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Series on the Safety of Novel Foods and Feeds No.2

**CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW VARIETIES OF
SOYBEAN: KEY FOOD AND FEED NUTRIENTS AND ANTI-NUTRIENTS**

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No. 1, Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola) (2001)

No. 2, Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Antinutrients (2001)

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OECD Environmental Health and Safety Publications

Series on the Safety of Novel Foods and Feeds

No. 2

**CONSENSUS DOCUMENT ON COMPOSITIONAL CONSIDERATIONS FOR NEW
VARIETIES OF SOYBEAN: KEY FOOD AND FEED NUTRIENTS AND ANTINUTRIENTS**

Environment Directorate

Organisation for Economic Co-operation and Development

Paris 2001

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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 consensus document addresses compositional considerations for new varieties of soybeans by identifying the key food and feed nutrients and anti-nutrients. A general description of these components and the main toxicants are provided. Also included are an overview of the production and processing of soybeans and the suggested analysis of new varieties of food and feed products.

The United States served as the lead country in the preparation of this document.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

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PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD Member countries. 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 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 current 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 Harmonization of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, 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.

The Role of Comparative Approach as Part of a Safety Assessment

In 1990, a joint consultation of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (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.

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 and key toxicants and anti-nutrients for the food source in question. The comparison of critical components should be between the modified variety and non-modified comparators with an appropriate history of safe use. The data for the non-modified comparator can be the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO and WHO, 1996). The comparator used to detect unintended effects for all critical components 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 of Soybeans¹

The soybean is grown as a commercial crop in over 35 countries world-wide. Of the major oilseeds traded in international markets, the soybean, *Glycine max* (L.), dominates (Smith and Huyser, 1987; ASA, 1997). The major producers, U.S., Argentina, Brazil, and China, account for 87% of the total production (Table 1; USDA-ARS, 1997). Most soybean meal, 97%, is used in animal feed, with 46% going to poultry, 32% to swine, and 9% each going to dairy and beef cattle feed, respectively. A sizeable amount is also used in pet food.

Table 1: Production and Export of Soybeans¹

| Country/Region | Production | Exports |
|----------------|------------|---------|
| United States | 72.8 | 23.5 |
| Brazil | 31.0 | 9.4 |
| Argentina | 18.5 | 2.8 |
| China | 14.0 | 2.8 |
| European Union | 1.4 | 0.1 |
| Paraguay | 3.0 | 2.4 |
| Other | 13.7 | 2.8 |
| Total | 154.4 | 41.0 |

¹(in Million tons, USDA, Nov. 1999)

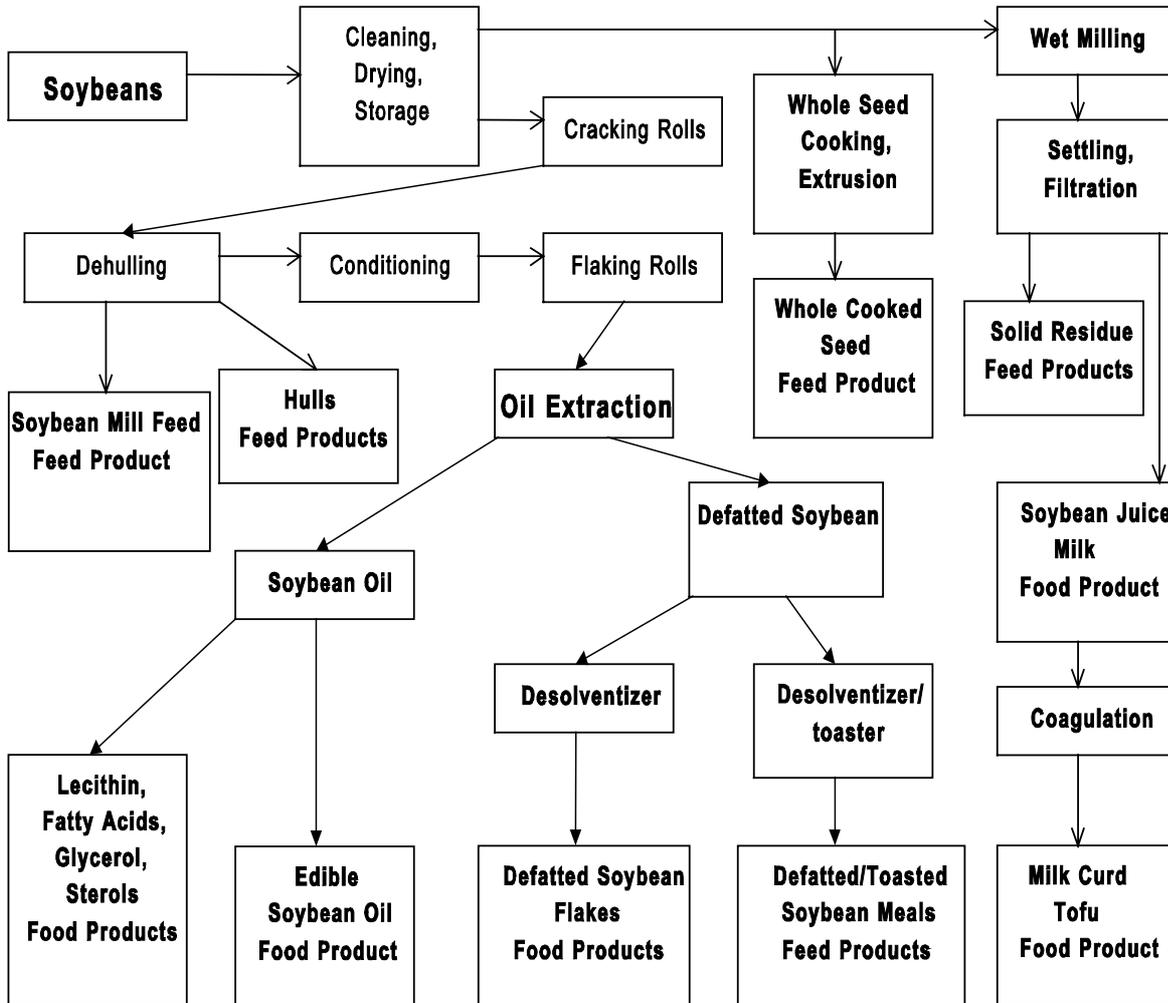
B. Processing of Soybeans

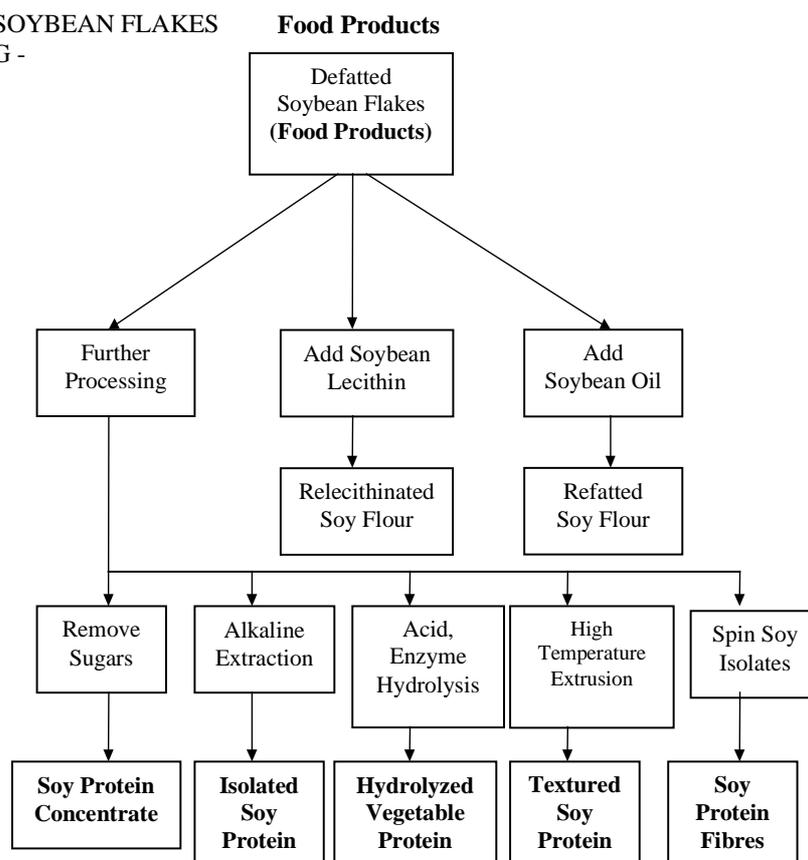
Today, there are three major soybean commodity products: seeds, oil, and meal. A bushel (27.2 kg) of soybeans yields about 21.8 kg of protein-rich meal and 5.0 kg of oil. There is only limited animal feed use, and no food use for unprocessed soybeans, since they contain anti-nutrient factors, such as trypsin inhibitors and lectins. Adequate heat processing inactivates these factors. Whole soybeans are used 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 the use of whole oil 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. Soybean meal is rich in the essential amino acids, particularly lysine, leucine, and isoleucine, which are required supplements in animal diets for optimum growth and health.

The processing steps used to produce the various soy products are shown in the following schematic:

1. For information on the environmental considerations for the safety assessment of soybeans, see OECD Consensus Document on the Biology of *Glycine max* (L.) Merr. (Soybean).

WHOLE SOYBEAN PROCESSING



DEFATTED SOYBEAN FLAKES
PROCESSING -

C. Appropriate Comparators for Testing New Varieties

This paper suggests parameters that soybean seed developers should measure. Measurements made in the new varieties should be compared to those obtained from the parental line/strain and/or a referenced soybean standard. A comparable food/feed should be selected and the comparable food/feed product should be compared to its counterpart derived from the parental line/strain and/or a referenced standard. A developer can also compare values obtained from new varieties with the literature values present in this paper.

D. Typical Characteristics Screened by Soybean Seed Developers

Phenotypic characteristics provide important information related to the suitability of new varieties for commercial distribution. Briefly, plant breeders developing new varieties of soybeans evaluate 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, and general disease resistance. The latter disease screening depends on the maturity and area in which the seeds are being grown. Later on, as a variety gets closer to commercialisation, breeders measure yield, first at one site, then in larger sites at increasing numbers of sites. Some factors that are considered in evaluating new varieties 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 in certain components, and the plant breeder would be expected to analyse for such components.

Section II. – Nutrients in Soybeans and Soybean Products

A. Seeds¹

The soybean seed is harvested at physiological maturity. The moisture content will usually be between 5.6 and 11.5%. Soybeans can be harvested at a slightly higher moisture content, and subjected to mechanical drying to bring the moisture content within the usual range for safe storage. In France, the commercial standard for soybean moisture content is 14%. Literature reports indicate that the ranges for crude protein, fat, ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), and carbohydrate content are 32 - 43.6%, 15.5 - 24.7%, 4.5 - 6.4%, 10.0 - 14.9%, 9.0 - 11.1%, and 31.7 – 31.8%, respectively on a dry matter (DM) basis (Ensminger et al., 1990; Human Nutrition Information Service [HNIS], 1989; University of Missouri, 1998; USDA-ARS, 1999; NRC, 1996; NRC, 1998; Novus, 1996). The essential amino acid profile of the soybean, plus cystine which can partially replace methionine, is shown in Table 2 (NRC, 1982; NRC, 1998, Ensminger et al., 1990; Han et al., 1991; USDA-ARS, 1999; Padgett et al., 1996; AgrEvo, 1998; Dupont, 1996). Fatty acid content of the seed as reported in these literature sources is shown in Table 3.

Table 2: Amino Acid Composition of Soybean Seed

| Amino Acid | Range, % of Dry Matter |
|---------------|------------------------|
| Arginine | 2.45 - 3.1 |
| Cystine | 0.45 - 0.67 |
| Histidine | 1.0 - 1.22 |
| Isoleucine | 1.76 - 1.98 |
| Leucine | 2.2 - 4.0 |
| Lysine | 2.5 - 2.66 |
| Methionine | 0.5 - 0.67 |
| Phenylalanine | 1.6 - 2.08 |
| Threonine | 1.4 - 1.89 |
| Tryptophan | 0.51 - 0.67 |
| Valine | 1.5 - 2.44 |

1. For those who would like information on the vitamin and mineral content of mature soybean seeds, please refer to the USDA Nutrient Database for Standard Reference, Release 13 (November 1999).

Table 3: Fatty Acid Composition of Soybean Seed

| Fatty Acid | Range, % of Dry Matter |
|------------------|------------------------|
| Palmitic, C16:0 | 1.44 - 2.31 |
| Stearic, C18:0 | 0.54 - 0.91 |
| Oleic, C18:1 | 3.15 - 8.82 |
| Linoleic, C18:2 | 6.48 - 11.6 |
| Linolenic, C18:3 | 0.72 - 2.16 |
| Arachidic, C20:0 | 0.04 - 0.7 |

B. Oil

Triglycerides make up 99% of soybean oil. Soybean oil is noted for its high content of the polyunsaturated fatty acids, linoleic (C18:2) and linolenic (C18:3) acids. It also contains sizeable amounts of another unsaturated fatty acid, oleic (C18:1) and moderate amounts of the saturated fatty acids, palmitic (C16:0) and stearic (C18:0). The range in fatty acid composition in soybean oil is shown in Table 4 (Maynard et al., 1979; CRC, 1982; Food Chemicals Codex, 1996).

Table 4: Fatty Acid Composition of Soybean Oil

| Fatty Acid | Range, % of Oil |
|------------------|-----------------|
| Palmitic, C16:0 | 7 - 12 |
| Stearic, C18:0 | 2 - 5 |
| Oleic, C18:1 | 19 - 34 |
| Linoleic, C18:2 | 48 - 60 |
| Linolenic, C18:3 | 2 - 10 |
| Arachidic, C20:0 | < 1.0 |

C. Isolated Soy Protein

Isolated soy protein is used in the production of infant formula and other food products as a source of amino acids. It is expected that the amino acid ratios in these products will be similar to that of the seed except as affected by processing. The amino acid composition of soy protein isolate is presented in Table 5 (USDA, 1999).

Table 5: Amino Acid Composition of Soy Protein Isolate¹

| Amino Acid | Value, % of Dry Matter |
|---------------|------------------------|
| Arginine | 6.67 |
| Cystine | 1.05 |
| Histidine | 2.3 |
| Isoleucine | 4.25 |
| Leucine | 6.78 |
| Lysine | 5.33 |
| Methionine | 1.13 |
| Phenylalanine | 4.59 |
| Threonine | 3.14 |
| Tryptophan | 1.12 |
| Valine | 4.1 |

¹Protein content on dry matter basis is about 80%.

D. Feed - Meal

Soybean meals as present in the marketplace are normally defatted, and toasted to a moisture content of 9 - 11%. Two types of meals are ordinarily produced. One is 44% crude protein and has hulls added. The other is a higher 49% crude protein meal, which has no 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 6 (Ensminger et al., 1990; Dale, 1998; USDA, 1997; Novus, 1996). The ranges in amino acid concentrations are shown in Table 7 (Ensminger et al., 1990; USDA, 1997; Dale, 1998; AgrEvo, 1998).

Table 6: Proximate nutrient content of soybean meal (Ranges, % of Dry Matter)

| Nutrient | Soybean Meal | |
|---------------------------|--------------|-------------|
| | 44% | 49% |
| Crude Protein | 43.8 - 49.9 | 52.8 - 56.3 |
| Crude Fat (Ether extract) | 0.55 - 3.00 | 1.00 - 3.30 |
| Ash | 5.6 - 7.2 | 5.2 - 9.1 |
| Crude Fibre | 4.3 - 7.2 | 3.14 - 4.10 |
| NDF | 12.3 - 18.9 | 7.4 - 12.2 |
| ADF | 8.9 - 11.9 | 5.2 - 6.7 |
| NFE ¹ | 34.3 | 33.2 |

¹Nitrogen Free Extract as determined by difference.

Table 7: Amino Acid Composition of 44% Protein Soybean Meal¹

| Amino Acid | Range, % of Dry Matter |
|---------------|------------------------|
| Arginine | 3.49 - 3.78 |
| Cystine | 0.66 - 0.75 |
| Histidine | 1.21 - 1.32 |
| Isoleucine | 2.15 - 2.78 |
| Leucine | 3.66 - 3.92 |
| Lysine | 2.99 - 3.22 |
| Methionine | 0.6 - 0.69 |
| Phenylalanine | 2.35 - 3.0 |
| Threonine | 1.89 - 2.03 |
| Tryptophan | 0.66 - 0.75 |
| Valine | 2.24 - 2.67 |

¹Meal was solvent extracted. Protein content on dry matter basis is about 50%.

E. Hulls/Forage/Hay

Soybean hulls are generally removed from the beans before oil extraction. In animal feeds hulls are mainly used as carriers, i.e., to provide bulk in animal feed supplements. Soybean forage is usually harvested between the time when the plants reach the sixth node stage to the beginning of pod formation. Soybean hay is usually harvested at mid-to-full bloom, before the bottom leaves begin to fall and when pods are approximately 50% developed. The forage is allowed to sun-cure to about 11% moisture. The proximate nutrient content for soybean hulls, soybean forage and soybean hay is shown in Table 8.

Table 8: Proximate Nutrient Content of Soybean Hulls, Soybean Forage and Soybean Hay (% of Dry Matter)

| Nutrient | Soybean ¹ Hulls | Soybean ¹ Forage | Soybean ^{1,2} Hay |
|---------------------------|-------------------------------|--------------------------------|-------------------------------|
| Moisture | 9 | 74-79 | 11 |
| Crude Protein | 10.8 | 11.2-17.3 | 15.8-21.2 |
| Crude Fat (Ether Extract) | 2 | 3.1-5.1 | 2.5-5.4 |
| Ash | 4.6 | 8.8-10.5 | 8.0-8.8 |
| NDF | 59.4 | 34-40 | 34.6-35.6 |
| ADF | 42.4 | 32-38 | 42.6-43.3 |
| NFE | 37.0 | 62-66 | 58.5-56.6 |

¹Ensminger et al., 1990; ²AgrEvo, 1998

Section III. - Antinutrients in Soybeans

A. Trypsin Inhibitors

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. Trypsin inhibitor activity ranging from 100 to 184 Trypsin Units Inhibited (TUI)/mg protein has been reported (Kakade et al., 1972). The activity of these inhibitors is destroyed when the bean or meal is toasted or heated during processing. [Note: Some reports show trypsin inhibitor levels as measured by urease activity. Swiss regulations (Ordinance on the Feedstuffs Manual, Annex 1, SR 916.307.1) limit soy products to 0.4 urease units. Still others measure trypsin inhibitors as mg/g. Thacker and Kirkwood (1990) report a range for trypsin inhibitors of 21.1 to 31.1 mg/g, and this can be expected to vary with the source of the soybean. The same authors report that the urease measurements correlate well with the trypsin inhibitor content.]

B. Lectins

Lectins are proteins that bind to carbohydrate-containing molecules. Lectins in raw soybeans can inhibit growth and cause death in animals. It is expected that similar effects would occur in humans (Liener, 1994). The ability of lectins to act as hemagglutinins that cause blood clotting, is the basis for most quantitative analytical methods. Soybean lectin is sometimes referred to as soybean hemagglutinin. Lectin levels can vary from 37 to 323 Hemagglutinating Activity Units (HU)/ mg protein (Kakade et al, 1972). 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 (1999) in his review found research to indicate that soy lectin is quite resistant to dry heat.

C. Phytoestrogens

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). However, it is not universally accepted that the isoflavones are antinutrients as they have also been reported to have beneficial anti-carcinogenic effects (Messina and Barnes, 1991).

The isoflavones in soybeans and soy products have three basic types: daidzein, genistein, and glycitein. Each of these three isomers, known as aglucones or free forms, can also exist in three conjugate forms: glucoside, acetylglucoside, or malonylglucoside (Wang and Murphy, 1994a; Liu 1997; AgrEvo, 1998). Therefore, totally, there are 12 isomers of isoflavones in soybeans. The isoflavone content of soybeans is greatly influenced by many factors, including variety, growing locations, planting year, planting date and harvesting date (Wang and Murphy, 1994b; Douglas, 1996; Aussenac, 1998. Murphy et al. 1998; USDA, 1999; Kikuchi et al. 2000). For example, a recent study conducted at Iowa State

University indicated that the total isoflavone content of a simple variety, Vinton 81, ranged from 84.4 to 163.6 mg/100g raw seeds among 8 locations in 1995, and from 160.8 to 284.2 mg/100g in 1996 (Hoeck, 2000). In another report (Wang and Murphy 1994b), a single variety grown in different locations or crop years can have up to 5 fold differences in isoflavone concentration. It appears that environmental effect is much greater than genetics.

Furthermore, differences in analytical methods and reporting conventions can also contribute significantly to variation in isoflavone values found in the literature. In some studies, total isoflavone is expressed as the sum of all 12 isomers (Wang and Murphy 1994b). In other studies, only free (aglycones) or bounded (conjugated) forms are tested and expressed (Coward et al. 1993; Taylor et al. 1996). Still, in other studies 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 later case, because the molecular weight of the glucosides is 1.6 to 1.9 greater than the aglycones, the total isoflavone amount can be significantly less than the value of non-normalised data (Murphy et al. 1998).

When the amount is adjusted to corresponding aglycones, the concentrations for total daidzein, genistein, and glycitein have a range of 20.2 – 206 mg, 31.5-268 mg, and 10.9-107 mg per 100 g raw seed, respectively (Douglas, 1996; Wang et al., 1994; USDA 1999). When the total isoflavone content is expressed without normalisation to aglycones, a recent report revealed a range of 44 - 910 mg per 100g raw seeds among 319 soybean cultivars tested (Kikuchi, et al. 2000).

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 (Wang and Murphy, 1996; Padgett, 1996; USDA, 1999). Soybean sprouts have also been reported to contain coumestrol (Liener, 1994; Wang et al., 1994).

D. Stachyose and Raffinose

The low molecular weight carbohydrates, stachyose and raffinose, are considered antinutrients due to the gas production and resulting flatulence caused by their consumption (Rackis, 1974). These compounds are present in defatted toasted soybean meal, as well as in raw soybeans (Padgett et al, 1996). The raffinose content of soybean seeds ranges from 0.1-0.9 g per 100g on fresh weight basis, while stachyose content is from 1.4-4.1 g per 100 g (Douglas, 1996; Hymowitz et al. 1972). Further processing of soybean meal into concentrate or isolate, reduces or removes, these oligosaccharides (Mounts et al. 1987).

E. Phytic Acid

Phytic acid (myo-Inositol 1,2,3,4,5,6-hexakis [dihydrogen phosphate]) is present in soybeans. Liener estimates that two-thirds of the phosphorus in soybeans is bound as phytate and unless freed is mostly unavailable to animals (Liener, 2000). This compound chelates mineral nutrients including calcium, magnesium, potassium, iron, and zinc, rendering them unavailable to monogastric animals consuming the beans (NRC, 1998; Liener, 1994). In fact, 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, Plumlee, and Beeson, 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. 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). However, higher values, up to 2.74 g per 100 g, have also been reported (Douglass, 1996).

F. Identification of Allergens

Saline extracts of soybeans have been reported to contain several antigenic proteins which can stimulate the rabbit systemic immune system after injection and/or orally sensitise guinea pigs, calves, pigs, and humans (Pedersen, 1988). The presence of these allergenic proteins in the diet of hypersensitive individuals can cause severe adverse reactions in the gastrointestinal tract. The allergenic effect is attributed to the globulin fraction of soybean proteins that comprise about 85% of total protein (Shinbasaki et al., 1980). When compared to soybean seeds, sprouts exhibit similar ability to bind IgE from soy-allergic individuals (ILSI, 1999; Herian et al., 1993). The effects on agronomic conditions, heating, and processing on allergenicity of soybeans have been discussed, see ILSI (1999), Taylor and Lehrer (1996). A number of immunological or immunochemical tests have been developed to examine allergenic proteins usually based on sera from sensitive subjects.

Section IV – Food Use

A. Identification of Key Soybean Products Consumed by Humans

The world-wide human consumption does not appear to be well documented. However, the average U.S. human consumption of soybeans is low (Human Nutrition Information Service, 1989, 1992, 1993, 1994). Soybean oil makes up 94% of the soybean food ingredients consumed by humans. Soy-based foods make up about 1.5% of an infant's diet and about 0.8% of a child's (ages 1 - 6) diet. Of the soy-based foods consumed by infants and children, the oil makes up about 80% and 97% of the total, respectively. Infants consume 20% of their soy foods as soybean flake (flour) products. In Japan, the daily consumption of soybean based products is 69.9 g per day, including tofu, miso and others. The per person consumption of oils including soybean oil is 8.6 g per day. Soybean oil and lecithin are used in all types of infant formulas, while soy protein isolated from soybean flour is used in the non-dairy lactose-free formulations. The amino acid and fatty acid profiles of infant formulas are very important. The essential amino acids required by humans are present in soybean proteins (American Soybean Association, 1996). Soy protein products are also added to a number of meat, dairy, bakery and cereal products as protein extenders (Liu, 1999). This usage accounts for the small amount of soybean flour, 1.9% of the total protein (USDA, 2000), consumed by people other than infants. Soy sprouts, baked soybeans, roasted soybeans, full fat soy flour and traditional soy foods (miso, soy milk, soy sauce and tofu) round out the soy-based products present in the human diet.

B. Identification of Key Products and Suggested Analysis for New Varieties

It appears scientifically justified to limit the testing to three products: seed, oil and isolated soy protein. The suggested key nutritional and anti-nutritional parameters to be analysed in soybean matrices for human food use are shown in Table 9.

Table 9: Suggested Nutritional and Compositional Parameters to be Analysed in Soybean Matrices for Human Food Use

| Parameter | Seed | Soybean Oil | Isolated Soy Protein | Lecithin |
|--------------------|------|-------------|----------------------|----------|
| Proximate analysis | X | | | |
| Amino acids | X | | X | |
| Fatty acids | X | X | | |
| Phytic acid | X | | | |
| Trypsin inhibitors | X | | | |
| Lectins | X | | | |
| Isoflavones | X | | | |
| Phosphatides | | | | X |

Section V –Feed Use

A. Identification of Key Soybean Products Consumed by Animals

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 a 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. These products are usually limited to about 10% of the diet (Thacker and Kirkwood, 1990) and are used commonly as feed in some European countries. The main soybean product fed to animals is the defatted/toasted soybean meal. 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).

The U.S. Environmental Protection Agency (EPA) has estimated the maximum possible contribution of soybean products to the diets of beef cattle, dairy cattle, poultry, and swine. These estimates were made with the intent to determine the maximum possible consumption of the soy products and are not an indication of the actual amount of usage. The maximum possible inclusion in animal diets for each fraction is shown in Table 10.

Table 10: Estimated Maximum Possible Inclusion of Soybean Fractions to Animal Feeds¹

| Soybean Fraction | Maximum contribution to the Animal Diet, % | | | |
|---------------------------|--|--------------|---------|-------|
| | Beef cattle | Dairy Cattle | Poultry | Swine |
| Aspirated Grain Fractions | 20 | 20 | - | 20 |
| Forage | 30 | 30 | - | - |
| Hay | 30 | 30 | - | - |
| Hulls | 20 | 20 | 20 | - |
| Meal | 15 | 15 | 40 | 25 |
| Seed | 15 | 15 | 20 | 25 |
| Silage | 30 | 30 | - | - |

¹ United States Environmental Protection Agency. 1996. Residue Chemistry Test Guidelines, OPPTS 860.1000. EPA 712-C-96-169 August 1996.

European considerations for the inclusion rate of seeds in swine diets are less than estimated by the EPA table. The soybean product should not be included at a rate higher than that supplying 2% soybean oil in order to guarantee a high bacon quality (<12 - 15 % polyenic fatty acids of total fatty acids in bacon).

Also, in Europe, feeding recommendations are that the incorporation rate of soybeans in swine feed should not exceed 10%. Also, due to the high content of polyenic fatty acids (soybean oil contains 50 - 70%), restriction of seeds seems to be necessary for dairy cattle too, because rumen fermentation is disturbed, resulting in decreased milk fat content and an increase in the trans-fatty acid concentration of milk fat. It has been suggested that the amount of polyenic fatty acids in dairy cattle diets be limited to 200 g/day (About 1% of the dry matter of the diet). Under consideration of a certain supply, with polyenic fatty acids from the basal components of the rations (about 100 g/day) maximum amounts of 1 kg soybean seeds (about 5% of total DM of the diet) are adequate to be incorporated in rations of dairy cattle. Further, 15% of soybean seeds in beef cattle diets may be too high as the high portions of trans-fatty acid could affect body fat composition and firmness.

B. Identification of Key Products and Suggested Analysis for New Varieties

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 of the fibre has been removed from the 49% product. Soybean meal contains relatively high levels of certain essential amino acids that are deficient in many other common feedstuffs. Essential amino acids¹ must be added to the diet as the animal cannot produce them at all, or in enough quantities sufficient to meet its physiological need. When soybean meal is used as a protein supplement in swine diets which are usually corn-based, the methionine content of the meal is the first consideration, since methionine is the most limiting essential amino acid in corn. However, when the meal is used as a protein supplement in poultry diets where lysine is the first limiting essential amino acid, meal lysine content is the first consideration. In cattle and sheep, where microbial protein from the rumen has been considered the primary protein source for the animal, there is increased interest in proteins that escape rumen fermentation, particularly in high producing dairy cattle. Thus, nutritionists are taking a closer look at the potential for cattle to also have certain limiting amino acids. Methionine, lysine, phenylalanine, and threonine have been suggested as being limiting amino acids for cattle.

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.

There is general agreement that the trypsin inhibitors are the primary soybean antinutrients 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. Isoflavones do not appear to be concerns when soybean meal is used in formulating livestock diets.

When one considers 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 for all the products

1. The essential amino acids are very important constituents of the soybean and its meal. The ten essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Cystine is also an important amino acid as it can partially substitute for methionine.

that could be fed. The suggested nutritional and compositional parameters to be analysed in soybean matrices for animal feed use are shown in Table 11.

Table 11: Suggested Nutritional and Compositional Parameters to be Analysed in Soybean Matrices for Animal Feed Use

| Parameter | Seed | Meal | Forage |
|------------------------|------|------|--------|
| Proximate ¹ | X | X | X |
| Amino acids | X | X | |
| Fatty acids | X | | |
| Phytic acid | X | X | |
| Trypsin inhibitors | X | X | |
| Lectins | X | | |

¹ ADF and NDF should be substituted for crude fibre.

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