
Microbially Fermented Soybean Meal as Natural Fertilizer: A Review

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Abstract

*Soybeans (*Glycine max L.*) have been a significant source of plant origin proteins for both the livestock feed and humans industries for many years. Soybean meal is the most popular protein source in the animal feed industry because of its high protein content and wide availability. Fermented soybeans are not only highly digestible and nutritious by contributing important nutrients including calcium, vitamin A and B vitamins, but fermented soybeans also have functional properties, such as immunomodulatory and anti-cancer effects. Since, fermentation can vastly improve the palatability of soy proteins along with increasing its digestibility, it is a very promising processing method for the industry. The present review paper briefly explained the role of microbially fermented soybean meal as natural biofertilizer. This present review composed of the following topics: Soybean, Agriculture and nitrogen sources, Soybean meal as a natural fertilizer, Processing methods of soybeans, Toasting treatment of soybean and Fermentation of soybean meal.*

Key words: Soybean, Fermentation, Microorganisms, Agriculture, Nitrogen source and Natural fertilizer.

Organic agriculture is one of the fastest growing segments of our Indian agriculture today. In 2015, domestic sales of organic products topped \$7.8 billion, with fresh produce the top-selling organic category. The growing popularity of organically grown foods has generated new market opportunities for both wholesale and direct-

market organic produce farmers. Organic fertilizer amendments can be expensive when used in large quantities, so they are often used only for supplementary nutrition, if they are used at all. In a 1994 nationwide survey of 300 certified organic growers, when asked about fertilizer sources, 78 % reported using animal manure, 77 % used legume cover crops and 61 % used compost in their farm operations.

Soybean (*Glycine max* L.) is one of the most important pulse crop in the world. It has a dual benefit of supplying about 43.3 per cent protein and 19.5 per cent oil hence termed as “Miracle bean”. Soybean is indigenous to China and was introduced to India in 1950’s (Caldwell, 1973). In India, the area and productivity have been rapidly increasing over the recent years. In Tamil Nadu, it is cultivated as an irrigated crop in an area of 31,000 hectares with the annual production crop of 8000 metric tonnes and has multiplicity of use as pulses, oil seeds, vegetarian meat, milk and also as an antibiotic. Soya protein is the only vegetable source of complete protein, of a quality comparable to meat and egg, which contains all the essential amino acids required by human and animals. So, there is a pressing need to improve the yield of soybean in order to meet the protein malnutrition and the edible oil needs of our country.

Soybean is a high nutritional value legume, its beans contains upto 30 % of proteins. They provide all the essential amino acids except methionine. This deficiency can be compensated by diet combinations with cereals as recommended in classical dietary procedures (Grupo, 2004). Soybean is also used as protein supplement for animal feed. In the last years, the world soybean production surpassed 250 million tons, led by US, Brazil, Argentina, China, India, Paraguay and Canada (Clive, 2010). In Cuba, soybean production recently arose as an economical policy priority, in order to substitute pod import, with a remarkable raise in the number of areas destined to soybean crops and the introduction of mechanized sowing. It has been estimated that the soybean plant requires up to 80 kg of assimilable nitrogen to produce a ton of pod, accounting for 240 kg/ha in average. Nitrate or ammonia becomes available in soil by organic nitrogen mineralization, chemical fertilization, and biological nitrogen fixation. The later process is essential for nitrogen incorporation to the biosphere. The conversion of atmospheric nitrogen into ammonium conducted by microorganisms bearing the enzyme nitrogenase is an intrinsic non-contaminating process that prevents soil impoverishment (Singh, 2005). At the same time, high concentrations of nitrate or ammonium in soil inhibit the nitrogen biological fixation.

Soybean has significant agronomic and nutritional relevance because of the high concentrations of protein and oil in its pods. Concomitant with the high protein content, the legume shows a strong demand for nitrogen for optimal development and pod productivity (Graham and Vance, 2008). Although, atmospheric N₂ is abundant, no eukaryotic organism is able to directly assimilate it, due to the strong triple bond linking the atoms (Brechenmacher *et al.*, 2008; Oldroyd *et al.*, 2011). However, when growing in nitrogen depleted soils, much of soybean’s need for nitrogen can be obtained *via* biological nitrogen fixation (BNF) in root nodules, through the symbiotic association with bacteria, collectively called *Rhizobia*, belonging mainly to the species *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* (Hungria *et al.*, 2006).

Nitrogen is one of the major important nutrient essential for plant growth. The economic and environment importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria (*Rhizobium* or *Bradyrhizobium* species). Like most legumes, soybeans performs N₂ fixation by establishing a symbiotic relationship with the rhizobia. *Bradyrhizobium japonicum* is a slow growing root nodule symbiont, which is widely used as an inoculant in soybean fields throughout the world. Generally, soybean inoculated with *Bradyrhizobium japonicum* forms highly effective nodules and frequently increased soybean yields, especially in fields where soybeans are cultivated for the first time (Caldwell and Vest, 1970).

Soybean (*Glycine max* L.) meal (SBM), a byproduct of soy oil extraction, is allowed as a non-synthetic plant or soil amendment. There is still debate concerning the use of SBM derived from Genetically modified organism (GMO) soybeans, such as those with the Round-Up Ready gene. The United States Drug Administration (USDA) does not allow the use of methods to genetically modify organisms or to influence growth and development by means that are not possible under natural conditions (USDA, 2002). The use of GMO seeds in organic production, therefore, is prohibited, but the regulations do not specifically prohibit fertilizing with meals made from GMO seeds.

Soybeans have been a significant source of plant origin proteins for both the livestock feed and human industries for many years. Soybean meal is the most popular protein source in the animal feed industry because of its high protein content and wide availability (Easter and Kim, 1999; Baker, 2000). Unfortunately, the use of soybean meal in animal diets was primarily limited to adult animals due to the inefficient digestibility of soy proteins by young animals and the susceptibility of young animals to antinutritional compounds in soybeans that are either not properly processed or undercooked (Jiang *et al.*, 2000; Baker, 2000). These antinutritional compounds include trypsin inhibitors, lectins, flatulence producing compounds, and many other allergenic proteins (Kim and Baker, 2003; Baker, 2000; Dunsford *et al.*, 1989). These antinutritional compounds can be denatured by fermentation thereby enabling the use of soybean meal (Feng *et al.*, 2007).

Fermented soybean meal can successfully replace animal-derived protein sources such as plasma protein and dried skim milk in piglet nursery diets without adversely affecting the growth performance of the piglets (Kim *et al.*, 2009). Fermented soybeans are not only highly digestible and nutritious by contributing important nutrients including calcium, vitamin A and B vitamins, but fermented soybeans also have functional properties, such as immunomodulatory and anti-cancer effects (Lee, 1998). Since fermentation can vastly improve the palatability of soy proteins along with increasing its digestibility, it is a very promising processing method for the industry.

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digestibility of soy proteins by young animals and the susceptibility of young animals to antinutritional compounds that are present in soybeans that are either not properly processed or undercooked (Jiang et al., 2000; Baker, 2000). These antinutritional compounds include trypsin inhibitors, lectins, flatulence producing compounds, and many other allergenic proteins (Kim and Baker, 2003; Baker, 2000; Dunsford et al., 1989). These antinutritional compounds can be denatured by fermentation thereby enabling the use of soybean meal in young animal diets (Feng et al., 2007).

Fermentation of soybean meal not only improves digestion and destroys antinutritional factors like trypsin inhibitors, glycinin, and β -conglycinin, but also releases many bioactive components that are immunomodulatory, antipathogenic, and enhance phagocytosis (Lee, 1998; Kim et al., 2009, Magalhaes et al., 2008; Hong et al., 2004). Fermented soybean meal also contains live microorganisms that are beneficial to the intestinal tract health (Kim et al., 2009). The bioactive components released by microbial fermentation potentially could increase overall calf health, mostly because of their immunomodulatory and intestinal tract benefits.

Fermented soybean meal can successfully replace animal-derived protein sources such as plasma protein and dried skim milk in piglet nursery diets without adversely affecting the growth performance of the piglets (Kim et al., 2009). In addition, piglets fed the fermented soy responded with increased feed intake, higher nutrient digestibility and absorption, improved growth performance, and reduced diarrhea compared to piglets fed other animal-derived protein sources (Kim et al., 2009). The researchers speculated that these fermented soy products could be incorporated into diets of pre-ruminant calves, ruminants, pets, as well as aquaculture diets (Kim et al., 2009).

Soybean

Soybean (*Glycine max* L.) is a globally important commercial crop, grown mainly for its protein, oil and nutraceutical contents. The seeds of this legume are 40 % protein and 20 % oil. Each year soybean provides more protein and vegetable oil than any other cultivated crop in the world. Soybean originated in China, where it has been under cultivation for more than 5000 years (Cui et al., 1999). The annual wild soybean (*G. soja*) and the current cultivated soybean (*G. max*) can be found growing in China, Japan, Korea and the far east of Russia, with the richest diversity and broadest distribution in China, where extensive germplasms are available. The National Gene Bank at the Institute of Crop Germplasm Resources, part of Chinese Academy of Agriculture Sciences (ICGR-CAAS), Beijing, contains close to 24,000 soybean accessions, including wild soybean types.

Soybean was introduced into North America during the 18th century, but intense cultivation started in the 1940s – 1950s and now North America is the world's largest producer of soybean (Hymowitz and Harlan, 1983; Qui and Chang, 2010). Although, grown worldwide for its protein and oil, high value added products such as plant functional nutraceuticals, including phospholipids, saponins, isoflavones, oligosaccharides and edible fibre, have gained importance in the last decade. Interestingly, while genistein and diadzein are signal molecules involved in the root

nodulation process, the same compounds can attenuate osteoporosis in post-menopausal women. The other isoflavones have anti-cancer, anti-oxidant, positive cardiovascular and cerebrovascular effects (Lui, 2004). More recently soybean oil has also been used as an oil source for biodiesel (Mandal *et al.*, 2002; Du *et al.*, 2003; Mushrush *et al.*, 2006; Huo *et al.*, 2009).

Soybean is a well-known nitrogen fixer and has been a model plant for the study of Biological nitrogen fixation (BNF). Its importance in BNF led to the genome sequencing of soybean; details of the soybean genome are available at soybase.org (*G. max* and *G. soja* sequences are available at NCBI as well). Although considerable work has been conducted on other legumes with respect to biological nitrogen fixation (Shiraiwa *et al.*, 2006). The efficiency of BNF depends on climatic factors such as temperature and photoperiod (FAO, 2009). The effectiveness of a given soybean cultivar in fixing atmospheric nitrogen depends on the interaction between the cultivar's genome and conditions such as soil moisture and soil nutrient availability (Jung *et al.*, 2008) and the competitiveness of the bacterial strains available, relative to indigenous and less effective strains, plus the amount and type of inoculants applied, and interactions with other, possibly antagonistic, agrochemicals that are used in crop protection (Campo and Hungria, 2004). The most important criteria, however, is the selection of an appropriate strain of *Bradyrhizobium japonicum* since specific strains can be very specific to soybean cultivar, and subject to influence by specific edaphic factors (Alves *et al.*, 2003; Abaidoo *et al.*, 2007). Under most conditions, soybean meets 50-60% of its nitrogen demand through BNF, but it can provide 100% from this source (Salvagiotti *et al.*, 2008).

Agriculture and Nitrogen sources

Nitrogen (N) is an essential element for animal and plants. In the latter, it plays extremely important roles as part of a number of organic molecules including chlorophyll, amino acids, proteins and enzymes. Even though the atmosphere contains about 78 % N and cultivated soils contain between 1,350 and 6,700 kg N ha⁻¹, N is often regarded in agricultural production as the most limiting element (Nasholm *et al.*, 2009; Robertson and Vitousek, 2009; Luce *et al.*, 2011; Sawyer, 2012; Xu *et al.*, 2012). While in natural ecosystems free amino acids, peptides- and protein-bound amino acids make up the largest fraction of soil organic N, in agricultural systems NO₃⁻ and NH₄⁺ are commonly found in higher concentrations than free amino acids due to addition of synthetic fertilizers and other agricultural management practices that influence soil N transformations (Olk, 2007; Jamtgard *et al.*, 2010; Vinnall *et al.*, 2012).

Nitrogen (N) from inorganic fertilizers has been recognized as a key factor for continued high yields and yield improvements (Tilman *et al.*, 2002; Duvick, 2005). Modern grain production systems rely heavily on external inputs, with N from inorganic fertilizers one of the major drivers for the high productivity of these systems (Grassini *et al.*, 2015) and fertilizer N is the most widely applied nutrient in agricultural production systems in the US. In 2010, about 60 % of the total fertilizers applied to the US farmland was N, with application of about 13 x 10⁶ tones of synthetic N fertilizer, with corn receiving 46 % of the total N used in 2011 (ERS, 2011). Iowa typically has

the largest amount of land planted to corn and the greatest average yield. In 2013, the Iowa corn crop represented (NASS, 2013).

The majority of nitrogenous fertilizers are applied either in NH_4^+ (e.g., anhydrous ammonia, ammonium nitrate, ammonium sulfate) or urea forms worldwide (IFA, 2015). Organic N is the most abundant form of N in agricultural soils, but few crop plants are able to utilize organic N directly. Instead, NH_4^+ and NO_3^- are the major N sources for crop production systems (Havlin *et al.*, 2005; Mikkelsen and Hartz, 2008). Although these two forms of inorganic N represent the sources of N taken up by crop plants, their presence in agricultural soils is below that required for intensive grain crop production (Cassman *et al.*, 2002; Sawyer, 2012).

Inorganic N forms are the preferable forms for uptake of N for plants; thus, mineralization of organic N is considered as a crucial step to achieve optimal plant N nutrition (Lipson and Näsholm, 2001; Fageria and Baligar, 2005; Havlin *et al.*, 2005; Mikkelsen and Hartz, 2008; Farrel *et al.*, 2013; Li *et al.*, 2013). Nonetheless, direct absorption of soil amino acids by plants is a topic that has gained attention and is still a subject of debate (Nasholm *et al.*, 2000; Owen and Jones, 2001). Because modern high-yielding cropping systems rely heavily on N fertilizer inputs, their use has greatly increased during the last four decades. Along with the continued increase of synthetic N fertilizers, environmental concerns like air and ground water pollution have arisen as well.

The unquestionable benefits of N fertilizer utilization has made possible much of the large increase in the supply of food to the world's growing population. On the other hand, the negative implications of low N recovery by crop plants have led to environmental degradation related to serious water and air contamination issues (Tilman 1998; Tilman *et al.*, 2001; Robertson and Vitousek, 2009; Sutton *et al.*, 2011). Nitrogen losses are related to microbial transformations of N in soils. Leaching and denitrification are the main processes that lead to N losses in agricultural systems (Subbarao *et al.*, 2012).

Soil application of amino acid biosynthesis byproducts from industrial processes may improve plant productivity by altering soil processes and plant physiological attributes when used as N sources or soil amendments in agricultural systems. The organic N in these amino acids byproducts may influence plant N metabolism and improve N use efficiency in crop plants. In a review article by Halpern *et al.* (2015), amino acids are classified as biostimulants, which are defined as substances capable of modifying physiological processes in plants. Furthermore, the N in biosynthesis byproducts may be less vulnerable to loss than N in inorganic fertilizers, and that may decrease N exports to surface and groundwater bodies. The amino acids TRP and LYS are hydrophobic and polar positive charged, respectively. Both characteristics of these amino acids should make their N less susceptible to losses by leaching.

Amino acids may represent an alternative source of N for crop plants. Moreover, many studies and reviews have shown that numerous grass and tree species growing under managed or natural ecosystems can take up amino acids directly from soils (Näsholm *et al.*, 2000; Lipson and Nasholm, 2001; Warren and Adams, 2007).

Despite the considerable amount of evidence that plants absorb organic N from simple molecules such as amino acids or oligomers including di-, tri- and tetra- peptides (Näsholm *et al.*, 2000; Paungfoo-Lonhienne, 2012; Vinall *et al.*, 2012; Farrel *et al.*, 2013), the relevance of these organic molecules for N nutrition of plants remains unclear. Experiments using ^{15}N , confirmed that free amino acids are a readily available form of N for higher plants (Ohlund and Näsholm, 2004; Warren, 2006). Although, the majority of studies investigating amino acids as N sources have been carried out in natural ecosystems, amino acid uptake evidence in some crop plants has been demonstrated.

Nasholm *et al.* (2000) reported that four commonly used pasture grasses in European grasslands were able to absorb organic N from the simple amino acid glycine under field conditions. Vinall *et al.* (2012) reported that sugar cane has the ability to take up N from amino acids and that N supplied from amino acids produced similar biomass to N supplied by inorganic sources in axenic culture and pot studies. Similarly, in a growth chamber study, corn seedlings grown with high amino acid concentrations became better competitors for amino acids than the soil microbial community (Jones *et al.*, 2005).

Concentration of amino acids in the soil solution was found to play a major role in plant amino acid uptake. But, it remains unclear what are the optimum amino acid concentration levels in soils required for plant uptake. In this sense there is a wide range of amino acid concentrations that have been proposed as optimal and they vary significantly between and within plant species (Jamtgard, 2010). In a recent study, Hill *et al.* (2011) reported that wheat (*Triticum aestivum* L.) was able to take up and metabolize amino acids at rates comparable to those when inorganic N sources were utilized. Their findings suggest it is necessary to reconsider the current assumptions about plant available N forms. Another desirable characteristic of using amino acid biosynthesis byproducts for agricultural purposes is that their chemical composition includes other important nutrients in addition to N, including P, K, S and Fe (Martinez and Tabatabai, 1997).

Diversity in amino acid biosynthesis byproducts chemical composition depends upon the raw materials and the fermentation conditions used to produce the pure feed amino acids. Henning (2007) reported that biosynthesis byproducts are N-rich, but they also contain other nutrients at similar or higher levels than N as in the case of Cl and S for two LYS by-products. High S and P concentration in biosynthesis byproducts was reported by Zhu *et al.* (1995), making them a potentially good soil amendment for agricultural soils. Others beneficial aspects of soil applied amino acids encompass improvement of soils chemical and physical characteristics, root growth and increase activity of NO_3^- assimilation enzymes (Walch-Liu *et al.*, 2006; Garcia-Martinez *et al.*, 2010; Halpern *et al.*, 2015).

Soybean meal (SBM) as a natural fertilizer

Soybean is one of the most widely cultivated plants in the world today and is valued for both its oil and meal (Hasegawa *et al.*, 2002). Soybean meal (SBM) is a by-product of soybean oil extraction. Processing a bushel (60 lbs. or 27 kg) of soybeans

produces 10.5 pounds (5 kg) of oil and 48 pounds (22 kg) of meal and hulls; the rest was waste and water (INFO source, 2002). SBM contains 44 % to 47 % protein and is a leading source of protein in poultry, swine, dairy, and beef diets (Erickson, 1995). It was also used in pet foods and aquaculture. In the last century, SBM was used as a slow release N fertilizer (Kubo *et al.*, 1994). With the advent of chemically synthesized fertilizers, the use of organic fertilizers declined (Rubins and Bear, 1942). Today, there is renewed interest in SBM as a fertilizer in organic vegetable production systems.

SBM has been found to increase biomass production in tomatoes. In a test of 13 organic fertilizers for the production of greenhouse tomato transplants, SBM increased shoot dry weight 40% above the unfertilized control (Gagnon and Berrouard, 1994). In another study looking at the use of seed crop meals to control nematodes, SBM applied at 1000 to 2000 lb acre⁻¹ (1121 to 2242 kg ha⁻¹) significantly increased the foliar fresh weight and dry weights of tomatoes compared to the control (Hafez and Sundararaj, 1999). There is evidence that high rates of SBM, though, can have phytotoxic effects on weeds and vegetables. At a rate of 4000 lb acre⁻¹ (4484 kg ha⁻¹) tomatoes suffered severe stunting, necrosis, and death (Hafez and Sundararaj, 1999). In another study, when SBM was used as a comparison to corn gluten meal (CGM), SBM inhibited perennial ryegrass (*Lolium perenne* L.) growth at 3046 lb acre⁻¹ (3414 kg ha⁻¹) and completely stopped growth at higher levels compared to the control (Liu *et al.*, 1994).

Many other organic materials have shown similar inhibition of germinating seeds. Cottonseed meal (CSM) mixed into soil at 200 and 300 lbs acre⁻¹ (224 and 336 kg ha⁻¹) reduced corn seed germination by 75 % compared to soil alone (Sherwin, 1923). The seeds germinated but died before reaching the soil surface. The absence of root hairs and the widespread decay of roots were attributed to the CSM decomposing fungus attacking the plant root system.

Chopped alfalfa at 22 and 44 t acre⁻¹ (4600 and 9200 g m⁻²) inhibited germination and seedling growth of cucumbers (Ells *et al.*, 1991). The authors attributed the damage to toxic levels of ammonia produced during the decomposition of the alfalfa in the soil. Manure extracts of 10 % and 30 % inhibited cress seed germination and root growth (Hoekstar *et al.*, 2002). The damage was ascribed to phytotoxic nitrogenous compounds, such as cyanide, amines, and phenolic compounds, and high salinity levels (EC). Corn gluten meal (CGM), another grain byproduct, was found to inhibit vegetable and weed seed germination (Liu and Christians, 1997).

Research with SBM should also investigate management practices that would utilize SBM's phytotoxic properties for weed control and its nutrient value as a slow release fertilizer. Research could also focus on development of fertilizer products derived from SBM. Hasegawa *et al.* (2002) found that SBM degraded with *Bacillus circulans* increased root hair density of Chinese cabbage (*Brassica campestris* L.) to three times that of the untreated SBM and increased yield of potato (*Solanum tuberosum* L.) by 37 % compared to a chemical fertilizer. As the public's concern over the long-term ecological effects of synthetic agricultural chemicals continues to grow, there will be more interest in natural products, such as SBM, for weed control and fertilizers.

Processing methods of Soybeans

Many other processing methods are used in addition to those that create byproducts of soybeans to improve soybean utilization. Expansion, extrusion, popping, heating, boiling, acid/alkali treatment, and toasting are a few processing methods that have been studied. Micronization, ultrafiltration, thermoalkali treatment, supplementation, microwaving, fermentation, soaking and cooking are more processes that have been studied. Regardless of the processing method utilized, the process should minimize activity of antinutritional factors and achieve maximum availability of nutrients for digestive enzyme access to maximum protein utilization (Abdelgadir *et al.*, 1984).

Boiling of soybeans inactivates up to 98% of the trypsin inhibitor, thus improving soy protein digestibility (Collins and Sanders, 1976). Extruded soybeans were equal to soybean meal as a source of protein for young calves (Daniels *et al.*, 1973; Stutts, 1982), and expanded, extruded soybeans were digested more efficiently in calves than soybean meal plus fat (Daniels and Flynn, 1976). Micronization processing offers a rapid alternative method for processing whole soybeans that effectively destroys urease activity and trypsin inhibitors while increasing protein digestibility (Hutton and Foxcroft, 1974). A micronizing temperature of between 200° and 225° C is required for optimal processing of whole soybeans (Hutton and Foxcroft, 1974). With soy proteins, increasing drying temperature to double the rate of destruction of trypsin inhibitor would increase the destruction of many nutrients by four- to fivefold (Rackis, 1974). Ultrafiltration is a processing method that is a separation technique and effectively removes phytate with little or no loss of protein (Okubo *et al.*, 1975).

Heating of Soybeans

Heating is one method of processing, and even though trypsin inhibitors are readily inactivated by steam heat (Rackis, 1966), heating of soybeans is not the most effective processing method to achieve this goal. This is because many of the antinutritional factors present in soybeans require such a high temperature to become denatured that the proteins and amino acids present in the soybeans also become denatured (Logenecker *et al.*, 1964; Rios Iriarte and Barnes, 1966; Arnold *et al.*, 1971). Since extreme heat denatures nutrients, soy protein that is heat treated to improve digestibility and remove deleterious factors often needs further processing to further improve the digestibility (Vest *et al.*, 1966). With soybean protein, unless the heat treatment was followed by very fine grinding or flaking, the maximum feeding potential of the whole soybeans cannot be achieved (Arnold *et al.*, 1971).

Miller and Ramsey (1978) found that for calves fed a milk replacer containing soy flour as the only source of protein, maximum calf growth was obtained when the flour was heated for 90 minutes. This treatment gave better results than the commercially available “fully-cooked” soy flour product. Hansen and Johnson (1976) concluded that the highest pepsin and trypsin digestion rates for soy flour proteins were for flour processed with 13% moisture content at 108 °C for 2 min. They also found that more severe processing resulted in a progressive reduction in digestion rates, probably due to denaturation of the protein.

Rackis (1974) found that processing by the form of short cooking time in an extruder minimizes damage to nutritional properties, but adequately destroys the growth inhibitors. The processing method of moist heat has a beneficial effect upon the nutritive value of soy protein isolates (Rackis, 1974). When treating defatted soybean meal with steam to inactivate antinutritional substances, the nutritive value of the product still remained markedly inferior to milk protein (Gorrill and Thomas, 1967).

The effectiveness of heat treatment on the nutritional characteristics of soy protein largely depends on water activity, pH, heating time, and processing temperature (Johnson *et al.*, 1980). Certain combinations of these factors create products that, when included in calf diets, promote weight gains and feed efficiencies superior to those of the raw protein (Arnold *et al.*, 1971). Nearly all vegetable proteins and products derived from them are consumed after some degree of heat treatment (Rackis, 1966). Processing of soybeans alters flavor, color, texture, and other functional properties of the proteins,

Alkali Treatment

Soy protein sources treated with alkali improves calf performance (Barr, 1981). This additional processing removes antigenic properties and enhances utilization (Barr, 1981). Alkaline processing conditions were found to render trypsin inhibitors more heat-labile and therefore easier to destroy during heat-processing (Badenhop and Hackler, 1970). The conditions of the ethanol treatment, such as time, temperature, and relative amounts of ethanol and water, may affect the extent of removal of the deleterious factors (Smith and Sissons, 1975). Borowska and Kozłowska (1986) found that a pH of 8.2 was optimal for soybean flour extraction. Treatment with hot aqueous ethanol has also improved soybean protein utilization (Sissons *et al.*, 1979). Additionally, moist heating under mild alkaline conditions improves soy protein quality, but this processing method can form a toxic amino acid, lysinoalanine, during the process (Woodard and Short, 1973).

Sissons *et al.* (1982) concluded that glycin and β -conglycinin levels were best denatured when soy protein was treated in 65 % ethanol at a temperature of 78 °C. In studies done by Kilshaw and Sissons (1979), it was furthermore shown that the antigenic activity of soy protein can be completely eliminated by treatment with hot aqueous alcohol. Several studies have proven that calves given a milk replacer containing protein from soy protein concentrate prepared by ethanol extraction had significantly better performances than calves receiving a milk replacer containing protein from heated soy flour (Gorrill and Thomas, 1967; Gorrill and Nicholson, 1969; Nitsan *et al.*, 1971). Wolf (1970) concluded that this improvement was due to the removal of oligosaccharides, which are soluble in aqueous alcohol, from soybean meal. Additionally, phytase activity is decreased by 50-70% with alkaline environments compared to non-modified soybean isolate (Borowska and Kozłowska, 1986).

Acid Treatment

Colvin and Ramsey (1968) have found that calves fed acid-treated soy flour grew at nearly twice the rate of those receiving untreated soy flour. They observed

improved calf performance when fully cooked soy flour was treated at pH of 4 versus 6.4. Sudweeks and Ramsey (1972) found similar results in that acid treatment did improve calf growth rates on soy flour based milk replacers, but they did not see any differences between acid-treated diets at different pHs. They also found that the carbohydrate fraction of the soy flour was not any more available to calf digestion, and therefore was not involved in the improved growth rates observed. Trypsin inhibitor, but not other detrimental factors, was degraded by proper treating with acid (Barr, 1981). Wilson and Ramsey (1972) also found that soy flour treated with anhydrous hydrochloric acid had improved nutrient value; however, they could not establish an optimum level of acid treatment. Nutritional quality of soy protein concentrate is improved by thermoalkali processing for 5 min, but increasing the length of time of processing to 25,

Toasting treatment of Soybean

Processing using dry roasting, or toasting, produces a very palatable, nutritious food from soybeans (Badenhop and Hackler, 1971). Poor results from feeding trials utilizing raw soybean meal are related to trypsin and chymotrypsin inhibitors which are inactivated by toasting (Nitsan *et al.*, 1971). The process of toasting additionally improves digestibility and availability of both protein and carbohydrates (Nitsan *et al.*, 1971).

During the roasting process, the temperature attained was considerably higher than needed to destroy trypsin inhibition and may indeed be detrimental to the nutritional quality of the protein (Badenhop and Hackler, 1971). Since, palatability is the number one factor in early weaning consumption, this protein loss is balanced by the benefit of increased consumption. The toasting process includes a rapid dehydration followed by a partial pyrolysis (Badenhop and Hackler, 1971). Soybeans toasted at 146 °C are better digested than untoasted soybean meal (SBM) (Abdelgadir, 1996).

Trypsin inhibitor activity is very low in toasted soybean meal and in isolated protein commercially processed (Rackis, 1966). Kunitz-type trypsin inhibitors, which have activity against trypsin, and Bowman-Birk type trypsin inhibitor, which inhibit both trypsin and chymotrypsin, accounted for most residual trypsin inhibitor activity of toasted soybean flour (Sessa and Bietz, 1986; Steiner and Frattali, 1969). Soybeans processed at 138 °C with or without tempering or at 171 °C are all similar in digestibility (Abdelgadir *et al.*, 1984). Additionally, calves consuming the starters containing soy protein processed at 171 °C consumed more feed, gained weight faster, had lower fecal scores, and less mortality than calves consuming soybeans processed at 138 °C with or without tempering (Abdelgadir *et al.*, 1984). Digestibility has been shown to be higher in toasted soybeans than microwave-cooked soybeans (Prasad and Morrill, 1976).

Fermentation of Soybean meal

Fermentation is another processing method that produces a highly palatable product. Fermentation also often increases the availability of nutrients and amino acids such as lysine, methionine, and tryptophan in the blends (Chompreeda and Fields,

1984). In addition to reducing antinutritional factors and eliminating trypsin inhibitors (Feng *et al.*, 2007), fermentation also reduced raffinose and stachyose in soybean meal (Chompreeda and Fields, 1984), which increases nutrient digestibility.

The most important factor in having a product ideal for fermentation is the heat treatment given to soy proteins at any stage during its preparation before inoculation with the organisms for fermentation (Patel *et al.*, 1980). The best heat treatment found by Patel *et al.* (1980) was treatment at 100 °C for 20 minutes. Certain minimal heat treatment was necessary to eliminate the lipoxylase activity and to destroy trypsin inhibitor found in raw soybean meal whether the product is being further processed by fermentation or not (Patel *et al.*, 1980).

Soybean processing treatments like soaking and cooking, which are often employed in soymilk preparation, substantially reduce the content of fermentable, soluble carbohydrates needed for the fermentation microorganisms (Patel *et al.*, 1980). Fortification of soy proteins with certain sugars like lactose and glucose is imperative to increase the sugar substrates present for the microorganisms to utilize (Patel *et al.*, 1980). Sucrose supplementation seems to be particularly suitable for certain lactobacilli like *Lactobacillus acidophilus*, alone or in combination with *Streptococcus thermophilus* (Patel *et al.*, 1980).

Fermentation of soybean meal using several *Bacillus* spp. has increased digestibility of soy proteins as well (Kiers *et al.*, 2003). Kiers *et al.* (2003) also found that complete breakdown of 3 subunits from β -conglycinin and both polypeptides from glycinin occurred after fermentation with *Bacillus subtilis*. Feng *et al.* (2007) also found that fermentation improved the nutritional value of soybean meal and reduced or eliminated some important antinutritional factors, such as glycinin and β -conglycinin. Active trypsin inhibitors have been shown to be liberated from a heat-resistant, inactive, bound form during fermentation by *R. oligosporus* proteases (Wang *et al.*, 1972); however, this trypsin inhibitor was readily inactivated by heat.

Conclusion

For agriculture, organic farming is more effective which can be done by usage of natural sources like soybean seeds which helps in the supplement of Nitrogen source to plants. We have proven the sharp increase in the growth of the plants when compared to the treatment using chemical fertilizers. Organic farming unalters the pH of the soil, does not cause any deficiency in the nutrients and also avoids the pest attack. Organic fertilizers are very easy for the plants to absorb than the chemical fertilizers. It is very cheap source and environmental friendly and helps in increasing the yield of the plants.

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