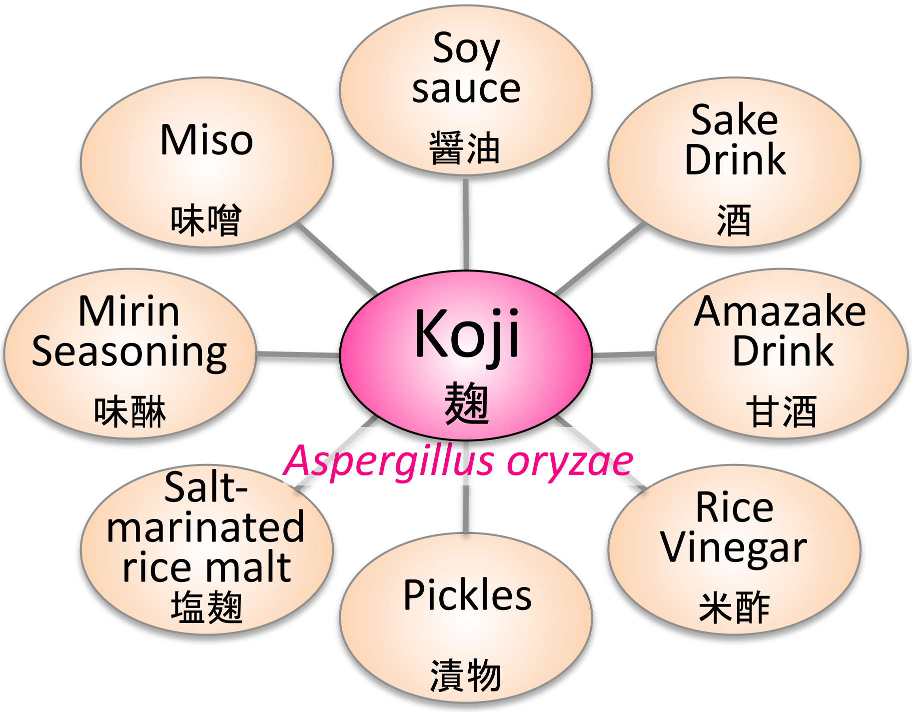
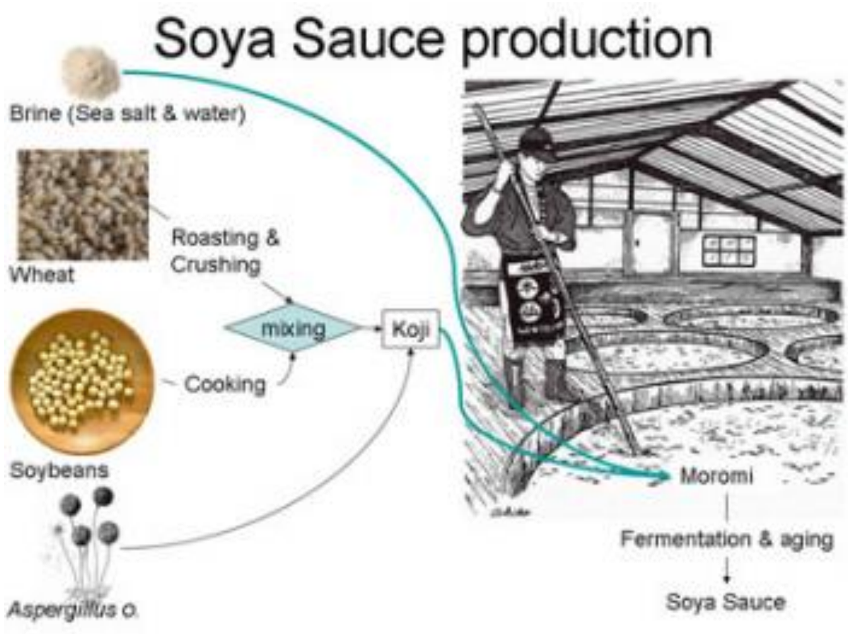
What is Koji ?

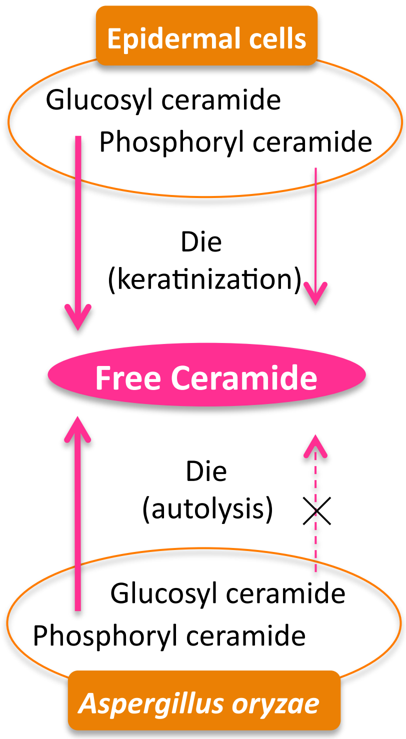
Japanese traditional food production, “the culture”





How ceramide is created ?

A phenomenon similar to the skin has occurred in the koji mold



Koji Fermentation

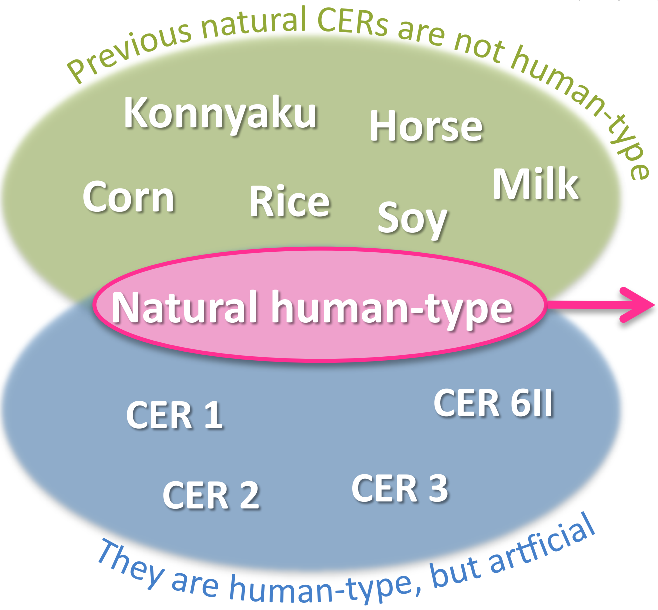
Carbohydrate → Simple sugars
Protein → Amino acids

Umami (good taste)

Raw materials of yeast fermentation

Natural Human-type Ceramide

Human skin identical



The world's first ceramide created through traditional Japanese food fermentation process

Fermentation lees (residue)
: Soy sauce, Sake, Shochu, Black vinegar etc.

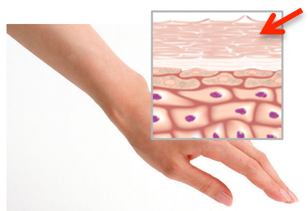
100 years have passed since the renown brightening ingredient, Kojic acid was discovered from koji mold, *Aspergillus oryzae*, the naturally occurring culture has been used for centuries in Japan to make soy sauce, sake and miso.

We are proud to announce that we have discovered our revolutionary ceramides from Koji.

It is safe and stable.

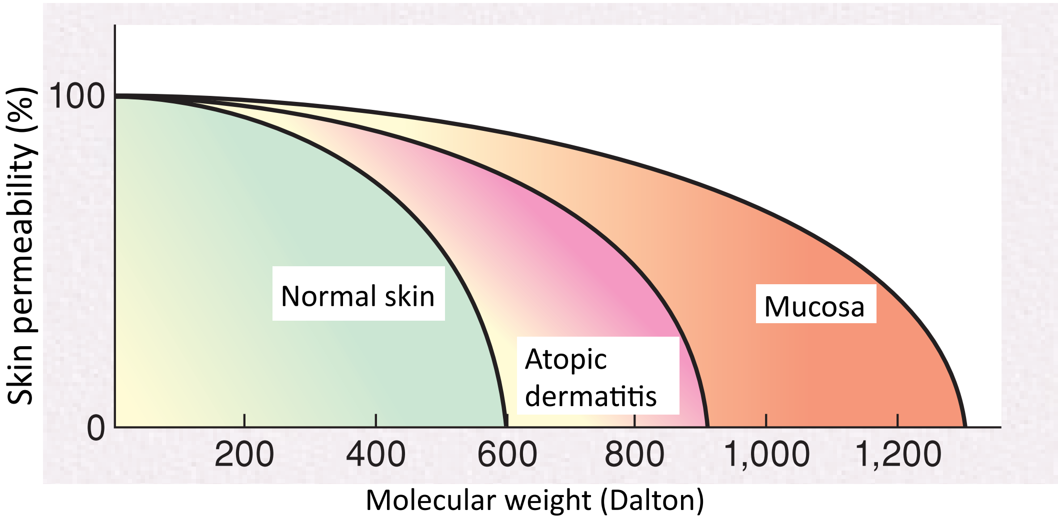
	Natural ceramide	Synthetic human-type ceramide	Natural human-type ceramide
Component	･Glycosyl ceramide (Cerebroside) ･Sphingomyelin	N-acylsphingoid (free ceramide) ※ Single component	N-acylsphingoid (free ceramide) ※ Various molecular species and very long-chain acyl type
Use	Foods	Cosmetics	Cosmetics ･ Foods

Conditioning ingredients

Occlusive	Emollient	Humectant	Biocomponent
閉塞剤	皮膚軟化剤	湿潤剤	ヒト角層成分（ヒト型）
Mineral oils Vaseline	Oil, wax, ester, silicon (mainly natural origin)	Hyaluronic acid Collagen	Ceramide Cholesterol, fatty acid NMF
Film	**Feeling**	**Sponge**	**Intercelluarlipid**
(Impermeable)	(Partially permeable) Oil-soluble	(Impermeable) Water-soluble	(Permeable)
Prevention of water loss	Prevention of water loss	Moisturizer	Human skin identical Internal skin moisturizer Natural protective barrier
			

Comparison of molecular weight and size of beauty components

Material	Molecular weight	Size
Collagen	100,000	300 nm
Hyaluronic acid	1,000,000	1~10 μm
Nano hyaluronic acid (Low molecular)	5,000	10~50 nm
Ceramide	600	1~5 nm
Sucrose	342	0.9 nm



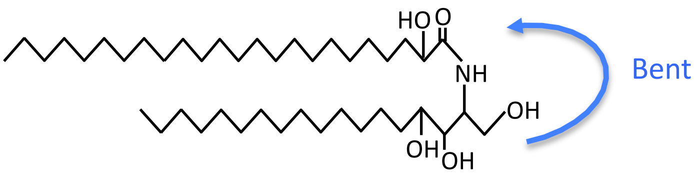
Relationship between skin permeability and molecular weight

【Epidermal permeability】

・Low molecular
(In general, components, more than 600 Dalton, do not pass)
・Lipophilic (oil-soluble)

<u>Gap between the corneocytes: 40～60nm (In case of healthy skin)</u>

・Ceramide is small size because of the bent structure



Ceramide AP with C24 very long chain fatty acid
(molecular weight 684)

Bio-barrier "Ceramide"

Genuine

Metabolic pathway of ceramide in the epidermis

Degrading system (Dead cells)

Free ceramide

Hydrolytic enzyme

Glucosylceramide

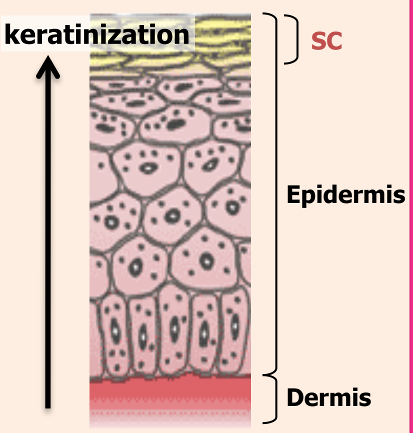
Sphingomyelin

Synthetic system (Living cells)

Free ceramide

Acyl

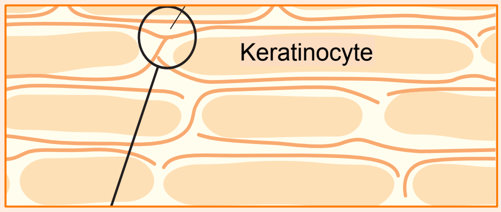
Sphingoid base

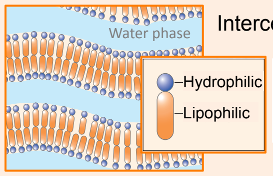


Membrane lipids in living cells are converted to intercellular ones in dead cells.

Ceramide bilayer in the stratum corneum

The largest tissue of human body is "Skin", most outer layer of which is SC.





Intercelluler lipids "Ceramide"

Ceramide accounts for 50% of intercellular lipids, in the SC.

Classification and nomenclature of human corneous ceramide

Human corneous ceramide		
INCI	OH	%
Ceramide EOS	2	8.3
Ceramide NS	2	20.5
Ceramide NP	3	18.0
Ceramide EOH	2	5.0
Ceramide AS	3	19.7
Ceramide AP	4	8.6
others		19.9

Ponec M, et al.（*J Investig Dermatol* 2003）

Ceramide XY
X: fatty acid
Y: sphingoid

N: non-hydroxy
A: α-hydroxy
EO: ω-hydroxy

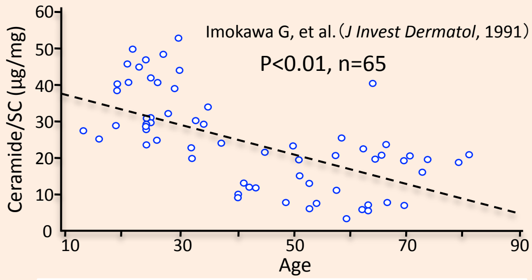
Y \ X	N	A	EO
S	NS(2)	AS(5)	EOS(1)
DS	NDS(2)	ADS	EODS
P	NP(3)	AP(6II)	EOP
H	NH	AH	EOH(4)

Parentheses: previous ceramide nomenclature

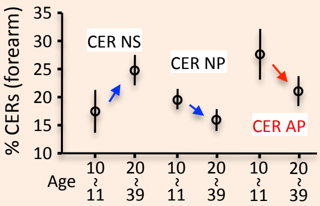
S: sphingosine
DS: dihydrosphingosine
P: phytosphingosine
H: 6-hydroxysphingosine

Human SC ceramides：diverse molecules (more than 300 species by large classification)

Decrease in ceramide with age



Significant decrease in ceramide level after 30 years old



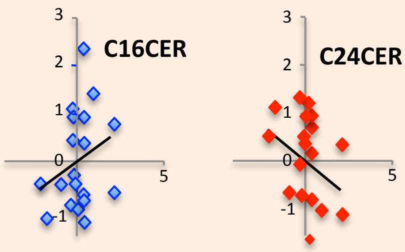
In female, CER AP significantly decreases with aging

Modified by Denda M, et al.（*Arch Dermatol Res*, 1993）

Ceramide is important as a beauty ingredient

Relationship between very long chain ceramide and skin barrier function

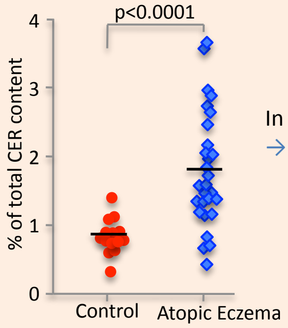
Joo K, et al.（J Dermatol Sci, 2010）



Increase in ceramides with long chain fatty acids such as C16 and C18 causes rise of TEWL.
→ Decrease of barrier function

Increase in ceramides with very long chain fatty acids such as C24 causes decrease of TEWL.
→ Increase of barrier function

Correlation (C16-CER) and reverse-correlation (C24-CER) vs TEWL

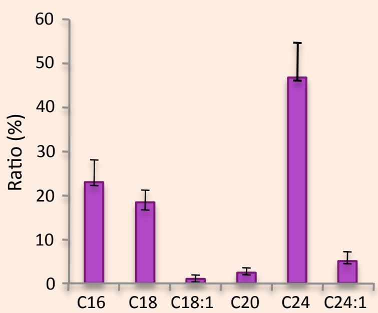


Total C34 CERs (C16-CER)

In skin of AE patients, short C34 CERs were increased.
→ Short CERs, which are often included in synthetic compounds, cause decrease of epidermal barrier function.

Janssens M., et al.（J Lipid Res, 2012）

Fatty acids in human SC ceramide NS



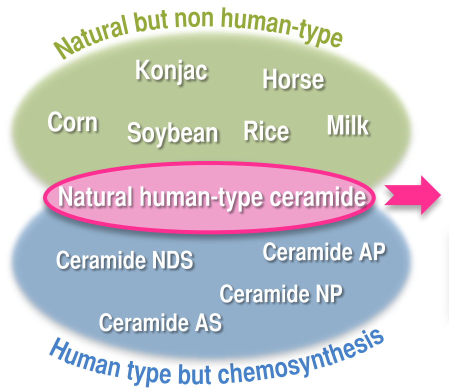
There is many C24-CER in the SC

Ceramides with very long chain fatty acid strengthen a ceramide bilayer and improving barrier function

Natural Human-type Ceramide

World first！Beauty material

~Very Long Chain Phytoceramide~



Natural ceramide (Glycosylceramide)

Synthetic ceramide (Free ceramide)

Phytoceramide (AP&NP) purified from fermentation residue

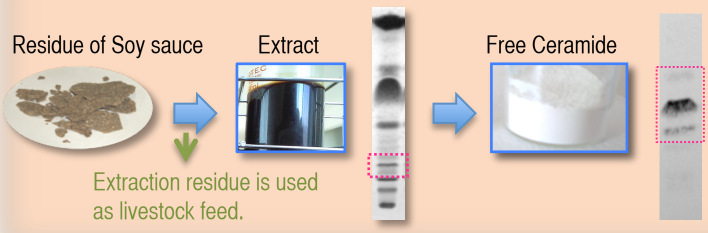
Fermentation residue: Soy sauce, Sake, Shochu Awamori, etc.

Molecular species of natural phytoceramide

Ceramide NP (2~10%) 3 OH	t18 - C22	C40
	t18 - C24	C42
	t18 - C25	C43
	t18 - C26	C44
Ceramide AP (75~93%) 4 OH	t18 - C18h	C36
	t18 - C20h	C38
	t18 - C22h	C40
	t18U - C22h	C40
	t18 - C23h	C41
	t18U - C23h	C41
	t18 - C24h	C42
	t18U - C24h	C42
	t18 - C25h	C43
	t18U - C25h	C43
	t18 - C26h	C44
	t18U - C26h	C44
	t19 - C24h	C43
	t20 - C22h	C42
	t20 - C24h	C44
	t20 - C25h	C45
Ceramide DP (5~15%) 5 OH	t18 - C24h2	C42
	t19 - C24h2	C43
	t18 - C25h2	C43
	t20 - C24h2	C44
	t18 - C26h2	C44

- More than 25 molecular species
- Very long chain fatty acid such as C24
- Phytosphingosine (trihydroxy)

Recycling of soy sauce residue which focused on ceramide production

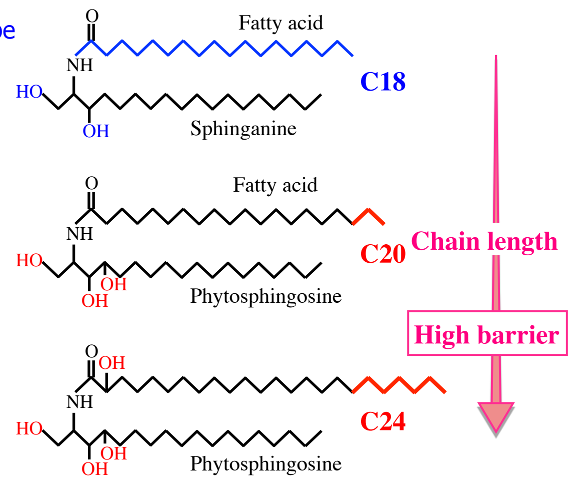


Extraction residue is used as livestock feed.

Synthetic human type ceramide NDS 1 specie

Natural human type ceramide NP 4 species（13%）

Natural human type ceramide AP 16 species



Superiority of natural ceramide than synthetic one

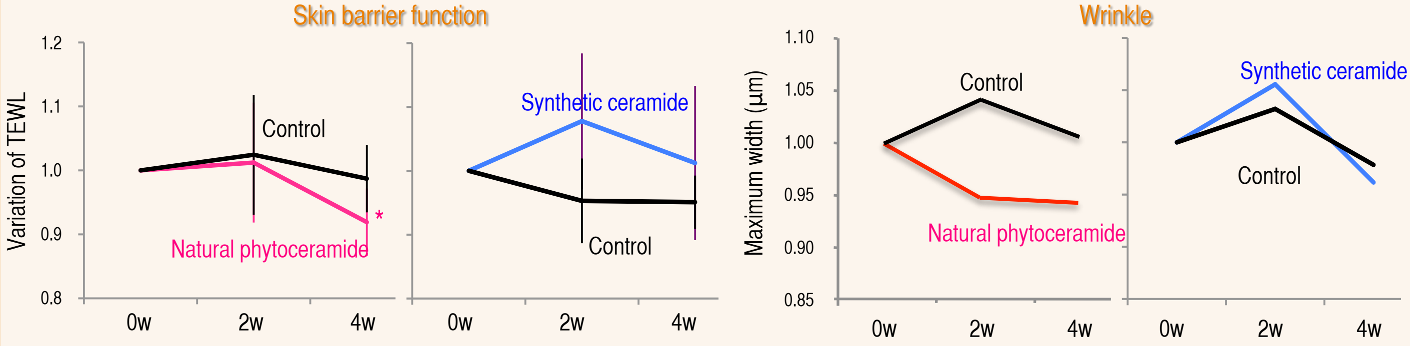
More than 300 species in skin

Molecular diversity

Rich in skin — **Very long chain**

Decreasing with age — **Poly-hydroxy**

- ·Epidermal barrier
- ·Moisture retention
- ·Smooth skin



*P<0.05 (vs 0w)

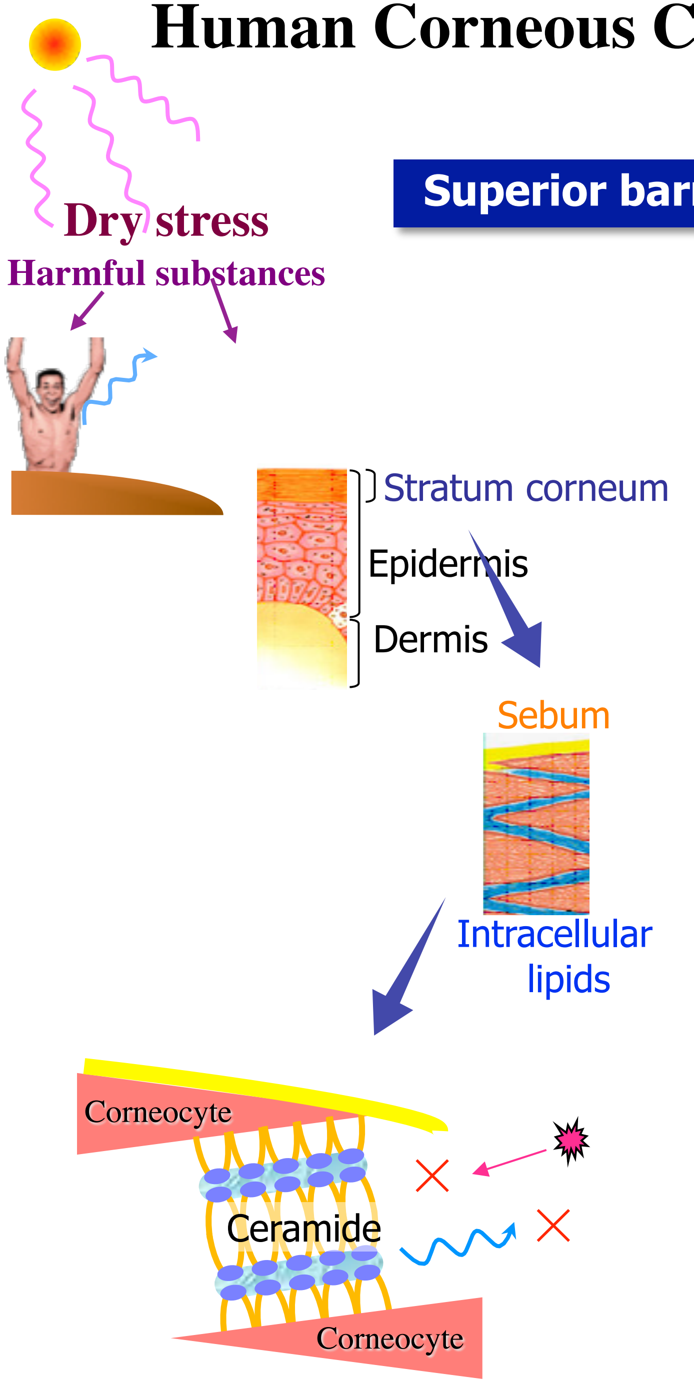
Subjects 11 (right face: with Cer, left face without Cer)

Natural phytoceramide significantly improved barrier function in the fourth week compared to the first day, but synthetic ceramide did not.

Genuine R&D, Co. Ltd./Imas Office 5F, 1–18–11, Hamamatsu-cho, Minato Ward, Tokyo, Japan/TEL+81.3.5452.2086/FAX+81.3.5452.2087（http://www.genuinerd.co.jp）

Human Corneous Ceramides

Superior barrier function acquired in evolutionary history on land



Intracellular lipids

Ceramides	54%
Free FA	21%
Cholesterol	16%
Cholesteryl ester	8%

Glucosylceramide → Glucocerebrosidase → Free Ceramides

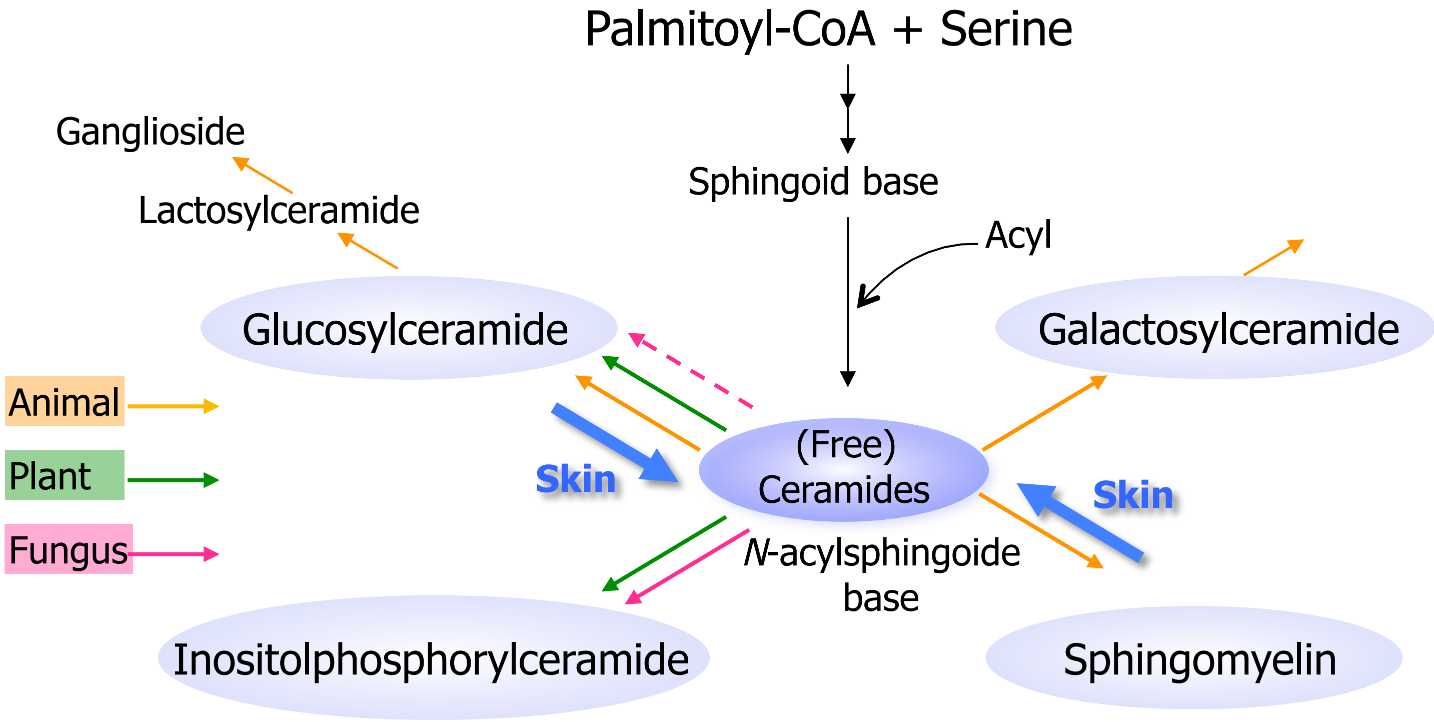
Sphingomyelin → Sphingomyelinase → Free Ceramides

- Epidermis (Living cells)
- Cornification (Lamella granule)

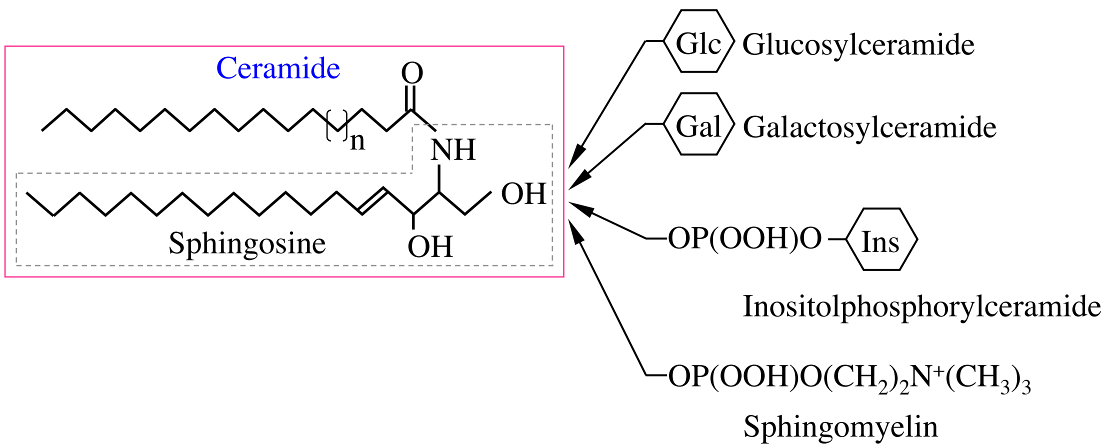
Formation of free ceramides

- SC (Dead cells without nucleus)

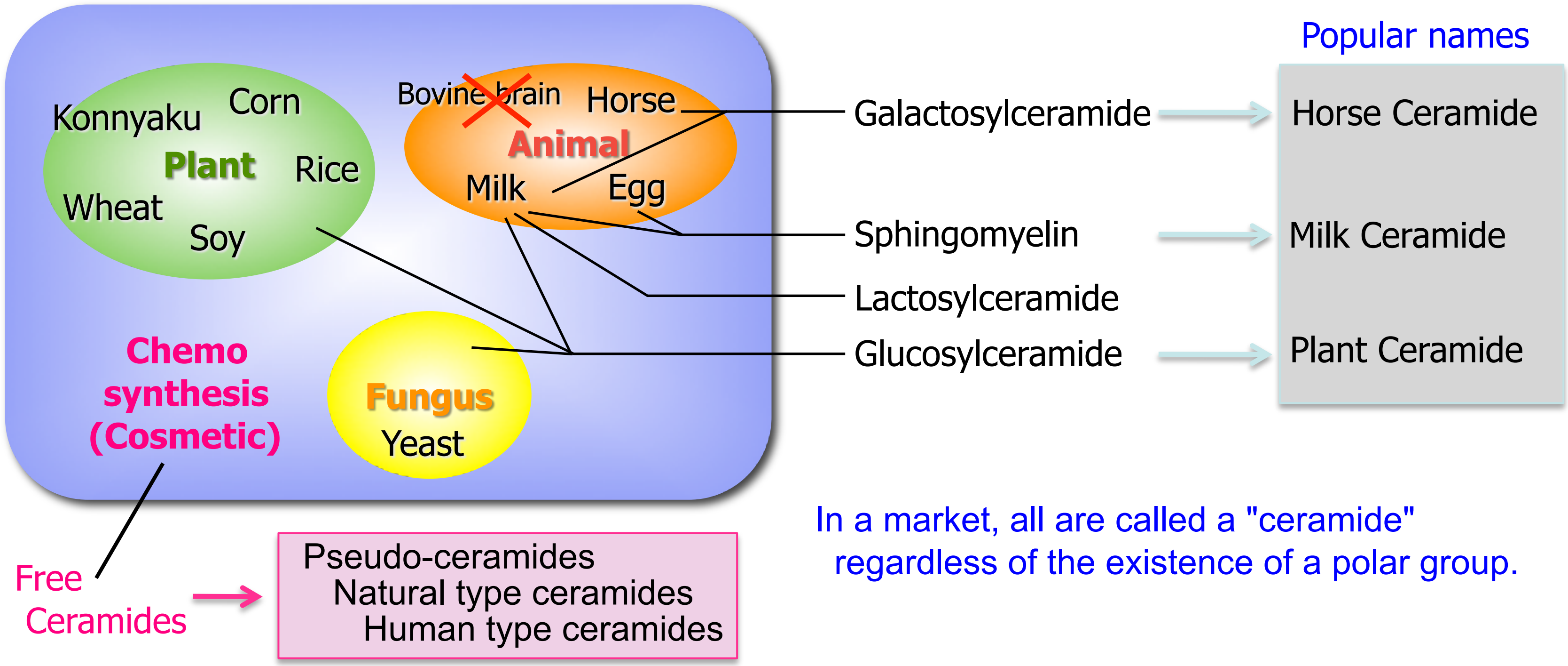
Synthetic Pathway of Sphingolipids



Free ceramides hardly exist in nature, since it is an intermediate in the synthetic pathway

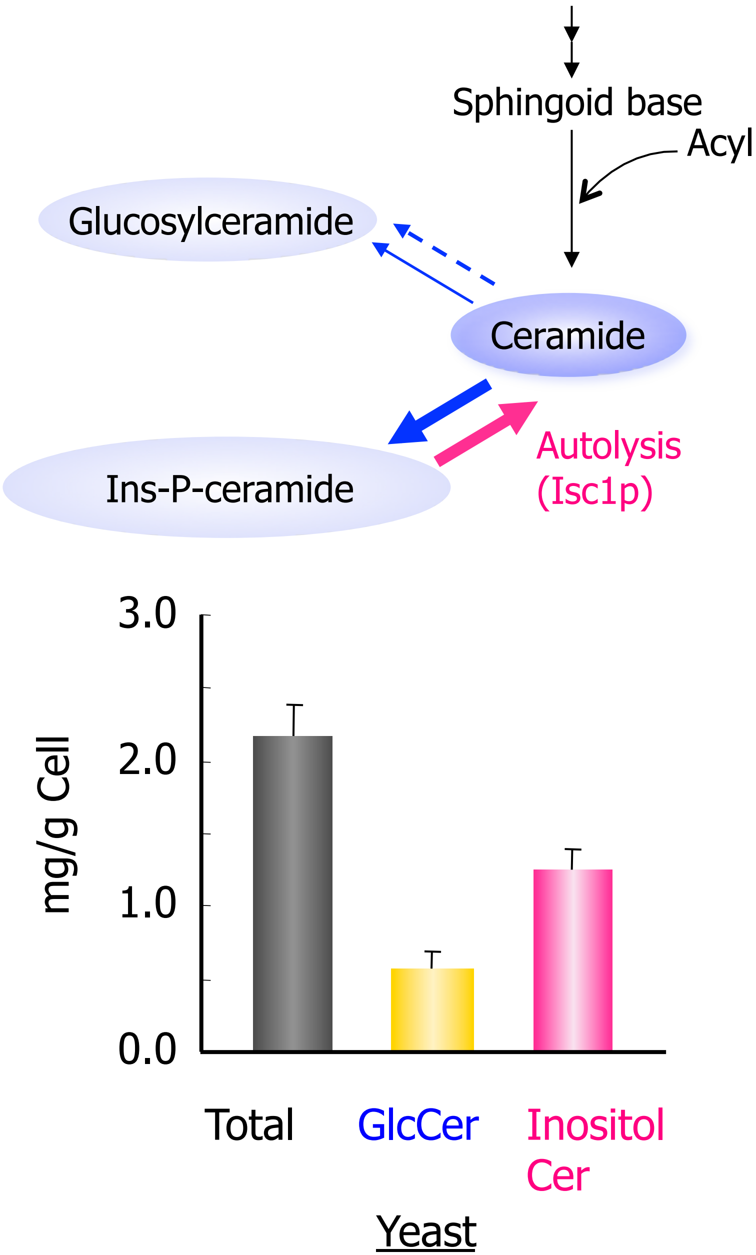


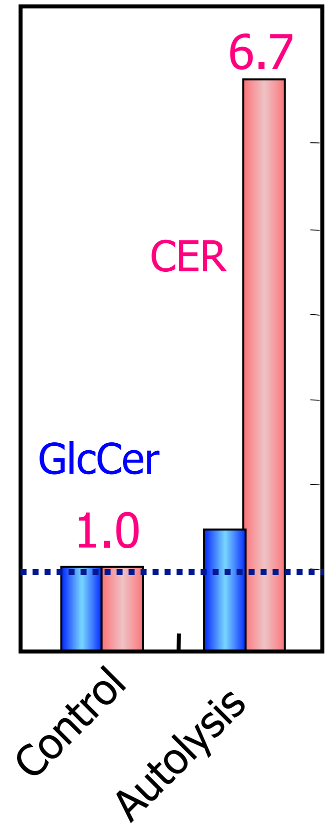
Commercially Available Sphingolipids: Ceramide-related Compounds



There was no effective natural ceramides as external use

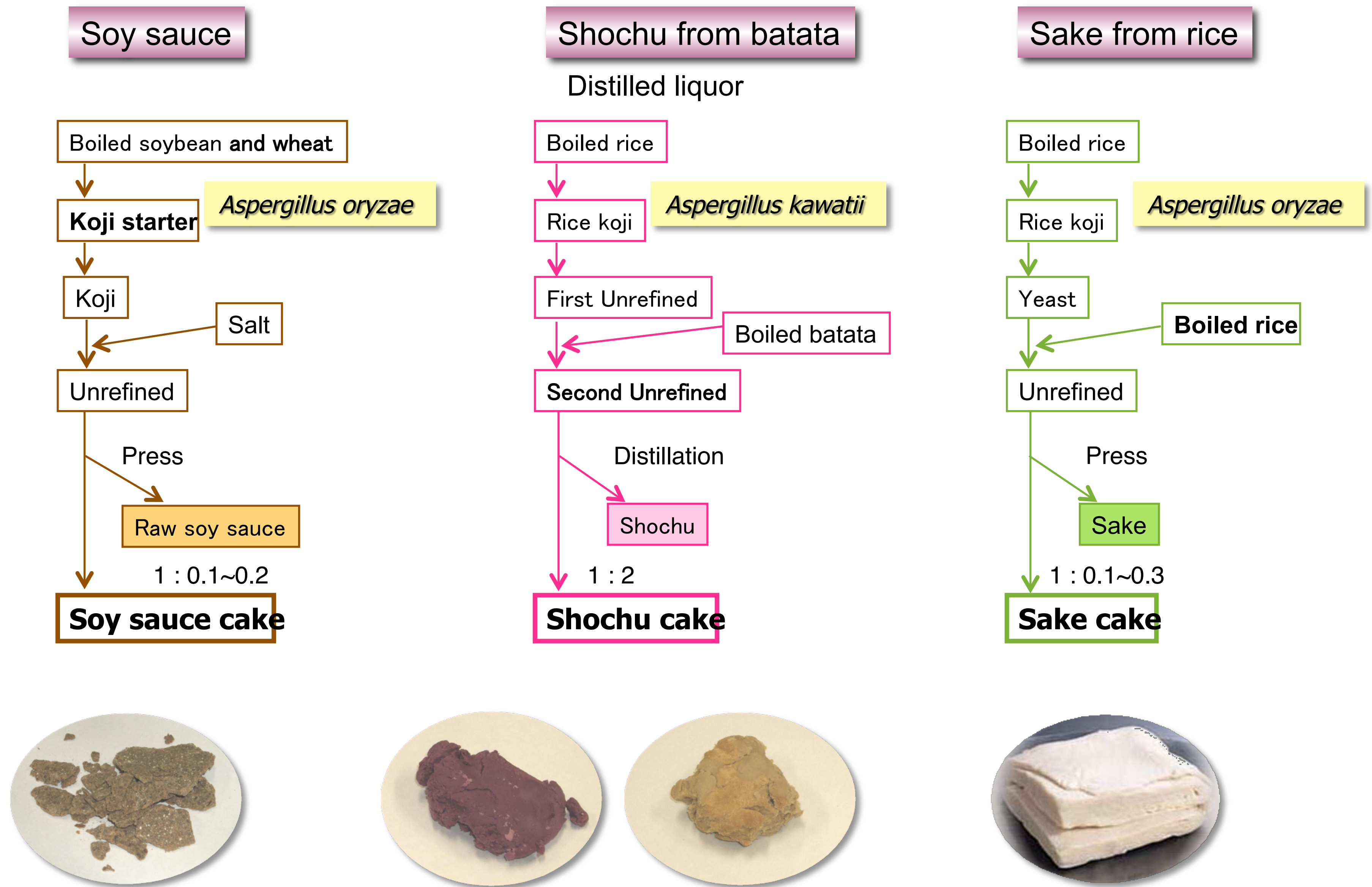
Production of Free Ceramides by Yeast





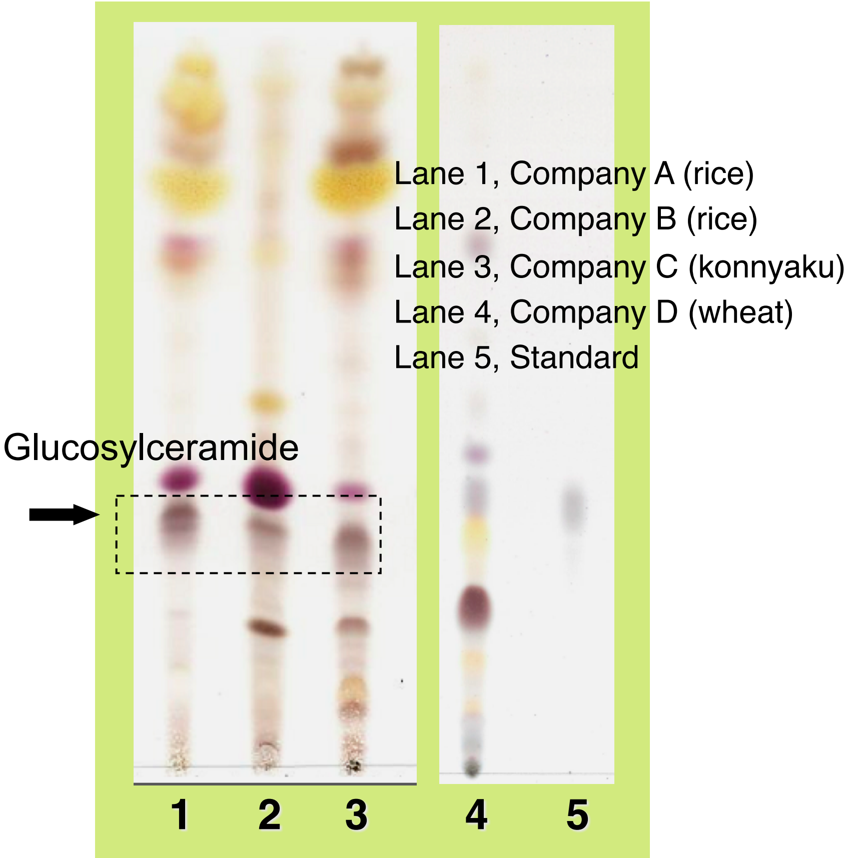
Yunoki et al. "Production of free ceramide from acidic sphingolipids by yeast", in JSBA (2007, P156)

Fermentation Cakes with Koji and Yeast



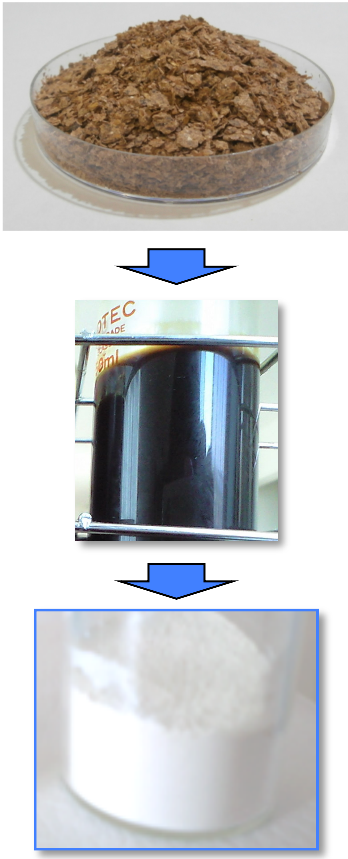
※After pressing

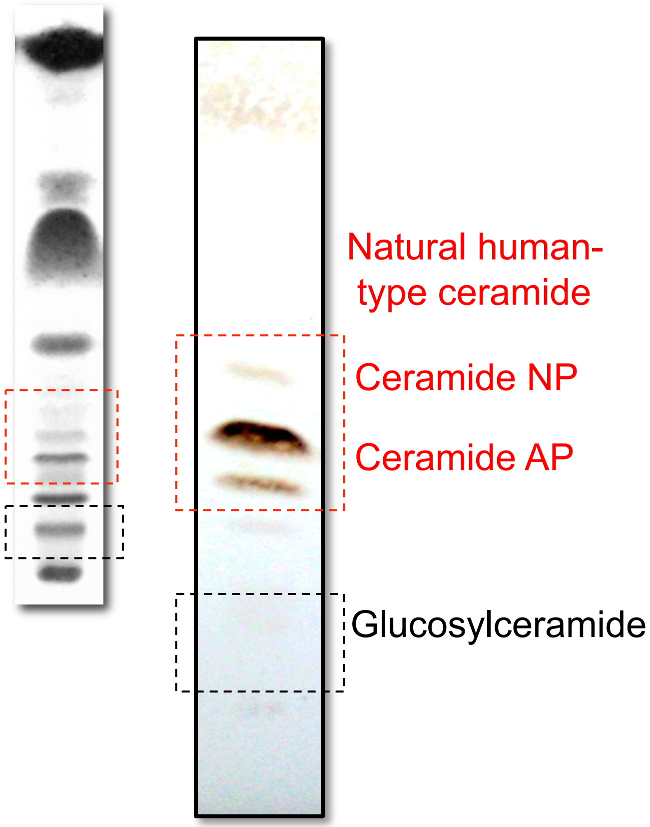
High Purity Ceramide



Crude natural product (from plant)

Soy sauce cake



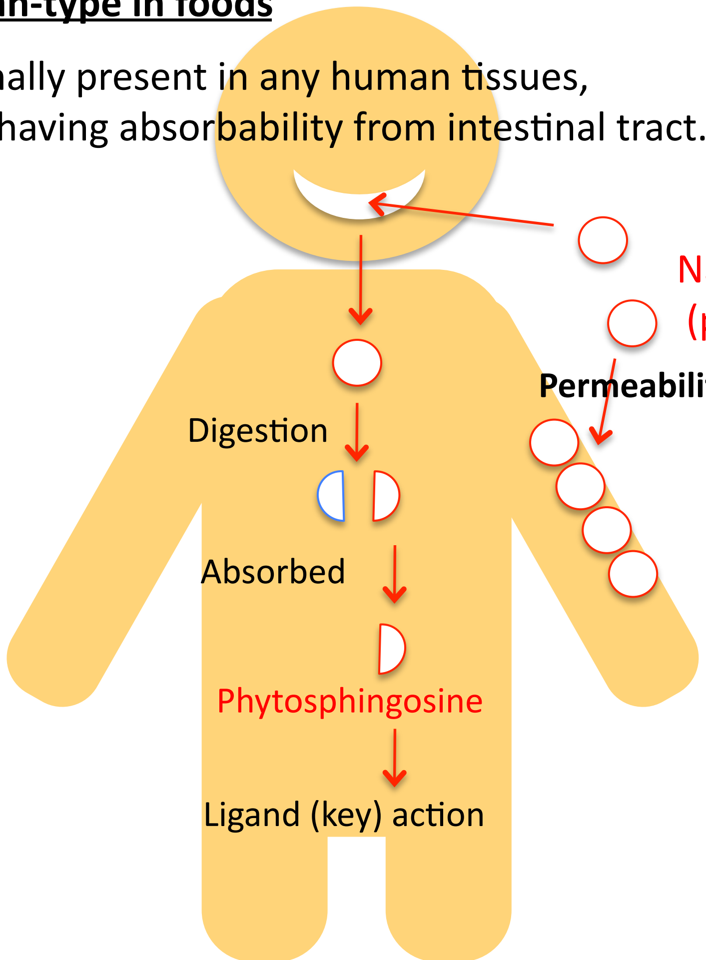


Pure natural product

What is the human-type ?

<u>Human-type in foods</u>

Originally present in any human tissues,
and having absorbability from intestinal tract.



<Inner and outer beauty material>

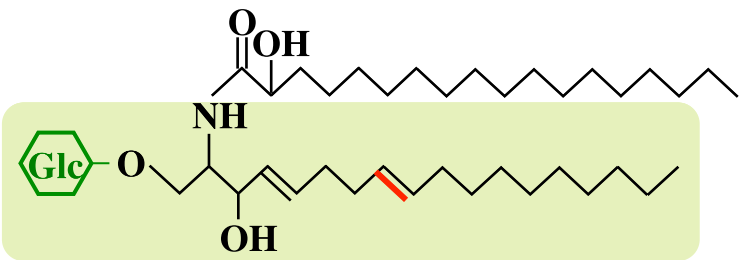
Natural human-type ceramide
(phytoceramide: Ceramide AP)

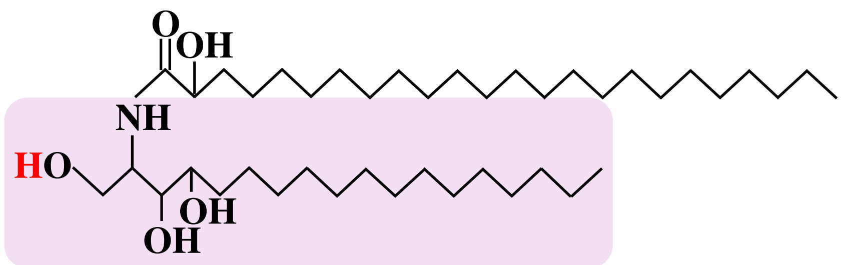
<u>Human-type in cosmetics</u>

Having the same structure as the ceramide present in human epidermis.
Human skin identical.

Priority of Natural Human-type Ceramide Compared to Plant Ceramide

Conventional Natural Ceramide	Natural Human-type Ceramide
Glucosylceramide mainly from plant ↓ Complex digestive system after oral ingestion	Free ceramide (with no glucoside) ↓ Simple digestive system after oral ingestion
Non human-type ↓ In is hardly absorbed after ingestion	Human-type (intestine, kidney, skin) ↓ The absorbability is proven
Low purity (3~5%) ↓ We do not know what has worked	High purity (>90%) ↓ The active ingredient is a clear





Manufacture of Polyphenolipids

Sweet potato (batata)
in Southern Kyushu, Japan

Shochu
Distilled liquor

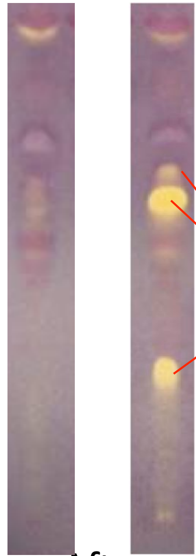
Cake after distillation

Separation

Dry

Extraction

Purification



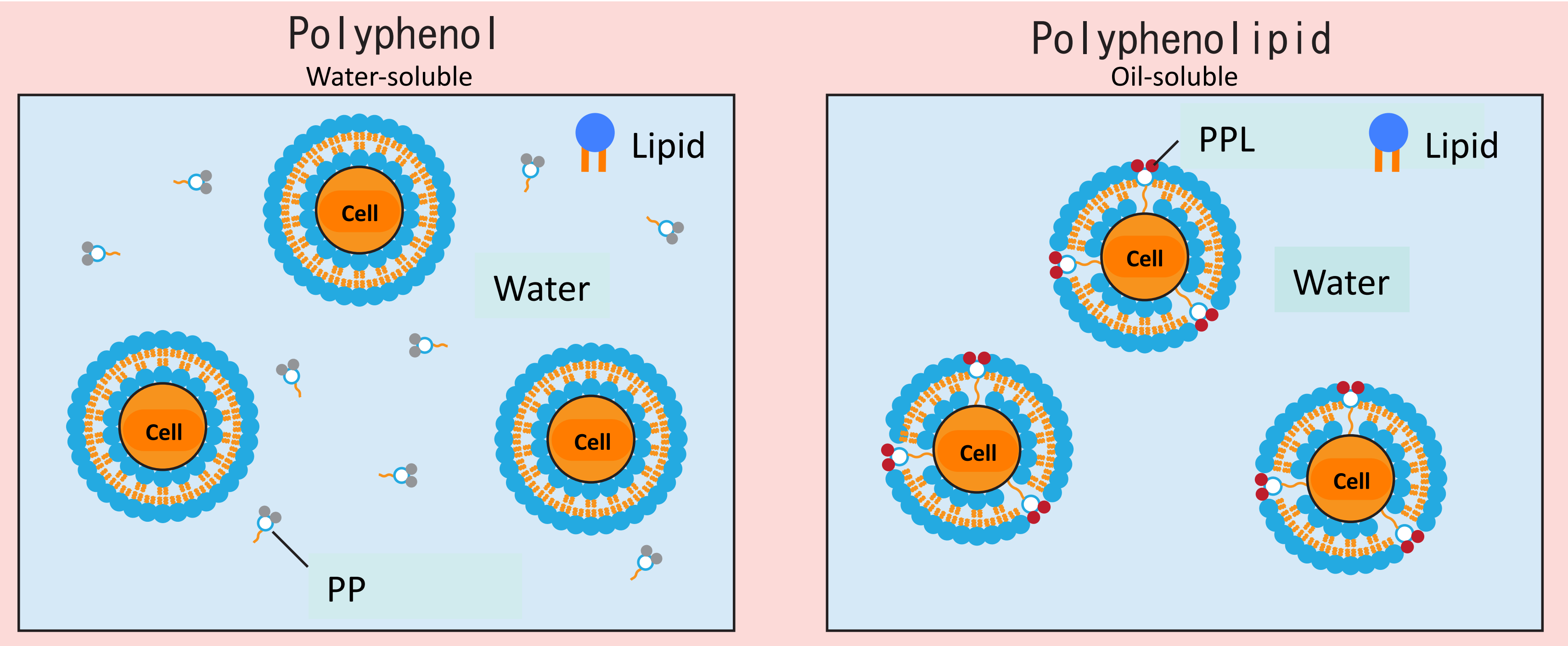
Hydophilic

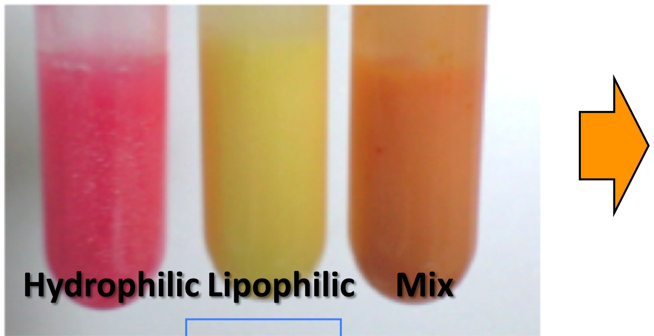
Oil

Polyphenolipid

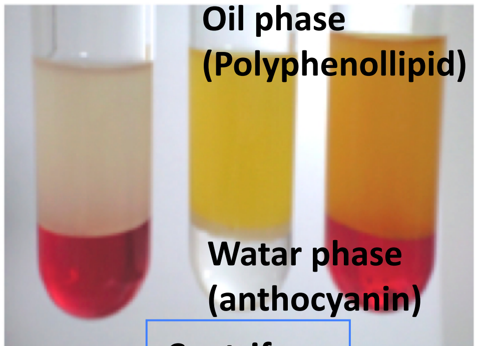
Ceramide

Characteristics of Polyphenolipids



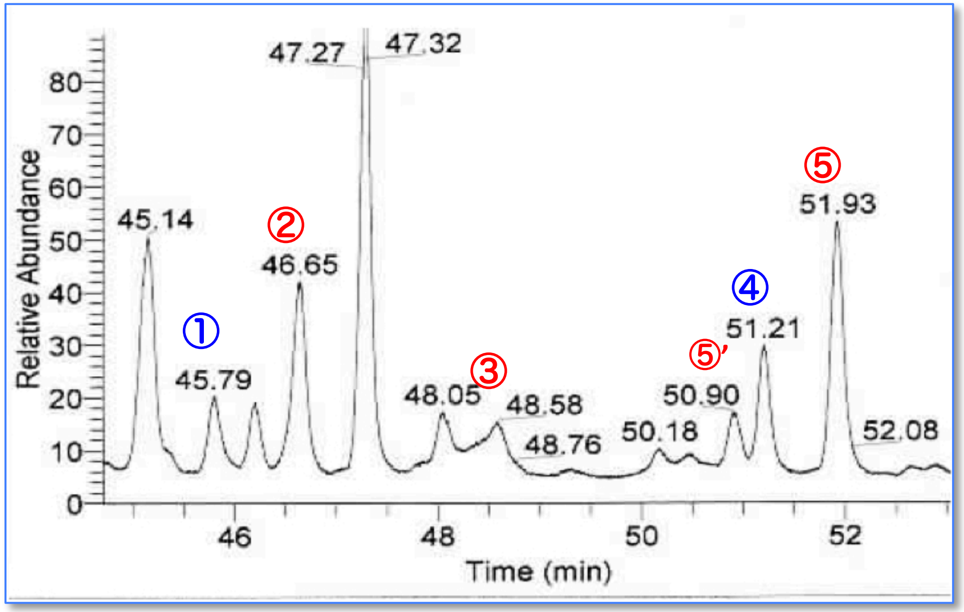


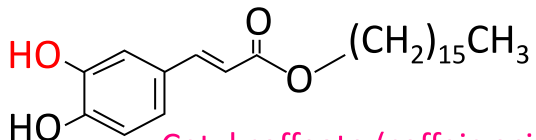
Stirring



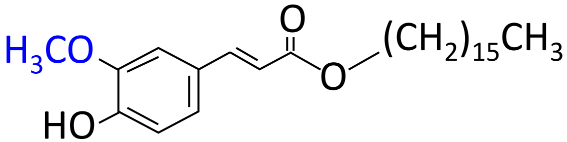
Centrifuge

Analysis of Polyphenolipid





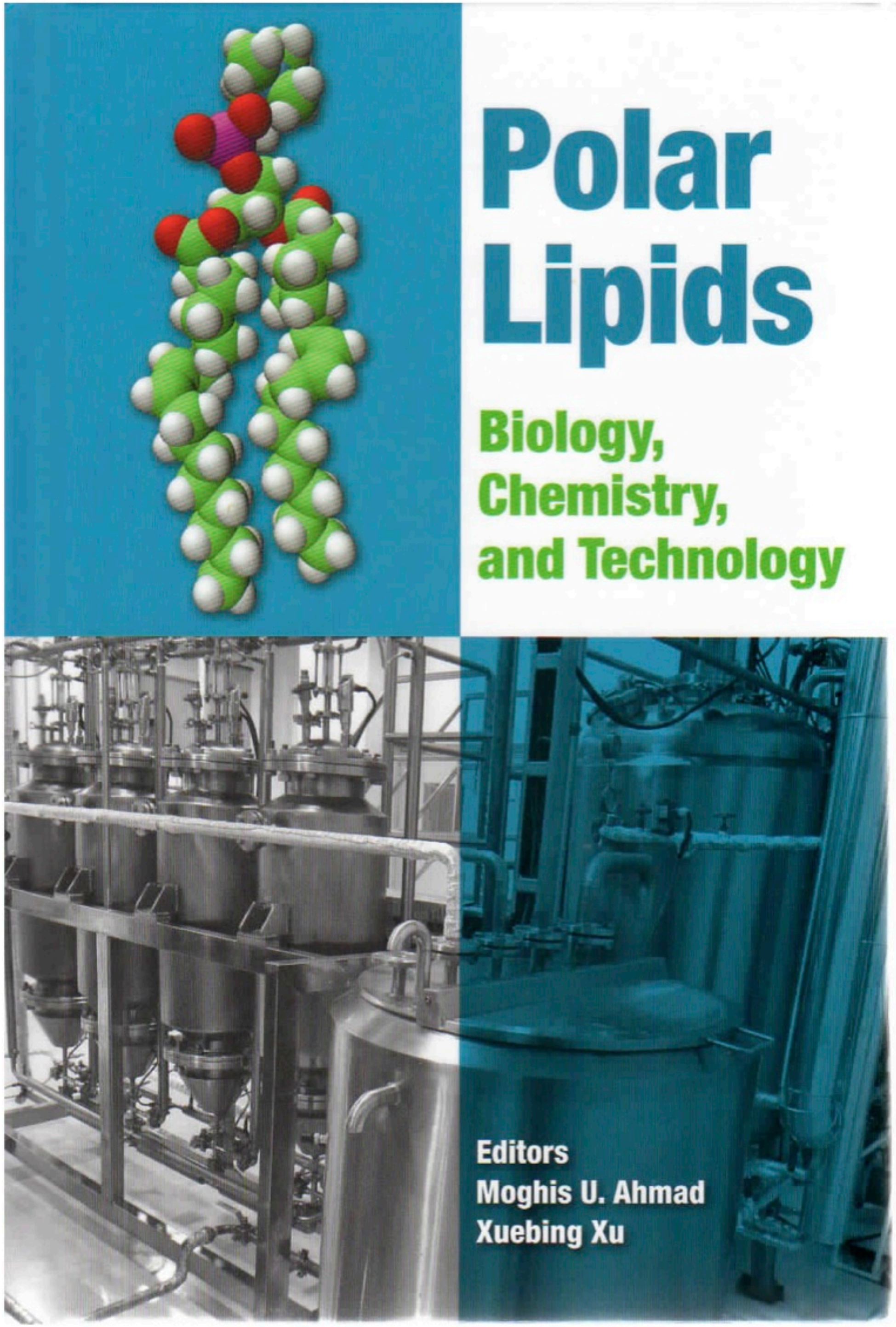
Cetyl caffeate (caffeic acid cetyl ester)



Cetyl ferulate (ferulic acid cetyl ester)

FA : ferulic acid
CA: caffeic acid
①: FA-16
②: CA-16
③: CA-17
④: FA-18
⑤: CA-18 (⑤')

***As a major component, CA-2 (ethyl caffeate) is also included.**



Phenolipids as New Antioxidants: Production, Activity, and Potential Applications

Derya Kahveci ■ *Department of Food Engineering, Faculty of Engineering, Yeditepe University, Istanbul, Turkey*

Mickaël Laguerre and Pierre Villeneuve ■ *UMR IATE, Montpellier, France*

Introduction

Phenolics are secondary metabolites widely found in plants; they have several biological roles and they contribute to the defense system of the host. Among them, the phenolic acid family is one of the most important classes and is mainly composed of the cinnamic and benzoic acid derivatives (Figure 7.1). The interest in these compounds from a nutritional point of view has risen due to their potential properties, including antioxidant, anti-inflammatory, antiallergic, antimicrobial, antiviral, and anticarcinogenic (Fernandez-Panchon et al., 2008; Haminiuk et al., 2012; Sun-Waterhouse, 2011).

Because phenolic acids have a rather low solubility in oils, improvement of hydrophopicity of these compounds by chemical or enzymatic lipophilization has been applied extensively in order to render these functionalized compounds, so-called phenolipids, active in the oil–water interphase. This chapter focuses on the synthesis of phenolipids derived from phenolic acids, their physicochemical and biological activity, and their potential applications.

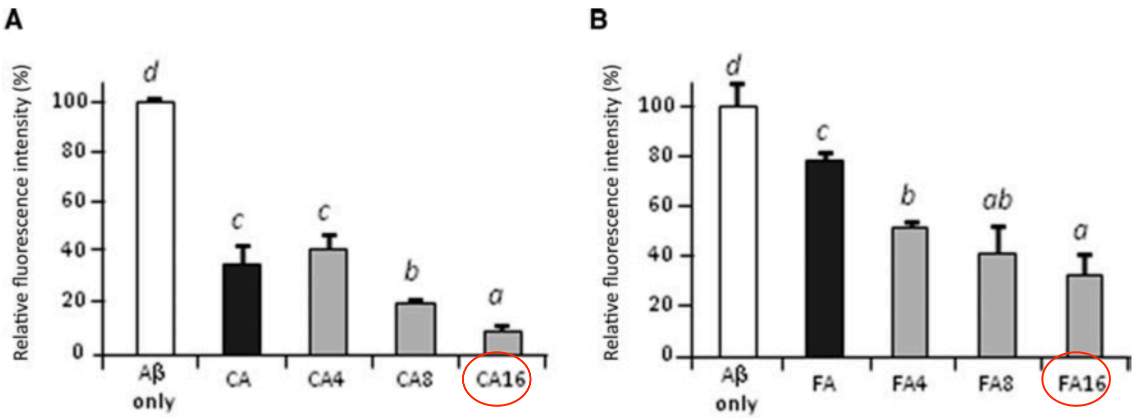
In American oil chemistry,
“phenolipids” has lately attracted considerable attention.

This is the target in the research fields
of drug discovery and functional food.

The “polyphenolipid” is only as a natural product.

Anti-Alzheimer’s Disease Activity of Polyphenolipids

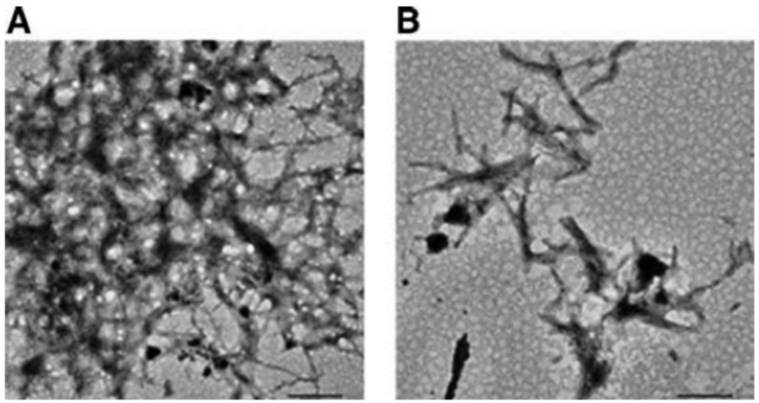
Alzheimer's disease is said to be about one million people in Japan and five million people in the United States.
Development of treatments and diagnostic methods has become a major challenge in the aging society.



Amyloid β peptide in Alzheimer's disease (Aβ) is a peptide consisting of 40 to 42 residues.
The fibrillar aggregates (the amyloid fibrils) is formed by self-organization, and is deposited in the brain to form senile plaques.
Neurodegeneration is caused by the formation of senile plaques.

Cetyl caffeate (CA16) and cetyl ferurate (FA16) as the main components of polyphenolipid strongly inhibit the fibrosis of Aβ
→ inhibit the formation of senile plaque responsible for neurodegeneration

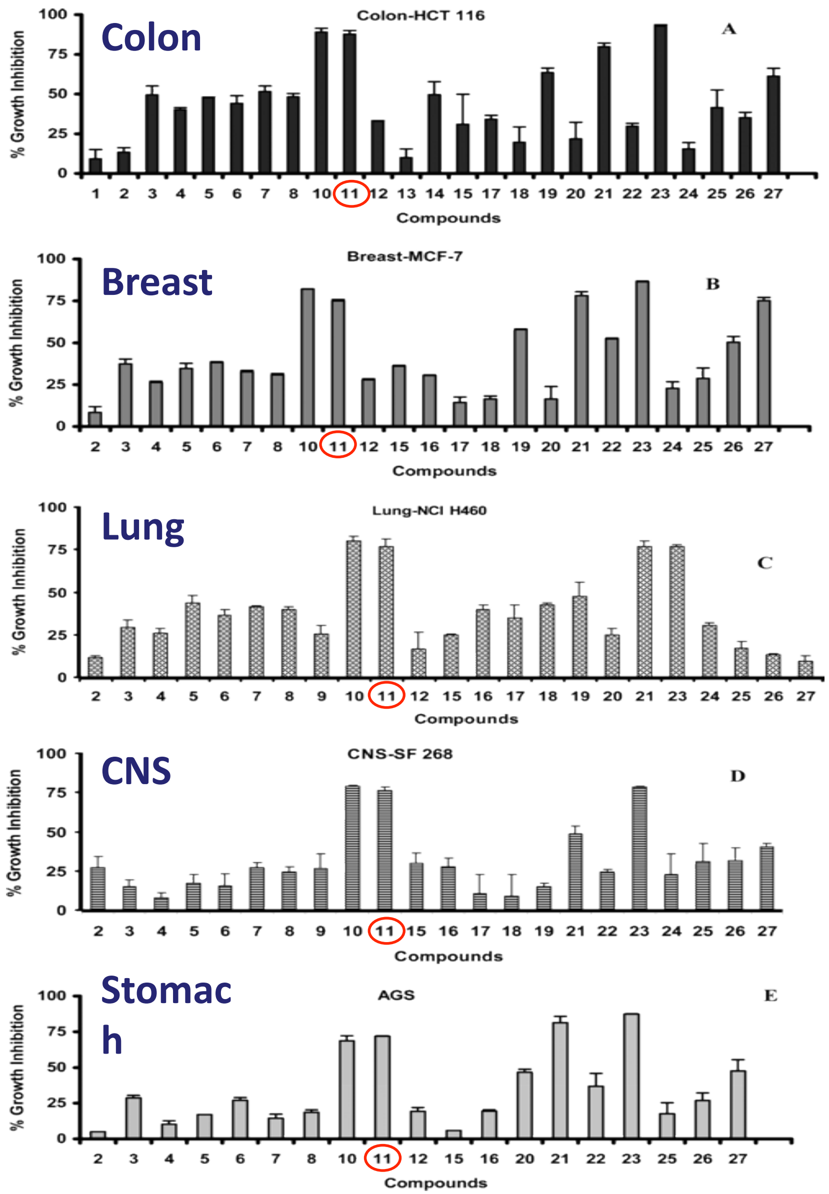
Kondo H, et al, Biotechnol Appl Biochem (2014)



Control CA16

Electron micrographs of Aβ

Anti-tumor Activity of Polyphenolipid



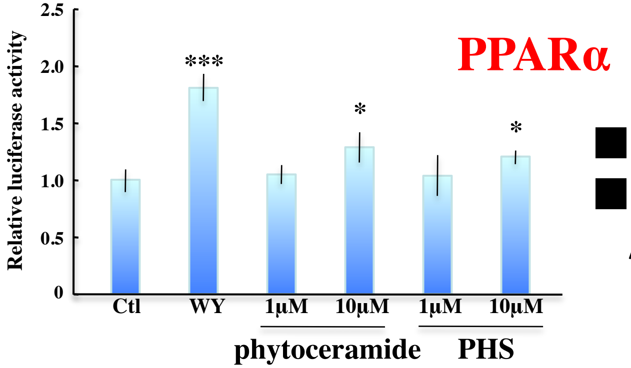
The various long chain alkyl groups are chemically bonded to a caffeic acid and ferulic acid to assess the anti-tumor activity in various cancer cell lines.

Jayaprakasam B, et al, J. Agric. Food Chem. 2006 54:5375.

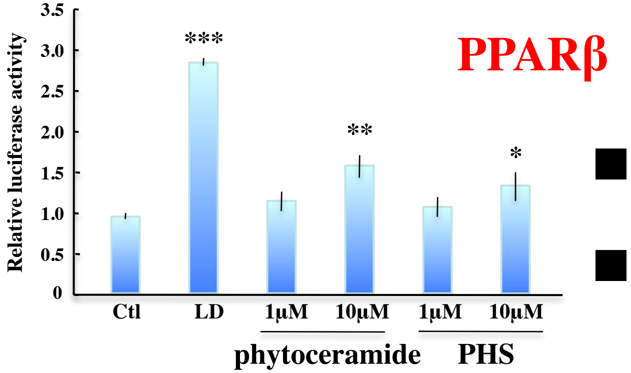
Caffeic acid cetyl ester, which is the main component of the polyphenolipid significantly inhibits all of the growth of 5 cancer cell lines.

Ligand Action of Phytoceramide

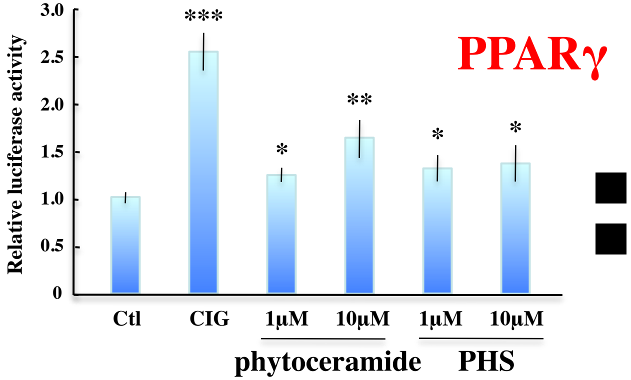
PPAR:
One of nuclear receptor.
Transcription factors that are closely involved in the cellular metabolism and differentiation of hydrocarbons, lipids and proteins.



- Improvement of lipid metabolism → Prevention of hyperlipidemia
- Maturity of epidermal barrier
  Activation of sebaceous cell → skin beauty
  Promotion of protein production



- Improvement of carbohydrate metabolism → Prevention of diabetes
- Differentiation of sebaceous cell and keratinocyte → skin beauty



- Improvement of insulin sensitivity → Prevention of diabetes
- Differentiation of sebaceous cell → skin beauty

Murakami et al. Lipids Health Dis. 2011

Commissioned Analysis

Genuine R&D

Lipid Analysis

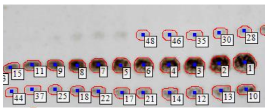
- Screening
- Detection
- Identification
- Structure
- Quantification
- Development
- Purification
- Fractionation

Neutral-lipids
- wax
- triacylglycerol
- cholesterol
- diacylglycerol
- fatty acid

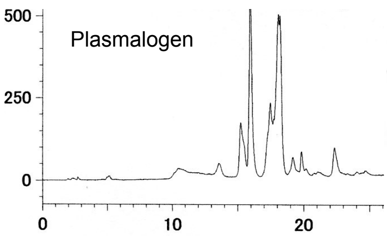
Complex-lipids
- glycerophospholipids
- plasmalogen
- glyceroglycolipids
- sphingophospholipids
- sphingoglycolipids
- ceramide

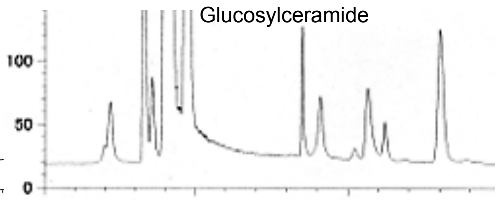
TLC

Densitometry



HPLC-ELSD





GC-MS