2nd Workshop Argentina-Japan
"Bioscience and Biotechnology for the Promotion of Agriculture and Food Production"
-November 8th and 9th 2010-
Tokyo

Area: Food Science

Acceptance of soybean food products in Japan
-From traditional foods to functional foods-

Prof. Yasuki Matsumura, Kyoto University

In the East Asia, soybean has been utilized as traditional foods for more than 1,000 years. In Japan, now, about one million tons of soybeans are consumed directly as food products such as Tofu, Natto, Miso, Shoyu. Yuba, etc.

Firm tofu

Silken tofu

Natto (traditional type) wrapped by straws

Miso

Shoyu

Yuba

Background in Japan

Soybean is one of the most popular food materials in Japan, and it is regarded as a key stuff for the long life and the low risk of some diseases such as various cancers and arteriosclerosis of Japanese people. A lot of research projects have been carried out from the fundamental and practical viewpoints. Not only traditional foods but also many functional foods are produced and commercialized from soybean. The information about soybean food products and their benefits for human health are open to the public via databases, website, TV programs, publications, etc.

Aim of this presentation

- Introduce the frontier of research and application to food products of soybean in Japan
- Introduce the present situation of the acceptance of soybean food products in Japan

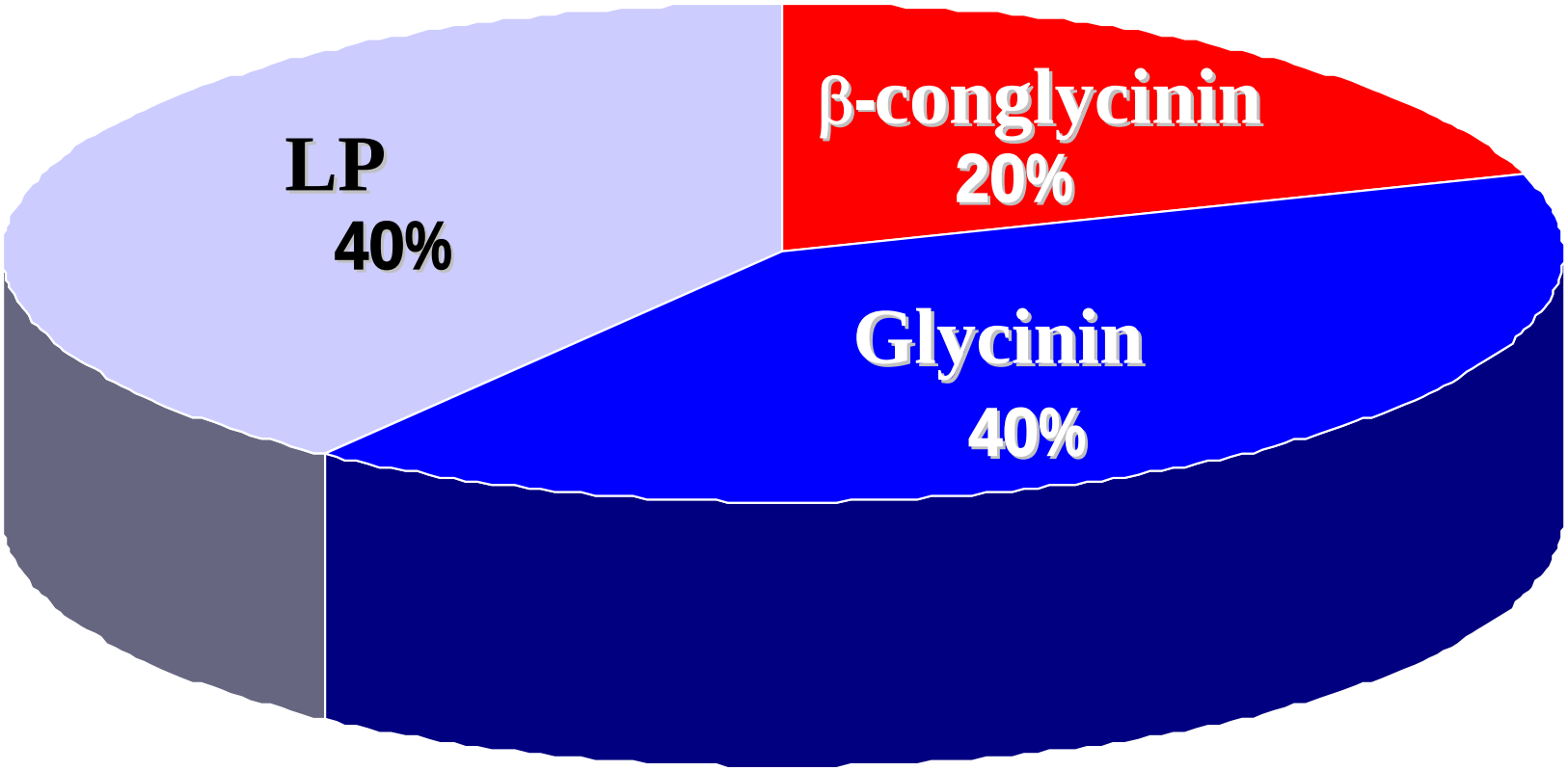
Contents of the presentation

1. Recent research on soybean components
2. New insight into traditional soybean food products
3. Present situation of acceptance of soybean food products in Japan

Recent research on soybean components

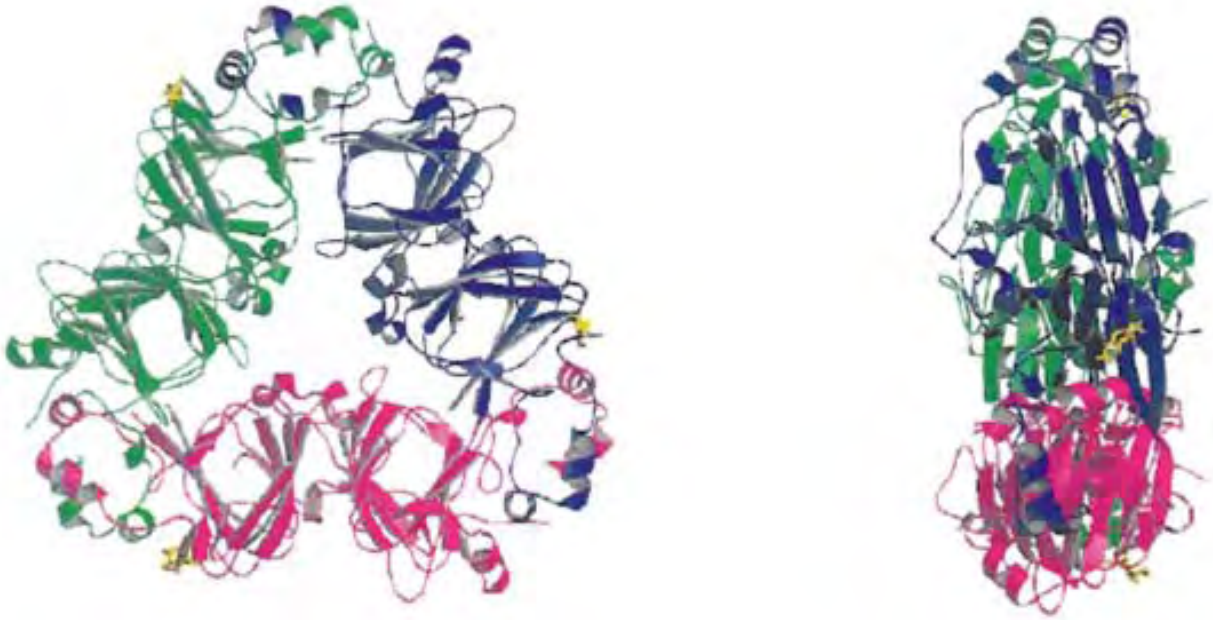
1) Protein
2) Lipid
3) Saccharide (Oligosaccharide, Polysaccharide)
4) Isoflavone
5) Saponin
6) Vitamin
7) Mineral

Composition of commercial soy protein isolate

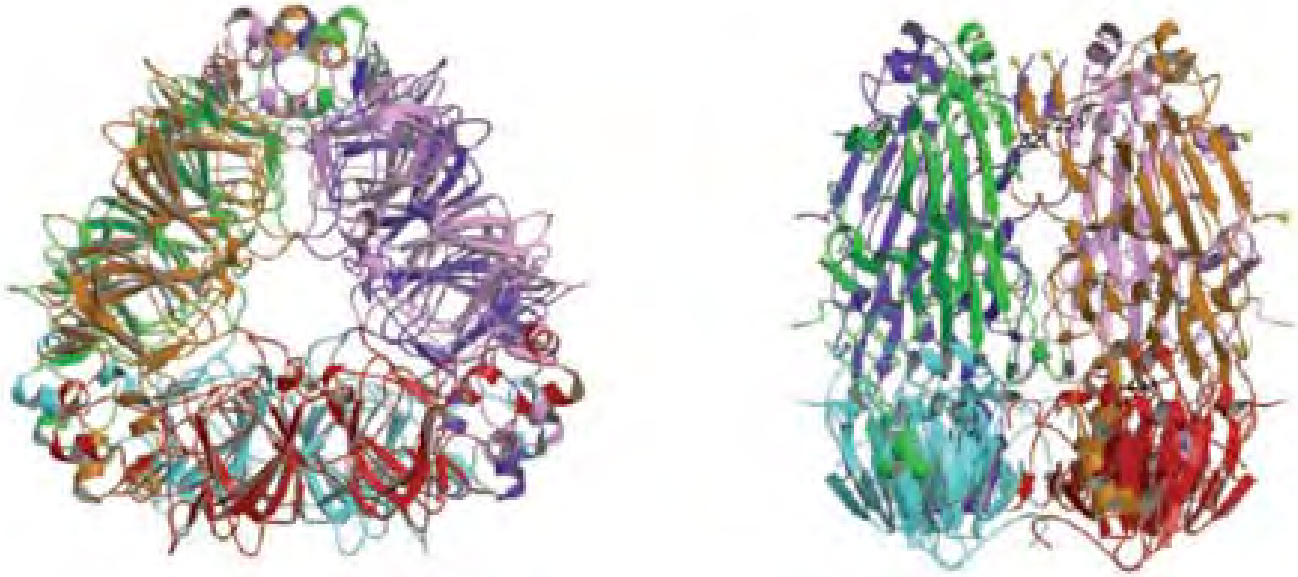


Samoto et al., Food Chem., 102, 317-322 (2007)

Three dimensional structure of β-conglycinin β subunit (Trimer)

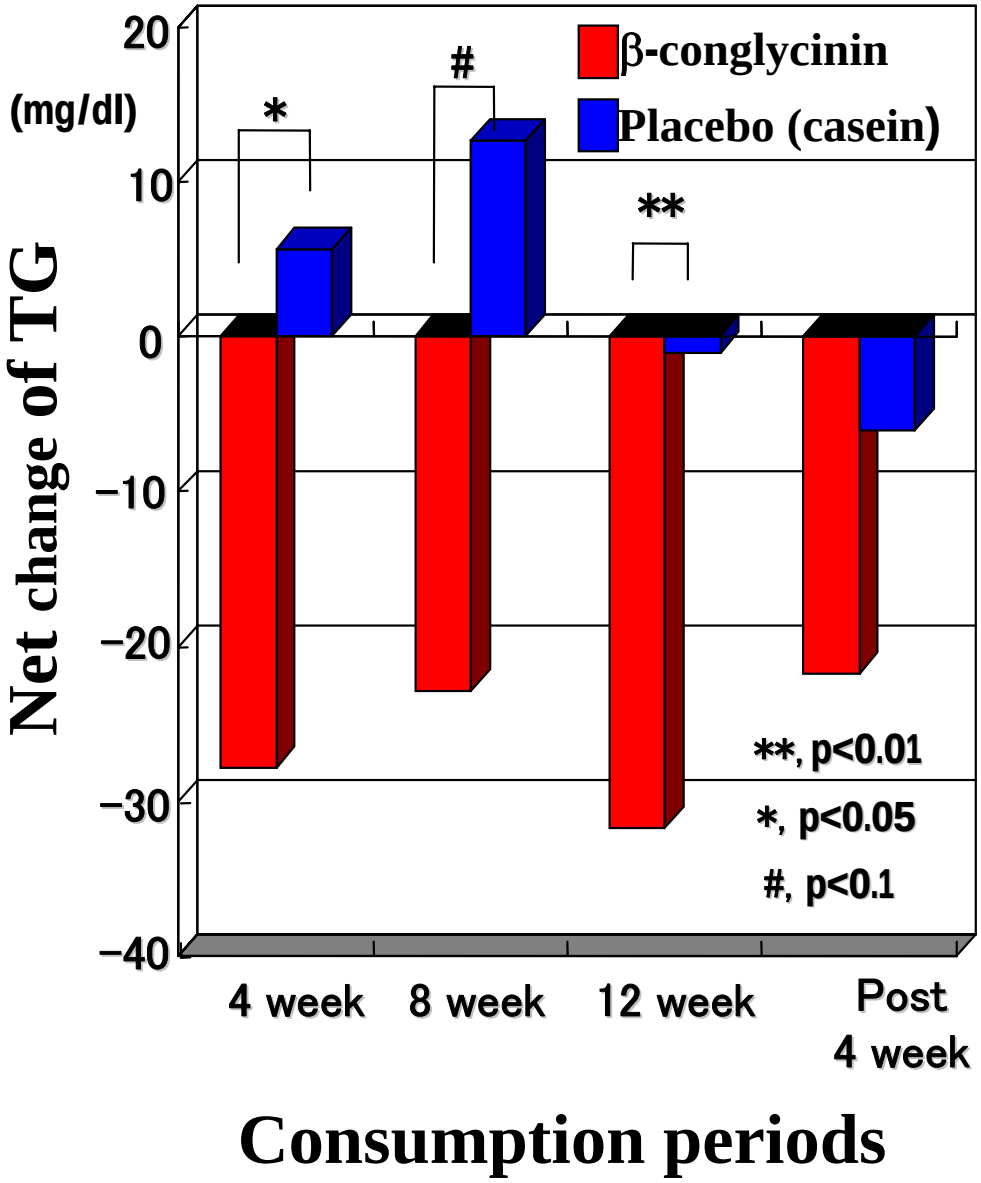


Three dimensional structure of glycinin A3B4 subunit (Hexamer)



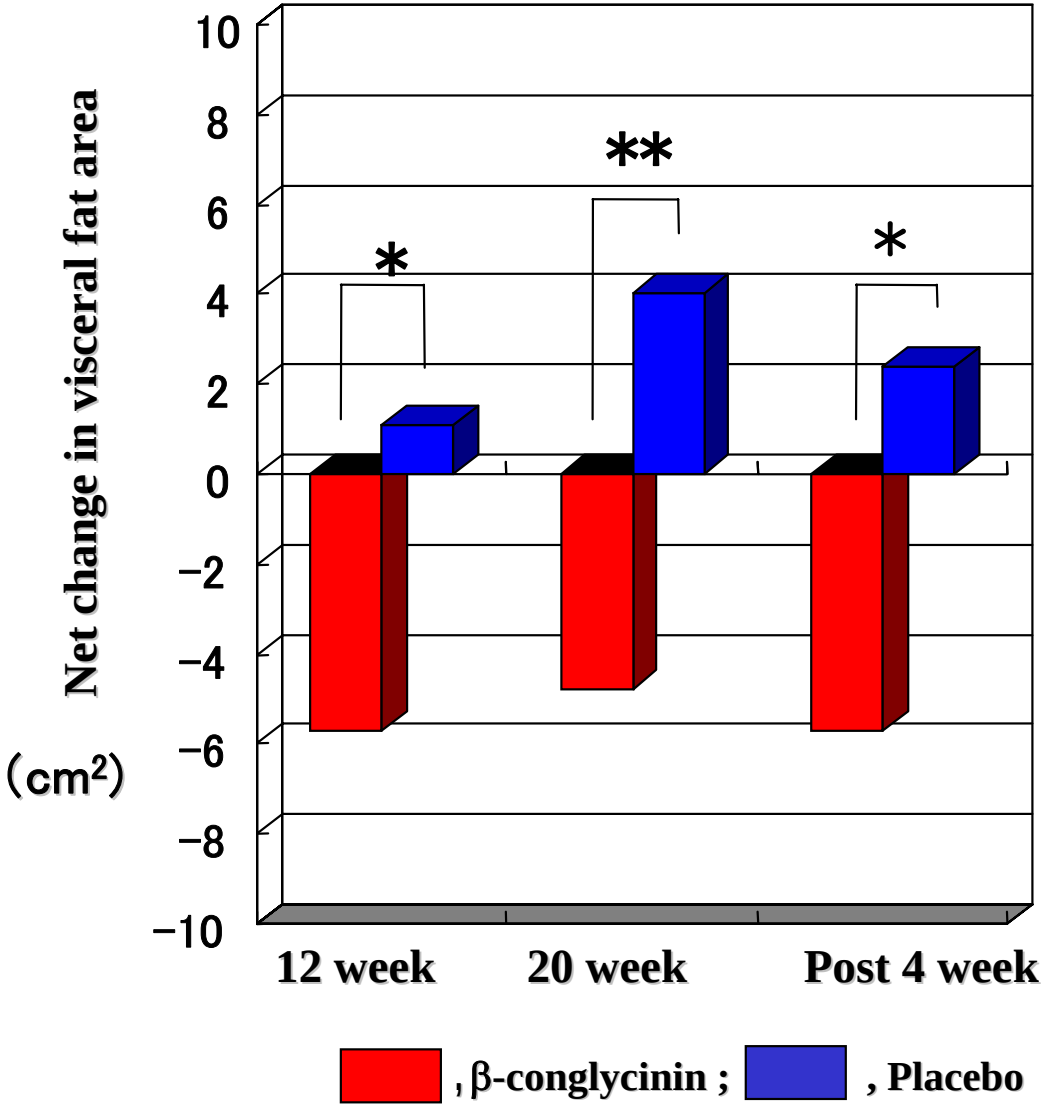
Structure of these subunits was reported by the group of Prof. Shigeru Utsumi

Reduction of Serum Triacylglycerol by β-conglycinin

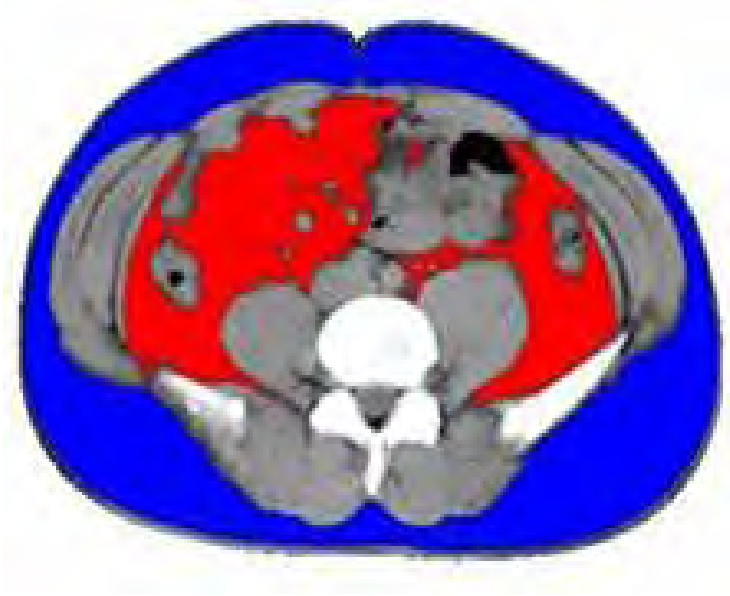


FOSHU of β-conglycinin

Net change in visceral fat area



**、p<0.01； *、p<0.05

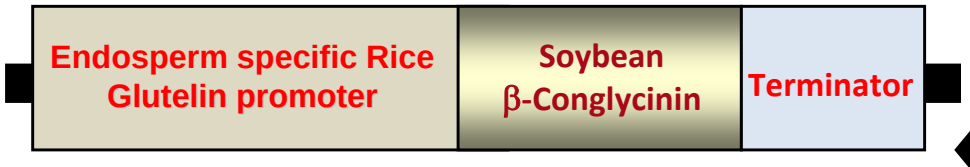


CT scan of abdominal circumference

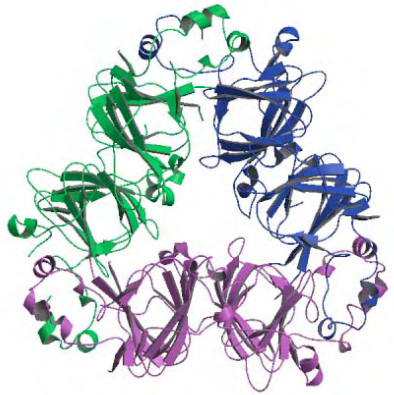
Red ; Visceral fat
Blue ; Subcutaneous fat

Development of transgenic rice to prevent lifestyle related disease

Transgenic Rice

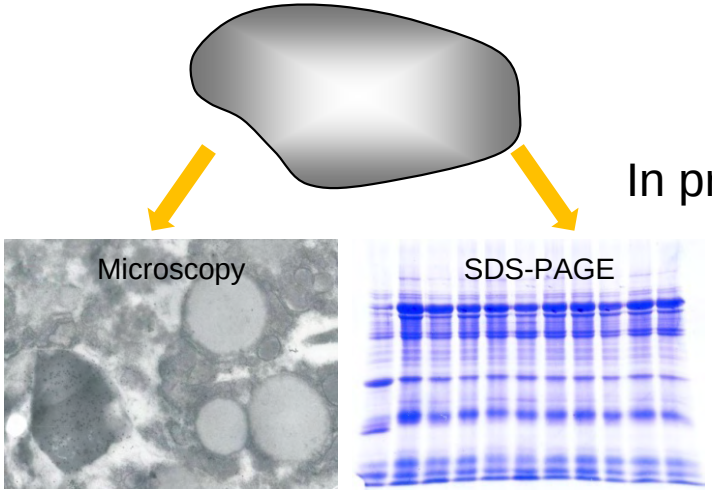


vector for an expression in rice endosperm



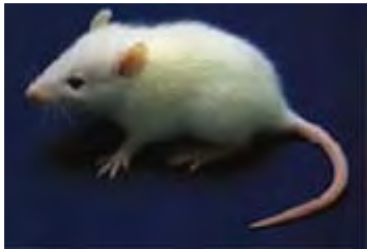
Soybean β-Conglycinin

Soybean β-conglycinin has a function to regulate a serum lipid level!



Accumulation of β-conglycinin in rice

In progress



Evaluation about a functionality

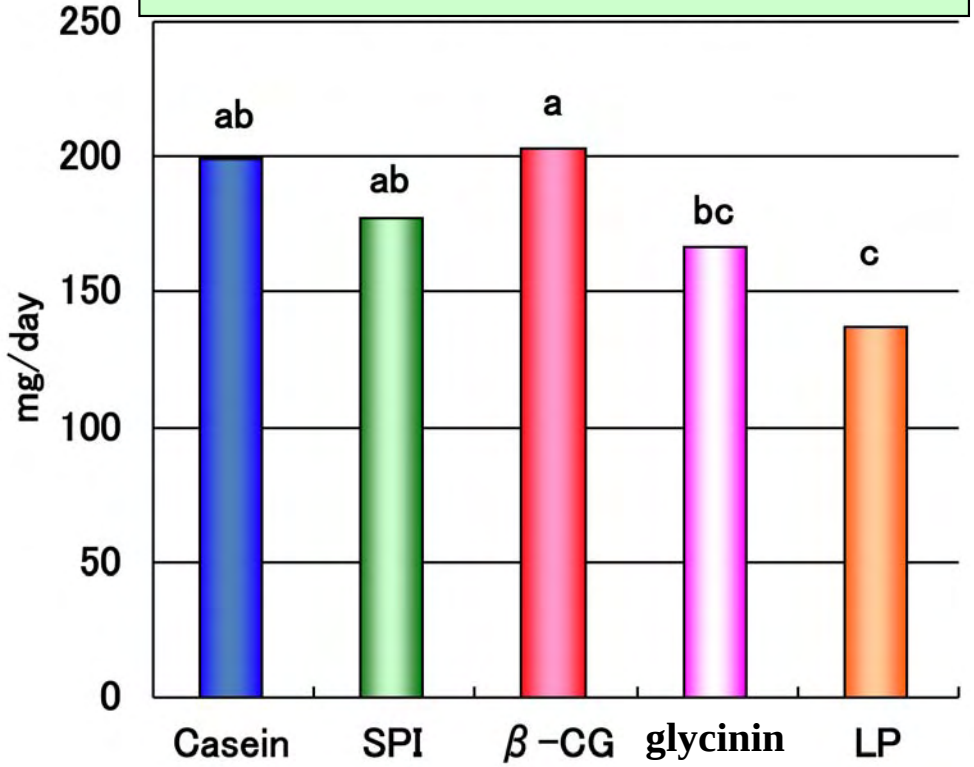
Test in a green house and in a field

Product

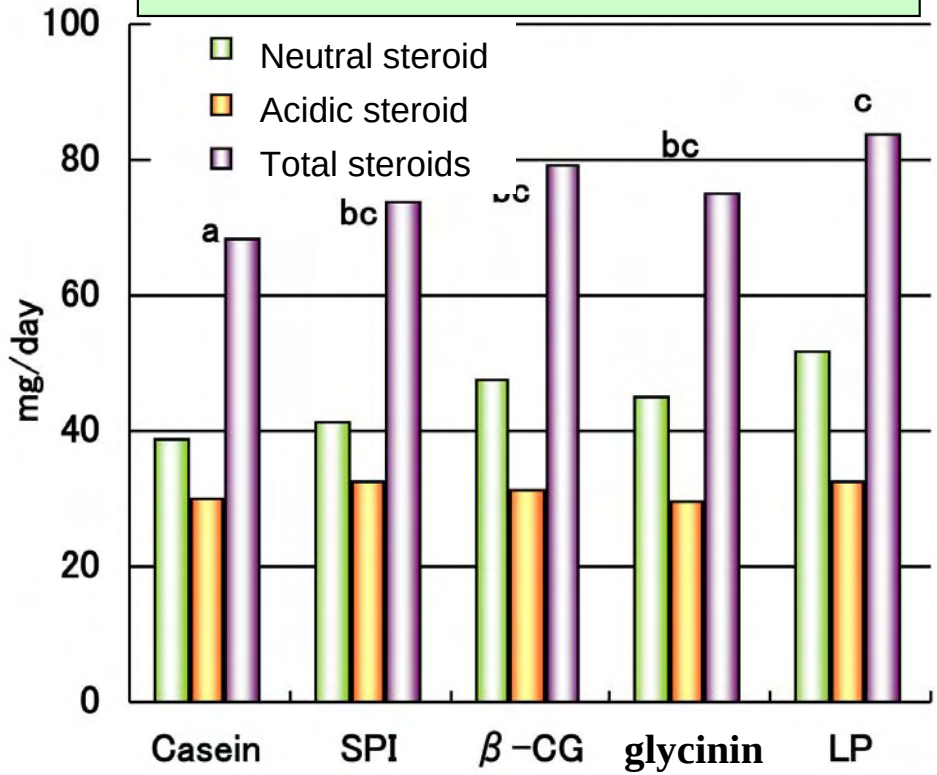
(Dr. Nobuyuki Maruyama)

LP has a function of reducing cholesterol

Effects of dietary proteins on plasma cholesterol in rats



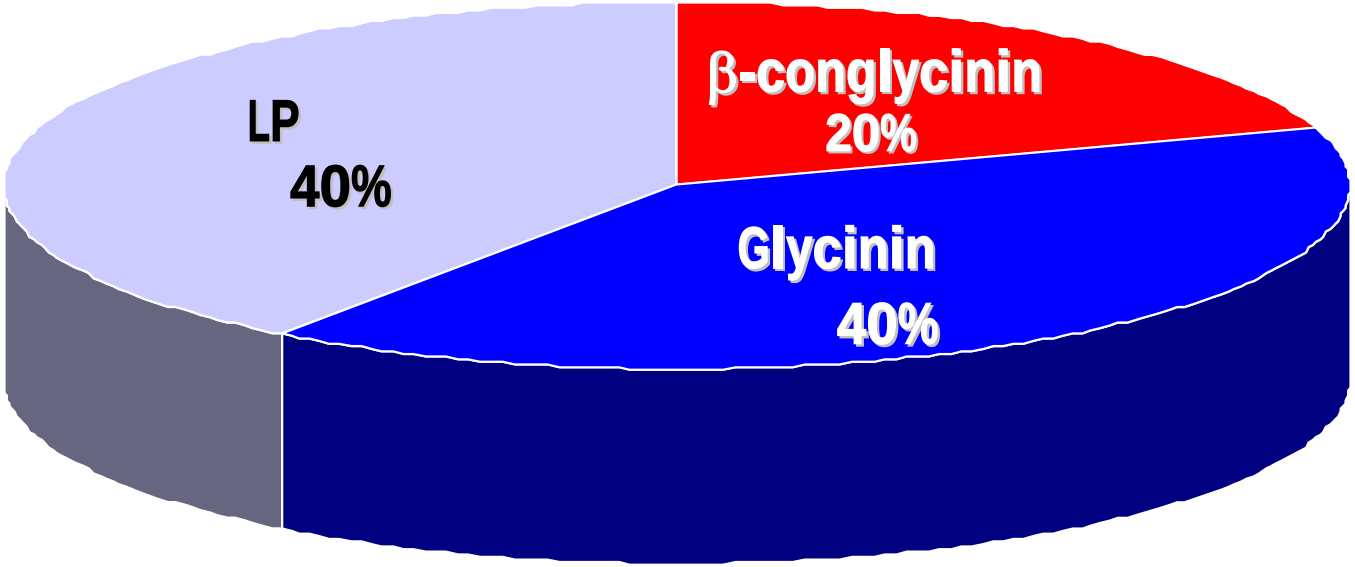
Effects of dietary proteins on fecal cholesterol in rats



LP plays a major role in the reduction of cholesterol

Soy Protein Research, Japan, **10**, 83-87 (2007)

Protein Composition of Soy Protein Isolate



β-conglycinin、Glycinin
= Soy Storage Protein/Globulin

LP (Lipophilic Proteins)
= Membrane Protein etc

Samoto M et al. Food Chemistry, 102, 317-22 (2007)

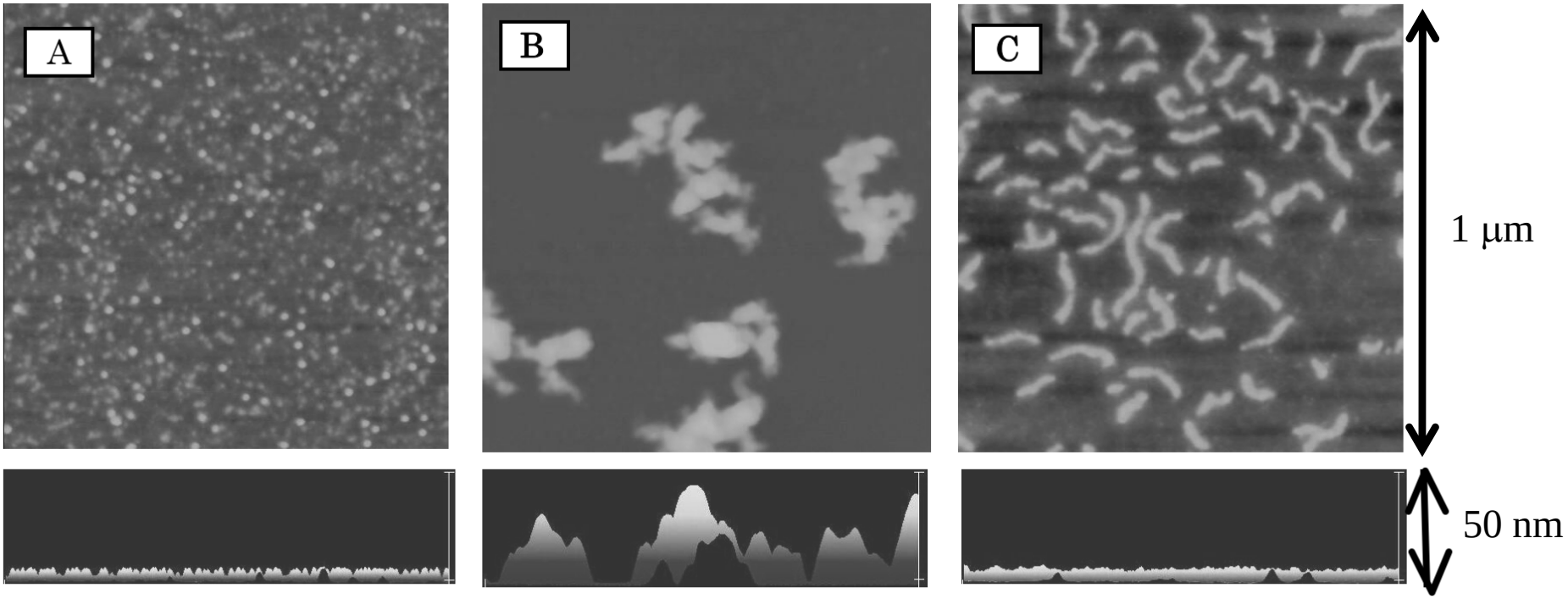
Physiological Properties of Fractionated SPI

β-conglycinin : Reduction of Serum Triacylglycerol Level

LP : Reduction of Plasma Cholesterol Level

Glycinin is of great importance from the viewpoints of nutrition and food processing.

Observation of Glycinin Structure by Atomic Force Microscopy



(A) : No heating, (B) : Heating for 1 min, (C) : Heating for 1 min in the presence of NEM
Upper: View from the upper
Lower: View from the side

Nakamura, Matsumura, Utsumi, “All of Soybean” (ed. K. Kitamura), 121-128, Science Forum, Tokyo (2010)

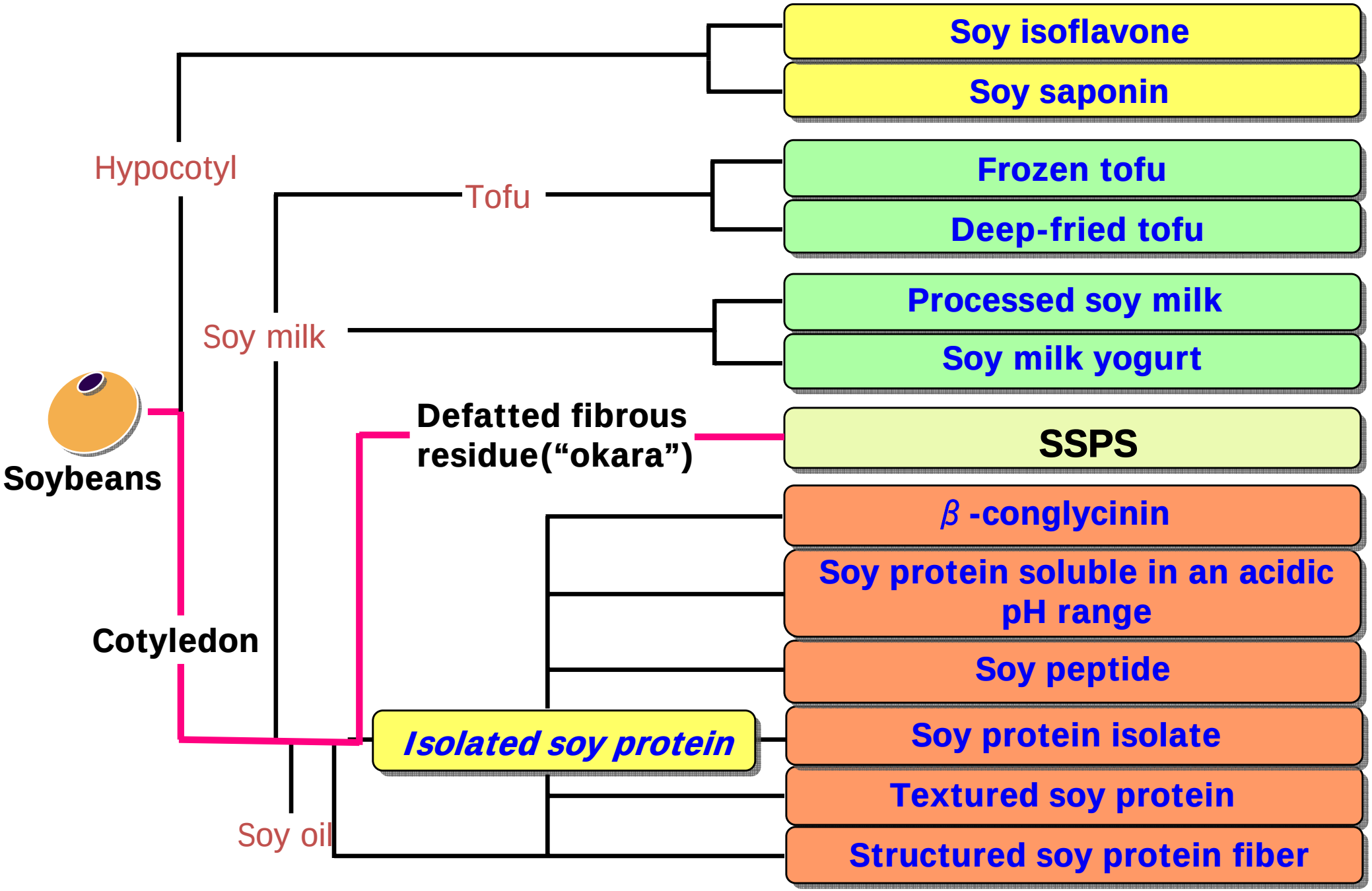
DNA microarray

Functional classification of genes of which expression level was significantly changed in livers of rats fed the SPI diet

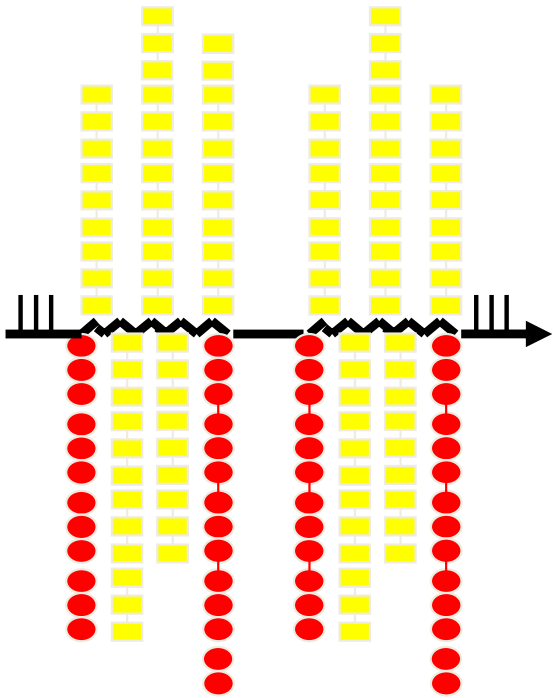
Function	Increase	Decrease
amino acid metabolism	4	10
antioxidant	9	2
cell growth and/or maintenance	6	11
energy metabolism	4	7
fatty acid metabolism	0	3
immunity	3	0
signal transduction	7	5
steroid metabolism	12	0
structural molecule	0	5
transcriptional regulator	4	4
others	12	2
Total	61	54

Tachibana et al., J. Agric. Food Chem., 53, 4253-57 (2005)

Production Process of soluble soybean polysaccharide (SSPS)



Structure and Functions of Soluble Soybean Polysaccarides (SSPS)



Main chain

Galacturonan

Rhamnogalacturonan

Side chain

Arabinose

Galactose

- SSPS is a water soluble polysaccharide prepared from soybean "Okara" (insoluble residues after soymilk preparation).

- SSPS has various functions relating to …

Food Processing

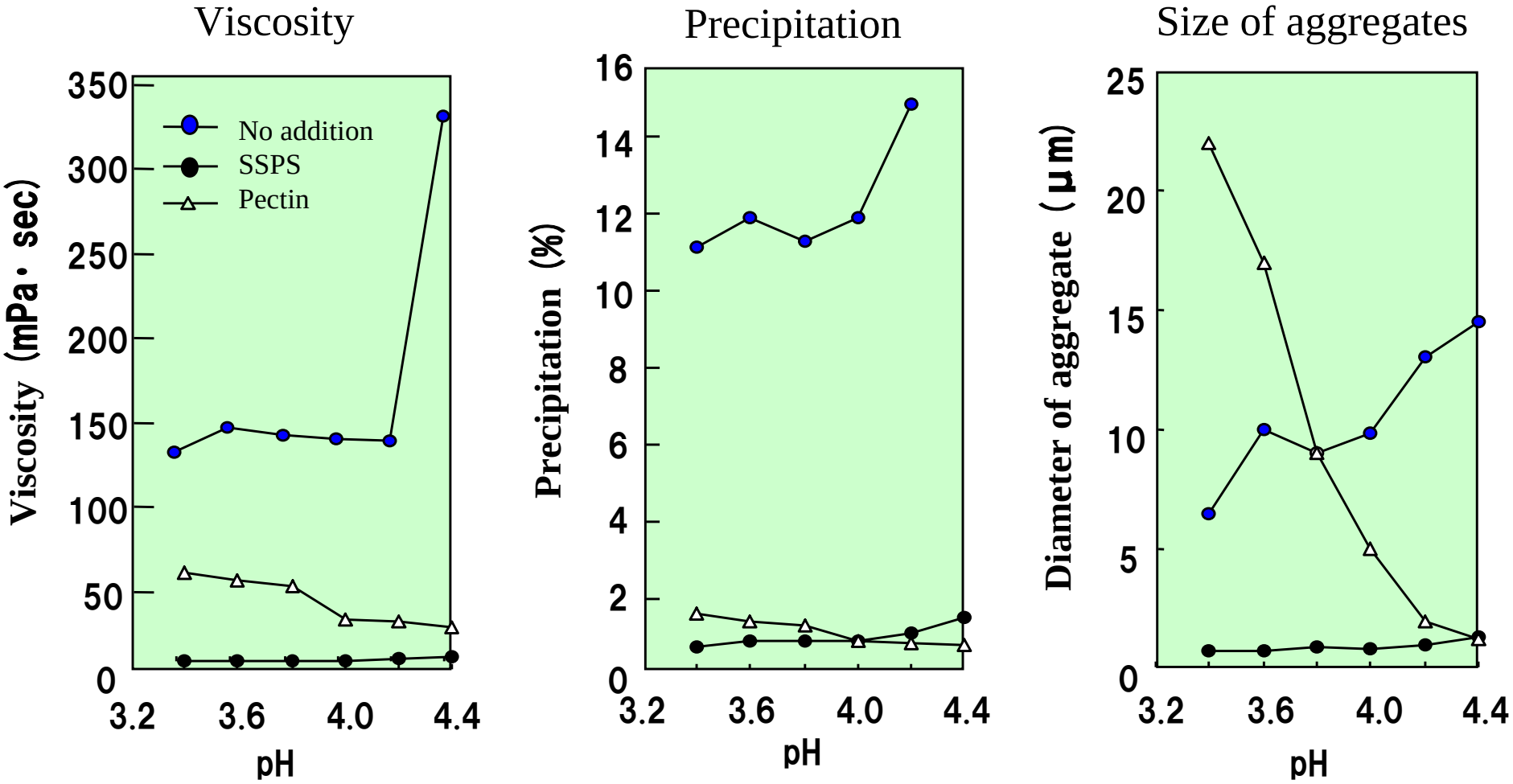
stabilization of protein dispersions, emulsification, film-formation, anti-sticking effects, texture modifying

Biology or Physiology

anti-microbial action, funcition as dietary fiber, increase of Ca intake, prevention of lipid oxidation

- Like pectin, SSPS contains galacturonic acids and can be used as a stabilizer for acidic beverages including proteins.

Milk protein dispersions stabilized by SSPS and pectin: Comparison of stability of dispersions at various pH



SSPS prevented increases of viscosity and aggregate size more effectively especially at lower pH regions as compared to Pectin.

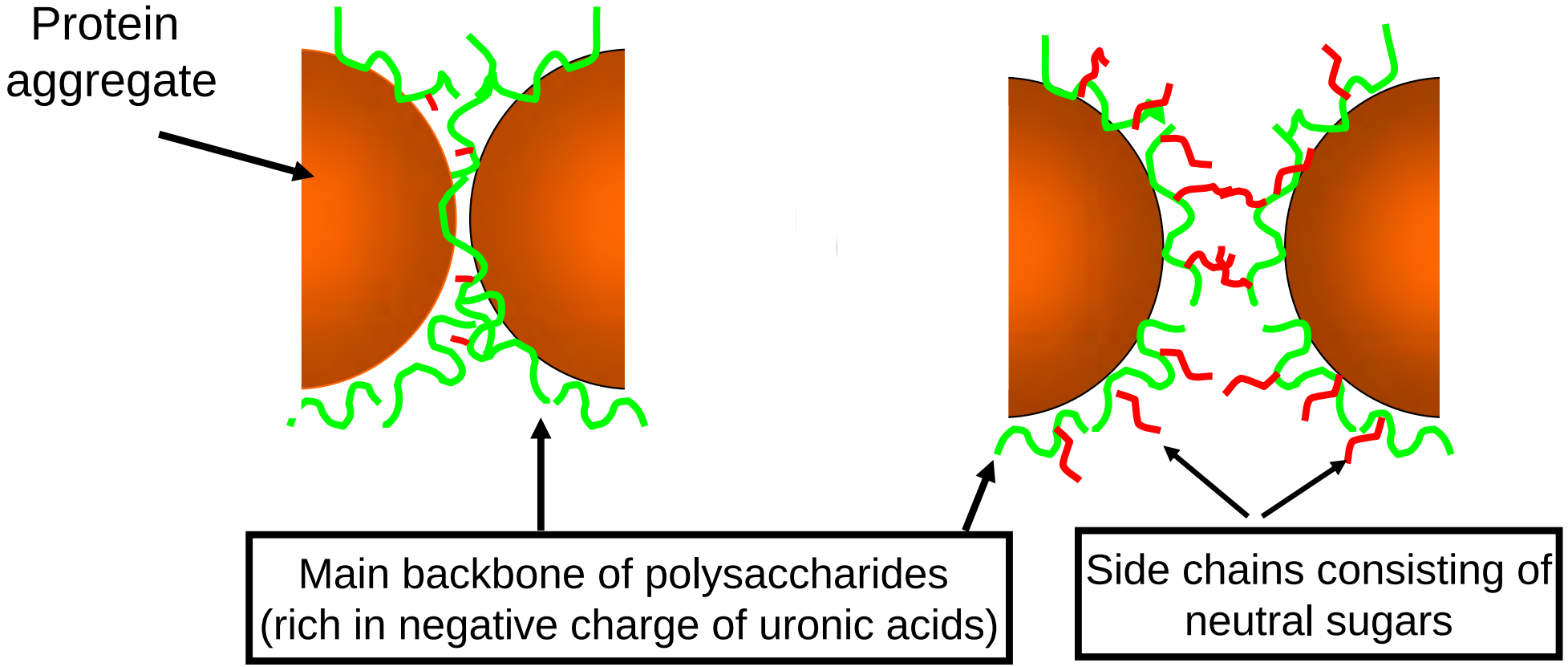
Stabilizing mechanism of protein aggregates by Pectin and SSPS

Pectin

Zeta potential : large
Steric hindrance : weak

SSPS

Zeta potential : small
Steric hindrance : strong



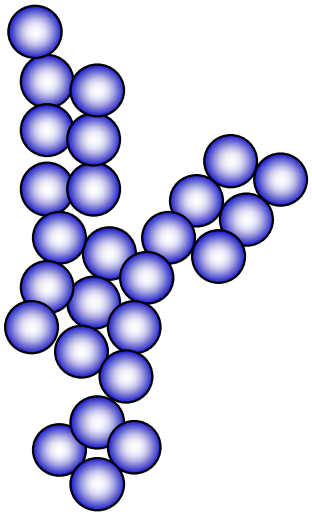
Schematic Representation of Gelation of Glycinin in the absence or presence of SSPS

(–)SSPS

glycinin

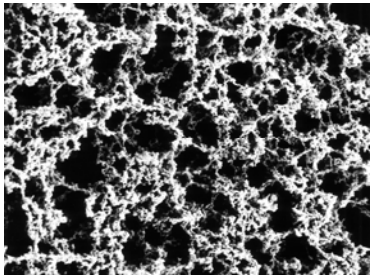
heating

Excess counter ions screen surface charges of glycinin



Formation of thick strands via hydrophobic interaction and SS bonding

Network formation (Rigid gel)



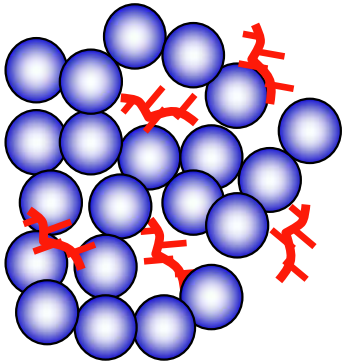
Microstructure (5% glycinin gel)

(+)SSPS

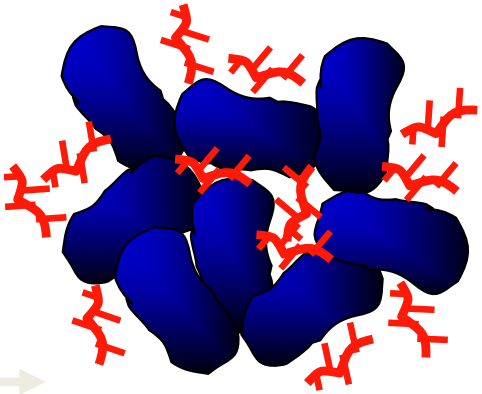
glycinin

SSPS

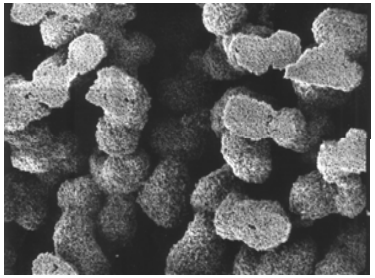
heating



Random aggregation of glycinin (Inhibition of regular association to form strands by SSPS)



Phase separation of gigantic particulates of glycinin and SSPS matrix (Soft gel or sol)



Microstructure (5% glycinin + 3% SSPS gel)

Prospective Cohort Study on Isoflavone (http://epi.ncc.go.jp/en/index.html)

In the presentation of the workshop, figures from the website of Japan Public Health Center-based Prospective study (JPHC) in National Cancer Center were introduced. Original publications are as follows.

(1) Effects on Lung Cancer

Hazard ratios (HR) for lung cancer incidence according to intake of isoflavones by smoking status (*American Journal of Clinical Nutrition*, **91**, 722-728 (2010))

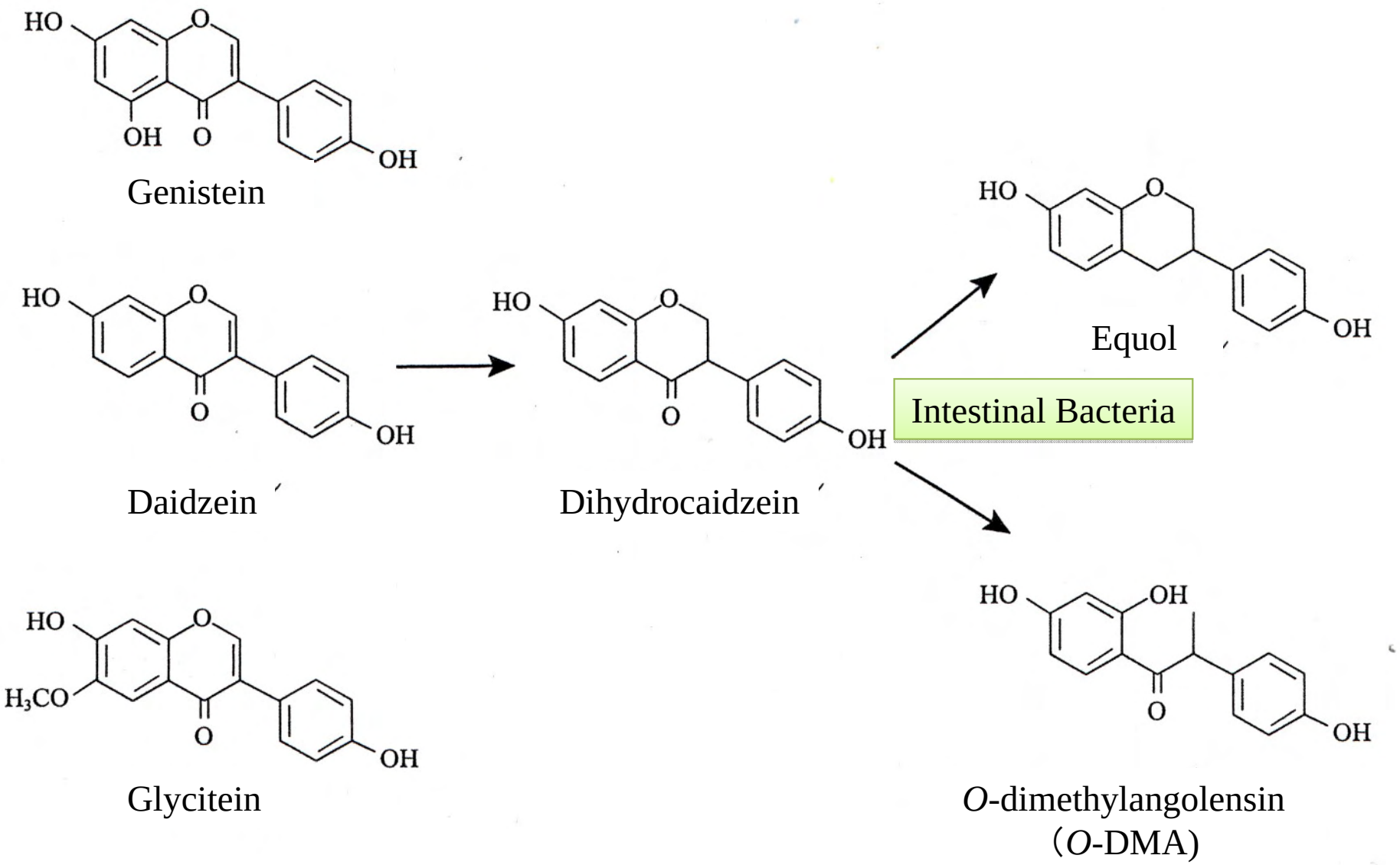
(2) Effects on Breast Cancer

Relationship between Isoflavone content in blood and the risk of breast cancer (*Journal of Clinical Oncology*, **26**, 1677-1683 (2008))

(3) Effects on Cerebral and Myocardial Infarctions

- Effects of soybean intake on the risk of cerebral and myocardial infarctions, and ischemic cardiovascular disease mortality
- Changes in hazard ratios (HR) for the incidence of Cerebral and Myocardial Infarctions according to dietary intake of isoflavone (*Circulation* November 27, 2007)

Major soybean isoflavones and metabolites of Daidzein



Comparison of Percent Equal Producers between Prostate Cancer Patients and Controls (*Jpn. J. Clin.Oncol.*, **34**, 86-89, (2004))

Table 2. Median blood soybean isoflavones levels and equol producers compared among Japanese, Korean and US subjects

(Patients/Control)	Japan		Korea		USA	
	(n = 133/162)	P value[a]	(n = 61/61)	P value[a]	(n = 24/21)	P value[a]
Daidzein						
Patient	25.6 (1.6–607.1)		29.9 (0.8–371.9)		3.0 (0.5–34.3)	
Control	24.4 (0.7–424.0)	0.273[a]	30.3 (2.3–940.6)	0.518[a]	3.0 (0.6–94.5)	0.592[a]
Genistein						
Patient	84.9 (7.2–1355.8)		67.5 (1.7–973.6)		1.8 (0.6–57.3)	
Control	84.5 (3.4–1056.8)	0.339[a]	64.9 (6.3–448.0)	0.574[a]	2.7 (0.6–140.0)	0.552[a]
Equol producer						
Patient (%)	29		30		17	
Control (%)	46	0.004[b]**	59	0.001[b]**	14	0.826[b]

[a]Wilcoxon's test. [b]Chi-square test. **Statistically significant ($P < 0.01$).

The active isoflavone level was markedly lower and the percentage of equol producer was also lower for Americans as compared to the Japanese and Koreans.

However, recently, the percentage of equol producer was decreased for young generation even in Japan and Korea (*Prostate Cancer Prostatic Dis.*, **11**, 252-257 (2008)).

Contents of the presentation

1. Recent research on soybean components
2. New insight into traditional soybean food products
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Bioavailability of Ca in Ca-fortified soy milk for young women (Soy Protein Research, Japan, 12, 1-10 (2009))

Ca content of soymilk is lower than that of cow's milk. The fortification of Ca into soymilk is difficult because glycinin is very sensitive to Ca^{2+}. The soymilk rich in β-conglycinin can be fortified with Ca.

In this research, the bioavailability of Ca in the Ca-fortified soy milk was compared to that of cow's milk for young female subjects

Energy and nutrients contained in 5 d cycle menus of cow's milk and soy milk diets excluding dessert*

	Cow's milk diet	Soy milk diet
Energy (kcal)	1,709 ± 26	1,655 ± 26
Protein (g)	66.3 ± 3.2	66.3 ± 3.2
Lipids (g)	44.8 ± 1.6	42.4 ± 1.7
Carbohydarate (g)	269.9 ± 7.6	262.1 ± 7.6
Ca (mg)	568.7 ± 13.1	568.7 ± 13.1
Mg (mg)	175.2 ± 8.3	187.2 ± 8.3
P (mg)	854.9 ± 20.1	710.9 ± 20.1
Vit. D (μg)	4.5 ± 0.1	4.2 ± 0.1
Fiber (g)	11.8 ± 0.8	12.1 ± 0.8
PFC energy ratio (%)	15 : 23 : 62	16 : 22 : 62

*eans ± SD/day

Bioavailability of Ca in Ca-fortified soy milk for young women (Soy Protein Research, Japan, 12, 1-10 (2009))

Table 5. Ca balance and apparent Ca absorption rate of soy milk and cow's milk

	Test meal	
	Ca fortified Soy milk+basal diet	Cow's milk +basal diet
Intake (mg/d)	578 ± 4	578 ± 5
Feces (mg/d)	469 ± 207	466 ± 166
Urine (mg/d)	99 ± 46	101 ± 46
Balance (mg/d)[1)]	9 ± 201	10 ± 168
Retention rate (%)[2)]	1.7 ± 24.7	1.8 ± 29.0
Apparent absorption (mg/d)[3)]	109 ± 206	112 ± 165
Apparent absorption rate (%)[4)]	18.8 ± 35.6	19.4 ± 28.5

1) Balance (mg/day)=Intake − (Feces+Urine)
2) Retention rate (%)= Balance /Intake × 100
3) Apparent absorption (mg/d)=Intake − Feces
4) Apparent absorption rate (%)=Apparent absorption/Intake × 100

*Mean ± SD (n=12). Significant differences were not observed between the two groups by Wilcoxon Signed Ranks Test.

The bioavailability of Ca in soymilk was similar to that of cow's milk.

The Ca-fortified soymilk rich in β-conglycinin is useful from the nutritional viewpoints.

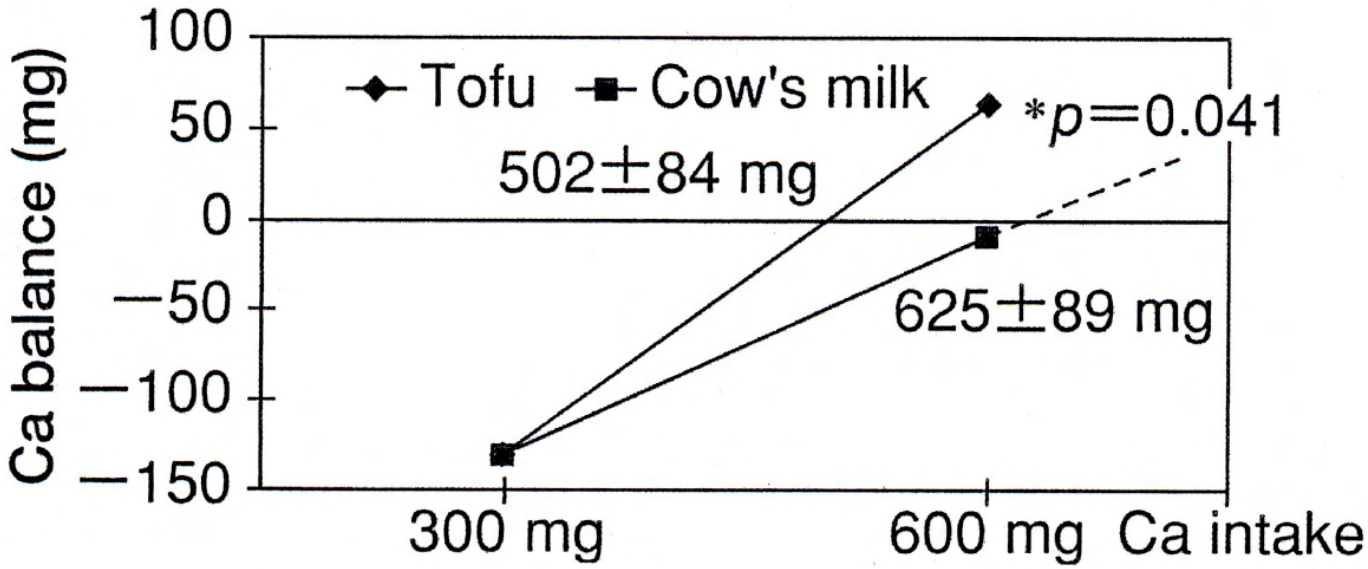
Absorption rate and requirement of Ca in Tofu for post-menopausal women in Vietnam (*Soy Protein Research, Japan*, **11**, 15-19 (2008))

Ca absorption rates and balances observed in 12 post-menopausal women by 3 dietary treatments[1)]

	dietary treatments		
	TOFU + basal diet	cow's milk + basal diet	basal diet
intake (mg/d)	634±10[a]	634±10[a]	330±8
feces (mg/d)	426±200[a]	498±193[b]	310±95
urine (mg/d)	145±42	146±54	150±36
balance (mg/d)[2)]	64±210[a]	−10±203[b]	−130±102
retention rate (%)[3)]	10.0±33.1[a]	−1.6±32.0[b]	−39.4±30.8
apparent absorption (mg/d)[4)]	208±200[a]	136±193[b]	20±95
apparent absorption rate (%)[5)]	32.8±31.6[a]	21.4±30.5[b]	6.1±28.8

1) Mean±SD (n=12). Different alphabets in the same line indicate significant differences between the two dietary treatment groups (Tofu+basal diet and Cow's milk+basal diet) by Wilcoxon Signed Ranks Test ($p<0.05$).
2) Balance (mg/day)=Intake − (Feces+Urine)
3) Retention rate (%)=Balance/Intake×100
4) Apparent absorption (mg/day)=Intake − Feces
5) Apparent absorption rate (%)=Apparent absorption/Intake×100

Absorption rate and requirement of Ca in Tofu for post-menopausal women in Vietnam (*Soy Protein Research, Japan*, **11**, 15-19 (2008))

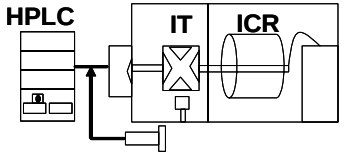


Regression lines of Ca balance between basal diet and test diets. Mean±SD. *Significant difference in values between the two groups by Wilcoxon Signed Ranks Test ($p<0.05$).

The results suggest that Tofu could be a important Ca source for post-menopausal women, particularly in Vietnam (and probably other East Asian countries) where people scarcely drink cow's milk.

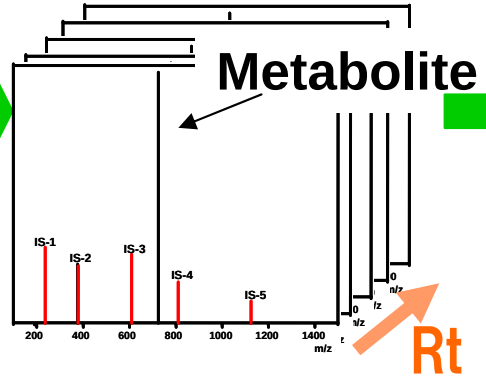
Procedure of high-through-put analysis of metabolites in Kazusa DNA Research Institute

LC-FTICR/MS analysis

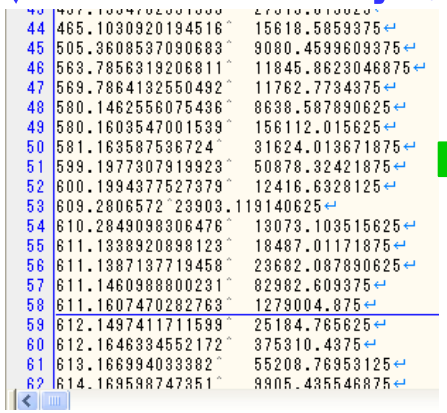


Internal standard mix (m/z 235-1123, 4-5 compounds)

Raw data acquisition



m/z calibration by I.S.

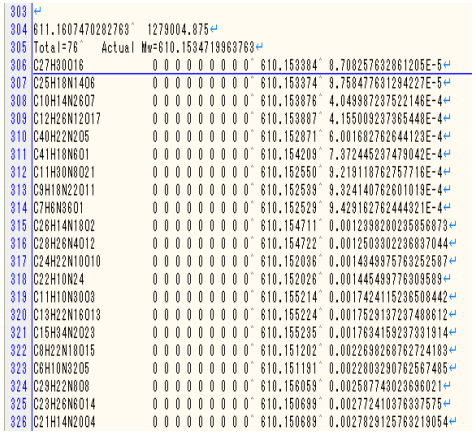


44	465.1030920194516	15618.5859375
45	505.3608537090683	9080.4599609375
46	563.7856319206811	11845.8623046875
47	569.7864132550492	11762.7734375
48	580.1462556075436	8638.587890625
49	580.1603547001539	156112.015625
50	581.163587536724	31624.013671875
51	599.1977307919923	50878.32421875
52	600.1994377527379	12416.6328125
53	609.2806572	23903.119140625
54	610.2849098306476	13073.103515625
55	611.1338920898123	18487.01171875
56	611.1387137719458	23682.087890625
57	611.1460988800231	82982.609375
58	611.1607470282763	1279004.875
59	612.1497411711599	25184.765625
60	612.1646334552172	375310.4375
61	613.166994033382	55208.76953125
62	614.1695987473351	9905.435546875

Extract m/z peak groups

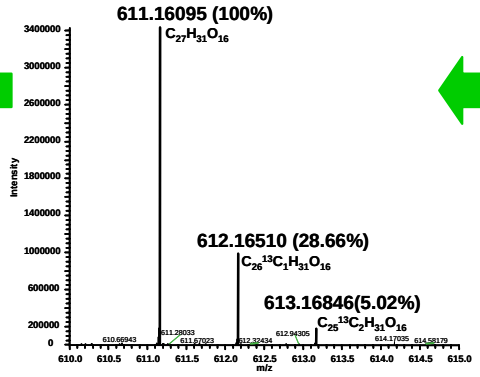
Detection of isotopic peak pairs

MF calculation



303													
304	611.1607470282763									1279004.875			
305	Total=76	Actual								Mw=610.1534719963763			
306	C27H30O16	0	0	0	0	0	0	0	0	610.153384	8.708257632861205E-5		
307	C25H18N1O6	0	0	0	0	0	0	0	0	610.153374	9.758477631294227E-5		
308	C10H14N2S07	0	0	0	0	0	0	0	0	610.153876	4.049887237522146E-4		
309	C12H26N12O17	0	0	0	0	0	0	0	0	610.153887	4.155009237365448E-4		
310	C40H22N2O5	0	0	0	0	0	0	0	0	610.152871	6.001682762644129E-4		
311	C41H18N6O1	0	0	0	0	0	0	0	0	610.154209	7.372445237479042E-4		
312	C11H30N8O21	0	0	0	0	0	0	0	0	610.152550	9.219118762757716E-4		
313	C9H18N2O11	0	0	0	0	0	0	0	0	610.152539	9.324140762601019E-4		
314	C7H6N3S6O1	0	0	0	0	0	0	0	0	610.152529	9.429162762444321E-4		
315	C26H14N1S02	0	0	0	0	0	0	0	0	610.154711	0.001239828023585687 3		
316	C28H26N4O12	0	0	0	0	0	0	0	0	610.154722	0.001250330223863704 4		
317	C24H22N10O10	0	0	0	0	0	0	0	0	610.152036	0.001434897576325258 7		
318	C22H10N24	0	0	0	0	0	0	0	0	610.152026	0.001445489776309589		
319	C11H10N3O03	0	0	0	0	0	0	0	0	610.155214	0.001742411523650844 2		
320	C13H22N16O13	0	0	0	0	0	0	0	0	610.155224	0.001752913723748861 2		
321	C15H34N2O23	0	0	0	0	0	0	0	0	610.155235	0.001763415923739191 4		
322	C8H22N18O15	0	0	0	0	0	0	0	0	610.151202	0.002269828876272418 3		
323	C8H10N3Z05	0	0	0	0	0	0	0	0	610.151191	0.002280329076256748 5		
324	C29H22N8O8	0	0	0	0	0	0	0	0	610.156059	0.002587743023696021		
325	C23H28N6O14	0	0	0	0	0	0	0	0	610.150699	0.002772410376337575		
326	C21H14N2O04	0	0	0	0	0	0	0	0	610.150689	0.002782912576321905 4		

Narrow down candidate formulas by isotopic pattern



Manual assignment

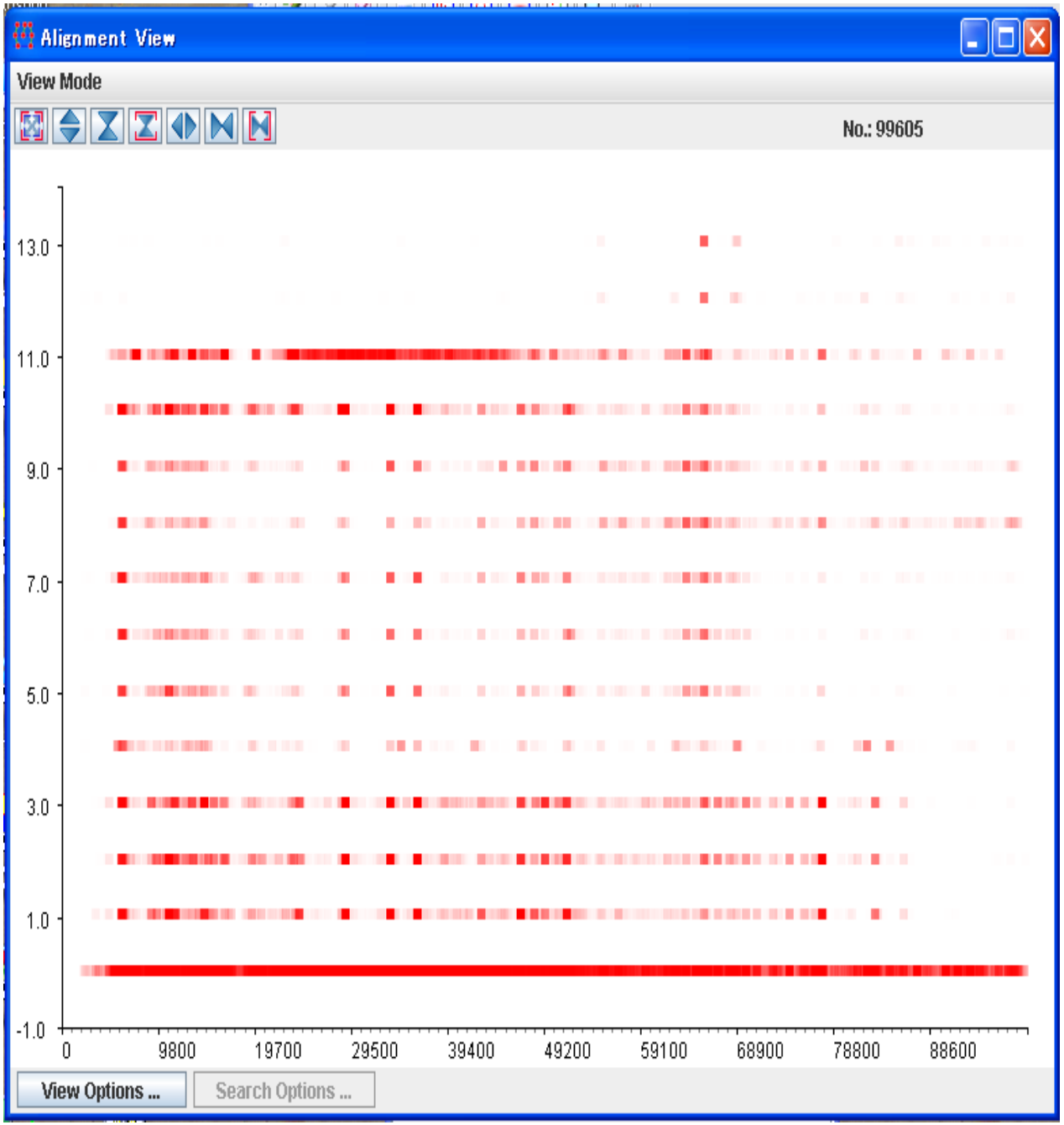
Metabolite annotations

- MS^n
- PDA
- Database hit
- Literature

MF confirmation

$C_nH_mO_p$

Table of components (metabolites) of soybean and soybean products



Natto, Fermented Soybean
Yuba, sheeted soy product
Fried tofu B
Fried tofu A

Tofu B
Tofu A

Okara, Soy Residue

Soy Milk

Imported
Domestic B
Domestic A

Soybean

Domestic

imported

Contents of the presentation

1. Recent research on soybean components
2. New insight into traditional soybean food products
3. Present situation of acceptance of soybean food products in Japan

Soybean Food Products as “Food for Specified Health Use (FOSHU)”

Approximately 900 foods are approved as FOSHU at present by the Ministry of Health, Labour and Welfare (The FOSHU system is now under the control of the Consumer Affairs Agency). The list of all FOSHU in exel file is available. The number of FOSHU including soybean components is 190.

Of soybean components, five components, that is, proteins (peptides), oligosaccharides, isoflavones, phytosterol, vitamin K are permitted to be used as the ingredients of FOSHU.

For the past five years, only 12 components are approved to be the ingredients for FOSHU. Soybean β-conglycinin obtained the permission for the health claim that it reduces the neutral lipids in June of 2007.

Texturized soy protein

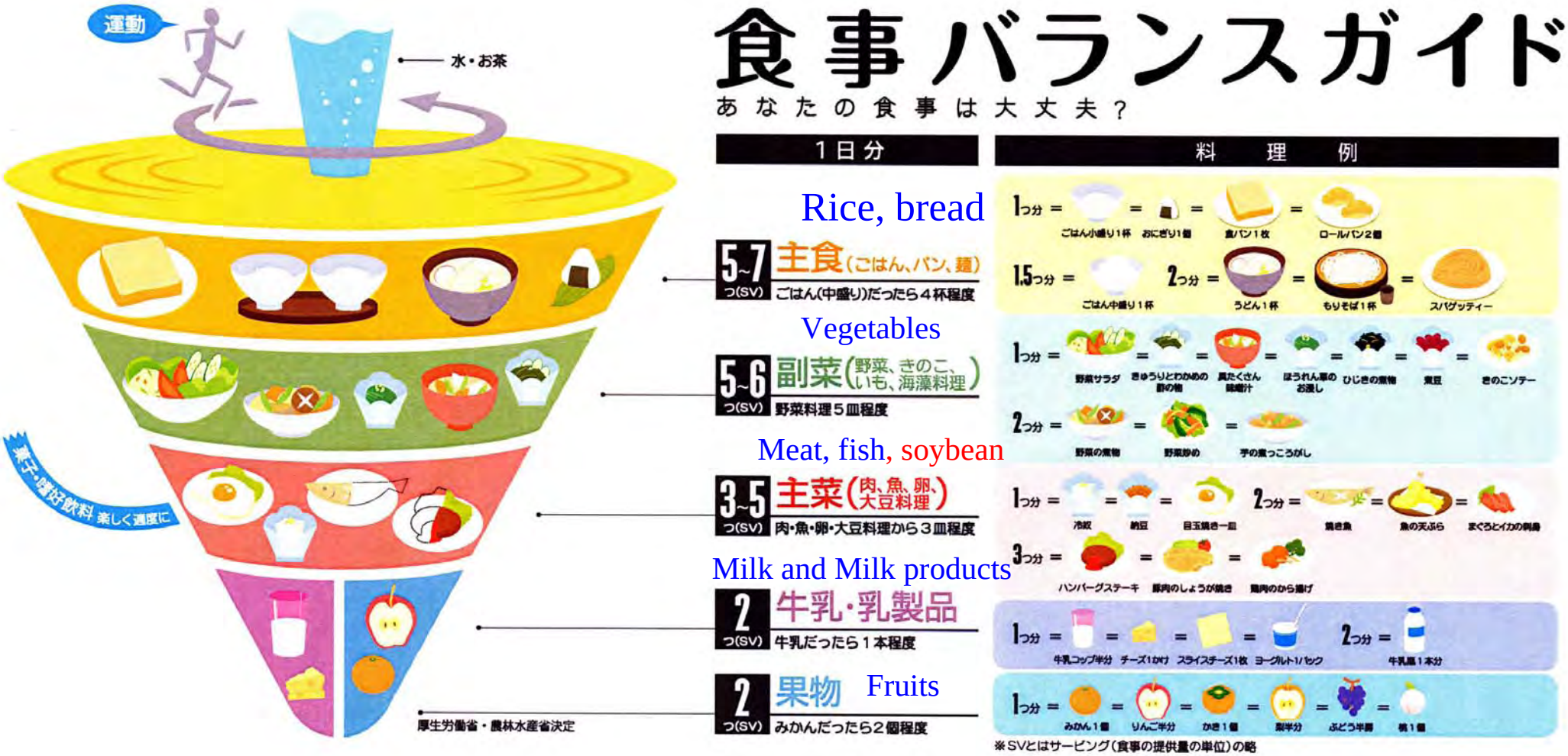
Fortified soymilk

Soy yohgurt

FOSHU of β-conglycinin

Numerous soybean food products or supplements except FOSHU are also available in Japan, and the information about the items were introduced in the Workshop.

The Ministry of Agriculture, Forestry and Fisheries and the Ministry of Health, Labour and Welfare made the guideline on the good balance of diet



The intake of soybean is highly recommended!

http://www.maff.go.jp/j/balance_guide/b_report/gaiyou.html

http://www.kenkounippon21.gr.jp/

Website and Database Relating to Soybean

Website

http://www.maff.go.jp/j/seisan/ryutu/daizu/index.html

(Homepage of soybean at the Ministry of Agriculture, Forestry and Fisheries)

http://www.daizupeptide.jp/english/index.html (Soy peptide health forum)

http://www.tofu-as.jp/english/index.html (Japanese Tofu Association)

http://epi.ncc.go.jp/en/index.html

(Research Center Cancer for Prevention and Screening, National Cancer Center: You can look at the results of JPHC studies on various foods including soybean foods)

Database

http://www.jircas.affrc.go.jp/DB/guide-eng.html

(Database of soybean genetic resources in Northeast China in JIRCAS)

http://www.grainnet.ne.jp/

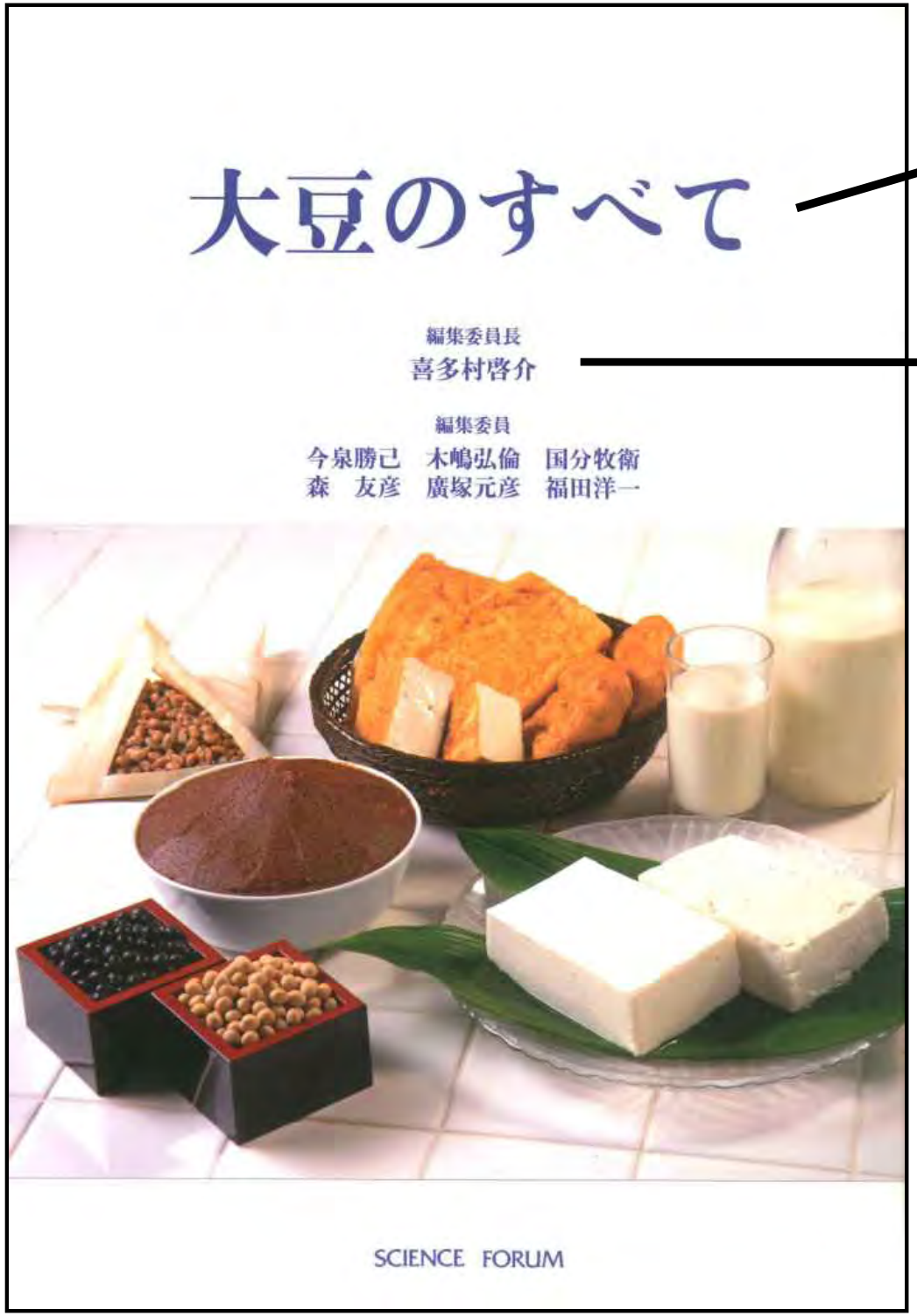
(Gainnet: Database on soybean products : only in Japanese)

http://www.hinshitsusekkei.kais.kyoto-u.ac.jp/Soybean/

(Soybean Proteome Database: Please ask Dr. Maruyama about the contents

http://133.11.220.243/nutdb.html

(Nutrigenomics database in Tokyo University: including data on soybean components)



All of Soybean

Chief Editor
Prof. Keisuke Kitamura

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