FDE 303
FOOD CHEMISTRY
WEEK-5

Amino Acids, Peptides and Proteins

---Continued---

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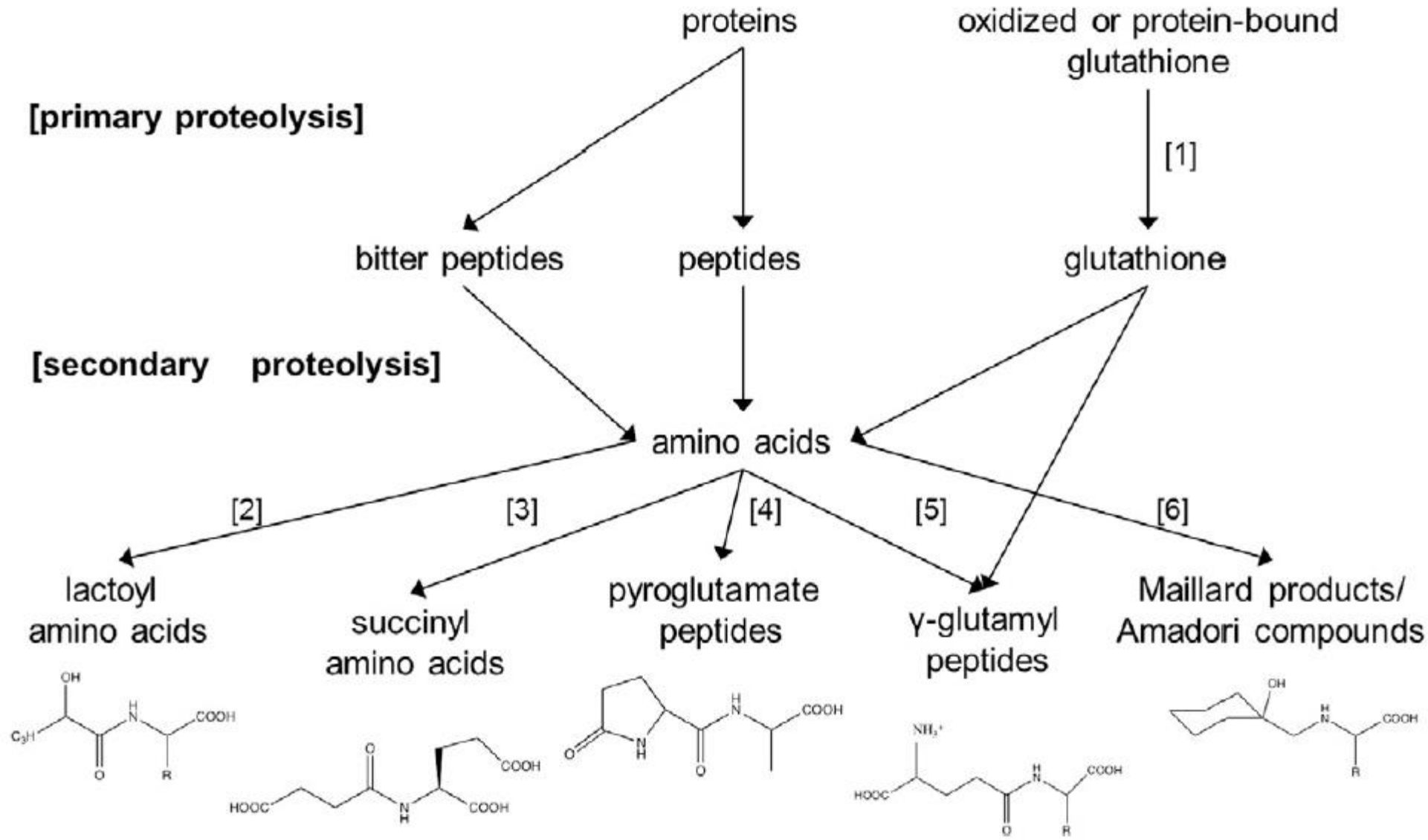
Overview on the generation of taste compounds from proteins during food fermentation



Zhao et al. 2016

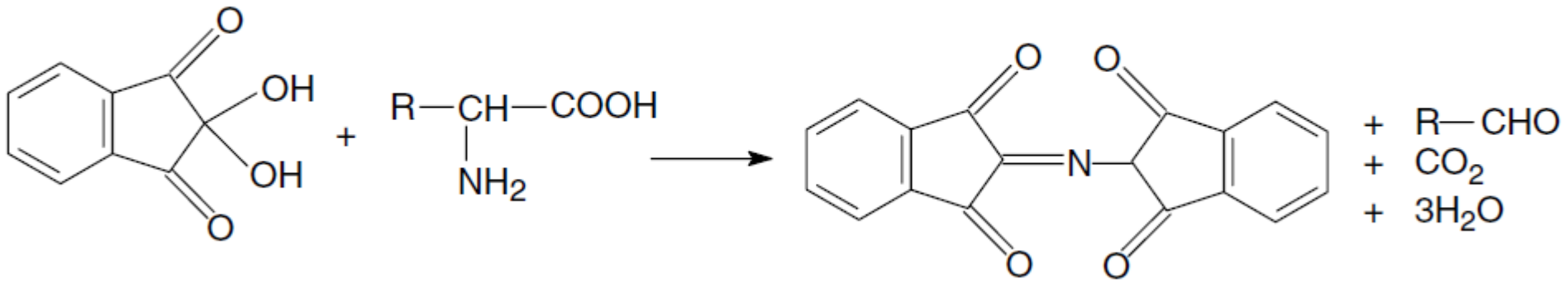
Chemical Reactivity of Amino Acids

- Several reactions for various side-chain groups can be used to alter the hydrophilic and hydrophobic properties and the functional properties of proteins and peptides.
  - may occur during storage, processing or cooking
  - can be used to alter protein ingredient functionality
  - can be used to analytically measure proteins
- Some of these reactions also can be used to quantify amino acids and specific amino acid residues in proteins.
  - For example, reaction of amino acids with ninhydrin, *O*-phthaldialdehyde, or fluorescamine is regularly used in the quantification of amino acids.

Chemical Reactivity of Amino Acids

Reaction with ninhydrin:

- This reaction is often used to quantify free amino acids. When an amino acid is reacted with an excess amount of ninhydrin, one mole each of ammonia, aldehyde, CO_2, and hydrindantin are formed for every mole of amino acid consumed:



- The liberated ammonia subsequently reacts with one mole of ninhydrin and one mole of hydrindantin, forming a purple color product known as *Ruhemann's purple*, which has maximum absorbance *at 570 nm*.

Chemical Reactivity of Amino Acids

Reaction with ninhydrin:

- *Proline and hydroxyproline* give a *yellow color* product, which has maximum absorbance *at 440 nm*.

- These color reactions provide the basis for colorimetric determination of amino acids.

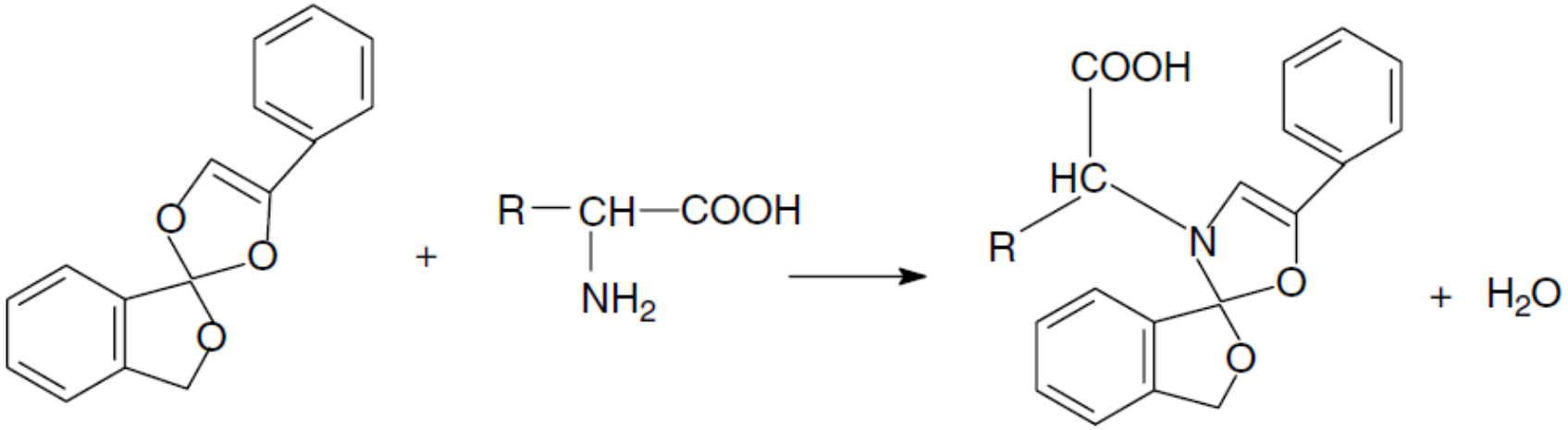
- The use of ninhydrin reaction to determine the amino acid composition of proteins:

  - In this case, the protein is first acid hydrolyzed to the amino acid level.
  - The freed amino acids are then separated and identified using ion exchange/hydrophobic chromatography.
  - The column eluates are reacted with ninhydrin and quantified by measuring absorbance at *570 and 440 nm*.

Chemical Reactivity of Amino Acids

Reaction with fluorescamine:

- Reaction of amino acids, peptides, and proteins containing primary amines with fluorescamine yields a highly fluorescent derivative with fluorescence emission maximum at 475 nm when excited at 390 nm.



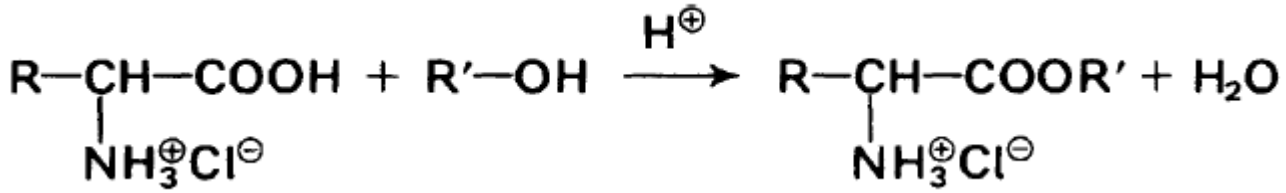
Fluorescamine

- This method can be used to quantify amino acids as well as proteins and peptides.

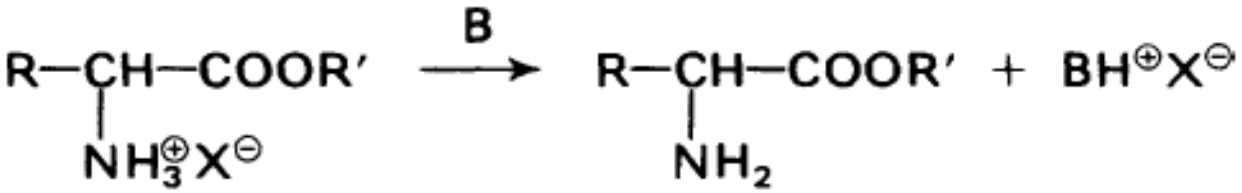
Chemical Reactivity of Amino Acids

Esterification of Carboxyl Groups:

- Amino acids are readily esterified by acid catalyzed reactions. An ethyl ester hydrochloride is obtained in ethanol in the presence of HCl:



- The free ester is released from its salt by the action of alkali. A mixture of free esters can then be separated by distillation without decomposition. Fractional distillation of esters is the basis of a method introduced by *Emil Fischer* for the separation of amino acids:



- Free amino acid esters have a tendency to form cyclic dipeptides or open-chain polypeptides.

Chemical Reactivity of Amino Acids

Reactions of Amino Acids at Higher Temperatures

- Important during the preparation of food
  - Frying, roasting, boiling and baking develop the typical aromas of many foods in which amino acids participate as precursors.
- Characteristic odorants are formed via the *Maillard* reaction.
  - they are subsequent products, in particular of cysteine, methionine, ornithine and proline.

Chemical Reactivity of Amino Acids

Reactions of amino acids at higher temperatures

Acrylamide:

- One of the volatile compounds formed during the heating of food.
- This toxic compound is produced in reactions of *asparagine* with reductive carbohydrates or from the resulting cleavage products (e. g., 2-butanedione, 2-oxopropanal).
- The formation is promoted by temperatures >100◦C and/or longer reaction times.
- *Cysteine and methionine* also form acrylamide in the presence of glucose, but the yields are considerably lower than those from asparagine.

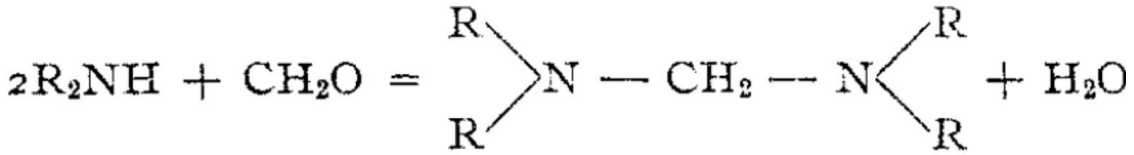
Mutagenic Heterocyclic Compounds (Heterocyclic Aromatic Amines):

- Pyrolyzates of amino acids and proteins are responsible for that effect.
- Mutagenic compounds isolated from amino acid pyrolyzates
  - **pyridoindoles, pyridoimidazoles and tetra-azafluoroanthenes**.
- They can also be formed at lower temperatures.

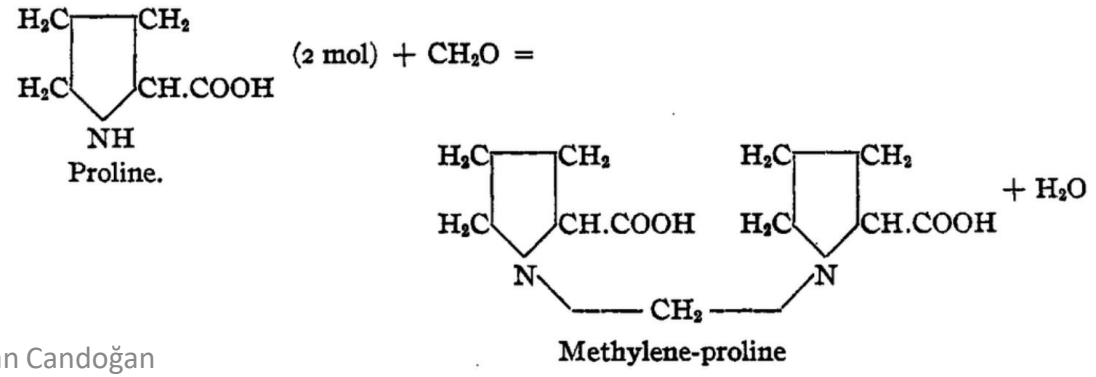
Sørensen formol titration

- Titration of an amino acid with a base such as sodium hydroxide in the presence of formaldehyde
  - Formaldehyde reacts with the amino group of an amino acid to form methylene amino (R-N=CH_2) group. Remaining acidic carboxylic acid group can then again be titrated with a base.
- Formol titration: after the addition of formaldehyde to a solution of the sample at pH 8–9, the free amino acids are determined by titration with sodium hydroxide solution
- The formol number (or formol index) is used to evaluate the quality of fruit juices and wines

Formol titration equation for secondary amines



Formol titration equation for proline



Solubility of amino acids

- The solubilities of amino acids in water are highly variable.
  - Besides the extremely soluble proline, hydroxyproline, glycine and alanine are also quite soluble.
  - Other amino acids are significantly less soluble.
  - Cystine and tyrosine have particularly low solubilities.
- Addition of acids or bases improves the solubility through salt formation.
- The presence of other amino acids, in general, also brings about an increase in solubility.
  - Thus, the extent of solubility of amino acids in a protein hydrolysate is different than that observed for the individual components.
- The solubility in organic solvents is not very good because of the polar characteristics of the amino acids.
  - All amino acids are insoluble in ether.

Nutritional properties of proteins

- The “quality” of a protein is related mainly to its essential amino acids content and digestibility.

- High quality proteins are those that contain all the essential amino acids at levels greater than the FAO/WHO/UNU reference levels, and a digestibility comparable to or better than those of egg white or milk proteins.

- Animal proteins are of better “quality” than plant proteins.

Classification of amino acids based on nutritional/physiological roles

- ***Essential amino acids:*** Among all amino acids, 8 of them have to be provided in the diet for adults. These amino acids are defined as 'essential' or 'indispensable' amino acids:
  - Leucine, Isoleucine, Valine, Threonine, Methionine, Phenylalanine, Tryptophan, Lysine

- ***'Conditionally' essential amino acids:*** In children, arginine, histidine, cysteine, glycine, tyrosine, glutamine and proline are also considered to be essential (indispensable) amino acids, because children are unable to make enough to meet their needs.

  - There may also be certain disease states during adult life when a particular amino acid becomes conditionally essential.

- The other amino acids are known as 'non-essential' or 'dispensable' amino acids.
  - They do not have to be provided by the diet.

Source: British Nutrition Foundation (https://www.nutrition.org.uk/)

Classification of amino acids based on nutritional/physiological roles

- Proteins of major cereals and legumes are often deficient in at least one of the essential amino acids.
  - Cereals proteins, such as rice, wheat, barley, and maize are very low in lysine and rich in methionine
  - Legumes and oilseeds proteins are deficient in methionine and rich or adequate in lysine
  - Some oilseed proteins, such as peanut protein, are deficient in both methionine and lysine
- The essential amino acids whose concentrations in a protein are below the levels of a reference protein are termed «*limiting amino acids*».

Digestibility of Various Food Proteins in Humans

- Protein quality also depends on the extent to which these amino acids are utilized in the body.
- Digestibility (bioavailability) of amino acids can affect the quality of proteins.

- Food proteins of animal origin are more completely digested than those of plant origin.
- Several factors affect digestibility of proteins.

Protein Source	Digestibility (%)	Protein Source	Digestibility (%)
Egg	97	Millet	79
Milk, cheese	95	Peas	88
Meat, fish	94	Peanut	94
Maize	85	Soy flour	86
Rice (polished)	88	Soy protein isolate	95
Wheat, whole	86	Beans	78
Wheat flour, white	96	Corn, cereal	70
Wheat gluten	99	Wheat, cereal	77
Oatmeal	86	Rice cereal	75

Chemical Alteration of Amino Acids

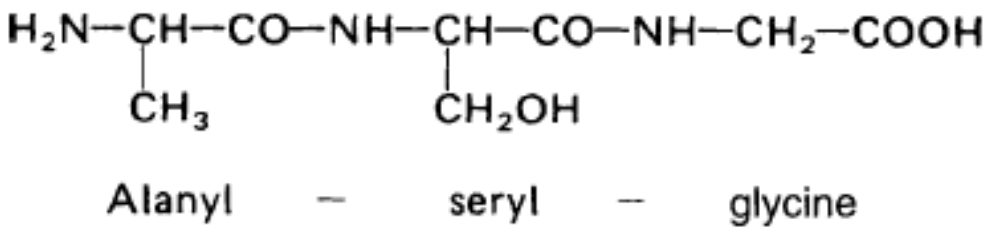
- Proteins undergo several chemical changes when processed at high temperatures:
  - Racemization
  - Hydrolysis
  - Desulfuration
  - Deamidation
- Most of these chemical changes are irreversible, and some of these reactions result in formation of modified amino acid types that are potentially toxic.

Peptides

- Peptides are formed by binding amino acids together through an amide linkage.
- Peptides are denoted by the number of amino acid residues as di-, tri-, tetrapeptides, etc., and the term “oligopeptides” is used for those with 10 or less amino acid residues.
- Higher molecular weight peptides are called polypeptides.
- The transition of “polypeptide” to “protein”: in general the limit is commonly assumed to be at a molecular weight of about 10 kdal, i. e., about 100 amino acid residues are needed in the chain for it to be called a protein.

Peptides

- Peptides are interpreted as acylated amino acids:



Ala Ser Gly or **ASG**

- The first three letters of the amino acids are used as symbols to simplify designation of peptides.

Sensory Properties

- Peptides, except for the sweet dipeptide esters of aspartic acid are neutral or bitter in taste with no relationship to configuration

❖ Taste of amino acids

Sweet amino acids are in general D- amino acids.

L-amino acids are bitter.

Sweet amino acids: Ala, Gly, Lys,Ser, Thr

Bitter amino acids : Arg, His, Ile, Leu, Phe, Trp, Tyr

Neutral amino acids: Asn, Asp, Cys, Gln

Umami: Glu

Sulphurous: Met

Sweet/Bitter: Pro

Peptides

- Peptides are widespread in nature.
  - They are often involved in specific biological activities (peptide hormones, peptide toxins, peptide antibiotics).
  - A number of peptides of interest to food chemists

- Glutathione (γ-L-glutamyl-L-cysteinyl-glyci-ne)
- Carnosine: predominant in beef muscle tissue
- Anserine: predominant in chicken meat
- Balenine: a characteristic constituent of whale muscle
- Nisin: is formed by several strains of *Streptococcus lactis*, has antimicrobial activity
- Lysine Peptides
  - Examples: Gly-Lys, Ala-Lys, Glu-Lys
  - These peptides substantially retard the browning reaction with glucose, hence they are suitable for lysine fortification of sugar-containing foods which must be heat treated.