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REVISED CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF SOYBEAN [Glycine max (L.) Merr.]: KEY FOOD AND FEED NUTRIENTS, ANTI-NUTRIENTS, TOXICANTS AND ALLERGENS

Series on the Safety of Novel Foods and Feeds No. 25

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No. 25

Revised Consensus Document on Compositional Considerations for New Varieties of SOYBEAN [*Glycine max* (L.) Merr]: Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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Also published in the Series on the Safety of Novel Foods and Feeds:

[No. 1, Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola) (2001) – ***REPLACED** with revised Consensus Doc. No. 24 (2011)*

No. 2, Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients (2001) – ***REPLACED** with revised Consensus Doc. No. 25 (2012)*]

No. 3, Consensus Document on Compositional Considerations for New Varieties of Sugar Beet: Key Food and Feed Nutrients and Anti-nutrients (2002)

No. 4, Consensus Document on Compositional Considerations for New Varieties of Potatoes: Key Food and Feed Nutrients, Anti-nutrients and Toxicants (2002)

No. 5, Report of the OECD Workshop on the Nutritional Assessment of Novel Foods and Feeds, Ottawa, Canada, February 2001 (2002)

No. 6, Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites (2002)

No. 7, Consensus Document on Compositional Considerations for New Varieties of Bread Wheat (*Triticum aestivum*): Key Food and Feed Nutrients, Anti-nutrients and Toxicants (2003)

No. 8, Report on the Questionnaire on Biomarkers, Research on the Safety of Novel Foods and Feasibility of Post-Market Monitoring (2003)

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No. 10, Consensus Document on Compositional Considerations for New Varieties of Rice (*Oryza sativa*): Key Food and Feed Nutrients and Anti-nutrients (2004)

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No. 18, Consensus Document on Compositional Considerations for New Varieties of Cassava (*Manihot esculenta* Crantz): Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens (2009)

No. 19, Consensus Document on Compositional Considerations for New Varieties of Grain Sorghum [*Sorghum bicolor* (L.) Moench]: Key Food and Feed Nutrients and Anti-nutrients (2010)

No. 20, Consensus Document on Compositional Considerations for New Varieties of Sweet Potato [*Ipomoea batatas* (L.) Lam.]: Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens (2010)

No. 21, Consensus Document on Compositional Considerations for New Varieties of Papaya (*Carica papaya* L.): Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens (2010)

No. 22, Consensus Document on Molecular Characterisation of Plants Derived from Modern Biotechnology (2010)

No. 23, Consensus Document on Compositional Considerations for New Varieties of Sugarcane (*Saccharum* spp. hybrids.): Key Food and Feed Nutrients, Anti-nutrients and Toxicants (2011)

No. 24, Revised Consensus Document on Compositional Considerations for New Varieties of Low Erucic Acid Rapeseed (Canola): Key Food and Feed Nutrients, Anti-nutrients and Toxicants (2011)

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FOREWORD

The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based ***consensus documents***, which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents are being published on the nutrients, anti-nutrients or toxicants, information of its use as a food/feed and other relevant information.

This document updates and revises the original *Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-Nutrients* issued in 2001. The revised Consensus Document addresses compositional considerations for new varieties of soybean (*Glycine max*) by identifying the key food and feed nutrients, anti-nutrients, toxicants and allergens. A general description of these components is provided. In addition, there is background material on the production, processing and uses of soybean, and considerations to be taken into account when assessing new varieties of these crops. The text also suggests the constituents to be analysed related to food use and to feed use.

The United States served as the lead country in the preparation for the document, and the draft has been revised on a number of occasions based on the input from other member countries and stakeholders.

The Task Force endorsed this document, which is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

TABLE OF CONTENTS

Tables

Figures

PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD member countries and elsewhere. The need has been identified for detailed technical work aimed at establishing appropriate approaches to the safety assessment of these products.

At a Workshop held in Aussois, France (OECD, 1997), it was recognised that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (e.g., key nutrients, key toxicants and anti-nutritional compounds) on a crop-by-crop basis, which should be considered in the comparison. It is recognised that the components may differ from crop to crop. The Task Force therefore decided to develop Consensus Documents on phenotypic characteristics and compositional data. These data are used to identify similarities and differences following a comparative approach as part of a food and feed safety assessment. They should be useful to the development of guidelines, both national and international and to encourage information sharing among OECD member countries.

These documents are a compilation of currently available information that is important in food and feed safety assessment. They provide a technical tool for regulatory officials as a general guide and reference source, and also for industry and other interested parties and will complement those of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, OECD member countries. They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach. In assessing an individual product, additional components may be required depending on the specific case in question.

In order to ensure that scientific and technical developments are taken into account, member countries have agreed that these Consensus Documents will be reviewed periodically and updated as necessary. Users of these documents are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered. Comments and suggestions can be sent to:

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THE ROLE OF COMPARATIVE APPROACH AS PART OF A SAFETY ASSESSMENT

In 1990, a joint consultation of the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO, 1991).

In 1993 the Organisation for Economic Co-operation and Development (OECD) further elaborated this concept and advocated the approach to safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology (as well as other methods of modifying a host genome including tissue culture methods and chemical or radiation induced mutation). In 2000 the Task Force concluded in its report to the G8 that the concept of substantial equivalence will need to be kept under review (OECD, 2000).

The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 concluded that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterise hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework.

A previous Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety (1996) elaborated on compositional comparison as an important element in the determination of substantial equivalence. A comparison of critical components can be carried out at the level of the food source (*i.e.* species) or the specific food product. Critical components are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question. The comparison of key nutrients should be between the modified variety and non-modified comparators with an appropriate history of safe use. Any difference identified would then be assessed against the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO, 1996). The comparator used to detect unintended effects should ideally be the near isogenic parental line grown under identical conditions. While the comparative approach is useful as part of the safety assessment of foods derived from plants developed using recombinant DNA technology, the approach could, in general, be applied to foods derived from new plant varieties that have been bred by other techniques.

SECTION I - BACKGROUND

A. Production

1. The soybean[1] [*Glycine max* (L.) Merr.] is grown world-wide as an important staple and commercial crop. The soybean accounted for 56% of the main world oilseed crops production in 2011, being also the dominant species traded in international markets among all major oilseeds (ASA, 2012). The five major soybean producers in 2011: United States, Brazil, Argentina, China and India, accounted for 90 % of the total production (Table 1).

Table 1. Production and export of soybeans in 2011 (million metric tonnes)

Country/Region	Production	Exports
United States	83.2	34.7
Brazil	72.0	37.8
Argentina	48.0	8.9
China	13.5	-
India	11.0	-
Canada	4.2	2.9
Paraguay	6.4	5.0
Others	13.1	3.5
Total	**251.5**	**92.8**

Source: from ASA, 2012

B. Uses

2. The major soybean commodity products are seeds, oil, and meal. A bushel (27.2 kg) of soybeans yields about 21.8 kg of protein-rich meal and 5.0 kg of oil (ASA, 2012). Unprocessed soybeans are not suitable for food and their use for animal feed remains limited because they contain anti-nutritional factors such as trypsin inhibitors and lectins. Adequate heat processing inactivates these factors. Whole soybeans are utilized to produce soy sprouts, baked soybeans, roasted soybeans, full fat soy flour and the traditional soy foods (miso, soy milk, soy sauce, and tofu). In addition to whole oil used for human consumption, refined soybean oil has many other technical and industrial applications. Glycerol, fatty acids, sterols and lecithin are all derived from soybean oil. Soy protein isolate is used as a source of amino acids in the production of infant food formula and other food products. Soybean meal is rich in essential amino acids, particularly lysine and tryptophan, which are required supplements in animal diets for optimum growth and health. Soybean meal is used in diets for poultry, swine, dairy cattle, beef cattle and pets. Being rich in hydrocarbon, soybean oil is used for biodiesel fuel production (soy methyl esters). Approximately 4.8 kg of soybeans are required to produce 1 litre of biodiesel (ASA, 2012).

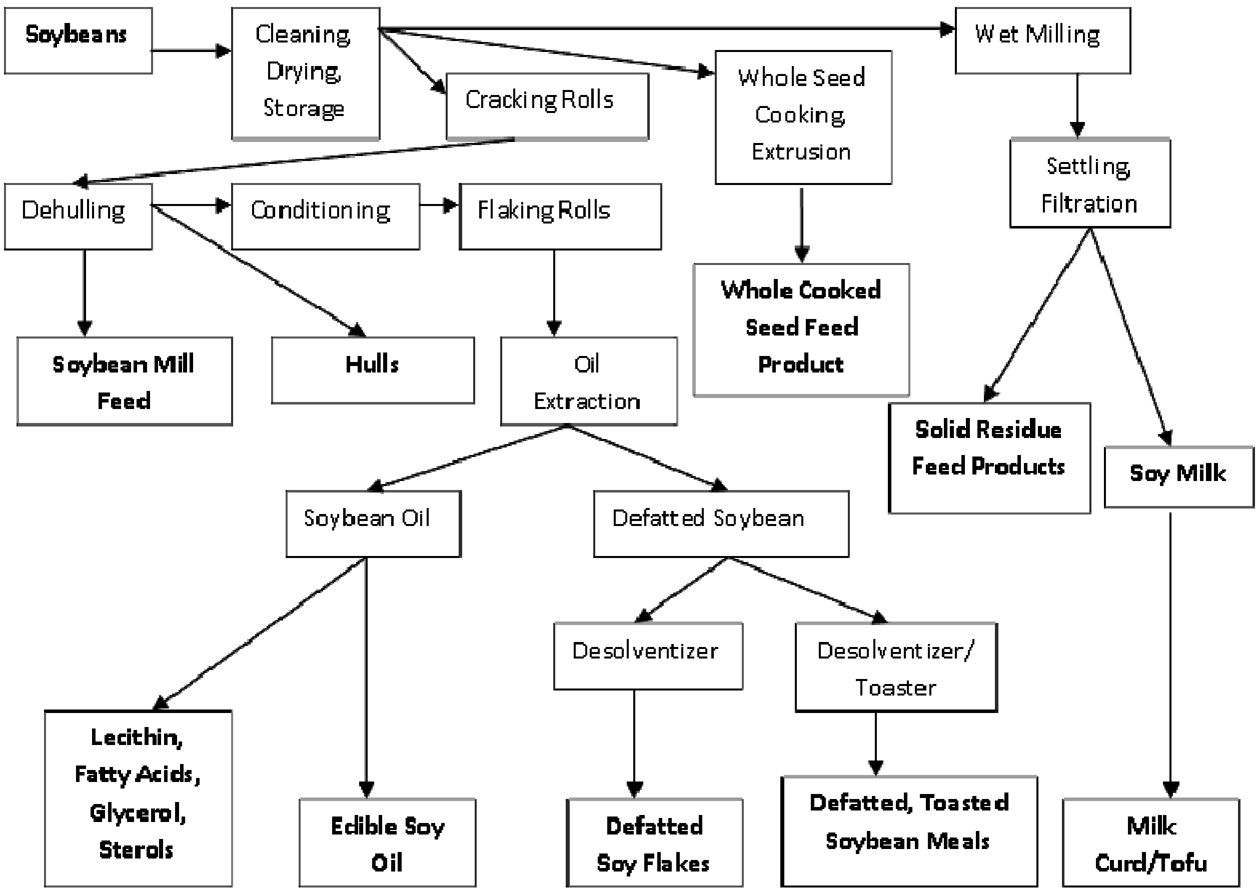
1 For information on the environmental considerations for the safety assessment of soybean, see the OECD Consensus Document on the Biology of *Glycine max* (L.) Merr. (Soybean), Series on Harmonisation of Regulatory Oversight in Biotechnology No. 15 (OECD, 2000).

C. Processing

3. Historically, the oil extraction process was conducted on a small scale using mechanical or hydraulic presses after the soybeans were rolled into flakes and properly conditioned by heat treatment. Gradually, the screw press (expeller) has replaced the hydraulic press; however, the hydraulic press is still efficiently used on small scale individual farms for organic production and in developing countries. Large scale solvent extraction facilities produce the bulk of soybean oil (Johnson, 2008). The solvent hexane is used to extract the oil from flaked soybeans (Lusas, 2000).

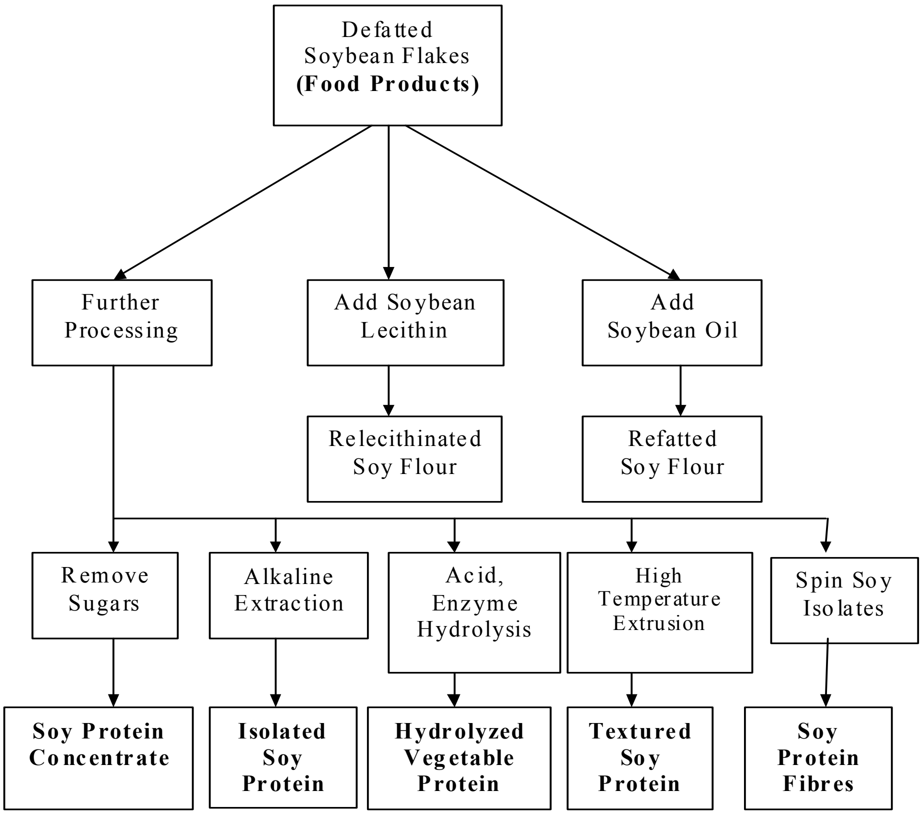
4. The processing steps used to produce the various soy products are schematized in Figure 1 and Figure 2.

Figure 1. Whole soybean processing



Source: adapted from Waggle and Kolar, 1979

Figure 2. Defatted soybean flakes processing



Source: adapted from Sipos, 1988

D. Appropriate comparators for testing new varieties

5. This document suggests parameters that soybean breeders should measure when developing new modified varieties. The data obtained in the analysis of a new soybean variety should ideally be compared to those obtained from an appropriate near isogenic non-modified variety, grown and harvested under the same conditions.[2] The comparison can also be made between values obtained from new varieties and data available in the literature, or chemical analytical data generated from other commercial soybean varieties.

6. Components to be analysed include key nutrients, anti-nutrients, toxicants and allergens. Key nutrients are those which have a substantial impact in the overall diet of humans (food) and animals (feed). These may be major constituents (fats, proteins, and structural and non-structural carbohydrates) or minor compounds (vitamins and minerals). Similarly, the levels of known anti-nutrients and allergens should be considered. Key toxicants are those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health.

[2] For additional discussion of appropriate comparators, see the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants CAC/GL 45/2003 of the Codex Alimentarius Commission (paragraphs 44 and 45).

Standardized analytical methods and appropriate types of material should be used, adequately adapted to the use of each product and by-product. The key components analysed are used as indicators of whether unintended effects of the genetic modification influencing plant metabolism have occurred or not.

E. Breeding characteristics screened by developers

7. Phenotypic characteristics provide important information related to the suitability of new varieties for commercial distribution. Plant breeders developing new varieties of soybeans consider many parameters at different stages in the developmental process. In the early stages, breeders evaluate flower colour, plant standability, stand count, relative maturity, plant habit, pubescence colour, hila colour, pod wall colour, plant morphology, time of flowering, emergence, tolerance to low temperatures, and general disease resistance. The latter disease screening depends on the maturity and area in which the seeds are being grown. Tolerance to low temperatures during flowering and early pod setting would be more important in developing genotypes for cooler soybean growing regions.

8. Later on, as a new variety gets closer to commercialisation, breeders measure yield, first at one site, then in larger plots and at increasing numbers of sites. Some of the factors considered in the evaluation process include maturity, height, lodging, flower colour, pubescence colour, pod wall colour, canopy width, leaf colour, hypocotyl elongation, emergence score, shattering score, seed size, seed quality, percent oil, and percent protein. Plants are also screened for resistance to various diseases. In some cases, plants are modified for specific increases/decreases in certain components, and the plant breeder would be expected to analyse for such components. For plants modified for changes in specific compositional components, it is noted that careful consideration may be needed to determine an appropriate comparator. [3]

[3] For additional discussion of appropriate comparators for plants that have been modified for changes in specific compositional components, see the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants CAC/GL 45/2003 of the Codex Alimentarius Commission (paragraph 51).

SECTION II - NUTRIENTS

9. Since the first issue of this publication in 2001, several new sources of valuable information have become available. Data from the International Life Sciences Institute (ILSI, 2010), the National Agricultural and Food Research Organization (NARO, 2001) and its National Food Research Institute (NFRI-NARO, 2011) in Japan, the *Food Composition and Nutrition Tables* (Souci *et al*., 2008), the *Danish Food Composition Databank* (revision 7.0, Saxholt *et. al*, 2008)*,* The Swedish National Food Administration's database (2011), Food Standards Australia New Zealand's *Nutrient Tables for Use in Australia (NUTTAB, 2010),* and the *USDA Nutrient Database for Standard Reference* (United States Department of Agriculture, Agricultural Research Service, USDA-ARS, 2008) have been incorporated into this revised version of the original publication.[4]

A. Seeds

10. Tables 2 to 6 provide data regarding the composition of soybean seed including proximates and fibre analysis, amino acids, fatty acids, minerals, and vitamins. It should be noted that soybean varieties that contain high levels of oleic acid have been developed, but corresponding data are not included in this document. Some data sources only report fatty acid content based on the percent of the total fatty acids; data from these sources is reported under the 'Oil' section of this document.

[4] On occasion, data from the original source may have been rounded to promote consistency in data presentation in the summary tables, and/or when specified, units were converted from a fresh weight to a dry weight basis.

Table 2. Proximates and fibre analysis of soybean seed

Reference	ILSI 2010		NRC Beef, 2000[1]	NRC Dairy, 2001[2]	Ensminger *et al.*, 1990	Souci *et al.*, 2008[3]		USDA-ARS, 2008[4]	NFRI-NARO, 2011[5]	
	Mean	*Range*	*Mean*	*Mean*	*Mean*	*Mean*	*Range*	*Mean*	*Mean*	*Range*
(g/100 g fresh weight)										
Moisture	10.1	4.7-34.4	13.6	10.0	8.0	8.4		8.5	11.1	9.2-13.7
(g/100 g dry matter)										
Crude Protein	39.5	33.2-45.5	40.3	39.2	41.7	41.7		39.9	42.1	35.8-46.2
Crude Fat	16.7	8.1-23.6	18.2	19.2	18.7	20.0	17.9-23.3	21.8	24.2	21.0-27.4
Ash	5.3	3.9-7.0	4.56	5.9	5.6	6.1		5.3	5.6	5.0-6.5
ADF	12.0	7.8-18.6	11.1	13.1	11.0					
NDF	12.3	8.5-21.3	14.9	19.5						
TDF						24.0		10.2	18.7	15.9-22.9
Crude Fibre	7.8				5.8					
Carbohydrates (by calculation)	38.2	29.6-50.2			26.0[5]			32.98	31.7	27.8-35.9[6]
Sugar (CHO-TDF)									13.1	9.0-16.4

1 NRC (2000) *Nutrient Requirements of Beef Cattle*
2 NRC (2001) *Nutrient Requirements of Dairy Cattle*
3 Data converted from fresh weight to dry weight basis using given moisture level
4 Data converted from fresh weight to dry weight basis using given moisture level. Data may include results from genetically engineered soybeans
5 Data converted from fresh weight to dry weight basis using given moisture content
6 Carbohydrate (by calculation) = 100%-(crude protein% + crude fat% + ash% + moisture%)

Table 3. Amino acid composition of soybean seed (g/100 g DM)

Reference	ILSI, 2010		NRC Dairy, 2001[1]	Ensminger *et al.*, 1990	USDA-ARS, 2008[2]	Souci *et al.*, 2008[3]		NFRI-NARO, 2011[3]	
	Mean	*Range*	*Mean*	*Mean*	*Mean*	*Mean*	*Range*	*Mean*	*Range*
Arginine	2.84	2.28-3.4	2.95	2.86	3.45	2.58	2.19-2.91	3.04	2.44-3.62
Cystine/Cysteine	0.59	0.37-0.81	0.57	0.45	0.72	0.64	0.57-0.72	0.66	0.52-0.73
Histidine	1.04	0.87-1.17	1.08	1.00	1.20	0.91	0.85-0.96	1.11	0.97-1.26
Isoleucine	1.81	1.53-2.07	1.73	1.76	2.16	1.94	1.72-2.16	1.85	1.59-2.06
Leucine	3.04	2.59-3.62	2.90	2.95	3.62	3.10		3.17	2.69-3.49
Lysine	2.56	2.28-2.83	2.34	2.52	2.96	2.07	1.56-2.54	2.61	2.55-2.87
Methionine	0.55	0.43-0.68	0.58	0.52	0.60	0.63	0.53-0.74	0.57	0.49-0.62
Phenylalanine	1.98	1.63-2.34	1.96	1.91	2.32	2.15	2.00-2.35	2.11	1.72-2.45
Threonine	1.47	1.14-1.86	1.55	1.58	1.93	1.63	1.47-1.81	1.66	1.42-1.79
Tryptophan	0.43	0.36-0.50	0.51	0.61	0.65	0.49	0.44-0.56	0.55	0.49-0.63
Valine	1.91	1.59-2.20	1.84	1.75	2.22	1.92	1.55-2.12	1.94	1.70-2.19
Glycine	1.69	1.46-1.99		1.55	2.06	1.55		1.77	1.52-1.94
Tyrosine	1.32	1.01-1.61		1.40	1.68	1.36	1.29-1.45	1.40	1.24-1.56
Serine	2.02	1.1-2.48		2.16	2.58	1.84		2.14	1.77-2.46
Proline	2.00	1.68-2.28			2.60	1.99		2.12	1.78-2.41
Alanine	1.72	1.51-2.10			2.09	1.67		1.78	1.59-1.95
Aspartic acid	4.49	3.81-5.12			5.59	4.36		4.79	3.95-5.36
Glutamic acid	7.09	5.84-8.20			8.61	7.09		7.73	6.21-8.60

1 NRC, (2001) *Nutrient Requirements of Dairy Cattle*
2 Data converted from fresh weight to dry weight basis using given moisture level. Data may include results from genetically engineered soybeans.
3 Data converted from fresh weight to dry weight basis using given moisture level

Table 4. Fatty acid composition of soybean seed (g/100 g DM)

Reference		USDA-ARS, 2008 [1]	ILSI, 2010 [2]		Souci *et al.*, 2008 [2]		NFRI-NARO, 2011 [2]	
		Mean	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*
Palmitic	C16:0	2.31	1.87	0.67-2.78	1.89	0.44-2.01	2.59	2.24-2.89
Stearic	C18:0	0.78	0.68	0.28-1.13	0.63	0.46-1.30	0.76	0.42-1.18
Oleic	C18:1	4.75	3.46	1.36-6.56	4.35	4.08-5.81	5.46	3.93-8.95
Linoleic	C18:2	10.85	8.91	3.46-13.36	10.71	9.40-11.58	12.15	10.34-13.60
Linolenic	C18:3	1.45	1.40	0.30-2.19	1.02	0.9-1.09	1.87	1.26-2.73
Arachidic	C20:0		0.06	0.02-0.11	-	0.09-0.46	0.07	0.05-0.09

1 Data converted from fresh weight to dry weight basis using given moisture level. Data may include results from genetically engineered soybeans.
2 Data converted from fresh weight to dry weight basis using given moisture level

Table 5. Mineral composition of soybean seed

Reference	USDA-ARS, 2008[1]	NRC Beef, 2000[2]	NRC Dairy, 2001[3]	Ensminger *et al.*, 1990	ILSI, 2010[4]		Souci *et al.*, 2008[4]	
	Mean	*Mean*	*Mean*	*Mean*	*Mean*	*Range*	*Mean*	*Range*
(g/100 g DM)								
Calcium	0.30	0.27	0.32	0.27	0.22	0.12 - 0.31	0.22	0.22 - 0.24
Phosphorus	0.77	0.65	0.60	0.65	0.71	0.50 - 0.94	0.60	0.52 - 0.70
Magnesium	0.31	0.27	0.25	0.29	0.26	0.22 - 0.31	0.24	0.23 - 0.31
Potassium	1.96	2.01	1.99	1.80	2.06	1.87 - 2.32	1.97	1.97 - 1.99
(mg/100 g DM)								
Iron	17.00	20.00	10.00	10.00	8.00	6.00 - 11.00	10.00	
Sodium	2.00	40.00	10.00	0.00			10.00	
Selenium	0.020		0.028	0.012			0.02	0.01 - 0.08
Manganese	2.75	34.5[5]	2.9	3.96			2.95	0 - 5.90
Copper	1.81	1.46	1.3	1.98			1.31	0.11 - 1.53
Zinc	5.35	5.9	4.9	6.18			4.59	1.09 - 6.77

1 Data converted from fresh weight to dry weight basis using given moisture level. Data may include results from genetically engineered soybeans.
2 NRC, (2000) *Nutrient Requirements of Beef Cattle*
3 NRC, (2001) *Nutrient Requirements of Dairy Cattle*
4 Data converted from fresh weight to dry weight basis using given moisture level
5 NRC (2000) *Nutrient Requirements of Beef Cattle* indicates that manganese is present in soybean seeds at 345 mg/kg soybean seed. In contrast, NRC (1984) *Nutrient Requirements of Beef Cattle* indicates that manganese is present in soybean seed at 39 mg/kg soybean seed (i.e., 3.9 mg Mn/100 g DM).

Table 6. Vitamin composition of soybean seed

Reference	Units/ 100 g DM	ILSI 2010[1]		Ensminger *et al.*, 1990	USDA-ARS 2008[2]	Souci *et al.*, 2008[3]		NFRI-NARO 2011[4]	
		Mean	*Range*	*Mean*	*Mean*	*Mean*	*Range*	*Mean*	*Range*
Folic acid	mg	0.36	0.24-0.47			0.27			
Vitamin A	IU			160	24.05				
β Carotene	mg				0.014	0.42	0.37-0.44		
Vitamin B_1	mg	0.20	0.10-0.25	0.12	0.96	1.12	0.95-1.30	0.99	0.79-1.31
Vitamin B_2	mg	0.27	0.19-0.32	0.32	0.95	0.50	0.25-1.42	0.33	0.27-0.51
Vitamin E (α-tocopherol except when noted)	mg	1.91	0.19-6.17	0.37	0.93	16.38[3]		3.97	1.25-10.75
Vitamin K	mg				0.051	0.04	0.03-0.05	0.017	0.00-0.046
Niacin	mg			2.4	1.78	2.95	2.62-3.28	2.26	0.79-3.23
Vitamin B_6	mg			0.12	0.41	1.09	0.66-1.31	0.64	0.37-1.18

[1] www.cropcomposition.org, (2010)
[2] Data converted from fresh weight to dry weight basis using given moisture level. Data may include results from genetically engineered soybeans.
[3] Data converted from fresh weight to dry weight basis using given moisture level. Vitamin E is reported as total tocopherol.
[4] Data converted from fresh weight to dry weight basis using given moisture level

B. Oil

11. Triglycerides are the main constituents (99%) of soybean oil. Soybean oil is noted for its relatively high content of unsaturated fatty acids, oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. Soybean oil contains relatively lesser amounts of the saturated fatty acids, palmitic (C16:0) and stearic (C18:0) acids (Wang *et al*., 1997). Arachidic and Behenic acid are also present, but at only low levels. The range of fatty acid composition in soybean oil is shown in Table 7.

12. In the human diet soybean oil is considered a source of vitamins K and E, but not provitamin A. The vitamin composition of soybean oil has been described in several publications. Data suggest that there may be partial loss of vitamin K in vegetable oils due to refining processes (Gao and Ackman, 1995). The Food and Agriculture Organization and the World Health Organization of the United Nations (FAO and WHO, 2002) have noted that certain vegetable oils, including soybean oil, represent biologically available sources of vitamin K. Soybean oil is a source of vitamin E even though some of the vitamin E may be lost during the processing of soybean oil (Frankel, 1996). The vitamin K_1 (phylloquinone) and vitamin E (reported as α-tocopherol) content of soybean oil is shown in Tables 8 and 9, respectively.

Table 7. Fatty acid composition of soybean oil (% of total fatty acids)

Reference		USDA-ARS, 2008[1]	Codex Aliment. Commis., 2009[2]	Souci *et al.*, 2008		MECSST, 2005[3]	Danish Food Composit. Databank, (2008)[4]	Swedish National Food Adm., (2011)	NUTTAB (2010)[5]	Padgette *et. al.*, 1996	
		Mean	*Range*	*Mean*	*Range*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Range*
Palmitic	C16:0	10.87	8.0-13.5	11.44	7.11-15.39	10.67	9.79	10.56	10.7	12.18	11.57-12.71
Stearic	C18:0	4.61	2.0-5.4	3.98	0.53-9.45	4.31	3.65	4.28	3.9	4.45	4.19-4.95
Oleic	C18:1	23.45	17.0-30.0	20.79	15.18-30.46	23.72	22.30	23.01	19.1	21.46	16.35-33.95
Linoleic	C18:2	52.98	48.0-59.0	58.42	38.74-61.35	53.90	55.70	53.45	57.7	57.16	47.92-59.82
Linolenic	C18:3	7.06	4.5-11.0	8.47	2.02-15.60	6.58	7.19	7.74	7.5	8.73	5.53-11.17
Arachidic	C20:0	0.38	0.1-0.6	0.55	0.11-0.96	0.38	0.625	0.31	0.4	0.39	0.34-0.47

1 Data converted from g/100 g to percent of total fatty acid. Total fatty acid calculated as the sum of total saturated fatty acids, total monounsaturated fatty acids, and total polyunsaturated fatty acids. Data may include results from genetically engineered soybeans.

2 Non-detects (ND) are ≤ 0.05 % total fatty acids.

3 Ministry of Education, Culture, Sports, Science and Technology (MECSST), (2005) *Standard Tables of Food Composition in Japan*
Data converted from mg/100g to percent of total fatty acids using given total fatty acid content.

4 Saxholt *et al*., (2008)

5 Food Standards Australia New Zealand, (2010)

Table 8. Vitamin K_1 levels (µg per 100 g of oil) **in commercially available soybean oil as measured by various types of HPLC-based analytical methodologies**

Reference	Sample Type	Vitamin K_1 (phylloquinone)	
		Mean	*Range*
Ferland and Sadowski (1992)	Commercially available oil	193	139-290
Gao and Ackman (1995)	Retail expeller oil	250	
Shearer *et al.* (1996)		173	
Piironen *et al.* (1997)	Refined oil	145	
Cook *et al.* (1999)	Commercially available oil	114.2* 102.5**	
Bolton-Smith *et al*. (2000)	Aged soybean oil	112	
	Fresh soybean oil	150	
Kamao *et al.* (2007)	Retail oil	234	
USDA-ARS (2008)	Salad or cooking oil	183.9	
Booth and Suttie (1998)		193	

* Sample prepared by enzymatic digestion and extraction
** Sample prepared by direct extraction

Table 9. Vitamin E (α-Tocopherol) levels (mg/100 g oil) **in soybean oil as measured by different analytical methodologies**

Reference[1]	Sample Type	Analytical Method	α-Tocopherol	
			Mean	*Range*
Shahidi (2002)		HPLC		10.1–10.2
Yuki and Ishikawa (1976)	Commercial refined, bleached and deodorized oil sample	TLC-GLC	4.8	
Codex Alimentarius Commission (2009)		HPLC		0.9-35.2
NUTTAB (2010)[2]			8.3	
USDA (2008)[3]		GLC, HPLC	8.2	
Swedish National Food Administration, (2011)			12	
Danish Food Composition Databank, (2008)[4]	Refined Oil		6.1	2.7 - 9.5
Jung et al. (1989)	Crude Oil	HPLC-UV	4.6	
	Degummed Oil		5.3	
	Refined oil		4.3	
	Bleached oil		4.6	
	Deodorized oil		4.0	

1 Many of these references provide additional data on the β-tocopherol, γ-tocopherol, δ-tocopherol and total tocopherol content of the samples.
2 Food Standards Australia New Zealand (2010)
3 Value is converted to mg amounts based on the conversions of vitamin E in IU to mg as defined by the DRI report, 1 mg of α-tocopherol = IU of the RRR-α-tocopherol compound × 0.67, where RRR-α-tocopherol compound is natural vitamin E (Gebhardt *and Holden* [2006]).
4 Saxholt *et al*. (2008)

C. Soybean meal

13. Soybean meals, as present in the marketplace, are normally defatted, and toasted to obtain a moisture content of approximately 9-11% (Table 10). Two types of meals are ordinarily produced. One is 44% crude protein on an as-is basis, with further addition of hulls. The other is a higher 49% (as-is basis) crude protein meal, without hulls. The reported ranges for protein, fat, ash, crude fibre, NDF, ADF, and carbohydrate (given as nitrogen-free extract [NFE]) content are shown in Table 10. The ranges in amino acid concentrations are shown in Table 11.

Table 10. Proximate and fibre content of soybean meal (% of dry matter)

Reference	*44% Meal*					*49% Meal*					*Meal*	*Meal (Dehulled, solvent extracted)*
	NRC-Beef, 2000[1]	**NRC-Dairy, 2001**[2]	**NRC-Swine, 1998**[3]	**NRC-Poultry, 1994**[4]	**Ensminger *et al.*, 1990**[5]	**NRC-Beef, 2000**[1]	**NRC-Dairy, 2001**[2]	**NRC-Swine, 1998**[3]	**NRC-Poultry, 1994**[4]	**Ensminger *et al.*, 1990**[5]	**NARO, 2001**[6]	**NARO, 2001**[6]
	Mean	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*
Moisture	10.9	10.9	11	11	11.0	10.1	10.5	10	10	10.0	11.7	9.8
Crude Protein	49.9	49.9	49.21	49.44	49.8	54.0	53.8	52.78	53.89	54.6	52.2	56.2
Crude fat (ether extract)	1.6	1.6	1.69	0.90	1.7	1.1	1.1	3.33	1.11	1.4	1.5	1.3
Ash	7.2	6.6			7.2	6.7	6.4			6.8	6.7	7.0
Crude fibre				7.87	7.0				4.33	4.1	6.3	3.7
NDF	14.9	14.9	14.94		14.0	7.79	9.8	9.89		7.4	14.3	
ADF	10.0	10.0	10.56		10.0	6.10	6.2	6.00		6.9	8.9	
NFE					34.3					33.2	33.3	31.8

1 NRC, (2000) *Nutrient Requirements of Beef Cattle*: 44% meal = Seed, meal solvent extracted; 44% protein, 49% meal = Seeds without hulls, meal
2 NRC, (2001) *Nutrient Requirements of Dairy Cattle*: 44% meal = Meal, solvent, 44% CP; 49% meal = Meal, solvent , 48% CP
3 NRC, (1998) *Nutrient Requirements of Swine*: 44% meal = meal, sol. extr.; 49% meal = meal without hulls, sol. extr. Data converted from fresh weight to dry weight basis using given moisture level.
4 NRC, (1994) *Nutrient Requirements of Poultry*: 44% meal = seeds, meal solvent extracted; 49% meal = seeds without hulls, meal solvent extracted. Data converted from fresh weight to dry weight basis using given moisture level.
5 44% meal = Seeds, Meal Solv Extd, 44% Protein; 49% meal = Seeds without Hulls, Meal Solv Extd, 49% Protein
6 The target amount of protein was not specified in this source.

Table 11. Amino acid composition of soybean meal (% of dry matter)

Reference	***44% Meal***				***49% Meal***				***Meal***	***Meal (Dehulled, solvent extracted)***
	NRC-Dairy, 2001[1]	**NRC-Swine, 1998**[2]	**NRC-Poultry, 1994**[3]	**Ensminger *et al.*, 1990**[4]	**NRC-Dairy, 2001**[1]	**NRC-Swine, 1998**[2]	**NRC-Poultry, 1994**[3]	**Ensminger *et al.*, 1990**[4]	**NARO, 2001**[5]	**NARO, 2001**[5]
	Mean	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*	*Mean*
Arginine	3.68	3.63	3.56	3.65	3.94	3.87	3.94	4.03	3.88	4.24
Cystine	0.76	0.79	0.75	0.75	0.81	0.82	0.81	0.83	0.80	0.85
Histidine	1.38	1.31	1.33	1.27	1.49	1.42	1.45	1.43	1.38	1.49
Isoleucine	2.28	2.24	2.22	2.38	2.45	2.40	2.40	2.60	2.39	2.61
Leucine	3.90	3.84	3.84	3.92	4.20	4.07	4.23	4.20	4.02	4.21
Lysine	3.13	3.18	3.05	3.20	3.38	3.36	3.35	3.44	3.21	3.53
Methionine	0.72	0.69	0.70	0.67	0.77	0.74	0.76	0.74	0.73	0.73
Phenylalanine	2.62	2.45	2.45	2.51	2.83	2.66	2.65	2.76	2.67	2.83
Threonine	1.99	1.94	1.95	2.03	2.13	2.06	2.12	2.23	2.07	2.21
Tryptophan	0.63	0.69	0.84	0.69	0.68	0.72	0.84	0.78	0.71	0.76
Valine	2.34	2.31	2.35	2.66	2.50	2.52	2.51	2.77	2.53	2.67
Glycine			2.15	2.36			2.32	2.66	2.26	2.34
Serine			2.60	2.66			2.81	3.08	2.68	2.89
Tyrosine		1.90	2.17	1.80		2.02	2.21	2.18	1.86	1.96
Crude Protein	49.9	49.21	49.89	49.8	53.8	52.78	53.73	54.6	52.21	56.21

1 NRC, (2001) *Nutrient Requirements of Dairy Cattle*: 44% meal = Meal, solvent , 44% CP; 49% meal = Meal, solvent, 48% CP.

2 NRC, (1998) *Nutrient Requirements of Swine*: 44% meal = meal, sol. extr.; 49% meal = meal without hulls, sol. extr. Data converted from fresh weight to dry weight basis using given moisture level.

3 NRC, (1994) *Nutrient Requirements of Poultry*: 44% meal = sees, meal solvent extracted; 49% meal = seeds without hulls, meal solvent extracted. Data converted from fresh weight to dry weight basis using given moisture level.

4 Seeds, Meal Solv Extd, 44% Protein; 49% meal = Seeds without Hulls, Meal Solv Extd, 49% Protein

5 The target amount of protein was not specified in this source.

D. Hulls and forage

14. Soybean hulls are generally removed from the beans before oil extraction. In animal feeds, hulls may be used as carriers and as a source of fibre. Soybean forage is usually harvested around the full seed (R6) stage. Soybean hay is produced from the harvested forage. The hay is allowed to sun-cure to about 11% moisture. The proximate nutrient content for soybean hulls, soybean forage and soybean hay are shown in Tables 12, 13, and 14, respectively.

Table 12. Proximate and fibre content of soybean hulls

Reference	Ensminger *et al.*, 1990[1]	NARO, 2001	NRC Dairy, 2001[2]	NRC Beef, 2000[3]
	Mean	*Mean*	*Mean*	*Mean*
(g/100 g fresh weight)				
Moisture	9.0	10.3	9.1	9.7
(g/100 g dry matter)				
Crude protein	11.9	17.6	13.9	12.2
Crude fat (Ether extract)	2.2	5.6	2.7	2.10
Ash	5.1	4.9	4.8	4.9
NDF	65.6	54.4	60.3	66.3
ADF	46.8	41.8	44.6	49.0
NFE[5]	40.9	40.2		

1 Data listed as soybean “seed coats”
2 NRC, (2001) *Nutrient Requirements of Dairy Cattle*
3 NRC, (2000) *Nutrient Requirements of Beef Cattle*. Data listed as soybean “seed coats”

Table 13. Proximate and fibre content of soybean forage

Reference	Ensminger *et al.*, 1990[1]	NARO, 2001	ILSI, 2010	
	Mean	*Mean*	*Mean*	*Range*
(g/100 g fresh weight)				
Moisture	77	77.1	77.0	73.5-81.6
(g/100 g dry matter)				
Crude protein	17.9	18.8	19.38	14.37-24.71
Crude fat (Ether extract)	4.0	2.2	3.14	1.30-5.13
Ash	10.5	8.3	9.04	6.71-10.78
Crude Fibre	27.3	32.3	22.67	13.58-31.73
NDF		47.2		
ADF		37.6		
NFE[1]	40.3	38.4		

[1] Data reported as fresh forage

Table 14. Proximate and fibre content of soybean hay

Reference	Ensminger *et al.*, 1990[1]	NARO, 2001
	Mean	*Mean*
(g/100 g fresh weight)		
Moisture	11.0	12.8
(g/100 g dry matter)		
Crude protein	15.8	18.1
Crude fat (Ether extract)	2.5	1.6
Ash	8.0	6.8
NDF		
ADF	40.0	
NFE	39.3	34.9

[1] Data reported as sun-cured hay

SECTION III - OTHER CONSTITUENTS

A. Anti-nutrients and toxicants

Oligosaccharides

15. Soybean meal contains stachyose and raffinose oligosaccharides, which limit the energy availability for this co-product in swine and poultry. These two low molecular weight carbohydrates are considered anti-nutrients due to the gas production and resulting flatulence caused by their consumption (Rackis, 1974).

16. These compounds are present in defatted, toasted soybean meal as well as in raw soybeans (Padgette *et al*., 1996). Further processing of soybean meal into concentrate or isolate, reduces or removes, these oligosaccharides (Mounts *et al*., 1987). Data regarding the oligosaccharide content of soybean seed are shown in Table 15.

Table 15. Oligosaccharide content of soybean seed (g/100 g dry matter)

Reference	ILSI 2010		Hymowitz *et al.*, 1972		NFRI-NARO[1] 2011	
	Mean	*Range*	*Mean*	*Range*	*Mean*	*Range*
Raffinose	0.36	0.21-0.66	0.39	0.1-0.9	0.91	0.61-1.60
Stachyose	2.19	1.21-3.50	2.79	0.6-5.1	3.82	2.58-4.96

[1] Data converted from fresh weight to dry weight basis using given moisture level.

Trypsin inhibitors

17. Protease inhibitors, *i.e*. the Kunitz inhibitor and the Bowman-Birk inhibitor, are active against trypsin, while the latter is also active against chymotrypsin (Liener, 1994). These protease inhibitors interfere with the digestion of proteins resulting in decreased animal growth. The activity of these inhibitors is destroyed when the bean or meal is toasted or heated during processing. Data on trypsin inhibitor content from soybean seed are shown in Table 16.

Lectins

18. Lectins are proteins that bind to carbohydrate-containing molecules. Lectins in raw soybeans can inhibit growth in animals (Liener, 1994). Soybean lectin is sometimes referred to as soybean hemagglutinin. Lectins are rapidly degraded upon heating. In one study, lectin levels dropped approximately 100-fold when the raw soybean was processed into defatted, toasted soybean meal (Padgette

et al., 1996). However, Liu (1997) in his review found research to indicate that soy lectin is quite resistant to dry heat. Data regarding the lectin content of soybean seed are shown in Table 16.

Phytic acid

19. Phytic acid [myo-Inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate)] is present in soybeans. Liener (2000) estimates that two-thirds of the phosphorus in soybeans is bound as phytate and is mostly unavailable to non-ruminant animals. This compound chelates mineral nutrients including calcium, magnesium, potassium, iron, and zinc, rendering them unavailable to non-ruminant animals consuming the beans (NRC, 1998; Liener, 1994). Some processing steps such as boiling, steaming, or fermenting may reduce the phytate content of soybeans (Reddy and Pierson, 1994). For example, during tempeh fermentation, the phytase action of *Rhizopus* sp. hydrolyzes phytate. Reddy and Pierson report that approximately 32.9 – 54.5 % of the phytate in soybeans may be hydrolyzed during tempeh fermentation.

20. Phytic acid chelation of zinc present in corn-soybean meal diets used for growing swine requires supplements of zinc to avoid a parakeratosis condition (Smith *et al.*, 1962). It is becoming common for feed formulators to add a phytic acid degrading enzyme, phytase, to swine and poultry diets to release phytin-bound phosphorus, so that the amount of this mineral added to the diet can be decreased, potentially reducing excess phosphorus in the environment.

21. Phytic acid naturally occurs in soybeans (and most soybean products) and can make up to 1–1.5 g per 100 g of the dry weight (Liener, 1994). Data on the phytic acid content of soybean seed are shown in Table 16.

Table 16. Anti-nutrient content of soybean seed

Reference	ILSI, 2010		NFRI-NARO, 2011		Kakade *et al.*, 1972[3]	Liener, 1994
	Mean	*Range*	*Mean*	*Range*	*Range*	*Range*
Lectins	1.72 HU[1]/ mg DM	0.11–9.04 HU/ mg DM			0.11 - 9.4 HU/mg DM[4]	
Phytic acid, g/100 g DW	1.12	0.63–1.96	1.63	0.8-2.5		1.0-1.5
Trypsin inhibitor	48.33 TIU[2]/ mg DM	19.59–118.68 TIU/ mg DM	7.57 µg/mg protein	4.23-10.64 µg/mg protein	100-184 TIU/mg protein[4]	

1 HU = Hemagglutination Units
2 TIU = Trypsin Inhibitor Units
3 Aqueous extractable proteins derived from defatted soybean seed extracts
4 Activity reported is in the protein content of the crude defatted soybean seed extract

B. Other Compounds

Isoflavones

22. Soybeans naturally contain a number of isoflavone compounds reported to possess biochemical activity, including estrogenic, anti-estrogenic, and hypocholesterolemic effects, in mammalian species. These compounds have been implicated in adversely affecting reproduction in animals fed diets containing large amounts of soybean meal (Schutt, 1976). The effect of isoflavones in humans is an active area

of research, including research on both the safety of isoflavones as well as the potential health benefits of isoflavones (Messina, 2010).

23. The isoflavones in soybeans and soy products have three basic types: diadzein, genistein and glycitein. Each of these three isomers, known as aglycones or free forms, can also exist in three conjugate forms: glucoside, acetylglucoside, or malonylglucoside (Wang and Murphy, 1994a; Liu 1997). Therefore, there are a total of twelve known isoflavone aglycones and glycosides in soybeans.

24. The isoflavone content of soybeans is greatly influenced by factors such as variety, growing locations, planting year, planting date and harvesting date (Wang and Murphy, 1994b; Aussenac *et al*, 1998; Murphy *et al*., 1998;). For example, a study indicated that the total isoflavone content of a single variety, Vinton 81, ranged from 84.4 to 163.6 mg/100 g raw seeds among eight locations in 1995, and from 160.8 to 284.2 mg/100 g in 1996 (Hoeck *et al*., 2000). The total isoflavone content of raw soybean seeds of an individual variety has been shown to range three- to five-fold depending on location and year of growth (Wang and Murphy, 1994b; Hoeck *et al.*, 2000, Aussenac *et al*, 2008).

25. Furthermore, differences in analytical methods and reporting conventions can also contribute significantly to variation in isoflavone values found in the literature. In some reports, total isoflavone is expressed as the sum of all twelve known isoflavone aglycones and glycosides (Wang and Murphy, 1994b). In other studies, only free (aglycones) or bound (conjugated) forms are tested and expressed (Coward *et al*., 1993; Taylor *et al*., 1996). Still, other sources describe that isoflavones are hydrolysed to their aglycone forms or the amount is normalised by molecular weight to the aglycone forms (Wang and Murphy, 1996). In the latter case, because the molecular weight of the glucosides is 1.6 to 1.9 times greater than the aglycones, the total isoflavone amount can be significantly less than the value from non-normalised data (Murphy *et al*., 1998).

26. Processing also significantly affects the retention and distribution of isoflavone isomers in the final products (Coward *et al*., 1993; Wang and Murphy, 1994a; Wang and Murphy, 1996; Liu, 1997). Toasted soybean meal appears to have similar levels of phytoestrogens as the raw seed (Padgette *et al*., 1996; Wang and Murphy, 1996). Soybean sprouts have also been reported to contain coumestrol (Liener, 1994; Wang and Murphy, 1994a).

27. Table 17 contains data on various isoflavones found in soybean seeds.

Table 17. Isoflavone content of soybean seed (mg/kg dry matter, unless noted)

Reference	ILSI, 2010		Kim *et al.*, 2005	Lee *et al.*, 2003	Wang & Murphy, 1994a[1]	Aussenac *et al.*, 1998	USDA, 2008[2]
	Mean	*Range*	*Range*	*Range*	*Range*	*Range*	*Mean*
Diadzin			13.1-83.6	310.5-608.9	180-690	503.3-96398.7	
Malonyldaidzin			61.9-558.1	1204.3-1806.3	241-300	1104-3889.7	
Daidzein			0.1-21.2	32.2-153.5	7-26	0.8-3.5	
Total Daidzein	834.8	60.0-2453.5		1568.0-2568.8	240-600		620.7
Genistin			11.7-143.0	493.2-773.5	394-852	378.6-957.7	
Malonylgenistin			135.5-603.4	153.5-1981.3	738-743	1407.7-3752.4	
Acetylgenistin			0.1-21.0	-	2-9	-	
Genistein			0.5-22.6	9.3-31.5	17-29	1.1-5.5	
Total Genistein	976.8	144.3-2837.2		1751.2-2646.6	648-954		809.9
Glycitin			1.1-33.5	56.4-218.3	53-56	228.7-411.7	
Malonylglycitin			6.6-71.2	134.1-463.0	50-61	58.0-210.5	
Glycitein				6.7-58.7	17-29	-	
Total Glycitein	156.6	15.3-310.4		208.7-502.4	82-107		149.9
Total Isoflavones	2123.8	678.7-3688.9		3764.5-5594.9	995-1636	3911.0-9797.9	1545.3

1 Data converted from fresh weight to dry matter (DM) basis assuming average DM of 90%

2 USDA Database for the Isoflavone Content of Selected Foods, (2008). Data source NBD No. 16108: Soybeans, mature seeds, raw (all sources). Data reported as mg/kg on a fresh weight basis.

Phospholipids

28. Phospholipids have been investigated for their medical and product stability characteristics (Hildebrand *et al*., 1984; O'Brien and Andrews, 1993). Soybean lecithin is known to contain the primary phospholipids identified as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid (Rydhag and Wilton, 1981). NFRI-NARO (2011) reported levels of these phospholipids and two other phospholipids, phosphatidylserine and phosphatidylglycerol, which are present in soybean seed at much lower levels. These results are reported in Table 18.

Table 18. Phospholipids content of soybean seed (g/100 g dry matter)

Reference	NFRI-NARO 2011[1]	
	Mean	*Range*
Phosphatidylcholine	0.41	0.21-0.55
Phosphatidylethanolamine	0.24	0.11-0.37
Phosphatidylinositol	0.28	0.19-0.42
Phosphatidylserine	0.04	0.02-0.08
Phosphatidylglycerol	0.05	0.01-0.10
Phosphatidic Acid	0.2	0.08-0.29
Lecithin	1.40	1.0-1.79

[1] Data converted from fresh weight to dry weight basis using given moisture levels

Sterols

29. Sterols are the other non-saponifiable components of vegetable oils besides tocopherols. Total sterols range from 180 to 450 mg/100 g of oil (Codex Alimentarius Commission, 2009). The proportions of major sterols are presented in Table 19.

Table 19. Sterol levels in soybean oil (% of total sterols)

Reference	Codex Alimentarius Commission, 2009[1]	Souci *et al.*, 2008
	Range	*Mean*
Cholesterol	0.2-1.4	0.25
Brassicasterol	ND-0.3	-
Campesterol	15.8-24.2	10.00
Stigmasterol	14.9-19.1	10.92
Beta-sitosterol	47.0-60	29.85
Delta-5-avenasterol	1.5-3.7	-
Delta-7-stigmastenol	1.4-5.2	-
Delta-7-avenasterol	1.0-4.6	-
Others	ND–1.8	49.23[2]

[1] Non-detects (ND) are ≤ 0.05 % total sterols.
[2] Reported as 'free sterols'

Saponins

30. Saponins are a diverse group of structurally related compounds containing a steroidal or triterpenoid aglycone linked to one or more oligosaccharides present in numerous plant families (Liener, 1994; Guclu-Ustundag and Mazza, 2007). Saponins from soybean have been shown to have no adverse effects when fed to laboratory animals at high levels (Liener, 1994). Unlike other plant saponins, soy saponins have only a weak effect on intestinal permeability and therefore have little impact on active nutrient transport (Liener, 1994). Consequently, soybean saponins are not considered to be true anti-nutrients. Total saponin content of soybeans ranges from to 0.09-0.53 g/100 g dry matter (Anderson and Wolf, 1995).

C. Allergens

31. Soybean is one of eight foods that account for 90% of all IgE-mediated food allergies (Taylor and Hefle, 2000). The prevalence of soybean allergy in the general population is reported to be between 0.3% and 0.7% (Becker *et al.,* 2004; Roehr *et al.* 2004; Sicherer and Sampson, 2006; Zuidmeer *et al.*, 2008; Boyce *et al.*, 2010), with an increased prevalence reported in children with atopic eczema (Becker *et al.*, 2004). Many cases of soy allergy are outgrown during childhood (Bock, 1987; Sampson and Scanlon, 1989; Host and Halken, 1990; Sicherer *et al.*, 1998; Becker *et al.*, 2004; Savage *et al*, 2010; Boyce *et al.*, 2010). Allergic reactions resulting from soybean consumption are similar to those elicited by the other food allergens; however, the most severe allergic reactions, anaphylaxis and death, seem to be rare (Sicherer *et al.*, 2000). Radioallergosorbent test (RAST) and skin prick test are both used in the diagnosis of soybean allergy; but these methods yield a far higher incidence of soybean allergy as compared with results from double-blind placebo controlled food challenges (Becker *et al.,* 2004).

32. A number of immunological or immunochemical tests have been developed to examine allergenic proteins usually based on sera from sensitive subjects. There are a number of proteins in the soybean (see Table 20) that are considered potential allergens due to their IgE binding ability (L'Hocine and Boye, 2007; WHO/IUIS, 2011). These proteins are involved in storage, enzymatic and protective functions. Some of the proteins are associated with inhalation induced allergy, such as Gly m 1, Gly m 2 and Kunitz trypsin inhibitor. Other proteins are associated with food allergy and include P34, β-conglycinin and glycinin. IgE binding to all subunits of β-conglycinin and glycinin was recently demonstrated using sera from soybean allergic subjects (Holzhauser *et al.*, 2009). The P34 allergen is considered as an immunodominant soybean allergen, *i.e.* responsible for a large percentage of the food allergy reactions to soybean (Wilson *et al.*, 2005). Some soybean proteins are also known to cross-react with certain allergens present in legumes (*e.g.* peanut, green pea, green bean) (Herian *et al.*, 1990). When compared to soybean seeds, sprouts exhibit similar ability to bind IgE from soy-allergic individuals (Herian *et al.*, 1993; ILSI, 1999).

33. The effects of agronomic conditions, heating, and processing on allergenicity of soybeans have been discussed in ILSI (1999), and Taylor and Lehrer (1996). Heating and other processing may increase or decrease the potency of soybean allergens (Taylor and Lehrer, 1996; Wilson *et al.*, 2005).

34. Soybean products such as soybean oil and soybean lecithin contain low levels of soy protein. Soybean oils, particularly unrefined oils, may contain allergenic proteins (Bush *et al.*, 1985; Paschke *et al.*, 2001). Soy lecithin, which is largely composed of phospholipids, may also contain allergenic proteins (Porras *et al.*, 1985; Awazuhara *et al.*, 1998; Gu *et al.*, 2001). Highly refined soybean oil has been studied in soy-allergic individuals; results from this study and other studies are consistent with the expectation that the amount of protein present in highly refined soybean oil does not elicit an allergic reaction in the overwhelming majority of these people (Bush *et al.*, 1985; Awazuhara *et al.*, 1998; Paschke *et al.*, 2001).

Table 20. Potential soybean allergens

IgE-binding proteins	Allergen nomenclature	Molecular weight (kDa)	Family
Hydrophobic proteins	Gly m 1[1]:	7.0-7.5	Lipid transfer protein
Defensin	Gly m 2[1]	8.0	Storage protein
Profilin	Gly m 3[1]	14	Profilin
SAM22	Gly m 4[1]	16.6	Pathogenesis related protein PR-10
P34	Gly m Bd 30 K	34	Protease
Unknown Asn-linked glycoprotein	Gly m Bd 28 K	26	Unknown
β-Conglycinin (vicilin, 7S globulin)	Gly m 5[1]	140–170	Storage protein (with subunits)
Glycinin (legumin, 11S globulin)	Gly m 6[1]	320–360	Storage protein (with subunits)
2S albumin	Not assigned	12	Prolamin
Lectin	Not assigned	120	Lectin
Lipoxygenase	Not assigned	102	Enzyme
Kunitz trypsin inhibitor	Not assigned	21	Protease inhibitor
Unknown	Not assigned	39	Unknown
Unknown	Not assigned	50	Homology to chlorophyll A-B binding protein
P22-25	Not assigned	22–25	Unknown

Source: adapted from L'Hocine and Boye, (2007); updated with information from WHO/IUIS (2011)

1 WHO/IUIS (2011) Allergen nomenclature recognized by WHO and IUIS

SECTION IV - SUGGESTED CONSTITUENTS TO BE ANALYSED RELATED TO FOOD USE

A. Key products consumed by humans

35. Soybeans are consumed in non-fermented and fermented forms (International Food Information Council Foundation, 2009; Liu, 2008). Non-fermented soy foods include dairy analogues (*e.g.* soymilk), meat analogues (*e.g.* "veggie burgers"), tofu, soy sprouts, yuba (soymilk film), okara (soy pulp), soy flours, soy protein (including isolates and concentrates), boiled soybeans (edamame), and baked soybeans ("soy nuts") Fermented foods include soy sauce, miso, natto, tempeh, soy yogurt, sufu (fermented tofu), and fermented whole soybeans (Liu, 2008). Soybean oil, soy lecithin, and soy protein isolate [5]are used in infant formulas. Soy protein products are also added to a number of meat, dairy, bakery and cereal products as protein extenders (Liu, 1997). Approximately 2% of soy protein is consumed by humans; the large majority of the remaining 98% is processed into soybean meal for livestock feed (Goldsmith, 2008). The daily intake of soy-based foods in Japan is generally estimated to be between 63.2 to 70.2 g per person (Food Safety Commission of Japan, 2006). In Korea, consumption of soybean and soybean-based products, including tofu, soymilk, sprouts, soybean paste, and other foods is estimated to be 21 g per person per day (Kim and Kwon, 2001).

36. Soybean oil is used in a wide variety of foods and is the predominant soybean-based product consumed by humans. Soybean oil makes up 94% of the soybean food ingredients consumed by humans. Yearly consumption of soybean oil *per capita* is 30 kg in Brazil, 4 kg in China, and 27 kg in the United States (Goldsmith, 2008).

B. Suggested analysis for food use of new varieties

37. Soybeans can be used in the diet to provide protein. Protein is evaluated in relationship to its biological value which is markedly influenced by the relative amounts of indispensable (essential) and dispensable (non-essential) amino acids and the form of nitrogen in the diet (WHO, 2007). The World Health Organization (WHO) and Institute of Medicine (IOM) list the following nine amino acids as indispensable, *i.e.* those that have carbon skeletons that cannot be synthesized to meet body needs from simpler molecules; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Additionally, IOM lists six amino acids as "conditionally indispensable", *i.e.* those amino acids requiring a dietary source when endogenous synthesis cannot meet metabolic needs; arginine, cysteine, glutamine, glycine, proline and tyrosine. However, WHO indicated that the requirement for indispensable amino acids is not an absolute value, and one must consider the total nitrogen content of the diet, including the dispensable amino acids particularly at lower levels of nitrogen consumption (IOM, 2005; WHO, 2007).

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[5] The composition of soy protein isolate would, in effect, be considered when one considers the composition of the seed or meal from which the protein isolate would be derived. Any safety or nutritional issues associated with soy protein isolate would be expected to be detected from an analysis of the seed or meal. Additionally, the composition of the soy protein isolate could depend on the composition of the starting materials and the process used to obtain the isolate.

38. Soybeans are also used as a source of fat for human diets. Soybean oil is evaluated primarily for its fatty acid content, particularly for its unsaturated fatty acids: oleic, linoleic and linolenic acids. Linolenic and linoleic fatty acids have been recognized as essential, those that cannot be synthesized by the body (IOM, 2005). Soybean oil is also a source of vitamins E and K.

39. Soybeans contain several anti-nutrients that are relevant to nutrition and human health. For example, soybeans contain phytic acid, stachyose, raffinose, lectins, and isoflavones.

40. The suggested key nutritional and anti-nutritional parameters to be analysed in soybean matrices for human food use are shown in Table 21. Fatty acids, vitamin E, and vitamin K may be measured in seed or oil.

Table 21. Suggested nutritional and compositional parameters to be analysed in soybean matrices for food use

Parameter	Seed	Soybean Oil
Moisture[1]	X	
Crude Protein [1]	X	
Crude Fat (Ether Extract)[1]	X	
Crude Fibre[1]	X	
Carbohydrate[2]	X	
Ash[1]	X	
Amino Acids	X	
Fatty Acids[3]	X	X
Vitamin E[3] (α-tocopherol)	X	X
Vitamin K_1[3]	X	X
Phytic Acid	X	
Stachyose	X	
Raffinose	X	
Lectins	X	
Isoflavones	X	

1 These components should be measured using a method suitable for the measurement of proximates.
2 Carbohydrate (by calculation) = 100%-(crude protein% + crude fat% + ash% + moisture%)
3 Measurement of this component can be conducted in either seed or oil.

SECTION V - SUGGESTED CONSTITUENTS TO BE ANALYSED RELATED TO FEED USE

A. Key products consumed by animals

41. Several whole and processed fractions of soybeans contribute to the total animal diet. Toasted soybeans (whole cooked seed feed product) are fed to cattle and swine on a limited basis, but the oil in toasted seeds causes the fat in pigs to take on an undesirable soft texture (Ensminger *et al*., 1990). Grummer and Rabelo (2000) suggest that whole cooked soybeans are a palatable protein and fat supplement that has the potential to increase lactation performance of dairy cattle when included at a rate of up to 24% of dry matter of the diet. Other methods of heating full-fat soybeans include jet-sploding, micronization and extrusion.

42. The main soybean product fed to animals is the defatted/toasted soybean meal (Thacker and Kirkwood, 1990). However, aspirated grain fractions, forage, hay, hulls, seed, and silage are also fed to a limited extent, primarily to cattle. In some instances, bakery products containing soybean oil are also fed to cattle. It has been reported, however, that hay and hulls are also fed to poultry, and soybean aspirated grain fractions, hulls, and seeds have been fed to swine (Ensminger *et al*., 1990).

43. The Environmental Protection Agency of the United States (US-EPA, 2008) has provided estimates on potential contribution of soybean products to the diets of high-producing beef cattle, dairy cattle, poultry and swine in the United States. US-EPA provides these estimates as percentages of feedstuffs in livestock daily rations for mature and market animals based upon production data of livestock meat, milk, and eggs for human consumption. Estimated inclusion in animal diets for soybean fractions, based on the feedstuff's classification as roughage (R) or protein concentrate (PC), is shown in Table 22.

Table 22. Estimated possible inclusion of soybean fractions to animal feeds

Soybean Fraction	Classification[1]	DM%	Percent of Diet (%)			
			Feedlot Beef	Mature, Lactating Dairy	Laying Hen	Finishing Swine
Seed	PC	89	5	15	20	15
Forage/Silage[2]	R	35	NU[3]	20	NU	NU
Hay[2]	R	89	NU	20	NU	NU
Meal	PC	92	5	10	25	15
Hulls	R	90	15	20	NU	NU

Source: US-EPA, 2008: Table 1 Feedstuffs (June 2008)

1 Classification of the soybean fraction as Roughage (R) or Protein Concentrate (PC)
2 Label restrictions about feeding may be allowed
3 NU indicates the feedstuff is not used or is used at less than 5% of diet

B. Suggested analysis for feed use of new varieties

44. Soybean meal is fed to animals primarily as a source of protein, and is normally marketed as either a 44% protein product with hulls or a more refined 49% protein product with hulls removed. The amino acid profile of the two products is essentially the same, with the difference being that more fibre has been removed from the 49% protein product. Soybean meal contains relatively high levels of certain essential amino acids that are deficient in many other common feedstuffs. However, addition of essential amino acids to the diet may still be needed to meet the essential amino acid requirements for swine and poultry (NRC, 1994 and 1998).

45. Proximate analyses are commonly conducted on animal feedstuffs. The process determines amounts of nitrogen, ether extract, ash, and crude fibre present in the feedstuff. Crude protein is calculated by multiplying the nitrogen content by 6.25, a conversion factor based on the average amount of nitrogen in protein. Fat is considered to be acid ether extractable material. For the ruminant animal, the traditional proximate analysis, crude fibre, is considered obsolete and has now been replaced by acid detergent fibre and neutral detergent fibre (NRC, 2001).

46. There is general agreement that the trypsin inhibitors are the primary soybean anti-nutrients that should be minimised in animal diets. However, the amount of lectins in the raw soybean and phytic acid levels are other important considerations. As previously discussed, toasting or heating reduces the content of trypsin inhibitors and lectin, and also will decrease urease concentrations. The oligosaccharides, raffinose and stachyose, because of their adverse effect on energy availability in swine and poultry, may also be important. Isoflavones do not appear to be a concern when soybean meal is used in formulating livestock diets.

47. When considering the remainder of the soybean products that could be fed to animals, the composition of the soybean seed, soybean meal, and the forage appear to be indicators of the safety and nutritional value of products derived from these matrices. The suggested nutritional and compositional parameters to be analysed in soybean matrices for feed use are shown in Table 23. For all analytes, except fatty acids and lectins, analytes can be measured in seed or meal.

Table 23. Suggested nutritional and compositional parameters to be analysed in soybean matrices for feed use

Parameter	Seed[1]	Meal[1]	Forage
Moisture[2]	X	X	X
Crude Protein[2]	X	X	X
Crude Fat (Ether Extract)[2]	X	X	X
NDF	X	X	X
ADF	X	X	X
Carbohydrates[3]	X	X	X
Ash[2]	X	X	X
Amino Acids	X	X	
Fatty Acids	X		
Calcium	X	X	
Phosphorus	X	X	
Stachyose	X	X	
Raffinose	X	X	
Phytic Acid	X	X	
Trypsin Inhibitors	X	X	
Lectins	X		

1 Parameters that are shared between seed and meal can be measured in either matrix.
2 These components should be measured using a method suitable for the measurement of proximates.
3 Carbohydrate (by calculation) = 100%-(crude protein% + crude fat% + ash% + moisture%)

SECTION VI - REFERENCES

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