REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Soybean (*Glycine max*), native to China, has been cultivated from the wild and consumed as a staple food for many centuries in China and adjacent Far Eastern countries (Huan and Bao, 1989). In the light of shortage of animal protein, and the functional versatility and nutritional significance of soybeans, a variety of modern technology in the Western world has been developed to utilise soybean. Many soy products such as full-fat flour , defatted flour , protein concentrate, protein isolate, texturised soy protein and spun soy protein, have resulted in the fabrication of various new products (Circle and Smith,1972 ; Visser and Thomas, 1987). Using soy products in dairy analogues and meat substitutes is the most common application of soybean as a non-traditional ingredient (Thulin and Kuramoto, 1967 ; Schaefer and Holdt, 1992).

This review highlights the traditional foods made from soybean, for consumption in the orient and its slowly increasing popularity in the developing countries, where simple traditional technologies are being used for incorporating soy bean in their conventional foods. Review also deals with the basic approaches to overcome the limitation of using soybean as a substitute or replacement for these conventional products in the developing countries applying various simple processing technologies.

Relevant literature on these and related aspects are reviewed under the following sub sections :

2.1) Traditional soybean foods of the Orient

2.2) Problems associated with the utilisation of soybean

2.3) Overcoming the limitations of using soybean

2.1 Traditional Utilization of Soybean

Historically, the consumption of soybean in China dates back to 16th century, where the beans and leaves were simply cooked and eaten as staple food. The bean sprouts were used for medicine and food. The consumption pattern of soybeans underwent major changes with the invention of tofu and related products between 207 B.C - A.D 960, and its recognition as a source of oil during A.D 960 - 1127 (Huan and Bao, 1989). The progress in the processing innovations resulted in a variety of new products, such as Soy sauce , Miso , Sufu, Natto etc,. Many of these traditional products have already gained popularity in the Western countries.

The forms of the different traditional foods prepared from soybean in the Far East as summarised by Byrne (1985), is presented in Table 2.1. Traditionally , most of these products

Table 2.1 Traditional Soy foods of the Orient*

SOYFOODS	LOCAL NAMES	DESCRIPTION	USES
	I. Oriental	Non Fermented Soyfooods	
Fresh green soybeans	Mao-tou (Chinese) Edamame (Japanese)	Picked before mature, firm, bright green	Boiled or steamed in the pods and shelled. Served as hors d'oeuvre (often with wine or beer or shelled before cooking served as fresh green vegetable.
Soybean sprouts	Huang-tou-ya(Chinese) Daizu-no-moyashi (Japanese)	Bright yellow beans with 3-5 cm sprouts	Served as vegetable in dishes or in salad (parboiled)
Soynuts	Hueh-huang-tou (Chinese) Iri-mame (Japanese)	Roasted whole soybeans or cotyledons by deep-frying or dry heat. Seasoned by salt,garlic or coated with chocolate	Served as snack.
Soymilk	Tou-chiang or tounai (Chinese) To-nyu (Japanese) Kongkuk (Korean)	Water extract of soybeans, resembling cow's milk, with plain, sweetened and flavoured	Served hot as breakfast drink or served cold as soft drink.
Soyflour	Huang-tou-fen (Chinese) Kinako (Japanese)	Ground roasted dry soybeans with nutty flavour	Used as filling or coating for pastries.
Soy protein lipid film	Tou-fu-pi (Chinese) Yuba (Japanese)	Creamy - yellow film formed over the surface of simmering soymilk, and moist sheets, flakes or sticks	Cooked and used as meat with chewy texture.
Soybean curd	Tou-fu (Chinese) Tofu (Japanese) Tubu (Korean) Taboo(SE Asia)	White curd cubes coagulated from soymilk with a calcium or magnesium salt soft to firm (by pressed curds)	Served as dishes with or without further cooking.

(Contd.,)

Table 2.1 Traditional Soy foods of the Orient* (Contd.,)

SOYFOODS	LOCAL NAMES	ORGANISMS USED	DESCRIPTION	USES
		II. Oriental Fermented Soyfoods		
Soy Sauce	Chiang-Yu (Chinese) Shoyu (Japanese) Ketjap (Indonesian) Kanjang (Korean) Toyo,see-ieu (S.E. Asia)	*Aspergllius*, *Pediococcus*, *Torulopsis* and *Saccharomyces*	Whole soybeans or defatted soy flake and wheat	Dark brown liquid,salty taste suggesting the quality of meat extract, a flavouring agent.
Miso	Tou-chian, wei-cheng (Chinese) Miso (Japanese) Doenjang (Korean) Tau-cho (Indonesian) Soybean Paste	*Aspergillus*, *Pediococcus* *Saccharomyces*, *Torulopsis* and *Streptococcus*	Whole soybean, rice or reddish brown paste.	Light yellow to dark smooth or chunky,salty and flavoured. A flavouring agent.
Tempeh	Tempeh (Indonesian) Tempei (Japanese)	*Rhizopus*	Whole soybeans	Cooked soft beans bound together by mycelia as cake, a clean fresh and yeasty odour Cooked and served as main dish.
Natto	Natto (Japanese) Tau-nou or Thua-no (Thialland) Kenima (Nepal)	*Bacillus natto*	Whole soybeans	Cooked beans bound together and covered with viscous, sticky substance, produced by the bacteria, ammonium odour musty flavour. Served with cooked rice as a dish.
Fermented tofu	Tou-fu-ru or Tao-ru (Chinese) Fu-nyu (Japanese) Sofu (Indonesian) Tohuri (Phillippiname) Chinese cheese	*Actinomucor*, *Mucor*	Soybean curd (firm tofu)	Creamy cheese-type cubes salty A condiment, served with or without further cooking.
Soy Nuggets	Tou-shih (Chinese) Tao-si (Philippinese) Tau-cheo (Malaysian) Tauco (Indonesian) Hamanatto (Japanese)	*Aspergillus*, *Streptococcus*, and *Pediococcus*	Whole soybeans Wheat flour	Nearly black soft bean, salty flavour resembling soysauce. A condiment.

* Byrne (1985)

utilised only soybean as a starting material, which was further replaced with defatted flakes in some of the products such as, shoyu, miso etc. Eventhough there have been many subsequent developments, namely the use of pure cultures, appropriate fermentation techniques and improvements in production and quality standards, the basic product manufacturing process remains the same (Hasseltine, 1983). Besides the germinated and roasted soybeans, soybean was consumed in the form of beverage (soy milk) , non fermented and fermented curds prepared from soy milk (tofu, sufu), mould fermented whole soy bean (natto and tempeh), and in the form of fermented seasoning such as shoyu and miso.

2.1.1 Soymilk

Soymilk is one of the important, basic product derived from soybean. Besides being consumed as liquid, this is also a starting material for the manufacture of various other soy products viz. Tofu, Sufu, etc (Ang and Kwit, 1985). Soymilk and their derivatives have played a vital role for many centuries as a source of protein in the diets of millions of people in Asia (Hang and Jackson, 1967).

Traditional preparation of soymilk involves atleast 8 - 10 hrs soaking of whole soybean in water, grinding of soaked soybean with water and filtration of ground mass through a cheese cloth. Several variations exist in the preparation of soymilk, mainly in terms of its extraction procedure(Ang and Kwit, 1985 ; Miskovsky and Stone, 1987). Soymilk traditionally prepared in China using cold filtration method was not accepted in Japan due to its strong flavour and was replaced with a hot filtration method to obtain soymilk with bland taste (Beddows and Wang, 1987). The milk thus obtained is flavoured and consumed as a drink or utilized in the preparation of various other products such as Tofu, Sufu etc., (Ang and Kwit,1985). However, such a product would still not be acceptable to the population of West and also in countries such as India, where people are not accustomed to soy bean (Mital and Steinkraus, 1979). Soy milk commercially available contains 1.1 - 3.6 % with a fat content of 1.5 %. Soymilk is low in sugars compared to dairy milk and therefore is often fortified with sugars (De Man *et al.*, 1987).

2.1.2 Tofu

Tofu or "bean-curd" , which is coarse or fine textured "silken" or "soft Tofu", is consumed as an every day food in oriental countries (Watanabe *et al.*, 1964). Preparation of Tofu from soymilk begins with the coagulation of soymilk with calcium salts. The coagulated mass is then laddled into baskets, to promote the expulsion of whey and the formation of cakes.

Modification in the method results in three basic types of tofu, namely soft tofu , hard tofu and dry tofu, varying in moisture content between 79 - 89 % . **Soft tofu** has a very soft texture and is generally consumed directly with a little sauce. In the preparation of **hard tofu** ,the soy curd is poured into wooden trays lined with cheese cloth, wrapped and pressed into blocks. **Dry tofu** is the firmest variety. The hard tofu is boiled in a mixture of soy sauce and seasoning to make savoury tofu (Shurtleff and Aoyagi, 1979 ; Tsai *et al.,* 1981). The protein content of tofu ranges between 5.5 % to 14 % depending on the length of time and pressure applied during its preparation (Mathur *et al.*, 1991).

2.1.3 Sufu

Sufu is a mould fermented product prepared with hard tofu and consumed only in China (Fukushima, 1979). In the case of utilizing tofu for the preparation of sufu, moisture content of tofu is kept below 70% , compared to the ordinary tofu consumed at 90% moisture. In the preparation of sufu the hard tofu is cut into 3 cm cubes and heated. To this heat treated tofu, moulds belonging to the genus of *Mucor* or *Actinomucor elegans* are usually used for fermentation to take place within 2 - 7 days. After the mould fermentation, the tofu is brined and left for aging, the duration of which ranges from 1 to 12 months, depending upon the variety of solutions used for brining (Tsai *et al.*, 1981, Beuchat, 1984).

2.1.4 Natto

"Natto" or fermented whole soybean is native to Japan (Fukushima 1979). A product similar to Natto is called "tu-su" in China and "tao-si" in Phillipines (Hasseltine and Wang,1967). Natto is prepared by fermenting the whole cooked soybean with *Aspergillus* or *Bacillus natto* resulting in two types of natto such as **Itohiki-natto** and **Hama-natto. Yukiwari natto** is another type of natto prepared by mixing itohiki - natto with rice, salt and ageing for 2 weeks at 25 - 30^{o}C Among these, Itohikki-natto or Bacillus natto , prepared with a strain of *B. subtilis* , is popular. Manufacturing of natto involves the fermentation of cooked soybean with bacillus natto for the duration of 14-18 hrs, this gives distinctive viscous fluid coating on the soybean. Hama natto, which resembles Miso in taste, is prepared by fermenting cooked whole soybean with the mold, *Aspergillus oryzae*. Natto is normally served with Shoyu and mustard (Kiuchi *et al.*, 1976 ; Reddy *et al.*, 1982).

2.1.5 Tempeh

Tempeh is a popular fermented soybean product of Indonesia. The traditional procedure for making tempeh includes removal of hulls by soaking soybean and the pressed soybeans are added with small amount of tempeh from a previous batch or with ragi tempeh or with a commercial starter containing sponrangiophores of *Rhizopus oligosporous*. The inoculated soybeans are then spread on bamboo frames (baskets), wrapped in banana or other suitable leaves and fermented for 1-2 days, during which the white mycelia of the mold invade and cover the substrate mass to bind the soybean. Tempehs are eaten within a day of its preparation, otherwise the release of ammonia due to the breakdown of mycelia and soy protein makes the product inedible . Consumption of tempeh is usually in the form of slices dipped in salt solution and deep fat fried. Another similar fermented product consumed in Nepal, Sikkim and Darjeeling districts in India is **Kenima** which is prepared in the similar way as tempeh is prepared (Reddy *et al.*, 1982 Beuchat, 1984).

The solid waste material or **okara** obtained as a residue after soymilk is filtered out from the wetground whole soybean and water mixture, is used in the preparation of fermented product similar to tempeh, called **Meitauza**. For the preparation of meitauza, the okara is moulded in to small cakes, inoculated with a mould (*Mucor mitauza* or *Actinomucor elegans*) and fermented for 10 - 15 days. During the fermentation, the mycelia of the mould covers the surface, and the fermented cakes are further dried and fried in oil or cooked with vegetables. (Reddy *et al.*, 1982).

2.1.6 Soy sauce

Soy sauce or (Chiang - Yu) originated in China. Due to its commercial value, the process knowhow was guarded as a family art passed on from one generation to the next with the variation in the method of preparation. The traditional preparation of soy sauce in China involves two major steps namely **koji** making and brine fermentation. In koji making cooked soybeans and roasted and cracked wheat in equal proportion are fermented with *A. Oryzae*. This koji is used as a seed mould for fermenting fresh batches. In brine fermentation the fermented koji is mixed with the salt solution to obtain **moromi**, which is left for fermentation for 6 - 8 months. The solid portion is eliminated and resultant liquid is filtered and used as soy sauce. This was later introduced in Japan by Budhist priests as a vegetarian seasoning alternate to the popular seasoning prepared from meat and fish (Smith and Circle, 1972 ; Fukushima, 1979).

The differences in aromas observed between Chinese and Japanese varieties of soy sauce are mainly due to the proportion of wheat used and the method of processing. The Japanese soy sauces have more proportion of wheat. Refining process which includes filtering and pasteurisation is a major processing difference included by the Japanese (Beuchat, 1984). Due to the popularity of vegetarianism in Japan, soy sauce further transformed into special soy preparations such as Miso (Fukushima, 1979 ; Reddy *et al.*, 1982).

2.1.7 Fermented Soybean paste

Fermented soybean pastes are also a fermentation product of soybeans and cereals in the presence of salt and are known as **Chaing** in China, **Jang** or **Doenjang** in Korea, **Miso** and **Shoyu** in Japan, **Tauco** in Indonesia, **Tao-chico** in Thailand and **Too-si** in Phillippines. Among these, Miso in Japan is manufactured commercially and available in three different types i.e. rice miso, barley miso and soybean miso. According to their taste they are further grouped as sweet, semi-sweet, and salty miso (Fukushima, 1979, Beuchat, 1984).

The manufacturing process of miso is somewhat similar to the preparation of soy sauce which involves two important processes such as 1) Koji making and 2) Brine fermentation without the refining process employed in shoyu preparation. Koji making in miso is carried out only with the carbohydrate source of ingredients such as rice or barley and mixed with non fermented cooked soybean, and undergoes lactic/yeast fermentation. This fermented mix further undergoes aging for 5 days to 20 days according to the variety of miso. After the ageing process the fermented mass is mashed and used as a soup base. Consumption of rice miso is much higher compared to barley or soybean miso (Beuchat, 1984 ; Fukushima, 1989).

The "soybean culture" created and developed by Oriental people, especially Chinese , Japanese and Koreans, encouraged the direct consumption of vegetable protein (Huan and Bao, 1989). This culture has brought a strong influence in popularising soybean to the other parts of the world where soybean was unknown.

The traditional ways of consuming soybean directly and processing soybean to milk type products such as soymilk, tofu and sufu, meat like products namely tempeh , and seasoning such as shoyu have contributed towards the development of various modern day foods.

This brief review on the traditional utilisation of soybean in the Orient clearly indicates that the fermentation has been a common approach in the development of various conventional products. Recent devlopments in the introduction of these conventional foods to Western and other countries mainly involve the use of pure culture strains , in order to improve product characteristics, and better control on the fermentation process.

2.2 Problems associated with the utilization of soybean :

Although soy products have been a major source of human food in the orient for more than 5000 years, the incorporation of soybean in the form of traditional Oriental products has not been of much success in the Western and other national diets. The West and other developed countries have attempted to use soybean in distinctly different forms , where soybean and its derivatives serve the role of ingredients in the formulation of the conventional foods of these populations. In order to understand the problems associated with the utilisation of soybean in these countries it is necessary to understand the approach of incorporating soybean in their various conventional foods. The ways in which soybeans are utilised in these countries, a distinct differences in approach may be noticed between the Western and other developing countries (Anon., 1981).

2.2.1 Utilisation of soybean in the West

Cultivation of soybean in substantial quantities in the West was taken up in the 1920s . The early interest was limited to the production of soybean oil for use in the manufacture of products such as shortening,cooking oil,margarine , dressings etc,. The defatted meal was used as animal feed (Wolf and Cowan, 1975).

The early introduction of soybean as a meat substitute did not receive a favourable response mainly due to the prejudice of what they called soybean, a "pig food". However, the introduction of soybean in various prepared and processed meat containing foods such as pies , puddings, stews,casseroles were considered acceptable (Anon., 1981 ; Anon., 1984).

Due to the nutritional significance of soybean and the world wide shortage of animal protein in the 1950's, technologies were developed to utilise soybean in several form of protein isolate, protein concentrate,texturised soy protein and spun soy protein etc, (Circle and Smith, 1972). Negative publicity and consumer awareness regarding saturated animal fats and cholesterol in foods of animal origin have also contributed towards the increasing popularity of these products (Barraquio and De Voort, 1988).

Western world is already well adapted to the use of various derivatives of soybeans such as full-fat flour, defatted flour , soy grits etc., in the preparation of soy isolates and soy concentrates (Morr, 1979). Improved oil extraction procedures yield defatted soyflakes with desirable functional properties (Waggle and Kolar, 1979 ; Lillford, 1983). These derivatives are further modified in the subsequent processing steps such as extrusion (Boyer, 1954), spun fibre texturisation (Hoer,1972) and controlled enzymatic hydrolysis or plastein formation (Fujimaki *et al.*, 1970) to yield products or analogues to conventional foods with good flavour profile and functional characteristics (Kinsella, 1979) . Examples in this category would include dairy analogues such as coffee whitener,sweet and sour cream,cheese,frozen dessert and whipped toppings and meat analogues namely frankfurters,bologna,sausages, meat patties etc.

Apart from the utilisation of soybean in meat and dairy products, soy protein products have found wide application in bakery, infant foods, hospital feeding and protein supplement products (Wolf and Cowan, 1975 ; Morr, 1979 ; Waggle and Kolar, 1979).

In addition to the developments in soy bean processing technologies in the West, the powerful marketing promotion of soy products has resulted in their increased utilisation (Byrne, 1985).

2.2.2. Soybean utilisation in India

In contrast to the importance of soy protein products in Western countries, it is yet to create an impact in India, inspite of its predominant protein shortage (Patni,1985).

Eventhough, the black variety of soy bean has been cultivated in India for many years in the Kumaon and Garwhal regions of U. P. and on the foothills of Himalayas, the cultivation of yellow varieties in the 1970's created a major impact in the oil processing Industry which had its modest beginning in 1975 (Shroff *et al.*, 1986). The industrial processing facilities for soy bean are established in India mainly for the solvent extraction of edible oil from soybean and to treat the deoiled, desolventized cake as a product for export.

The cultivation of soybean is steadily increasing since its beginning in 1975, with the exception of the drought year 1987 - 1988. The area and production of soybeans have increased from

6.22 lakh hectares and 4.66 tonnes in 1981 - 82 to 22.74 lakh hectares and 23.30 lakh tonnes, respectively during 1990 - 91. The soybean processing industry is mainly concentrated in the states of Madhya pradesh , Maharastra , Gujarat and Uttar Pradesh. Cultivation of soy bean is also being encouraged in the states of Tamilnadu, West bengal, Andhra Pradesh and Karnataka (Patni, 1985 ; Sulebule, 1991).

To derive nutritional benefits from soy protein various attempts had been made to introduce soybean in Indian diets. Kale (1985) formulated various recipies for the utilisation of soybean in Indian diet. The systematic efforts on the product development work from co- ordinated research projects set up at Pantnagar (Singh and Mital, 1984 ; Nasim *et al.*, 1986), Bhopal (Ali and Gandhi, 1988) and at the institutions such as National Dairy Research Institute Karnal (Rajor and Patil,1983; Patil and Gupta, 1981) and from Agricultural and other institutions (Kanchana *et al.*, 1990 a,b ; Changade and Tambe, (1992) has resulted in many possible applications of soybean suitable for Indian palate.

The incorporation of soybean in Indian diets have adapted essentially two major approaches namely, using soybean as a substitute for pulses and as analogues of dairy and meat products. Legumes, meat and milk products being considered as regular items of foods in the Indian diet, substitution of these items with soybean would have a greater potential in increasing the consumption of soybean. Soybean based Indian food items, developed through last few decades of research have been reviewed by Naik (1995) , and presented in Table 2.2. These studies indicate the extent of substitution (Full / partial) possible without significantly altering the acceptability characteristics of the conventional products.

Among the cereal - pulse based fermented products such as Idli, Khaman and Dhokla, soybean has been demonstrated to successfully replace the entire amount of the traditionally used pulses such as black gram and bengal gram dal (Cherian, 1969 ; Akolker and Parekh, 1983). Products such as "sweetball" , high protein savouries, rajma and chole also utilised 100 % soy dal (Chakraborty *et al.*, 1985 ; Kanchana *et al.*, 1990 a,b). In other products, the substitution level ranged somewhere between 15 % to 60 % in the preparation of chapaties, bread and biscuits.

In case of milk based products the substitution level was above 25 % . Dairy milk was completely replaced with soy milk in the development of paneer like products (Nasim *et al.*, 1986). The inclusion of soybean in milk based products has always been

Table 2.2 Soybean in Indian foods*

NAME OF THE PRODUCT	FORMS OF SOYBEAN	LEVEL USED	REFERENCES
I. Fermented products (non-dairy)			
Khaman and Dhokla	Soaked & Dehulled	100%	Cherian (1969)
Idli	Soaked & Dehulled	25,50,100%	Cherian (1969);Thomas & Kamath (1988) Vaidehi (1988) Geervani et al., (1988) Akolkar & Parekh (1983)
Dosa	Soaked & Dehulled	50%	Geervani et al., (1988)
II. Sweet products			
Halwa	Okara	50-100%	Parihar et al., (1977) Kaur (1992)
	full fat flour	50%	Ali & Gandhi (1988)
	Flaked	20%	Desikachar (1988)
Burfi, Mysore Pak	Defatted and full fat flour	50%	Ali & Gandhi (1988)
Payasam	Flaked	20%	Desikachar (1988)
Rava Kheer	Okara	50-100%	Kaur (1992)
Sweet ball	Soydal	100%	Kanchanaet al.,(1990a)
III. Bakery products			
Bread	Soyflour	15%	Surana et al.,(1973);Khadar(1983) Raidi & Klein (1983)
Biscuit	Defatted and full fat flour	50,20%	Geervani (1988) Vaidehi (1988) Gupta & Singh (1991)
Cake	Okara	-	Parihar et al.,(1977)

(Contd.,)

Table 2.2 Soybean in Indian foods* (Contd.,)

NAME OF THE PRODUCT	FORMS OF SOYBEAN	LEVEL USED	REFERENCES
IV. Meat Substitutes			
Sausages,Meat Chunks	Okara	50-100%	Kaur (1992)
Meal Extenders	extruded soyflour	100 %	Ruchi Industries (Nutrella) Voltas Industries (Meal maker)
V. Milk substitute			
Paneer	Soydal Soydal Soybean	60% 100% 100%	Vijayalakshi & Vaidehi (1982) Nasim *et al.*,(1986);Gangopadhyay and Chakraborti (1989)
Soycurd	Soybean	25%	Changade and Tambat (1992)
Chana	Soybean	30%	Katara and Bhargava (1992)
Protein rich beverage	Soybean	75%	Patil and Gupta (1981)
Softy Icecream	Soybean	Partial	Rajor *et al.*, (1983)
Icecream	Soybean	Partial	Rajor and Patil (1983)
Lassi/cultured drink	Soybean	70%	Deka *et al.*, (1984)
Frozen yoghurt	Soybean	50%	Rajasekaran and Rajor (1989)
Cheese Spread	Soybean	64%	Singh and Mital(1984) Kumari and Singh (1985)
Kulfi	Soyflour	50%	Rajor and Vani (1991)
Low fat spread	Soybean	partial	Patel and Gupta (1988)
Flavoured Soymilk (Greatshake)	Soydal	100 %	Godrej India Ltd

(Contd.,)

Table 2.2 Soybean in Indian foods* (Contd.,)

NAME OF THE PRODUCT	FORMS OF SOYBEAN	LEVEL USED	REFERENCES
Others			
Chuvda	Soaked & Dehulled	50%	Geervani *et al.*, (1988) Desikachar (1988)
Chutney Powder	Soaked & Dehulled	50%	Geervani *et al.*, (1988)
Rajma and Chole type	Soydal	100%	Chakraborty *et al.*, (1985)
Pongal and Khitchidi	Flaked Soydal	20%	Desikachar (1988)
Chapati, Puries	Soyflour	50-60%	Desikachar (1988)
Roti, tandoori, nans	Soyflour	15%	Ali & Gandhi (1988) Ebeler & Walker (1983)
Samosa, Sev kurma	Okara	-	Parihar *et al.*, (1977)
Upama, Chutneys	Okara	50-100%	Kaur (1992)
High protein Savouries	Soydal	100%	Kanchana *et al.*, (1990)

*Adapted from Naik (1995).

faced with the criticism of imparting beany flavour , which otherwise is bland in taste (Morr, 1979). Lactic fermentation which is commonly practiced in the Indian household for making "dahi" from dairy milk, was adapted in improving the flavour of soy products (Mital and Steinkraus,1976). Since most of these products developed utilise whole soybean or defatted flour as a starting material, and use simple processing technologies,cost of these products is likely to be lower than the traditional products with similar nutrient content.

Although several recipies formulated with soybean has been published in the literature, relatively few products have been exploited on a commercial basis.Important ones include,flavoured soymilks, marketed as "Soyso" and "Great shake", and meat extenders under the brand name "Nutrella" and "Meal maker" by Ruchi and Voltas India Ltd respectively. These products have yet to create any substantial impact in the Indian dietary habits, mainly due to their unattractive cost, compared to those for dairy milk or meat products, and lack of aggressive marketing.

It is thus clear that the advanced food proceessing technological innovations utilised by the West has already given them a series of soybean preparations of improved nutritional and functional quality more suitable for their type of products. In contrast developing world's approach has been to use simpler traditional food technologies with varying degrees of modifications for utilising essentially the whole soybean for their type of conventional products.

2.2.3. Constraints in soybean Utilisation

Development of new products utilising soybean imposes three important constraints namely antinutritional factors in soybeans, characteristic beany or objectionable off flavours in soy products, and functional compatibility of soy products in food systems (Rackis *et al.*, 1979;Liener, 1979;Kinsella, 1979).

2.2.3.1 Antinutritional Factors

The presence of antinutritional compounds is a common difficulty in the utilisation of legumes in general. Their significance varies according to their concentration levels, intensity of their effects, and to the possibility of their inactivation, reduction, or elimination in different stages of food preparation and processing conditions (Jood *et al.*, 1985).In this category, a large number of compounds have been implicated, including favism inducing factors, trypsin inhibitors and tannins, antinutritional amino acids, flatulant factors, lectins, cyanogenic factors, and alkaloids (Monte and Grillo, 1983).

The antinutritional factors detected in soybean include trypsin inhibitors, and haemagglutinins, both of which are heat labile, and oligosaccharides (flatus producing), goitrogens, estrogens and allergens (Anderson *et al.*, 1979). Among these trypsin inhibitors, which belong to a broad class of proteins known to inhibit proteolytic enzymes, have been shown to exert significant adverse effects on animals (Rackis, 1965).

2.2.3.1.1 Trypsin inhibitors

The importance of trypsin inhibitor in soy bean was realised for the first time with the observations of Osborne and Mandel (1917) regarding the growth depressing effects of soybean in rats. Although more than five types of trypsin inhibitors have been isolated (Wolf and Cowan, 1975) , only two major water soluble proteins in the whey protein fraction of soybean (25 - 28 %) such as Kunitz trypsin inhibitor with a molecular weight of 21 k. Da. (Kilo dalton) and Bowman - Birk trypsin inhibitors, with a comparitively lower molecular weight of 8 K. Da. (Tan-Wilson and Wilson,1986) have been extensively studied . These two inhibitors are also different interms of their structural and inhibitory activity. The presence of six disulfides linkage in Bowman - Birk inhibitors make the molecular structure more stable and resistant to denaturation by heat and acid or pepsin, compared to Knuitz inhibitors which are easily inhibited by these treatments (Wolf and Cowan, 1975). The knuitz inhibitors strongly inhibit trypsin at a single specific reactive site and weakly inhibit chymotrypsin at two reactive sites whereas, Bowman - Birk inhibitors simultaneously inhibit both the protease molecules (Tan-Wilson and Wilson, 1986), thereby interfere with the regulation of pancreatic secretion and cause pancreatic enlargement and growth inhibition. A specific Arg (64)-Ile (65) peptide bond cleavage in the virgin inhibitor has been proposed to be the primary stage in the formation of active modified trypsin inhibitor (Finkenstadt and Laskowski, 1967). However alternative mechanism such as the specificity of aminoacids on the affinity of enzymes has also been suggested by Haynes *et al.*, 1967.

The mechanism whereby the trypsin inhibitor induces pancreatic enlargement in man is still not fully understood (Liener, 1979 ;Grant, 1989). However, the suppression of negative feed back regulation of trypsin and chymotrypsin for the growth inhibition postulated in rat has also been suggested in humans (Grant, 1989). The pancreatic secretion is controlled by a mechanism of feedback inhibition which depends upon the level of trypsin and chymotrypsin present in the small intestine at any given time. When the level of these enzymes fall below a certain critical threshold value, the cholecystokinin is released from the intestinal mucosa which further induces the pancreas to produce trypsin and chymotrypsin. The supression of negative feed back inhibition can occur if the trypsin is complexed with the inhibitor or by the undenatured protein itself (Fushiki and

Iwai, 1989). The improper digestion of soy protein combined with the loss of essential aminoacids present in trypsin and chymotrypsin explains the growth inhibition effect of soybean (Liener, 1979).

The fractionation of soybean to soy protein concentrates and isolates substantially reduces the trypsin inhibitor activity (Kotter, 1970 ; Churella *et al.*,1976 ; Liener, 1979).

Many soybean products available in the Western market are made from soy bean isolate , which depending on their mode of preparation, retains no more than 30% of the trypsin inhibitor activity of raw soybean (Liener, 1979).

Kotter *et al.*, (1970) reported that canned frankfurter-type sausages containing 1-5% soy isolate were devoid of any trypsin inhibitor after the canning process. Several meat analogues developed with soy isolate found to have antitrypsin activity ranging from 6.5-10.2 units (Trypsin Inhibitor Units g dry solids x 10^{-3}) of trypsin inhibitor compared to anti-trypsin activity of 86.4 units of unheated soy flour (Liener, 1979).

Nordal and Fossum (1974) reported that the trypsin inhibitor activity, especially Bowman-Birk inhibitor being rich in disulfide, is more labile to heat inactivation due to some components in meat ingredients.

Blesa *et al.*, (1980) found that the presence of cow's milk (60 %) in soy milk (40 %)accelerated the inactivation of trypsin inhibitor activity.

Churella *et al.*, (1976) demonstrated that the heat treatment involved in the processing and sterilisation of infant soy formulas prepared with soy isolate had only 10 % of original activity of the isolate.

In products prepared with whole soybean as a starting material and using traditional processing methods, maximum inactivation of trypsin inhibitors has been observed.

Manorama and Sarojini (1982) studied the trypsin inhibitor activity in soybeans subjected to different heat treatments, and found that the pressure cooked soybeans retained very little of the initial activity, followed by boiling, puffing and roasting . Authors also reported that the trypsin inhibitors of soybean was completely inactivated in products like "dosa" and "vada". In all moist heating methods , soaking soybeans enabled faster destruction of trypsin inhibitor activity.

Kanchana *et al.*, (1990 a & b) developed savouries and sweet products completely free from trypsin inhibitor using conventional processing methods such as frying , pressure cooking and puffing of soaked soybeans.

2.2.3.1.2 Oligosaccharides

Another important group of antinutritional factor limiting the direct consumption of soybean is the low molecular weight oligosaccharides. The oligosaccharides such as raffinose and stachyose containing alpha 1-6 galactosidase linkage have been implicated as causative factor in digestive disturbances (Steggerda *et al.*, 1966). The level of raffinose and stachyose in soybeans are 1.1 and 3.8 % respectively (Kawamura, 1967) and both these sugars are non - reducing in nature. Due to the absence of alpha-galactosidase in the human intestinal tract, these are not utilised (Steggerada *et al.*, 1966, Cristofaro *et al.*, 1974). The undisgested sugars pass into the large intestine, undergo anerobic fermentation by alpha-galactosidase producing bacteria which results in the production of gas (Rackis *et al.*,1970). This increase in pressure in rectal gas in acute cases leads to various vegetative signs of discomfort, such as headache, dizziness, slight mental confusion, reduced ability to concentrate (Cristofaro, 1974).

Flatus activity of soyprotein products vary due to the form in which they are prepared and consumed. The fractionation procedures involved in the preparation of soy protein products reduces these undesirable compounds (Visser and Thomas, 1987). Full- fat and defatted soy flours cause increased flatulance compared to soy protein isolate. Similarly soy protein concentrate prepared from soyflour extracted with 80 % ethanol show less flatus activity (Staggerda *et al.*, 1966 ; Rackis *et al.*,1970).

However the utilisation of unprocessed soybean has a limitation of retaining higher amounts of these flatulance factors in the final products. In order to overcome the ill effects of oligosaccharides in the products prepared from unprocessed soybean , two possibilities namely removal or hydrolysis of oligosaccharides has been conidered. Hydrolysis of oligosaccharides is considered to be more appropriate approch since it retains the monosaccharide galactose (Maiti and Paul, 1992). Since these oligosaccharides are water soluble,hydration and softening which occur during soaking in water facilitates leaching of these compounds in to wash water (Wang *et al.*, 1979). Due to the activation of alpha galactosidase during germination helps in the hydrolysis of oligosaccharides (Hsu *et al.*, 1973). Hydrolysis of oligosaccharides with lactic acid producing bacteria has yielded successful results (Mital and

Steinkraus, 1975). Apart from these conventional means, processes such as ultrafiltration (Ang *et al.*, 1986), and enzymic hydrolysis (Delente and Ladenburg, 1972) have also been attempted.

2.2.3.1.3. Other anti-nutritional factors

In addition to protease inhibitors and oligosaccharides, soybeans also contain other undesirable factors such as lectins or phytohemagglutinins like most other legumes (Stiren *et al.*, 1985). Several terms like phytohemagglutinin, phytagglutinins and lectins have been used interchangably to refer plant proteins that agglutinate red blood cells. Soybean contain several lectins consisting 5 - 7 % (w/w) of the whey protein fraction, is a carbohydrate binding glycoprotein which is specific for terminal N-acetyl-D-galactosamine and to a lesser extent D _ galactose. It has a molecular weight of 20 ,000 , is a trimer composed of identical subunits and has two N - acetyl - D - galactosamine binding sites per molecule (Liener, 1981). Inclusion of purified soybean lectin found to result in the inhibition of growth mainly due to reduction in food intake and interference with local and systemic metabolism, which leads to elevated loss of N and dry matter in the faeces (Liener, 1981 ; Santidrian,1980). Soybean lectin also induces pancreatic enlargement and has been considered to have synergistic effect on the trypsin inhibitor activity (Grant, 1989).

However, soybean lectins are heat sensitive and destroyed like trypsin inhibitors and causes relatively minor dificulty (Turner and Liener, 1975). The lectins were also found to be inactivated following digestion invitro with pepsin. It has been considered that only very little lectin is likely to survive passage through small intestine (Liener, 1981).

Other antinutritional factors such as saponins,goitrogenic factors and allergens contribute very little to the antinutritional value of soybeans (Anderson, 1979).

Whole soybean contain about 0.5 % saponin. The isolated saponin did not exihibit any harmful effect when fed at level three times higher than the level in diets containing soy flour to chicks, rats and mice (Liener, 1972). Eventhough goitrogenic effect of soybean was considered to be eliminated during processing (Block *et al.*, 1961), the goitrogenicity of soybean was not positively demonstrated with the consumption of soybean as a supplement to a iodine free basal diet (Scolow and Suzuki, 1964). Estrogenicity could be a possible problem only in the livestock and under circumstances where the ration is comprised almost completely of soybeans. Introduction of soybean as a new food product might possibly develop some allergic reactions in some but there is not enough data to conclude its allergenicity (Anderson,1979).

2.2.3.2 Flavour Factors

The characteristic beany flavour and various flavour compounds formed during the processing of soybean is a major deterrant in their utilization, particularly when they are considered in the imitation of dairy milk and meat products.

A number of investigators have reported on the volatile and non volatile flavour compounds identified from soybean and its derivatives (Arai *et al.*, 1966 ; Sessa *et al.*, 1976; Rackis *et al.*, 1979; Huang *et al.*, 1981). Various flavour categories with the components and precursors responsible for them as summarised by Visser and Thomas (1987) are presented in Table 2.3.

Of these objectionable off flavours identified in soy based products, green beany and grassy odour developed through the lipoxygenase hydroperoxide lyase and the other astringent, bitter and chalky flavour which are generated through beta-glucosidases have been considered to be the major flavour defects of soybean (Oliver *et al.*, 1981 ; Gandhi, 1994).

Table 2.3. Off flavours present in soy protein products*.

Off- flavour	Compounds responsible	precursors
Bitter taste	?	Phosphotidyl cholines
Sweet taste	Sucrose	---
Green,grassy odour	Carbonyl compounds	Polyunsaturated fatty acids + lipoxygenase
Cooked soybean odours	p-Vinyl phenol p-Vinyl guatacol	p-Coumaric acid Ferulic acid
Burnt flavour	Ketones, aldehydes, furans Sulfur compounds,pyrazines	Amino acids + Carbohydrates (Maillard reaction)
Catty odours	4-methyl-4-mercapto-2-pentanon	Acetone + Hydrogen sulfide
Fusel note	Long chain alcohols	----

* Visser and Thomas (1987)

Fujimaki *et al.*, (1965) reported the presence of n-hexanal in raw soybean amounting to the level of 10 ppm. Pentanol and Hexanal found in soybean (Arai *et al.*, 1967) and soymilk (Wilkens and Lin, 1970) were identified to be resonsible for the green bean odour of soy based products. Sessa *et al.*, (1976) reported that hexanal, acetaldehyde and acetone were the major volatile carbonyl compounds of both full fat and defatted soybean flakes.

Goosens (1974) indicated that among alcohols pentanol, hexanol and 1-octen 3-ol are more crucial in developing off flavour than ethanol.

Arai *et al.*, (1970) studied the mechanism of formation of hexanol in soy protein concentrate and concluded that the formation of hexanal during the preparation of soy protein concentrates was found to be mainly due to lipoxygenase.

On the other hand, ethyl vinyl ketone and pentyl furan has been reported by Mattick and Hand (1969) and Oliver *et al.*, (1981) in defatted soyflour, as being responsible for the green bean odour of soaked soybeans. Ethyl vinyl ketone was also found to be present in soybean oil (Hill and Hammond, 1965 ; Wilkens and Lin, 1970) and commercial soy protein isolate (Quist and Von Sydow, 1974). These compounds were postulated to be decomposed products of soy lipids present in soy flour.

Rackis *et al.,* (1979) in their review sugested that n-Hexanal, 3-cis hexanal, n-pentyl furan and ethyl vinyl ketone as key flavour compound responsible for grass-beany and green flavours of soybean. The astringent, bitter and after-taste of soy-based products is mainly due to glucosides such as saponins present in soybean.

Another glycosidic group is isoflavone glycosides, which are phenolic compounds mainly consisting of daidzin and genistein (Walz, 1931; Ahluvalia ,1953).

Arai *et al.*, (1966) identified nine phenolic acids from defatted soy flour possesing bitter, sour astringent tastes which might have an influence on soybean flavour.

Huang *et al.*, (1981)reported that a bound oxidised fatty acids in the lecithin molecule instead of phosphocholine, was responsible for bitter taste. Murphy (1982) studied the isoflavone compound concentrations in germinated soybean sprouts and in tofu and reported that genistein the agylcone of genistein increased during the soaking of soybean. Matsuura *et al.*,(1989) reported that beta-glucosidase was related to the production of deidzein and genistein which increased during soaking of soybean were found to be responsible for objectionable after taste of soybean.

Huang *et al.*, (1981) showed that the isoflavones such as genistein, daidzein present in soyflour are responsible for undersirable bitter or after taste of soybean.

Matsuura and Obata (1993) confirmed that beta-glucosidases in the soybeans, hydrolysed isoflavone glucosides in soymilk.

Their experiments showed that genistein was more easily hydrolyzed than daidzein by beta - glucosidase B and C.

Ethyl-D-galactopyranosie and L-tryptophan found in soy flakes were related to be responsible for the bitter and after taste of soy products (Honig *et al.*, 1971).

It is clear from the literature that the flavour problems recognized in soy products is mainly due to the presence of lipid and its associated (Oliver *et al.*, 1981 ; Arai *et al.*, 1970). Poor extraction processes and procedures used in the preparation of soy flour, soy isolate and soy protein concentrate also leads to the formation of various off flavour compounds (Kinsella, 1979). However the difficulty in flavour characteristics is more prominent when soy products are intended for the imitation of dairy products which are otherwise bland (Morr,1979). Eventhough masking approach is not very well appreciated in soy products, the procesing innovations utilised in the development of meat based products have resulted in more successful products (Rackis *et al.,* 1979).

2.2.3.3 Functional properties

Eventhough, soybean with their versatile functional properties are being accepted as an alternate food ingredient in many new food formulations,their functional characteristics in these products are not necessarily to the optimum level (Schmidt and Morris,1984) mainly due to their flavour binding, thermal denaturation, poor solubility characteristics, and pH sensitivity (Kinsella and Damodaran, 1980 ; German *et al*., 1982; Schutte, 1983).

Functional properties of food products are , those physical and chemical properties which affect their behaviour in food systems during processing, storage, preparation and consumption. These characteristics ultimately decide the stability of the sensory and keeping quality characteristics of final products (Kinsella, 1979). Important functional properties of food ingredients such as solubility, water absorption, fat absorption, gelation, emulsification, foaming and viscosity determine their suitability for application in various food systems (Martinez, 1979). It is unlikely that any single ingredient would posses all the functional properties required for a particular food system. Similarly, not all the functional properties are critical to all food systems. However in many food systems a range of functional properties are desired, eg., solubility, clarity, turbidity , viscosity in liquid foods, while water holding, emulsion stabilisation and gellability are important in meat and cheese products (Kinsella, 1979).

Functional properties and the technical difficulties involved in the utilisation of soybean in various simulated dairy and meat products are the subject of intensive research (Kinsella, 1979 ; Morr, 1979 ; Wolf and Cowan, 1975 ; Schmidt and Morris, 1984 ; Barraquio and De voort, 1988). However these reviews are concerned with the development of products utilising soy protein isolate and soy protein concentrate. Although carbohydrates and lipid may have some role in water binding, swelling, viscosity and emulsification , protein fraction is considered to be the principal functional component (Visser and Thomas, 1987).

The functional properties of soyproteins such as solubility, waterholding, fat binding, emulsification, foaming, gelling property are considered important for the successful inclusion of soy protein in new food products. These properties of soy proteins mainly depends on the form and the method of processing applied in their preparation, and get modified due to their concentration, presence of other ingredients, acidity, ionic strength, temperature and processing time etc (Kinsella,1979 ; Schmidt and Morris, 1984).

Soybeans make their greatest contribution in the production of emulsified or comminuted meat products, mainly due to fat emulsifying and waterholding properties (Wilcke *et al.*, 1979).

The difficulties in their utilization in milk systems, however, are more complicated. This is mainly due to the bland taste, flavour properties required as well as their difference compared to milk proteins (Morr, 1979 ; Barraquio and De voort, 1988).

Morr (1979) in his review attributed the technical problems encountered with the utilization of soyprotein products in milk system mainly to their molecular complexity, susceptibility to denaturation, and their variability due to the difference in manufacturing condition.

The fuctional , chemical and physical properties of soy proteins are extensively reviewed by Wolf (1970) ; Kinsella (1979) ; Schmidt and Morris (1984) ; Barraquio and De Voort (1988). On the basis of sedimentation rates soyproteins can be fractionated into 2S,7S, 11S and 15S fractions with the major fraction being the 7S globulin (B and x conglycin) and the 11S globulin (glycinin) (Wolf, 1970).

The major chemical structural difference between soy proteins and milk proteins summarised by Schmidt and Morris (1984) is presented in Table 2.4 .

The soy proteins are considerably large molecules compared to milk proteins with a molecular weight of 1,86,000 to 2,10,000

Table 2.4 Physico chemical and structural differences between casein, whey, soy proteins*.

Chemical Structural properties	Caseins	Whey proteins	Soy proteins
Major components	xs B X	B -lg x - la serum albumin	2s 7s 11s 15s
Molecular weight	15.000 - 26000	18,000 (B - lg) 16,000 (x - la)	generall > 1.00,000 2s
Predominating amino acids	proline,negatively charged and hydrophobic amino-acids	sulphur containing amino acids	hydrophobic and hydrophilic aminoacids
Presence of conjugated proteins	all are phosphorylated to varying degrees;X-caseins are glycosylated	not phosphorylated and rarely glycosylated	contain some carbohydrate but only hemaglutinin and 7s globulin are glycoprotein
Dependance on disulfide bonding and presence of sulfydryl groups	minimal structural dependance on disulfide bonding and negligible amount of sulfydryl groups	one intramolecular disulfide and one sulfydryl group per B-lg monomer: 4 - disulfides per x-la monomer with no sulfydryl groups	some dependance on disulfide and sulfydryl groups
Heat stability	stable	stable except B - lg	stable
pH stability	stable	stable	unstable in acidic range

* Barraquio and De Voort (1988)

daltons for the 7s and 3,50,000 daltons for 11s fraction. Soy proteins are heterogenous and undergo dissociation - association reactions which depend mainly upon the ionic condition of the solution (Thanh and Shibasaki, 1978). The soy protein also contain carbohydrate and are not phosphoproteins, their hydrophobic and hydrophilic aminoacids are randomly distributed in their primary structure (Morr, 1979).

The fractionation procedure involved in the preparation of soy protein products such as soyprotein concentrate, soyprotein isolate has been considered to play a keyrole in the functional properties of final products. Functional properties of soy protein concentrates and isolates in comparison to that of milk proteins reviewed by Barraquio and De Voort (1988) are summarised in Table 2.5.

The beany after taste and poor hydrophillic properties limit soy protein in the incorporation of dairy milk products. Eventhough the thermal stability of soyproteins are considered as limiting factors in its utilization , this property manifest itself into more important functional property such as gelation, which makes it suitable in the replacement of milk proteins (Barraquio and De Vourt 1988). Gelation of soy protein follows a mechanism which involves initial unfolding and dissociation of the protein followed by reversible aggregation and formation of a progel intermediate depending on covalant bonding. Further heating causes the disruption of progel and the covalent bonding

Table 2.5. Functional properties of milk proteins, soy concentrates and isolates*

Functional Properties	Milk proteins	Soy concentrates	Soy isolates
Taste	good	little beany taste in acid washed:improved in alcohol washed	less beany taste than concentrate
Product appearance	no effect on colour	may decrease or enhance colour	may decrease or enhance colour
Hydration	more hydrophilic	less hydrophilic	less hydrophilic
Solubility in water	Na and K caseinates are soluble;coprecipitate and lactalbumin are insoluble	soluble	flocculates at low pH
Emulsification	good	improved in acid washed less in alcohol washed	good
Whipping and Foaming	good	whippable	whippable
Heat gelation	caseinate no ; whey protein yes	improved in acid washed less in alcohol washed	good
Water holding capacity of gel	less	higher	higher
Texturisation	can be texturised	good	good

* Barraquio and De Voort (1988)

(disulfides) results in the formation of irreversible gel structure (Catsimpoolas and Meyer, 1970). The development and maintanence of gel structures require a minimum concentration of 8 % of soy protein (Catsimpoolas and Meyer, 1970 ; Utsumi and Kinsella, 1985).

An important feature of soy protein gelation property include high water holding capacity which has limited their utilization in the preparation of hard cheese type of products where synerisis is desirable. Tofu is the best example among the traditionally prepared oriental food which contains more than 80 % moisture compared to 50 % for rennet casein curd (Lee and Marshall, 1979). However this property has been taken for its advantage in yoghurt like products where synerisis is undersitable (Schmidt and Morris, 1984). Soy proteins being calcium sensitive, the firmness of the gel is altered with the incorporation of calcium due to the lowering of water holding capacity (Saio *et al.*, 1969).

The solubility properties of soy protein products are mainly attributed to the treatments involved in their preparation. This property has an important role when soy protein is utilised in beverages. The solubility properties of soyprotein depends mainly on the heat treatment, solvent used in extraction procedures and also depends on further processing of defatted flour in the preparation of soyproteins and soy isolates. Acid precipitation process used in the isolation of of soyprotein results in acid denaturation, adversely affecting the solubility characteristics (Anderson, 1974).

Solubility of soy proteins also depends on the degree of agglomeration which occurs during the centrifugation and drying steps during the isolation of soy protein . The protein complex formed during centrifugation required for the isolation of soy protein is of tan to brown colour and readily binds oxidised lipids and off-flavours (Anderson and Warner, 1976). This limits its utilisation in coffee whiteners and beverages (Kinsella , 1979). Eventhough the treatment of soy protein products with combinations of heat and organic solvent extraction improves flavour (Honig *et al.*, 1971) but results in unsatisfactory solubility characteristics. The poor solubility characteristics of soy proteins inturn causes problems such as high viscosity and poor stability characteristics when intended for the use in beverages (Nsofar and Anyanwu, 1992).

In general, emulsifying properties of soy isolates are similar to caseinates, their utilisation in mayonnaise and yoghurt are limited due to the, pH sensitivity, close to the isoelectric point (Schutte, 1983). Soy isolates show a greater emulsifying capacity compared to soy protein concentrate. However, blending of soy isolate with sodium caseinate had limited success due to the preferential coating of oil globules with a thin layer of sodium caseinate and preventing the adsorption of soy isolate at the interface (Aoki *et al.*, 1984).

Soy protein preparations particularly isolates, exihibit good foaming properties compared to flours or concentrates (Fleming *et al.*, 1974) , provided they are completely free from residual lipids which otherwise destabilises the resultant protein film.

Overall, soy beans have gained popularity in the West with the utilisation of soy protein concentrates and isolates having excellent functional properties such as emulsification, whipping, foaming, and gelation . Limiting functional properties are mainly solubility, pH and ion sensitivity (Barraquio and De Voort, 1988).

2.3 Overcoming the limitations of using soybean

The progress in the use of soybean in the diets of nations not used to the traditional soybean foods of the Orient has been based largely on the success of incorporating soybean in their conventional products using appropriate processing technologies. In this regard there has been essentially two approaches namely, a) the use of soy derivatives , ranging from the unrefined defatted flakes, grits, flour to the refined protein concentrates and isolates and b) the use of whole soybean as a starting material (Wolf and Cowan, 1975).

Manufacture of texturally modified products from defatted flakes, protein concentrates and isolates forms a major approach in the utilisation of soy bean in the West. The utilisation of soy bean as a starting material in the formulation of several conventional products, on the other hand is considered to be an important approach for the developing countries, due to its simplicity and cost saving approach (Wilcke *et al.*, 1979 ; Patil and Ali,1990). In both these approaches the utilisation of proper ingredients and the application of appropriate processing methods play a key role in the development of successful products.

2.3.1 Derivatives of soybean

Preparation of soy derivatives begin with the desolventisation of defatted flakes coming out of the solvent extraction process. Although, the extraction procedure is a milder one, the severity of the desolventisation step can affect the functional properties of the soy proteins. The earlier practices of steam desolventisation was aimed at preparing soymeal for animal feed purpose. The alteration in the functionality of soy proteins, particularly the solubility characteristics in steam desolventisation process makes it less suitable for products where special functional properties are required. Improved desolventisation procedures yield soy flakes with minimum alteration in the functional properties of native protein (Waggle and Kolar, 1979). Defatted flakes with minimum denaturation characteristics are obtained using vacuum or flash desolventiser or deodouriser (Visser and Thomas,1987).

After desolventisation, defatted soy flakes are used in the preparation of **grits** with different particle size (10 - 80 US mesh) or **flours** with a mesh size of 100 or finer. Soy grits and flour are used directly in several preparations such as bakery, and meat products, or can further undergo refinement processes for the concentration of protein components (Wolf and Cowan, 1975).

The major use of soy grits have been in the categories of frankfurters,bologna sausages and in meat substitutes,where the emulsion formation, emulsion stability and fat absorption are the important functional properties. Flour can also be used where grits are used, but in addition they have wider usage in a variety of food groups including bakery products, soups etc,. for its functional characteristics such as fat and water absorption, and texture and colour control (Kinsella, 1979 ; Wolf and Cowan, 1975) .

In the manufacture of isolates or concentrates, the methods or procedures used is considered to be appropriate only if most of the protein contained in the isolate or concentrate remains in a native state i.e., that the protein processes used do not substantially denature the protein (Giddey, 1983). The fractionation scheme depends on the differential solubility of protein, sugar and undesirable or toxic molecules in water , pH and temperature and ionic composition of the media (Lillford, 1983). As a result successive stage of refininement helps the elimination of oligosaccharides,water soluble sugars, polysaccharides and other minor compounds (Visser and Thomas, 1987).

Important processing steps involved in the preparation of soy protein concentrates and isolates are outlined in figure 2.1. Soy **concentrates** with 70 % protein are prepared from defatted soy flakes or undenatured soy flour. The processes used in the preparation of concentrates mainly differ in the methods used to insolubilise the major proteins. The insolubilisation of proteins is achieved by a) using 60 - 80 % aqueous alcohol leach, b) a dilute acid leach or c) moist heat followed by water leach (Wolf,1970). The concentrate is dried at its neutral pH incase of precipitation with alcohol or water leach , or neutralised before drying if the acid precipitation is employed. The precipitation process involved in the preparation of concentrates eliminates oligosaccharides, part of the ash and some of the minor compounds (Barraquio and De Voort, 1988).

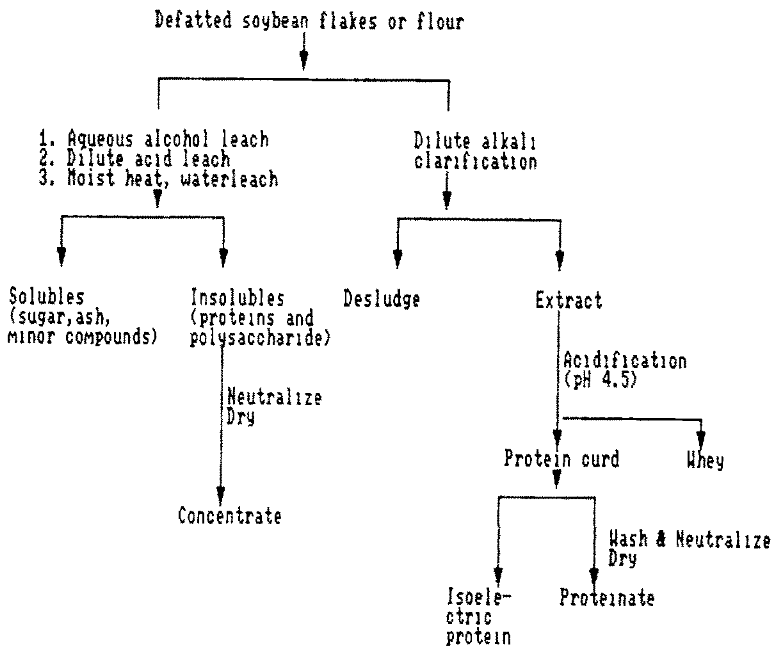

Fig. 2.1 Outline for the preparation of soy protein and concentrate (Barraquio and De Voort 1988)

In order to improve the functionality of soy protein concentrates comparable to that of isolates and to improve the yield and texturisation characteristics , various processing improvements including high temperature shock, heating of aqueous dispersions ,followed by spray drying have been incorporated in the preparation of soy protein concentrates (Roefs *et al.*, 1994).

Most refined forms of soy protein preparation available industrially are the isolates. They are processed one step further than the concentrates by removing water insoluble polysaccharides as well as water soluble sugars and other minor compounds. Soy isolates with 90 % protein are prepared from minimum heat treated (undenatured) , defatted flakes by dissolving the protein in dilute alkali (pH 7 - 9) at 50 - 55^{o}C. Water insoluble polysaccharides are then removed by screening, centrifugation and filtration. The protein component in the extract is precipitated by bringing the extract to its isoelectric pH of 4.5. After filtering and centrifuging to remove the solubles, the protein curd is washed with water. Direct spray drying of curd yields the **isoelectric protein** while spray drying after neutralisation yield curd in the **proteinate** form which is more dispersible (Circle and Smith, 1972).

Isolates have a wider use, compared to the concentrates especially in products such as simulated meats, whipped topping, frozen desserts etc., in which fibre formation, elasticity, cohesion, fo aming and aeration are important (Kinsella, 1979 ; Morr, 1979 ; Wolf and Cowan, 1975).

2.3.1.1 Chemical and Enzymic modifications

Although isolated soy proteins can have minimal residual beany flavour and good functional properties, their functionality is lost in acidic pH range, close to their isoelectric point (Schutte, 1983). This difficulty is overcome by several methods such as hydrolysis by proteases (Alder-Nissen *et al.*, 1983 ; Gunther, 1979) , acetylation (Melnychyn and Stapley, 1973), succinylation (Franzen and Kinsella, 1976) and phosphrylation yield products with good fuctional properties in the wide range of pH (Hirotsuka *et al.*, 1984).

In the improvement of flavour characteristics, Fujimaki *et al.*, (1970) studied the plastein reaction of soymilk. In this process, proteolytic enzymes are used to cause reversible changes in the proteins, which are hydrolysed enzymatically. Of the reversal of changes, the hydrolysates form high molecular weight compounds known as plasteins, which are somewhat different from native protein (Smith and Circle, 1972). Plastein formation has been found to eliminate bitter and off-flavour characteristics of soyproteins (Fujimaki *et al.*, 1968 ; Fujimaki *et al.*, 1970). In addition to the improvement in flavour properties , nutritional improvement using plastein reaction was also studied. The incorporation of methionine and cystein indicate the possibility of preparing plastein products with amino acid composition similar to an ideal protein (Yamashita *et al.*, 1970).

Partial proteolysis has been shown to improve the foaming properties of soy proteins (Horiuchi *et al.*, 1978). Succinylation markedly improves foaming and emulsification properties (Franzen and Kinsella, 1976). Soy proteins modified with acetic and succinic anhydrates have lower viscosities and iselectric points as well as less off-flavour (Melnychyn and Stapley, 1973). Sulfite , thiols and mercaptoethanol used for limited rupture of intra peptide disulfide bonds to facilitate protein unfolding and interfacial film formation further improves the stability of foams (Horiuchi *et al.*, 1978).

2.3.2 Texturisation of soy proteins

The textured products are fabricated ingredients which are made up as fibers, shreds, chunks, bits, granules slices or other forms , when prepared for consumption by hydration, cooking, retorting and other procedures they retain their structural integrity and chracteristic chewy texture (Smith and Circle, 1972).

According to Giddey (1983) the texturisation techniques include two essential steps : 1. creation of a molecular or macromolecular body in the hydrated medium characterised by a directional structure and 2. insolubilisation of compounds of the molecular body and formation of sufficiently coherent reticulate system able to immobilise the hydrated phase of the product.

Work on the texturisation of soy protein has received considerable attention with the introduction of several meat substitutes (Thulin and Kuramoto, 1967 ; Wilcke *et al.*, 1979 ; Wolf and Cowan, 1975). Soy proteins make their greatest contribution in the production of emulsified and comminuted meat products. This includes sausage type products, such as frankfurters, mortadella, bologna, fish balls, fish-paste type products (Wilcke *et al.*, 1979) where texturisation is employed effectively.

The texturised products can include the products which are artificially stabilised in the structure using ordinary thermal coagulation or the products in which structural stabilisation is achieved with special texturisation methods such as spin fibre texturisation (Thulin and Kuramoto, 1967 : Wilcke *et al.*, 1979). Among many methods known to exist for industrial use, the important ones utilised for soy protein products include, extrusion cooking and wet spinning.

2.3.2.1 Extrusion cooking

The extrusion process combines blending , high sheer mixing, elevated temperatures and pressures, resulting in the conversion of the form of the proteinaceous materials into a continuous phase. When passed through a die, the release of pressure allows the super heated moisture to expand and flash off, leaving a porous and fibrous cellular matrix which provides the desired texture (Wilcke *et al.*, 1979). Thermal stabilisation in the extrusion cooking process takes place during and immediatety after the expansion phase. According to the characteristics of extruders and its outlet head, the operation process, produces lamellar structure including macroscopic fiber elongation (Giddey, 1983).

In the extrusion process of cooking, defatted soy flakes, grits and flours are used as starting materials. The starting material is preconditioned with water or steam which initiates protein-carbohydrate hydration, reduces the stickiness of the blend.

Dunning *et al.*, (1972) developed direct steam texturization process. Soy flour in this process is metered in blender to which water, colours, flavours, and other ingredients are added. The finely divided particulate protein material is texturized by passing the material through an elongated cylinder at elevated pressure and temperature. Then the textured particles are separated from the steam and then cut to the desired size. The resulting flake like blend product is used in meat patties and ground meat formulations.

For the utilization of soy protein isolate, Hoer (1972) patented a structuring process in which protein isolates are made into a thick slurry in the isoelectric pH region and pumped at high pressure (50 to 500 psi) through heat exchanger . This heat denatured proteinaceous material is then forced through a narrow orifice to obtain small,discrete textured particles or a continous filaments. The structured product obtained by this method is used to provide increased bite or texture in deboned red meats, poultry, and fish , and to provide desirable texture in crabmeat, turkey and chicken rolls.

2.3.2.2 Spin fibre texturisation

Unlike the thermal stabilisation of proteins in other texturisation methods , coagulation results from neutralisation of nagative charges and reticulation (cross linking) promoted by the low pH in case of spin fibre texturisation. This is often reinforced by a combination with reactive cation of calcium or mineral salts contained in the spinning bath. In the spin fibre texturisation process, first the globular proteins are solubilised in the alkaline bath and passed through spinnerettes in to the acid coagulating bath. The insolubilisation of the components results from the acid coagulation of the protein, which inturn stabilizes the linear structure of macromolecules (Giddey, 1983 ; Wolf and Cowan, 1975).

Boyer (1954) patented a process for the texturization of protein fibers. In this process soy protein after extraction, acid precipitation and washing is reslurried in water and metered to a mixing pump where alkali is added to form an alkaline spinning dope. This filtered dope is then forced through spinnerettes into an acid coagulating bath to form filaments or fibres. The fibres are washed, stretched to alter the texture, which can range from tender to tough by controlling the stretching. The stretched fiber are then treated with binders to form bundles of fibers to which fat, flavour, colour, stabilizers are added. After cutting to the desired size the fibrous products are used to produce simulated bacon, ham and other familiar meat cuts .

2.3.3 Utilisation of whole soybean

Eventhough the approach of utilising soy bean in the form of soy protein products combined with texturisation processes has resulted in the development of many successful products, its direct utilization or consumption as a pulse is limited, due to various inherent problems associated with soybean.

Apart from the products developed using advanced processes, where the raw material mainly consisting of soy protein concentrates, flours, grits and isolates are prepared with desirable properties to suit the requirement of different functional requirements, some of the traditional processing methods also have certain advantages in overcoming the limitations of soy bean. These processes have cost saving potential for the developing countries. The processes used in the development of conventional foods which overcome the inherent difficulties in the utilization of soybean can be grouped as : 1) Dry heat processing, 2) wet heat processing, 3) Coagulation and fermentation, and 4) the extrusion cooking process (Patil and Ali, 1990), discussed earlier.

Preprocessing steps such as soaking, blanching and germination which are included in the preparation of conventional pulse based products are also found to exert some beneficial effects on soybean.

2.3.3.1 Soaking

Soaking of soybean in tap water is the initial step in the preparation of many soybased traditional products, including tempeh and tofu (Steinkraus, 1983).Soaking in plain water is an essential step when whole soybean is utilised for the preparation of dairy product analogues. Development of milk substitutes with soymilk has received worldwide interest in order to overcome the relatively limited milk supply particularly in developing countries (Metwalli *et al.*, 1982) .

Hydration and softening of the bean which occur during soaking facilitate leaching or extraction of bean components in water. Lo *et al.*, (1968) observed the presence of sucrose, stachyose and raffinose in soak water at the end of 24 hours soaking of soybeans. Wang *et al.*,(1979) also noted that the concentrations of stachyose, raffinose, sucrose and fructose within the beans decreased during soaking, even in the presence of antibiotics that controlled microbial growth.

Leaching of carbohydrate components from beans into the soak-water due to enzymic hydrolysis has an importance in eliminating flatulance factors such as stachyose and raffinose (Mulyowidarso *et al.*, 1991).

However , the off-flavours developed due to beta-glucosidases in the formation of isoflavone compounds such as daidzein and genistein, is considered to be a limitation of the soaking step (Matsuura *et al.*, 1989).

Matsuura *et al.*, (1989) showed that the addition of glucono -delta -lactone, a competitive inhibitor of beta-glucosidase, added to the soak water at 0.0015 - 0.15 % (w/w) strongly inhibits the production of daidzein and genistein. Alkaline treatment of beans during soaking appears to enhance the inactivation of lipoxygenase, a primary causative factor for beany odour of soybean.

Nelson *et al.*, (1976) developed a method for the preparation of soymilk in which soybeans were soaked in 0.5%. Sodium bicarbonate solution found to develop soymilk with bland taste. Ashraf and Snyder (1981) found that soaking of soybean in 0.1 M NaOH before the preparation of soymilk, yielded soymilk with bland taste without painty off flavour.

2.3.3.2 Moist Heat

Hydration of soybeans during soaking also have an important role in the inactivation of antinutritional factors in the subsequent moist heat treatment, such as blanching. The blanching process is performed either by cooking the split soybeans in boiling water or application of steam to wet beans (Patil and Ali,1990).

Albrecht *et al.*, (1966) observed that the atmospheric steaming for 20 minutes destroys trypsin inhibitors in whole soybeans, after it is tempered to contain 25 % Moisture. Further increase in the moisture content of soybeans helps in reducing the time required to inactivate trypsin inhibitors. Boiling for only 5 minutes was found to be sufficient to inactivate the inhibitor in soybeans containing moisture content above 60 %.

Blanching of soybeans before milling and by presoaking the beans in aqueous ethanol and by treating soybean with hot water has also been suggested in reducing the beany off flavour of soymilk (Schroder and Jackson,1972).

Moist heat treatment is more effective in eliminating antinutritional factors compared to dry heat and have a greater role, particularly in the inactivation of lipoxygenase enzyme (Nelson *et al.*, 1976). However subjecting whole soybean or defatted flakes to moist heat denatures the protein rapidly and causes poor extractability of proteins (Wolf,1970). Limited heat treatment given in the form of blanching improves the flavour characteristics (Schroder and Jackson, 1972).

Moist heat treatment is effectively utilised in the preparation of various dairy analogues where the ground soy bean is in the form of slurry or dispersion. Most of the modifications introduced in the improvement of nutritional and flavour characteristics of soy milk, utilise variations in the heat treatment.

Wilkens *et al.*, (1967) found that the application of steam or boiling water during grinding yielded fairly bland soymilk.

Nelson *et al.*, (1976) developed hot wet grinding and filteration method for the preparation of soy milk.

Johnson *et al.*, (1981) developed steam infusion cooking method to extract a high quality bland soymilk with adequate inactivation of lipoxygenase and trypsin inhibitors.

Miskovsky and Stone (1987) observed that soymilk processed by the rapid hydration hydrothermal cooking yielded soymilk with higher protein and thiamin compared to traditionally extracted soymilk.

How and Morr (1982) suggested activated carbon treatment for the elimination of phenolic compounds in aqueous extracts of defatted soy flour . However this was found ineffective in the elimination of objectionable flavour characteristics of soyyogurt prepared with soaked and wet ground soybean (Lee *et al.*,1990).

2.3.3.3 Dry heat treatment

The dry heat treatment approach employed in the preparation of salted or sweetened nuts inactivates some of the antinutritional factors but found to impart dark brown colour alongwith nut like flavour to the product (Patil and Ali, 1990).However, this flour has found a successful application in the substitution of wheat in bakery products (Hoover, 1979).

Toasting raw intact soybean at 200^{o}C for 30 min was found to inactivate lipoxygenase (Wilkens *et al.*, 1967) and in turn reduced major off-flavour components of soybean such as n-hexanol and 1,oceten-3ol between 10-20 minutes of roasting (Kato *et al.*, 1981). This process is not applied in the preparation of soy milk since protein extraction is greatly reduced in soymilk (Johnson *et al.*, 1981). However ,toasting of soy flour did not alter the functional characteristics when used in soup formulations and found to enhance the organoleptic characteristics (Valencia *et al.*, 1983).

2.3.3.4. Germination

Germination of soybeans has also received attention because of the potential improvement in flavour and nutritional qualities. The Okamura-Wilkinson (Ang and Kwit, 1985) Process utilizes sprouted soybeans for soymilk extraction in the improvement of flavour characteristics of soymilk.

Bau and Debry (1979) found that the 30% of trypsin inhibitor activity in raw soybeans was eliminated during germination for 3 days along with an increase in vitamin C.

Boralkar and Reddy (1985) observed that the germination of soybeans for the duration of 24-48 hours caused significant increase in the *invitro* digestibility of protein.

2.3.3.5 Lactic fermentation

In the preparation of dairy based analogue lactic fermentation has gained much popularity due to its overall improvement on product characteristics especially in beverages or yoghurt and cheese like products (Hang and Jackson, 1967 ; Pinthong *et al.*, 1980b ; Metwalli *et al.*, 1982; Singh and Mital,1984). The advantages of utilizing lactic fermentation in soymilk was evaluated in two aspects : 1) development of acidity in improving organoleptic characteristics and 2) utilization of oligosaccharides.

Soymilk fermented with lactobacillus acidophillus, resulting in a butter-like product, was the first attempt in the utilization of lactic fermentation (Kellogg, 1934).

Gehrke and Weiser (1947, 1948) compared the lactic fermentation in soymilk with that of cow's milk and found that the lactic cultures used produced less acid in soymilk than in cow's milk.

Acid production by various lactic cultures in soy and soy-skimmilk with or without added fermentable sugars has been studied (Angeles and Marth, 1971 ; Pinthong, 1980a). Improvement in acid production found to increase with the fermentable sugar supplementation indicating the limitation of sugars present in soybean and their utilization by the lactic cultures. Amount of acid produced is not similar with all the lactic cultures used in the fermentation. Mital *et al.*, (1974) observed that S.thermophilus, L.acidophilus, L.cellobios and L. Plantarum utilised sucrose efficiently and produced greater amounts of acid among various lactic cultures used.

Apart from the lack of fermentable carbohydrate and its utilization by lactic acid bacteria other factors such as total solids have been reported to play an important role in acid development.

Chang and Stone (1990) suggested that when soymilk is used as a substrate for the growth of lactic acid cultures, factors such as protein and carbohydrate in soymilk required adjustment in optimizing the growth of specific strain. Their studies indicated that the acid production was decreased at higher or lower extraction ratios than 1 : 9 .

Delente and Ladenburg (1972) found that the treatment of soybean meal with a mixture of invertase and alpha-galactosidase resulted in quantitative conversion of oligosaccharides into monosaccharides.

The galactosidases of lactobacilli were found to be active between pH 4.5 and 8.0 and exhibit pH optima in a narrow range of 5.2-5.9 (Mital *et al.*, 1973). Mital and Steinkraus (1975) fermented soymilk with *St.thermophilus* and *L.fermenti* and found complete utilization of stachyose and raffinose. However, Pinthong *et al.*, (1980b) showed that *St.thermophilus* contributes to the unpleasant flavour of fermented soymilk due to the production of n-pentanal and L.bulgaricus reduces the beany odour by partial removal of n-hexanal. They also found that the combinations of *St.thermophilus* and *L.bulgarius* produced lesser acid compared to *L.bulgaricus* when used alone with the fermentable supplements.

Therefore combinations of L.fermenti and L.bulgaricus with the supplementation (0.1% yeast extract and 1% glucose) were utilized in the fermentation of soymilk, which resulted in only 16% reduction in the amount of stachyose present with the complete utilization of raffinose (Pinthong *et al.*, 1980c).

Development of the yoghurt type products using lactic fermentation has been successful in terms of colour and physical properties. However these products were not completely free from residual beany flavour and lacked yoghurt flavour. Therefore further attempts were initiated to promote the flavour characteristics of yoghurt.

Lee *et al.*, (1990) formulated yoghurt from soymilk containing whey protein concentrate or non fat dry milk and fermented with commercial yoghurt cultures. Both WPC and NFDM were found to function well as ingredients but they did not cause any further improvement in yoghurt flavour.

Murti *et al.*, (1992) incorporated *Bifidobacterium ssp* with yoghurt cultures in the fermentation of soymilk. The presence of bifidobacteria in soymilk favoured only *streptococci* but reduced *lactobacilli* and the development of volatile compounds in soy-yoghurt with bifidobacterium was found to improve the sensory characteristics.

Sugimoto *et al.*,(1981) in the improvement of organoleptic quality of yoghurt incorporated propylene glycol alginate as emulsion stabilizer in the lactic fermented beverage and found propylene glycol alginate decreased the emulsion stability and the defect was overcome with the addition of calcium lactate.

2.3.3.6 Combining lactic fermentation and heat treatment for soybean based dessert

This review on soybean points out that the utilisation of soybean in countries such as India would depend on its successful incorporation in our conventional foods. Since milk and dairy foods have an universal appeal in this country, developing soybased dairy analogues,starting with whole soybean is likely to result in its substantial use in the Indian diet.

The utilisation of lactic fermentation as an essential step in the development of milk substitutes using soybean has the advantage of overcoming antinutritional factors and in improving the organoleptic qualities of the product.

Lactic fermentation in the preparation of "dahi" is a common household practice in India, and is consumed in everyday foods. Lactic fermented "dahi" also forms as a basic ingredient for various other products such as "lassi", "misti dahi" and "shrikhand" etc,. Among these, shrikand is a popular sweet delicacy of Gujarat and Western India and is prepared with "muska" obtained by draining of lactic fermented curd . After the removal of whey from dahi the concentrate or "muska" is utilised in the preparation shrikhand with the addition of sugar and flavouring agents (Patel and Chakraborty, 1988).

Cheese cake is another product similar to shrikhand interms of sweetened acidic taste, and is popular in the West. The published information on cheese cake manufacturing process is rather limited (Linton-Smith and Siebenmann, 1960 ; Sultan, 1986). This product utilises soft cheese or cheese curd as the basic ingredient. Use of egg as a coagulating agent, and the in-container heat treatment given to the mix makes it different from shrikand interms of textural characteristics. Moreover , the final heat treatment has the potential of improving the shelf-life characteristics.

Utilisation of soybean in egg or starch thickened products is rather limited (Schaefer and Holdt, 1992). Since sweet products in the Indian context have a special role to play , the introduction of such a product would have greater potential in the utilisation of soy bean in India.