
Enzymes in food technology

Starch industry

Acid hydrolysis (Kirchhoff, beginning of 19th century)

Disadvantages

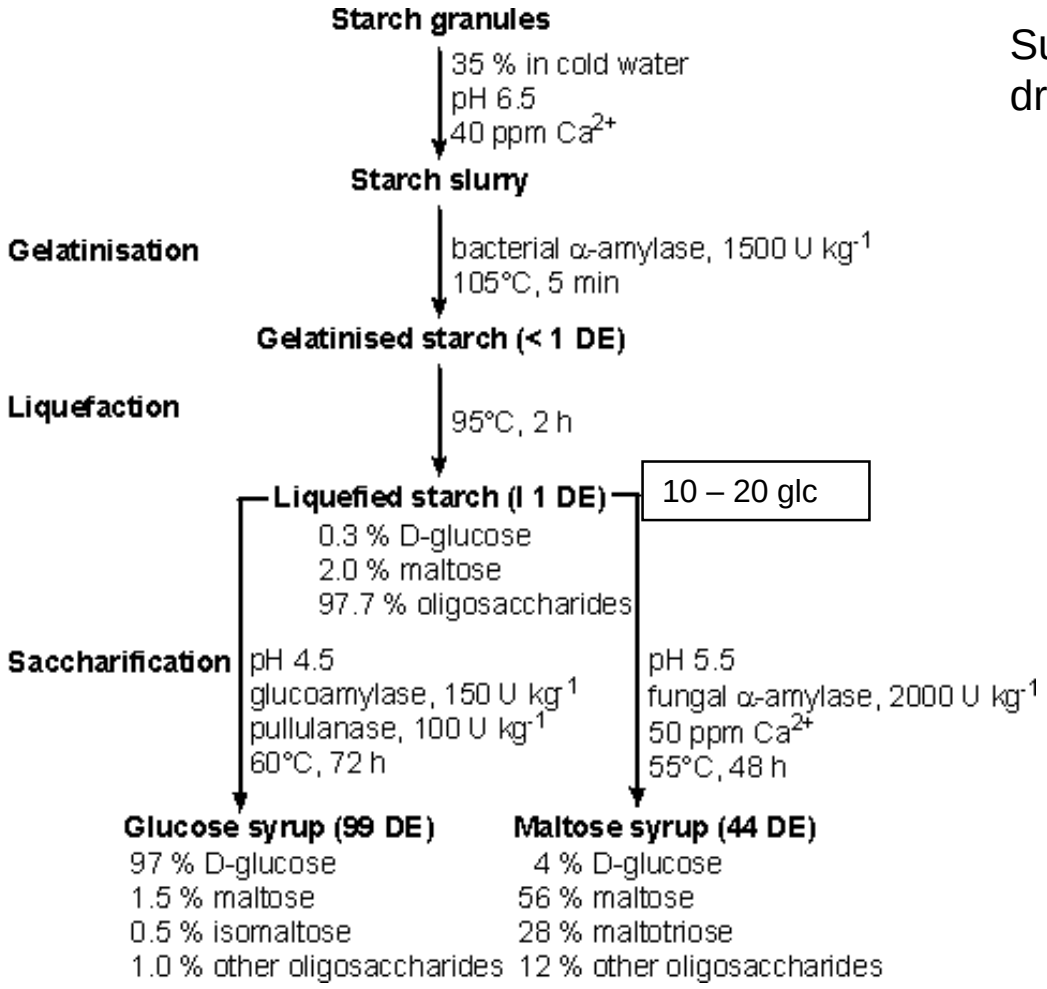
- side products
- possible to adjust only degree of hydrolysis, not the product composition
- Expensive technological device (conc. sulphuric acid, temperature ~ 150 °C)

Enzyme hydrolysis – avoids most of the drawbacs

!

Enzymes are used also in starch separation – enzymes degrading plant cell walls (pectinases, xylanases, celulases + proteases, lipases)

Starch degradation



Substrate: 20 -40% of dry matter (starch)

DE - dextrose equivalent = (amount of red.saccharides put as glucose/overall amount of polysaschariide)x100
DE of starch = 0, DE of glucose = 100

Specificity of applied enzymes determines the composition of products

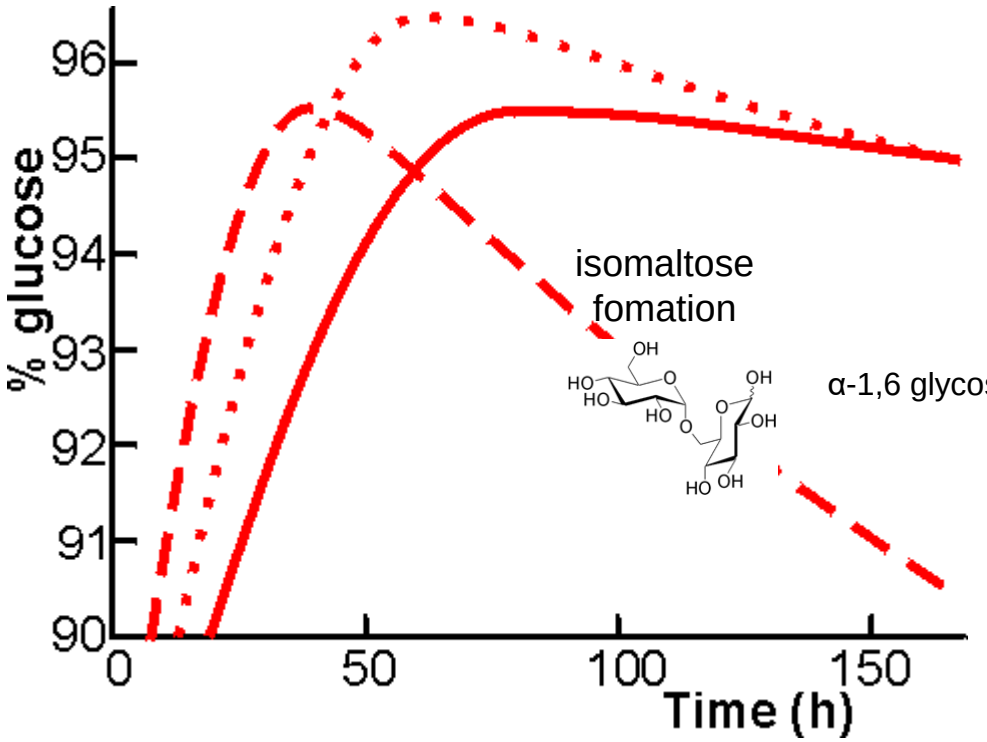
%	*Bacterial α-amylase*	*Fungal α-amylase*	*Malt β-amylase*	*amyloglucosidase*
Glucose	4	3	1	**83**
Maltose	10	**50**	**60**	7
Maltotriose	18	26	8	3
dextrins	**68**	21	31	7

Production of glucose sirup:

-Liquified starch DE 8 – 12, conc. 30% ⟶ DE 96 – 98

-pH 4 - 4,5, 60 °C

DE 100 only in diluted solutions



200 U kg-1 *A. niger* **glucoamylase** plus 200 U kg-1 *B.acidopullulyticus* **pullulanase**

200 U kg^{-1} *A. niger* glucoamylase

α-1,6 glycosidic bond

400 U kg^{-1} *A. niger* glucoamylase

The % glucose formed from 30% (w/w) 12 DE maltodextrin, at 60°C and pH 4.3, using various enzyme solutions. The relative improvement on the addition of pullulanase is even greater at higher substrate concentrations.

Production of HFCS

Glucose isomerase, (D-xylose ketolisomerase, EC 5.3.1.5)

Tetramer, Mg, Mn, Co

glucose sirup $\xrightarrow{\text{GI, } Mg^{2+}}$ 42% fru $\xrightarrow{\text{ionex}}$ 55% fru

Sweetzyme , Streptomyces murinus, immobilized cells

Operational stability of immob. GI – half-life 200 days

One column filling used within 3x half-life (end point- 12,5% of the starting activity)

HFCS - Substitute saccharose

- less expensive
- higher solubility
- lower crystalization
- consumption10 million tons/year

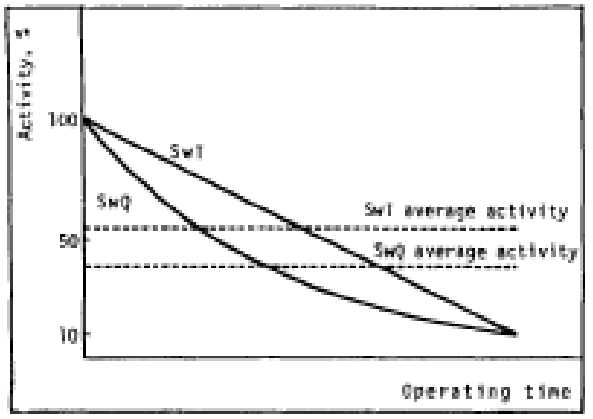


Figure 5. Activity decay modes for Sweetzyme Q* and T*.

TABLE 4. Immobilized GIs of commercial importance

Source organism(s)	Trade name	Manufacturer	Immobilization method
Cell-free enzyme			
S. olivochromogenes	G-zyme G-994	CPC (Enzyme Biosystems)	Adsorption on an anion-exchange resin
S. rubiginosus	Spezyme	Genencor International	DEAE-cellulose agglomerated with polystyrene and TiO_2
S. rubiginosus	Optisweet II	Solvay	Adsorption of specific SiO_2 particles followed by cross-linking with glutaraldehyde
S. olivochromogenes	Ketomax 100	UOP	Polyethyleneimine-treated alumina with glutaraldehyde cross-linked GI
Whole cells			
Actinoplanes missouriensis	Maxazyme	IBIS	Cells occluded in gelatin followed by glutaraldehyde
Flavobacterium arborescens	Takasweet	Solvay	Polyamine glutaraldehyde cross-linked cells extruded and granulated
S. griseofuseus	AGIS-600	Godo-Shusei	Chitosan-treated glutaraldehyde cross-linked cells
S. phaeochromogenes	Sweetase	Nagase	Heat-treated cells bound to anion-exchange resin
S. murinus and *Bacillus coagulans*	Sweetzyme T	Novo-Nordisk	Glutaraldehyde cross-linked cells extruded

Usage of other starch hydrolysis products

Food industry, textile and paper industry

SHP (DE 5 - 8) - termoreversible gels, replacement of fats and starch, stabilizers

Maltodextrins (DE 3 - 20) - stabilizers, thickeners, fillers

nutrition for convalescents

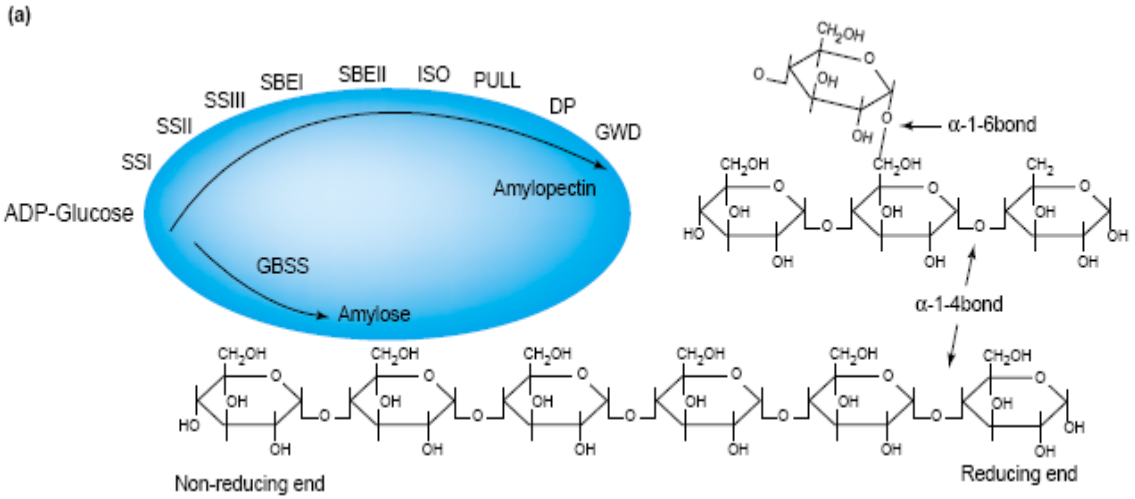
Maltose and glucose sirups

Cyclodextrins – shown previously

Production of modified starches

Amylose 20 - 30%

Starch synthesis



Amylose: GBSS – granule bound starch synthase

↓ X

Waxy

Better gelatinizes, constant viscosity:

- filler stabilizer

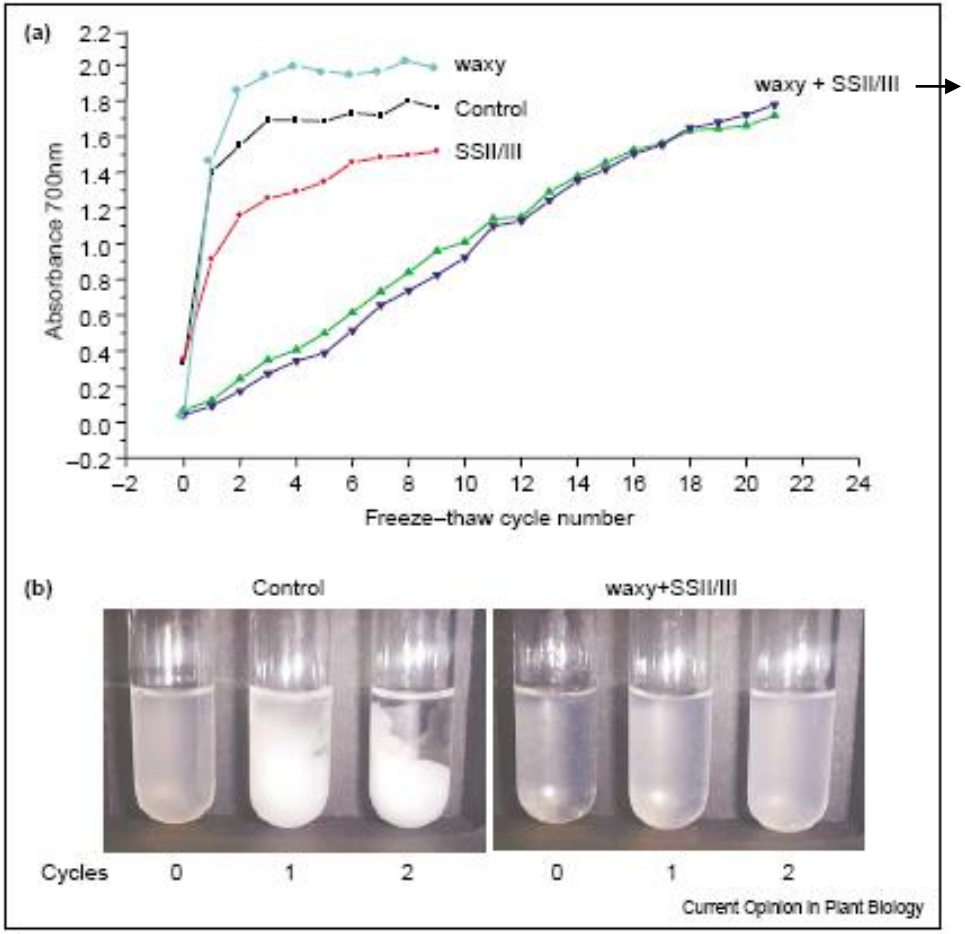
Amylopectin:
SS – starch synthase
SBE – starch branching enzyme
GWK – glucan water dikinase – fosforylation of glucose units on C3 a C6 → high swelling of starch and viscosity

} Targets of genetic modifications

Chemical modification of starch

- Crosslinking of polyglucan chains - ↓ swelling
- introduction of charged groups – gel stabilization

Gene ingeneering:



Amylopectin with short side chains

Multiple freezing and thawing of products – sedimentation of starch

antisense SBEI a II - 50 -90% amylose – preparation of surface films on fried food products

Resistant starch – high content of amylose – suitable for diabetics

GWD mutants – control of viscosity – i.e. starch for paper industry

Protein hydrolysis

Goal:

- Changes of physico-chemical properties
  - Solubility → extractability
  - emulgation properties
  - foaming
  - capability to bind water
- Changes in nutritional and sensoric properties
- Changes in texture of raw materials
- Decrease of allergenicity

Protein hydrolysates are broadly used in numerous food technologies:

Proteins	**Changes in properties**	**Application/advantages**
Plant		
soybean	solubility	Increase of digestibility, nutritional value, replacement of egg white, beverages
wheat	sensor hydration/rheology solubility	Food additives Baking industry Enhancement of digestibility
maize	solubility	Feed industry
Animal		
Meat (fish and others)	Texture Solubility, senzory	Tenderization of meat Food additives Cleaning of bones
milk	coagulation solubility	Cheese production Increase of nutritional value
blood	Solubility, senzory	Food additives
Leather	texture	Lether industry
Microbial		
yeasts	Solubilty, senzory	Food additives, feed industry. Fermentation media
bacteria	solubility	Feed industry

Risk of protein hydrolysis – formation of bitter peptides

Proteins are not bitter !!

Hydrophobic AA have bitter taste: Leu, Pro, Phe, Tyr, Ile, Trp

- leu, phe (15 – 20 mM) leu-phe, leu-leu - 10 times lower conc.

bitter taste of peptides:

↑ with the hydrophobicity of C terminus,

↓ pro

Quantitation of protein and peptide hydrophobicity:

Q = free energy of the transport of AA side chain from ethanol to water

Limit for bitter taste: Q > 1400 cal/mol
Size of peptides < 6 kDa

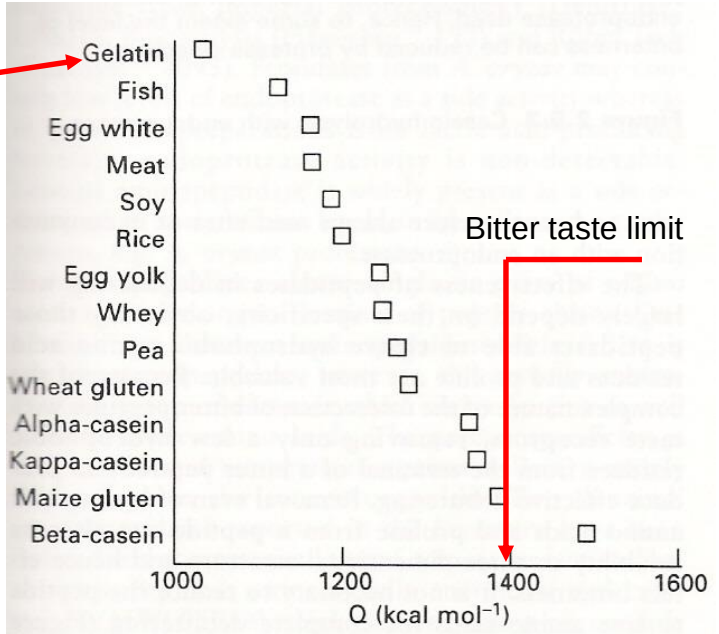
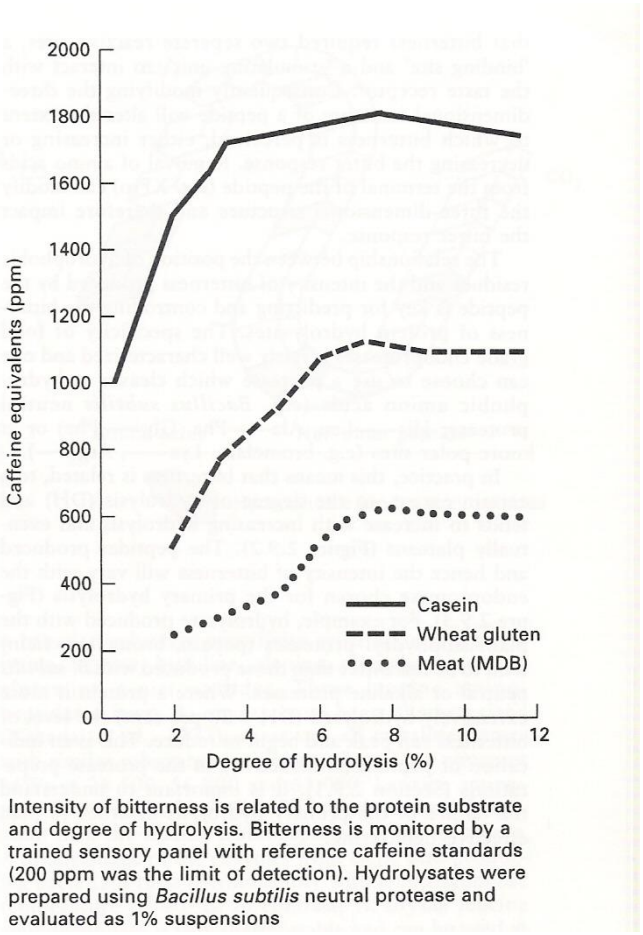


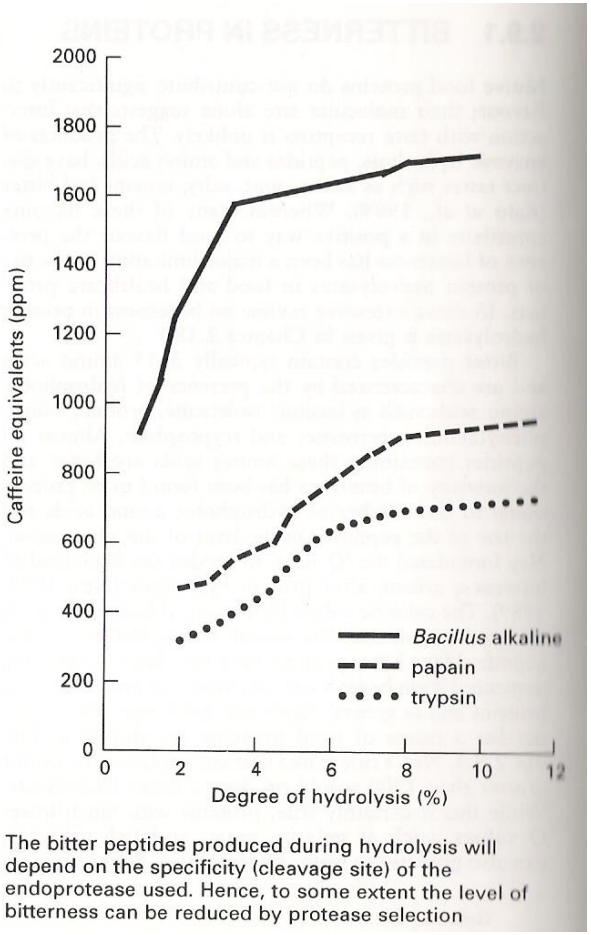
Figure 2.9.1 Hydrophobicity of food proteins. The more hydrophobic food proteins (i.e. higher Q value) will have a greater tendency to produce bitter flavours on hydrolysis

Formation of bitter peptides in hydrolysates depends on the protein and proteases used

Evaluated as caffein equivalent – limit of detection = 200 ppm



Intensity of bitterness is related to the protein substrate and degree of hydrolysis. Bitterness is monitored by a trained sensory panel with reference caffeine standards (200 ppm was the limit of detection). Hydrolysates were prepared using *Bacillus subtilis* neutral protease and evaluated as 1% suspensions



The bitter peptides produced during hydrolysis will depend on the specificity (cleavage site) of the endoprotease used. Hence, to some extent the level of bitterness can be reduced by protease selection

Caffein standard – 200 ppm is a standard limit of bitter taste

Removal of bitter peptides

Classical:

- Adsorption on charcoal (activated carbon?)
- Chromatography, extraction by alcohols
- masking of bitter taste (polyphosphates, aspartate, glutamate, cyclodextrins)

Enzymatic:

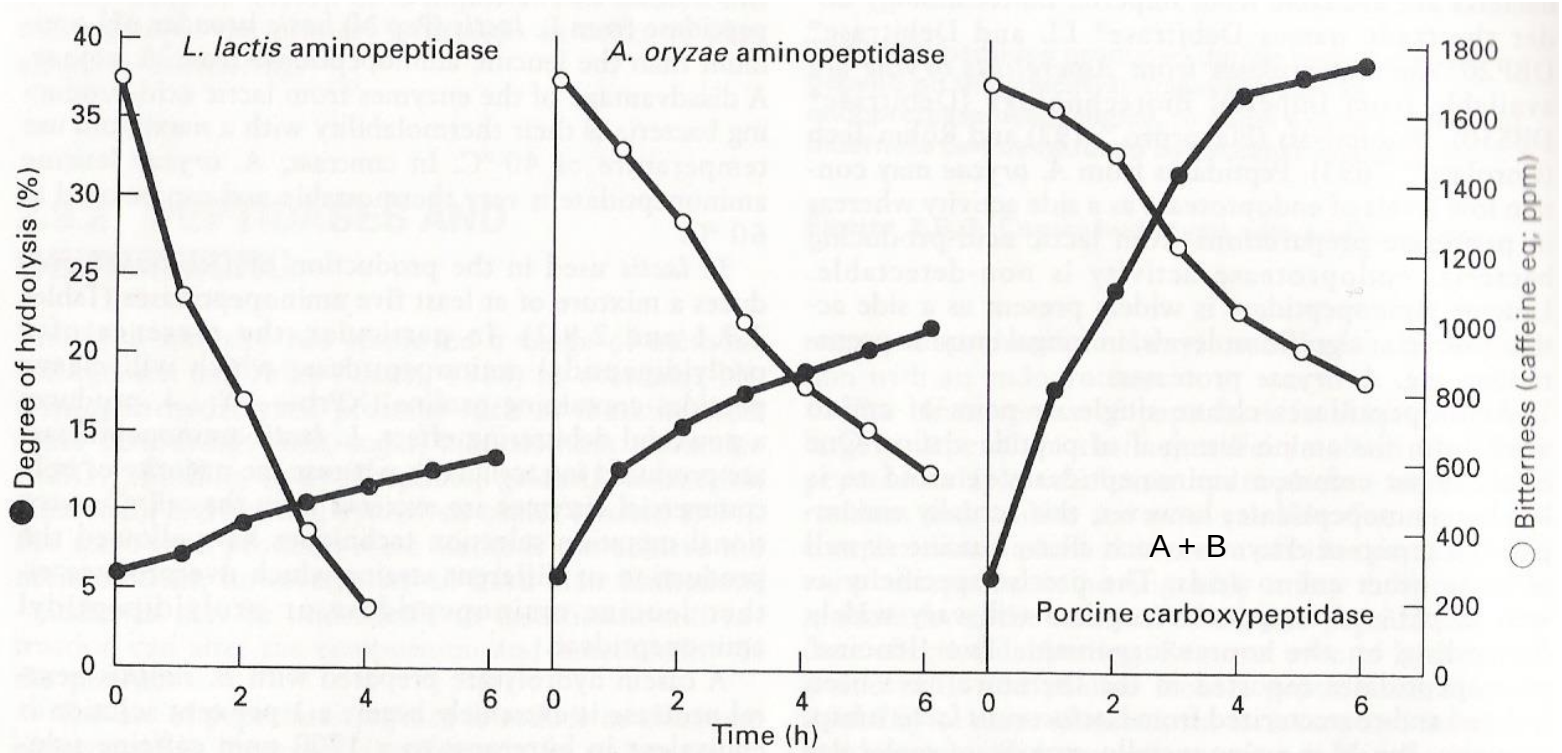
- Exopeptidases, aminopeptidases
- Crosslinking of peptides - transglutaminase (lower solubility

-X-Pro-Y

-Leu-

A: -Leu (Ile,Phe)

B: -basic AA

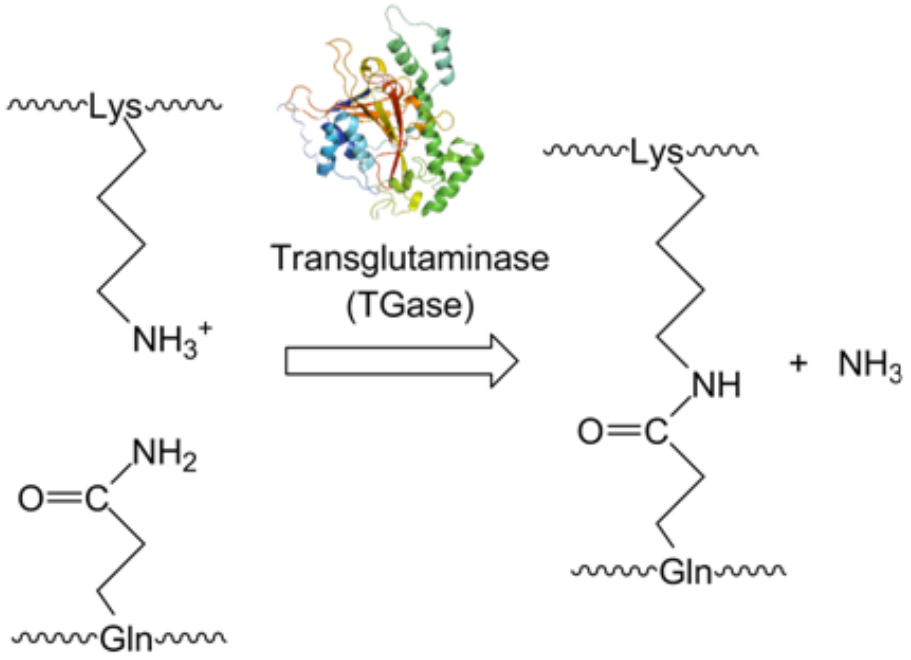


Debittering a casein hydrolysate with *L. lactis* aminopeptidase (Debitrase™ LL), *A. oryzae* aminopeptidase (Debitrase™ DBS50) and porcine carboxypeptidase A and B. Initial casein hydrolysate was 5 per cent DH and had a level of bitterness equivalent to 1720 caffeine equivalents as a 1 per cent solution. Limit of bitterness detection is 200 ppm

Crosslinking– transglutaminase – (protein-glutamine gamma-glutamyltransferase, EC 2.3.2.13)

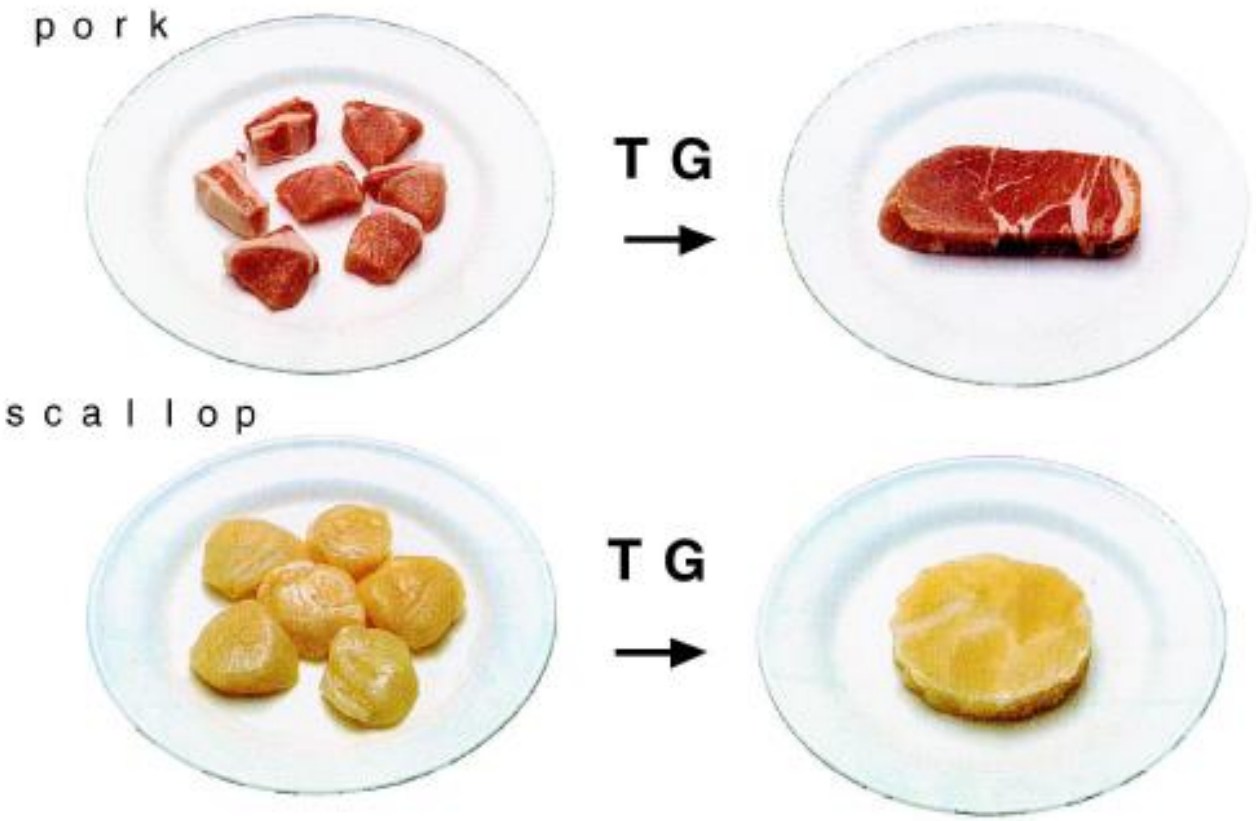
– „food glue“

- changes in texture of proteins
- gel formation

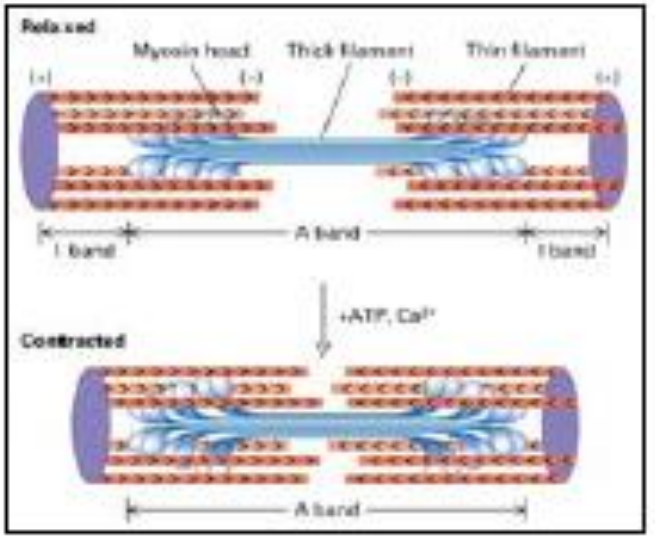


Protein glutamine + alkylamine <=> protein N(5)-alkylglutamine + NH_4^+

Examples…..



Tenderization (maturation) of meat



After slaughter:
- rigor mortis – muscle contractility decreases

- release of proteases (calpains, cathepsins) – cleavage of filaments → tender meat

- To speed up the process: aplication of plant proteases (papain, bromelain, ficin)
  post mortem: multi injection
  pre mortem: intravenous application 2-10 min before slaughter

Dairy industry

1. Chees production

- clotting
- ripening

2. Production of lactose free milk

3. Whey processing

Milk components

Water (87 – 90%)

Proteins (2- 3%) – caseins (mycellar 75 – 85%)

- whey (lactoglobulin, lactalbumin, 15 – 22%)

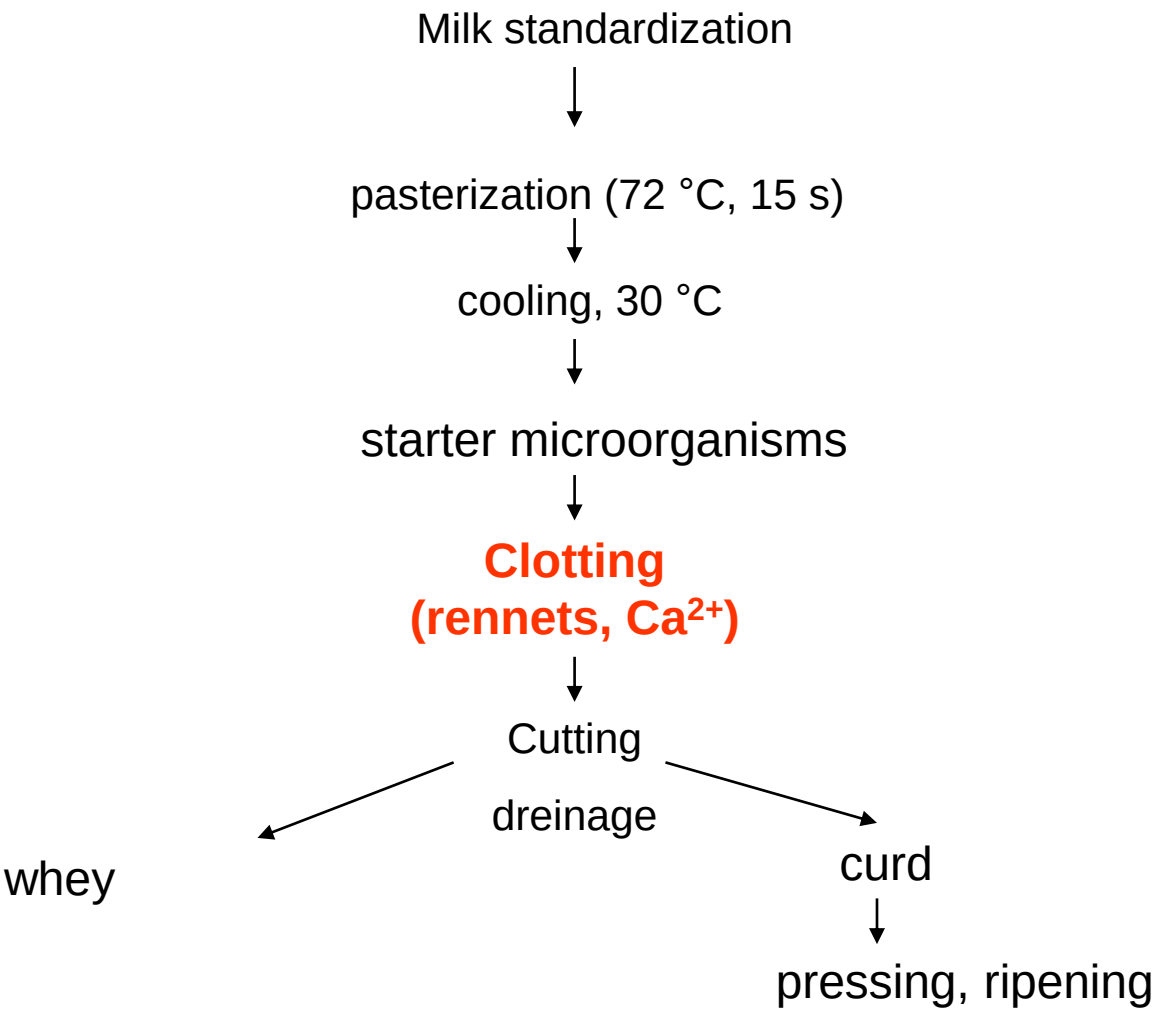
- peptides (2 – 4%)

Lipids (1- 4%) – TAG, phospholipids, cholesterol

Lactose (4,5 – 7%)

Vitamins

Cheese production



Substrates for rennets – casein mycelles

αS1-casein: (molecular weight 23,000; 199 residues, 17 proline residues)
Two hydrophobic regions, containing all the proline residues, separated by a polar region, which **contains all but one of eight phosphate groups. It can be precipitated at very low levels of calcium**.

αS2-casein: (molecular weight 25,000; 207 residues, 10 prolines)
Concentrated negative charges near N-terminus and positive charges near C-terminus. It can also be precipitated at very low levels of calcium.

ß -casein: (molecular weight 24,000; 209 residues, 35 prolines)
Highly charged N-terminal region and a hydrophobic C-terminal region. Very amphiphilic protein acts like a detergent molecule. Self association is temperature dependant; will form a large polymer at 20° C but not at 4° C. Less sensitive to calcium precipitation.

κ-casein: (molecular weight 19,000; 169 residues, 20 prolines)
Very resistant to calcium precipitation, stabilizing other caseins. Rennet cleavage at the Phe105-Met106 bond eliminates the stabilizing ability, leaving a hydrophobic portion, para-kappa-casein, and a hydrophilic portion called kappa-casein glycomacropeptide (GMP), or more accurately, **caseinmacropeptide** (CMP).

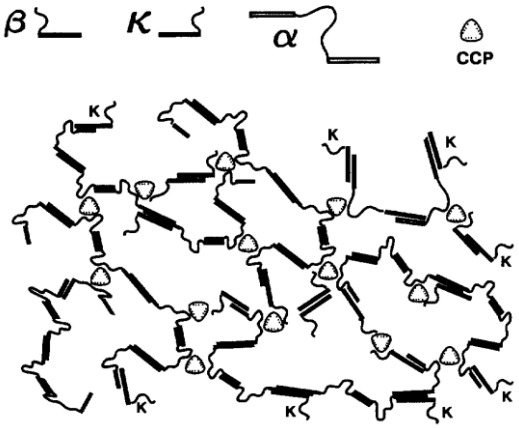
Chymosin - rennet

Aspartate protease,

pH optimum 3,5 - 6,5

Specificity: κ-casein

Casein mycelles



Surface of casein mycelle

-glu-…..-his-(pro-his)$_2$-leu-ser-phe-met-ala ……val

1 105 106 169

Para-κ-casein

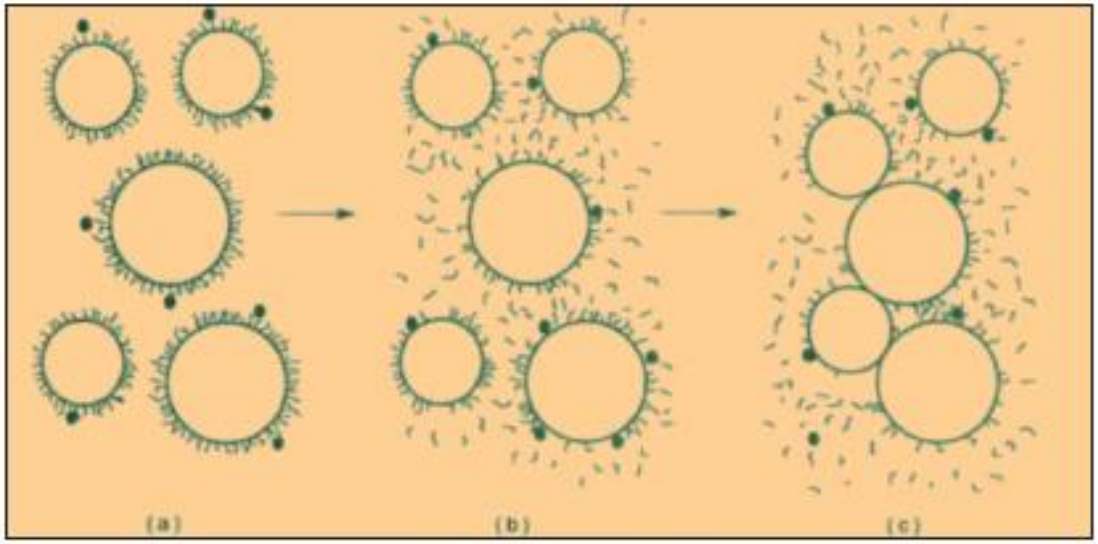
hydrophobic

Casein macropeptide

hydrophilic

Recombinant: mRNA from calf abomasum - production MO - E.coli, B. subtilis, S.cerevisiae, K. lactis, A. niger

Coagulation of casein mycelles (acid x enzymatic)



Coagulation proceeds only in the presence of calcium ions

Depends on temperature and degree of hydrolysis

Too proteolytic activity - continuing of hydrolysis in later phases is undesirable

Rennets – chymosin, but also other proteolytic enzymes (pepsin) with milk clotting activity

Properties of rennets:

- ✓ limited hydrolysis of β-casein – risk of bitter peptides formation
- ✓ i.e. specificity and properties (temperature, pH) close to chymosine
- ✓ limitation of the proteolysis during later phases – temperature stability

Microbial rennets: higher ratio of proteolytic and coagulation activity

higher termostability – risk of bitter peptides

changes in technology (pH, temp., Ca^{2+})

Recombinant -

Rennet preparations

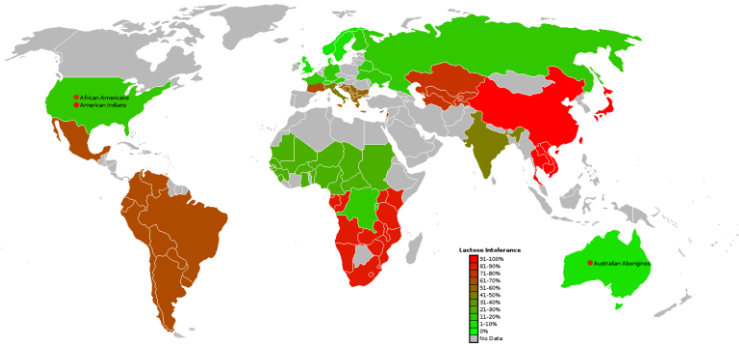
rennet	source	Commercial prep.	comment
animal	bovine	Stabo	100% pepsin
	bovine + calf	Cabo	60 – 100% chymosin
	calf		
	goat	Grandine	
microbial	Mucor pusillus	Emporase, Renzyme	
	Rhizomucor miehei	Fromase, Rennilase	
	Cryphonectria parasitica	Superen, Thermolase	
recombinant	A.niger	Chymogen, Chymostar	Not allowed in all countries
	A.oryzae	Novoren	Used intensivelly from 1994
	E.coli	Chymax	Not allowed in all countries
	Kluyveromyces marxianus	Maxiren	Not allowed in all countries
Plant	Annanas - bromelain		Too proteolytic
	papaya - papain		Too proteolytic
	Cynara cardunculus	Cardoon	Serra de Estrela Portugalsko

Chees ripening - complex biochemical processes

- Rennets (up to 30% in curd cheese)
- endogenous enzymes – plasmin,xanthin oxidase, acid phosphatase, lipases
- enzymes of starter cultures and secondary microorganisms (blue cheese)

lactate metabolism, citrate metabolism, proteases, lipases – low Mw FA

- exogenous enzymes - proteases, lipases in some special types of cheese



β-galactosidase

1. Cleavage of lactose in milk
   - ➢ Lactose free milk and products
   - ➢ Adjustment of milk for production of condensed milk, yogurts and ice creams
2. Hydrolysis of lactose in whey
   - ➢ Free enzyme – batch process with ultrafiltration
   - ➢ Immobilized enzyme –
     - batch (4 h, 37 °C resp. 24 h, 8 °C)
     - continual

Usage of hydrolyzed whey

1. **Sweetener in dairy industry, confectionery, bakery and production of non alcoholic beverages**

1. **Acceleration of fermentation in yogurts, curd cheese, beer,**
2. **Controlled browning in bakery and confectionery products**
3. **Substitution of milk in ice creams**
4. **Lactose free products**
5. **Production of ethanol**
6. **Feed for pets and farm animals**

Production of beverages

- brewing industry
- wine production
- Fruit and vegetable processing (canning industry)

Brewing process

1. Malting- barley grains soaking in water and germinate “sprout”. This allows the tough starch molecules to be softened and begin to convert them to sugars. Sprouts are dried in a kiln; the temperature affects the flavor of the finished brew.
2. Milling- malted grains are ground - allows better extractability of sugars when mixed with water.
3. Mashing- ground malted grain is mixed with water - most of the remaining starches are converted to sugars due to enzymes present in the malt and the sugars then dissolve into the water. The mix is gradually heated to 75℃
4. The slurry is then filtered to remove the majority of particulates. This filtered sugary liquid is called wort.
5. Brewing- the wort is brought to a boil for roughly 1–2 hours. During this time, other grains and hop that will contribute flavor, color, and aroma to the brew are added. Boiling allows for several chemical reactions to occur and reduces the water content in the wort,
6. Cooling- The wort is filtered to remove the majority of the grains and hops and then immediately cooled to allow the yeast to survive and grow in the next step.
7. Fermentation - cooled wort is saturated with air and yeast is added within the fermentation tank. Different strains of yeast will create different styles of beer. This step takes around ten days.
8. Maturation — The freshly fermented un-carbonated beer is then placed into a conditioning tank and is allowed to age. If this step is rushed the beer will have an off flavor (acetaldehyde). During this process of aging, the majority of the residual particulates will settle to the bottom of the tank.
9. Finishing — The beer is filtered last time; carbonated and moved into a storage tank for either bottling or kegging.

Brewing

Traditional process, without enzyme addition …….but in special cases exogenous enzyme addition is applied

1. **Wort** – substitution with starch - α-amylase (BAN), proteases – release of β-amylase from grains
2. **Fermentation** – exceptionally –

production of„lite“ i.e. light beers (alcohol free, lower calory value):

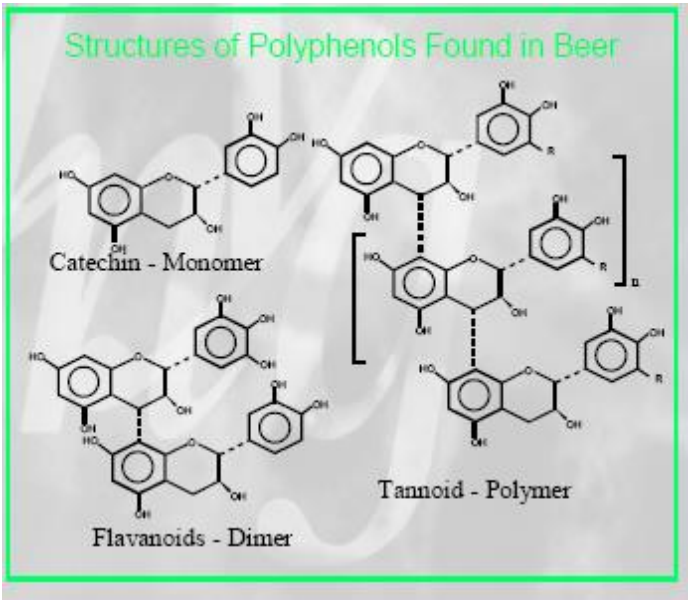
increase of fermentable saccharide content - pullulanase, amyloglucosidase

alcohol is evaporated

3. **Filtration** – gum formation when cleavage of plant cell wall polysaccharides - β-glucans (β-1,3, β1,4) is unsufficient – endogenous β-glucanases, lower temperature stability
4. **Stabilization of beer** – **removal of haze**
5. **Maturation**

Chill haze → permanent haze

Interaction of coloid particles :



Proteins rich in proline

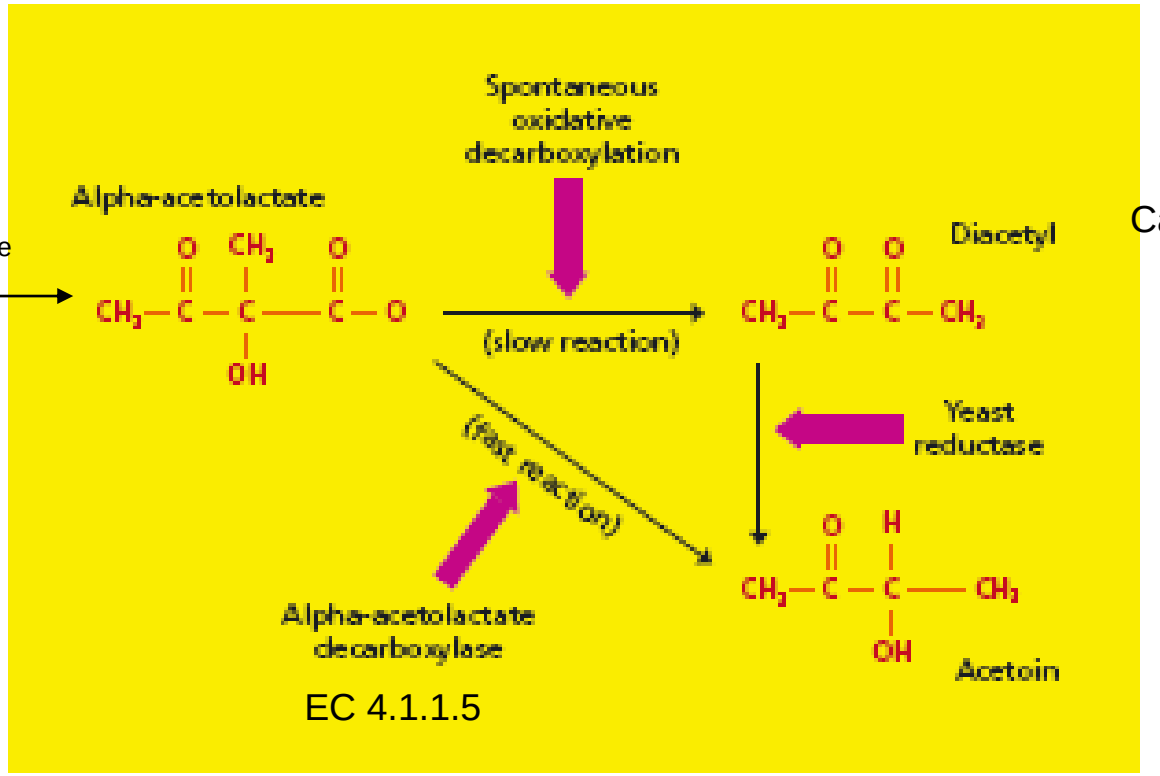
Removal of haze

….. papain

Maturation

2 pyr

Acetolactate synthase



Caramel or butter taste

EC 4.1.1.5

Maturex (B.brevis)

Wine productiom

1. Improvement of must extraction- pectolytic enzymes
2. Improvement of wine aroma - terpenols bound to saccharides

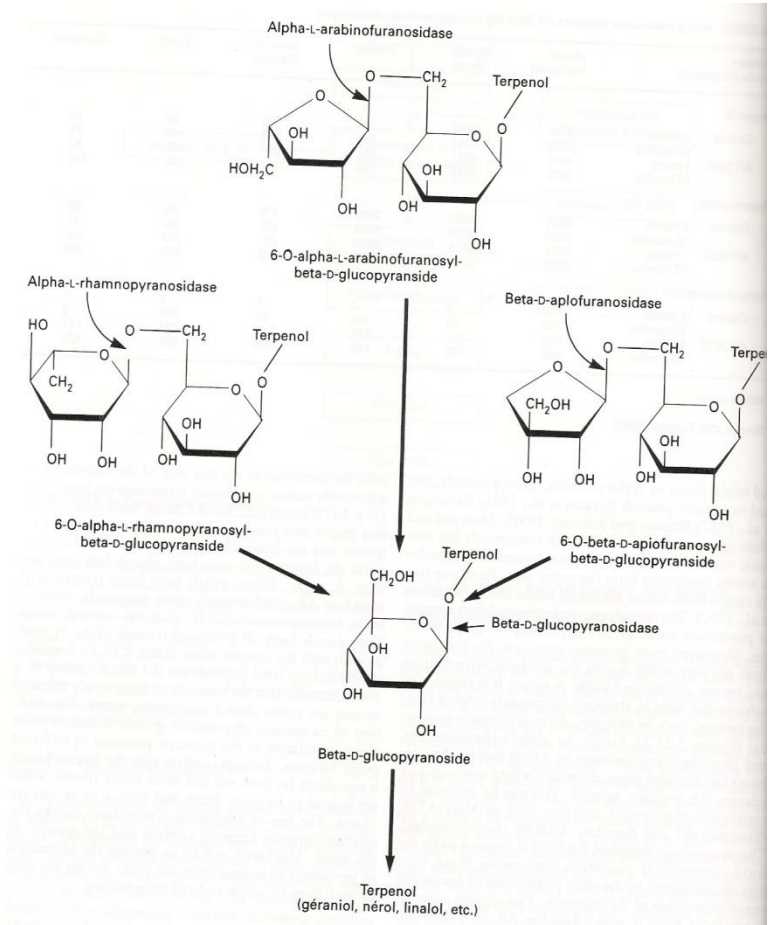
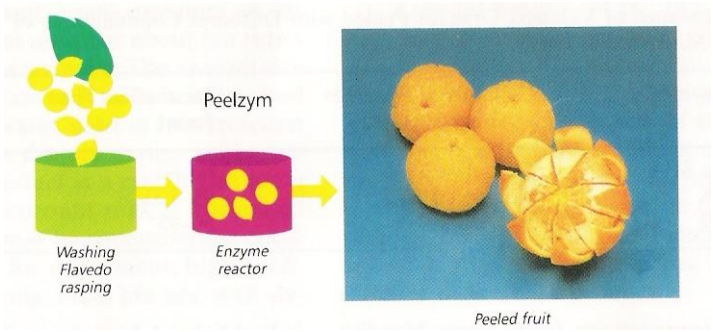


Figure 2.22.3 Hydrolysis of terpene glycosides

Processing of plant row material

- Peeling of citrus fruits
- Pulp maceration → cell suspensions



fruit juice production

- clarification
- filtration
- Adjustment of sensory properties

Formation of nonsedimenting haze in fruit juices (cloudy juice)

High viscosity – intact, higly esterified pectin -

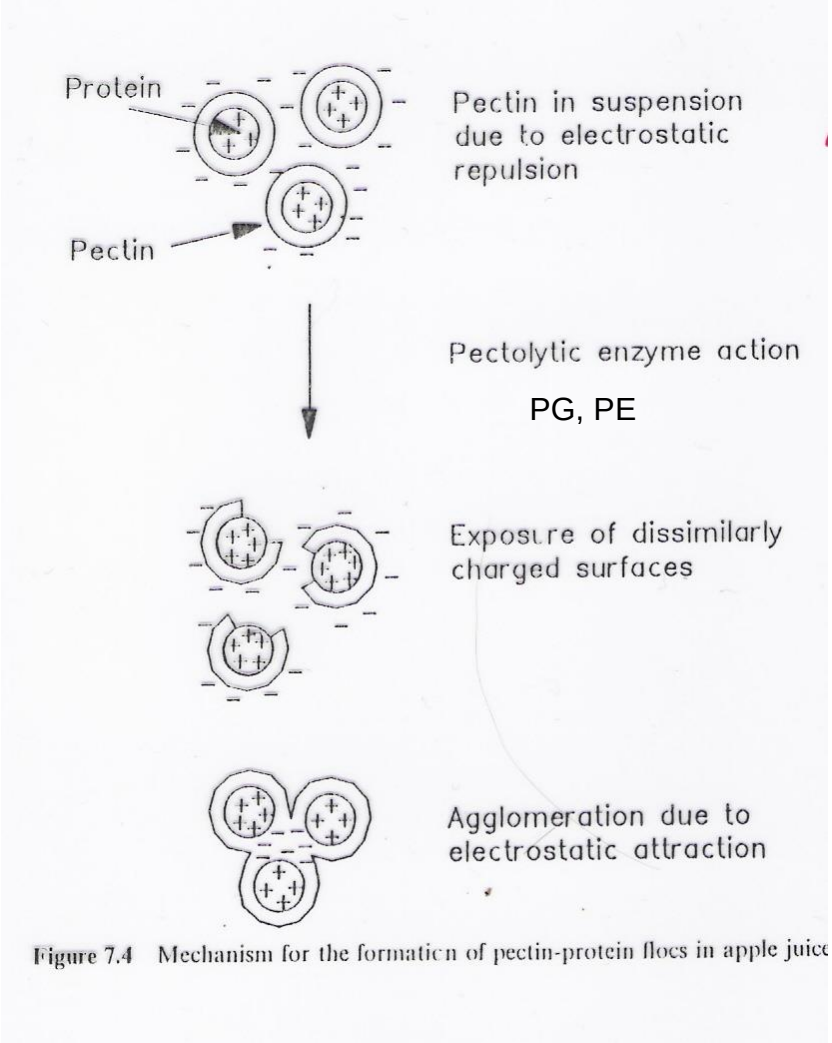
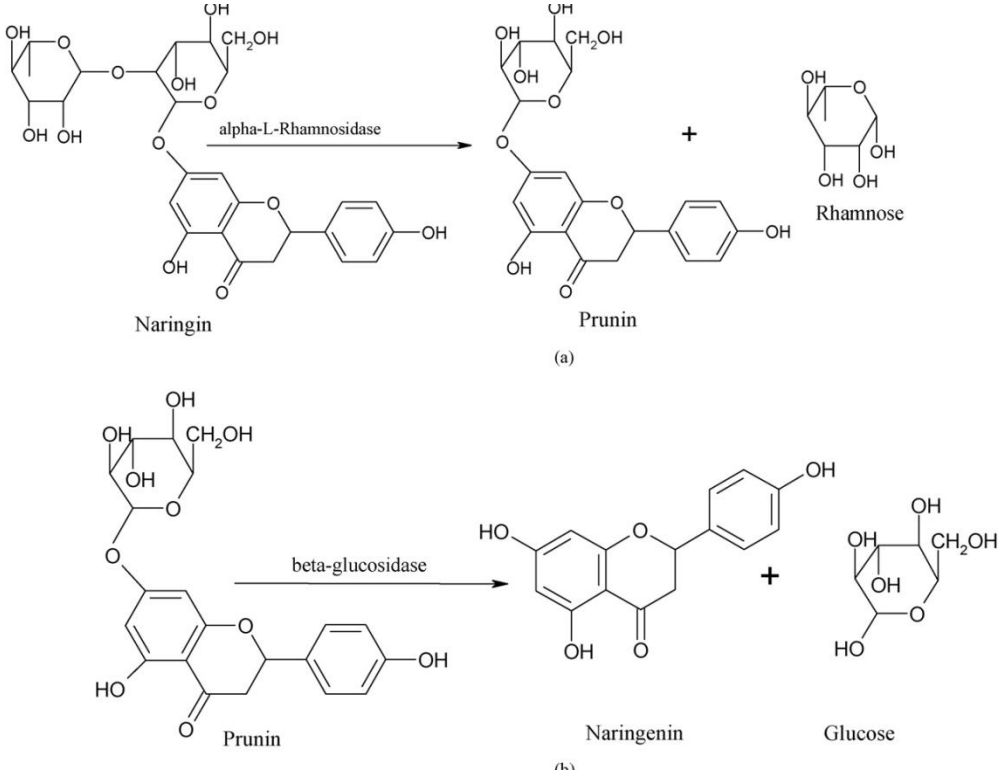


Figure 7.4 Mechanism for the formation of pectin-protein flocs in apple juice

pH 3,5

Reduction of bitterness in citrus juices

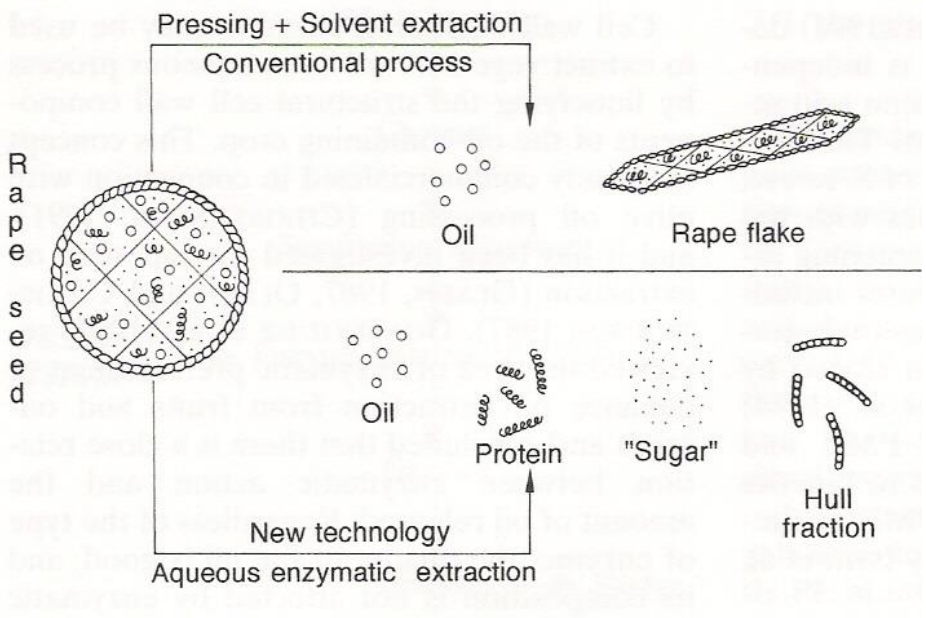
Naringin – threshold level 1,5 - 20 ppm (1-6 g in piece)



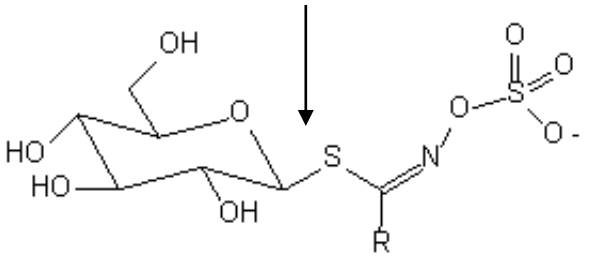
Oil processing

Rape seed oil: extraction by organic solvents (hexane)

Possibility to avoid:



Glucosinolates in classical process:



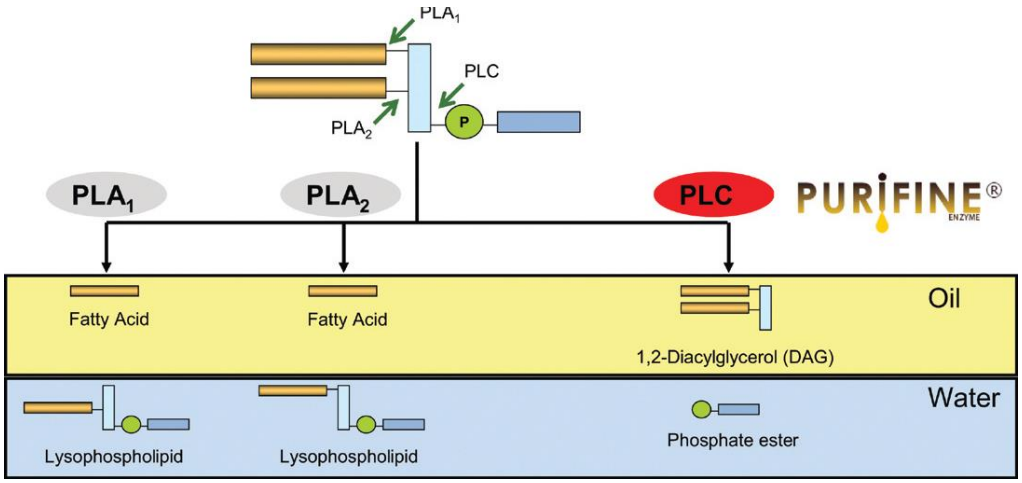
Oil processing

Processing of olive oil degradation of pant cell walls - *Olivex* – pectolytic enzymes

Removal of non hydratable components of oil (degumming) – PA, FFA, phospholipids, metal ions, dyes, aromatic compounds,

1.Chemical refining - NaOH,
2. Water vapour + acid
3. With enzymes – phospholipases A, C

Lecithase



Transesterification – retailoring of fats

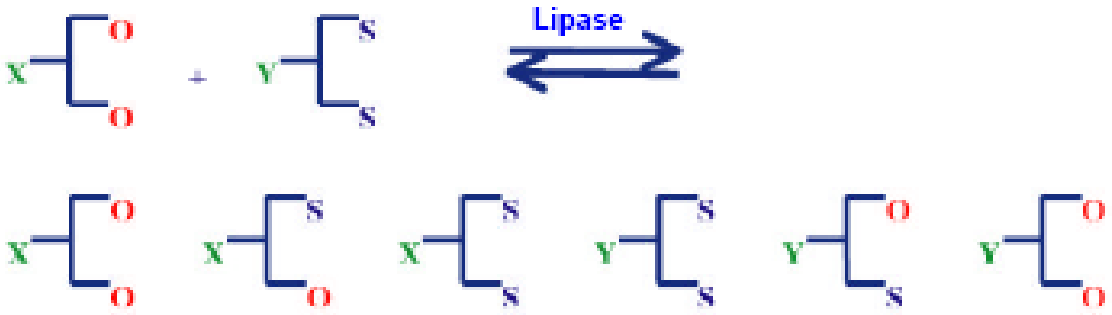
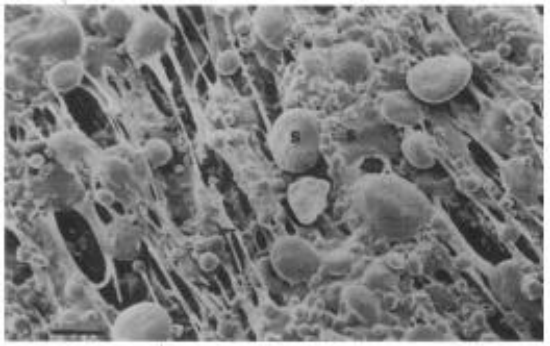


Figure 4. Interesterification using a 1,3-specific lipase.

Baking industry



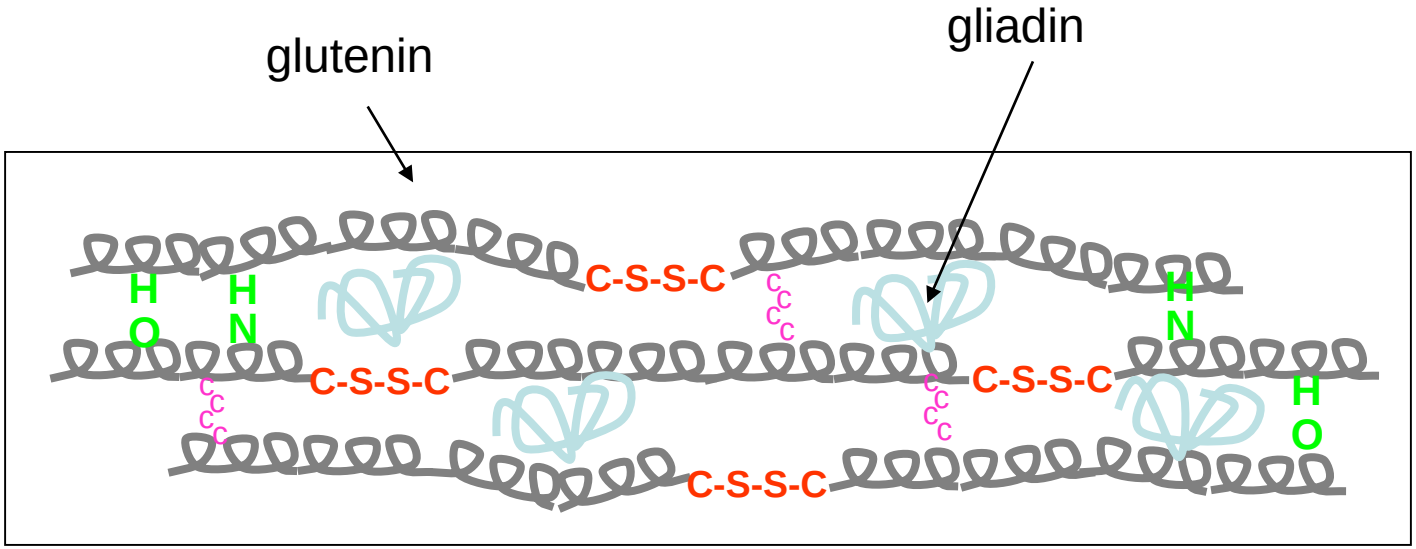
1. Standardization of flours – Taka-amylase

2. Production of dough, **gluten quality - rheology**

Proteases (fungal, plant, transglutaminase)

GOD/CAT – oxidation of SH groups of gluten

β-glucanases, pentosanases – water content -



Gln, Pro, Cys, hydrophobic AA,
mostly beta structures → dough elasticity

4. Rising of the dough – production and retention of CO_2

α-amylases (fungal)

glucoamylase

5. Appearance of products

LOX – color of the crumb

Amylases – crumb structure, antistaling effect