REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The main objective of the present study was to develop cheese spreads utilising soybeans. Soy cheese spreads being a relatively new product, published information on the manufacturing technology is rather limited on the subject.

Considering the fact that yellow varieties of soybean had been introduced in India as early as in the 1970's, its utilization in our diets still remains to be somewhat limited (Randhawa, 1988). Realising the importance of soybean in the supplementation of our existing diets, the nutritional role of soybean as a food is highlighted in this review.

In recent years, attempts have been made to develop cheese both cured and uncured types of products from soybean through the use of lactic cultures, and technology employed in the manufacture of cheeses prepared from cow's milk. The review is also concerned with the technology of cheese and cheese spreads, and the use of soybean in the preparation of these products.

Literature on these various aspects are reviewed under the following heads:

(1) Soybean production in India

(2) Significance of soybean as a food.

(3) Technology of cheese and cheese spreads , and

(4) Utilization of soybean in cheese type products.

2.1 Soybean production in India:

Eventhough soybean production and soy processing industry in India started in the early 1970's, it is the last decade which has witnessed a progress in terms of soybean production, processing, and export trade in India (Jain, 1992). In the world 53 million ha. of land goes in for soybean production, the yield being 88 million tonnes (Verma, 1991). The total area under production in India in 1992 was estimated to be 3 million ha, producing approximately 3 million tonnes of soybean (Jain, 1992).

Eventhough the soybean production as well as processing have assumed a place of pride in national perspective (Agrawal, 1992), India is lagging far behind the world both in terms of total production, and yield/ha. According to Jain (1992) India's share in the world soybean production is only 2.37% with USA maintaining the first position, with a production of 50.6%, followed by Brazil with 15.67%, China, 10.93%, and Argentina 10.16%. The world average yield of soybean is 1.88 tonnes/ha, other countries have achieved spectacular average as can be seen from the the figures for Italy, 2.91 tonnes/ha, Canada, 2.63 tonnes/ha, Greece, 2.57 tonnes/ha, and USA, 2.29 tonnes/ha. The Indian average for 1992 is around 1.25 tonnes/ha (Jain, 1992; Agrawal, 1992).

2.2 Significance of soybean as a food:

Soybean is generally produced for its commercial value as a source of oil, protein, and lecithin (Ali, 1992). The advancement in technology has made soybean available in various forms (Smith and Circle, 1978), which in turn have created an array of products in the market ranging from beverages to meat sausages, meat extenders, meat analogues, bakery products, breakfast cereals, infant and junior foods, pet foods, confectionery items, and dairy type products (Wolf, 1979; Waggle and Kolar, 1979; Gandhi et al., 1992).

Primary importance of soybean as a source of protein has been emphasised because of its presence in amounts as high as 40% and its quality (essential amino acids profile), which is far superior to that in other legumes and oilseeds (Orr and Watt, 1957; Circle and Johnson, 1958). Earlier effort to promote soybean in India has been essentially to substitute/supplement the legumes in the diet (Randhawa, 1988). The significance of soybean as an article of food has been reviewed under the following sub heads:

(a) Composition of soybean.

(b) Soy bean products.

(c) Soybean in traditional foods.

(d) Soybean in Indian foods.

(e) Soybean in fabricated foods and food analogues.

2.2.1 Composition of soybean:

The composition of soybean has been studied extensively by Tombs (1967), Wolf (1970), Millner (1971), Smith and Circle (1978). On the weight basis, soyean consists of about 8% seed coat, 2% hypocotyl, and 90% cotylendons, all the nutrients being concentrated in cotylendons which amounts to 41.3% protein, 18% fat, and 4.4% ash. (Tombs, 1967; Smith and Circle, 1978). Soybean ranks highest among pulses for having maximum calories (432 Kcals), protein (43.2 g%), iron (10.4 mg%), minerals (4.6 g%), only 20.9 g% of carbohydrates (Gopalan et al., 1993). According to Arnold and Choudhary (1962), Kellor (1974), and Vaidehi (1988), soybean differs from other legumes principally in the amount and quality of nitrogenous substances, oil content, and negligible amounts of starch.

The varietal and agro-climatic conditions influence the physical (colour, shape, and size), and chemical composition of soybean with particular reference to protein (30-46%) and oil (12-24%) contents (Cartter and Hooper, 1942; Smith and Circle, 1978; Gandhi et al., 1988).

2.2.1.1 Protein System:

The protein content varies between 30 - 46% in the mature soybean seed. Soy protein is found to be composed of several fractions. Moving boundary electrophoresis have showed that iso-electrically precipitated soy protein is a mixture of atleast 4-5 components (Briggs and Mann, 1950, Smith and Circle, 1978). In the ultracentrifuge, acid precipitated proteins separates into 4 distinct fractions with sedimentation co-efficient of 2,7,11, and 15 S; the sedimentation pattern differed only slightly from the pattern for the unfractionated proteins obtained by extraction of meal with 10% sodium chloride (Naismith, 1955).

The earliest investigations of Osborne and Mendle (1917) on the amino acids make up of the proteins of soybean revealed their similarity to those of cow's milk. Soybean contains small quantities of peptides, and amino acids having variable molecular dimensions, which may occur as the residue of imcomplete protein synthesis or degradation. Muller and Armbrust (1940) reported that a protein free extract of mature soybeans contained adenosine, arginine, choline, glycine, betonine, trigonellin, and guanidine. These minor nitrogen bearing compounds are classified as non-protein nitrogen. Murmatan (1970) reported that water soluble fractions of soybean protein contained 84% of globulins, 5.4% albumin, 4.4% proteose, and 6.6% non-protein nitrogen.

The amino acid profile of soy protein is unusually well rounded for a plant protein (Erdman and Fordyer, 1989). Comparison of variety of soy proteins with ideal patterns published by Foods and Nutrition Board, USA (1980) or the Food and Agriuculture Organization (FAO, 1985) demonstrates adequate quantities of essential amino acids, particularly lysine concentration, which is high. However, sulfur containing amino acids such as methionine, cystein, fall below the recommended pattern in most soy proteins.

Excellent reviews on the protein quality of various soy proteins for human nutrition were published by Scrimshaw and Young (1979), Forman and Ziegler (1979), Torun (1981), and Erdman and Fordyer (1989). Research on the protein quality has been particularly focussed upon adults, children and infants in the above studies. Results of a few rat studies that used the PER assay (Torun et al., 1981, and Rackis et al., 1975) suggested that protein quality of adequately processed soybean protein is 62-92% that of casein. The true digestibility of 92-98% has been reported by Wayler et al. (1983) for heat processed soy protein isolates. Desikachar et al. (1946) have indicated a digestibililty co-efficient of 91, and a biological value of 79 for soybean protein. This can be improved by supplementing the limiting amino acid viz., methionine and cystein to the total level of 900 mg of sulfur containing amino acids or 3 g of protein nitrogen (Kapoor and Gupta, 1975; Zezulka and Calloway, 1976 ; Scrimshaw and Young, 1979).

Soy proteins are reported to have many additional benefits. Feeding soy bean proteins as soy concentrate, soy isolates and flours in contrast to animal protein (meat, dairy products) to hyperlipidemic subjects in controlled human feeding studies reduced serum cholesterol (Sirtori et al., 1977, 1979, 1983; Carroll et al., 1978) and triglycerides in those subjects with high levels of these lipids. According to Kritchersky (1979) and Sirtori et al.(1983) the beneficial effects may be due to the amino acid make up of soy protein. In addition, soybean cotyledons polysaccharides containing both digestive carbo - hydrate, and fiber, reduced serum cholesterol (Shoney et al., 1985), and caused a 20% reduction in insulin requirement to an oral glucose load (Lo et al., 1986) in hypercholesterolemic and hyperglyceridemic patients respectively. However, Van Raaij et al. (1981. 1982), and Lovati et al. (1985) have failed to find marked hypocholesterolmic effect of soy protein in hyperlipdimic patients. Further research is needed to establish the role of soy protein per se, or in combination with fiber in reducing cholesterol level in human subjects.

2.2.1.2 Lipid System:

Fat in soybean varies from 13.24 to 20%. The fatty acid composition compiled by Denel (1951) indicated a high unsaturated fats level in soy lipid representing 86.1%, the linoleic content being 50.7%, linolenic, 6.5%, and, oleic 28.9%. The ratio of 86.1% unsaturataed to 13.9 % saturated fat is 6.1, which is fairly constant, irrespective of the total amount of oil present in the seed (Dollear et al., 1940; Gopalan et al., 1993). The digestibility co-efficient of soybean oil has been worked out to be 97.5 in human subjects, similar to that of other edible oils (Langworth and Holmes, 1915). Soybean contains 1.65 to 3.05% lecithin, which is much higher than that found in any vegetable fat (Denel Jr., 1951).

2.2.1.3 Carobohydrate System:

The carbohydrate content varies between 17.9 to 30.2%, almost all being non-starchy polysaccharides. Starch content is as low as 1-3% (Smith and Circle, 1978). The principle sugars of soybeans are disaccharides, sucrose (4.5%), tri-saccharides, raffinose (1.1%), and tetra-saccharides, stachyose (3.7%) (Kwamura, 1967). It is clear from the above composition that the carbohydrate fraction does not contribute factor to the calories.

2.1.1.4 Mineral System:

Minerals play an important role towards the physical stability of proteins, and is therefore, important to the phenomenon of heat stability, gelation, and curd forming properties (Gandhi, 1988). The total ash content of soybean is fairly high ranging from 3 to 6%. Soybean contains about 0.51 to 0.73% phytic phosphates. It is also rich in calcium (240 mg), iron (10.4 mg), megnesium (175 mg), and copper (2 to 11 mg) per 100 g sample (Ramashastri, and Balasubramaniam, 1976; Gandhi, 1988, Gopalan *et al*., 1993).

Much like other plant foods, the bio-availbililty of minerals such as zinc (Erdman Jr. and Forbes, 1981; Cossock and Prasad, 1983; Solomons *et al*., 1984) and iron (Cook *et al*., 1981; Morck *et al*., 1982) from soy products are found to be lower than that reported for animal food systems.

2.2.1.5 Vitamins:

Soybean does not contain vitamin A, D, and B_{12} (Harries *et al.*, 1950). Moinichen (1952) and Smith and Circle (1978) reported that the bean is a good source of B-group vitamins particularly thiamine (0.73 mg%).

2.2.1.6 Anti-nutrients:

Inspite of its excellent nutrient make up, soybean hase been known to contain several anti-nutrients. The important ones reported by several investigators along with the effect of these consitituents on physiology and treatments suggested for their elimination are detailed in table 2.1.

Anti-nutritional effects of soybean have been extensively reviewed by Smith and Circle (1978), and Grant (1989). Liener (1981) and Gandhi (1988) reported the presence of a variety of anti-nutrients such as enzymes, protease inhibitors, hemagglutinins, phytates, flatus causing oligosaccharides, saponins, sterols, alcohols, goitrogens, and phenolic compounds. The level and/or activities of anti- nutrients can be either reduced or totally eliminated depending upon the processing conditions (Kakade *et al.*, 1973, Baggalich *et al.*, 1980; Boralkar and Reddy, 1985). Several investigations with animals and human subjects reveal that the consumption of unprocessed soybean results in growth retardation, and indigestion which can be eliminated by proper thermal processing of the bean.

Table 2.1 Anti-nutritional components of soybean and related factors.

Anti-Nutrients	Level present	Effect on physiology	Method of elimination	References
Oligo-saccharides	5-15%	Flatulence	Fermentation Germination	Hang & Jackson, (1967); Cristofaro et al. (1974); Pinthog et al. (1980); Boralker & Reddy (1985); Buono et al.(1990)
Hemagglut-inins	3%	Interfere with normal defence mechanism of intestinal cells.	Heat treatment Fermentation	Gandhi et al. (1988)
Trypsin-inhibitors	8.1-38.5 IUI/mg	Growth retardation	Heat treatment Fermentation	Liener (1967) Jaffe (1969) Grant et al.(1980); Barr (1981); Liener (1981).
Saponins	0.5%	Exact effect not known	-	Jaffe (1979) Fenwick & Oakenfude, (1981); Gandhi et al. (1988).
Phytates	0.51-0.73mg%	reduce protein solubility & calcium absorption.	Fermentation Germination	Pons & Guthric (1941); Boralkar & Reddy (1985)
Goiterogens	-	Thyroid enlargement	Heat treatment	Liener (1981).
Phenolic compounds	-	Interfers in mineral absorp-ion	Fermentation	Narasinga Rao (1983).
Lectin	5-7%	Growth retardation organ enlargment	Pepsin digestion	Liener (1981).

Presence of factors such as lipoxygenase associated with oxidised paint like off-flavour, other compounds (phytates etc.) and trypsin inhibitors can reduce the sensory and nutritional quality of soy products. These however, are relatively heat labile (Rackis, 1976). Several anti-nutritional factors can be eliminated by fermentation (Grant, 1989; Buono et al, 1990; Goyal and Khetarpaul, 1991; Chauhan and Grenal, 1991). Thomas and Kamath (1986) and Erdman and Fordyer (1989) reported the presence of a group of protein that bind to the proteolytic enzymes, thus reducing the intestinal digestive process, which can be inactivated by proper heat treatment and fermentation. Hang and Jackson (1967) and John and Erdman Jr (1986) reported the presence of flatulence producing oligosaccharides, which responded to heat treatment and fermentation. Several processing like roasting, and germination, were found to enhance significantly the digestibility of starch and protein in soybean (Boralkar and Reddy, 1985).

2.2.3 Soybean products:

Intensive technological developments in the West have made available a variety of products derived from soybean which have a wide application in food industries. The major soy products are listed in table 2.2.

Edible soy grits and flours are made from dehusked beans and are classified according to particle size as coarse, 10-20 mesh size; medium, 20-30 mesh size; fine, 50-80 mesh size, and flour

Table 2.2 Major soy products and their proximate composition.

Soy products	Moisture	Protein	Fat	Fiber	Ash
Wholė soybean	9.0	41.0	20.0	2.3	5.4
Grits & Flours:					
Full fat flour	5.0	41-41.8	21.0	2.1-2.8	3.3-5.2
Low fat flour	5.5	46.0	6.3-6.5	2.1	5.5
Defatted flour	5.0	50-53	1.5-0.9	2.9-3.8	5.8-6.0
Lecithinated flour (15%)	5.5	45.2	16.4	2.4	5.3
Soy protein concentrate	3.1-6.7	66-72	0.3-1.2	3.5-5.0	3.5-6.5
Protein isolate	4.7-7.6	92-95	0.1-1.0	0.1-0.2	2.0-3.8
Okara(fresh)	20.6	1.58	1.1	0.70	0.25
Soy oil					
Lecithin					

Source: Thulin & Karamato (1967); Meyer (1970); Wolf (1979); Cowan (1981);Bressani (1981); Gandhi et al.(1992); Ali (1992), Kaur (1992).

100 mesh size or finer (Wolf, 1979). In the commercial preparation of full fat products, the beans are cleaned, cooked, dried, cracked, dehulled, ground and screened (Pringle, 1974). Defatted flours are made from solvant extracted soybean flakes after desolventizing, grinding, and screening (Becker,1971; Smith and Circle, 1972; Horan, 1974 and Wolf, 1979).

A major application of grits and flours is in the bakery products (Cotton, 1974). The added soy flours, besides increasing the protein content of the bakery products, help in contributing to the water holding capacities (due to proteins), and helps in bleaching, due to catalytic reaction of lipoxygenase (raw flours) with poly unsaturated fatty acids (Wolf, 1979). Accoridng to Rakosky (1974) soy flours are added to processed meats for improved functional properties in terms of binding, emulsion, stabilization, and fat absorption.

The differences between the full fat, low fat and defatted flours are mainly in their oil contents. Full fat flour has oil content (21%) similar to that of original bean. In defatted flour, the oil content is as low as 0.9 to 1.5%. The lecithinated flour contains 15% lecithin in defatted soy flour, and is used in bakery products where extra emulsification is desired (Meyer, 1970).

Soy protein concentrates are made from defatted flours by removing soluble sugars, along with some ash and minor constituents (Sair, 1959; Mc Anelly, 1954; Mustakas and Griffin, 1966). According to Burnett (1951) and Wolf (1979) soy protein concentrates find similar uses as flours. A major outlet for concentrates are in processed meats, sausages, meat balls etc., for functional characteristics such as moisture absorption and fat binding, in ready-to-eat break fast cereals, infant foods etc.

Rakosky (1974) and Wolf (1979) considered the isolated proteins as the most refined form of soybean protein available commercially. By definition, they must contain a minimum of 90% protein (Wolf, 1979). Like concentrates, isolates are made from defatted flakes or flours. The food uses of isolates are similar to those for concentrates and flours. Isolates are often used to replace the higher priced sodium caseinate in dairy type items such as whipped toppings, liquid coffee whiteners, and frozen desserts. Instant cocoa mixes, instant break fast preparations, and milk replacers are examples of beverage powder products containing protein isolates (Wolf, 1970; Waggle and Kolar, 1971; Raoksky, 1974; Smith and Circle, 1978).

The literature on soy continues to emphasise the flavour as the factor that limits the use of these different soybean protein products (Cowan et al., 1973; Dutton, 1978; Rackis et al., 1979; Sessa, 1979). Kalbrener et al. (1981) surveyed 19 commercial soy products including concentrates, isolates, and flours for odour, and flavour characteristics. The ranges in scores (in 10 point scale) for the four types of products were 4.2 to 6.7 for flours, 5.3 to 6.7 for textured flours, 4.4 to 5.9 for concentrates, and 5.9 to 5.9 to 6.4 for isolates. The authors concluded that most of the samples evaluated showed flavour improvement relative to raw, defatted soy flour. According to Warner _et al_. (1983) the objectionable flavour characteristics of raw soy are retained in the soy concentrates, isolates, grits and flours, and detrimental off flavours were generated during processing. Indepth research has been undertaken since then to solve the soy flavour problem which include: extracting with hexane-alcohol azeotrope (Honig _et al_., 1976 and Rackis _et al_., 1973), wet milling with ethanol (Eldridge _et al_., 1963), extracting defatted soy flour with ethanol, methanol, and isopropyl alcohol (Baker _et al_., 1979), soaking of soybeans in acidified water to prevent off flavour developement (Kon _et al_., 1970).

Significant quantities of lecithinated full fat and defatted soy flours and soy concentrates have been used in the baking industry for many years. According to Pringle (1974), enzyme active soy flours are used to improve the crumb colour of bread, and other yeast raised products. Low levels of the full fat soy flour added to wheat flour (half to 1%) increases the bread crumb softness and keeping quality. Soy flour added at 6 to 12% levels in wheat flour has been used to produce special high protein breads (Hoover, 1974). Pomeranz _et al_.(1969) and Tsen and Hoover (1973) demonstrated that bread with acceptable volume, grain, and texture could be prepared with 25% substitution of wheat flour with soy flour.

Soy flours have also been used in doughnuts, cakes (Cotton, 1974), and cookies (Tsen and Hoover, 1973 and Levinson and Lemarnsik, 1974, Geernvani, 1988, Ali and Gandhi, 1992).

It is worth noting that products such as soy protein concentrates and isolates are used much less in India, even at the commercial levels due to their non-availbility, and high cost (Gandhi _et_ _al_., 1992).

The edible oil products manufactured from soybean fats in India are essentially by solvant extraction method, while the soy meal is largely exported. In 1991-92 about 1.7 million tonnes of soybean were processed which resulted in 0.3 million tonnes of oil (Gandhi et al., 1992). The edible oil products manufactured from soybean fats are soybean refined oils, blend oils, shortening, salad oils, and vanaspati (Ali, 1992).

According to Harby and Denel Jr (1951), one of the important by products of soybean fat which has gained a number of important commercial application is the phospholipid, lecithin, widely used for its surface active properties in margarines, candies, and bakery products.

2.2.5 Soybean in traditional foods:

Soybeans were used as a food in South East Asian countries long before the existence of written records. The first Chinese records which mention soybean date back to 2838 B.C. (Kale, 1936; Smith and Circle, 1978). Later, soybean cultivation and utilisation spread to other Asian countries such as Japan, Indonesia, Malaysia, and Thailand. The present century marks the extensive technological development of soybean processing and utilisation, particularly in the developed world (Gandhi et al., 1988.).

Steinkraus (1977), Hesseltine (1983), and Shurtleff and Aoyagi (1983, 1985) have extensively reviewed the fermentation of soybean with specific reference to indigenous foods. The traditional recipies prepared by orientals involved mainly the process of fermentation. The commonly consumed soybean products of the orientals are **miso,** a fermented soybean paste, (Shibasake and Hesseltine, 1962), **tofu,** a soybean curd (Muto et al., 1969), **natto,** a cooked and fermented soybeans (Arimotio, 1961), and **tempeh,** a mold cured Indonesian food (Saono et al., 1977), and non-fermented soybean products such as soymilk (Steinkraus, 1977).

Miso have been manufactured in Japan for about 1000 years. It is a salty, smooth to chunky paste with a meat flavour. It is made by the fermentation of soybeans with or without the addition of rice or barley, using mold A. oryzae, or A. soyae and yeast S.rouxii. Pediococcus halophilus and S. fecalis, are also sometimes involved in the same fermentations. Miso colour ranges from light yellow to dark brown or even black, with wide range of flavours which may be predominantly sweet, salty, or meat like (Ebine, 1977, and Shurtleff and Aoyagi, 1983).

Tempeh kedele is a very popular fermented soybean food consumed in Indonesia, Malaysia, Holland, and now even in the west (Steinkraus, 1977), though originated hundred of years ago in Central and East Jawa. Tempeh consists of tender cooked soybeans bound together by dense cottony mycelia of Rizopus mold (R.oligosporus, R.stolonifer, R.oryzae, R.arrhizhs) into compact white cakes or patties (Hesseltine et al., 1963 and Shurtleff and Aoyagi, 1979). It owes its good flavour, sliceable meat like texture, and excellent nutritional properties, much to the process of fermentation. Freshly prepared tempeh has a clean, mushroom like aroma. Following deep frying, the flavour becomes nut like, and peppery,due in part to the presence of free fatty acids. The qualities of tempeh depends on the substrates. Lower quality tempeh are also prepared by soybean flour, soybean curd waste (okara), and soybean hulls (Gandjar and Hermana, 1972).

Chinese **sofu** is a highly flavoured, creamy, bean paste made by overgrowing soybean curd with a mold belonging to genus Actinomucor rhizopus or mucor, and fermenting the curd in a salt brine/rice wine mixture. The salty flavour of sofu is suggestive of anchovies, and it is a soft, and pale yellow. The mold plus the wine or fermented rice mash in which the sofu is cured and stored impart additional flavour (Lin, 1977).

Natto is another ancient food, prepared in Japan (Thua-Nao in Thailand). The beans are covered with a viscous sticky fluid during fermentation with Bacillus subtilis. The fluid has the property of forming long, stingy threads when touched with the fingers, the longer the strings, the better the quality of the natto (Piper and Morse, 1943; Smith and Circle, 1978).

Shoyu or soy sauce, a widely used condiment, is a dark brown liquid made by fermentation of a soybean and cereal mix, (usually wheat). According to Komiya (1964) shoyu originated during Chan dynasty in 1134-246 B.C.

Hamanatto is made by fermentation of whole soybean and is produced in a limited area of Japan. It has a pleasant flavour resembling that of miso or shoyu, but is sweeter (Sundhagel et al., 1972; Ota, 1977; and Hayashi, 1977).

Tao Tjo and **Kochu Chang** (Smith , 1949) are small scale fermented products in Indonesia, Thailand, and Korea.

The principle non-fermented soybean foods include soybean milk (Smith and Beckel, 1946), **tofu**, and bean nuts and sprouts (Waggle and Kolar, 1979). The soybean milk or **Fu Chang** in Chinese is reported to have been developed and used in China before the christian era. The traditional milk is made by soaking the beans in water overnight, wet grinding the beans, heating the wet mash to improve flavour, and nutritional value, and filtering. The soymilk prepared by the traditional method has strong flavour. Today, soymilks are prepared both at commercial and household levels in several South Asian countries (Smith and Circle, 1978).

Tofu or bean curd is a cottage cheese like product formed into a cake, which is precipitated from soymilk by calcium salts or concentrated sea water (Smith and Circle, 1978, Shrutleff and Aoyagi, 1983)..

It is interesting to note that many oritental soybased products have mixtures of soybeans with cereals as in miso, shoyu, and involve the process of fermentation using molds, yeasts, or microorganisms (Sakari and Nakano, 1961), due to which undesirable beany flavours are destroyed, trypsin inhibitors are inactivated, and factors causing flatulence are eliminated (Hesseltine, 1983).

2.2.3 Soybean utilisation in India:

Soybeans are being used in increasing amounts around the world as a low cost, high quality protein supplement, to impart desirable functional properties to the products (Mosaiya et al., 1978). Although a black variety of soybean as **kalatur'** was known for a long time in India, a renewed interest to use soybean in Indian dietaries was developed only after the introduction of yellow variety in 1970's, basically due to its nutritional significance, in providing both high quality protein and, PUFA containing oil. Initial attempts in reintroducing soybean was through the substitution of pulses (Geervani, 1988; Randhawa, 1988).

Studies on product developement and processing taken up under the co-ordinated research project at Pantnagar, Jabalpur, and

Bangalore as well as under PL 480 projects at Pantnagar, led to the standardization of techniques for making dehulled dal, full fat flour, wheat flour fortification up to 15% with defatted soy flour, an soymilk (Randhawa, 1988; and Agrawal, 1992). This initiation further resulted in the development of over 300 soy based recipes of Indian dishes which are published in several books (Singh, 1970; Kanthamani, 1970; Kapoor, 1979; Lingaiah et al., 1975; Krishnamurthy and Shivashankar, 1975; and Vaidehi, 1988).

The soybean in its various forms has been found useful in the preparation of bread, biscuits, cakes, pastries, soups, omlets, sprouts, roasted beans, pulav, dal, chevda (roasted and spiced cereal and pulse combination), shev (extruded type of product prepared by deep fat frying), bhajiya (pakodas), usal (boiled whole pulse), and many other tasty dishes (Kale, 1936, Gandhi, 1992). The table 2.3 presents the reported reserach studies where soybean is used in its various forms in several Indian recipies.

Defatted soy flours have been extensively used in the manufacture of doughnuts, due to the low absorption of fat during frying. Several bakeries at Bangalore, Coimbatore, Madras, Bombay, and Delhi are using soy flours to the extent of 1-2% in bread (Singhal, 1992). The Government of India has recently permitted the blending of soy flour 10% with 90% wheat flour. The blended soy-wheat flour will have 50% higher protein than

Table 2.3 Research on selected soybean based Indian recipes

Name of the product	Level used	References
A. Soybean: (soaked and dehulled)		
Idli	25%, 50%, 100%	Cherian (1969) Thomas & Kamath (1988); Vaidehi, (1988); Geervani *et al.* (1988); Akolkar & Pareekh (1983).
Dosa	50%	Geervani (1988); Gouramma *et al.* (1988).
Khaman	100%	Cherian (1969)
Dhokla	100%	Cherian (1969)
Cooked dal	50%	Gouramma *et al.*
Mashed dal	50%	(1988).
Sambar	50%	
Vadai	50%	
Soy sprouts		Gandhi *et al.*
Soy peanut crisp	50%	(1988).
Chuduva	50%	Geervani *et al.* (1988);Desikachar (1988).
Chutney powder	50%	Geervani *et al.* (1988)
B. Flaked soy dal:		
Payasam	20%	Desikachar (1988)
Halwa	20%	
Pongal	20%	
Kichidi	20%	
C. Soy flour:		
Chapati, puries, roti, tandoori, nans	50-60% 15%	Desikachar (1988) Gandhi & Ali (1988) Ebeler & Walker (1983)
Bread	15%	Surna *et al.*(1973) Khadar (1988)
		Raidi& Klein(1983)

..... Contd.

Table 2.3 contd...

Name of the product	Level used	References
Biscuit	50%, 20% defat and full fat flour	Geervani (1988) Vaidehi (1988) Vani & Rajor(1991) Gupta & Singh (1991).
Kulfi	50%	Vani & Rajor(1991)
Burfi, Mysore pak	50%	Gandhi & Ali(1988)
Halwa	50%	
D. Soy residue (Okara):		
Cake, Samosa, Sev	-	Parihar *et al*(1977)
Kurma, Halwa	-	
Rava kheer, Rava Halwa Upama, Sausages, Chutneys Meat chunks	50 to 100%	Kaur (1992)
E. Beverage:		
Soy protein nector	30 % Apricot pulp in 70% aqueous extract of soybean	Chauhan *et al*. (1991)

in 100% wheat flour, and also higher protein efficiency ratio (Singhal, 1992).

Geervani _et al_. (1988) incorporated whole bean/ soy flour at 50% level in several traditional cereal, millet, and legume recipies such as idli, dosa, dhokla, chapaties, rotis, sambar, biscuits, chivada, and pakoras. The organoleptic and consumer evaluation indicated that all the mentioned products were acceptable without any significant differences among them. Besides increasing the nutritive value of the products, incorporatin of soy flour reduced the cost of the items.

Gouramma _et al_. (1988) prepared several typical products like cooked dhal, mashed dhal, dosai, and vadai, which were typical Karnataka dishes from soy in place of traditional pulses. It was found that these products were acceptable even when the pulses were substituted up to 50%.

Gandhi and Ali (1988, 1992) evaluated the acceptance of full fat soy flour (incorporated in amounts ranging from 50 to 60%) in various indigenous products such as chapaties, puris, pakoras, sev, soypeanut crisp, burfi, mysore pak, and halwa. The results indicated that all were acceptable with no significant differences among the products.

According to Desikachar (1988), the bitterish beany taste of soybean was greatly reduced by fermentation as normally practiced for **idli**, and **dosa**. Soybean could replace black gram by 50% in **idlis**, and even fully in dosa. Further, flaked soy dhal was found to be excellent for making **pongal** (sweet kichidi), **kichidi** or **payasam**, and thus can replace greengram dhal (Desikachar, 1988).

Vaidehi and Rathnamani (1990) introduced nutritious fermented **soy-sunflower tempeh** in the rural areas of Karnataka and prepared products that can be used for supplementary programmes for preschool and school age children. All the blended tempeh products showed a high percentage (90%) of acceptability. The **toffee** scored the highest (100%),only 6% of the children disliked the **sweet chips**, and 4%, the **salt chips**, and 2% the **stew**.

The literature on the food use of okara (soybean residue) is rather limited. Parihar et al.(1977) satisfactorily incorporated okara in the preparation of **sev**, **khurma**, **samosa**, and cake. Kaur (1992) successfully used okara in many Indian dishes in amounts ranging from 50 to 100% of the major cereal or pulse ingredients without seriously affecting the sensory qualities. The fermented foods such as **idli**, **dosa**, and **handwa** (fermented and baked cereal-pulse product) were accepted very well. However, dishes such as **chutney**, **upama**, **halwa**, **kheer** which required significant water binding properties scored low due to colour and appearance.

2.2.4 Soybean in fabricated food and food analogues:

The increasing cost of foods of animal origin such as milk, egg, meat, particularly in the underdeveloped world have created a growing interest in the alternative sources of protein. Altschul (1974), Fox and Cameron (1970), Milner (1971), Pyke, (1971), Smith and Circle (1978), Pirie (1980), and Subramanian (1980) have reviewed exhaustively the available conventional and unconventional alternative plant food as a source of protein for human nutrition. Search for unconventional food sources in the conventional form perhaps gave rise to the development of imitation foods and food analogues.

Food fabrication has been the part of our society since the beginning of recorded history. The earliest known fabricated food has been the bread (Glicksman, 1975). Inglett (1975) defines fabricated food as foods made by structuring, shaping, or blending various ingredients into finished food products.

The fabricated foods according to Inglett (1975) has several advantages over traditional foods by being novel, convenient, economical, and nutritionally improved due to the blending of several ingredients.

Wolf (1975) explains that fabricated food is prepared by combining the three basic components of food i.e. protein, fats and carbohydrates, in ways to provide convenience, texture, flavour, and other desirable characteristics.

The essence of fabrication of any food lies in the choice of ingredients, based on their nutritional value, and functional properties, using the prevailing/advanced techonologies that will help to create shape, colour, body and texture, and appearance, similar to familiar foods (Wolf, 1975, Pyke, 1971). Many existing fabricated foods are the reproductions of existing foods (Inglett, 1975), which are undergoing a transition from a poor imitation of an existing food to a sophisticated fabricated food.

The functional properties of foods such as gelation, viscosity, emulsification, water absorption, dough formation, visco-elasticity, adhesion, cohesion, aeration, solubility are essential for satisfactory fabrication (Waggle and Kolar, 1979). In this regard, soybean satisfies the basic functional properties required for the fabrication of a variety of foods. With particular reference to protein, the functional properties of soybean, as discussed by Bressani (1979), are presented in table 2.4.

The major technology of fabrication using soy protein ranges from simple methods such as mixing, blending, and heat application (eg. bread, Glicksman, 1975; hamberger, Wolf, 1979) to utilising sophisticated technology such as spinning operations as in the case of meat analogues (Inglett, 1975; Wolf, 1979). The fabricated products prepared by using different processing technologies using soybeans as listed by Wolf (1979) are presented in table 2.5.

A major technology available for fabricating textured vegetable proteins is by extrusion of protein mix under heat and pressure (Strommer and Beck, 1973; Wolf, 1975). Smith and Giddy (1975) defines extrusion cooking as a process by which moistened,

Table 2.4 Functional properties of soy proteins products.

Functional	Mode of action	Food system	Products
Solubility	Protein solvation pH dependent	Beverages	F, C, I, H
Water absorption and binding	Hydrogen bonding of water entrapment	Meat, sausages	F, C
Viscosity	Thickening, water binding	Soup, gravies	F, C, I
Gelation	Protein matrix formation and setting	Meat, curds, cheeses	C, I
Cohesion and adhesion	Protein action adhesive materials	Meat sausages, baked foods	F, C, I
Elasticity	Disulfide links in deformable gels	Meats, bakery items	I
Emulsification	Formation and stabilization of fat emulsion	Sausages	F, C, I
Fat absorption	Binding of free fat	Meat, Sausages donuts	F, C, I
Flavour binding	Absorption, entrapment release	Simulated meat bakery items	C, I, H
Foaming	Forms film to entrap gas	Whipped toppings desserts, cakes	I, W, H
Colour control	Bleaching	Breads	F

F - Full fat soy flour, C - Concentrates, I - isolates,
H - Hydrolysed proteins, W - soy whey.

(Source: Bressani, 1979)

Table 2.5 Fabricated foods using soy products

Method employed	Fabricated products
Extrusion	Textured vegetable products Breakfast cereals Snacks Infant foods Dry soup mixes Breadings Poultry stuffings Pasta products Beverage powders Hot breakfast gruels
Spinning	<u>Meat analogues</u> Bacon like bits Simulated sausages Simulated ham chunks Simulated chicken chunks Simulated bacon slices Meat extenders
Blending & Mixing	<u>Meat extenders</u> Hambugerger meat loaf Chilli, soups stews, Salads, pizza, sausages, etc. <u>Dairy type foods</u> Coffee whiteners Desserts Beverage powders Pizza toppings Margerine Blend oils

Source: (Wolf, 1975 and 1979).

expansile, starch and/or proteinaceous material(s) are plasticized in a tube by a combination of pressure, heat, and mechanical shear. This results in elevated product temperature within the tube, gelatinization of starchy components, denaturation of proteins, the stretching or restructuring of tactile components, the shaping of the end product and exothermic expansion of the extrudate (Smith and Giddy, 1975).

Defatted soy flour, or soy concentrates are made into chewy textured soy proteins products by an extrusion cooking process (Smith, 1975). Extruded soy flours are made by mixing soy flour with other starchy flours, water, plus flavours, colours, and supplementary nutrients, if desired, and then passing through a cooker-extruder (Wolf, 1979).

Soy proteins are also subjected to complex spinning operation to prepare a variety of meat analogues eg. hams, beef, chicks, and sea foods. These products have the advantage of being more meat like in texture (Wolf, 1974; Waggle and Kolar, 1975; Inglett, 1975). Pioneering work on the production of spun protein fibers was published by Boyer (1940, 1954). This method made possible for the first time the fabrication of meat like product (Giddy, 1975).

Several meat analogue products are being produced that utilise soy flour, soy concentrates, and isolated proteins. The production of meat analogues and processing of textured products is described by Rosenfield and Hartman (1979), and Horan (1974).

The isolated soy proteins are used in meat products mainly for their emulsifying capacity, emulsion stabilising effect, water absorption and binding, elasticity, fat absorption and property of increasing viscosity, and forming gels on heating, the meat products (Schweiger, 1974). The functional properties of soy proteins which make them particularly suitable in emulsified meat product are their low flavour, and odor, contribution to gel like property, in a manner similar to meat proteins (Bressani, 1979).

Textured soy flour and concentrates are also used extensively in coarse, ground meat products and convenience food items. The textured soy products can also help in some water and fat absorption properties in these products in addition to their chewy and other textural characteristics. They are also used in pizza toppings, chilli products, meat balls, meat pattis, tacos, meat spreads, poultry products, and fish patties.

Isolated proteins are used in combinations with textured soy flour, and concentrated products to provide binding, adhesive, and cohesive properties (Waggle and Kolar, 1979). The isolated soy proteins are also used as binders in sectioned and formed hams (Hawley et al., 1976) as stabilisers of emulsions in fish sausages, as binders in sausages for water retention, as protein supplement.

The soy flour and grits, concentrates, and isolates are used to give soybean proteins a texture that resemble specific types of meat (Wolf, 1979). These items range from extenders to be used with ground meat to complete meat analogues.

A few commercially available fabricated food products using soybean proteins in India are Nutrinuggets', Nutrella,'and Meal Maker', which are chunks and granules, manufactured and marketed by Ruchi', and Volta' Industries, Ltd. Bombay (Mukherjee, 1988 and Gandhi and Ali, 1992). These extruded products are becoming popular in India and the present production is around 35,000 tonnes/annum, and it is expected that by end of this decade the production would grow to 75,000 tonnes/annum (Gandhi and Ali, 1992). Kaur (1992) attempted to fabricate products using soy residue (okara). The sausages, and meat chunk analogues prepared entirely with okara using essentially the heat gelation technique was found well accepted.

One of the most commercially significant fabricated food using soy oil is margarine, which is usually prepared by soy oil and lecithin with varying percentages of other oil such as cotton seed oil (Black and Mattil, 1951, Inglett, 1975). Mayonnaise, butterin, are the other products fabricated with soybased fats. The use of soybean fat in the fabricated foods depends on its colloidal, interfacial, emulsifying, softening, anti-oxidant, and physiological properties (Bodman and James, 1951; Denel, 1951; and Black and Mattil, 1951).

In the field of dairy analogues, soy proteins are used in the products such as non-dairy coffee whiteners, and whipped toppings. The use of isolated soy protein to replace approximately 50% of sodium casecinate in spray dried coffee whiteners is described by Cho and Kolar (1977). Soy proteins have also been used for dairy analogue such as yogurt, sour cream, frozen desserts (Ice cream), cheese, and dip-type products, and infants formulae (Clans (1974)).

Soymilk though a popular traditional product among orientals, has not been accepted in the west because of its strong beany flavour, and an objectionable aftertaste, and more so because of the belief that they cause flatulence (Mital and Steinkraus, 1975). Several attempts to reduce the beany flavour of the soymilk have been made through altering the processing conditions. Mastuura et al. (1989) identified the diadzein and genistein as compounds responsible for the objectionable flavour in soymilks. These compounds have been found to increase by the action of β-glucosidases in soybeans during soaking, the first step of soymilk manufacturing. The action of lipoxygenase on the unsaturated lipid as the cause of beany flavour have also been documented by several investigators (Wilkin et al., 1967; Mital and Steinkraus, 1974 and Smith and Circle, 1978).

Subramanian (1980) suggested the debittering of the bean by sodium-bi-carbonate, steam deodourization of the milk, and use of anti-oxidants. The use of sodium-bi-carbonate in soaking water and hot grinding of soaked bean as a measure of reducing beany flavour has been suggested by Mital and Steinkraus (1974), Pinthong _et al_. (1980), Mastuura _et al_. (1989) and Reddy and Mital (1992).

Fermentation as a technique has been suggested to improve the acceptability of soy milk by Hang and Jackson (1967), Matsouka _et al_. (1968), and Mital and Steinkraus (1975). Angeles and Marth (1971) reported that soymilk with added sugars serves as an excellent medium for the growth of lactic acid bacteria.

The popular fermented beverage is yogurt/curd type products (Schmidt _et al_., 1986). However, by using soy alone it is difficult to obtain a product with nutritional and organoleptic qualities of yogurt. Growth and acid production in soymilk by lactic acid bacteria is limited due to the rather low sugar content (Mital and Steinkraus, 1975), therefore, it becomes necessary to add fermentable glucose (Pinthrong _et al_., 1980), fructose (Buono _et al_., 1990), lactose (Cheng _et al_., 1990) or milk products (Paolielo _et al_., 1987, Rao _et al_., 1988, Buono _et al_., 1990), inspite of the fact that oligosaccharides like stachyose and raffinose can be utilised to some extent by certain lactic acid bacteria, lactobacillus in particular (Mital _et al_., 1973; Mital and Steinkraus, 1975 and Pinthong _et al_., 1980).

The fermentation of soymilk with _L. bulgaricus_ has revealed that they do not grow well in soy milk without supplementation with sugars (Pinthong _et al_., 1980). Mital and Steinkraus (1975) fermented soymilk with _St. thermophilus_, and _L.fermenti_ with apparently complete utilisation of stachyose and raffinose.

However, work of Pinthong et al. (1980) have shown that St. thermophilus contributed to the unpleasant odour of fermented soymilk by the production of n-pentanal, whereas L.bulgaricus reduced the beany odour by partial removal of n-hexanal. Hence, Pinthong et al. (1980) and Rajor (1990) opine that the combination of L.bulgaricus and L. fermenti or L. acidophilus can produce a more acceptable product.

Shirai et al. (1992 a,b) prepared acceptable yogurt like products which composed of soymilk, oat flour and dried cheesy whey.

A series of products have been developed out of soybean in India, particularly at NDRI, Karnal, such as lassi, buttermilk (Deka et al., 1984 and Rajor, 1990), paneer (Vijayalakshmi and Vaidehi, 1985; Nasim et al., 1986; Grover and Tyagi, 1989; Gangopadhyay and Chakraborti, 1989; Barje et al., 1992; Ali, 1992), soy whey beverage (Patil and Gupta, 1983), ice cream, kulfi (Parihar et al., 1977; Vani and Rajor, 1991), puddings, custard, and milk shakes (Parihar, 1976). The acceptance of many of these products depends on the scope of adding suitable flavour and colour to improve the sensory parameters.

The sensory evaluation of these products has revealed that even after converting soymilk into various products, and blending with various flavouring agents to mask the beany flavour, the original flavour could not be totally masked (Parihar, 1976; Deka et al., 1984; and Rajor, 1990).

In many advanced countries, purified soy proteins such as soy concentrates, and isolates have been used to eliminate the objectionable beany flavour in soy based products (Hofi et al., 1974; Wolf, 1979; Waggle and Kolar, 1975). In this regard, the utilisation of soybean in cheese type of products has certain potential advantages over several other dairy analogues. Cured varieties of cheeses are particularly known to undergo extensive changes in sensory qualities such as flavour, body and texture, and appearance (Davies, 1965; Kosikowski, 1970; and Ecks, 1987). A few attempts have been made world wide to use soybean in cheese type of products. Before dealing with the technology adopted to use soybean in cheese type products, it is felt necessary to review the essentials of the technology of milk based cheese and cheese spreads.

2.3 Technology of cheese and cheese spreads:

Cheese belongs to the broad group of dairy products which involves the transformation of liquid milk to gel, and expulsion of whey from the gel. Some of the cheeses undergo extensive changes during the process of curing (Kosikwoski, 1966). The tech-nology of cheese making with particular reference to the cured varieties, involves essentially 3 basic steps, (a) coagulation, (b) release of whey through drainage, and (c) curing (Kosikwoski, 1966).

2.3.1 Coagulation of milk:

The conversion of liquid milk to curd is probably the first step in cheese making. Coagulation of milk is usually brought about by the action of coagulants such as enzymes, acid, and salts which bring about the physico-chemical changes in the casein micelles leading to the formation of coagulum or gel. The mechanisms for the formation of coagulation are different depending on whether they are induced by the action of enzymes, salts or acidification (Ecks, 1987). Sudden acidification by adding inorganic or organic acids, produce flocculation and form granular precipitate which separates from the whey. On the other hand, progressive gradual acidification effected by lactic acid fermentation leads to the formation of a smooth, homogenous three dimensional gel like structure.

Though cow milk is used for making many types of cheese for example, **cheddar, feta, roquforti, emmental,** milk from several other animals are also used for the preparation of specific cheese varieties such as buffalo milk for **mozzarella,** goats milk for **saint marcellin** or **soumaintrain,** ewe's and sheep milk for **gorgonzoals, fourme cheeses** (Kosiwoski, 1966).

The commercial preparation of cheese involves the standardization of milk as an initial step with particular reference to casein to fat ratio. The main aim of standardizing cheese milk is to entrap maximum available milk solids in the coagulated protein matrix of the cheese. Price and German (1931) found a casein to fat ratio of 0.7 in milk most suitable for cheddar cheese, which can be achieved by either separation of excess of fat or by adding protein in the form of snf.

Cheese making requires the presence of desirable microflora in sufficient number and of proper activity (Davis, 1965). The major role of culture is in the development of acidity, and during curing, release of enzymes to bring in desirable biochemical changes. The most effective method of adding microflora is in the form of lactic starter culture which are generally available in various combinations of types and strains (ranging from single strain to mixed type multiple strains). Lactic culture for cheese making are available as liquid, freeze dried, and concentrates. The concentrates can be added directly to the vat, while traditional method of propagating through stock, mother, and bulk cultures still continues. Various aspects of culture technology have been reviewed extensively by Whitehead and Cox (1935), Whitehead (1953), Kosikowski (1970), Wilster, (1964), Eck, (1987).

The microorganisms are also introduced during subsequent process stages in the form of surface smears as in brie cheese, or injected into the cheese as in mold-ripened roquforti or blue veined varieties (Pernodet, 1987).

A large number of proteolytic enzymes of animal, vegetable, or microbial origin have the ability to coagulate the casein complex. Rennet, a mixture of chymosin and pepsin is the best known milk clotting enzyme. The mode of action of rennin is well established (Lindquist, 1963, Garnier, 1968 and Bruie and Lenoir, 1987).

The action of rennet occurs in two phases, (a) a primary enzymatic phase in which rennin splits phenylalanine (105)-methionine (106) peptide bond of k-casein releasing glycomacropeptide (Waugh and Von-Hippel, 1956; Nitschmann et al., 1957; Jolles et al., 1968; Bruie and Lonior, 1987). and (b) a secondary non-enzymatic phase in which the paracasein system clots in the presence of calcium ions (Fox, 1970). Rennet is still widely used in cheese making. But in the face of growing worldwide shortage, a great interest is shown to substitutes that can be used industrially (Veringa, 1961; Sardirnas, 1972; Chakraborty and Tiwari, 1986, 1987). The success of rennet substitute is based on its close activity with rennet, having high milk clotting activity and low proteolytic activity towards the different casein fractions (Eck, 1987). Some of the substitutes of animal origin that are tried out are trypsin, chymotrypsin, pepsin (Ernstrom et al., 1977), the vegetable origin substitutes such as ficin from fig tree latex, papain from papaya, brolmelain from pineapple, which were not found very suitable in cheese making due to their high proteolytic actions and mircobial substitutes being bacterial, mold, fungi (Veringa , 1961; Sardinas, 1972; Ramet, 1987; Chakraborty and Tiwari, 1986).

The post coagulation process involves expulsion of whey from the curd, aided by (i) increased surface by cutting/shattering of the curd into smaller pieces, (ii) gradual increase in developed acidity and lowering of pH due to the lactic cultures and (iii) gradual raising of the temperature of the whey in which the curd is cooked (Davis, 1941; Gilles, 1976).

Cooking of the curd is followed by the drainage of whey, packing of the settled curd into blocks and cheddaring (in cheddar cheese) of these curd blocks until they develop the desired, cooked chicken breast' texture and a whey acidity level of 0.4 to 0.5% lactic acid. During cheddaring, certain structural changes in the body and texture of the curd take place, leading to further fusion of curd matrix (Chapman, 1974; Brooker, 1979) that results in the cooked chicken breast texture.

The curd fusion continues during subsequent milling, salting and pressing of the curd. At the end of cheddaring, the curd is shredded into smaller pieces using a curd mill. To promote further removal of whey, salt distribution and to prepare curd for pressing, the milling of curd at an acidity of more than 0.4% lactic has been suggested (Wilson,et al., 1945; Vanslyke and Price, 1952, Gills, 1976; Morris, 1962; Eck, 1987). The addition of salt to the milled curd has the effect of expelling more moisture from the curd, and controlling the growth of undesirable organisms in cheese, besides the seasoning effect. The salt can also be deposited on the surface of the cheeses (eg. Saint marcellin), or by immersion in brine (brie, camembert).

Some variations and alterations in these processing treatments are according to the varieties. The effect of variations in the steps of coagulation and subsequent processing steps determines the curing behaviour of the cheeses. In the manufacuring of cheeses such as **Tome de savoje, St. Neetaire,** and **Edam**, the curd after drainage is molded and submitted immediately to the pressure. As in the case of cheddar, the curd particles are allowed to mat together in **cheshire**, and **cantal** cheeses. In case of **parmesan, emmental**, or sbrinz varieties of cheeses the curd is cooked after cheddaring and prior to curing.

2.3.2 Curing of cheese:

Cheese curing involves a number of complex physico-chemical, and biochemical changes initiated by the milk clotting enzymes, starter organisms, native substrate microflora, and the enzymes liberated from these microflora. The technology of cheese ripening has been exhaustively reviewed by Schormuller (1968), Seitz (1974). Lawrence et al. (1984), Moskowitz and Noelck (1987), and Seitz (1990). The ripening technology varies between the cheeses such as bacteria ripened as in the case of **emmental, gruyere**, and **asiago**, with the formation of eyes or gas holes, mold ripened throughout the interior of the cheese as in **roqueforti, stilton**, or **gorgonzoals**, surface ripened principally by mold as in **camembert**, brie, surface ripened principally by bacateria, and yeast as in brick, port-salut, limburger, etc. (Bruce, 1970).

Some cheeses are given a protective covering with paraffin wax (as in cheddar), and are allowed to cure under controlled time - temperature, and humidity conditions. The curing period for different cheeses may vary from a few days (high moisture soft cheeses) to a year or two (parmesan). The popular cheddar cheese undergoes curing for a period that falls in between these time intervals, i.e. about 6-9 months (Schormuller, 1968).

The curing of cheeses involves the degradation of lactose, protein, and fat, in addition to the evaporation of moisture, and gradual change in pH. Of the three components, lactose is utilised initially, resulting in lactic acid production. This is followed by proteolytic and lipolytic activites (Schormuller, 1968). Lactose utilisation is assoicated with the initial lowering of pH in cheese. Then, gradual increase in pH occurs due largely to the liberation of basic amino acids during protein breakdown (Wang, 1974).

The breakdown of the lactose, protein, and lipid should progress during curing at a controlled rate for developing desirable sensory attributes, which are characteristics of different types of cheeses.

The extent and rate of protein degradation during curing depends on the method of manufacture of cheeses (Choisy , et al. 1987). The insoluble nitrogen contituents are to some extent changed to soluble forms. During the progress of proteolysis, the paracasein and the minor proteins are gradually converted to simpler nitrogenous compounds viz., proteoses, peptones, amino acids, and ammonia (Kosikowski, 1966). The rate and extent of proteolysis is influenced by several factors such as ripening temperature, amount of enzyme, moisture levels in cheese, salt content, size of cheese block, and the level of acidity (Eck, 1987; Schormuller, 1968).

The degradation of proteins contributes to the softening of cheeses (Hard cheeses), thereby altering the body and texture. The major role of protein in the texture of cheese has been studied with Electron-microscopy (Schormuller, 1968). In cheese body, the protein forms a 3-dimensional net work which traps the fat globules and whey. Any modifications of the proteins in the cheese, as during curing, influence its rheological properties. In firm textured cheeses, proteolysis results in a loss of firmness and elasticity. This phenomenon has been artificially amplified in some cheddar and gouda cheeses by adding proteases to the curd or to the milk from which the cheese is made (Vassal et al.,1982; Lawrence, et al., 1987). In certain types of soft cheeses, as the changes in texture progress, the cheese become very soft and even in some cases semi-fluid (eg. camembert cheese).

The lipid fraction of cheeses contributes more to the development of flavour than any other compounds (Schormuller, 1968) particularly in cheddar cheese. The lipolytic changes in fat release free fatty acids which are brought about by enzymes during ripening (Choisy et al., 1987), and oxidative changes resulting in the release of carbonyl compounds such as methyl ketones (Stokoe, 1928) that contribute to the flavour.

During the curing session, the cheeses depending on the varieties, undergo extensive changes in flavour. The flavour characterisitics of ripened cheese is derived from a blend of several odours, and taste imparted by several volatile and soluble substances. The aroma or flavour of cheese is produced in the course of ripening through a series of mechanisms, mostly enzymatic, which transform the various constituents of the curd, protein and lipid in particular. The proportions and nature of the various flavour components vary according to the progress of curing biochemistry and microbiology, which in their turn depend on the technology employed (Law, 1981).

Schormuller (1968) and O'Keeffe et al., (1978) demonstrated that proteases, peptidases, transaminases, decarboxylases, and phosphatases originated from the starter bacteria, are involved in the formation of product responsible for flavour development in soft and hard variety of cheeses. Microbiological and chemical reactions and compounds related to flavour have been reviewed by Mabbit (1955), Marth, (1963), Fryer, (1969), Moskowitz and Noelck, (1987) and Seitz (1990).

Although the knowledge on the flavour development is some what incomplete, it is possible to identify certain components which are assoicated with the development of characteristic flavours of a number of cheeses (Bedings and Neeter, 1980).

Among the several cheeses, the flavour of the cheddar has been studied extensively (Kristoffersen, 1979). Several studies have identified and emphasised different compounds responsible for typical cheddar flavour such as free fatty acids (Orhan and Tuckey, 1968), gama and delta-lactones (Wong et al., 1973), and methanethiol (Manning, 1979). Several sulfur compounds are thought to contribute to cheddar flavour such as dimethyl sulfide (Patton et al., 1958), hydrogen sulfide (Walker, 1961), active -SH groups (Bells, 1967; Kristoffersen and Nelson, 1954; Kristoffersen et al., 1964, 1979).

The Oct-l-en-3-ol is identified as a flavour indicator in camembert cheese (Dumont et al., 1976), diacetyl and propionic acid in Swiss cheese (Langsend and Reinbold, 1973; Soda et al., 1990), hydrogen sulfide and methanethiol in limburger and tappist cheeses (Dumont et al., 1976). The different types of semi-soft mold ripened cheeses have in common a predominant odour due to the presence of alcohols (Anderson and Day, 1966; Godinho and Fox, 1981).

2.3.3 Accelerated Ripening:

In recent years, new cheese making technologies have emerged. The advancement in technology has covered several phases of cheese making eg. (i) treatment of milk, such as pre-concentration of milk through ultrafiltration, (ii) starter culture technology, e.g. direct addition of culture into the vat, (iii) use of rennet substitutes from non-animal sources viz. microbial enzymes, and (iv) mechanized cheese manufacturing systems including those for setting, cutting, and cooking curds, cheddaring, salting, milling, hooping, etc.

As cheese ripening is a slow process, involving high capital investment, and the running cost, lots of effort towards the advancement in cheese technology has been geared to speed up the ripening process. The rapid development of more intense cheese flavour in shorter period of time has been a long term goal of cheese makers (Mohamed et al., 1989). Strategies for accelerating the ripening of cheese include, (a) ripening at elevated temperature, (b) addition of enzymes, (c) addition of pre-ripened slurries/cheese, (d) addition of metals. Extensive reviews on accelerated ripening have been published by Law et al. (1979), El-Soda and Sadda, (1986), Kamaly and Marth (1989) and El-Soda and Pandian (1991).

Elevated temperature ripening has been dealt with in a limited way as one of the major strategies for the acceleration of cheese maturation. Law (1979), Fedrick et al. (1983), Nunez et al. (1986), Aston et al . (1983, 1985), and Gaya et al. (1989), have documented increased proteolytic, and lipolytic reactions, resulting in more than 50% reduction in the maturation time for Manchago, Ras, and Domiati cheese varieties.

The largest proportion of the research work related to the acceleration of cheese ripening deals with addition of cheese related enzymes (Barach et al., 1985; Mashaly et al. 1986, Brezina *et al.*, 1988; Hassan *et al.*. 1988; Tamime *et al.*, 1990), and enzymes from sources other than cheese (Fedriek *et al.*, 1980; Frey, *et al.*, 1986; Lin *et al.*, 1987; Alkhalaf *et al.*, 1987). These studies have recorded 10 to 60% reduction in cheese maturation time.

Several workers have found that pre-ripened slurry improved the quality of cheese, when added at 1% level and hastened the flavour development (Rabie, 1989; Hofi, *et al.*, 1989; Laila, *et al.*, 1989, Salam *et al.* (1989), and El-Soda (1991).

Ernstorm *et al.* (1980) achieved the production of cheese base from whole milk which could be used in a processed cheese blend. A blend of 80% cheese base and 20% matured cheddar cheese has been used in the manufacture of processed cheese. A similar method for the production of cheese base was reported by Madsen and Bjerre (1981) and Rubin and Bjerre (1983a, 1983b) who recommended proportions of cheese base and cheese similar or even lower. Several preparations for the production of similar blend, have been reported by Modler *et al.* (1985), Jameson and Sutherland (1986), Moran *et al.* (1989), and Tamime *et al.* (1990).

Dilaminan (1984) found that addition of manganese, zinc, copper, cobalt, and chloride to milk improved the quality and organoleptic scores of Swiss type of cheeses. Hofi *et al.* (1973) opined that the role of trace elements on ripening is probably due to the stimulation of certain microbial groups and/or to the activation of enzymes present in the cheese.

The method developed by Kristoffersen *et al*. (1967) for accelerating flavour development in cheddar cheese curd by increasing its moisture content, and incubating at 30oC has been successfully adopted to different cheeses such as Ras (Rbdel Baky *et al*., 1982; Hofi *et al*., 1989), Gouda cheese (Spangler *et al*. 1989), and Edam cheese (Salam *et al*. 1989).

Accelerated ripening products prepared by blend and slurry approaches have a particular use in secondary cheese based products, as in hamburgers, pizza, and sauces etc.

2.3.3 Processed cheese and cheese spreads:

As curing progresses, the cheeses reach an optimal sensory quality interms of flavour, body and texture. Any further curing tends to deteriorate the sensory characteristics of the cheese, and therefore cheese at this point be preferably consumed within a short time. The biochemical reactions responsible for bringing the changes during curing require to be checked by heat processing, resulting in an extended shelf life.

The economic and commercial significance of cheese and also its secondary use in several food items (eg. pizza, burgers, cheese rolls, spreads etc.) might have led to the innovation of processed cheese making techniques.

According to Meyer (1973) cheese processing offers a number of technical advantages in particular to the operational side, besides creating an array of procesed products which are manufactured by several combinations of foods.

The processed cheeses are available in several forms such as blocks, slices, dried powder, spreads, prepared from hard, semi-hard and soft cheeses. The processing provides an ample opportunity for using additives such as milk constituents (milk, whey, butter, curd), seasonings (dried herbs, spices, plant extracts), and other food items (meat, sea foods, mushrooms, alcohol, vegetables, fruit, fruit juice, jams, coffee, chocolate, yeast, vitamins) in the cheeses. (Meyer, 1973; Prajapati et al., 1992; Santos et al., 1989). The manufacture of dried cheese fecilitates to extend the use of cheese in bakery foods, as binding agent in sausages, mayonnaise, dips and soups etc. (Meyer, 1973). Conventionally processed cheese is manufactured by giving an appropriate heat treatment to a blend of cheeses differing in age, texture, and flavour, along with several emulsifiers (citrates and phosphates), stabilisers (pectin, agar agar etc) to impart physico-chemical stability . Several additives are known to be used while processing cheese such as colour, flavouring agents, preservatives, fungicides, bactericides (sorbates, sodium sulphites etc.), oxidising agents to raise the redox potentials such as hydrogen peroxide, nitrates, chlorates, bromates, iodates, persulphates etc.) and biological inhibiting substances such as nisin (Meyer, 1973).

Cheese after processing is filled hot in several types of containers such as cans, tubes etc., sealed hermitically and stored between 5 to 10o C.

Good, packaging material is essential to ensure protection against outside influences during the period that begins with packaging and ends with the consumption of the product. According to Stenk (1987), the choice of packaging materials depends on the type of the product, and possible storage that the nature of the product permits. Depending on the need, several packaging materials such as water proof/ grease proof paper,, paper coated with paraffin (for soft cheese), and card boards, plastics (low density polyethylene, high density polyethylene, polystyrene), aluminium (as foils), laminated packaging materials glass jars, and lacquered tins are available for packing varieties of cheese products (Stenk, 1987). Goyal and Babu (1981) studied the microbiological quality of procesed cheese packaged in small lacquered tins (LT), polystyrene cups (PC), and also low density polyethylene tubes (PT), and stored at 31oC / 60% RH and 7 to 8oC / 80% RH, respectively. The results revealed that LT was found to be the best package, followed by PT and PC for storing processed cheeses.

Spreads are gaining more popularity at present due to its being an alternative to butter, and providing a pleasant mild flavour. Spreads are plastic products used for table purposes such as for applying on with bread slices, crackers, and the likes. Primary ingredients for spreads are dairy products. The best known spreads is butter. Due to the current aversion against the consumption of saturated animal fats in high amounts, other sources of fat (vegetable) and proteins paricularly of vegetable origin are becoming increasingly popular (Patel and Gupta, 1988; Madsen, 1989).

Imitation cheese spreads using vegetable sources such as peanut milk (Krishnaswamy and Johar, 1960; Krishnaswamy and Patel, 1968; Guirguis et al., 1985), peanut isolate (Chen et al., 1979), peanuts (Upchurch, 1978; Sanots et al., 1989) have been developed as a potential cheese substitutes. Peanut butter is popular both in India and West (Santos et al., 1989, Patel and Gupta, 1988).

Soybean has also been a principal non-dairy ingredient that has found use in low-fat-high-protein spreads, due to its functional and nutritional characteristics. Prajapati et al. (1992) developed a cheese flavoured spread which contained 40% fat (84.75 parts of a 1:1 blend of hydrogenated fat, and soybean oil, 15.25 parts of milk fat, (contributed by added cheddar cheese), 15% snf (from cheddar cheese and reconstituted dried skim milk), 0.1% carrageenan, 0.3% glycerol, monostearate, 1% tri sodium citrate, 1.5% salt and 0.25% annatto butter colour. Use of 20% ripened cheddar cheese was found most suitable for the desired cheese flavour, spreadability, and other sensory attributes.

2.4 Utilisation of soybean in cheese type of products:

Several attempts have been made to utilise soybean in cheese type products particularly, in last three decades,through the use of cultures, and technology employed in the manufacture of regular cheese from cow's milk. Although these new products have not become commercially available, these studies have resulted in several publications and patents (Kenkyusho, 1965; Hang and Jackson, 1967; Obara, 1968; Kim and Shin, 1971). Normally, the coagulation of soymilk is accompanied by the action of lactic acid bacteria. The development of cheese like flavour is contributed by suitable enzyme preparations, bacterial and/or mold.

Soybean has been used in its various forms in several types of cheeses such as protein isolates (Bennett and Ohren, 1974, Caric *et al*., 1989), soymilk (Hang and Jackson, 1967), soy curd (Rosenan and Herrick, 1981); soy flour (Dorderic and Caric, 1968), and slurries (Mittal and Singh, 1984; Kumari and Singh, 1986; Kumari and Singh, 1990).

Smith (1968) considered tofu as a suitable product for making fermented cheese, and is, in fact, used for this purpose in China. Wai (1964) described a process used for preparing Chinese soybean cheese. The resulting cheese had a longer shelf life than tofu, but this was achieved only by adding large amounts of salts and alcohol.

Kenkyusho (1965) prepared a cheese like product from soybean. Soymilk was supplemented with casein, glucose, butter fat, and vegetable oil. The mixture was treated with S. faecalis, rennet extract and calcium chloride. The resulting curd was processed according to the conventional method of cheese making.

The preparation of cheese like product from soymilk using S. thermophilus as a fermenting organism was investigated by Hang and Jackson (1967a). The investigators concluded that the lactic fermentation resulted in a satisfactory product in terms of textural parameters. Hang and Jackson (1967b) modified their product with the addition of rennet extract and skim milk; and found that rennet improved the body and flavour of the cheese. In the sample of cheese there was a gradual loss of moisture, occuring most rapidly during the first week.

Obara (1968) developed a cheese like product by treating soy curd directly with proteinases rather than by using traditional process. He reported no satisfactory product from soymilk prepared by the conventional cheese making process. Among the enzymes tested, papain gave the best results with respect to flavour and texture. Trypsin and molisin were found to be unsatisfactory because of poor digestibility and inferior taste. A combinations of enzymes showed a higher rate of activity than a single one. The combination of papain, bioprase and pronase exhibited higher rate of ripening as well as desirable flavour and texture than other enzyme combinations tested.

Matsuoka et al. (1968) reported that S.thermophilus produced far greater amount of acid in soymilk than S.lactis, and L. bulgaricus. However, the cheese like product made from soymilk using S.thermophilus darkened significantly during ripening.

Dorderic and Caric (1969) studied the feasibility of manufacturing cheese from milk with the additions of soybean flour and reported that with increasing concentration of flour, the coagulation time increased and milk failed to coagulate, when the concentration exceeded 4%. With the addition of $CaCl_2$ at 0.04% of milk even with 5% soy flour was coagulated.

Kim and Shin (1971) studied the preparation of cheese like product from soybeans. Soymilk and reconstituted dried skim milk were combined in the ratio of 7:3, and were subjected to lactic acid fermentation for 24 hrs. The resulting product had high protein, and low moisture content. On the basis of these results, a proceedure was developed: A cheese was prepared from the mixture of soybean milk and reconstituted skim milk (combined in a ratio of 7:3, heated at 121oC for 20 mins.) and by additions of S.thermophilus using incubation temperature 37oC for 24 hrs. The curd was then cooked, hooped and pressed for 24 hrs. Penicillium caseiocolium and sodium chloride were spread on the surface, and the curd was ripened at 15oC and 85-90% RH for 21 days. Penicillium caseiocolium developed considerably after 7 days. The pH of the product increased, and proteolytic acitivity reached a peak after 14 days. After 7 days of ripening total water soluble nitrogen, water soluble protein nitrogen, and amino nitrogen had begun to increase which reached the level of 52%, 32%, and 14% total nitrogen respectively, after 21 days of ripening.

Schroder and Jackson (1971) attempted to improve the flavour and texture of soy cheese by incorporating skim milk and mold. Addition of skim milk did not improve the flavour and texture of the finished cheese, because of the dominating effect of beany flavour, and fibrous matters of soybean. Mold ripening brought about desirable changes in the texture of the product but they were offset by the development of bitter flavour.

Benckisser-Knapsack (1972) studied a process for preparing a spread similar to cheese spread. The process involved heating of soy cheese at 90oC in the presence of an emulsifer.

Lundstedt and Lo (1973) developed a cheese like product from soybean curd by fortifying it with butterfat, and milk snf, and by inoculating it with *Penicillium requeforti* and also *S. diacetylactis*. A blue cheese like product was developed with same flavour, taste, and blue veins, as in roqueforti cheese. A new curd from soybean milk was also prepared by adding 1 to 10% fat, 0.5 to 5% skim milk solids to soymilk and by the use of lactic acid bacteria as starter. The resulting product was heat stable, and suitable for the preparation of a metltable cheese (60 to 80% moisture).

Snow Brand Milk Products Co. Ltd. (1974) in Japan patented a cheese product from extracted soybean proteins. The product had the taste and texture of natural cheese.

Pulle (1974/1975) showed that cheese prepared from soy protein extract was superior to cheese made from other plant proteins (green gram and red gram) with regard to protein, fat, flavour, body and texture, approaching the cheddar cheese in quality.

Bennett and Ohren (1974) carried out investigations to extend milk protein with a moidified soy protein isolates, in order to develop a snack spread base. The resultant product was found to be spreadable both at refrigerated and room temperature.

Abou-elnago et al. (1974) prepared cheddar cheese, using soybean oil in place of butter fat.

El-Safty and Mohanna (1977) reported that the addition of soymilk to buffalo milk decreased the curd tension of milk at the cutting time of cheddar cheese. Increasing the amount of rennet, and addition of $Cacl_2$ improved the curd characteristics. They concluded that the addition of 20% soymilk to buffalo milk improved the quality of cheese.

Abou El-Ella et al. (1977) prepared a **Karish** cheese using buffalo milk and soy milk in the ratio of 4:1. The cheese containing soymilk had a slightly higher acidity and salt content than the control (buffalo milk - skim milk cheese)

cheese. The addition of 20% soymilk was found to have no adverse effect on ripening index, total volatile fatty acids, total nitrogen, soluble nitrogen contents, and sensory qualities of karish cheese.

Yu et al. (1978) prepared cheese like product from defatted soymilk, using S. lactis, and coagulants such as megnesium chloride, calcium chloride, and curing for 6 weeks.

El-Safty et al. (1979) reported that the addition of 20% soymilk to buffalo milk improved the quality of ras cheese. The addition of soymilk was found to increase the lipase activity in the milk mixture, and in the resultant cheeses.

Manufacturing of soybean cheeses, and chemical changes during ripening have been reported by Jeong and Choi (1980). Addition of skim milk and/or rennet resulted in higher ripening index.

Taranto and Yang (1981, 1982) compared the morphology and texture of mozzarella cheese analogs prepared from soybean proteins with that of control. Soybean mozzarella cheese exhibited morphological features resembling control cheese, i.e. a protein matrix in which fat and other ingredients are embedded and dispersed. The texture profile analysis (Weissenberg test) exhibited textural characterisitics in the solid and gelled starch, and stretching properties in the melted state, comparable with natural mozzarella cheese. More close textural and structural properties were exhibited when soybean proteins concentrates were used.

Lee and Marshall (1981) revealed that the replacement of 10% cheese proteins with native or boiled soy protein reduced the emulsifying extension capabilities of casein, the hardness of the processed cheese was reduced but cohesiveness was increased. Replacement with 20% protein, reduced both hardness and cohesivenss of rennet coagulated milk. Scanning Electron microscopy micrographs showed that soy protein particles tended to aggregrate. Compared with native soy protein, the heat denatured soy proteins, had a more marked destructive effect on structure and texture of processed cheese.

Metwalli et al. (1982 a,b) prepared satisfactory domiati cheese using 4:1 ratio of buffalo milk with soymilk, soaked beans, and soy flour. Cheese made from soymilk had an unacceptable nutty flavour, when fresh, but an acceptable flavour after ripening for one month.

Similarly, Abou-El-Ella (1983) added soy milk (5 to 10%) in cow milk for the manufacture of domiati cheese. Soymilk addition increased the ripening indices, probably due to stimulation of microflora. While addition of 5% soy milk improved the sensory qualities of the cheese, 10% addition was found to lower the sensory scores.

According to Ghaleb et al. (1983) replacement of hard cheeses with up to 50% flour, **bashkack** with up to 66% soy flour, or natural **samne** with up to 75% soy bean oil produced satisfactory results. However, combined replacement of 50% hard cheese, and

75% natural samna or 66% bashkeek, and 75% natural samna had delterious effects.

Mittal and Singh (1984) , Kumari and Singh (1985, 1990) prepared acceptable soy cheese spread using soy slurry (64% soy solids) , skim milk, cream using slurry approach.

Ras, Kuribayashi (Shenata et al., 1985) and **Qquasi** (sueoka, 1989) cheeses were prepared by utilsing soybean milk. Sano and Kizaki (1989) patented a method to prepare cheese like product using soybean protein, casein, oil/fats, and water. The method involved thorough mixing of soy protein with water, and melting the casein by steam, and the heating the system to 80oC with agitation.

The application of isolated soy protein as a substitute for cheese in processed cheese production was examined by Caric et al. (1989). Results gained during laboratory studies on an industrial scale showed that substitution was successful up to 15%.

El-Ssayed et al. (1990) prepared **Zabadi** cheese using 10 to 30% soymilk along with buffalo milk. The milks were inoculated with 2% Zabadi starter (L. bulgaricus and S. thermophilus) and incubated at 42oC for 4 to 4.5 hrs. The Zabadi cheese prepared by higher concentration of soymilk (30%) scored very low in sensory qualities due to beany off flavour.

Due to its high protein and oil content several attempts have been made worldwide to utilise soybean and its various fractions. It is apparent from the above reviews that the objectionable flavour of soybean could not be totally elimiated, unless purified soybean proteins (eg. isolates) were incorporated. In the Indian context, the utilisation of whole soybean is more practical.

However, attempts in India to utilise soybean in dairy analgoues has not been successful due to the beany flavour problem. Among the dairy analgoues, the cured cheese type of products have some scope since these are known to extensively modify the flavour, body and texture of the final product. The use of whole soybean in cheeses prepared by covnentional techniques has been rather limited. A slurry approach (high moisture) developed by Kristoffersen _et al_. (1967) has the advantage of reducing the curing period from several months to a few days. The slurry type products are particularly suitable for preparing spreads. The blending of natural cheese with soy curd can also be an alternative approach for spread making. The cheese spreads are being favoured as an alternative to butter even in India.

Spread also provides scope for the addition of several spices and condiments. Keeping these factors in mind, the present study was planned to develop a mild flavoured cheese spreads employing both slurry and blend approaches, using whole soybean in the starting material.