Enzymatische Modifikationen von Lebensmittelinhaltsstoffen: Verfahren zur Beeinflussung des Produktdesigns

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Global enzyme markets

($ Millions)

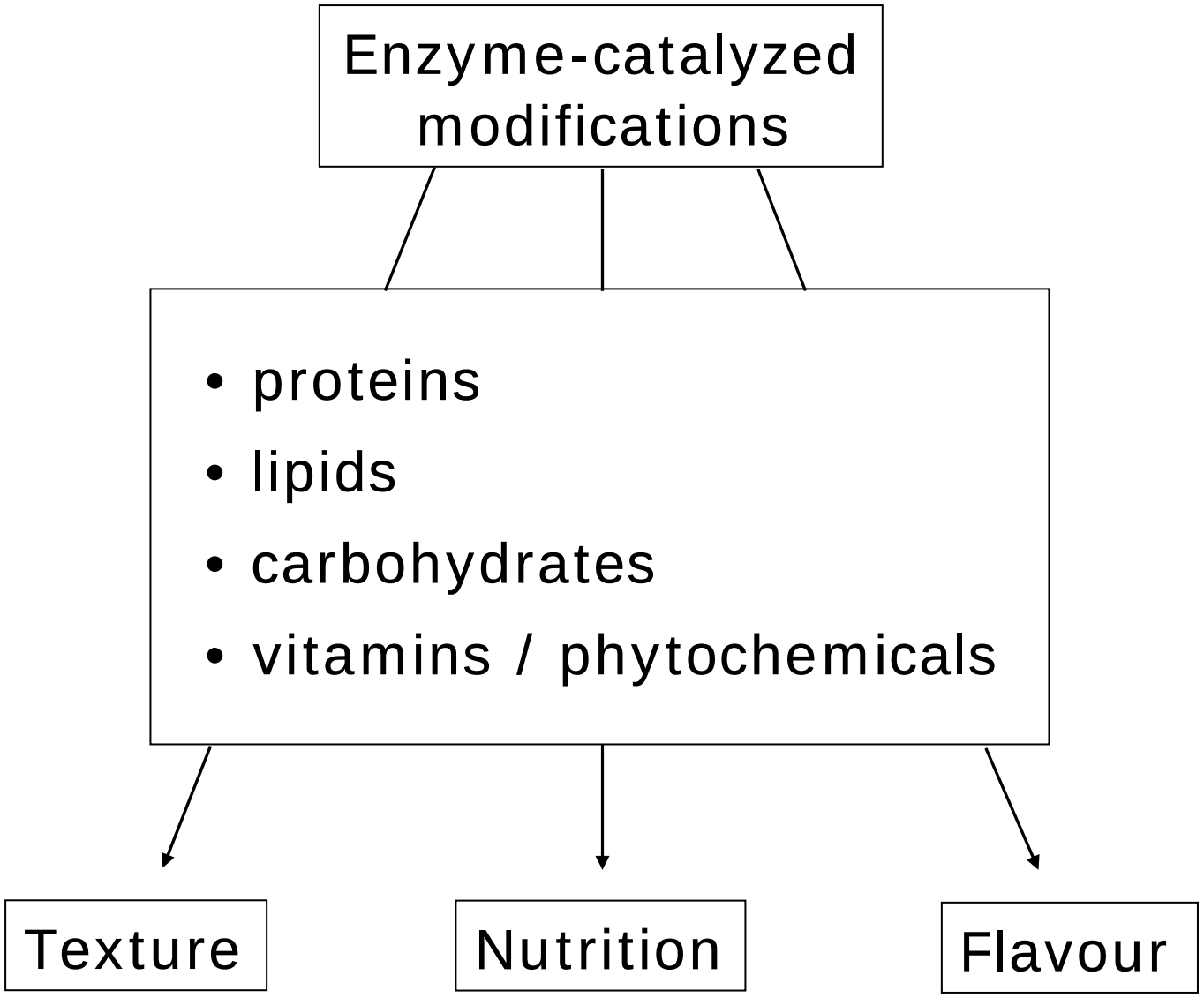
	2005	2006	2007	2012
Technical Enzymes	1,075	1,105	1,140	1,355
Food Enzymes	775	800	830	1,010
Animal Feed Enzymes	240	260	280	375
Total	2,090	2,165	2,250	2,740

Source: BCC, Inc.

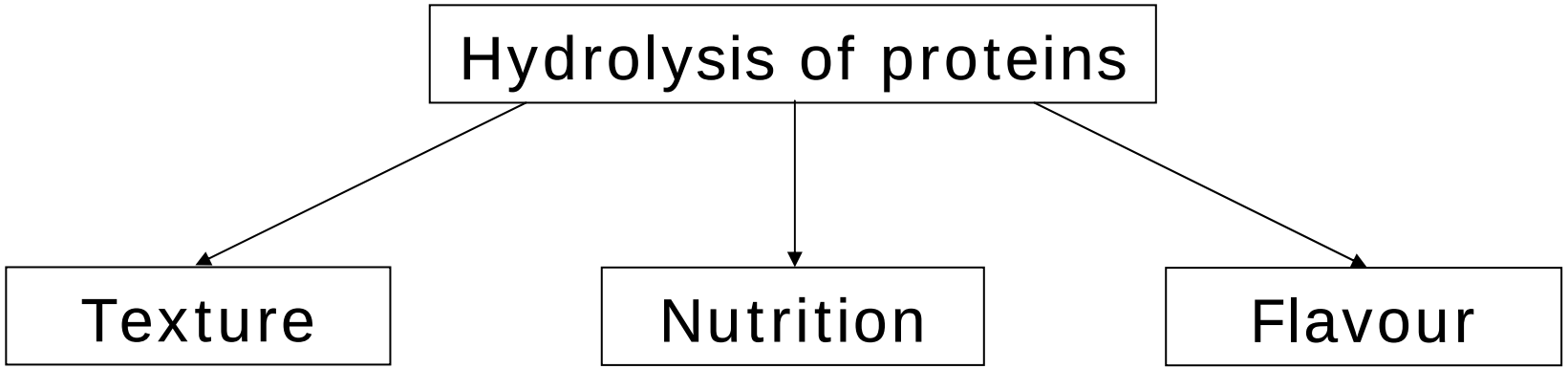
Industrial enzyme market

Conversion / enzyme type	Sales (%)	Main applications
Protein hydrolysis		
Alkaline proteases (e.g. subtilisin)	37.0	Detergents
Rennets (calf, microbial)	10.5	Cheesemaking, dairy
Plant proteases (papain, bromelain)	0.7	Food processing
Pancreatic proteases	1.0	Leather processing
	49.2	
Carbohydrate conversion		
Amylases	16.7	Starch processing, baking, brewing, textile, detergents
Pectinases	3.0	Beverages
Cellulases	15.3	Detergents, textile, baking
Xylanases / β-glucanases	4.5	Animal feed processing, brewing
Glucose isomerase	2.0	Food and beverages
Pullulanases	0.5	Starch
Phytases	1.2	Animal feed processing
Lactases	0.9	Dairy
	44.1	
Fat hydrolysis		
Lipases	7.0	Fat processing, dairy, detergents, organic synthesis

Food design


Enzyme-catalyzed modifications
• proteins
• lipids
• carbohydrates
• vitamins / phytochemicals
Texture
Nutrition
Flavour

Proteases



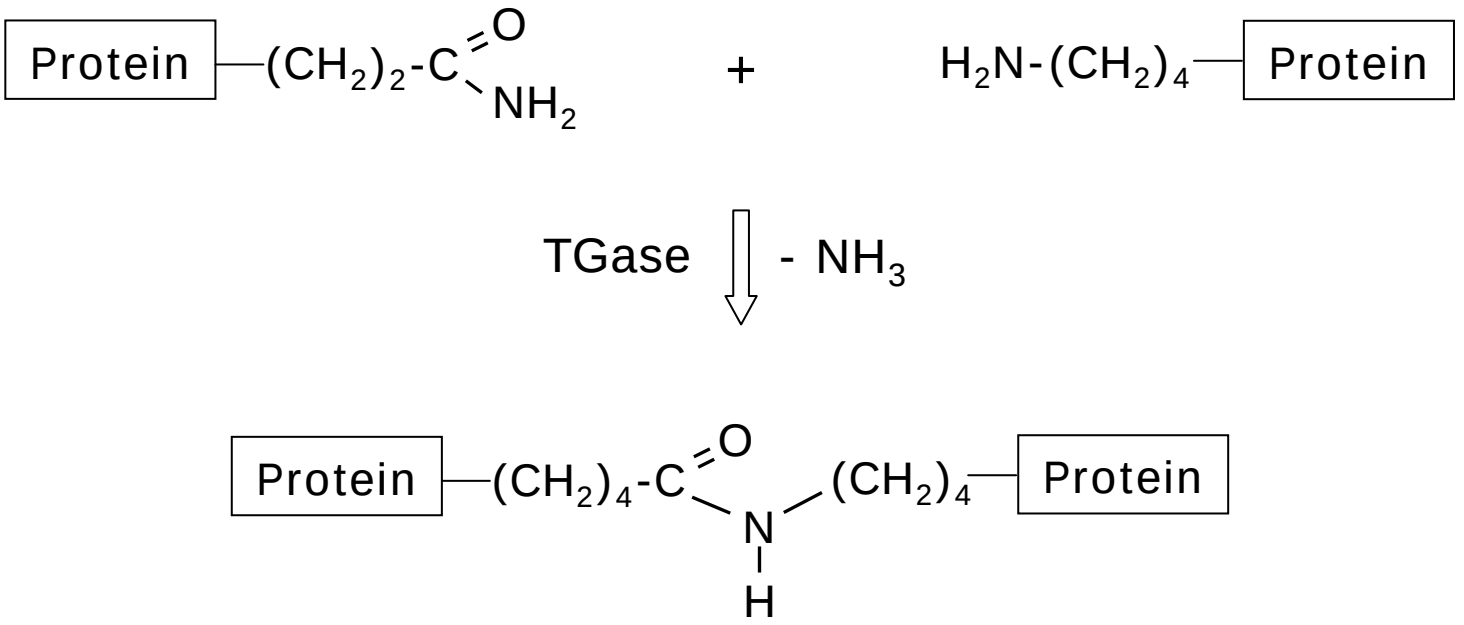
- Coagulation
- Foaming
- Water retention
- Emulsification

- Fat replacer
- Bioactive peptides
- Hypoallergenic foods

- Hydrolyzed vegetable protein
- Yeast extracts

Crosslinking of proteins

- Formation of isopeptide bonds



- Microbial Transglutaminase (*Streptoverticillium mobaraense*)

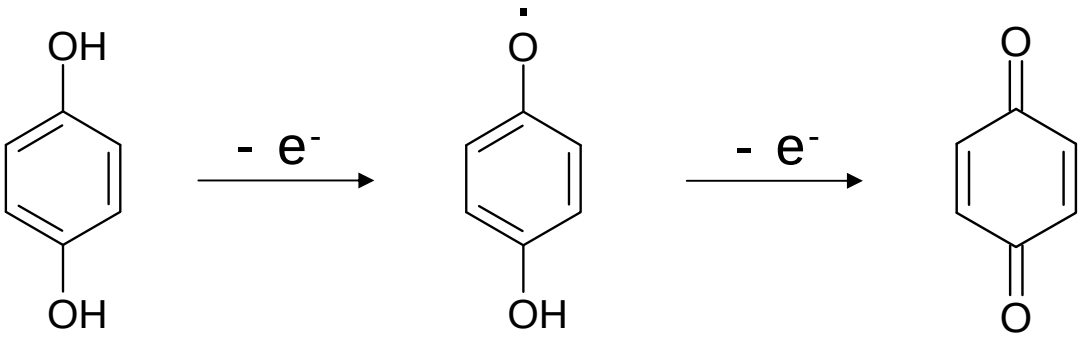
Applications of transglutaminase

- viscosity
- gelation
- water retention
- syneresis

- meat products
  - sausages
  - re-structured meat
- dairy products
  - yoghurt
  - cheese
- bakery products
- pasta

Enzymatic crosslinking

Laccase (*p*-diphenol oxidase)



$$O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O$$

- Wheat arabinoxylan gels (Carvajal-Millan et al., 2005)
- Sugar beet pectin gels (Kuuva et al., 2003)

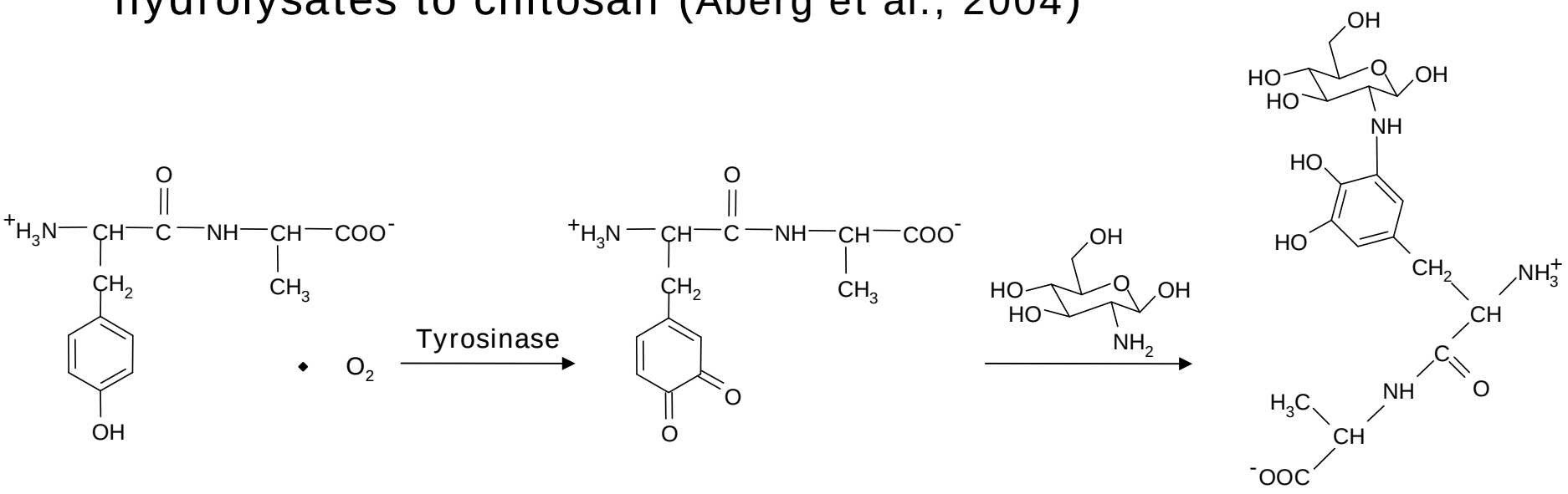
- Gelatin-catechin conjugates (Chung et al., 2003)

Enzymatic crosslinking

Tyrosinase

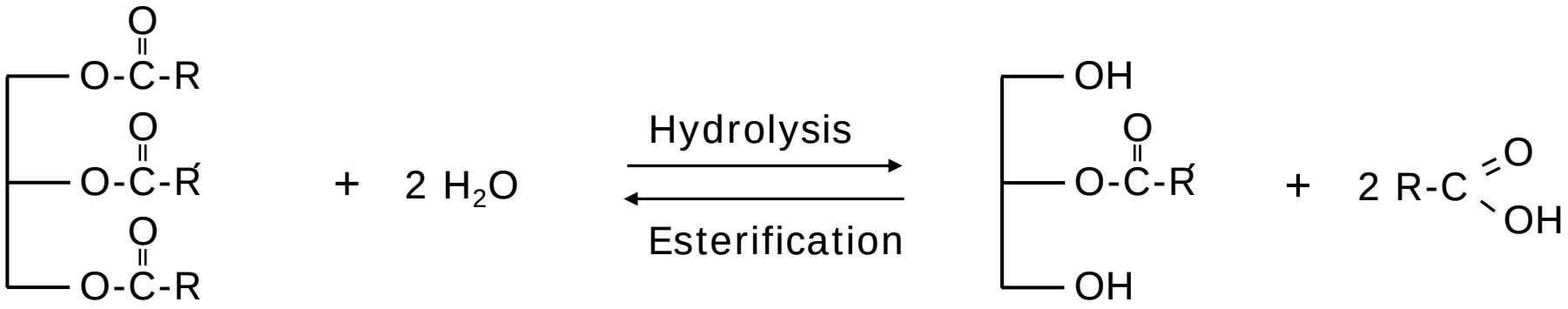
- Oxidation of peptide-bond tyrosine to quinones
- reaction with free –SH and -NH_2 groups
- tyrosine–cysteine and tyrosine–lysine crosslinks

- Enzymatic grafting of peptides from casein hydrolysates to chitosan (Aberg et al., 2004)



Proposed tyrosinase-initiated reaction between glucosamine and Tyr-Ala

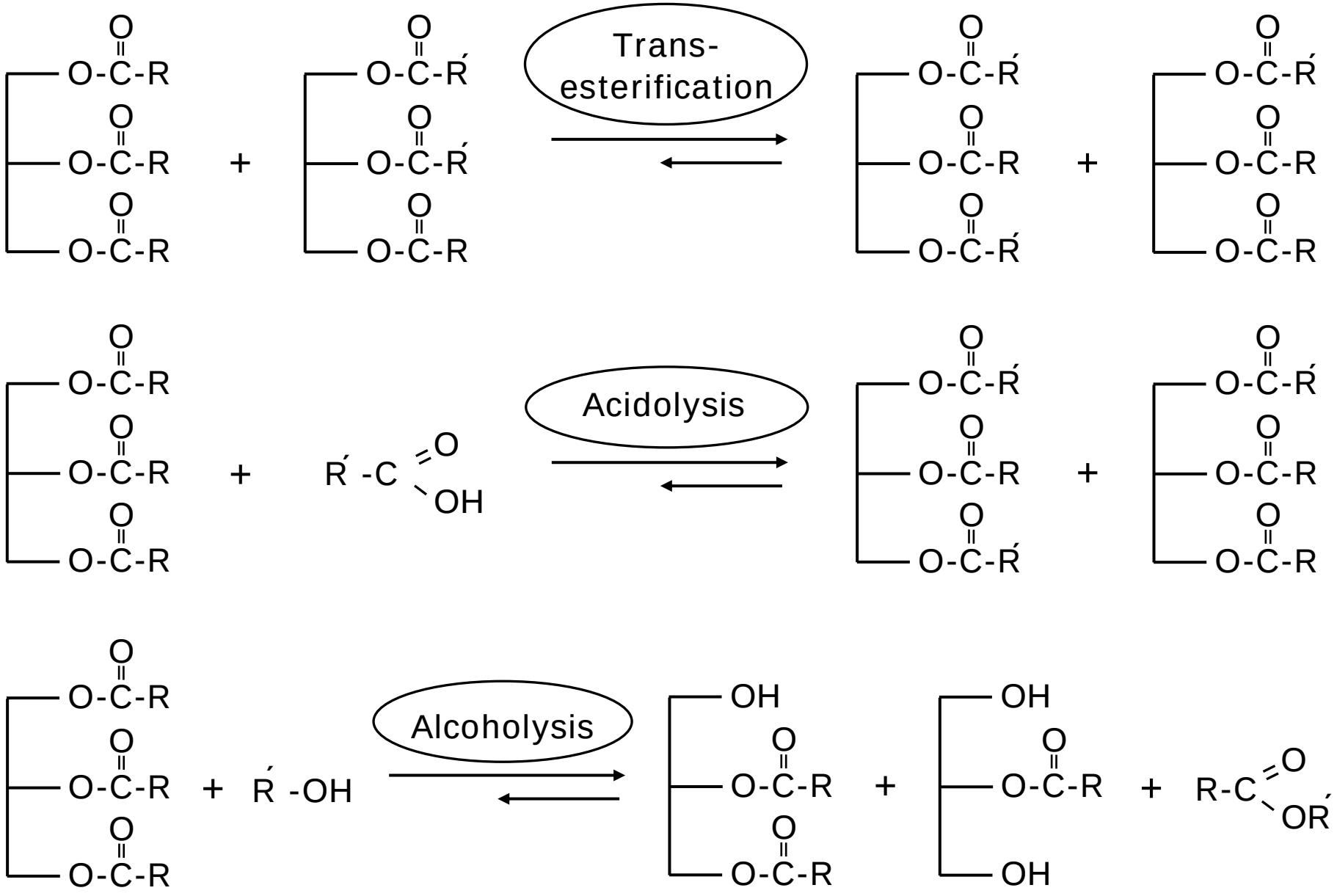
Lipases



Specificities

- position of fatty acid (e.g. 1,3-regiospecific)
- chain length of fatty acid
- number / position of double bonds in fatty acid

Interesterifications (1,3-specific lipases)

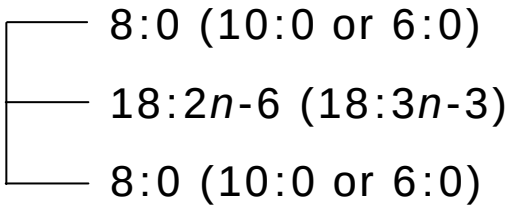


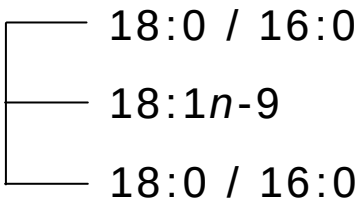
Designer lipids

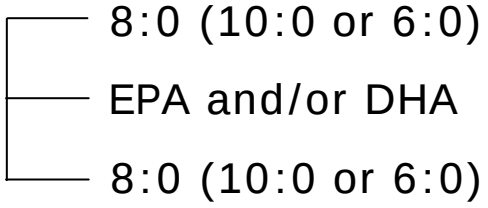
- Structured
  - triglycerides
  - mono-/ diglycerides
  - phospholipids
  - sphingolipids
- Incorporation of
  - short / medium chain fatty acids
  - polyunsaturated fatty acids (EPA, DHA, CLA)
- Positioning of fatty acids

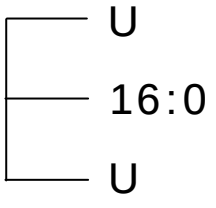
⇨ improved / adjusted nutritional value

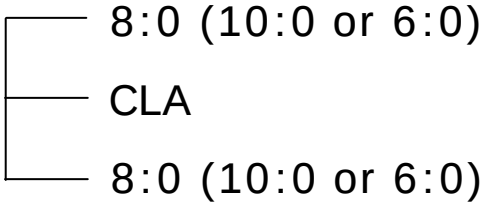
Structured lipids

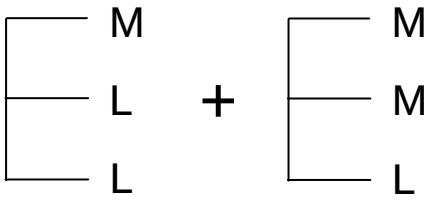












Commercially available immobilized lipases

Name	Supplier	Organism	Specificity	Matrix
Lipozyme TL IM	Novozyme A/S	*Thermomyces lanuginose*, TLL-1	sn-1,3 specific	Silica granules
Lipozyme RM IM	Novozyme	*Rhizomucor miehei*, RML	sn-1,3 specific	Macroporous ion exchange resin
Novozyme 435	Novozyme	*Candida antartica* lipase B	non-specific	Macroporous acrylic resin
Lipase PS-C	Amano	*Pseudomonas cepacia* lipase, PCL	non-specific	Ceramic particles
Lipase AK-C	Amano	*Burkholderia cepacia* lipase (formerly *Pseudomonas fluorescens* lipase PFL)	sn-1,3 specific	Ceramic particles

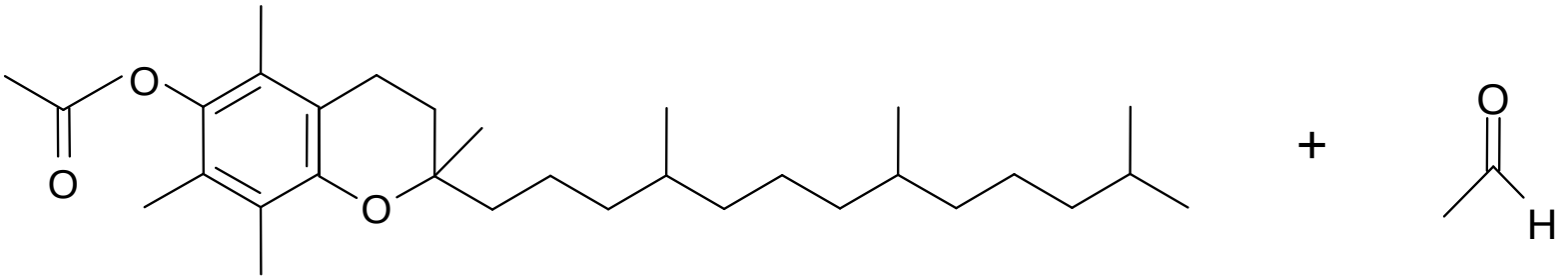
Acetylation of tocopherols

α-tocopherol (25 – 100 mM)

+ vinyl acetate (100 – 400 mM)

C. antarctica
lipase B (Novozym 435)

60°C; solvent: 2-methyl-2-butanol



(30%, 8 days)

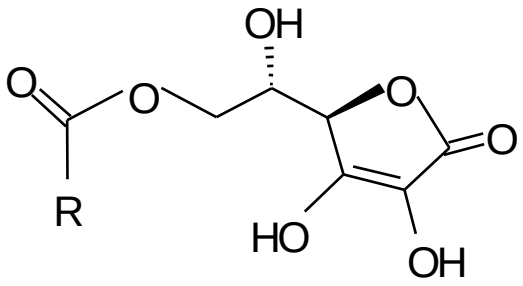
Torres et al. Process Biochemistry *43*, 145, (2008)

Enzymatic modifications of L-ascorbic acid

L-ascorbic acid

+ vinyl palmitate

Candida antarctica lipase B (Novozym 435)



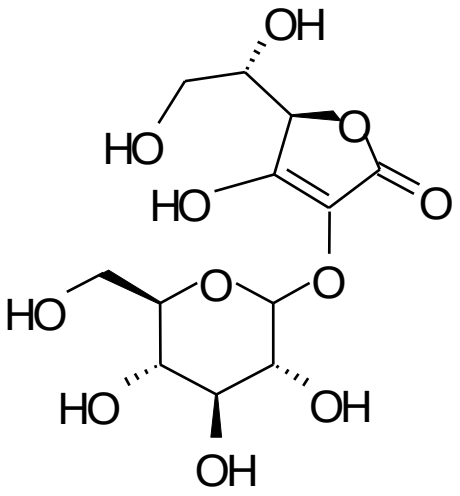
6-*O*-palmitoyl ascorbate

(Torres et al., 2007)

L-ascorbic acid

+ maltotriose

Bacillus stearo-thermophilus maltogenic amylase



ascorbic acid 2-glucoside

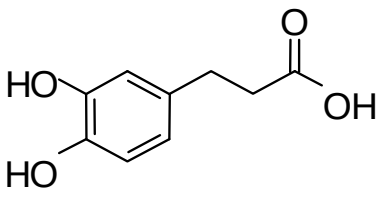
(Bae et al., 2002)

Enzymatic synthesis of fatty acid derivatives of L-ascorbic acid

Acyl donor	Solvent	Biocatalyst	Yield [%]
Methyl palmitate	2-methyl-2-butanol	Novozym 435	68
Phenylbutyric acid	*t*-butanol	Novozym 435	22
Methyl palmitate, EPA and DHA ethyl esters	2-methyl-2-butanol	Novozym 435	≤ 40
Saturated fatty acids	2-methyl-2-pentanol	Novozym 435	≤ 65
Vinyl esters (C8-C16)	*t*-butanol	Chyrazyme L-2	≤ 91
Palmitic acid	hexane	Lipase from *Bacillus stearothermophilus*	≤ 97
EPA	acetone	Chyrazyme L-2	47
L-Methyl lactate	2-methyl-2-butanol	Novozym 435	80
Oleic acid, Palmitic acid	2-methyl-2-butanol	Novozym 435	71-87
Saturated fatty acids (C10-C14)	acetone	Chyrazyme L-2	≤ 60
Oleic acid, Linoleic acid, Linolenic acid	acetone	Chyrazyme L-2	60
Oleic acid	ionic liquid	Novozym 435	61
Oleic acid, Linoleic acid	2-methyl-2-butanol	Novozym 435	≤ 45

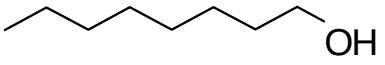
Torres et al. The Open Food Science Journal *2*, 1-9 (2008)

Esterification of phenolic acids



dihydrocaffeic acid

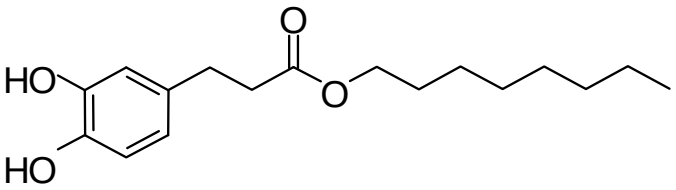
+



octanol

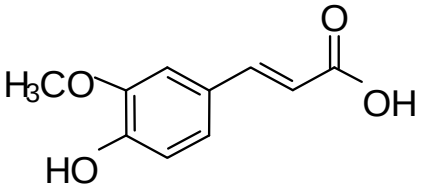
C. antarctica lipase

60 °C, solvent-free



+ H_2O

80%, 8 days



ferulic acid

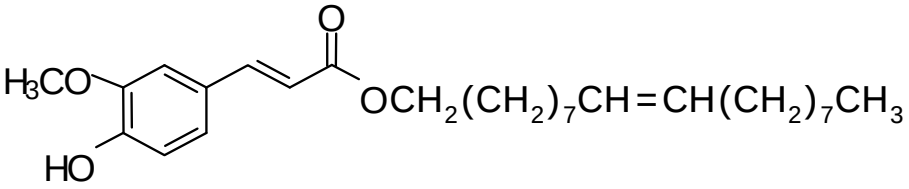
+

$CH_3(CH_2)_7CH=CH(CH_2)_7CH_2OH$

oleyl alcohol

C. antarctica lipase

60 °C, solvent-free

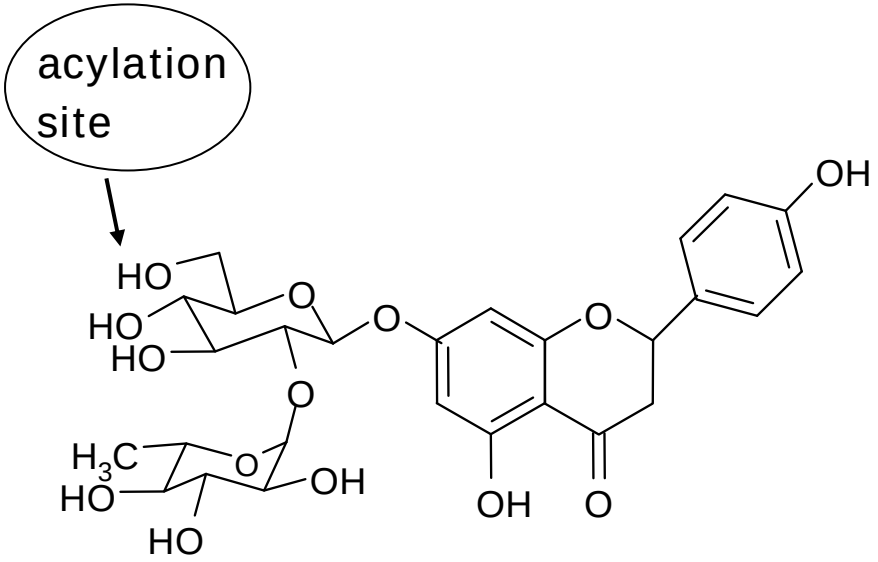


+ H_2O

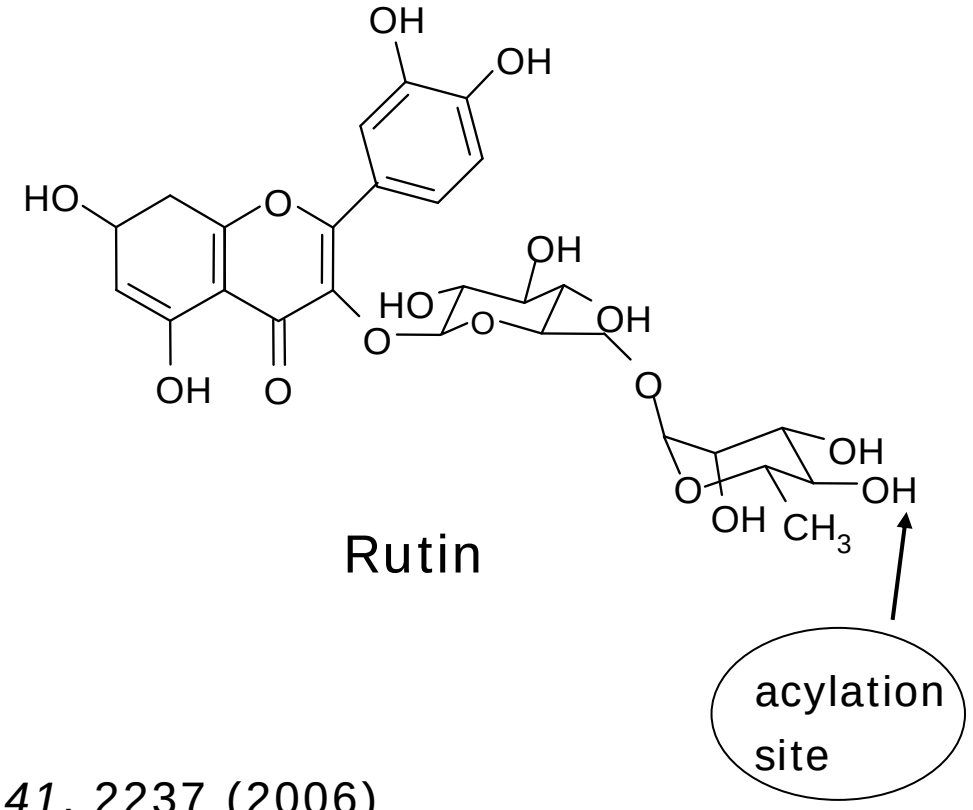
7%, 8 days

Enzymatic acylation of flavonoids

- *C. antarctica* lipase B (Novozym 435)
  - oleic, linoleic, linolenic acid
  - solvents: acetone, *tert*-butanol



Naringin



Rutin

For review: Chetoit et al.
Process Biochemistry *41*, 2237 (2006)

Functional oligosaccharides

Plant cell wall materials

- Arabinoxylans (soy, wheat, sorghum)
- Rhamnogalacturans (apple)
- Arabinan (sugar beet)

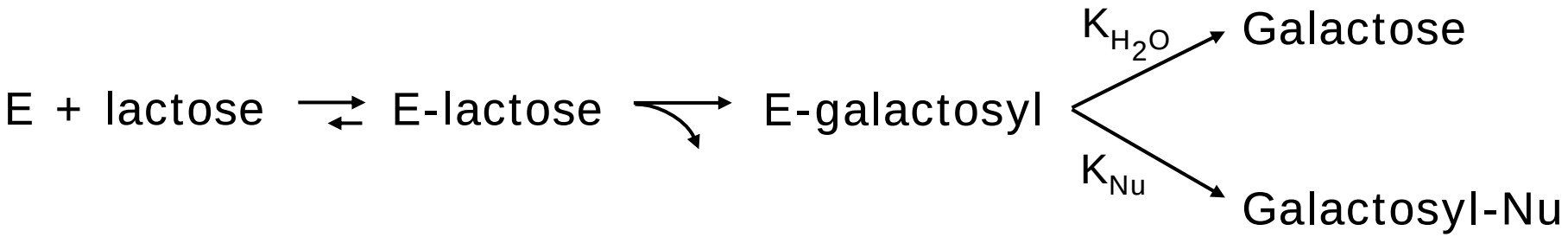
- endo-xylanase (*A. tubigensis*)
- endo-galactanase (*A. aculeatus*)
- rhamnogalacturonase (*A. aculeatus;* Ultra-sp.)
- endo-arabinase (*A. aculeatus*)

→ new types of non-digestible oligosaccharides

Van Laare et al. J. Agric. Food Chem. *48*, 1644 (2000)

β-Galactosidase (lactase)

- hydrolysis of lactose
- formation of galacto-oligosaccharides (GOS)
  - transgalactosylation activity in lactose-concentrated solutions



GOS: α-D-Glu (1→4)-[ß-D-Gal (1-6)]$_n$

n = 2 - 10

Enzymes used for functional oligosaccharide production

Trivial names	Systematic name	Raw material	Functional oligosaccharide
Levansucrase (beta-fructosyl-transferase)	Sucrose:2,6-beta-D-fructan 6-beta-D-fructosyltransferase EC 2.4.1.10	Sucrose	Fructo-
Inulosucrase	Sucrose:2,1-beta-D-fructan 1-beta-D-fructosyltransferase EC 2.4.1.9	Sucrose	Fructo-
Beta-galactosidase	beta-D-Galactoside galactohydrolase EC 3.2.1.23	Lactose	Galacto-
Beta-xylanase	1,4-beta-D-Xylan xylanohydrolase EC 3.2.1.8	Corn cob	Xylo-
Beta-fructofuranosidase	beta-D-Fructofuranoside fructohydrolase EC 3.2.1.26	Sucrose Lactose	Lacto-fructo
Beta-amylase	1,4-alpha-D-Glucan maltohydrolase EC 3.2.1.2	Starch	Isomalto-
Pullulanase	Pullulan alpha-1,6-glucanohydrolase EC 3.2.1.41		
Transglucosidase (Alpha-glucosidase)	alpha-D-Glucoside glucohydrolase EC2.4.1.20		
Isomaltulose synthase	Sucrose glucosylmutase EC 5.4.99.11	Sucrose	Isomaltulose (palatinose)
Endoinulinase (inulinase)	2,1-beta-D-Fructan fructanohydrolase EC 3.2.1.7	Inulin	Inulo
Dextransucrase	Sucrose:1,6-alpha-D-glucan 6-alpha-D-glucosyltransferase EC 2.4.1.5	Sucrose	Isomalto-

Selection / optimization of enzymes

- Isolation of enzyme variants from organisms living in appropriate environments (extremophiles)
- Recombinant DNA technology
  - heterologous expression in suitable host
- Rational protein design
- Directed evolution

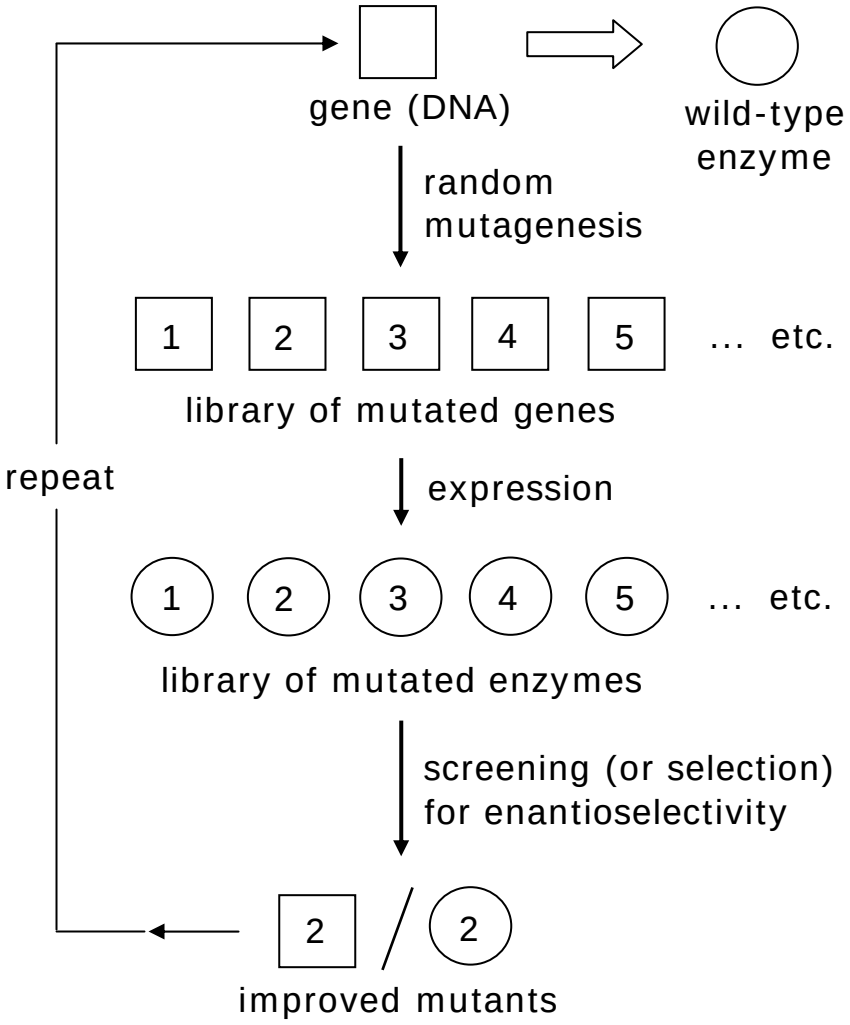
Protein engineering

- Rational protein design
  - development of a molecular model on protein structure and function
  - generation of mutants by site-directed mutagenesis
  - transformation of vectors into host organisms
  - expression, purification and analysis of recombinant protein

Directed evolution

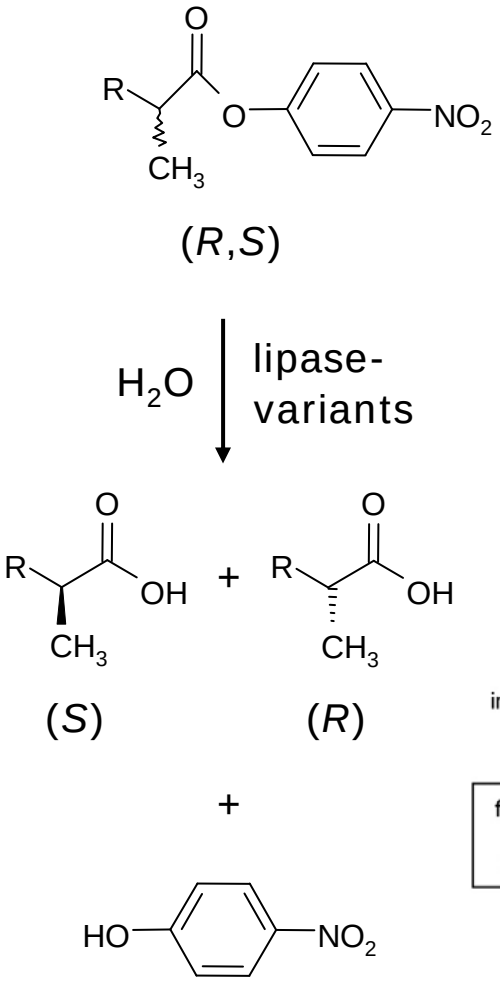
- Diversification
  - random mutagenesis (error prone PCR)
  - fragmentation
  - reassembling (DNA shuffling)

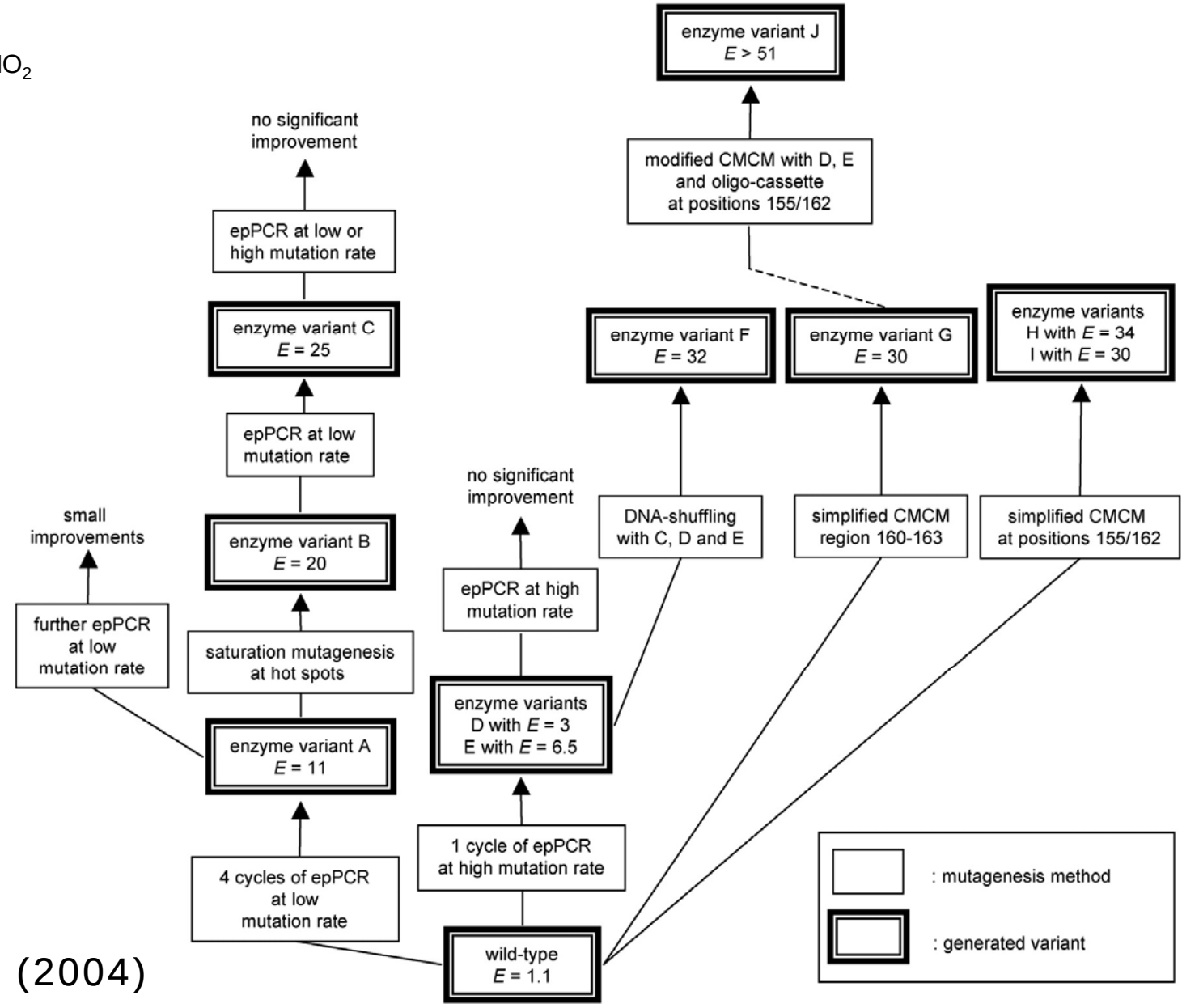
  ⇨ mutant library
- Selection and screening
- Amplification



Reetz, M. T.
PNAS *101*, 5716 (2004)

Directed evolution of enantioselectivity





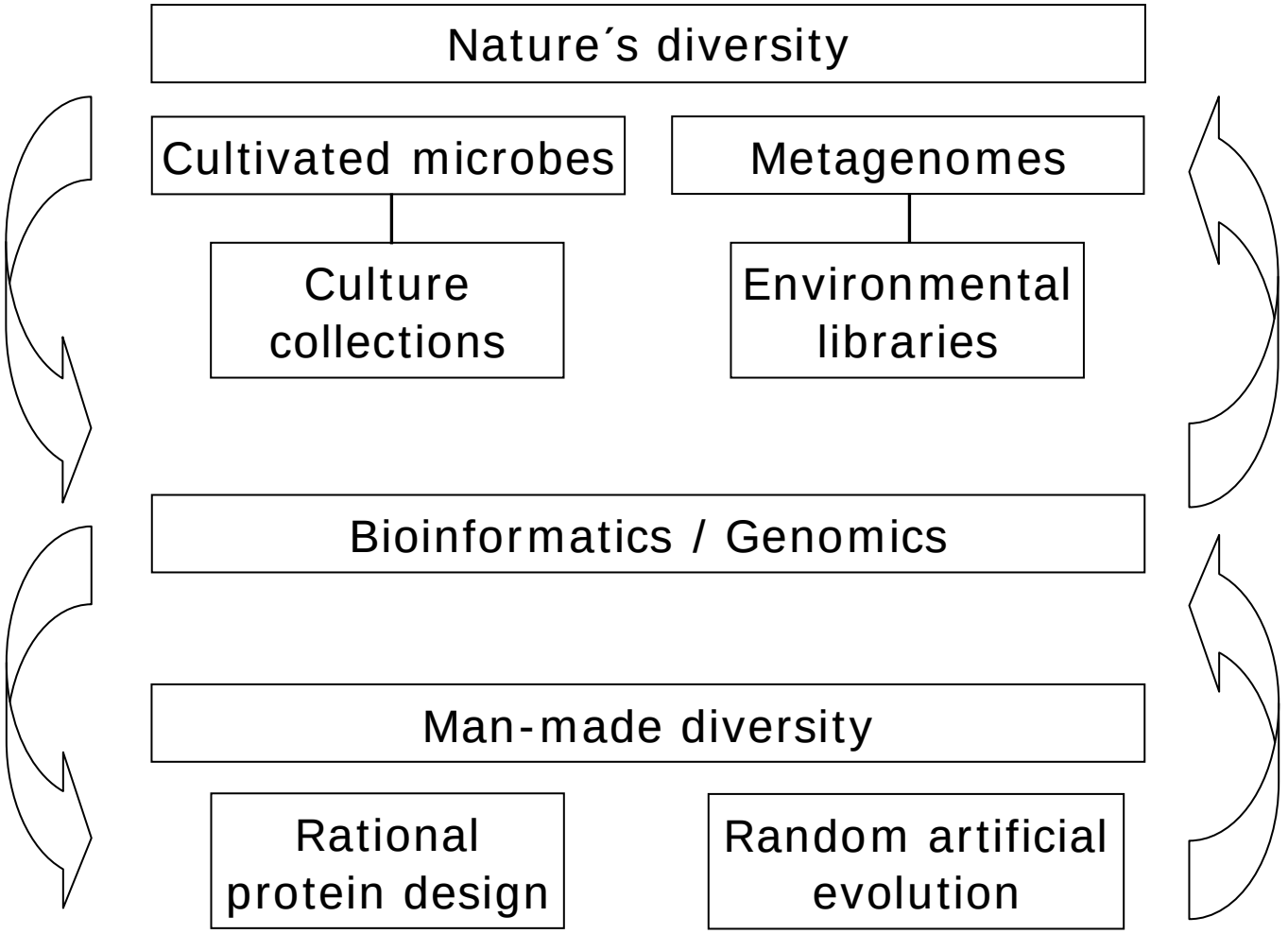
Reetz, M. T.
PNAS *101*, 5716 (2004)

Microbial lipases improved by directed evolution technologies

Microorganism	Desired property	Mutagenesis method
Pseudomonas aeruginosa PA01	Increase in enantioselectivity toward 2-methyldecanoic acid *p*-nitrophenylester	Error-prone PCR
Bacillus thermoacetenulatus BTL2	Conversion of lipase to phospholipase activity	Random mutagenesis
Staphylococcus hyicus	Improvement in phospholipase activity	Error-prone PCR + DNA shuffling
Thermomyces lanuginosus	Improvement of properties in presence of detergents	Combinatorial protein engineering / phage display approach
Bacillus subtilis	Enantioselectivity toward *meso*-1,4-diacetoxy-2-cyclopentene	Various mutagenesis methods

Houde et al. Appl. Biochem. Biotechnol. 118, 155 (2004)

Discovery of new enzymes



T. Schäfer, In: Novel enzyme technology for food applications (R. Rastall, Ed.), 2007