Food Enzyme Technology

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EPFL Course ENG-436

Only for Teaching Purposes
Personal Copy

https://youtu.be/ZRKqGrFEeFo?feature=shared

Examples of dietary digestive enzymes

https://www.dsm.com/content/dam/dsm/human-nutrition/pdfs/DS_Tolerase_L_Infographic.pdf

Helping digest gluten with Tolerase® G

https://www.dsm-firmenich.com/content/dam/dsm-firmenich/health-nutrition-care/human-nutrition/pdfs/ds-tolerase-g-onepager.pdf

https://youtu.be/ttHsRLQSa14?feature=shared

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NURICA™ All-in-One Enzyme Solution

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

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

A comprenensive 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.**

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

Promoting digestive health
1.6% of GOS fiber generated in situ

Reduced sugar content
35% sugar reduction

https://www.youtube.com/watch?v=7OwCGq4GTE8

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 thermo-labile 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

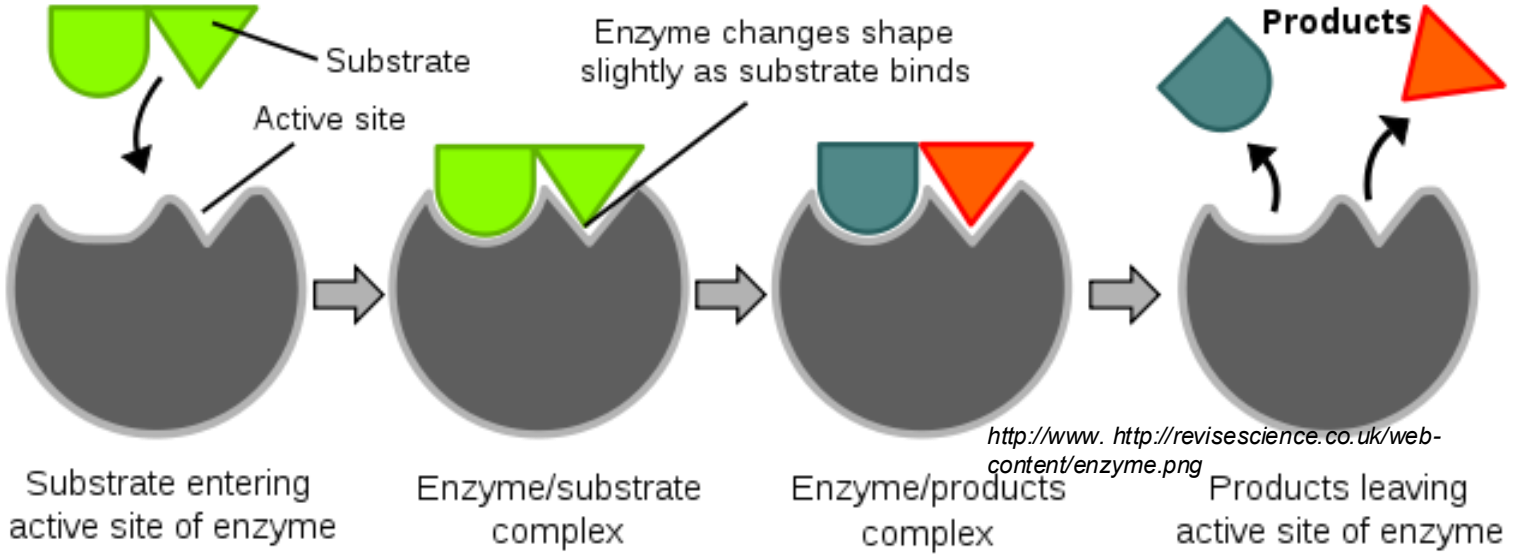
Advantages and Disadvantages of Biocatalysts

- **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 un 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-aquous 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

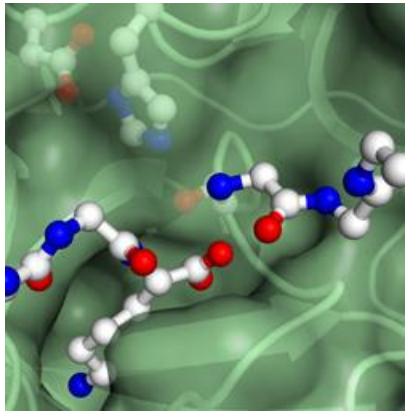
- **Disadvantages**
  - Enzymes are provided by Nature in only one enantiomeric form
    - Almost all enzymes are made from L-amino acids and thus are chiral catalysts. In some reactions only 50% of substrate is converted (one enantiomer)
  - Enzymes require narrow operations parameters
    - Extreme 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



http://www. http://revisescience.co.uk/web-content/enzyme.png

- **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

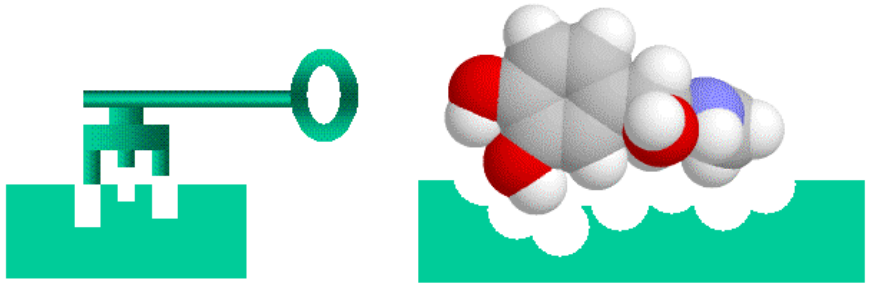
Enzyme Specificity & Stability

- Most enzymes are **highly selective (chemo, regio, and enantioselectivity),** but some are also unselective („promiscuous“) enzymes

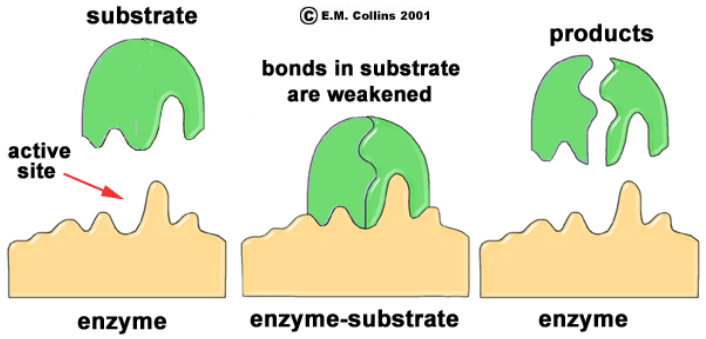
- **Usually enzymes loose activity at temperatures above 40 °C**, but there are also enzymes which remain active at temperatures up to 80 °C (extremophiles)

- **Enzymes can be further stabilized** through additives,

  e. g. glycerol or sucrose, or by immobilization onto carrier materials



http://www.acrazychicken.blogspot.com

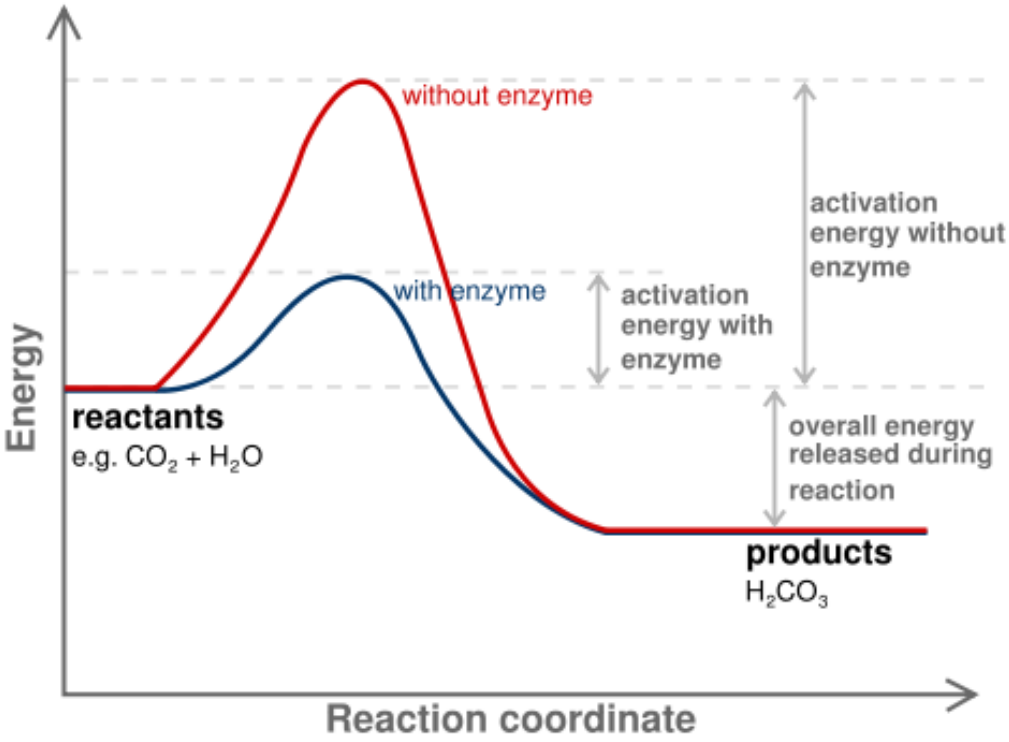


http://www.waynesword.palomar.edu

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

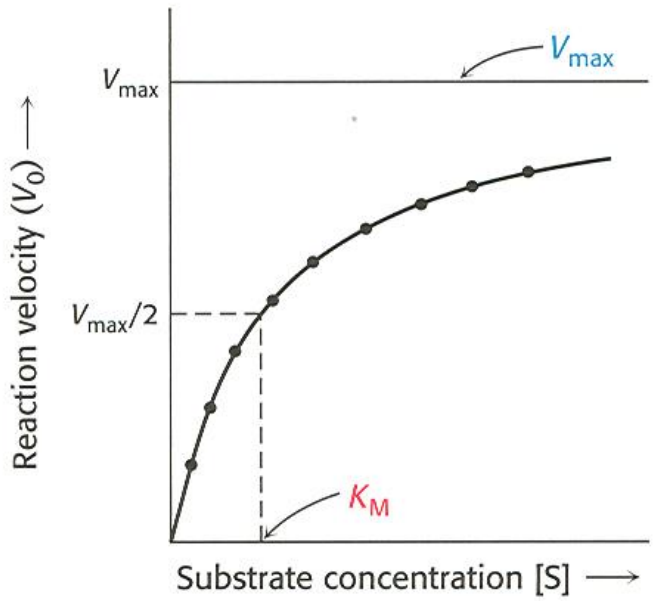
There is no difference in free energy release (ΔG) between catalyzed and uncatalyzed reactions

Enzyme reactions: Kinetics

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

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

V_o is the reaction velocity.
V_{max} is the maximal rate of the reaction.
S is the substrate concentration.

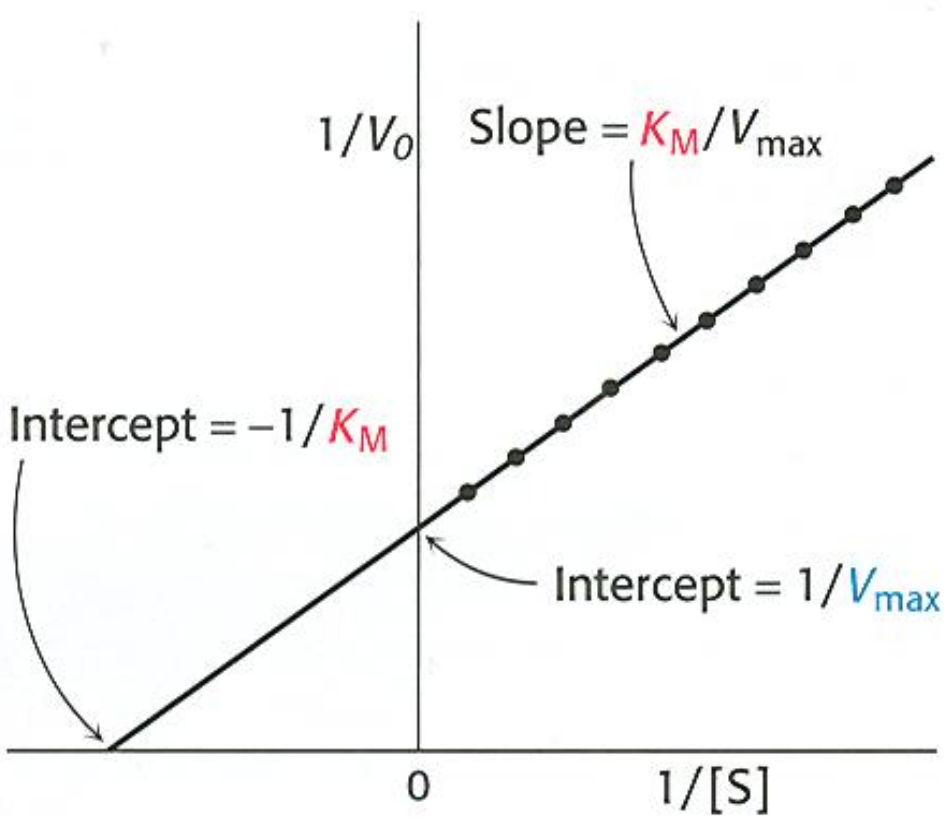


http://stevegallik.org/sites/all/images/enzyme_03.jpg

- **<u>Michaelis-Menten constant (Km),</u> which is the substrate concentration required for an enzyme to reach one-half its maximum velocity.**
- **Each enzyme has a characteristic Km for a given substrate, and this can show how tight the binding of the substrate is to the enzyme.**
- **Another useful constant is <u>kcat</u>, which is the number of substrate molecules handled by one active site per second.**
- **Km 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 Km indicates a large binding affinity whereas a high Km indicates that the enzyme does not bind as tightly with the substrate.**

Enzyme reactions: Kinetics (2)

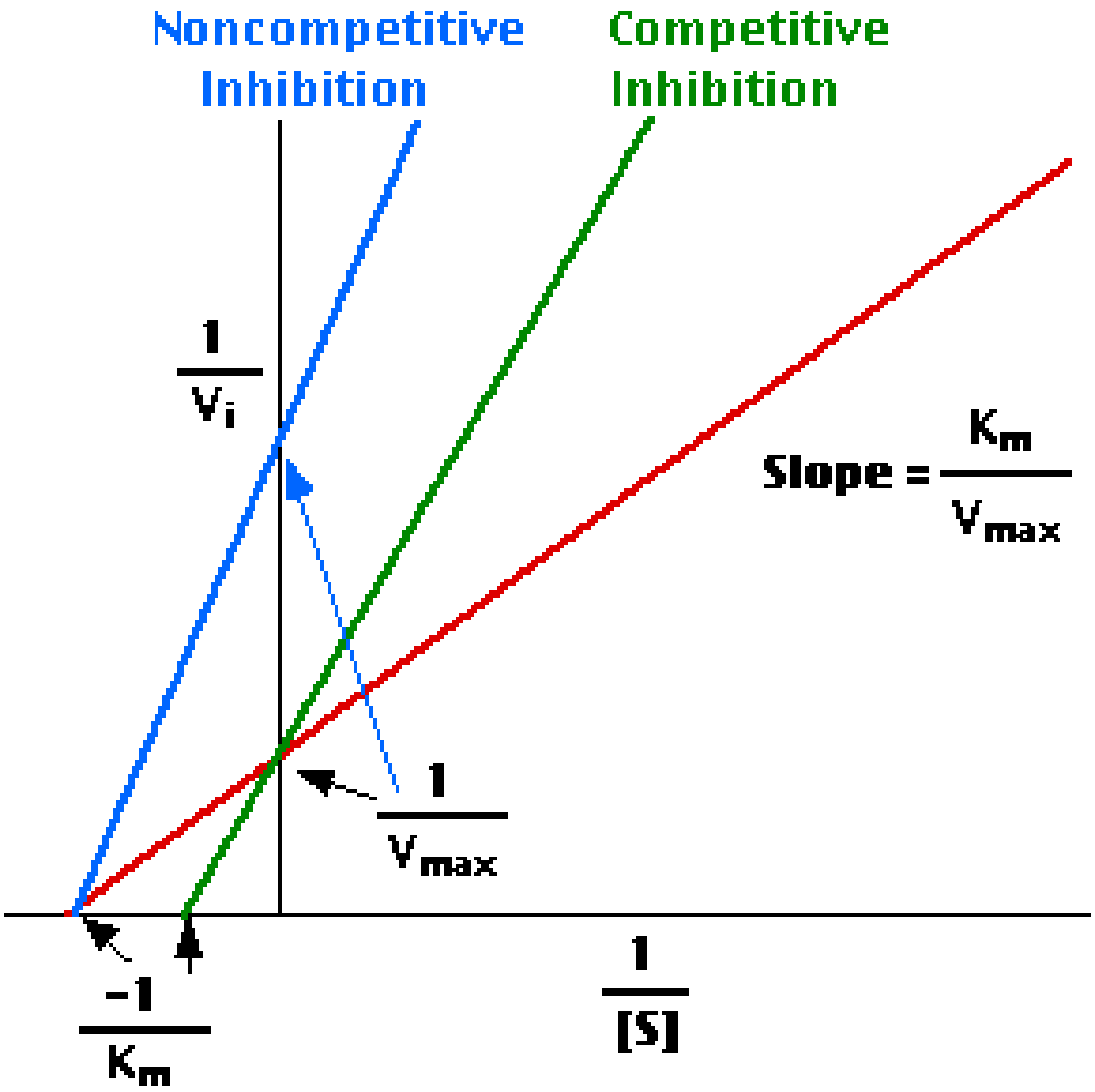
- **The Lineweaver–Burk plot** was widely used to determine important terms in enzyme kinetics, such as Km and Vmax, before the wide availability of powerful computers and non-linear regression software.
- As the y-intercept of such a graph is equivalent to the inverse of Vmax; the x-intercept of the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



http://en.wikipedia.org/wiki/Lineweaver%E2%80%93Burk_plot

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/medbiochem_mi/cources/biochemistry/1st_year_medicine/enzymes/files/lecture_03.htm

http://en.wikipedia.org/wiki/Lineweaver%E2%80%93Burk_plot

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

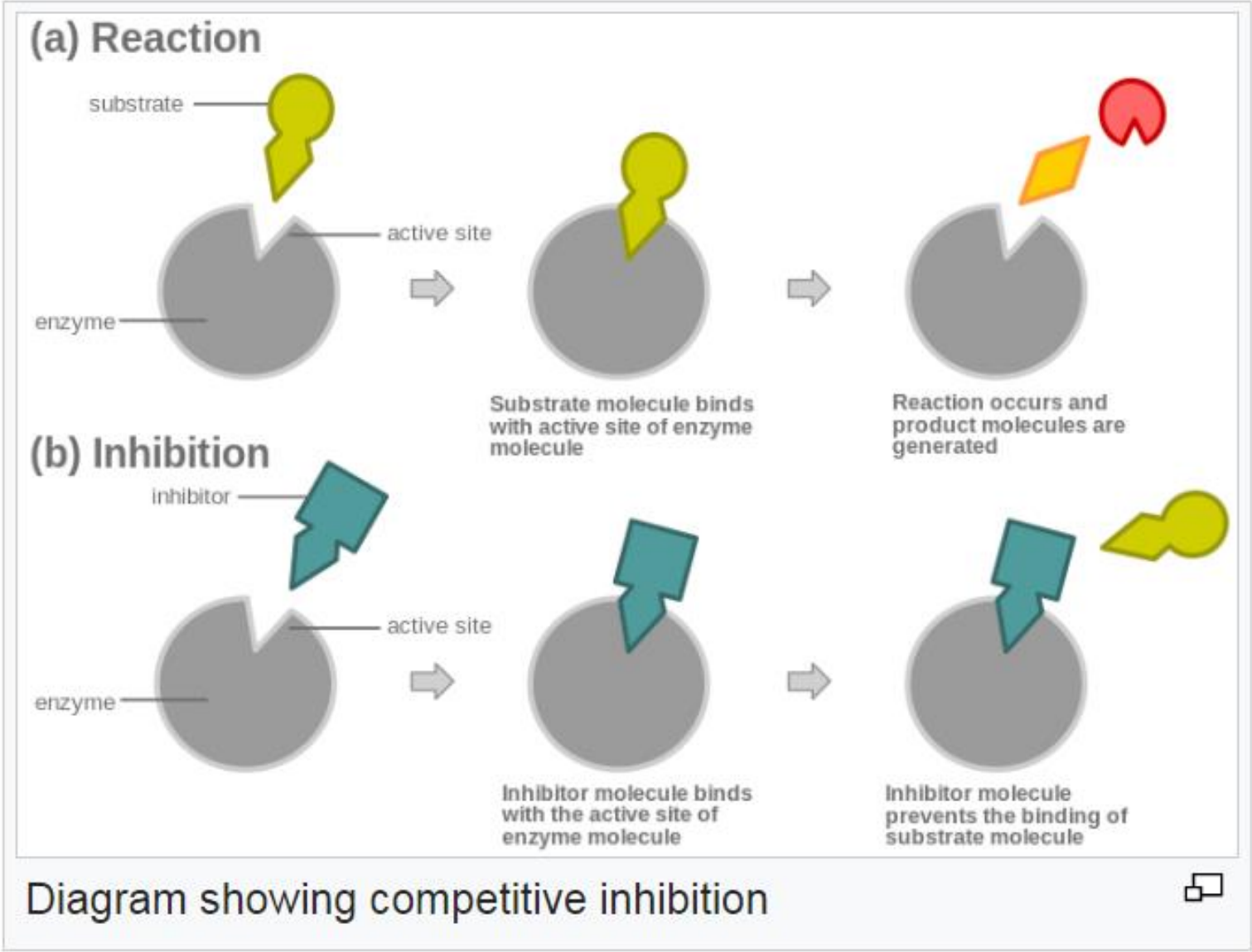
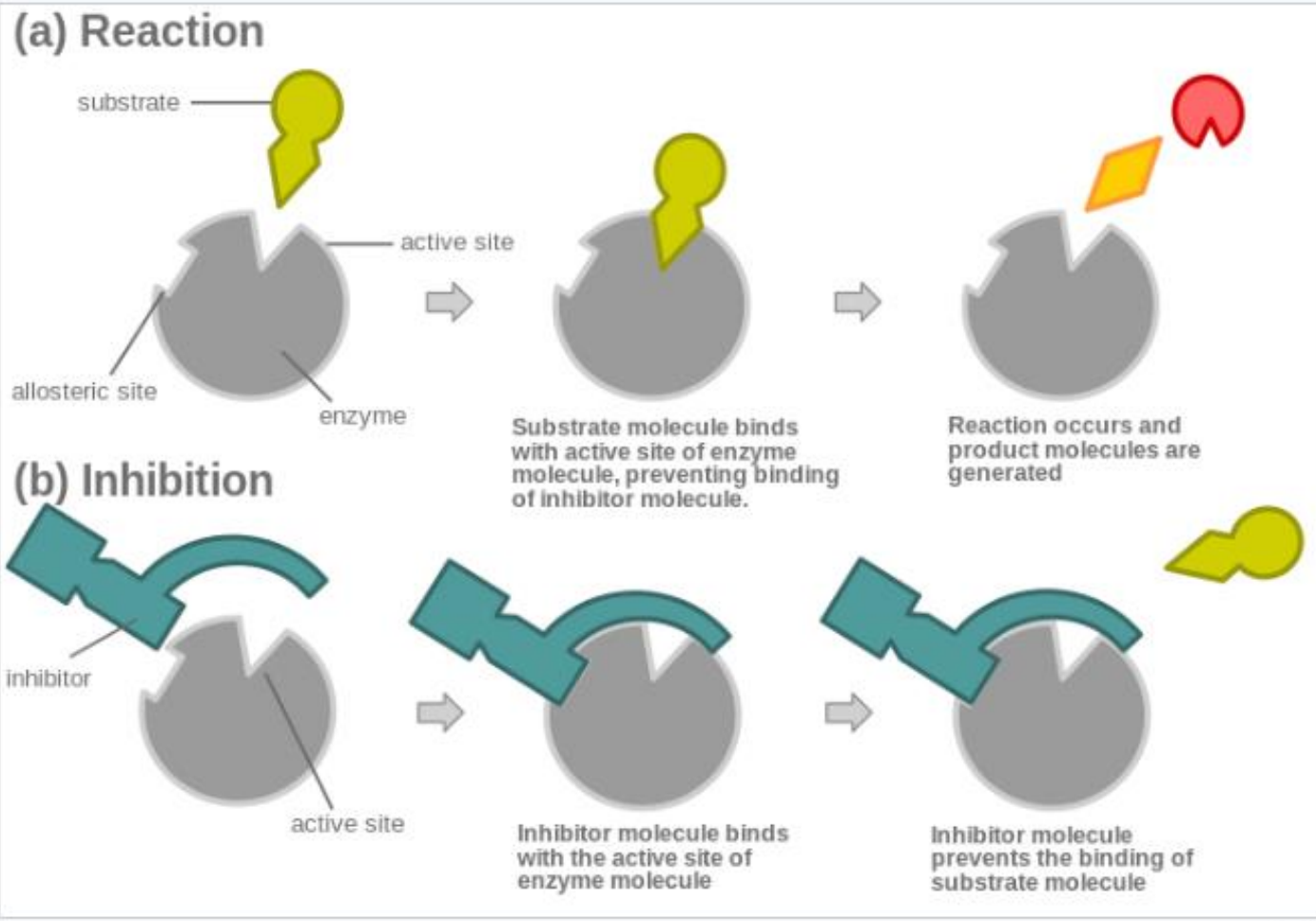
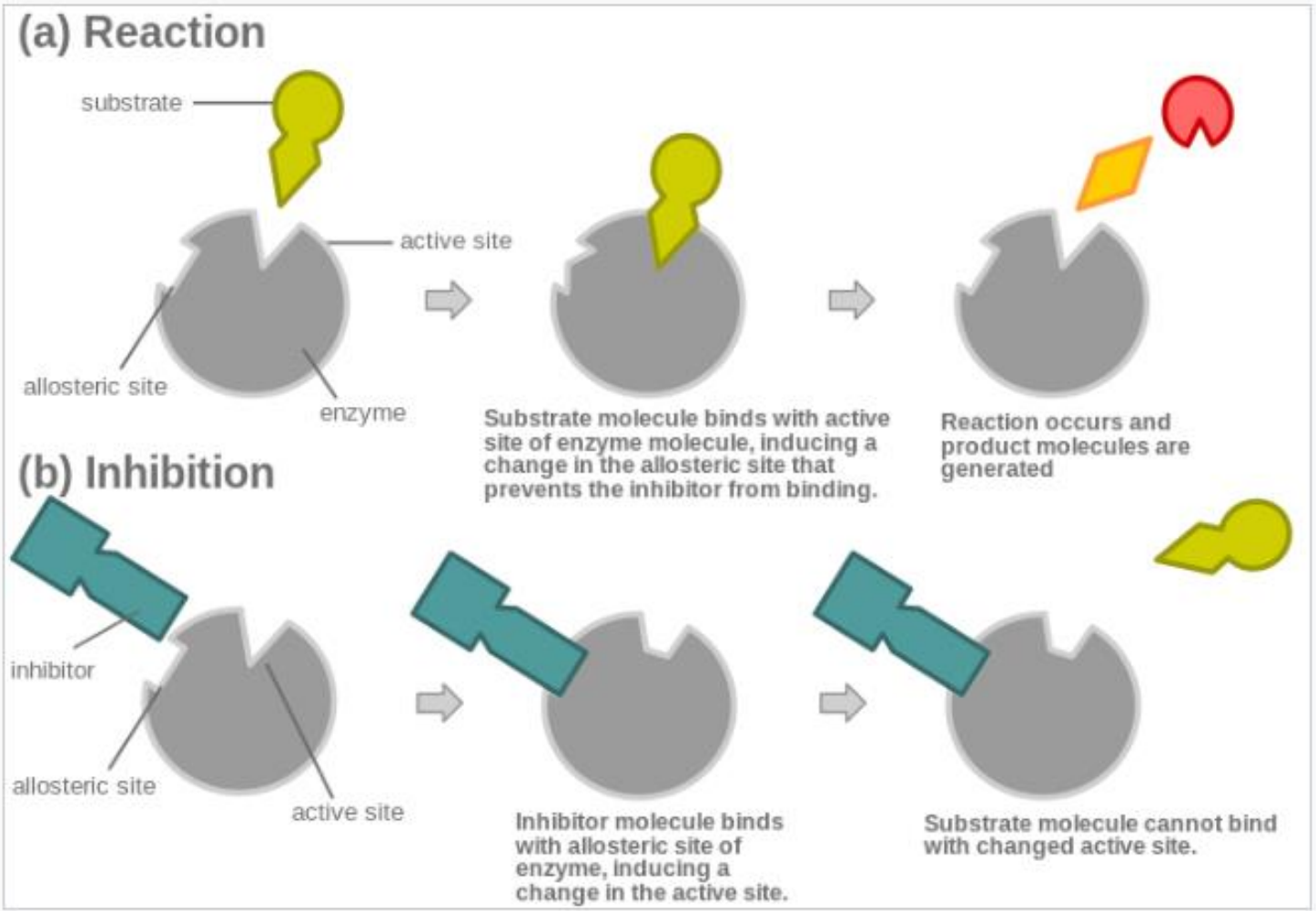


Diagram showing competitive inhibition



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



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

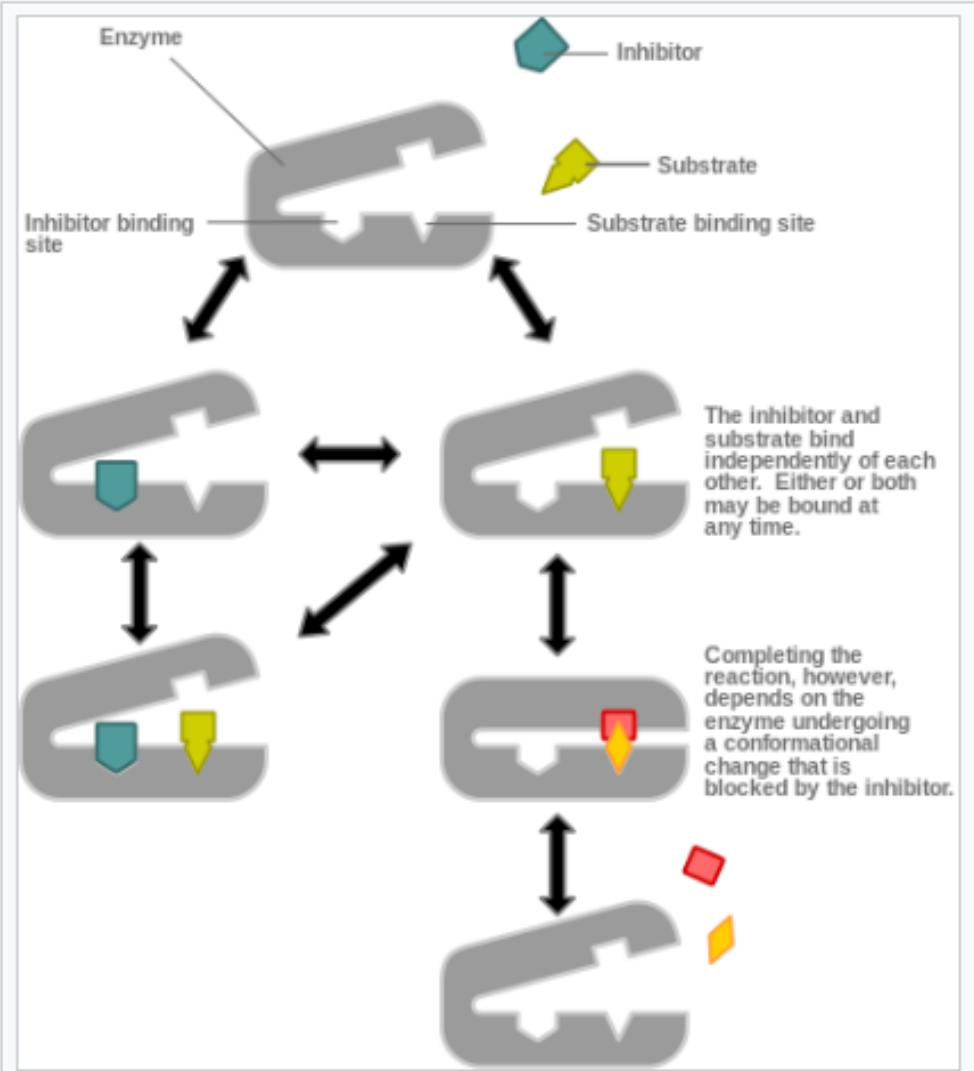


Illustration of a possible mechanism of non-competitive or mixed inhibition.

Enzyme reactions: Kinetics (3)

- The rate constant **kcat** 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**

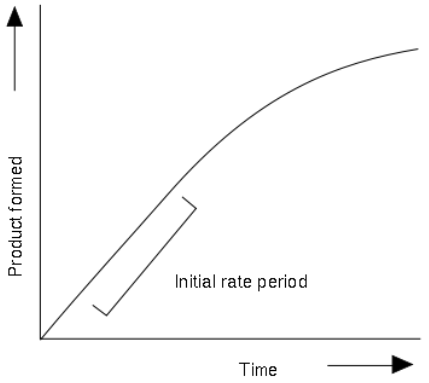
	turnover per second
carbonic anhydrase	600.000
acetylcholinesterase	25.000
penicillinase	2.000
lactate dehydrogenase	1.000
chymotrypsin	100
lysozyme	0.5

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 × 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 µmol/min.
- The specific activity of an enzyme is the activity per mg of total protein (expressed: µmol/min/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/File:Enzyme_progress_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[4]

Group	Reaction catalyzed	Typical reaction	Enzyme example(s) with trivial name
EC 1 *Oxidoreductases*	To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	AH + B → A + BH (reduced) A + O → AO (oxidized)	Dehydrogenase, oxidase
EC 2 *Transferases*	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	AB + C → A + BC	Transaminase, kinase
EC 3 *Hydrolases*	Formation of two products from a substrate by hydrolysis	AB + H_2O → AOH + BH	Lipase, amylase, peptidase
EC 4 *Lyases*	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	RCOCOOH → RCOH + CO_2 or [x-A-B-Y] → [A=B + X-Y]	Decarboxylase
EC 5 *Isomerases*	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	AB → BA	Isomerase, mutase
EC 6 *Ligases*	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 → 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^+ as acceptor	sub group 1
- alcohol: NAD^+-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^{+}

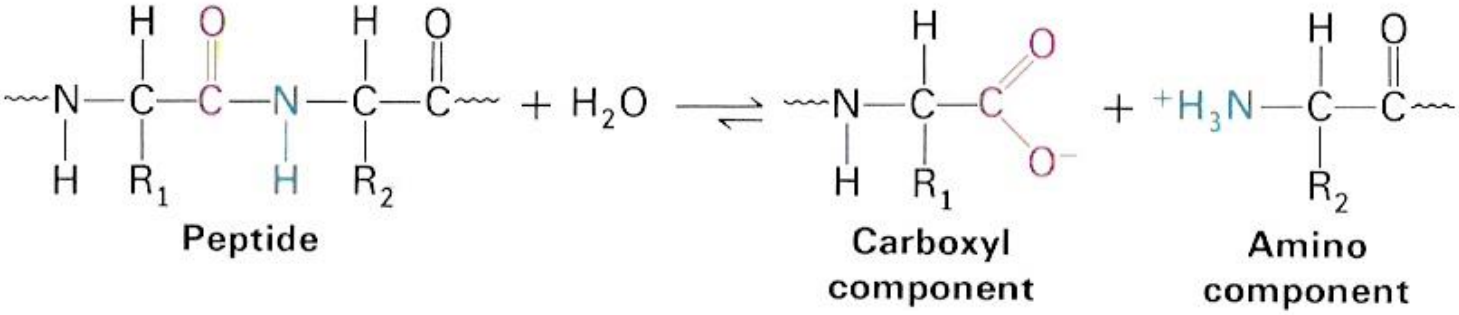
Coenzyme	**Reaction type**
NAD^{+}/NADH, $NADP^{+}$/NADP	removal or addition of hydrogen
ATP	phosphorylation
SAM	C_1-alkylation
Acetyl-CoA	C_2-alkylation
Flavines	oxygenation
Pyridoxal-phosphate	transamination

Proteases: Activity & Specificity

Proteases are :

✓ Proteins also named proteinases, peptidases or proteolytic enzymes

✓ The "protein-cutting machinery" ……
….catalyzing the **<u>hydrolytic breakdown of proteins</u>** into peptides or amino acids by cleavage of peptide bonds between amino acids. This process is called *<u>proteolytic cleavage</u>*. 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

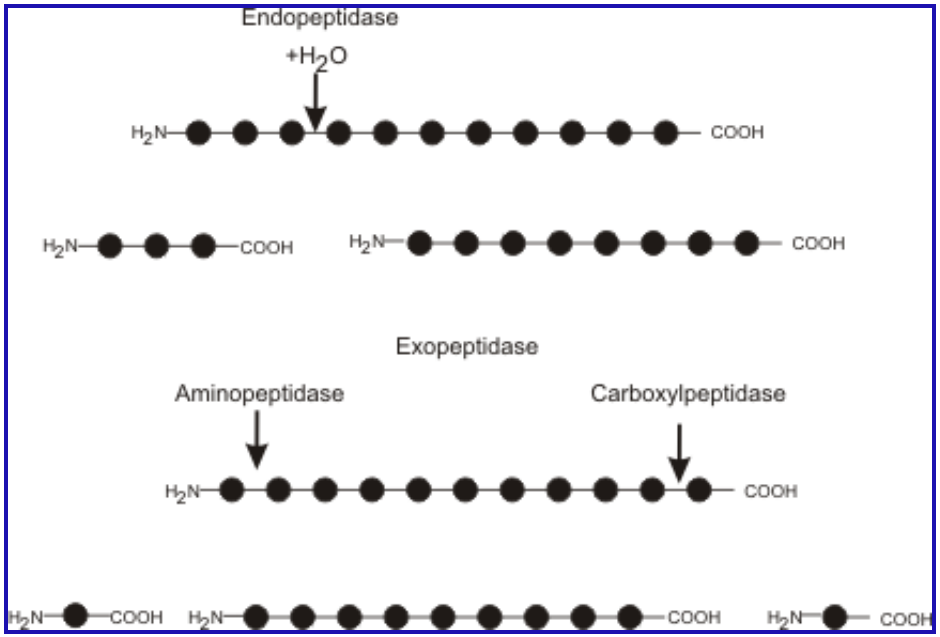
No need to know EC numbers by heart

Exopeptidases
EC 3.4.11.- to 3.4.19.-

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

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:

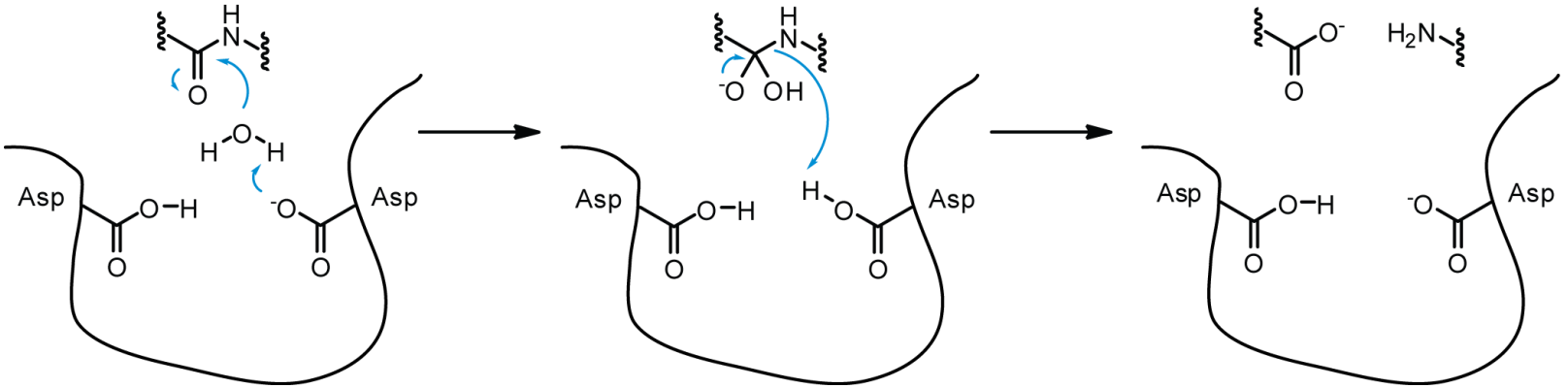


- ❖ **Serine endopeptidases** **EC 3.4.21**
- ❖ **Cysteine endopeptidases** **EC 3.4.22**
- ❖ **Aspartic endopeptidases** **EC 3.4.23**
- ❖ **Metallo endopeptidases** **EC 3.4.24**
- ❖ **Threonine endopeptidases** **EC 3.4.25**
- ❖ **Endopeptidase of unknown catalytic mechanism** **EC 3.4.99**

http://en.wikibooks.org/wiki/Medical_Physiology/Gastrointestinal_Physiology/Digestion_%26_Absorption

Aspartic Protease

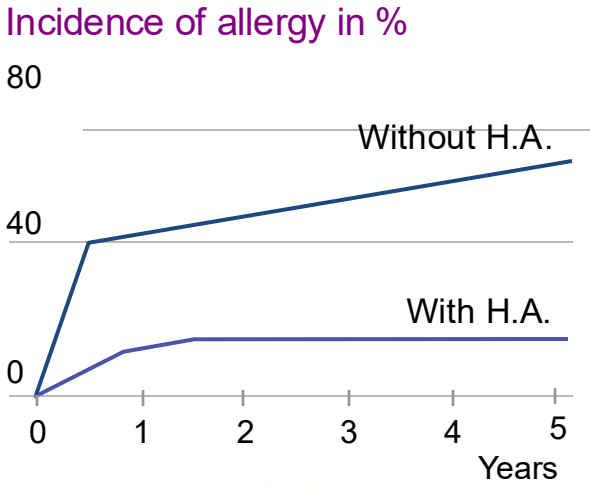
- Aspartic proteases are a family of protease enzymes that use an aspartate residue for catalysis of their peptide substrates.
- In general, they have two highly-conserved aspartates in the active site
- Optimally active at acidic pH
- Nearly all known aspartyl proteases are inhibited by pepstatin
-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



http://en.wikipedia.org/wiki/File:Aspartyl_protease_mechanism.png

Application of proteases
Example 1: Reduce the Risk of Allergy

The Nestlé H.A. Infant Formula



Goal:

- Preventive Formula for Infants with allergy risks

Science:

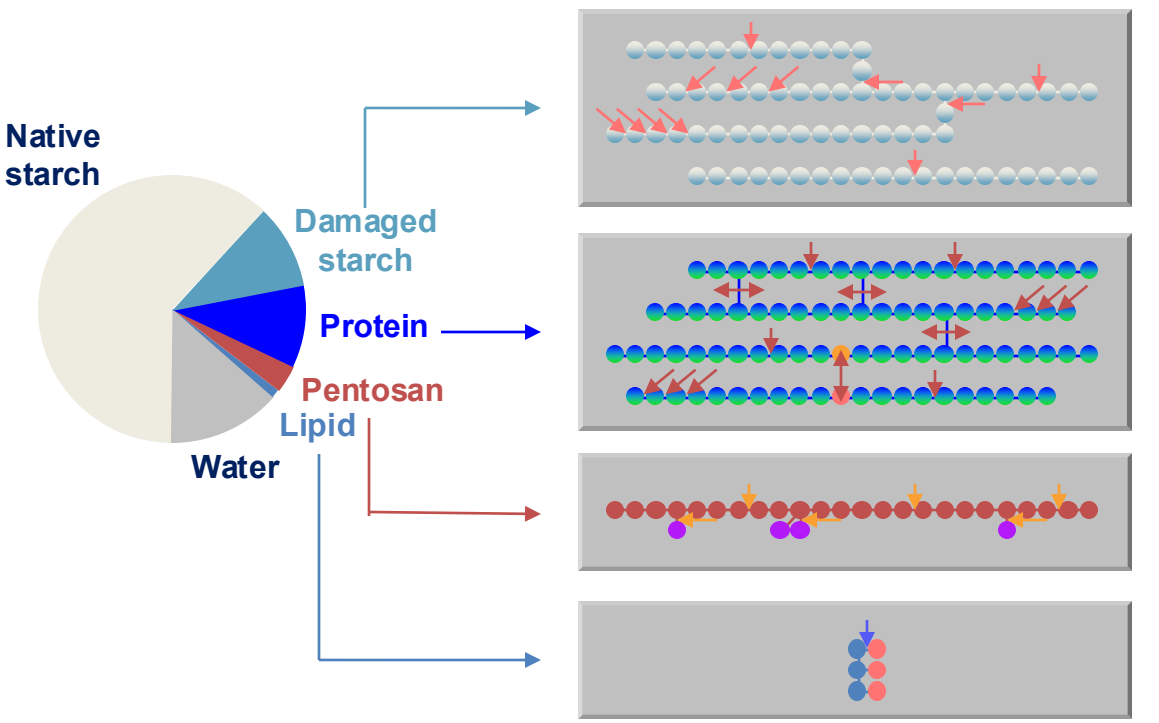
- Moderate enzymatic hydrolysis of milk whey proteins
- No sensitization of immune system
- Various clinical studies show:

  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

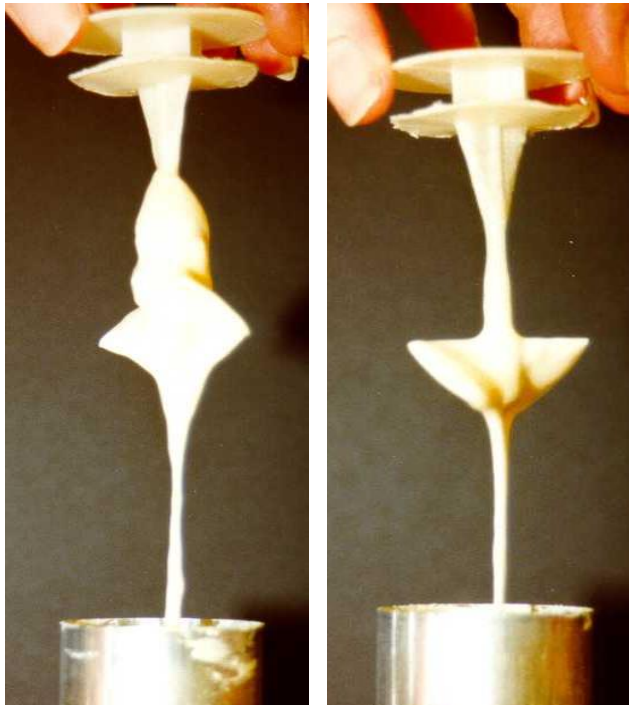
Application of enzymes
Example 2: Modification of cereal flour components

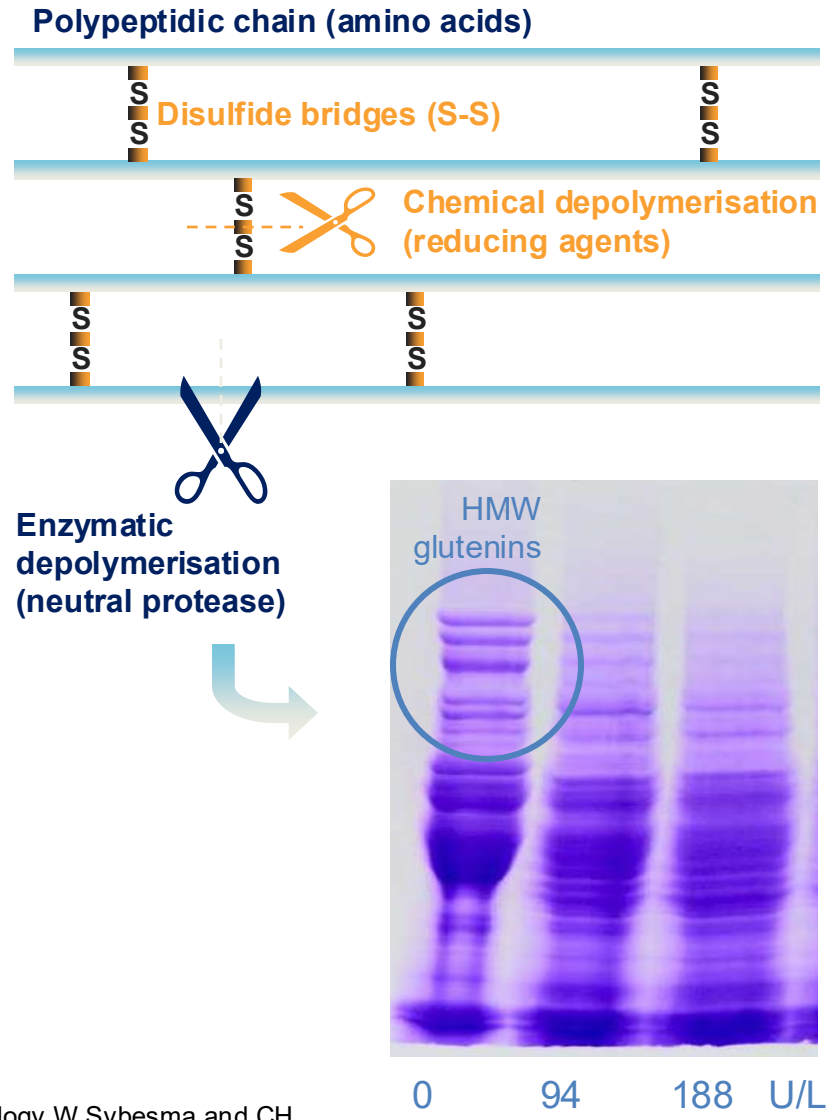


Application of proteases
Example 3: Prevent lumps formation

Goal: improve batter machinability

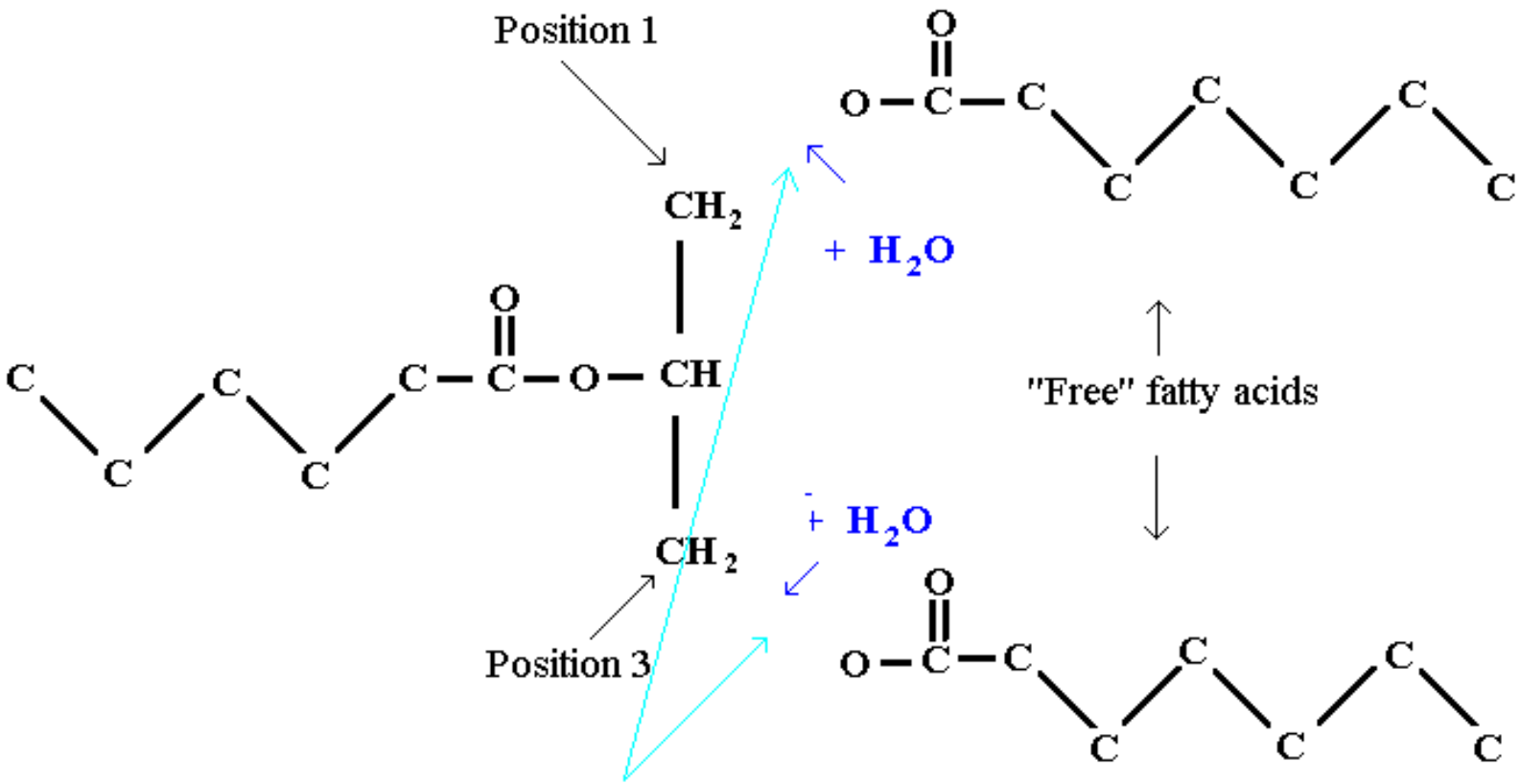
Gluten tends to form aggregates. The use of a neutral protease produces batter without lumps





LIPASES

Lipase action on Triglycerides

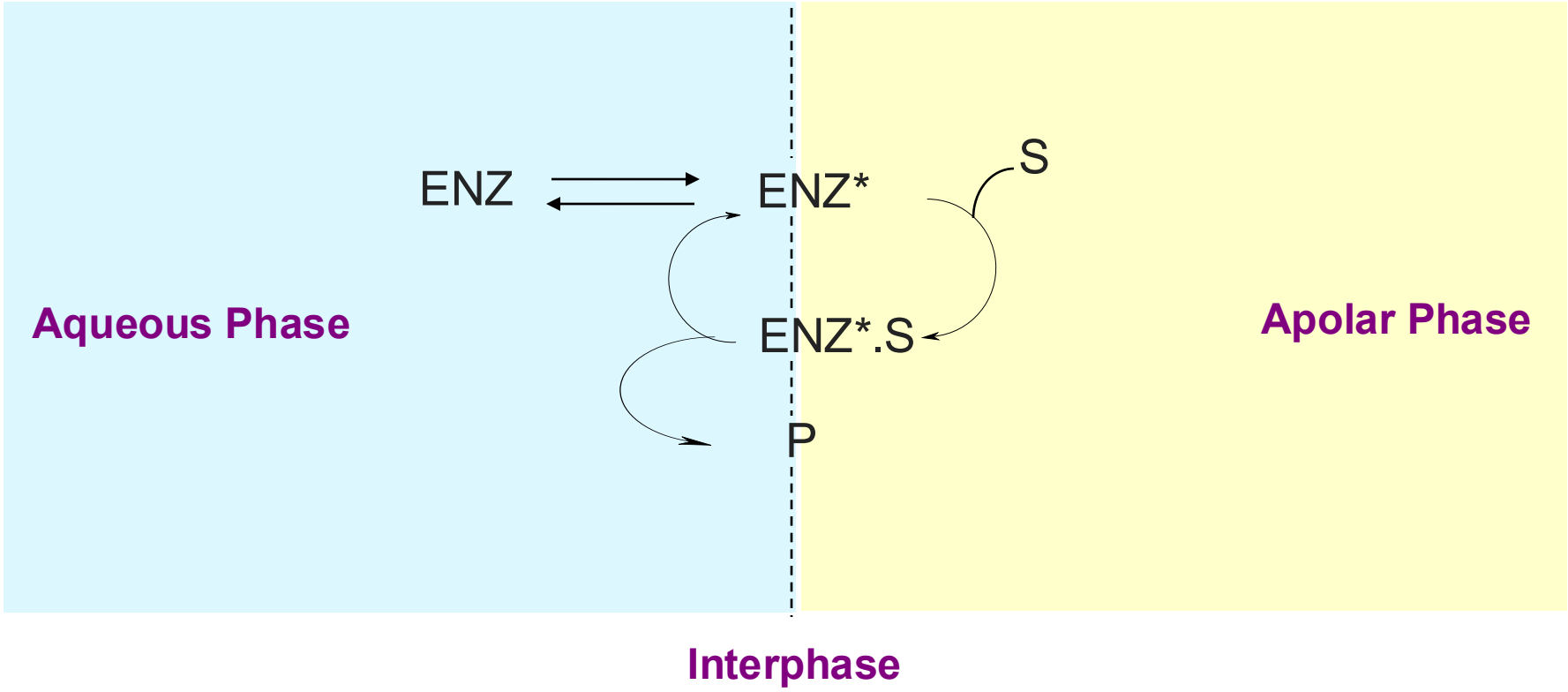


Pancreatic lipase is an enzyme that breaks the bonds between glycerol and the fatty acids at positions 1 and 3, liberating the 2 fatty acids.

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

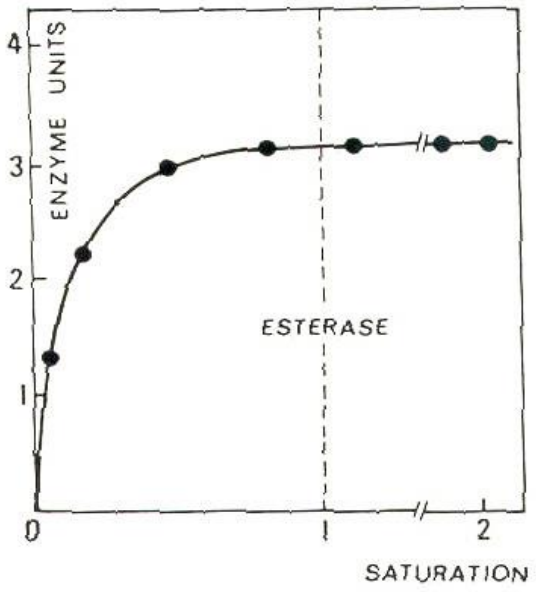
DEFINITION OF LIPASES

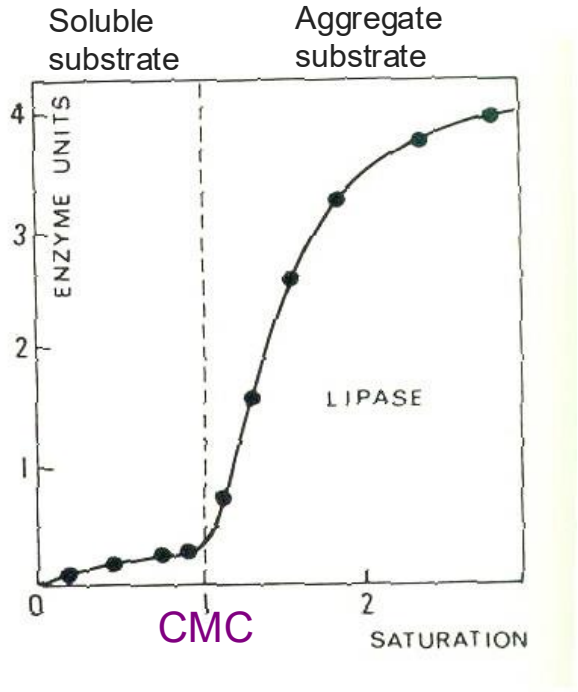
Lipases are acyl hydrolases that catalyze the hydrolysis of triglycerides at the lipid-water interface



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

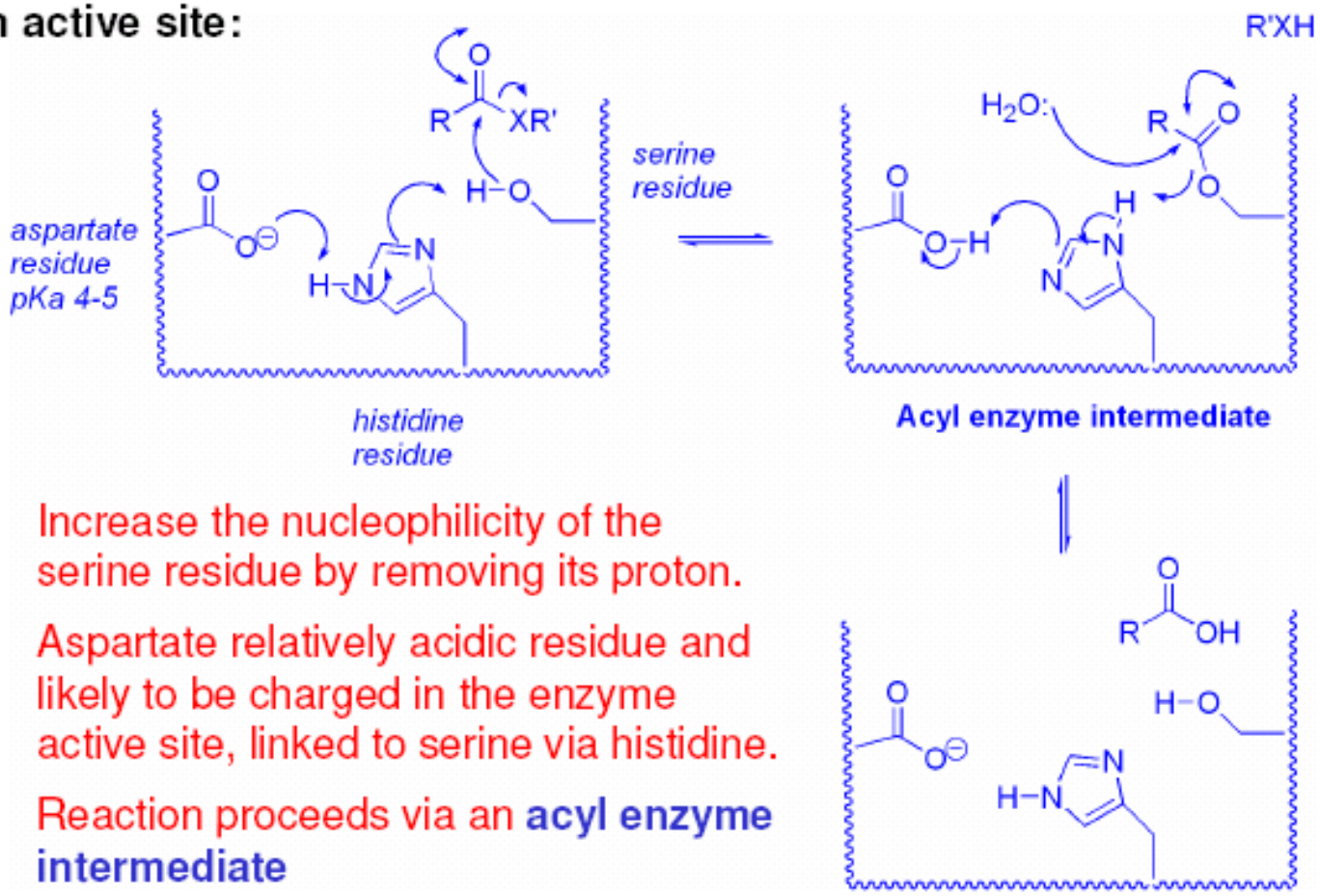
- A sharp increase in activity is observed when the substrate concentration is increased beyond its Critical Micellar Concentration (CMC)

Lipases: Reaction Mechanism

No need to understand reaction mechanism

2. Hydrolytic Enzymes - Catalytic Triad Mechanism

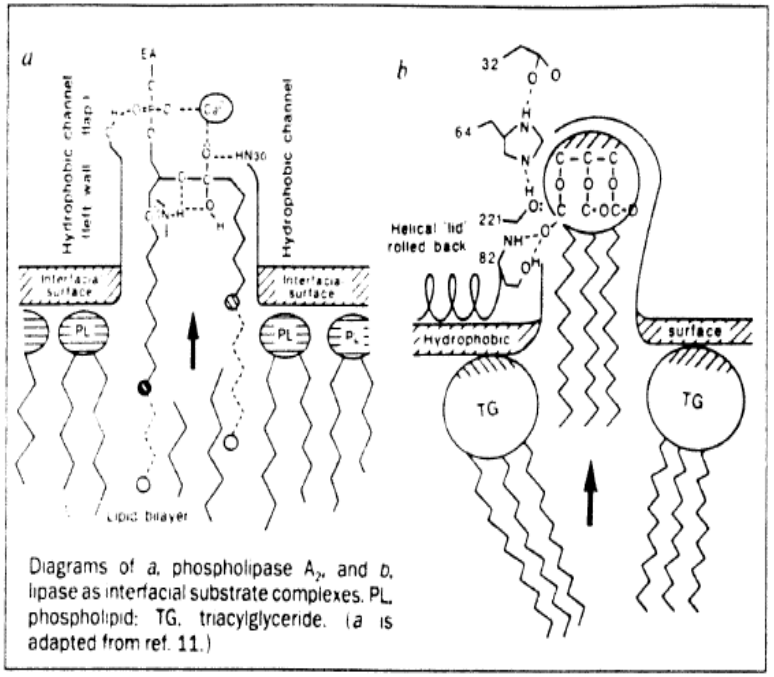
In active site:



- 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 burried 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 encirceling 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 partlially withdraw the substrate, leaving its fatty-acid side chains projecting into the lipid



Diagrams of *a*, phospholipase A_2, and *b*, lipase as interfacial substrate complexes. PL, phospholipid; TG, triacylglyceride. (*a* is adapted from ref. 11.)

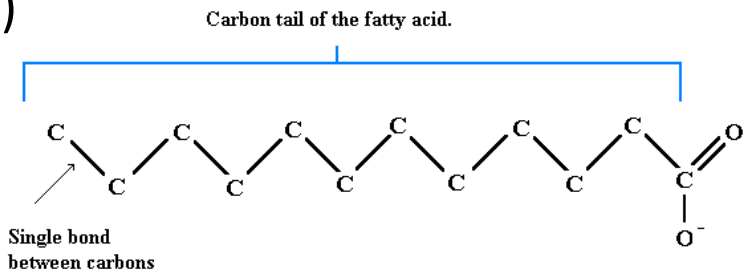
NATURE · VOL 351 · 6 JUNE 1991

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)

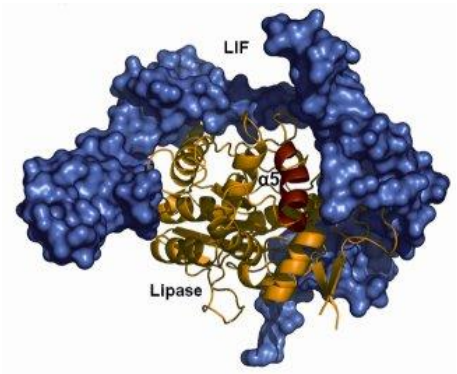
Classification of Lipases

- 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

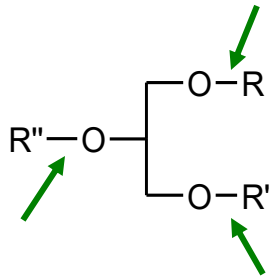
- Classification of lipases based on:
  - Molecular weight
  - Amino-acid sequences
  - Sequence motifs



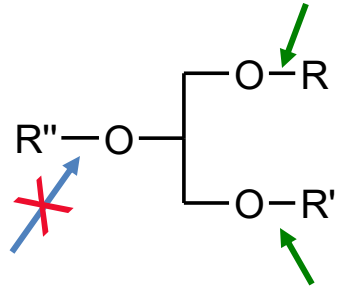
http://ultr23.vub.ac.be/ultr/images/research/lipase.jpg

Specificity of Lipases

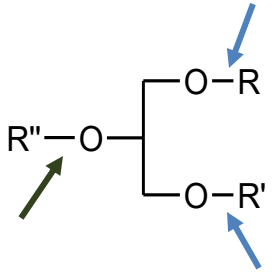
Non-specific



1,3-Specific



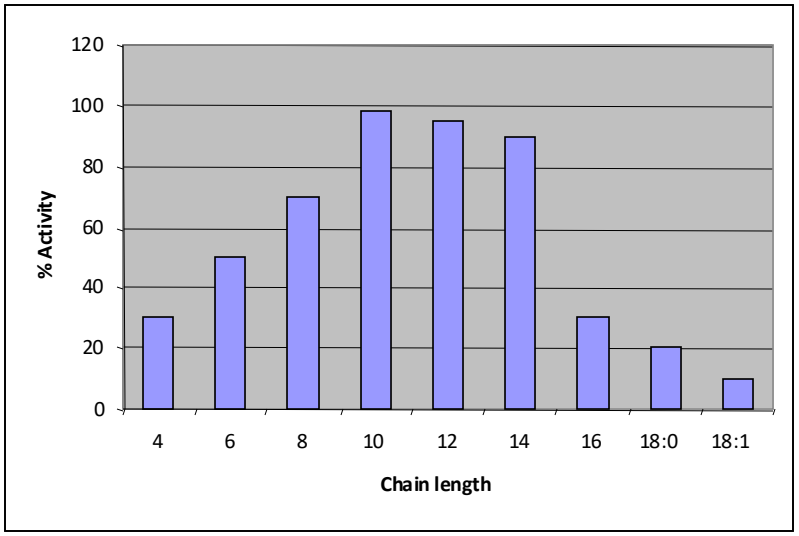
Preference for *sn*-2 position



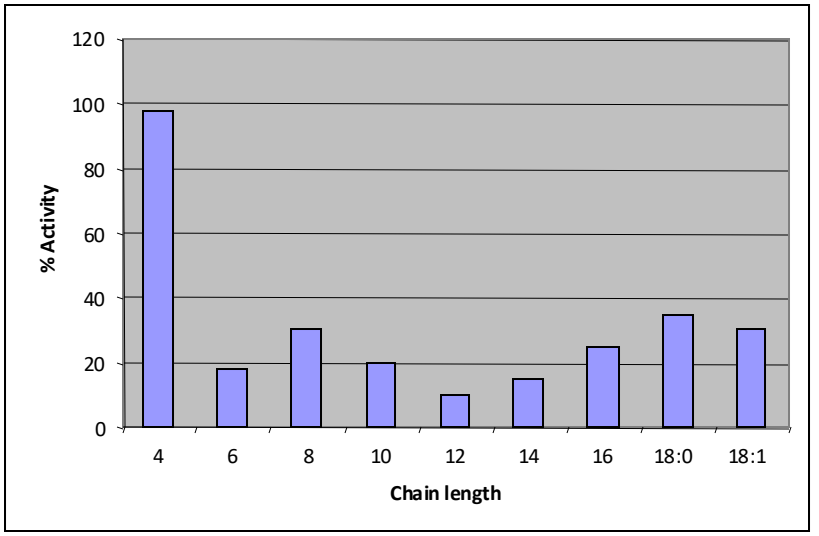
Specificity of Lipases

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



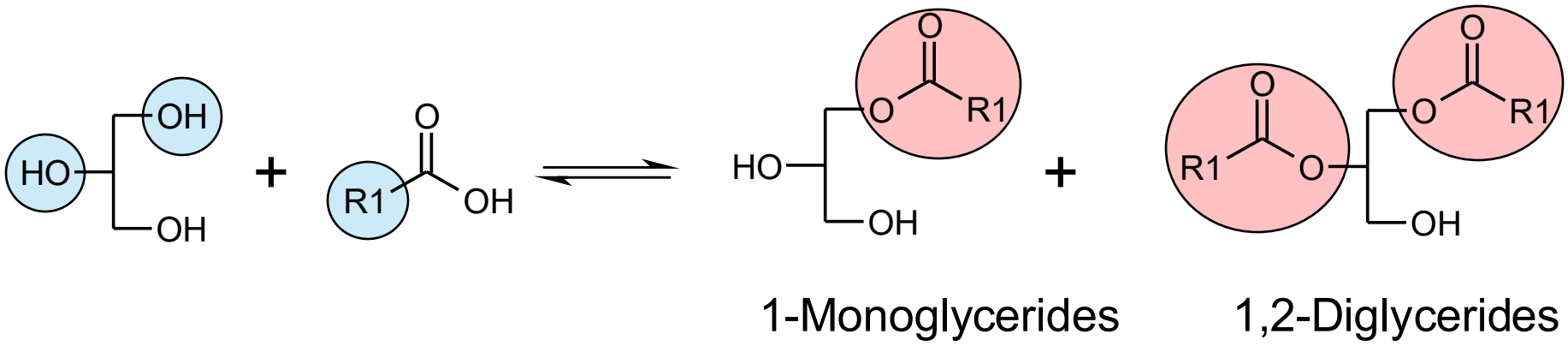
Lipase from *Humicola lanuginosa*



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, Ca^{2+}) in different concentrations influence lipase activity. 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

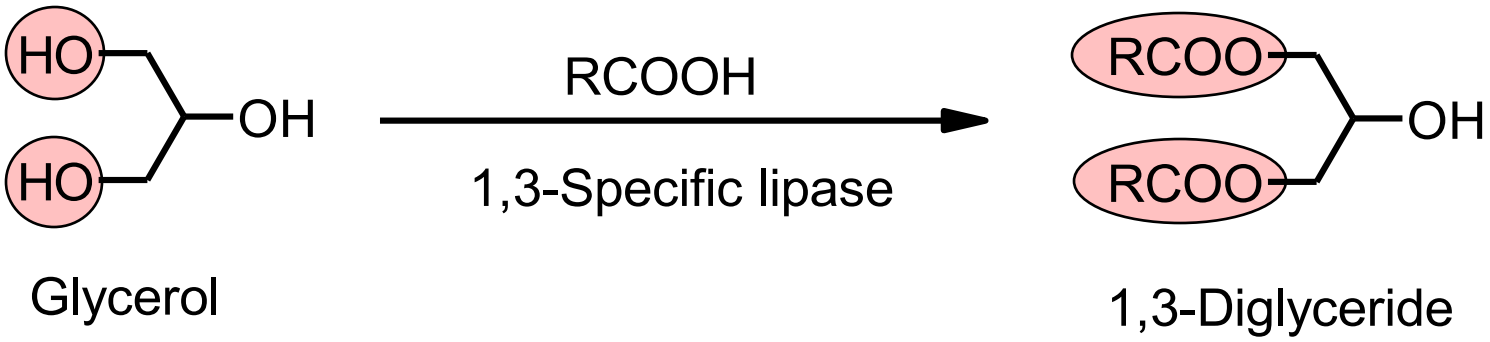
Application of Lipases: ENOVA™ OIL

http://www.fasebj.org/doi/abs/10.1096/fasebj.22.2_supplement.737

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)

The product (from Kao, ADM) has been stopped due to potential risks for cancer
http://www.nishoren.org/en/?p=458

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



JOURNAL ARTICLE

Production of trans-free margarine fat by enzymatic interesterification of soy bean oil, palm stearin and coconut stearin blend

Ruttiya Lakum, Sopark Sonwai

International Journal of Food Science and Technology, Volume 53, Issue 12, December 2018, Pages 2761–2769, https://doi.org/10.1111/ijfs.13888

Published:

03 August 2018

Article history

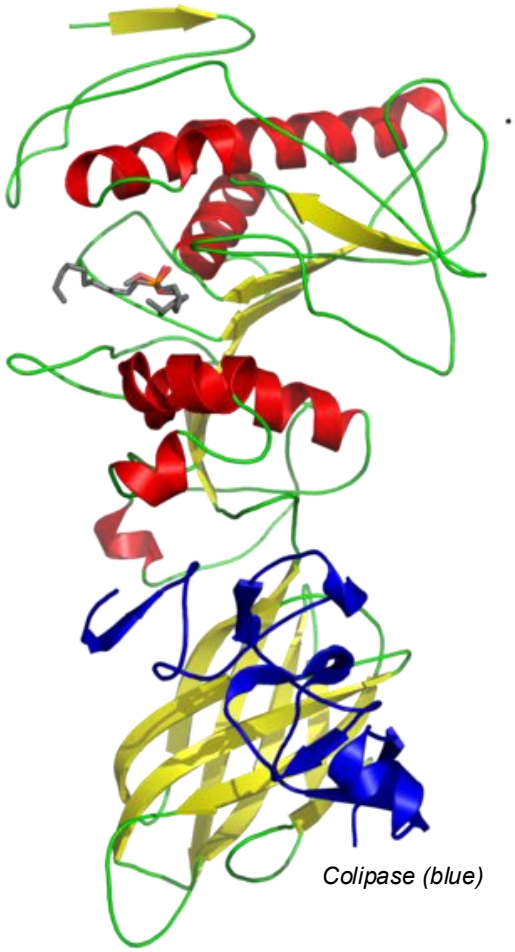
Trans-free structure lipids (SLs) were prepared from enzymatic interesterification (EI) of soybean oil (SBO), palm stearin (PS) and coconut stearin (CNS) blends in different ratios (0:70:30, 10:60:30, 20:50:30, 30:40:30 and 40:30:30% wt.) using Lipozyme RM IM at 65 °C for 8 h. After EI, the fatty acid composition of the SLs exhibited no change from the physical blends (PBs). Medium-chain fatty acid was incorporated into the triacylglycerol of the SLs. The melting completion temperature decreased from 50–54 °C of PBs to 32–44 °C of SLs with a reduction in solid fat content. The small needle-shaped crystals were observed in the SL samples. SL obtained from EI of 20:50:30 (SBO:PS:CNO) blend exhibited the highest potential to be used as a *trans*-free margarine fat as it showed a similar SFC curve to the commercial margarine fat and crystallised into β′ polymorphic form.

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.

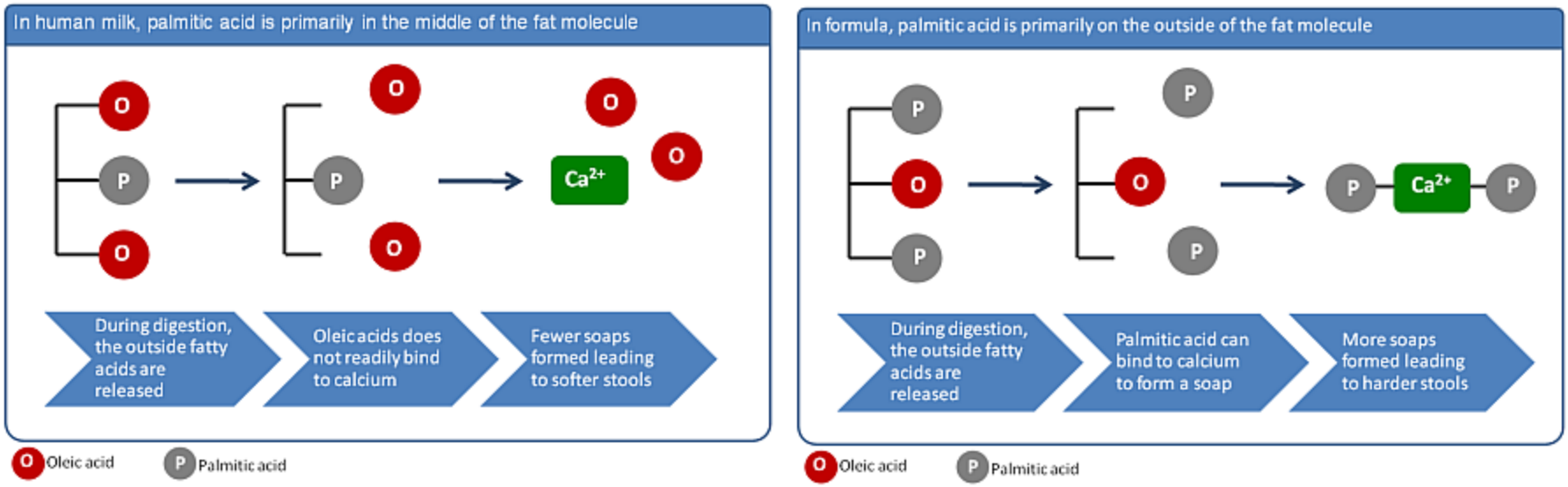


Colipase (blue)

http://en.wikipedia.org/wiki/File:Pancreatic_lipase%E2%80%93colipase_complex_with_inhibitor_1LPB.png

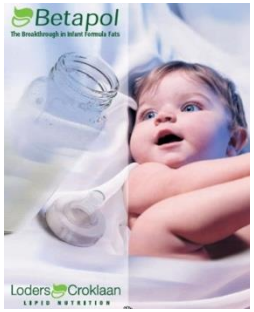
Digestion and absorption of fat

- 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.

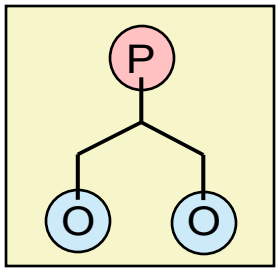


https://www.wyethnutritionacademy.org/Wyeth-Nutrition/resources-hcps/nutrition-for-newborns/human-milk-lipid/information#

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	1
Rat	TG			6	19	14	12	21	2	3	13	10	1
	1			3	10	10	10	20	2	5	24	14	1
	2			6	20	16	18	29	2	1	3	5	1
	3			10	26	15	9	13	2	2	12	12	1
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).

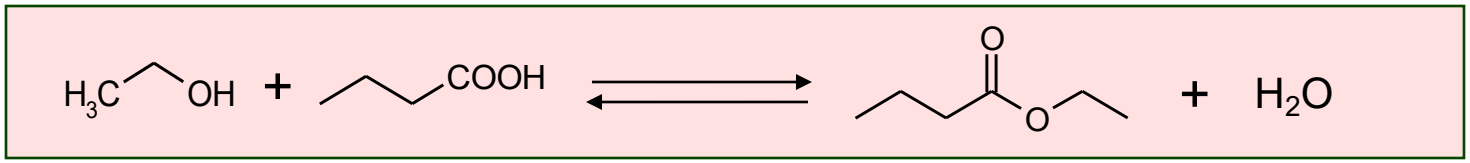
http://lipidlibrary.aocs.org/Lipids/tag1/index.htm

- **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

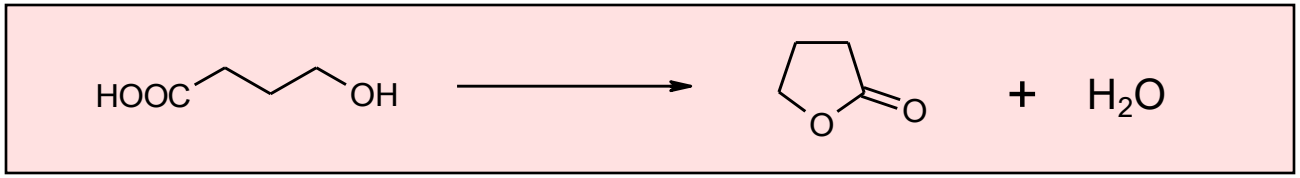
Alcohol + Acid ⇌ Ester + Water



Fruity

Intra-molecular cyclisation/ transesterification

Hydroxycarboxylic acid → Lactone + Water

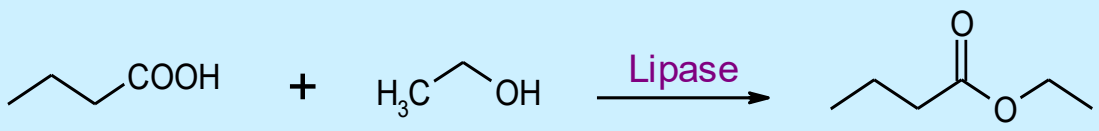


Savoury

Application of Lipases: Generation of Flavours

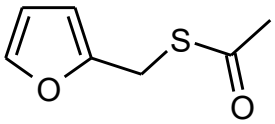
Butter fat —Lipase→

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

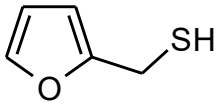


Butyric Acid + Ethanol —Lipase→ Ethyl butyrate (Ethereal, Fruity)

Application of Lipases: Generation of Flavours

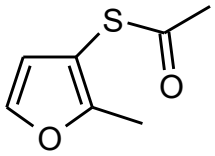


Candida rugosa lipase

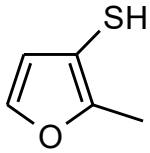


2-furfurylthiol (FFT)

Roasted

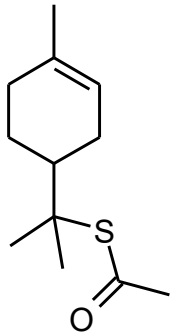


Candida rugosa lipase

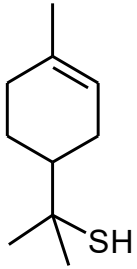


2-methal-3-furanthiol (MFT)

Boiled Meat

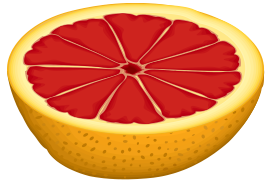


Candida rugosa lipase



1-*p*-Menthene-8-thiol

Grapefruit juice



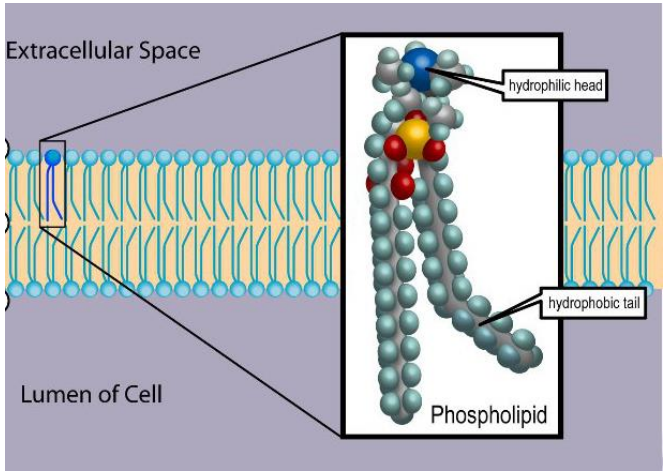
No need to remember details

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

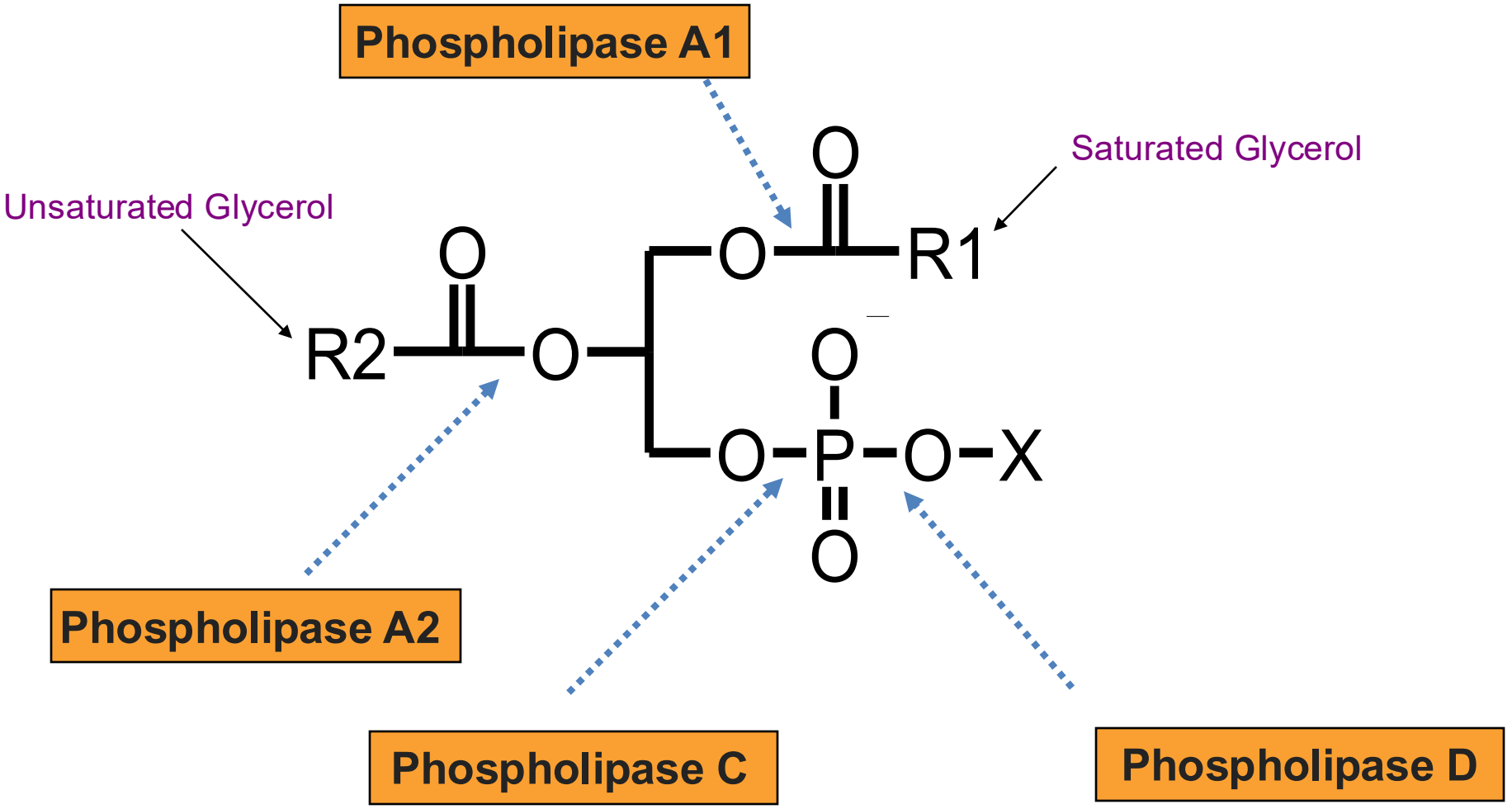
- **Enzymatically modify phospholipids to yield lysophospholipids**

  - 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**

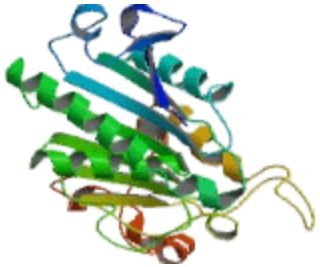
  - Improve the stability of emulsions
  - Or reduce or replace emulsifiers and chemical stabilizers due to *in-situ* enzymatic formation of lysophospholipid.

Phospholipases

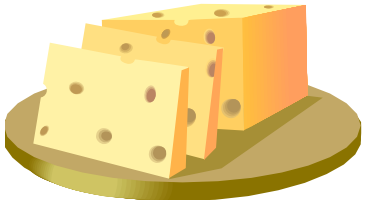


Phospholipase A1

- 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.



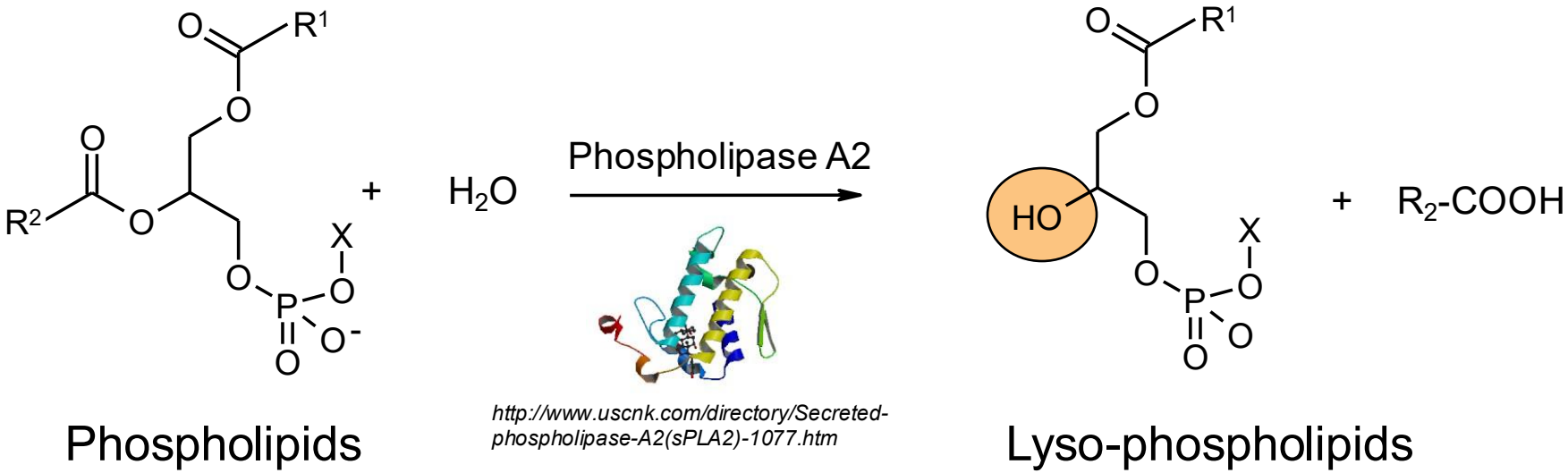
http://www.uscnk.com/directory/Phospholipase-A1(PL-A1)-1151.htm



- 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

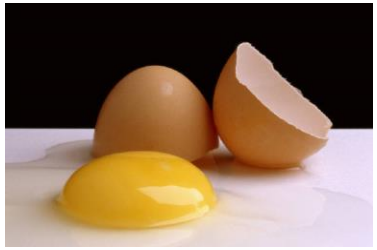
- Phospholipase A2 represents a class of heat-stable, calcium-dependent enzymes



Phospholipids

http://www.uscnk.com/directory/Secreted-phospholipase-A2(sPLA2)-1077.htm

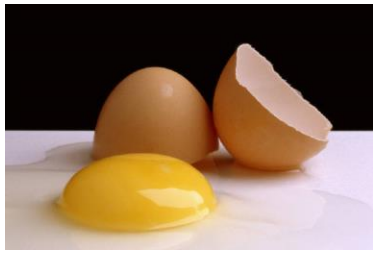
Lyso-phospholipids



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

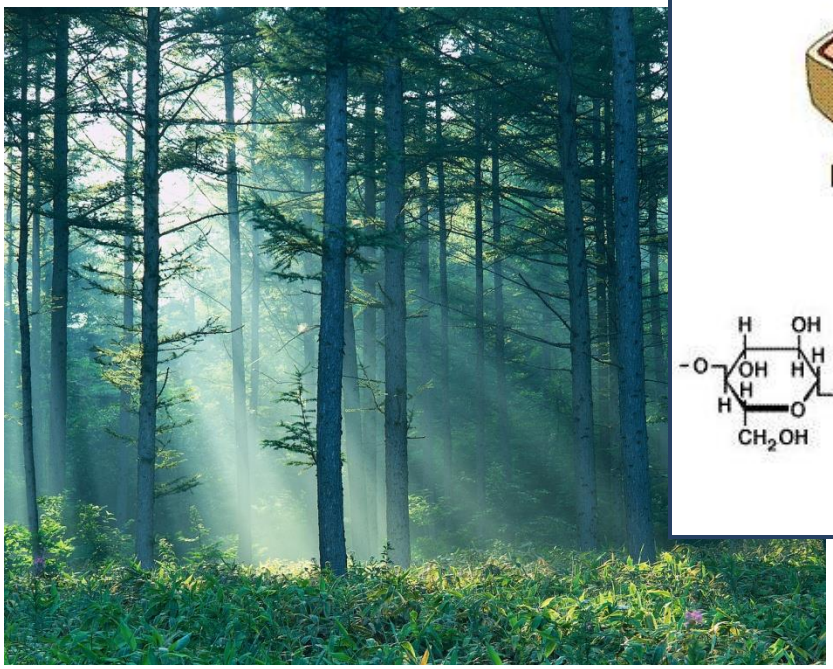
- Modified egg yolk treated with PA2 has demonstrated
  - Superior stability at retort temperatures above 150°C when used in certain food systems (i.e., mayonnaise). This stability allows for the incorporation of pasteurization into processing, which is critical for microbial quality and which also contributes to increased shelf life.
  - Increased viscosity is another benefit offered by modified egg yolk, which is particularly important in the production of mayonnaise.

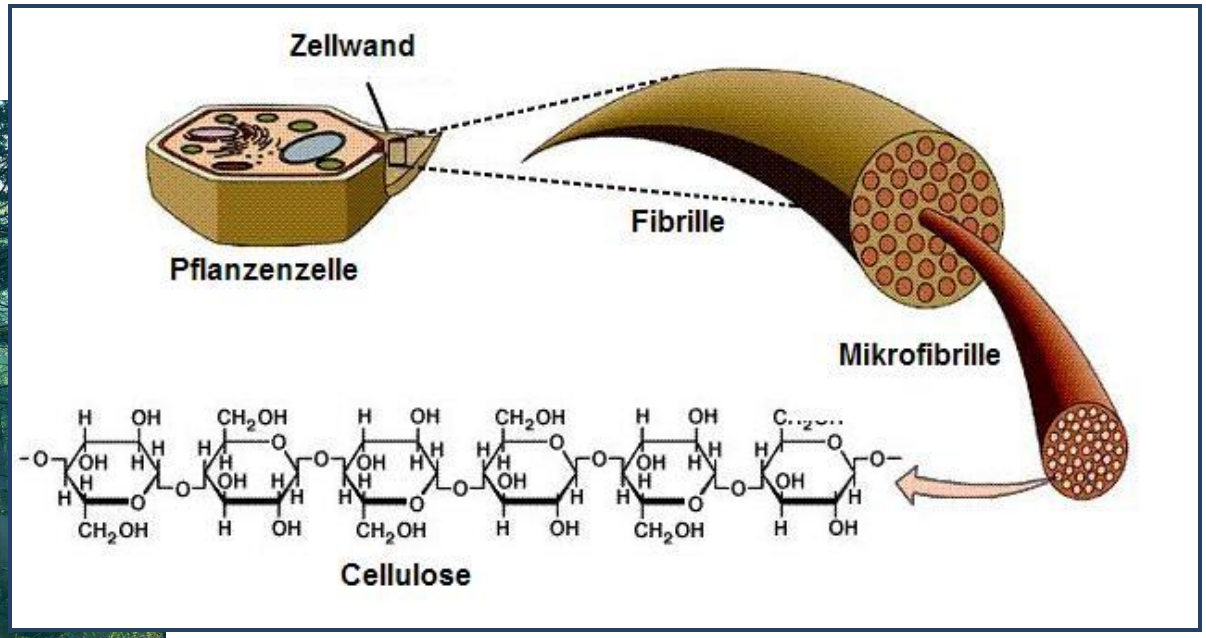


Carbohydrases

Introduction to carbohydrates

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

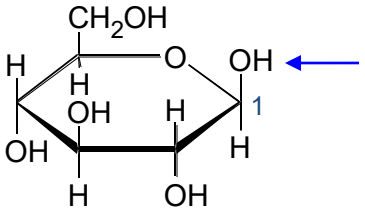




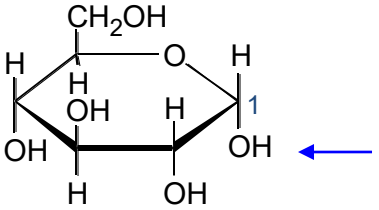
http://www.lookfordiagnosis.com/images.php?term=cellulose&lang=4&from2=60

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:
  - CHOs can also contain other elements such as S,P or N

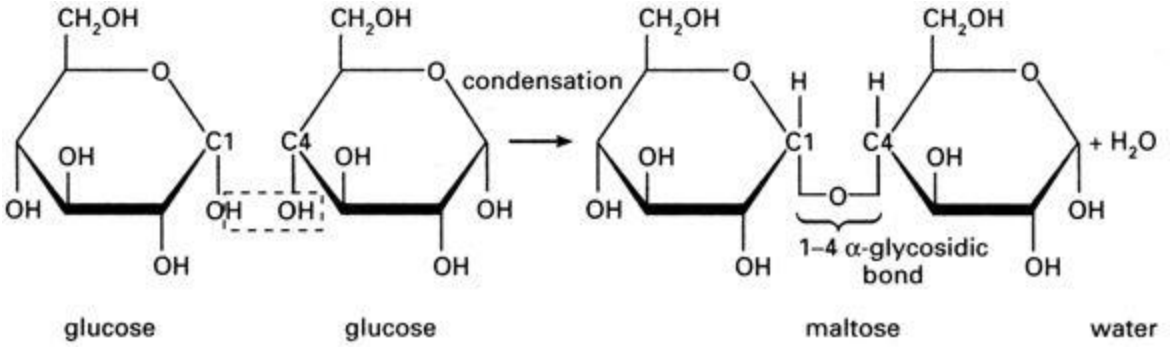


β-D-Glucose



α-D-Glucose

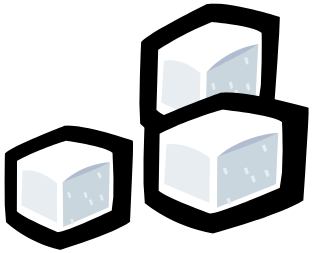
- A glycosidic bond usually joins two monosaccharides.
  - A glycosidic bond is formed between the hemiacetal group of a saccharide (or a molecule derived from a saccharide) and the hydroxyl group of an other molecule.
  - A substance containing a glycosidic bond is a glycoside

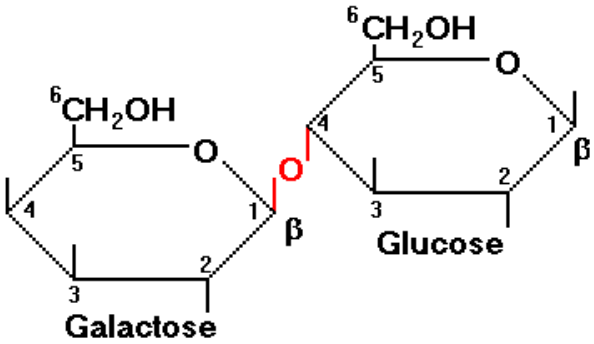


http://www.answers.com/topic/glycosidic-bond-1

Introduction to carbohydrates
Disaccharides

- Lactose
  - Lactose is a disaccharide containing one galactose and one glucose residue linked by a β-(1,4) glycosidic bound.

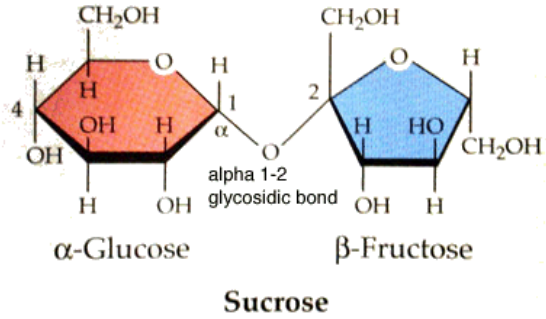




http://www.rpi.edu/dept/chem-eng/Biotech-Environ/IMMOB/lactose.html

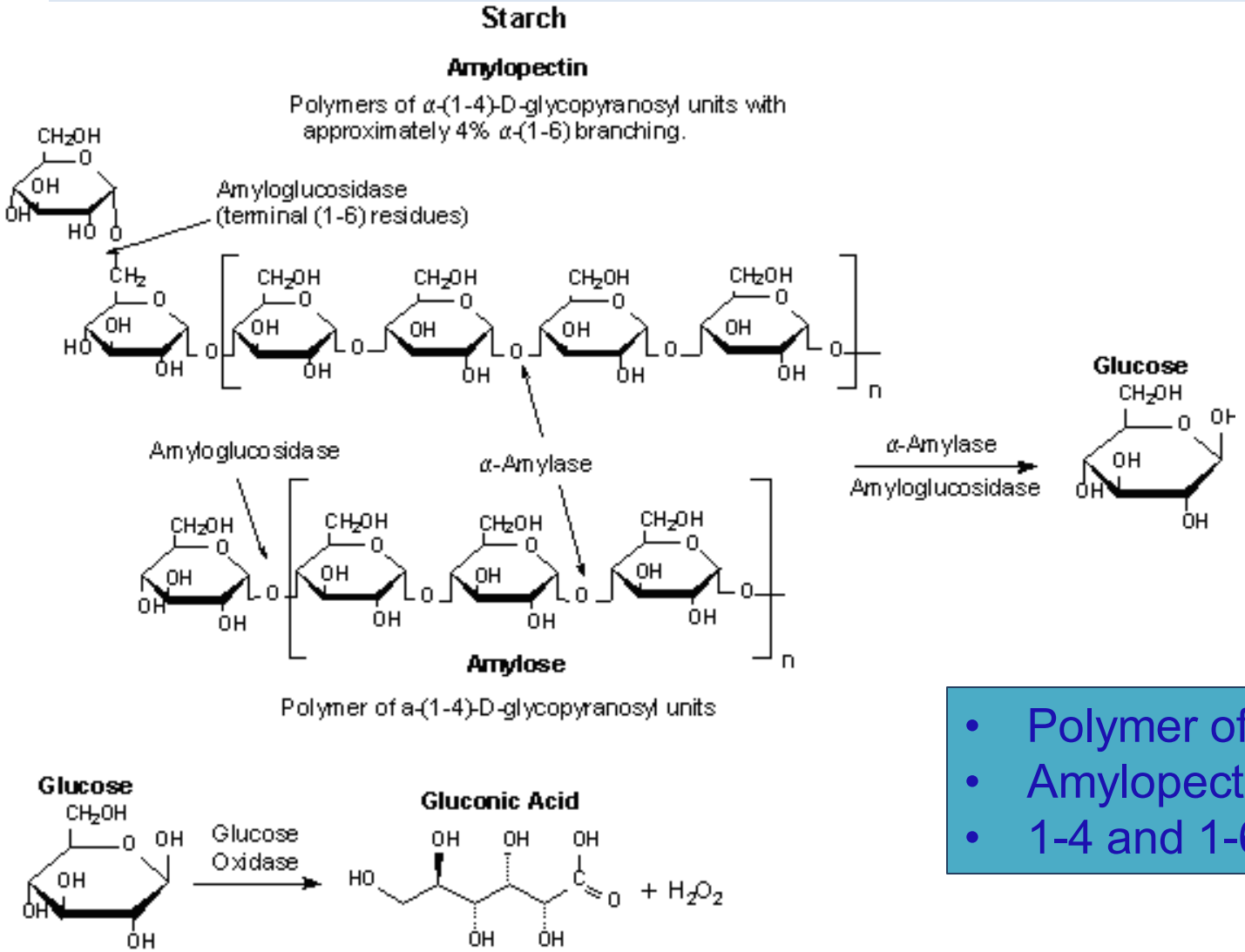
- Sucrose
  - Sucrose consists of two monosaccharides, α-glucose and β-fructose, joined by a (1,2)-glycosidic bond between carbon atom 1 of the glucose unit and carbon 2 of the fructose unit.
  - No reducing end

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



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

Starch

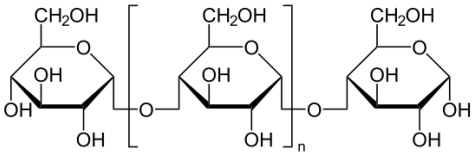


- Polymer of glucose
- Amylopectin & Amylose
- 1-4 and 1-6 linkages

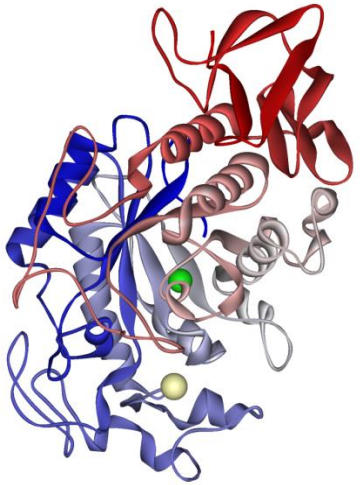
Starch degrading enzymes as an example

1. **Glycosyl hydrolases**
   - Endo-hydrolases
     - α-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, α-amylase is faster than β-amylase
     - β-amylase: Working from the non-reducing end. Catalyzes hydrolysis of the second α-1,4 glycosidic bond, cleaving off two glucose units (maltose)
   - Exo-hydrolases – Amyloglucosidase: will cleave α-1,6 glycosidic linkages, as well as the last α-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)



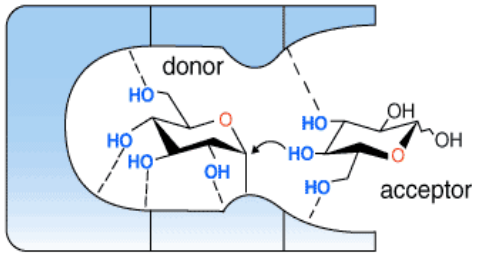
Dextrins are mixtures of polymers of D-glucose units linked by α-(1,4) or α-(1,6) glycosidic bonds.



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

Glycosyl Hydrolases: Three Types of Active Sites

A

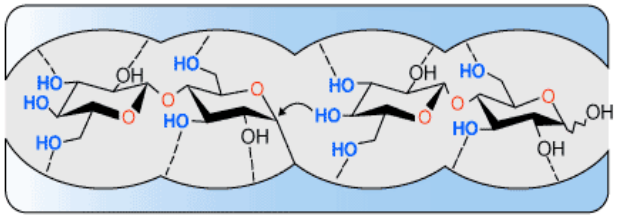


GlycoWord

Pocket/Crater:

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

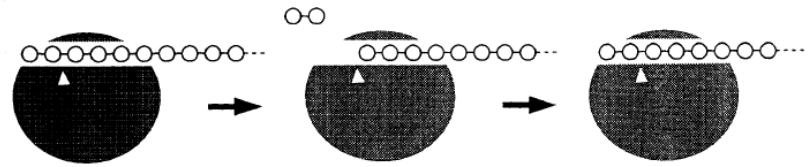
B



Cleft/Groove:

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

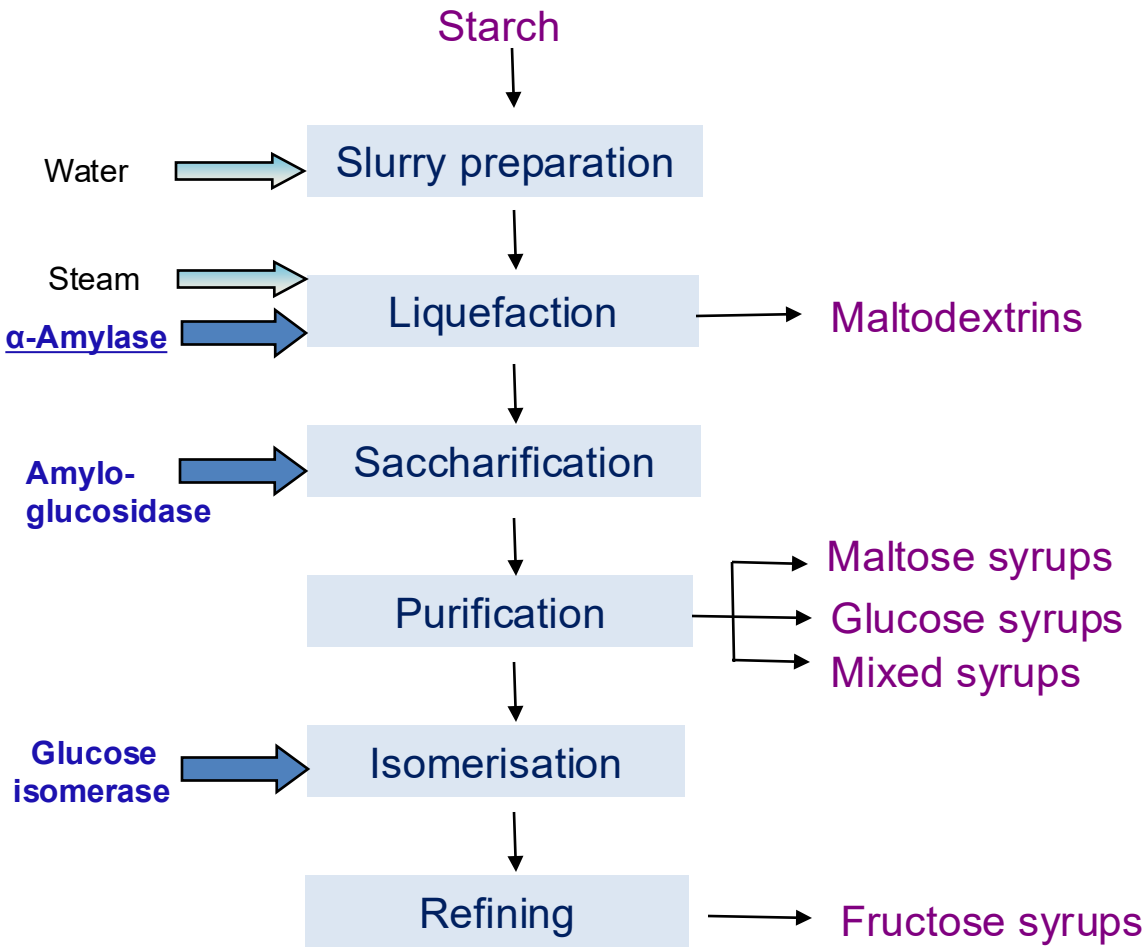
C

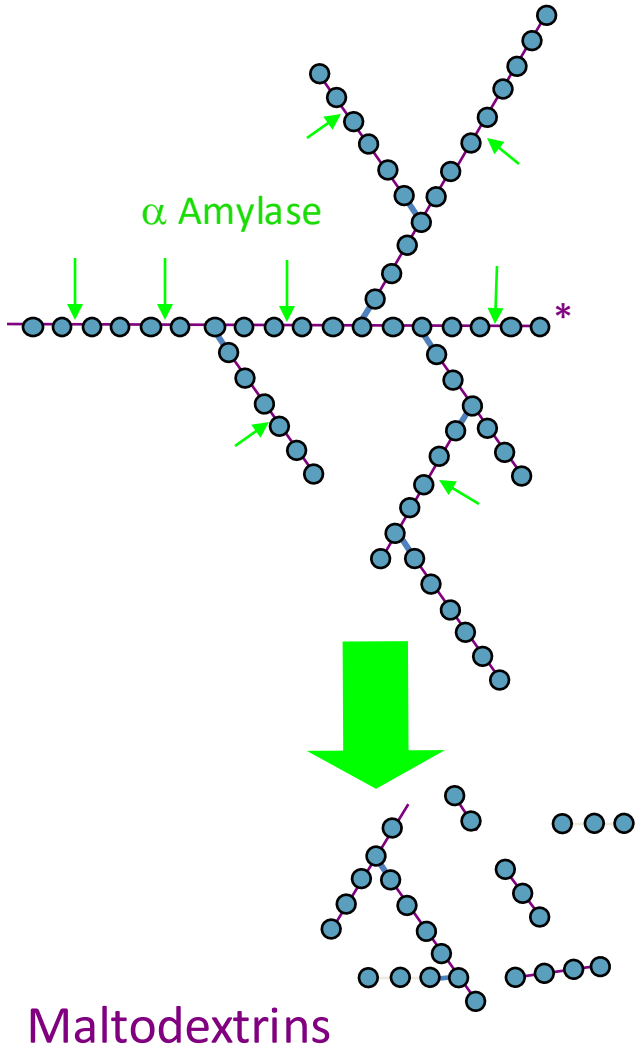


Tunnel:

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

Major steps in enzymatic degradation of starch





Major steps in enzymatic degradation of starch

Starch

Water → Slurry preparation

Steam → Liquefaction → Maltodextrins

α-Amylase → Liquefaction

Saccharification

Amylo-glucosidase → Saccharification

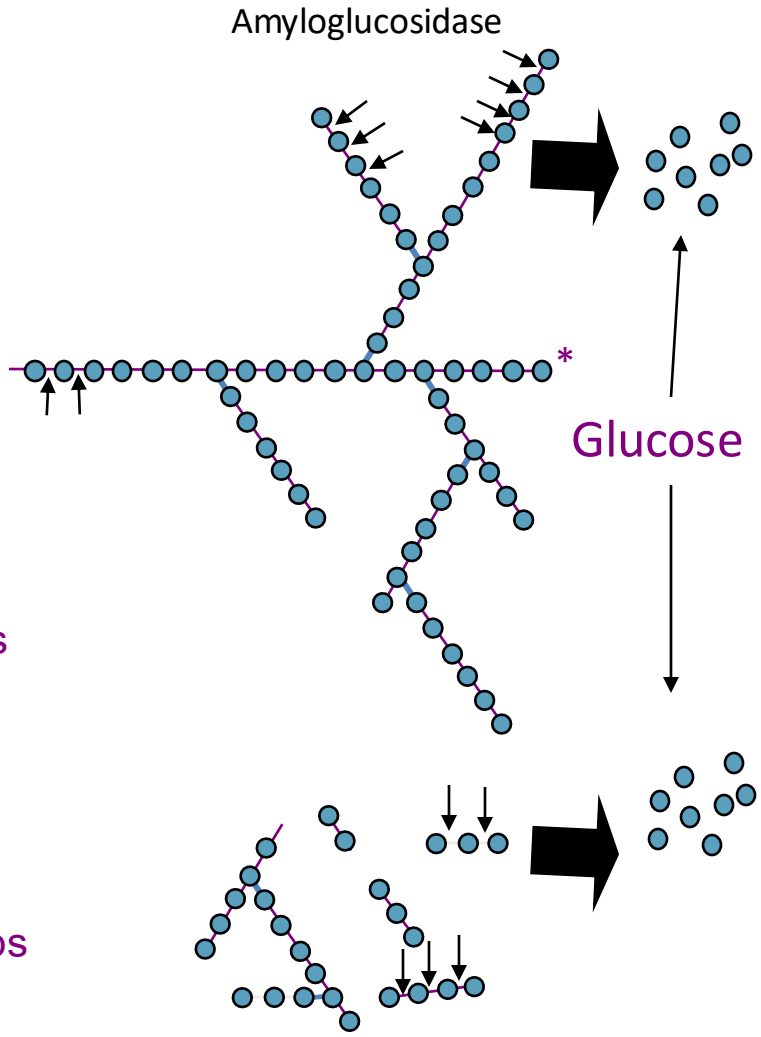
Purification → Maltose syrups

Purification → Glucose syrups

Purification → Mixed syrups

Glucose isomerase → Isomerisation

Refining → Fructose syrups



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:

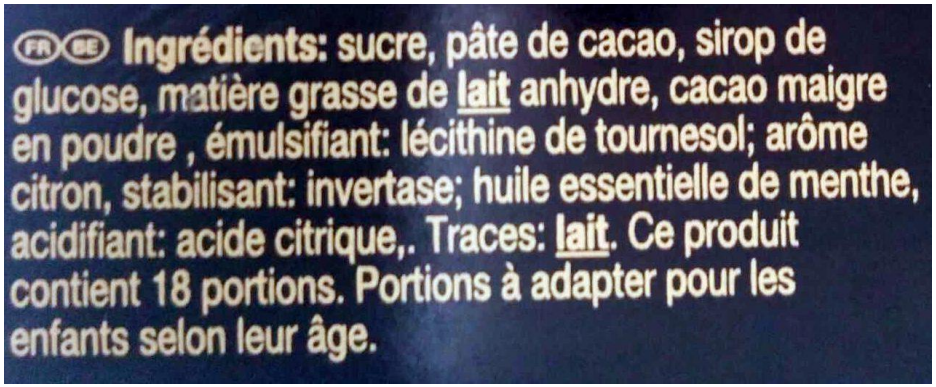
$$DE = 100x\left(\frac{\text{Number of glycosidic bonds cleaved}}{\text{Initial number of glycosidic bonds present}}\right)$$

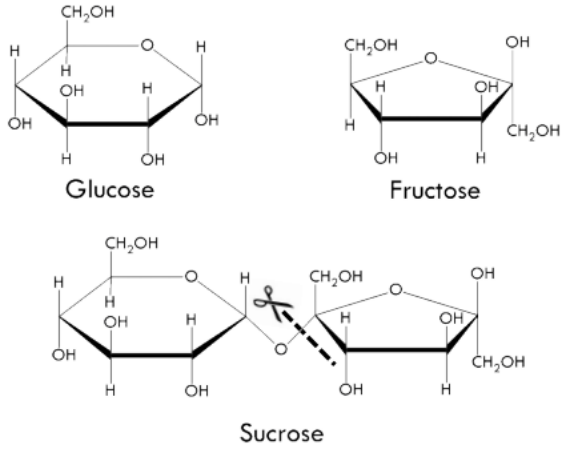
In practice, this is usually determined analytically by use of the closely related, but not identical, expression:

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

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 Nestlé After Eight has liquefied itself inside the chocolate coating all thanks to an enzyme.





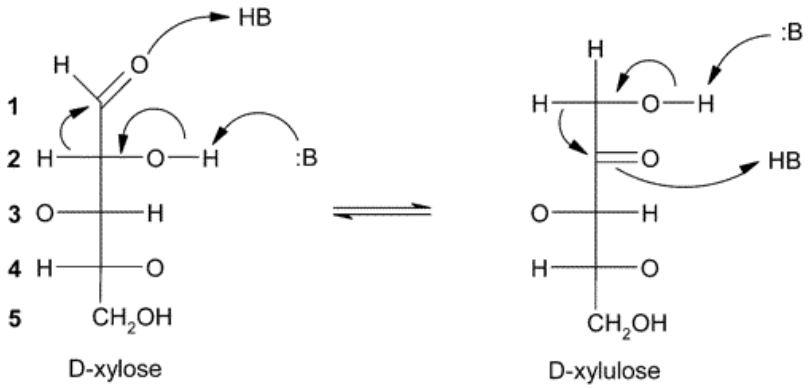
- Invertase is an enzyme used in **After Eight** chocolates to create their signature liquid fondant center. Here's how it works:
- 1. **Initial Fondant Filling** – When the chocolates are made, the filling inside starts as a **firm, sugary paste** made from sucrose (table sugar), glucose syrup, and water.
- 2. **Invertase Addition** – Invertase is added to this fondant mixture before it's sealed inside the chocolate shell.
- 3. **Enzymatic Reaction** – Over time (usually a few days to weeks), the invertase **breaks down sucrose into glucose and fructose**. This reaction, called **inversion**, turns the solid fondant into a **smooth, syrupy liquid**.
- 4. **Chocolate Shell Protection** – The outer chocolate layer remains intact, keeping the liquid inside. When you bite into an After Eight, the thin chocolate shell breaks, releasing the liquid center.
-

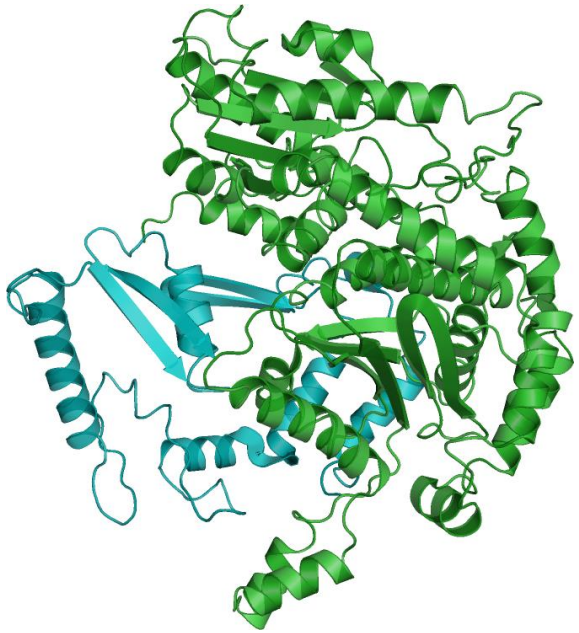
- **Why Use Invertase?**
- • It creates the **distinctive runny texture** inside After Eight.
- • The reaction happens **gradually**, allowing chocolates to be stored and shipped with a firm center before transforming.
- • Fructose and glucose are **sweeter than sucrose**, enhancing the taste.
-

- This enzymatic process is commonly used in confectionery to make **liqueur-filled chocolates** and other soft-centered sweets!

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)

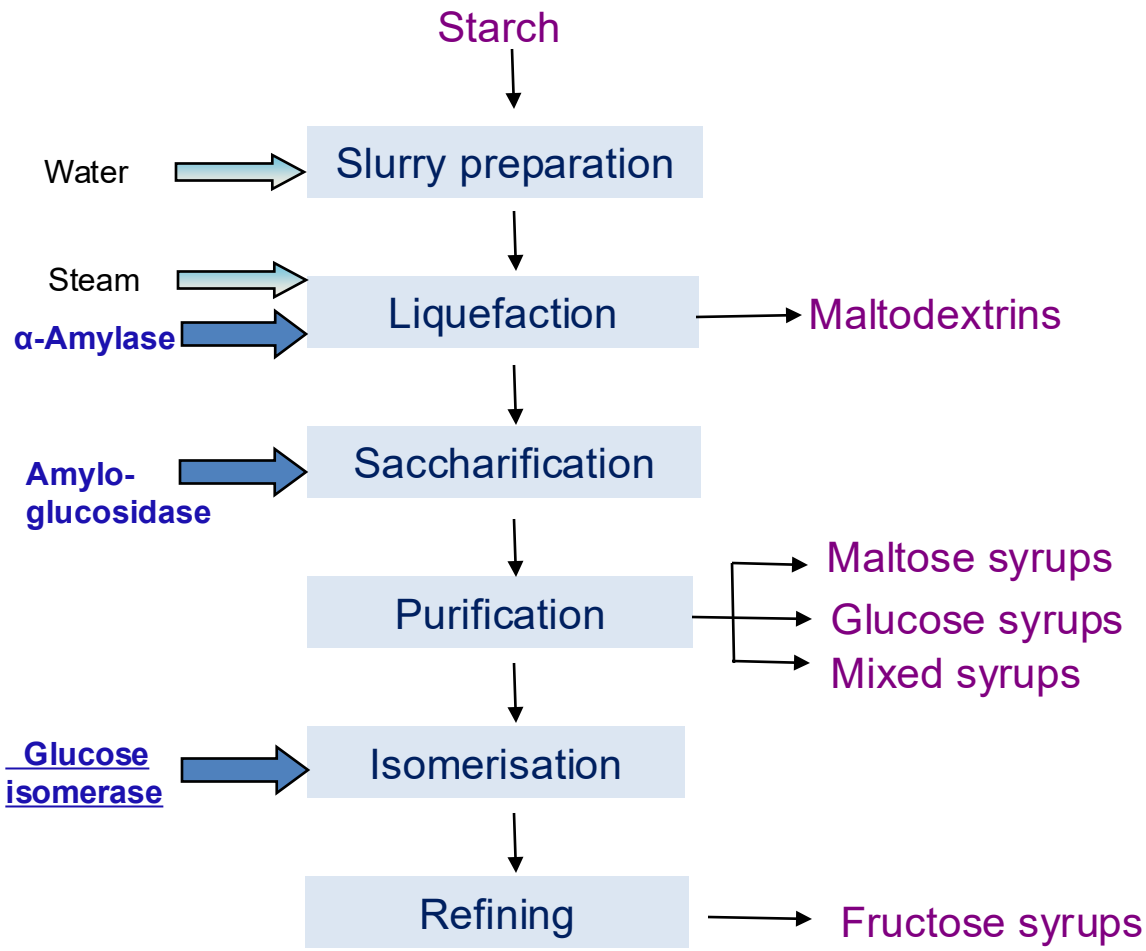


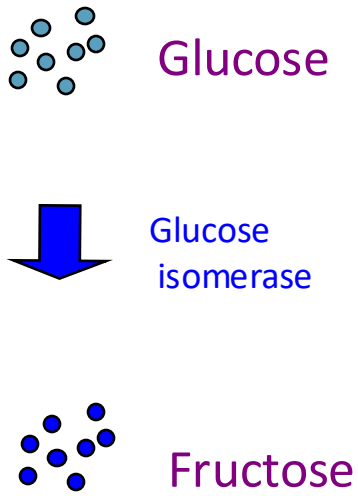


http://en.wikipedia.org/wiki/File:Glucose-6-phosphate_isomerase_wpmp.png

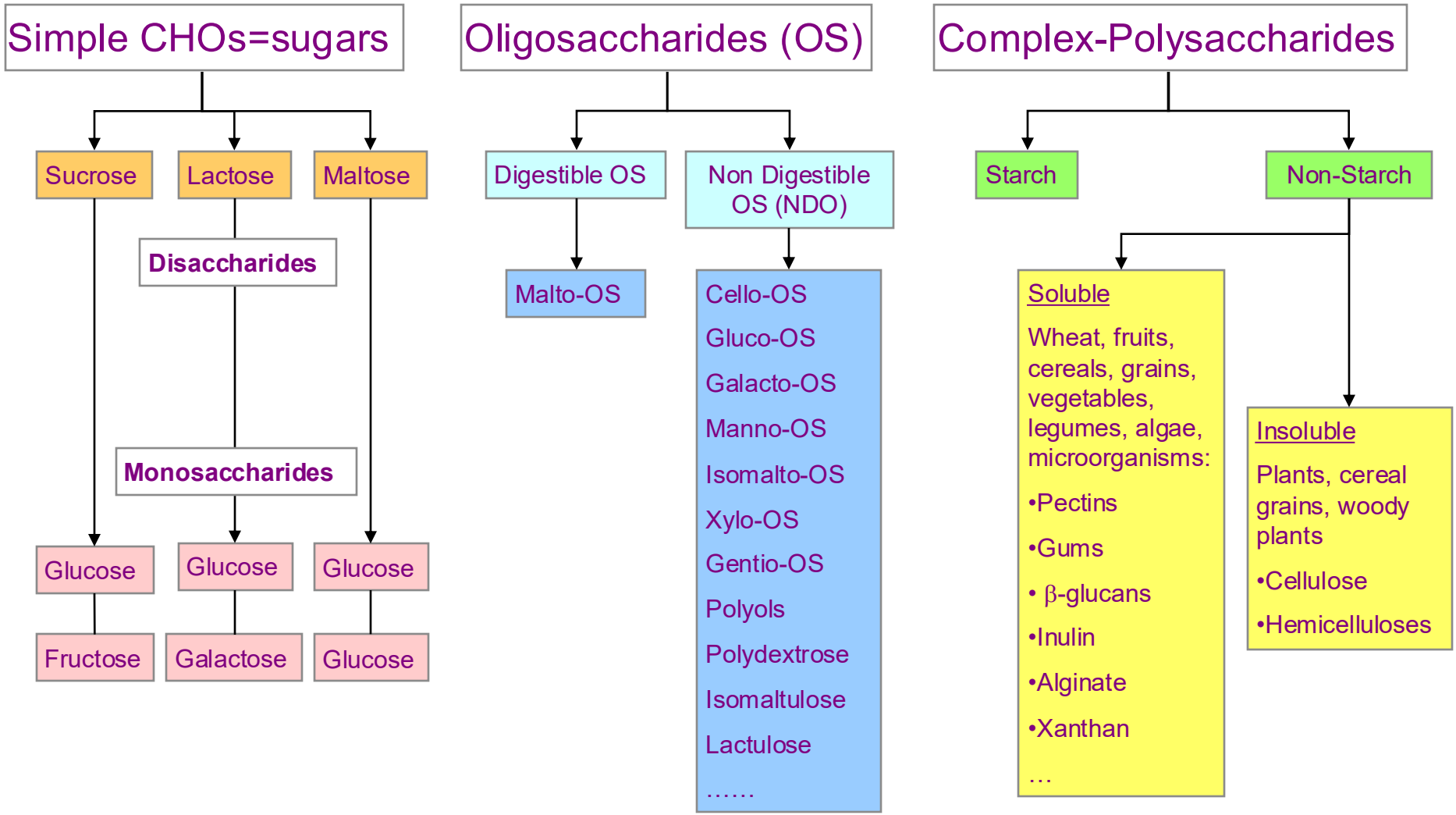
  - Interconversion of xylose to xylulose and glucose to fructose

Major steps in enzymatic degradation of starch



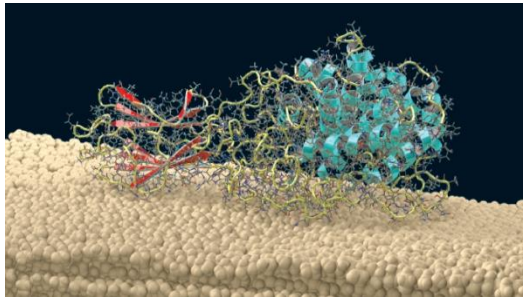


Carbohydrates in Food

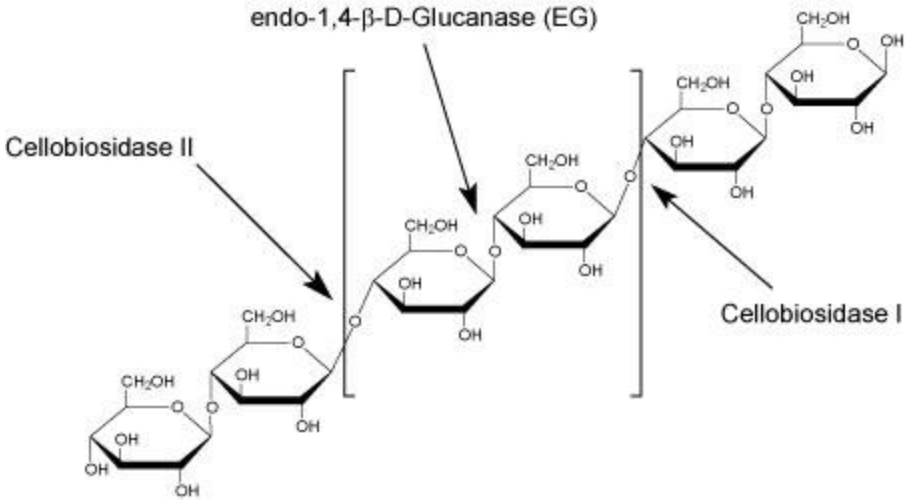


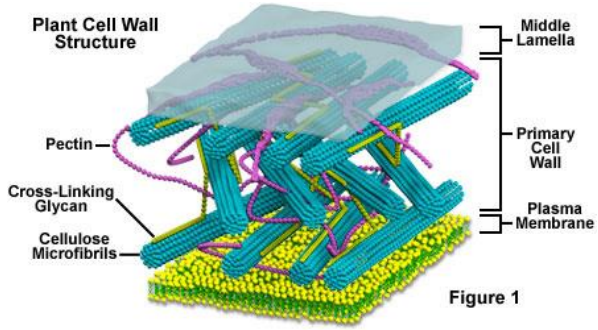
Cellulases

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



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





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

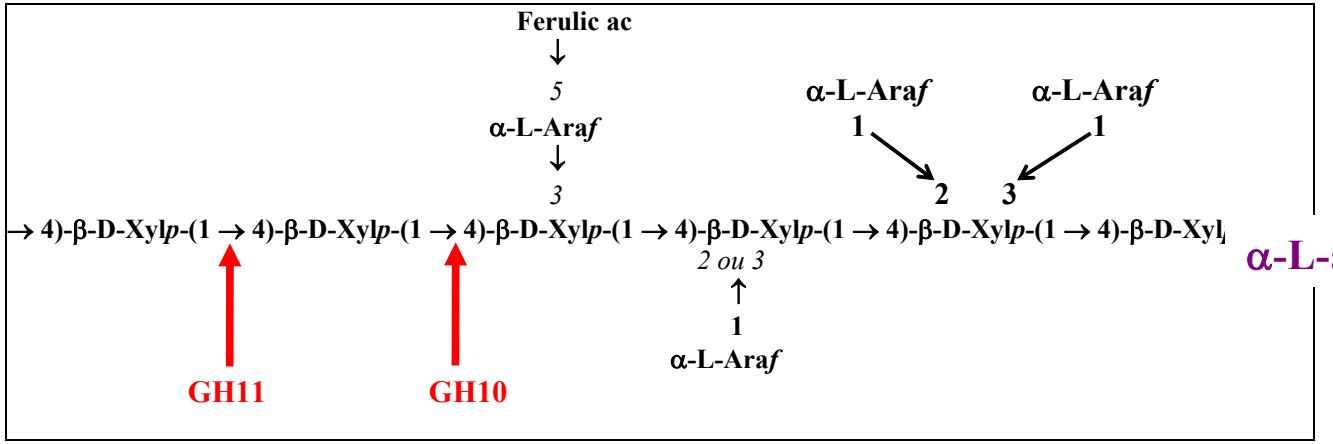
Structural unit of cellulose:
Cellulose is an isoluble linear polymer of ß-(1,4)-D-glucopyranose units

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

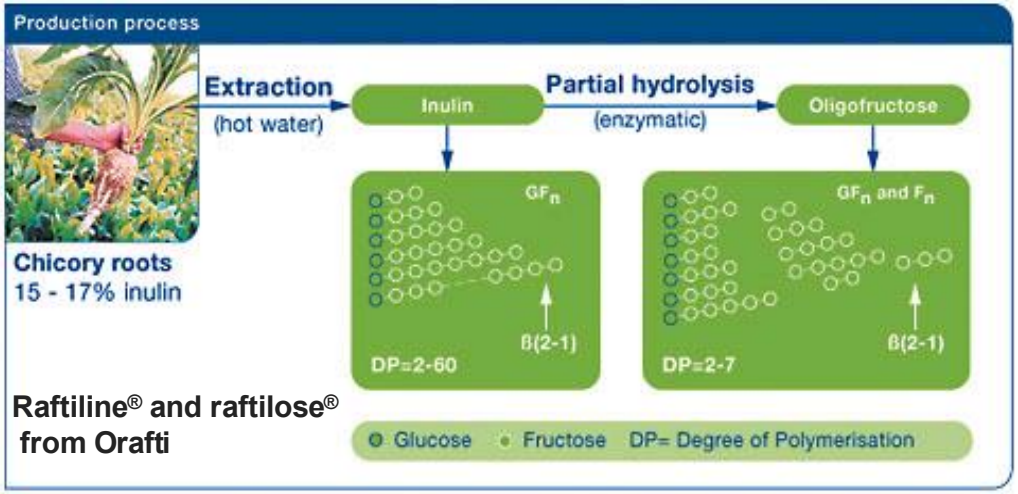
Xylanases

- Fungal xylanases are used in bread making (with α-amylases) and juice industry (with pectinases)

- Endo-1,4-β-xylanhydrolase
- Catalyze the random hydrolysis of (1,4)-β-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



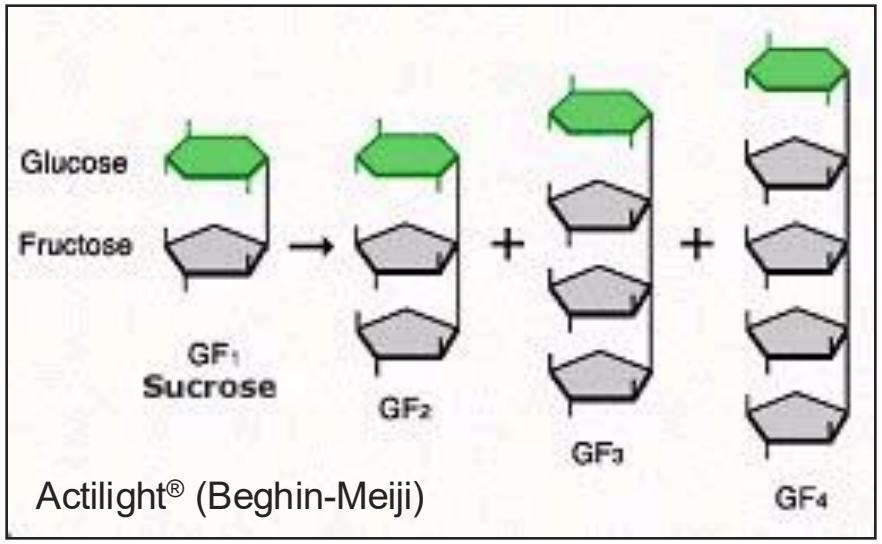
Production of Fructo-Oligosaccharides



Raftiline® and raftilose® from Orafti

Inulinase

Production of FOS from inulin



Beta-fructofuranosidase

Production of FOS from sucrose

(Reverse hydrolysis)

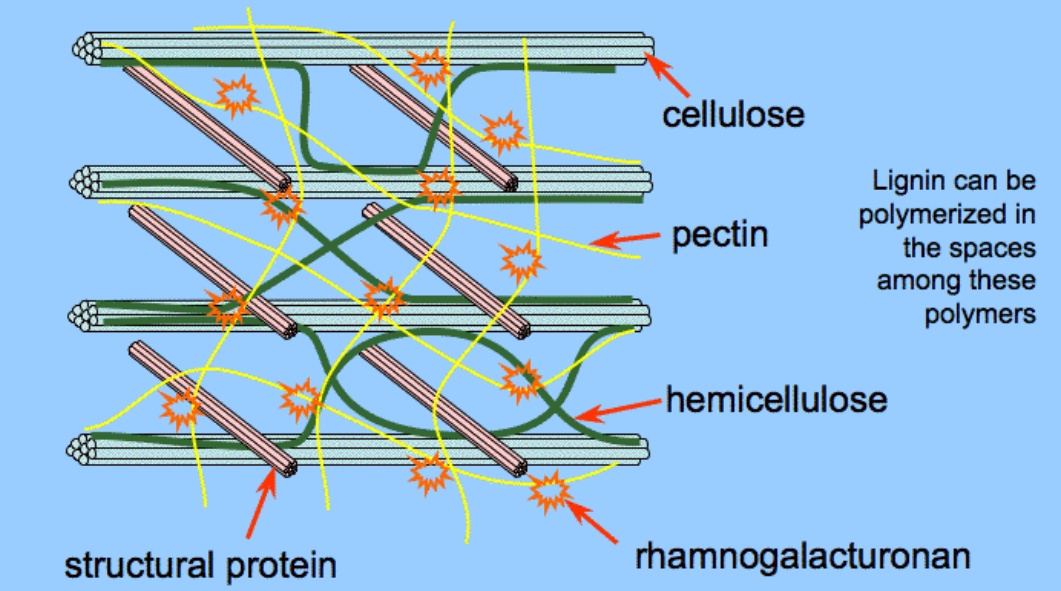
An ingredient company launches a new processing aid for dairy applications - How does this work?

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

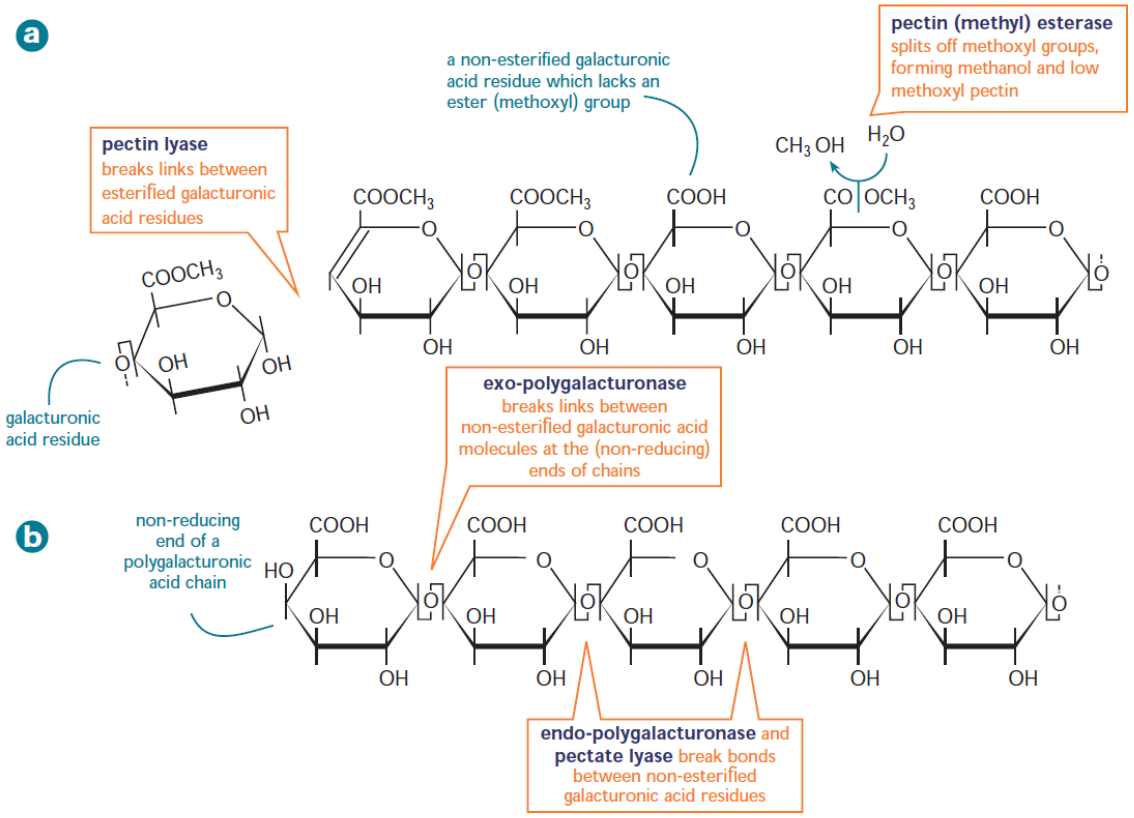
Pectic enzymes and use in fruit juice processing (1)



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

- 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, rhamno-galacturonan, 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)



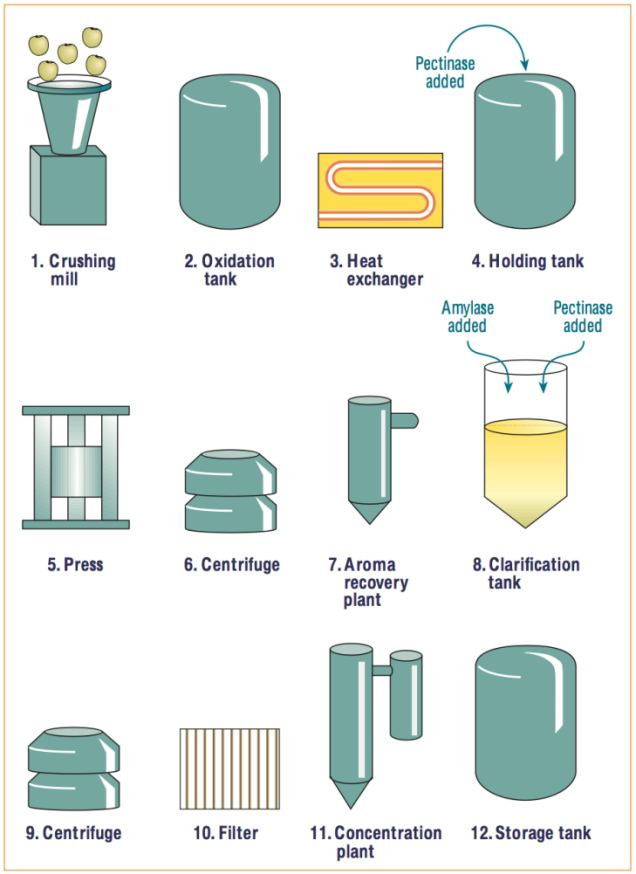
Polygalacturonase	EC 3.2.1.15	Responsible for the random hydrolysis of 1,4-a-D-galactosiduronic linkages.
Pectinesterase	EC 3.2.1.11	Releases methanol from the pectyl methyl esters, a necessary stage before the polygalacturonase can act fully.
Pectin lyase	EC 4.2.2.10	Cleaves the pectin, by an elimination reaction releasing oligosaccharides with non-reducing terminal 4-deoxymethyl-a-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.

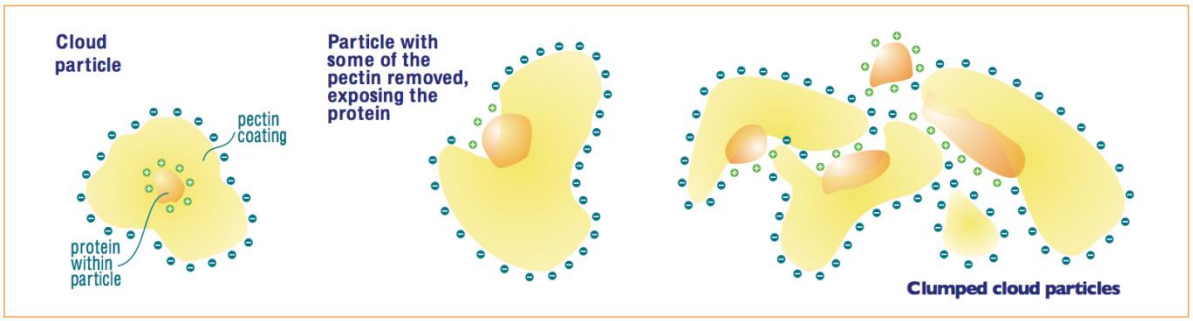
Pectic molecules are composed of chains of between 300 – 1000 galacturonic acid residues.

a) Upper Chain: high methoxyl pectin
b) Lower chain: low methoxyl pectin

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

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=2ZXumhwzolE

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

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 dextrins 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	*Bacillus licheniformis* *Aspergillus nidulans* *Trichoderma reesei*	40	1
starch hydrolysis	α-amylase	*Bacillus amyloliquefaciens*	5	3, 4
glucose iso-merization	glucose isomerase	*Streptomyces venezuelae*	7	1, 3
beer brewing	amylase	*Bacillus subtilis*	3	3, 4
fruit processing, wine	cellulases, hemicellulases, pectinases	*Aspergillus niger*	5	3, 4, 5, 6
flour, bakery goods	α-amylase, proteases	*Aspergillus oryzae*	8	1, 3
cheese manufacture, aroma	proteases, chymosin, lipases	animal rennin, *Rhizomucor miehei*, *Saccharomyces cerevisiae*	12	2
silage and animal feed	phytases	*Aspergillus niger*	8	3
paper and textiles	α-amylase, lipase	*Bacillus, Humicola*	2	4
leather treatment	proteases	*Aspergillus oryzae*	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

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

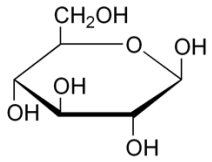
https://www.youtube.com/watch?v=PVihOW3SmRo

Sugar Uptake and Metabolism

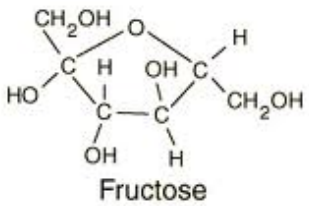
Main dietary digestible carbohydrates

Simple carbohydrates

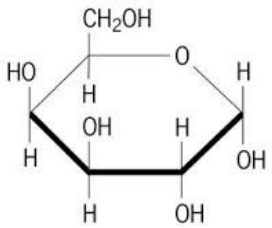
Monosaccharides



Glucose

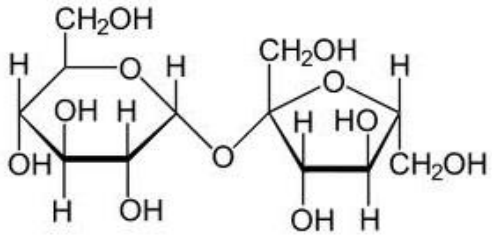


Fructose



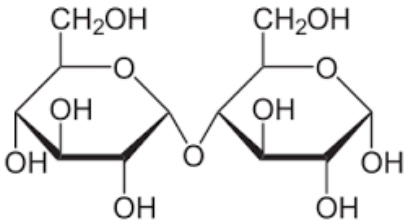
Galactose

Disaccharides



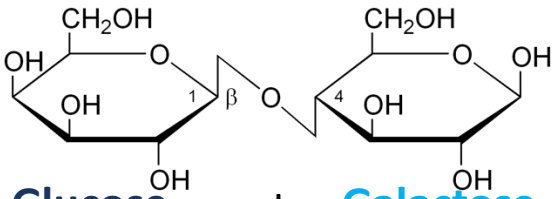
Glucose + Fructose

Sucrose



Glucose + Glucose

Maltose

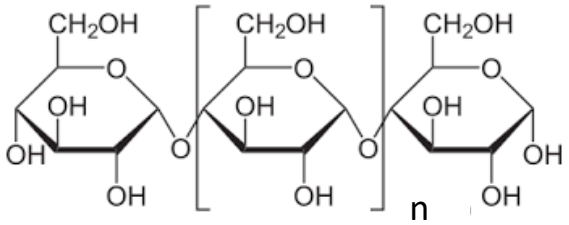


Glucose + Galactose

Lactose

Complex carbohydrates

Polysaccharides



Glucose $[Glucose]_n$ Glucose

Maltodextrins n=3-9

Starch n=300-600

Sugar sources: Raw ingredients and Products


Whole fruit (150 g)


Fruit juice (250 mL)


Honey (10g)


High-Fructose Corn Syrup


Refined sugar

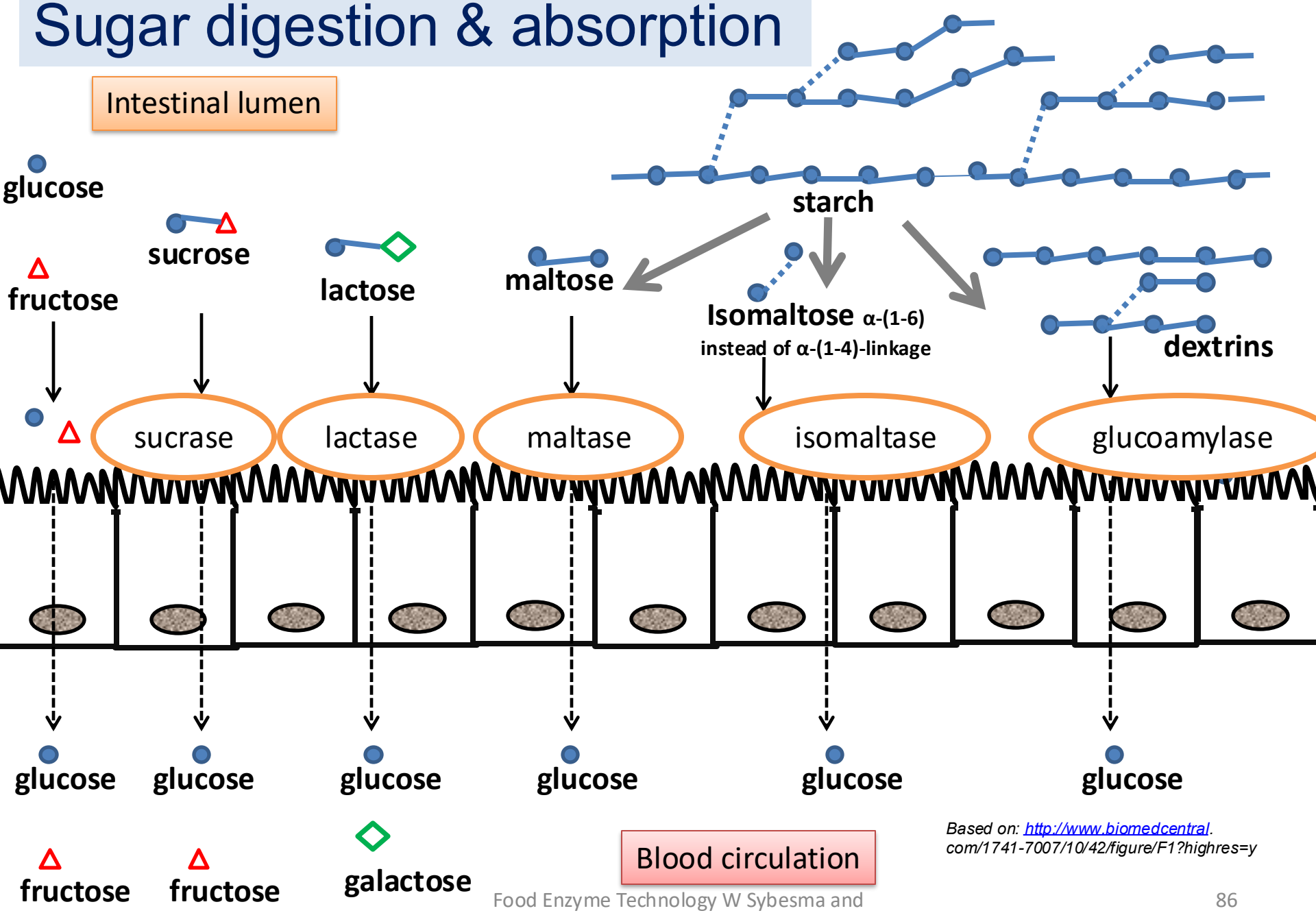
Soft drink (250 mL)


Milk (250 mL)

	Whole fruit (150 g)	Fruit juice (250 mL)	Honey (10g)	High-Fructose Corn Syrup	Refined sugar	Soft drink (250 mL)	Milk (250 mL)
Fructose △	✓	✓	✓	✓			
Glucose ●	✓	✓	✓	✓			
Sucrose ●—△	✓	✓	<1%		✓	✓	
Lactose ●—◇							✓
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

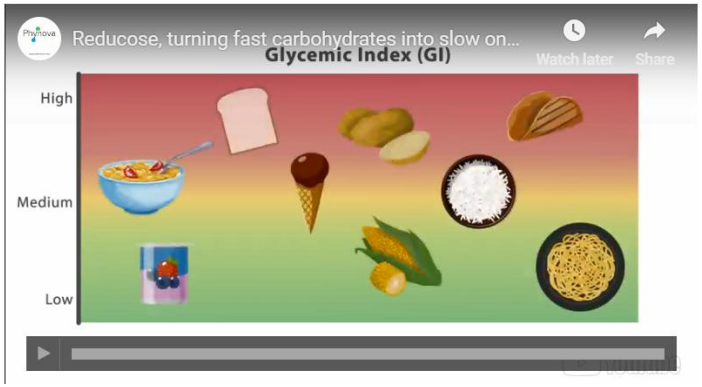


Based on: http://www.biomedcentral.com/1741-7007/10/42/figure/F1?highres=y

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).

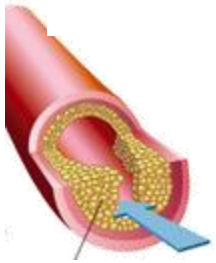
https://www.youtube.com/watch?time_continue=2&v=c6HfK01PWDU&feature=emb_logo

Good to watch this movie once



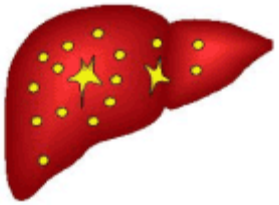
Carbohydrates bigger than monosaccharides are too large to be directly absorbed by the body and during digestion are broken down into monosaccharides such as glucose or fructose by enzymes. This breakdown starts in the mouth with salivary amylase. After swallowing the salivary amylase is inactivated 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)



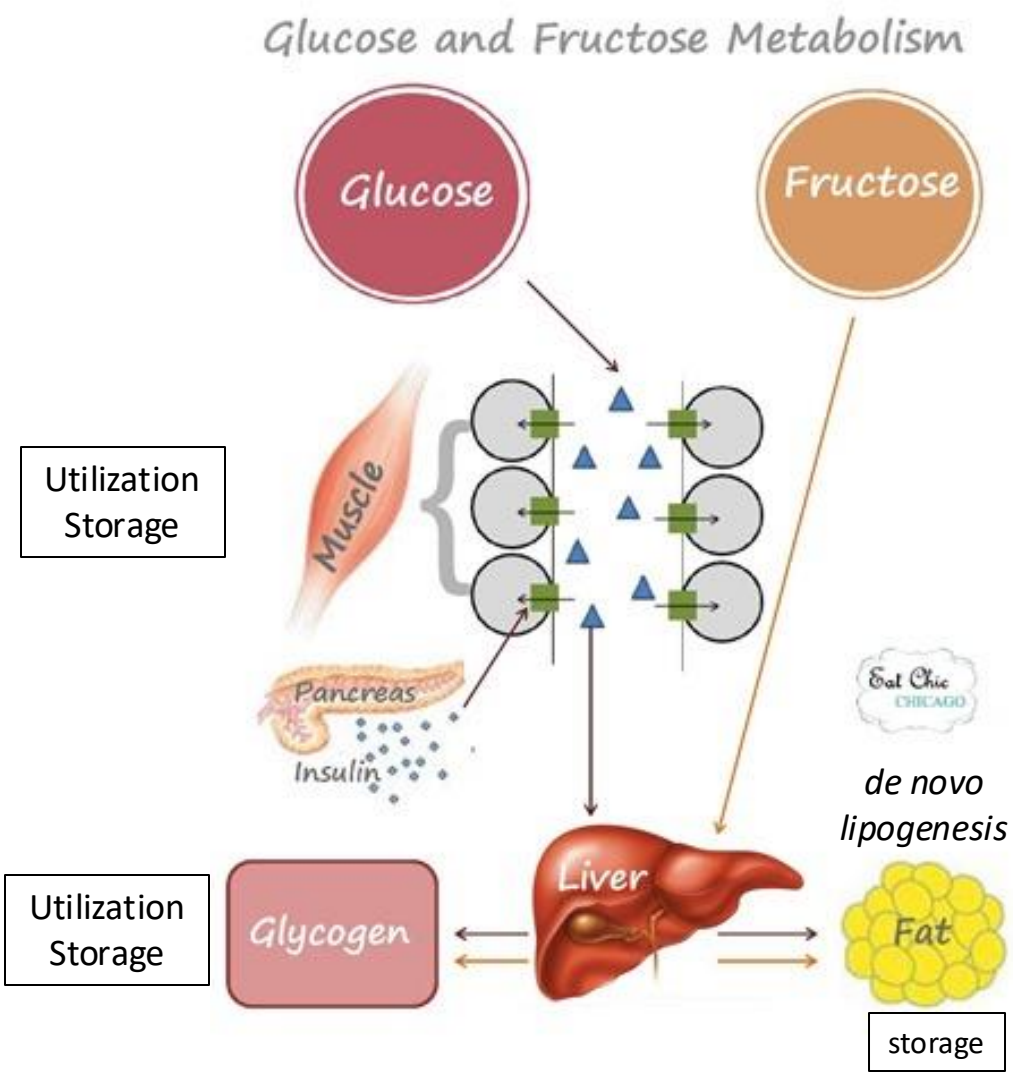
Liver fat content:

- High sugar intake (≥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/GlucoseandFructoseMetabolismInforgraphic.jpg

- 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

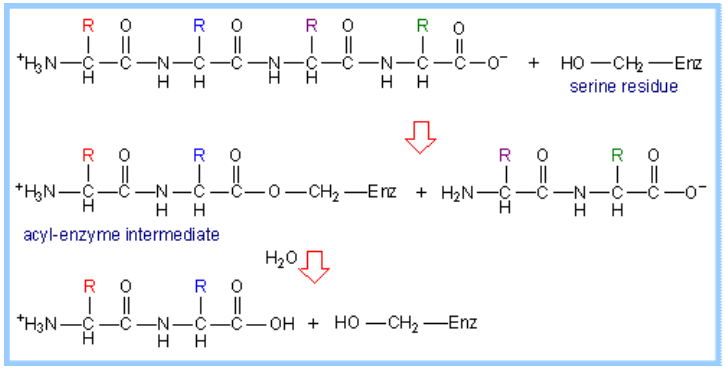
Back up slides enzymes

Enzyme databases

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

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.



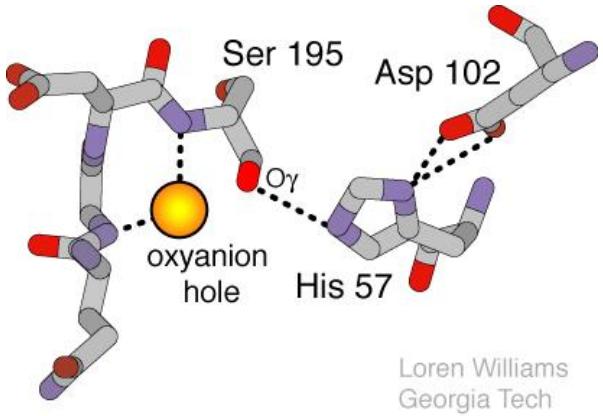
http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/protease.htm

Example of Serine Proteases mechanism

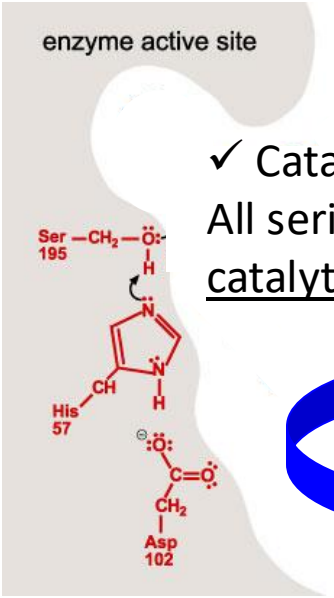
✓ Substrate specificity

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://ww2.chemistry.gatech.edu/~lw26/structure/protein/serine_protease/triad_1.html



✓ Catalytic site

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

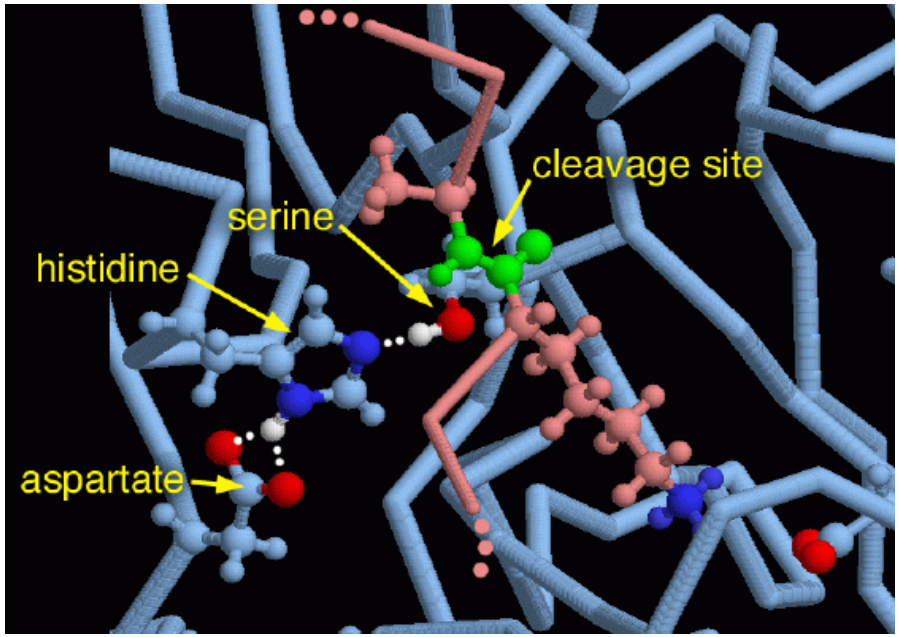
- Ser 195 [side-chain CH_2-OH]: combined action of the triad makes it a better **nucleophile.**
- His 57: base [accept a proton]
- Asp 102: acid [donate a proton]

Cooperative action among the catalytical triad amino acids

Structure of serine protease

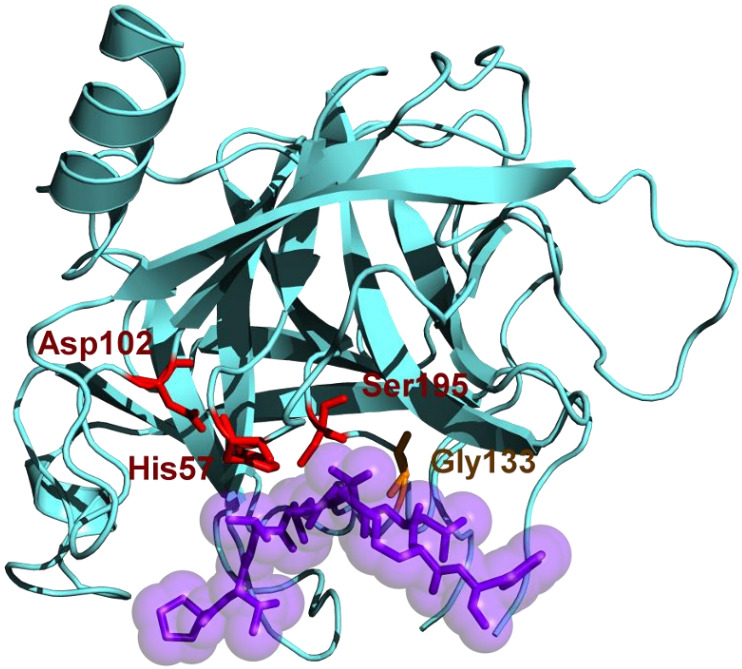
Structure of the active sites of Serine Proteases

Trypsin



http://www2.rcsb.org/pdb/education_discussion/molecule_of_the_month/images/2ptc-rasmol.gif

Chymotrypsin

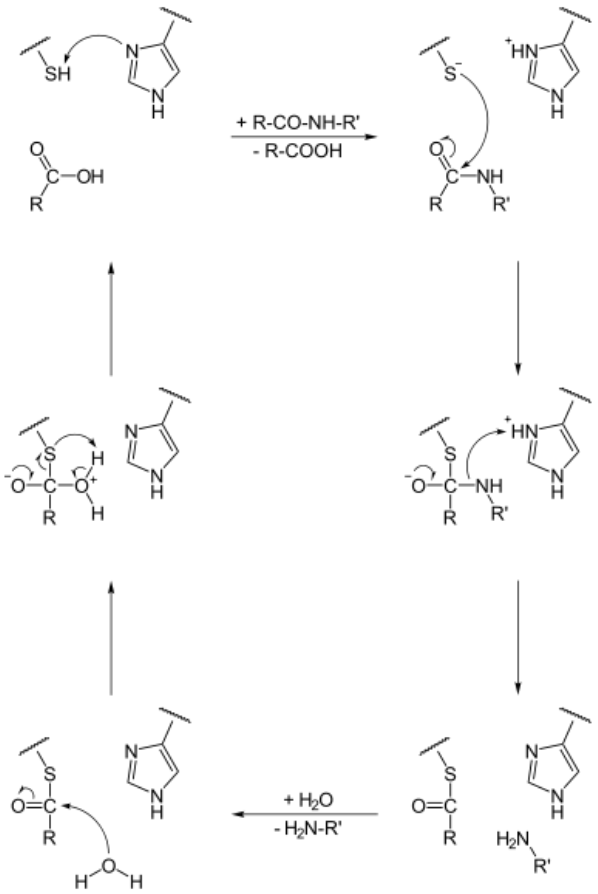


http://upload.wikimedia.org/wikipedia/commons/2/2e/Chymotrypsin_4cha.png

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

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

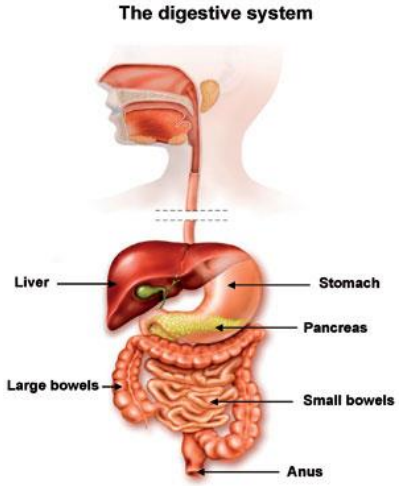
- All living cells maintain a particular rate of protein turnover by continuous control and regulation of protein composition, size, shape, turnover and ultimate destruction: all is done by proteases.
- Catabolism of proteins by proteases provides a pool of amino acids used as precursors of the protein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



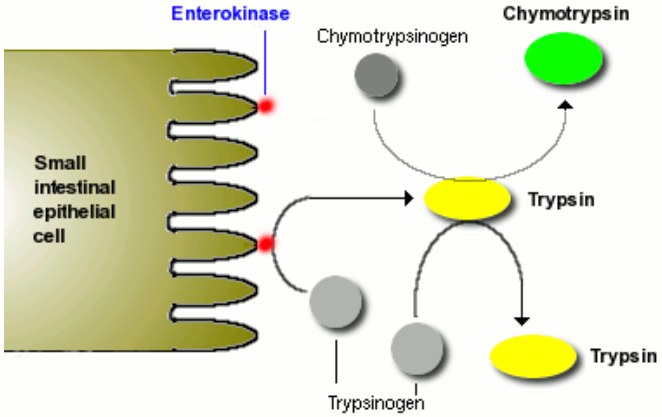
- Proteases assist the hydrolysis of proteins & large polypeptides into smaller peptides and amino acids, thus facilitating their absorption by the intestinal cells

Pepsin

Trypsin
Chymotrypsin
AminoPeptidases
CarboxyPeptidases

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

Physiological Regulations (cascade processes)

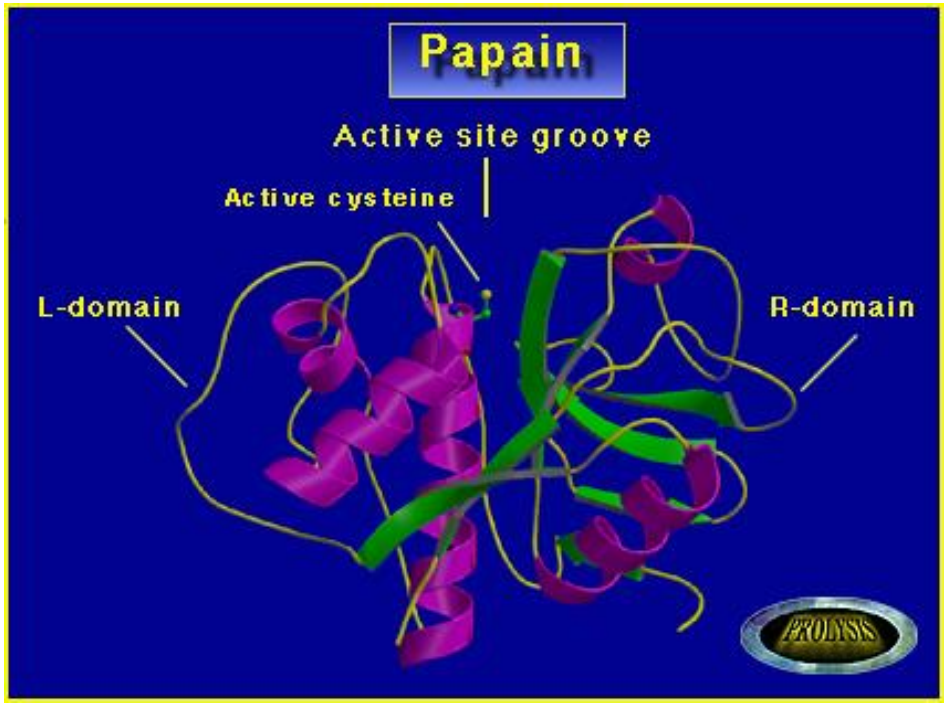


- Activation of precursors forms of enzymes by specific proteases represents an important step in the physiological regulation of many rate-controlling processes:
  - Digestion: activation of Trypsinogen and Chymotrypsinogen
  - Generation of proteins hormones / bioactive peptides
  - Regulation of blood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

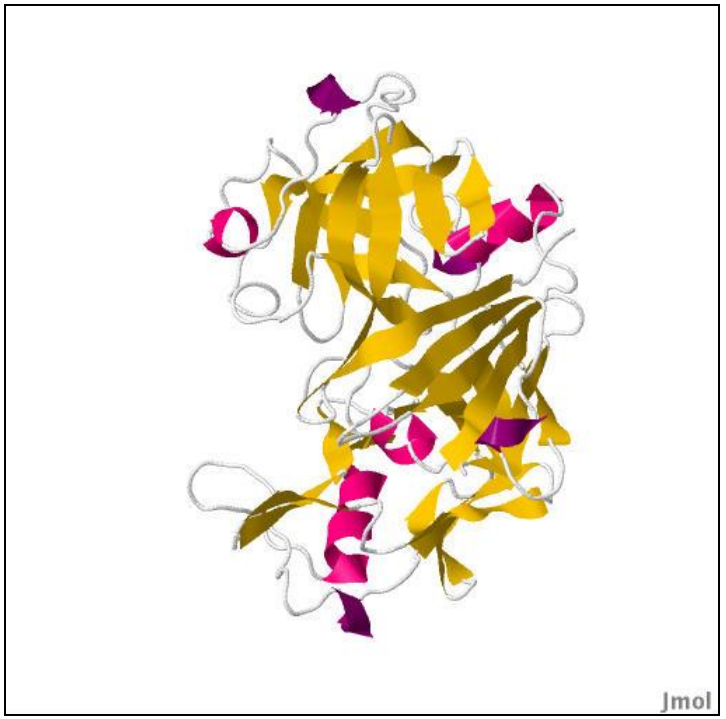
http://www.vivo.colostate.edu/hbooks/pathphys/digestion/pancreas/exocrine.html

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://en.wikipedia.org/wiki/File:CHYMOSIN_COMPLEX_WITH_THE_INHIBITOR_CP-113972.jpg

Sources of Proteases

No need to remember details

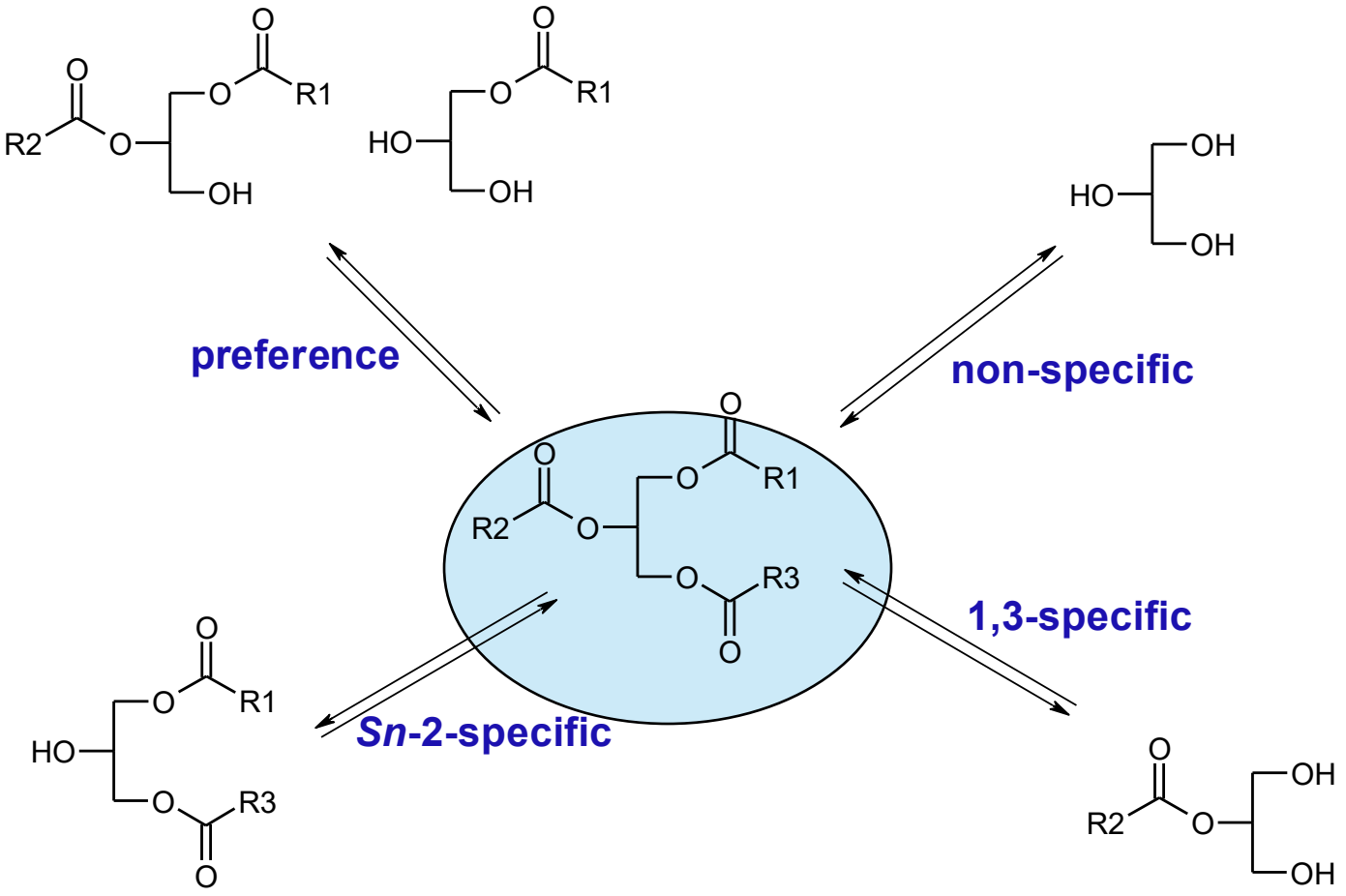
- ✓ Since proteases are physiologically necessary for living organisms, they are found in a wide diversity of sources such as plants, animals and microorganisms.

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

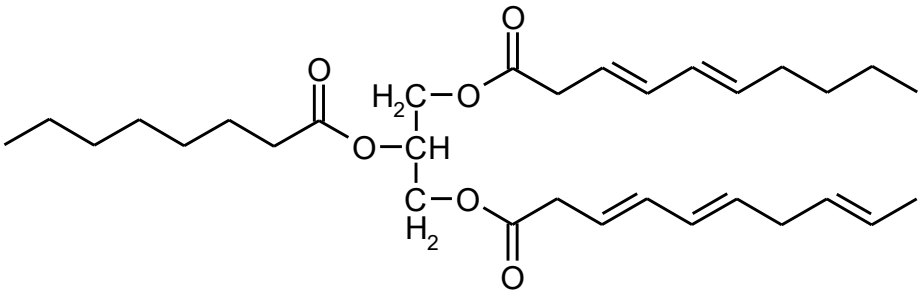
Reactions Catalyzed by Lipases: Hydrolysis

- 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.

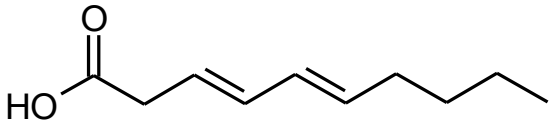


Application of Lipases: Generation of Flavours



Saturated or unsaturated fatty acids
Short chain fatty acids

Lipases



lipoxygenases

Esters, Alcohols, Acids, Aldehydes

- 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_2 = 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

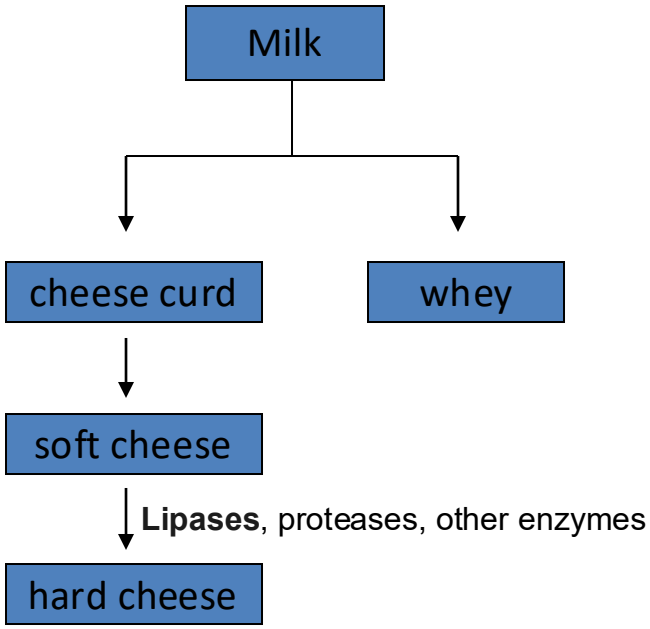
- Lipase increases gluten strength
- Gluten from wheat flour dough treated with lipase is significantly stronger, with an increased G', and more elasticity
- Gluten treated with lipase has an increased relaxation time
- *In-situ* generation of more hydrophilic emulsifiers (mono-acylglycerides)

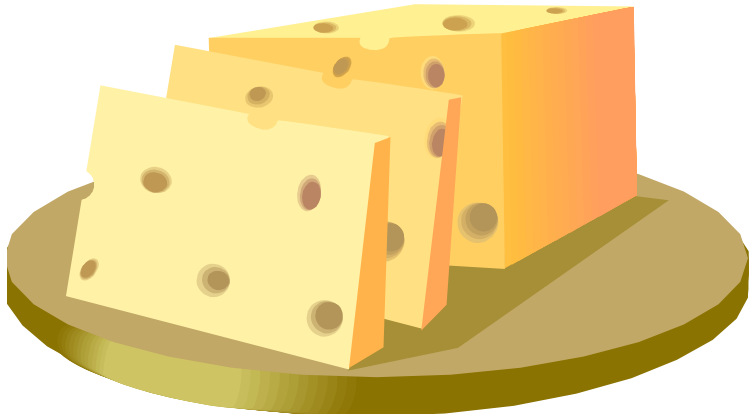
Improved processing, volume and quality of bread

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

Application of Lipases in Cheese

Cheese Processing



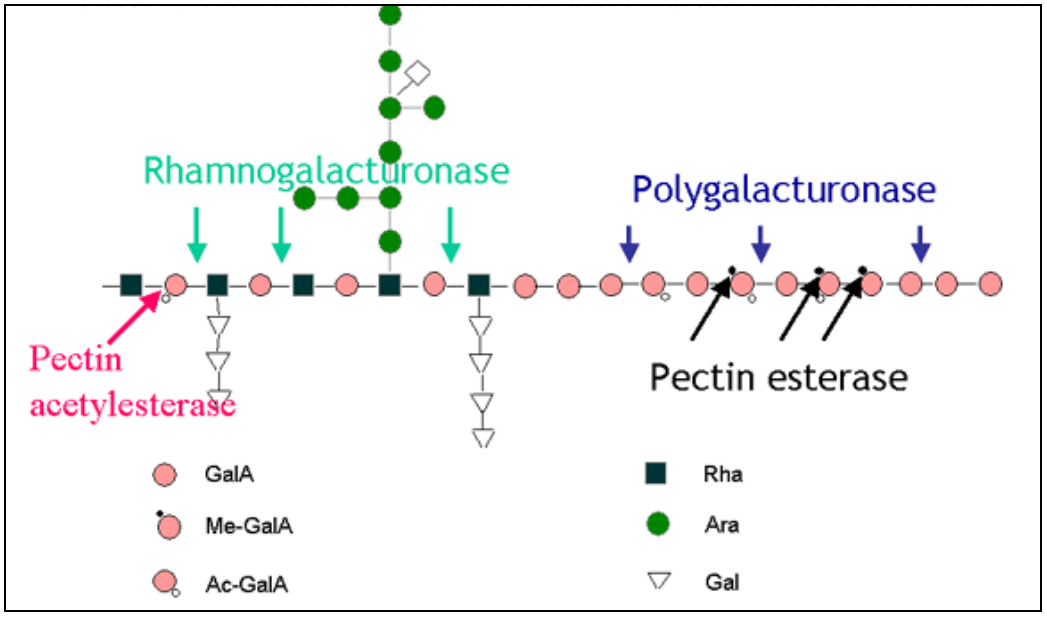


Cheese Aroma

The short and medium-chain fatty acids occuring 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

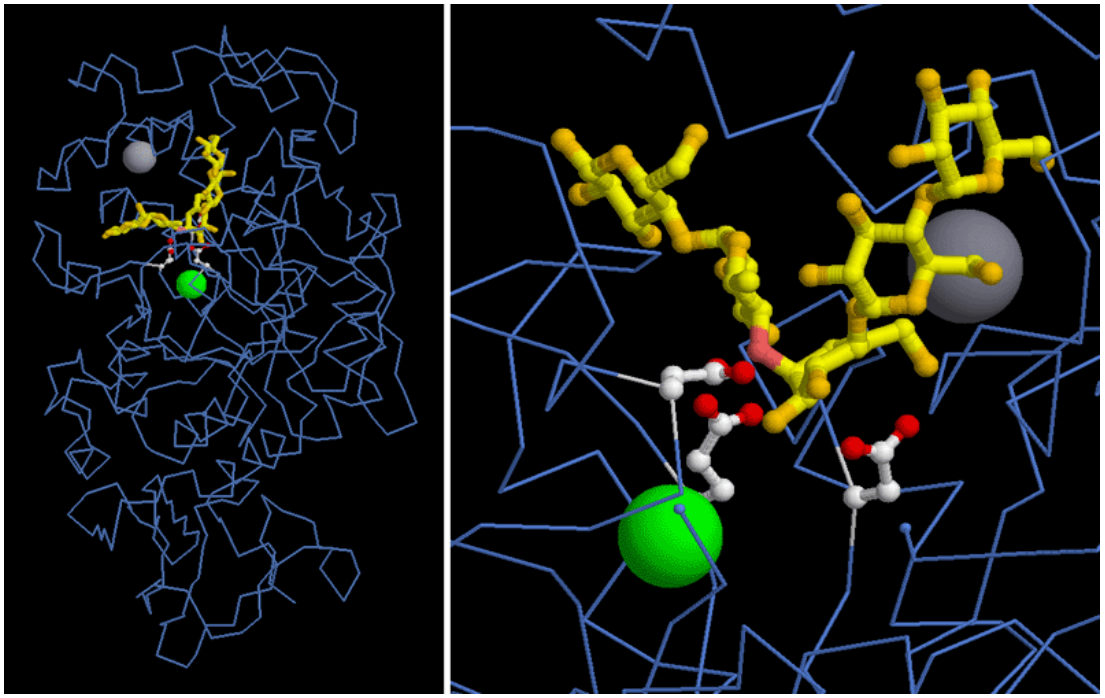
A wide diversity of substrates and enzymes

- 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

α-Amylase – Active site



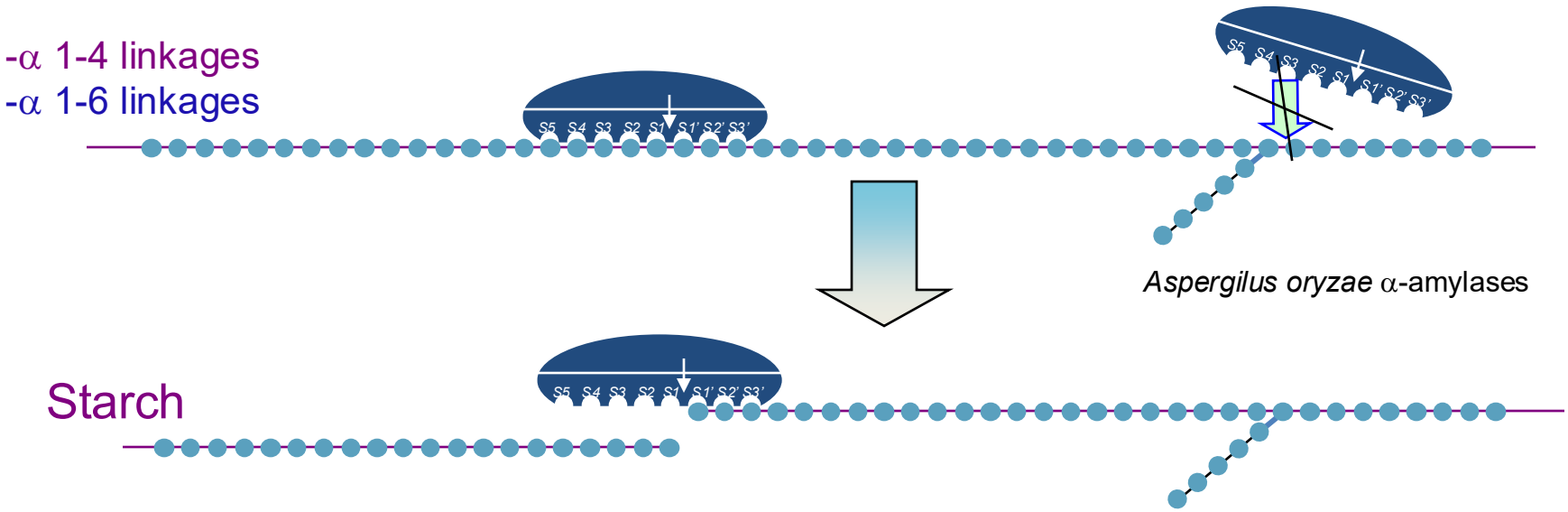
No need to understand mechanisms

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.

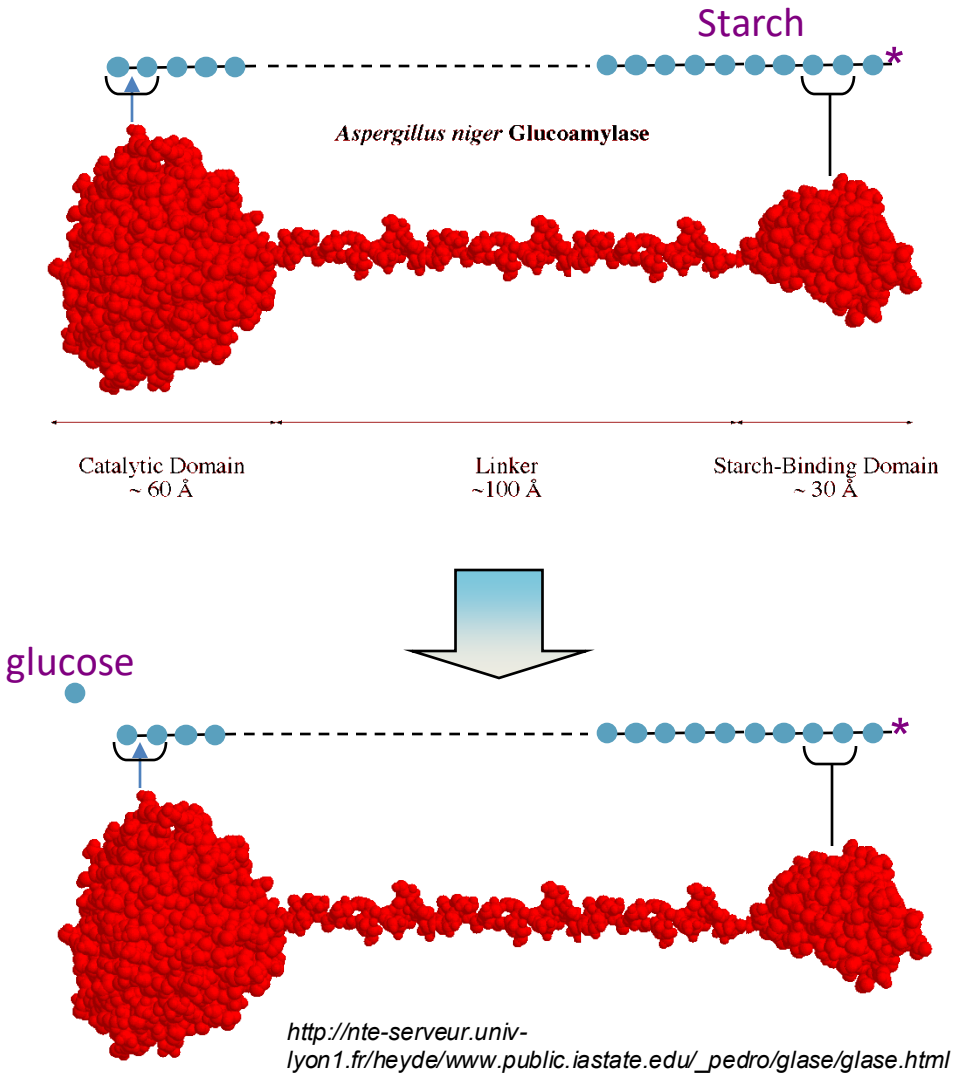
α-Amylase is an Endo-hydrolase

- Catalytic domain of hydrolases is specific:



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

Glucoamylase is an Exo-hydrolases



http://nte-serveur.univ-lyon1.fr/heyde/www.public.iastate.edu/_pedro/glase/glase.html

- The glucoamylase or amyloglucosidase (AMG) cleaves α-(1,4)-glucose from the non-reducing terminus of starch and maltose to yield β-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 α-(1,4)- and α-(1,6) glycosidic linkages may be assumed to hydrolyze starch theoretically 100% into glucose