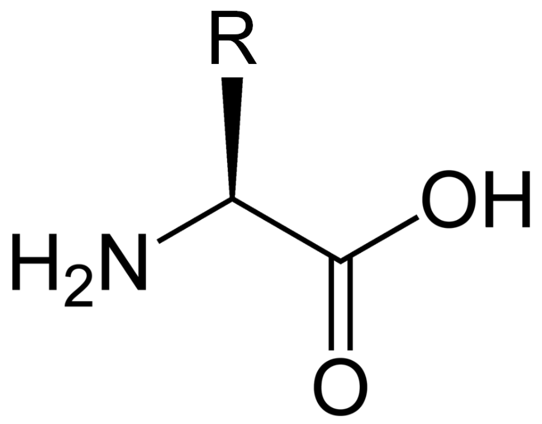
EPFL

FOOD CHEMISTRY

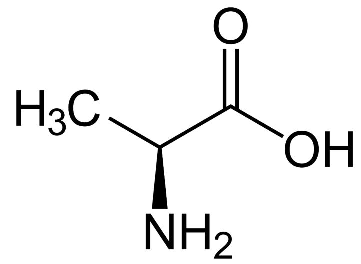
Chapter 5 : Amino acids and proteins

5.1 STRUCTURE OF AMINO ACIDS

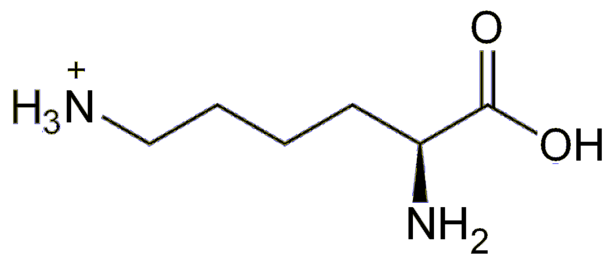
General formula of amino acids



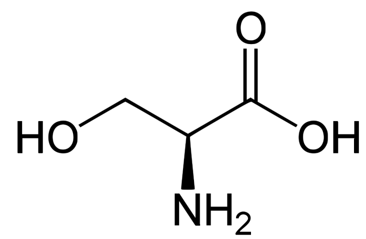
Amine in α-position



Non polar side chain
Alanine



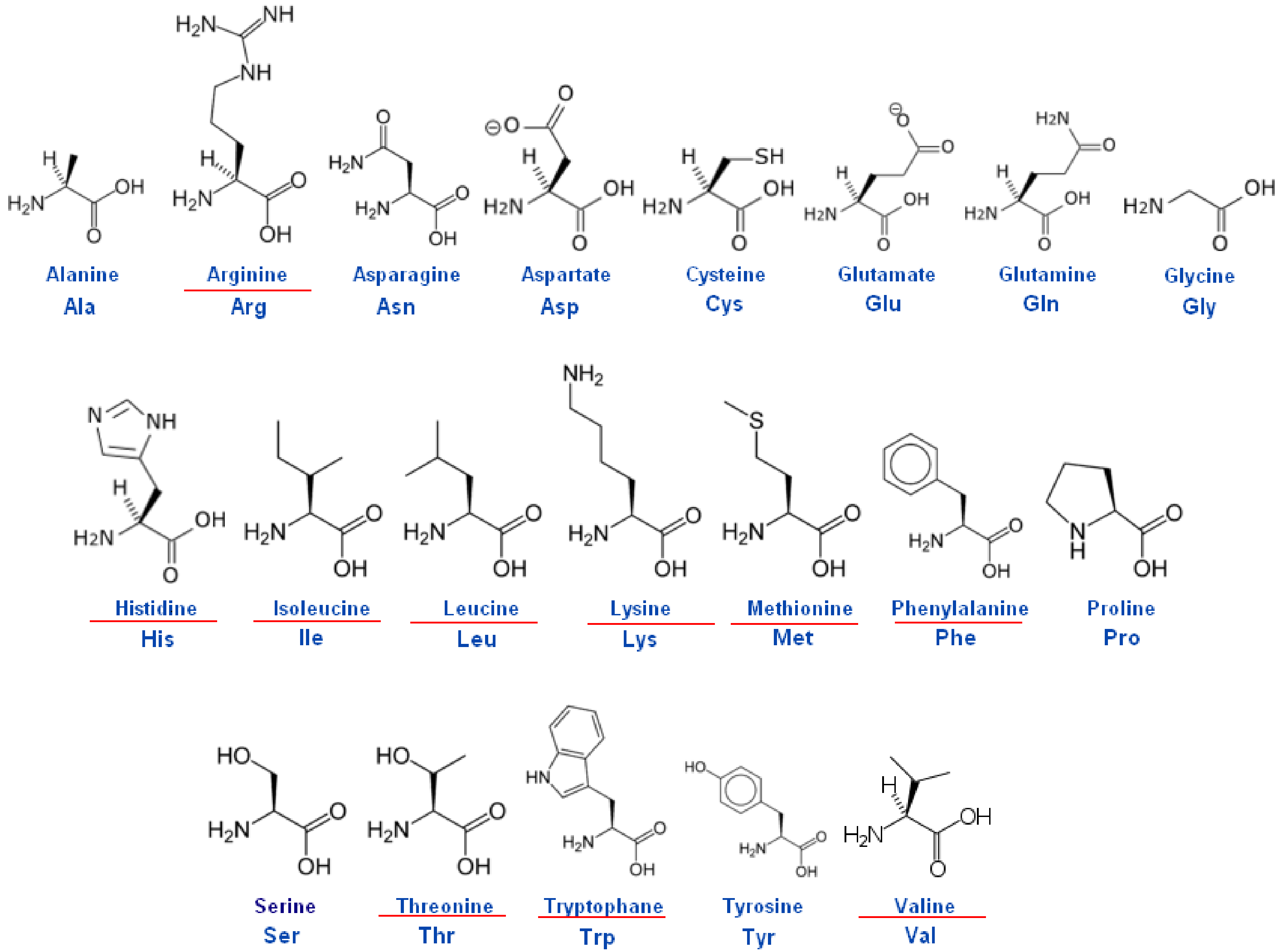
Charged side chain
Lysine



Polar side chain
Serine

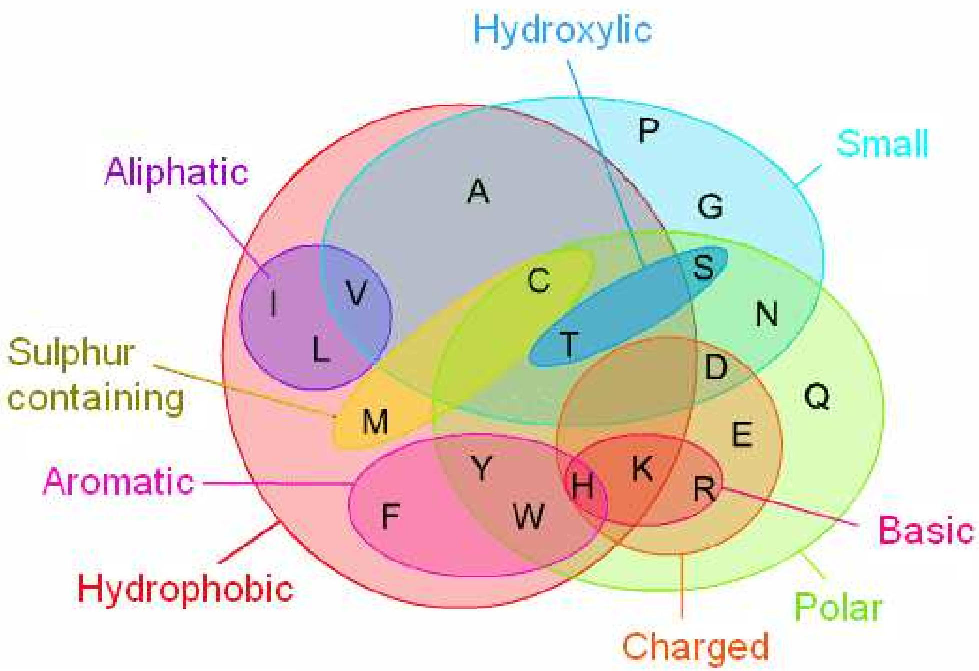
5.1 STRUCTURE OF AMINO ACIDS

Natural amino acids



5.1 STRUCTURE OF AMINO ACIDS

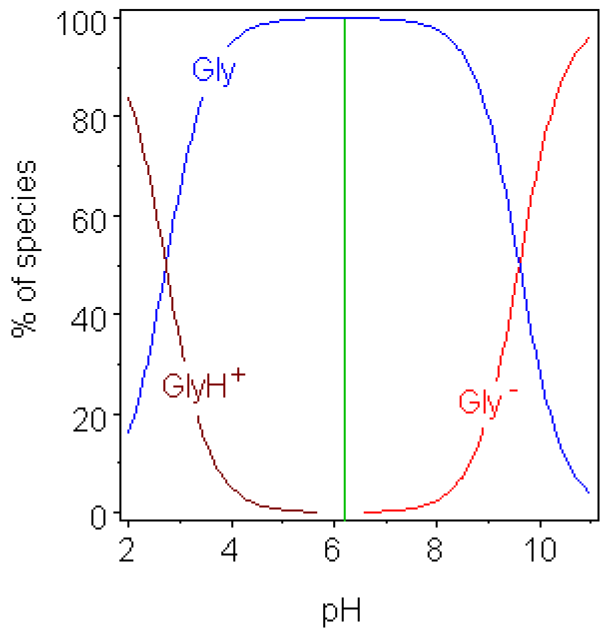
Classification of amino acids

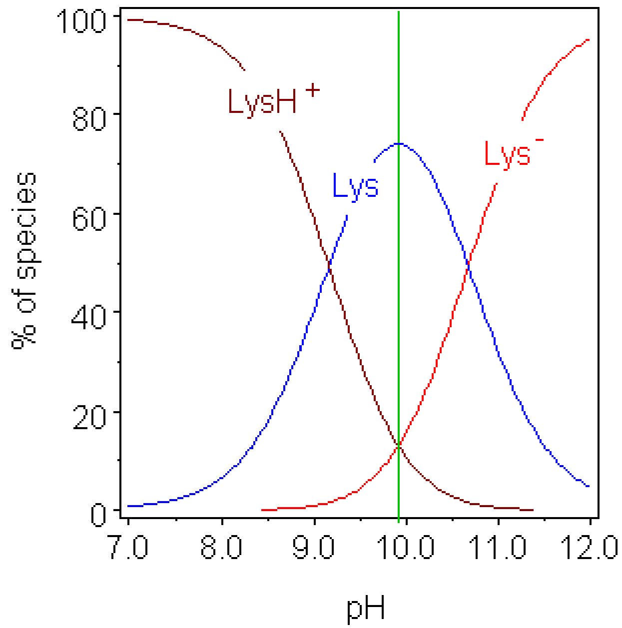


A alanine
R arginine
N asparagine
D aspartic acid
C cysteine
Q glutamine
E glutamic acid
G glycine
H histidine
I isoleucine
L leucine
K lysine
M methionine
F phenylalanine
P proline
S serine
T threonine
W tryptophan
Y tyrosine

5.2 PHYSICAL PROPERTIES OF AMINO ACIDS

Isoelectric point



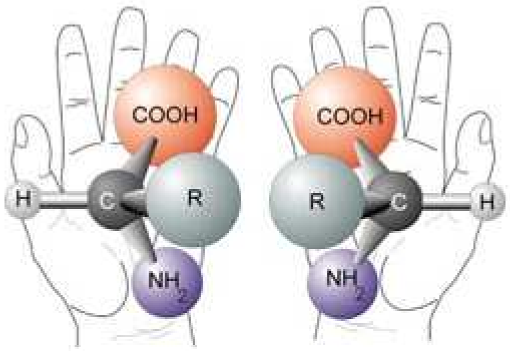


$H_3\overset{+}{N}CH_2COOH$	$pK_1 = 2.72$
$H_3\overset{+}{N}CH_2COO^-$	$pI = 6.1$
$H_2NCH_2COO^-$	$pK_2 = 9.60$

$H_3\overset{+}{N}(CH_2)_4CH(\overset{+}{N}H_3)COOH$	$pK_1 = 2.15$
$H_3\overset{+}{N}(CH_2)_4CH(\overset{+}{N}H_3)COO^-$	$pK_2 = 9.16$
$H_3\overset{+}{N}(CH_2)_4CH(NH_2)COO^-$	$pI = 9.7$
$H_2N(CH_2)_4CH(NH_2)COO^-$	$pK_3 = 10.67$

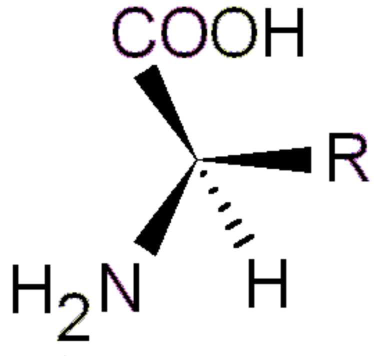
5.2 PHYSICAL PROPERTIES OF AMINO ACIDS

Optical activity



The « CORN » rule :

With H atom away from the viewer, « CORN » is the D-form (the natural one is the L-form).

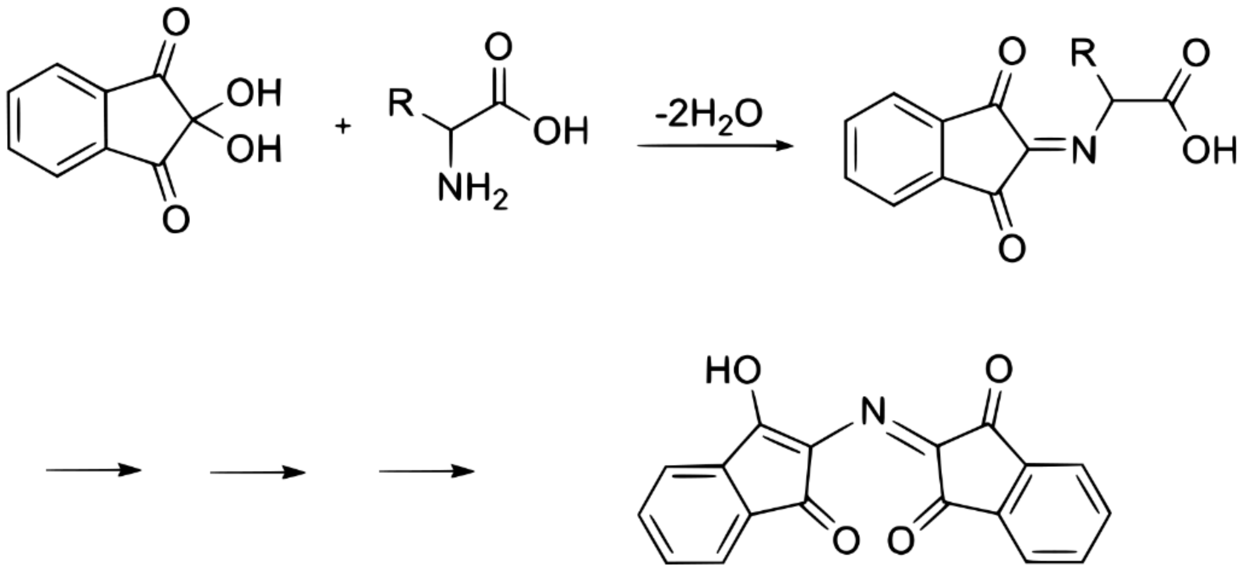


This has nothing to do with the rotation of the plane of polarized light.

(+) alanine
(-) cysteine
(-) tyrosine
(+) valine

5.3 REACTIONS OF AMINO ACIDS

Ninhydrin reaction

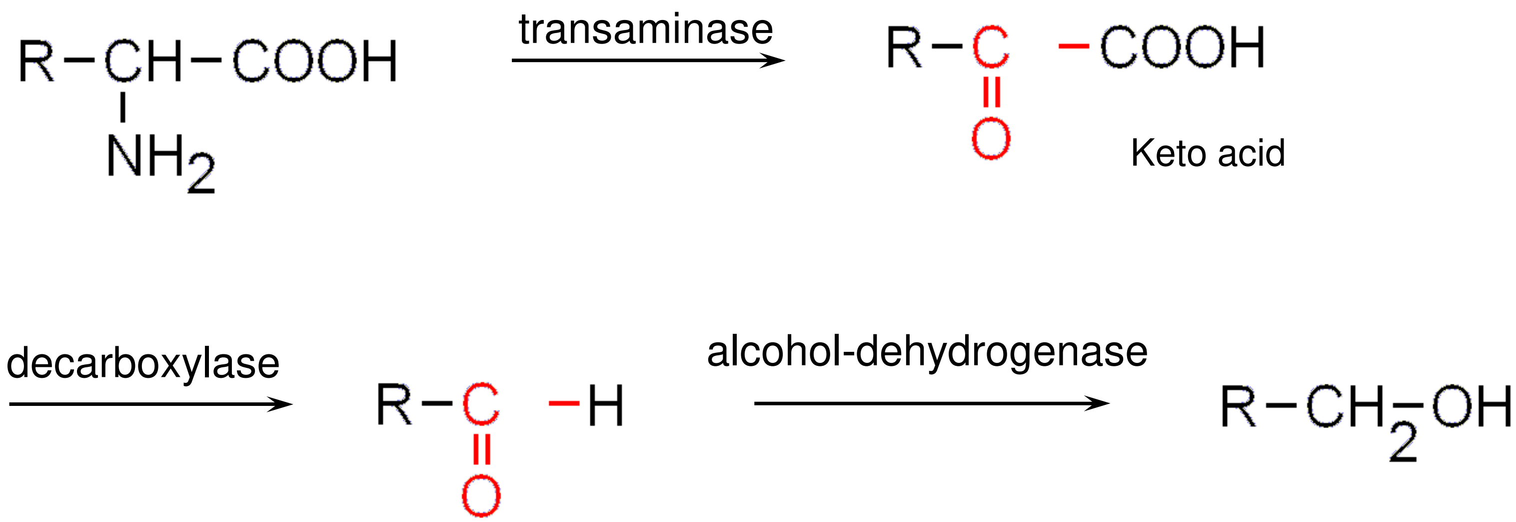


Ruhemann's purple

Allows to detect minute amounts of proteins (eg fingerprints).

5.3 REACTIONS OF AMINO ACIDS

Formation of higher alcohols



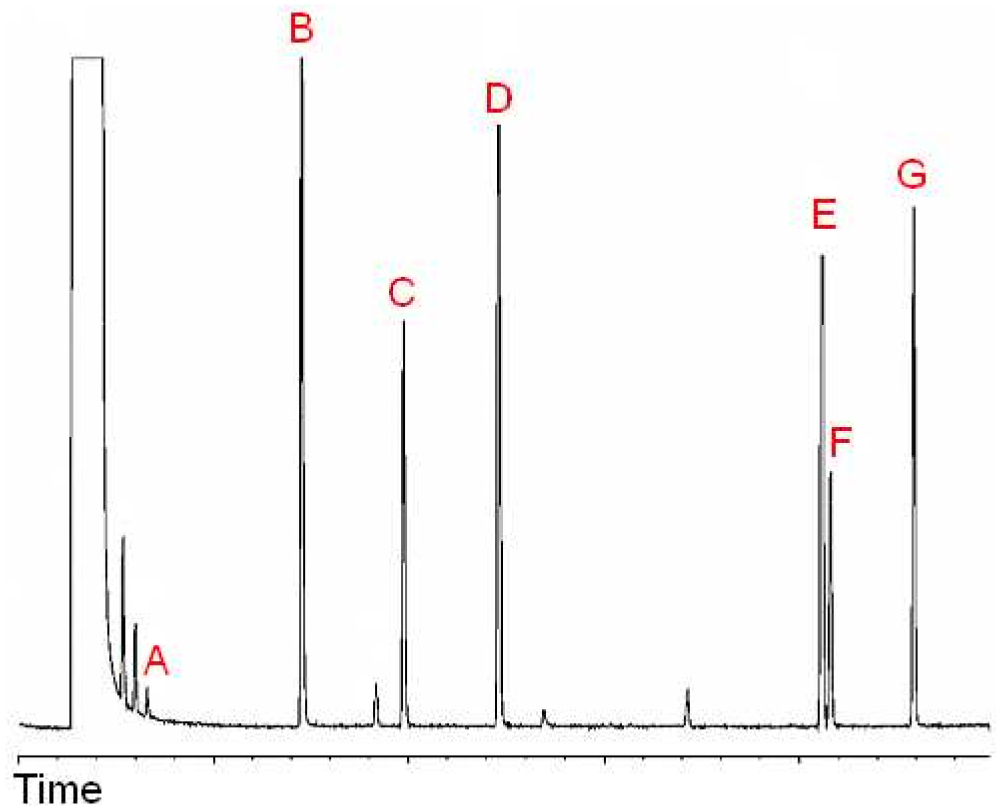
Isoleucine → 2-methyl-butanol

Leucine → 3-methyl-butanol

Phenylalanine → 2-phenyl-ethanol

Valine → Isobutanol

5.3 REACTIONS OF AMINO ACIDS
Chromatogram of higher alcohols in Rhum



A 2-Propanol

B n-Propanol

C n-Butanol

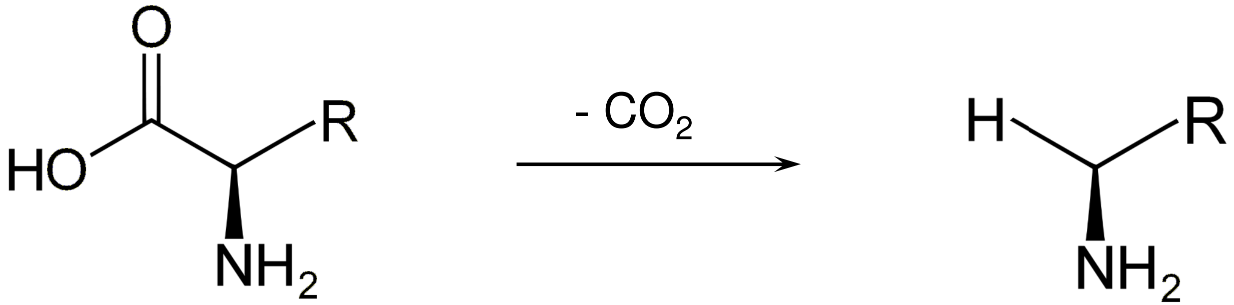
D 2-Methyl-propanol

E 3-Methyl-butanol

F 2-Methyl-butanol

G n-Pentanol

5.3 REACTIONS OF AMINO ACIDS
Decarboxylation leading to biogenic amines



Tryptophan → Serotonin

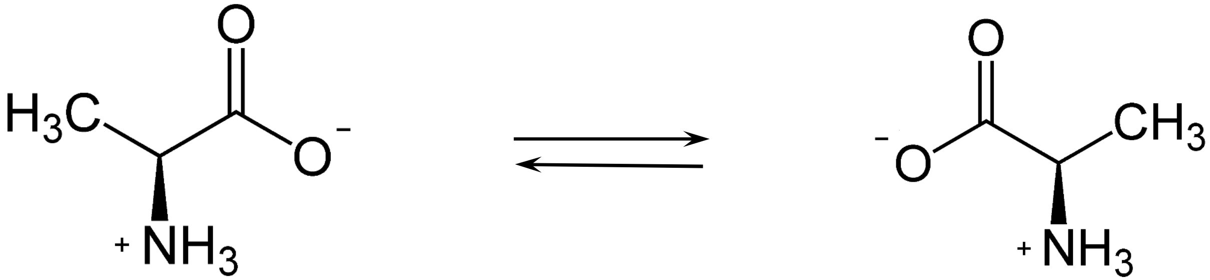
Histidine → Histamine
Lysine → cadaverin
arginine → putrescin

Tyrosine → Tyramine

Tyrosine → Dopamine

5.3 REACTIONS OF AMINO ACIDS

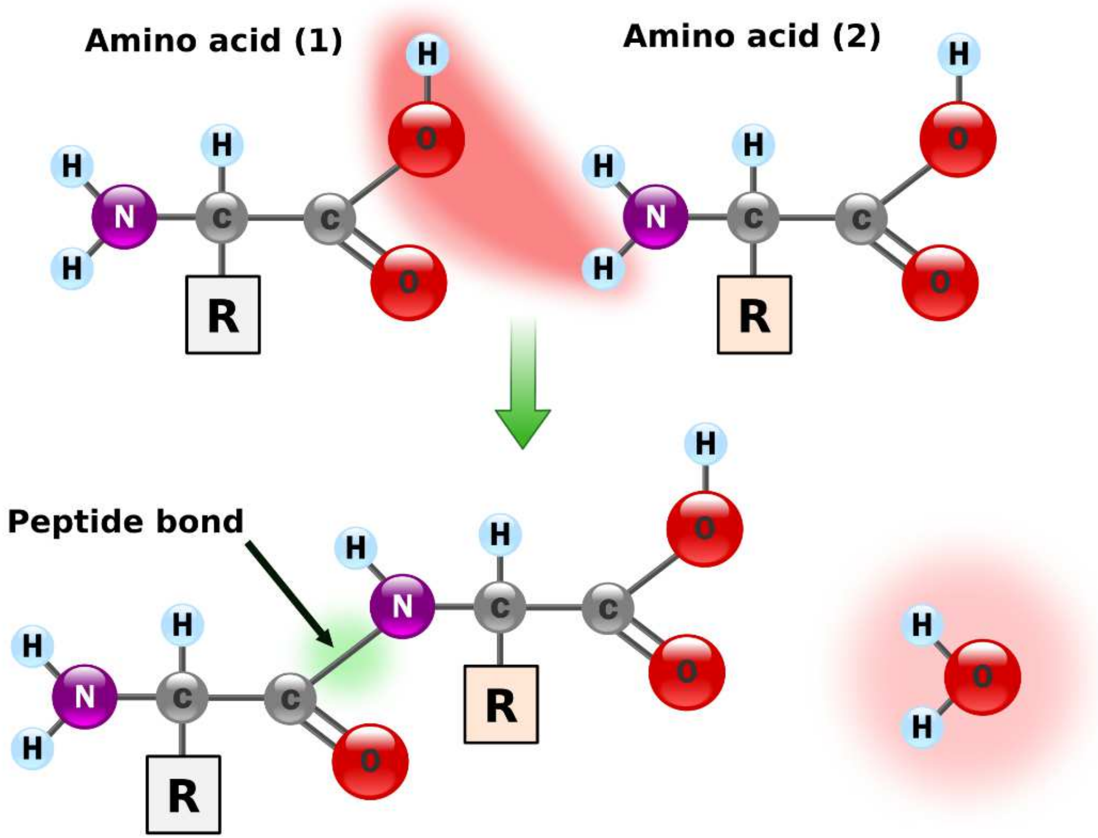
Racemisation



- Asparagine racemizes relatively quickly and has frequently been used to date materials from the present back to around 25000 BP.
- Isoleucine racemizes much more slowly, and has been used to date materials from 5000 to 2 million years of age.

5.4 PEPTIDES

The peptide bond



5.4 PEPTIDES

Some definitions

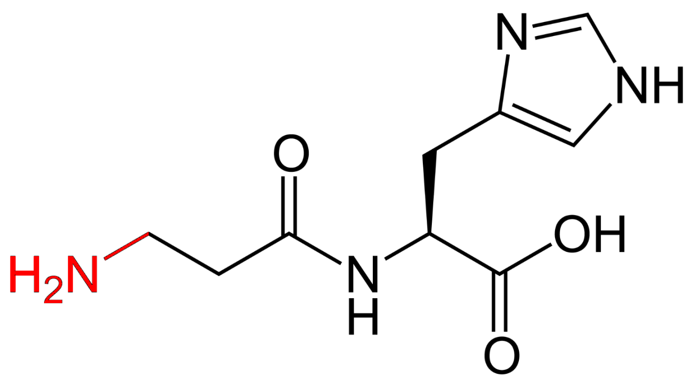
Peptides are short polymers of amino acids linked by peptide bonds.

A polypeptide is a single linear chain of amino acids.

An oligopeptide is a polypeptide less than 30-50 amino acids long.

A di-, tri- or tetrapeptide is a chain with 2, 3 or 4 amino acids.

Notation :



Carnosine

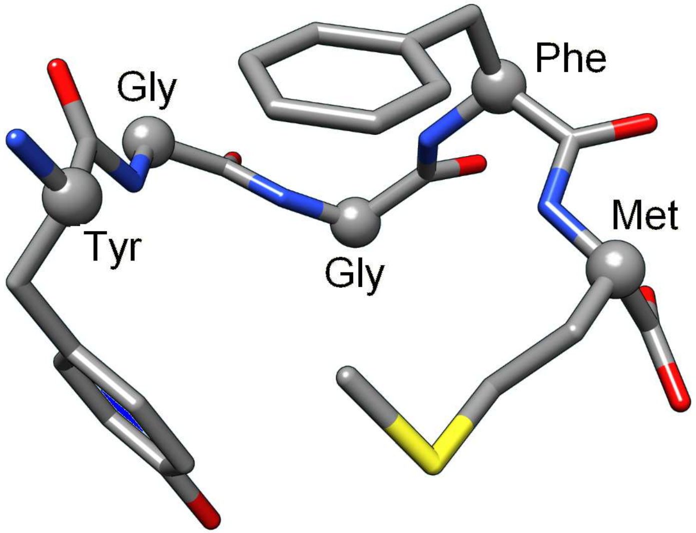
β−alanyl-L-histidine

*A*la-His

Always start with N-terminal aa !

5.4 PEPTIDES

Opioid peptides



Met-enkephalin

Tyr-Gly-Gly-Phe-**Met**

Leu-enkephalin

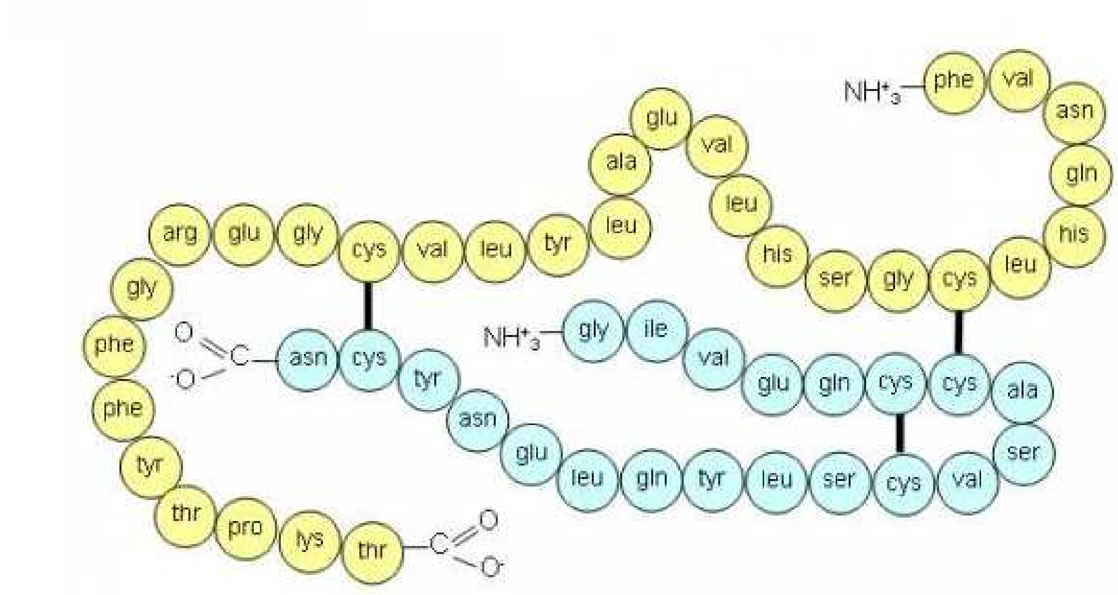
Tyr-Gly-Gly-Phe-**Leu**

Opioid peptides are short sequences of amino acids that bind to opioid receptors in the brain. They may be produced by the body itself, for example endorphins. Brain opioid peptide systems are known to play an important role in motivation, emotion, response to pain, and the control of food intake.

5.5 STRUCTURE OF PROTEINS

Primary structure

Sequence of amino acids chain, with disulfur bridges



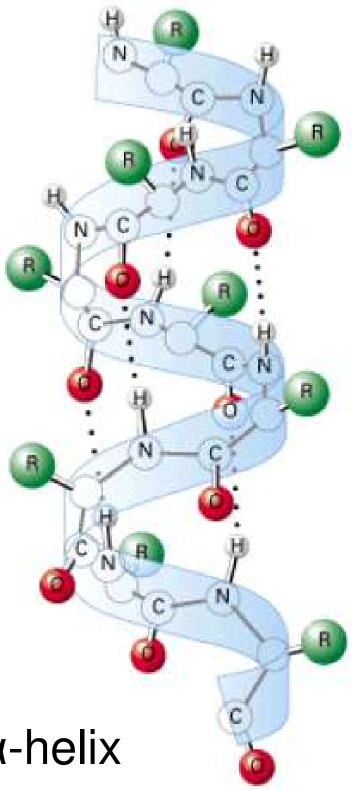
Human insulin

Chain A (21 aa)

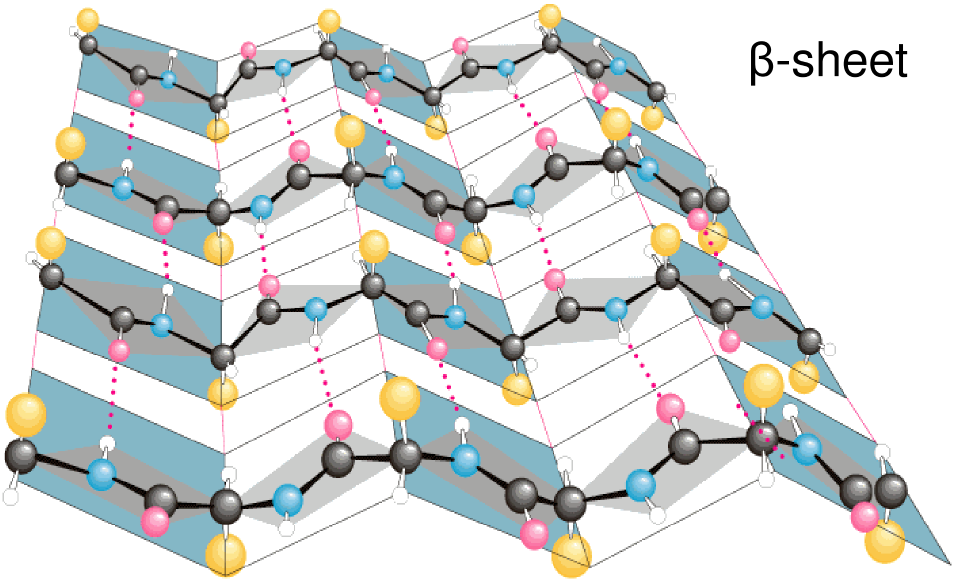
Chain B (30 aa)

5.5 STRUCTURE OF PROTEINS
Secondary structure

Three-dimensional form of local segments due to H-bonds



α-helix

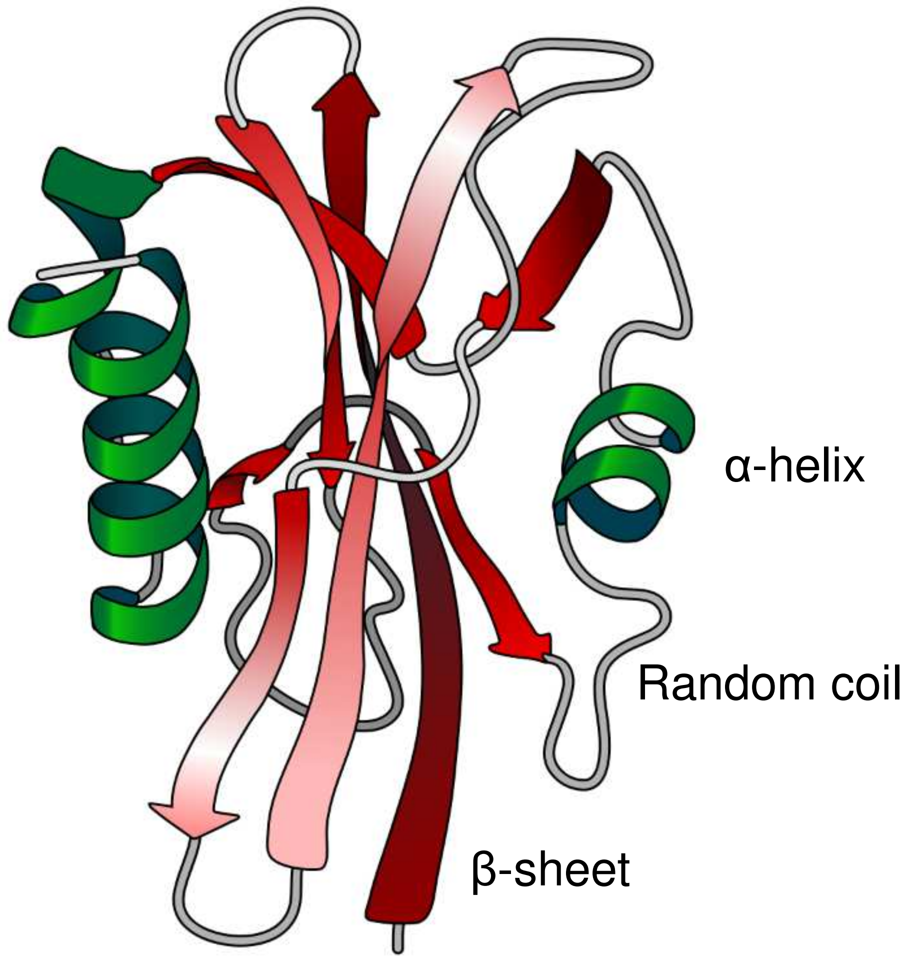


β-sheet

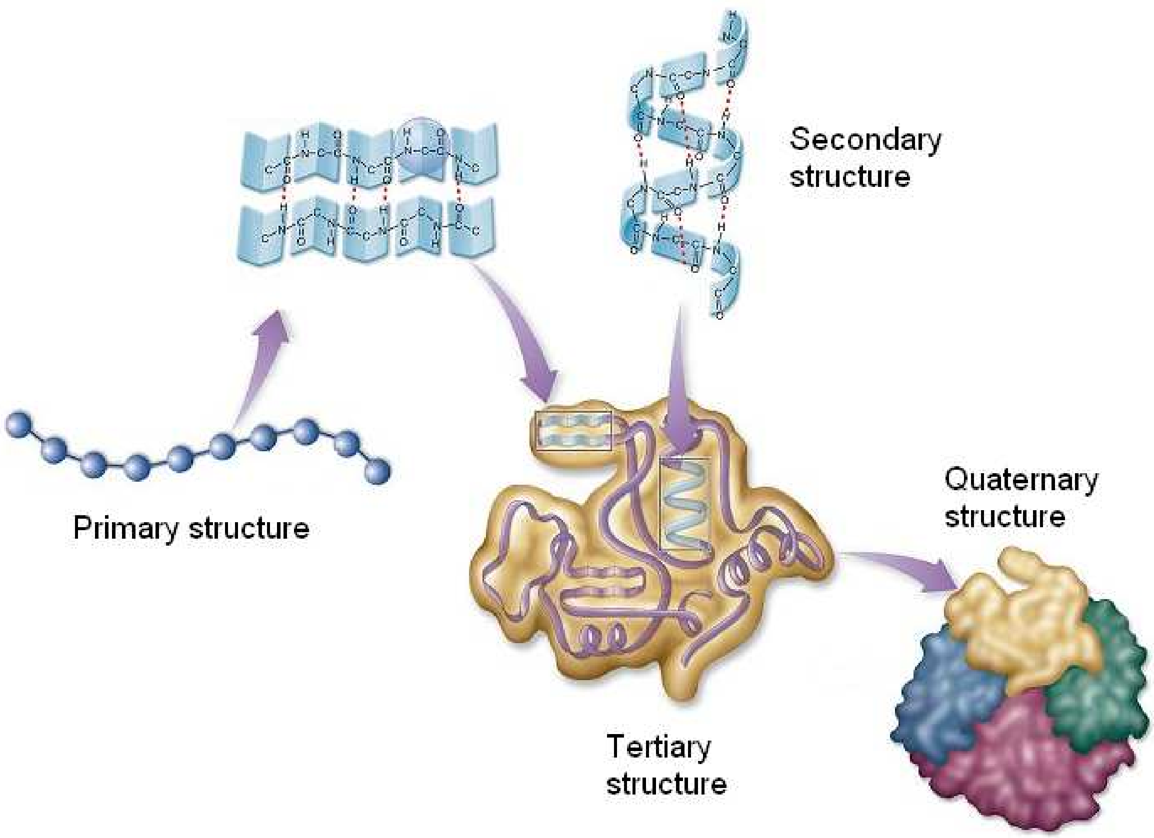
5.5 STRUCTURE OF PROTEINS

Tertiary structure

Three-dimensional structure of a protein. The alpha-helices and beta-sheets are folded into a compact globule. The folding is mainly driven by hydrophobic interactions and the burial of hydrophobic residues from water.



5.5 STRUCTURE OF PROTEINS
General overview



5.5 STRUCTURE OF PROTEINS

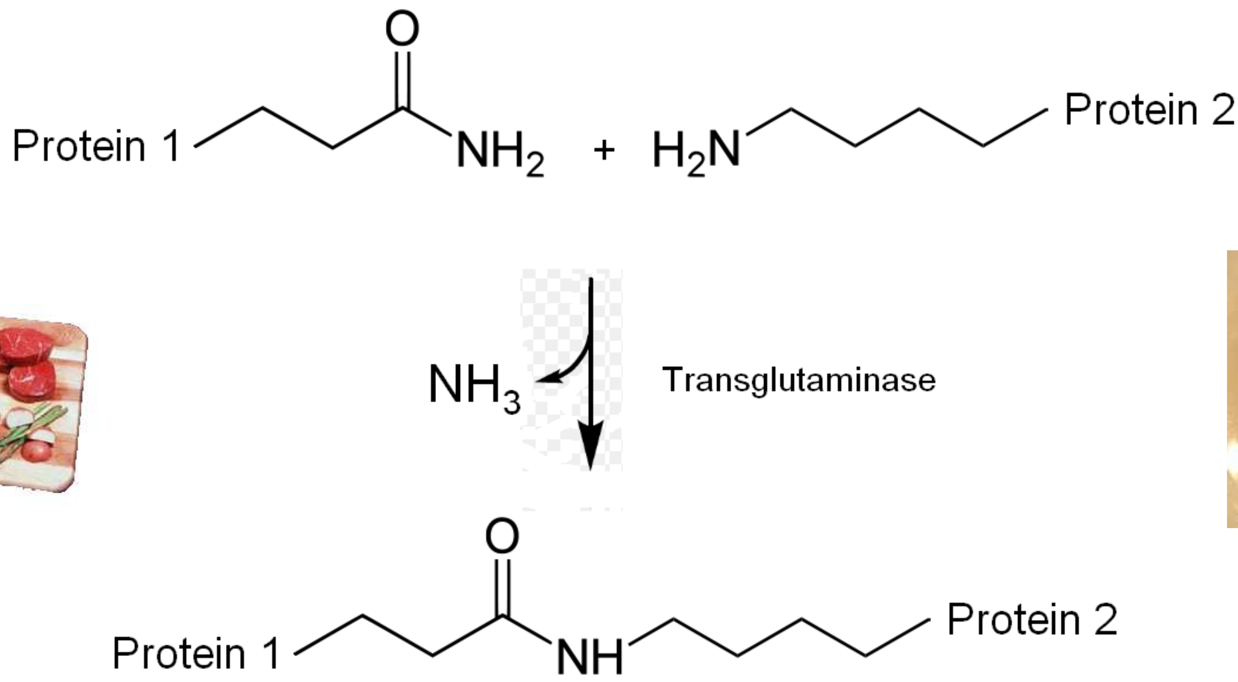
Texturisation of proteins

Heat is very often used in food industry to obtain a structure that provides the texture of a solid, but also to stabilize foams or emulsions. The various stages of protein processing are the following :

Denaturation	Transition from an ordered to a disordered state without breaking of covalent bonds : it is the unfolding of the protein
Polymerization	Formation of large aggregates
Precipitation	Formation of large aggregates with total loss of solubility
Flocculation	Unordered aggregation without denaturation
Coagulation	Protein-protein aggregation with denaturation
Gelification	Formation of a more or less ordered three dimensional network polymer. It is the result of the balance between cohesive and repulsive forces.

5.5 STRUCTURE OF PROTEINS

Texturisation through transglutaminase



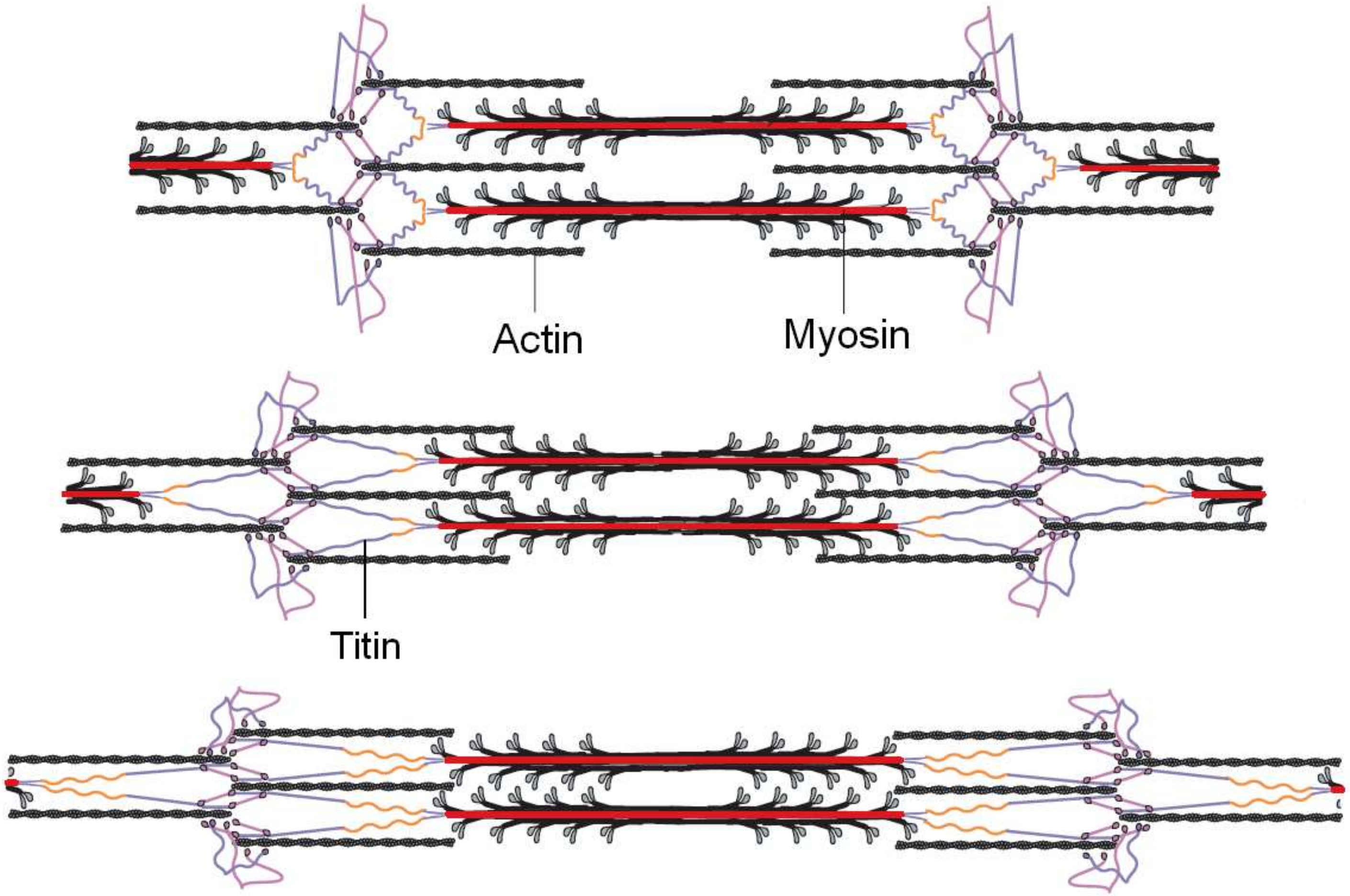
5.6 MEAT PROTEINS
Composition of meat

Type of meat	***Moisture***	***Proteins***	***Fat***	***Minerals***
Pork shoulder	74.9	19.5	4.7	1.1
Beef steak	74.6	22.0	2.2	1.2
Chicken breast	74.4	23.3	1.2	1.1

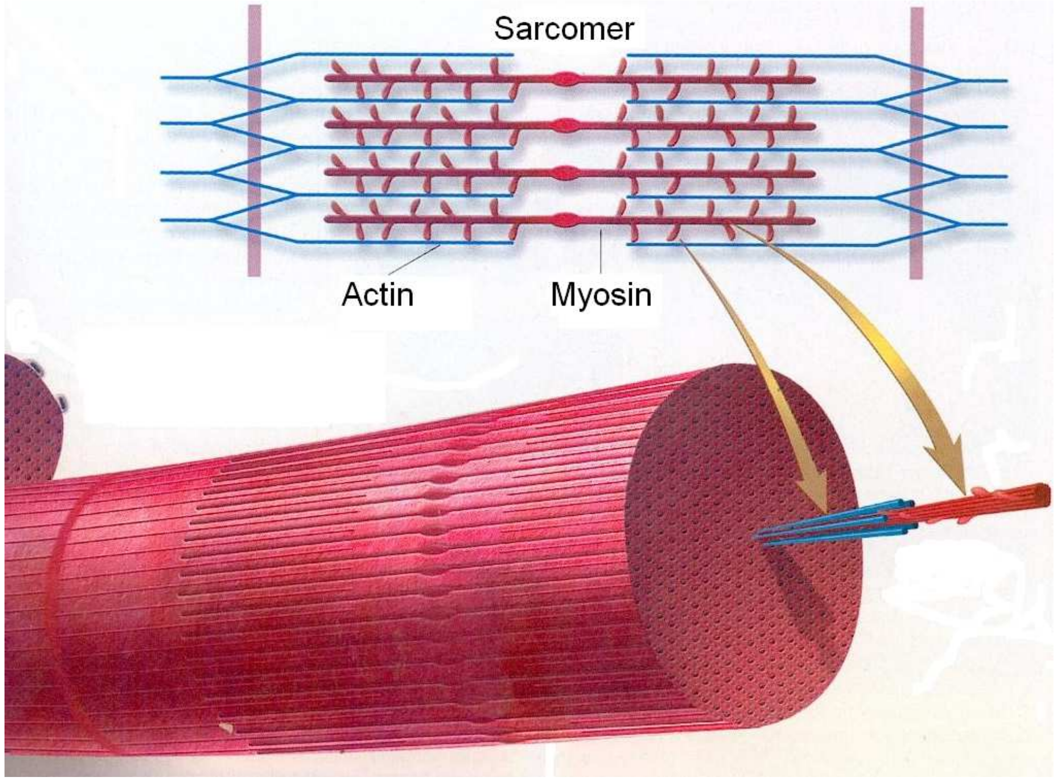
Type of proteins	***Proteins***	***Percentage***
Myofibrillar	Myosin	29
	Actin	13
	Total	61
Sarcoplastic	Hemoglobin	4
	Enzymes	24
	Total	29
Connective tissues	Collagen	6
	Elastin	1
	Total	10

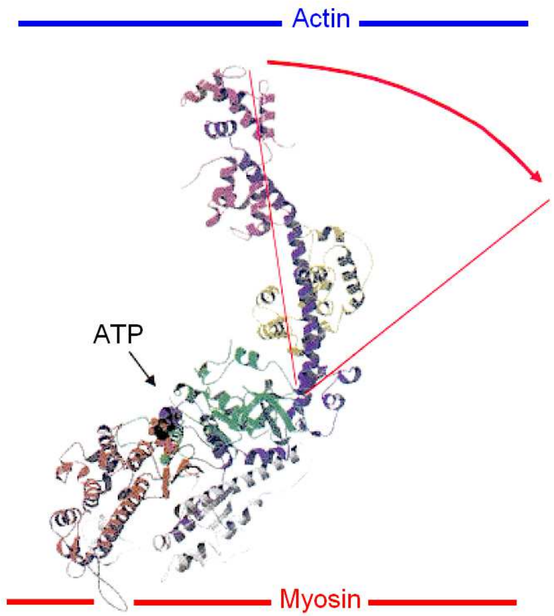
5.6 MEAT PROTEINS
Muscle constriction I

Sarcomere: striated muscle

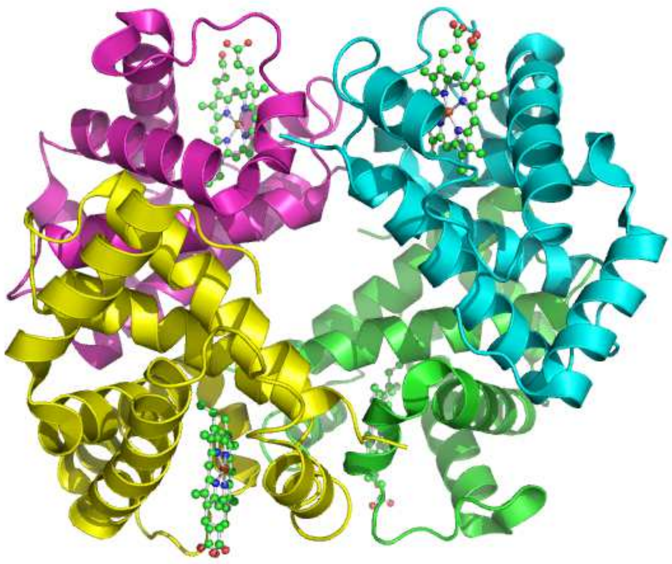


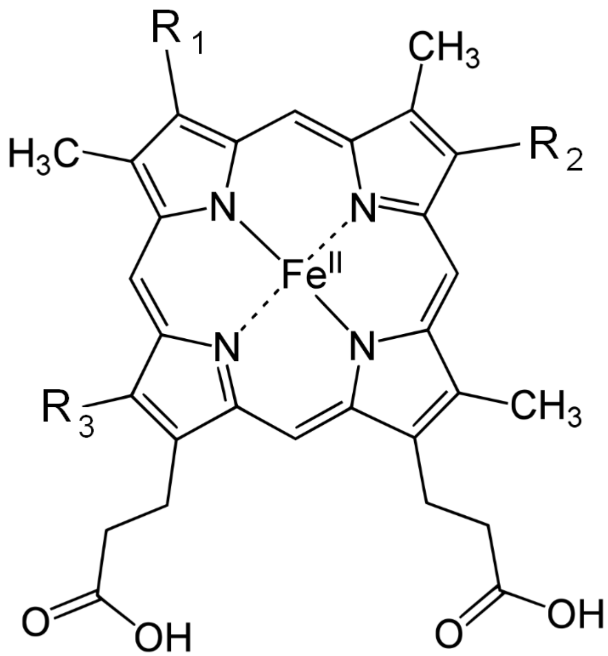
5.6 MEAT PROTEINS
Muscle constriction II





5.6 MEAT PROTEINS
Structure of hemoglobin

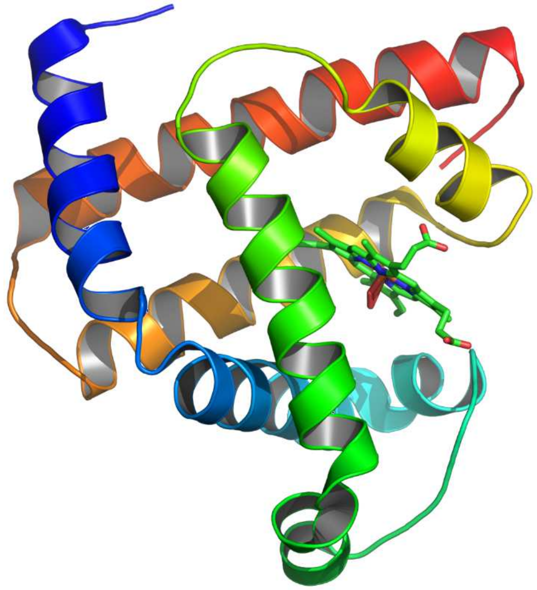




Hemoglobin is the iron(II)-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates. It has a quaternary structure with four sub-unit globular proteins.

The heme group consists of an iron ion held in a porphyrin ring. Iron coordinates with the four porphyrin nitrogens and to an histidine residue of the globular protein. The sixth position can reversibly bind O_2, CO, CO_2 NO or NO_2^- by a weak covalent bond.

5.6 MEAT PROTEINS
Structure of myoglobin



Myoglobin is a single-chain globular protein of 153 amino acids and stores oxygen in muscle tissues. It is not found in the blood stream (except in case of muscle injuries).

Other types of heme-proteins :

► Hemocyanin (Copper center)

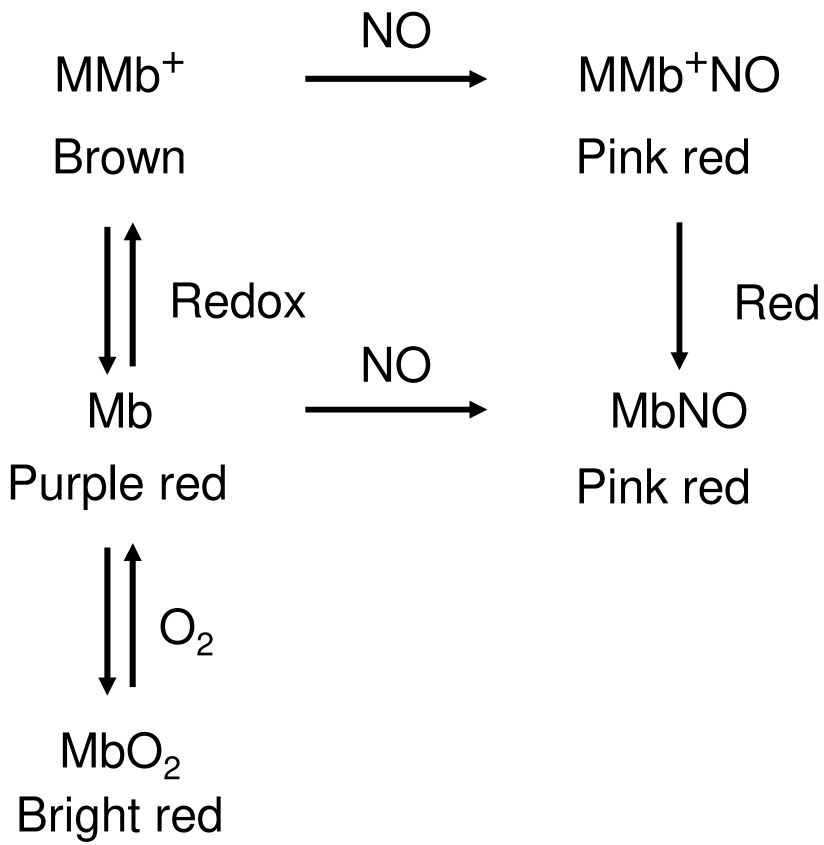
Transport and storage of oxygen in marine organisms and invertebrates.

► Chlorophyll (Magnesium center)

Absorption of light, first stage of photosynthesis.

5.6 MEAT PROTEINS

Myoglobin and meat colours

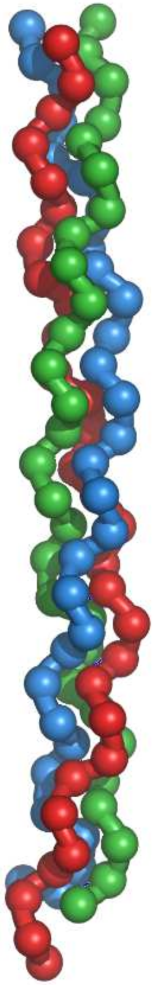


Center	*Ligand*	*Name*
Fe(II)	H_2O	Myoglobin (Mb)
	O_2	Oxymyoglobin
	NO	Nitrosomyoglobin
	CO	Carboxymyoglobin
	CN	Cyanmyoglobin
Fe(III)	OH^-	Metmyoglobin (MMb^{+})

NO is provided by meat curing : $NO_3^- \rightarrow NO_2^- \rightarrow NO$

5.6 MEAT PROTEINS

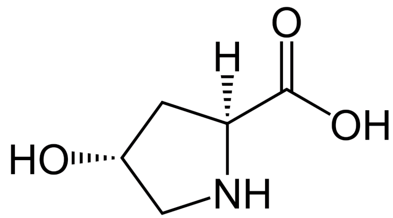
Connective tissues and collagen



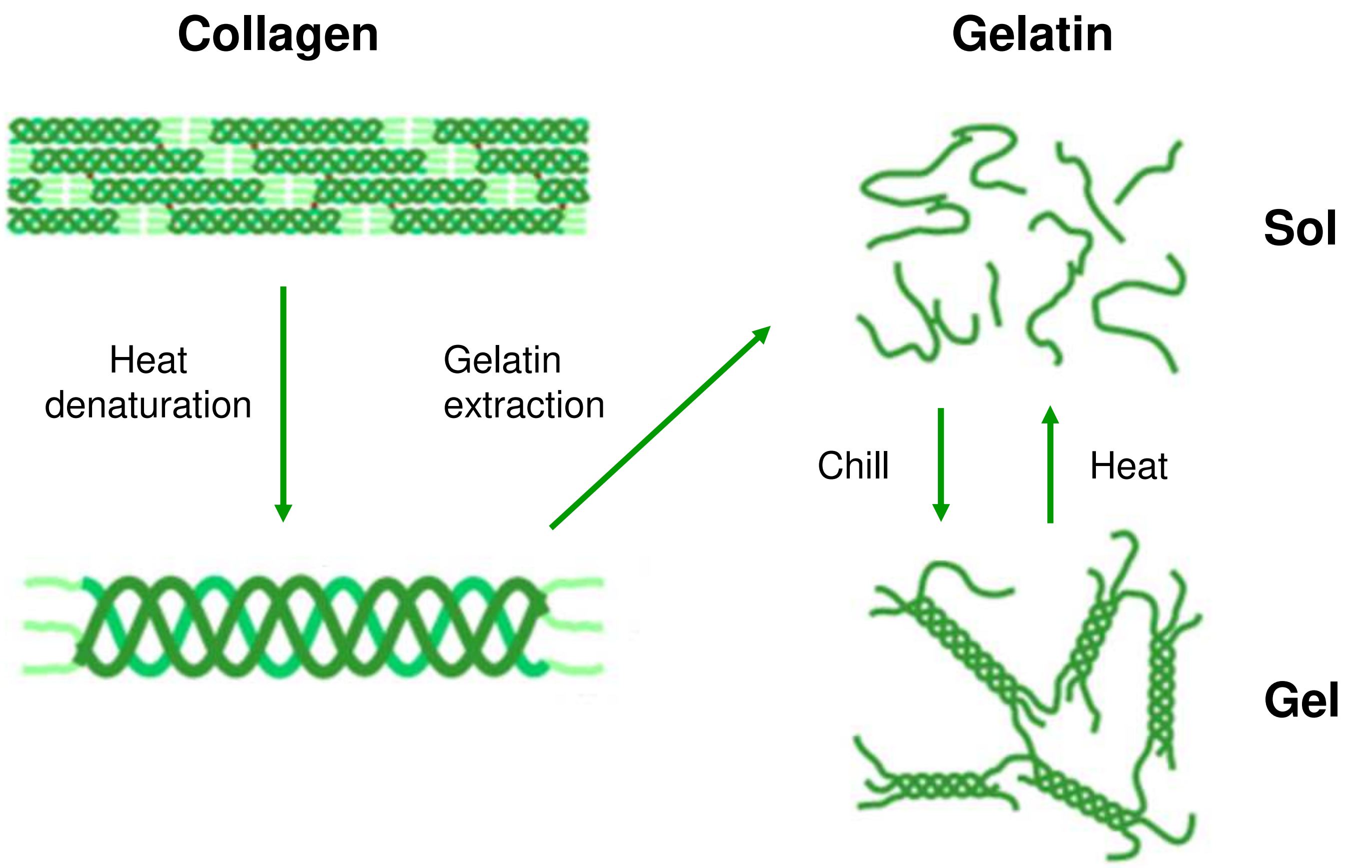
Connective tissues makes up a variety of physical structures including tendons,cartilage, bone and lymphatic tissue.

The main protein in connective tissues is collagen. The tropocollagen subunits are twisted together into a right-handed coiled coil, a cooperative quaternary structure.

Connective tissues are the only ones that contain hydroxyproline. This analysis is used to quantify the amount of connective tissues in meat products.



5.6 MEAT PROTEINS
Collagen and gelatin formation



5.6 MEAT PROTEINS
Production of surimi

Fish meat is ground in a mechanical separator that eliminates skin and bones. The pulp obtained is washed with sodium bicarbonate 0.5%, water and finally with saline. These washes are designed to eliminate low molecular weight and sarcoplasmic proteins, and supernatant fat brown muscle.

The remaining mass consisting mainly of actomyosin is pressed and supplemented with cryoprotectants such as sorbitol. It is called surimi.

Gelation sets up a protein network that is carried out in two stages, first denaturation followed by aggregation. Aggregation must be done very slowly so that proteins can associate and be properly oriented. The gel formed is then stabilized by steaming and is called kamaboko.

Cooking can also be done on thin films to be rolled into a cylinder to give artificial crab sticks.

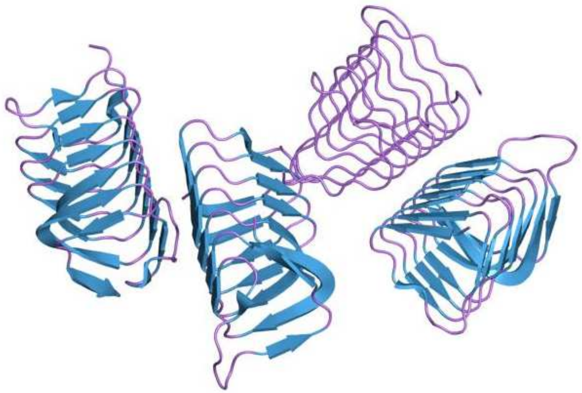
5.6 MEAT PROTEINS

Effects of ice structuring proteins (ISP)

Or **Antifreeze proteins**

- ISPs are naturally occurring proteins that bind to ice and are found mainly in fish, but also in vegetables, lichens, and bacteria

- The freezing point-depression activity of ISP is a non-colligative mechanism, thereby minimizing the effect on the osmotic pressure of cells



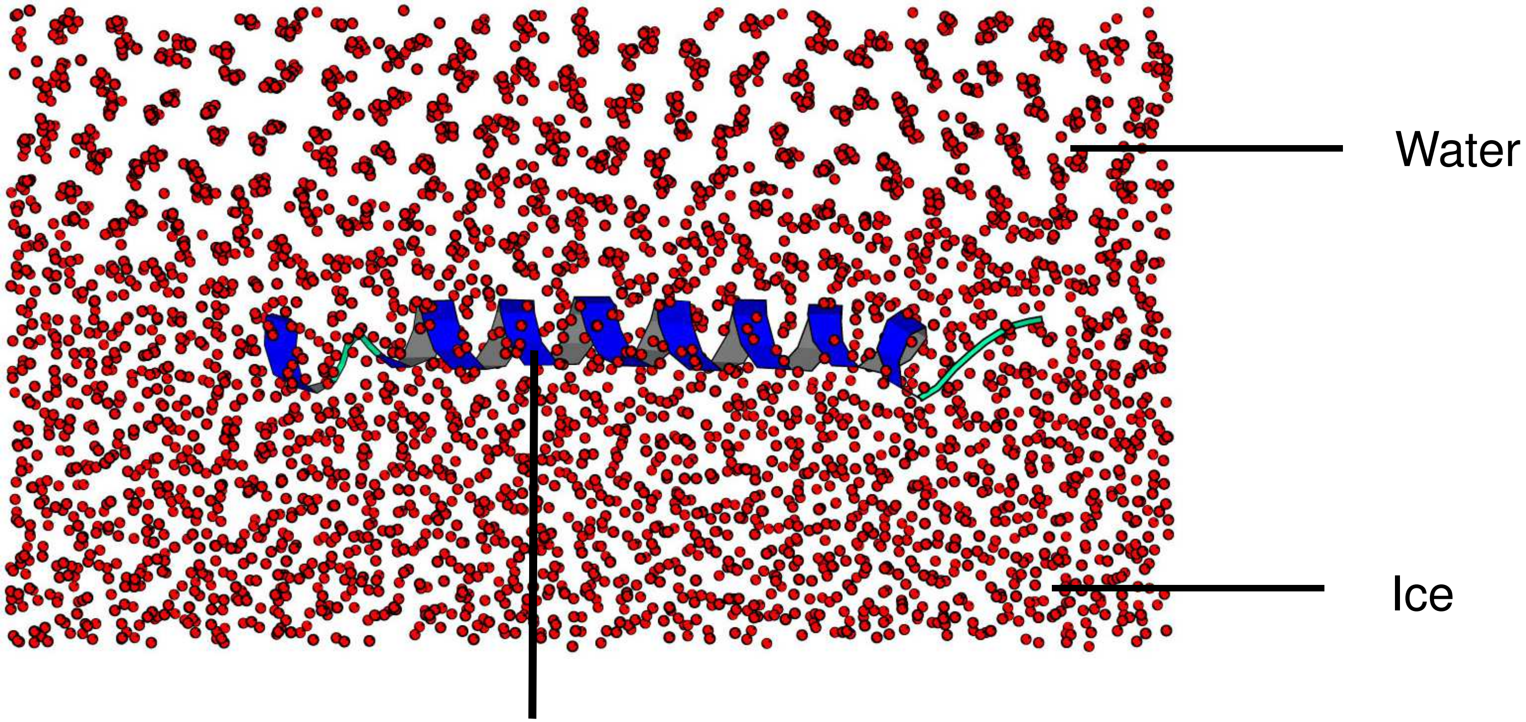
Spruce budworm ISP (polypeptides)

- They are 200 to 500 times more effective than sodium chloride

- Ice structuring proteins exert their effect by binding directly to the growing ice crystal and thereby modifying its size and morphology

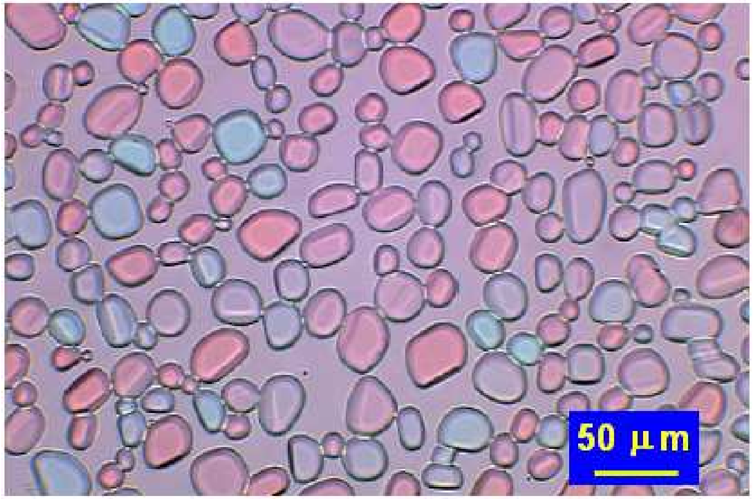
5.6 MEAT PROTEINS
Action of ice structuring proteins

Molecular dynamics simulation

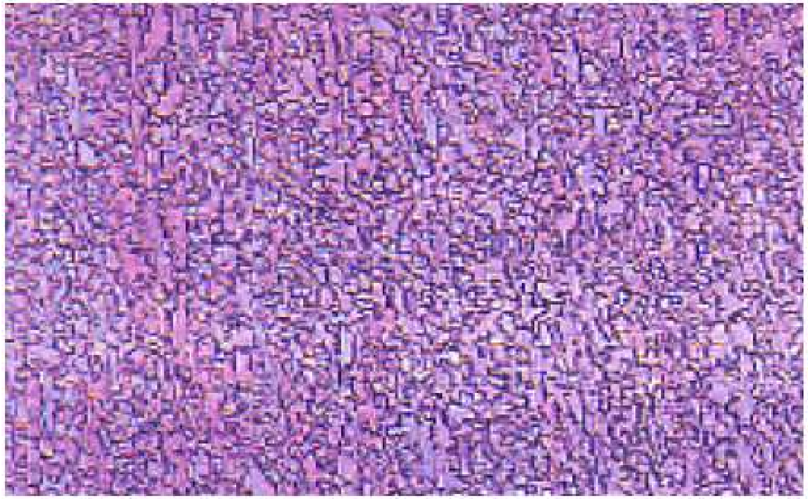


5.6 MEAT PROTEINS
Effects of ice structuring proteins

30% sucrose without ISP



30% sucrose with ISP

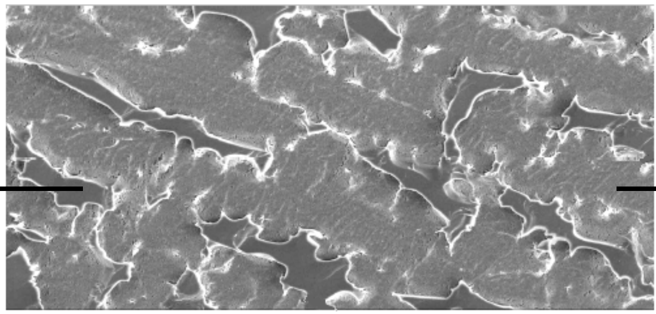


5.6 MEAT PROTEINS
Use of ice structuring proteins in ice cream production

Ice cream is a complex, frozen, aerated emulsion, made up of four phases: air, ice, fat, and matrix (matrix is the unfrozen part containing sugar, protein, stabilisers, flavours, and emulsifiers)

ISP ice creams are softer and mellower. They provide new texture and creaminess

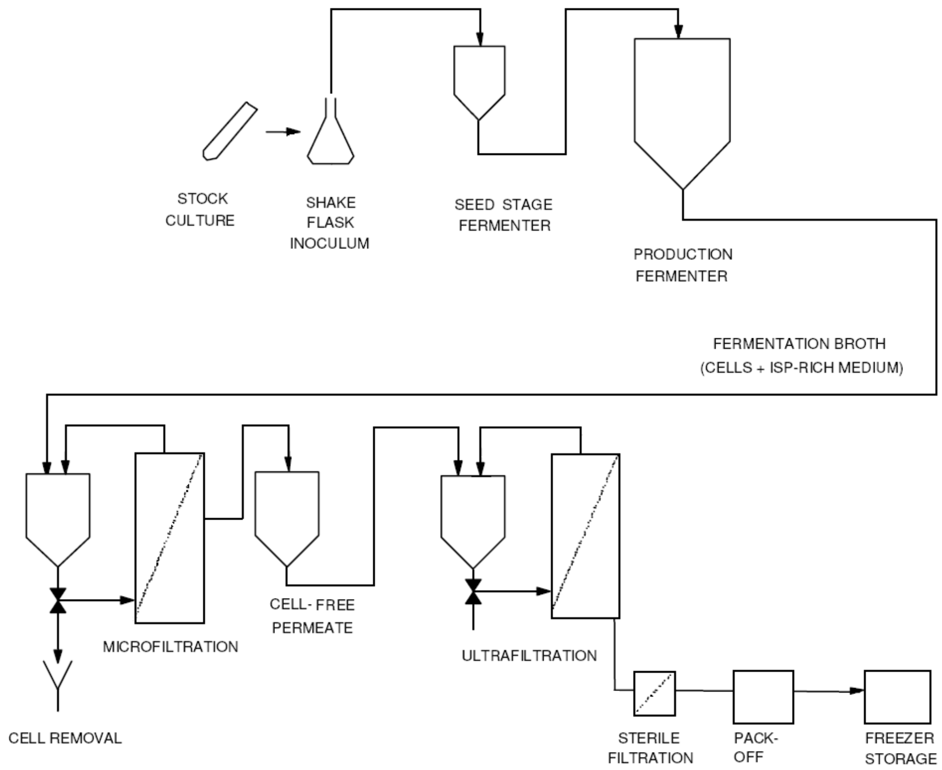
Ice structure with ISP



5.6 MEAT PROTEINS

Industrial ISP production (Unilever)

Fermentation system using a genetically modified baker's yeast (*Saccharomyces cerevisiae*) carrying a synthetic gene encoding for the ISP Type III HPLC 12 from ocean pout (*Macrozoarces americanus)*



5.7 MILK PROTEINS

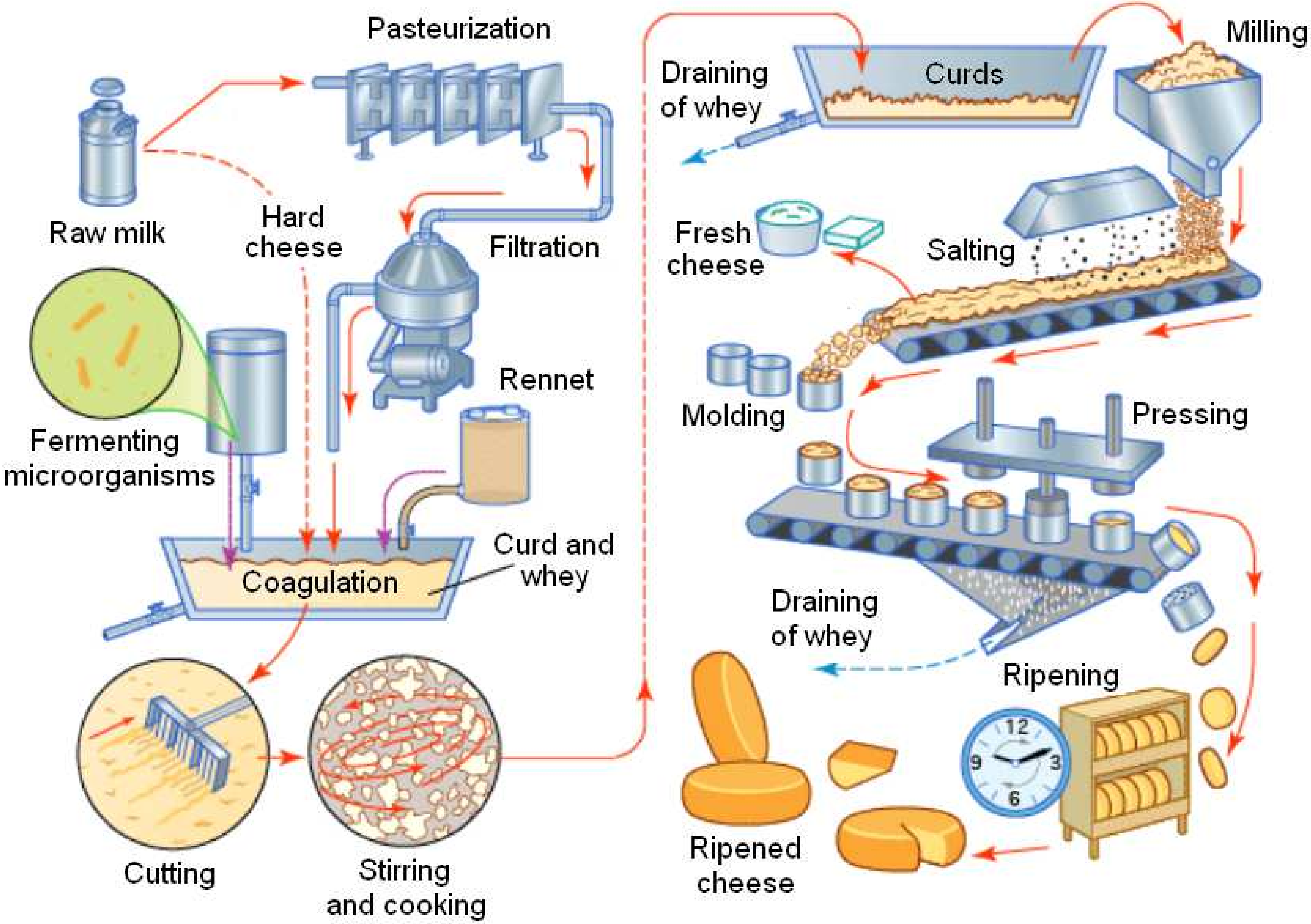
Proteins of bovine milk

Proteins	***Percentage***	***Molecular weight (kD)***
α-Casein	42	23.6 - 25.2
β-Casein	25	24.0
γ-Casein	4	11.6 - 20.5
κ-Casein	9	19.0
β-Lactoglobulin	9	18.3
α-Lactalbumin	4	14.2
Immunoglobulins	2	162 - 950

Caseins 80% (insoluble in acid media)

Whey proteins 20% (soluble)

5.7 MILK PROTEINS
Cheese production



5.7 MILK PROTEINS
Swiss cheese fondue

Cheese, consist mainly of insoluble calcium paracaseinate and fat globules. By chelating the calcium from the protein structure, the emulsifying salts contributes to enhance their emulsifying properties.

Calcium caseinate (insoluble)

$$\downarrow \begin{array}{l} -\ H^+ \\ +\ \text{Calcium ligands} \\ \Delta \end{array}$$

Monomers (soluble caseins)

Ca ligands : sodium citrate (lemon juice)
sodium tartrate (acidic wine)

pH adjustment : $NaHCO_3$

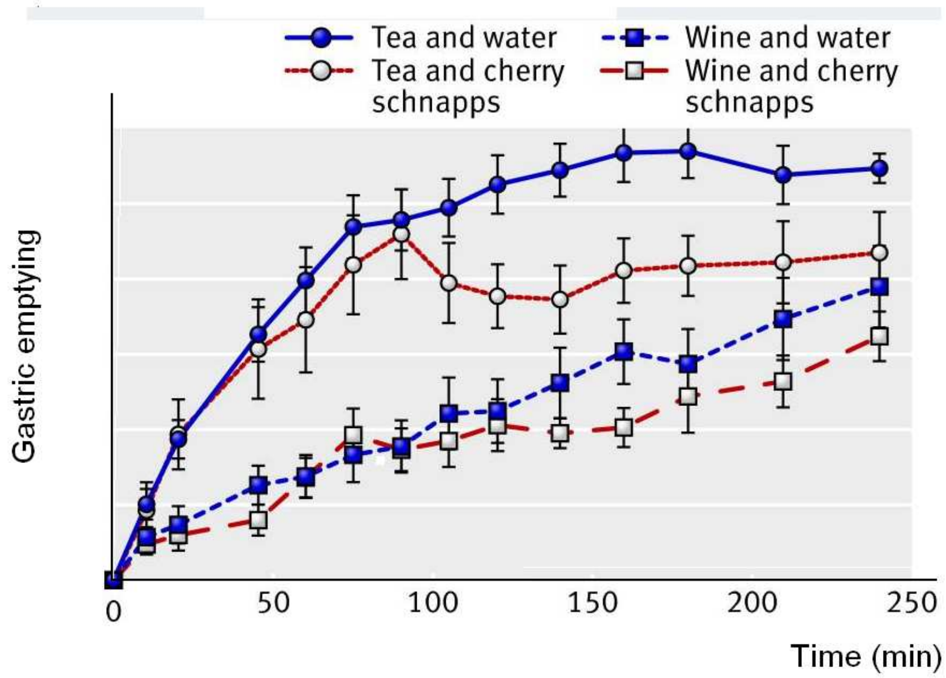
In aged cheese, caseins are partially degraded and present better emulsifying properties

Starch addition contributes to thicken the water phase, and prevents the coalescence of fat globules

5.7 MILK PROTEINS
What can we drink with a swiss cheese fondue ?

Cheese fondue (3260 kJ) labelled with 150 mg C^{13}-Na octanoate was consumed with 300 ml of white wine (13%) or black tea in randomised order, followed by 20 ml kirsch (40%) or water in randomised order.

20 healthy volunteers



*H. Heinrich & al., British Med. J., **341**, c6731 (2010)*

5.8 CEREALS PROTEINS

Separation of grain proteins

Osborne (1907) : 4 fractions based on sequential solubility

1. Water → Albumins
2. NaCl 0.4 mol/l → Globulins
3. EtOH 70% → Prolamins
4. Residue → Glutelins

Fractions	***Wheat***	***Rye***	***Oats***	***Barley***	***Corn***	***Rice***
Albumins	Leukosin					
Globulins	Edestin		Avenalin			
Prolamins	Gliadin	Secalin	Avenin	Hordein	Zein	Oryzin
Glutelins	Glutenin	Secalinin		Hordenin	Zeanin	Oryzenin

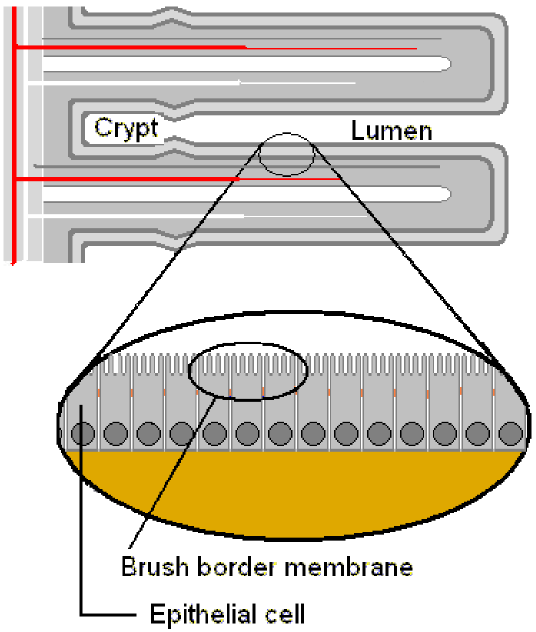
5.8 CEREALS PROTEINS

Bread production

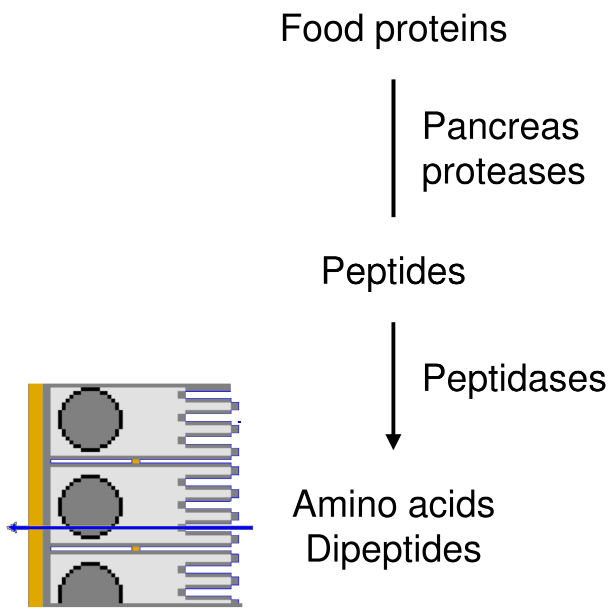
Mixing	Homogeneization of ingredients
Kneading	Degradation of starch through α-amylase Crosslinking of glutenin (backbone) and gliadin (viscosity) Formation of –s–s– bonds between gluten proteins
Fermentation	Production of CO_2 and EtOH through alcoholic fermentation Formation of a foamy structure (CO_2 bubbles embedded in gluten membranes)
Baking	Formation of a sponge-like structure (expansion of CO_2 bubbles) Solidification of the membranes (proteins denaturation) Formation of aroma compounds (Maillard reactions)

5.8 CEREALS PROTEINS

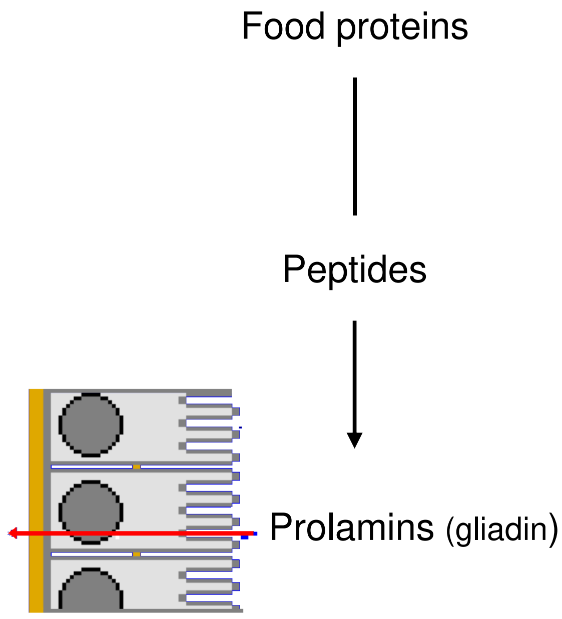
Coeliak disease



Normal intestine



Normal gut



Coeliak gut

Prolamins (glutamine&proline) cause membrane leaking, allowing peptides to enter the cells, causing inflammations and cell disruption. (truncating of the villi)

5.9 SOYBEAN PROTEINS
Soybean products

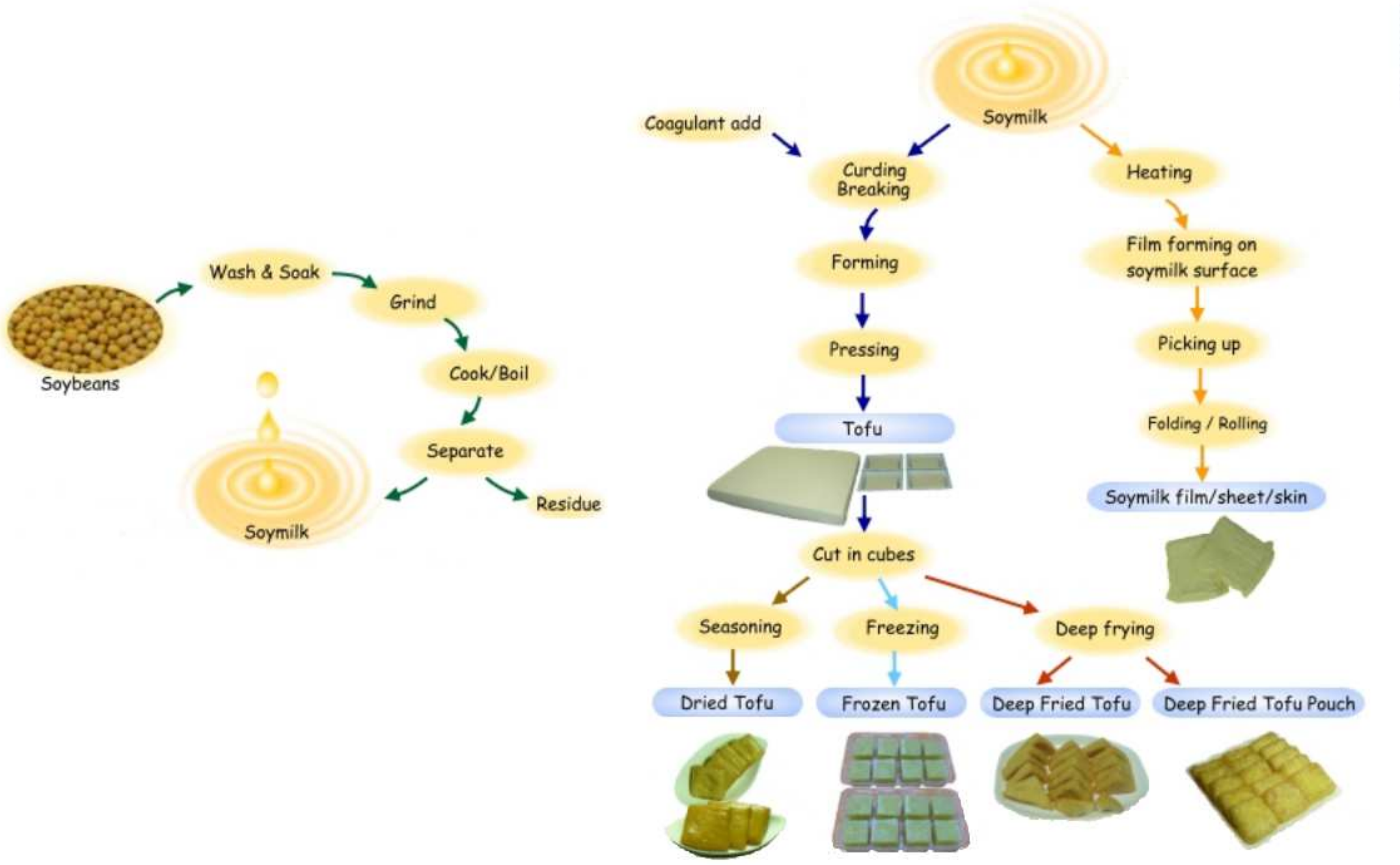
Soybean is one of the legumes with the highest protein contents (40%)

It contains two Osborne fractions, albumins (10%) and globulins (90%)

The globulin fraction is mainly formed of Vicilin, Glycinin and Conglycinin

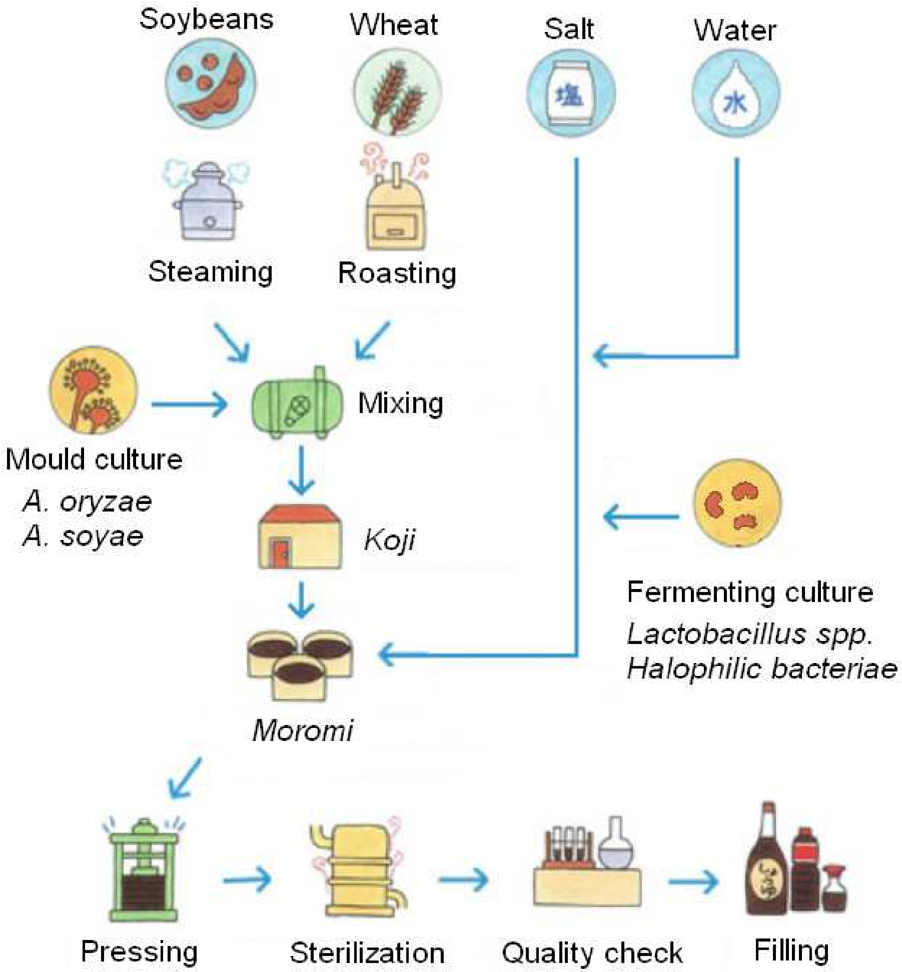
Soy milk	Stable emulsion produced by grinding soaked soybeans with water
Tofu	Coagulated soy milk, pressed into soft white blocks
Soy sauce	Condiment produced by fermenting soybeans with water and salt
Miso	Japanese seasoning produced by fermenting soymeal

5.9 SOYBEAN PROTEINS
Soymilk and tofu production



5.9 SOYBEAN PROTEINS

Soy sauce production



5.10 NUTRITIONAL ASPECTS
Essential amino acids

Amino acids	***RDA (mg per kg BW)***
Isoleucine	20
Leucine	40
Lysine	30
Methionine + Cysteine	15 (total)
Phenylalanine + Tyrosine	25 (total)
Threonine	15
Tryptophan	5
Valine	25

Recommended Dietary Allowance

5.10 NUTRITIONAL ASPECTS

Measures of proteins nutritional quality

Biological value (BV)

Measure of the proportion of absorbed protein which becomes incorporated into the proteins of the body.

$$\boxed{BV = (N_r / N_a) * 100}$$

N_a = nitrogen absorbed in proteins on the test diet
N_r = nitrogen incorporated into the body on the test diet

Protein efficiency ratio (PER)

Weight gain of a test subject divided by its intake of a particular food.

$$\boxed{PER = \text{Gain in body mass} / \text{Protein intake}}$$

5.10 NUTRITIONAL ASPECTS
Protein Digestibility Corrected Amino Acid Score

Evaluation of the protein quality based on both the amino acid requirements of humans and their ability to digest it.

$$\text{PDCAAS} = \frac{\text{Mass of limiting aa in test protein}}{\text{Mass of same aa in reference protein}} \times \text{digestibility percentage}$$

Adopted by the US Food and Drug Administration (FDA) and the Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) in 1993 as the preferred best method to determine protein quality.

5.10 NUTRITIONAL ASPECTS
Comparison of protein quality measures

Food	***BV***	***PER***	***PDCAAS***	***Limiting aa***
Chicken egg	94	3.9	1.00	
Cow's milk	91	3.1	1.00	Met
Fish	76	3.5	0.95	Thr
Beef	74	2.3	0.92	Met
Soybeans	73	2.3	0.91	Met
Rice	86	2.2	0.59	Lys, Tyr
Wheat	54	1.5	0.42	Lys, Thr

5.10 NUTRITIONAL ASPECTS
Tryptophan and post-Thanksgiving dinner drowsiness

Tryptophan can be metabolized into serotonin and melatonin, neurotransmitters that exert a calming effect and regulates sleep

➡ High tryptophan contents of turkey has been cited as the cause of « post-Thanksgiving dinner drowziness »

⇨ Turkey : 0.33 % Trp   Chicken : 0.28 % Trp (no sleep-inducing effect)

⇨ Carbohydrate rich meal induces insulin production, which in turn enhances Trp concentration in the brain, thus producing more serotonin

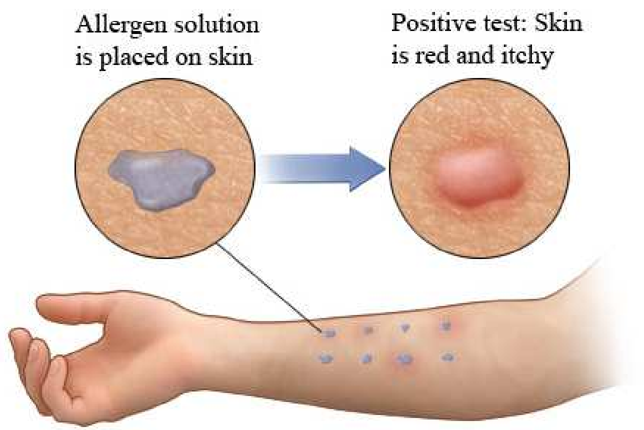
⇨ Fat rich meal take a lot of energy to digest, so blood will be redirected to the digestive system, meaning less bloodflow and less energy elsewhere

⇨ Drinking alcohol (a central nervous system depressant) during the meal will add to the nap-factor

5.10 NUTRITIONAL ASPECTS
Food allergies due to proteins

Immunological intolerances		Estimated frequencies
Coeliac disease		1 %
Food allergies	Adults	2 – 4 %
	Children up to 17 years	3 – 4 %
	Babies	2 – 6 %

Pollen	Food reponsible of cross-reactions
Birch	Apricots, almonds, peanuts, carrots, celery, cherries, kiwis, nectarines, hazelnuts, peaches, pears, apples, potatoes, plums, soy
Grass	Peanuts, chard ribs, melons, oranges, potatoes, tomatoes
Mugwort	Bananas, cucumbers, zucchini, melons
Plane tree	Peanuts, green beans, kiwi, lettuce, corn, melons, nuts, peaches, chickpeas, apples



5.11 WORLD CONSUMPTION OF PROTEINS

Environmental effects of meat and vegetable proteins

Environmental impact	*Arbitrary effect of proteins from food based on soybean*	*Relative effect of proteins from food based on meat and dairy*
Land use	1	6 - 17
Water	1	4 - 26
Fossil fuel	1	6 - 20
Fertilizers	1	7
Pesticides	1	6

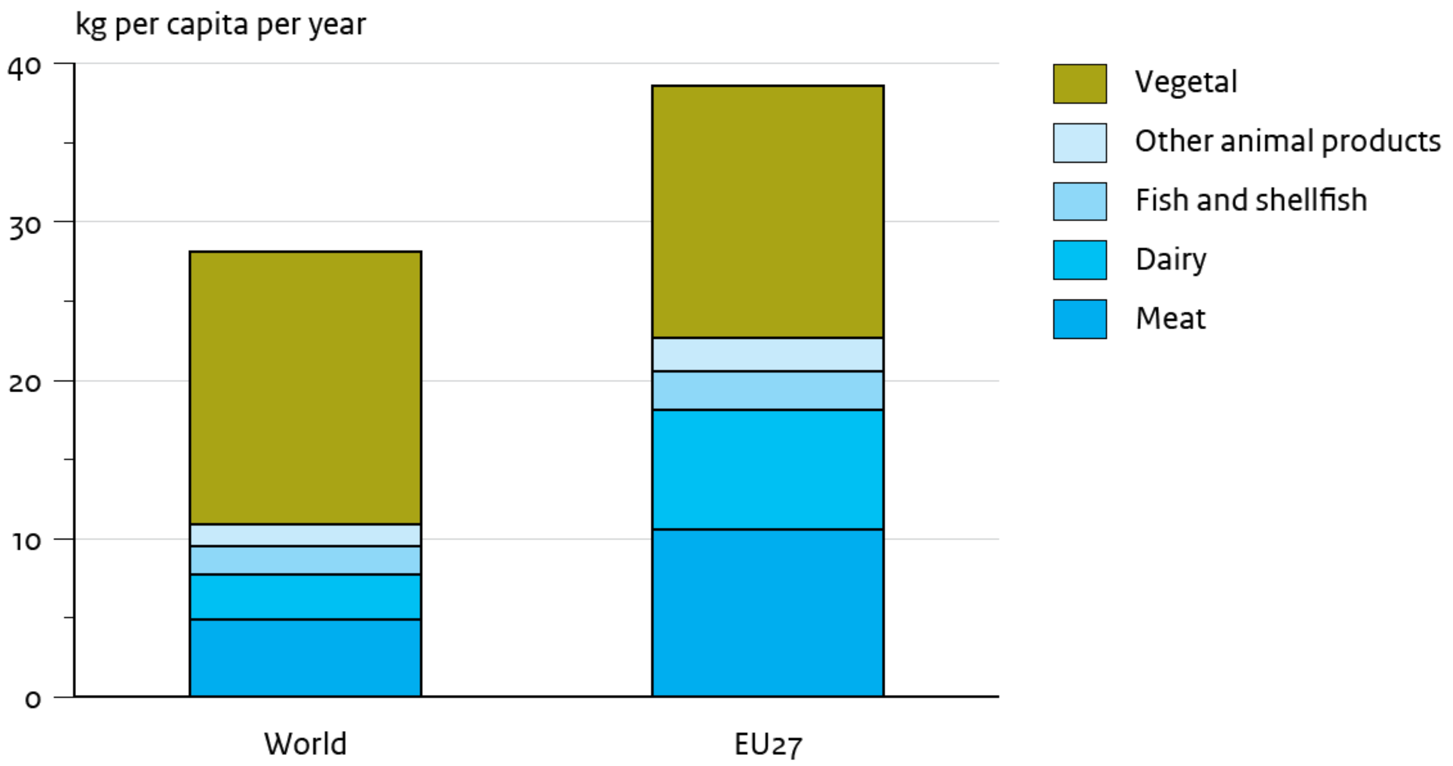
5.11 WORLD CONSUMPTION OF PROTEINS

Proteins productivity

	Soybean	***Rice***	***Milk***	***Wheat***	***Eggs***	***Maize***	***Beef***
Edible proteins (g/m^2)	40	29	9.2	15.0	8.5	24.0	2.2
Usable proteins (g/m^2)	29	25	8.4	8.1	8.0	7.7	1.7

5.11 WORLD CONSUMPTION OF PROTEINS

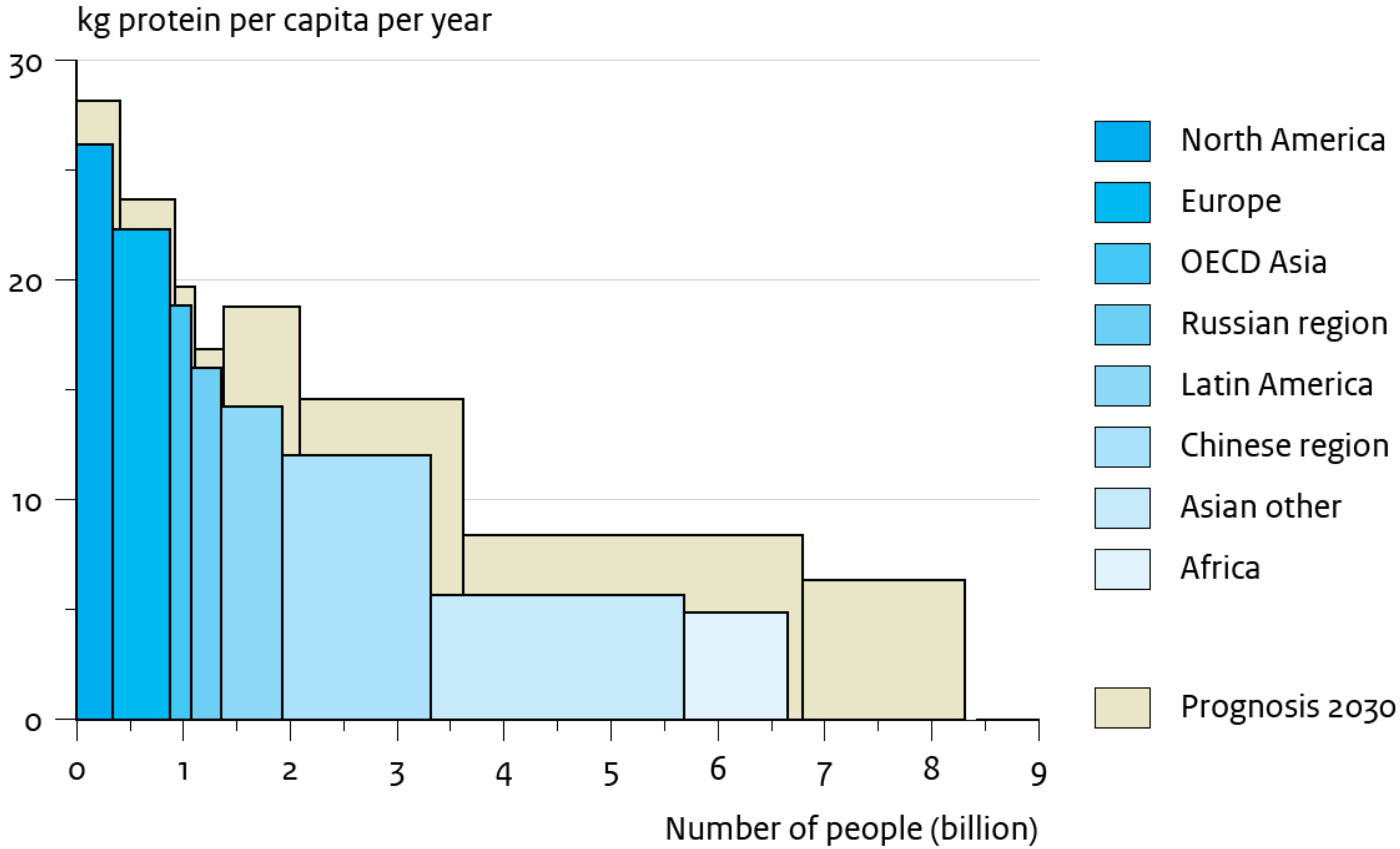
Protein supply (FAO, 2007)



Livestock production uses around 80% of global agricultural land !

5.11 WORLD CONSUMPTION OF PROTEINS

Global intake of animal proteins (FAO, 2007)



5.11 WORLD CONSUMPTION OF PROTEINS

Global protein production