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SOYBEAN: A Multifaceted Legume with Enormous Economic Capabilities

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1. Introduction

Soybean is the broad bean classified under plant legume with its scientific name as *Glycine max*. It is known for more than 3000 years in Southeastern Asia. Due to its versatility and exceptional health benefits it is being cultivated across the whole world. Different varieties of this truly amazing legume are available throughout the year. It is found in varied of sizes as well as seed coat colors, right from black, brown, blue, yellow or mottled. Like other beans, soybeans grow in pods and come under category of edible seeds.

About 85% of the world's soybeans are processed annually into soybean meal and oil. Approximately 98% of the soybean meal is crushed and further processed into animal feed with the balance used to make soy flour and proteins. Of the oil fraction, 95% is consumed as edible oil; the rest is used for industrial products such as fatty acids, soaps and biodiesel. It is one the most widely researched and health-promoting cheapest food. The key benefits are related to their excellent protein content (contains all 8 essential amino acids), high levels of essential fatty acids, numerous vitamins and minerals, isoflavones, and fiber. Most of the soy products act as perfect replacements for meat and dairy products specifically required for vegetarians. For children, soybeans are effective in their growth and development. Soybeans have considerable amounts of α -linolenic acid, omega-6 fatty acid and isoflavones (genistein and daidzein) (Messina, 1995). While the isoflavones present reduces the risk of developing cancer of breast, cervical, ovarian, lung and colon, protein helps in lowering cholesterol levels, thus helpful in reduction heart and blood pressure associated diseases.

Soybean is one of the important crops taken into consideration as candidature of genetically modified (GM) foods due to its great demand worldwide. GM organisms have had specific changes introduced into their DNA by genetic engineering techniques. These techniques are much more precise than mutagenesis and breeding where an organism is exposed to radiation or chemicals to create a non-specific but stable change. The herbicide resistant gene has been taken from bacteria and inserted into soybean that has led to resistance to glyphosate or glufosinate herbicides. United States (US) stands first in using GM soybean and also it is one of the major exporters across the world. There are various issues associated with genetic modification, particularly its long term affects on environment. The technique seems to be analogous to nuclear power, nobody loves it, but climate change has made genetic modification, a most important requisite. It offers both faster crop adaptation and a biological, rather than chemical approach leading to tremendous increase in crop yield.

Soybean contains about 30% of soluble and insoluble carbohydrates. The primary soluble carbohydrates in the soybean are: stachyose, raffinose and sucrose. These sugars play significant role in determining viability and germinating ability of soybean seeds. The amounts of these sugars vary according to the variety of soybean and its growing conditions (Rotundo & Westgate, 2009). The oligosaccharides raffinose and stachyose are significantly important for human health by promoting growth of bifido bacteria present in the lower intestine. Bifido bacteria has significant role in reducing the incidence of many diseases of lower tract including colon cancer (Rada *et al.*, 2002).

Soybean is a nutritious meal with tremendous food value. It has been explored worldwide for its inclusion in variety of food and is also an important constituent for animal feed. Soybean has been explored for variety purpose to have its better economic capabilities. It is the source of various enzymes, particularly important in seed imbibition and germination. Many of these enzymes have industrial significance and due to their plant origin are more acceptable, particularly by food industry. The present chapter is based on economic capabilities of soybean in variety of fields and its emerging significance in enzyme based industries.

2. History

Linguistic, geographical and historical evidence suggest that the soybean emerged around 11th century B.C. (during Shang dynasty) in the eastern half of north China. The plant's wild precursor was a recumbent vine, *Glycine max* var. *ussuriensis*. During the early centuries of domestication, the soybean was nothing like as important in the Chinese diet as it is today. In fact it may well have been far more useful as a fertilizer than as a food-ploughed back into the soil to enriching it for other crops such as wheat or millet. The soy plant belongs to member of a family of plants that has the ability to draw nitrogen from the air, impart it into the soil through its roots thus enriching poor soils.

By the first century A.D. it reached central and south China, as well as peninsular Korea. The movement of the soybean within the primary gene center is associated with the development, consolidation of territories, and degeneration of Chinese dynasties. From about the first century A.D. to the Age of Discovery (15-16th century), soybeans were introduced into several countries and land races developed in Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal and north India. These regions comprise the secondary gene center. The movement of the soybean throughout this period was due to the establishment of sea and land trade routes and the rapid acceptance of the plant as a staple food by other cultures like Indonesians. For centuries, the soybean has been the cornerstone of East Asian nutrition. In the late 16th century and throughout the 17th century European visitors to China and Japan had noted in their diaries the use of a peculiar bean from which various food products were produced. They noted that the Japanese flavor fish dishes with a certain sauce called misol, what Europeans call gravy is made from a bean grown in various localities of Japan. In 1665, Friar Domingo Navarrete described tofu as a common and cheapest food of China. In the 17th century soy sauce was a common item of trade from the East to the West. In 1679, John Locke noted that mango and soy are two sauces brought to England from the East Indies. It was not until 1712, when Engelbert Kaempfer, who lived in Japan during 1691 and 1692 as a medical officer of the Dutch East India Company, published in his book *Amoenitatum Eroficum*, that the Western world fully understood the connection between the cultivation of soybeans and its utilization as a food plant. It reached

the Netherlands before 1737 as Linnaeus described the soybean in the *Hortus Cliffortianus* which was based on plants cultivated in the garden at Hartecamp. In 1739, soybean seeds sent by missionaries in China were planted in the Jardin des Plantes, Paris, France. In 1790, soybeans were planted at the Royal Botanic Garden at Kew, England and in 1804 they were planted near Dubrovnik, Yugoslavia. The soybeans were grown for taxonomic or display purposes in Netherlands, France and England. However, the soybeans grown in Yugoslavia were harvested, cooked, mixed with cereal grain and then fed to chickens for increased egg production.

Soybeans made their first appearance in the US in the 18th century, planted by an American who brought them back from China. American farmers began planting soybeans in the 19th century; yet, it was not until the early 20th century, when nutrition pioneers such as George Washington Carver and John Harvey Kellogg began discovering and promoting the health benefits of soybeans to receive greater public attention. Interest in the nutritional benefits of soybeans has steadily increased only in the past few years. This has come about in response to the recent scientific research that is supporting the numerous ways that soybeans can promote health. Today, the US is the world's leading commercial producer of soybeans (www.soyinfocenter.com, www.fas.usda.gov).

3. Soybean in meals

Soybeans have been a part of traditional food for human population in Asian and Eastern part of the world for several decades. Recently it has become popular in other parts of the world. They are high in protein, fiber and unsaturated fat, and rich in vitamins and minerals. They also show many anticarcinogenic properties related to the unique benefits of soy isoflavones, phytochemicals which exert biological effects in humans and other animals (de Mejia *et al.*, 2003). In recent years, breakthroughs in food science and processing have made it possible to use soybean ingredients in new ways, creating foods that are familiar to consumers incorporating parts of the soybean for functional or nutritional purposes. This has greatly expanded the food processing industries to use soybeans and soy-based ingredients.

3.1 Traditional Soy-Foods (Liu, 2004)

3.1.1 Tofu

It is a regular part of the diet in many Asian nations and is available across the US and in most Western nations. It is soft, white and almost cheese-like food favored for its versatility, mild flavor and high nutritional value. Since it is naturally processed it retains the soybean's important nutrients such as the isoflavones. It is made from soymilk by adding a coagulant (calcium sulfate) to the milk to form curds that are shaped and pressed into cakes. Depending on the coagulant used, tofu is rich in minerals and is an excellent source of high-quality protein (10-15%), polyunsaturated fats *viz.* linoleic and linolenic acids (5-9%) and B vitamins. Tofu is versatile and nutritious as it can be used in soups, salads, pastries, sandwiches, and spreads. It is relatively low in carbohydrates and in fiber (as the pulp was removed), making it easy to digest.

3.1.2 Soy-milk

Liquid extract of soybean used in the preparation of tofu, or as a nutritious beverage. It can be consumed by people who are dairy sensitive (lactose intolerant) or by strict vegetarians who eat no animal proteins. Soymilk is an excellent source of protein, B-vitamins and iron.

There are also a number of soymilks available that are fortified with vitamins and minerals, such as β -carotene, calcium, docosahexaenoic acid and omega-3 fatty acid. It has low levels of saturated fat and no cholesterol. The popularity of soymilk has grown in US and Europe since 1980s when refrigerated soymilks came onto the market.

3.1.3 Green vegetable soybeans

This simple and nutritious soyfood is really just the whole soybean picked at its peak of green maturity, at a time when it is high in sucrose and chlorophyll, and has a firm texture. It is harvested in the pod, and sold either in the pod or shucked, after being blanched and frozen. Because they are picked when their sugar levels are high, green vegetable soybeans are very sweet. Green vegetable soybeans contain ~13% protein same as in tofu, and are naturally high in calcium.

3.1.4 Tempeh

Traditional fermented soyfood from Indonesia and is quite unique in its texture, flavor and versatility. It is made from the whole soybean, which has been de-hulled, cracked and cooked in water with added vinegar to reduce the pH. Once cooked, the soybeans are mixed with the spores of the *Rhizopus oligosporus* fungus and left to incubate for 24 h at around 88°F. Various grains or seeds may be mixed in during processing to vary the taste and texture of the final product. It contains ~19% protein, is higher in fiber than tofu, and is a significant source of vitamins and minerals.

3.1.5 Miso

It is a rich and flavorful paste made from fermented and aged whole soybeans, or from soybeans in combination with wheat, barley or rice using fungal starter *Aspergillus oryzae* under specific conditions. This salty paste is a treasured soup-base and flavoring ingredient used throughout Japan, Korea, Taiwan, Indonesia and China. It has unique medicinal properties and is believed to help reduce the effects of environmental poisons on the body, and contains enzymes and bacteria that can aid in digestion. It is high in protein but contains large amount of sodium therefore should be consumed sparingly.

3.1.6 Soy sauce

It is the most well-known and popular traditional soyfoods, used extensively as a flavoring ingredient in most Asian cooking. There is variety of soy sauce in the market: tamari (naturally processed), shoyu (processed with a fermented wheat starter) etc. Much of the soy sauce sold today is made with hydrolyzed vegetable protein (HVP) with added sugar, color and preservatives. All soy sauces are high in sodium due to which it has been precluded to use as nutritional food. From a nutritional prospective, tamari has highest protein level followed by shoyu and then the HVP-based soy sauce.

3.1.7 Natto

It is a whole soybean food (very popular in Japan) that is produced by fermenting small, cooked soybeans with *Bacillus natto* until they develop a sticky, viscous coating. It has a strong taste and aroma and is definitely for the adventurous eater. It can be found frozen, or fresh, and will last about a week refrigerated.

3.1.8 Okara

It is the fibrous remains of the soybean after it has been processed to make soymilk. It is very high in moisture content and contains the insoluble carbohydrates and dietary fiber of the soybean, as well some remaining protein and fat. If fully cooked, it is blend in flavour and is an excellent ingredient to add to breads and other baked goods. It is not usually sold in stores, as it is very wet, heavy and highly perishable.

3.1.9 Soy Sprouts

Fresh, crisp sprouts of germinated soybeans sold after having grown for 5 to 7 days. They are a traditional food of Korea and eaten either raw or in prepared food dishes. They are high in protein and fiber, and contain vitamin C.

3.1.10 Soybean oil

It is produced from soybeans by mechanical or solvent extraction. Crude soybean oil is further filtered to produce salad and cooking oils. In the US, most of what is sold as “vegetable oil” in the stores is really pure soybean oil. In other countries, it is often labeled as soybean oil, or soya oil. It is the primary oil used in the food processing industry and is found in vegetable oil shortenings and margarine. Soybean oil is high in polyunsaturated fats, low in saturated fats, and a good source of linoleic and linolenic acids, the omega-3 and omega-6 fatty acids also found in fish oils.

3.2 Second generation Soy-foods

Soy-foods manufacturers have been very responsive to consumer demands for convenient, healthful foods. As a result, a wide array of “second generation” soy-foods is available in the marketplace today, catering to different tastes and use preferences. Here are some second generation soy-foods that are currently available:

3.2.1 Soy-nuts

Crunchy nuts have been prepared by dry roasting or oil roasting of whole or split soybeans that have first been soaked in water. They can be sold with salt or other flavoring ingredients added as a coating. Soy-nut butter is a paste of ground soy-nuts that have been prepared in a similar manner to peanut butter, and may have salt, sweeteners and additional oil added.

3.2.2 Meat alternatives

This is the category made from tofu, tempeh, textured soy flour, textured soy concentrate, isolated soy protein and wheat gluten. Products may take the shape of burgers, hot dogs, sausages, luncheon meats, ground meat and meatballs. Most products are made with a combination of vegetable protein ingredients to achieve the best texture and are flavored for a particular use.

3.2.3 Cheese alternatives

Block, sliced, spreadable and grated cheese alternatives may be made from soymilk, tofu or other vegetable protein ingredients. They can be flavored with cheese like American cheese, mozzarella, cheddar, Monterey jack, Parmesan etc. Most of these products are made with some amount of casein (from cow's milk) which is responsible for melting action in cheese when heated.

3.2.4 Soymilk yogurt

Soymilk yogurt is made in the same manner as cow's milk yogurt by inoculating pasteurized soymilk with cultures like *Acidophilus*, *Bifidos* etc. It tastes very similar to cow's milk yogurt and is available in a variety of styles and flavors. It is very high in protein, a great source of isoflavones and can be used as it is or in recipes calling for yogurt.

3.2.5 Nondairy frozen desserts

Nondairy frozen desserts are produced in the same manner as their dairy counterparts. They may be prepared from a base of soymilk, soymilk yogurt, tofu or isolated soy protein. Some brands, such as the pioneering Tofutti brand, have created a loyal niche for themselves, but the category as a whole still struggles to reach a broad market.

4. Soybean as source of nutrition

(www.soyinfocenter.com, www.fas.usda.gov, www.whfoods.com)

4.1 A Health-promoting meat replacer

Soybeans are regarded as equal in protein quality to animal foods. Just one cup of soybeans provides 57.2% of the Daily Value (DV) for protein for less than 300 calories and only 2.2 g of saturated fat. **Table 1** shows the nutritional values of 100 g of soybean. Soy protein tends to lower cholesterol levels, while consuming protein from animal sources tends to raise them, since they also include saturated fat and cholesterol. In addition to healthy protein, some of soybeans' nutritional high points include a good deal of well-absorbed iron: 49.1% of the DV for iron in that same cup of soybeans; plus 37.0% of the DV for Nature's relaxant, magnesium; and 41.2% of the DV for essential omega-3 fatty acids.

4.2 Stay lean with soy

Active isoflavone, genistein found in soy helps in staying lean as it produces fewer and smaller fat cells. It was found that when laboratory animals have eaten diets containing 500-1500 ppm of genistein led to decrease in fat content by ~40% than the animals eating regular chow. Comparable amount of genistein in humans could easily be consumed by simply including traditional soy foods, such as tofu, soy milk, tempeh, and miso as part of their diet which provides about 30-40 mg per serving.

4.3 Eat whole soy foods, not purified soy products for optimal health

A cancer-preventive ability of soy foods markedly reduced in highly purified soy products and supplements. Further, processed soy foods can stimulate the growth of pre-existing estrogen-dependent breast tumors. Soy foods contain complex mixtures of bioactive compounds that interact with one another to promote health, while the partially purified isoflavone-containing products consumed lost many of the biologically active components compare to whole soy foods. In one of the study, laboratory animals were divided into four groups, one of which received no soy and served as the control group, while the others were given whole soy flour, soy extract, a mixture of isoflavones, or genistein in pure form. In the animals given minimally processed soy food *viz.* soy flour, tumors neither grew nor regressed. But in the animals given dietary soy products containing isoflavones in more purified forms, the tumors grew. It is most important for postmenopausal women with estrogen-responsive breast cancers. Avoid processed soy products and supplements that contain isoflavones in more purified forms (Cassidy *et al.*, 1994).

4.4 Soybean oil: Nutritional analysis

Soybean oil is 61% polyunsaturated fat and 24% monounsaturated fat, which is comparable to the total unsaturated fat content of other vegetable oils (~ 85%). Like other vegetable oils, soybean oil contains no cholesterol.

4.4.1 Polyunsaturated vs Saturated fats

Nutrition experts recommend limiting total fat consumption to 30% or less of the total daily calories and limiting saturated fats to 10% or less. Saturated fatty acids raise blood cholesterol which can thicken arterial walls and increase the risk of heart disease. Populations with diets low in saturated fats have the lowest death rates. As a result, the replacement of saturated fats with reasonable amounts of polyunsaturated fats, such as those found in soybean oil, is recommended.

4.4.2 Essential fatty acids

Soybean oil is rich in polyunsaturated fatty acids, including the two essential fatty acids: linoleic (50%) and linolenic (8%). They aid the body's absorption of vital nutrients, precursors to hormones that regulate smooth muscle contraction, blood pressure, and the growth of healthy cells in human.

4.5 Soy protein: Nutritional benefits

Almost 40% of the calories in soybeans are derived from protein causing soybeans to be higher in protein than other legumes and many animal products. The quality of soy protein is highly notable and approaches the quality of meat and milk. Unlike many other good sources of protein, soybeans are low in saturated fat, low-density lipoproteins, triglycerides and are cholesterol-free. Soybean is the best and cheapest source for protein supplementation with respect to other plant and animal sources. It is a healthy adjunct to a varied diet high in fiber, fruits and vegetables and moderate in fat helpful in losing weight and improves sports performance. There is a huge debate regarding importance of soy-protein over other sources, especially whey protein is the most important matter of concern. Both of them are high-quality proteins and have a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1.0. Soy protein isolate is 90% protein on a dry-weight basis, is highly digestible (97%) and allows the essential amino acids *viz.* valine, isoleucine and leucine absorbed across the gastrointestinal tract. Processing of soy protein requires a water-washed process allowing isoflavones to remain intact and removal of all gas-producing carbohydrates and fat. In contrast to whey protein, which suffers two major disadvantages; primarily its high lactose content and expensive processing. It is estimated that 75% of adults worldwide show some decrease in lactase activity during adulthood. The frequency of decreased lactase activity ranges from nearly 5% in Northern Europe, up to 71% for Southern Europe, to more than 90% in some African and Asian countries. Further, processing of whey protein requires two processes: micro-filtration and ion exchange. Both of these processes are very expensive, due to which whey protein is the most costly protein source in the market.

According to comparative analyses of whey and soy protein, later has almost double content of glutamine and arginine. Glutamine is the primary carrier of nitrogen to skeletal muscle and other tissues in the body, helps in buffering lactic acid buildup in the blood and muscles, boosts muscle protein activity, increases growth hormone levels and strengthens immune capacity. Arginine plays a key role in stimulating the release of anabolic hormones

that promote muscle formation, reducing physiological stress, and maintaining a strong and healthy immune system. Soy protein has 18% of branched chain amino acids (BCAA) while whey concentrate has 20%. BCAA are used as an energy source during exercise. During endurance activity, nitrogen is removed from the BCAA and converted to alanine, which is transported *via* the bloodstream from the muscle to the liver where it is converted to glucose. Glucose from the liver returns to muscle to supply energy for fueling exercise (www.soybean.org, www.bodybuildingforyou.com).

5. Physiological significance of carbohydrates

During seed germination of soybean, the total soluble carbohydrate content of the cotyledons and embryo axis declines rapidly during the first 3 days of germination. Depletion began earlier in the embryo axis with respect to the cotyledon. The total carbohydrate content of the cotyledons of plants grown in light and dark was approximately the same for the first 7 days of germination. Between day 9 and 13 the total carbohydrate content of the cotyledons of soybean seedlings grown in dark was higher than that of plants grown in light. The reducing sugar content of light-grown soybean cotyledons increased by ~5-fold during the first 9 days of germination, whereas that of dark-grown soybean cotyledons increased more slowly during this interval. Reducing sugars in the embryo increased during the early stages of germination until they approximately equaled the total carbohydrate. Between day 4 and 13, oil was depleted more rapidly in the cotyledons of seedlings grown in light than those grown in the dark. The reserve carbohydrates of soybean embryos and cotyledons consisted primarily of low molecular weight oligosaccharides, particularly sucrose, stachyose, and raffinose. These compounds decreased rapidly during germination. Stachyose and raffinose declined rapidly in the cotyledons by day 3 and disappeared by day 9 of germination. Sucrose increased slightly during the first 3 days but decreased steadily after the 3rd day. Fructose and glucose appeared in day-1 cotyledons, with the former reaching a maximum value on the 5th day and the latter on the 9th. Stachyose, raffinose, and sucrose were also the major sugars found in the 0-day embryo axes. Stachyose and raffinose were depleted completely by around day 3 in the embryo axes while sucrose decreased between day 5 and day 13. Fructose and glucose appeared in day-1 embryo axes, continued to increase through day-5 of germination, and decreased thereafter (Tsung Min Kuo *et al.*, 1990; Bernal-Lugo & Leopold, 1992; Sitthiwong *et al.*, 2005; Dierking & Bilyeu, 2009). Raffinose, stachyose and verbascose were thought to be involved in seed protection during the desiccation process by stabilization of the membrane. Raffinose, stachyose in combination with phytin plays significant role towards protection against imbibitional chilling. Sucrose is exceptionally effective in protecting membrane integrity in dry systems, as well as being one of the best vitrifying sugars. Raffinose is known to enhance the protective effects of sucrose by limiting crystallization. In quiescent seeds, the main soluble embryonic carbohydrate reserves are sucrose, usually associated with lesser amounts of the oligosaccharides, raffinose, stachyose, and/or verbascose. The decline of seed quality during storage leading to decrease in the growth rate of the germinating axis termed as “vigor” and subsequently loss of germinating ability (germinability) (Sharma *et al.*, 2007). The major factors leading to decline of vigor and germinability of the seed are:

- Decline in soluble carbohydrate contents with seed aging lead to limited availability of respiratory substrates for germination.

- Depletions of disaccharides lessen the protective effects of sugars on structural integrity of membranes as well as limit the ability of the seeds to maintain the vitrified state, a non-crystalline liquid state of high viscosity.
- The presence of reducing sugars also leads to deterioration of protein components through Amadori and Maillard reactions.

6. Source of various enzymes having industrial significance

Soybean has enormous economical significance due to its high food value, role in oil industries as well in bio-diesel production. Leguminous plants are source of various industrial enzymes, particularly found during seed imbibitions and germination as well as those involved in nitrogen metabolism (Dey, 1984). Recently, the approach is emphasizing to use soybean which is a legume for extraction of enzymes at industrial scale helpful for its economic significance. Most importantly, extract prepared from soybean used for enzyme isolation while leaving behind the residues is useful for various purposes *viz.* animal fodder, oil extraction, bio-diesel production etc. For various industrial applications, enzymes so extracted from leguminous seeds are immobilized onto suitable matrices leading to improvement in physico-chemical properties (Das *et al.*, 1997; Kayastha & Srivastava, 2001; Tripathi *et al.*, 2007; Dwevedi *et al.*, 2009; Kumar *et al.*, 2009). **Figure 1 (A, B)** show immobilized β -galactosidase from *Pisum sativum* onto Amberlite MB150 beads and gold nanoparticles (Dwevedi and Kayastha, 2009) being used for lactose hydrolysis at industrial scale. Implementation of soybean at industrial scale as source of various enzymes will make it a multifaceted crop with enormous economic capabilities. Following are few enzymes known from soybean having specific industrial applications.

6.1 Cellulases (Kemmerer & Tucker, 1994)

It is used commercially for food processing in coffee, textile industry, in laundry detergents, paper and pulp industries. They are even used for pharmaceutical applications, fermentation of biomass into biofuels, used as treatment for phytobezoars, a form of cellulose bezoar found in the human stomach.

6.2 α - and β -Amylases (Tripathi *et al.*, 2004; Kumari *et al.*, 2010; Kumari & Kayastha, 2011)

Amylases find application in bread making and in clothing and dishwasher detergents to dissolve starches from fabrics and dishes, production of sugars from starch, such as in making high-fructose corn syrup.

6.3 Proteases (Oh *et al.*, 2004)

They are used to dissolve gelatin off scrap film, allowing recovery of its silver content, remove cloudiness produced during storage of beers. They are used in manufacturing biscuits in lowering the protein level of flour.

6.4 Phytases (Hamada, 1996; Hegeman *et al.*, 2001)

They have been used as animal feed additive in diets largely for swine and poultry, and to some extent for fish. They help in improving the utilization of phosphate from phytate.

Therefore, including adequate amounts of phytase in the diets for simple-stomached animals reduces the need for orthophosphate supplementation of the feed. As a result, the environment is protected from pollution by phosphorus containing manure because of the reduced (~50%) faecal phosphate excretion by the animals. There is also a great potential for the use of phytases in processing and manufacturing of food for human consumption, but up to now, no phytase product for a relevant food application has found its way to the market. Technical improvements by adding phytases during food processing have been reported for bread-making, production of plant protein isolates, corn wet milling and the fractionation of cereal bran.

6.5 Transglutaminases (Kang & Cho, 1996; Lilley *et al.*, 1998)

They have significant role in food industry, used in cold bond meat pieces, attach bacon to the surface of meat, improves texture of cheese and yogurt by reducing water loss, etc.

6.6 Ureases (Kumar *et al.*, 2009)

The enzyme is used in the field of diagnosis to determine urea in the blood serum, to decompose urea in the artificial kidneys. In food industry, urea has been marked as an undesirable substance, particularly in biologically fermented food products such as sake, beer, wine, soy sauce etc. Presence of urea in food products gives bitter taste, cause coloring or deterioration of the flavor and cause lowering of food safety. Urease is being used in treatment of waste water plant particularly from agriculture, rich in urea, in secondary and tertiary oil recovery etc.

6.7 Peroxidases (Chatfield & Dalton, 1993)

They are used for treatment of industrial waste waters, *viz.* phenols (important pollutants) are oxidized to phenoxy radicals, which participate in reactions leading to production of polymers and oligomers that are less toxic than phenols by the enzyme. Furthermore, peroxidases can be an alternative option of a number of harsh chemicals, eliminating harsh reaction conditions. There are many investigations about the use of peroxidase in many manufacturing processes like adhesives, computer chips, car parts, and linings of drums and cans.

6.8 α -Galactosidases (Herman & Shannon, 1985)

The enzyme works in the digestive tract to break down the complex or branching sugars (polysaccharides and oligosaccharides) in foods such as legumes (beans and peanuts) and cruciferous vegetables (cauliflower, broccoli, cabbage, brussels sprouts, among others). The enzyme breaks those complex sugars into simple sugars, making these foods somewhat more digestible, and reducing intestinal gas. The polysaccharides and oligosaccharides found in these foods otherwise pass through the small intestine unaffected. Once in the large intestine, those sugars may be metabolized by intestinal flora, fermenting to produce the gases that cause discomfort. The approach is moving to use oral α -galactosidase for solving gastric problems.

7. Agricultural status and economic importance of soybean

Soybean stands first in the world as edible oil and occupies important place in the economy. Soybean de-oiled cake is being exported and earning foreign exchange. Soybean

cake is edible and is used for preparation of different by-products such as Nutri Nuggets. Soybean de-oiled meal is recommended as animal feed. From soybean whole seed, soy-milk obtained and considered to be the best health drink for infants and soya cheese is also considered as health food. Recent advancements in food processing have transformed the bitter, gray, beany-tasting Asian beverage into a product that Western consumers will accept.

The world soybean price has increased tremendously by ~50% in the year 2007-2008 (remarkable year in the soybean history) with respect to previous years due to strong world demand (**Figure 2**). Area expansion and yield improvement have greatly stabilized its price for the last few years. Brazil's soybean area is expanded annually by about 3% for the last few years due to increment in its price. It has been estimated that by 2017, Brazil will surpass the US to become the leading producer in the world. Falling real domestic prices and expansion of urban area have led to very limited growth of soybean production in China. On the other end, robust economic growth has encouraged domestic consumption of soybean by 40% over the baseline period. Import of oilseeds and domestic crush make China, the world's leading soybean importer for the last 10 years (**Figure 3**). This strong demand for soybeans becomes a focus of attention for major exporting countries (Brazil and the US dominate the soybean net exports market). Paraguay has emerged as an important soybean net exporter and holds a 7.6% share of the world market. Argentina, the leading soybean meal net exporter, ships 98% of its production to the world market because of its differential export tax.

World soybean meal production grows by 2.5% per year in response to rising feed demand. China increases its consumption by 3.4% annually because of strong expansion in its livestock sector whereas that of US increases by 19% (**Figure 4**). The volume of net exports in the soybean meal market increases by 32% in the whole world. Significance of soybean oil in the production of biodiesel has led to increment industrial use of soybean oil in countries like Argentina, Brazil, and the US by 187%, 208%, and 101%, respectively over the year 2007-2008. Despite the domestic biodiesel mandate, Argentina still dominates world soybean oil exports, satisfying 72% of the world market. Brazil and the US together account for 21% of world soybean oil net exports by 2017-2018. China and India's combined share of net imports holds 48% of the world market.

All soybean producers pay a mandatory assessment of 0.5% to 1% of the net market price of soybeans. About \$80 million annually has been invested in United Nations soybean's program to strengthen the position of soybeans in the marketplace and expand domestic as well as foreign markets for uses for soybeans and its products. State soybean councils from Maryland, Nebraska, Delaware, Arkansas, Virginia, North Dakota and Michigan provide another \$2.5 million for the purpose of research. Private companies like Archer Daniels Midland (ADM) also contribute their share. ADM spent \$4.7 million for advertisement. Soy milk has posted the biggest gains, soaring from \$2 million in 1980 to \$300 million in the US in 2001 (www.soygrowers.com, www.agmrc.org, www.soyconnection.com, www.agbioforum.org).

8. Soybean in biotechnology

The GM foods are most commonly used to refer to crop plants created for human or animal consumption using the latest molecular biology techniques. These plants have been modified in the laboratory to enhance desired traits such as increased resistance to

herbicides and pest, cold, drought and salinity tolerance, improving nutritional content, in pharmaceuticals and phytoremediation. The enhancement of desired traits can be traditionally undertaken through breeding but it is very time consuming and are often not very accurate. US is the largest commercial producer of GM crops (~99 million acres are devoted to GM crops) with respect to 13 other countries (Argentina, Canada, China, Australia, Bulgaria, France, Germany, Mexico, Romania, South Africa, Spain, and Uruguay). Soybeans and corn are the top two most widely grown GM crops followed by cotton, rapeseed (or canola) and potatoes.

Approximately 70% of the GM soybeans are produced in the world. Currently, in the United States GM soybeans have reached the marketplace to be use in animal feed. The major question aroused about the effect of GM soybeans on the animals. As reviewed from the results of 23 research experiments conducted over the past few years have revealed that there was negligible effects. In one of the study, separate groups of chickens, dairy cows, beef cattle and sheep were fed with GM as well as traditional soybean as a portion of their diet. Each experiment independently confirmed that there is no significant difference in the ability of animals to digest the GM crops and no significant difference in the weight gain, milk production, milk composition, and overall health of the animals when compared to animals fed with traditional crops. It was also concluded that human consumption of milk, meat and eggs produced from animals fed GM crops would be as safe as products derived from animals fed conventional crops (www.biotechknowledge.monsanto.com). The soybeans used in the studies were produced by inserting a gene that causes the plant to be tolerant to the environmentally friendly herbicide glyphosate. This tolerance to glyphosate allows farmers to spray and kill weeds without killing the soybeans. In other studies the nutritional value of GM soybeans were compared with traditional ones. These studies showed significantly negligible difference in the nutritional composition of the beans (Herd, 2006).

Biotechnologists feel that GM crops hold the answer of feeding the growing population of the world. It has been estimated that the supply of food required to adequately meet human nutritional needs over the next 40 years. It is quantitatively equal to the amount of food previously produced throughout the entire history of humankind. With the current world population at about six billion, and the estimated 10 billion people expected by the year 2040, modern methods of biotechnology must be used to produce enough feed for livestock and food for humans. GM foods have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. Yet there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy and food labeling. Many people feel that genetic engineering is the inevitable wave of the future and that we cannot afford to ignore a technology that has such enormous potential benefits. However, we must proceed with caution to avoid causing unintended harm to human health and the environment as a result of our enthusiasm for this powerful technology (Bonny *et al.*, 2008).

9. Soybean in treatment of various diseases

9.1 Lowers blood pressure and cholesterol levels, helpful for treatment of heart related diseases

Effect of soy protein and soy isoflavones on blood pressure and cholesterol levels were studied in 61 middle-aged men, at high risk of developing coronary heart disease. For five

weeks, half the men consumed diets containing at least 20 g of soy protein and 80 mg of soy isoflavones per day. The effects on their blood pressure, cholesterol levels, and urinary excretion of isoflavones were measured, and then compared to those of the other half of the men who were given a placebo diet containing olive oil. The men consuming soy in their diet were found to have significant reductions in both diastolic and systolic blood pressure. Not only was their total blood cholesterol significantly lower, but their HDL cholesterol level significantly increased (Candy, 1996).

Soy protein has been shown in some studies to be able to lower total cholesterol levels by 30% and lower LDL levels by as much as 35-40%. This is important because high levels of cholesterol, especially LDL cholesterol, tend to become deposited into the walls of blood vessels, forming hard plaques. If these plaques grow too large or break, they can cause a heart attack or stroke. Some studies have even shown that soy protein may be able to raise HDL cholesterol levels. HDL cholesterol travels through the body collecting the cholesterol that has been deposited in the arteries, so it can be taken away and removed by the liver. One of the main goals of atherosclerosis treatment and prevention, therefore, is to lower LDL cholesterol levels while raising HDL levels. And soy is one food that may be able to do both at once. In addition, soybeans also contain very good amounts of fiber. When eaten, the fiber in soybeans binds to fats and cholesterol in food, thus less is absorbed. In addition, soybeans' fiber binds to bile salts and removes them from the body. Since the liver gets rid of cholesterol by transforming it into bile salts, their removal by fiber forces the liver to use more cholesterol to form more bile salts, leads to lowering of overall cholesterol levels (Beretz *et al.*, 1988).

A recently discovered bioactive peptide found in soybeans, *lunasin* is likely to be a key factor in soy's cholesterol lowering actions. A study in which *lunasin* was added to human-liver cells demonstrated the compound's potential to significantly lower cholesterol by inhibiting the expression of the gene responsible for body's internal production of cholesterol and increasing the expression of a gene that reduces levels of LDL cholesterol in the blood. When added to a cell culture of HepG2 liver cells, lunasin slashed HMG-CoA reductase expression by 50%. HMG-CoA is the gene that directs production of HMG-CoA reductase, the enzyme responsible for cholesterol biosynthesis. At the same time, *lunasin* increased by 60% the expression of the gene which produces LDL cholesterol receptors that help clear plasma cholesterol (Anthony, 2000).

9.2 Increased nitric oxide production

Soy protein protects against atherosclerosis by increasing blood levels of nitric oxide which helps in blood vessel dilation, inhibit oxidative damage of cholesterol and the adhesion of white cells to the vascular wall. It was found that when laboratory animal which are apolipoprotein-E deficient (a condition leading to development of atherosclerosis) are fed with soy protein have significant increase in blood level of nitric oxide. Intensive study has concluded that soy protein has led to increased blood levels of L-arginine (the amino acid that the body uses to produce nitric oxide) and nitric oxide metabolites. Thus, soy protein rich diet helps in protection against atherosclerosis (Henrotin *et al.*, 2003).

9.3 Stabilize blood sugar at healthy levels, lowers diabetes risk and protect against diabetes-related kidney and heart disease

Soybeans can be very beneficial for diabetics, particularly type 2 diabetes. The protein in soybeans, and also in other legumes, is excellent for diabetic patients. The protein and fiber

in soybeans prevent high blood sugar levels and help in keeping blood sugar levels under control. Diabetes patients are especially susceptible to atherosclerosis and heart disease, which is the number one killer of persons with diabetes. Keeping cholesterol levels low with soybeans may be useful for preventing these heart problems. In addition, soybeans have been shown to lower high triglyceride levels. Triglyceride levels tend to be high in diabetic patients, and high triglyceride levels are another factor of diabetics' increased risk for heart disease. A study was conducted on 64,227 middle-aged Chinese women with no previous history of diabetes, cardiovascular disease or cancer for 5 years. It was revealed an inverse association between eating legumes and incidence of type-2 diabetes. A high intake of all legumes resulted in a 38% reduction in risk, while a high intake of soybeans, specifically, was associated with a 47% risk reduction. Soy yogurt plays an important role in controlling high blood pressure in type-2 diabetic patients. High blood pressure is one of the problem for which diabetics are at increased risk. Soy yogurt contains angiotensin-I converting enzyme (ACE-I), which plays important role in the constriction of blood vessels and is a target of blood pressure-lowering medications (ACE-inhibitors) (Villegas *et al.*, 2008).

A small clinical trial was conducted on type 2 diabetes patients with nephropathy (diabetes-related kidney damage) suggested that soy protein can help diabetics hearts and kidneys from damage caused by the disease. The study was conducted on 14 type 2 diabetes male and female patients receiving medical care at an educational university hospital and private kidney disease clinic. For the first seven weeks, patients followed a diet (0.8 g/kg of protein, based on 70% animal and 30% vegetable protein) typically recommended to control nephropathy. After a washout period they were re-admitted for another 7-week cycle consuming a diet containing 35% soy protein and 30% vegetable protein. Following the soy diet, all patients experienced significant reductions in total cholesterol, triglyceride and LDL-cholesterol, while levels of beneficial HDL cholesterol remained stable and renal function improved. Specifically, the patients' urinary urea nitrogen (a protein component that is not normally leaked into the urine) and proteinuria (protein in the urine, another indicator that the kidneys are beginning to fail) were both much lower on the soy protein diet (Teixeira *et al.*, 2004).

9.4 Minifies chronic inflammation

Egg yolks are the richest source of choline, followed by soybeans. Intake of diet rich in choline (~300 mg per day) containing food leads to reduction of inflammatory markers like C-reactive protein, interleukin-6, tumor necrosis factor alpha by ~20%. Each of these markers of chronic inflammation has been linked to a wide range of conditions including heart disease, osteoporosis, cognitive decline and Alzheimer's, and type-2 diabetes. It has also been found that choline in association with betaine (found naturally in vegetables such as beets and spinach) work together in cellular process of methylation, which is not only responsible for the removal of homocysteine, but is involved in turning off the promoter regions of genes involved in inflammation. Recommended daily intakes of choline is 550 mg per day for men and 425 mg a day for women (www.whfoods.com).

9.5 Promote gastrointestinal health

A sphingolipid called soy glucosylceramide found in soybeans reduces colon cancer risk. When laboratory animals exposed to carcinogen were given a diet containing 1% soy glucosylceramide, the proliferation of colon cancer cells were dropped by 56%. When the

same diet was given to a strain of animals bred to spontaneously develop colon cancer, the rate at which tumors formed was dropped by 37%. Soy sphingolipids provided this protection by affecting the expression of 96 different genes in the cells that form the lining of the intestines. Soy's effects on these genes resulted in a decrease in the production of two factors associated with cancer initiation and promotion: hypoxia-induced factor 1 alpha and transcription factor 4. Other plants also contain sphingolipids but soy contains relatively high amounts of glucosylceramide due to which it is much effective in cancer prevention. The fiber in soybeans also provides preventative therapy for colon cancer. Fiber is able to bind to cancer causing toxins and remove them from the body, so they can't damage colon cells (www.florahealth.com).

9.6 Protection against cancer of prostate, breast and endometrium

A 9-year Japanese study involving 43,509 men ranging in age from 45 to 74 years found that eating soy food lead to significant lowering of localized prostate cancer risk. Among the men who were older than 60, the protective effect was strongest. It has been suggested that isoflavone found in soybeans protectively alter men's metabolism of estrogen (men do produce some estrogen! the prostate is the primary locus of estrogen production.). Soy's effect of increasing the amount of 2-hydroxy estrogen produced in relation to the amount of 16-hydroxyestrone made in prostate helps in prevention of prostate cancer. Earlier research linking soy to protection against prostate cancer has suggested that soy's isoflavones reduce testosterone levels and inhibit 5-alpha-reductase (converts testosterone to its most potent form, DHT) which are linked to prostate growth and male baldness. A recent study of human prostate cancer cells demonstrated some of the mechanisms behind genistein's anti-prostate cancer effects. Genistein blocks cell cycling and prevent the proliferation of cancerous cells in the prostate by inducing *apoptosis* of abnormal cells. Another study found that genistein protected cells in healthy men from an increase in free radical production by inhibiting the activation of an important inflammatory agent called NF-kappa B and by decreasing levels of DNA adducts (a marker of DNA damage). In addition to genistein, another isoflavone found in soybeans called *daidzen* has also demonstrated protective action against prostate cancer.

Soy's isoflavones also contribute to protective effect against breast cancer in women. The women, who ranged in age from 40 to 59 years, filled out a dietary questionnaire that included questions about soy consumption and were followed for 10 years. Whether prior postmenopausal, women who reported eating three or more cups of miso soup per day had a 40% lower risk of developing breast cancer compared to women who reported consuming less than one cup per day. Women with the highest intakes of isoflavones compounds in soyfoods that can bind to estrogen receptors in the body and block out human estrogen, thus lessening its effects had a 54% lower risk of developing breast cancer compared to those whose intake of isoflavones was lowest.

Soy foods may also reduce risk of endometrial cancer (cancer affecting the lining of the uterus). Eating soy foods is one of the reason due to which Asian women have the lowest incidence of endometrial cancer in the world. The study included over 800 women (aged 30-69 years) with endometrial cancer, and a disease-free, matched control group of over 800 women. When the data was evaluated, a significant inverse association was found between frequency of eating soy foods and endometrial cancer risk. Soybeans contain isoflavones genistein and *daidzein* (1000 times less potent than human estrogen) that can bind to

estrogen receptors in the body, blocking out human estrogens and providing a much more gentle estrogenic effect. Moreover, minimally processed soy foods are rich in dietary fiber, which has been shown to lower estrogen levels (Arjmandi *et al.*, 1998; Tong *et al.*, 1999; Miyazawa *et al.*, 1999; Zhou *et al.*, 1999).

9.7 Leads to healthy transition through menopause and reduction in various symptoms during post-menopause in women

It has been found that consuming isoflavone-containing soy foods significantly inhibits bone loss and stimulates bone formation in menopausal women. Women whose daily diets provided soy isoflavones had much lower amounts of deoxypyridinoline (Dpyr, a bone resorption marker) in their urine, and much higher amounts of bone-specific alkaline phosphatase (BAP, a bone formation marker) in their blood (Welty *et al.*, 2007).

Women's risk for cardiovascular disease (CVD) increases after menopause, in part because levels of risk factors including homocysteine and excess body iron tend to rise. In this study, researchers looked at the effect of soy protein, specifically soy's isoflavones and phytate, on CVD risk factors in 55 postmenopausal women. To do so, they randomly assigned the women to 1 of 4 soy protein (40 g per day) groups: soy containing both its native phytate and native isoflavones, native phytate but low isoflavone, low phytate and native isoflavone, or low phytate and low isoflavone. After just 6 weeks, women in the groups given soy protein with its native phytate had significant reductions in homocysteine and excess iron concentrations. Soy protein with native isoflavones had no effect. Most interesting about this study is the fact that the phytate in soy, a compound which, because of its mineral-binding effects, has sometimes been considered problematic, that is responsible for some of the CVD-protective effects soy offers postmenopausal women (Kritz-Silverstein & Goodman-Gruen, 2002).

Eating soy nuts also resulted in significant improvements in the women's scores on the menopausal symptom quality of life questionnaire: a 19% average decrease in vasomotor symptoms score, 12.9% reduction in psychosocial symptoms score, 9.7% decrease in physical symptoms score, and a 17.7% reduction in sexual symptoms score (Welty *et al.*, 2007).

Despite the fact that the isoflavones found in soy have only 1/1,000th the potency of human estrogens, and epidemiological studies indicate that populations consuming diets high in soy have lower rates of breast cancer, the safety of consuming soy has been questioned because hormone replacement therapy has been found to increase breast cancer risk. Now, an animal study conducted at Wake Forest University Baptist Medical Center, has found that consuming the amount of soy phytoestrogens that would be ingested when soyfoods are included in the diet (in women, about 129 mg/day of isoflavones) does not increase risk of breast or uterine cancer, and appears to be protective. The researchers measured a number of markers of cancer risk: breast density, numbers of dividing breast and uterine cells, sex steroid receptor expression, and blood levels of estrogen. In the monkeys receiving Premarin, levels of all cancer markers increased significantly. In contrast, the monkeys given soy with or without its isoflavones had no increase in any of the cancer markers. And animals receiving soy with isoflavones actually had significantly lower levels of estrogen than the animals given soy from which the isoflavones had been removed. The researchers concluded that the high dietary levels of soy isoflavones did not increase markers related to uterine and breast cancer risk in the laboratory animals studied. Wood's research team has now begun investigating whether soy isoflavones can be used to block breast cell proliferation triggered by estrogen replacement therapy. The theory is that since soy

Nutrients	Nutritional Value
Water	8.5 g
Energy	416 kcal
Protein	36.5 g
Fat (total lipid)	19.9 g
Fatty acids, saturated	2.9 g
Fatty acids, mono-unsaturated	4.4 g
Fatty acids, poly-unsaturated	11.3 g
Carbohydrates	30.2 g
Fiber	9.3 g
Ash	4.9 g
Isoflavones	200 mg
Calcium	277 mg
Iron	15.7 mg
Magnesium	280 mg
Phosphorus	704 mg
Potassium	1797 mg
Sodium	2.0 mg
Zinc	4.9 mg
Copper	1.7 mg
Manganese	2.52 mg
Selenium	17.8 µg
Vitamin C (ascorbic acid)	6.0 mg
Thiamin (vitamin B ₁)	0.874 mg
Riboflavin (vitamin B ₂)	0.87 mg
Niacin (vitamin B ₃)	1.62 mg
Panthotenic acid (vitamin B ₅)	0.79 mg
Vitamin B ₆	0.38 mg
Folic acid	375 µg
Vitamin B ₁₂	0.0µg
Vitamin A	2.0µg
Vitamin E	1.95 mg

Table 1. Nutritional Values of Soybeans (per 100g)

isoflavones, but not estrogens, are similar enough in structure that they can bind to estrogen receptors, they can prevent the much more powerful human estrogen from doing so, thus reducing its effects in the body. Until this research provides more answers, it looks like soyfoods may, at the very least, reduce cancer risk in postmenopausal women with healthy breast tissue.

One of the more popular uses of soybeans lately has been in the treatment of menopausal symptoms. Soybeans contain active compounds called *isoflavones* that act like very weak estrogens in the body. These phytoestrogens bind to estrogen receptors and may provide enough stimulation to help eliminate some of the uncomfortable symptoms that occur when natural estrogen levels decline. Studies have shown that women who consume soy foods report a significant reduction in the amount of hot flashes that they experience. There is also some evidence that soy foods may even be able to help reduce the bone loss that typically occurs after menopause. And as women's risk for heart disease significantly increases at menopause, soybeans numerous beneficial cardiovascular effects make it a particularly excellent food to consume frequently as menopause approaches (Wood *et al.*, 2004).

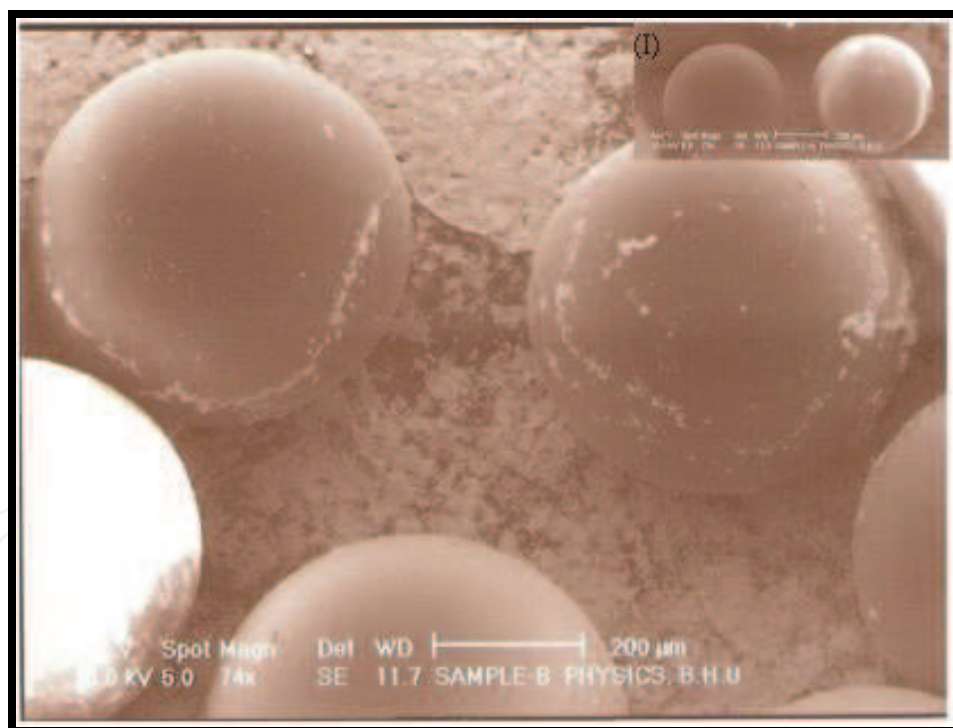


Fig. 1A. Scanning electron micrograph of *PsBGAL* (β -galactosidase from *Pisum sativum*) immobilized on Amberlite MB-150 beads and its control (inset) with scale bar of 200 μ m (adapted from Dwevedi & Kayastha, 2009).

10. Future perspectives

Soybean has been the most studied legume due to its enormous economical significance as well as health benefits. Intensive research has been going on to understand soybean at its genetic level, particularly understanding of the organization, complexity, and distribution of the gene and topography of its repetitive sequences. Although, the distribution of genic and repetitive sequences in soybean is known but a detailed analysis is lacking. This gap could be filled by a combination of cytogenetic analyses, targeted sequencing and functional genomic analyses. Phenotypical functional genomics systems, particularly gene knockout systems and improvements in transformation efficiencies are needed.

Soybean is the number one oilseed crop in the world and provides a multi-billion-dollar source of high-quality protein. The rich genomic resources available for soybean make it a model crop legume. The gene discovery stemming from structural and functional genomics research in soybean will certainly lead to new products and to varieties with improved nutritional and agronomic characters.

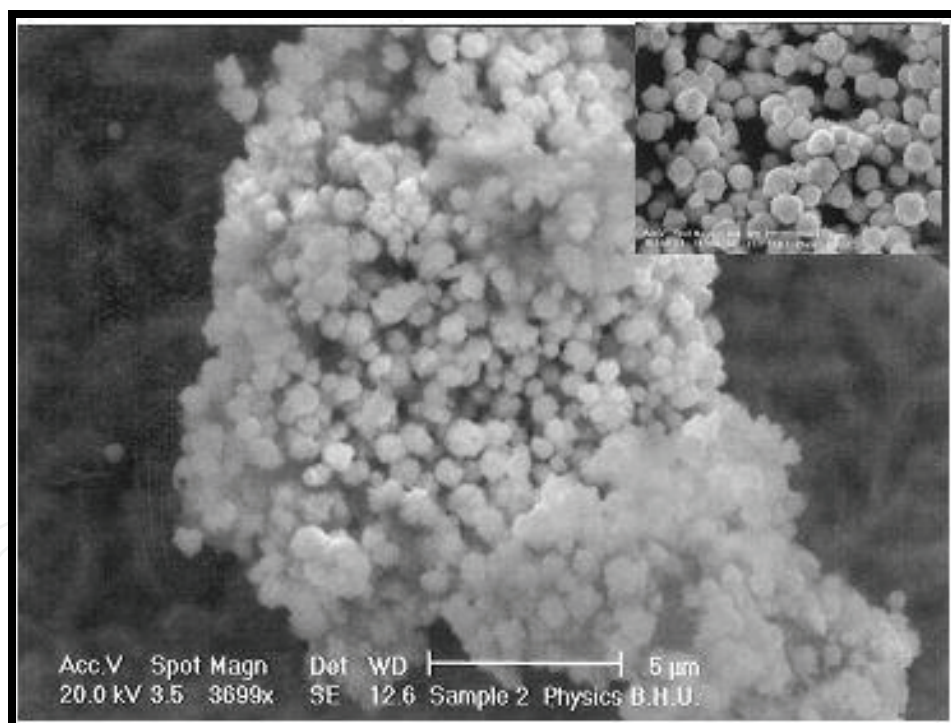


Fig. 1B. Scanning electron micrograph of immobilized *PsBGAL* onto gold nanoparticles and its control (inset) without enzyme with scale bar of 5 μm , respectively (adapted from Dwevedi *et al.*, 2009).

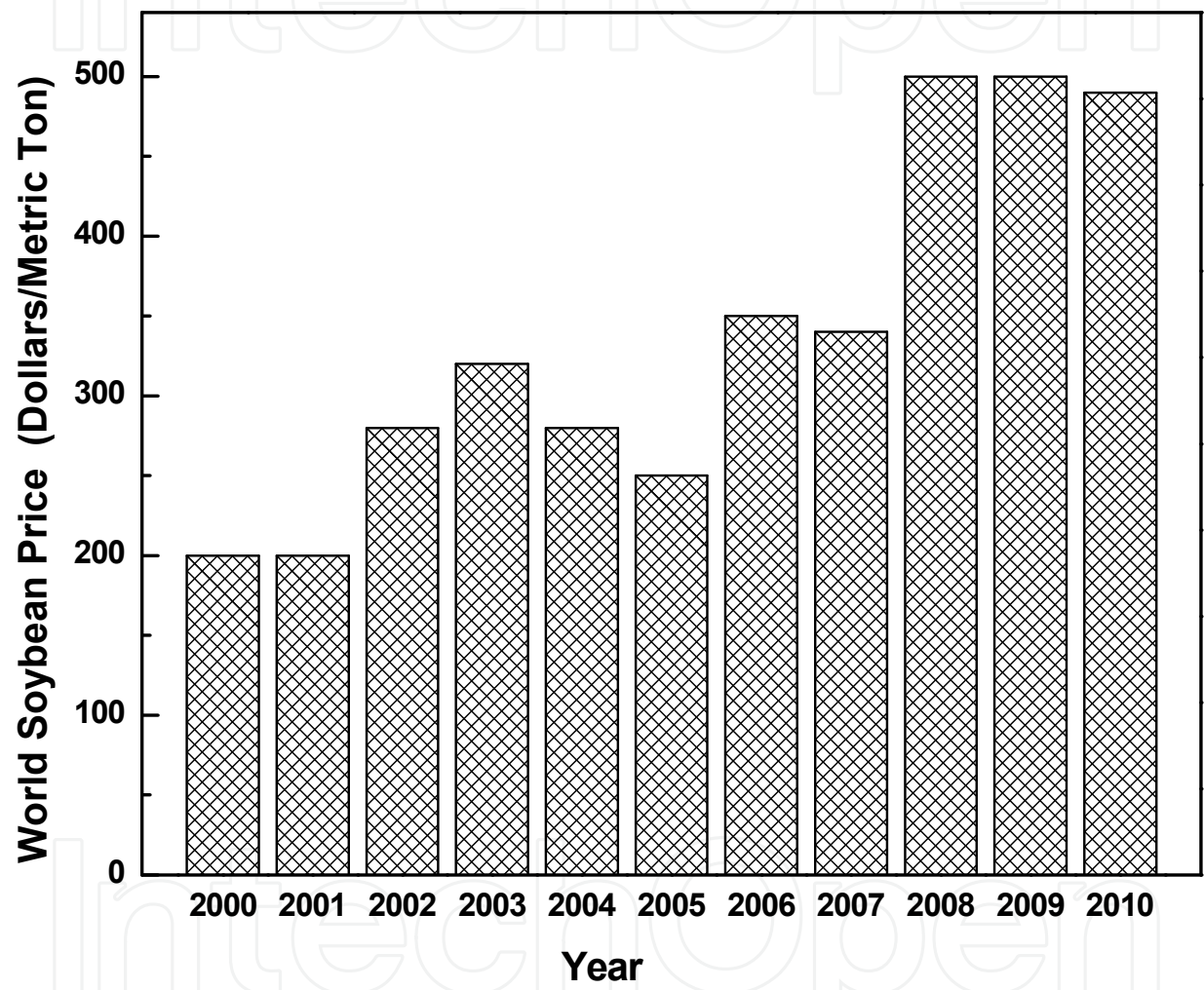


Fig. 2. Increase in the price of Soybean across the world for the last 10 years

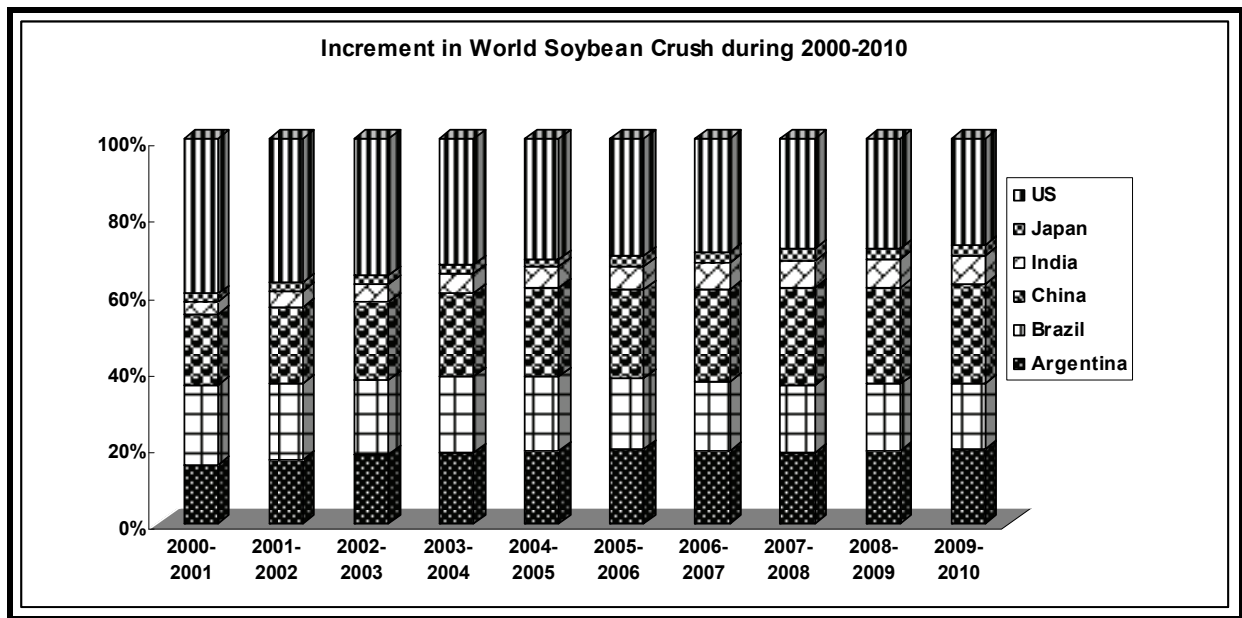


Fig. 3. Increase in world Soybean crush for variety of purpose for the last 10 years

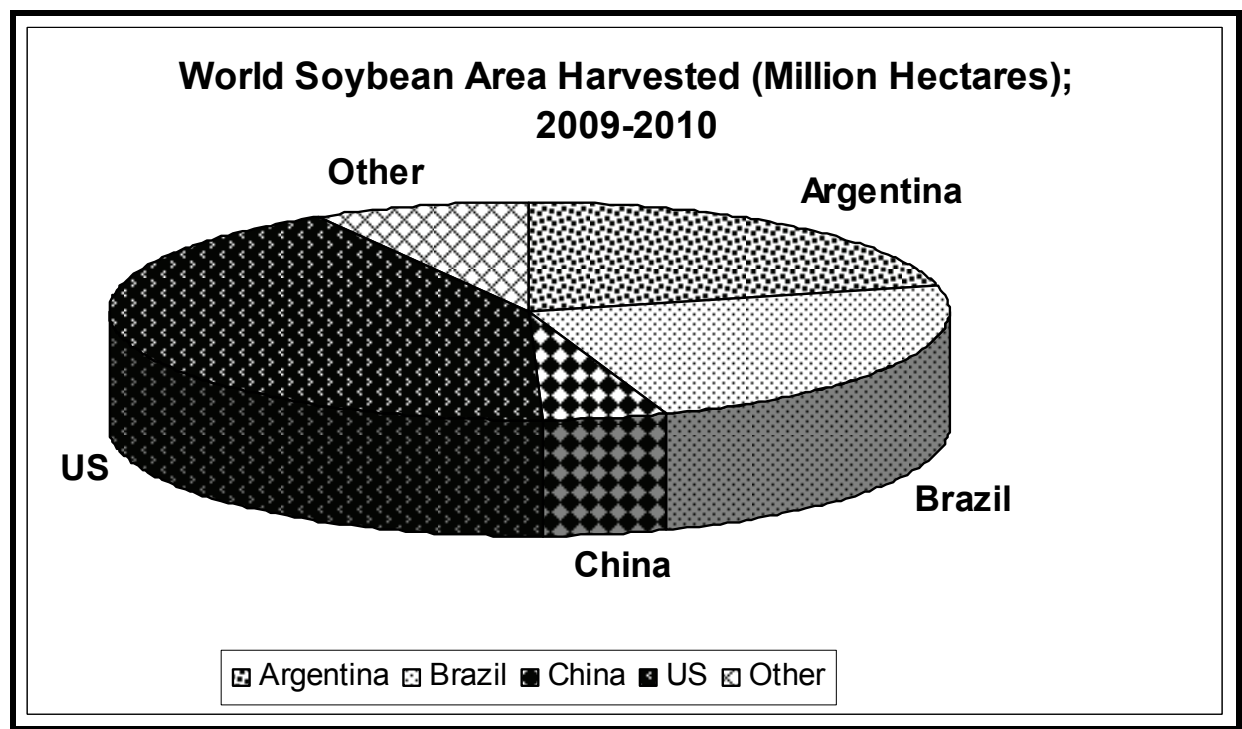


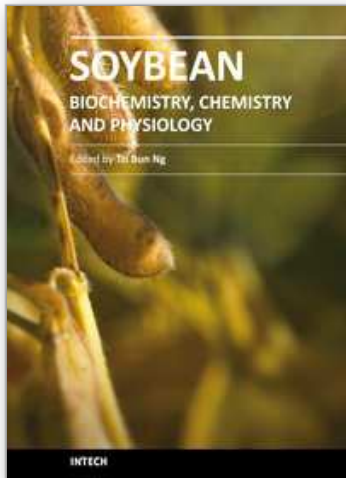
Fig. 4. Area which is used for Soybean cultivation for the last year (2009-2010)

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Soybean is an agricultural crop of tremendous economic importance. Soybean and food items derived from it form dietary components of numerous people, especially those living in the Orient. The health benefits of soybean have attracted the attention of nutritionists as well as common people.

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