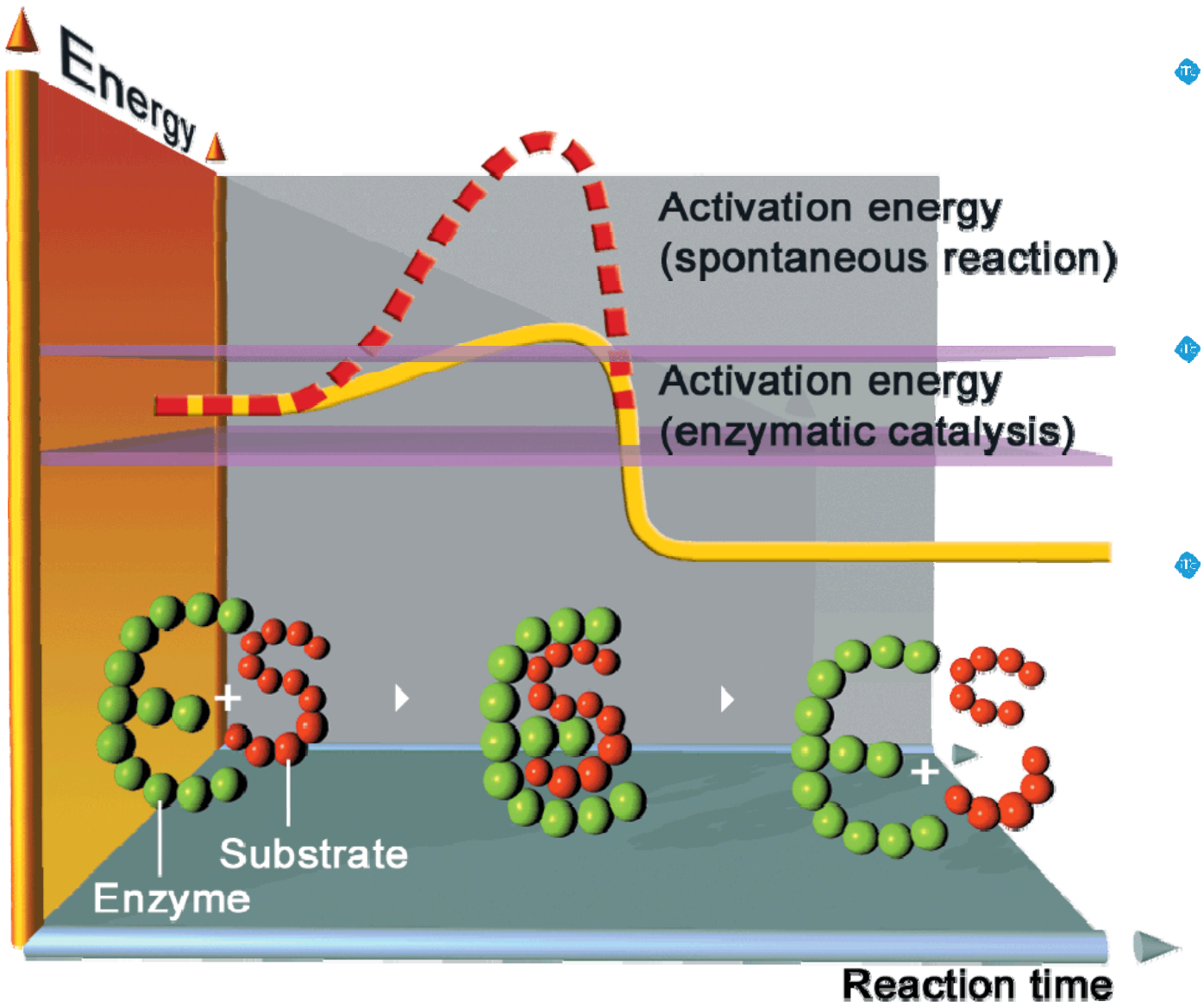
Enzyme Introduction

Enzymes are Bio-Catalysts



- Enzymes are proteins that are capable to accelerate chemical reactions – just like catalysts do.
- Enzymes are derived from the living nature → bio-catalysts
- With the help of enzymes, reactions can take place without the addition of chemicals or thermal exergy.

Enzyme Diversity

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- **Based on genes, it can be predicted that there are around 25,000 enzymes. Of these enzymes, only 5,000 have been characterised, so there are a great many that we do not yet know. Of these few thousand, only 1-2% are used for commercial applications and only a handful are used on a large scale.**

  [Professor Willem van Berkel, Professor of Molecular Enzymology at Wageningen University, 2011]

Enzymes in Food Applications, Examples

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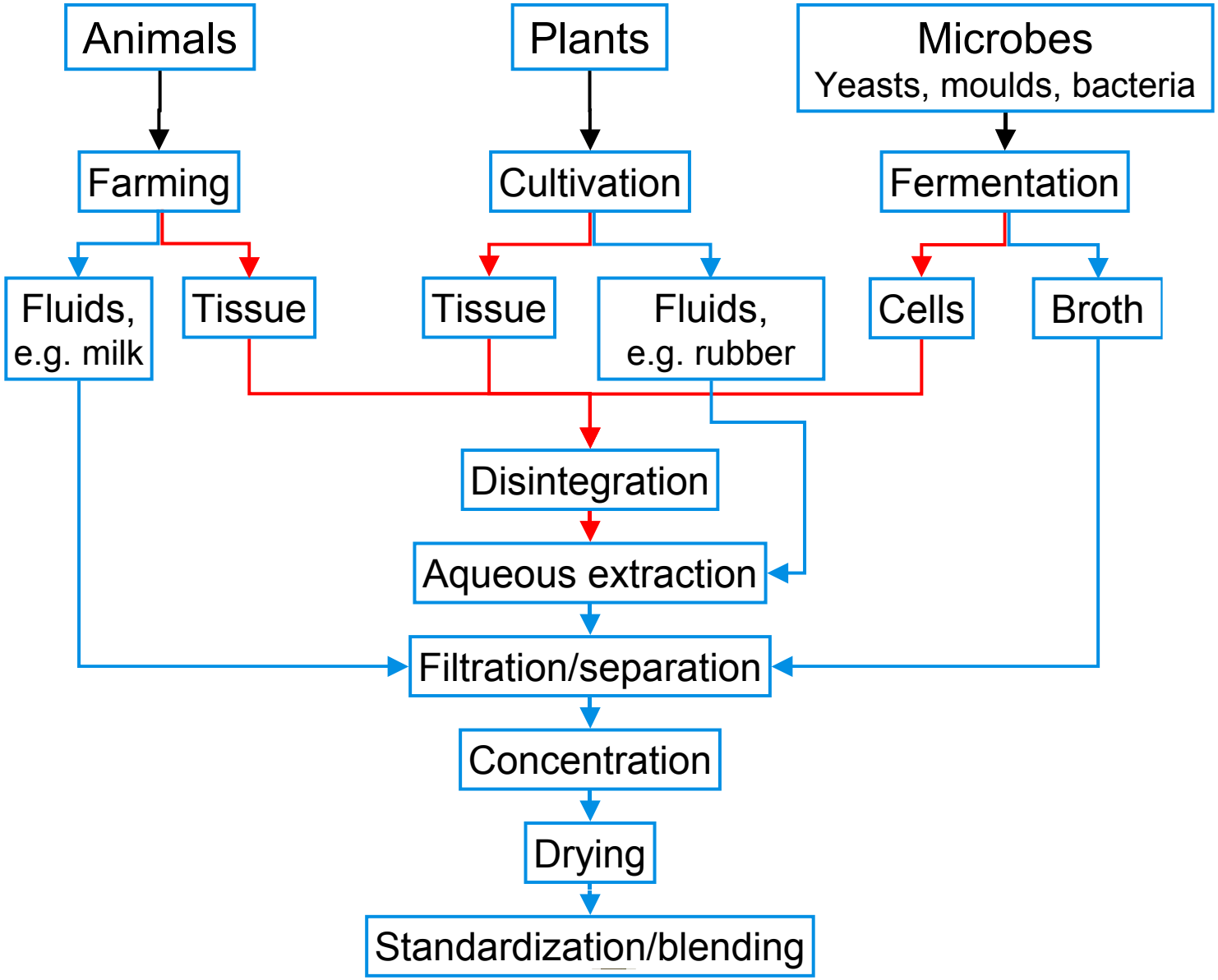
Application	Enzyme examples	Purpose
Baking	Amylase, xylanase, protease, carboxyl esterase, lipoxygenase, oxidase	Volume yield, processing properties, dough stability, bleaching, shelf-life
Brewing	Amylase, glucanase, protease	Fermentation, stability
Cheese	Protease, lipase, phospholipase, peroxidase, lysozyme	Structure, flavor, yield, bleaching, preservation
Confectionery	Invertase	Structure, shelf-life
Egg	Glucose-oxidase, phospholipase	Glucose removal, heat stability, emulsifying properties
Flavors	Lipase, lipoxygenase	Formation of free fatty acids, aldehydes
Fruit & vegetables	Pectinase, pectinmethyl esterase	Softening, firming
Juices	Pectinase, arabinase, amylase	Yield, clarification, stabilization
Lipids	Lipase, phospholipase	Transesterification, hydrolysis, degumming
Meat & fish	Protease, transglutaminase	Softening, firming
Milk	Lactase	Lactose removal
Sugar	Dextranase, amylase	Viscosity reduction, clarification
Wine	Pectinase, protease, laccase, lysozyme	Clarification, stabilization, flavor, removal of off-flavors, preservation

Enzyme Production: from Natural Sources only



Enzyme Activity Depends on Many Factors

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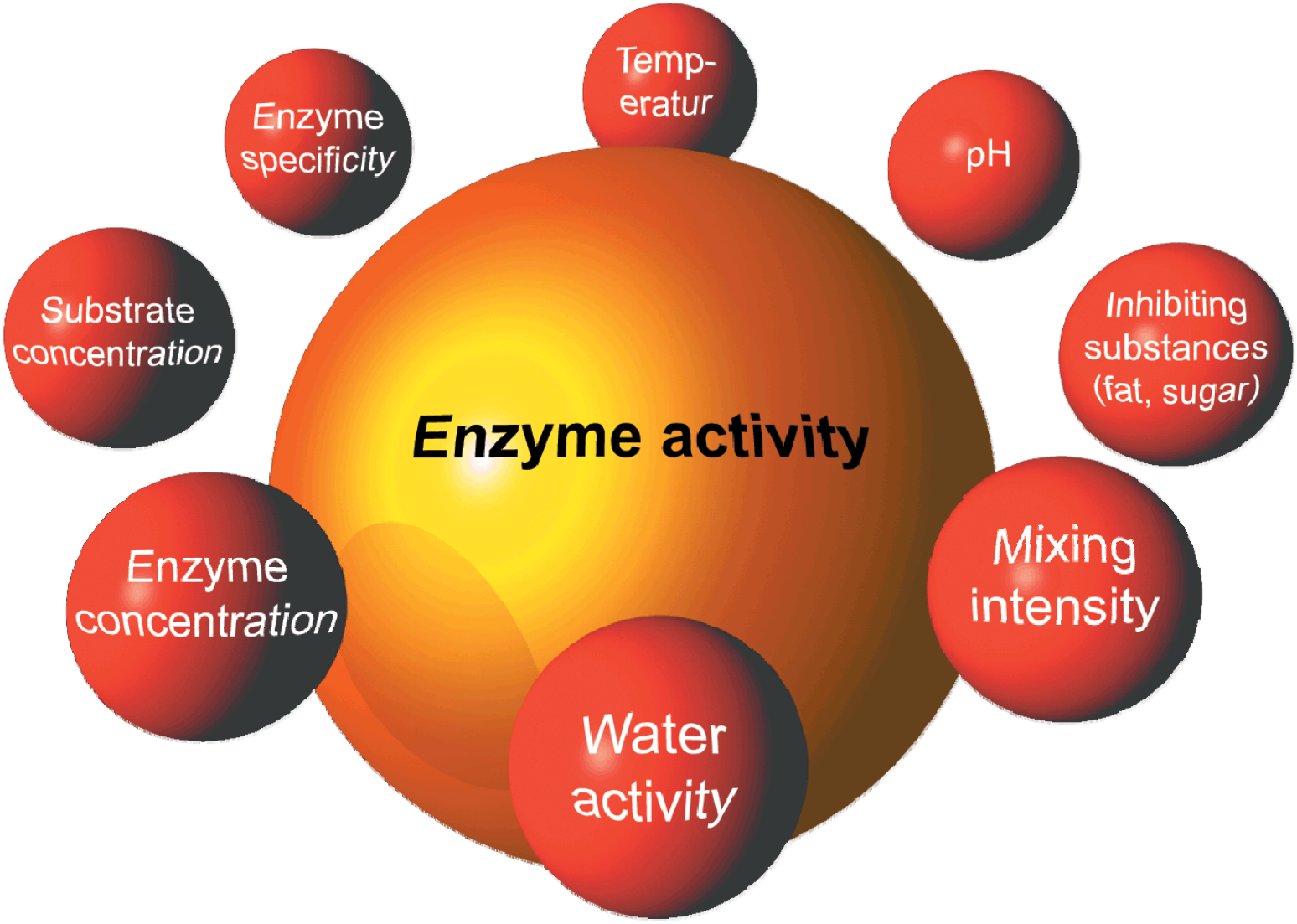
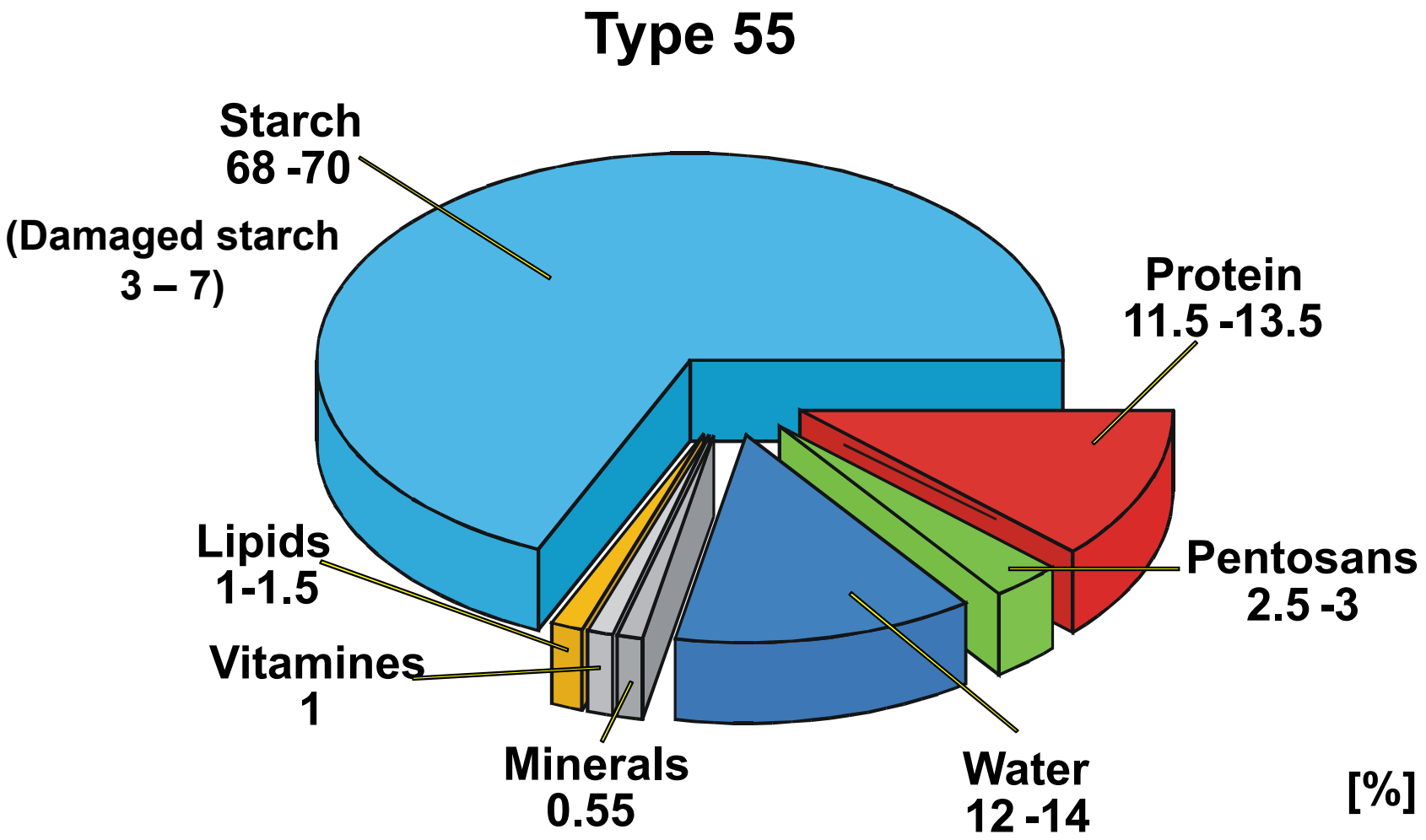
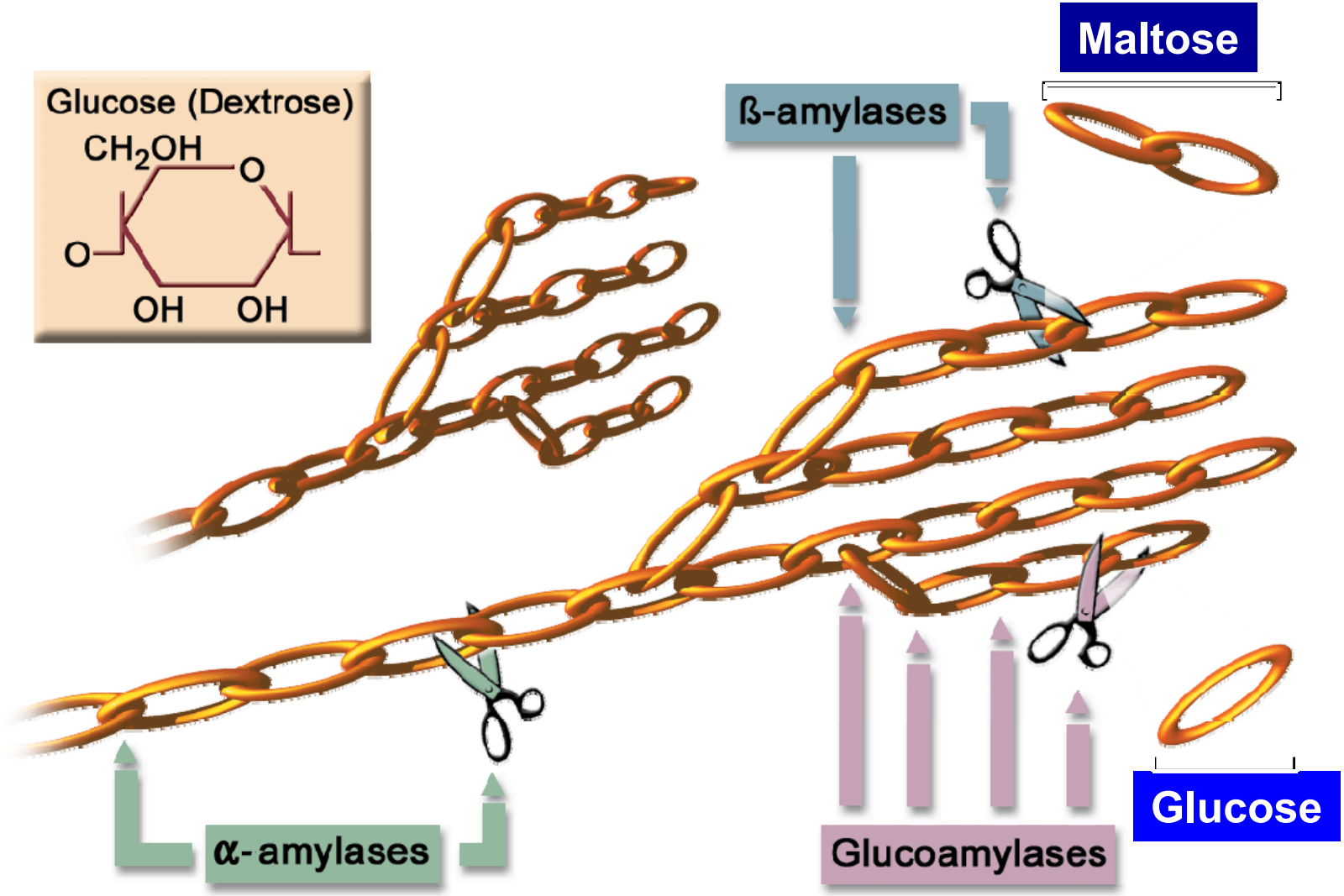


Wheat Flour Composition



Amylolytic Enzymes

Amylolytic Enzymes used in Baking



Effect of Amylases on Dough and Baked Good

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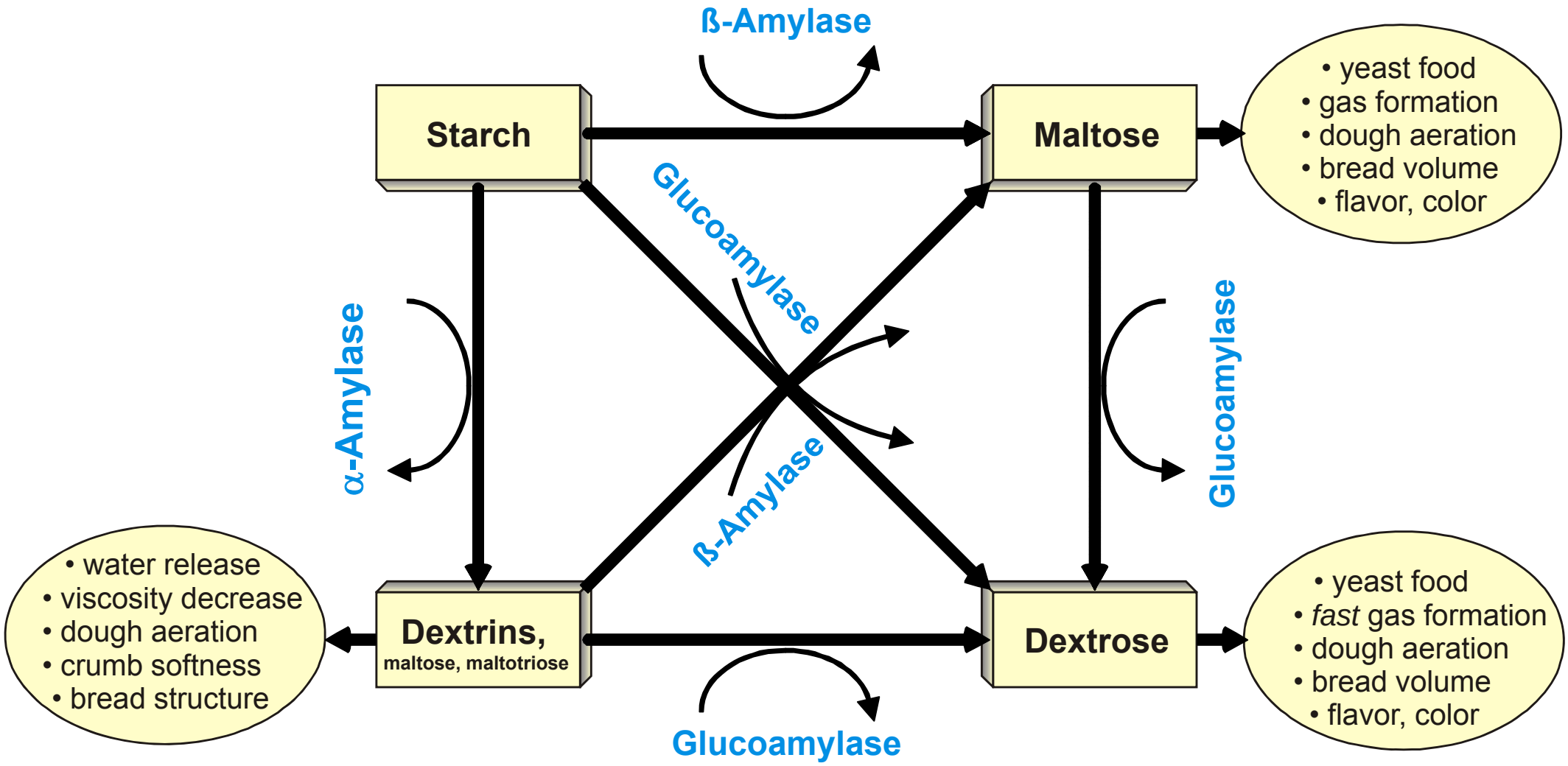

Effect of α-Amylase on Dough and Baked Good

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- **Break-down of hydrated starch (only mechanically or thermally damaged starch)**
- **Release of water**
  - Reduction of dough viscosity/consistency
  - Improved extensibility
  - May cause stickiness if used in excess
- **Produces “limit dextrins” (branched fragments) and short linear dextrins and finally maltose from linear sections of the starch molecule**
  - Improved browning
  - Improved shelf life
  - Better fermentation
- **Enhanced volume yield and bread aspect**

Dosage Recommendation for Fungal α-Amylase

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- **Minimum dosage (ppm) of Alphamalt VC 5000 (5,000 SKB/g) estimated from Falling Number and extraction rate**

Falling number	Type 405 / 550, 70-75 % extraction	Type 812 / 1050, 80-85 % extraction
220 – 240	20	0
240 – 260	25	0
260 – 280	40	20
280 – 300	45	40
300 – 320	55	45
320 – 350	65	> 55
350 – 380	80	-
>380	> 100	-

- **Strong gluten allows for higher dosages**

Hemicellulases

Pentosanases, Xylanases and Co.

The Family of Hemicellulases

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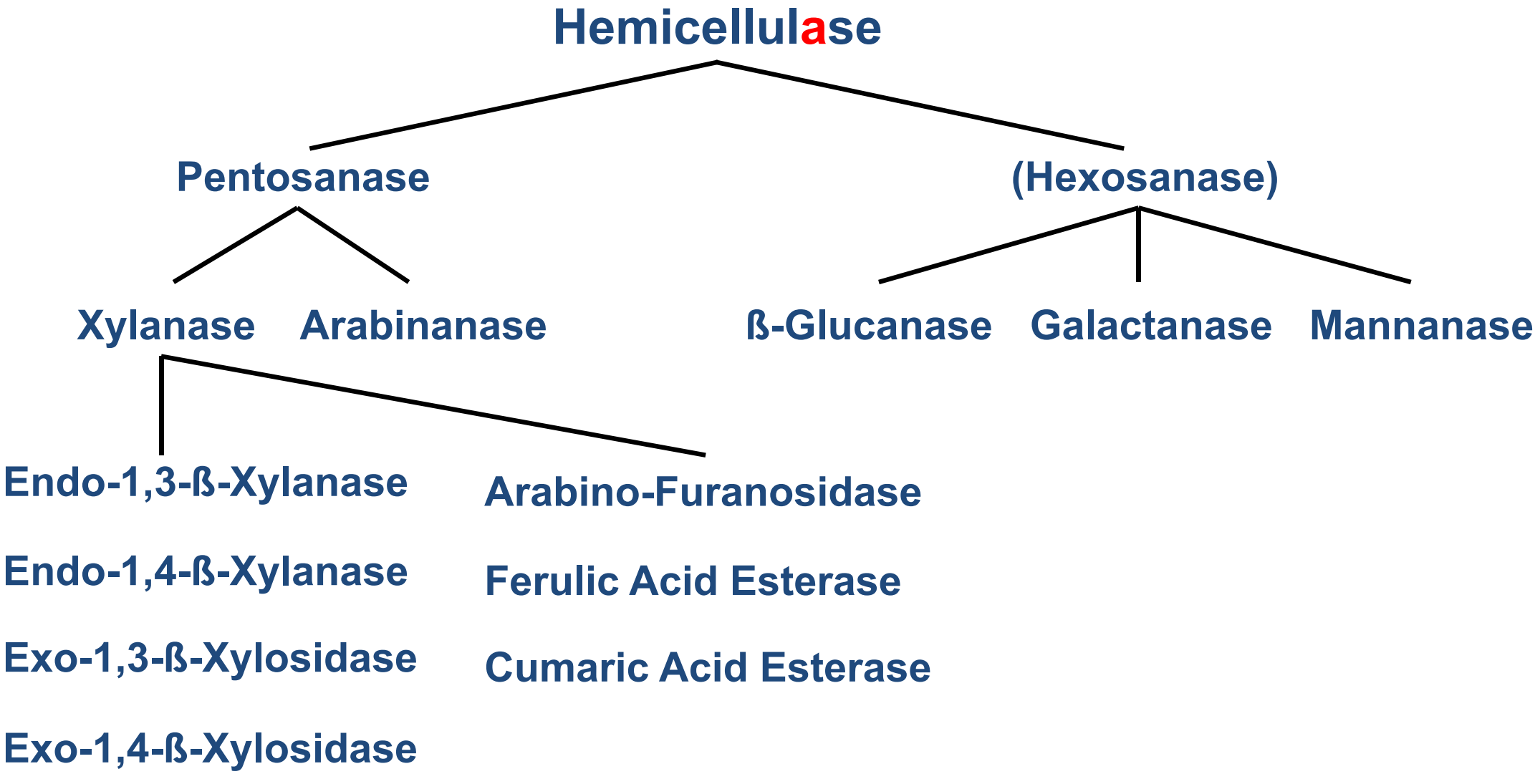

Enzymatic Hydrolysis Sites in Wheat Xylan

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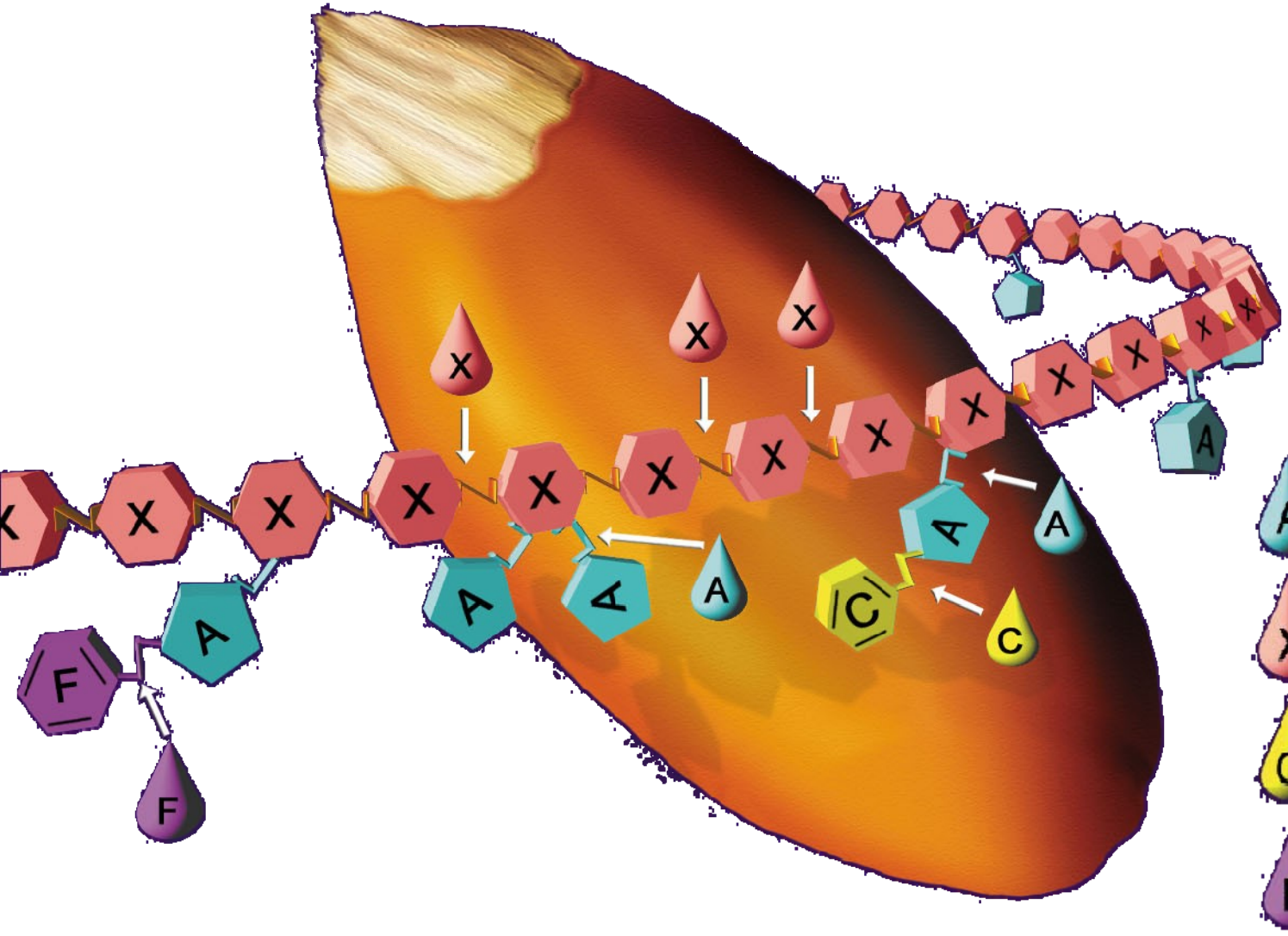


A Arabinose

X Xylose

C Coumaric acid

F Ferulic acid

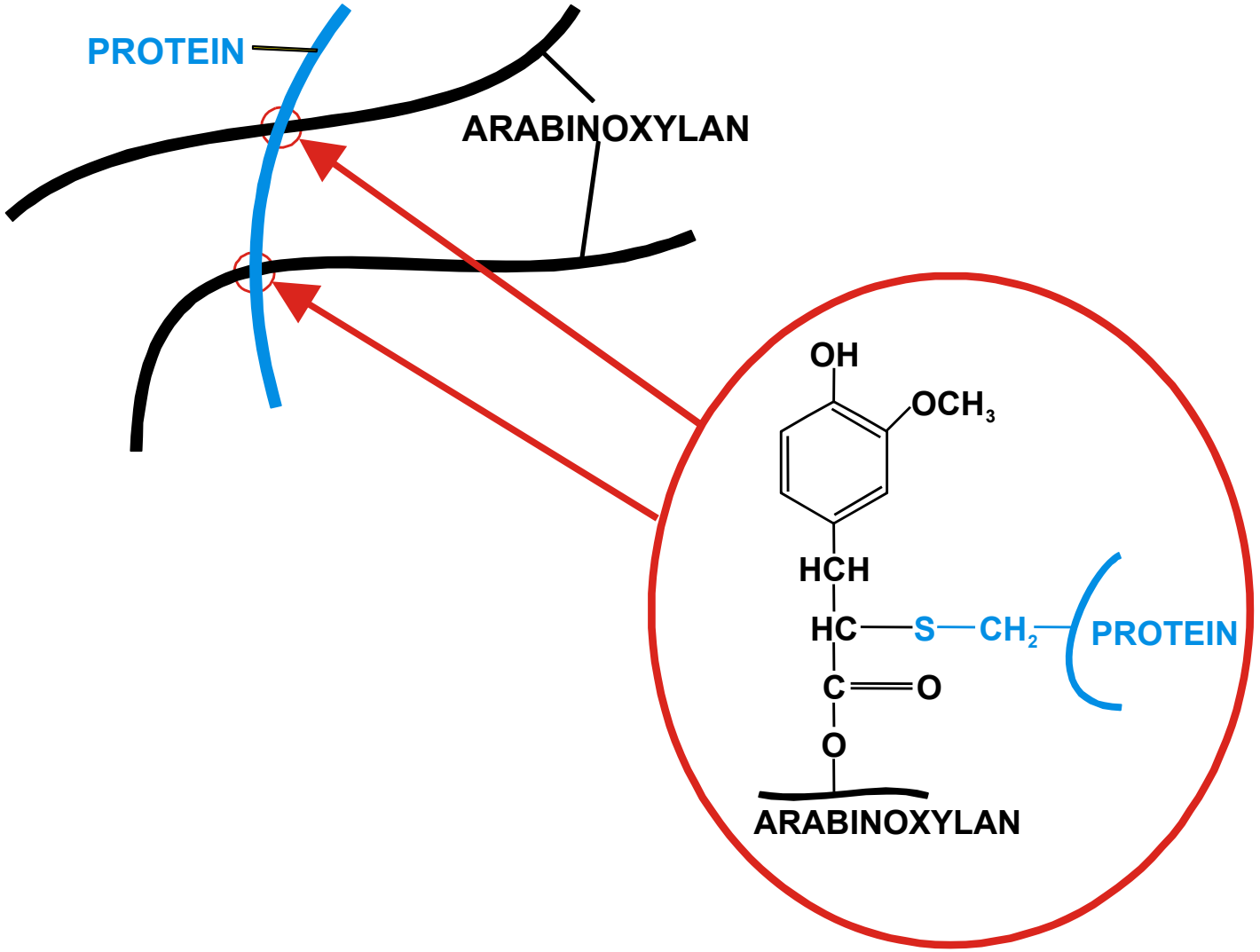
A α-L-Arabinofuranosidase

X Endo-1,4-ß-xylanase

C Coumaric acid esterase

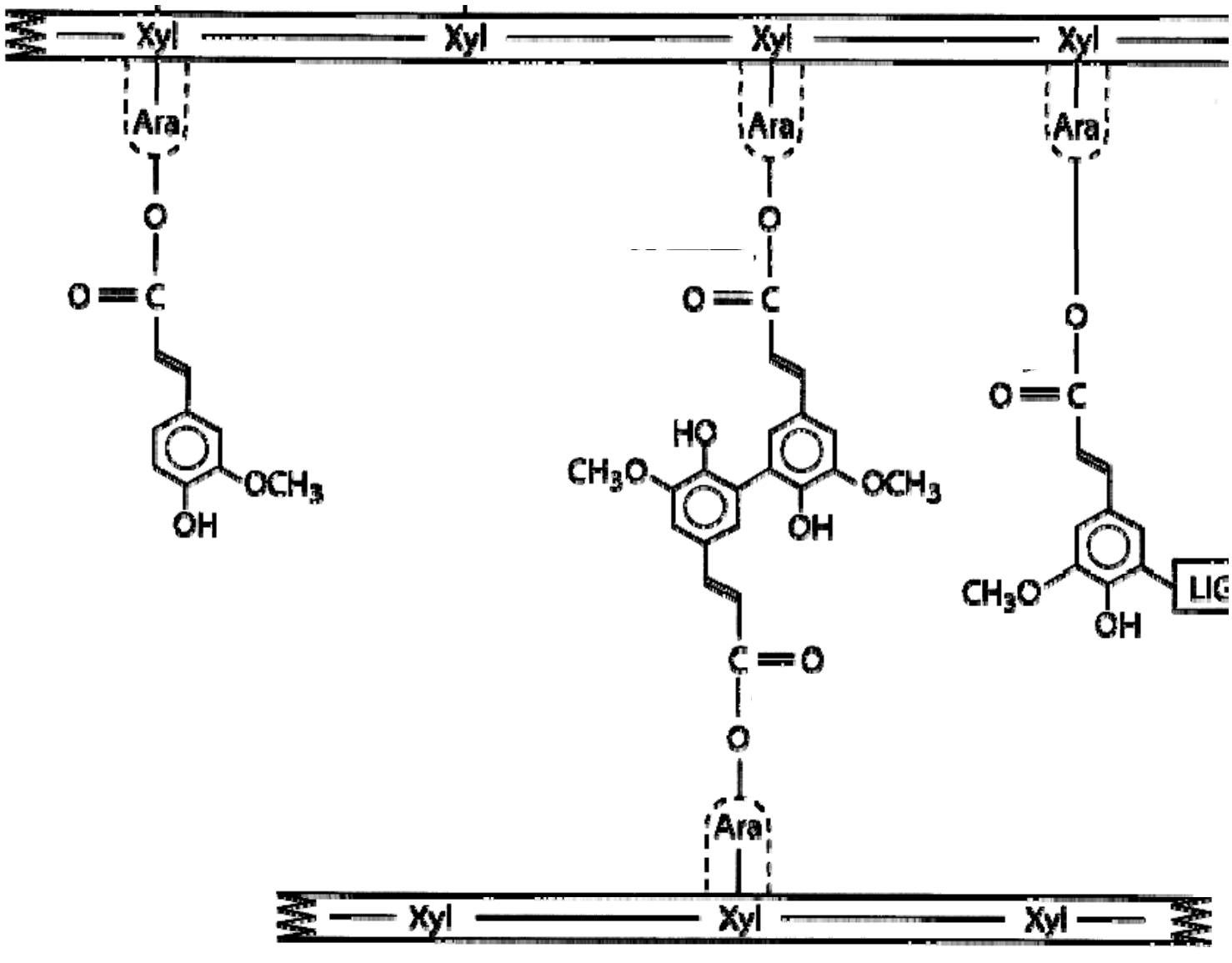
F Ferulic acid esterase

Cross-Linking of Gluten and Hemicellulose



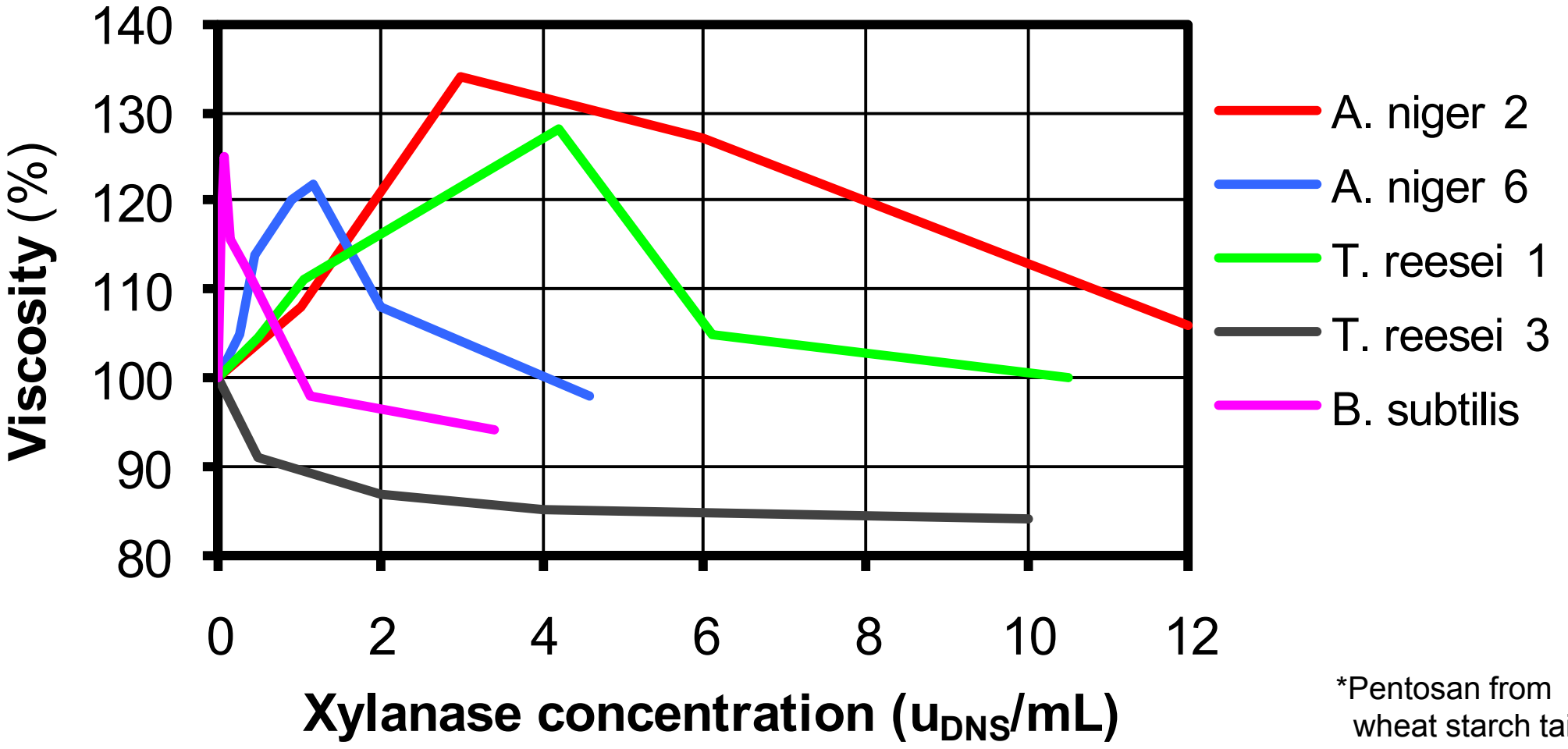
Adapted from Hoseney & Faubion, 1981

Interaction of Feruloyl Side-Chains in Arabinoxylan



Modif. from Williamson *et al.*, 1998

Effect of Various Xylanases on Pentosan* Viscosity



*Pentosan from wheat starch tailings

Summary of the Effects of Xylanases

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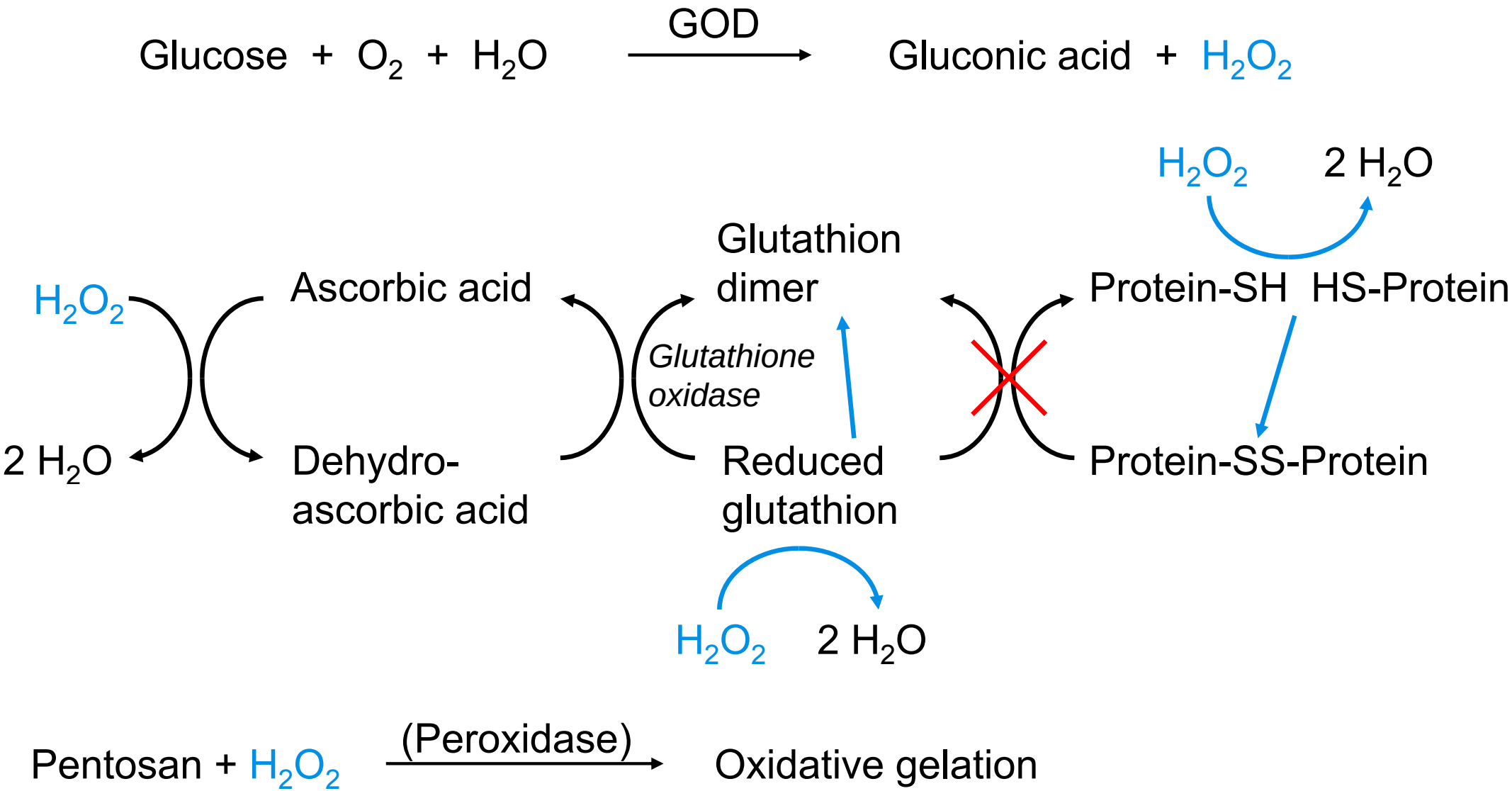
- **Break down xylan backbone**
- **Soften gluten-xylan network**
- **Hydrolyse soluble and insoluble pentosans**
  - initial increase of water absorption → dough drying
  - release of water → softening of gluten
- **Improve extensibility**
- **Dough softening**
- **Volume increase of baked goods**
- **Can be used to achieve finer or coarser crumb**
- **May cause stickiness if not suitable or overdosed**

Oxidases

Some Oxidizing Enzymes

- Glucose oxidase
- Galactose oxidase
- Hexose oxidase
- Sulfhydryl oxidase
- Phenoloxidase (laccase)
- Peroxidase
- Katalase

Effects of Glucose Oxidase in Dough



Effect of Glucose-Oxidase on Dough Development

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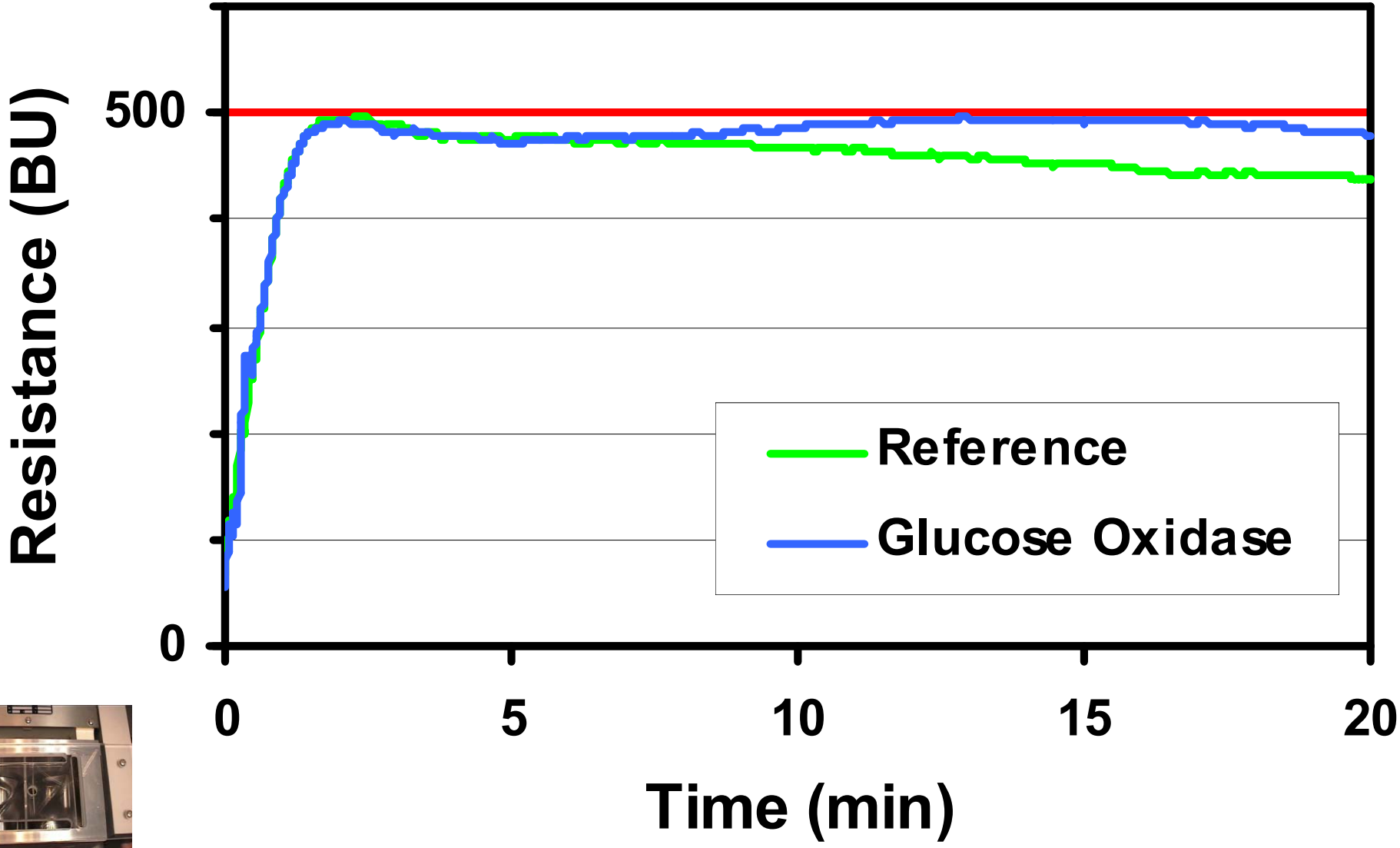

Glucose Oxidase in German Breakfast Rolls

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Stress test by over-proof of dough pieces

Wheat flour: German soft wheat; rolls

Summary of the Effects of Oxidases

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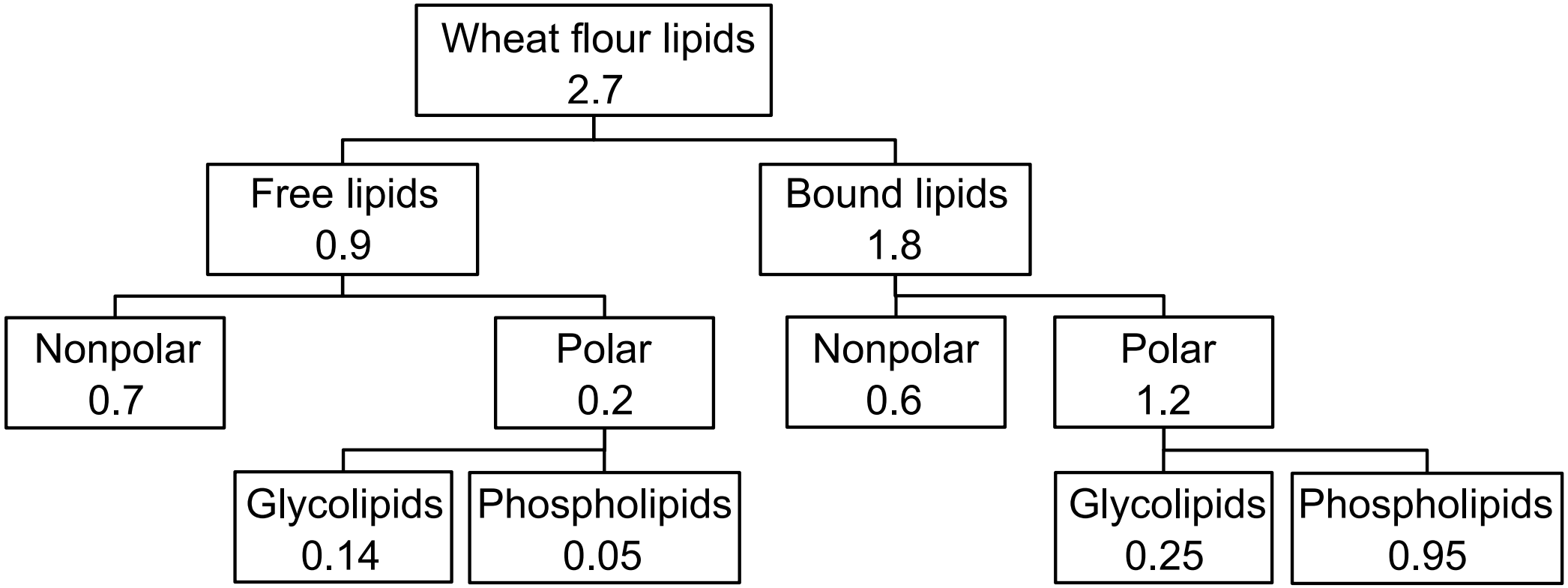
- Create hydrogen peroxide
- Cause cross-linking of proteins and pentosans
- “Inactivate” softening (reducing) substances such as cysteine or glutathione
- Increase water absorption
- Result in dryer dough surfaces and hence better handling properties
- Improve the opening of the cut, f.i. of baguette
- Improve dough stability
- Help to preserve the dough shape in long fermentations

Carboxylester Hydrolases

Lipase, Phospholipase, Galactolipase & Co.

Simplified Classification and Distribution of the Main Lipids in Wheat Flour (averages; % d.s.)



Modif. from Pomeranz & Chung, 1978, using data from Chung & Ohm, 2009

Action of Lipolytic Enzymes

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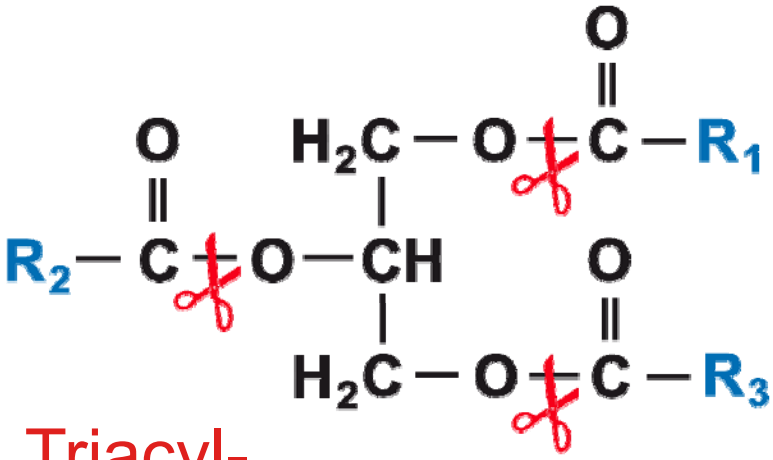
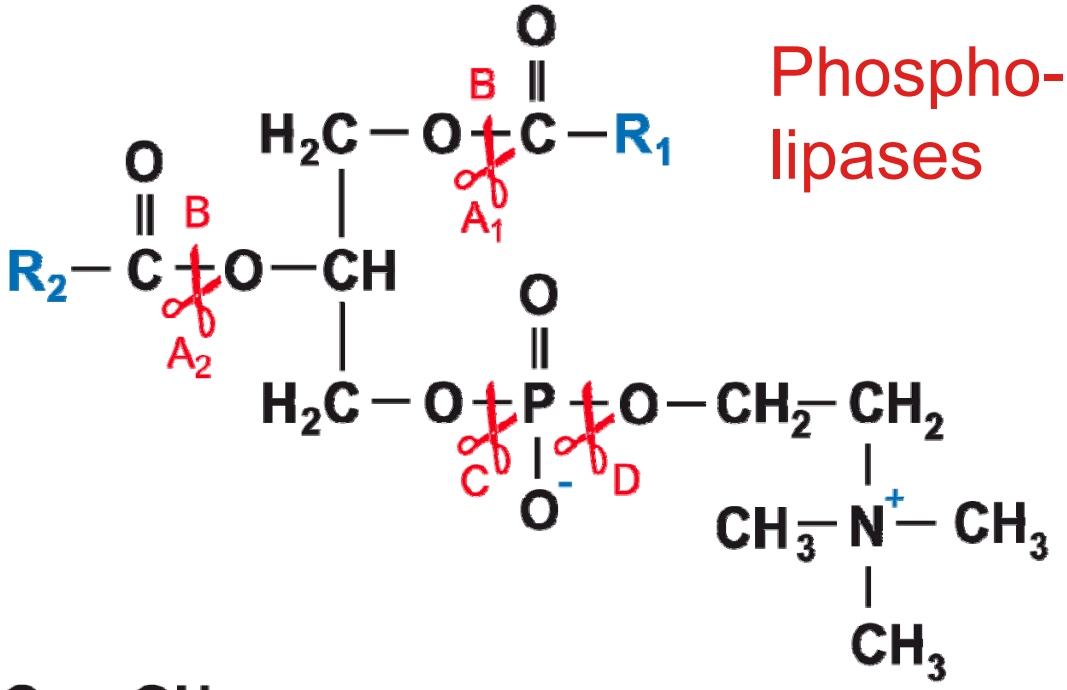
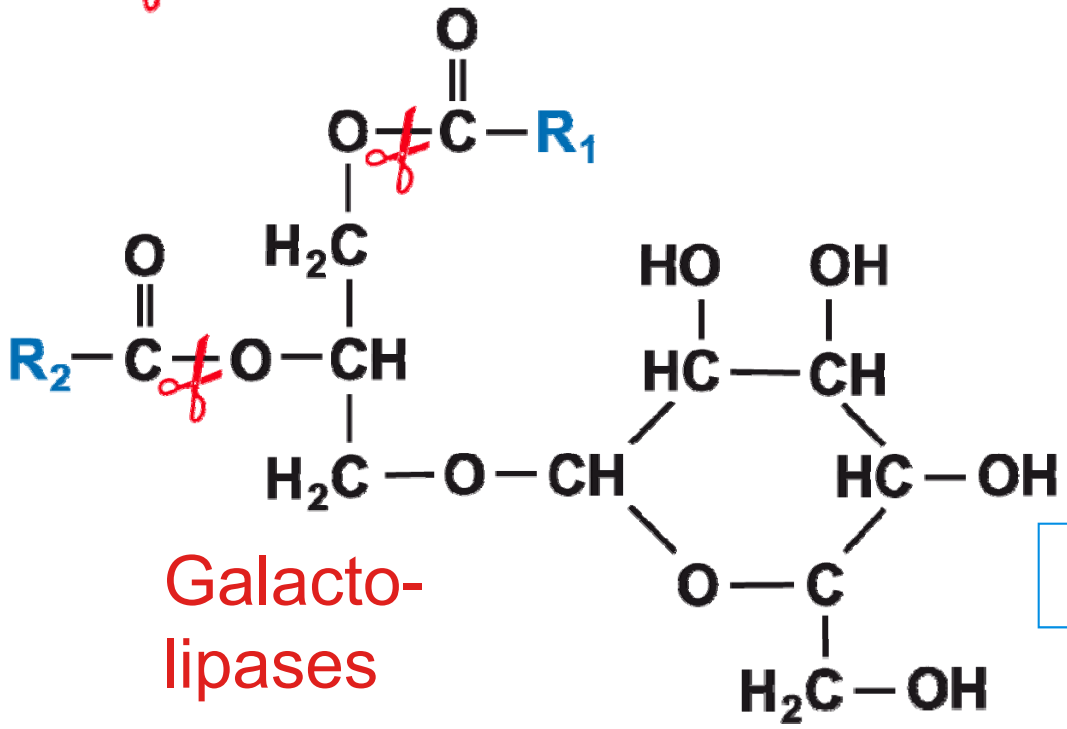


Triacyl-lipases



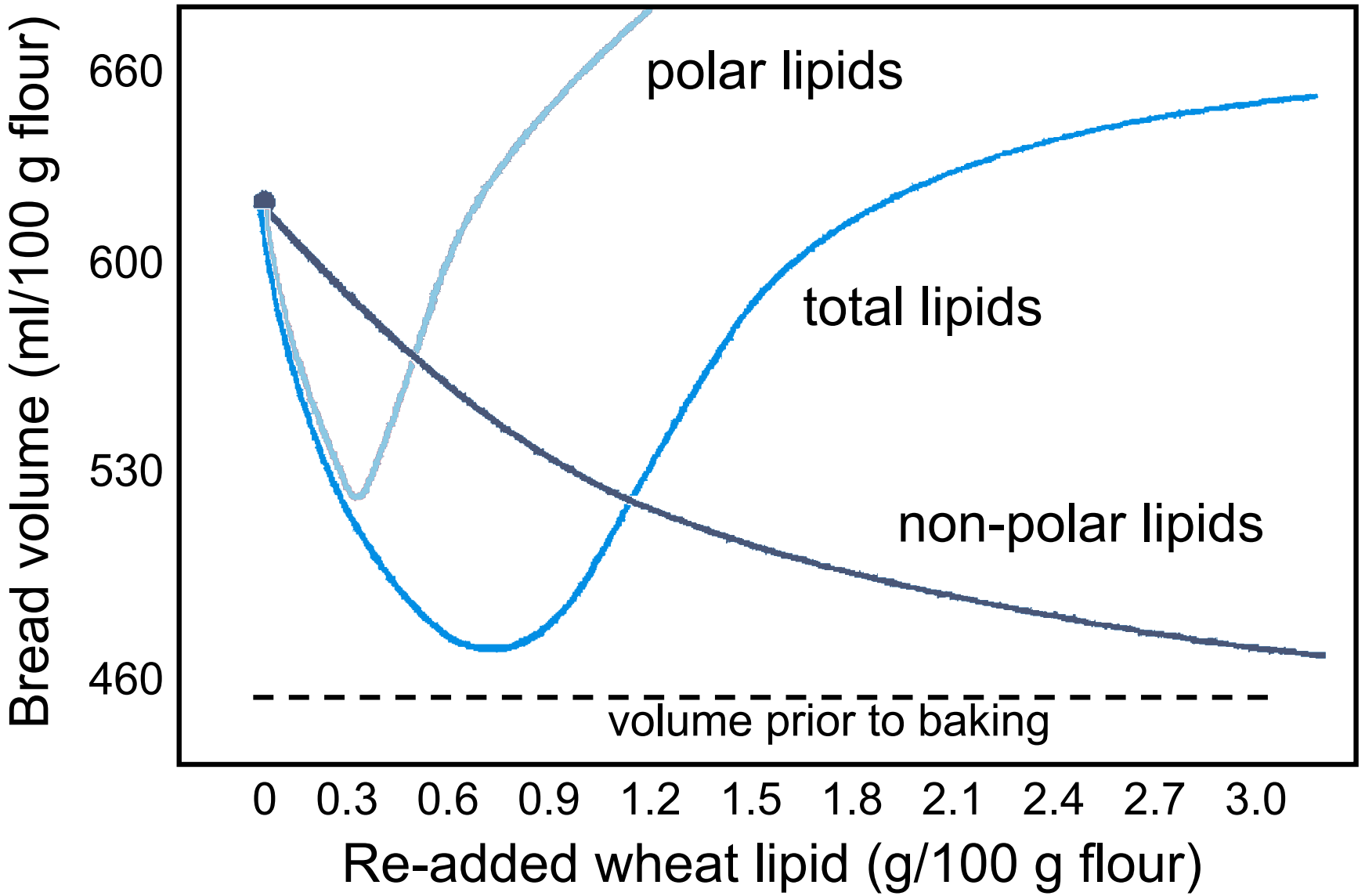
Phospho-lipases



Galacto-lipases

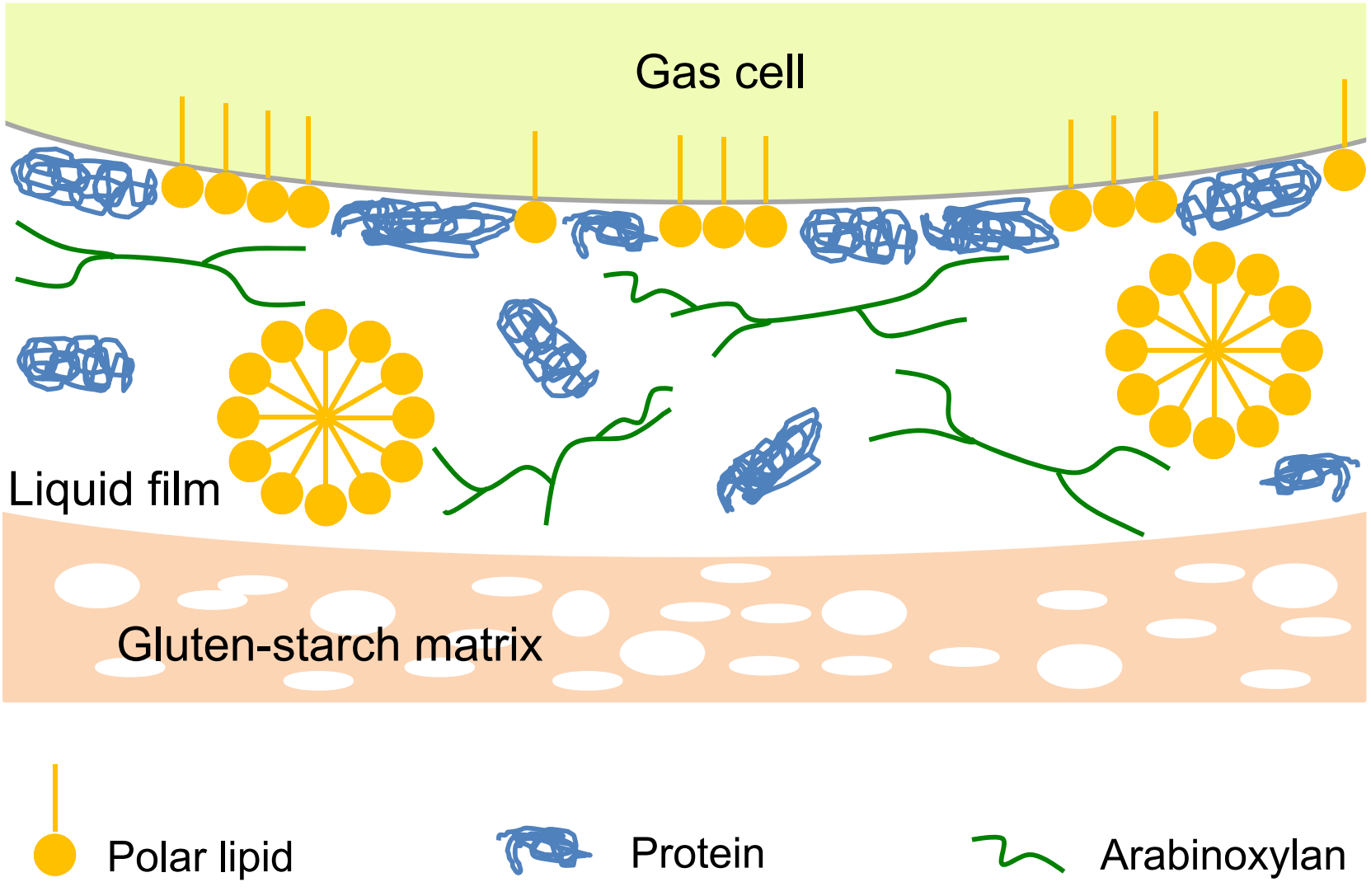
R_1, R_2, R_3 = fatty acid residues

Effect of Wheat Lipids on Volume Yield of Defatted Wheat Flour



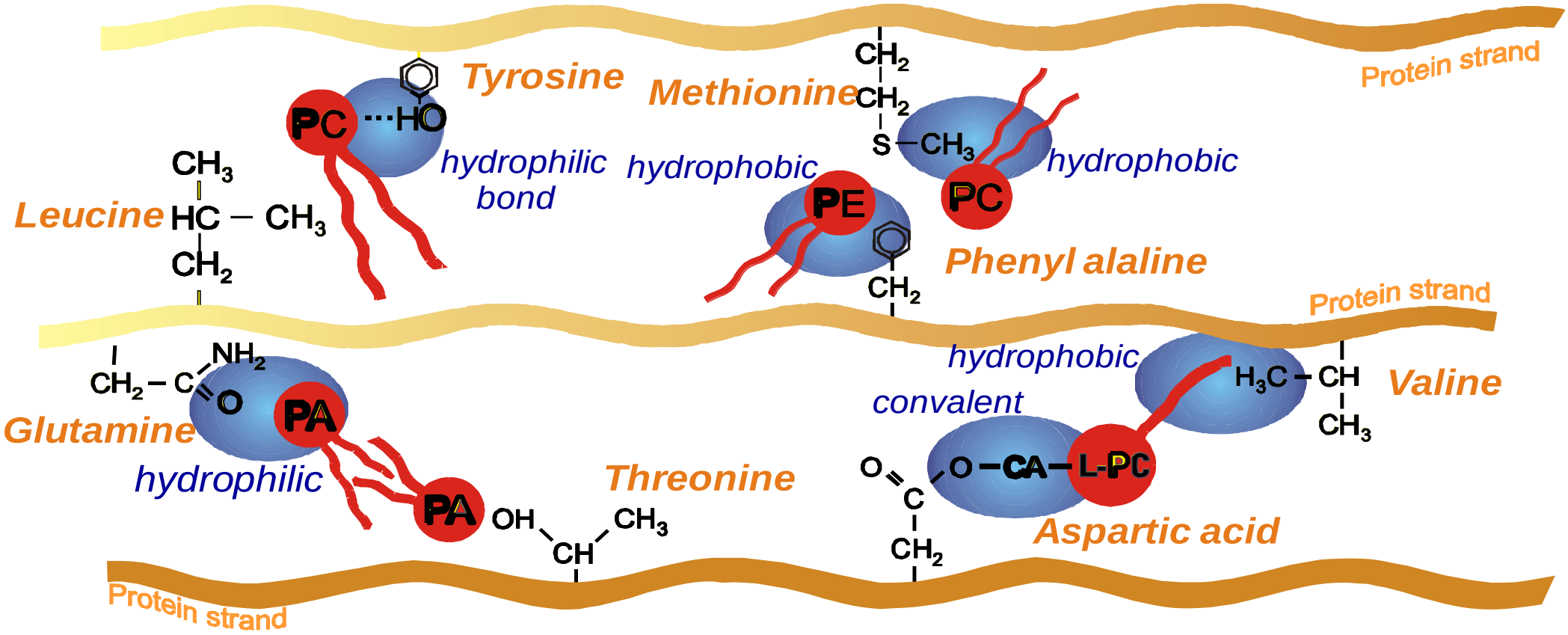
Modif. from MacRitchie & Gras, 1973

Gas Cell Stabilization by Proteins, Lipids and Arabinoxylans



Modif. from Sroan and MacRitchie, 2009

Formation of Lipoprotein Complexes



Summary of the Properties of Carboxylester Hydrolases

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- Produce emulsifier-like substances from lipids
- Enhance dough stability
- Increase volume yield
- Result in finer pore structure
- Enhance crumb whiteness be physical (shallower shadows) and chemical (indirect bleaching) effects
- Improvement of initial crumb structure & bread volume →
- Improved crumb softness after storage
- May cause off-flavour if not compatible with involved lipids

Enzymes Résumé

Carboxyl Esterase Boosts the Baking Results

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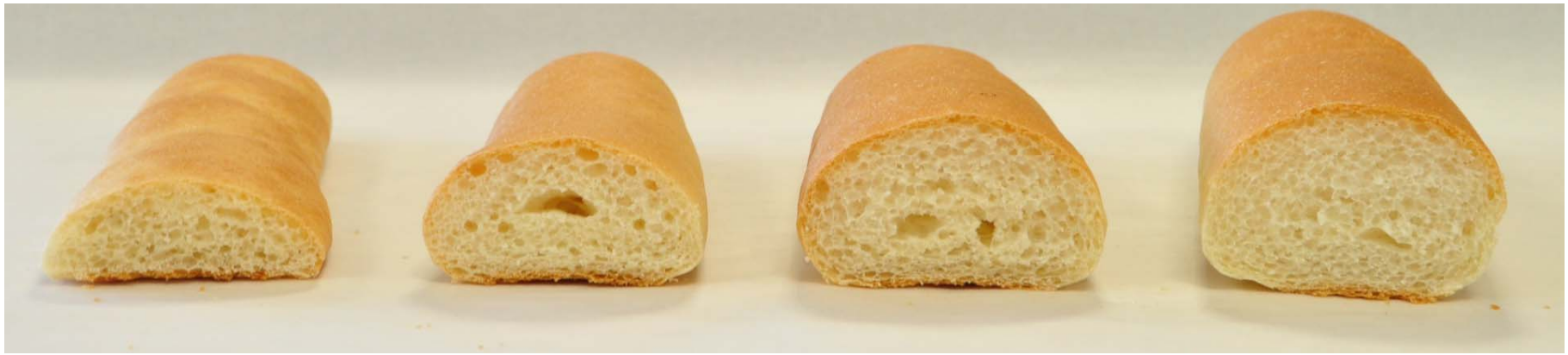
ELCO C 100K:	Ascorbic acid, 100 %
Alphamalt A 15140:	Amylase, 140,000 SKB/g
Alphamalt HC 13045:	Hemicellulase
Alphamalt Gloxy 14080:	Glucose oxidase
Alphamalt EFX Mega:	Carboxyl esterase



Reference	ELCO, 50 ppm A 15140, 10 ppm	ELCO, 50 ppm A 15140, 10 ppm HC 13045, 30 ppm	ELCO, 40 ppm A 15140, 10 ppm HC 13045, 30 ppm Gloxy 14080, 20 ppm EFX Mega, 10 ppm

Typical Effects of Enzymes on Bread Quality
used at common dosages

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Enzyme	WA[1]	Volume[2]	Stability[3]	Cut[4]	Colour[5]	Crumb[6]	Shelf-life[7]
α-Amylase, fungal	o	++	-	+	+	-	+
α-Amylase, cereal	-	+	--	-	++	--	+
α-Amylase, bacterial	-	(+)	(-)	o	o	-	+
α-Amylase, maltogenic	o	o	o	o	o	o	++
$Xylanase_{WUX}$	+	++	+	+	o	+	(+)
$Xylanase_{WEX}$	-	+	-	-	o	-	o
Protease	o	(+)	(+)/-	+	o	(-)	o
Oxidase	++	+	++	++	o	+	(+)
Carboxylesterases	+	++	+	+	o	++	+
Transglutaminase	o	o	+	+	o	o	o

(1) Water absorption (2) Baking volume yield (3) Shape stabilty (4) Opening of the cut, shred (5) Crust colour (6) Crumb fineness (7) Non-microbial shelf-life

Enzymes Suggested for Bread and Flour Improvers

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Enzyme	Claimed Effect
α-Amylase	Energy supply for yeast, dough viscosity, shelf life
Amyloglucosidase (glucoamylase)	Energy supply, colour, flavour
Ascorbate & amino acid oxidase	Gluten strengthening
Branching enzyme (glucotransferase)	Water binding
Cellulase	Water binding
Furanosidase, arabinofuranosidase	Dough structure, water binding
Ferulic & cumaric acid esterase	Dough structure, water binding
Glutathion oxidase	Gluten strengthening
Glycolipase, galactolipase	Dough stability & volume yield
ß-Glucanase	Structure, liquefaction
Glucose / galactose / hexose oxidase	Gluten strengthening
Hemicellulase, xylanase, pentosanase	Dough structure, water binding, volume yield
Laccase, monophenol oxidase	Dough strengthening
Lipase (triacyl lipase)	Flavour, emulsification, dough stability & vol. yield
Lipoxygenase, lipoxidase	Dough structure, decolorization
exo-Peptidase	Colour, flavour
Peroxidase	Gluten strengthening
Phospholipase	Pore structure & volume yield
Polyphenol oxidase	Gluten strengthening
Protease, proteinase, peptidase	Protein relaxation, liquefaction
Pullulanase	Structure, water binding
Sulfhydryl oxidase & transferase	Gluten strengthening
Transglutaminase	Protein cross-linking, gluten stabilization

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Thank you very much for your attention!