

动物营养中的蛋白质水解物: 工业生产, 活性肽和功能上的意义

Protein Hydrolysates in Animal Nutrition: Industrial Production, Bioactive Peptides, and Functional Significance

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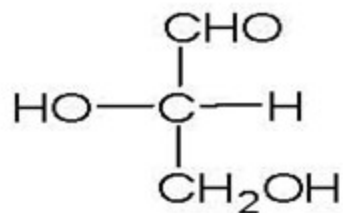
氨基酸，肽和蛋白质的定义

I. Definitions of Amino Acids, Peptides, and Protein

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猪业国际论坛

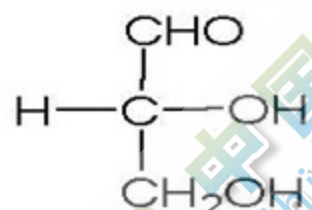
氨基酸的化学结构

Chemical Configurations of Amino Acids



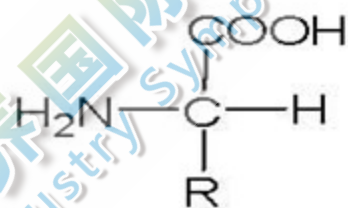
L-Glyceraldehyde

L-甘油醛



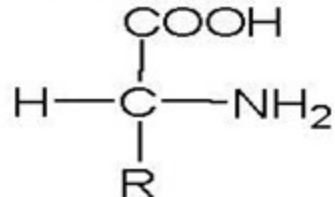
D-Glyceraldehyde

D-甘油醛



L-Amino Acid

L-氨基酸

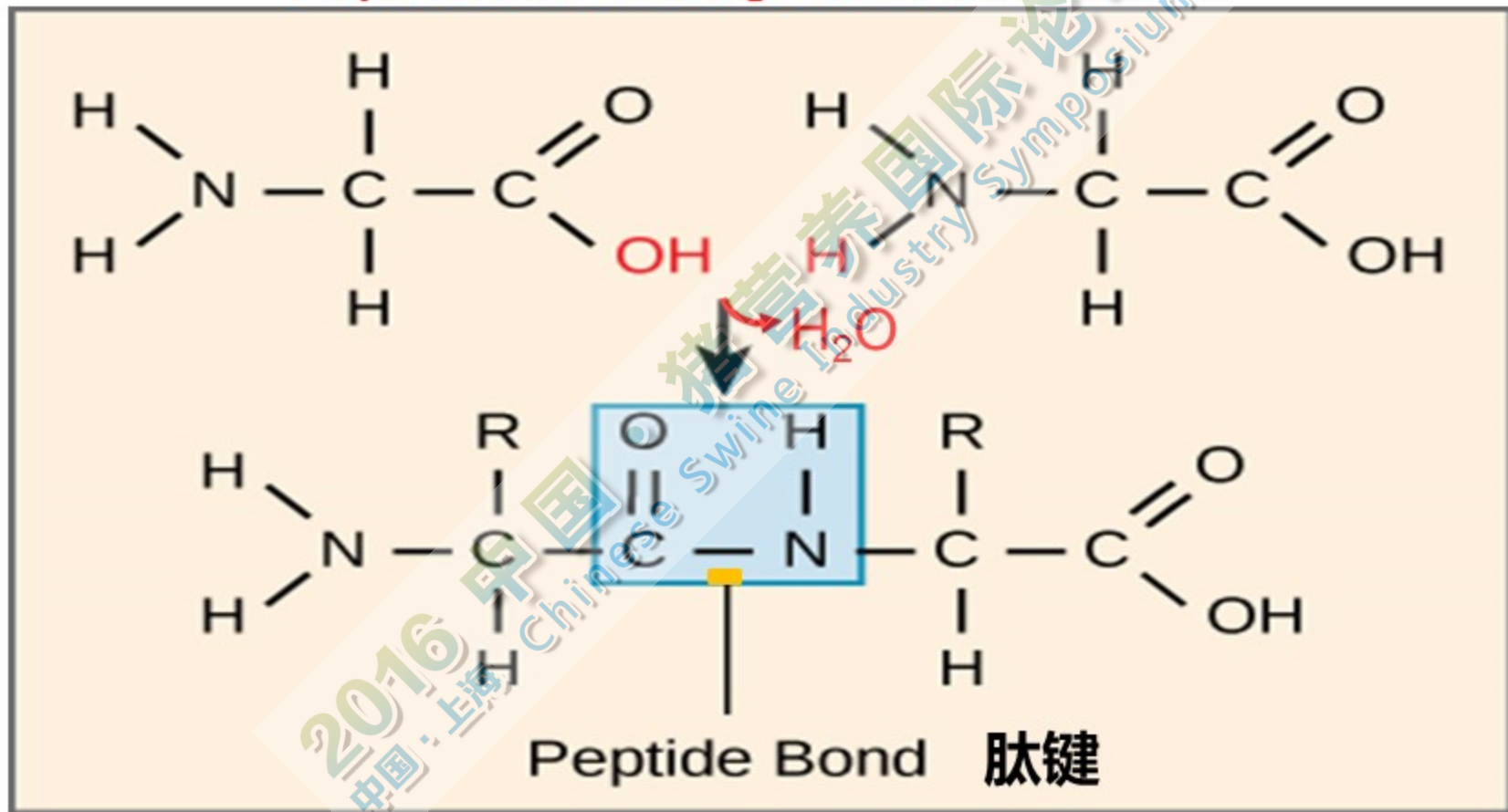


D-Amino Acid

D-氨基酸

由氨基酸组成的肽

Peptides Consisting of Amino Acids



肽的分类 Classification of Peptides

小寡肽 : ≤ 10 氨基酸

Small oligopeptide: ≤ 10 amino acids

大寡肽 : 10~20 氨基酸

Large oligopeptide: 10 to 20 amino acids

多肽 : > 20 氨基酸

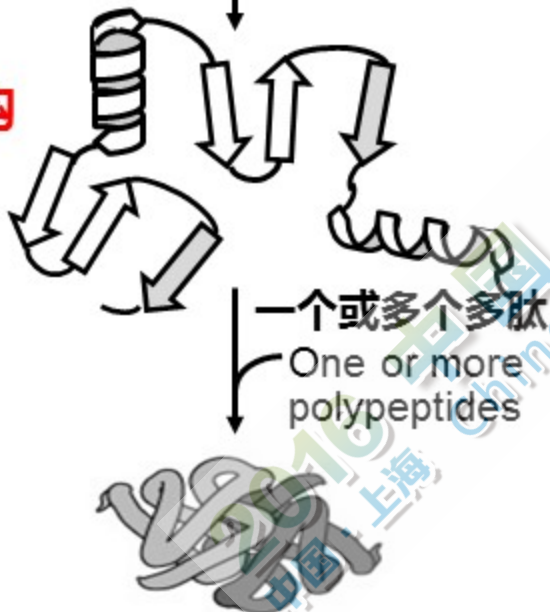
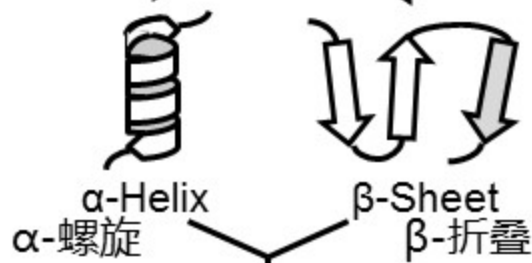
Polypeptide: > 20 amino acids

蛋白质 : 具有3D结构的多肽

Protein: Polypeptide with 3-dimensional structure

蛋白质和多肽间的分界线通常是它们的分子量。一般来说，一个多肽的分子量不低于8000道尔顿（即不少于72个氨基酸）。

The dividing line between proteins and polypeptides is usually their molecular weight. Generally speaking, a polypeptide has a molecular weight of $\geq 8,000$ Daltons (i.e., ≥ 72 amino acids).



蛋白质结构

蛋白质的一级结构 Primary protein structure
(多肽中的氨基酸序列) (sequence of amino acids in a polypeptide)

蛋白质的二级结构 Secondary protein structure
(多肽链中由氢键连接的氨基酸) (linkage of amino acids within strands of a polypeptide by hydrogen bonds)

蛋白质的三级结构 Tertiary protein structure
(多肽中 α -螺旋和 β 折叠间的吸引力; 蛋白质折叠) (attractions between the α -helices and β -sheets of a polypeptide; protein folding)

蛋白质的四级结构 Quaternary protein structure
(蛋白质中不同多肽的空间排列) (spatial arrangements among different polypeptides of a protein)

Separation of Proteins from Peptides and Free Amino Acids (AAs)

分离肽和游离氨基酸中的蛋白质

1. Trichloroacetic acid (TCA; the final concentration of 5%)

三氯乙酸 (TCA; 最终浓度为5%)

2. Perchloric acid (PCA; the final concentration of 0.2 M)

高氯酸 (OCA; 最终浓度为0.2 M)

3. Ethanol (the final concentration of 80%)

乙醇 (最终浓度为80%)

肽&游离氨基酸
Peptide & free AAs

Solution 溶解

TCA, PCA or Ethanol
TCA, PCA或乙醇

Protein precipitate ↓ 蛋白质沉淀

蛋白质水解物的工业生产

II. Industrial Production of Protein Hydrolysates

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猪营养国际论坛

蛋白质来源的原材料

Raw Materials of Protein Sources

动物蛋白：酪蛋白，乳清，肠道，肉

Animal protein: Casein, whey, intestine, and meat

植物蛋白：大豆，小麦，水稻和棉籽蛋白

Plant protein: Soy, wheat, rice, pea, and cottonseed proteins

蛋白质水解的方式：蛋白酶，微生物，酸或碱

Methods of protein hydrolysis: Proteases, Microorganisms, Acids, or Bases

动物产品或植物性饲料中的蛋白质 Proteins in animal products or plant feedstuffs

↓ 水 (无杂质) Water (free of any contamination)

增溶 Solubilization

调节pH, 温度, 离子浓度和反应物压力
Adjust pH, temperature and ion concentration and reactor pressure

↓ 化学水解 (如HCl或NaOH) Chemical hydrolysis (e.g., HCl or NaOH)
酶解 (如胰蛋白酶或胃蛋白酶) Enzymatic hydrolysis (e.g., trypsin or pepsin)
微生物水解 (如细菌, 真菌或酵母) Microbial hydrolysis (e.g., bacteria, fungi or yeast)

Controlled hydrolysis for a specific period of time (e.g., 4 to 48 h)

控制特定时间段内的水解 (如4~48hr)

↓ 降低温度 (化学水解) Reducing temperature (chemical hydrolysis)
升高温度 (酶解或微生物水解) Increasing temperature (enzymatic or microbial hydrolysis)
调节pH Adjust pH

终止水解 Terminate hydrolysis

Pasupuleki VK, Braun S.
State of the art manufacturing of protein hydrolysates. 2010.

↓ 过滤, 离心和分离
Filtration, centrifugation, and separation

肽 Peptides

↓ 巴斯德氏杀菌, 蒸发, 喷雾干燥和包装
Pasteurization, evaporation, spray-drying and packaging

动物饲料成品 Products for animal feeding

水解度 Degree of Hydrolysis

水解度：断裂肽键的数量除以蛋白质中的肽键总数。

Degree of hydrolysis: the number of peptide bonds cleaved, divided by the total number of peptide bonds in a protein.

断裂肽键的数量：游离氨基酸的摩尔数加上可溶于TCA或PCA的肽的摩尔数。

Number of peptide bonds cleaved: the moles of free amino acids and plus the moles of TCA- or PCA-soluble peptides (non-protein small and large peptides).

水解度 Degree of Hydrolysis

游离态氨基酸的比例 (%) = (游离氨基酸的总数/蛋白质中的氨基酸总数) x 100%

Percentage of AAs in the free form (%) = (Total free AAs/Total AAs in protein) x 100%

肽中氨基酸的比例 (%) = (肽中氨基酸的总数、蛋白质中氨基酸的总数) x 100%

Percentage of AAs in peptides (%) = (Total AAs in peptides/Total AAs in protein) x 100%

氨基酸 AAs = amino acids

蛋白质水解的产物 Products of Protein Hydrolysis

产物：游离氨基酸，小肽，以及大肽

Products: free amino acids, small peptides, and large peptides.

影响因素：蛋白质的来源

Affecting factors: Sources of proteins

水的质量

Quality of water

蛋白酶的种类

Type of proteases

微生物的种类

Species of microbes

分析方法：

Methods of analysis:

高效液相色谱

HPLC

质谱法

Mass spectrometry

离子交换色谱法

Ion-exchange chromatography

蛋白质的酸水解 Acid Hydrolysis of Protein

条件: 6 M HCl, 110°C, 2~6hr **Conditions:** 6 M HCl, 110°C, 2 to 6 hours.

主要目的: 生产增香剂 (调味产品, 如水解植物蛋白)

Main purpose: Produce flavor enhancers (e.g., flavoring products, such as hydrolyzed vegetable protein).

优势: 成本低 **Advantage:** low cost.

劣势: 色氨酸: 完全损坏

蛋氨酸: 部分丢失

谷氨酰胺: 转化成谷氨酸

天冬酰胺: 转化成天冬氨酸

强酸条件

Disadvantages: Tryptophan: complete destruction.

Methionine: partial loss.

Glutamine: conversion to glutamate

Asparagine: conversion to aspartate

Strong acid conditions

2016
中国: 上海

蛋白质的碱水解

Alkaline Hydrolysis of Protein

条件：碱剂，如氢氧化钙，氢氧化钠，或氢氧化钾（如4 M, 55 °C, 4~8hr）

Conditions: Alkaline agents, such as calcium, sodium, or potassium hydroxide (e.g., 4 M); 55 °C, 4 to 8 h)

主要目的：发泡剂（如卵蛋白的替代品）

Main purpose: foaming agents (e.g., substitutes for egg proteins)

优势：成本低；无色氨酸损失

Advantages: low cost; no loss of tryptophan

劣势：大多数氨基酸：完全损坏；

Disadvantages: Most amino acids: complete destruction;

很多氨基酸：部分丢失

Many amino acids: partial loss

强碱条件

Strong alkaline condition

蛋白质的酶解 Enzymatic Hydrolysis of Protein

条件：蛋白酶 Conditions: Proteases

来源于动物的蛋白酶（特别是猪）：胰酶，胰蛋白酶，胃蛋白酶，羧基肽酶和氨基肽酶
Proteases of **animal sources** (particularly pigs): pancreatin, trypsin, pepsin, carboxypeptidases and aminopeptidases

来源于植物的蛋白酶：木瓜蛋白酶和菠萝蛋白酶
Proteases of **plant sources**: papain and bromelain

来源于细菌和真菌的蛋白酶：很多外肽酶和内肽酶
Proteases of **bacterial and fungal sources**: numerous exopeptidases and endopeptidases

（如链霉蛋白酶：从细胞外液的灰色链霉菌分离出的蛋白酶混合物）
(e.g., **Pronase**: a mixture of protease isolated from the extracellular fluid of *Streptomyces griseus*)

蛋白质的酶解 Enzymatic Hydrolysis of Protein

目的：产生多种生物活性肽

Purpose: Produce various biologically active peptides

优势：特异性，条件温和，无氨基酸损失，并且容易分离蛋白质水解物

Advantages: specific, mild conditions, no loss of amino acids, and easy to isolate protein hydrolysates

劣势：成本相对较高；蛋白原料中可能存在酶抑制剂

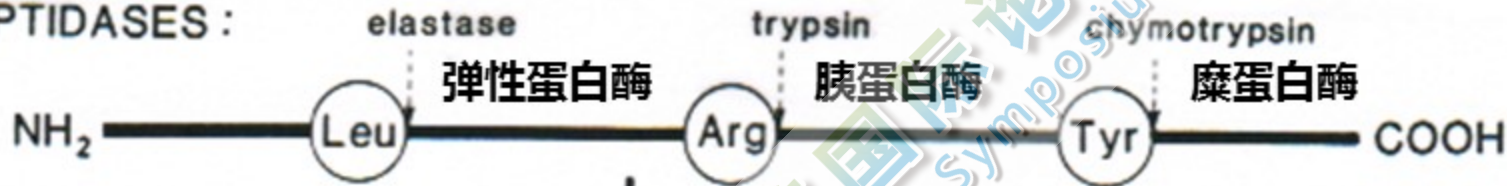
Disadvantages: relatively high cost; the potential presence of enzyme inhibitors in the raw protein materials

Cleavage of Specific Peptide Bonds by Some Proteases

由一些蛋白酶分离的特定肽键

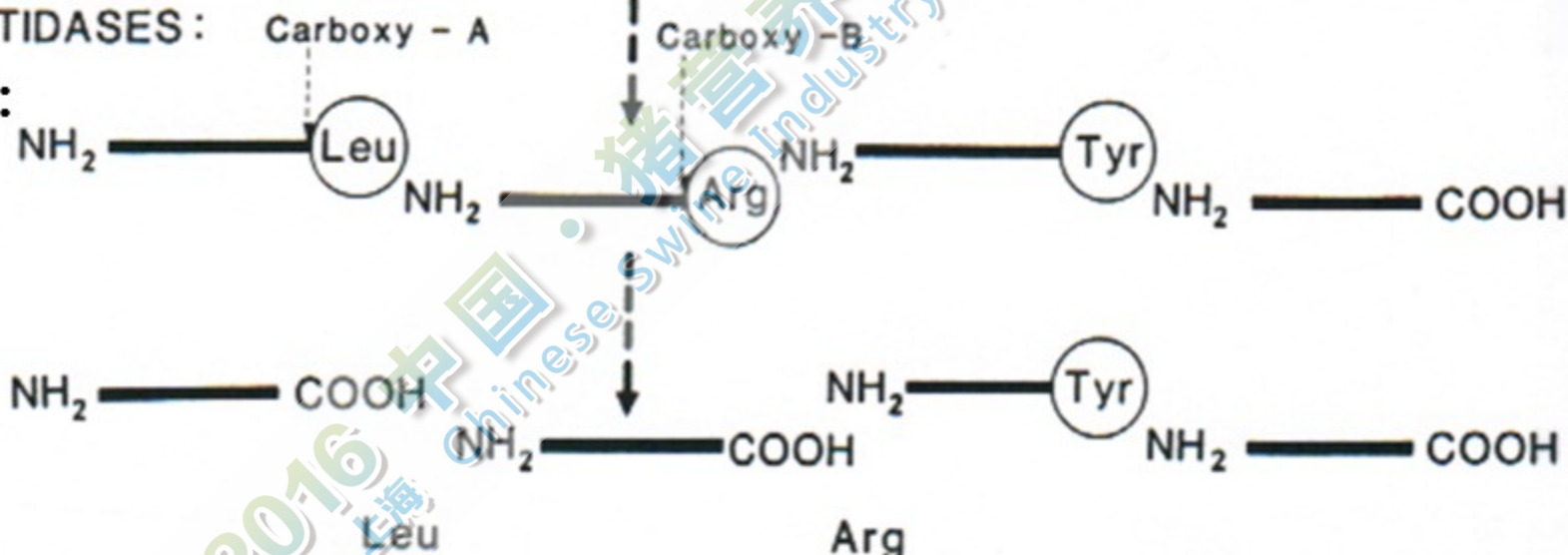
ENDOPEPTIDASES :

内肽酶 :



EXOPEPTIDASES :

外肽酶 :



蛋白质的微生物水解 Microbial Hydrolysis of Protein

液态发酵：在高水分发酵条件下水解的蛋白质底物

Liquid-state fermentation: Hydrolysis of protein substrates under high-moisture conditions fermentation

固态发酵：在低水分发酵条件下水解的蛋白质底物

Solid-state fermentation: Hydrolysis of protein substrates under low-moisture fermentation conditions

(例如)**An example** :

酱油：起源于公元后第二世纪的中国

Soy sauce: originated in China in the 2nd century AD.

原材料：煮黄豆，炒谷，盐水，米曲霉，和酱油曲霉（真菌的一种）

Raw materials: boiled soybeans, roasted grain, brine, and *Aspergillus oryzae* or *Aspergillus sojae* (a genus of fungus)

用于蛋白质水解的微生物种类

Microbial Species Used for Protein Hydrolysis

植物源性蛋白 Plant-source proteins:

Lactobacillus rhamnosus BGT10 and *Lactobacillus zeae* LMG17315 for pea proteins; *Bacillus natto* or *B. subtilis* for soybean; fungi *A. oryzae* or *R. oryzae* for soybean.

动物源性蛋白 Animal-source proteins:

乳酸菌，如乳杆菌和乳球菌 Lactic acid bacteria, such as *Lactobacillus* and *Lactococcus* species

用于蛋白质水解的微生物种类

Microbial Species Used for Protein Hydrolysis

优势：条件温和；将蛋白质分解为肽，同时去除原料中的高敏或抗营养因子

Advantages: mild conditions; Break down proteins into peptides, while removing **hyper-allergic or anti-nutritional** factors in ingredients

(如胰蛋白酶抑制剂，大豆球蛋白， β -伴大豆球蛋白，植酸，寡糖棉子糖和水苏糖，大豆皂)

(e.g., trypsin inhibitors, glycinin, β -conglycinin, phytate, oligosaccharides raffinose and stachyose, saponins, in soybeans).

劣势：成本相对较高；在多种条件下改变微生物活性；因此在肽的生产中不一致。

Disadvantages: Relatively high costs; changes in microbial activity under various conditions; therefore, inconsistency in the production of peptides.

III. 蛋白质水解物中的生物活性肽

Bioactive Peptides in Protein Hydrolysates

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Chinese Swine Industry Symposium



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Chinese Swine Industry Symposium

生物活性肽 Bioactive Peptides

生物活性肽：蛋白中氨基酸序列的片段，除其营养价值外还有其他生理功效。

Bioactive peptides: fragments of amino acid (AA) sequences in a protein that confer biological functions beyond their nutritional value.

功能：抗菌，抗氧化，抗高血压，以及免疫调节。

Functions: antimicrobial, antioxidant, antihypertensive, and immunomodulatory.

长度：通常2~20个氨基酸，部分>20个氨基酸

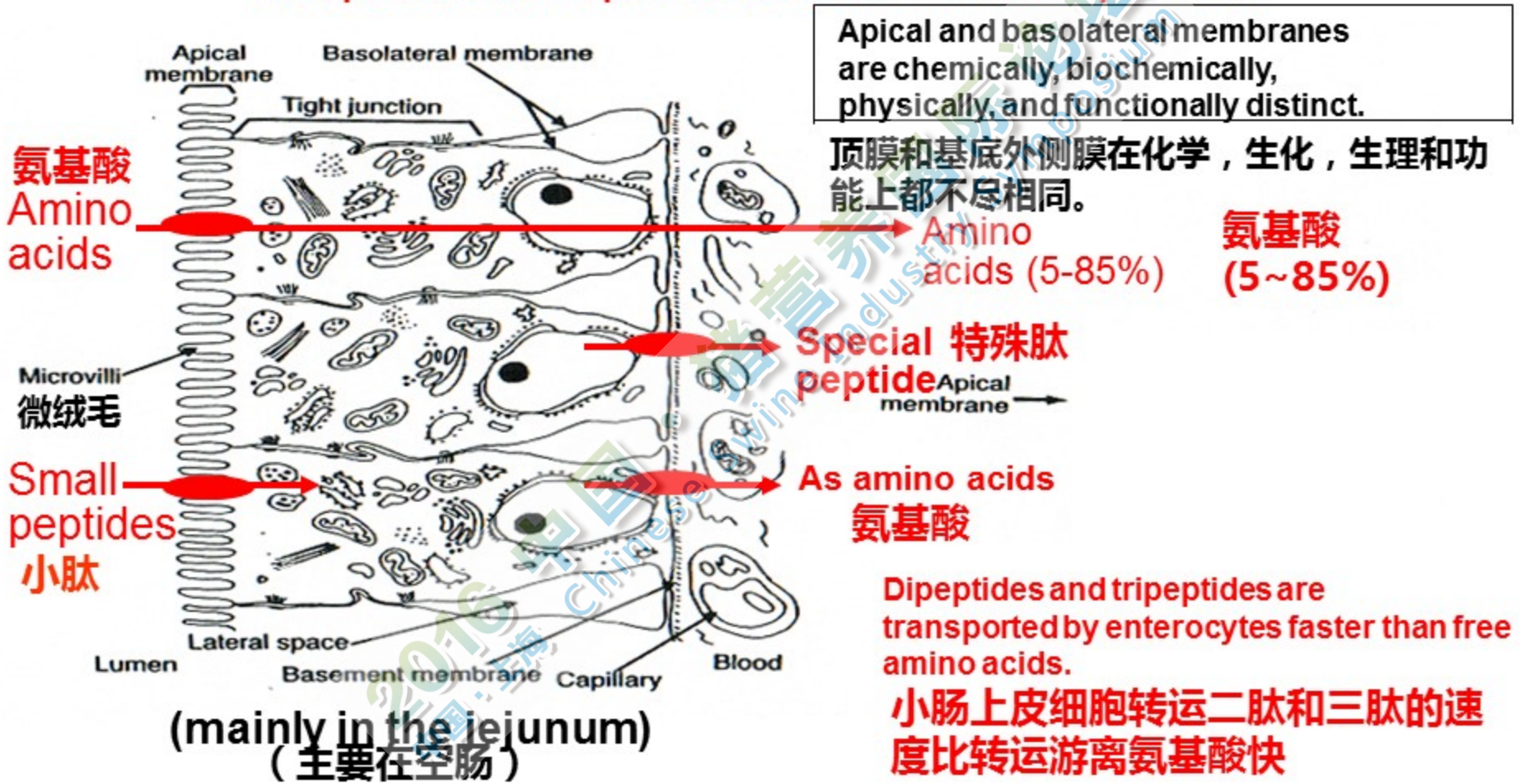
Length: usually 2–20 AA, but some > 20 AA

一般结构：含有较多的**疏水性氨基酸**，其中有**精氨酸，赖氨酸，和脯氨酸**

Common structure: high abundance of **hydrophobic amino acids**, as well as the presence of **Arg, Lys, and Pro**

小肽在小肠上皮细胞中的运输

Transport of Small Peptides in the Small-Intestinal Epithelium

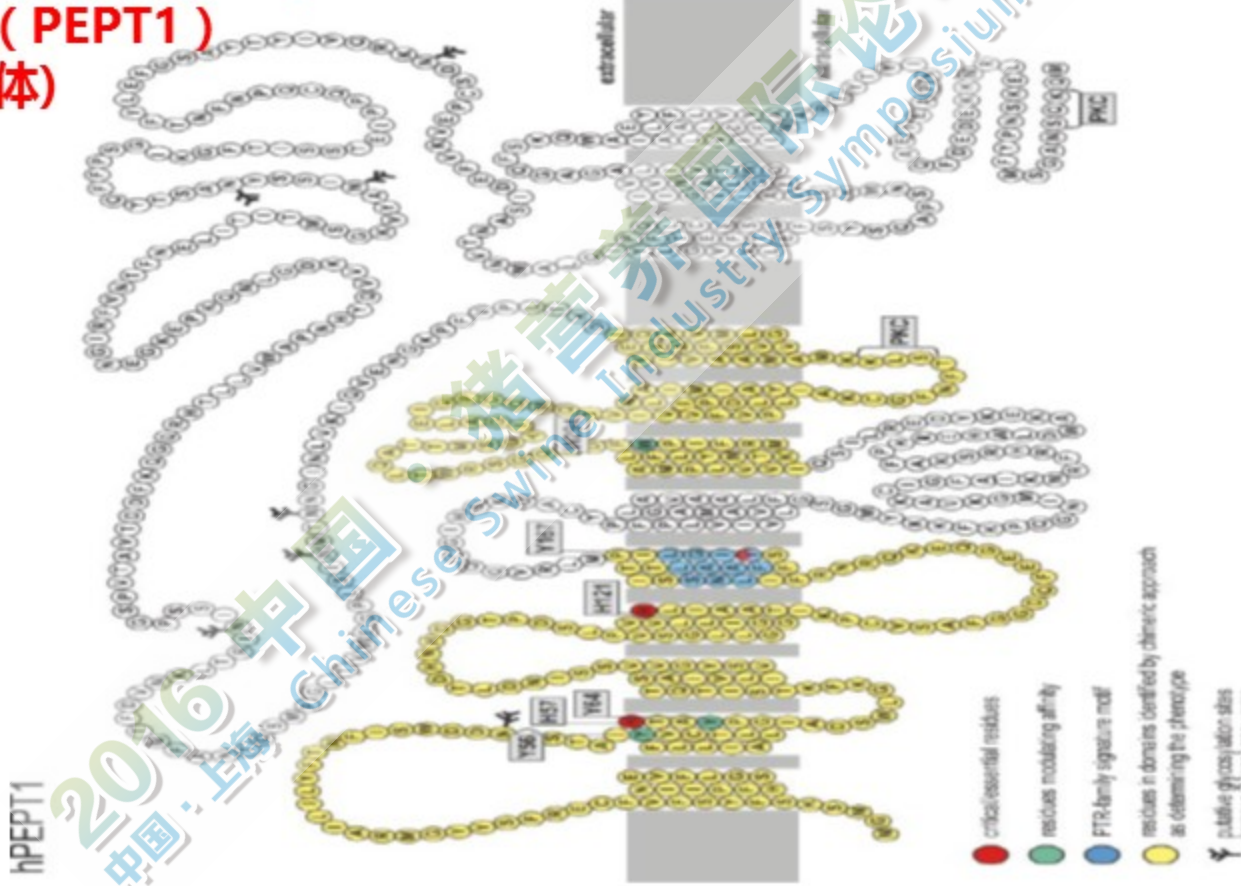


Peptide Transporter 1 (PEPT1) on the apical membrane

(H⁺-Driven Transporter)

顶膜上的肽载体 1 (PEPT1)

(H⁺驱动载体)



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小肠里的肽转运载体1和2 (PEPT1和PEPT2)

Peptide Transporters 1 and 2 (PEPT1 and PEPT2)

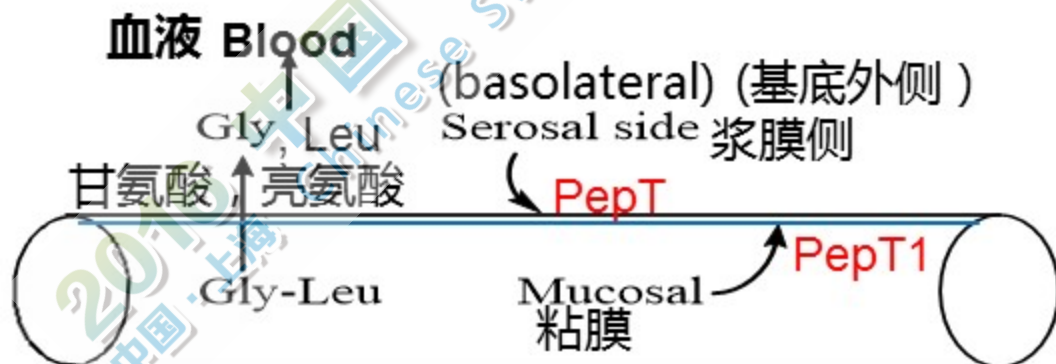
In the Small Intestine

PEPT1 位于小肠的顶膜上。

PEPT1 is localized on the apical membrane of the small Intestine.

另一个肽转运载体位于基侧膜上

Another peptide transporter is located on the basolateral membrane.

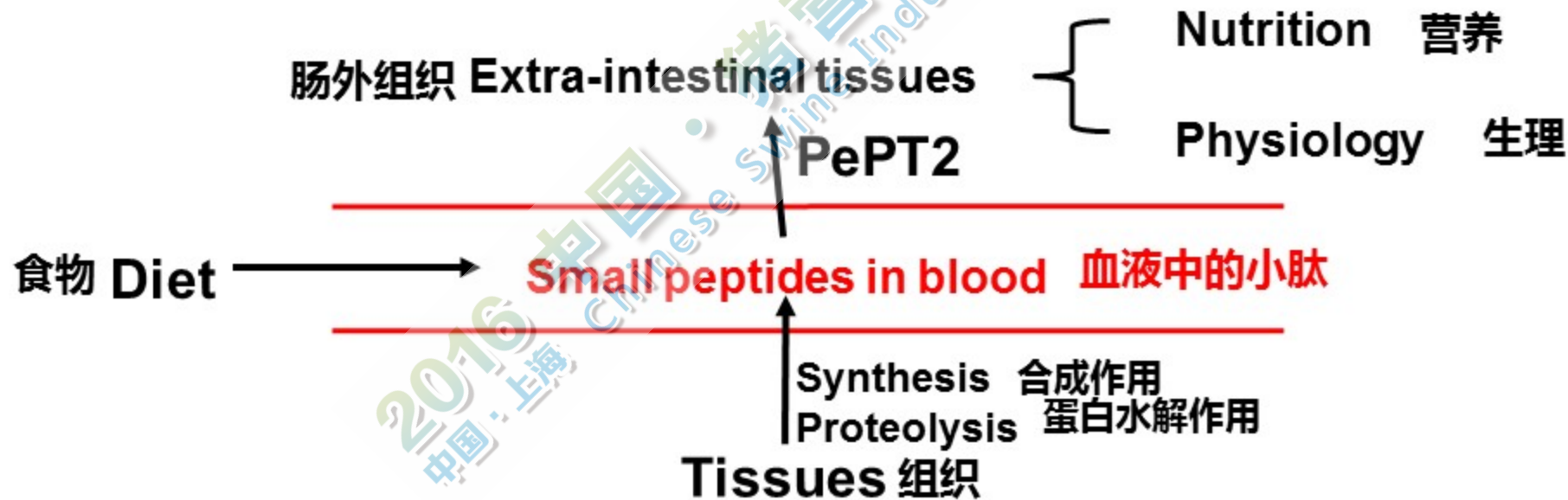


肠外组织中的主要肽转运载体 (PEPT2)

Major Peptide Transporter in Extra-Intestinal Tissues (PEPT2)

血液含的小肽: 来自于食物和内源性来源 (合成和蛋白水解作用)。

这些小肽由肠外组织的PePT2转运。 Blood contains small peptides derived from diets and endogenous sources (synthesis and proteolysis). These small peptides are taken up by extra-intestinal tissues through peptide transporter-2 (PePT2).



Presence of Small Peptides in Animals

动物体内小肽的分布

Common name 统称	Composition 组成	Storage in major tissue 主要分布的组织
Dipeptides 二肽		
Anserine 鹅肌肽	β -Alanyl-L-1-Methylhistidine	Skeletal muscle and brain (Birds)
Balenine 鲸肌肽	β -Alanyl-L-3-Methylhistidine	Skeletal muscle and brain
Carcinine 肌肽胺	β -Alanyl-Histamine	Brain and heart of vertebrates
Carnosine 肌肽	β -Alanyl-L-Histidine	Skeletal muscle and brain
Homocarnosine 高肌肽	γ -Aminobutyryl-L-Histidine	Brain 脑
Homoanserine 高鹅肌肽	γ -Aminobutyryl-L-1-Methylhistidine	Brain of vertebrates 脊椎动物的脑
Tripeptides 三肽		
Glutathione 谷胱甘肽	γ -Glu-Cys-Gly	Cells, bile acid, pancreatic juice & uterine fluid 细胞, 胆汁, 胰液和子宫液
Collagen peptide 胶原蛋白肽	Gly-Pro-Hydroxyproline	Milk and plasma 奶和血浆

含有动物产品的饲料可以提供这些重要的抗氧化肽类

ACE-抑制肽的工业生产

Industrial Production of ACE-inhibitory Peptides

血管紧张素转换酶 ACE = angiotensin-I converting enzyme

来源于乳蛋白 (被木瓜蛋白酶水解) Derived from milk proteins (hydrolysis of papain)

Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP)

来源于猪肉 (被胃蛋白酶水解) Derived from porcine meat (hydrolysis by pepsin)

Ile-Thr-Thr-Asn-Pro

Lys-Arg-Val-Ile-Thr-Tyr

Val-Lys-Arg-Gly-Phe

Lys-Arg-Gln-Lys-Tyr-Asp-Ile

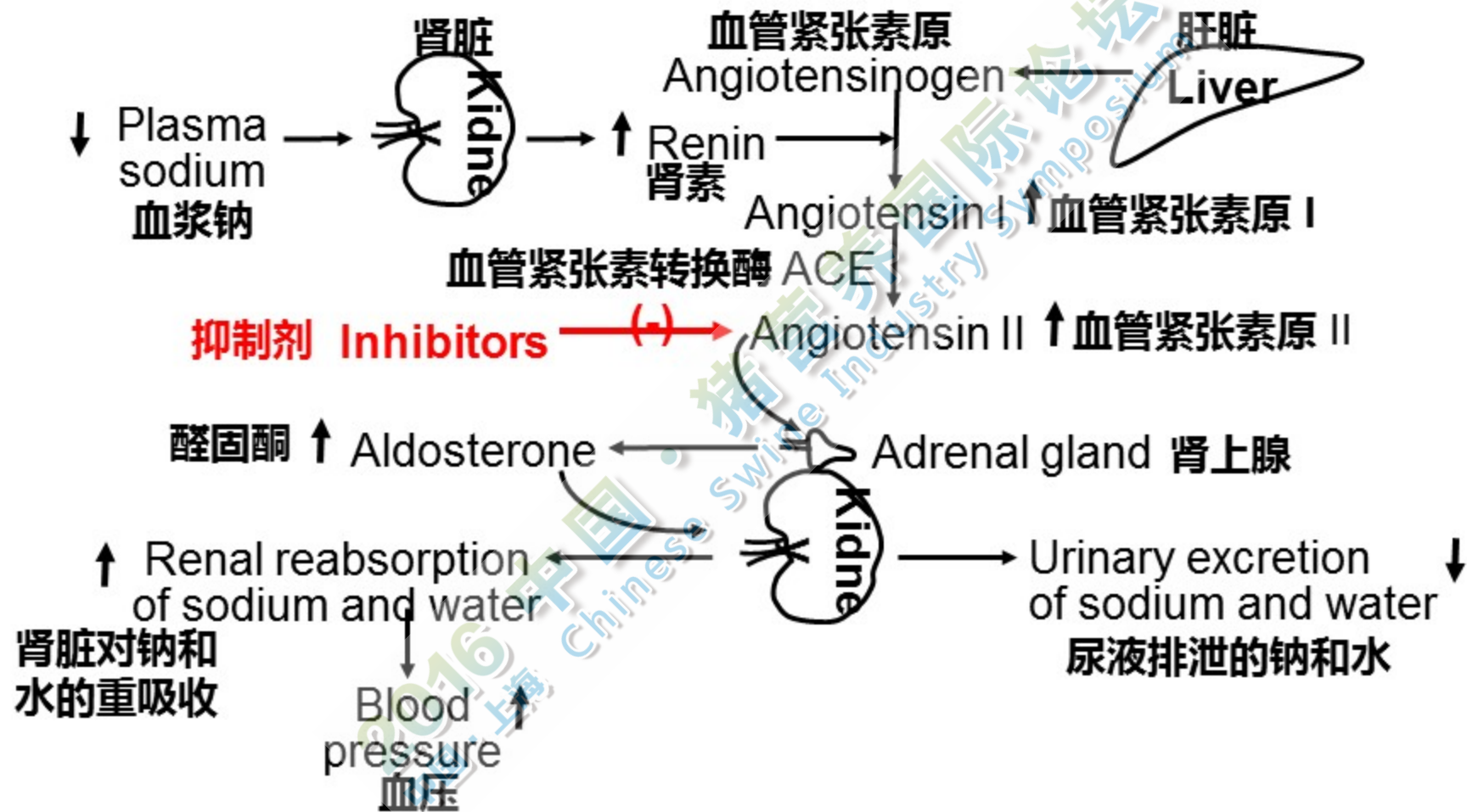
Lys-Leu-Pro

Arg-Pro-Arg

来源于蛋黄 (被胃蛋白酶水解) Derived from egg yolk (hydrolysis by pepsin)

Tyr-Ile-Glu-Ala-Val-Asn-Lys-Val-Ser-Pro-Arg-Ala-Gly-Gln-Phe

Tyr-Ile-Asn-Gln-Met-Pro-Gln-Lys-Ser-Arg-Glu



抗氧化肽的工业生产

Industrial Production of Antioxidative Peptides

来源于乳蛋白 (被胰蛋白酶水解) Derived from milk proteins (hydrolysis by trypsin)

Tyr-Phe-Tyr-Pro-Glu-Leu

Phe-Tyr-Pro-Glu-Leu

Tyr-Pro-Glu-Leu

Pro-Glu-Leu

Glu-Leu

来源于猪肉 (被木瓜蛋白酶+肌动蛋白酶E水解)

Derived from porcine meat (hydrolysis by papain + Actinase E)

Asp-Ser-Gly-Val-Thr

Ile-Glu-Ala-Glu-Gly-Glu

Asp-Ala-Gln-Glu-Lys-Leu-Glu

Glu-Glu-Leu-Asp-Asn-Ala-Leu-Asn

Val-Pro-Ser-Ile-Asp-Asp-Gln-Glu-Glu-Leu-Met

来源于蛋黄 (被胃蛋白酶水解) Derived from egg yolk (hydrolysis by pepsin)

Tyr-Ile-Glu-Ala-Val-Asn-Lys-Val-Ser-Pro-Arg-Ala-Gly-Gln-Phe

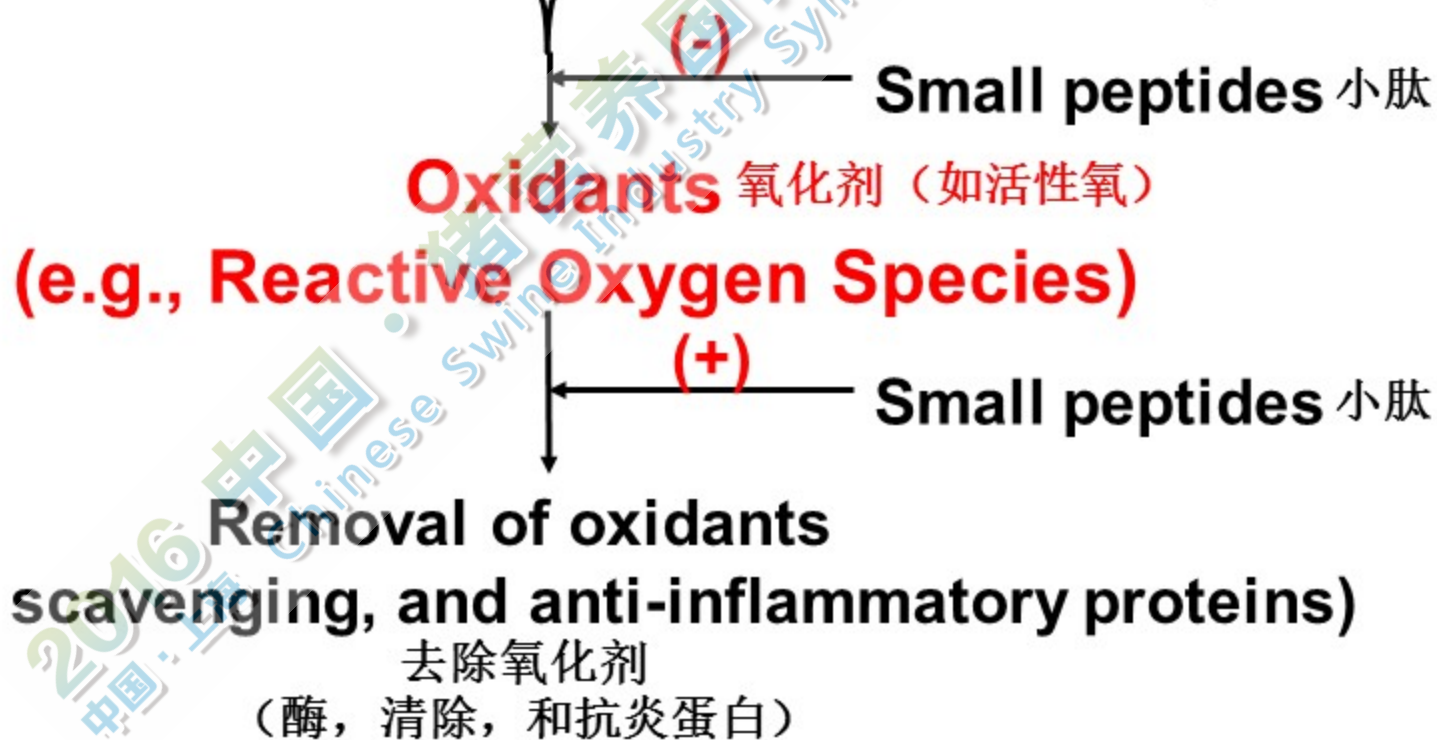
Tyr-Ile-Asn-Gln-Met-Pro-Gln-Lys-Ser-Arg-Glu

脂肪酸的氧化
Oxidation of
fatty acids

脂质过氧化
Lipid
peroxidation

氨基酸合成和氧化
AA synthesis
and oxidation

热应激
Heat
stress



抗菌肽的工业生产

Industrial Production of Antimicrobial Peptides

来源于牛肉 (被木瓜蛋白酶+肌动蛋白酶E水解)

Derived from bovine meat (hydrolysis by papain + Actinase E)

Gly-Leu-Ser-Asp-Gly-Glu-Trp-Gln

Inhibits both gram-positive bacteria (*Bacillus cereus* and *Listeria monocytogenes*) and gram-negative bacteria (*Salmonella typhimurium* and *Escherichia coli*)

抑制革兰氏阳性菌 (*Bacillus cereus* and *Listeria monocytogenes*)

和革兰氏阴性菌 (*Salmonella typhimurium* and *Escherichia coli*)

来源于蛋黄 (被木瓜蛋白酶和胰蛋白酶水解)

Derived from egg yolk (hydrolysis by papain and trypsin)

Asn-Thr-Asp-Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg

抑制革兰氏阳性菌和革兰氏阴性菌

Inhibits both gram-positive and negative bacteria

Lopez-Barros et al. J Food Sci. 2014;79:R273-83;

Bevins et al. Nature Rev Microbiol. 2011;9:356-68.

抗菌肽是如何运作的？

How Do Antimicrobial Peptides Act?

- (1)破坏细胞膜上的细菌 Damaging the cell membrane of bacteria;
- (2)干扰细菌胞内蛋白的功能 Interfering with the functions of intracellular proteins in bacteria;
- (3)诱导细菌内的细胞质蛋白聚集 Inducing the aggregation of cytoplasmic proteins in bacteria;
- (4)影响细菌的代谢 Affecting bacterial metabolism.

Lima et al. J Food Sci Technol. 2015;52:4459-66.

Osman et al. LWT-Food Sci Technol 2016;65:480-86.

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阿片肽的工业生产 Industrial Production of Opioid Peptides

来源于乳蛋白 Derived from milk proteins

Tyr-Pro-Phe-OH
Tyr-Pro-Phe-Pro-OH
Tyr-Pro-Phe-Pro-NH₂
Tyr-Pro-Phe-Pro-Gly-OH
Tyr-Pro-Phe-Pro-Gly-Pro-Ile-OH
Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-OH

来源于面筋蛋白 Derived from gluten protein

Gly-Tyr-Tyr-Pro-Thr-OH
Tyr-Gly-Gly-Trp-OH
Tyr-Pro-Ile-Ser-Leu-OH
Tyr-Pro-Gln-Pro-Gln-Pro-Phe-OH

来源于大豆 Derived from soybeans

Tyr-Pro-Phe-Val-Val-OH
Tyr-Pro-Phe-Val-Val-NH₂
Tyr-Pro-Phe-Val-Val-Asn-OH
Tyr-Pro-Phe-Val-Val-Asn-Ala-OH

利用猪小肠中的酶生产阿片肽。
Using enzymes from the **pig small intestine** to produce opioid peptides.

与大脑中的阿片受体结合，从而影响猪的肠道功能，行为，和食物摄取。
Bind to opioid receptors in the brain to affect the **gut function, behavior & food intake** of pigs.

IV.蛋白质水解物中的生物活性肽

Bioactive Peptides in Protein Hydrolysates

2016 中国
中国·上海

Chinese Swine Industry Symposium

· 精品猪业国际论坛
Symposium

植物源性肽 Plant-Derived Peptides

例子 Examples :

3%或6%的发酵豆粕 **3% or 6% Fermented soybean meal**
提高断奶仔猪的生长性能和饲料效率，与同比例的脱脂奶粉效果相当
Improve growth performance and feed efficiency of weanling pigs;
Comparable to that of the same percentage of dried skim milk

4.9%发酵豆粕 **4.9% Fermented soybean meal**
替代断奶仔猪饲料里3.7%的血浆蛋白粉 Replace 3.7% spray-dried plasma protein in diets of weanling pigs

50%大豆蛋白质水解物 **50% Soybean protein hydrolysate**
替代真鲷幼鱼饲料中50%的鱼粉 Replace 50% of fish meal in the diet of juvenile red sea bream

1%或2%水解全小麦蛋白 **1% to 2% Hydrolyzed wheat gluten proteins**

作为宠物饲料的香味添加剂 Used as flavor additives in the diets of companion animals

动物源性肽 Animal-Derived Peptides

例子 Examples :

1.5 ~ 6%的肠膜蛋白粉 (SDPI) 1.5 to 6% spray-dried porcine intestine hydrolysate (SDPI):

与血浆蛋白粉或乳清粉相比, 饲喂1.5~6%SDPI的断奶仔猪有更好的生长性能

Better growth performance, compared with spray-dried plasma or dried whey in weanling pigs

6%猪血和禽类组织的酶水解蛋白
poultry tissues

6% enzymatically hydrolyzed proteins of swine blood and

与同比例的断奶仔猪血球粉作用相当
cells in weanling pigs

Comparable to the same percentage of spray-dried blood

2.5%, 5%或7.5%水解猪肠粘膜
使生长鸡具有更好的生长性能

2.5% 5% or 7.5% hydrolyzed porcine mucosa:

Better growth performance in growing chicks

10%的鱼副产物蛋白水解物 10% fish by-product protein hydrolysate:

促进欧洲海鲈鱼苗在鳃弧菌环境下的肠道健康和生长
larvae challenged with *Vibrio anguillarum*

Improve gut health and growth in European sea bass

总结 Summary

这些蛋白质水解物的工业生产涉及：强酸或强碱条件，酶法，或细菌发酵。
Industrial production of these protein hydrolysates involves: strong acidic or alkaline conditions, enzymatic methods, or microbial fermentation.

植物性和动物性蛋白水解物可提供极易消化的肽和生物活性肽，对猪有营养性，抗菌，抗氧化，抗高压，和免疫调节的作用。

Plant- and animal-protein hydrolysates provide highly digestible peptides and bioactive peptides to confer nutritional, antimicrobial, antioxidant, antihypertensive, and immunomodulatory roles in pigs.

这些进展提供了一个经济的方法，将动物副产物或植物性饲料转换成高质量的蛋白水解物原料，从而饲喂家畜，家禽，鱼，和宠物。

These advances provide a cost-effective approach to converting animal by-products or plant feedstuffs into high-quality protein-hydrolysate ingredients to feed livestock, poultry, fish, and companion animals.

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