

# Food Enzyme Technology

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**EPFL Course ENG-436**

**Only for Teaching Purposes  
Personal Copy**

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LabTube

<https://www.labtube.tv/video/MTA3Mzg1>

# Examples of dietary digestive enzymes



[https://www.dsm.com/content/dam/dsm/foodandbeverages/en\\_US/documents/hnh/tolerase-l-leaflet.pdf](https://www.dsm.com/content/dam/dsm/foodandbeverages/en_US/documents/hnh/tolerase-l-leaflet.pdf)



Helping digest gluten with Tolerase® G



[https://www.dsm.com/content/dam/dsm/foodandbeverages/en\\_US/documents/hnh/Tolerage-G\\_Global%20leaflet\\_August%202017.pdf](https://www.dsm.com/content/dam/dsm/foodandbeverages/en_US/documents/hnh/Tolerage-G_Global%20leaflet_August%202017.pdf)

<https://www.youtube.com/watch?v=8UKMz5RMVT4&t=8s>



## NURICA™ All-in-One Enzyme Solution

Enables low sugar, high fiber and lactose-free dairy products

### A comprehensive solution

While existing health-focused dairy products may address one of these elements, DuPont™ Danisco's® new unique Nurica™ enzyme solution makes it possible to offer **all three at once**.



Reduced sugar content  
35% sugar reduction

Lactose-free  
reduction of lactose content to **<1%**

Promoting digestive health  
1.6% of GOS fiber generated in situ

Nurica™: nutrição saudável & redução de açúcar combinados | DuPont Nutrition & Biosciences

<https://www.youtube.com/watch?v=70wCGq4GTE8>

# Origin of enzymes

- Earlier researchers observed a relationship between enzyme action and what was produced by yeast during fermentation – thus the name ***ferment***
- Opposing views by Justus Liebig who saw fermentation processes related to chemical substances and Louis Pasteur who maintained that fermentation was inseparable from living cells.
- The words ***unorganized ferments*** and ***organized ferments*** were used to describe what is now called extracted enzymes and microorganisms, respectively.
- Controversy since pepsin and malt extracts were active without living cells, whereas fermentation took place only in presence of living cells.
- In 1878, Kühne proposed the name **Enzyme** (Greek: “In yeast”) to avoid use of unorganized ferments and organized ferments.
- The Liebig - Pasteur controversy came to an end when Büchner in 1897 succeeded to obtain **fermentation system using a cell free extract**

# Food Enzymes - History

- 1833 Payen & Persoz recognized that alcohol extract of malt extract contained a thermolabile substance that converted starch into sugar. It was called diastase, because it was able to separate soluble dextrines from the insoluble envelope of stratch grains (now called amylase)
- 1836 Theodor Schwann discovers the digestible enzyme Pepsin. (substance responsible for “albuminous” digestion in stomach).
- 1855 Schoenbein described peroxidase, an enzyme from plant that in presence of hydrogen peroxide, caused a solution of gum guaiac to turn from brown to blue
- 1856 Schoenbein described another enzyme in mushroom, polyphenoloxidase, that in presence of molecular oxygen, caused aerobic oxidation of certain compounds.
- 1860 Berthelot discovered, in yeast, an enzyme that was called invertase since it was able to change the optical rotation of a sucrose solution by hydrolysis into glucose and fructose
- 1874 Christian Hansen DK produces rennet by calf stomach extraction
- 1894-1913 Emil Fischer developed theory of enzyme catalysis. Victor Henry stated that enzyme-substrate complex is an essential step in enzymic catalysis. Michaelis & Menten described enzyme kinetic mathematically.
- 1926 James Sumner (USA) demonstrated that enzymes are proteins by first crystallization of an enzyme.
- 1940-1943 Novozymes : Production of Insulin and Trypsin from porcine pancreas
- 1960s Glucoamylase launched to completely break down starch into glucose
- 1963-1967 Novozymes : Microbial screening for new alkaline serine proteases within the Subtilisin group for detergents : launched the Alcalase protease
- 1984-1986 Novozymes developed first enzyme from a genetically modified organism for starch

# Advantages and Disadvantages of Biocatalysts

- **Advantages**

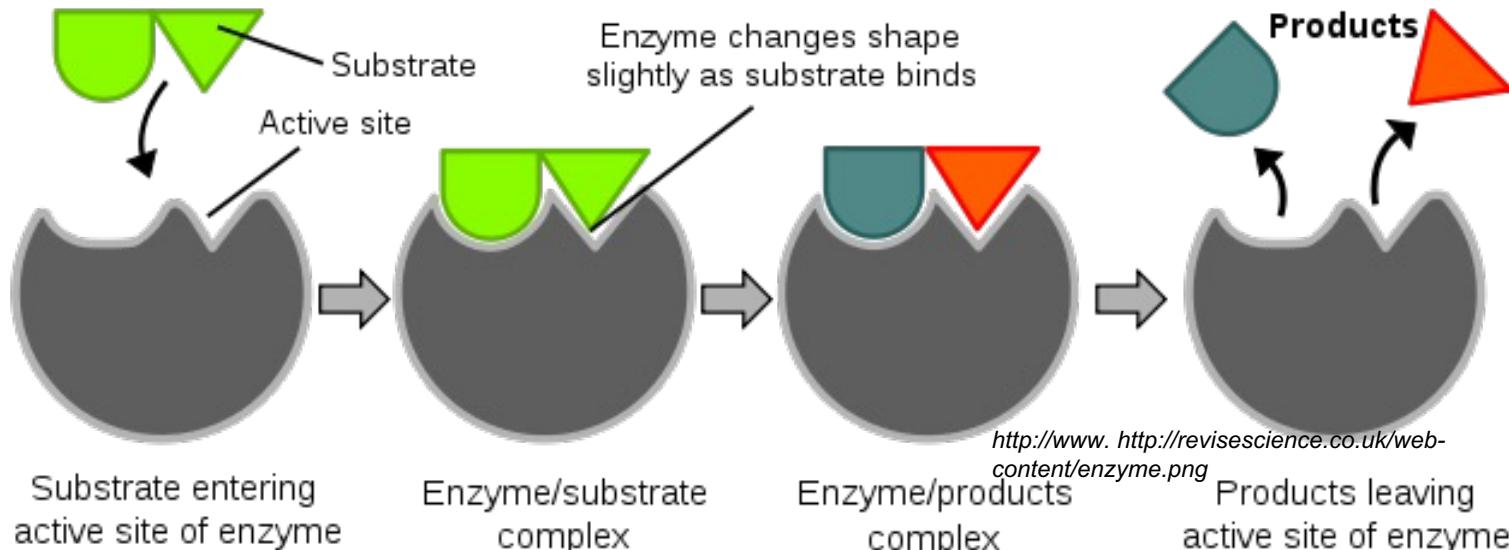
- Enzymes are very efficient catalysts
  - Typically the rates of enzyme-mediated processes are accelerated by a factor of  $10^8$ - $10^{10}$  are compared to non-enzymatic reactions
- Enzymes are environmentally acceptable
  - Unlike heavy metals, for instance, biocatalysts are degraded in the environment
- Enzymes act under mild conditions
  - Enzymes act in a pH range of about 5-8, typically around 7, and in a temperature range of 20-40°C
- Enzymes are compatible with each other
  - Several biocatalytic reactions can be carried out in one flask (similar conditions)
- Enzymes are not bound to their natural role
  - High substrate tolerance by accepting a large variety of unnatural substrates and often they can also act in non-aqueous environments
- Enzymes can catalyze a broad spectrum of reactions
  - There is an enzyme-catalyzed process equivalent to almost every type of organic reaction

# Advantages and Disadvantages of Biocatalysts

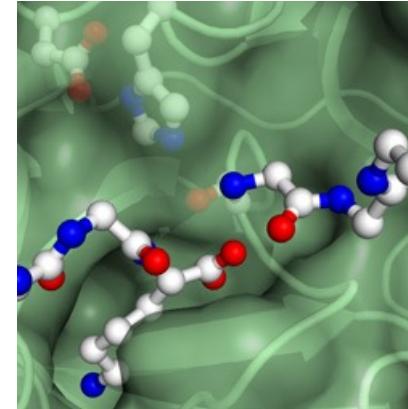
- **Disadvantages**

- Enzymes are provided by Nature in only one enantiomeric form
  - Almost all enzymes are made from L-amino acids and thus are chiral catalysts. In some reactions only 50% of substrate is converted (one enantiomer)
- Enzymes require narrow operations parameters
  - Extreme pH, high temperature and high salt concentration deactivate almost all enzymes.
- Enzymes are prone to inhibition phenomena
  - Many enzymatic reactions are prone to substrate or product inhibition limiting the efficiency of the process
- Enzymes may cause allergies
  - These may be minimized if enzymes are regarded as chemicals and handled with the same care

# Enzyme Structure



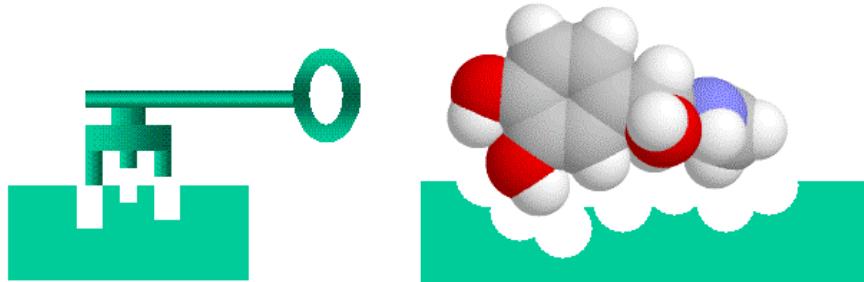
- **Globular proteins** – specific 3D shape set by the arrangements of their amino acids
- Always **soluble** due to hydrophilic R-groups
- Only have tiny functional region called the **active site**
- Substrate molecule held within the active site by **temporary bonds** between substrate and R-groups
- This structure is called the **enzyme-substrate complex**



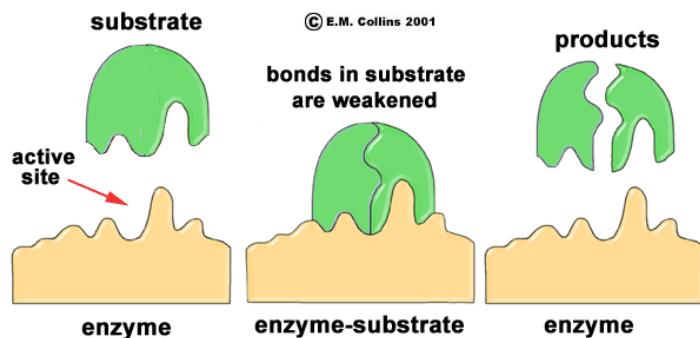
Zakharova E, Horvath MP, Goldenberg DP (2009)  
Structure of a serine protease poised to resynthesize a peptide bond. Proc. Natl. Acad. Sci. USA

# Enzyme Specificity & Stability

- Most enzymes are **highly selective (chemo, regio, and enantioselectivity)**, but some are also unselective („promiscuous“) enzymes
- **Usually enzymes loose activity at temperatures above 40 °C**, but there are also enzymes which remain active at temperatures up to 80 °C (extremophiles)
- **Enzymes can be further stabilized** through additives, e. g. glycerol or sucrose, or by immobilization onto carrier materials



<http://www.acrazychicken.blogspot.com>

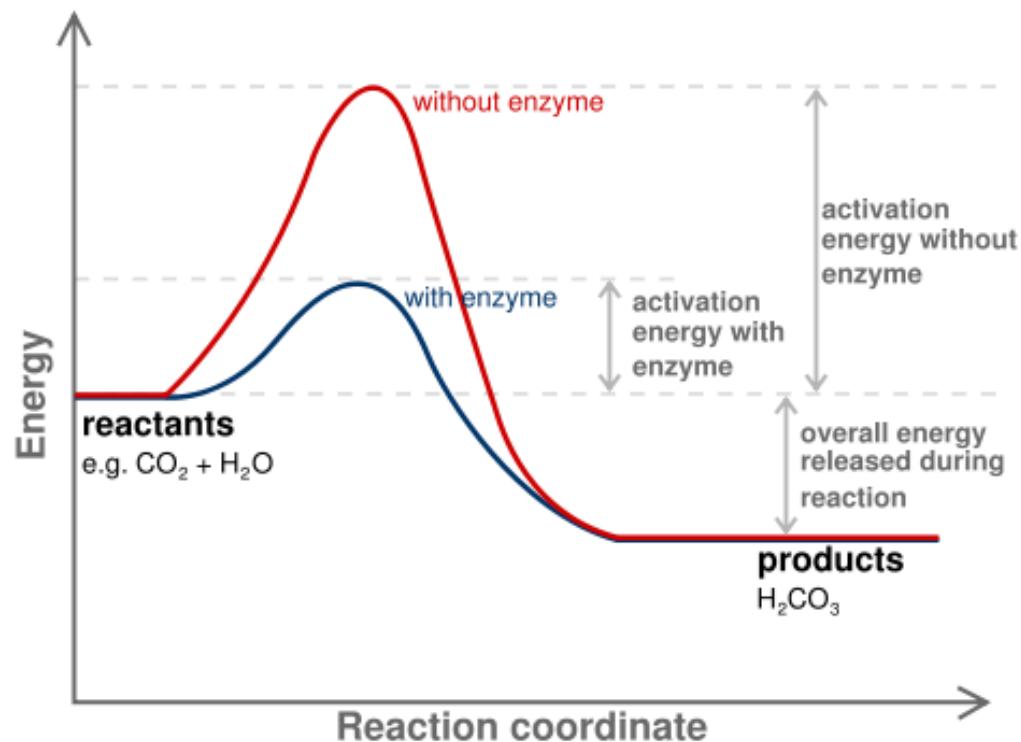


<http://www.waynesword.palomar.edu>

The active site on the enzyme attaches to a substrate molecule (such as a disaccharide) forming an enzyme-substrate complex. While attached to the substrate, the enzyme causes a weakening of certain chemical bonds in the substrate molecule, resulting in a breakdown (hydrolysis) of the substrate into two smaller product molecules (such as two monosaccharides). The enzyme is unaltered during the reaction and is free to catalyze the breakdown of another substrate molecule. If the active site on the enzyme is blocked by a poison molecule, this vital hydrolysis reaction cannot occur.

# Enzyme reactions: Thermodynamics

- Substrates need a lot of energy to reach a transition state
- Enzymes **lower the activation energy** of a reaction through binding their substrate in an activated complex
- **The enzyme stabilizes the transition state, reducing the energy needed to form products**
- Enzymes may catalyze reactions in **reverse**, if thermodynamics permit



*Wikipedia*

**There is no difference in free energy release ( $\Delta G$ ) between catalyzed and uncatalyzed reactions**

# Enzyme reactions: Kinetics

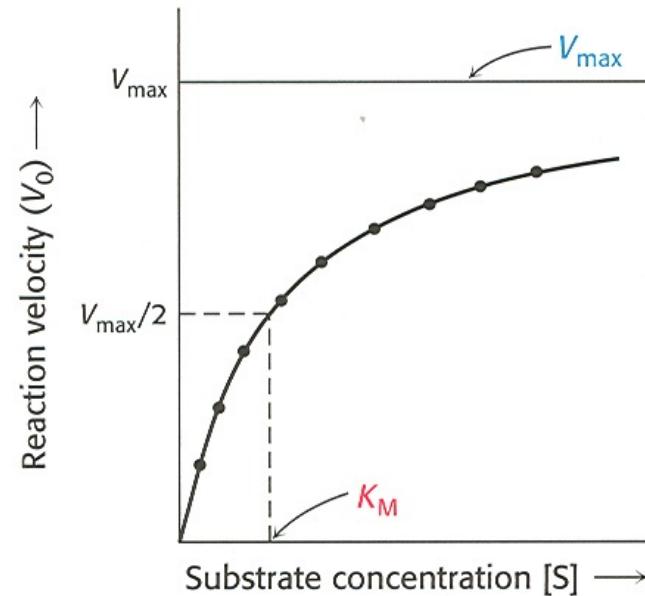
- Their kinetic behaviour is described by the **Michaelis-Menten equation**

$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$

$V_0$  is the reaction velocity.

$V_{\max}$  is the maximal rate of the reaction.

$S$  is the substrate concentration.

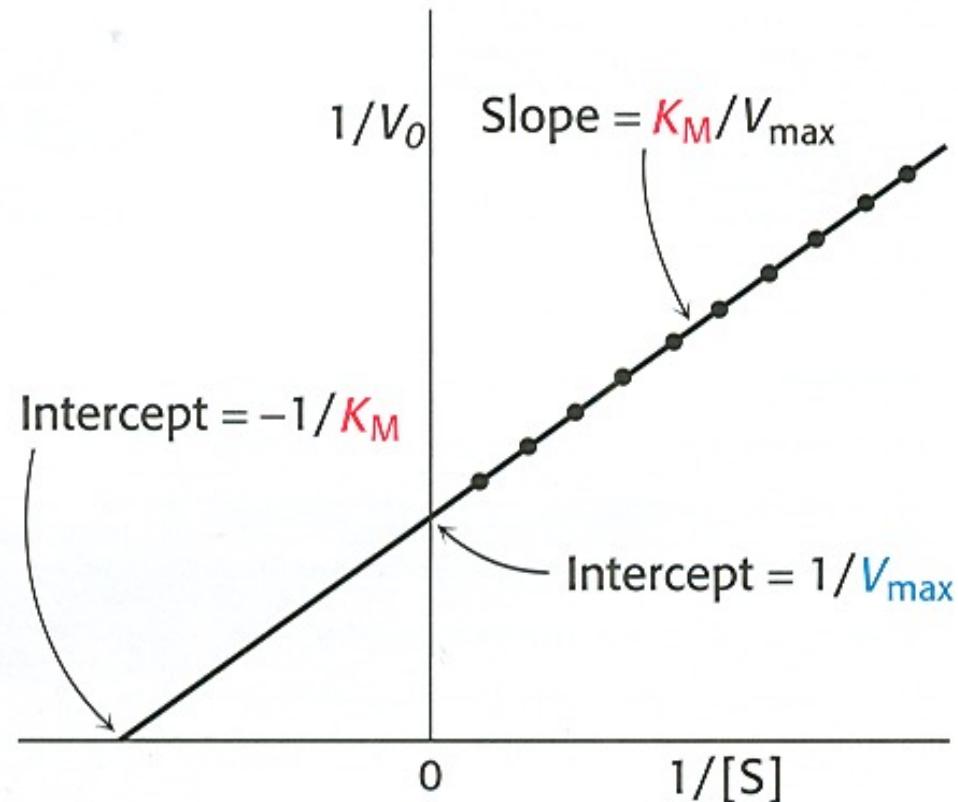


[http://stevegallik.org/sites/all/images/enzyme\\_03.jpg](http://stevegallik.org/sites/all/images/enzyme_03.jpg)

- Michaelis-Menten constant (K<sub>m</sub>)**, which is the substrate concentration required for an enzyme to reach one-half its maximum velocity.
- Each enzyme has a characteristic K<sub>m</sub> for a given substrate, and this can show how tight the binding of the substrate is to the enzyme.
- Another useful constant is **k<sub>cat</sub>**, which is the number of substrate molecules handled by one active site per second.
- K<sub>m</sub> shows the concentration of the substrate when the reaction velocity is one half of the maximal velocity for the reaction. It can also be thought of as a measure of how well a substrate complexes with a given enzyme, otherwise known as its binding affinity. A low K<sub>m</sub> indicates a large binding affinity whereas a high K<sub>m</sub> indicates that the enzyme does not bind as tightly with the substrate.

# Enzyme reactions: Kinetics (2)

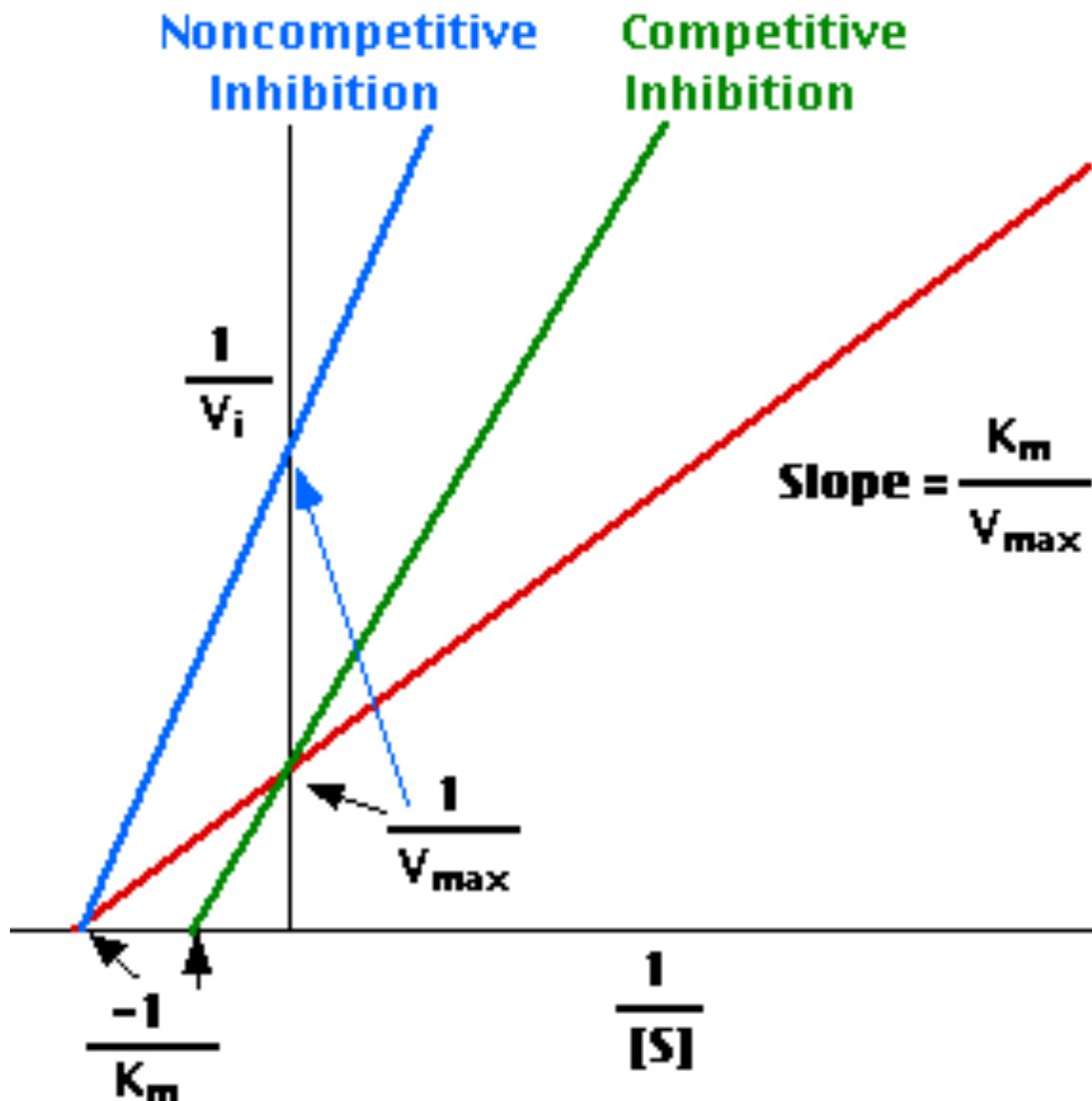
- The Lineweaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as  $K_m$  and  $V_{max}$ , before the wide availability of powerful computers and non-linear regression software.
- As the y-intercept of such a graph is equivalent to the inverse of  $V_{max}$ ; the x-intercept of the graph represents  $-1/K_m$ .
- It also gives a quick, visual impression of the different forms of **enzyme inhibition**



[http://en.wikipedia.org/wiki/Lineweaver-Burk\\_plot](http://en.wikipedia.org/wiki/Lineweaver-Burk_plot)

[http://osp.mans.edu.eg/medbiochem\\_mi/cources/biochemistry/1st\\_year\\_medicine/enzymes/files/lecture\\_03.htm](http://osp.mans.edu.eg/medbiochem_mi/cources/biochemistry/1st_year_medicine/enzymes/files/lecture_03.htm)

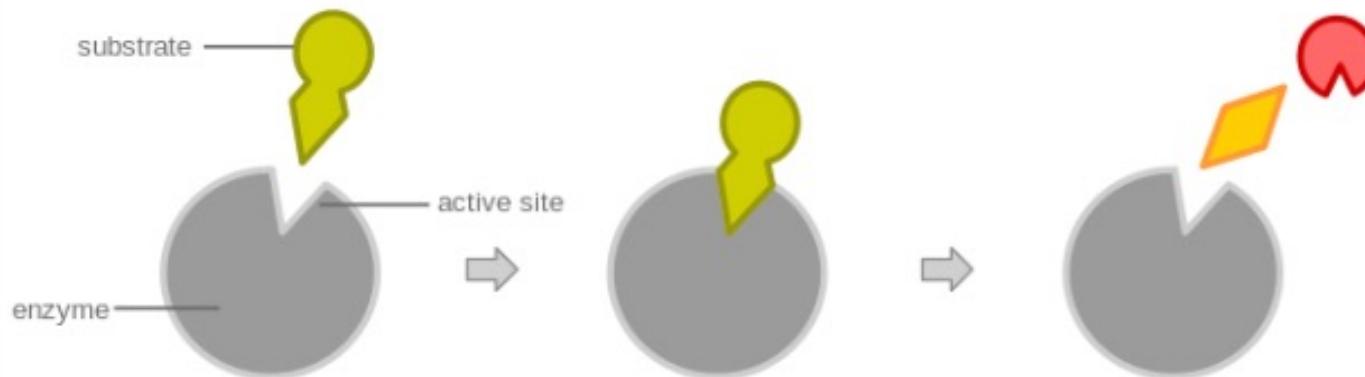
# Enzyme reactions: Kinetics (2)



[http://osp.mans.edu.eg/med\\_biochem\\_mi/courses/biochemistry/1st\\_year\\_medicine/enzymes/files/lecture\\_03.htm](http://osp.mans.edu.eg/med_biochem_mi/courses/biochemistry/1st_year_medicine/enzymes/files/lecture_03.htm)

In competitive inhibition, the maximum velocity of the reaction is unchanged, while the apparent affinity of the substrate to the binding site is decreased

### (a) Reaction



### (b) Inhibition

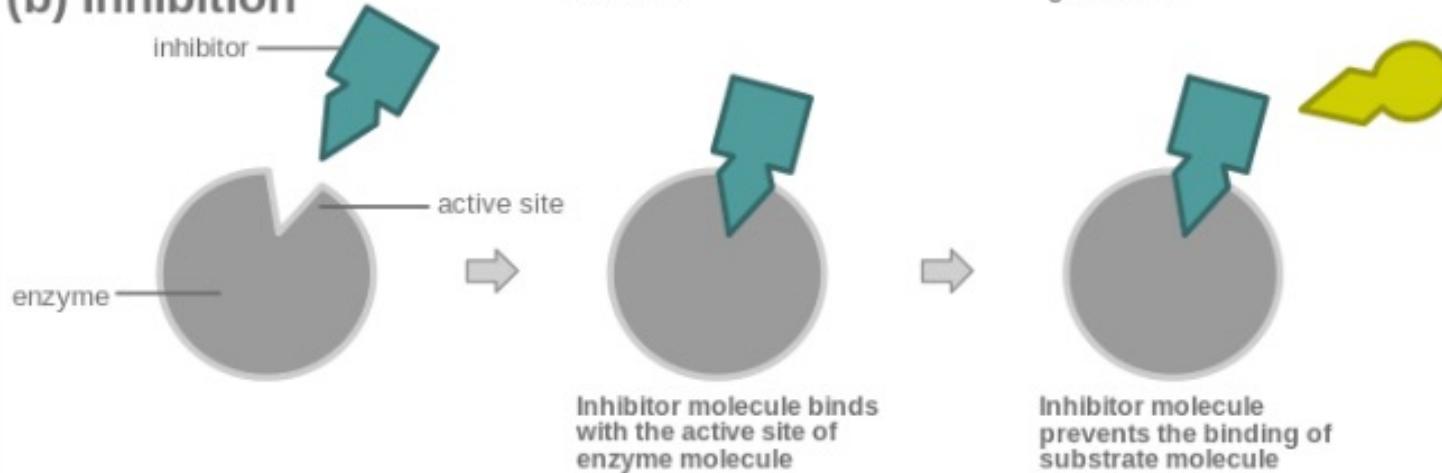
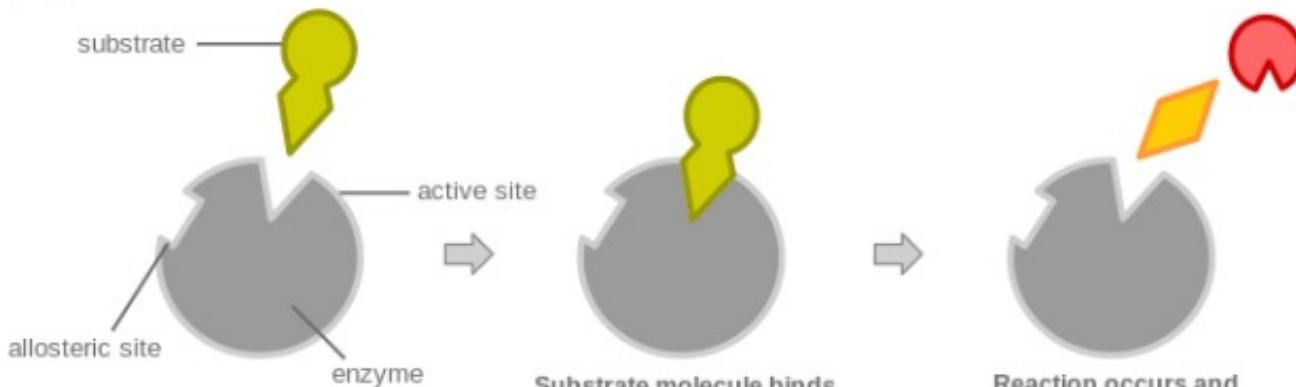
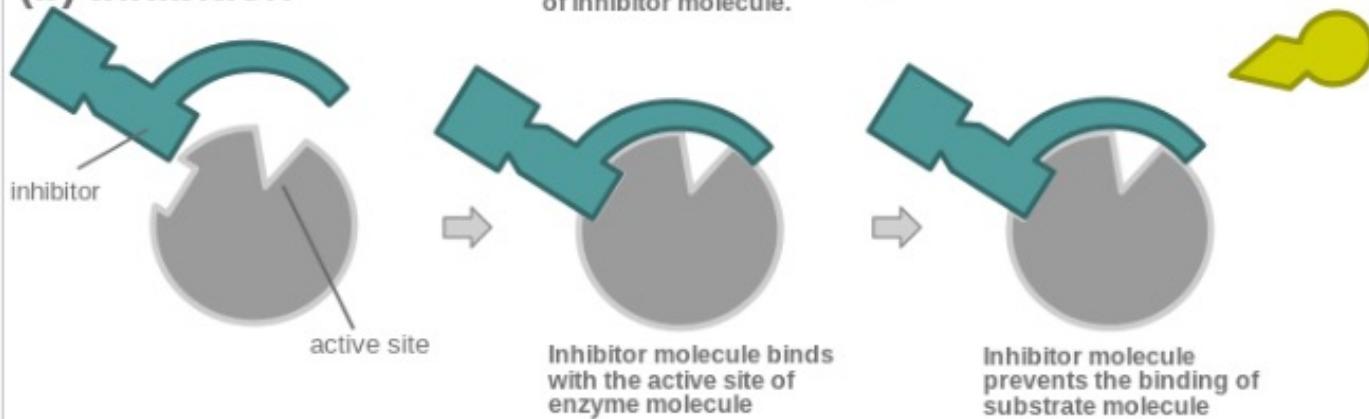
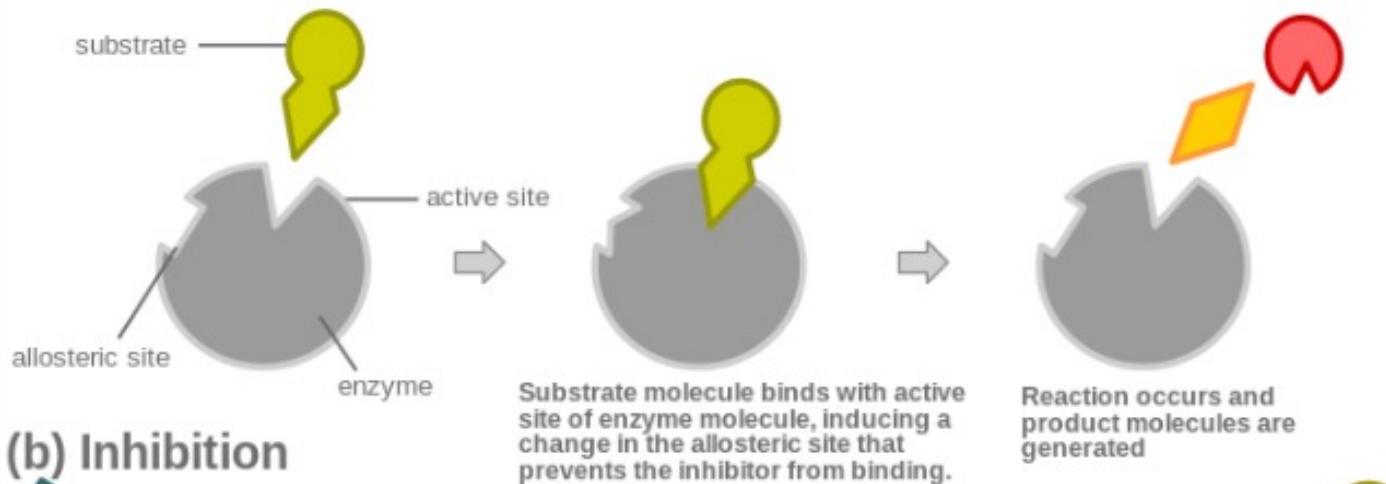
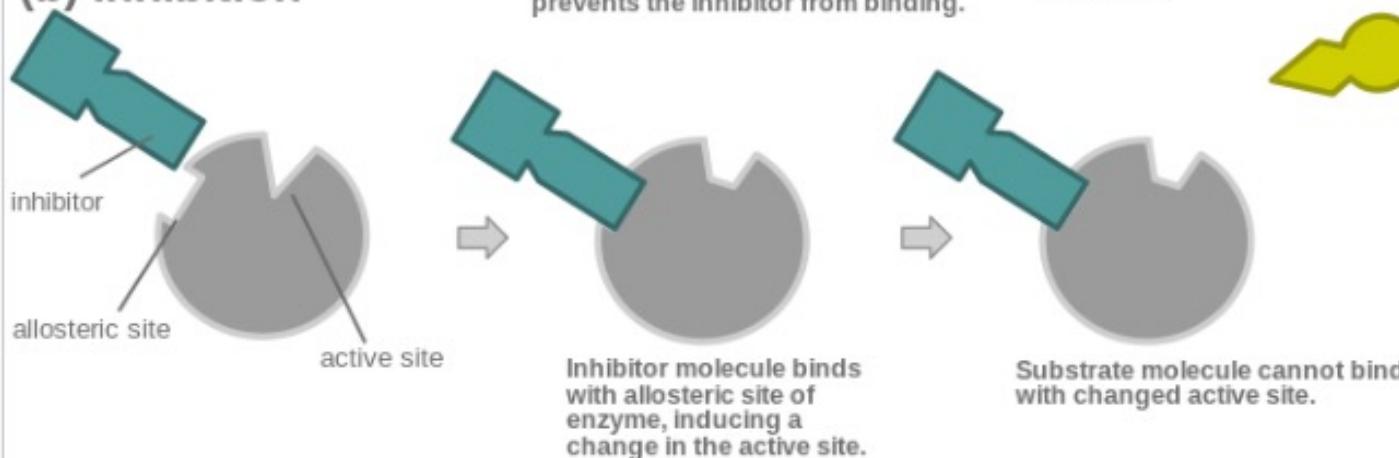


Diagram showing competitive inhibition



**(a) Reaction****(b) Inhibition**

Competitive inhibition can also be allosteric, as long as the inhibitor and the substrate cannot bind the enzyme at the same time.

**(a) Reaction****(b) Inhibition**

Another possible mechanism for allosteric competitive inhibition.



Non competitive inhibition reduces the maximum rate of a chemical reaction without changing the apparent binding affinity of the catalyst for the substrate

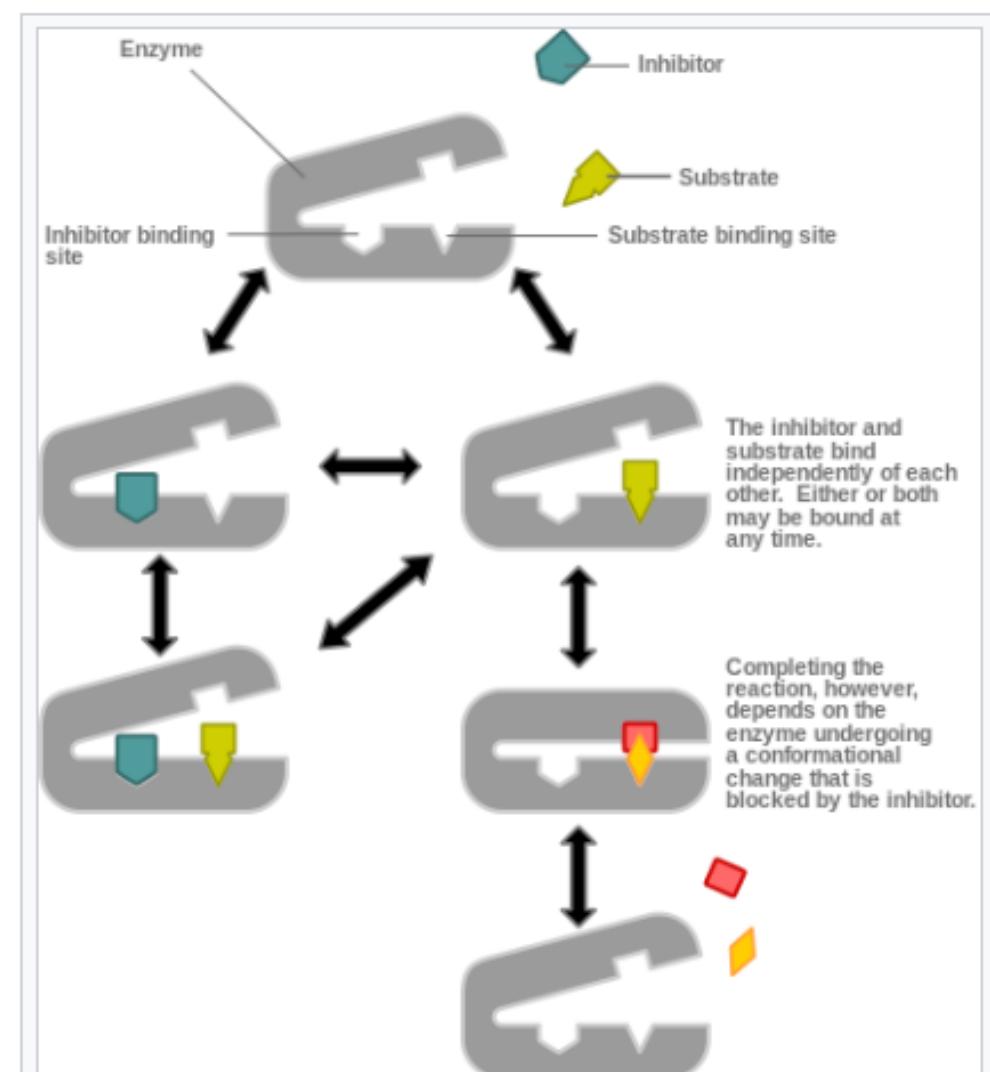


Illustration of a possible mechanism of non-competitive or mixed inhibition.

# Enzyme reactions: Kinetics (3)

- The rate constant  **$k_{cat}$**  is also called turnover number and denotes the **maximum number of enzymatic reactions catalyzed per second**
- The **turnover may reach many thousand cycles per second**

	turnover per second
carbonic anhydrase	600.000
acetylcholinesterase	25.000
penicillinase	2.000
lactate dehydrogenase	1.000
chymotrypsin	100
lysozyme	0.5

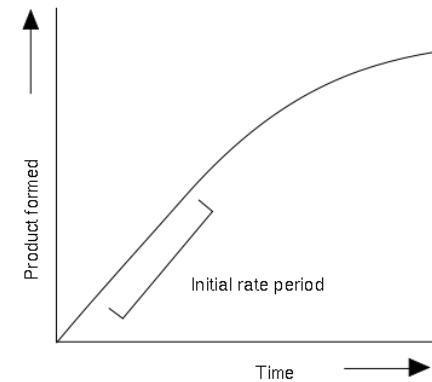
*Pocket Guide to Biotechnology and Genetic Engineering  
(ed. R. Schmid, Wiley-VCH, 2003)*

# Enzyme Assays

- Enzyme assays are laboratory methods to measure enzyme activity. Key to study enzyme kinetics & inhibition.
- Enzyme activity = moles of substrate converted per unit time = rate  $\times$  reaction volume.
- Enzyme activity is a measure of the quantity of active enzyme present. Dependent on specified conditions.
- Commonly used: 1 enzyme unit (U) = 1  $\mu\text{mol}/\text{min}$ .
- The specific activity of an enzyme is the activity per mg of total protein (expressed:  $\mu\text{mol}/\text{min}/\text{mg}$ ). It is the amount of product formed by an enzyme in a given amount of time under given conditions per mg of total protein.
- Assays: E.g. spectrophotometric, fluorimetric, radiometric, chromatographic



[http://en.wikipedia.org/wiki/Enzyme\\_assay](http://en.wikipedia.org/wiki/Enzyme_assay)



[http://en.wikipedia.org/wiki/File:Enzyme\\_process\\_curve.svg](http://en.wikipedia.org/wiki/File:Enzyme_process_curve.svg)

# Enzyme classification and nomenclature

- Enzymes are **classified into 6 groups**
- For technical applications, **hydrolases are the most important group of enzymes**, as they do not require any cofactors. This group includes proteases, lipases, carbohydrases



Top-level EC numbers <sup>[4]</sup>			
Group	Reaction catalyzed	Typical reaction	Enzyme example(s) with trivial name
EC 1 <i>Oxidoreductases</i>	To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	$AH + B \rightarrow A + BH$ (reduced) $A + O \rightarrow AO$ (oxidized)	Dehydrogenase, oxidase
EC 2 <i>Transferases</i>	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	$AB + C \rightarrow A + BC$	Transaminase, kinase
EC 3 <i>Hydrolases</i>	Formation of two products from a substrate by hydrolysis	$AB + H_2O \rightarrow AOH + BH$	Lipase, amylase, peptidase
EC 4 <i>Lyases</i>	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	$RCOCOOH \rightarrow RCOH + CO_2$ or $[x-A-B-Y] \rightarrow [A=B + X-Y]$	Decarboxylase
EC 5 <i>Isomerases</i>	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	$AB \rightarrow BA$	Isomerase, mutase
EC 6 <i>Ligases</i>	Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP	$X + Y + ATP \rightarrow XY + ADP + Pi$	Synthetase

No need to understand details

# Enzyme classification and nomenclature

For its identification every enzyme has got a 4-digit number **EC A.B.C.D** where EC stands for “Enzyme Commission” with the following properties encoded:

- A denotes the main type of reaction, e.g. oxidoreduction (1), transfer (2) hydrolysis (3)
- B stands for the subtype, indicating the substrate type or the type of transferred molecule
- C indicate the nature of co-substrate
- D is the individual enzyme number

**Example: E.C. 1.1.1.1**

an enzyme converting an alcohol to an aldehyde or ketone using NAD as coenzyme would be classified as:

- oxidoreductase	main class 1
- acting on CH-OH group	sub class 1
- using NAD <sup>+</sup> as acceptor	sub group 1
- alcohol: NAD <sup>+</sup> -oxidoreductase	E.C.1.1.1.1

# Coenzymes/Cofactors

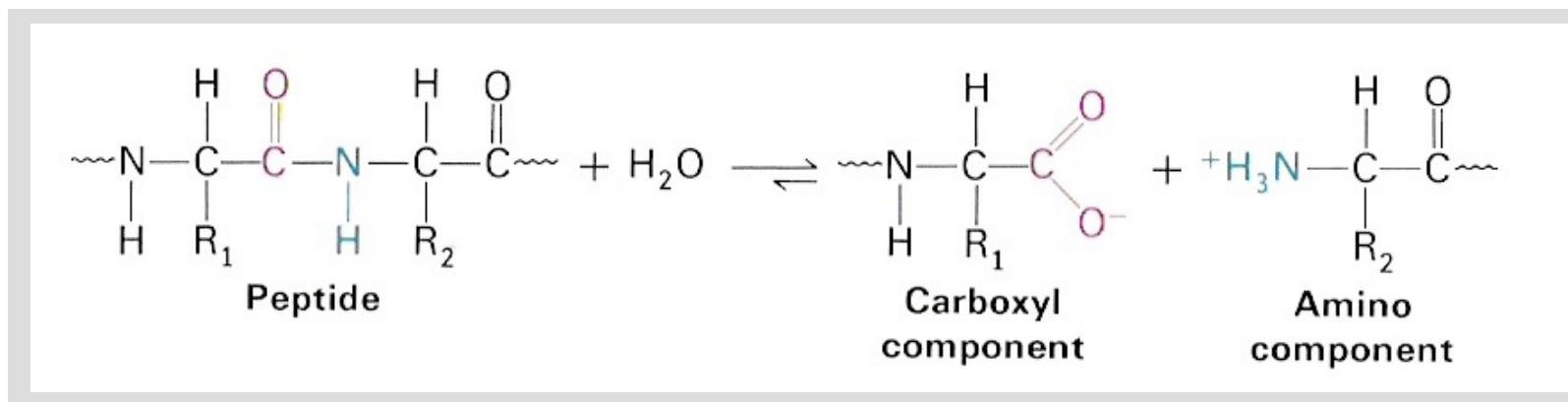
- Fraction of synthetically useful enzyme-catalyzed reactions require cofactors (coenzymes).
- Cofactors are compounds of relatively low molecular weight compared to the enzyme
- Cofactors provide either redox-equivalents (e.g. Hydrogen, oxygen, electrons) and carbon-units
- Some enzymes contain or require metal ions:  $Zn^{2+}$ ,  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Cu^{2+}$ ,  $K^+$ ,  $Na^+$

<u>Coenzyme</u>	<u>Reaction type</u>
$NAD^+$ / $NADH$ , $NADP^+$ / $NADP$	removal or addition of hydrogen
ATP	phosphorylation
SAM	$C_1$ -alkylation
Acetyl-CoA	$C_2$ -alkylation
Flavines	oxygenation
Pyridoxal-phosphate	transamination

# Proteases: Activity & Specificity

Proteases are :

- ✓ Proteins also named proteinases, peptidases or proteolytic enzymes
- ✓ The “protein-cutting machinery” ....  
....catalyzing the **hydrolytic breakdown of proteins** into peptides or amino acids by cleavage of peptide bonds between amino acids. This process is called **proteolytic cleavage**. Proteases are using a molecule of water for this.



- ✓ Enzymes essential for all life, playing important role in conception, birth, digestion, growth, maturation, ageing and death of all organisms.

# Class 3: Hydrolases EC 3

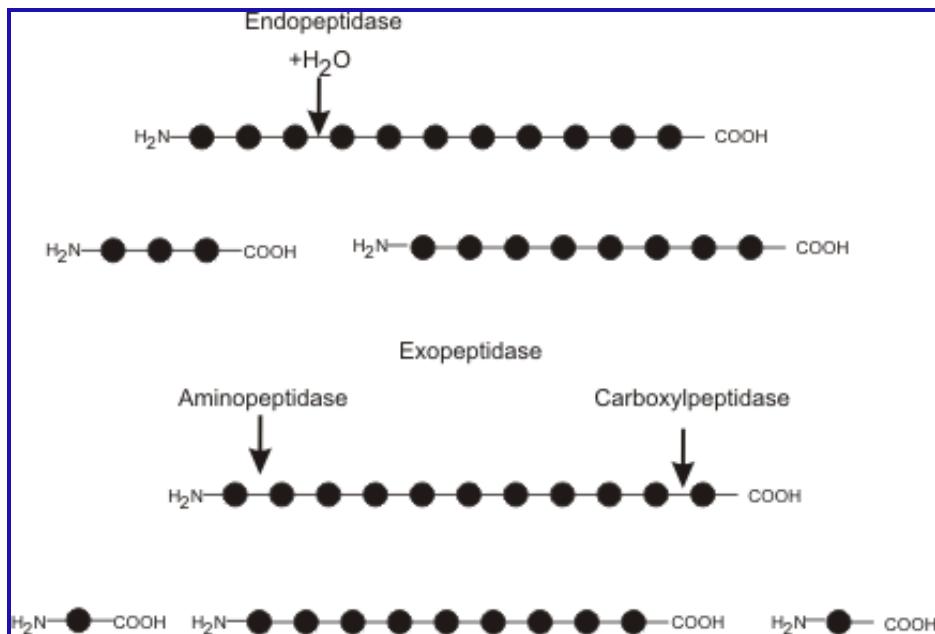
## Peptidases or proteases family EC 3.4

Only

### Exopeptidases

EC 3.4.11.- to 3.4.19.-

- Act only near the ends of polypeptide chains at the N or C terminus.



No need to know EC numbers by heart

### Endopeptidases (or proteinases)

EC 3.4.21.- to 3.4.99.-

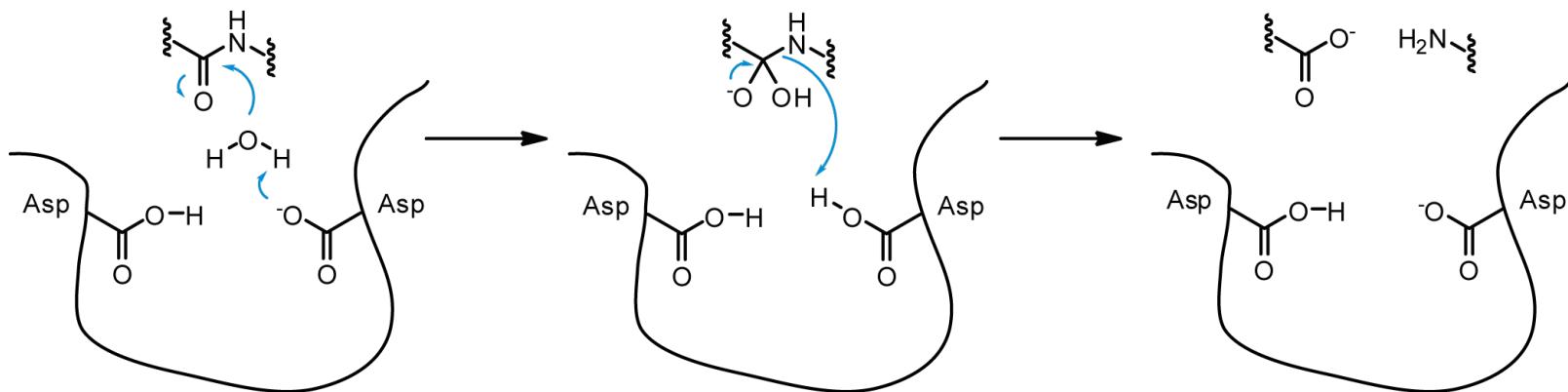
- Act preferentially in the inner regions of peptide chains away from the N or C terminus:



- ❖ Serine endopeptidases EC 3.4.21
- ❖ Cysteine endopeptidases EC 3.4.22
- ❖ Aspartic endopeptidases EC 3.4.23
- ❖ Metallo endopeptidases EC 3.4.24
- ❖ Threonine endopeptidases EC 3.4.25
- ❖ Endopeptidase of unknown catalytic mechanism EC 3.4.99

# Aspartic Protease

- Aspartic proteases are a family of protease enzymes that use an aspartate residue for catalysis of their peptide substrates.
- In general, they have two highly-conserved aspartates in the active site
- Optimally active at acidic pH
- Nearly all known aspartyl proteases are inhibited by pepstatin
- E.g. Pepsin, Chymosin (rennin), HIV-1 Protease



[http://en.wikipedia.org/wiki/File:Aspartyl\\_protease\\_mechanism.png](http://en.wikipedia.org/wiki/File:Aspartyl_protease_mechanism.png)

### The Nestlé H.A. Infant Formula

Incidence of allergy in %

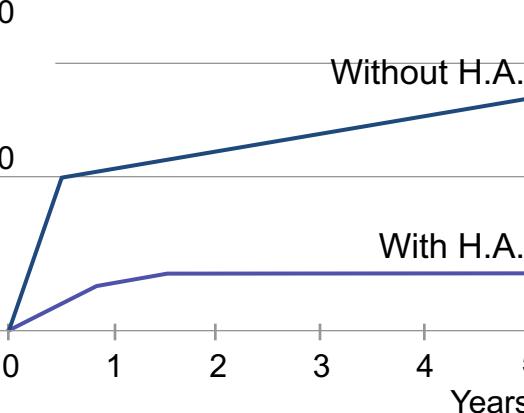
80

40

0

Without H.A.

With H.A.



#### Goal:

- Preventive Formula for Infants with allergy risks

#### Science:

- Moderate enzymatic hydrolysis of milk whey proteins
- No sensitization of immune system
- Various clinical studies show:

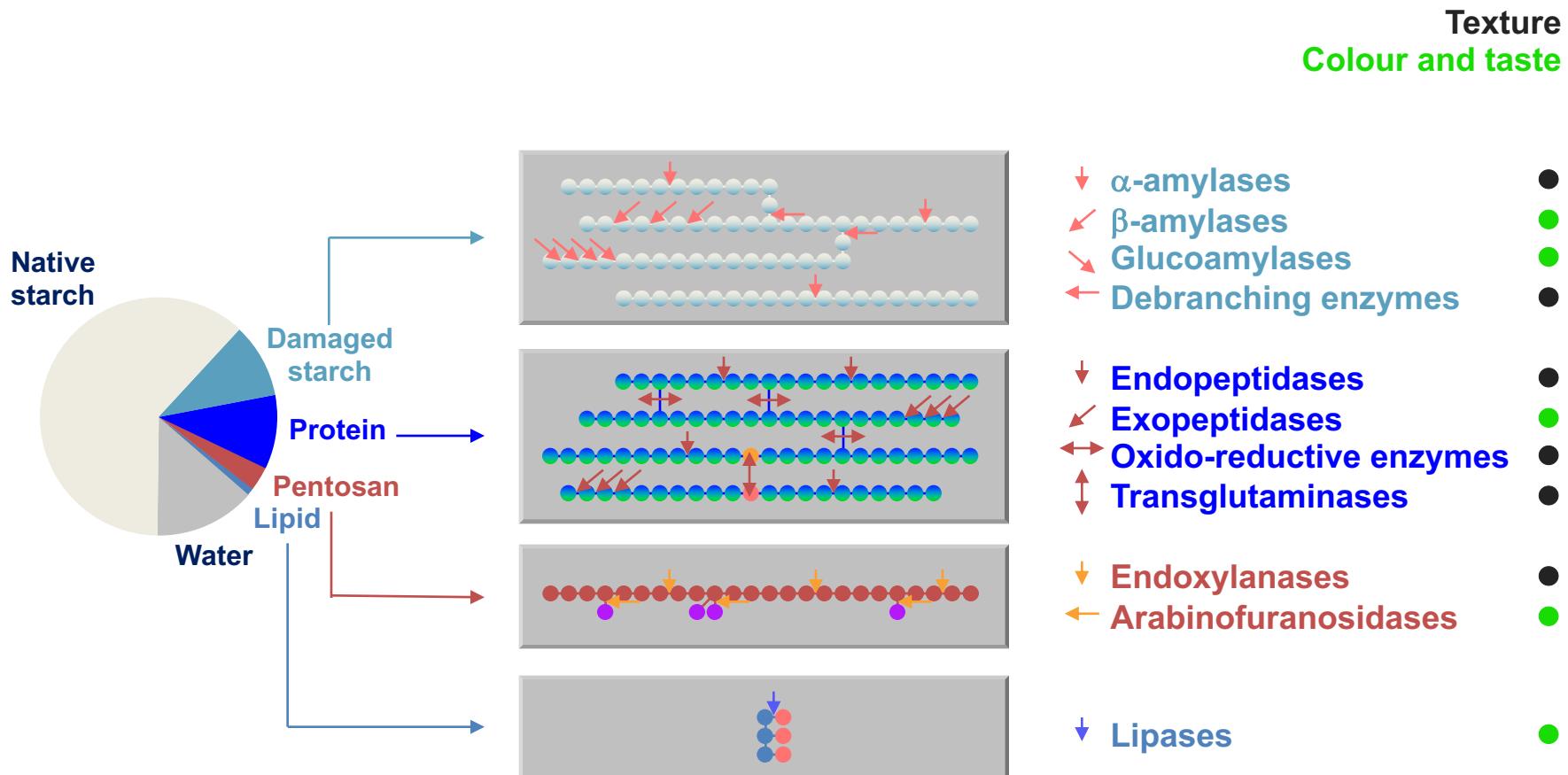
H.A. Formula during the 6 first months of life reduce risk of allergies over years (similar to breastfed)

#### Benefit:

- Clear reduction of atopic symptoms in infants with risk of allergies

# Application of enzymes

## Example 2: Modification of cereal flour components

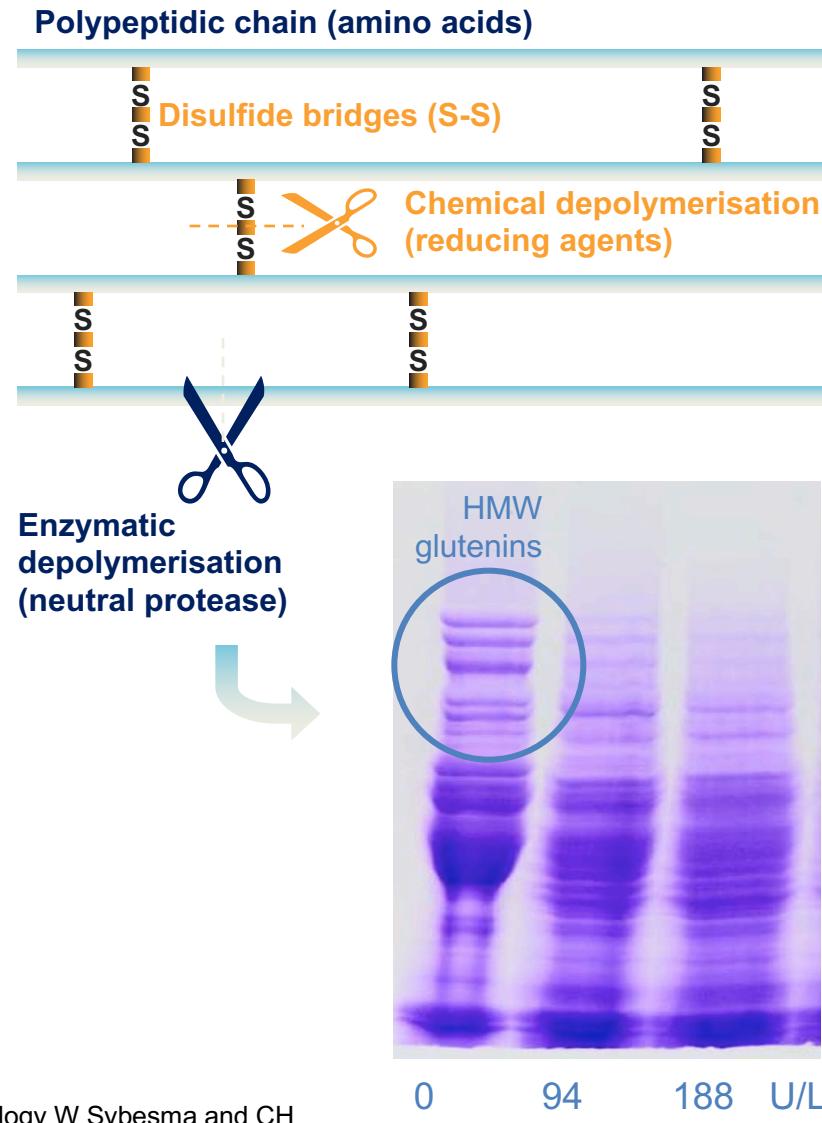
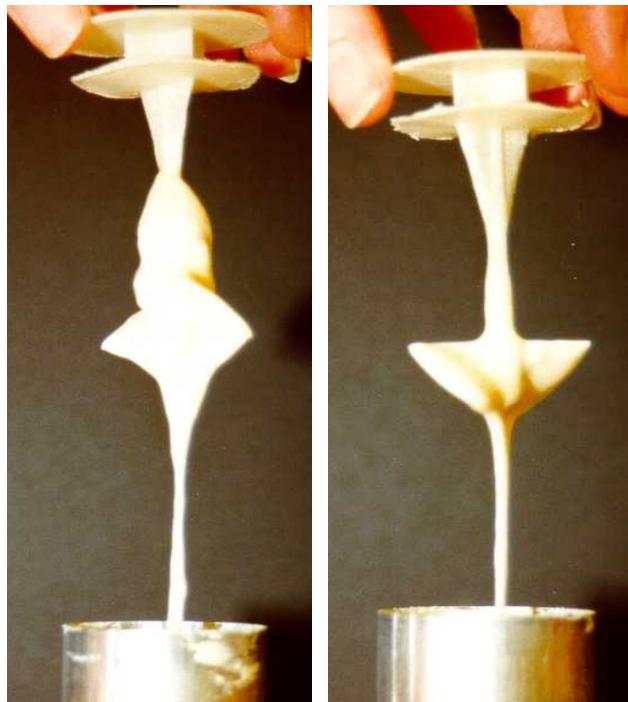


# Application of proteases

## Example 3: Prevent lumps formation

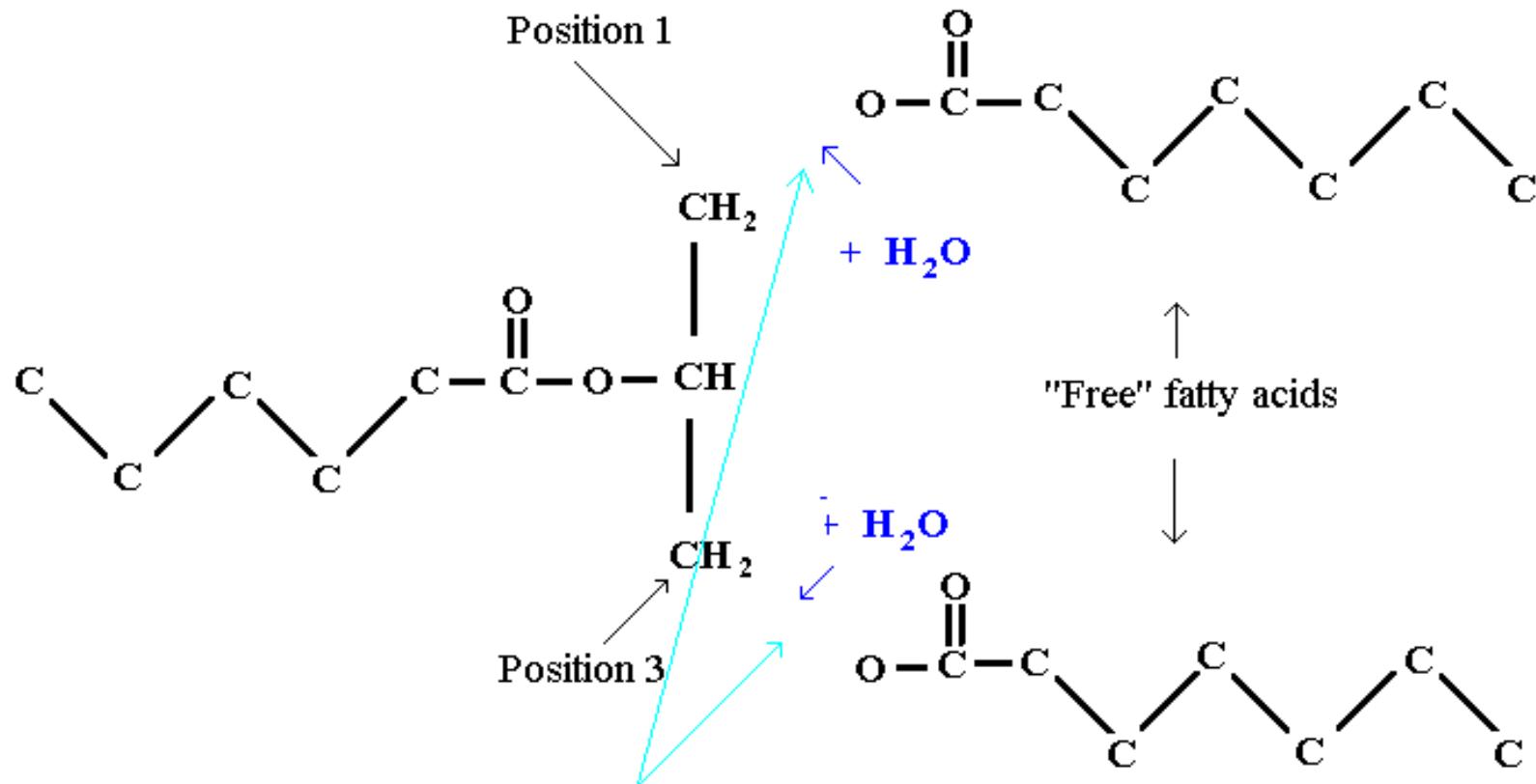
**Goal: improve batter machinability**

**Gluten tends to form aggregates.  
The use of a neutral protease  
produces batter without lumps**



# LIPASES

# Lipase action on Triglycerides

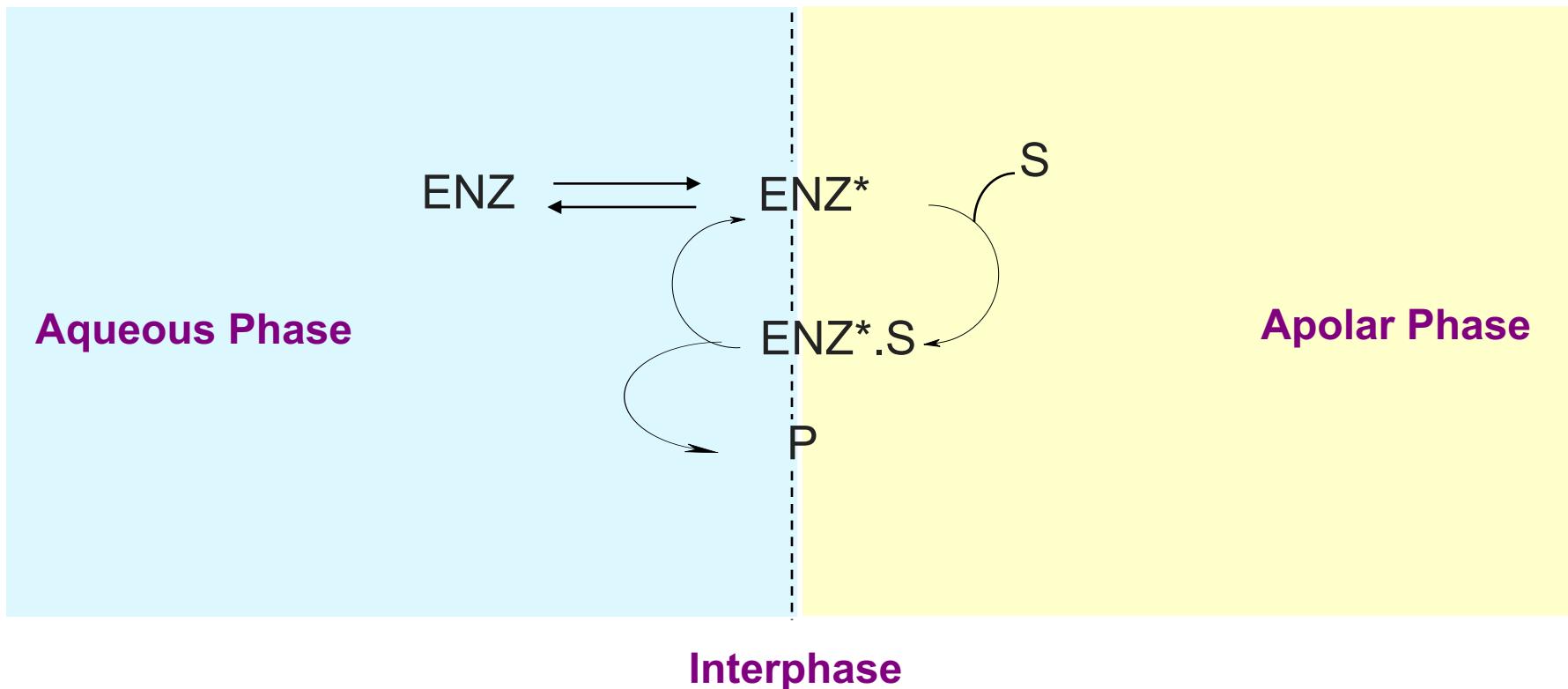


Pancreatic lipase is an enzyme that breaks the bonds between glycerol and the fatty acids at positions 1 and 3, liberating the 2 fatty acids.

<http://www.google.ch/imgres?imgurl=http://www.nutriology.com>

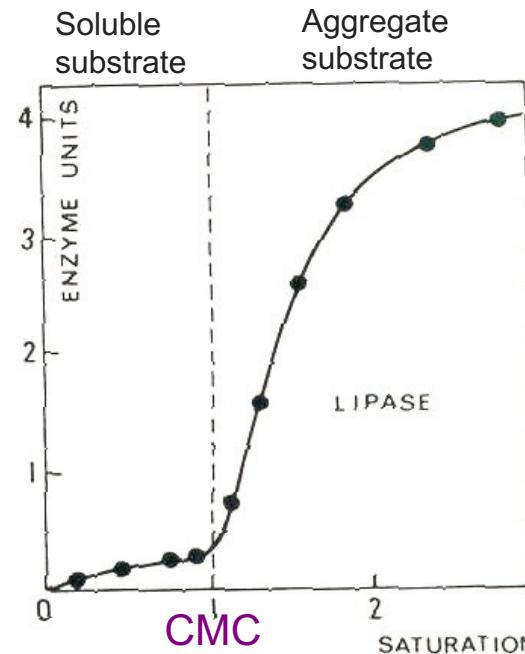
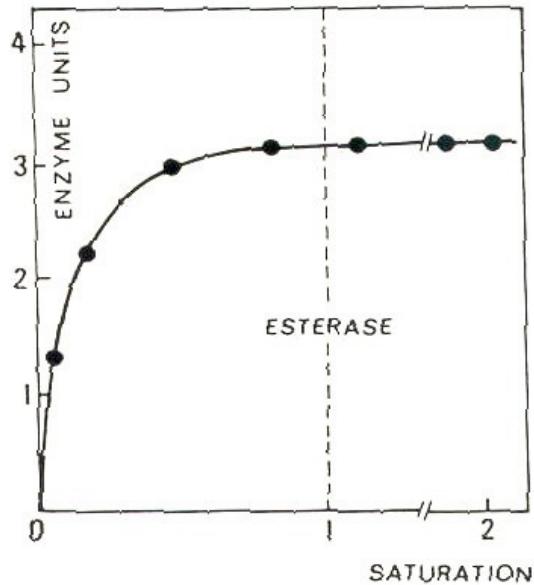
# DEFINITION OF LIPASES

Lipases are acyl hydrolases that catalyze the hydrolysis of triglycerides at the lipid-water interface



# Difference Between Lipases and Esterases

- Unlike esterases, which show a normal Michaelis-Menten activity, lipases display little activity in aqueous solutions with soluble substrates



Verger et al., 1973

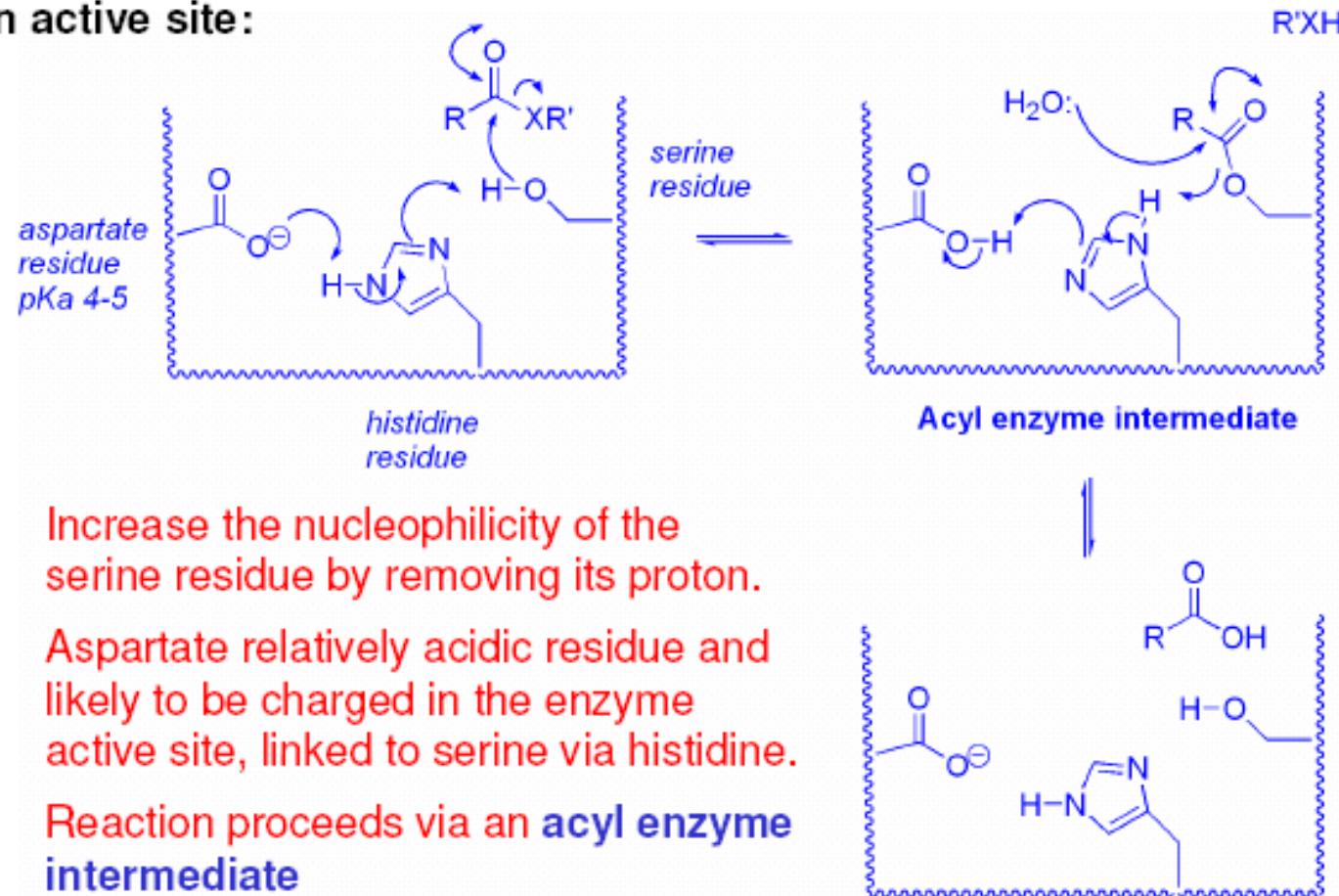
- A sharp increase in activity is observed when the substrate concentration is increased beyond its Critical Micellar Concentration (CMC)

# Lipases: Reaction Mechanism

No need to understand reaction mechanism

## 2. Hydrolytic Enzymes - Catalytic Triad Mechanism

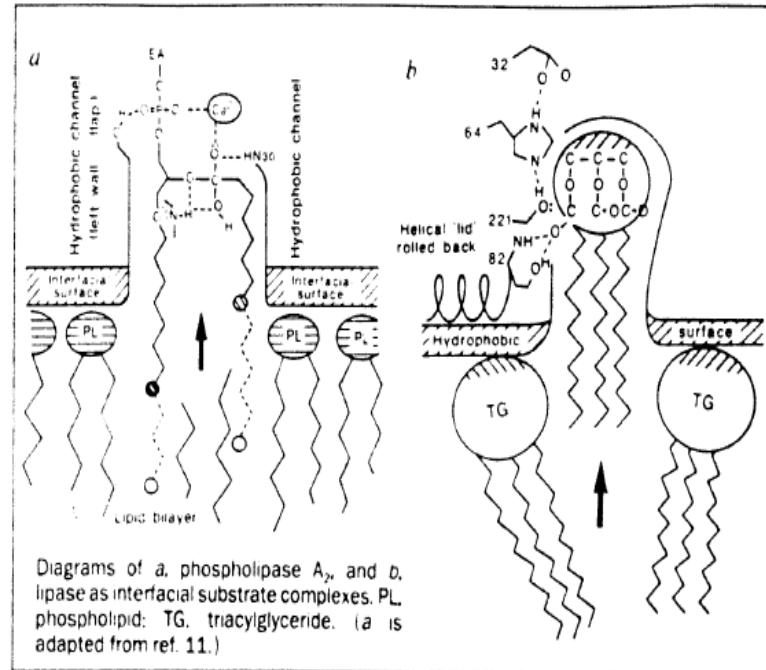
In active site:



- Increase the nucleophilicity of the serine residue by removing its proton.
- Aspartate relatively acidic residue and likely to be charged in the enzyme active site, linked to serine via histidine.
- Reaction proceeds via an acyl enzyme intermediate

# Lipases: Reaction Mechanism

- The active site is buried inside the enzyme
- A helical lid (hydrophobic flap) composed of His, Asp, Ser opens to form a hydrophobic channel
- TAG (tri-acyl glycerides) bind, resulting in major change of the protein backbone – moving a seven aa helix which lies over the active site as a lid. This creates a new hydrophobic surface surrounding the active site
- A non-polar surface is encircling the active site to form a hydrophobic seal to allow the lipid to enter the active site without interacting with the bulk water
- The enzyme will only partially withdraw the substrate, leaving its fatty-acid side chains projecting into the lipid



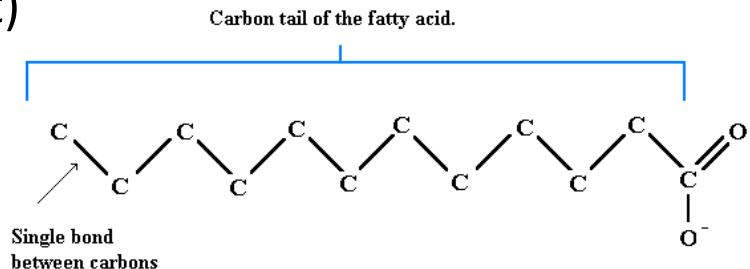
NATURE · VOL 351 · 6 JUNE 1991

# Characteristics of Lipases

- Lipase (triacylglycerol hydrolases) are the **most frequently used** enzymes in bio-organic synthesis
- They are **widely found in nature** (animals, man, bacteria, yeasts, fungi, plants)
- Their natural function is the **hydrolysis and re-esterification of triglycerides** (natural fats & oils)
- The reaction is catalyzed by **catalytic triad composed of Ser, His, and Asp** (sometimes Glu)

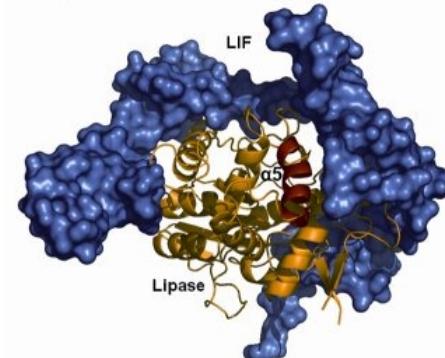
# Classification of Lipases

- Classification of lipases based on substrate specificity:
  - Non-specific lipases
  - Sn*1,3-regiospecific lipases (e.g. in GI tract)
  - Chain length
  - Saturation specificity



<http://www.nutriology.com/TGmetab.html>

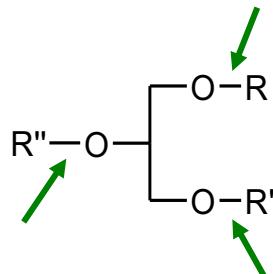
- Classification of lipases based on:
  - Molecular weight
  - Amino-acid sequences
  - Sequence motifs



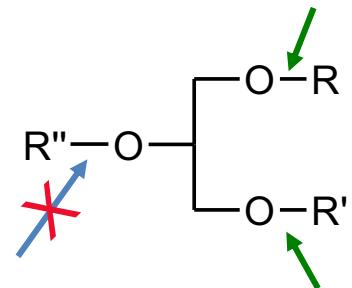
<http://ultr23.vub.ac.be/ultr/images/research/lipase.jpg>

# Specificity of Lipases

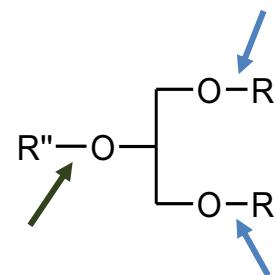
Non-specific



1,3-Specific



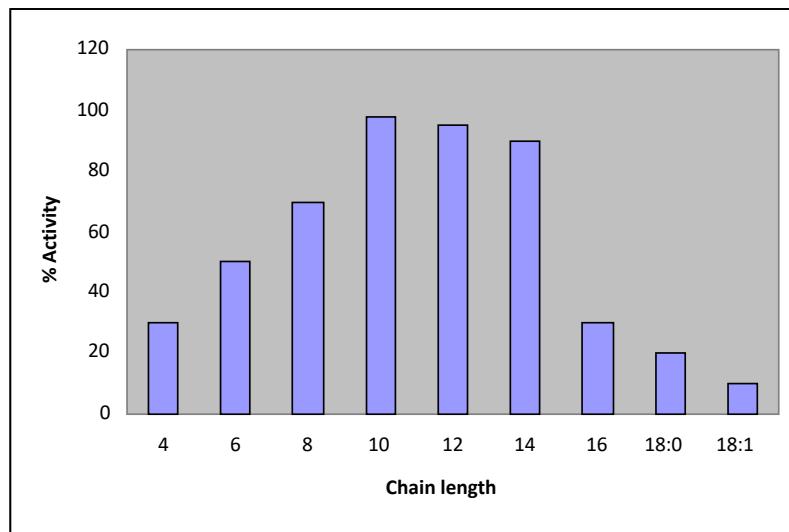
Preference for *sn*-2 position



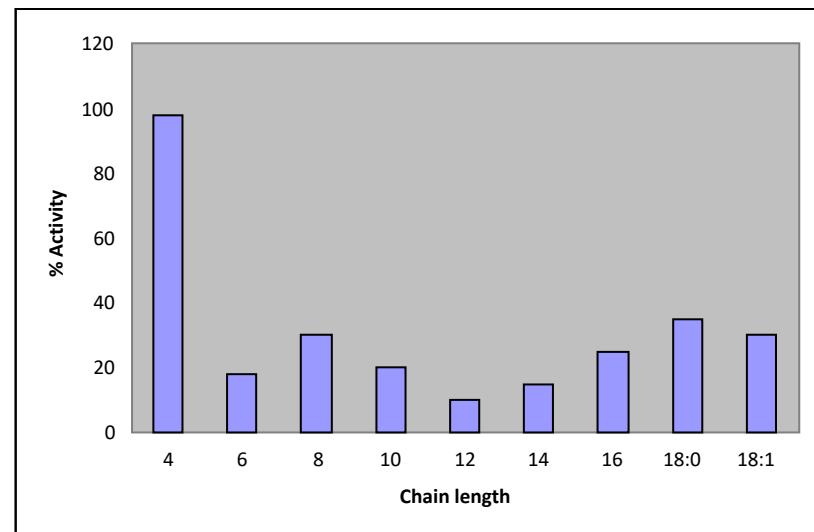
# Specificity of Lipases

## Chain Length Specificity

- Many microbial lipases show only a low specificity in the hydrolysis of natural oils and fats (fish oils and butterfat are the exception)
- Differences in the specificity of different microbial lipases can be observed; some prefer to cleave short-chain fatty acids, whereas others prefer the long-chain fatty acids



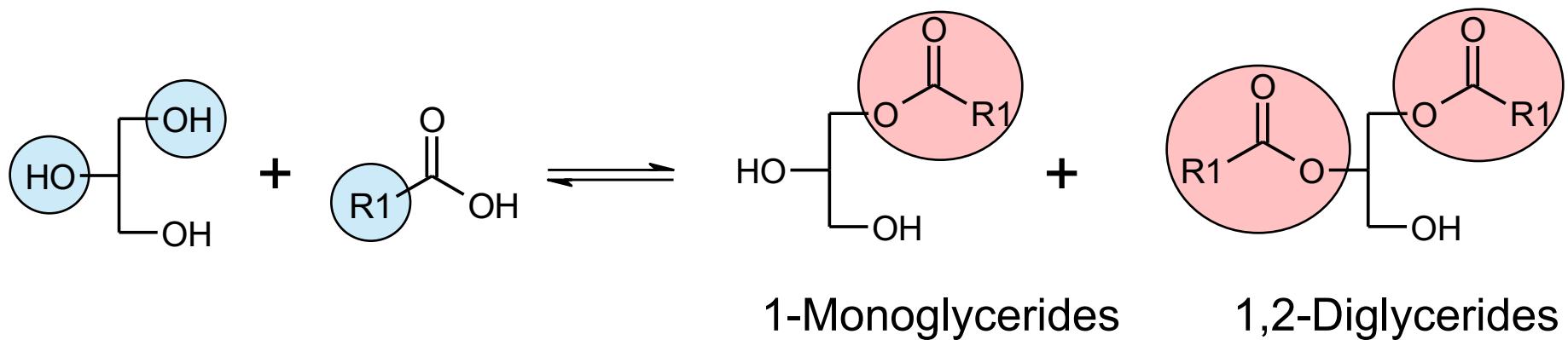
Lipase from *Humicola lanuginosa*



Lipase from *Candida rugosa*

# Reactions Catalyzed by Lipases: Esterification

Generally performed using immobilised lipases in a fixed bed reactor, if the reaction partners are miscible as such, or in an organic solvent. In such systems, water content and water activity plays an important role on the reaction equilibrium.



# Factors Influencing Lipase Activity

Lipid hydrolysis depends on different parameters such as pH, temperature, water content, and the phase boundary area

- **pH**
  - The pH optimum of most lipase lies between 7.5 and 9.0
  - Different microbial lipases are active between 5.5 and 8.5
  - Milk lipases are active between 4.1 and 6.5
- **Temperature**
  - For most lipases, temperature optimum is between 40°C and 60°C
  - Some lipase activity has been detected in frozen foods at – 30°C
  - Denaturation at temperature > 80°C
- **Salts**
  - Salts (bile salts,  $\text{Ca}^{2+}$ ) in different concentrations influence lipase activity.  $\text{NaCl}$  is required to activate porcine pancreatic lipase (PPL)
- **Co-lipase**
  - Helps to anchor pancreatic lipase at the interphase to avoid inhibition by bile salts
- **Interface**
  - Lipase activity depends strongly on interfacial composition of an oil-water emulsion

# Application of Lipases: ENOVA™ OIL



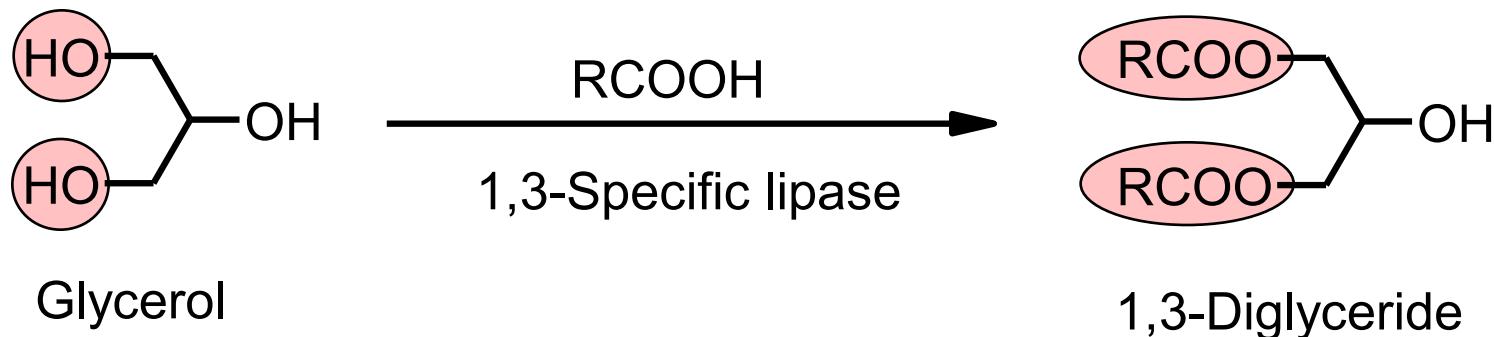
While most cooking and salad oils are triglyceride-based, Enova™ oil is 80% diglycerides.

Studies show (?) that consumption of DAG-rich oil may result in lower serum triglyceride levels after a meal (difficult to re-esterify glycerol into triglycerides compared to sn-2 monoglyceride)



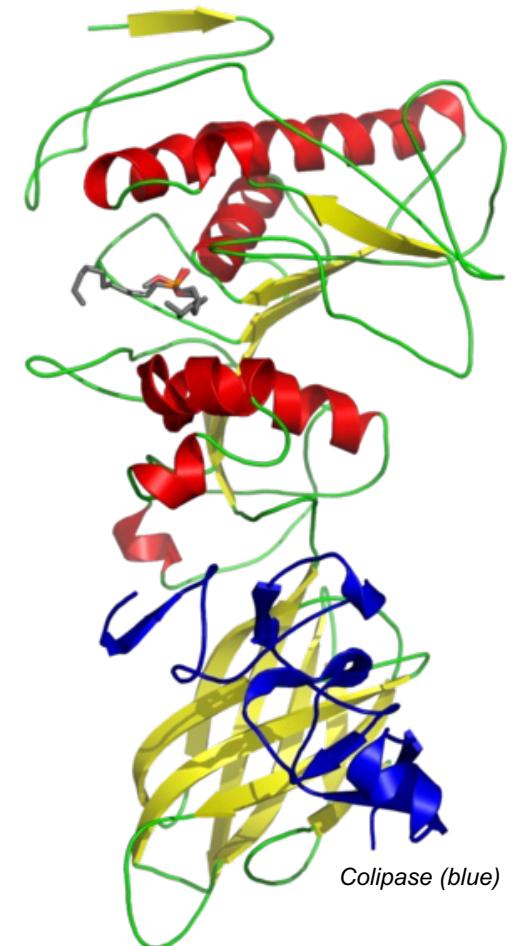
[http://www.fasebj.org/doi/abs/10.1096/fasebj.22.2\\_supplement.737](http://www.fasebj.org/doi/abs/10.1096/fasebj.22.2_supplement.737)

<https://pgio.info/project/enova-oil/>



# Pancreatic Lipase

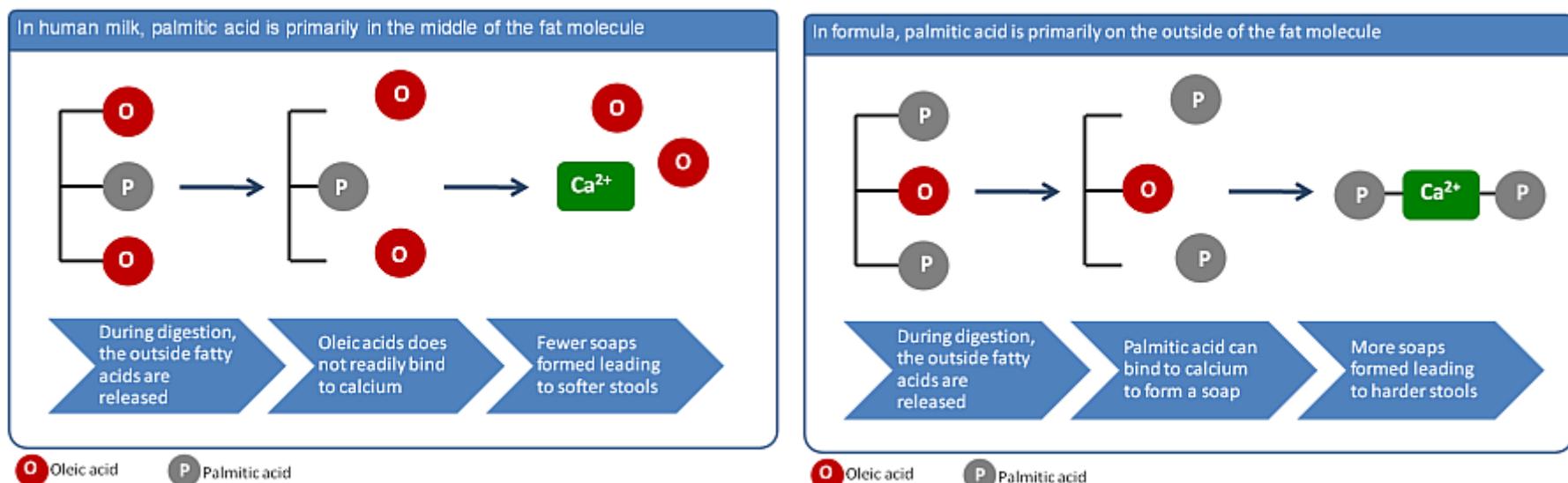
- Pancreatic lipase (pancreatic triacylglycerol lipase) is secreted from the pancreas, and is the primary lipase that hydrolyzes dietary fat in the human digestive system, converting triacylglycerols found in ingested fats and oils to monoacylglycerols and free fatty acids.
- Bile salts secreted from the liver and stored in the gallbladder are released into the duodenum where they coat and emulsify large fat droplets into smaller droplets, thus increasing the overall surface area of the fat, which allows the lipase to break apart the fat more effectively. The resulting monomers (2 free fatty acids and one 2-monoacylglycerol) are then moved by way of peristalsis along the small intestine to be absorbed into the lymphatic system by a specialized vessel called a lacteal.
- Unlike some pancreatic enzymes that are activated by proteolytic cleavage (e.g. trypsinogen), pancreatic lipase is secreted in its final form. However it only becomes efficient in the presence of a colipase in the duodenum.



[http://en.wikipedia.org/wiki/File:Pancreatic\\_lipase%280%93colipase\\_complex\\_with\\_inhibitor\\_1LPB.png](http://en.wikipedia.org/wiki/File:Pancreatic_lipase%280%93colipase_complex_with_inhibitor_1LPB.png)

# Digestion and absorption of fat

- Once human milk or an infant formula is ingested, pancreatic lipase hydrolyses the triacylglycerols and releases free fatty acids from the *sn*-1 and *sn*-3 positions, leaving one fatty acid attached to the glycerol molecule at the *sn*-2 position. These metabolites are called *sn*-2 monoacylglycerols and are easily absorbed into the small intestinal cells regardless if the fatty acid attached is saturated or unsaturated. Besides, the coefficient of absorption of free fatty acids is relatively low for long chain saturated fatty acids (C12:0 to C18:0) such as palmitic acids (74%) compared to medium chain fatty acids (C6:0 to C10:0; > 88%) and unsaturated fatty acids (> 90%). The free palmitic acids, resulting from hydrolysis of an infant formula, have a tendency to form soaps with minerals such as calcium (very low water solubility) at the pH of the intestine.

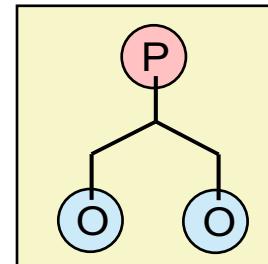


<https://www.wyethnutritionacademy.org/Wyeth-Nutrition/resources-hcps/nutrition-for-newborns/human-milk-lipid/information#>

# Example BETAPOL™



<http://www.betapol.com/HealthBenefits/Howbetapolworks/>



- Mother milk is unique, as an unusual large proportion – about 60%-70% – of the most abundant saturated fatty acid, palmitic acid, is attached to the middle ('beta' or *sn*-2 position) of the triacylglycerols. The 'alpha' (*sn*-1) and 'gamma' (*sn*-3) positions are primarily occupied by unsaturated fatty acids.
- The Betapol, a commercial version of an OPO-Fat, contains, about 48% total palmitic acid of which 54% is esterified in the *sn*-2 position.
- It is manufactured from palm oil triacylglycerols and oleic acid by using a 1,3-specific lipase for enzymatic inter-esterification. This results in a blend of OPO, remaining high melting triacylglycerols mainly of the tripalmitin type and free fatty acids. The free fatty acids are removed by distillation, whereas the high melting triacylglycerols are removed by fractionation.

# Fatty acid profiles of the milk fat of various species

Table 3. The composition of the fatty acids esterified to each position of the triacyl-*sn*-glycerols in the milk fats of various species.

Species	Position	Fatty acid											
		4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Cow	TG	12	5	2	4	4	11	24	2	7	24	3	
	1	-	1	1	2	5	10	34	2	10	30	2	
	2	-	1	1	3	6	18	32	4	10	19	4	
	3	35	13	4	6	1	6	5	1	1	23	2	
Human	TG				tr	3	26	27	6	7	36	11	1
	1				tr	1	3	16	4	15	46	11	tr
	2				tr	2	7	58	5	3	13	7	1
	3					1	6	7	6	8	2	50	15
Rat	TG				6	19	14	12	21	2	3	13	10
	1				3	10	10	10	20	2	5	24	14
	2				6	20	16	18	29	2	1	3	5
	3				10	26	15	9	13	2	2	12	12
Pig	TG						4	32	9	5	39	10	1
	1						2	22	7	7	50	11	1
	2						7	58	11	1	15	8	1
	3						4	15	10	6	52	12	2

tr = trace (< 0.5 %). TG = intact triacylglycerols.

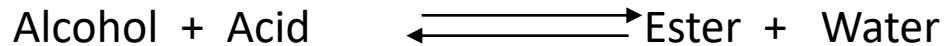
Data from - Christie, W.W. and Moore, J.H. *Biochim. Biophys. Acta*, **210**, 46-56 (1970); Christie, W.W. *J. Dairy Res.*, **52**, 219-222 (1985); Christie, W.W. and Clapperton, J.L. *J. Soc. Dairy Technol.*, **35**, 22-24 (1982); Breckenridge, W.C. et al., *Canad. J. Biochem.*, **47**, 761-769 (1969).

<http://lipidlibrary.aocs.org/Lipids/tag1/index.htm>

- Overall fatty acid compositions of the triacylglycerols are different for various species
- Palmitic acid (16:0) is generally found in position *sn*-2
- Cow's milk (34-32-5) is different from human milk (16-58-6) for palmitic acid (16:0) in positions (*sn*-1, *sn*-2 and *sn*-3)
- A high content of palmitic acid (>50%) in position *sn*-2, as found in human and pig, is not common in mammal milk triacylglycerols

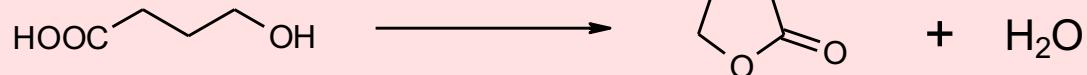
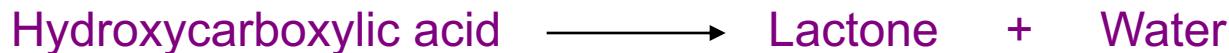
# Application of Lipases: Generation of Flavours

## Esterification/ Hydrolysis



Fruity

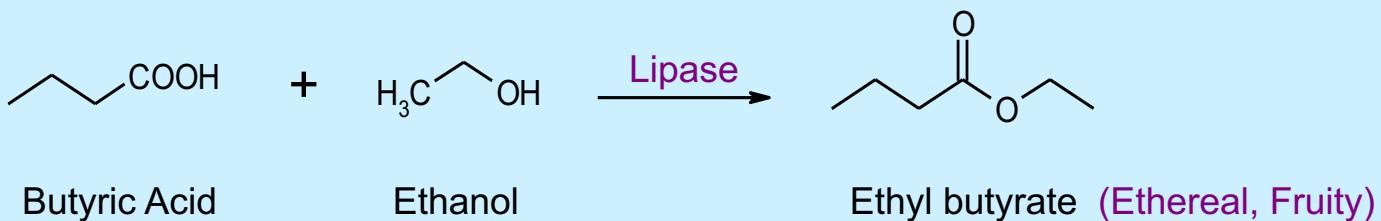
## Intra-molecular cyclisation/ transesterification



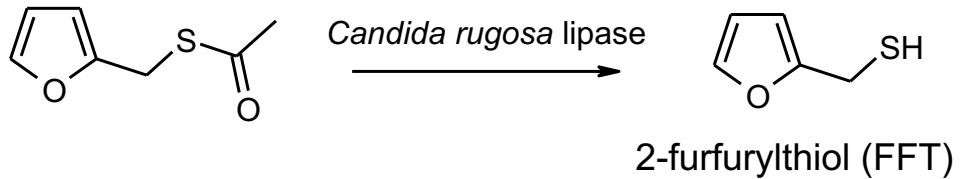
Savoury

# Application of Lipases: Generation of Flavours

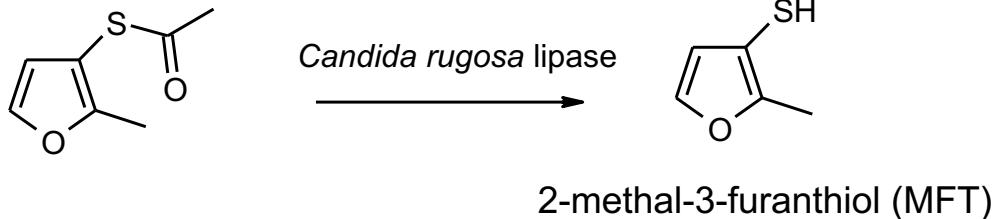
Butter fat	Butyric Acid	(Sour, Rancid)
	Caproic Acid	(Fatty-rancid, Sweet-like)
	Caprylic Acid	(Oily-rancid, Sweet-like)
	Capric Acid	(Sour-fatty, Rancid)



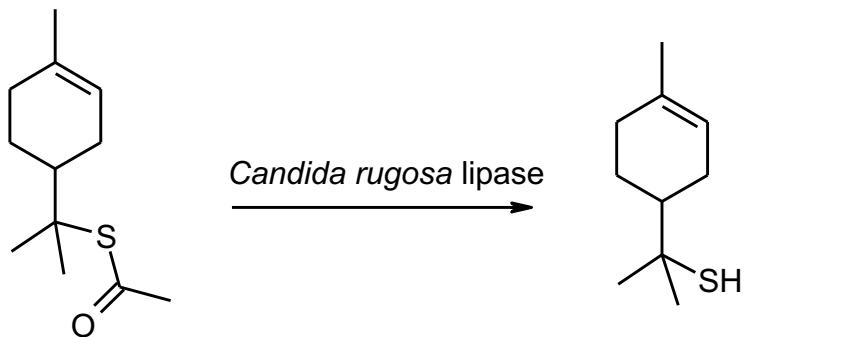
# Application of Lipases: Generation of Flavours



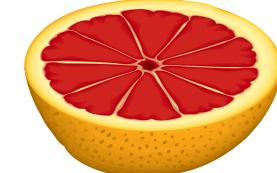
Roasted



Boiled Meat



Grapefruit juice



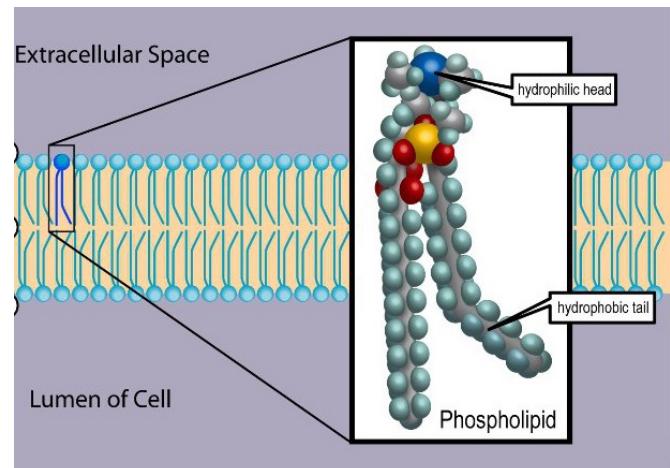
No need to remember details

# Phospholipids

## Phospholipids are:

- Natural emulsifiers found within the biological membranes of all living organisms
- Commercially used phospholipid emulsifiers:
  - Mainly obtained as a by-product from vegetable oil refining
  - Widely used as an additive in various processed foods to stabilize emulsions alone or together with proteins.

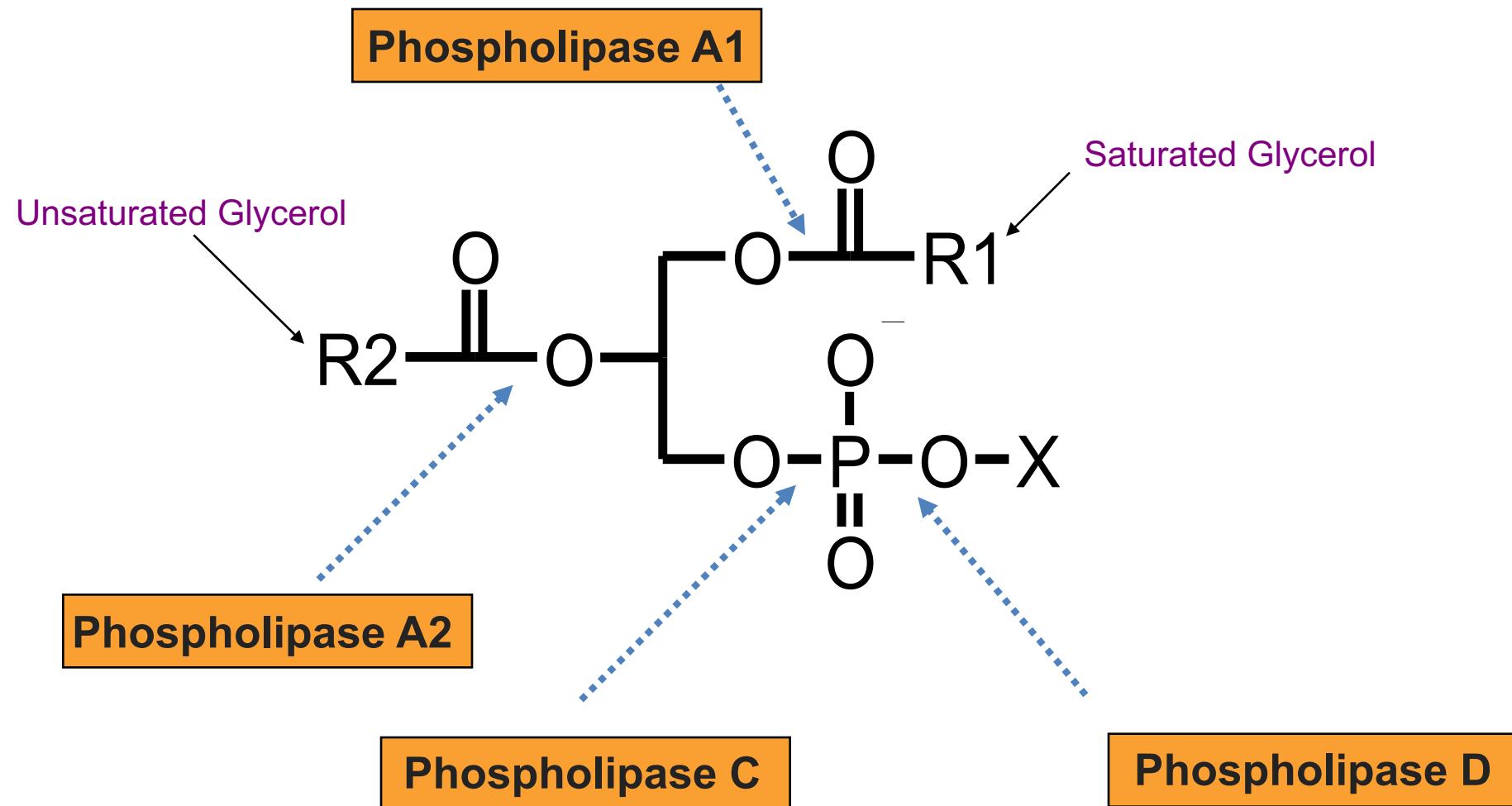
- Phosphatidylglycerol
- Phosphatidylcholine
- Phosphatidylethanolamine
- Phosphatidylinositol
- Phosphatidylserine
- Lysophospholipids
- Plasmalogens
- Sphingomyelins (only sphingolipid that are also phospholipids)
- Glycosphingolipids



# Phospholipases

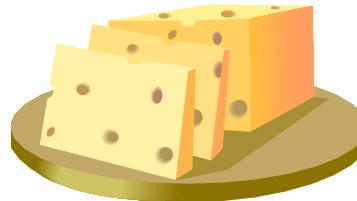
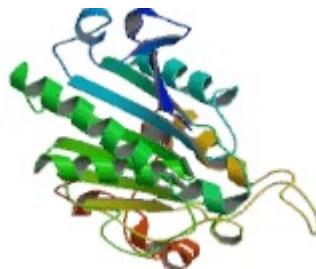
- **Enzymatically modify phospholipids to yield lysophospholipids**
  - Lysophospholipids are more water-soluble and provide improved functionality in some applications, e.g., in margarines and heat-treated emulsions.
  - Hydrolysis in GI tract to assure uptake
- **Employed for modification of phospholipids directly in different food materials during processing**
  - Improve the stability of emulsions
  - Or reduce or replace emulsifiers and chemical stabilizers due to *in-situ* enzymatic formation of lysophospholipid.

# Phospholipases



# Phospholipase A1

- The main use of phospholipase A1 (from *A. oryzae*) is in the dairy industry for cheese manufacture to improve process efficiencies and cheese yields.
  - The enzyme acts on phospholipids to form a lysophospholipid and a free fatty acid. These reaction products have improved emulsifying properties which produce an approximate 2% increase in cheese yield.

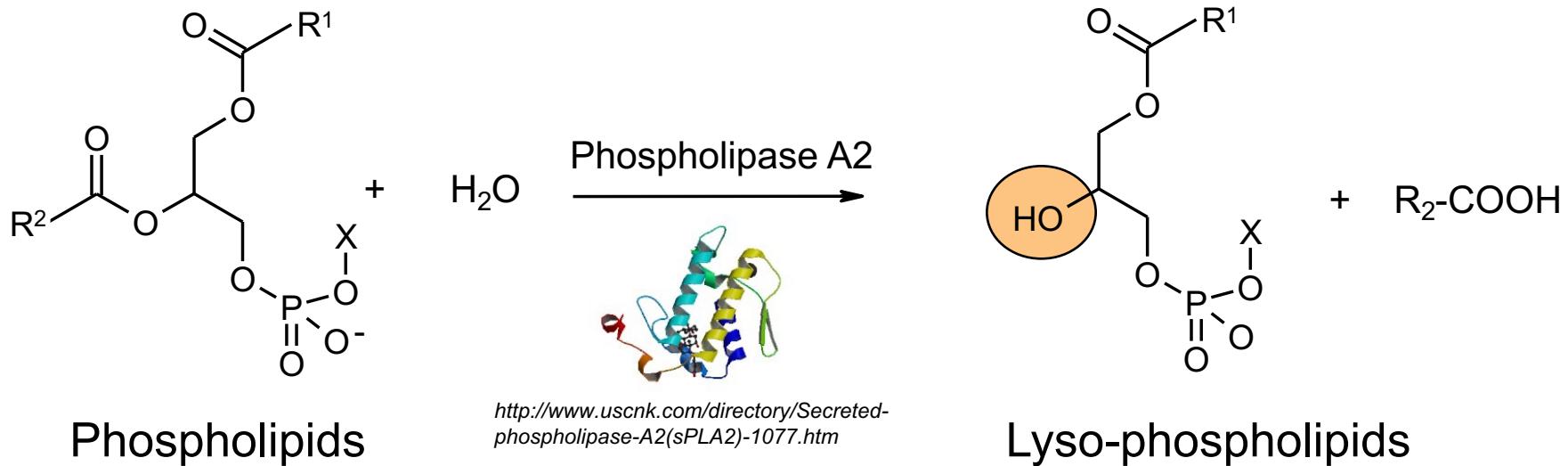


[http://www.uscnk.com/directory/Phospholipase-A1\(PL-A1\)-1151.htm](http://www.uscnk.com/directory/Phospholipase-A1(PL-A1)-1151.htm)

- Phospholipase A1 is also used
  - In oil processing for vegetal oil degumming (The removal of gum from a material, especially the removal of sericin from silk or phospholipids etc. from vegetable oils).
  - To enrich fish oil with n-3 PUFA using phospholipase A1

# Phospholipase A2

- Phospholipase A2 represents a class of heat-stable, calcium-dependent enzymes



# Phospholipase A2

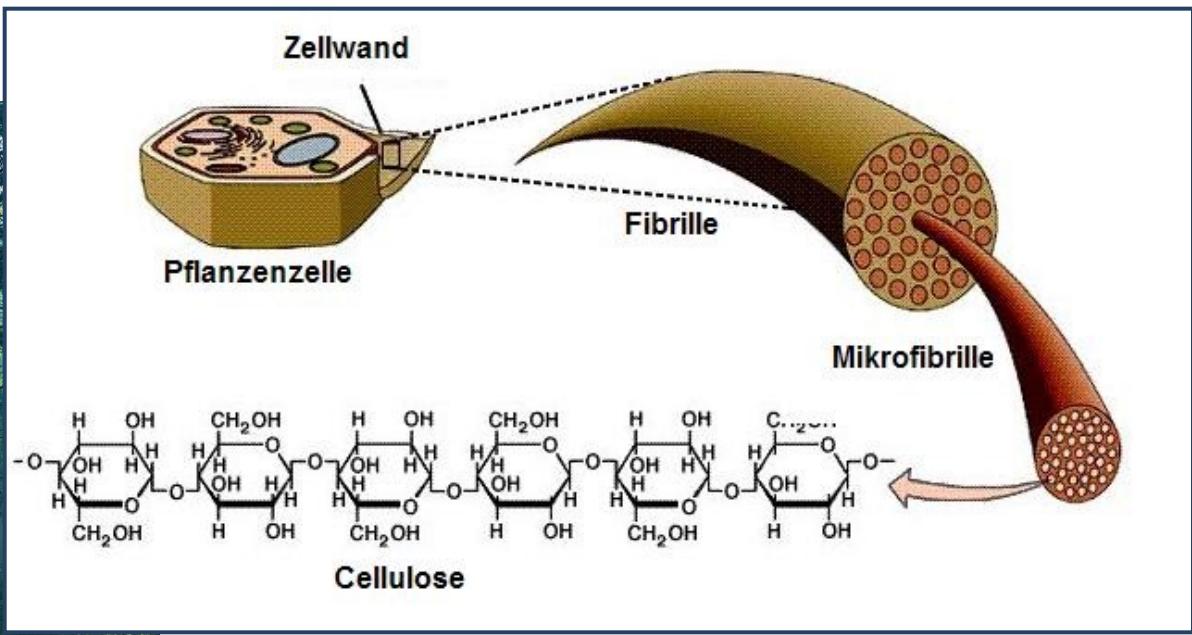
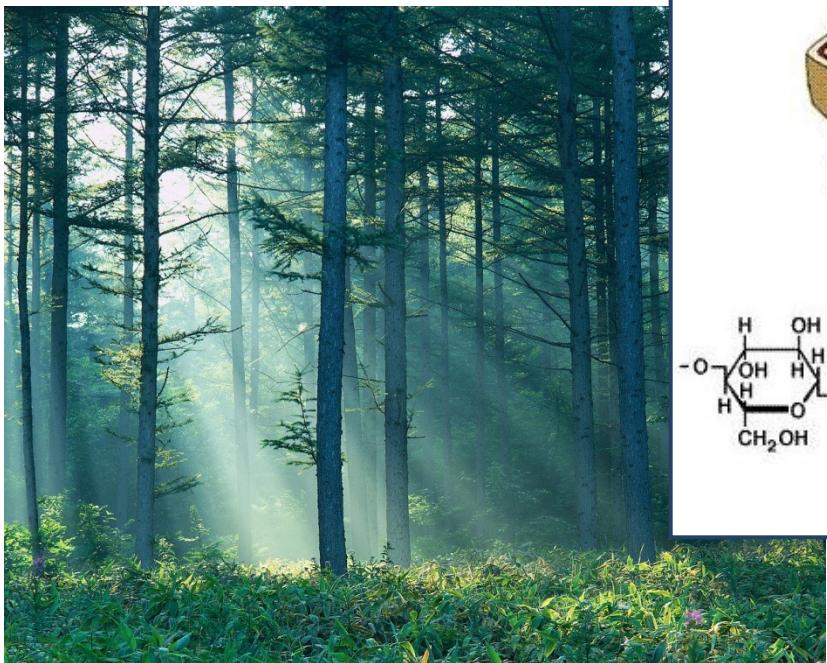
- Sources for industrial application:
  - Porcine pancreatic lecitase™ (Novozymes).
  - LIPOMOD (Biocatalyst)
  - Lecitase® Ultra: a kosher- and halal-certified liquid phospholipase preparation for use in vegetable oil degumming and in hydrolysis of lecithin
- Modified egg yolk treated with PA2 has demonstrated
  - Superior stability at retort temperatures above 150°C when used in certain food systems (i.e., mayonnaise). This stability allows for the incorporation of pasteurization into processing, which is critical for microbial quality and which also contributes to increased shelf life.
  - Increased viscosity is another benefit offered by modified egg yolk, which is particularly important in the production of mayonnaise.



# Carbohydrases

# Introduction to carbohydrates

- Carbohydrates are the most abundant and diverse class of organic compounds occurring in nature!

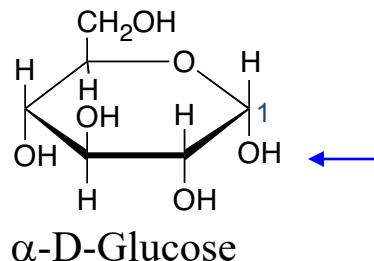
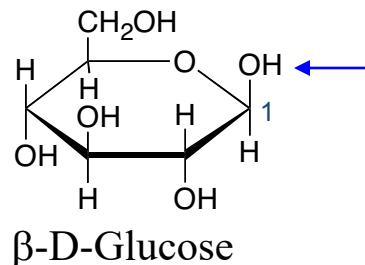


<http://www.lookfordiagnosis.com/images.php?term=cellulose&lang=4&from2=60>

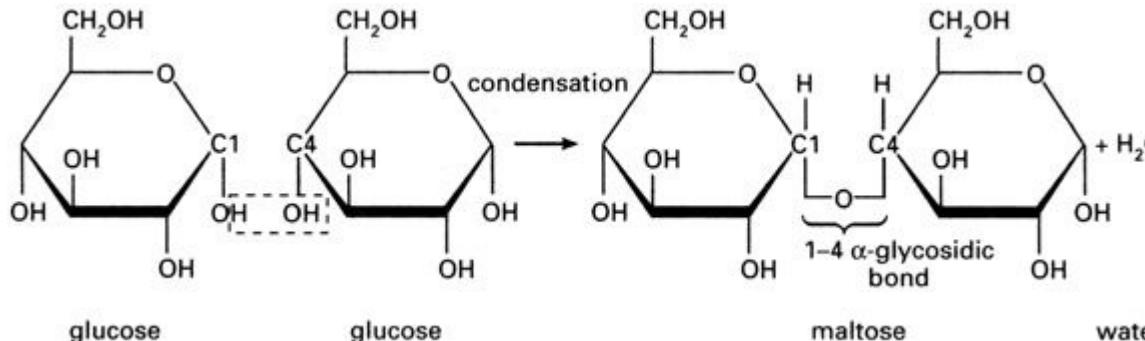
# Introduction to carbohydrates

## Monosaccharides

- Carbohydrates (CHOs) consist of monosaccharide sugars, of varying chain lengths, that have the general chemical formula  $C_n(H_2O)_n$  or are derivatives of such:
  - CHO<sub>s</sub> can also contain other elements such as S, P or N



- A glycosidic bond usually joins two monosaccharides.
  - A glycosidic bond is formed between the hemiacetal group of a saccharide (or a molecule derived from a saccharide) and the hydroxyl group of an other molecule.
  - A substance containing a glycosidic bond is a glycoside



<http://www.answers.com/topic/glycosidic-bond-1>

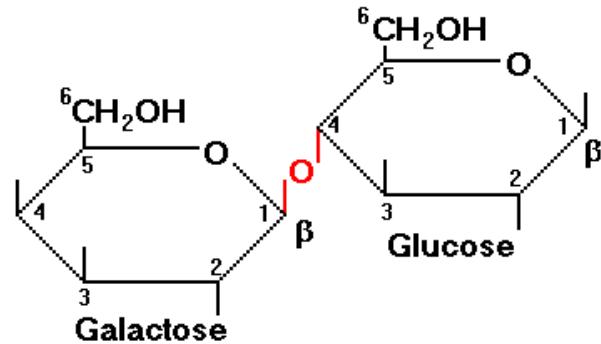
# Introduction to carbohydrates

## Disaccharides

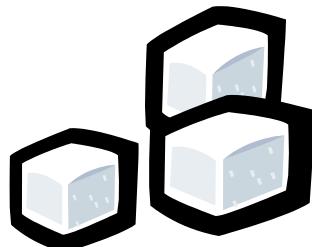
se Only

- Lactose

- Lactose is a disaccharide containing one **galactose** and one **glucose** residue linked by a  $\beta$ -(1,4) glycosidic bond.



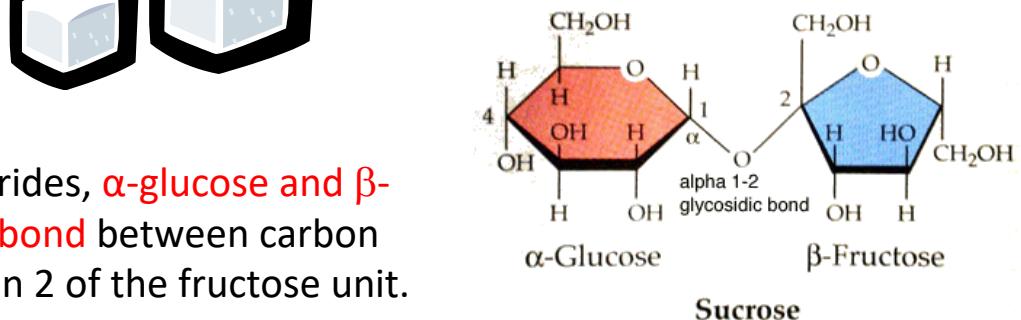
<http://www.rpi.edu/dept/chem-eng/Biotech-Environ/IMMOB/lactose.html>



- Sucrose

- Sucrose consists of two monosaccharides,  **$\alpha$ -glucose** and  **$\beta$ -fructose**, joined by a **(1,2)-glycosidic bond** between carbon atom 1 of the glucose unit and carbon 2 of the fructose unit.
- No reducing end

Sucrose has the molecular formula  $C_{12}H_{22}O_{11}$



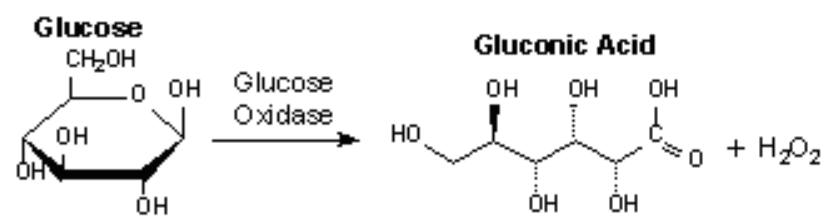
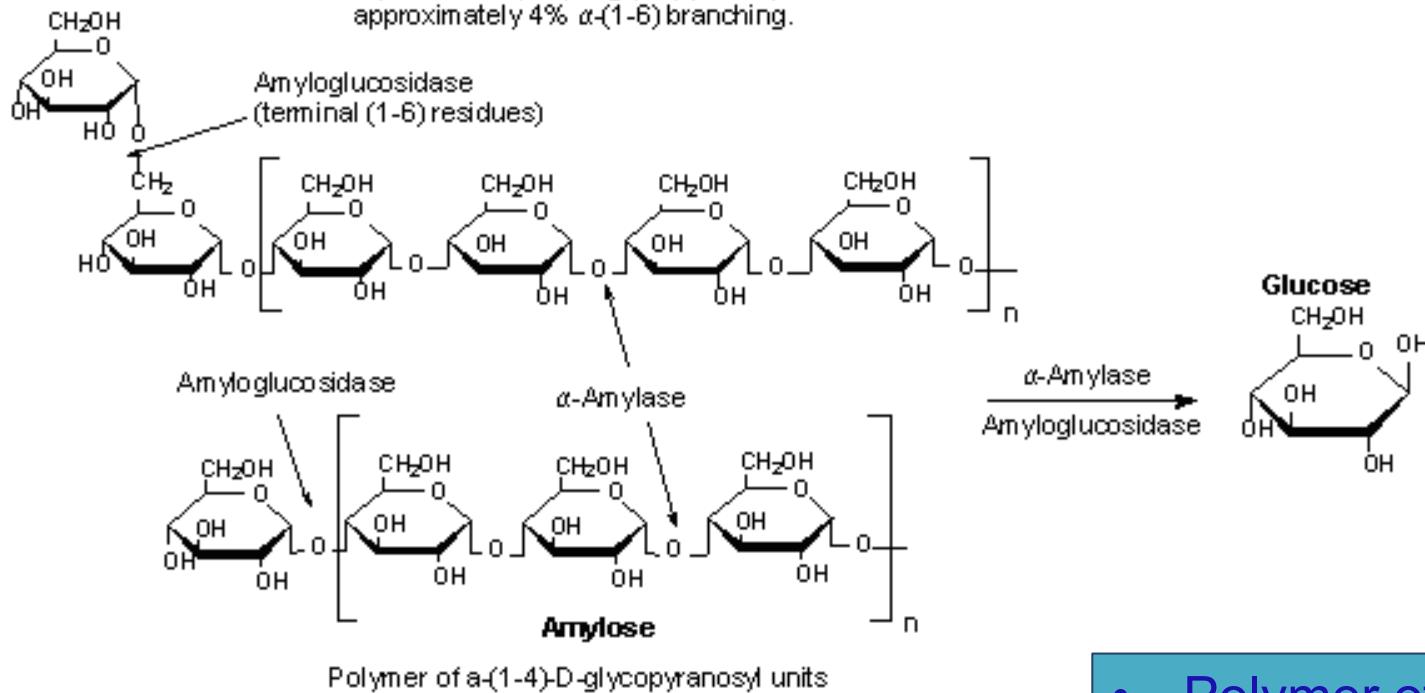
<http://www.chm.bris.ac.uk/motm/glucose/glucose.htm>

# Starch

## Starch

### Amylopectin

Polymers of  $\alpha$ -(1-4)-D-glycopyranosyl units with approximately 4%  $\alpha$ -(1-6) branching.



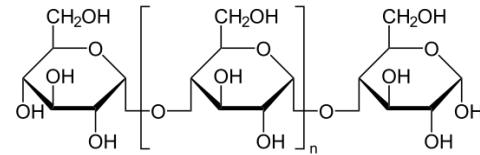
- Polymer of glucose
- Amylopectin & Amylose
- 1-4 and 1-6 linkages

# Starch degrading enzymes as an example

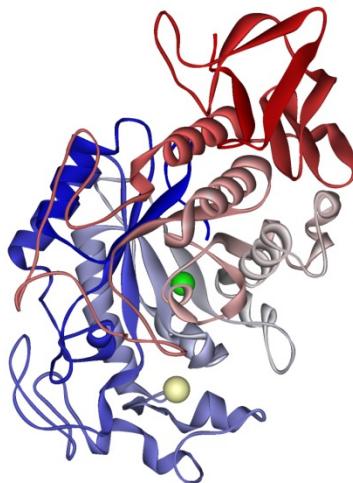
## 1. Glycosyl hydrolases

- Endo-hydrolases
  - $\alpha$ -amylase: acting at random locations along the starch chain. Breaks down long-chain carbo-hydrates, yielding maltotriose and maltose from amylose, or maltose, glucose and dextrins from amylopectin. Because it can act anywhere on the substrate,  $\alpha$ -amylase is faster than  $\beta$ -amylase
  - $\beta$ -amylase: Working from the non-reducing end. Catalyzes hydrolysis of the second  $\alpha$ -1,4 glycosidic bond, cleaving off two glucose units (maltose)
- Exo-hydrolases – Amyloglucosidase: will cleave  $\alpha$ -1,6 glycosidic linkages, as well as the last  $\alpha$ -1,4 glycosidic linkages at the non-reducing end of amylose and amylopectin, yielding glucose.

## 2. Carbohydrate isomerases: Catalyzes intramolecular rearrangements on monosaccharides (e.g. glucose to fructose)



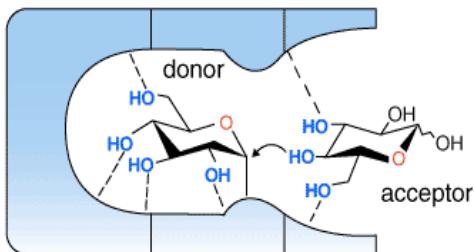
*Dextrins are mixtures of polymers of D-glucose units linked by  $\alpha$ -1,4 or  $\alpha$ -1,6 glycosidic bonds.*



<http://www.glucide.wikibis.com/amylase.php>

# Glycosyl Hydrolases: Three Types of Active Sites

A

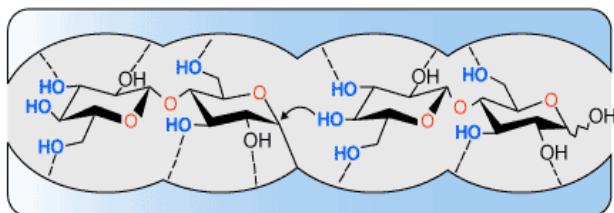


GlycoWord

## Pocket/Crater:

for the recognition of non-reducing extremity of saccharides, found in glycosidases that hydrolyze the glycosidic bond of oligosaccharides

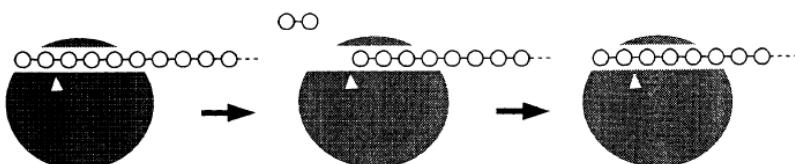
B



## Cleft/Groove:

allows the random binding of several sugar units in polysaccharides, found in endoglycanases

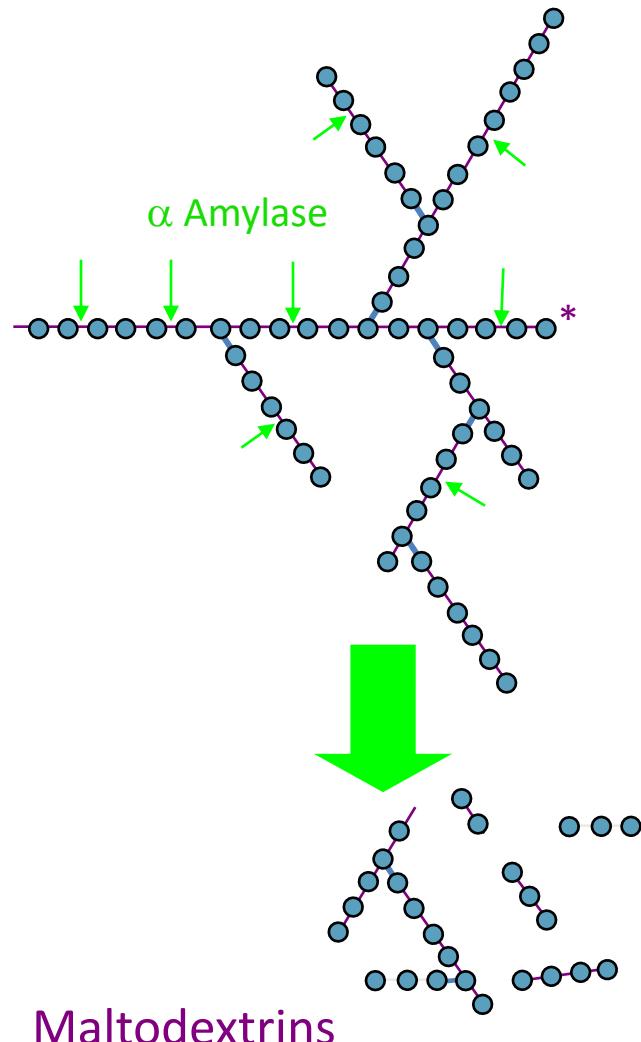
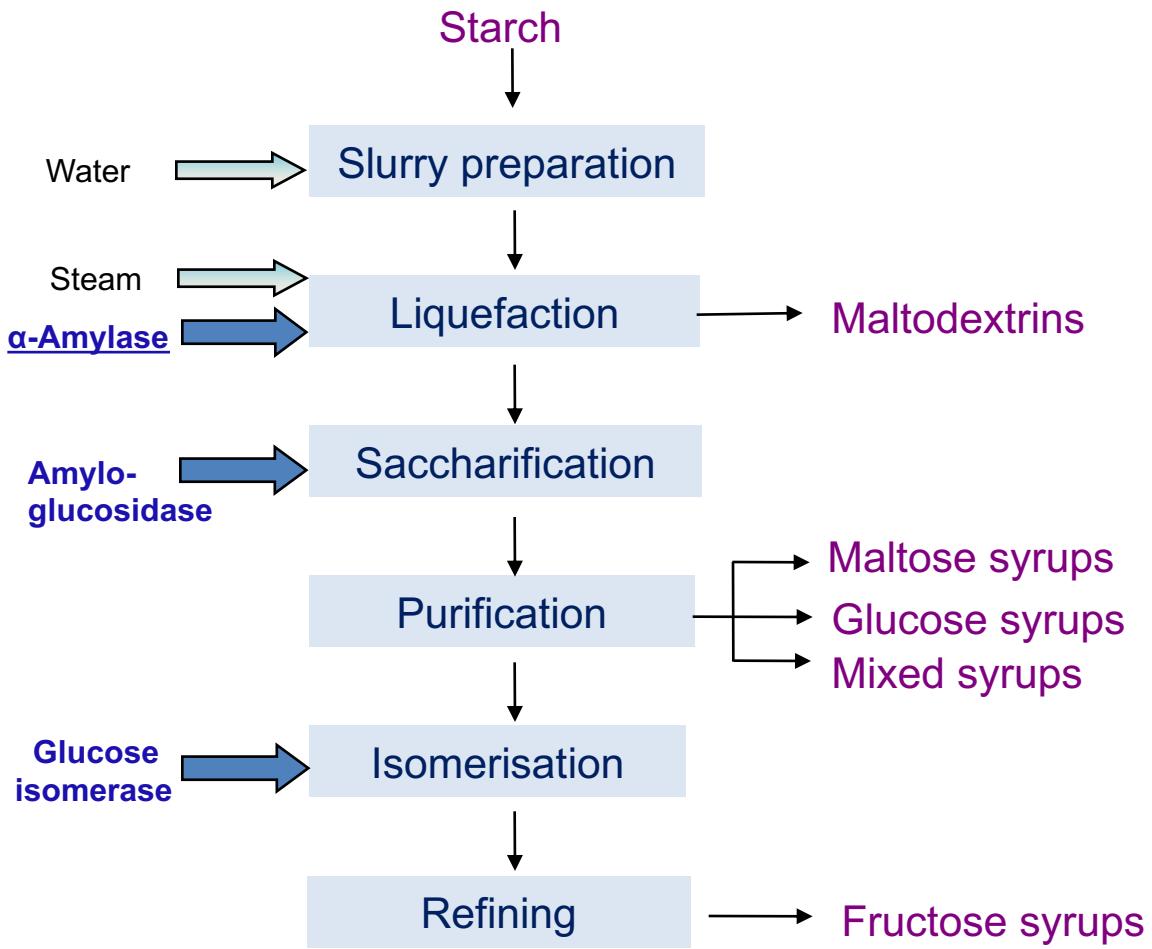
C



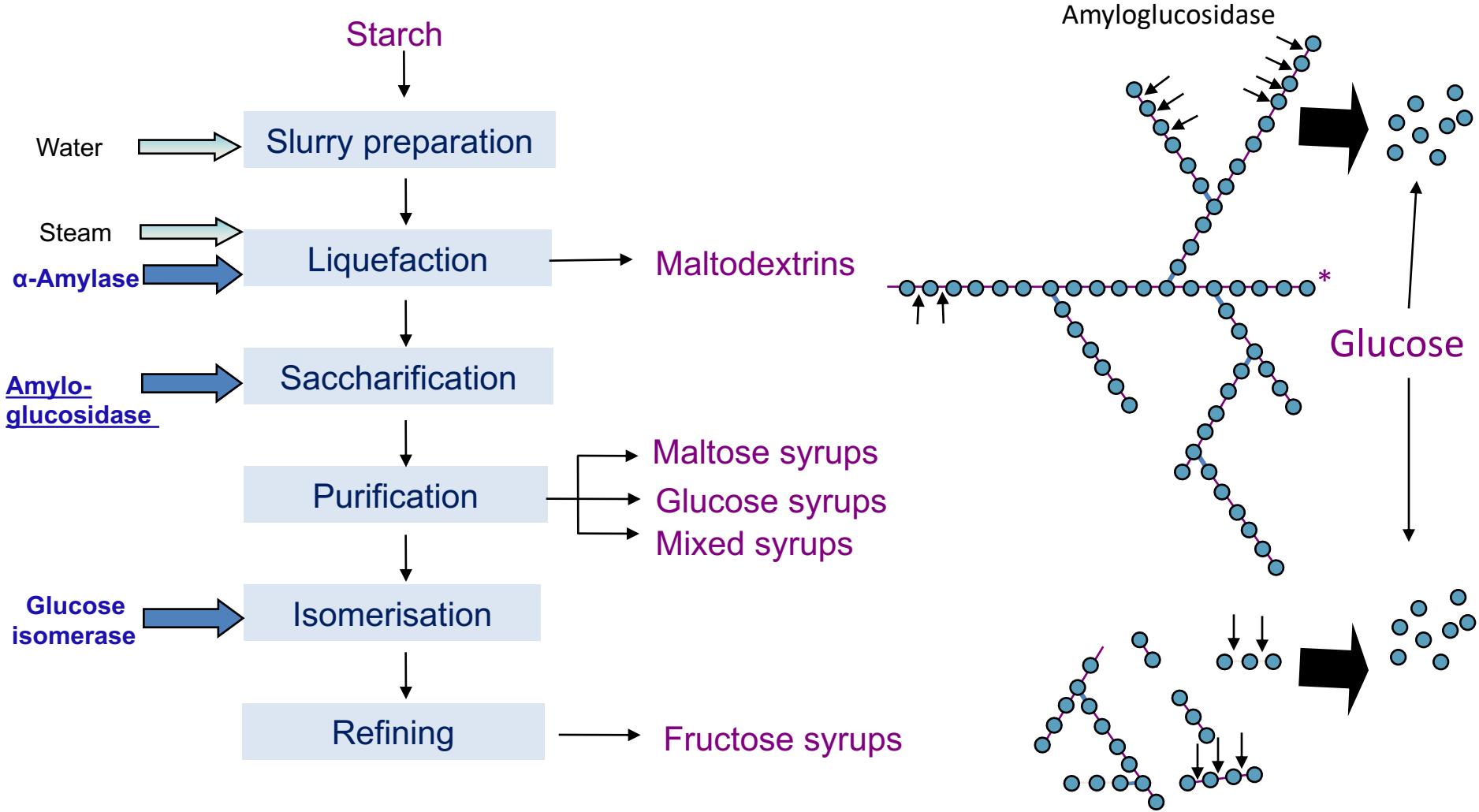
## Tunnel:

enables a polysaccharide chain to be treated through it, found only in cellobiohydrolases

# Major steps in enzymatic degradation of starch



# Major steps in enzymatic degradation of starch



# Percentage of Hydrolysis

The starch and glucose syrup industry uses the expression dextrose equivalent or DE, similar in definition to the DH units of proteolysis, to describe its products, where:

$$DE = 100 \times \left( \frac{\text{Number of glycosidic bonds cleaved}}{\text{Initial number of glycosidic bonds present}} \right)$$

In practice, this is usually determined analytically by use of the closely related, but not identical, expression:

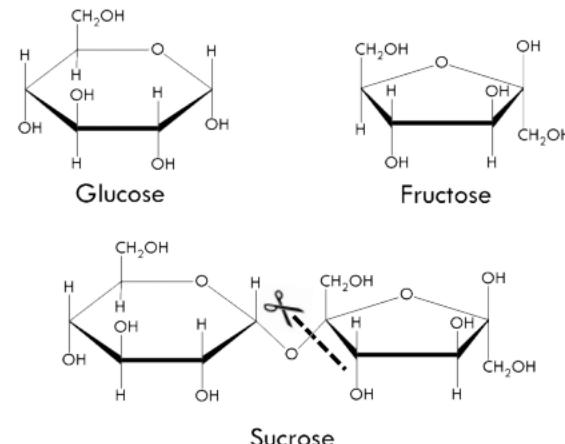
$$DE = 100 \times \left( \frac{\text{Reducing sugar, expressed as glucose}}{\text{Total carbohydrate}} \right)$$

# Carbohydrases in food: Invertase



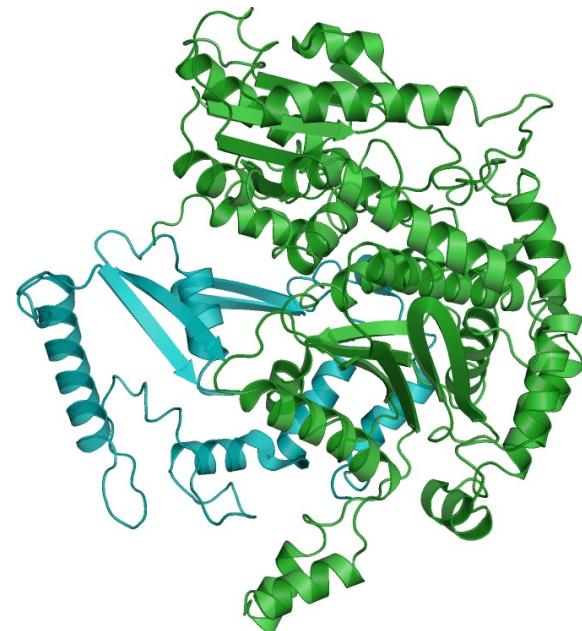
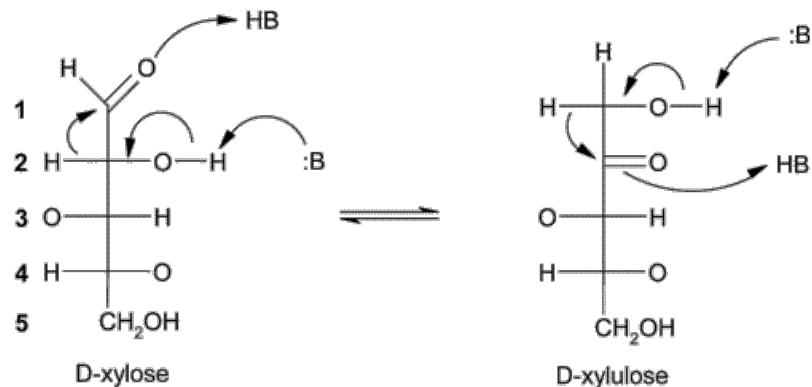
Invertase's job is to hydrolyse sucrose, cleaving the O-C bond (shown below). Sucrose, the ordinary sugar you use in baking, is a double sugar called a disaccharide and invertase splits this into its component parts - glucose and fructose. Glucose and fructose are smaller monosaccharides (single sugars) and have a more liquid-like consistency. So the minty interior of a Rowntree After Eight has liquefied itself inside the chocolate coating all thanks to an enzyme.

**FR/BE** **Ingédients:** sucre, pâte de cacao, sirop de glucose, matière grasse de lait anhydre, cacao maigre en poudre, émulsifiant: lécithine de tournesol; arôme citron, stabilisant: invertase; huile essentielle de menthe, acidifiant: acide citrique,. **Traces:** lait. Ce produit contient 18 portions. Portions à adapter pour les enfants selon leur âge.



# Isomerases

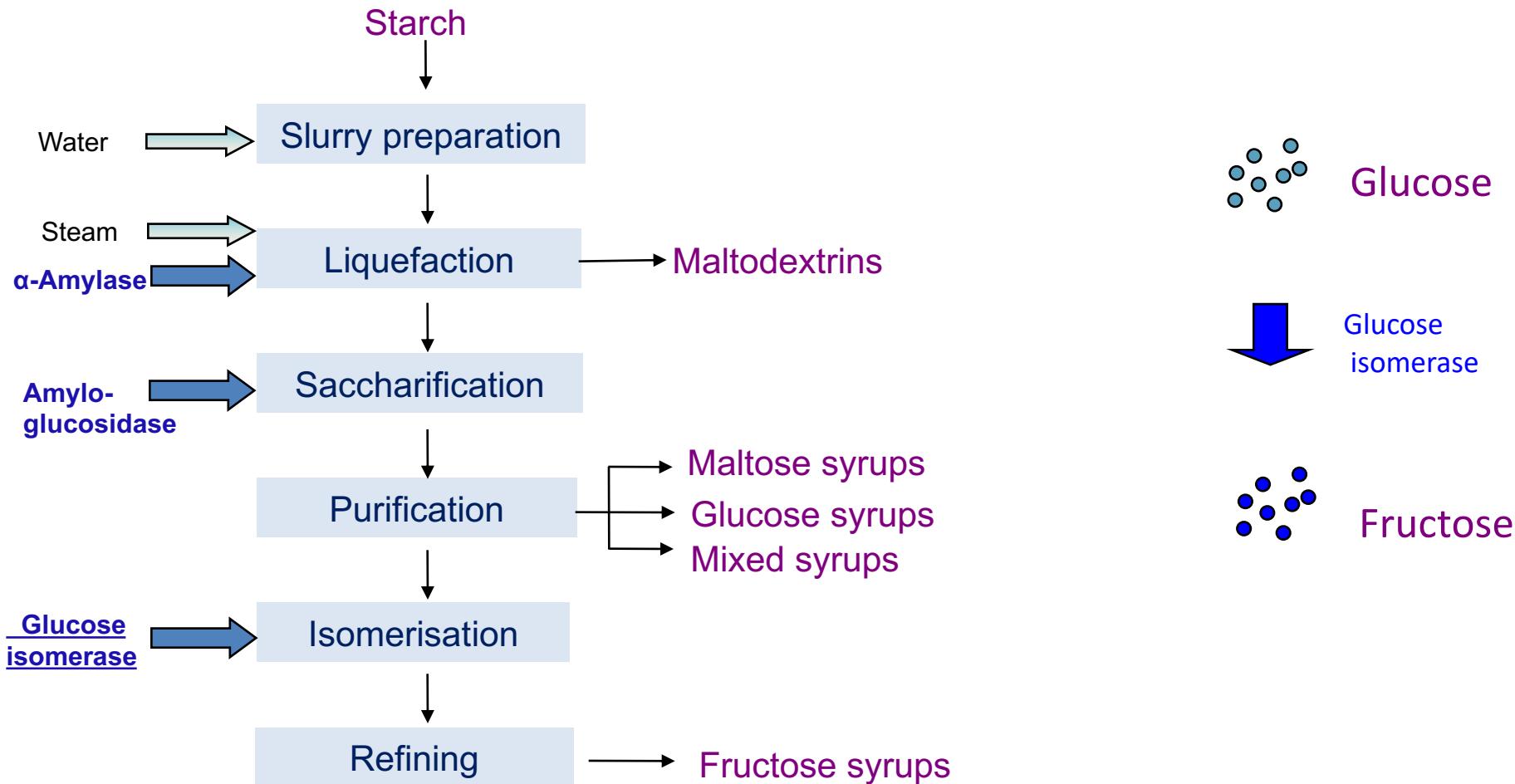
- Catalyze intramolecular rearrangements on monosaccharides
- A major constituent in sugar metabolism
- Example: Xylose isomerase
  - Mechanism of Xylose isomerase (E.C. 5.3.1.5)



[http://en.wikipedia.org/wiki/File:Glucose-6-phosphate\\_isomerase\\_wpmp.png](http://en.wikipedia.org/wiki/File:Glucose-6-phosphate_isomerase_wpmp.png)

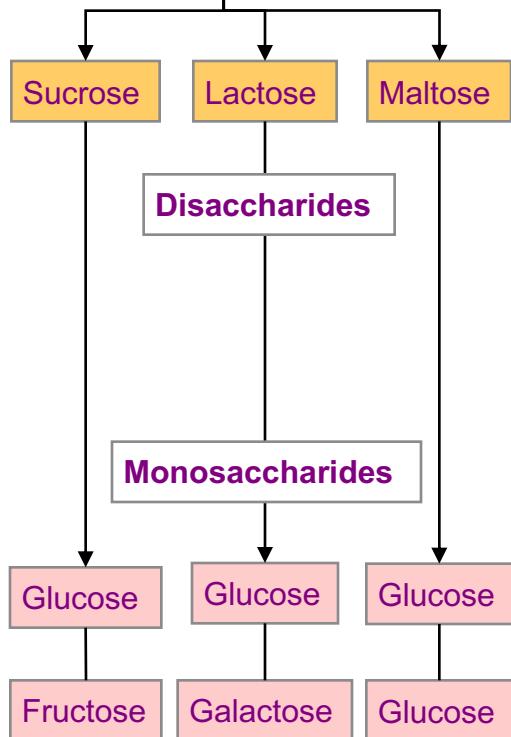
- Interconversion of xylose to xylulose and glucose to fructose

# Major steps in enzymatic degradation of starch

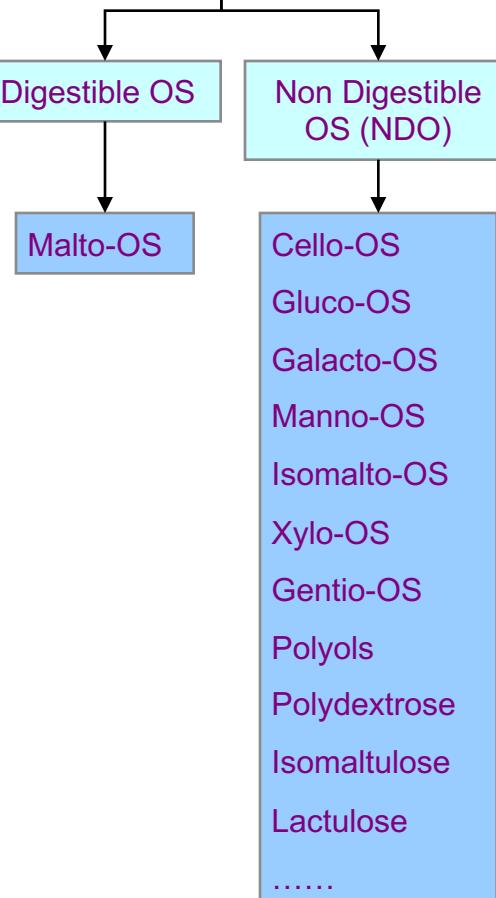


# Carbohydrates in Food

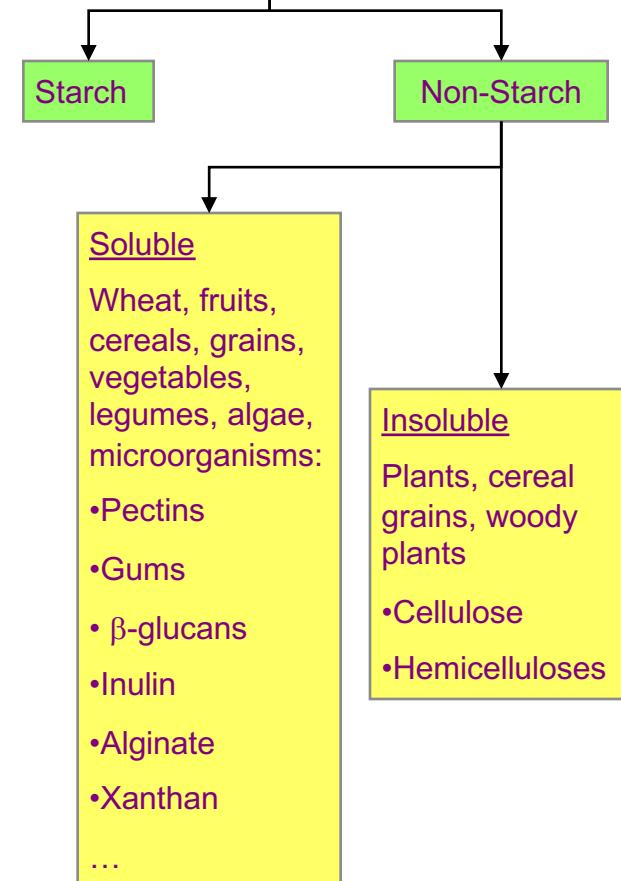
## Simple CHOs=sugars



## Oligosaccharides (OS)

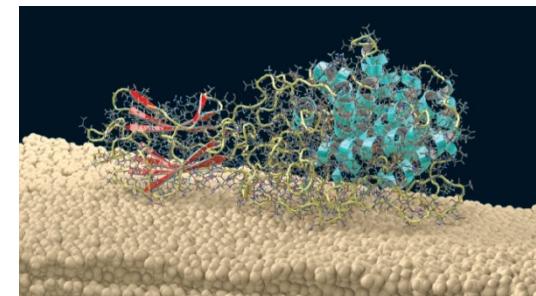


## Complex-Polysaccharides



# Cellulases

- Used in fruit juice preparation
- Endo-(1,4)- $\beta$ -glucanase, Endoglucanase
- Hydrolysis of (1,4)- $\beta$ -D-glucosidic linkages in cellulose, lichenin and cereal  $\beta$ -D-glucans
- Together with cellobiohydrolases and  $\beta$ -glucosidases



<http://www.scidacreview.org/0704/html/hardware.html>

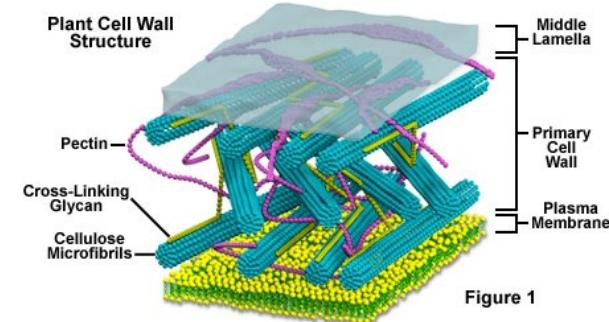
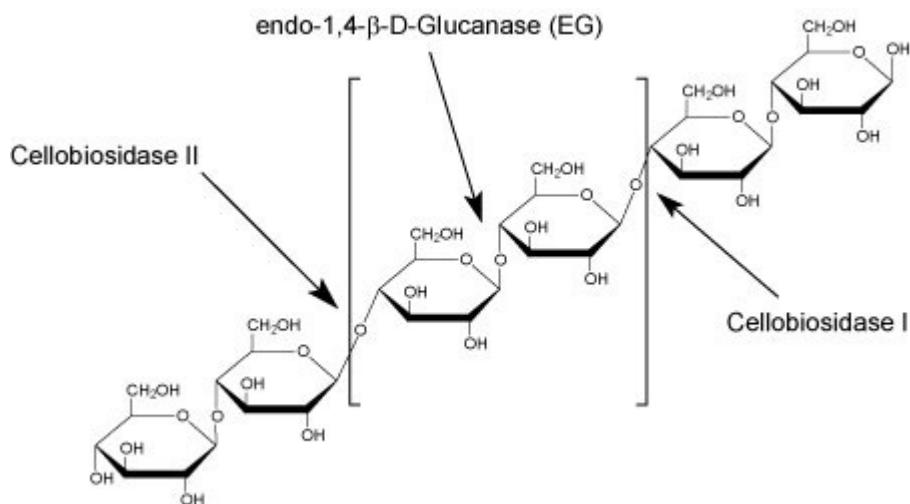


Figure 1

<http://micro.magnet.fsu.edu/cells/plants/cellwall.html>

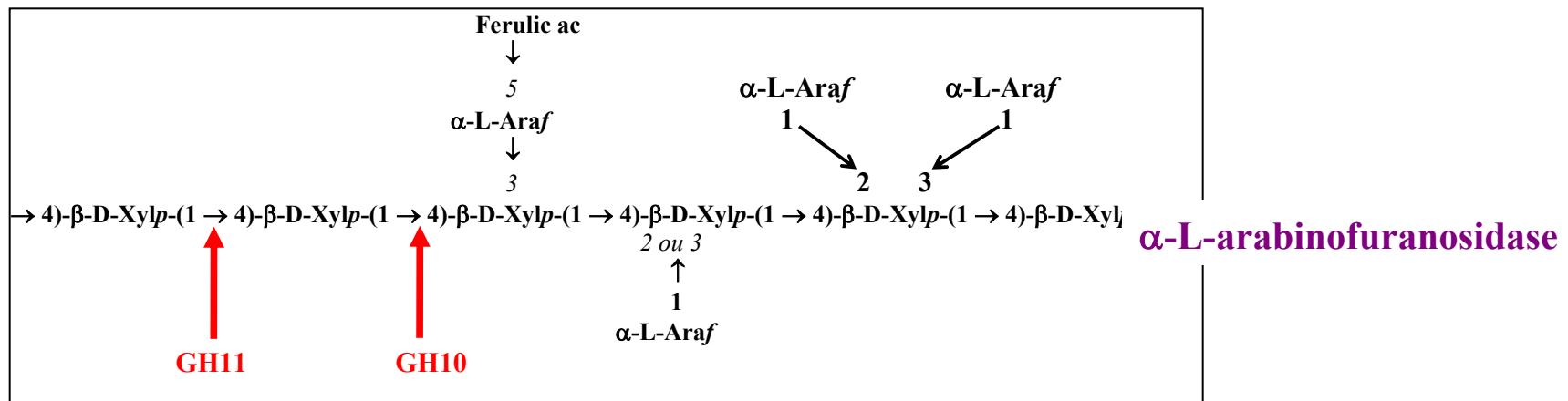
## Structural unit of cellulose:

Cellulose is an insoluble linear polymer of  $\beta$ -(1,4)-D-glucopyranose units

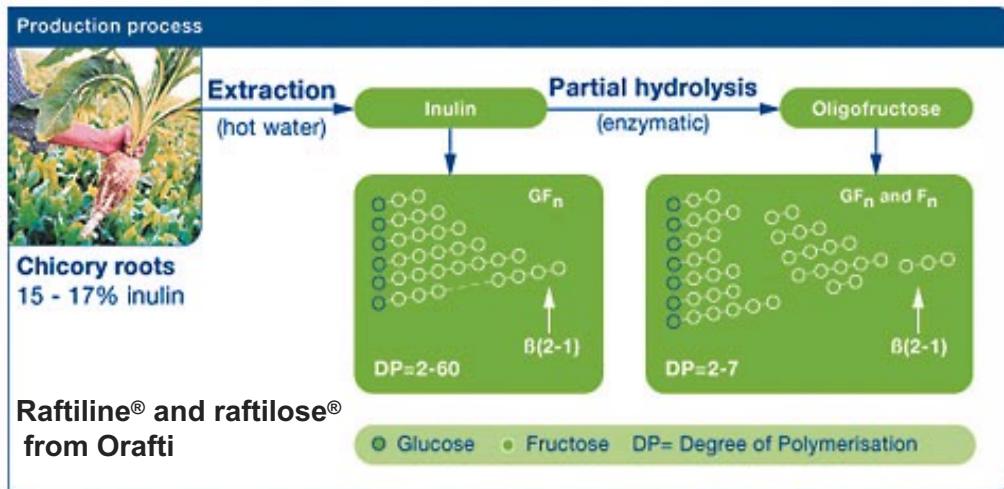
<http://www.biocatalysts.com/2016/06/improve-yield-clarity-and-stability-of-fruit-juice-with-the-use-of-enzymes/>

# Xylanases

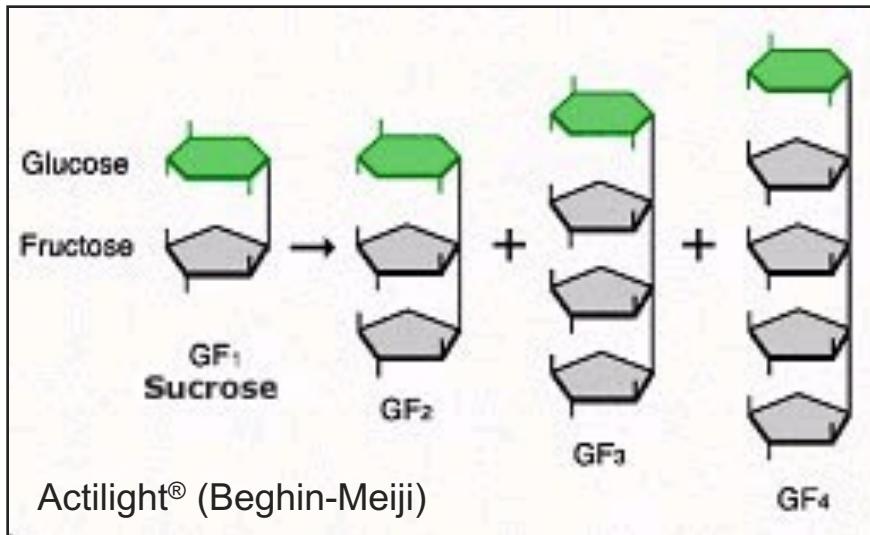
- Fungal xylanases are used in bread making (with  $\alpha$ -amylases) and juice industry (with pectinases)
- Endo-1,4- $\beta$ -xylanhydrolase
- Catalyze the random hydrolysis of (1,4)- $\beta$ -D-xylosidic linkages in xylans, arabinoxylans In plants, fungi, bacteria
- Optimal pH: 4.5 to 6, Optimal temperature: 50-60°C
- Specificity varies with family:
  - GH11 xylanases hydrolyze unsubstituted regions of xylan
  - GH10 enzymes are able to attack decorated forms of the polysaccharide



# Production of Fructo-Oligosaccharides



## Inulinase Production of FOS from inulin



## Beta-fructofuranosidase Production of FOS from sucrose (Reverse hydrolysis)

# An ingredient company launches a new processing aid for dairy applications - How does this work?

Reduce total sugar by more than 35%  
Source of prebiotic fiber  
Suitable for lactose-free diets  
Appealing taste and texture  
Supports mineral absorption, digestive health and weight management



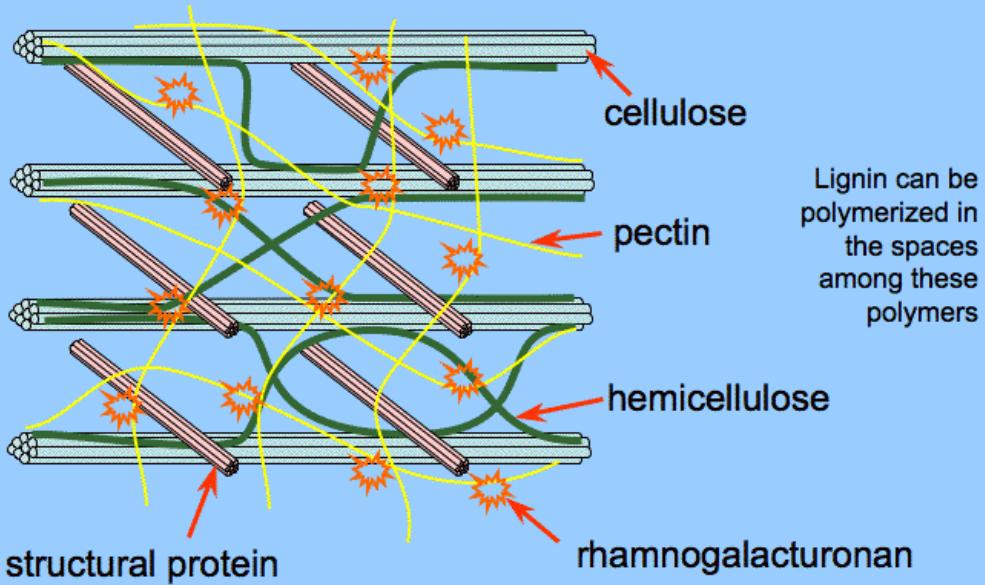
# More info

<https://www.dairyreporter.com/Article/2019/10/31/DuPont-launches-new-lactases>

# Pectic enzymes and use in fruit juice processing (1)

## Primary Plant Cell Wall:

cross-linked polymers of various sugars and protein

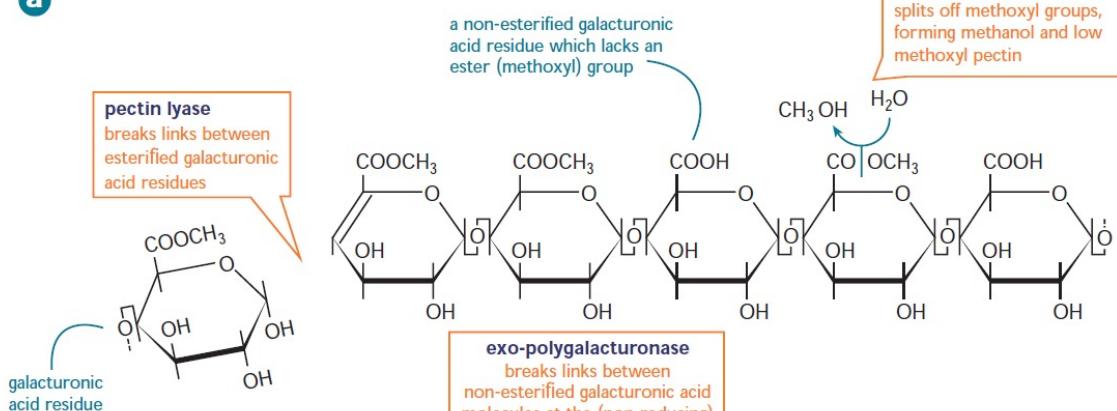


[www.ncbe.reading.ac.uk](http://www.ncbe.reading.ac.uk). Enzymes in fruit juice production.  
In a jam and out of juice. Version 1.0. December 2000.  
[http://plantphys.info/plant\\_physiology/basiccytology1.shtml](http://plantphys.info/plant_physiology/basiccytology1.shtml)

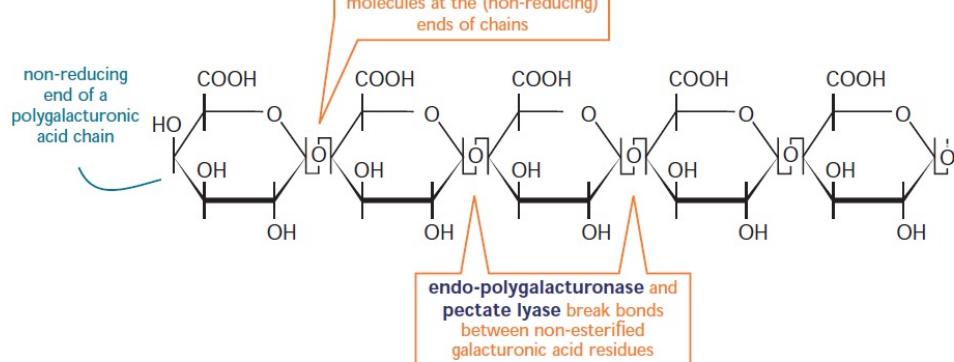
- The cell wall is made of layers of variously arranged/aligned cellulose microfibrils.
- In addition, cell walls have a range of various polymers (e.g. Hemicellulose, rhamnogalacturonan, pectins)
- Hemicellulose provides cross-linking of cellulose microfibrils
- Pectins are the “glue” that holds cells together. Middle-lamella is comprised of much pectin.
- In some woods, up to 40% can be attributed to lignins (polymer of polyphenols).

# Pectic enzymes and use in fruit juice processing (2)

a



b



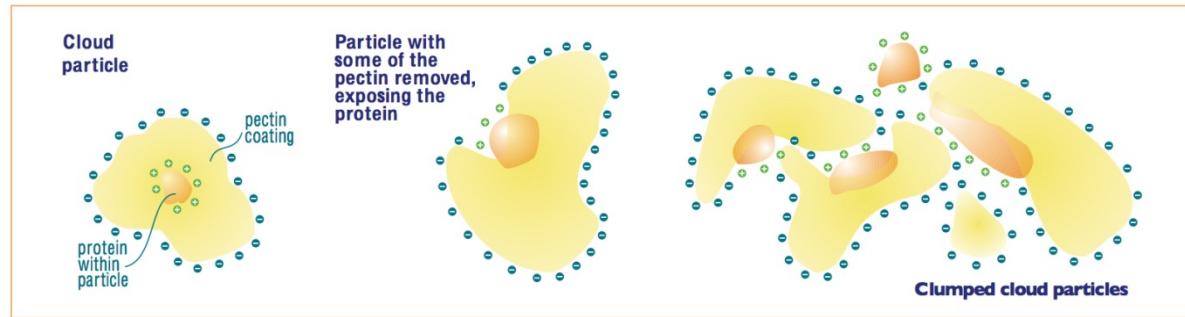
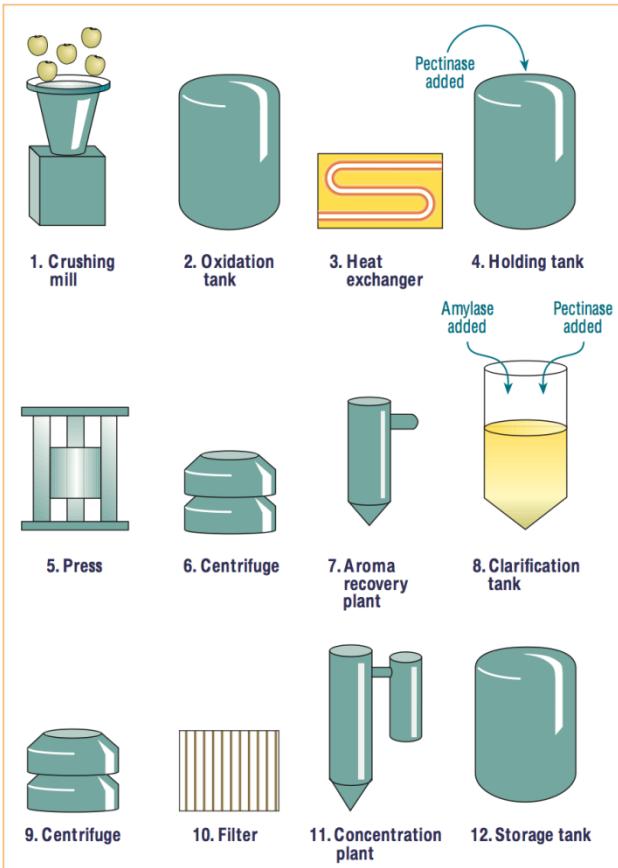
Pectic molecules are composed of chains of between 300 – 1000 galacturonic acid residues.

- Upper Chain: high methoxyl pectin
- Lower chain: low methoxyl pectin

Polygalacturonase	EC 3.2.1.15	Responsible for the random hydrolysis of 1,4- <i>a</i> -D-galactosiduronic linkages.
Pectinesterase	EC 3.2.1.11	Releases methanol from the pectyl methyl esters, a necessary stage before the polygalacturonase can act fully.
Pectin lyase	EC 4.2.2.10	Cleaves the pectin, by an elimination reaction releasing oligosaccharides with non-reducing terminal 4-deoxymethyl- <i>a</i> -D-galact-4-enuronosyl residues, without the necessity of pectin methyl esterase action.
Hemicellulase	A mixture of EC 3.2.1.32, EC 3.2.1.37 & EC 3.2.1.55	Strictly not a pectinase but its adventitious presence is encouraged in order to reduce hemicellulose levels.

[www.ncbe.reading.ac.uk](http://www.ncbe.reading.ac.uk). Enzymes in fruit juice production. In a jam and out of juice. Version 1.0. December 2000. [http://plantphys.info/plant\\_physiology/basiccytology1.shtml](http://plantphys.info/plant_physiology/basiccytology1.shtml)

# Pectic enzymes and use in fruit juice processing (3)



- **Depectination has two effects:**
  - Degrades the viscous soluble pectins
  - Causes aggregation of «cloud particles»
  - A second centrifugation and filtration to give clear juice
- **Advantages:**
  - Increases yield
  - Texture, sweetness and clarity
  - More environmentally friendly
  - Allows concentrate production

Movie about pectins:

<https://www.youtube.com/watch?v=2ZXumhwzoIE>

[www.ncbe.reading.ac.uk](http://www.ncbe.reading.ac.uk). Enzymes in fruit juice production.

In a jam and out of juice. Version 1.0. December 2000.

[http://plantphys.info/plant\\_physiology/basiccytology1.shtml](http://plantphys.info/plant_physiology/basiccytology1.shtml)

# Most Used in Food Applications

Important slide!

Enzyme	Application
Beta-amylase	saccharification , conversion of starch to maltose (malt syrups)
Alpha-amylase	flour processing, production of maltodextrins, glucose syrups, glucose and sweeteners
Xylanase	bread improvement, production of xylooligosaccharides
Cellulase	fruit and vegetable processing
Pectinase	clarification of fruit juices, production of carrot puree
Hemicellulases	clarification of fruit juices
Amyloglucosidase	saccharification, hydrolysis of starch dextrans to glucose
Alpha-glucosidase (maltase)	production of glucose from starch
Glucose isomerase	conversion of glucose syrups to high-fructose syrups
Invertase	production of fructose from sucrose (confectionery)

*Pocket Guide to Biotechnology and Genetic Engineering*  
(ed. R. Schmid, Wiley-VCH, 2003)

# Enzyme applications in Food, Feed, Detergent and Textile Industry

## Enzymes as Processing aids

application	enzyme type	organisms (examples)	market size (% of total)	economic advantage
detergents	proteases, cellulases, lipases	<i>Bacillus licheniformis</i> <i>Aspergillus nidulans</i> <i>Trichoderma reesei</i>	40	1
starch hydrolysis	$\alpha$ -amylase	<i>Bacillus amyloliquefaciens</i>	5	3, 4
glucose isomerization	glucose isomerase	<i>Streptomyces venezuelae</i>	7	1, 3
beer brewing	amylase	<i>Bacillus subtilis</i>	3	3, 4
fruit processing, wine	cellulases, hemicellulases, pectinases	<i>Aspergillus niger</i>	5	3, 4, 5, 6
flour, bakery goods	$\alpha$ -amylase, proteases	<i>Aspergillus oryzae</i>	8	1, 3
cheese manufacture, aroma	proteases, chymosin, lipases	animal rennin, <i>Rhizomucor miehei</i> , <i>Saccharomyces cerevisiae</i>	12	2
silage and animal feed	phytases	<i>Aspergillus niger</i>	8	3
paper and textiles	$\alpha$ -amylase, lipase	<i>Bacillus</i> , <i>Humicola</i>	2	4
leather treatment	proteases	<i>Aspergillus oryzae</i>	10	1, 7

## important goals in application technology

- 1 higher product quality
- 2 improved taste
- 3 better yields
- 4 reduced process costs
- 5 better filtration
- 6 better conservation
- 7 improved working conditions, reduced environmental load



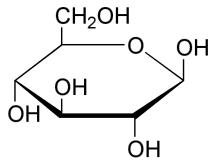
<https://www.youtube.com/watch?v=PVihOW3SmRo>

# Sugar Uptake and Metabolism

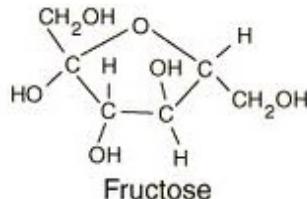
# Main dietary digestible carbohydrates

## Simple carbohydrates

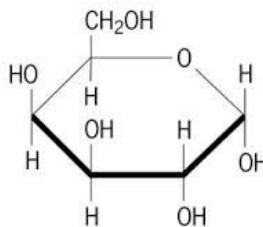
### Monosaccharides



Glucose

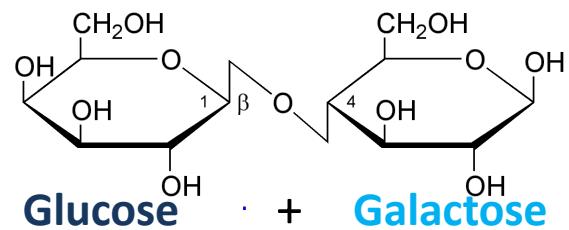
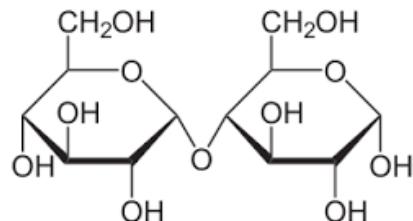
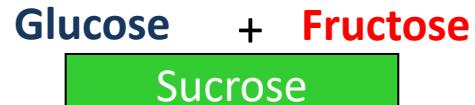
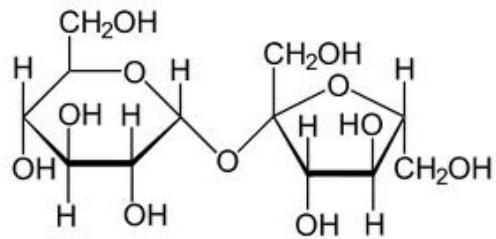


Fructose



Galactose

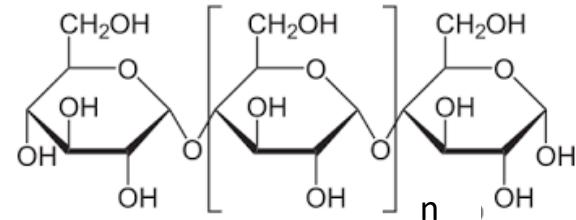
### Disaccharides



Lactose

## Complex carbohydrates

### Polysaccharides



# Sugar sources: Raw ingredients and Products

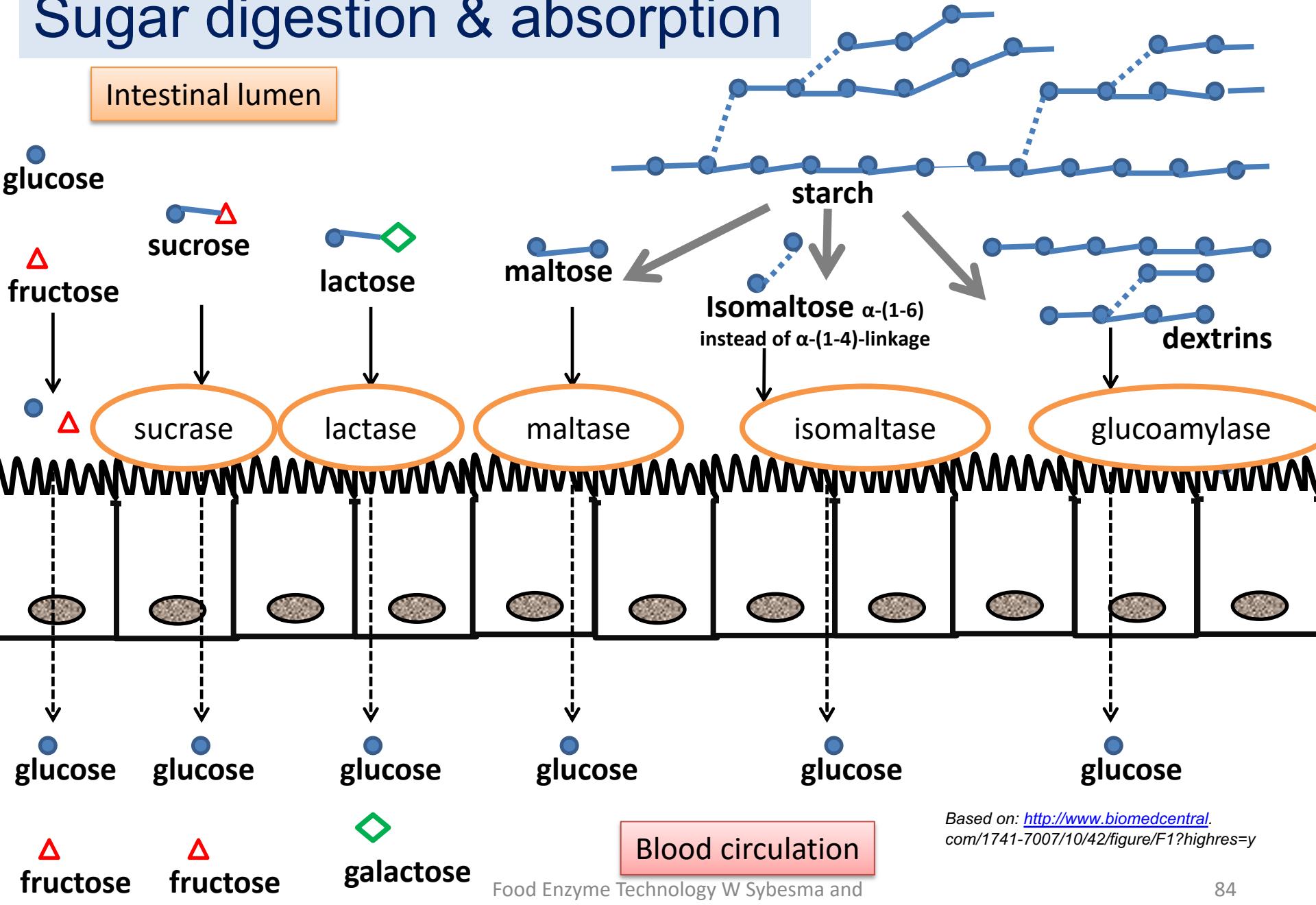


Whole fruit (150 g)	Fruit juice (250 mL)	Honey (10g)	High-Fructose Corn Syrup	Refined sugar	Soft drink (250 mL)	Milk (250 mL)
------------------------	-------------------------	----------------	-----------------------------	------------------	------------------------	------------------

Fructose <span style="color:red">△</span>	✓	✓	✓	✓			
Glucose <span style="color:blue">●</span>	✓	✓	✓	✓			
Sucrose <span style="color:blue">●</span> <span style="color:red">△</span>	✓	✓	<1%		✓	✓	
Lactose <span style="color:blue">●</span> <span style="color:green">◇</span>							✓
g per 100g	8.3	8.5	75	96	100	9.5	4.5
g per serving	12.4	21.2	7.5	-	-	24	11.2

Kim-Anne Lê / Nestlé, Research Center, Lausanne

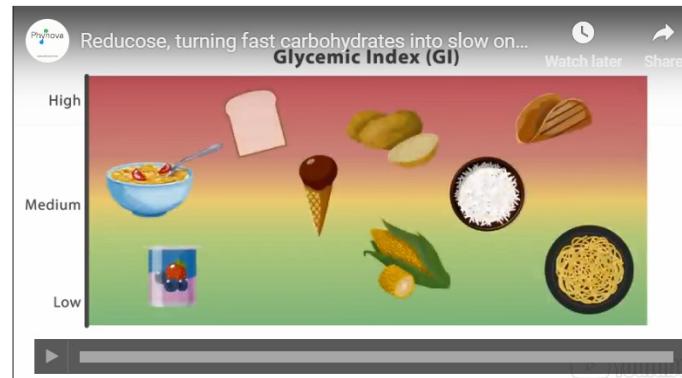
# Sugar digestion & absorption



Reducose is not a sugar replacement, but is rather a functional food ingredient that helps turn fast carbs and sugars into slow ones. Carbohydrates, also called saccharides, are a group of biological molecules that includes sugars, starch and cellulose. The simplest carbohydrate unit is called a monosaccharide and includes molecules like glucose and fructose. Monosaccharides are the building block for more complex carbohydrates, which are classified as disaccharides (sugars), oligosaccharides, and polysaccharides (includes starch).

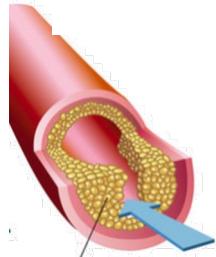
[https://www.youtube.com/watch?time\\_continue=2&v=c6HfK01PWDU&feature=emb\\_logo](https://www.youtube.com/watch?time_continue=2&v=c6HfK01PWDU&feature=emb_logo)

Good to watch this movie once



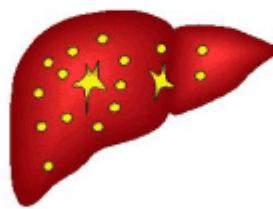
Carbohydrates bigger than monosaccharides are too large to be directly absorbed by the body and during digestion are broken down into monosaccharides such as glucose or fructose by enzymes. This breakdown starts in the mouth with salivary amylase. After swallowing the salivary amylase is inactivated by stomach acid and so the breakdown continues after food leaves the stomach in the small intestine. Several different enzymes are involved in this breakdown including pancreatic amylase, which breaks polysaccharides like starch into disaccharides, enzymes such as sucrase and alpha-glucosidase that break disaccharides into monosaccharides for absorption. Many foods are capable of inhibiting these enzymes, with mulberry leaves being a good example. Reducose is an extract of mulberry leaves and is able to inhibit these enzymes such that the carbohydrate chains remain unbroken and so fewer glucose molecules are able to get into the body. This allows people to enjoy their favourite foods and maintain blood glucose levels within a healthy range.

# Will high sugar consumptions increase the risk for Cardiovascular Disease (CVD)?



- Plasma lipids: Total- and VLDL-TG

(Very low-density lipoprotein tri glycerides)



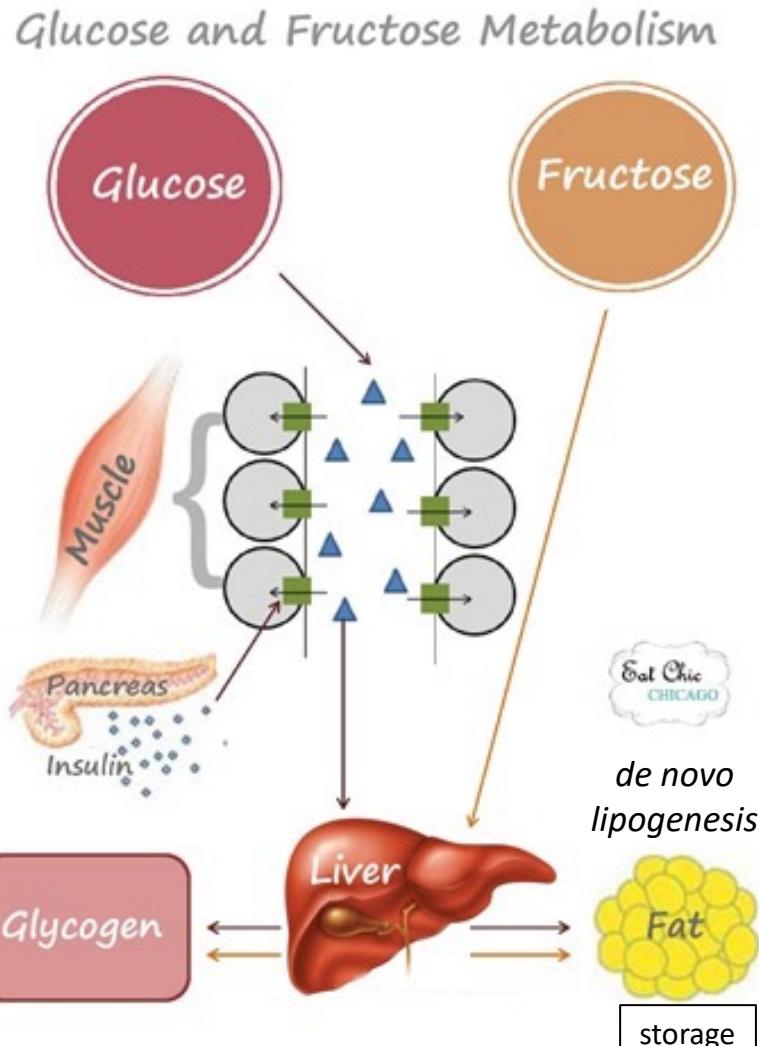
## Liver fat content:

- High sugar intake ( $\geq 106$  g/day) in overweight subjects is associated with high liver fat
- Mostly associated with energy intake

- Positive association between sugar consumption and dyslipidemia / fatty liver disease
- Threshold unknown; probably comprised between 50-100 g/d

Welsh, 2011; Maersk, 2012

# Differences in glucose and fructose metabolism



<http://www.eatchicchicago.com/wp-content/uploads/2014/01/GlucoseandFructoseMetabolismInfographic.jpg>

Food Enzyme Technology W Sybesma and  
CH Hansen EPFL

- Fructose is metabolized almost exclusively in the liver and independently of insulin, in contrast to glucose
- Excess fructose consumption can lead to production of triglycerides and liver fat accumulation through the de novo lipogenesis pathway

# Back up slides enzymes

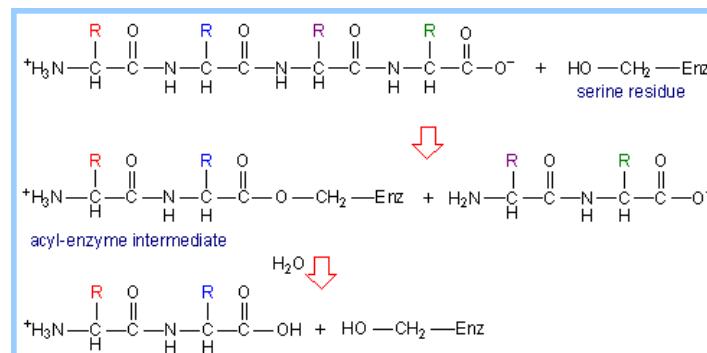
# Enzyme databases

- At present, there are more than 3500 types of enzymes [EC numbers], with ten thousands of individual varieties
- BRENDA is the most comprehensive enzyme database
- The PDB (protein data base) now contains over 50.000 enzyme structures

The screenshot shows the BRENDA enzyme database homepage. The URL in the address bar is <http://www.brenda-enzymes.org/>. The page title is "Enzyme Database - BRENDA - Windows Internet Explorer provided by S.N.O.W. Workstation". The main content area includes a search bar with fields for EC-Number, Enzyme Name, Organism, Protein, Full text, and Advanced Search. Below the search bar, a message says "New BRENDA release online since 2nd July 2010". A table lists various enzyme parameters under categories like Nomenclature, Isolation & Preparation, Stability, Reaction & Specificity, Functional Parameters, Organism-related information, Enzyme Structure, and Disease & References. The sidebar on the left contains links for Quick Search, Fulltext Search, Advanced Search, Substructure Search, TaxTree Explorer, EC Explorer, Sequence Search, Genome Explorer, Ontology Explorer, Functional Enzyme Parameters NEW, SBML Output NEW, and Download. It also includes a "Tutorial/Training NEW", "BRENDA input", "Propose new enzyme NEW", "Introduction/References", "News", "Impressum/Team/Errors", "Jobs", "Copyright", "Related Links", "Help", "Acknowledgements", "BRENDA Professional", and "Commercial Version". The footer includes a "Webmaster Maurice Scheer" email address (m.scheer@tu-bs.de), a note about Javascript and frames, and a "Food Enzyme Technology W Sybesma and CH Hansen EPFL" credit. The browser status bar at the bottom right shows "Internet | Protected Mode: On" and "100%".

# Serine Protease

- Serine proteases are proteases in which one of the amino acids in the active site is serine
- Found in both eukaryotes and prokaryotes
- Serine proteases were originally digestive enzymes. In mammals, they evolved by gene duplication to serve functions in blood clotting, the immune system, and inflammation.
- Serine proteases are paired with serine protease inhibitors
- Chymotrypsin-clan
  - Chymotrypsin cleaving peptide bonds following a bulky hydrophobic amino acid residue. Preferred residues include **phenylalanine**, **tryptophan**, **tyrosine**, which fit into a snug hydrophobic pocket.
  - Trypsin cleaving peptide bonds following a positively-charged amino acid. Instead of having the hydrophobic pocket of the chymotrypsin, there exists an aspartic acid residue (position 189) at the base of the pocket. This can then interact with positively-charged residues such as **arginine**, **lysine** on the substrate peptide to be cleaved.
  - Elastase cleaving peptide bonds following small neutral amino acid, such as **Alanine**, **glycine**, **valine**. (These amino acids form much of the collagen in meat). The pocket that is in "trypsin" and "chymotrypsin" is now partially filled with valine and threonine, to accommodate these smaller amino acid residues.



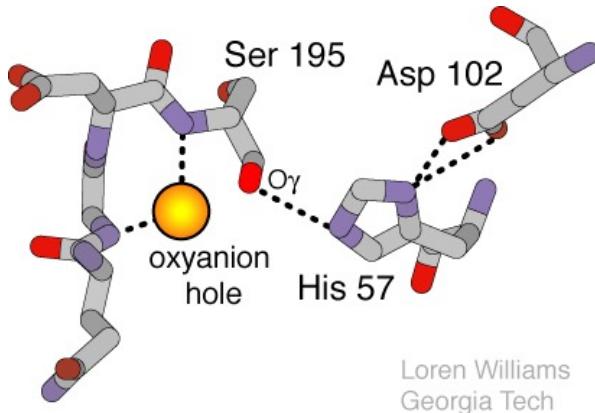
<http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/protease.htm>

# Example of Serine Proteases mechanism

## ✓ Substrate specificity

No need to understand mechanism by heart

- Trypsin hydrolyses peptide bonds to the C-terminal side of basic amino acids Lys and Arg
- Chymotrypsin cleaves peptide bonds after aromatic amino acids Phe, Tyr, Trp [and to a lower extent, Leu]

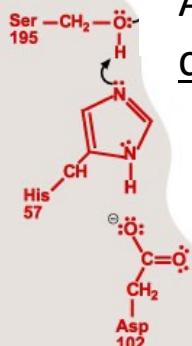
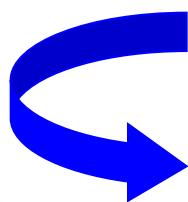


[http://www2.chemistry.gatech.edu/~lw26/structure/protein/serine\\_protease/triad\\_1.html](http://www2.chemistry.gatech.edu/~lw26/structure/protein/serine_protease/triad_1.html)

enzyme active site

## ✓ Catalytic site

All serine proteases have in the active site 3 amino acids known as catalytic triad:



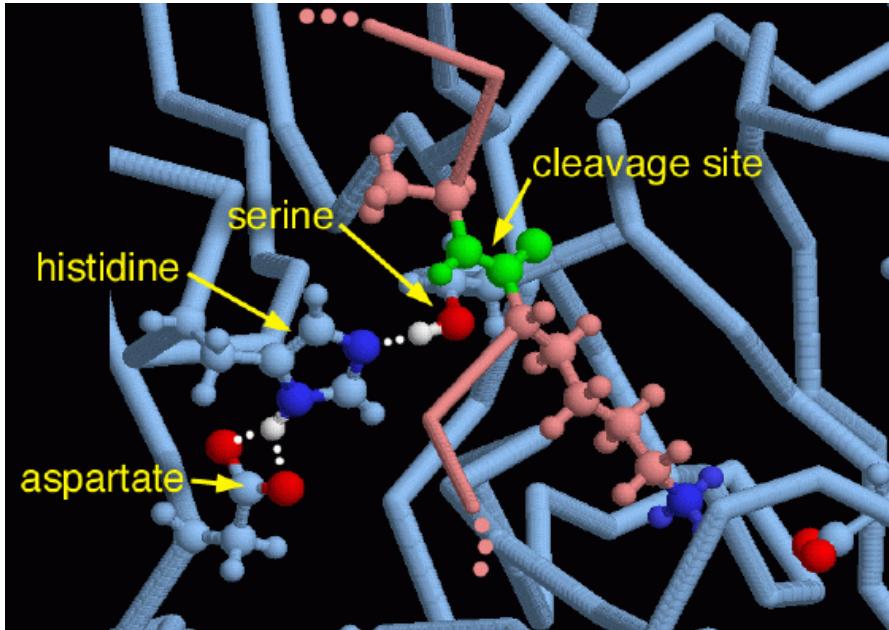
- Ser 195 [side-chain  $\text{CH}_2\text{-OH}$ ]: combined action of the triad makes it a better **nucleophile**.
- His 57: base [accept a proton]
- Asp 102: acid [donate a proton]

Cooperative action among the catalytical triad amino acids

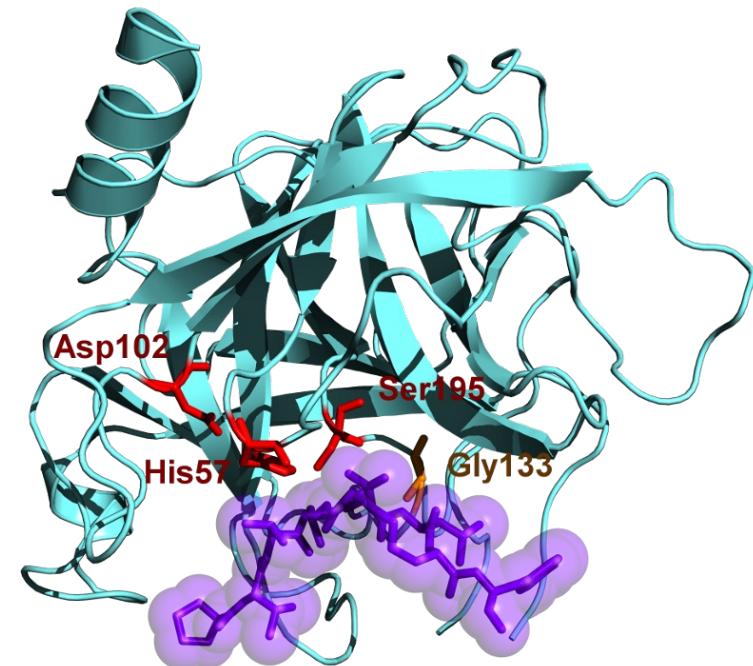
# Structure of serine protease

## Structure of the active sites of Serine Proteases

Trypsin



Chymotrypsin



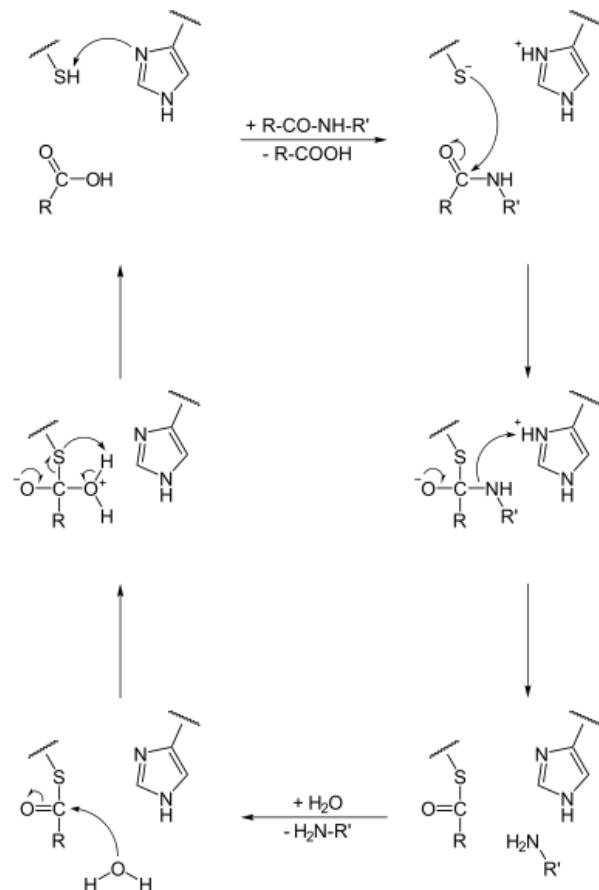
[http://www2.rcsb.org/pdb/education\\_discussion/molecule\\_of\\_the\\_month/images/2ptc-rasmol.gif](http://www2.rcsb.org/pdb/education_discussion/molecule_of_the_month/images/2ptc-rasmol.gif)

[http://upload.wikimedia.org/wikipedia/commons/2/2e/Chymotrypsin\\_4cha.png](http://upload.wikimedia.org/wikipedia/commons/2/2e/Chymotrypsin_4cha.png)

# Cysteine Protease

No need to understand mechanism by heart

- Cysteine proteases have a common catalytic mechanism that involves a nucleophilic cysteine thiol in the catalytic site
  - First step is deprotonation of a thiol in the enzyme's active site by an adjacent amino acid with a basic side chain, usually histidine
  - Next step is nucleophilic attack by the deprotonated cysteine's anionic sulfur on the substrate carbonyl carbon. A fragment of the substrate is released with an amine terminus, the histidine residue in the protease is restored to its deprotonated form, and a thioester intermediate linking the new carboxy-terminus of the substrate to the cysteine thiol is formed.
  - The thioester bond is subsequently hydrolyzed to generate a carboxylic acid moiety on the remaining substrate fragment, while regenerating the free enzyme.
- Found in fruits (papaya, pineapple, fig, kiwifruit)
- Cysteine proteases are used in meat tenderizers.



[http://en.wikipedia.org/wiki/File:Cysteinprotease\\_Reaktionsmechanismus.svg](http://en.wikipedia.org/wiki/File:Cysteinprotease_Reaktionsmechanismus.svg)

# Proteases execute a large variety of important physiological functions: some examples

## Regulation of gene expression

- Modulation of gene expression is mediated by proteases [e.g. proteolysis of a Gene repressor; regulation of translation by modification of ribosomal proteins by proteases].
- Proteases are essential in viruses, bacteria, parasites for their replication and the spread of infectious diseases

## Protein Turnover

- All living cells maintain a particular rate of protein turnover by continuous control and regulation of protein composition, size, shape, turnover and ultimate destruction: all is done by proteases.
- Catabolism of proteins by proteases provides a pool of amino acids used as precursors of the protein synthesis.

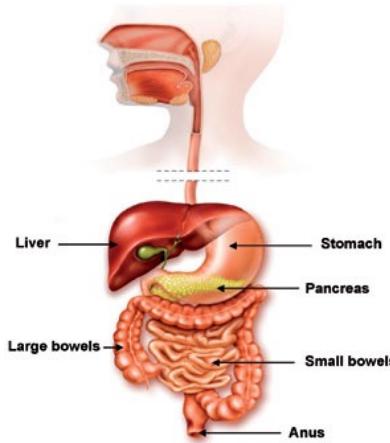
## Formation & germination of spores in microorganisms

- Spore formation: intensive protein turnover that requires protease.
- Germination: degradation of proteins in dormant spores by protease (serine endoproteinase) makes amino acids and nitrogen available for biosynthesis of new proteins and nucleotides.

# Proteases execute a large variety of important physiological functions: some examples

## Digestion

The digestive system



- Proteases assist the hydrolysis of proteins & large polypeptides into smaller peptides and amino acids, thus facilitating their absorption by the intestinal cells

Pepsin

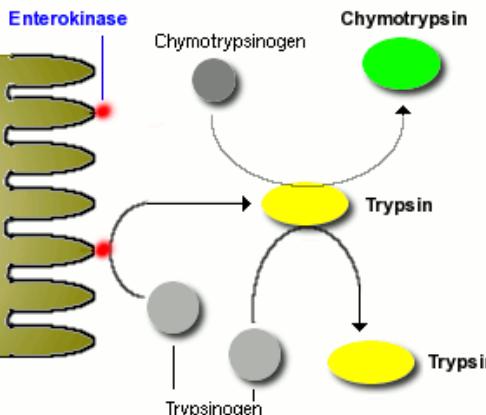
<http://digestion.ygoy.com/digestive-enzyme-function-in-the-process-of-digestion/>

Trypsin

Chymotrypsin

AminoPeptidases  
CarboxyPeptidases

## Physiological Regulations (cascade processes)

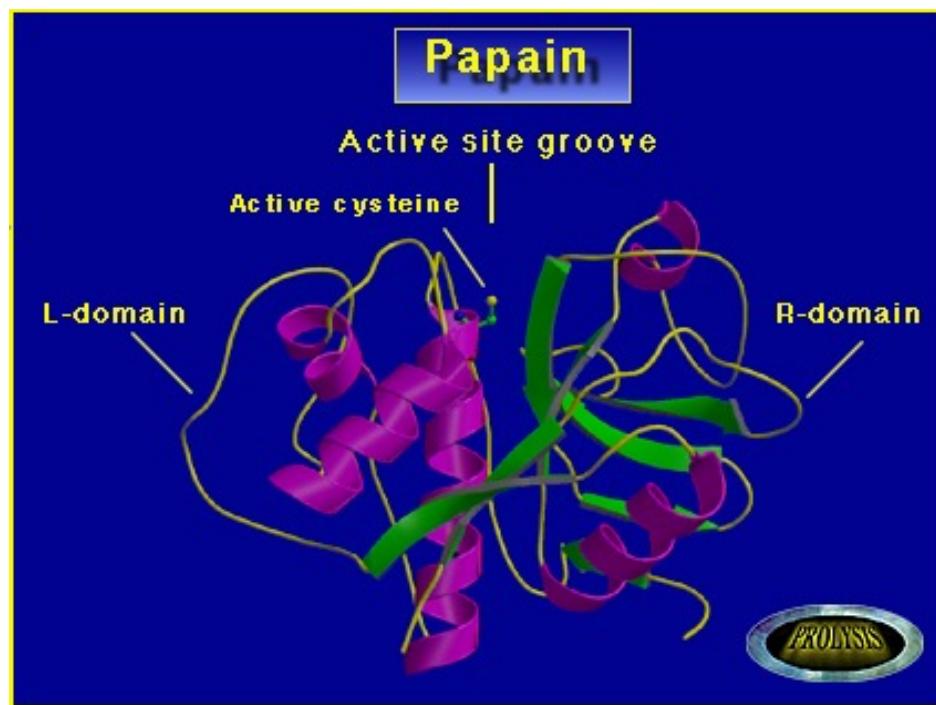


- Activation of precursors forms of enzymes by specific proteases represents an important step in the physiological regulation of many rate-controlling processes:
  - Digestion: activation of Trypsinogen and Chymotrypsinogen
  - Generation of proteins hormones / bioactive peptides
  - Regulation of blood coagulation and inactivation of fibrin clot formation: role of Thrombin.
  - Proteolytic Inactivation of enzymes.

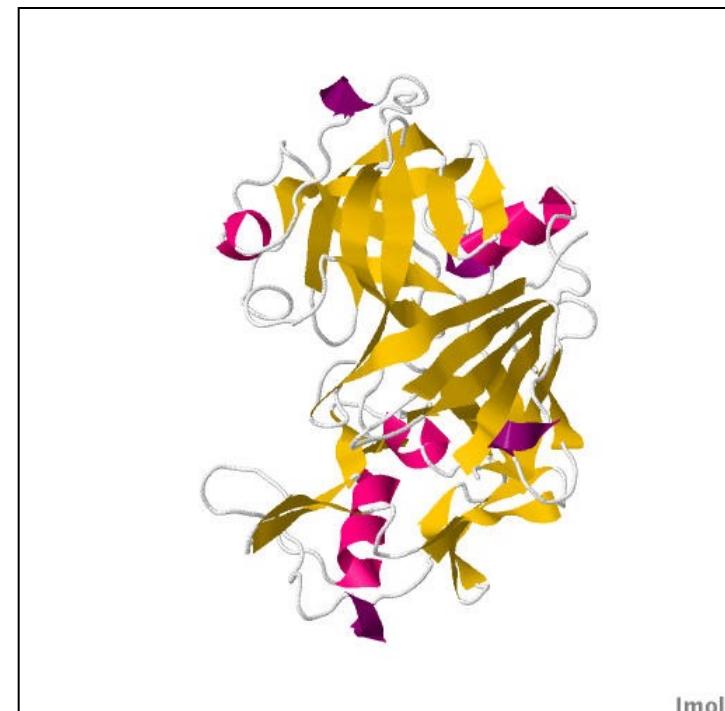
<http://www.vivo.colostate.edu/hbooks/pathphys/digestion/pancreas/exocrine.html>

# Examples: Active site

## Structure of the active sites of Cysteine- & Aspartic-endoproteases: Examples of Papain Chymosine



[http://linus.chem.ku.edu/hewlett/Chem188/Enzyme/enzyme\\_background.htm](http://linus.chem.ku.edu/hewlett/Chem188/Enzyme/enzyme_background.htm)



[http://en.wikipedia.org/wiki/File:CHYMOSIN\\_COMPLEX\\_WITH\\_THE\\_INHIBITOR\\_CP-113972.jpg](http://en.wikipedia.org/wiki/File:CHYMOSIN_COMPLEX_WITH_THE_INHIBITOR_CP-113972.jpg)

# Sources of Proteases

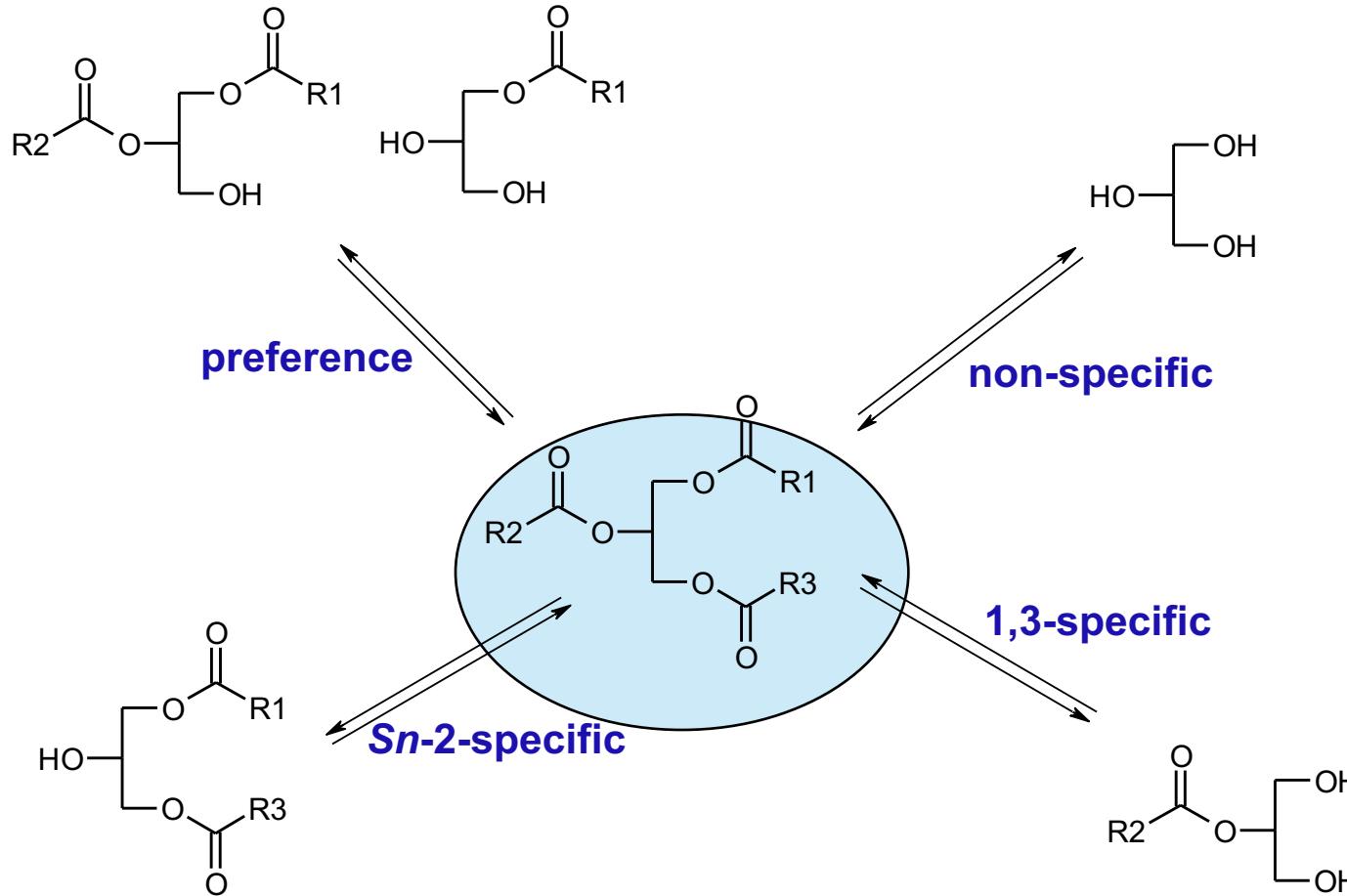
No need to remember details

✓ Since proteases are physiologically necessary for living organisms, they are found in a wide diversity of sources such as plants, animals and microorganisms.

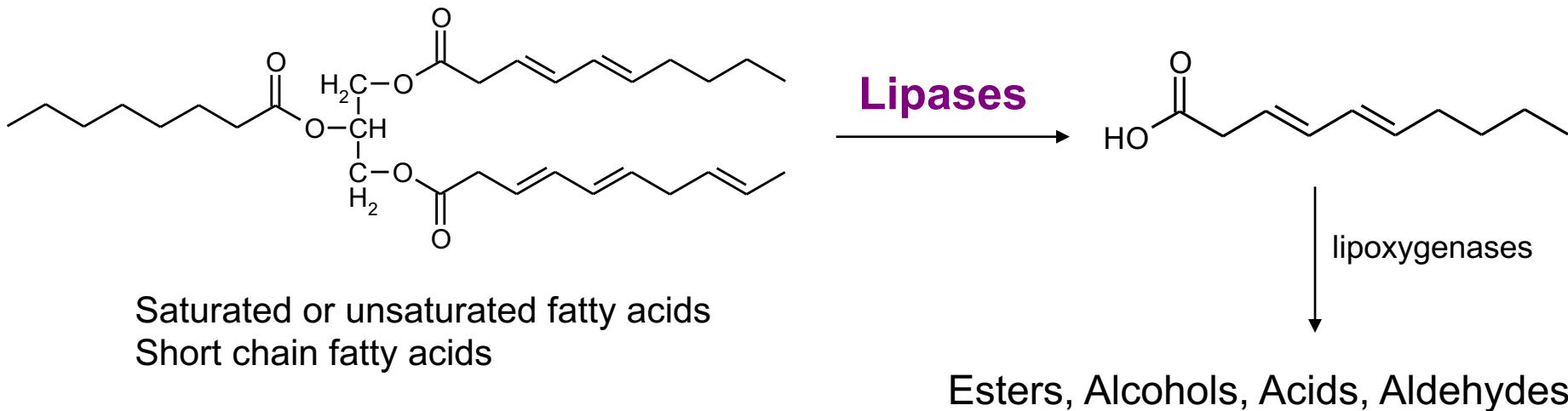
Source	Common names tradenames	Type of Protease	Typical pH range	Preferential specificity	Industrial use
<b>Animals</b>					
Ox, pigs gastric mucosa	Pepsin, pepsin A	Aspartic protease	pH 1-4	Aromatic-COOH and-NH2 Leu-, Asp-, Glu-COOH	
pancreas	Trypsin Chymotrypsin Pancreatin	Serine protease Serine protease Mixture of trypsin chymotrypsin, elastase and carboxypeptidase A or B	pH 7.9 pH 8.9 pH 7.9	Lys-, Arg-COOH Phe-, Tyr-, Trp-COOH Very broad specificity	
Calf	Chymosin, rennin	Aspartic protease	pH 3-6	Rennet specificity	Cheese-making
<b>Plants</b>					
Papaya fruit	Papain pure Papain crude	Cysteine protease Mixture of Papain, chymopapain & lyzozyme	pH 5.7 pH 5.9	Lys-, Arg-, Phe-X-COOH Broad specificity	
Fig latex Pineapple stem & juice	Ficin Bromelain	Cysteine protease Cysteine protease	pH 5-8 pH 5-8	Phe-, Tyr-COOH Lys-, Arg-, Phe-, Tyr-COOH	Baking industry
<b>Microorganisms</b>					
<b>Bacteria</b>					
<i>Bacillus amyloliquefaciens</i>	Neutrase	Metalloprotease	pH 6-8	Leu-, Phe-NH2 and other	
<i>Bacillus licheniformis</i>	Subtilisin Carlsberg, Alcalase	Serine protease	pH 6-10	Broad specificity, mainly "hydrophobic"-COOH	
<i>Bacillus sp alkalophilic</i>	Esperase	Serine protease	pH 7-12	Broad specificity, mainly "hydrophobic"-COOH	
<i>Bacillus thermoproteolyticus</i>	Thermolysin	Metalloprotease	pH 7-9	Ile-, Leu-, Val-, Phe-COOH	
<b>Fungi</b>					
<i>Aspergillus oryzae</i>	Flavourzyme	Mixture of aspartic-, serine-, metallo-proteases & carboxypeptidases	pH 5-7	Very broad specificity	
<i>Aspergillus niger</i>		Aspartic protease, usually mixed with carboxypeptidase	pH 2.5-5	Pre asp. Protease: as pepsin Mixture: very broad specificity	
<i>Mucor miehei</i>	Rennilase; Fromase	Aspartic protease	pH 3-6	Rennet specificity	
<i>Mucor pusillus</i>	Emporase; Meito rennet	Aspartic protease	pH 3-6	Rennet specificity	Cheese-making
<i>Streptomyces griseus</i>	Pronase	Mixture alkaline & neutral proteases - amino- & carboxy-peptidases	pH 7-9	Very broad specificity	Cheese-making

# Reactions Catalyzed by Lipases: Hydrolysis

- Hydrolysis is carried out in a biphasic mixture consisting of the lipophilic phase (acylglycerols & fatty acids) and the hydrophilic phase (glycerol & lipase in aqueous buffer).
- The reaction occurs at the interface and the regio-specificity depend on the fatty acid.



# Application of Lipases: Generation of Flavours



- Lipoxygenases (EC 1.13.11.-) are a family of iron-containing enzymes that catalyse the dioxygenation of polyunsaturated fatty acids in lipids containing a *cis,cis*-1,4- pentadiene structure.
- It catalyses the following reaction: fatty acid + O<sub>2</sub> = fatty acid hydroperoxide
- Lipoxygenases are found in plants, animals and fungi. Products of lipoxygenases are involved in diverse cell functions.

# Application of Lipases in Bread

- Lipase increases gluten strength
- Gluten from wheat flour dough treated with lipase is significantly stronger, with an increased  $G'$ , and more elasticity
- Gluten treated with lipase has an increased relaxation time
- *In-situ* generation of more hydrophilic emulsifiers (mono-acylglycerides)

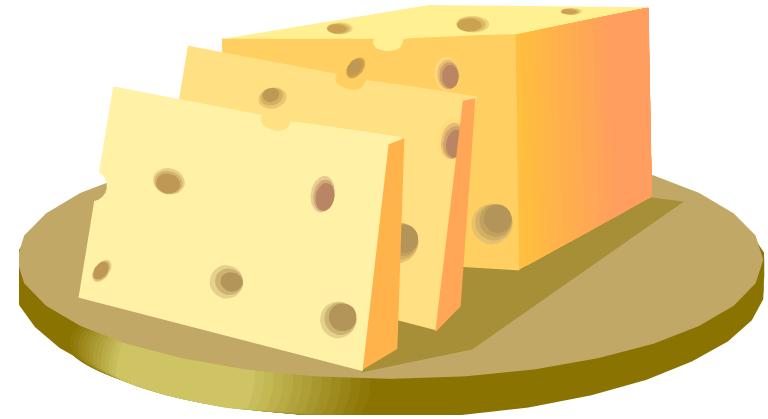
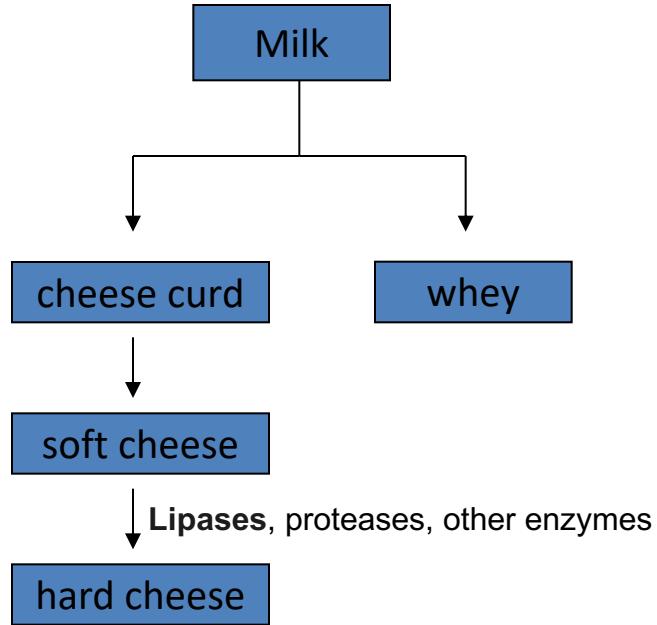


Improved processing, volume and quality of bread

<http://onlinelibrary.wiley.com/doi/10.1111/1541-4337.12085/full>

# Application of Lipases in Cheese

## Cheese Processing



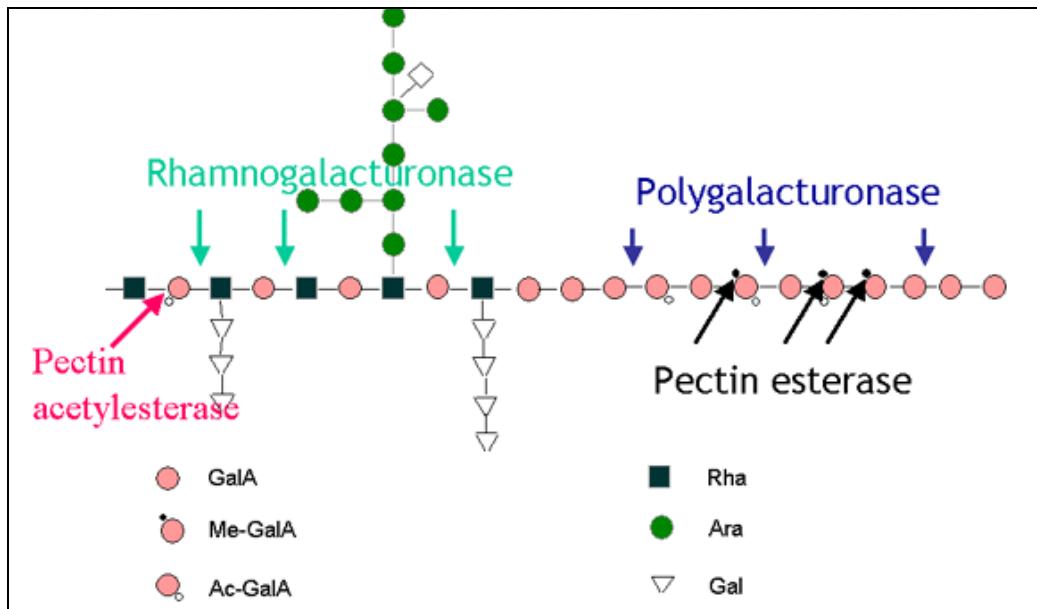
## Cheese Aroma

The short and medium-chain fatty acids occurring in butterfat can be partially hydrolyzed by lipases to a product mixture useful for their aroma in cheese production (enzyme-modified cheeses, EMC).

Depending on the chain-length specificity of the lipase used, various aroma notes can be produced

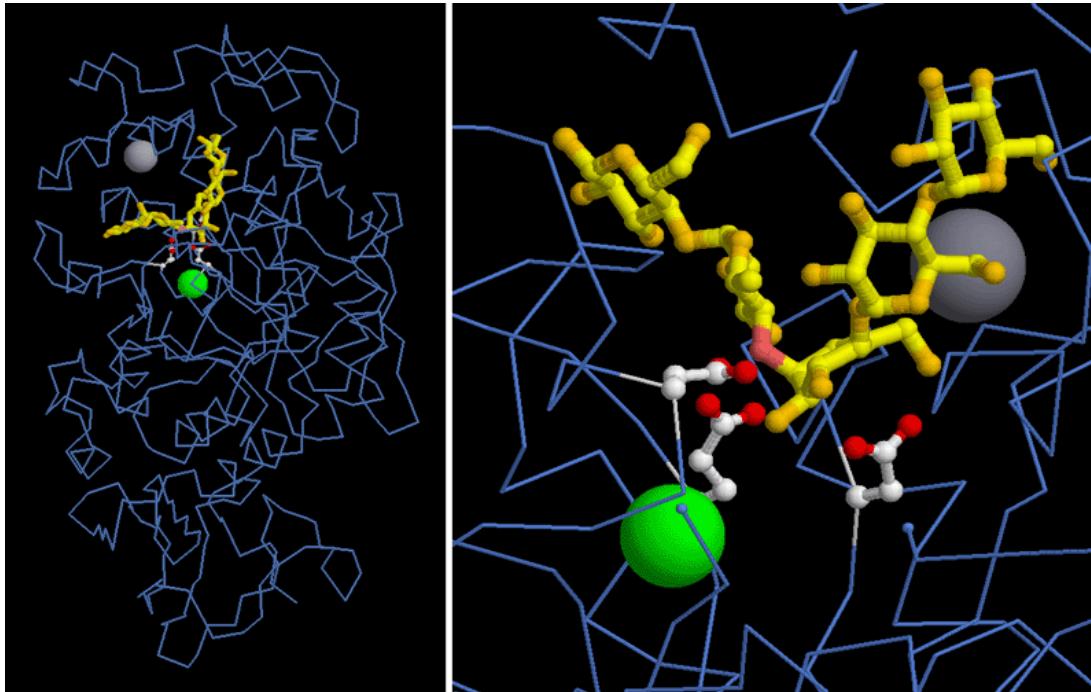
# A wide diversity of substrates and enzymes

- Carbohydrates play an essential role in many biological processes
- Carbohydrates show a wide stereochemical variation
- As a consequence/cause of saccharide diversity, there is a great variety amongst the enzymes that modify carbohydrates
- The glycosidic bond is one of the most stable chemical bonds, enzymes that catalyze the hydrolysis of this bond are among the most "powerful" catalysts



<https://www.adisseo.biz/Productguides/RovabioGuide/versatility.aspx>

# $\alpha$ -Amylase – Active site



No need to understand mechanisms

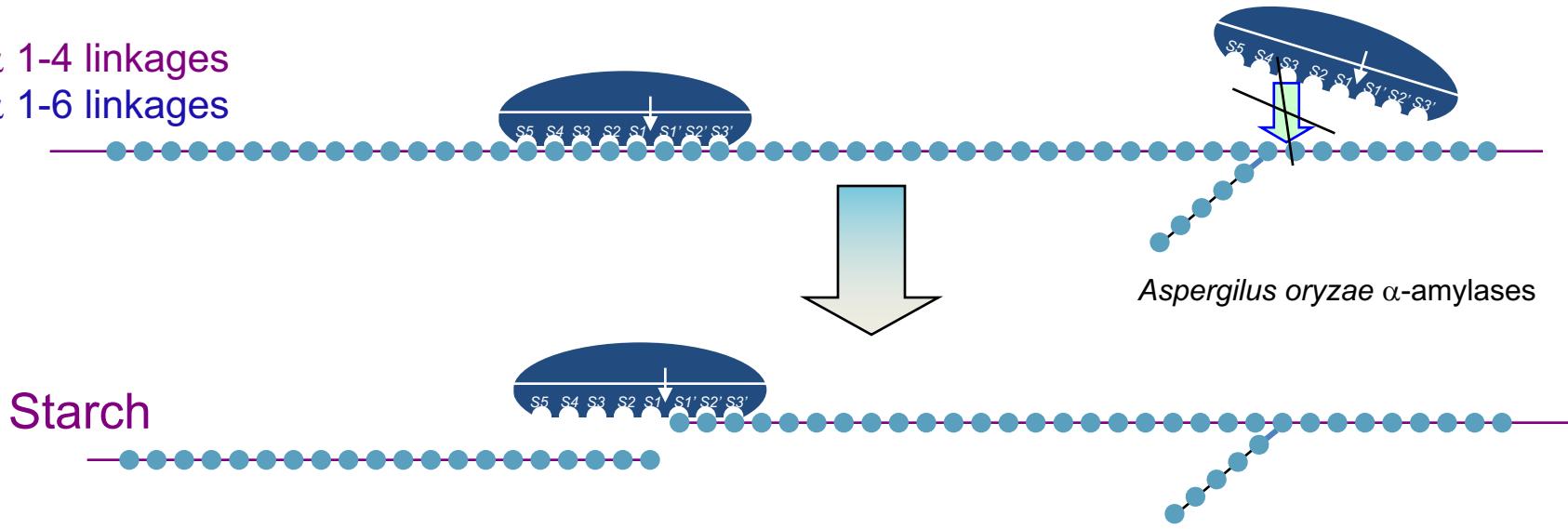
[http://www.pdb.org/pdb/101/motm  
.do?momID=74](http://www.pdb.org/pdb/101/motm.do?momID=74)

- The active site of alpha-amylase contains a trio of acidic groups (colored white and red)
- In the amylase shown here, glutamate 233, aspartate 197, and aspartate 300 work together to cleave the connection between two sugars in a starch chain. This structure contains a short chain of five sugar units (colored yellow and orange) bound in the active site. The site of cleavage is shown in pink.
- A calcium ion, shown as the large gray sphere, is found nearby where it stabilizes the structure of the enzyme. A chloride ion, shown as a green sphere, is bound underneath the active site in many amylases, where it may assist the reaction.

# $\alpha$ -Amylase is an Endo-hydrolase

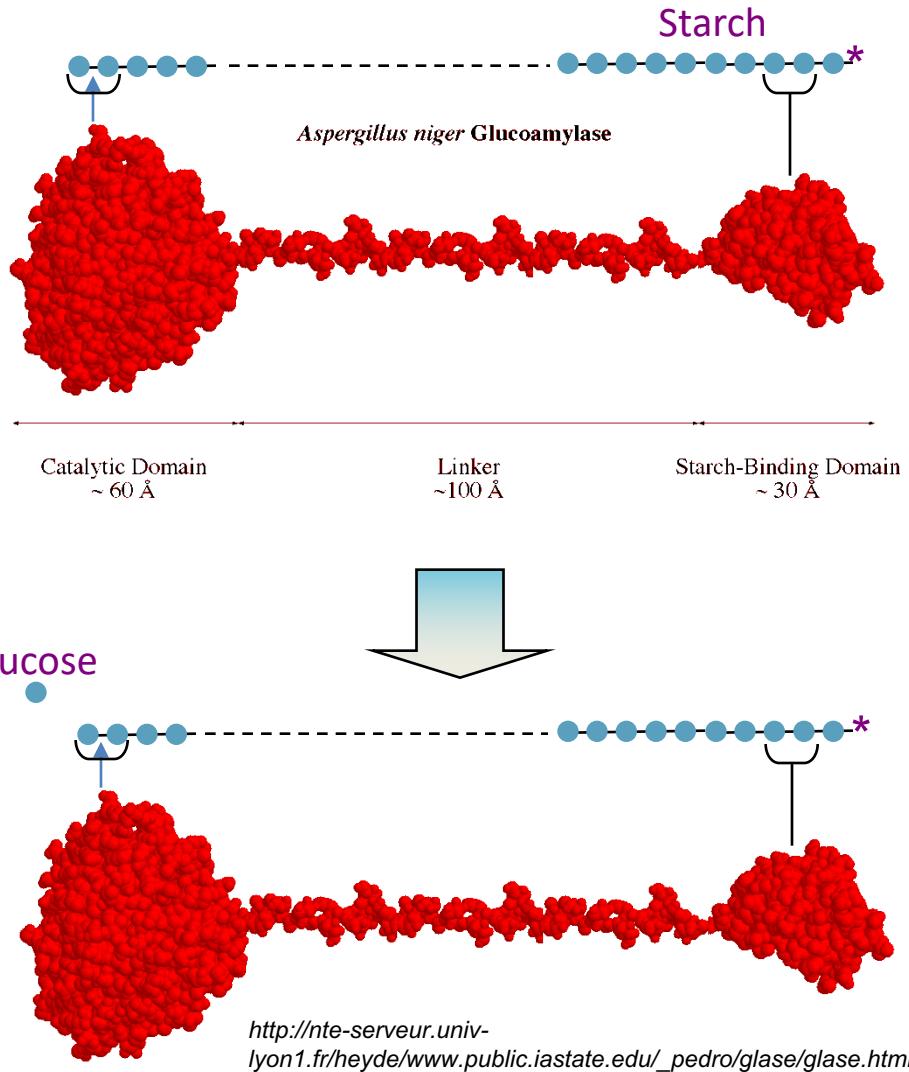
- Catalytic domain of hydrolases is specific:

- $\alpha$  1-4 linkages  
- $\alpha$  1-6 linkages



- Specificity of BAN:
  - Hydrolysis of  $\alpha$ -(1,4)-glycosidic linkages in amylose, amylopectin
  - End reaction product are maltodextrines, maltose and glucose in defined ratios

# Glucoamylase is an Exo-hydrolases



- The glucoamylase or amyloglucosidase (AMG) cleaves  $\alpha$ -(1,4)-glucose from the non-reducing terminus of starch and maltose to yield  $\beta$ -D-glucose and an n-1 sugar polymer.
- The mechanism of action is a classical example of an inversion of configuration during glycoside hydrolysis
- Glucoamylase that cleaves both  $\alpha$ -(1,4)- and  $\alpha$ -(1,6) glycosidic linkages may be assumed to hydrolyze starch theoretically 100% into glucose