

**Karunya** INSTITUTE OF TECHNOLOGY AND SCIENCES

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# SOYA IN HUMAN NUTRITION

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# Soybean - overview

- ▶ Soybean (*Glycine max* Leguminosae) is an important grain legume that is not only a valuable oil crop but also used as feed for livestock and aquaculture has been cultivated in China for more than 4,000 years.
- ▶ Commercial crop and grown in over 35 countries as the major oil seed. Globally 38% of total soybean crop is grown in US; followed by Brazil (25%) Argentina (19%), China (7%), India (3%), Canada (2%) and Paraguay (2%).
- ▶ The major producers in the world of soybean products are the United States, Brazil, Argentina and India.
- ▶ Extensively used as important source of dietary protein and oil throughout the world.

## Soy bean oil



- ▶ Main product of processing of soybean crop.
- ▶ Contains about 15.65% saturated fatty acids, 22.78% monounsaturated fatty acids, and 57.74% polyunsaturated fatty acids (7% linolenic acid and 54% linoleic acid).



# Overview of soya protein

- ▶ Soybean protein is one of the least expensive sources of dietary protein.
- ▶ Considered to be a good substituent for animal protein.
- ▶ Nutritional profile except sulfur amino acids (methionine and cysteine) is almost similar to that of animal protein.
- ▶ Biological value of soy protein is similar to many animal proteins such as casein if enriched with the sulfur-containing amino acid methionine.

- ▶ According to the standard for measuring protein quality, Protein Digestibility Corrected Amino Acid Score, soybean protein has a biological value of 74, whole soybeans 96, soybean milk 91, and eggs 97.
- ▶ Soybeans contain two small storage proteins known as glycinin and beta-conglycinin.





# Nutrient composition of soybean and products

Nutrient	Soybean				
	Flour	Protein concentrate	Seed heat processed	Meal solvent extracted	Seed without hulls, meal solvent extracted
Protein%	13.3	84.1	37.0	44.0	48.5
Fat%	1.6	0.4	18.0	0.8	1.0
Linoleic acid %	-	-	8.46	0.40	0.40
Crude fiber%	33.0	0.2	5.5	7.0	3.9
Calcium%	0.37	0.0	0.25	0.29	0.27
Total phosphorus%	0.19	0.80	0.58	0.65	0.62
Non phytate phosphorus %	-	0.32	-	0.27	0.22
Potassium %	1.50	0.18	1.61	2.00	1.98
Chlorine%	0.02	0.02	0.03	0.05	0.05
Iron (mg/kg)	-	130	80	120	170
Magnesium %	0.12	0.01	0.28	0.27	0.30
Manganese(mg/kg)	29	1	30	29	43


## Soybean

Nutrient	Soybean				
	Flour	Protein concentrate	Seed heat processed	Meal solvent extracted	Seed without hulls, meal solvent extracted
Sodium %	0.25	0.07	0.03	0.01	0.02
Sulfur %	0.06	0.71	0.22	0.43	0.44
Copper (mg/kg)	-	7	16	22	15
Selenium (mg/kg)	-	0.10	0.11	0.10	0.10
Zinc (mg/kg)	-	23	25	40	55
Biotein(mg/kg)	0.22	0.3	0.27	0.32	0.32
Choline(mg/kg)	640	2	2.860	2794	2731
Folacin (mg/kg)	0.30	2.5	4.2	1.3	1.3
Niacin(mg/kg)	24	6	22	29	22
Pantothenic acid (mg/kg)	13.0	4.2	11.0	16.0	15.0
Pyridoxine(mg/kg)	2.2	5.4	10.8	6.0	5.0
Riboflavin(mg/kg)	3.5	1.2	2.6	2.9	2.9
Thiamin(mg/kg)	2.2	0.2	11.0	4.5	3.2
Vitamin B12 (µg/kg)	-	-	-	-	-
Vitamin E (mg/kg)	-	-	40	2	3

# Conventional soy products

- ▶ **Soy sauce** - Condiment produced from fermented paste of boiled soybean, roasted brine, grain and *Aspergillus oryzae* or *Aspergillus sojae* molds. Soy sauce is conventional ingredient in East and Southeast Asian countries.
- ▶ **Natto** - Traditional Japanese breakfast food made from fermented soybean with *Bacillus subtilis*. As a rich source of protein. It has acquired taste.
- ▶ **Misco** - Traditional Japanese flavor food produced by soybean with salt and the fungus kojikin.
- ▶ **Douche** - Used for making black bean sauce. Product are similar to ogiri and iru (African fermented bean products).



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- ▶ **Tempeh** - traditional soy product. Made by fermentation process. it is unique product a rich protein.
  - ▶ **Gouchuisng** - salty and strong fermented traditional food made of red chilli, glutinous rice, fermented soybean and salt a rich source of carbohydrate and protein.
  - ▶ **Tofu** - also known as bean curd it is made of coagulant soy milk. It contain large amount of protein and high iron
  - ▶ **Stinky tofu** - form of fermented tofu that has strong smell made of fermented milk.

- ▶ **Soy milk** - Traditional staple of Asian countries. it contain protein, fat and carbohydrate.
- ▶ **Tamari** - Type of soy sauce, darker in appearance and richer in flavor.
- ▶ **Soy biscuit** - Biscuits contain minimum 12% protein, 5% fat and maximum 4% crude fiber, 6.5% ash and 10% moisture. Color of biscuit is creamy to yellow and nutty and crunchy taste and texture of biscuit.
- ▶ **Soy bread** - Contain minimum 30% protein, 1.5% fat, maximum 4% crude fiber, 40% carbohydrate, 6.5% ash and 40% moisture. Color of soy bread is creamy to yellow and nutty and soft taste and texture of bread.





# Soybean Proteins and peptides

- Fermentation and germination are two commercial ways of improving soybean meal properties

**Table 1.** Effects of fermentation and germination on soybean antihypertension compounds

Compound	Fermentation (inoculated microbes/fermentation condition) or germination	IC <sub>50</sub> Sequences peptides Amount of phenols	Effect
Peptides	Germinating soybean in two temperature (30°C and 40°C)	<p>IC<sub>50</sub> value of none germinated soybean= 0.174 mg ml<sup>-1</sup></p> <p>IC<sub>50</sub> value of (30°C)= 0.098 mg ml<sup>-1</sup></p> <p>IC<sub>50</sub> value of(40°C)= 0.025 mg ml<sup>-1</sup></p> <p>ACE I of none germinated soybean= 33.28 % ACE I of (30°C)= 66.20% ACE I of (40°C)= 83.53%</p>	<p>- Increasing low molecular weight</p> <p>- Increasing ACE I<sup>1</sup> up to 83.5% by Germinating at 40°C</p> <p>- Germination at 40°C was more effective for releasing ACE I.</p>
Isoflavone and peptides	Inoculating germinated soybeans and germinated black soybeans with 3% (v w <sup>-1</sup> ) spore of <i>A. oryzae</i> was for 5 days at 25°C	ACE I%= 58.69%	<p>- The isoflavone contents in GS and GBS-doenjang were slightly higher than in RS-doenjang in the early stage of fermentation.</p> <p>- The ACE I activity increased as the fermentation period progressed.</p>

Phenols, flavonols, tannins

Investigating effect of germination time (3 and 6 days) and temperature (20 and 30°C) on the phenolic constituents, and bioactive compounds

\*NR

- The ACE I activity were significantly higher in GBS-doenjang (58.69%) than in other preparations after 75 days of fermentation.

- Doenjang prepared using GBS (GS: GBS= 7:3) showed greater ACE I activity than RS doenjang.

Germination at 30°C for 3 days resulted in highest total phenols, flavonols, tannins, saponins, ascorbic acid and tocopherols.

Isoflavone aglycones (daidzein and genistein) and saponin

Determining the effect of germination time and temperature on producing bioactive compounds

\*NR

- Optimal increases in the concentrations of isoflavone aglycones (daidzein and genistein) and saponin glycosides were observed with a 63 h germination time at 30°C.

- Both germination time and the temperature had an influence on the composition and concentration of bioactive compounds in germinated soybean flour.



Peptides	NR	Valyl-prolyl-proline (Val-Pro-Pro), isoleucyl-prolyl-proline (Ile-Pro-Pro) and (Tyr-Pro)	Exhibited ACE I activity and blood pressure lowering effect in spontaneously hypertensive rat.
Peptides	Hydrolyzing by <i>B. subtilis</i> Protease	$IC_{50}$ = 26.5 $\mu$ M Pro-Gly-Thr-Ala-Val-Phe-Lys	Identifying and introducing PGTAVFK sequence with antihypertensive effect
Peptides	Inoculating soybean with 4 lactobacilli strains: <i>L. fermentum</i> , <i>L. plantarum</i> , <i>L. fructosus</i> , and <i>L. casei</i> spp. <i>pseudopiantarum</i> at different time period (12, 18, 24, 36 and 48 h) and temperature (25, 37, 40, and 50°C)	1- $IC_{50}$ = 17.2 $\mu$ g ml <sup>-1</sup> 2- $IC_{50}$ value of Leu-Ile-Val-Thr-Gln= 0.087 $\mu$ g ml <sup>-1</sup>	- The <i>L. casei</i> spp. <i>pseudopiantarum</i> , LAB strain, exhibited maximum protease activity (37°C/ 36 h).  - Purified peptide fractions showed ACE I potency.  - Synthesized different peptide analogs LIVTQ, LIVT with ACE I activity
Peptides	<i>B. subtilis</i> protease digestion (40°C, 1 h)	$IC_{50}$ values= 0.1964 $\mu$ g ml <sup>-1</sup>	The $IC_{50}$ value of hydrolyzed with <i>B. subtilis</i> protease was less than pepsin digestion $IC_{50}$ .
Peptides	Isolating 3 fractions (F535A, F535B, and F535C) from fermented soybean paste	$IC_{50}$ (F535A, F535B, and F535C)= 2.1 to 3.0 $\mu$ g ml <sup>-1</sup>  His-His-Leu ( $IC_{50}$ = 2.2 $\mu$ g ml <sup>-1</sup> )	- Fraction F53 exerted a strong ACE I activity in vitro tests.

1)

- Peptide with His-His-Leu sequences showed lowering activity in vivo tests.

- Small decrease in ACE activity accompanied by a strong reduction of blood pressure

Peptides

Fermenting soybeans by *B. natto* and *B. subtilis* CH-1023

Ala, Phe and His

ACE I activity and antihypertensive effects

Peptides

Comparing Fermentation douchi qu pure-cultured by *A. Egyptiacus* for 48 h and 72 h with douchi secondary-fermented for 15 d

ACE I for 24h= 7.8%  
ACE I for 48h= 66.2%  
ACE I for 72h= 72.4%  
ACE I for 15d= 86.3%

Improving ACE I activities following the fermentation

Several compounds

Making Natto by fermenting steamed soybeans with *B. subtilis natto*

$IC_{50} = 0.27 \text{ mg ml}^{-1}$

total ACE I activity in lyophilized natto (100 g)=  $3.7 \times 10^5$  units

Reduction the blood pressure of rats



Peptides	Pre-fermenting Douchi by <i>A. oryzae</i> , <i>Mucor wutungkiao</i> , <i>B. subtilis natto</i> and <i>B. subtilis</i> B1	<p><math>IC_{50}</math> of <i>A. oryzae</i>= 0.4991 mg ml<sup>-1</sup></p> <p><math>IC_{50}</math> of <i>Mucor wutungkiao</i>= 0.3535 mg ml<sup>-1</sup></p> <p><math>IC_{50}</math> of <i>B. subtilis</i> Natto= 0.2294 mg ml<sup>-1</sup></p> <p><math>IC_{50}</math> of <i>B. subtilis</i> B1= 0.0901 mg ml<sup>-1</sup></p>	<p>- ACE I</p> <p>- The type of starter cultures affected significantly the production of ACE I activity in douchi qu, and that <i>B. subtilis</i> B1 was the best starter.</p>
Peptides	Fermenting Mao-tofu by <i>Mucor</i> spp. for 3-9 days		<p>- The extract exhibited the highest ACE I activity in vitro.</p> <p>- Hydrophobic amino acids, especially Pro, are usually found in ACE I peptides.</p>
Peptides	Obtaining miso paste and natto extracts by using distilled water with the same method as that used for the tofuyo extract, while soybean sauce was used directly	<p>The <math>IC_{50}</math>= 1.77 mg ml<sup>-1</sup></p> <p>Ile-Phe-Leu (<math>IC_{50}</math>= 44.8 μM)</p> <p>Trp-Leu (<math>IC_{50}</math>= 29.9 μM)</p> <p>The <math>IC_{50}</math> of Soybean sauce = 3.44 mg ml<sup>-1</sup></p> <p>The <math>IC_{50}</math> of Miso paste= 1.27 mg ml<sup>-1</sup></p> <p>The <math>IC_{50}</math> of Natto = 0.16 mg ml<sup>-1</sup></p> <p>The <math>IC_{50}</math> of Tofuyo= 1.77 mg ml<sup>-1</sup></p>	<p>- ACE I activity was observed in a tofuyo.</p> <p>- The ACE I activity of Trp-Leu was completely preserved after treatment with pepsin, chymotrypsin or trypsin.</p> <p>- Ile-Phe-Leu and Trp-Leu isolated from tofuyo were expected to contribute the antihypertensive effect via an in vivo transport system.</p>

Oligo-peptides

Chungkookjang fermentation

$IC_{50}$  of Lys-Pro=  
0.083 mg (100 g)<sup>-1</sup>

- Chungkookjang had  
ACE I and antihyper-  
tensive effect.

- Systolic blood pressure  
dropped by 15 mmHg  
and diastolic blood  
pressure by 8 mmHg 2 h  
after a single  
administration of 20 g of  
fermented soybean.

Peptides

Fermenting by *B. natto* or *B. subtilis*

Val-Ala-His-Ile-Asn-Val-Gly-  
Lys or Tyr-Val-Trp-  
Lys

Releasing several ACE I  
Peptides

Peptides

Preparing different fermented soybean products from various locations  
Chinese

$IC_{50}$  value of tempeh = 0.51 mg ml<sup>-1</sup>

$IC_{50}$  value of tofuyo = 0.66 and 1.77 mg ml<sup>-1</sup>

$IC_{50}$  value of miso = 2.38-1.27 mg ml<sup>-1</sup>

$IC_{50}$  value of natto = 0.16-0.44 mg ml<sup>-1</sup>

$IC_{50}$  value of soybean past = 0.012 mg ml<sup>-1</sup>

The lowest ACEI belonged to fermented soybean past.

Peptides

Modified soybean past fermentation, termed Fermented Soybean Seasoning (FSS),

$IC_{50}$  value of fermented soybean seasoning = 454  $\mu$ g ml<sup>-1</sup>

$IC_{50}$  regular soybean sauce = 1620  $\mu$ g ml<sup>-1</sup>

The ACE I peptides isolated from FSS were

Alae-Trp  $IC_{50}$  = 10  $\mu$ g ml<sup>-1</sup>

Alae-Tyr  $IC_{50}$  = 48  $\mu$ g ml<sup>-1</sup>

Glye-Trp  $IC_{50}$  = 30  $\mu$ g ml<sup>-1</sup>

Sere-Tyr  $IC_{50}$  = 67  $\mu$ g ml<sup>-1</sup>

Glye-Tyr  $IC_{50}$  = 97  $\mu$ g ml<sup>-1</sup>

Vale-Pro  $IC_{50}$  = 480  $\mu$ g ml<sup>-1</sup>

Alae-Phe  $IC_{50}$  = 190  $\mu$ g ml<sup>-1</sup>

Alae-Ile  $IC_{50}$  = 690  $\mu$ g ml<sup>-1</sup>

Vale-Gly  $IC_{50}$  = 1100  $\mu$ g ml<sup>-1</sup>

- Fermented soybean seasoning showed low  $IC_{50}$  compared to regular soybean sauce.

- Several dipeptides with ACE I potency were recognized.



## Peptides

Hydrolyzing soybean and the soybean-fermented foods were dephosphorylated, deglycosylated and digested with a variety of endoproteases (pronase, trypsin, Glu C protease, plasma proteases, and kidney membrane proteases) to generate oligopeptides

$IC_{50}$  = 0.1, 0.3, 0.5 and 0.7 mM

- Peptide with ACE I potency was produced by a tryptic-like cleavage with a C-terminal arginine.

- The proteases of lower specificity produced showed more oligopeptides and a higher percentage of bioactive peptides than the proteases of higher specificity, namely trypsin, and Glu C.

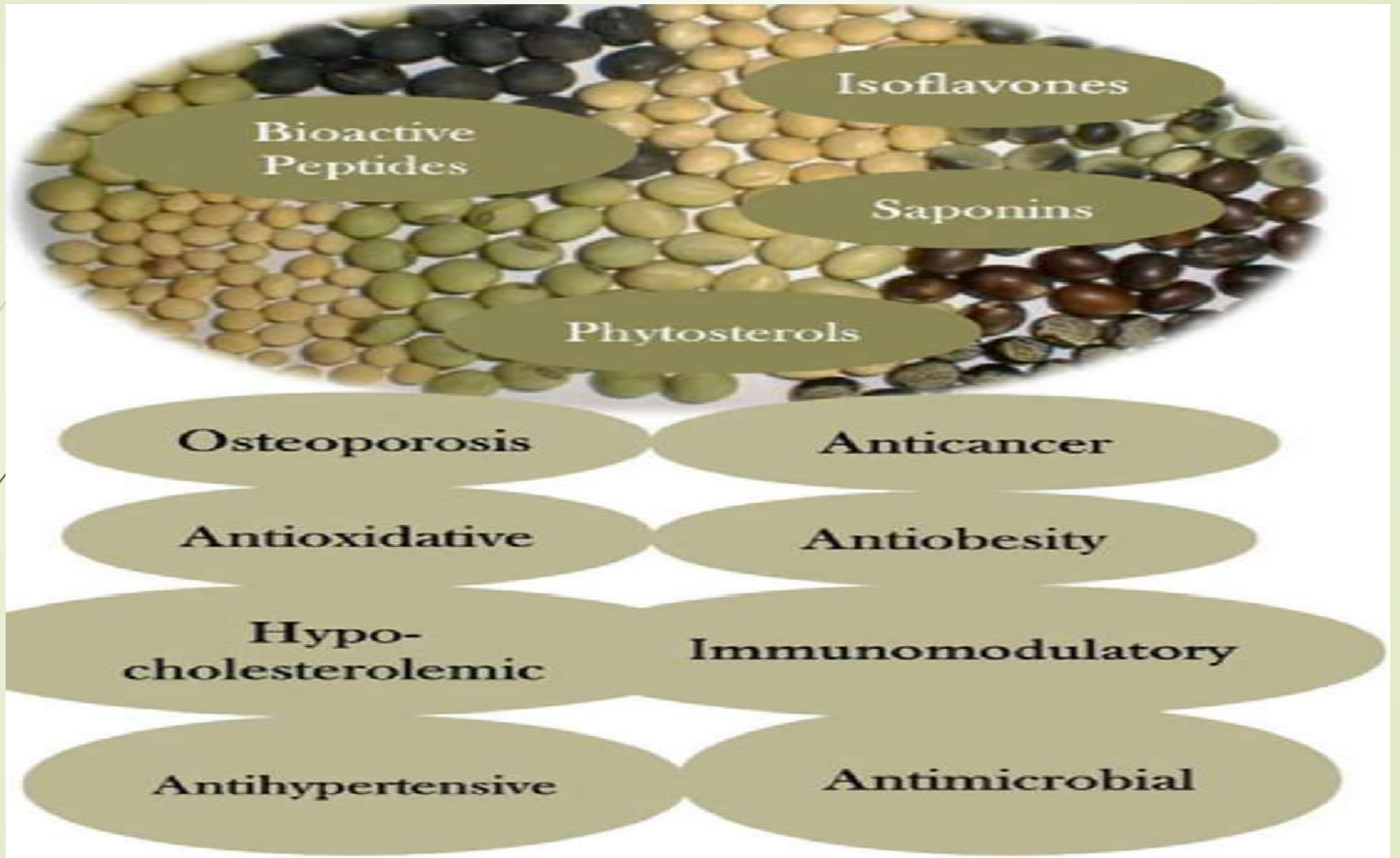
## Isoflavonoids and peptides

Fermenting Meju for 20d or 60 d), without the use of salt

Amount of two kinds isoflavone after 60 d fermentation:  
Daidzein: 152  
Genistein :170

- Longer fermentation period (60 d) enhanced the antidiabetic effect of soybeans.

- After 60 d fermentation: 15 kd (96.9%)



**Bioactive Peptides**

**Isoflavones**

**Saponins**

**Phytosterols**

**Osteoporosis**

**Anticancer**

**Antioxidative**

**Antiobesity**

**Hypocholesterolemic**

**Immunomodulatory**

**Antihypertensive**

**Antimicrobial**


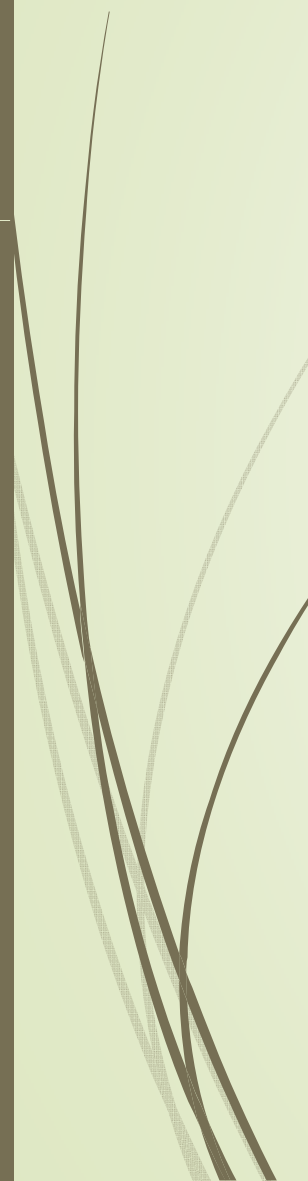
# Health benefits of soya Proteins and Peptides

## **Lunasin - Hypocholesterolemic effect**

43-amino acid peptide sequence.

- Discovered in the year 1996 through collaborative efforts at Dr. De Lumen's lab at UC Berkeley.
- Anti cancer -It can block cell division by binding to specific chromosomal proteins called "hypoacetylated histones."
- FDA has allowed a health claim on food labels stating that consuming 25 g soy protein/day may lower the cardiovascular problem.



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- Effect of lunasin is identical to statins but differs in the mode of action.
  - Seems to inhibit expression of HMG-CoA, which leads to increased LDL receptor expression at transcriptional level and not inhibiting its enzyme activity.
  - Statins can often be too efficient and block too much of the HMG-CoA enzyme, which culminates into serious side effects like termination of cellular functions.
  - **Cholesterol-lowering property of lunasin has been studied in cell lines and animal models.**
  - **Attempts need to traverse clinical studies to conclusively prove its efficacy and safety in therapeutic application.**
  - Peptides accelerates wound healing.
  - It's anti-inflammatory.

## **Lunasin - Anti inflammatory and anticarcinogenic**

Inhibit 2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt radical scavenger,

- inhibit the release of proinflammatory cytokines
- potent scavenger of peroxy and superoxide radicals
- Might confer a significant chemoprotection against oxidative stress associated liver disorders by restraining ROS overproduction.
- acts as an adjuvant by targeting dendritic cells (DCs) and modulate the immune responses to vaccine antigens in human peripheral blood mononuclear cells.

# Bioavailability of lunasin

- ▶ Lunasin was found to resist the harsh environmental condition of gastrointestinal tract which was attributed to the protection provided by BBI.
- ▶ Combined protection by BBI and other natural protease inhibitors in soy and wheat protein plays a crucial role in preventing degradation of the peptide to make it bioavailable.



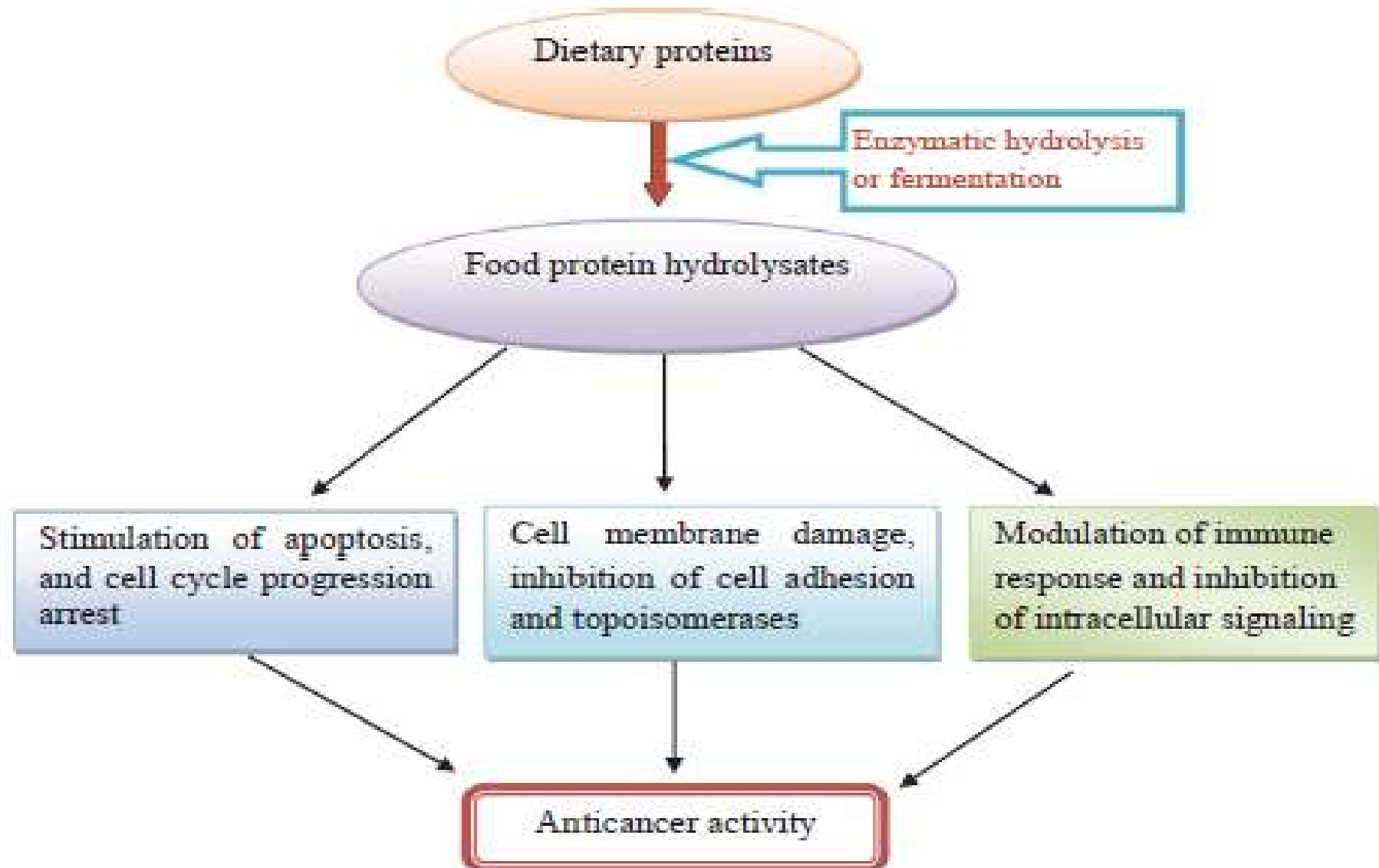
# Commercially available Lunasin Products

Brand name	Company	Nature	Country	Application	Health claims
LunaSoy™	Soy Labs®, LLC	Powder (protein complex)	USA (Jefferson City, Missouri)	Dietary supplement and as an ingredient for development of functional foods and nutraceuticals	<ul style="list-style-type: none"> <li>● Heart health</li> <li>● Immune modulation</li> <li>● Skin health</li> <li>● Anti-inflammatory</li> <li>● Antiaging</li> </ul>
Lunasin XP®	Soy Labs®, LLC	Powder (peptide extract)	USA (Jefferson City, Missouri)	Dietary supplement and as an ingredient for development of functional foods, beverages, and nutraceuticals	<ul style="list-style-type: none"> <li>● Antiaging</li> <li>● Heart health</li> <li>● Immune modulation</li> <li>● Skin health</li> <li>● Anti-inflammatory</li> </ul>
LunaRich®	Reliv International	Capsules (Highly pure and concentrated form)	USA	Dietary supplement	<ul style="list-style-type: none"> <li>● Support weight loss</li> <li>● Heart health</li> <li>● Overall cellular health</li> <li>● Improve immunity</li> <li>● Metabolic wellness</li> </ul>
FSP100™	Carefast® Products, Inc.	Soy drink mix	USA (Las Vegas)	Food supplement	<ul style="list-style-type: none"> <li>● Heart healthy</li> <li>● Highly nutritious</li> </ul>
Soy Guard™	Biotec-Foods	Capsules	USA	Dietary supplement	<ul style="list-style-type: none"> <li>● Highly bioavailable form of phytonutrient complex providing a complete spectrum of soy phytonutrients including genistein, daidzein, and phyto-estrogens (lunasin)</li> </ul>

# Reported to impart several health benefits such as

- ✓ lowering of plasma cholesterol
  - ✓ prevention of cancer
  - ✓ improvement in bone mineral density
  - ✓ provide protection against bowel and kidney disease
  - ✓ reduce the effects of menopause, promotes bone health,
  - ✓ improve metabolism, and
  - ✓ decrease the risk of diabetes.
- ▶ FDA authorized a health claim for soy protein that 25 g of soy protein per day may reduce the risk of heart disease.
  - ▶ Caused by the presence of isoflavone, saponins , protein and peptide.

# Anti cancer activity of soy proteins and peptides





# Soy isoflavones

ISF are major soy phytoestrogens present in soy foods

- Genistin, daidzin, and glycitein major isoflavones
- Soy foods and soy-based infant formulas are rich sources of ISF and contain ~4.2 mg ISF/g, whereas soy ISF supplements contain up to 500 mg ISF/g.
- consumption of soy protein or associated isoflavones (ISF) has beneficial impacts on the risk factors for cardiovascular disease including
  - lowering liver or blood triglyceride,
  - Lowering total and LDL cholesterol levels,
  - increasing HDL cholesterol and the ratio of HDL/LDL cholesterol.

# Isoflavone content of soy products

Food	mg per 100 g
Miso	41.45
Edamame	17.92
Natto	82.29
Soy cheese <sup>2</sup>	6.02–25.72
Soy flour, textured	172.55
Soy flour, defatted	150.94
Soy lecithin	15.7
Soy protein concentrate <sup>3</sup>	94.65
Soy protein concentrate <sup>4</sup>	11.49
Soy protein isolate	91.05
Shoyu (soy sauce)	1.18
Soy beans, roasted	148.5
Soy beans, raw	154.53
Yuba/ foo jook	44.67
Soy milk <sup>5</sup>	0.7–10.73
Sufu	13.75
Tempeh	3.82
Tofu <sup>6</sup>	13.1–34.78
Okara	9.39
Fuyu	45.51
Soybean oil	0

# Health benefits of soya phytochemicals

- **Phytoalexins** inducible chemicals involved in plants' self-defense system, have low molecular weight, possess anti-microbial activities, and are biosynthesized *de novo* in response to stress, including microbial attack, heavy metal salts, or UV radiation.
- Aside from the anti-microbial activity, some phytoalexins are also indicated to exhibit chronic disease prevention and health-promoting effects in humans
- **Glyceollins** are one of the major groups of phytoalexins in soy.
- Phenylalanine, daidzein, 7,2',4'-trihydroxyisoflavone, 3,9-dihydroxypterocarpan and glycinol are precursors.



# Health effects

- Antiproliferation
- Antiestrogenic
- Antibacterial
- Antinematode
- Antifungal activities
- Insulinotropic - glyceollins could improve insulin-stimulated glucose uptake and decrease triacylglycerol accumulation
- Slightly improved glucose-stimulated insulin secretion in Min6 pancreatic cells, and they potentiated insulinotropic actions in dysfunction cell.
- Attenuation of vascular contraction activity in the rat
- Anti-inflammatory
- ***Cholesterol-lowering***



**THANK YOU**