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Soya, Human Nutrition and Health

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1. Introduction

Popular advice for healthy diets that may promote health and longevity include the daily consumption of at least three servings of fruits or vegetables and the variation of foods to include items derived from different plants and those plants should belong to different botanical families (Thompson H.J. et al., 1999).

Ancient civilisations in the Middle East and in America included grain legumes and cereals in well-balanced diets. In the funeral offerings found in the Egyptian pyramids various legume seeds were present, including lentils and grass pea. Apparently, legumes were a food of special consideration to be offered to kings, in contrast to the present day reputation of being the meat of the poor, with 75% of all legumes now being produced in developing countries. Excavations of ancient settlements indicate the use of both cereals and legumes (Mahler-Slasky & Kislev, 2010). A well balanced food basket promoting health and strength may have given an evolutionary advantage. The benefits of legume cultivation for soil fertility were already recognised in the 4th century BC (Flint-Hamilton, 1999).

Legumes are important factors in the natural cycle of nitrogen, being able to fix atmospheric nitrogen in symbiosis with *Rhizobium* bacteria. This enables the leguminous plants to thrive on poor soil, which makes them essential partners in the maintenance of soil fertility, and to produce protein-rich seeds. However, maintenance of optimum rates of nitrogen fixation requires continued attention by plant breeders (Provorov & Tikhonovich, 2003).

Legumes are also unusually diverse in their defence against predators by producing a large array of secondary metabolites forming their chemical armoury. Those metabolites include anti-nutrients such as inhibitors of digestion and compounds interfering with predator's metabolism reaching as far as brain function and hormonal control (Rozaan et al., 2000). Interestingly, some of these metabolites are beneficial by their inhibition of human cancer cells or by antioxidant activity that can delay ageing.

Although legumes have many beneficial properties, they are not a well balanced food by themselves because of deficiencies in some essential amino acids, and should not be the sole component of the food basket. In combination with cereals that are richer in those essential amino acids which are deficient in legumes such as methionine, cysteine and tryptophan, legumes are beneficial for human health and for the world's ecology. The optimum protein quality is approximated when 60-70% cereals are mixed with 30-40% cooked legumes. This would produce a combined quality of protein comparable with meat (Bressani & Elias, 1974).

Deficiency of the sulphur amino acids methionine and cysteine in the diet during extended periods can have detrimental health effect. Cassava roots (*Manihot esculenta*) and grass pea seeds (*Lathyrus sativus*) are used as staple food, especially in drought prone areas of Asia and Africa. Both foodstuffs are notorious for their deficiency in essential sulphur amino acids and overconsumption during an extended period in unbalanced diets can result in irreversible crippling neurodegenerative diseases konzo or neurolathyrism (Bradbury & Lambein, 2011). Oxidative stress is an important factor in the aetiology of these diseases.

Both germination and fermentation have positive effects on the nutritional quality of legumes. Anti-nutritional factors such as trypsin and chymotrypsin inhibitors and the galactosidases as flatulence factors are reduced by germination or fermentation. Germination also reduces the level of fats and carbohydrates while increasing the dietary fibres and vitamins (Vidal-Valverde et al., 2002). Fermentation can increase the amino acid score as the micro-organisms involved can also produce essential amino acids. In some cases toxins present in the seeds can be reduced by fermentation (Kuo et al., 2000). Grain legumes are well known to contain deficient levels of sulphur amino acids. While this fact is widely recognised, a closer examination of several groups of antinutritional factors reveals that many of these factors target metabolic systems involving sulphur amino acids in their predators (Enneking, 2011).

Proteins that act as anti-nutritional factors such as trypsin inhibitor, chymotrypsin inhibitor and alpha-amylase inhibitor are destroyed by simple cooking (moist heat treatment) and only pose a problem when the seeds are consumed raw or are cooked insufficiently. Soybean seedlings (or more often mungbean seedlings sold as soy sprouts) are consumed raw in salads in Western cuisine, while in the East, from where the practice originated, seedlings are always heated either by deep-frying or in a wok before consumption.

The present chapter gives an overview of health and nutritional aspects of soybean for human consumption and the general description of soy foods. The potential of genetically improved varieties of soybean (GM soy) and the hurdles for its commercialization in Europe and the rest of the world are discussed.

2. Soybean, the healthiest legume

Soybean (*Glycine max*) is the legume with the highest amino acid score and closest to the standard set by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). The content of the sulphur amino acids methionine and cysteine is double when compared to grass pea (*Lathyrus sativus*), the commercial legume with the lowest content of these essential amino acids (Kuo et al., 1995). Nevertheless, American farmers are spending an estimated 100 million dollars each year to supplement the soybean based feed with synthetic methionine for optimal performance by their domestic animals (Imsande, 2001). Compared to other grain legumes, soybean produces the highest yields of seeds per ha. The reason for this may in part be the allocation of better land for soybean than for other cheaper pulses.

The recent update of the 'dietary guidelines', the US-government's evidence based nutritional guidance to promote health and reduce the risk for chronic diseases and the prevalence of obesity through improved nutrition, recommends increasing soy intake as fortified beverages and other soy products (USDA, 2010). An industry-sponsored Newsletter 'Soy & Health' is devoted to the popularization of information on soy foods (Soy & Health, 2011).

The main advantage of soybean for human health, besides the nutritional value provided by its energy and protein content, is the high level of isoflavones genistein and daidzein present in the seeds. These secondary metabolites play a role in the symbiosis of the plant with *Rhizobium* but also have beneficial effects on human health. The concentration of these isoflavones in soybean is several orders of magnitude higher than in other commercial legumes. This includes the mungbean seedlings that are marketed as soybean sprouts in some European countries.

Soybean isoflavones genistein and daidzein are used in medicine at daily doses of 50 mg for the prevention of prostate cancer and breast cancer. This is the equivalent of about 50 g of soybean products or about 30 kg of other legumes (Liggins et al., 2000). Isoflavones are also antioxidants that can regenerate vitamins E and C, and are considered as a factor in the longevity of people who regularly consume soybean products in various forms. In some Asian countries with high life expectancy, the daily intake of soy-isoflavones is estimated at 50-100 mg. These soy-products include the seeds used in various recipes, soybean milk, many products made from precipitated protein such as tofu, fermented foods such as soy sauce, tempeh and also the germinated seeds. Especially the incidence of pre-menopausal breast cancer is reduced by high consumption of soybean products during adolescence and adulthood (Lee et al., 2009). It is well established that the incidence of prostate cancer is reduced in populations regularly consuming soy products. In vitro, genistein inhibits a wide range of cancer cells and inhibits several critical enzymes involved in signal transduction (Messina et al., 1994). Although more controversial, the regular consumption of soy products also seems to lower the incidence of type 2 diabetes, at least in some populations (Villegas et al., 2008).

2.1 Nutritional benefits of soya

Soybean (*Glycine max*) is one of the most important crops worldwide for producing oil and protein.

The seeds of the soya plant are an excellent source of macronutrients (protein, carbohydrate and lipid). Its composition may vary depending on the varieties and the growing conditions. The proximate composition of the whole seed of soybean, soy milk, tofu and soy sprouts is shown in Table 1.

2.1.1 Protein

Characterization. As in all legumes, globulins are the bulk of soybean proteins. Based on sedimentation, four major fractions can be distinguished:

- β -conglycinin, the principal component and sugar containing globulin;
- glycinin, the principal protein of soybeans;
- trypsin inhibitors: Soybeans contain two types of trypsin inhibitors: Kunitz trypsin inhibitor (KTI) and Bowman Birk inhibitor. KTI is reported to be one of the abundant anti-nutritional proteins in soybean seeds and it has been characterized as a food allergen in humans (Quirce et al., 2002).
- enzymes and hemagglutinins: lipoxygenase is technologically the most important enzyme found in soybean because it catalyses the oxidation of the poly-unsaturated fatty acid by molecular oxygen, leading to the development of rancidity and beany flavor. Hemagglutinins (lectins), carbohydrate-binding proteins, have been found to exert specific physiological effects as antinutritional protein factors (Natarajan, 2010) but they are easily inactivated by heat.

per 100 g	Soybean	Soy milk	Tofu	Soy sprouts
Proteins (g)	38.0	3.7	12.0	5.5
Fat (total) (g)	18.0	2.2	7.0	1.0
saturated fatty acids (g)	2.5	0.4	-	-
mono-unsaturated fatty acids (g)	4.0	0.5	-	-
poly-unsaturated fatty acids (g)	10.7	1.3	-	-
-linoleic acid (Ω-6) (g)	9.8	1.2	-	-
-alpha-linolenic acid (Ω-3) (g)	0.9	0.2	-	-
Carbohydrates (g)	6.3	2.8	1.0	4.7
Fibers (g)	22.0	0.6	-	2.38
Calcium (mg)	201	120.0	87.0	32.0
Magnesium (mg)	220	-	99.0	19.0
Potassium (mg)	-	-	94.0	235.0
Vit B2 (mg)	-	0.2	-	-

Table 1. Overview of the nutritional composition of soybean, soymilk, tofu and soy sprouts (Adapted from: <http://www.sojanet.com/nutritional-benefits.html#c3501>)

β -conglycinin and glycinin are storage proteins found in the protein bodies within the cells of the cotyledons. Severe allergic reactions have been reported after human consumption of those soy proteins (Bardare et al., 1988). The main allergens found in soy protein are Gly m Bd 60K (a subunit of β -conglycinin), Gly m Bd 30K (previously described as the 34-KD vacuolar protein, P34) and Gly m Bd 28K (Ballmer-Weber et al., 2007).

Besides these allergens, soybeans like other legumes contain a number of anti-nutritional factors such as trypsin and chymotrypsin inhibitors. These enzyme inhibitors are associated with the depletion of sulfur amino acids in the consumer’s metabolism. A significant portion of the low cysteine content in grain legume seeds can be found in proteins designed to be active in hostile extracellular environments, where cysteine functions to stabilize these proteins by disulfide bridges. This cysteine depletion is enhanced by these digestive enzymes and manifests itself by an enlargement of the pancreas (pancreatic hypertrophy) and a depletion of sulfur amino acids, since digestive enzymes also require elevated levels of cysteine for stabilization in a hostile extra-cellular environment (Enneking, 2011). Generally, these stabilized proteins can be destroyed by moist heat during food preparation. However, depending on the effectiveness of the heat treatment [heat, moisture, time], some activity, particularly for the chymotrypsin inhibitors, may remain.

Nutritional value. The proximate protein content of soybean is very high (38%) but it is not only the total amount of protein that is important; the quality of the protein also has to be taken into account. All eight essential amino acids which are necessary for human nutrition and are not produced naturally in the body are found in soybean protein. When comparing the essential amino acids of soybean with those from the reference protein (FAO/WHO), methionine and cysteine (sulfur containing amino acids) are the limiting factor with a chemical score of 47, compared to 100 for an ideal protein for human nutrition. While sulfur amino acids are the limiting factor in soybean, lysine content is very high, and that makes soybean an excellent complement for cereals that are deficient in lysine but excellent sources of S-containing amino acids (Lambein et al., 2005).

The chemical score alone is not sufficient to evaluate the protein quality since it does not take into account the digestibility and the biological availability of the amino acid. The protein in most soybean products has a Protein Digestibility Corrected Amino Acid Score

(PDCAAS) that approaches 1.0 for soy protein and 0.92 for whole soybeans, indicating that both amino acid pattern and digestibility of soya protein are excellent for human nutrition. Consuming soya foods is an excellent way to increase the protein content of the diet. Although high protein diets may increase the risk of developing kidney disease in susceptible individuals, some studies indicate that soya protein favorably affects renal function in comparison to animal proteins (Sarwar, 1997).

	Soybean	FAO/WHO reference protein
ILE	4.6	4
LEU	7.9	7
LYS	6.5	5.5
MET + CYS	2.6	3.5
PHE + TYR	8.2	6
THR	3.9	4
TRY	1.3	1
VAL	4.9	5

Table 2. Soybean Essential Amino acids composition as % of total amino acids versus FAO/WHO reference protein (adapted from Lecerf & Fressin, 1995)

2.1.2 Lipids

The lipids (crude oil) content of soybeans is approximately 20% which consists of:

- Triglycerides representing about 96 % of the soybean lipids
- Phospholipids or lecithins (2%) which are used in medical and food industries as emulsifiers
- Unsaponifiable lipids (1.6 %) mainly tocopherols (Vit E) and sterols
- Free Fatty acids (0.5 %)

Soybean contains a heart-healthy balance of fatty acids, high in mono and polyunsaturated fatty acids (80 % of total fatty acids primarily in the form of linoleic acid) and low in saturated fatty acids (20 %) (Kris-Etherton et al., 1988). Linoleic acid, when substituting saturated fatty acids in the diet reduces blood cholesterol levels. Furthermore, soybean is one of the few good plant sources of the essential fatty acid, alpha-linolenic acid (ALA), an omega-3 fatty acid that may have independent coronary benefits (See the fatty acid profile of soybean in Table 3).

Fatty acid	%
Lauric acid (C12:0)	4.5
Myristic acid (C14: 0)	4.5
Palmitic acid (C16:0)	11.6
Stearic acid (C18:0)	2.5
Oleic acid (C18:1)	21.1
Linoleic acid (C18:2), Ω-6	52.4
Linolenic acid (C18:3), Ω-3	7.1

Table 3. Fatty acid profile of soybean oil (adapted from Weber et al., 2007).

The health claim that “Intake of 25 grams of soya protein a day, as part of a diet low in saturated fat, may reduce the risk of heart disease” has been approved by the American Food and Drug Administration and the British Joint Health Claim Initiative. Moreover, the important role of soy foods in displacing higher-saturated-fat foods from the diet, thereby helping to lower blood cholesterol levels was highlighted by the American Heart Association (Sacks et al., 2006). The cholesterol lowering effect of the poly-unsaturated fatty acids of soybean is mainly affecting the LDL-cholesterol, and also decreases the triglyceride level. Indeed, the excellent fatty acid composition of soya offers various advantages.

2.1.3 Carbohydrates

The total carbohydrate content of soybean is about 30%. Unlike other beans, soya is low in high molecular weight carbohydrates in soya. Soybeans contain 10-13% soluble carbohydrates of which sugars (sucrose, fructose, saccharose, raffinose and stachyose) represent 10-12% and starch 1%. Raffinose and stachyose are tri and tetrasacchide, respectively which are not broken down by human digestive enzymes but by the bacteria present in the small intestine with production of intestinal gas (flatulence). Soybean also contains 18% fibers, a mixture of cellulosic and noncellulosic structural components (cellulose, hemicellulose and pectin substances).

Considerable efforts are underway to further improve the nutritional quality of this healthy grain legume. For example of the 3 major oligosaccharides present in soybean only sucrose can be digested by monogastric animals. As stachyose and raffinose are indigestible and are considered as anti-nutritional factors which cause a reduction of energy in the diet and flatulence, research has focused on their removal from soybean meals. Recently, alleles of soybean raffinose synthase genes have been identified which are associated with a low stachyose and raffinose content in soybean plants and seeds. This identification in combination with molecular marker breeding is a promising tool to introduce the trait in soybean varieties (Dierking & Bilyeu, 2008; Schillinger et al., 2010). In cases where traditional breeding techniques have not yet succeeded, genetic transformation can be the method of choice for this endeavour.

2.1.4 Minerals

The mineral content of soybeans is about 5%. Major constituents are calcium, potassium and magnesium. Soybean milk is poor in calcium, only 12 mg/100 g. But the content of soya drinks is always enriched with calcium to be comparable to cow's milk (Zhao et al., 2005). Iron, copper and zinc are other minerals that are present in soybeans as trace elements.

2.2 Uses of soybeans as food

Raw soybeans cannot be eaten as such because of the presence of trypsin inhibitors that can disrupt digestion activities in the stomach, leading to cramps and associated discomfort. (Mccue & Shetty, 2004).

Soybean foods are typically divided into three categories: non-fermented, fermented and fortified.

Traditional non-fermented soya foods include fresh green soybeans, whole dry soybeans, soy nuts, soy sprouts, whole-fat soy flour, soymilk and soymilk products, tofu, okara and yuba.

Traditional fermented soy foods include tempeh, miso, soy sauces, natto and fermented tofu and soymilk products.

General description		Uses
Nonfermented soya foods		
Soy milk	Heated water extract of soybeans after grinding and filtration. Resembles dairy milk	Served hot or cold, as breakfast, beverage, or with other foods
Tofu	White protein curd precipitated from soya milk with salt or acid	Cooked with or without meat, vegetable, and seasonings and served as a main dish or soup
Soy sprouts	Germinated soybeans in dark yellow cotyledons with white sprouts	Cooked as vegetable or in soup
Soy film – Yuba	Creamy, yellowish protein-lipid film formed from surface of boiling soya drink. Sheets, sticks, or flakes	As delicacy, cooked with meat or vegetables, or in soups
Edamame	Green immature soybeans	Cooked in pod or pod removed. Served as snack or vegetable
Roasted soya beans	Dry roasted soy beans, seasoned or non-seasoned.	As a snack or made into powder.
Fermented oriental soya Foods		
Miso	Heated water extract of soybeans after grinding and filtration. Resembles dairy milk	All purpose seasoning for dishes or soups
Natto	Cooked whole soya beans fermented with <i>Bacillus natto</i> . Soft beans covered by viscous, sticky polymer, distinct aroma.	Seasoned and eaten with cooked rice.
Tempeh	Cooked and dehulled soya beans fermented with <i>Rhizopus oligosporus</i> . Soft beans bound by white mycelia, cakelike, nutty flavor	Fried or cooked as part of meal, snack, or in soups.
Shoyu (Soya sauce)	Dark-brown liquid extracted from a fermented mixture of soybeans and wheat and is based on the use of the <i>Aspergillus oryzae</i> strain.	All-purpose seasoning

Table 4. Different soya foods (Source: <http://www.sojanet.com/the-plant-the-food/different-soya-food.html>)

Fortified soy foods are frequently used in humanitarian programs to prevent and address nutritional deficiencies, partially precooked and milled soybean is used to provide protein in **Corn Soya Blend** (CSB), the main fortified blended food distributed to affected people but sometimes soya is also mixed with wheat in Wheat Soya Blend (WSB). Those fortified blended foods are also generally used to provide extra micronutrients and to complement the general ration in Supplementary Feeding, Mother and Child Health programs and during disaster or emergency operations by the World Food Program (2006)

2.3 Soybean sprouts or mung bean sprouts?

Sprouts of mung bean (*Vigna radiata*, called green gram in India, green bean in China) are often marketed as ‘soy sprouts’, creating confusion among nutritionists. In the East, mung bean and soybean sprouts are sold side by side and correctly labeled. In the West, the real

soybean sprouts are rare and mung bean sprouts are labeled as soy sprouts. Mung beans are easier to sprout, cheaper and more widely available than soy sprouts. Real soybean sprouts are much richer in the isoflavones genistein and daidzein and also have more protein than any other sprouts plus an appealing flavor and taste. Health conscious consumers with the intention to lower the risks for some cancers by consumption of high isoflavone containing foodstuffs are in fact cheated by this mislabeling. For sprouting purposes, small-seeded soybeans are preferred because of the relatively thicker seed coat than the large-seeded soybeans and also because of the higher rate of germination. One gram of dry beans yields about 8-10 grams of sprouts after 4-5 days of germination. The sprout (hypocotyl) is then about 3 cm long. In mung bean the cotyledons shrink after one week germination and the first leaves become visible. The cotyledons of soybean sprouts are bigger and more yellow. Although the level of anti-nutritional factors such as trypsin inhibitors is reduced during germination, while mungbean sprouts are often used in Western cuisine uncooked together with a variety of other sprouts, in the East the sprouts are always boiled in soups or fried in a wok. Unfortunately, prolonged heat also destroys some of the vitamin C (Wai et al., 1947). According to the history book by Gavin Menzies (2002) soybean was one of the foods taken on long ocean voyages by the Chinese in 1421, and sprouted on board. This may have protected those seamen from vitamin deficiency related diseases. Indeed, germination is a simple post-harvest processing technique that increases the levels of riboflavin by up to 642%, niacin by up to 443% and ascorbic acid by up to 467% (Kaushik et al., 2010).

Fig. 1. Comparison of sprouts of mungbean (*Vigna radiate*) and soybean (*Glycine max*) (left picture), with a commercial product of mungbean sprouts sold as soysprouts, (right picture). Notice the shrunken cotyledons in the commercial product and the intact yellow cotyledons in the soybean sprouts.

An early detailed scientific report was published by Schulze in 1889 "On Some Nitrogen-Containing Constituents in Soy Sprouts (or Etiolated Soy Shoots)" (cited in Shurtleff & Aoyagi, 2011). These observations were more botanical than culinary and were done with soybeans germinating in a field. Although the culinary use of soybean sprouts has an almost one thousand year tradition in China, the first known reference to soy sprouts for food use was by Li Yu-Ying in 1910 and 1912 (cited in Shurtleff & Aoyagi, 2011). By late 1910 or early 1911, he was producing soy sprouts in his soy foods plant near Paris. A detailed history of soy seedling production and consumption is described by Shurtleff and Aoyagi (2011). Bau et al. (1997) found that the trypsin inhibitor activity of soybeans was reduced by 30% during

sprouting and that the vitamin C content increased from 0 to 25 mg/100 gm. Fernandez-Orozco et al. (2008) compared the antioxidant capacity in soybean sprouts and mung bean sprouts and found a greater increase during sprouting of mung bean than in soybean, while the total tocoferol (vit E) content increased much more in soybean sprouts.

3. GM soy

3.1 History

Since the introduction of genetically modified (GM) crops in the nineties GM soy has been one of the most prominently cultivated GM crops. The first traits to be introduced in soy by means of genetic engineering were herbicide resistance to glyphosate and glufosinate. The GM soy plant acquires glyphosate resistance through the action of a genetically introduced enzyme from bacterial origin (Thompson C.J. et al., 1987) that acts as an inhibitor to the herbicide. Glyphosate (N-(phosphonomethyl)glycine), inhibits the 5-enylpyruvylshikimate-3-phosphate synthase (EPSPS) of plants and certain bacteria. This enzyme catalyses a key step in the synthesis pathway of the aromatic acids, hormones, plant metabolites, lignins and other phenolic compounds (Dill, 2005). Glufosinate (dl-homoalanin-4-yl(methyl)phosphinic acid) on the other hand inhibits glutamine synthase, required for the conversion of L-glutamic acid to L-glutamine in the presence of ammonia. Blocking this pathway in plants results in toxic ammonia levels and cell death (Duke, 1990).

Monsanto and Bayer CropScience have commercially developed and launched respectively GM glyphosate and glufosinate resistant soy lines, both of which have been approved for human and animal consumption. Since its first commercial cultivation in the US in 1996, Roundup Ready soy developed by Monsanto still is the principal biotech crop at global scale. The total herbicide resistant GM soybean area is cultivated on 73.3 million hectares, representing 50% of the total area of GM crops in the top-ten biotech crops. Countries that are growing GM soy are the USA, Brazil, Argentina, Paraguay, Uruguay, Bolivia, Mexico, Chile, and Costa Rica. In Paraguay and Bolivia GM soy accounts even for the total area of GM crops (James, 2010).

Herbicide tolerance is the only GM soybean trait that has been commercialized to date (see Table 5). Worldwide, only 3 different events of genetic modification can be found, commonly known as Roundup ready soy and Roundup ready 2 soy (Monsanto Company), both resistant to glyphosate, and Liberty Link soy (Bayer Crop Science) which confers resistance to glufosinate. Eleven additional GM soybean events have received regulatory approval in at least one country and are ready for commercialization. Amongst these are also the first GM soybeans modified to increase the value for industry by having an increased oleic acid content, and which are expected to be ready for global use by 2012 (Plenish, Dupont). Three other lines (Optimum GAT, Cultivance, Bt Roundup Ready 2 Yield) are also expected to be commercially launched within the next two to five years (Dupont, BASF Plant Science and Monsanto Company). Other GM soybean lines like 260-05 (Dupont), W62, W98, GU262 (Bayer CropScience) have already been regulatory approved in the nineties but have not yet been commercialized (James, 2010; Stein and Rodriguez-Cerezo, 2009).

3.2 Biotech soy in the pipeline

Besides the first generation of traits that have been commercialized in GM soybean, many other GM varieties with interesting traits have been developed in the lab, most of them in the private sector. Remarkably most of the notifications that have been submitted but have

not yet made it to the market. Four new lines are in advanced research phases, while 6 lines are pending regulatory approval in at least one country resulting in a total of 10 lines that could join the market within the coming years (Stein and Rodriguez-Cerezo, 2009, United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS), 2011) (see Table 5). So far only one stacked (with more than one genetic modification event) soybean variety, Dupont's DP305423 x GTS40-30-02, has been regulatory approved and is ready for commercialization (see Table 5). If in addition, all available single events would be combined into stacked varieties, this would result in a theoretical market of over 100 GM soybean lines (Stein and Rodriguez-Cerezo, 2009). Besides new herbicide tolerant soybean events, two new nutritionally enhanced soybean lines (Monsanto Company) are in the process of regularization in February 2011 (see Table 6). SDA omega-3 soybeans are developed to produce oil rich in omega-3 fatty acids, while the oil end product derived from Vistive Gold soybeans would contain levels of mono-unsaturated fatty acids similar to olive oil (Monsanto).

3.3 Nutritional fortification strategies

Biotechnology with its current high throughput bioinformatics technologies such as genomics, proteomics, phenomics, and metabolomics offers even more potential for developing useful GM crops. Recently the 1.1 gigabase soybean genome has been completely sequenced. This will allow the association of mapped phenotype effectors with the causal DNA sequence of important crop production traits and those important to seed quality for human nutrition, animal production, and biofuel production (Schmutz et al., 2010).

Apart from GM soy for industrial application, nutritionally ameliorated or enhanced GM soy will be the next generation. Despite the widespread use of soybeans and derived products in food and feed, associated nutritional problems remain an issue. Soybean contains multiple allergenic proteins: Gly m Bd 60 K, Gly m Bd 30 K, and Gly m Bd 28 K are the 3 main soy proteins triggering allergic reactions. Since mutagenesis and breeding could not reduce or remove all three allergens, genetic engineering was used to address removal of Gly m Bd 30 K by transgene-induced silencing of the corresponding gene. This approach can allow sensitive individuals to make use of soybean products in the future (Herman et al., 2003).

In addition to allergenicity, amino acid deficiencies are known to limit soybean nutritional quality as methionine, cysteine and threonine levels are below FAO/WHO recommended values. Genetic engineering could provide a solution for these issues by designing nutritionally fortified soybeans. Despite the fact that these amino acids can be supplemented in the diet, a more attractive solution would be to produce soybeans with an improved amino acid profile. One successful approach to increase the methionine content of soybean was the introduction of the Brazil nut albumin gene. However, as the Brazil nut albumin was identified as a major allergen, this product was not further developed and new approaches were explored (Clarke & Wiseman, 2000). A successful approach is the introduction of cereal storage proteins rich in methionine and cysteine (Li et al., 2005).

Although soy is a known phosphorus source, most phosphorus is present as phytic acid, which cannot be digested by monogastric animals such as poultry and swine. This does not only result in non-optimal levels of phosphorus and minerals, but also contributes to wastewater pollution. As supplementation is costly and seems to contribute to the pollution

problem, one might prefer to make use of a soybean strain expressing a bacterial phytase, resulting in the production of utilizable inorganic phosphorus (Bilyeu et al., 2008). Soybeans are also a known source of vitamin E and folate. Although vitamin E deficiency in humans does not result in a typical disease state or disorder, it might lead to an increased risk of atherosclerosis and other degenerative diseases (Bramley et al., 2000). Folate deficiency is known to cause anemia, high levels of plasma homocysteine hereby increasing the risk for

Product name	Developer	Trait	Status of commercialization
<i>Commercialized</i>			
Roundup Ready	Monsanto	Herbicide tolerance (to glyphosate)	commercialized
Genuity Roundup Ready 2 Yield	Monsanto	Herbicide tolerance (to glyphosate)	commercialized
Liberty Link (event A2704-12)	Bayer CropScience	Herbicide tolerance (to glufosinate)	commercialized
<i>Authorized in at least 1 country, ready for commercialization</i>			
260-05	Dupont	Crop composition (altered fatty acid and oil content)	not commercialized
Liberty Link (event A5547-127)	Bayer CropScience	Herbicide tolerance (to glufosinate)	2012
Optimum GAT	Pioneer Hi Bred	Herbicide tolerance (to ALS inhibitors and glyphosate)	2011-2012
Cultivance	BASF Plant Science and Embrapa	Herbicide tolerance (to imidazolinone)	2012
Bt Roundup Ready 2 Yield	Monsanto	Insect resistance and herbicide tolerance (to glyphosate)	2013
Plenish	Dupont	Crop composition (high oleic acid content)	2012
MON87701	Monsanto	Insect resistance (to Lepidoptera)	n/a
DP305423 x GTS40-30-02	Dupont	Crop composition (altered fatty acid and oil content) and herbicide tolerance	n/a
W62	Bayer CropScience	Herbicide tolerance (to glufosinate)	not commercialized
W98	Bayer CropScience	Herbicide tolerance (to glufosinate)	not commercialized
GU262	Bayer CropScience	Herbicide tolerance (to glufosinate)	not commercialized

Table 5. Commercial status of authorized GM soybeans worldwide (n/a: not available)

Product name	Developer	Trait
<i>Pending regulatory approval</i>		
n/a	n/a (China)	Insect resistance
SDA Omega 3	Monsanto	Crop composition (stearidonic acid content)
Vistive Gold	Monsanto	Crop composition (improved fatty acid profile)
Dicamba tolerant	Monsanto	Herbicide tolerance (to dicamba)
DHT	Dow AgroSciences	Herbicide tolerance (to 2,4D and glufosinate)
n/a	Bayer CropScience	Herbicide tolerance (to isoxaflutole and glyphosate)
<i>Commercial R&D pipeline</i>		
n/a	Syngenta	Nematode resistance
n/a	Syngenta	Herbicide tolerance (to HPPD inhibitors)
n/a	Bayer CropsScience	Herbicide tolerance (to isoxaflutole and glufosinate)
n/a	Monsanto	Higher yield

Table 6. GM soybeans in the pipeline worldwide (n/a: not available)

cardiovascular disease or stroke and, in association with the maternal status, spina bifida in infants (Scott et al., 2000). Therefore it might be interesting to look for strategies to enhance vitamin E and/or folate synthesis and accumulation.

Soybean has several other interesting nutritional properties. Its high level of isoflavones as antioxidants and phytoestrogens, have received much attention because of their positive effect on osteoporosis, cardiovascular disease and cancer prevention (Cassidy et al., 1994; Messina, 1999; Setchell & Cassidy, 1999). Although the beneficial health effects of soy isoflavones are strongly being debated (Erdman et al., 2000; Sebastian, 2005), soybean consumption is considered a factor in the longevity of people in the Orient. One may think of increasing the isoflavone content in soy as an edible disease-preventing drug. Also the calcium bioavailability, naturally present in beans (Messina, 1999), could be the subject for a GM upgrade in soybeans. All together these data indicate that soybean has much potential for further development, which may result in the availability of a variety of nutritionally enhanced soybeans in the future as an important source of functional food.

3.4 Public acceptance

Many independent studies have been published demonstrating the positive agro-economic impact and socio-environmental effects of biotech crops, including GM soy, in different parts of the world (Brookes & Barfoot, 2009; Carpenter, 2010; James, 2010; Park et al., 2011; Trigo & Cap, 2006). In most countries GM soy accounts for the largest share of benefits, mainly due to the fact that soy bean is the major biotech crop with the largest percentage of

acreage. Direct and indirect benefits, with as major beneficiaries the growers and technology developers, include higher farmer incomes, job generation, lower price for the consumers and environmental benefits, e.g. less tilling, better second-cropping practices, better water, pesticide and diesel usage as well as CO₂ measures. Despite the fact that biotech crops are the most fast adopted technology in modern agriculture (James, 2010), and have clear benefits, the global public acceptance still tends to be rather low. Although approved GM varieties that went through the regulatory systems are proven to be equal in nutrient composition to, and as safe as their traditional counterparts in terms of allergenicity and toxicity and that no significant harm to human, animal health and the environment has been reported, the public at large is still very reluctant and not aware of potential benefits of GM crops. The public perception however does strongly vary between different continents and from country to country. Key findings from the last Eurobarometer on Life Sciences and Biotechnology (Gaskell et al., 2010) on the attitude of European citizens towards GM food show that the values of acceptance of GM food are much higher in Member States where the crops are grown, demonstrating a clear link between private attitudes and public policies.

When it comes to the adaption of novel, innovative and, more importantly, life-saving technologies such as GM crops, an interesting paradigm shift is occurring which most likely will be reinforced during the next decades. Out of the 29 biotech crop countries in 2010, only 10 were industrialized countries. Strikingly the lead developing countries for the cultivation of GM crops are China, India, Brazil, Argentina, and South-Africa (James, 2010), that are equally emerging and rapidly expanding economies. Developing countries are also predicted to exceed the industrial countries in percentage of biotech crops cultivated before 2015 (James, 2010). Developing countries with the largest acreages of GM crops are also amongst the biggest growers of GM soy.

3.5 EU policy in contrast to global policy development

The EU Policy, with its more than five year moratorium (1998-2004) on GM crops under the pressure of NGOs and the public opinion, has always been in strong contrast with the US Policy since the beginning of the biotech crops era. While the USDA allowed unlimited planting of GM herbicide resistant soy and recently has approved GM soy with a higher oleic acid content developed by DuPont Canada Agricultural Products, Romania even had to stop growing Roundup Ready soya after having entered the EU. Until today only two GM crops are approved for cultivation (Bt maize and Amflora potato). In addition the EU has adopted a zero-tolerance policy towards imports containing even trace amounts of non-approved GMO's, which can cause disruptions on the agricultural import and export market.

Based on the principle of 'Freedom of Choice' the EU has introduced its labeling policy for food stuff that has been produced with the aid of genetic engineering. The Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 concerns the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (<http://eur-lex.europa.eu>). Despite the fact that processing of raw material often renders identification of GM traces in the end product impossible, labeling depends on the origin of the ingredients of the product (GM or non-GM). As a result all soy products must be labeled if the raw material at least partially consisted of GM soybeans. Technical unavoidable or unintentional admixtures of regulatory approved GM content below the 0.9

% threshold limit value do not fall into this category. Baked goods (bread, biscuits and snacks) containing flour, for example, and/or oil from GM soybean, sweets containing lecithin from GM soy, and fat products such as margarine, vegetable oils, mayonnaise require labeling (For a complete overview see Table 7) (GMO compass, 2011).

Soy ingredient	Use	Processing and testing products for GM content	Labeling
Oils and fats	Margarine, vegetable oils, mayonnaise, etc.	Refining soy oil by heating to 120°C under a vacuum to remove solvent residues and other unwanted substances. DNA and proteins destroyed during the process to such an extent that no GM testing is possible.	Yes
Lecithin, other emulsifiers	Chocolate, desserts, baked goods, other processed foods	Lecithin is extracted from soy oil. GM trace identification maybe possible when using non-refined soy oil as a source.	Yes
Tocopherol/Vitamin E	In fatty foods to prevent oxidation, vitamin fortified products	By-product of plant oils. GM trace identification identical to lecithin.	Yes
Soy protein additives, soy isolate	Prepared foods (soups, sauces), meat substitutes, diet foods, imitation milk products	From roasted, de-oiled soy flakes. Detection possible, but more difficult along with the processing into the final product.	Yes
Soy meal, semolina flour	Bread, snacks, pasta	Similar to soy additives Baking destroys often traces of GM content	Yes
Hydrolyzed soy protein	Soy sauce, seasonings	Protein chemically changed by acids or enzymes DNA usually destroyed along the process.	Yes
Products from whole soybeans	Tofu, soy drinks, miso, soy flour	GM traces detectable in products derived from whole soybeans	Yes
Animal feed	Indirectly for animal products as meat, eggs and milk	Animals that have been fed or not with GM feed cannot be distinguished because all DNA (GM and non-GM) is degraded during digestion.	Feed: Yes, Animal products: No

Table 7. Overview soy-based food and feed (Adapted from GMO compass)

The EU imports each year 18 million tons of soybeans and 20 million tons of soy meal from Brazil, the US, and Argentina (www.gmo-compass.org), the largest GM soy producers. According to the EU Observer (www.euobserver.com) the EU imported more than 51 million tons of animal feed of which half was GM soy from Argentina and Brazil. Despite the high dependence of the EU livestock industry on feed import, in 2009 Europe blocked 180,000 tons of GM soy from the US as a result of its zero-tolerance policy. Although the GM soy was approved for import, traces of non-approved maize were detected (Wager & McHughen, 2010). The current situation of asynchronous approvals, where the EU has only two approved GM soy varieties and one ongoing renewal of authorization (EU register of genetically modified food and feed), while 5 new soybean events are ready for commercialization (see Table 6) might even worsen the situation for EU imports. The zero-tolerance policy could lead to a net shortage between 3.3 million (minimal impact scenario) and 25.7 million tons (worst case scenario) of soybean meal resulting in increased feed costs and meat prices for consumers (Wager & McHughen, 2010). Therefore, the EU committee recently voted for an easing of the labeling policy with the allowance of 0.1% unapproved GM material but only in animal feed imports. Concerns are however being raised that it will be extremely costly to separate the material according to intended use (Dunmore, 2011).

3.6 Costs of compliance with biotechnology regulations

Despite the great potential of GM crops, only a limited number of traits have been commercialized (James, 2010, Table 6). The major reason for this is, aside from the often lacking regulatory framework in developing countries, the regulatory costs related to bringing a GM product to the market. These costs can be the direct result of the necessary compliance with biosafety regulations or can arise from delays during the regulatory approval process (Bayer et al., 2010). While the average time to gain import approval for example in North America and Brazil is one and even less than one year respectively. Europe lags behind requiring an average of 3 years to authorize GM imports (Wager & McHughen, 2010). The regulatory compliance costs can vary from tens of thousands to millions of dollars (Van Montagu, 2010) depending on the country, the GM specific event and the intended use or the developer (Bayer et al., 2010). Direct compliance for herbicide-tolerant corn in top producing and importing countries including US, Canada, Argentina and the EU, can go up to \$15,510,000. Sixty percent of these costs arise from the production of tissues to perform the biosafety studies, the compositional assessment, protein production and characterization and the molecular characterization. These costs do not even include preregulatory safety assessments, which are considered as research or private compliance costs resulting from regulatory delays (Kalaitzandonakes et al., 2007). The whole process starting from discovery and research over validation to the market phase of a GM crop is estimated to cost between 52 and 100 million US\$ (Monsanto). The costly regulatory framework renders the marketing of GM crops far more expensive than traditionally bred crops leaving public institutions and spin-offs, especially in developing countries, merely out of the loop when it comes to commercializing GM crops. As a consequence, the development of GM crops is mostly in hands of large private companies which turn to commercially interesting crops as corn and soybean, and successful traits with broad applications. As such, orphan crops with non attractive returns of investments but of importance to local populations in least developed countries are easily left behind (Van Montagu, 2010). Crops that are considered a life-insurance during drought for poor subsistence farmers such as grass pea (*L. sativus*), but without global economic importance, are ignored.

One example of a public research institution project that faced a more than 12 years delay as a result of regulatory burdens is the Golden Rice. Golden Rice was developed in 1999 in the lab to produce vitamin A precursor by inserting two genes, phytoene synthase and phytoene double-desaturase, would provide a way to reduce vitamin A deficiencies amongst rice-dependent poor populations prone to night-blindness. Although it was ready in 1999, it will only be commercially launched in 2012. The delay faced was due to long time required for field-testing approval (more than 2 years) and subsequent data gathering for the regulatory dossier (more than 4 years). In addition, the project had to be taken up into a public-private partnership with Syngenta to move the product development forward (Potrykus, 2010).

3.7 Soybean as a life saver on marginal lands

Due to climate change and water shortage, addressing water scarcity in arid and semi-arid rural areas will be one of the priorities in the coming decades in order to secure lives and secure their livelihoods (WHO and UNICEF, 2006; FAO/UN-Water, 2007). Moreover a significant increase for water irrigation of agricultural lands has been predicted (Bruinsma, 2009). Water shortage is also an important restrictive factor for crop yield. In the case of soya, drought stress strongly reduces vegetative branch and reproductive growth (Frederik et al., 2001), increases the rate of pod abortion during early development stage (Liu et al., 2004) and can as a result limit the total yield by up to 40% (Specht et al., 1999). Several physiological and biochemical approaches can be used for the development of drought resistant soy bean varieties such as modulation of root related traits (root morphology and plasticity, nitrogen fixation) or shoot related traits (stomatal conductance, epidermal conductance, leaf pubescence density, water use efficiency, osmotic adjustment) and soybean seed and grain-filling practices. Progress in (marker assisted selection) breeding strategies has however been rather limited as the main focus for soybean breeding programs was on biotic resistance in the past. Moreover drought resistance is a complex trait not only by the genotype, but also by the environment and their interaction. A constant selective pressure is required to be able to directly select for this trait which is not easy to achieve when it comes to drought stress (Lakshmi et al., 2009). By breeding for example, two soy lines were achieved which had an improved symbiotic nitrogen fixation and a yield advantage in environments with moderate soil water shortage. Unfortunately this trait would not allow maintaining the yield gain under very severe drought conditions (Sinclair et al., 2007). Therefore, genetically introducing drought resistance into soy may also be a promising strategy to cope with environmental changes, soil erosion and population growth.

Drought tolerance in soy could be addressed by genetic engineering of regulatory genes, analogous to the examples obtained by introducing the transcription factors ZmNF-YB2 and SNAC1 in maize and rice (Hu et al., 2006; Nelson et al., 2007). A different approach could be to genetic engineer functional genes, as for example trehalose-6-phosphate synthase/phosphatase in rice resulting in the accumulation of trehalose and increased drought stress tolerance (Garg et al., 2002). Soybean was genetically engineered to over-express L- Δ 1-pyrroline-5-carboxylate reductase resulting in proline accumulation and improved drought stress tolerance (De Ronde et al., 2004). Additional research will be required to identify and use drought stress related genes for a successful engineering strategy.

An alternative interesting source for such abiotic stress resistance genes would be grass pea (*L. sativus*) that is the most environment tolerant legume that thrives on poor soil with

minimal inputs without irrigation and the most efficient nitrogen fixer among commercial legumes (Campbell, 1997). The plant is also considered for phyto-remediation (Brunet et al., 2009) and was suggested to help removing heavy radioactive metals from the human body after the Tchernobyl accident (pers. comm.). The plant is also more resistant to biotic and abiotic stress than other legumes and needs little or no inputs. It is considered a life-saver during droughts and famine in areas of Africa and Asia prone to droughts (Campbell, 1997). This controversial legume has been cultivated since the Neolithic era and is appreciated by farmers for its easy cultivation and for its production of tasty seeds when other crops fail due to drought. However, overconsumption during extended periods in socio-economic settings dominated by illiteracy and poverty can lead to irreversible crippling or neurolathyrism. Recent epidemiological research has identified protective factors as literacy, the use of at least one third of cereals in the diet and the addition of antioxidant-rich condiments such as onion, garlic and ginger to the grass pea preparations (Getahun et al., 2003, 2005). In a well balanced diet grass pea is harmless. However, because of its toxic reputation grass pea is a neglected crop that receives little attention from major research institutions. The genes for this important and sometimes live-saving environmental tolerance are not yet known. The possibility that this environmental tolerance is linked to the presence of the neuro-excitatory amino acid β -ODAP (β -N-oxalyl- α , β -diaminopropionic acid) cannot be ruled out. This unusual amino acid is a good chelator for copper and zinc and may play a role in phytoremediation (Brunet et al., 2008).

The alternative strategy could be to introduce the biosynthetic pathway for genistein into legumes more tolerant to environmental stress. Grass pea (*L. sativus*) could then be genetically improved to become a health promoting crop that at the same time can improve marginal soil with minimal ecological cost. Efforts to improve the nutritional quality of grass pea are now directed at the lowering or elimination of a neuroexcitant amino acid beta-ODAP and at the improvement of the amino acid score by increasing methionine and cysteine in the seeds (Vaz-Patto et al., 2006).

4. Conclusions

We can safely propagate that the presence of various preparations of soybean in a food basket containing also a variation of cereal products and vegetables belonging to other plant families than the legumes is beneficial for human health and for the world ecology. Soybean has particular and proven advantages on human health and longevity. The consumption of soybean products such as tofu and soymilk are becoming more popular in the West, but it is disturbing that mungbean seedlings continue to be mislabeled as soy sprouts, giving fundamentally wrong information to health conscious consumers.

Untreated grain legume seeds can contain significant levels of digestive enzyme inhibitors to cause harm in the consumer. Especially for the poor who can not afford a well-balanced diet, prolonged overconsumption of foods that are deficient in essential sulfur amino acids, such as legumes or cassava, can have detrimental medical consequences (Bradbury & Lambein, 2011).

Several high-level reports have demonstrated the need for higher food productivity in the coming years on less arable land (Foresight, 2011; The Royal Society, 2009). Especially those living in drought prone areas of Africa and Asia need the attention of the scientific community and the authorities to break the vicious cycle of malnutrition, illiteracy and underdevelopment. GM crops, including GM soy, have been recognized to have the

potential to considerably contribute to the alleviation and reduction of poverty through the provision of food, feed and fibre security (FAO International Technical Conference 2010; James, 2010). Soybean and other legumes are important contributors for maintaining soil fertility and sustain agricultural production through their ability to fix atmospheric nitrogen, which is the most expensive fertilizer consuming most energy for its production.

Due to its nutritional qualities, especially high oil and protein content, soy is part of many humanitarian programs as one of the most important grain legumes. With the use of modern genetic technologies, soy has even more potential as functional food and a complete food like the WHO/FAO reference food. The GMO-soy presently commercialized or in the pipeline is mostly directed at improving economic benefits for the producer while there is little or no attempt to improve the nutritional quality of the seed as functional food. Technology transfer of biotechnologies and public sector research initiatives as well as government incentives are needed to develop soy with traits that are of importance to human health and to poor regions and less arable soil. GM crops may also facilitate sustainable agriculture development with reduced environmental footprint and helping the mitigation of climate change and reducing greenhouse gases (FAO International Technical Conference 2010; James 2010; Organization for Economic Co-operation and Development (OECD) 2009, World Wildlife Fund (WWF) report 2009). More in particular soybean could be the subject for genetic engineering to acquire drought stress tolerance and to be able to grow on marginal lands.

5. Acknowledgement

We thank Lansing Chen for finding the fresh soy sprouts and mungbean sprouts in the market at Taipei and making the picture for comparison.

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Soybean and Nutrition

Edited by Prof. Hany El-Shemy

ISBN 978-953-307-536-5

Hard cover, 476 pages

Publisher InTech

Published online 12, September, 2011

Published in print edition September, 2011

Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Sylvia Burssens, Ine Pertry, Delphin Diasolua Ngudi, Yu-Haey Kuo, Marc Van Montagu and Fernand Lambein (2011). Soya, Human Nutrition and Health, Soybean and Nutrition, Prof. Hany El-Shemy (Ed.), ISBN: 978-953-307-536-5, InTech, Available from: <http://www.intechopen.com/books/soybean-and-nutrition/soya-human-nutrition-and-health>

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