

**Contratto di ricerca tra DII-UNIPR e Natura Nuova s.r.l. sul tema:  
“Semilavorati testurizzati per nuovi prodotti a base di soia e di frutta” (marzo 2011-2013)**

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RELAZIONE SULLE ATTIVITÀ DI RICERCA  
EFFETTUATE NEL PERIODO: MARZO 2011 – DICEMBRE 2012

Parte A. Semilavorati Testurizzati per Nuovi Prodotti a Base di Soia

**Allegato A.5 – Aspetti nutrizionali e salutistici**  
sintesi della bibliografia richiamata

## Composizione

Soybeans vary widely in nutrient content based on the specific variety and growing conditions, but they typically contain 35% to 40% protein, 15% to 20% fat, 30% carbohydrate, and 10% to 30% moisture (Golbitz P, Jordan J. 2006. Soyfoods: market and products. In: Riaz MN, editor. Soy applications in food. New York: CRC Press. Pp. 1-21).

J.Heiser, T. Trentelman (1989) **Full-Fat Soya-Products – Manufacturing and Uses in Foodstuffs**. In: Vegetable Protein Utilization in Human Foods and Animal Feedstuffs. *American Oil Chemists' Society, Champaign, Illinois, pp. 52-61*

### Typical Composition of Soy Flour

Amino acid	(mg per 100 g)
Lysine*	3300
Methionine*	720
Phenylalanine*	2600
Proline	3040
Serine	2520
Threonine*	2170
Tryptophan*	740
Tyrosine	1700
Valine*	2730
Alanine	2220
Ammonia	1050
Arginine	3790
Aspartic Acid	5690
Cystine	720
Glutamic Acid	9160
Glycine	2100
Histidine	1350
Isoleucine*	2510
Leucine*	4075

\* Considered essential Amino Acids

### Carbohydrates

Acid polysaccharide complex	5-7%
Sucrose	8.2%
Stachyose	5.5%
Arabinan	1%
Neutral arabiongalactan	8-10%
Raffinose	1.2%

### Typical Analysis of Soybean for Mineral Content

Element	Content	
	Mg/100 gm	Percent RDA in 20 gm Protein <sup>a</sup>
<b>Macro-</b>		
Sodium Na <sup>+</sup>	0.9	—
Potassium K <sup>+</sup>	2090.0	—
Calcium Ca <sup>++</sup>	252	10.0
Magnesium Mg <sup>++</sup>	302	30.2
Phosphorus PO <sub>4</sub> <sup>-</sup>	740	29.6
Chloride Cl	0.07	—
<b>Micro-</b>		
Iron Fe <sup>+++</sup>	9.6	21.0
Cobalt Co <sup>++</sup>	10 <sup>-7</sup>	—
Copper Cu <sup>++</sup>	1.6	32.0
Manganese Mn <sup>++</sup>	3.6	—
Zinc Zn <sup>++</sup>	5.6	14.9
Aluminum Al <sup>++</sup>	0.9	—
Iodine I <sup>-</sup>	5 <sup>-5</sup>	1.0

<sup>a</sup> RDA of adults and those 4 years and older

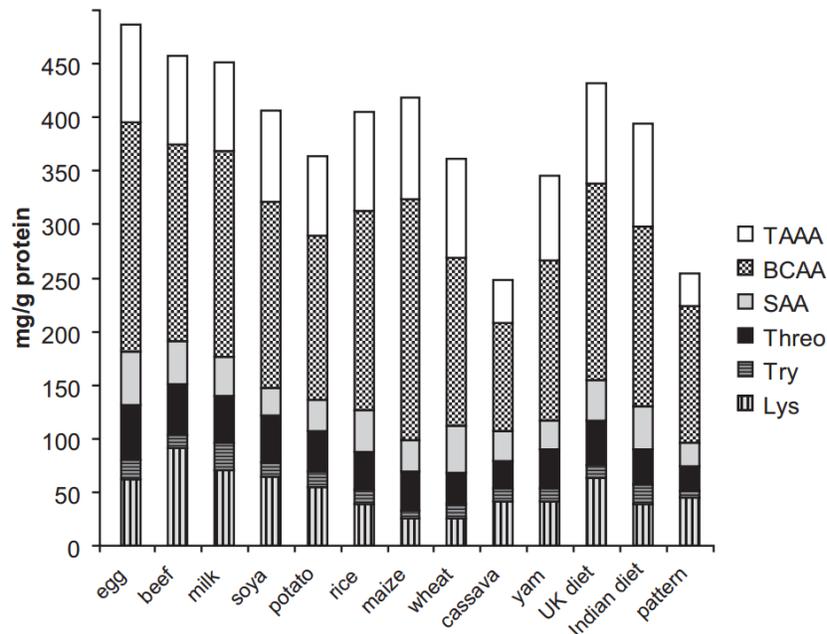
### Carbohydrate Constituents of Dehulled Defatted Soybean Meal

Carbohydrate	Source	Percent
<b>Monosaccharides</b>		
Glucose	Cotyledons	0.3
Arabinose	Hull	Trace-0.1
Ribose	Nucleic Acids	Trace-0.1
<b>Oleyosaccharide</b>		
Sucrose	Cotyledons	8.1
Maltose	Cotyledons	0.6
Raffinose	Cotyledons	1.1
Stachyose	Cotyledons	4.9
Verbascose	Cotyledons	Trace
<b>Polysaccharides</b>		
Arabinan	Cotyledons	15.0
Arabinogalactan	Cotyledons	5
Xylan (hemicellulose)	Hulls	3.5
Galactomannans	Hulls	Trace
Cellulose	Hulls	1-2

Vitamins of Defatted Soy Flour (5)		Mineral in Defatted Soy Flour (5)	
Vitamin	Quantity (mg/100 gm)	Mineral	Quantity (mg/100 gm)
Thiamin	1.10–1.50	Aluminum	2.33
Riboflavin	0.24–0.44	Calcium	220.00
Niacin	4.09–6.70	Cobalt	0.05
Vitamin B <sub>6</sub>	0.48–1.20	Copper	2.30
Folic Acid	0.03–0.09	Iodine	0.001
Vitamin B <sub>12</sub>	0.06–0.20	Iron	11.00
Biotin	0.17–0.66	Magnesium	309.00
Pantothenic Acid	1.3 –5.1	Manganese	2.8
Choline	2.2 –3.8	Phosphorus	680.0
		Potassium	2360.00
		Sodium	25.4
		Zinc	6.1

FAO/WHO/UNU (2007) **Protein and Amino Acid Requirements in Human Nutrition**. WHO Press, 2007  
[http://whqlibdoc.who.int/trs/WHO\\_TRS\\_935\\_eng.pdf](http://whqlibdoc.who.int/trs/WHO_TRS_935_eng.pdf)

Indispensable amino acids in food proteins and diets compared with the requirement pattern



TAAA: Total aromatic amino acids; BCAA Branched-chain amino acids  
 SAA Sulfur amino acids; Threo: Threonine; Try: Tryptophan; Lys: Lysine

The soybean - a legume - is a vegetable protein that contains zero cholesterol and is also low in saturated fat. Soybeans are a great source of iron, B vitamins, calcium, zinc, and dietary fiber-on par with the high-quality proteins found in milk, eggs, and meat (Lindsay SH, Claywell LG. (1998) *Considering soy: its estrogenic effects may protect women*. AWHONN Lifelines 2(1):1-44)

Most soy proteins are globulins.

Soybeans are the only vegetable food that contains all eight essential amino acids (Dudek S. G. 2001. *Nutrition essentials for nursing practice* (4th ed.). Philadelphia: Lippincott.; Morrison G, Hark L. 1999. *Medical nutrition and disease* (2<sup>nd</sup> ed.). Malden, MA: Blackwell Science.).

Golbitz P, Jordan J. 2006. **Soyfoods: market and products**. In: Riaz MN, editor. Soy applications in food. *New York: CRC Press. pp. 1-21*

The protein in the soybean contains all eight amino acids essential for human health, but until recently it was accepted that soy protein was lower in quality than many animal proteins. These earlier assumptions were based on an older method of evaluating protein quality, the protein efficiency ratio (PER), which is based on the growth rates of rats as measured in laboratory tests. However, rats require 50% more methionine (one of the amino acids found in soybeans) than humans do, making this particular method inappropriate for evaluating soy protein quality for human consumption. In order to make up for the shortcomings of the PER evaluations, the World Health Organization of the United Nations and the U.S. Food and Drug Administration have adopted a new method for evaluating protein quality called the protein digestibility corrected amino acid score (PDCAAS). This method uses an amino acid score, a comparison between the amino acid pattern of a protein and human amino acid requirements, and a factor for digestibility to arrive at a value for the quality of a protein. Using the new PDCAAS method, soy protein products generally receive scores of between 0.95 and 1.00, the highest value possible.

Soy protein is a rich source of lysine, which is the first limiting essential amino acid in most cereal proteins (Klein BP, et al. 1995. *Incorporating soy proteins into baked products for use in clinical studies*. J Nutr 125:666–74.)

Soybeans contain many phenolic compounds such as chlorogenic acid, caffeic acid, ferulic acid, and p-coumaric acid possessing antioxidant effect beneficial to human health (Kim, S. H., et al. 2005. *Analysis of isoflavone concentration and composition in soybean [Glycine max (L.)] seeds between the cropping year and storage for 3 years*. Eur. Food Res. Technol., 220:207–214.).

I fagioli di soia contengono mediamente su base umida circa:

- 35% di proteine (delle quali circa il 30% glicinina e 40% conglucina);
- 31% di carboidrati (comprendenti saccarosio, stachiosio, raffinosa, cellulosa, emicellulosa e pectina);
- 17% di olio (comprendente gli acidi grassi linoleico, oleico, palmitico, linolenico e stearico);
- 4,4% di ceneri.

Alcuni fattori antinutrizionali, quali le lectine (che agglutinano gli eritrociti del sangue), i fitati e altri inibitori della tripsina (che riducono la digeribilità delle proteine), sono inattivati dalla cottura e/o da altri trattamenti tecnologici come la fermentazione.

Anche l'enzima lipossigenasi, responsabile di aromi anomali nei prodotti derivati della soia per idrossiperossidazione degli acidi grassi insaturi, e gli oligosaccaridi non digeribili che provocano flatulenza (stachiosio e raffinosa) possono essere adeguatamente eliminati con trattamenti tecnologici.

The Ohio State University (2007) **Properties of Soybean Proteins**. *Food Science 822, Lecture Notes* <http://class.fst.ohio-state.edu/FST822/lecturesab/Soy.htm>

Defatted soy flour is used as a starting material and the soluble carbohydrate material is removed. The soluble sugars (mostly sucrose, raffinose and stachyose) can be removed by one of three procedures.

- The defatted soy flour can be washed with dilute acid at pH 4.5. This is the isoelectric point of the main soy protein and they are insoluble at this pH if the salt content is low.
- Another procedure is to soak the flour in a mixture of alcohol and water that contains enough alcohol to prevent the proteins from becoming soluble but enough water to remove the carbohydrate.
- In the final procedure, the soy proteins are denatured with steam to make them insoluble and the soluble carbohydrate is then removed by a water wash.

The precipitated proteins are called soy curd and the supernatant fraction is called whey.

The composition of the concentrate produced by all these procedures is about the same and a typical one is presented in Table 2. Use of alcohol or heat causes extensive loss of protein solubility in the finished products.

To produce soy protein isolates which by definition must contain greater than 90% protein on a dry basis, defatted flakes are utilized as a starting material. The soluble protein is removed by extraction with dilute alkali (pH 7-9) at moderate temperatures. The insoluble carbohydrate (fiber) and some proteins are left behind. The proteins are then recovered from the soluble carbohydrates and ash by isoelectric precipitation at pH 4.5. The precipitated proteins are called soy curd and the supernatant fraction is called whey. The curd is then washed, sometimes neutralized, and then dried. Table 2 contains a typical composition for a soy protein isolate.

Table 2. Proximate composition of soy products.

Component	Full Fat Flour	Defatted Flour	Concentrate	Isolate
Moisture	3.4	6.5	9.0	4.8
Protein	41.0	53.0	65.3	92.0
Fat	22.5	5.1	0.3	-
Fiber	1.7	3.0	2.9	0.25
Ash	5.1	6.0	4.7	4.0
PER	2.1	2.3	2.3	1.1-2.6

#### Classification of Soy Proteins

Most soy proteins are insoluble in water at their isoelectric point, but are solubilized in the presence of salt. The addition of NaCl to 0.7N will make most of the soy proteins soluble at their isoelectric points. This behavior is typical of proteins that have classically been classified as globulins and thus we frequently refer to the soy proteins as globulins.

2S Proteins. The 2S fraction has been reported to contain from 8 to 22% of the extractable soybean protein and consists of a number of enzymes. Probably the most studied protein of the 2S fraction are the trypsin inhibitors. While the feeding of non-heat treated soy proteins to animals has been demonstrated to have a detrimental affect on growth due to the trypsin inhibitor, they can be inactivated almost completely by commercial heat treatment.

7S Fraction. The 7S fraction of soy protein comprises about 35% of the soluble protein. It contains some enzymes, a number of hemagglutinins and a protein known as the 7S globulin. The enzyme of greatest commercial significance in this fraction is lipoxygenase. This enzyme causes the formation of hydroperoxides by the addition of oxygen to the double bonds that occur in linoleic and linolenic acid. Breakdown of these hydroperoxides can lead to further lipid oxidation and the creation of off-flavors in the soy protein. Much of the typically objectionable, beany flavor often associated with soy protein is thought to arrive from the oxidative deterioration of lipids that are tightly complexed with protein molecules.

For most uses of soy proteins, the minimal heat treatment required will be designed to inactivate this enzyme. In some applications, however, low-heat soy proteins are added that contain active lipoxygenase. These particular applications often require that a moderate amount of oxidation be allowed to take place. This oxidation can cause the destruction of pigments and thus a bleaching of the products. It can also cause the oxidation of free sulfhydryl groups to form disulfide bonds. Both of these functions can be of importance in the preparation of freshly ground flour for the baking industry.

The 7S fraction also contains a group of molecules called hemagglutinins. These carbohydrate containing proteins. These proteins have the ability to cause the aggregation of red blood cells in vitro and hence, their name. In some foods, hemagglutinins have been shown to lower the utilization of proteins in vitro, but they seem to have no affect on the protein quality of soybean proteins.

The majority of the 7S protein consists of the 7S globulins also called conglycinin.

At concentrations below 7%, total protein, soy protein are extremely heat stable. The proteins do not, however, precipitate from solutions after such heat treatments and are stable toward heat treatments in excess of 100C. As the ionic strength of the heating medium increases, the 7S globulin became more heat sensitive.

11S Fraction. The 11S fraction comprises from 31 to 52% of the soluble soy protein with about 85% of the total being, the 11S globulin. The 11S globulin is also called glycinin. This heat stability can be of benefit when it is desirable to obtain proteins that can be processed at high temperatures and maintain their solubility. In other cases where partial gelation of the proteins is desired, this heat stability can be a detriment.

15S Protein. The final fraction of the extractable soy protein is the 15S fraction. This material comprises about 5% of the total extractable protein. It is only poorly characterized and is thought to be composed of polymers of the other soy proteins.

<https://mospace.umsystem.edu/xmlui/bitstream/handle/10355/5099/research.pdf?sequence=3>

Table 2.1.1 Proximate Composition of Soybeans and Seed Parts (Wolf and Cowan 1975).

	% (Moisture-free basis)			
	Protein (N x 6.25)	Lipid	Carbohydrates (Include fiber)	Ash
Whole bean	40%	21%	34%	4.9%
Cotyledon	43%	23%	29%	5.0%
Hull	8.8%	1%	86%	4.3%
Hypocotyl	41%	11%	43%	4.4%

## U.S. Soybean Export Council

<http://www.ussec.org/why-u-s-soy/about-soy-2/soy-protein/textured-soy-protein-tsp/>

### *Textured Soy Protein (TSP)*

Textured soy protein (TSP) usually refers to products made from textured soy flour and textured soy protein concentrates. It is used as a meat extender or analog and can be added to a meal to increase its protein content. TSP has a texture similar to ground beef or other meat products and must be rehydrated with boiling water before use.

Textured soy flour is made by running defatted soy flour through an extrusion cooker, which allows for many different forms and sizes. It contains 50 percent protein as well as the dietary fiber and soluble carbohydrates from the soybean. When hydrated, it has a chewy texture. It is widely used as a meat extender. Often referred to as textured soy protein, textured soy flour is sold dried in granular and chunk style and is bland in flavor.

Textured soy protein concentrates are made by extrusion and are found in many different forms and sizes. These concentrates contain 70 percent protein as well as the dietary fiber from the soybean. When hydrated, they have a chewy texture and contribute to the texture of meat products.

It is available in as either a mince/crumble or chunk form. The former is similar in texture to ground beef, while the chunks resemble cubed meat used in stews. It is a dry product, easily rehydrated and takes no additional cooking time when used as an ingredient in another dish.

### Nutritional Values (TPS)

Macronutrient and micronutrient data per 100 grams of uncooked TSP (100% defatted soy flour)

(\*)

Proximate Data	Textured Soy Protein	Defatted Soy Flour (*)
Moisture, max, %	9	9
Protein (N x 6.25, mfb), min %	53	52
Ash, max %	6	6
Fat (pet. ether), max, %	1	1
Fat (acid hydrolysis), max, %	3	3
Crude Fibre, max	3.5	4
Total Dietary Fibre	18	18
Carbohydrates (including % TDF by difference)	30	30

Nutrient	Amount per 100 grams	Unit
Moisture	9	g
Protein (N x 6.25) moisture-free basis	52	g
Ash	6	g
Fat (petroleum ether)	1	g
Fat (acid hydrolysis)	3	g
Crude fiber	4	g
Total dietary fiber	18	g
Total carbohydrates	30	g
Energy	270	Kcals

### **Vitamins**

Vitamin A	trace	IU
Vitamin C	0	mg
Thiamine	0.62	mg
Niacin	2.6	mg
Vitamin D	trace	IU
Vitamin E	trace	IU
Vitamin B6	0.50	mg
Vitamin B12	trace	mg
Folic acid	0.30	mg
Biotin	0.07	mg
Pantothenic acid	2.1	mg
Riboflavin	0.31	mg

### **Minerals**

Sodium	15	mg
Potassium	2400	mg
Phosphorus	730	mg
Calcium	320	mg
Magnesium	300	mg
Iron	10	mg
Zinc	5	mg
Iodine	0.84	ppm

Coward, L., et al. (1998) **Chemical modification of isoflavones in soyfoods during cooking and processing.** *Am. J. Clin. Nutr.*, 68:1486S–1491S (Allegato A.5.1)

The principal chemical forms of isoflavones in soybean are their 6''-*O*-malonyl- $\beta$ -glucoside (6OMalGlc) conjugates. Analysis of soyfood products by reversed-phase HPLC–mass spectrometry revealed that defatted soy flour that had not been heat treated consisted mostly of 6OMalGlc conjugates; in contrast, toasted soy flour contained large amounts of 6''-*O*-acetyl- $\beta$ -glucoside conjugates, formed by heat-induced decarboxylation of the malonate group to acetate. Soy milk and tofu consisted almost entirely of  $\beta$ -glucoside conjugates; low-fat versions of these products were markedly depleted in isoflavones. Alcohol-washed soy-protein concentrates contained few isoflavones. Isolated soy protein and textured vegetable protein consisted of a mixture of all 3 types of isoflavone conjugates. Baking or frying of textured vegetable protein at 190 °C and baking of soy flour in cookies did not alter total isoflavone content, but there was a steady increase in  $\beta$ -glucoside conjugates at the expense of 6OMalGlc conjugates. Because the composition of the isoflavone glucoside conjugates will alter the rate of absorption and possibly the degree of further metabolism, these alterations in the chemistry of isoflavones need to be taken in account when interpreting data from clinical trials.

The proportion of each chemical type of isoflavone absorbed into the blood remains poorly understood, although it was proposed that the aglucones are absorbed readily from the upper small intestine, that the  $\beta$ -glucoside conjugates are absorbed from the distal small intestine after hydrolysis to the aglucone, and that the 6OMalGlc and 6OAcGlc conjugates are absorbed from the large intestine after hydrolysis (Barnes S, et al. 1996. *Soy isoflavonoids and cancer prevention: underlying biochemical and pharmacological issues*. In: Butrum R, ed. *Dietary phytochemicals and cancer prevention*. Plenum, 87-100).

Setchell KDR, et al. (2001) **Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements.** *J Nutr* 131: 1362S–1375S (Allegato A.5.2)

The systemic bioavailability of genistein is much greater than that of daidzein, and bioavailability of these isoflavones is greater when ingested as  $\beta$ -glycosides rather than aglycones as measured from the area under the curve of the plasma appearance and disappearance concentrations.

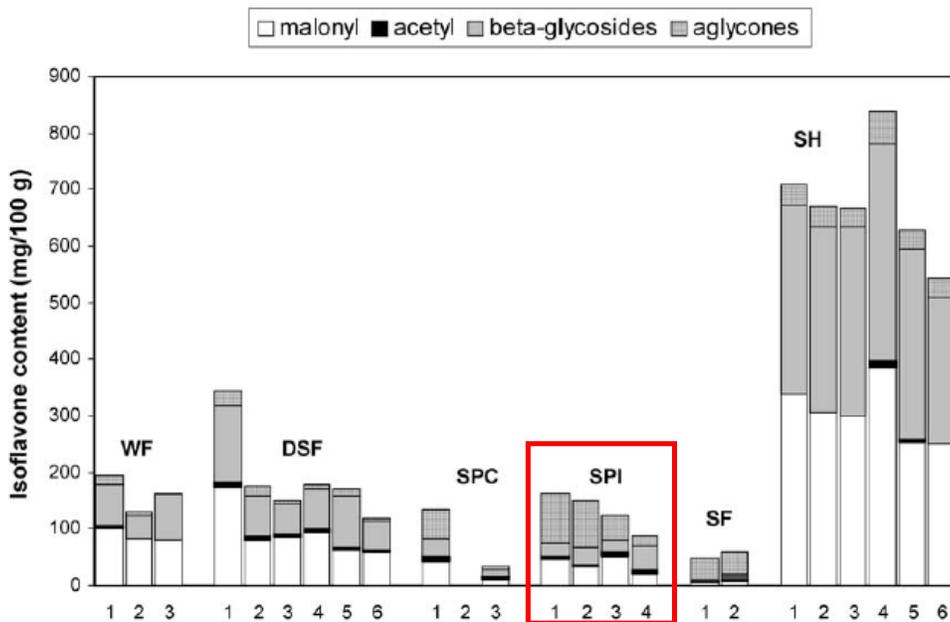
M. Messina (2002) **Brief Historical Overview of Isoflavone Research.** In: **Phytoestrogens and Health.** G. Sarwar Gilani and John J.B. Anderson, Editors, *AOCS Press*

Isoflavones are a subclass of a larger and more ubiquitous group of nutraceuticals called flavonoids. In comparison to most flavonoids, isoflavones have a very limited distribution in the plant kingdom. Flavonoids are found in many plant foods such as onions, apples, and grapes, whereas soybeans are the only food to contain nutritionally relevant amounts of isoflavones. The primary isoflavones in soybeans are genistein (4',5,7-trihydroxyisoflavone) and daidzein (4',7-dihydroxyisoflavone), and their respective ( $\beta$ -glycosides, genistin and daidzin. Typically, more genist(e)in exists in soybeans and soy foods than daidz(e)in. There are also small amounts of a third isoflavone in soybeans, glycitein (7,4'-dihydroxy-6-methoxy-isoflavone) and its glycoside, glycitin. In soybeans and nonfermented soy foods, isoflavones are present primarily as  $\beta$ -glucosides, esterified with malonic or acetic acid.

M. I. Genovese et al. (2007) **Commercial Soy Protein Ingredients as Isoflavone Sources for Functional Foods.** *Plant Foods for Human Nutrition*, 62:53–58 (Allegato A.5.3)

The results obtained here showed that the total isoflavone content varied significantly among products. For defatted and whole soy flours the total isoflavone content ranged from 120 to 340 mg/100 g, for soy protein isolates from 88 to 164 mg/100 g, and for commercial textured soy proteins, from 66 to 183 mg/100 g (wet basis, expressed as aglycones). The highest isoflavone content was found for soy hypocotyl flours, from 542 to 851 mg/100 g. Compared to hypocotyl and whole and defatted flours, soy ingredients presented a decrease of malonylglycosides and deesterified  $\beta$ -glycosides with a significant increase in the percentage of aglycones, mainly for soy fibers (65–76%). While defatting was shown to cause isoflavone concentration without altering conjugation, extrusion process caused destruction of isoflavones and a significant increase in the amount of acetylglycosides, but this effect was less intense for the concentrates. From the results obtained it can be concluded that differences in isoflavone concentration and profile may be related to oscillations in the isoflavone content present in the rawmaterial and to the type of processing.

Isoflavones represent the most common group of phytoestrogens and have been associated to beneficial effects in humans, such as prevention of cancer, cardiovascular diseases, osteoporosis and menopausal symptoms. These bioactive substances are found in particularly high levels in soybeans as glycosilated conjugates (malonylglycosides and underivatized  $\beta$ -glycosides), which, as a result of processing, form aglycones and acetylglycosides. The biological significance of the presence of these different forms in soybeans is not clear yet, but differences in their antioxidant activities and bioavailabilities were reported.

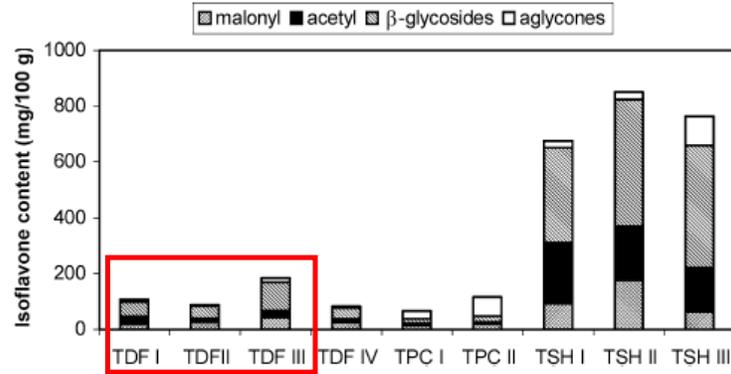


**Total isoflavone content** (mg/100 g FW) of commercial soy products.

WF, whole soy flours; DSF, defatted soy flours; SPC, soy protein concentrates;

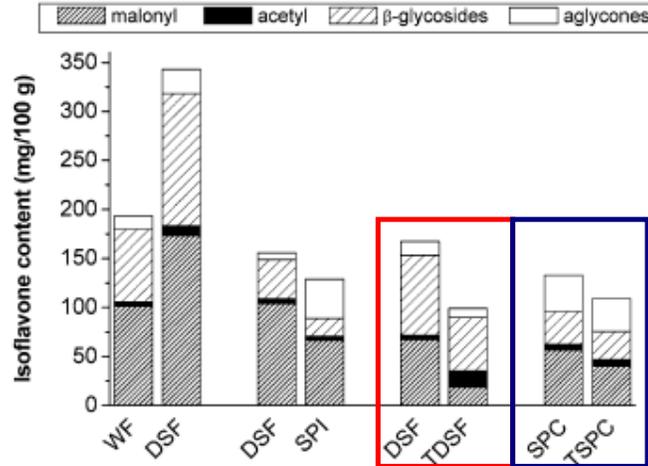
**SPI, soy protein isolates**; SF, soy dietary fibers; and SH, soy hypocotyl flours.

Numbers (1, 2, 3 etc.) indicate different samples.



**Total isoflavone content (mg/100 g FW) of textured soy products.**

**TDF, textured defatted soy flours;** TPC, textured soy protein concentrates; and TSH, textured soy hypocotyl flours. Numbers (I, II, III etc.) indicate different samples.



**Total isoflavone content (mg/100 g FW) of the starting materials**

and the respective derived products. WF, whole soy flour; **DSF, defatted soy flour;** SPC, soy protein concentrate; **TDSF, textured defatted soy flour;** **TSPC, textured soy protein concentrate;** SPI, soy protein isolate.

Poichè i β-glucosidi hanno maggiore biodisponibilità dei corrispondenti agliconi, è preferibile un testurizzato di farina di soia deoleata, anziché un testurizzato di proteine di soia concentrate (da proteine di soia isolate).

Sacks F.M., et al. (2006) **Soy protein, isoflavones, and Cardiovascular Health.** An American Heart Association Science Advisory for Professionals From the Nutrition Committee. *Circulation*, 113:1034-1044 (Allegato A.5.4)

Soy protein and isoflavones (phytoestrogens) have gained considerable attention for their potential role in improving risk factors for cardiovascular disease. This scientific advisory assesses the more recent work published on soy protein and its component isoflavones. In the majority of 22 randomized trials, isolated soy protein with isoflavones, as compared with milk or other proteins,

decreased LDL cholesterol concentrations; the average effect was ~ 3%. This reduction is very small relative to the large amount of soy protein tested in these studies, averaging 50 g, about half the usual total daily protein intake. No significant effects on HDL cholesterol, triglycerides, lipoprotein(a), or blood pressure were evident. Among 19 studies of soy isoflavones, the average effect on LDL cholesterol and other lipid risk factors was nil. Soy protein and isoflavones have not been shown to lessen vasomotor symptoms of menopause, and results are mixed with regard to soy's ability to slow postmenopausal bone loss. The efficacy and safety of soy isoflavones for preventing or treating cancer of the breast, endometrium, and prostate are not established; evidence from clinical trials is meager and cautionary with regard to a possible adverse effect. For this reason, use of isoflavone supplements in food or pills is not recommended. Thus, earlier research indicating that soy protein has clinically important favorable effects as compared with other proteins has not been confirmed. In contrast, many soy products should be beneficial to cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat.

R. S. MacDonald, et al. (2009) **Soy Protein Isolate Extruded with High Moisture Retains High Nutritional Quality.** *J. Agric. Food Chem.*, 57:3550–3555 (Allegato A.5.5)

This study compared the growth rate of male and female mice fed diets containing soy protein isolate without extrusion or with high-moisture extrusion. Overall, the extruded soy protein was equally nutritious as the unextruded soy protein for the animals. Hence, high-moisture extrusion may be considered a useful method to generate high-quality protein foods. A longer term feeding trial may be recommended to further define the nutritional adequacy of this protein.

M.A. Asgar, et al. (2010) **Nonmeat Protein Alternatives as Meat Extenders and Meat Analogs.** *Comprehensive Reviews in Food Science and Food Safety*, 9:513-529 (Allegato A.5.6)

The increasing importance of legume and oilseed proteins in the manufacturing of various functional food products is due to their high-protein contents. However, the greatest obstacle to utilizing these legumes and oilseeds is the presence of antinutrients; but these antinutrients can be successfully removed or inactivated by employing certain processing methods. In contrast, the potential negative impact of the antinutrients is partially balanced by the fact that they may have a health-promoting role.

Soybeans in food applications became popular after the U.S. Food and Drug Administration (FDA) approved the “Soy Protein Health Claim” on October 26, 1999. The FDA confirmed that 25 g of soy protein a day, may lower cholesterol and reduce the risk of coronary heart disease.

Soybean proteins are composed of 2 major components,  $\beta$ -conglycinin and glycinin.  $\beta$ -Conglycinin (Koshiyama and Fukushima 1976) has a sedimentation coefficient of 7S whereas glycinin (Mitsuda and others 1965) has a sedimentation coefficient of 11S.  $\beta$ -conglycinin is a trimeric protein composed of 3 subunits with a molecular mass ranging between 150 and 200 kDa (Thanh and Shibasaki 1977). In contrast, glycinin is a hexameric protein with a molecular mass ranging between 300 and 380 kDa (Staswick and others 1984; Sun and others 2008).

Saponins and isoflavones were previously considered as undesirable substances (Off-flavor), but now they are considered as useful substances because of their anticarcinogenic activities (Fukushima 2004).

Removal of undesirable components from soybeans to a satisfactory extent is possible through the creation of new soybean cultivars by genetic modification because these soybean components are controlled at a DNA level (Fukushima 2004). Alternatively, Katayama and Wilson (2008) reported that the disadvantage of the beany flavor that limited the incorporation of a large amount of textured

soy protein into meat-alternative products for human consumption had been overcome by the use of vegetable-based chicken flavor.

Soybean is a source of high-quality protein due to its relatively well-balanced composition of amino acids (Sun and others 2008). Soy protein products are used to complement cereal proteins (Singh and others 2008). The quality of the cereal protein is improved in soy/cereal mixtures because soy protein is a rich source of lysine, which is the first limiting essential amino acid in most cereal proteins (Dubois and Hoover 1981; Klein and others 1995).

#### Positive and negative physiological effect of antinutrients from plant origin.

Antinutrient	Positive effect	Negative effect	References
Protease inhibitors	Anticarcinogenic activities	Impairing intestinal protein digestion	Clemente and others (2004), Guillamon and others (2008)
$\alpha$ -Amylase inhibitors	Used as dietary supplements to control body weight in obesity therapy	Reduce the rate of digestion of starch	Mosca and others (2008), Karthic and others (2008)
Lectins (phytohemagglutinin)	Stimulation of lymphocytes (T cell)	Outbreaks of gastroenteritis, nausea, and diarrhoea	Gollob and others (1995), Harada (1999), Nasi and others (2009)
Polyphenols (especially tannins)	High antioxidant activity	Decreasing the activities of digestive enzymes and protein and amino acid availabilities	Oboh and others (2009), Troszynska and others (2006), Salunkhe and others (1982)
Phytic acid	Anticarcinogenic activities	Mineral depletion and micronutrient deficiency	Thavarajah and others (2009)

**Worldwide, the food market has become more complex, and the demand for more sophisticated meat alternatives has grown. Food manufacturers face the challenge of providing nutritious, economical, and healthy foods while at the same time ensuring that the product has an appealing taste and texture. An important reason for the increased acceptance of soy protein is their low cost. Soy protein will have an opportunity to get the attention it deserves as a highly nutritious and functional food ingredient. Consumers are changing their attitudes toward “soy as an ingredient or platform for health.” They should be viewed as vital components that will enable the food technologists to fabricate new foods.**

S. Singh, et al. (2007) **Nutritional aspects of food extrusion: a review.** *International Journal of Food Science and Technology*,42:916–929 (Allegato A.5.7)

Extrusion cooking, as a multi-step, multi-functional and thermal/mechanical process, has permitted a large number of food applications. Effects of extrusion cooking on nutritional quality are ambiguous. Beneficial effects include destruction of antinutritional factors, gelatinisation of starch, increased soluble dietary fibre and reduction of lipid oxidation. On the other hand, Maillard reactions between protein and sugars reduce the nutritional value of the protein, depending on the raw material types, their composition and process conditions. Heat-labile vitamins may be lost to varying extents. Changes in proteins and amino acid profile, carbohydrates, dietary fibre, vitamins, mineral content and some non-nutrient healthful components of food may be either beneficial or deleterious. The present paper reviews the mechanisms underlying these changes, as well as the influence of process variables and feed characteristics. Mild extrusion conditions (high moisture content, low residence time, low temperature) improve the nutritional quality, while high extrusion temperatures ( $\geq 200^{\circ}\text{C}$ ), low moisture contents ( $< 15\%$ ) and/or improper formulation (e.g. presence of high-reactive sugars) can impair nutritional quality adversely. To obtain a nutritionally balanced extruded product, careful control of process parameters is essential.

It was found that the retention of available lysine during processing of a cereal/soy-based mixture containing 20% sucrose In order to keep lysine losses within an acceptable range, it is necessary to avoid extrusion cooking above  $180^{\circ}\text{C}$  at water contents below 15%, and/or avoid the presence of higher amount of reducing sugars during the extrusion process.

The highest protein quality (as measured by protein efficiency ratio), corrected for a value for casein of 2.5 is 2.15 in extruded soy flour, obtained at a barrel temperature of 153°C, 20% moisture and 2 min residence time, coinciding with 89% reduction of trypsin inhibitors (Bjorck & Asp, 1983).

Extrusion has been shown to be very effective in reducing or eliminating lectin activity in legume flour (Alonso et al., 2000a,b). Thus, extrusion cooking is more effective in reducing or eliminating lectin activity as compared with traditional aqueous heat treatment.

Phenolic compounds, such as genistein and phytoestrogens in soy may help prevent breast cancer, yet extrusion texturisation of soy to produce more palatable soy foods might significantly reduce these compounds (Camire, 1998).

P. Singh, et al. (2008) **Functional and Edible Uses of Soy Protein Products.** *Comprehensive Reviews in Food Science and Food Safety*, 7:14-28 (Allegato A.5.8)

Soy protein supplies all 9 essential amino acids

Product	Protein digestibility corrected amino acid score (PDCAAS)
Casein	1.0
Egg white	1.0
Soy protein concentrate	0.99
Soy protein isolate	0.92
Beef	0.92
Pea flour	0.69
Kidney beans (canned)	0.68
Rolled oats	0.57
Lentils (canned)	0.52
Peanut meal	0.52
Rice	0.47
Whole wheat	0.42
Wheat gluten	0.25

The generic terms “texturized soy protein” or “TSP” typically mean defatted soy flours or concentrates, mechanically processed by extruders to obtain meat-like chewy textures when rehydrated and cooked. Coarse defatted soy particles (flakes and grits) and shaped soy protein isolates (sometimes called structuring proteins to differentiate them from extruded products) also are innovatively used to add texture and appeal to food products. Additional benefits of extrusion cooking are denaturing of the proteins, inactivation of trypsin inhibitors, control of bitter flavors, and the homogeneous bonding of ingredients that may include colors, chemicals, and other additives, which all can have an effect on appearance or textural quality (Bjorck and Asp 1983; Hayakawa and others 1988).

The FDA has agreed that food products which contain a minimum of 6.25 g of soy protein per serving and which are low in fat, saturated fat, and cholesterol may carry one of 2 label claims. The 1<sup>st</sup> option is 25g of soy protein per day as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease. The other permitted claim that manufacturers may use is diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease.

Genistein is thought to act against cancer in several ways: by interfering with cancer promoting enzymes, by blocking the activity of hormones in the body, and even by interfering with the process by which tumors receive nutrients and oxygen (Coward and others 1993; Sarkar and Li 2003). Genistein is found in soy, clover, and only a few other green plants; however, soy-based foods represent the only practical way consumers can incorporate genistein in their diet. As long as isoflavones are present, any type of soyfood - soymilk, tofu, tempeh, textured vegetable protein, or whole soybeans, may offer cancer prevention benefits (Broihier1997; Duncanand others1999).

Isoflavone-rich soy protein isolate eattenuates boneloss in the lumbar spine of perimenopausal women (Alekeland others2000).

M. Amigo-Benavent, et al. (2008) **Protein Quality, Antigenicity, and Antioxidant Activity of Soy-Based Foodstuffs.** *J. Agric. Food Chem.*, 56:6498–6505 (Allegato A.5.9)

Commercial soy-based foodstuffs, including beverages, cow's milk supplemented with soy isoflavones, snacks, and biscuits, were analyzed to find any link between alterations in protein quality, safety (antigenicity), functionality (antioxidant activity), and food processing. Lysine availability was higher in liquid soy beverages compared to that in other soy foodstuffs studied here. 7S and 11S soy allergens were detected by RP-HPLC and SDS-PAGE, respectively. Both data indicated changes in soy protein patterns due to processing of instant powdered soymilk, soy snacks, and biscuits. Immunoblotting assay showed modifications in the antigenic response of these foodstuffs based on soy, suggesting that their processing had altered the structure of soy allergens. Protein quality, antigenicity, and antioxidant activity of soy products can be affected as a function of the intensity of the thermal processing.

### Allergenicità

Ohishi. A., et al. (1994) **Detection of soybean antigenicity and reduction by twin-screw extrusion.** *Am. Oil Chenu*, 71:1391-1396

Antiserum toward soybean meal was prepared by feeding a soybean meal diet containing large amounts of antigens to calves. The antiserum was used to develop a sensitive detection method for soybean meal antigens by competitive inhibition enzyme-linked immunosorbent assay. With this method, the effectiveness of twin-screw extrusion cooking in reducing antigenicity in soybean meal was examined. Antigenicity was reduced to 0.1% of the original activity by extrusion cooking with screws containing kneading-disc elements and die-end temperatures exceeding 66°C. Electrophoretic analysis of the cooked meal indicated that the reduction in antigenicity was due to degradation of protein structures, particularly those with molecular weights exceeding 40 kDa.

**TABLE 1**

**Characteristics of Soybean Products**

Products	Moisture (%)	Fat (%)	CP (%) <sup>a</sup>	NSI <sup>b</sup>
Raw soybean (S)	12.2	19.5	35.8	90.3
Defatted soybean meal-1 (DSM-1)	12.8	1.0	45.7	22.9
Defatted soybean meal-2 (DSM-2) <sup>c</sup>	7.8	1.2	52.1	19.4
Parched soybean flour (PS)	6.7	19.2	38.3	12.9
Single-screw extruded soybean (SES)	9.1	20.7	35.8	15.6
Soybean protein concentrate (SPC)	7.1	—	68.4	4.2

<sup>a</sup>CP = crude protein.

<sup>b</sup>NSI = nitrogen solubility index.

<sup>c</sup>Dehulled and powdered soybean meal.

P. Frank, et al. (2002) **The allergenicity of soybean-based products is modified by food technologies.** *Inf. Arch. Allergy Immunol.*, 128:212-219 (Allegato A.5.10)

reported that texturized soy proteins, produced by a process that includes heating, mechanical pressure, and acid treatment (pH 4.5) led to the disappearance of the major allergen Gly m Bd 30K, whereas a 38-kDa allergenic band corresponding to the glycinin fragment remained intact. However, they suggested that further studies of texturized soy protein could lead to the development of modified technologies using a complementary enzymatic treatment in order to obtain more hypoallergenic products.

The texturized extract contained high proportions of 31- to 34- and 38-kD proteins. Sera recognizing the 38- and 50-kD proteins in texturized soy protein also recognized the 37- and 49-kD proteins in soybean flour and in soy milk, suggesting a protein glycation by texturization processes. The 30- to 34-kD band in texturized proteins was devoid of any allergenicity. This study seems to indicate that the 30-kD allergen (Gly m Bd 30) disappears during the production of texturized soy protein. Texturized protein could lack the major allergen Gly m Bd 30.

Sanshiroh Saitoh, et al. (2000) **Antigenicity in Soybean Hypocotyls and Its Reduction by Twin-Screw Extrusion.** *JAOCS*, Vol. 77(4):419-424 (Allegato A.5.11)

The purpose of the present study was to develop a simple method to make a low-antigenicity food and/or feed rich in isoflavones from soybean hypocotyls. The antigenicity of soybean hypocotyls for bovine antisoybean sera was assessed by enzyme-linked immunosorbent assay. Immunoblotting demonstrated that the antigenicity was derived from storage proteins, which were present in hypocotyls as glycinin and  $\beta$ -conglycinin, and from unknown proteins. Ground soybean hypocotyls (32-mesh sieve size) were passed through a twin-screw extruder to reduce the antigenicity to 1% of the original activity. The degradation of antigen proteins in soybean products was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Trypsin inhibitor and urease activity were also greatly reduced. The concentrations of isoflavones were unaffected.