The V. M. Gorbatov All-Russian Meat Research Institute (VNIIMP)
Moscow, 9th December 2015

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Bioactive peptides and probiotics for functional meat products

Keizo ARIHARA

School of Veterinary Medicine,
Kitasato University, JAPAN

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Our University & Laboratory

Lab. Food Function and Safety
School of Veterinary Medicine

Kitasato University

北里大学

Kita-sato University

Seven Schools

- Veterinary Medicine
- Pharmacy
- Medicine
- Fisheries
- Nursing
- Science
- Health Science

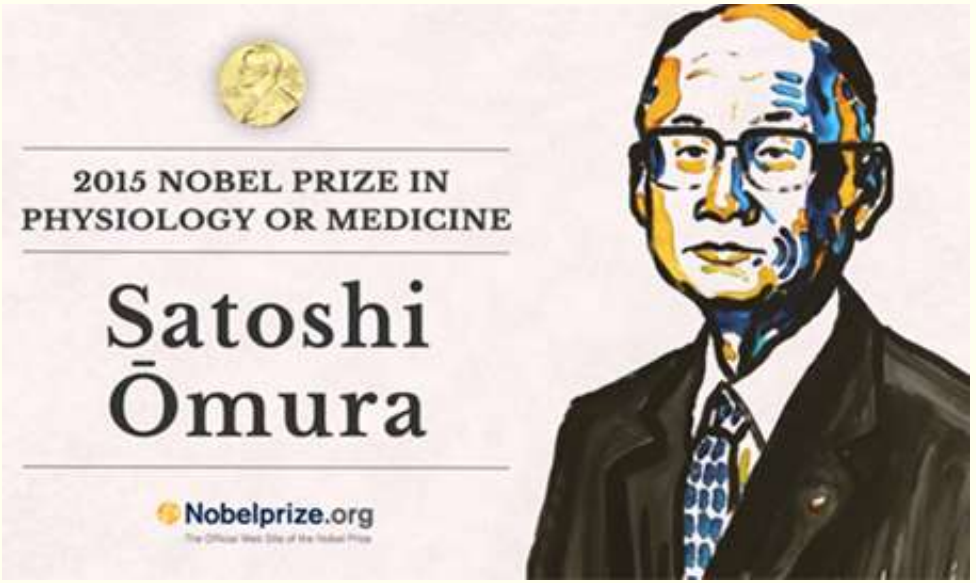
Life Science Institute
University Hospital

Dr. Shibasaburo Kitasato

The 2015 Nobel Prize in Physiology or Medicine

Dr. Satoshi OMURA

Professor Emeritus
Kitasato University



Congratulations! 2015 Nobel Prize in Physiology or Medicine!

School of Veterinary Medicine

University Farm

Towada Campus

Iwate Campus
(Fisheries)

Tokyo Campus
(Pharmacy)

Kanagawa Campus
(Medicine, Nursing, Science,
Health Science)

Views of Towada Area in Japan

Towada Campus

School of Veterinary Medicine

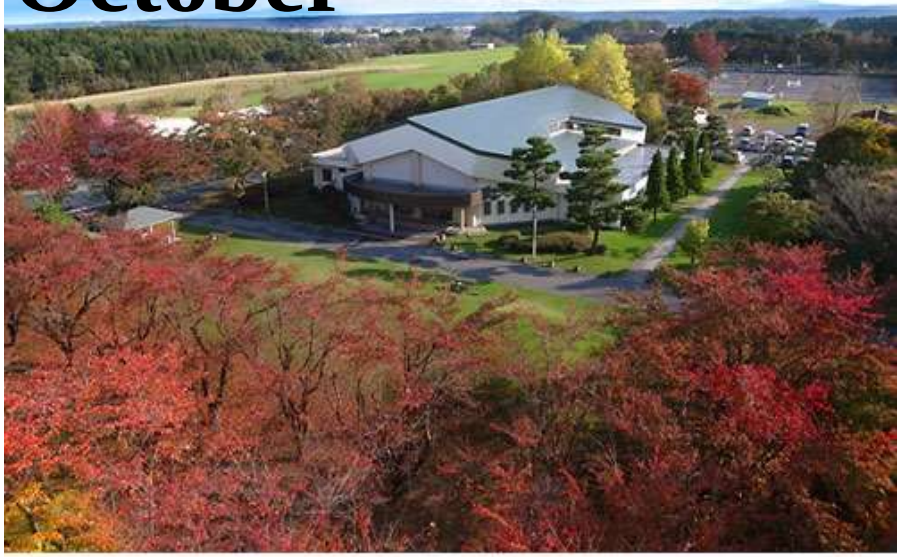
School of Veterinary Medicine

-Veterinary Medicine
-Animal Science
-Enviromental Science

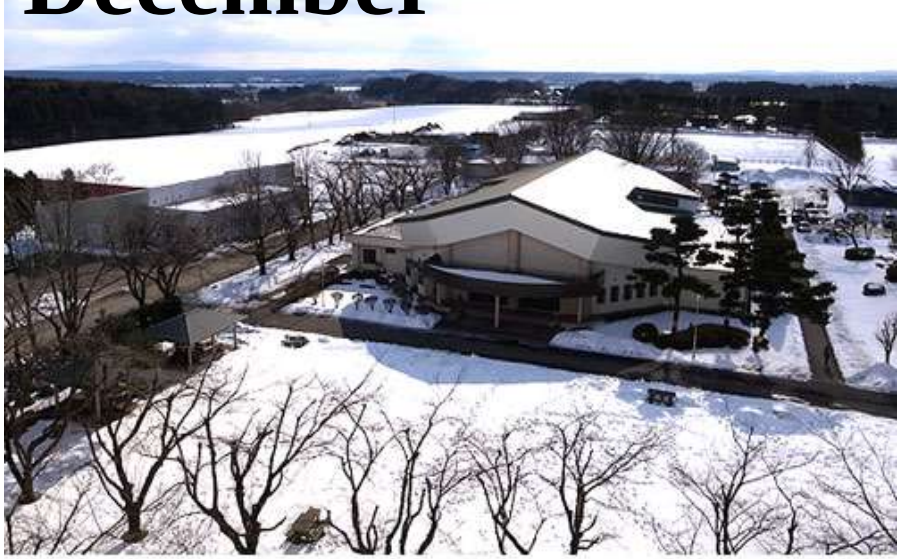
May

August

October



December



Views of Our Campus

Views of Our Campus (Spring)

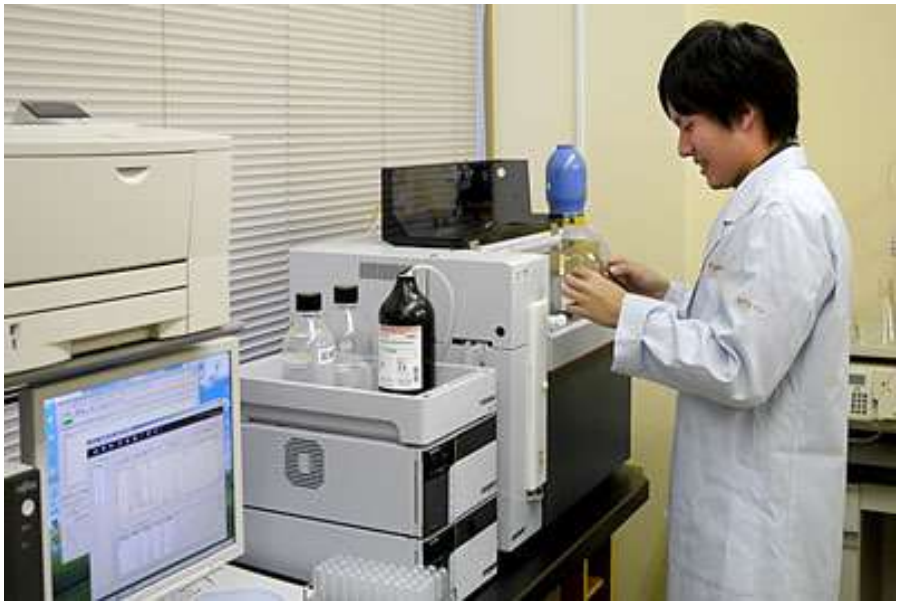
Views of Our Campus (Winter)

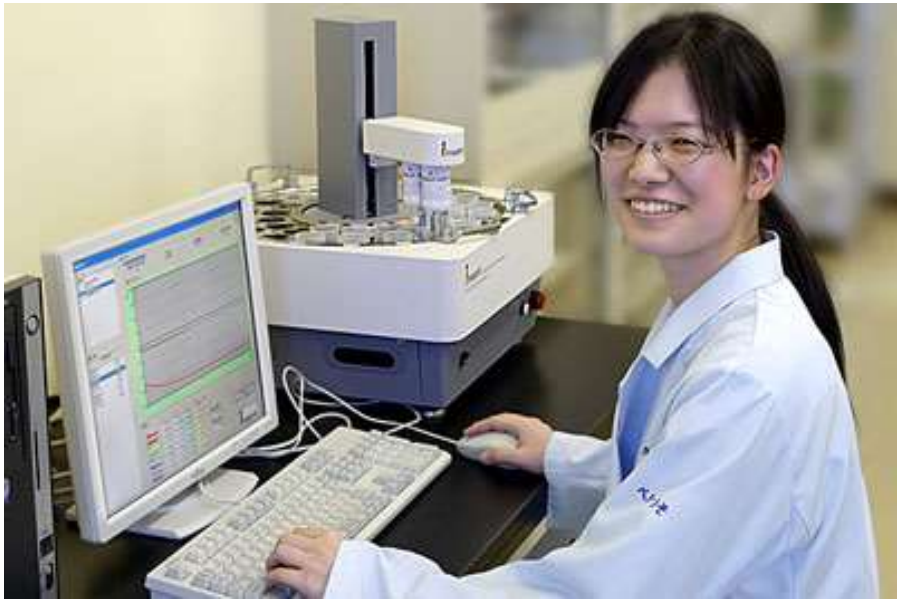
Members of our Lab

Professor	1
Assist. Professor	1
Graduate students	8
Under grad. Students	15
Visiting researcher	1

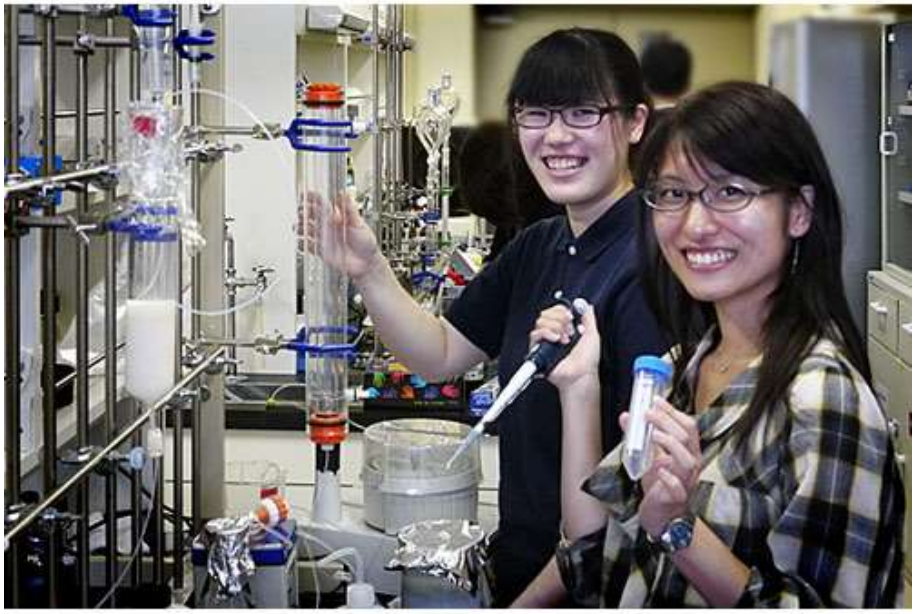
Research topics of our Laboratory

-Bioactivity of food protein-derived peptides
-Bioactivity of Maillard reaction products
-Probiotic bacteria and probiotic meat products
-Palatability of flavor components
-Bioactivity of flavor components
-Development of functional foods





Laboratory of Food Function & Safety



Laboratory of Food Function & Safety

Collaborative research with foreign institutes

Malaysia

Spain

China

Outline of This Presentation

1. Introduction
2. Peptides from Meat Proteins
3. Probiotics for Meat Products
4. Peptide-based New Ingredients
5. Concluding Remarks

Functional Foods

'Processed foods having disease-preventing and health-promoting benefits in addition to their nutritional value'

Much attention has been paid to physiological functions of foods. Progress has been made in the development of functional foods, such as functional dairy products. However, ---

Reformulation for Functional Meat Products

- Reduction of fat content
- Modification of fatty acid profile
- Reduction of cholesterol
- Reduction of calories
- Reduction of sodium content
- Reduction of nitrites
- Incorporation of functional ingredients

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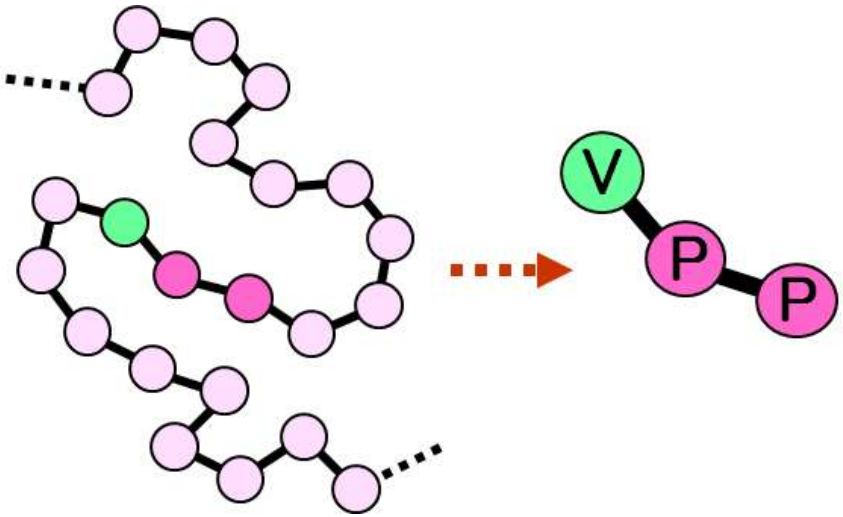
Generation of Bioactive Peptides from Meat Proteins



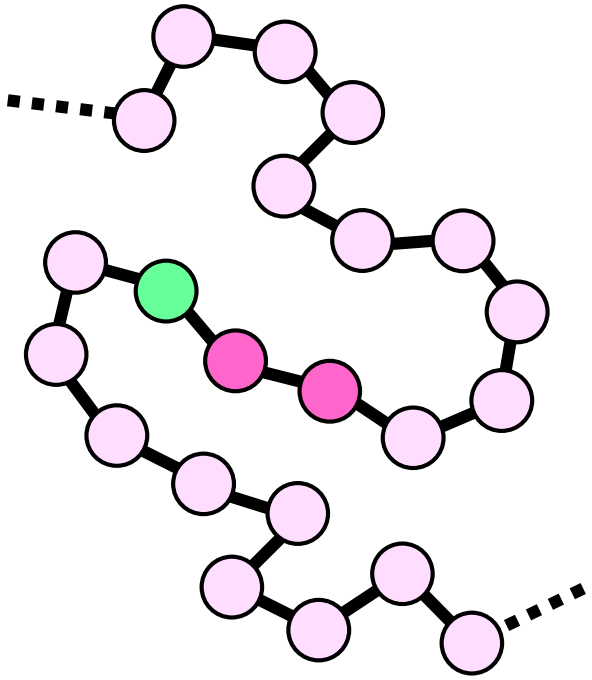
Bioactive Peptides from Food Proteins

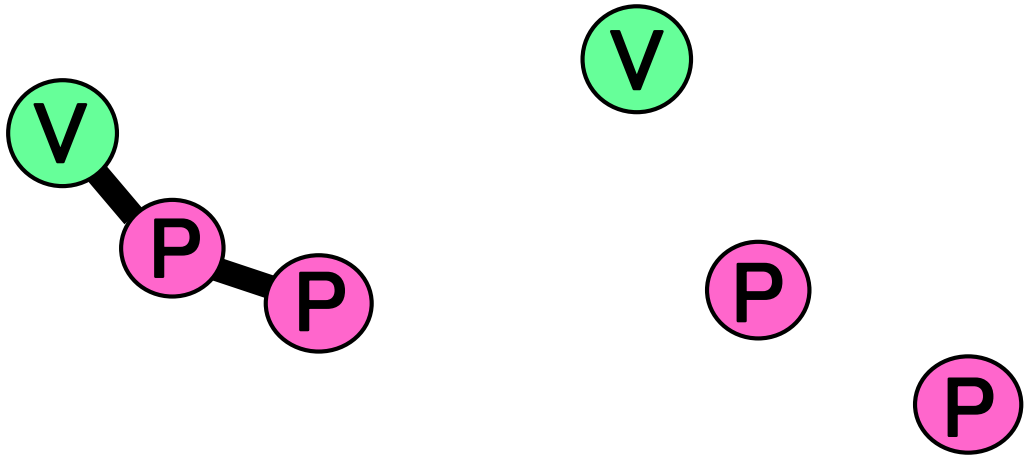
Food (meat) proteins

Enzymatic Digestion

Bioactive Peptides

These sequences are inactive within the parent proteins.





Proteins → Hydrolysis → **Bioactive Peptides** → Hydrolysis → **Amino Acids**

No Activity

Importance of the Structure

No Activity

Generation Ways of Bioactive Peptides from Meat Proteins

1. Gastrointestinal Proteolysis

pepsin, trypsin, chymotripsin, etc.

2. Aging and Storage

calpains, cathepsins, etc.

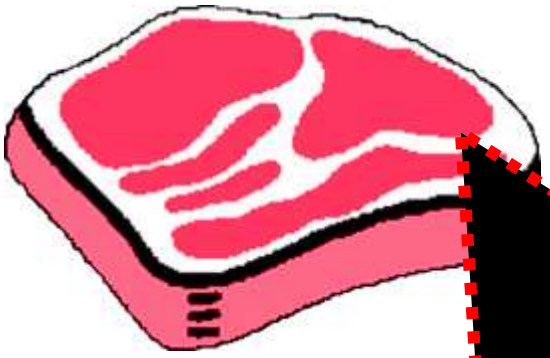
3. Fermentation

muscle enzymes, microbial enzymes

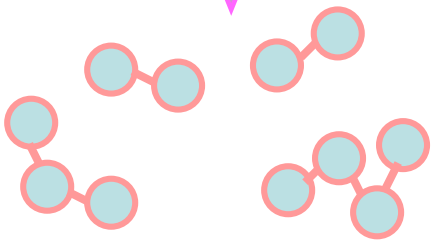
4. Enzymatic Treatment

various commercial proteinases

Generation of Peptides during Meat Aging

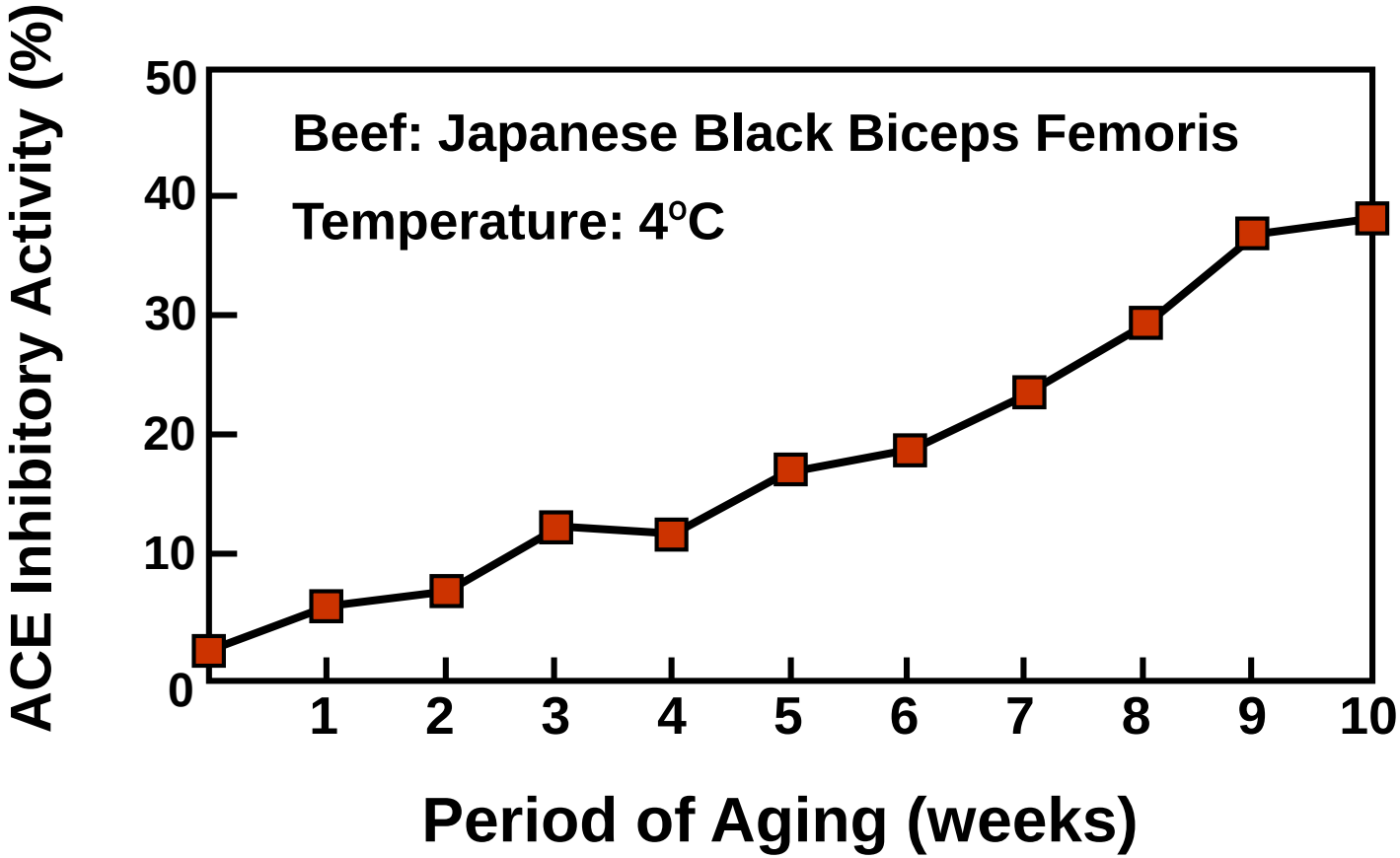


Aging ↓ **Proteolysis**



Peptides

Generation of Bioactive Peptides during Beef Aging



Antihypertensive peptides were identified in aged meat.

(e.g., Gly-Pro-Leu-Lys)

Representative Activities of Food Protein-derived Bioactive Peptides

- Antihypertensive
- Antioxidative
- Opioid agonistic
- Immunomodulatory
- Antimicrobial
- Prebiotic
- Mineral-binding
- Hypocholesterolemic

Products Utilizing Bioactive Peptides

Ameal-S
(Japan)

Evolus
(Finland)

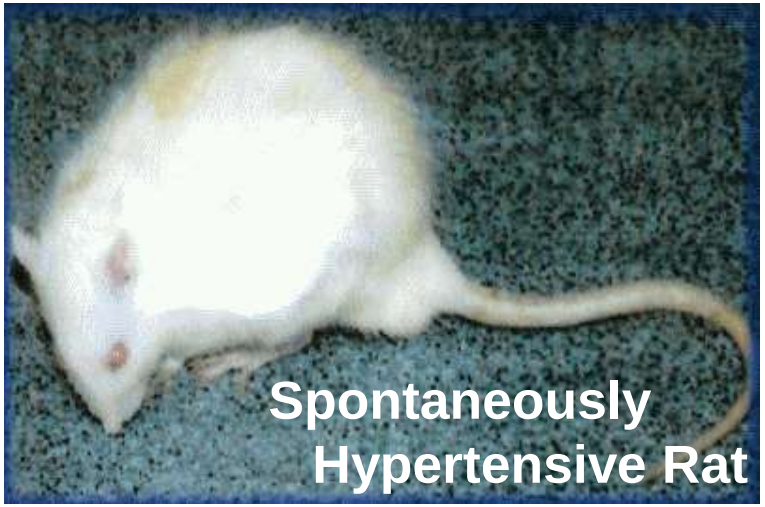
Antihypertensive peptides

Kotsukotsu Ca
(Japan)

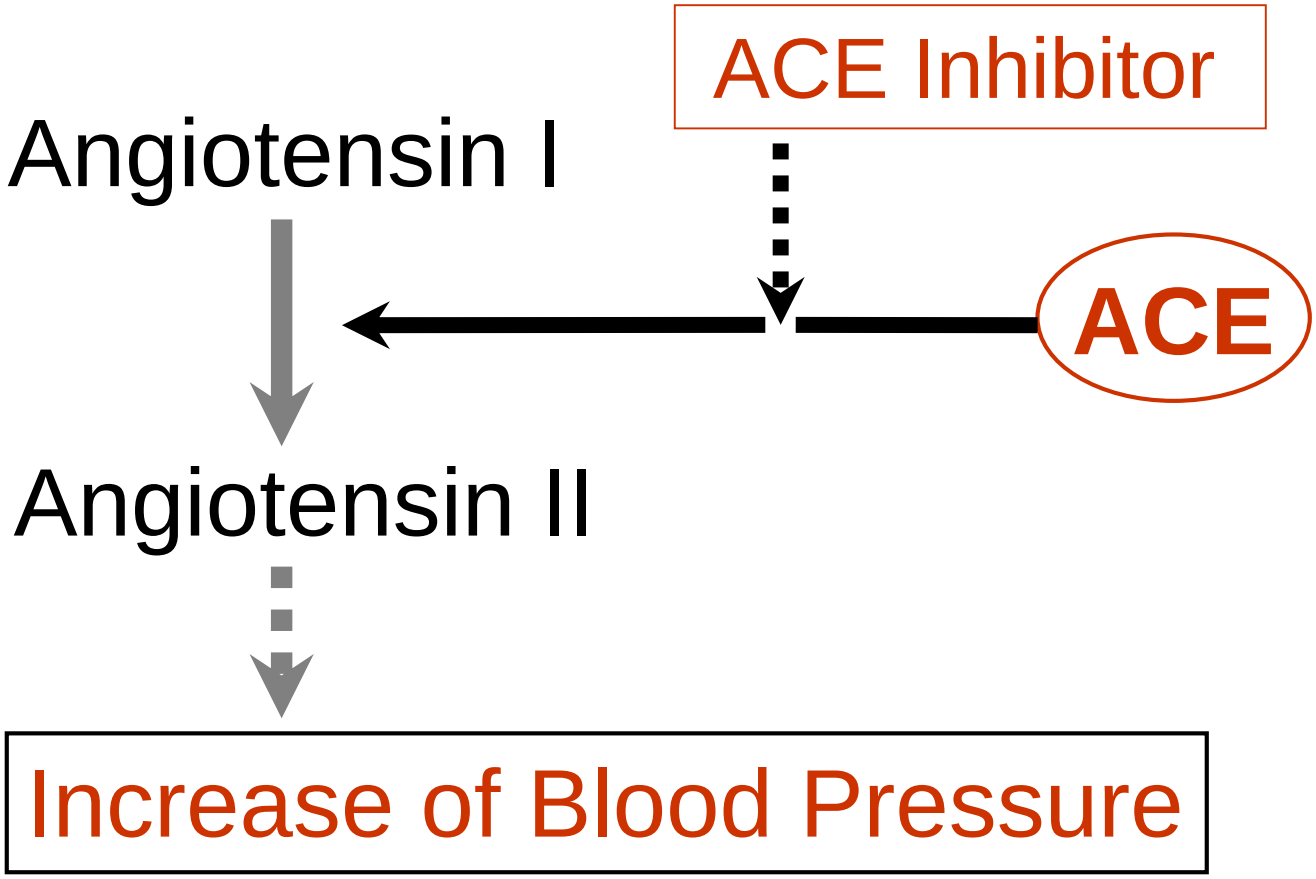
Caseinophosphopeptides

Antihypertensive Peptides from Meat Proteins

Among bioactive peptides from meat proteins, ACE inhibitory peptides have been studied extensively.



Role of ACE in Blood Pressure Regulation



ACE: Angiotensin I-Converting Enzyme

Identification of ACE Inhibitory Peptides

Pork proteins hydrolyzed by thermolysin

Purification & Identification

Met-Asn-Pro-Pro-Lys
Ile-Thr-Thr-Asn-Pro

Both sequences are found in myosin heavy chain.

Arihara et al., 2001

Both peptides showed antihypertensive activity when administered orally to SHR.

ACE Inhibitory Activities of Extracts Prepared from Fermented Sausages

Wine-flavored salami (USA)

Abruzzese (USA)

La. Estrella (Argentina)

Gold salami (Netherlands)

Cacciatore (Switzerland)

Salami Norcinetto (Italy)

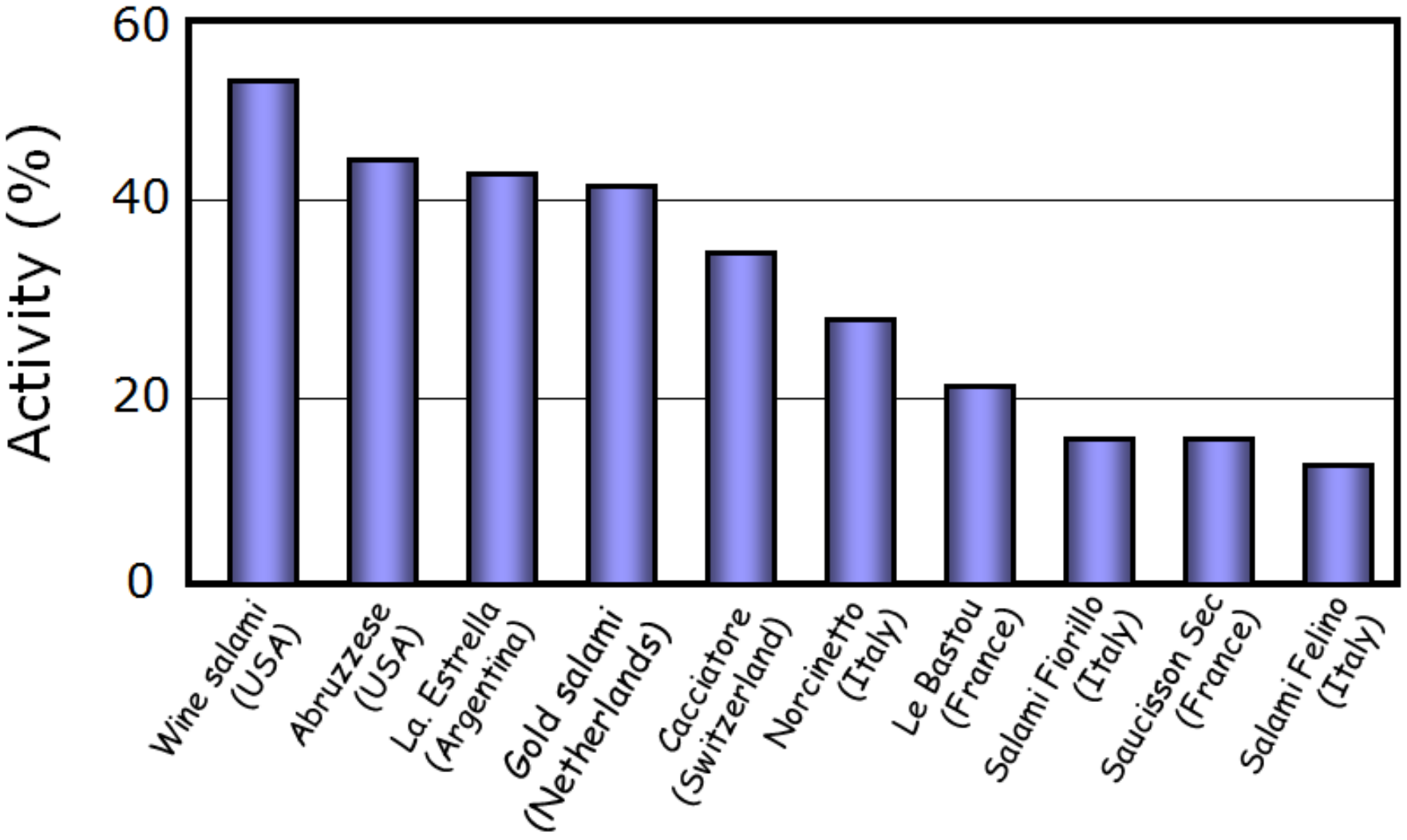
Le Bastou (France)

Salami Fiorillo (Italy)

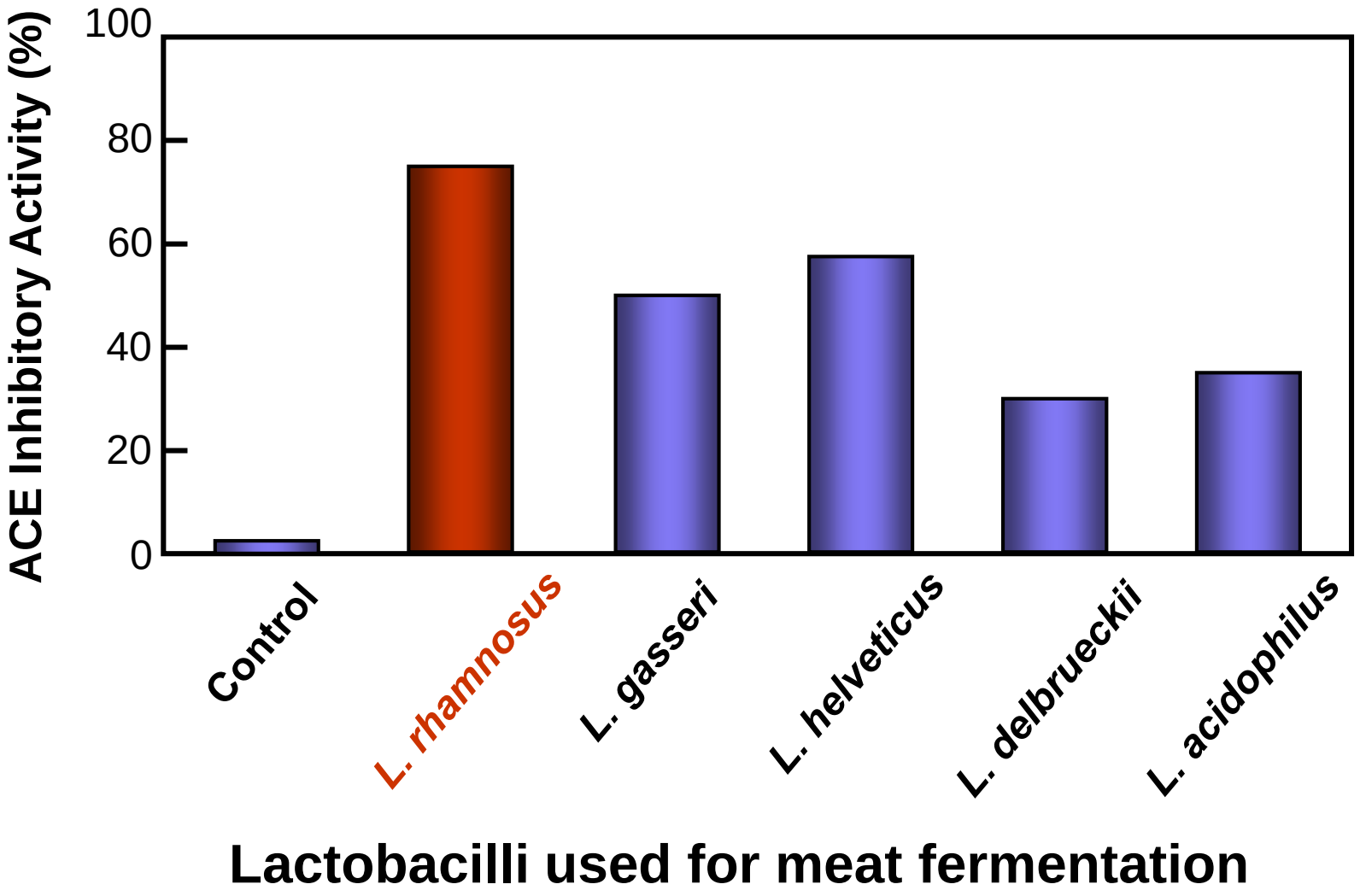
Saucisson Sec (France)

Salami Felino (Italy)

ACE Inhibitory Activities of Fermented Sausages

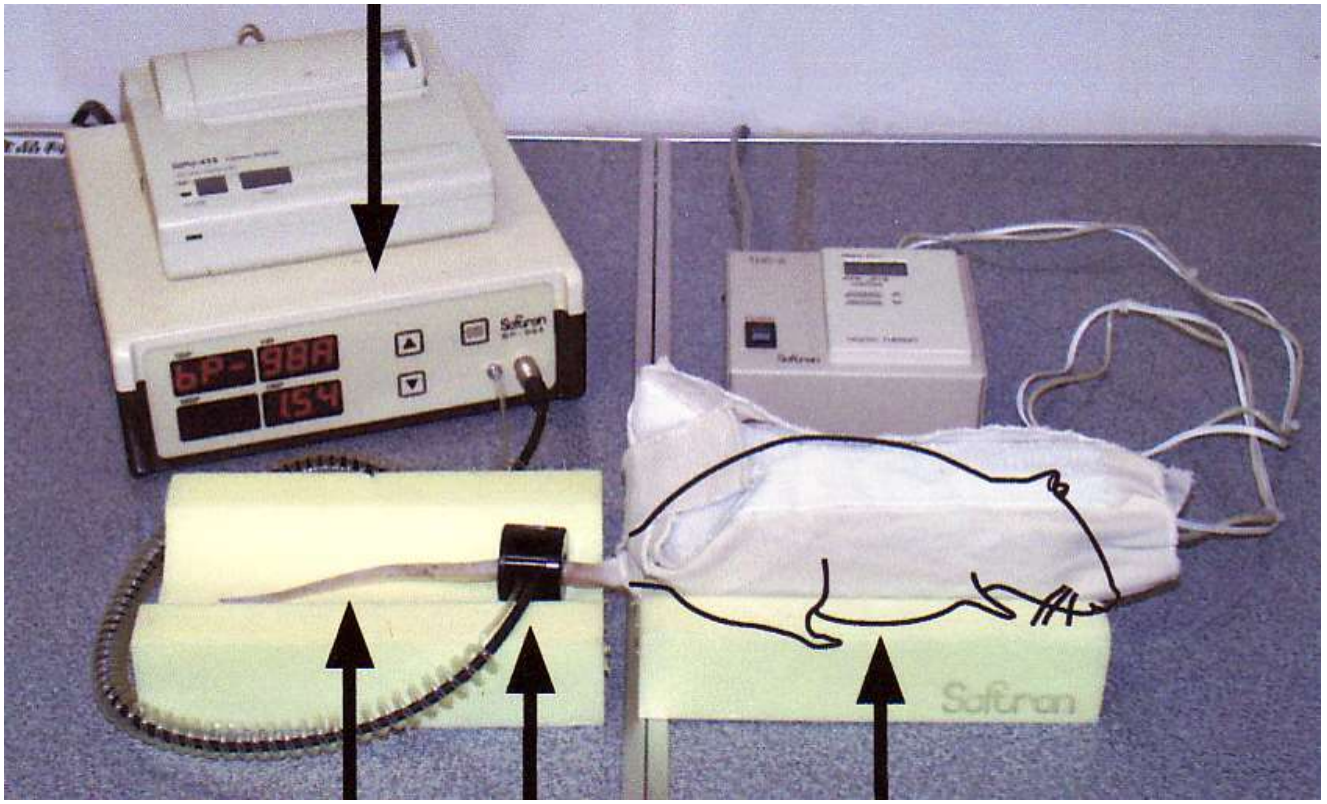


ACE Inhibitory Activities Generated from Meat by *Lactobacillus* sp.



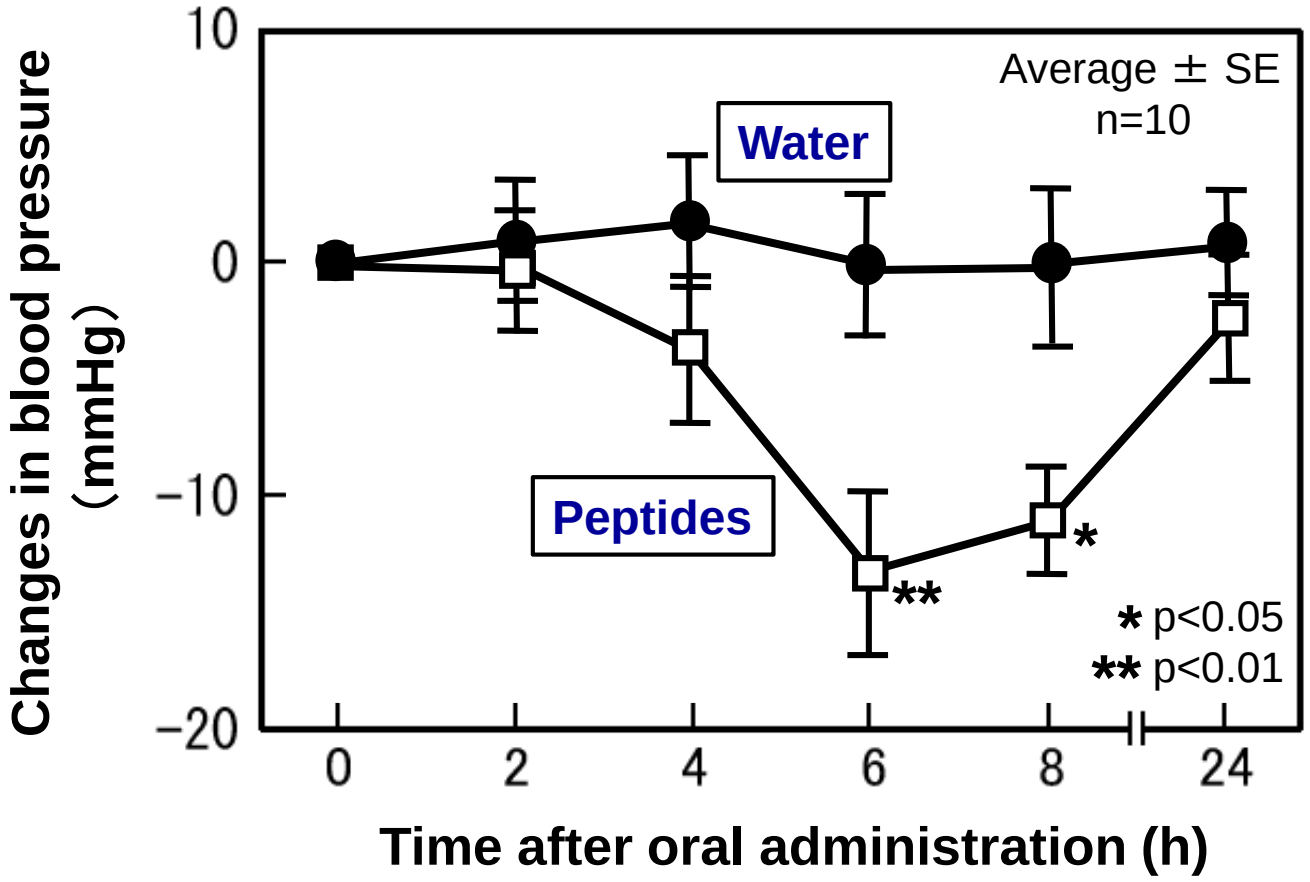
Measurement of Blood Pressure of Spontaneously Hypertensive Rat

Sphygmomanometer



Tail **Cuff** **Rat (SHR)**

Antihypertensive Activity of Fermented Meat by *Lactobacillus rhamnosus*

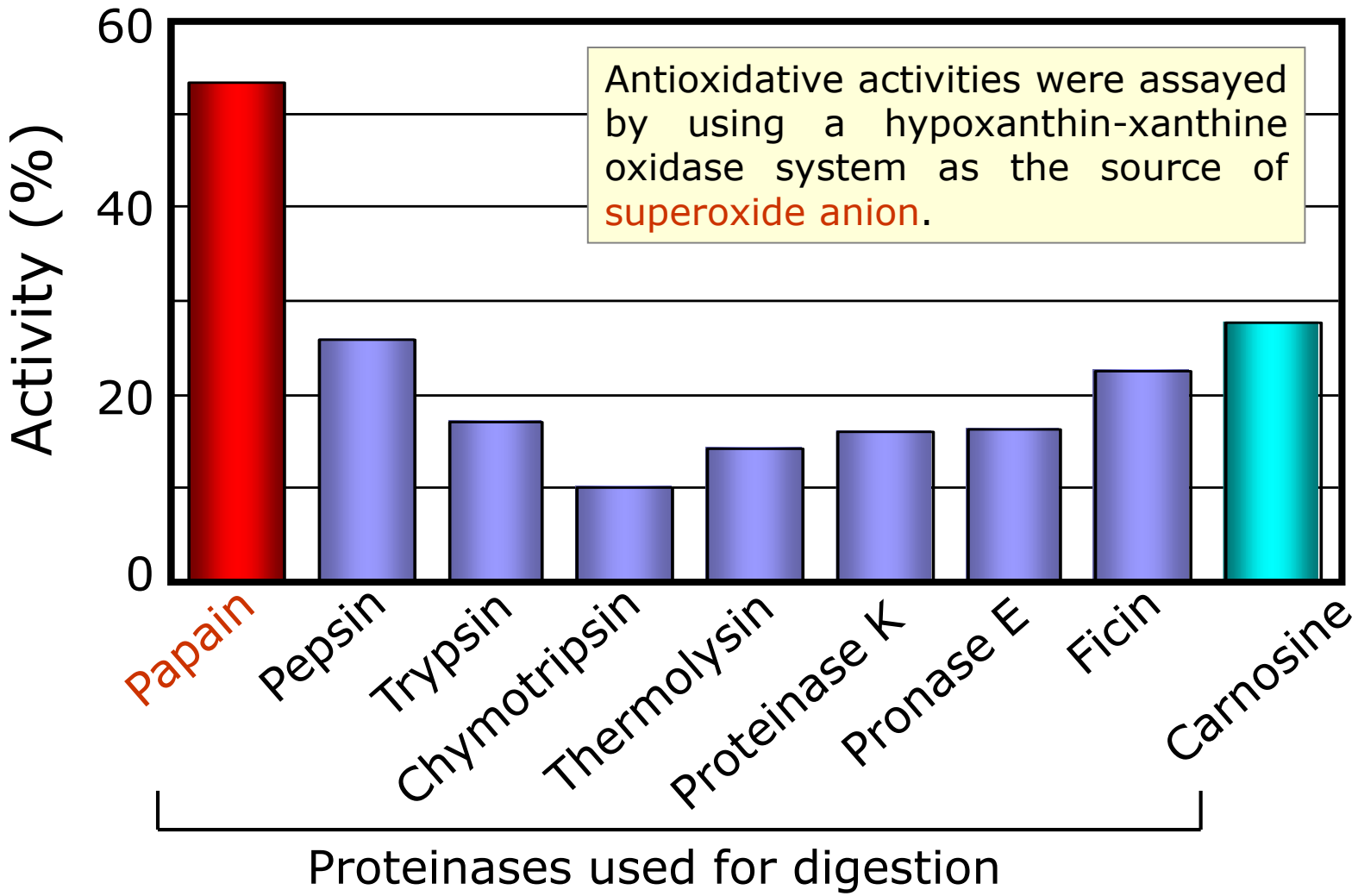


"Val-Phe-Pro-Met-Asn-Pro-Pro-Lys" was identified as an ACE inhibitory peptide.

Antioxidative Peptides Generated from Meat Proteins

Antioxidative substances have been reported to play many physiological roles, such as prevention of diseases related to oxidative stress.

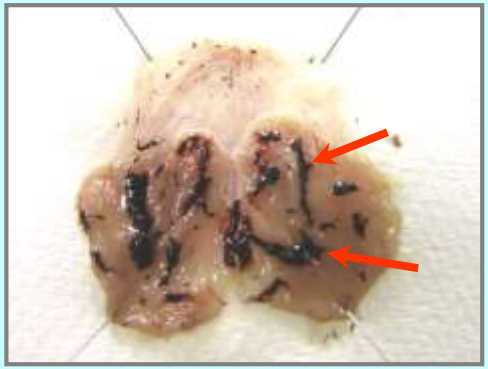
Antioxidative Activities of Pork Hydrolysates



Anti-stress Effect of Pork Protein Hydolysate

6-week-old male rats → Administration of samples (7days) → Water immersion stress (10hours) → Stomach extirpation

Normal stomach



Stressed stomach (ulceration)

Results of studies ⇢ Pork protein hydrolysates showed anti-stress activity

Identification of Antioxidative Peptides

Pork Protein Hydrolyzed by Papain

Purification

Asp-Leu-Tyr-Ala
Ser -Leu-Tyr-Ala
Val -Trp

These sequences are found in actin.

Arihara et al., 2005

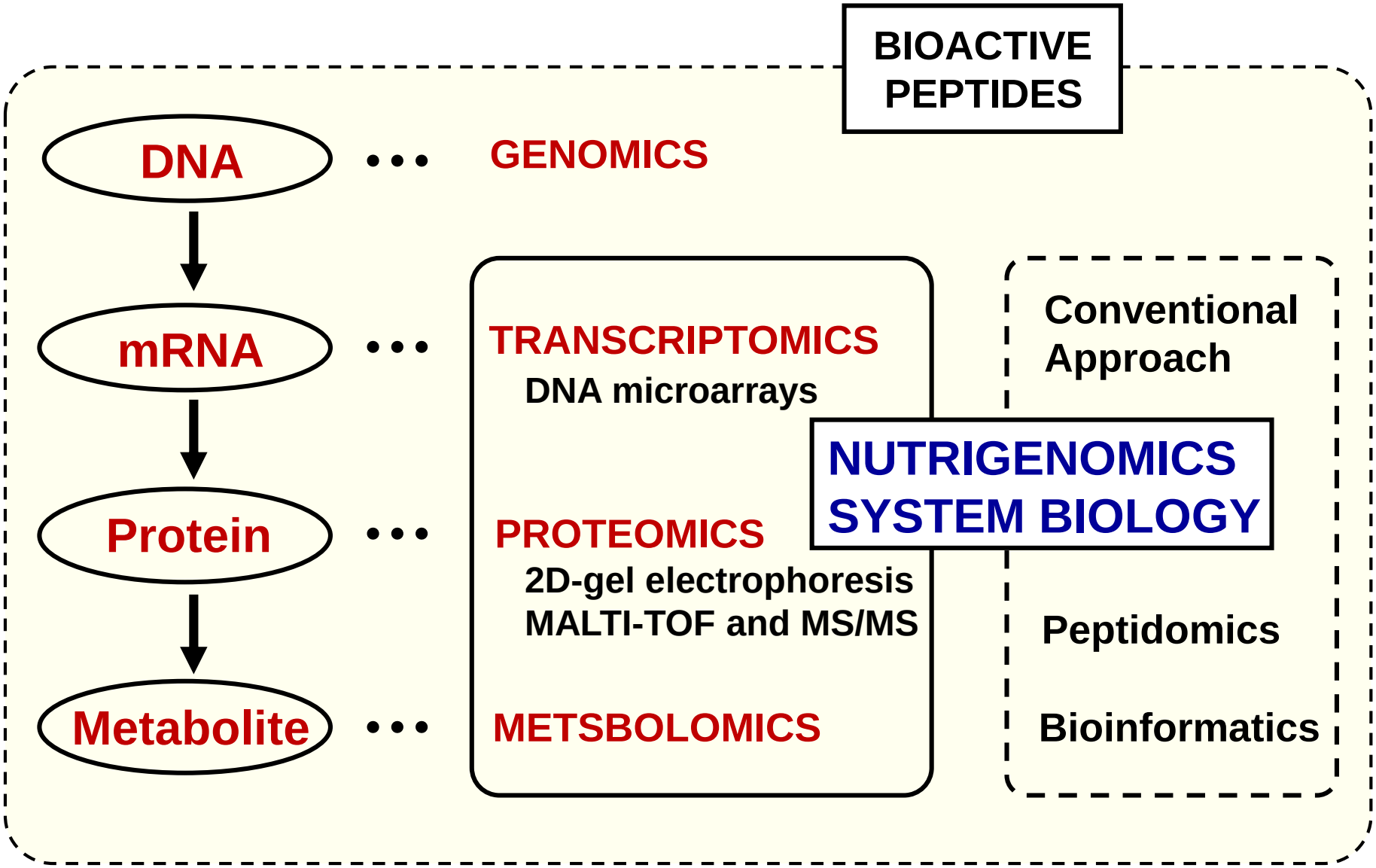
Synthesized **Asp-Leu-Tyr-Ala** showed strong **anti-fatigue** and **anti-stress** activities.

Antioxidative Peptides by Fermentation

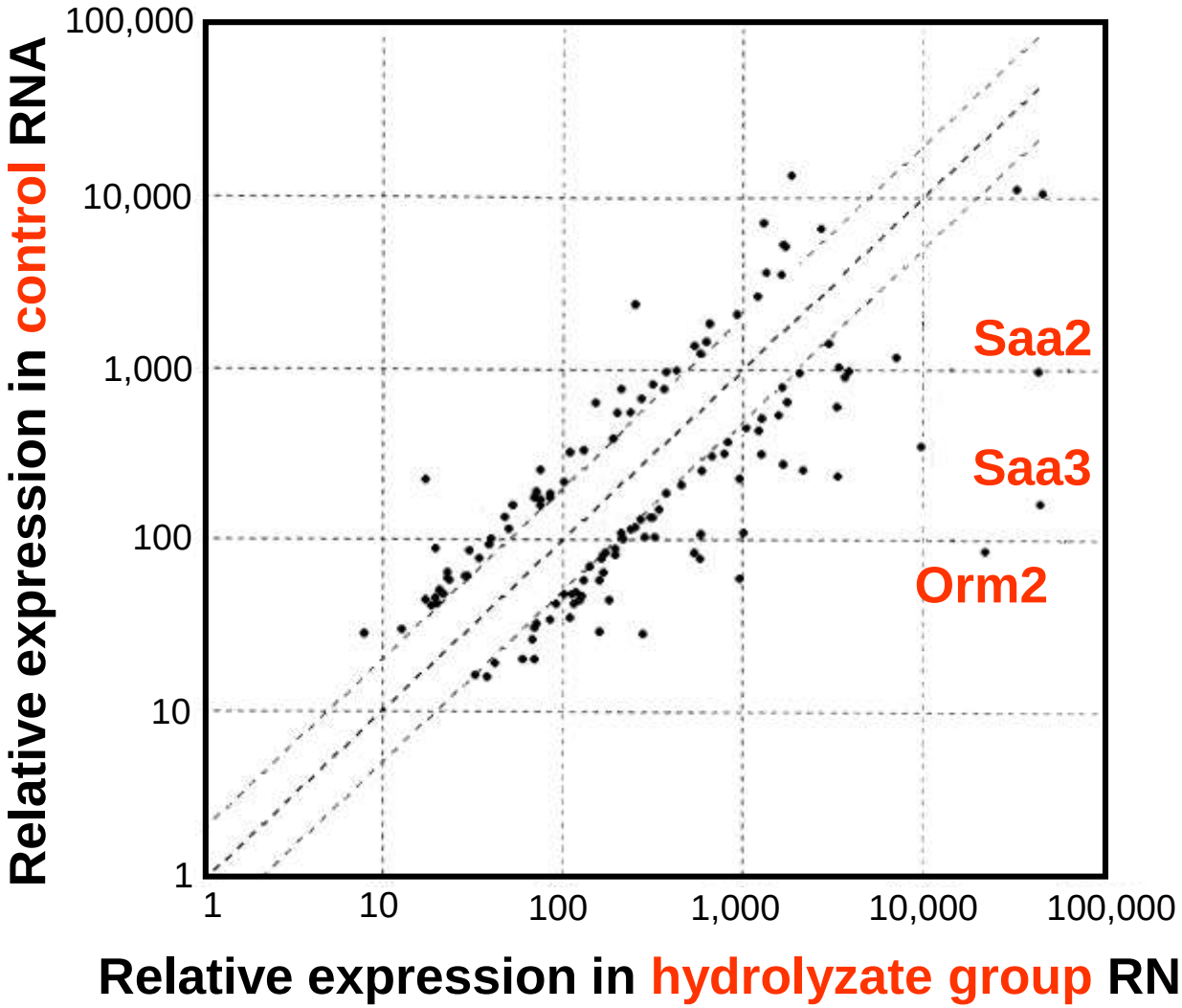
Antioxidative peptides have been detected in fermented sausages and dry-cured ham.

Such peptides would be generated by muscle or microbial enzymes from meat proteins.

Nutrigenomic Approach for Bioactive Peptides



Comprehensively Analysis of Gene Expression by DNA Microarray



Meat peptides were orally administrated to mice.

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Utilization of Probiotic Bacteria for Meat Fermentation

Definition of Probiotics

‘Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host’

Representative Probiotic Bacteria

Intestinal strains of
Lactobacillus* & *Bifidobacterium

Properties of Probiotic Bacteria

- Human origin
- Resistance to acid and bile toxicity
- Adherence to human intestinal cells
- Colonization of the human gut
- Antagonism against pathogenic bacteria
- Production of antimicrobial substances
- Immune modulation properties
- History of safe use in humans

Examples of Probiotic Dairy Products

Probiotic dairy products are popular in many countries, including Japan, Germany, Finland, France, Spain, USA, Korea, New Zealand, India, ---.

Development of probiotic meat products

The possibility of development of probiotic meat products has been discussed in the field of meat science and industry.

By using probiotic bacteria, potential health benefits can be introduced to meat products.

Target products with probiotic bacteria are mainly dry sausages, which are processed by fermentation without heat treatment.

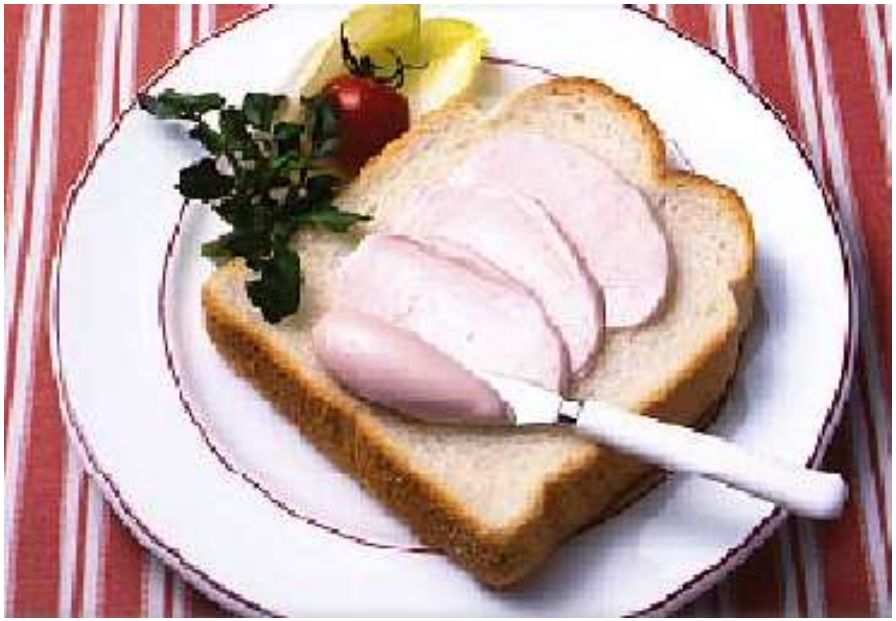
Screening of probiotic bacteria for meat

***Lactobacillus rhamnosus* FERM P-1520 has been selected from the collection of human intestinal lactobacilli for a probiotic meat starter culture.** *Arihara et al. (1998)*

In addition to the probiotic properties, screening was carried out to clear the following regulations in Japan.

- **use of 200 ppm nitrite & 3.3% NaCl**
- **processing at a temperature below 20ºC**

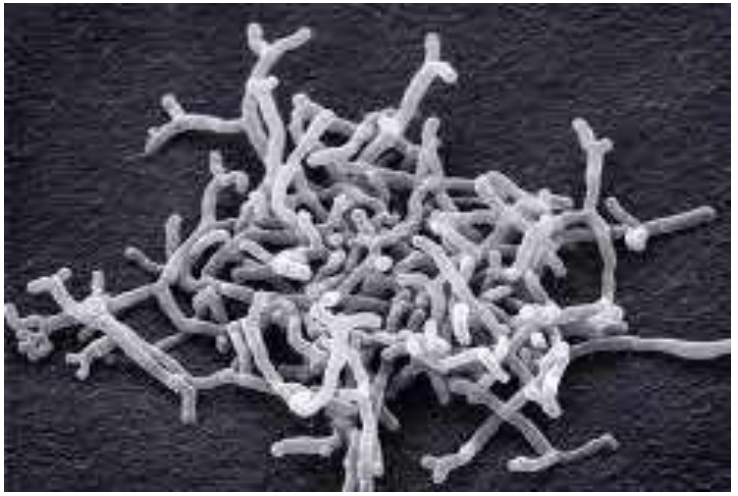
Developed probiotic meat product



Fermented meat spread product (“Breadton”, Prima Meat Packers Ltd, Japan) utilizing the human intestinal *Lactobacillus rhamnosus*.

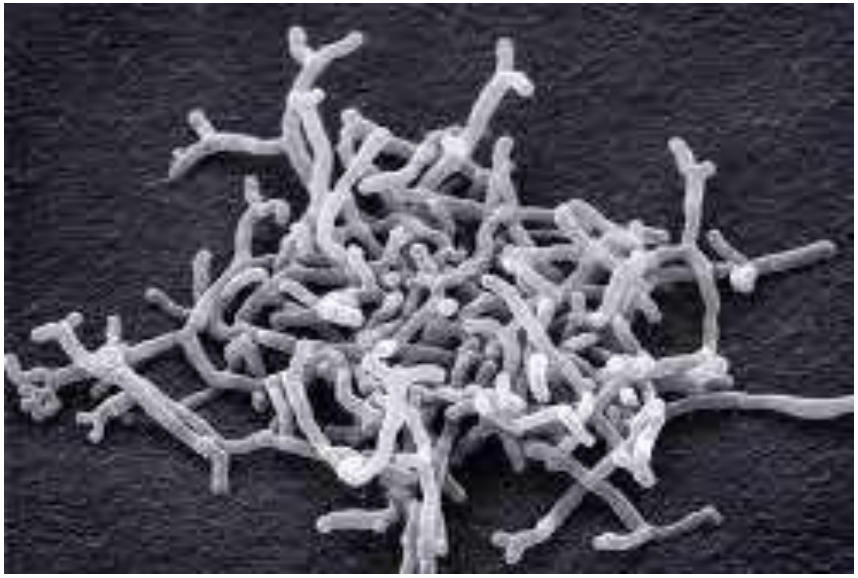
Utilization of *Bifidobacterium* sp.

Since *Bifidobacterium* is most significant bacteria contributing to the human health, utilization of this bacterium is desirable for producing a healthy meat product.



***Bifidobacterium bifidum* K202 was selected from our bacterial collection for meat fermentation. Although *B. bifidum K202* grow well in sausages, a single culture of *B. bifidum* give undesirable flavor due to its acetic acid production.**

Bifidobacterium Growth-promoting Peptide Isolated from Meat Protein Hydrolyzate



Bifidobacterium sp.

Prebiotics

‘Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve the health of the host’

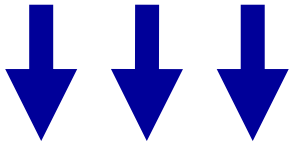
Prebiotic substances (oligosaccharides, dietary fibers) enhance the activity of probiotic bacteria.

PROBIOTICS — **PREBIOTICS**

Lactobacillus
Bacillus coagulans
Bifidobacterium

Oligosaccharides
Dietary fibers
Peptides

SYNBIOTICS

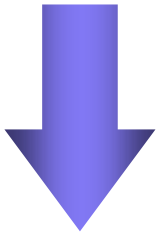


Intestinal Microflora

Health maintenance
Disease prevention

Objectives

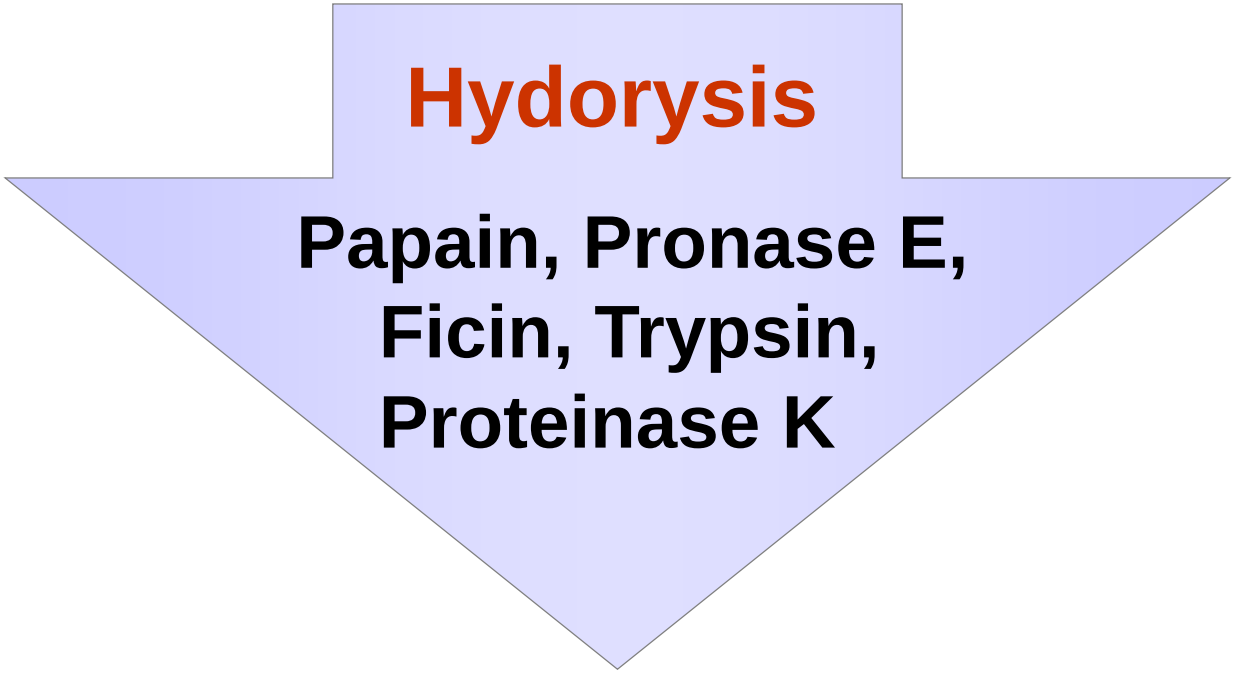
***Bifidobacterium* growth-promoting activities generated in meat protein hydrolyzates**



Identification and characterization of prebiotic peptides

Preparation of meat protein hydrolyzates

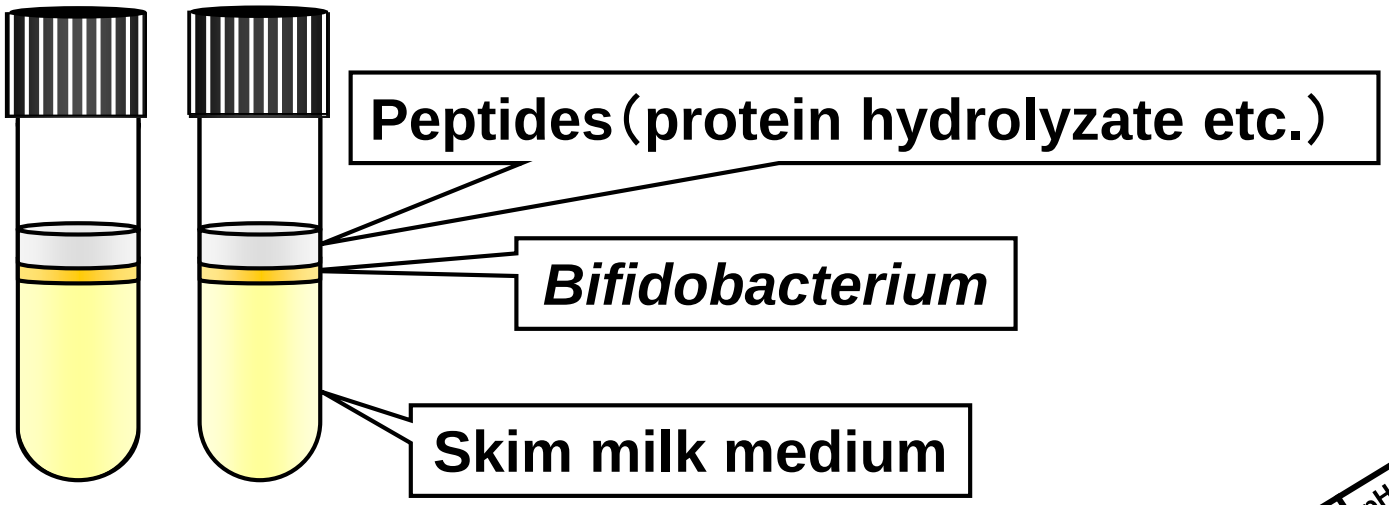
Pork Proteins

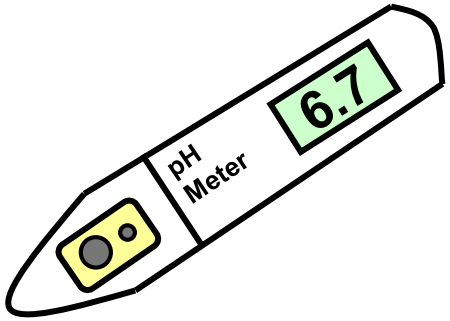


Pork Protein Hydrolyzates

Peptides

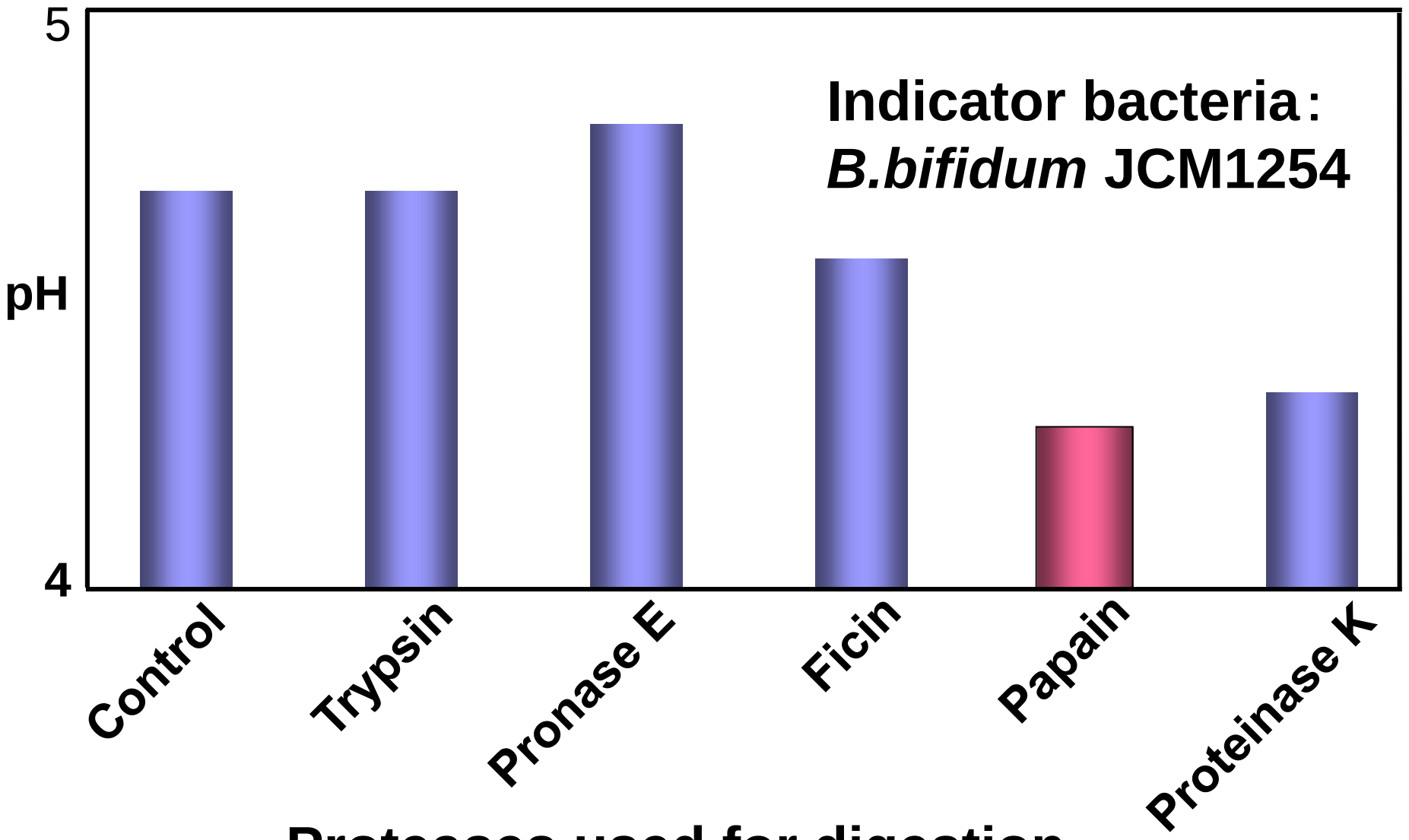
Measurement of *Bifidobacterium* growth-promoting activity



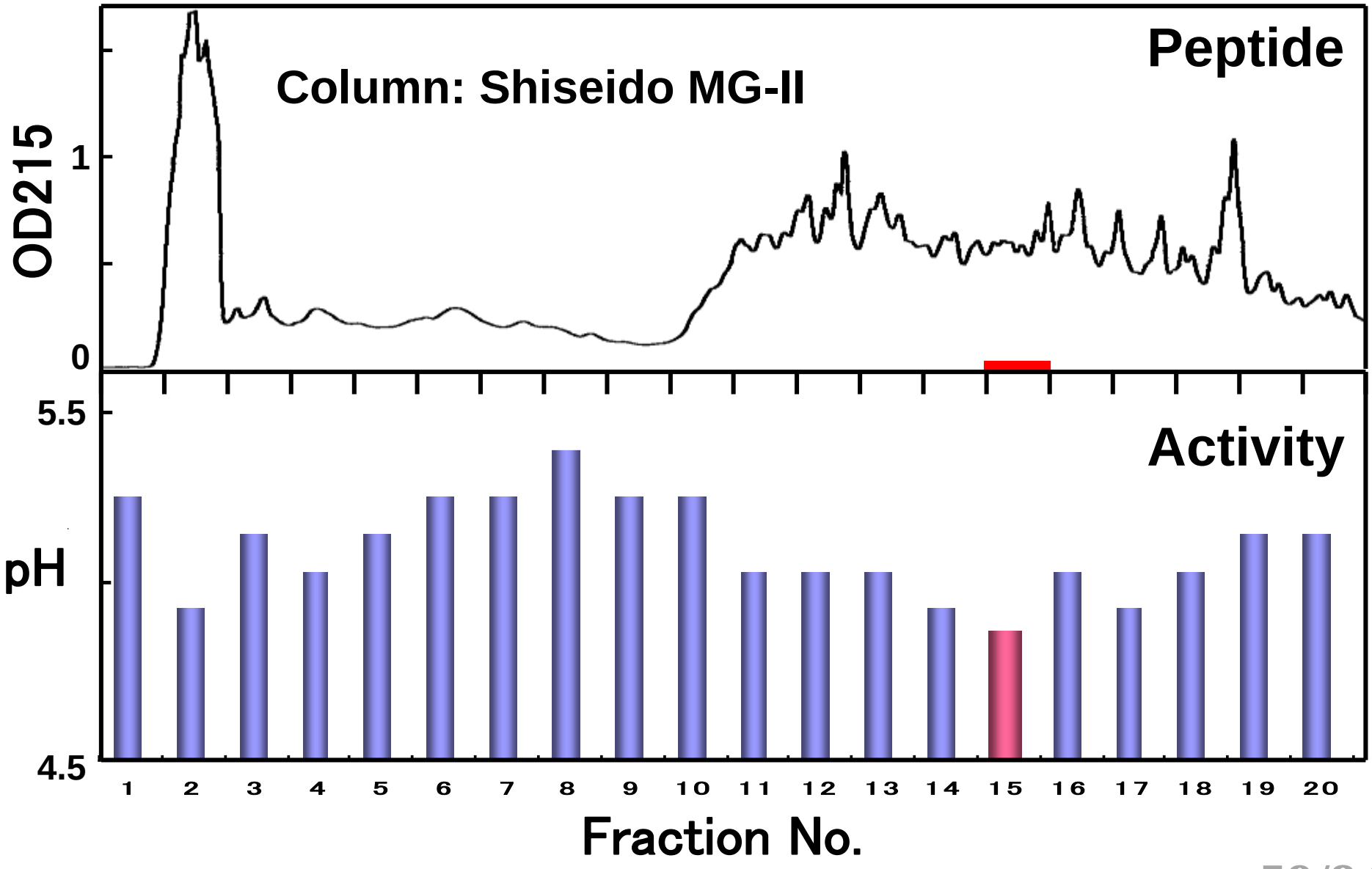


Incubation (37°C, 48h) → **pH measurement and CFU count**

Growth-promoting activity of pork protein digests

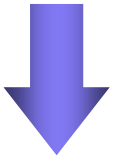


Fractionation by 1st RP-HPLC

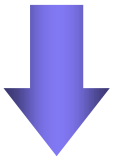


Identification of purified peptide

Purified peptide



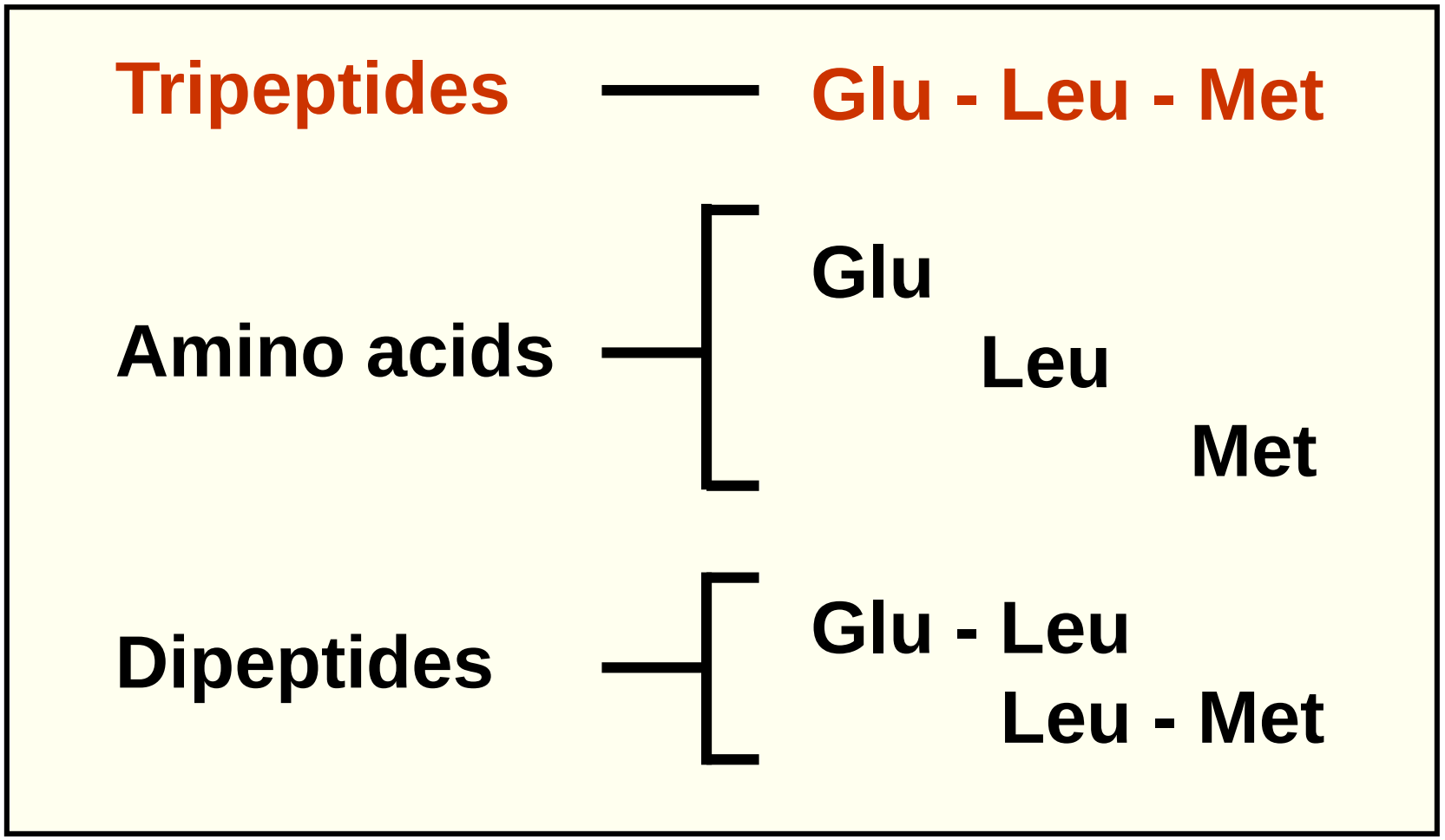
Mass Spectormetry
Protein sequencer



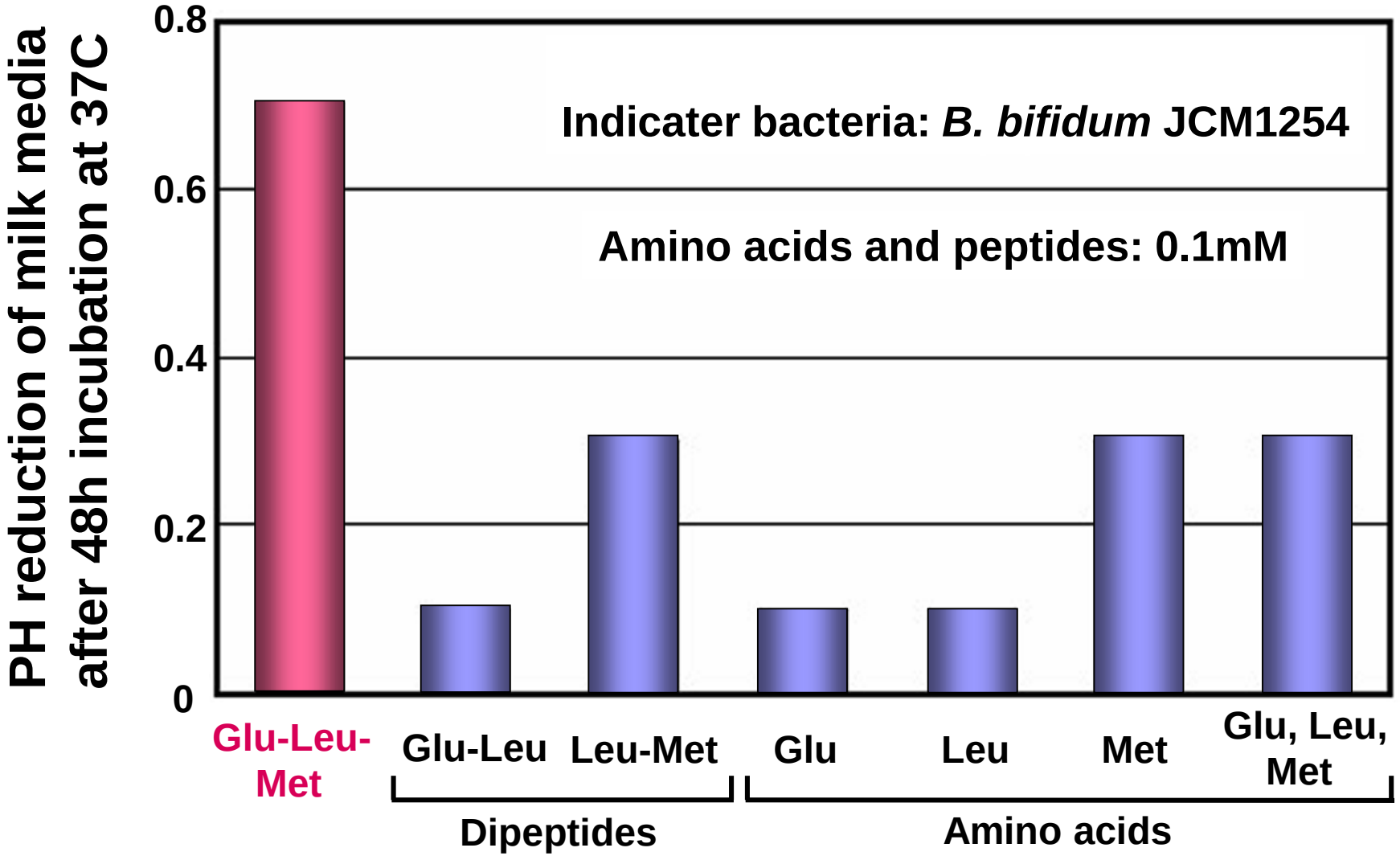
Glu - Leu - Met

Myosin heavy chain
(331-333)

Growth-promoting activities of Glu-Leu-Met and related dipeptides and amino acids



Growth promoting activities of Glu-Leu-Met and related dipeptides and amino acids



Activities of Glu-Leu-Met to bacteria

- ***Bifidobacterium*** **growth-promoting（11/26）**

B. bifidum	**3/5**	***B. breve***	**3/5**
B. adolescentis	**1/7**	***B. infantis***	**1/5**
B. longum	**3/3**	***B. catenulatum***	**0/1**

- **Against pathogenic bacteria（no effect）**

Salmonella enteritidis, Listeria monocytogenes, Yersinia entericolitica, Clostridium perfringens, Staphylodoccus aureus, Bacillus cereus, E. coli

Prebiotic Peptides

- **Meat protein hydrolyzates promote *Bifidobacterium* growth.**

- **Glu-Leu-Met is responsible for the growth-promoting activity.**

This activity would be useful for novel functional foods.

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Development of Peptide-based Ingredients by Maillard Reaction

Peptide

Sugar

⇢ **Millard Reaction Products**

Maillard Reaction in Foods

Non-enzymatic browning reaction

What is Maillard Reaction ?

Amino Acid
(amino group)

+

Reduced Sugar
(carbonyl group)

Amino–Carbonyl Reaction

⋯⋯▶ **Maillard Reaction Products**

Color, Flavor, Bioactivity

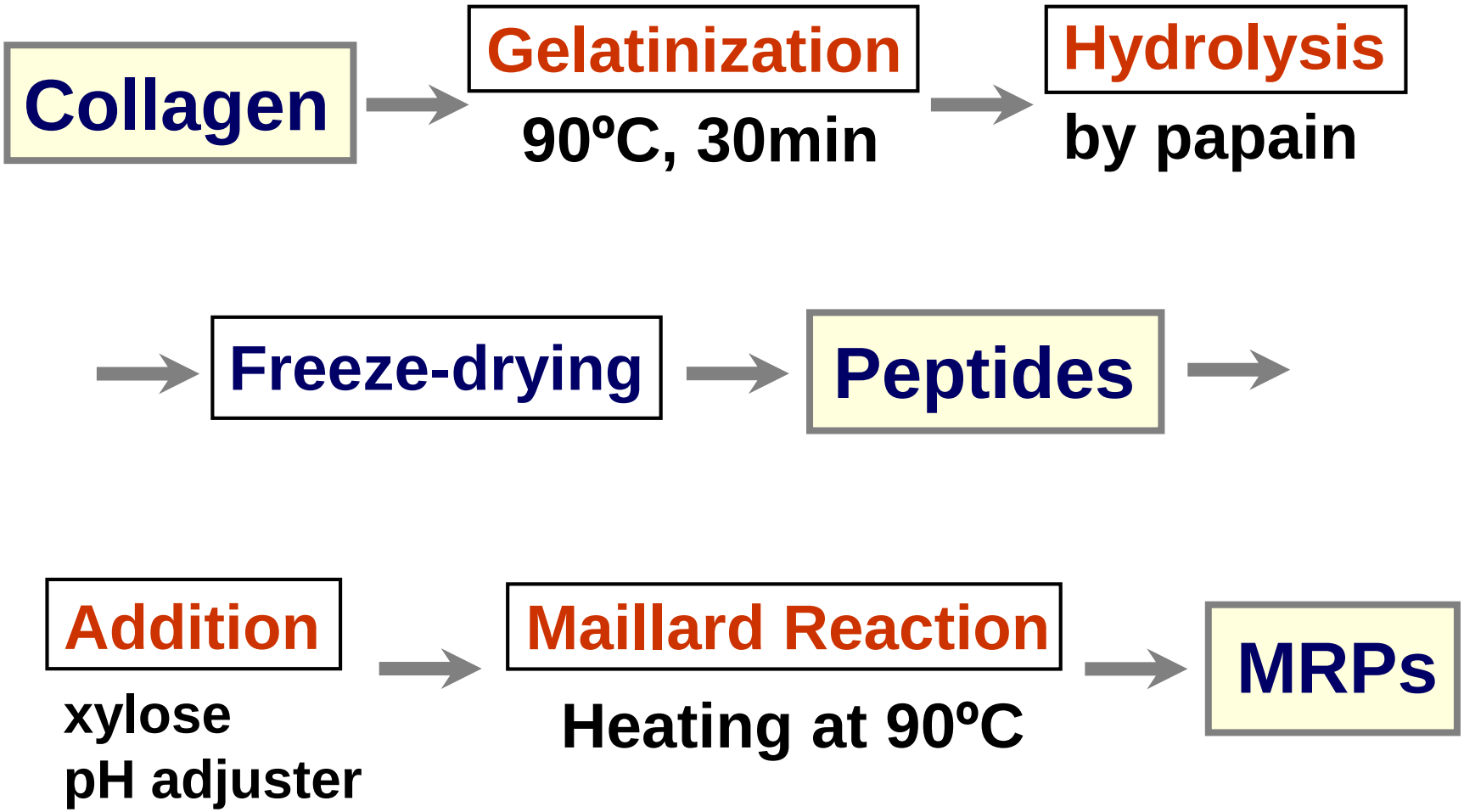
Utilization of Collagen for MRPs

- Byproducts with Collagen
  e.g., animal skin, fish scales
- High Glycine Content
- No Aspargine

MRPs from Collagen Peptides

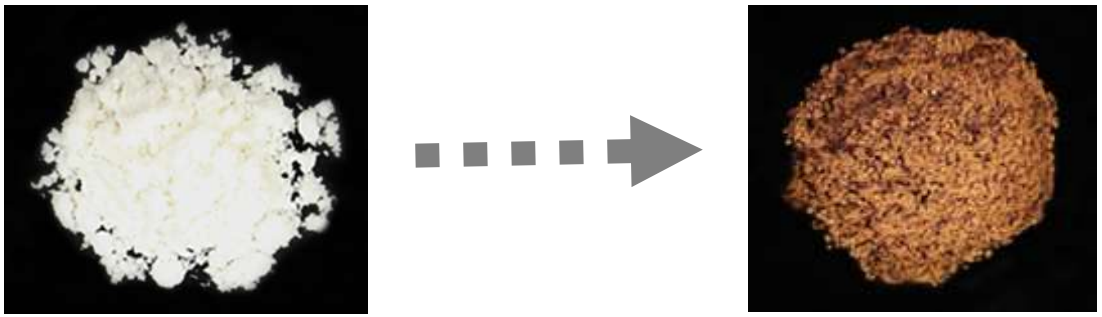
as Functional Ingredients

Preparation of MRPs from Collagen



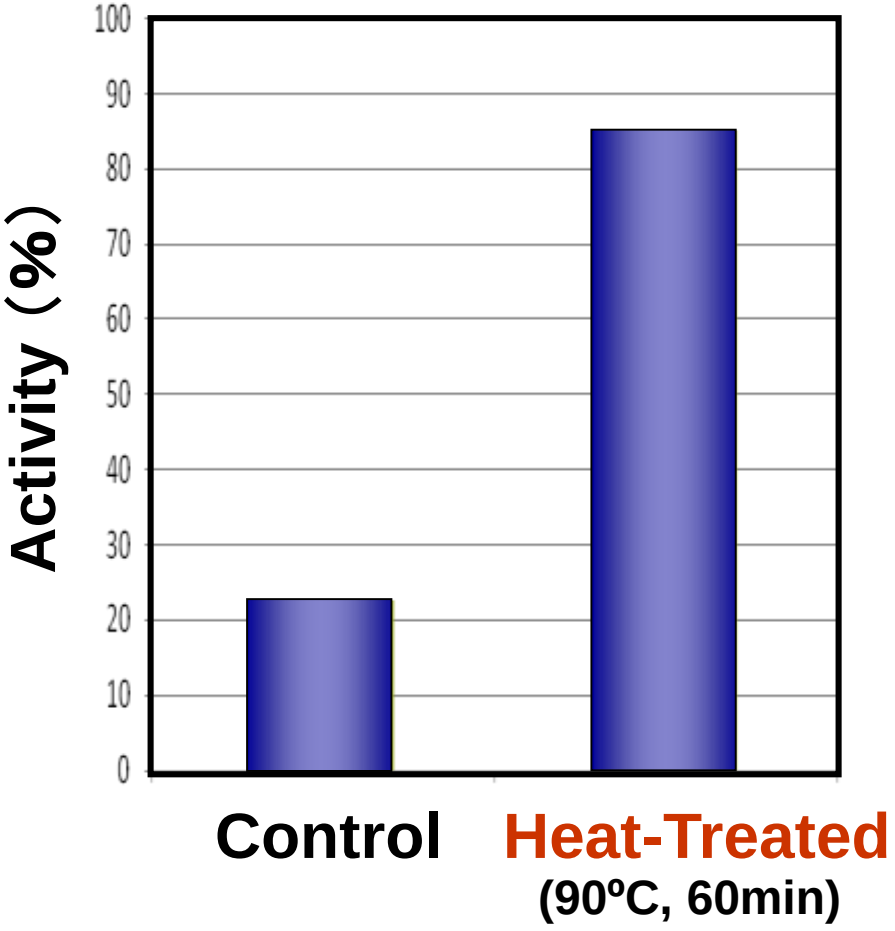
Conditions of Maillard Reaction

- **Peptide : Collagen hydrolysate（0.05g/ml）**
- **Sugar : Xylose（0.04g/ml）**
- **pH : 9.5 by sodium carbonate**
- **Temperature : 90ºC**
- **Time : 60 or 240min**

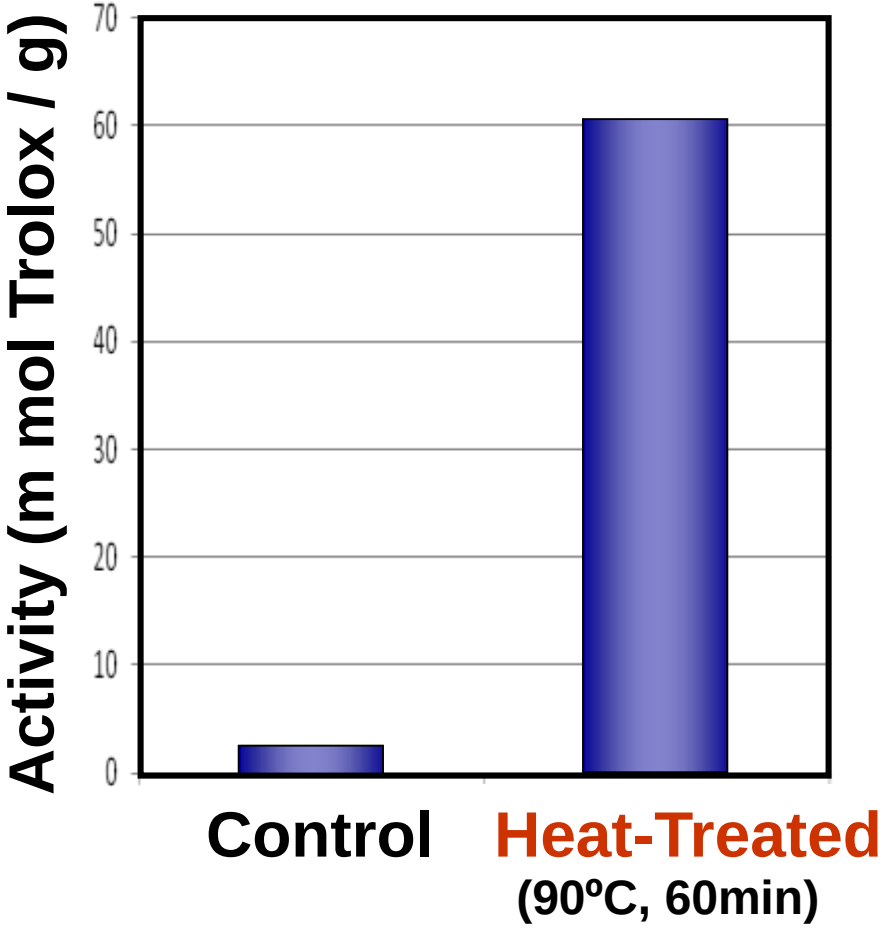


Antioxidative Activities MRPs

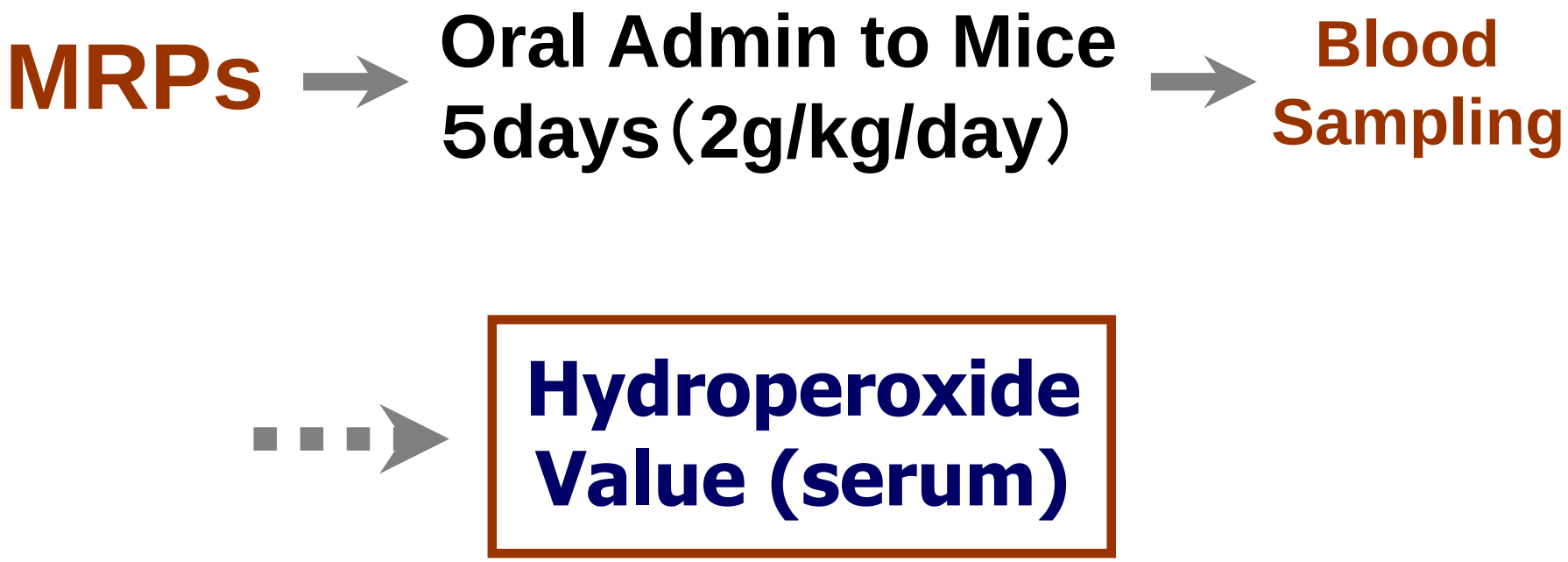
Superoxide Ion



DPPH Radical

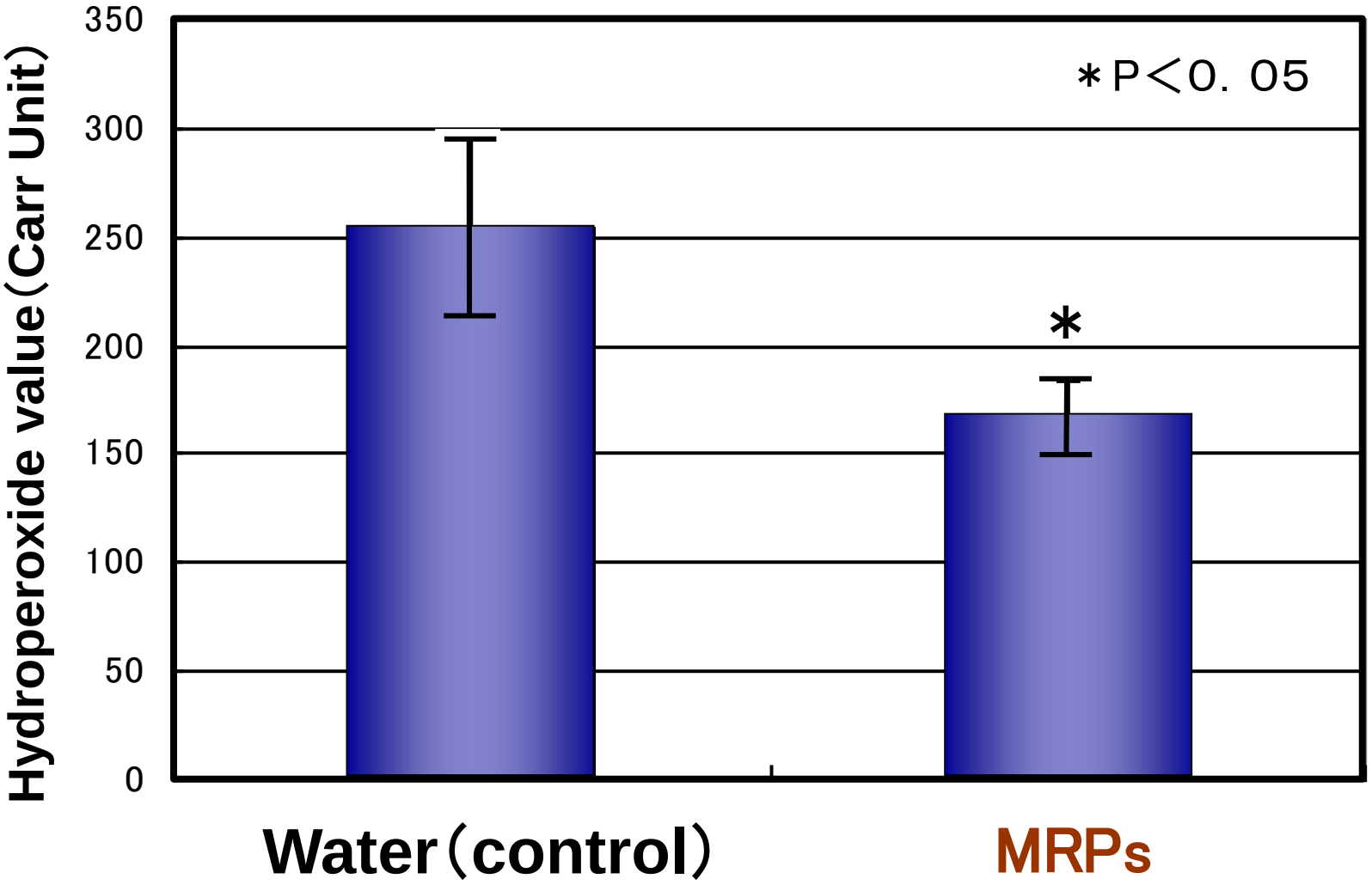


Effect of Oral Administration on Degree of Oxidative Stress

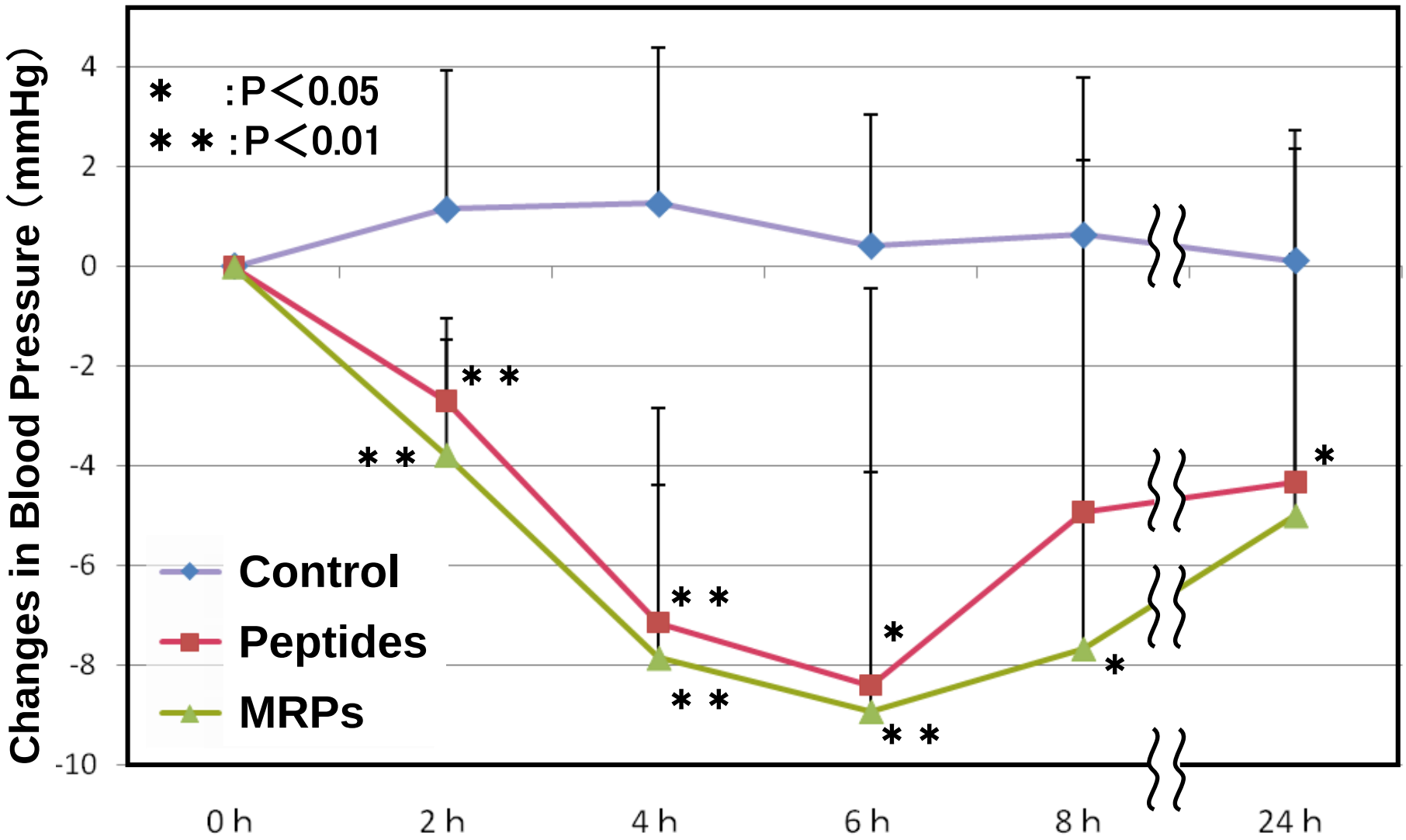


Measurement as Stress Marker

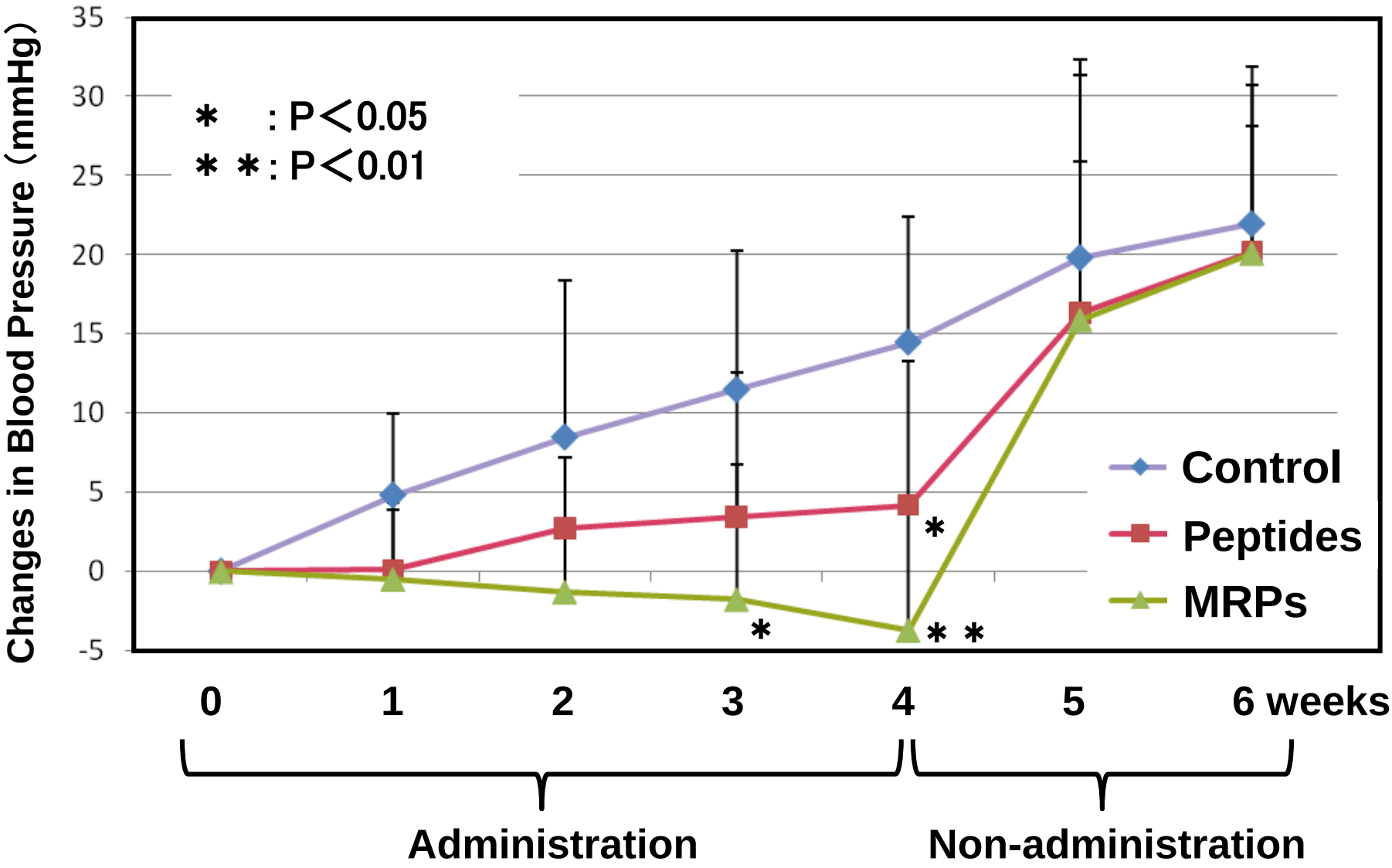
Hydroperoxide Value of Serum



Antihypertensive Activity by Single Administration

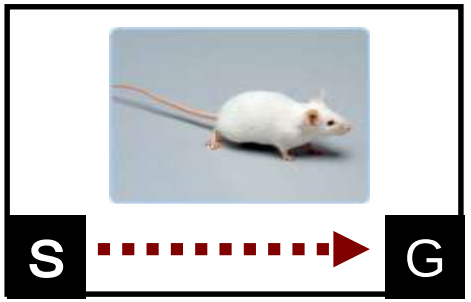


Antihypertensive Activity by Continuous Administration

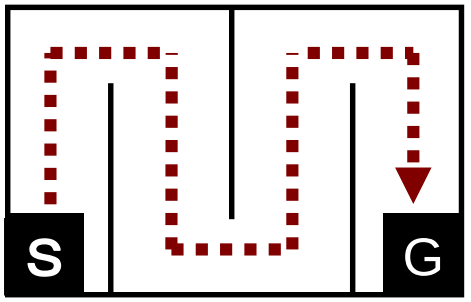


Water Maze Test with Mice

Training by direct swimming



Test by using water maze



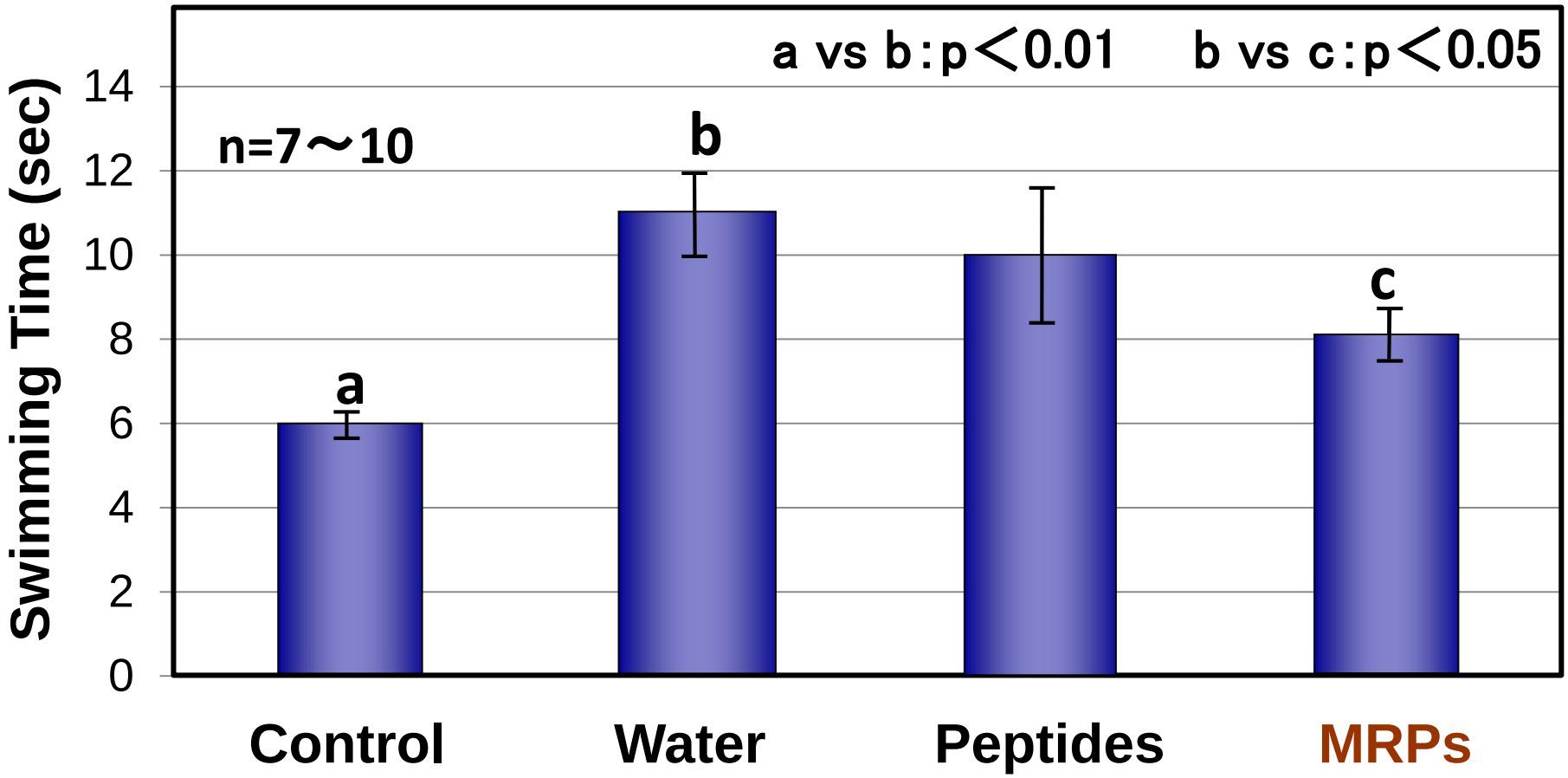
Sample Admin.

Scopolamine
(Defect of memory)

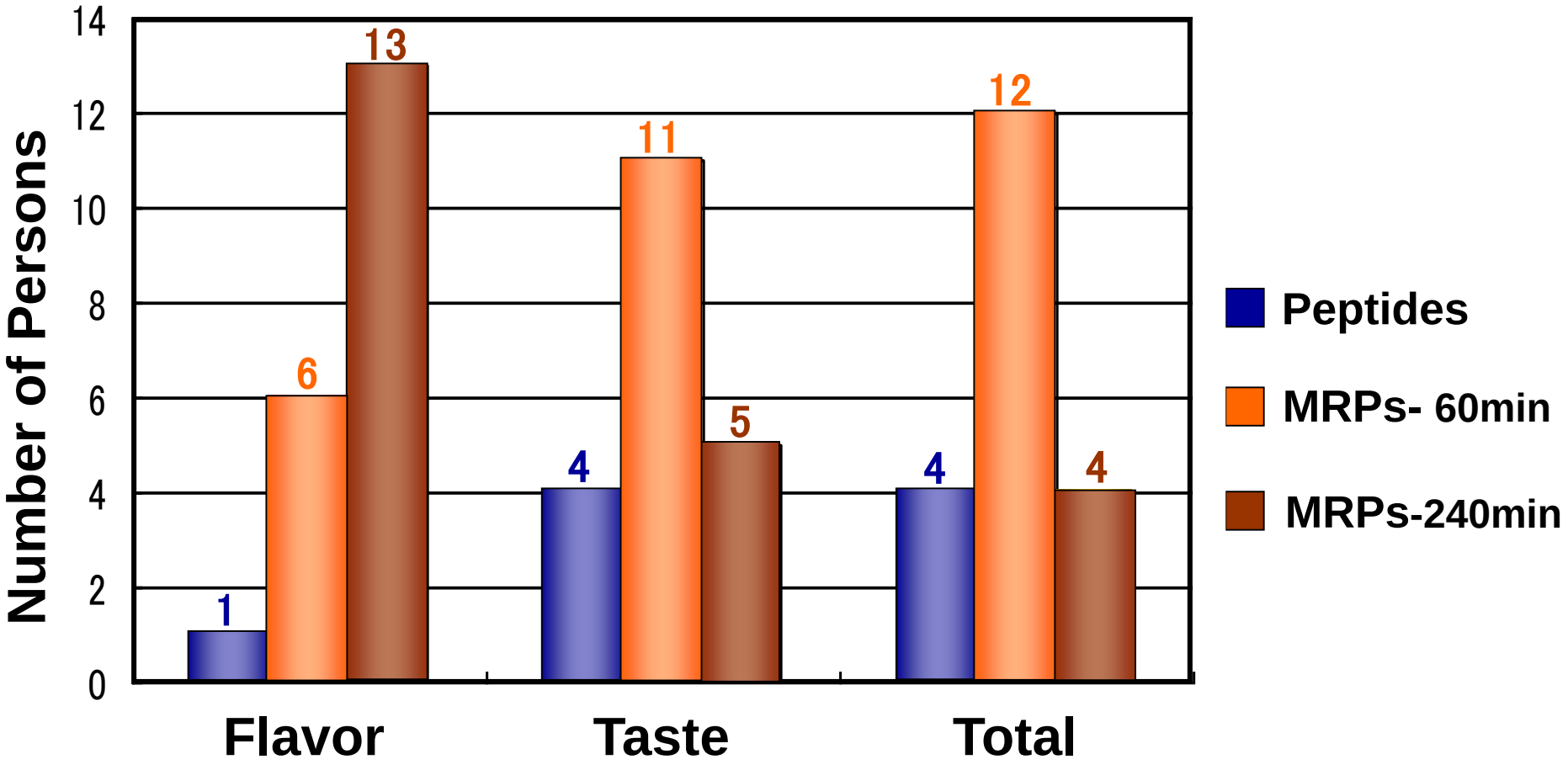
Measurement
(Swimming time)

30min **30min**

Results of Water Maze Test



Sensory Properties of MRPs



MRPs Generated from Collagen

- Good Functional Properties

- Good Sensory Properties

Possible Functional Food Ingredient

Kitasato 100×50
Pioneer the Next

Concluding Remarks

Functional Food Ingredients and Products

- Generation of bioactive peptides
- Utilization of probiotic bacteria
- Development of MRPs from peptides

- Appropriate selection
- Effective combination

Promising Functional Foods ?

Salchiochon


Casa Riera

Sumaia


Chorizo

- **Rediscovery of traditional fermented meats**
- **Development of novel foods (meat products)**

Hurdles in Developing and Marketing Novel Functional Meat Products

Such products are unconventional and consumers in many countries regard meat products to be bad for health.

1. Demonstrate the benefits for health
2. Inform consumers of the exact value
3. Ensure the safety of new products



Thank you for your kind attention.



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