EFFECT OF ADDITION OF SOY MILK ON THE PREPARATION OF PANEER

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Effect of addition of soy milk on the preparation of *paneer*

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This *dissertation* entitled *Effect of Addition of Soy Milk on the Preparation of Paneer* presented by Sanjeev Neupane has been accepted as the partial fulfillment of the requirement for the B. Tech Degree in Food Technology.

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Abstract

Soy *paneer* is a vegan friendly dairy product prepared by using soymilk as a principle ingredient. The aim of this research was to develop the formulation for soymilk incorporated paneer and to study the effect of blending of soymilk and cow milk on *paneer* quality. Design expert ® 10 was employed for formulating the recipe of *paneer*. The obtained 6 formulations of soy paneer coded as A, B, C, D, E and F with varying levels of soymilk and cow milk were prepared in lab where the ratio of cow milk: soy milk were in 100:0, 90:10, 80:20, 70:30, 60:40, 50:50. The samples were subjected to sensory evaluation. Microbiological status of the final optimum soy *paneer* was determined to study the effect of formulation on microbiology. Chemical analysis of the sensory optimized *paneer* sample was carried out.

From sensory evaluation, 70% cow milk and 30% soymilk were found to be significantly best ($p<0.05$). In most of the formulations, body, color, flavor, texture and overall acceptance were significantly affected ($p<0.05$) by variation in soymilk and cow milk. Soy *paneer* analyzed for moisture, fat, protein (%N×6.25), total solids, ash content, pH and acidity were found out to be 56.68%, 19.04%, 23.83%, 47.94%, 2.23%, 5.350 and 0.507 respectively while that of control sample was found out to be 55.97%, 18.98%, 19.93%, 48.65%, 1.45%, 6.52 and 0.41% respectively.

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List of abbreviations

Abbreviation	Full form
ADF	Acid Detergent Fibre
ANOVA	Analysis of Variance
AOAC	Association of Analytical Communities
APHA	American Public Health Association
CCUR	Centre for Crops Utilization Centre
CCT	Central Campus of Technology
DDC	Dairy Development Corporation
EVA	Ethylene vinyl chloride
FAO	Food and Agricultural Organization
GLY 1	Glicynine
GLY 1B	Beta- conglicynine
LDL	Low density lipoprotein
PDI	Protein dispersibility index
PVDC	Polyvinylidene chloride
SNF	Solid not fat
USSEC	United States soybean export council
WHO	World Health Organization

Part I

Introduction

1.1 General Introduction

Paneer, a popular indigenous food product of South Asia, is similar to an unripened variety of soft cheese. It is obtained by heat and acid coagulation entrapping all of the fat, casein complexed with denatured whey proteins and a portion of salt and lactose. *Paneer* is marble white in appearance, having firm, cohesive and spongy body with a close-knit structure and a sweetish-acidic-nutty flavor. It has a simple, fresh, versatile flavor which makes it highly useful in an assortment of recipes (Singh and Kanawjia, 2014).

Soybean (*Glycine max*), belonging to the family Leguminosae, is the world's most important seed legume which contributes to 25% of global edible oil, about two-thirds of world's protein concentrate for livestock feeding. Soybean contains number of nutraceutical compounds such as isoflavones, tocopherol, and lecithin besides 20% of oil and 40% of protein (Agarwal *et al.*, 2013). It is rich source of monosaturated, polysaturated, and saturated fatty acids. It has good emulsifying properties and low starch content (Foschia *et al.*, 2017).

Soybean can be processed into a wide range of products. Soymilk is a stable emulsion prepared by soaking and grinding soybean in water. Besides being rich in protein, vitamin and mineral, soymilk is an economical, lactose free, digestible and nutritious alternative to a dairy-centered diet (Ali, 2010). Soybean is one of the nature's wonderful nutritional gifts. Soybean provides high quality protein with minimum saturated fat. Soybean contains all the three nutrients viz., carbohydrate, protein and fat required for good nutrition, as well as fiber, vitamins and minerals. It has high PUFA content. Soybean has more than twice the amount of minerals, especially calcium, iron, zinc and phosphorus than any other legume. The 1990's FAO/WHO protein evaluation committee put Soy protein at par with egg and milk protein and ahead of beef protein (Venter, 2004).

Soy milk (also called soya milk, soymilk, soybean milk, or soy juice and sometimes referred to as soy drink/beverage) is a beverage made from soybeans In addition to being a rich source of nutrients, soybean has a number of phytochemicals, which offer health benefits such as cancer prevention, cholesterol reduction, combating osteoporosis and menopause regulation, Although Many soy products have limited human use in the Western hemisphere due to undesirable off flavors. soybeans are high in protein. . Since soy doesn't contain galactose, a product of lactose breakdown, soy-based infant formulas can safely replace breast milk in children with galactosemia. Like lactose-free cow's milk, soymilk contains no lactose, which makes it a good alternative for lactose-intolerant. Today, researchers are interested in both the nutritional value and the potential health benefits of soy (Anderson *et al.*, 1995). Fermented soy milk products may provide economic and nutritional benefits, because they can be prepared at higher protein levels at comparable or lower cost than regular fermented milk products (Karleskind *et al.*, 1991).

1.2 Statement of the problem

Paneer is a milk product prepared by the combined action of acid coagulation and heat treatment of cow or buffalo milk (Rao and Patil, 1999). The high cost of *paneer* has prohibited its consumption by many sections of the society. Therefore, to reduce the cost of *paneer*, it is necessary to replace the milk by cheap and nutritious non-conventional ingredients (Sutar *et al.*, 2010).

Consumption of soy foods and utilization of soy ingredients have been the rise because of knowledge of recent health effects and advancement in soy processing technology. A report released in 1995 estimated that over 12,000 food products were available that contained soy protein (Anderson *et al.*, 1995), and sales of soy beverages rose more than 82% in 1999 (Nestle, 2002).

Using soybeans to make milk instead of raising cows may be ecologically advantageous, because the amount of soy that could be grown using the same amount of land would feed more people than if used to raise cows (Shurtleff and Aoyagi, 2000). The blending of soymilk with cow and buffalo milk will reduce the cost and enhance the nutritional quality of the product as soymilk is a rich source of vitamin C and iron which will supplement the cow and buffalo milk as it is poor in vitamin C and iron content. Therefore, formulation changes that enhance the overall flavor and textural characteristics of soy beverages may be necessary to further increase soy consumption (Chaudhary, 2014).

1.3 Objectives

1.3.1 General Objectives

The general objective of the dissertation work is to prepare *paneer* from soy milk blended with cow milk and to conduct its quality evaluation in terms of color, body, texture, flavor and overall acceptability.

1.3.2 Specific Objectives

- To study the effect of addition of soy milk incorporation in *paneer* making.
- To study the physico-chemical properties of plain and soy- cow milk *paneer*.

1.4 Significance of the study

Paneer is a nutritious heat acid coagulated indigenous milk product. However its high cost has restricted its popularity particularly among middle class and poor people. Milk fat is costly and is a major contributive factor for the increasing occurrence of coronary complications. Hence, there is a considerable interest to reduce the milk fat in *paneer*. This requires the manufacture of *paneer* like products utilizing low milk fat from non-conventional food solids (like soybean), which are not only cheap but can also be converted to a product closely similar to the nutritional and textural qualities of *paneer* (Mathare *et al.*, 2009) .

The most acceptable form of soy protein for dairy applications is isolates because of its fine particle size and dispersibility. Soy proteins are used to form fat emulsions as a method for incorporating fat into the formulation and to provide protein for nutrition. The functional properties of emulsion, emulsion stability, color and flavor are critical factors in dairy applications. New soy products having better flavor and functional properties will play an increasingly greater role in dairy-type industries (Singh *et al.*, 2008).

1.5 Limitations of the work

- Rheological parameters (hardness, cohesiveness, chewiness) of *paneer* could not be estimated due to lack of texturometer.
- Only one variety of soybean (white variety) could be studied for preparation of soymilk

Part II

Literature review

2.1 History and development of *paneer*

People during the *Kusana* and *Saka Satavahana* periods (AD 75-300) used to consume a solid mass, whose description seems to be the earliest reference to the present day *paneer*. The solid mass was obtained by the admixture of heated milk and curd. The nomads of South West Asia developed distinct heat/acid varieties of cheese. Cheese manufactured using high heat and precipitation without resorting to use of starter culture was practiced in many countries of South Asia and Central South and Latin America. First several distinctive cheese varieties were developed by Nomads of South West Asia. One of the unique Iranian nomadic cheese was called '*Paneer-khiki'*. It was originally developed by the well-known '*Bakhtiari*' tribe that resided in *Isfahan* in summer and *Shraz* in winter. The literal meaning of '*paneer*' is container and '*khiki*' is skin (Khan and Pal, 2011).

White *paneer* is a staple food of Nomads in Afghanistan. It is referred to as '*Paneer-e-khom'* and '*Paneer-e-pokhta'* when made from raw and boiled milk respectively. A product similar to this is also found in Mexico and Caribbean islands. *Paneer* is indigenous to South Asia and was first introduced in India by Afghan and Iranian travellers (Mathur, 1991).

A product similar to *paneer* is white unripened cheese made from milk coagulated by rennet or acid referred to as *Kareish* in Egypt, *Armavir* in Western Caucasus, *Zsirpi* in Himalayas, *Feta* in Balkans and *Queso Criollo, Queso del Pais, Queso Lianero* etc. in Latin America (Torres and Chandan, 1981).

2.2 *Paneer*

Paneer represents a South Asian variety of soft cheese obtained by acid and heat coagulation of milk. It is non-fermentative, non-rennet, non-melting and unripened type of cheese. The unique feature of *paneer* is that it not only includes casein but also most of the whey proteins which get recovered during its manufacture while they are mostly lost in whey in case of other types of cheeses (Khan *et al.*, 2011).

It must have a characteristic blend of the flavor of heated milk and acid, i.e. pleasant, mildly acidic and sweet (nutty). Its body and texture must be sufficiently firm to hold its shape during cutting/slicing, yet it must be tender enough not to resist crushing during mastication, i.e. the texture must be compact and smooth; Its color and appearance must be uniform, pleasing white, with a greenish tinge in the case of buffalo milk *paneer* and light yellow in the case of cow milk *paneer*. It is used in culinary dishes, snacks and an excellent substitute of meat (Kumar *et al.*, 2014).

2.3 Composition of *paneer*

Paneer is made without starter culture or rennet and results from the acid precipitation of milk at high temperatures. The phenomenon of coagulation involves the formation of large structural aggregates of proteins in which milk fat and other colloidal and soluble solids are entrained with whey. Good quality *paneer* is characterized by a typical mild acidic flavor with a slightly sweet taste. It is a rich source of milk protein and milk fat and is one of the best methods of conserving milk solids in highly concentrated form. *Paneer* contains on an average approximately 54% moisture 27% milk fat, 17.5% protein, 1.5% minerals and lactose (Chawla *et al.*, 1985).

The chemical composition of *paneer* depends mainly on the type of milk, composition of milk, the conditions of coagulation, the technique of straining/ pressing and the losses of milk solids in the whey. An average chemical composition of *paneer* is given in Table 2.1.

Table 2.1 Approximate composition (%) of *paneer*

Product	Moisture (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Buffalo milk *paneer*	52.3	27	15.8	2.3	1.9
Cow milk *paneer*	52.5	25	17.3	2.2	2.0

Source: Chawla *et al.* (1985)

2.4 Standards of *paneer*

Today, there are many choices in *paneer* to cater a wide variety of consumer tastes and standards of identity as well as specification are set so that consumers will get a consistent product, no matter what brand or type they buy. The Dairy Development Corporation (DDC), Nepal specification for *paneer* are shown in Table 2.2.

Table 2.2 DDC specification of *paneer*

Characteristics	Requirement (g)
Moisture (%)	47.5
Protein (%)	19.7
Fat (%)	26
Carbohydrate (%)	0.6
Minerals (%)	1.9

2.5 Defects in *paneer*

Low quality milk, faulty method of production, unhygienic condition, lack of refrigeration facility and proper storage conditions are mainly responsible for defects in *paneer* (Kumar *et al.*, 2014).

- Flavor defects: It arises from poor quality coagulating agent, improper heating and temperature. Flavor defects includes following:
    - Sour flavor is due to use of milk having high titratable acidity and addition of excess amount of coagulating agent.
    - Smoky flavor is due to use of smoky fire for heating of milk.
    - Rancid flavor is the result of hydrolysis of fat by lipase enzyme or oxidation during storage at room temperature.
    - Stale flavor is caused by storage of *paneer* at low temperature for longer duration.
- Body and texture defects: Body refers to firmness while texture refers to fine structure of *paneer*.
    - Hard body is caused by low fat: SNF ratio in milk and excessively high coagulation temperature.

Coarse texture is due to use of high acidic milk and inadequate fat content in milk. Too low pH of coagulation also affects texture of *paneer.*

- Color and appearance defects:
    - Dry surface in *paneer* is due to high percentage of fat in the milk used.
    - Surface hardening is caused when *paneer* is exposed to atmospheric air for longer duration.
    - Mouldy surface is due to storage of *paneer* in humid condition and excessive moisture content in *paneer*.
    - Foreign matters are seen due to improper straining of the milk and transport of *paneer* in unhygienic manner (Kumar *et al.*, 2014).

2.6 Shelf life of *paneer*

The major hurdle in the production of *paneer* commercially is its low shelf life. *Paneer* could be stored for only 6 days at 10ºC without much deterioration in its quality, though the freshness is lost after 3 days. It is noticed that growth of micro-organisms on the surface of *paneer* leads to its spoilage. Formation of a greenish yellow slime on the surface is accompanied with discoloration and off flavor. Therefore, efforts have been made to increase the shelf life of *paneer* by checking the surface growth of micro-organisms. Dipping of *paneer* in brine solution may increase the shelf life from 7 days to 20 days at 6-8ºC (Kanawjia and Khurana, 2006).

2.7 Packaging of *paneer*

Use of packaging materials significantly increased the shelf life of *paneer*. Packaging provides protection against different physiochemical and microbiological changes maintaining its quality, sales appeal, freshness and consumer convenience. Use of saran coated packaging films helped in enhancing the shelf life of *paneer* to a greater extent (Sachdeva and Singh, 1990). Packaging of chemical preservative treated *paneer* with or without vacuum extended its shelf life up to 35 and 50 days, respectively at 8ºC. Vacuum packaging of cow milk *paneer* is reported to have enhanced its shelf life from 1 week to 30 days at 6ºC (Sachdeva and Prokopek, 1992).

Paneer packaged in high barrier film (EVA/EVA/PVDC/EVA) under vacuum and heat treated at 90ºC for 1 min had a shelf life of 90 days under refrigeration. Heat sterilization led to considerable extension in shelf life of *paneer*. *Paneer* packed in tins along with water/brine and sterilized in autoclave at 1kg/cm^2 for 15 min could stay well for 4 months at room temperature (Kanawjia and Singh, 2000).

2.8 Factors affecting the quality of *paneer*

The manufacture of *paneer* involves standardization of milk, heat treatment, coagulation, draining, pressing, dipping in chilled water and packaging. Some of the parameters that affect the quality of *paneer* are:

2.8.1. Milk composition and standardization

In order to obtain the product with uniform composition and maximum yield, milk needs to be standardized. Standardizing buffalo milk to 5.8% fat and 9.5% SNF (Fat: SNF: 1:1.65) for *paneer* making was also recommended according to (Sachdeva and Singh, 1988). Good quality *paneer* was also made from buffalo milk with lower levels of fat (3.5%); *paneer* did not comply with the PFA standards (Chawla *et al.*, 1987).

Cow milk with lower solid level (3.7% fat, 8.4% SNF) enabled preparing *paneer* conforming to the PFA standards (Pruthi and Koul, 1989). (Vishweshwaraiah and Anantakrishnan, 1986) used cow milk standardizing to 4.5% fat level. Adjusting both fat and SNF levels in milk for *paneer* manufacture was suggested by (Mistry *et al.*, 1992).

2.8.2 Heat treatment of milk

The yield and total solids recovery increases with the increase in heating temperature while solids in whey decreases. This is due to complex formation between whey proteins and casein. At higher temperatures casein acts as a scavenger for serum proteins, which are otherwise lost in whey (Walstra and Jenness, 1983).

Temperatures beyond 90ºC, however, cause deposition of milk solids on the heating surface resulting in an overall solids loss. Milk heated at 90ºC without any holding, results in *paneer* with a total solids recovery of about 66%. The recovery does not increase appreciably on holding the milk at 90ºC and is, therefore, not required (Muller *et al.*, 1967).

2.8.3 Type and strength of coagulant

Strong solutions of citric acid result in *paneer* with acidic taste, hard body and higher losses in whey. Dilute solutions (0.5% citric acid) give slightly better solids recovery but the volume of the coagulant required increases too much making handling difficult. A solution of 1% citric acid concentration is optimum for effective coagulation to get good quality *paneer*. Certain non-conventional, low cost coagulants can also be used in the manufacture of *paneer* without any loss of its yield and quality. These include inorganic acids such as hydrochloric and phosphoric (0.6% solutions) alone and acidophilus whey (Pal *et al.*, 1999).

The use of citric acid in partially soured whey instead of water reduces the requirements of citric acid and increases the solids recovery without any loss of *paneer* quality. Whey cultured with Lactobacillus acidophilus at 2% and incubated overnight at 37ºC can be effectively used as a substitute for citric acid (Deshmukh *et al.*, 2009).

2.8.4 Temperature of coagulation

The moisture and yield of *paneer* decreases consistently with the increase in coagulation temperature. The recovery of total solids increases directly with the coagulation temperature while the solids loss in whey decreases. *Paneer* obtained by coagulating milk at 70ºC had the best organoleptic quality and had desired frying quality namely integrity/shape retention and softness (Chandan, 2007).

The optimum temperature of coagulation differs for different types of milk and their composition, including fat. A coagulation temperature of 70ºC has been recommended for *paneer* making from buffalo milk. Coagulation temperature of 85ºC for low-fat buffalo milk was recommended by (Chawla *et al.*, 1985).

To obtain good quality *paneer*, most workers recommended higher coagulation temperature for cow milk. The suggested coagulation temperature for obtaining good quality *paneer* from cow milk was 80–85ºC (Vishweshwaraiah and Anantakrishnan, 1985). Low coagulation temperature of 60ºC has been used for preparing reduced-fat *paneer* by Sanyal and Yadav (2000).

2.8.5 pH of coagulation

The pH of coagulation affects the yield, solids recovery and quality of *paneer*. According to (De, 1980) with the fall in pH (5.5-5.0), the moisture retention and yield of *paneer* decreased. The moisture content and yield of *paneer* increased from 50 to 58.6% and from 20.8 to 24.8% respectively, when coagulation pH increased from 5.1 to 5.4. Sensory quality was best at pH 5.3–5.35 which is recommended for *paneer* making from buffalo milk (Sachdeva and Singh, 1988). The pH range of 5.20–5.25 was recommended for cow milk *paneer* according to (Sachdeva *et al.*, 1991).

2.8.6 Hooping and pressing

The straining and pressing of coagulated mass affect the body and texture of *paneer*, moisture retention and solids recovery in *paneer*. The coagulated mass should be collected in fine cloth or hoop with fine cloth and gently pressed with appropriate application of weight/pressure. Different workers have used different pressure for varied time period for *paneer* manufacture. (Bhattacharya *et al.*, 1971) applied pressure of 40–45 kg for 10–15 min for paneer hoop sized 35x28x10 cm for buffalo milk *paneer* with moisture around 56%. (Kumari and Singh, 1992) used 0.08 kg/cm^2 for *paneer* preparation from cow and buffalo milk which resulted in *paneer* with 47.9 and 42.7% moisture respectively. Higher weights of 70–100 kg on hoops for 10–15 min was recommended by (Aneja *et al.*, 2002).

2.9 Quality characteristics of *paneer*

2.9.1 Microbiology of *paneer*

The microbiological quality of *paneer* depends upon the post manufacture conditions, particularly, handling, packaging and storage of the product. Spoilage of *paneer* during storage is mainly due to the growth of spoilage organisms on the surface. Increase in total plate, yeast and mold and coliform counts in stored *paneer* were studied by several workers. Sachdeva and Singh (1990) observed the microbiological characteristics of *paneer* stored at 8–10ºC and reported that total plate count related well with its spoilage. The fresh *paneer* samples showed that the initial count ranged from 2.3×10^4 to 9.0×10^4 cfu/g. The total plate count of the spoiled samples ranged from 1.58×10^6 to 4.5×10^7 cfu/g. The initial yeast and mold count of fresh samples varied over a narrow range of

3.5×10^2 to 5.2×10^2 cfu/g, while at the time of spoilage it ranged from 5.3×10^3 to 6.3×10^4 cfu/g.

Vishweshwaraiah and Anantakrishnan (1985) carried out microbiological analysis of 8–24 h old market samples and laboratory made *paneer*. The microbiological standards for *paneer* is as shown in Table 2.3.

Table 2.3 Microbiological standards of *paneer*

Parameters	Count/g	Grade
Standard Plate Count	< 5,000	Excellent
	5,000 - 50,000	Good
	50,000 – 2,00,000	Fair
	> 2,00,000	Poor
Coliform Count	< 10	Satisfactory
	>10	Unsatisfactory

Source: Vishweshwaraiah and Anantakrishnan (1985)

2.9.2 Sensory quality of *paneer*

Milk fat exerts significant effect on the organoleptic quality of *paneer*. The sensory score increased with increasing fat (4 to 6%) levels (Arora and Gupta,1980). Chawla *et al.* (1985) reported that acceptable quality *paneer* could be obtained from milk possessing 3.5–6.0% fat. Such high temperature of coagulation also held true for *paneer* obtained from recombined milk added with 0.15% $CaCl_2$. A coagulation temperature of 85ºC has been recommended for *paneer* making from reconstituted milk (15.0% TS) (Singh and Kanawjia, 1992).

Arya and Bhaik (1992), found that *paneer* made from cow milk (2.2% fat) resulted in a product lacking in softness and typical flavor. (Arora *et al*.1996) observed that use of

0.05% $CaCl_2$ in milk diluted with water to 4.6% fat and 8.0% SNF resulted in *paneer* comparable to that made from normal milk (5.5% fat and 9.0% SNF).

Citric acid yielded sensorily superior *paneer* compared to malic acid; the body and texture of *paneer* obtained using malic acid was quite poor (Pal *et al.*, 1999). Kaur *et al.* (2003) found that *paneer* dipped in 3% brine had a good sensory score. *Paneer* made from buffalo milk heated at 85ºC yielded sensorily superior product then when heated at 80 or 90ºC (Masud *et al.*, 2007) .

2.9.3 Textural properties of *paneer*

Syed *et al.* (1992) observed that the hardness of *paneer* was highest for skim milk *paneer* when compared to cow and buffalo milk *paneer*. Kumari and Singh (1992) found that cow milk *paneer* had higher values for cohesiveness, gumminess and chewiness than buffalo milk *paneer*, whereas the hardness and springiness were greater in buffalo milk *paneer*. However, the *paneer* or channa from buffalo milk have been found to produce harder and chewy texture due to higher concentration of casein in the micelle state with bigger size, harder milk fat due to larger proportion of high melting triglycerides in it and higher content of total and colloidal calcium.

2.10 Preservation

Paneer blocks obtained after pressing are immersed in water for cooling. It is during this period that microorganisms establish themselves in the product and proliferate on storage later on. The dipping water is a potent source of contamination and its quality is very important. To curb the surface growth of microorganisms and thereby increase the shelf-life of *paneer*, the following practices can be successfully adopted.

2.10.1 Chilling

Rapid chilling of *paneer* is necessary to arrest the growth of microorganisms. If *paneer* is transferred to a refrigerator or cold store, it takes quite some time to cool down to the desired temperature. Microorganisms get fully established by that time and cause spoilage of the product. The bacteriological quality of chilled water should also be very high. It is essential that pasteurized chilled water should be used for cooling of *paneer* blocks (Kumar *et al.*, 2014).

2.10.2 Brining

Paneer dipped in 5 percent brine solution lasts for nearly 20 days as against control that is spoiled after 6 days of storage at 8-10ºC. The sensory attributes are rated higher for salted samples. Since *paneer* is mostly salted and spiced before consumption, the salting at the time of dipping can be advantageously used in extending the shelf life of *paneer*. For preparation of brine salt should be dissolved in pasteurized water (Kumar *et al.*, 2014).

2.10.3 Use of chemical preservatives

A shelf life of 32 days under refrigeration can be achieved when *paneer* is treated with a combination of delvocid and hydrogen peroxide. Shelf life of 40 days using benzoic acid (1200 ppm) under refrigeration conditions and 20 days at 37ºC has been reported. Further, enhanced shelf life of 36 days at room temperature by adding sorbic acid to milk (0.15%) and subsequent wrapping of *paneer* in sorbic acid coated paper can be achieved (Kumar *et al.*, 2014).

2.10.4 Freezing

On storage of *paneer* at sub-zero temperature i.e. –13ºC and –32ºC for 120 days, the flavor and appearance is not affected but its body and texture deteriorates and the product becomes crumbly and fluffy on thawing. Blast freezing has recently been used to enhance the shelf life of *paneer*. The *paneer* block is cut into pieces of approx. 1.5 x 1.5 x 1.5 cm size and blast frozen at a temperature below –20ºC. The product can be stored under frozen conditions (below –18ºC) for more than one year without any deterioration in its quality (Kumar *et al.*, 2014).

2.10.5 Vacuum packaging

Vacuum packaging of *paneer* in laminated pouches can help to increase its shelf life to about 30 days at 6±1ºC. The body and texture of *paneer* also improves on vacuum packaging as it becomes more compact and shows better sliceability. *Paneer* packaged in high film (EVA/ EVA/ PVDC/ EVA) under vacuum and heat treated at 90ºC for one minute is reported to have a shelf life of 90 days under refrigeration (Kumar *et al.*, 2014).

2.10.6 Heat sterilization

Although the refrigerated shelf life improves markedly by the various treatments given to raw *paneer*, the shelf life at room temperature does not improve noticeably. Heat sterilization of *paneer* is an effective treatment for improving its shelf life at room temperature. *Paneer* packed in tins along with water/ brine and sterilized in an autoclave at 15 psi for 15 min lasts for 4 months. The perception of an oxidized flavor renders the product unacceptable afterwards. A slight amount of cooked flavor accompanied by Maillard browning, the intensity of which increases slightly during storage, is noticed (Kumar *et al.*, 2014).

2.10.7 Grass additives

In order to enhance the keeping quality of *paneer*, the efficacy of four grass additives viz. cardamom, clove, cinnamon and ginger were investigated. The additives were individually added to milk at the time of coagulation. Ginger was added at the of 5, 9 and 11 g per kg milk and cardamom, clove and cinnamon were added at the rates of 1.0, 1.5 and 2.0 g per kg of milk respectively. *Paneer* samples containing ginger, cardamom, clove and cinnamon each for low, medium and high dose showed shelf life of 32, 36 and 40 days: 23, 32 and 36 days: 24, 28 and 32 days: 23, 28 and 32 days. Clove and Cinnamon treated samples exhibited same shelf life as per their respective dosage. On the basis of sensory evaluation and physico-chemical changes during storage, medium dosage of four spices were found to be most effective and in totality, the treatment of *paneer* with medium dose of ginger followed by medium dose of cardamom and clove/ cinnamon respectively was most effective (Kumar *et al.*, 2014).

2.11 Soybean

2.11.1 Introduction

Soybean (*Glycine max*) is one of the most commercial crops in many countries. It is a diploidized tetraploid (2n=40), in the family Leguminosae, the subfamily Papilionoideae, the tribe Phaseoleae, the genus Glycine Willd and the subgenus Soja (Moench). It is an erect, bushy herbaceous annual that can reach a height of 1.5 m. Also known as the king of legumes, it is grown primarily for the production of seed, has a multitude of uses in the food and industrial sectors, and represents one of the major sources of edible vegetable oil and of proteins for livestock feed use (Anon, 1996).

The major world producers of soybeans are the USA, China, North and South Korea, Argentina and Brazil. In Nepal, soybean is commonly known by the name *'Bhatmas'*. The agricultural farms of Khumaltar, Kakani and Rampur collected 138 samples of soybeans from the different districts of height from 500-1800 m and conclusion was derived that most dominant varieties of soybeans in Nepal are of white, brown, grey and black colors. It has different local name depending on the varieties, color of seeds and locations like *Nepale, Hardi, Saathiya, Darmali, Maily, Kalo, Seto* and so on (Lama, 2009).

While 10% of the world's soybean crop is used directly for human food, a stunning array of products is made from the bean. Many of these soy foods may utilize the soybean while some are made with a variety of soy protein ingredients like isolated soy-proteins, soy protein concentrate soy flour or soy milk. Soy foods are typically divided to two categories: fermented and non-fermented. Traditional fermented foods include natto, miso, tempeh and fermented tofu. Traditional no-fermented soy foods include soynuts, okara and tofu (Shrestha, 2017).

2.11.2 History of soybean and soy foods

The origin of soybean cultivation is China. China was the world's largest soybean producer and exporter during the first half of the 21^{st} century. The annual wild soybean *(Glycine soja)*, the kindred ancestor of the current cultivated soybean *(Glycine max)*, is found throughout Northeast China**.** The cultivated area of soybean in China in 2007 was 8.90 ha, the total production was 13.80 million and the yield per unit area was 1550 kg $ha^{-1.}$ China has used soybean as a human food for centuries (Qui and Chang, 2010).

In Japan, the first prohibition of meat eating was promulgated by Emperor Tenmu. Soy foods gradually began to supply the savory flavor and protein that formerly had come from meat. Not until the 1860s did meat-eating resume in Japan, and not until after World War II did it become part of Japanese culture. Soybeans were first cultivated in South Africa and several North and South American countries in between 1903 to 1908. In 1905, Sugita brewery started making soy sauce in San Jose and five tofu shops were owned and operated by the Japanese in California (Shurtleff and Aoyagi, 2014).

Although soy foods have been consumed for more than 1000 years, only for the past 15 years have they made an inroad into Western cultures and diets. Soy protein is one of the plant based complete proteins. Westerners have adopted some of these foods wholeheartedly, whereas others will undoubtedly take more time to accept. Early soy foods companies were often family run organizations that sold their tofu or soy milk door-to-door to small segments of population (Golbitz, 1985).

Americans, known for their ability to adapt foreign foods to their own tastes, have developed a whole new class of "second generation" soy foods, which includes such products as tofu hot dogs, tofu ice cream, veggie burgers, tempeh burgers, soymilk yogurt, soymilk cheeses, soy flour pancake mix and a myriad of other prepared Americanized soyfoods. Largely because of the great entrepreneurial spirit of many small American companies, sales of soyfoods in the United States have been growing steadily since 1980 and are projected to increase every year (Golbitz, 1985).

2.11.3 Composition of soy foods

Also known as the 'miracle crop', soybean has been one of the world's most valuable and economic agricultural commodities due to its unique composition. Generally soybean seeds content 5,6-11.5% of water, ranges for crude protein is from 32 to 43, 6%, for fat from 15.5 to 24.7%, for crude ash from 4.5 to 6.4%, for neutral detergent fiber (NDF) from 10 to14,9%, acid detergent fiber (ADF) from 9 to 11,1%, carbohydrates content from 31.7 to 31.85% on a dry matter basis. The soybean contain very little of starch (4.66-7%) and quite a lot of hemicellulose and pectins (Ensminger *et al.*, 1990).

Soybean is characterized the highest digestibility of protein, lysine and methionine. Protein of soybean products characterized much quantity of lysine, tryptophane, isoleucine, valine and threonine. Soy is used to produce edible oil and fat as its lipid content is considerably high. Lipid fraction of the soybean seeds contain about 99%of triglycerides, in which content of polyunsaturated fatty acids (linoleic and linolenic) and unsaturated – oleic acid is high. Soy provides significant amount of linoleic (48-60%), linolenic (2-10%), palmitic (7-12%), oleic (19-34%) and stearic (2-5%) acids. Soy shows high content of Calcium, potassium, Magnesium, Sodium and phosphorus among minerals and Vitamin E, folic acid, pantothenic acid, thiamin, riboflavin and niacin are the most present vitamins (Boye and Ribereau, 2011).

The composition of some soy based foods in shown in Table 2.4. Soybean contains isoflavones. This compounds have got biochemical activity, including estrogenic, anti-estrogenic and hypocholesterolemic effects. Total isoflavones content ranges from 160.8 to 284.2 mg/100 g . The isoflavones in soybean and soy products have three types: daidzein, genistein and glycitein in three isomers and three forms. Totally, there are 12 isomers of isoflavones in soybean. The concentrations of total daidzein , genistein and glicitein carried out of 20.2-206 mg, 31.5-268 mg and 10.9-107 mg per 100 g raw seed The raffinose content of soybean seeds ranges from 0.1 to 0.9 g/100 g on fresh weight basis and stachyose is from 1.4 to 4.1 g/100 g (Hymowitz *et al.*, 1972).

Table 2.4 Composition of some soy foods

Soy Product	Moisture %	Protein %	Fat %	Carbohydrate%	Ash %
Fresh soybean	68	13	6	11	2
Soybean (dry)	7.5-10.1	31.1-36.6	16.3-21.3	6.29	4.69
Soy milk	88.7	3.2	1.84	5.76	0.48
Soy flour (defatted)	6-8	52-54	0.5-1.0	30-32	5-6

Source: Boye and Ribereau (2011)

2.11.4 Physiological benefits of soy

Soy foods are no longer just for vegetarians, they are for everyone. Soy foods have long been praised for their high protein content and rich in phytochemicals. They are part of a balanced diet and have important beneficial effects on human health.

Soy foods can lower LDL cholesterol. The protein in soyfoods has lipid lowering effects. Soy protein reduces LDL cholesterol without reducing the HDL (or "good") cholesterol. Soy foods also lower serum triglycerides, another fat that, at high levels, is correlated with coronary heart disease. Soy foods may inhibit clot formation and arterial-plaque formation. Genistein, an isoflavone present in soy foods, plays an important part in keeping our arteries free of build-up. Clots and plaques can cause heart attack and/or stroke. Genistein is beneficial in that it is an antioxidant, preventing the attack on LDL cholesterol by free radicals (or unstable oxygen molecules). When LDL cholesterol is oxidized in this way, it accumulates in blood vessels (Messina, 2006)

Many soyfoods contain fiber, particularly insoluble fiber. This type of fiber helps to reduce serum cholesterol by binding it and preventing its absorption from the intestinal tract. Soluble fiber also controls blood sugar levels in diabetics. Isoflavones, saponins, phytates, protease inhibitors and phytosterols, present in soybeans have anticancer properties. These chemicals control cell growth and protect cells from damage. Lab studies suggest that isoflavones in soy reduce the risk of colon, prostate and breast cancers. The National Cancer Institute and the University of California, Los Angeles are two institutions studying the effects of a high-soy diet on prostate cancer (Melkus, 2011).

Isoflavones are considered weak estrogens. Isoflavones represent a possible alternative to hormone replacement for postmenopausal women. It has been shown that Asian women experience fewer hot flashes than Western women do, which may be an effect of a high soy diet. Isoflavones also help to retain calcium in the bones preventing from osteoporosis (Maskarinec *et al.*, 2008).

Soybean oil is the primary commercial source of vitamin E. Consuming enough Vitamin E has been linked to reduced risks of cataracts, premature aging and arthritis. Beta- sitosterol and its derivatives, called sitostanol esters, have been shown to decrease serum cholesterol. While soybean oil contains around 50% omega-6 fatty acids, this oil is one of the most concentrated sources of heart-healthy, polyunsaturated fat (Melkus, 2011).

2.11.5 Dietary intake and recommendation

In 1999, in the process of awarding a health claim for soyfoods and coronary heart disease based on the cholesterol-lowering effects of soy protein, the U.S. Food and Drug Administration established 25 g of soy protein per day as the threshold intake required for cholesterol reduction. However, this threshold intake has limited value as a guide for incorporating soy into the diet for general nutritional and health purposes and for proposed benefits unrelated to cholesterol reduction. Furthermore, it address only one component of the soybean (protein), it does not provide guidance regarding isoflavones (Xiao, 2008).

In Japan, the daily mean intake of soy protein among those consuming a traditional diet is approximately 7 to 10 g, which represents about 10% of the total dietary protein intake. In Shanghai, men consume as much as 12 to 13 g of soy protein per day, which represents about 15% of total protein intake. Women consume about 9 g/day. Mean isoflavone intakes range from about 30 to 50 mg per day. Individuals in the upper one-quarter of intake in Shanghai and Japan consume about 15 to 20 g soy protein daily (Messina *et al.*, 2006).

Amount of soy intake is associated with benefits in epidemiologic and clinical studies, a reasonable intake goal for adults is 15 g soy protein and about 50 mg total isoflavones per day. These amounts are provided by approximately two servings of traditional soy foods. Higher amounts may be needed for some specific effects, such as the 25 g/day soy protein thought to be needed for cholesterol reduction. In contrast, it may be that some health benefits can be achieved in response to a lower amount of soy when intake occurs over a prolonged period of time. Certainly, two servings of soy foods per day can contribute to meeting nutrient needs and is consistent with sound dietetic practice (USSEC., 2013)

2.11.6 Effects of soy

There is increasing interest in soy foods for optimization of diets and estimation of total quality nutrients. Hence, it is important to explore the safety regarding soy. A study found out that men who consume an average of half a portion of soy products everyday are more likely to have lower concentration of sperm. High levels of phytic acid, which binds to important nutrients like calcium, magnesium, iron and zinc during digestion(Balk *et al.*, 2005).

Although in general, soymilk is not suitable for babies or infants, there exist baby formulas based on soy proteins, that are used primarily in the case of lactose intolerant children, those allergic to cow’s milk, or parental preference for a vegan diet. These formulas commonly contain extra carbohydrate, fats, vitamins and minerals. However, care must be taken that children with ‘Soy protein intolerance’ are not fed soymilk. Heinz Soya Infant Formula is approved by the Vegan Society in the UK (Liu, 1997).

The nutritive value of soybean is limited mainly by trypsin and chymotrypsin inhibitors. They interfere with the digestion of proteins resulting in decreased growth. The level of the lectins in soybean (37 to 323 HU /mg of protein) increases the mortality rate. The phytates decreases the activity of enzymes (pepsin, trypsin and amylase) as well as availability of protein, amino acids, starch and energy. Oligosaccharides are substances can cause of flatulent problems, decrease of digestibility of nutrients and hypertrophy of intestines. They can also influence on quantity of microorganisms in intestines. Mycotoxins shows estrogenic activity which can cause disturbance in reproduction (Banaszkiewicz, 2011).

The allergenic effect is attributed to the globulin fraction of soybean proteins. In the soybean seeds the globulins comprise about 85% (80-90%) of total protein.The most important allergens of soybean are GLY 1 and GLY1B - glicynine and beta- conglicynine. Soybeans contain several antigenic proteins which can stimulate the immune system sensitive of human. Therefore, it is safe to consume soyfoods in limited amounts and rely on it for proteins. Consumption at larger amounts only leads to potential hazards.

2.11.7 Functional properties of soybean

Functional properties have been defined as "those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption". The functional behavior of proteins in food is influenced by some physicochemical properties of the proteins such as their size, shape, amino acid composition and sequence, net charge, charge distribution, hydrophobicity, hydrophilicity, type of structures, molecular flexibility/rigidity in response to external environment such as pH, temperature, salt concentration or interaction with other food constituents. Functional properties are important in determining the quality (nutritional, sensory, physicochemical and organoleptic properties) of the final product (Jideani, 2011).

2.11.7.1 Water holding capacity

Water holding capacity is the ability to retain water against gravity, and includes bound water, hydrodynamic water, capillary water and physically entrapped water. The amount of water associated to proteins is closely related with its amino acids profile and increases with the number of charged residues, conformation, hydrophobicity, pH, temperature, ionic strength and protein concentration. Germination, fermentation, soaking or thermal treatments (toasting/autoclaving) significantly improves water absorption capacity of protein meals (Jideani, 2011).

2.11.7.2 Viscosity

Solubility, hydrodynamic properties, hydrophobicity and microstructure of proteins have been reported to play an important role in the rheological properties of proteins. Apparent viscosity of soybean isolates depends on interaction between soluble and insoluble proteins with water and between the hydrated particles. Due to the increased interactions of hydrated proteins, the water absorption and swelling, viscosity increases exponentially with protein concentration. Knowledge of the viscosity and flow properties of protein dispersions are of practical importance in product formulation, processing texture control and mouth feel properties and in clarifying protein-protein interactions and conditions affecting conformational and hydrodynamic properties (Jideani, 2011).

2.11.7.3 Gelation

Protein gels are three-dimensional matrices or networks of intertwined, partially associated polypeptides with entrapped water; and are characterized by a relatively high viscosity, plasticity and elasticity. The ability of protein to form gels and provide a structural matrix for holding water, flavors, sugars and food ingredients is useful in food applications, and in new product development and provides an added dimension to protein functionality. Gelling property is the basis of many oriental textured food e.g. tofu (Jideani, 2011).

Properties of the gel are determined by the interactions between solvent and the molecular net resulting in transparent or coagulant gels. Soy flour and concentrates form soft, fragile gels, whereas soy isolates form firm, hard, resilient gels. Protein gelation is concentration dependent; a minimum of 8% protein concentration is necessary for soy isolates to form a gel. The general procedure for producing soy protein gel involves heating the protein solution at 80 to 90ºC for 30 min followed by cooling at 4ºC (Jideani, 2011).

2.11.7.4 Protein solubility

Protein solubility is influenced by the hydrophilicity/hydrophobicity balance, which depends on the amino acid composition, particularly at the protein surface. The presence of a low number of hydrophobic residues; the elevated charge and the electrostatic repulsion and ionic hydration occurring at pH above and below the isoelectric pH favour higher solubility. Protein solubility is also influenced by production method and in particular by denaturation due to alterations in the hydrophobicity/hydrophilicity ratio of the surface. A highly soluble protein is required in order to obtain optimum functionality required in gelation, solubility, emulsifying activity, foaming and lipoxygenase activity. Soluble protein preparations are easier to incorporate in food systems, unlike those with low solubility indices which have limited functional properties and more limited food uses (Jideani, 2011).

2.11.7.5 Emulsion stability

Emulsions are two phase systems commonly found in food systems, whose formation is significantly affected by protein surface activity. Emulsions are generated by mixing two immiscible liquids e.g. oil and water. The liquids are immiscible because of their relative polarities. When liquid of low polarity such as fat is mixed with water a strong driving forces limits the contact between the two liquids resulting to phase separation. Droplet size of emulsion significantly affects the stability of emulsions; emulsions with precisely controlled droplet size exhibit better stability. Reduction in droplet size also improves stability of an emulsion to separation due to gravity (Jideani, 2011).

The goal in food processing is to stabilize the emulsion thereby giving it a reasonable lifetime. The dispersed system can be stabilized against coalescence and phase separation by adding a component that is partially soluble in both phases. Such components are phospholipids (emulsifiers) which when mixed with lipid in an aqueous environment; the fatty acid portion of the molecule is inserted into the oil phase, while the phosphate ester head group remains in contact with the aqueous phase. The result is that the two immiscible phases are not in contact with each other and the total energy of the system is lower. Emulsifiers or foaming agents therefore reduce the interfacial tension and help to stabilize the oil-water and air-water interfaces (Jideani, 2011).

2.11.7.6 Other functional properties

Soy protein increases nutritional value and may impart anti-oxidant effects in food. It improves uniform emulsion formation and stabilization, reduces cooking shrinkage by entrapping-binding fat and water. Soy improves moisture holding and mouth feel, enhances binding of meat particles without stickiness. Gelation by soy protein improves firmness, palatability and texture. Functional properties are physical and chemical properties that influence the behavior of proteins in food systems (Shrestha, 2017).

Table 2.5 Functional properties of soy protein products in foods

Functional Property	Mode of Action
Water adsorption and binding	Hydrogen bonding of water, water entrapment
Viscosity	Thickening, water binding
Gelation	Protein matrix formation and setting
Solubility	Protein solvation, pH dependent
Cohesion- Adhesion	Protein acts as an adhesive
Emulsification	Formation and stabilization of fat emulsion
Foaming	Film formation to trap gas
Flavor binding	Adsorption, entrapment, release

Source: CCUR (1987)

2.11.8 Relevance for food industries

Soy protein utilization as well as processing and adoption of soy foods in diet are continuing to accelerate so as to create sustainable solutions for protein demands of people. Soy ingredients can be used both directly for food purpose and can be processed further for food applications. These include roasted soy nuts, enzyme-active-full-fat soy flour and grits, toasted-full-fat soy flour and grits, enzyme-active defatted soy flake or flour with protein dispersibility indices (PDI) of 90, 70 and 20, lecithinated soy flour, textured soy flour, refatted soy flour, soy concentrates, soy isolates, soy germ, chemically isolated soy flavones, soy fiber from hulls and organic soy flour and concentrates (Towmbly and Manthey, 2006).

The largest commercial food usage of soy flour in the U.S. is in bakery products. Commercial sales to the bakery trade in 1972 were estimated by one source to be 65 million lb soy flour and grits and 9 million lb soy concentrate. It has been found that by raising absorption, decreasing mixing time, increasing oxidant (bromate) treatment, and reducing fermentation time, the baking performance of flours to which defatted soy flour has been added will be improved. Soy flour will provide, functionally, better water absorption, and, at least, a good tenderizing effect, body, and resilience. The introduction of soy fortified flour into bakery products requires very little change in bakery technology and no changes at all in bakery equipment. Good breads have been made using straight dough, sponge dough, short time dough, and continuous procedures (Hoover, 1975).

Soy proteins have been extensively used in producing meat alternatives that include structure meat analogs, minces products, spun protein isolates, fibrous protein products by process called texturization due to their unique meat-like textures after hydration (Strahm, 2006b). Soy ingredient can be used as a raw material in order to create opportunities to develop a vast range of value added products such as cheese, yoghurt, tofu, frozen desserts, soy milk, reconstituted soy milk, soy milk powder, flavored beverages, sauce, gravies, soups, shakes, smoothies and juice blends (Debruyne, 2006).

In recent years, interest has increased in high-protein versions of normally starchy snacks. Production of protein enhanced snacks and cereal is driven by dietary trends and health recommendations although number of challenges exist in the field (Strahm, 2006a). Soy isolates are incorporated in pasta and noodles to give high protein content and cooked product weight as soy has high affinity for water but not more than 14% soy can affect the gluten matrix, color and firmness of the product (Towmbly and Manthey, 2006).

Many functional properties of soybeans can be utilized into several soy based ingredients based on its applications and demands of people.

2.11.9 Use of soymilk

Soy milk is the non-fermented, aqueous extract of cooked whole soybeans. Full-fat soy flour and soy protein isolate can also be used as the starting point. Soybean selection, processing, and storage methods as well as additives such as sugar, oil, salt, malto-dextrin, vitamins, minerals, and flavor affect soy milk’s chemical, physical, and sensory characteristics. Color and texture, specifically grittiness, chalkiness, and viscosity, vary affecting consumer acceptability. Soy milk flavor is often described as beany, grainy, chalky, and dry (Keast and Lau, 2006).

Soy milk contains high amount of calcium and iron. Your body relies on the calcium from your diet to maintain dense and strong bone tissue. Without it, your body draws on your bones as a source of calcium, which reduces your bone density over time. A cup of unsweetened plain soy milk boasts a calcium content of 299 mg, which contributes 30% toward your recommended daily calcium intake. The iron in soy milk helps your red blood vessels function properly, helping ensure that all the tissues throughout your body get the oxygen they need. Each serving of soy milk provides 1.1 mg of iron -14 and 6% of the daily iron intakes recommended for men and women, respectively (Swanson *et al.*, 2012).

Soy milk also helps to consume B-complex vitamins, and serves as an especially rich source of riboflavin, or vitamin B-2, and vitamin B-12. Getting enough vitamin B-12 in diet helps your cells produce DNA, aids in red blood cell function and also keeps your nerves healthy. A serving of soy milk provides 3 mg of vitamin B-12, more than the 2.4 µg needed each day. The riboflavin in soy milk helps your cells produce energy, and it also shields your DNA from damage. Drinking a cup of soy milk boosts your riboflavin by 0.51 mg -39% of the recommended daily intake for men and 46% for women. Being free of cholesterol, gluten and lactose, soymilk is suitable for lactose intolerant consumers, vegetarians and milk allergy patients (Liu and Lin, 2000).

Part III

Materials and methods

3.1 Raw materials

The materials collected for the formulation of soy *paneer* were as follows:

3.1.1 Milk

Fresh cow milk (fat = 3.8% and SNF = 8.7%) was collected from local area of Dharan.

3.1.2 Soybean

White variety of soybean *(Glycine max)* was collected from the local market of Dharan.

3.1.3 Soymilk

The white variety of *Glycine max* was soaked, dehulled, steamed and then subjected to grinding, boiling and filtering. The process outline for preparation of soymilk from soybeans is shown in Fig. 3.1.

3.1.4 Equipment and chemicals

The following equipment and chemicals used were provided by the CCT lab. The list of chemicals for the analysis is shown and the list of equipments is shown in Table 3.1

- Citric acid
- Catalyst Mixture (Mixture of 2.5 g of powdered SeO_2, 100 g K_2SO_4 and 20 g $CuSO_4.5H_2O$)
- Mixed Indicator Solution (Mixture of 10 ml of 0.1% bromocresol green and 2 ml of 0.1% methyl red solution which is prepared separately in 95% ethanol)
- Sodium bicarbonate ($NaHCO_3$)
- Sodium hydroxide (NaOH)
- Conc. sulphuric acid (H_2SO_4)
- Oxalic acid
- Amyl alcohol
- Gerber sulphuric acid

- Neutral boric acid
- Phenolpthalein
- Conc. nitric acid (HNO_3)
- Conc. Hydrochloric acid (HCl)
- Petroleum benzene

Table 3.1 List of equipments used

Physical apparatus	**Physical apparatus**
Heating arrangement	Grinding apparatus
Electric balance	Stainless steel vessels
Thermometer	Dessicator
Centrifuge	Kjeldahl digestion and distillation set
Muslin cloth	Refrigerator
Titration apparatus	Daily routine glassware
Soxhlet apparatus	Stirrer
Hot air oven	Muffle Furnace
Gerber Butyrometer	Pressing arrangement

3.2 Methods

3.2.1 Extraction of soymilk from soybean

The whole soybeans were soaked in water for 10 h. The puffed soybeans were dehulled by rubbing and then autoclaved at 121ºC for 15 min. The beans were washed with hot water at 70ºC and then with cold water. This was repeated for 2-3 times. It was then grinded with hot water 80ºC, brought to boil and left for 15 min. Finally extraction of soymilk was done by filtering through muslin cloth. The residue was grinded and filtered again to obtain soymilk.

One kg soybean gave about 3 kg soymilk using bean to water ratio 1:2. The obtained soymilk was used in calculated amount for each *paneer* recipe. The method of extraction soymilk from soybeans is shown in Fig. 3.1.

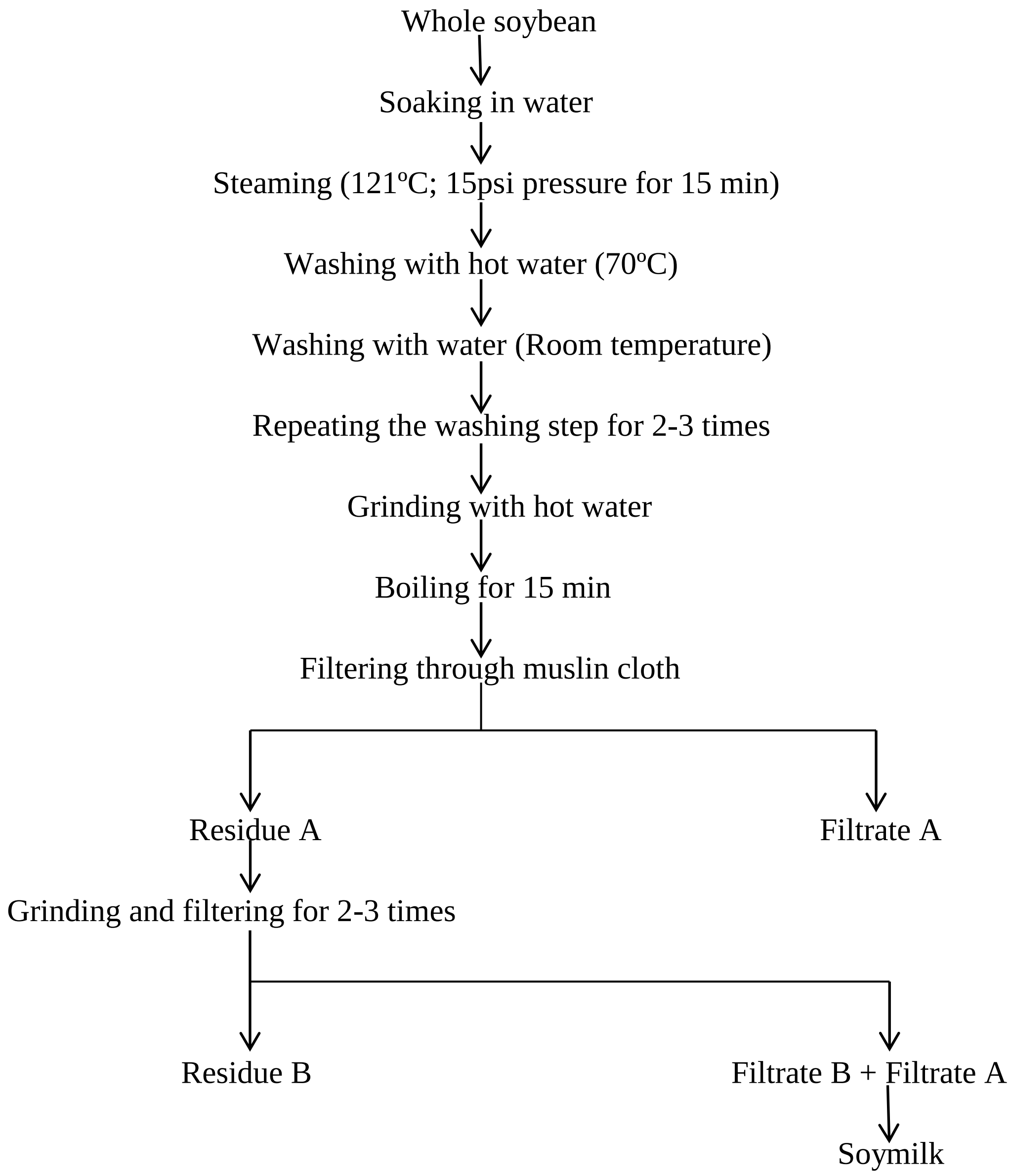

Fig. 3.1 Method for extraction of soymilk from soybean

Source**:** Gartade *et al.* (2009)

Note: Thus obtained soymilk was analyzed for fat and SNF content and was added in required proportion.

3.2.2 Experimental plan

Six samples were prepared coded as A, B, C, D, E and F as shown in Table 3.2. Each samples had different formulations based on results shown by Design Expert ® 10.

Table 3.2 Experimental plan

Samples	Cow milk	Soy milk
A	50	50
B	60	40
C	70	30
D	80	20
E	90	10
F	100	0

3.2.3 Methods of soy *paneer* preparation

The method of soy *paneer* preparation is shown in Fig. 3.2

Milk in different proportions

↓

Heating (80-85ºC) for 5 min

↓

Cooling (70^{o}C)

↓

Addition of coagulant (2% citric acid solution at 70ºC)

↓

Continuous stirring till clear whey separates out

↓

Settling for 10 min

↓

Draining of whey

↓

Pressing the coagulum after filling in muslin cloth lined hoops

↓

Removal of blocks and cutting into desired sizes

↓

Immersion of *paneer* blocks in chilled water (4ºC)

↓

Draining of water and wiping surface

↓

Storage at 4ºC

Fig 3.2 Flow diagram for preparation of soy *paneer*

Source: Chaudhary (2014)

3.3 Details of preparation

3.3.1 Heat treatment

Cow milk having 4% fat content was taken. The milk with different formulations (cow milk: soy milk= 100:0, 80:20, 70:30, 60:40, 50:50) was heated at 80-85ºC for 5 min and cooled to 70ºC.

3.3.2 Coagulation

It was coagulated with citric acid (2% solution), which was added slowly to the milk with continuous stirring.

3.3.3 Whey drainage

After coagulation of milk, coagulum (curd) is formed and clear whey is separated out. The mixture was allowed to settle down for 10 min and the whey was drained out through a muslin cloth.

3.3.4 Hooping and pressing

The curd was then collected and filled in a hoop (35×28×10 cm) lined with a clean and strong muslin cloth. The hoop had a rectangular frame with the top as well as bottom open. The frame was then rested on a wooden plank and filled with the curd before covering with another plank on the top of the hoop by placing a weight of 45 kg for about 15–20 min.

3.3.5 Dipping in chilled water

The pressed block of curd is removed from the hoop and cut into 6– 8” pieces and immersed in chilled water (4–6ºC) for 2–3 h. The chilled pieces of *paneer* are then removed and placed on a wooden plank for 10–15 min to drain occluded water. Afterwards, these pieces were wrapped in parchment paper, and stored at refrigeration temperature (4±1ºC). A schematic approach for the manufacture of soy *paneer* is depicted in Fig. 3.2.

3.4 Physico- chemical analysis of raw material, final product

The cow milk and soymilk were analyzed for fat content, acidity, protein, and total solids. In addition to this SNF was also determined for cow milk. The final soy *paneer* was analyzed for its moisture content, fat content, protein, total solids, acidity and ash content.

3.5 Microbiological analysis of final product

Total plate count, yeast and mold count, Staphylococcus and coliform count of *paneer* samples were determined as per the standard methods given in (APHA, 1992). Samples were inoculated in duplicate plates of suitable media and incubated at the recommended temperature (Table 3.2). At the end of incubation period, the plates were counted for number of colonies.

Table 3.3 Media and incubation condition for microbial examination

Determination	Medium	Incubation
Total plate count	Plate count agar	37ºC for 24-48 h
Yeast and mold count	Potato dextrose agar	22ºC for 72 h
Coliform count	Violet red bile agar	37ºC for 24-48 h

3.6 Sensory analysis

A panel consisting of 10 members was selected for sensory evaluation. Blended soy *paneer* samples were presented to panelists drawn from the faculty members and students of CCT, Hattisar. The panelists were asked to judge the samples for color, taste, flavor and overall acceptability using a 9-point hedonic scale rating (Ranganna, 2000) as per the performa (Appendix A).

3.7 Statistical analysis

The data obtained were analyzed statistically by using analysis of variance technique (ANOVA) to find if the differences were significant or not at 5% level of significance.

Part IV

Results and discussion

The experimental findings of utilizing soymilk for developing highly nutritious *paneer* by blending it with cow milk are presented and discussed in this part. Blends of soymilk with cow milk were heated and coagulated to prepare *paneer*. The results showing the effect of blending on chemical and sensory characteristics of cow milk and its *paneer* are presented.

4.1 Chemical composition of raw material

Proximate analysis provides inexpensive yet very informative, particularly from the nutritional and biochemical points of views. The results normally expressed in percentage and because of the fairly general nature of test employed for the determination, the term crude is usually used as a modifier; for instant, crude protein, crude fat and crude fiber etc. Therefore proximate constituent represent only a category of compounds present in biological material.

The proximate composition of raw soy milk and cow milk are given in Table 4.1.

Table 4.1 Proximate composition of soy milk and cow milk

Attribute	Soy milk	Cow milk
Moisture (%)	89.6±0.15	87.1±1.89
Crude fat (%)	2.28±0.15	3.8±0.15
Crude protein (%)	4.03±0.16	3.3±0.2
Ash (%)	0.58±0.16	0.7±0.3
Carbohydrate (%)	3.51±0.15	5.1±1.42

*Values are the means of three determinations ± standard deviations. Figures in the parentheses are the standard deviations.

The result presented in Table 4.1 revealed that the moisture, fat, protein, ash and carbohydrate content in soy milk were 89.6%, 2.28%, 4.03%, 0.58% and 3.51% respectively. It was observed that values obtained in the present investigation are similar to those reported by Ahmad *et al.* (2008) and Rehman *et al.* (2007).

The moisture, fat, protein, ash and carbohydrate content in cow milk were 87.1%, 3.8%, 3.30, 0.7 and 5.1%, respectively. The values are similar to those reported by Posati and Orr (1976) and Han *et al.* (2012) and any variation may be due to source or processing errors.

4.2 Sensory analysis of soy *paneer*

Sensory analysis of soy *paneer* was performed with the aid of ten semi- trained panelists evaluating color, flavor, body, texture and overall acceptance of prepared soy *paneer*. From the statistical analysis ($p < 0.05$), products were found significantly different in terms of all sensory parameters.

4.2.1 Effect of formulation on color

The mean sensory scores for color of samples A, B, C, D, E and F were found to be 6.3±0.67, 6.3±0.48, 7.2±0.63, 5.9±0.73, 6.4±0.84 and 6.9±1.28 respectively as shown in Fig. 4.1. The mean score was found to be highest for sample C which was nearly equal to control A. Samples C and D, D and F, D and E were found to be significantly different in color but samples A, B and E had the same mean score which indicates that average and complete soymilk gave the same color effect which indicates that panelists preferred the white milk color over the yellow tinge of soy *paneer*.

Babaje *et al.* (1992) found similar results where the scores decreased with increased soy content which has been attributed to the dark yellowish brown color in *paneer*. The inclusion of soymilk up to 30% did not decrease the color significantly ($p \leq 0.05$) and thereafter the increased levels of soymilk *paneer* lowered the color of blend significantly ($p \leq 0.05$). Adding soymilk would increase amine compounds which react with aldehydes through Maillard reaction to form dark pigments thus making color darker.

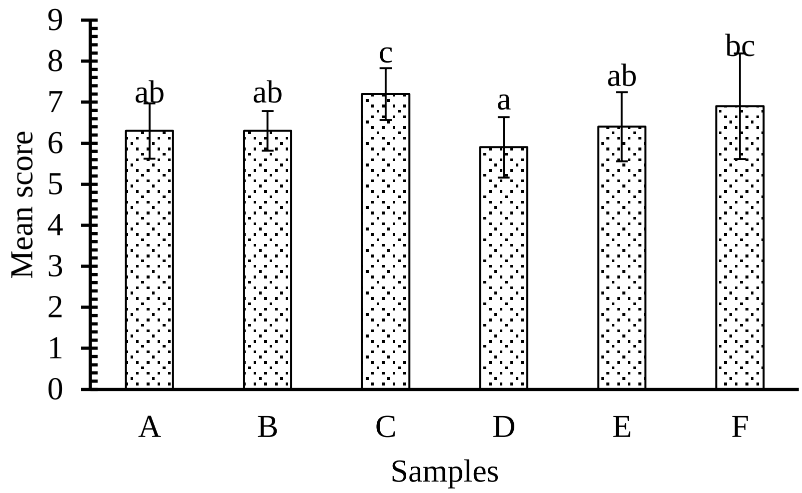

Fig. 4.1 Mean sensory score for color of soy *paneer*

Fig. 4.1 represents the mean sensory scores for color of soy *paneer*. Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.2.2 Effect of formulation on body

The mean sensory scores ± standard deviation for body of samples A, B, C, D, E and F were found to be 6.5±0.84, 7.3±0.67, 6.8±1.13, 6.2±1.31, 6.1±0.99 and 6.5±0.97 respectively. The mean score was found to be highest for sample B as shown in Fig. 4.2. Sample C had mean sensory score slightly greater than control F. Samples A and E, B and D were significantly different in body while other samples were not. The high score of sample B due to high proportion of cow milk and less soy milk and lowest for E because of higher proportion soy milk. It was reported that the body of soy *paneer* was hardened with increasing soy concentration.

Babaje *et al.* (1992) and Chowdhury *et al.* (2011) found out that 60:40 substitution of soymilk in milk created the highest consistency in *paneer*. This showed that slightly greater amount of soymilk resulted in tighter and better body in *paneer*.

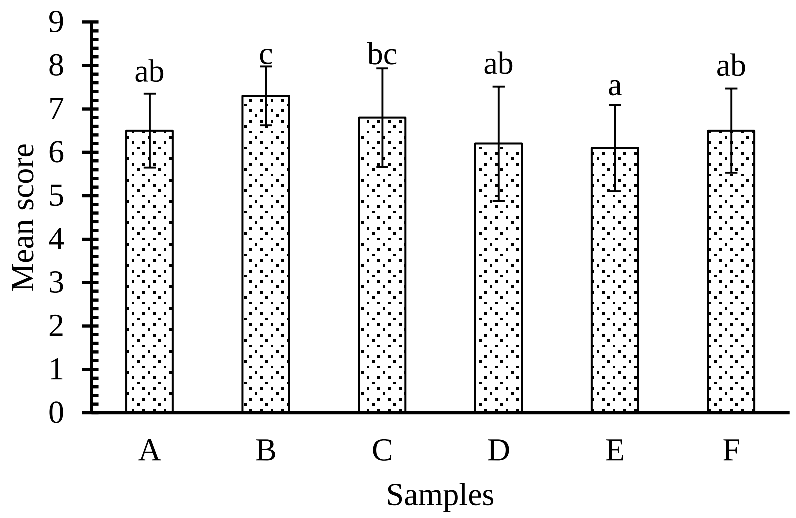

Fig. 4.2 Mean sensory score for body of soy *paneer*

Fig. 4.2 represents the mean sensory scores for body of soy *paneer*. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.2.3 Effect of formulation on texture

The mean sensory scores along with standard deviations for texture of samples A, B, C, D, E and F were found to be 6.3±0.94, 6.6±0.96, 7±0.67, 6±1.49, 5.8±1.03, and 6.8±1.31 respectively which is shown in Fig. 4.3.

The mean sensory score for texture was found to be highest for sample C. Sample C and control F obtained similar sensory scores. Samples A and B, D and E had no significant difference between them at 5% level of significance. It was indicated that too high soy concentration was not preferred by the panelists for texture.

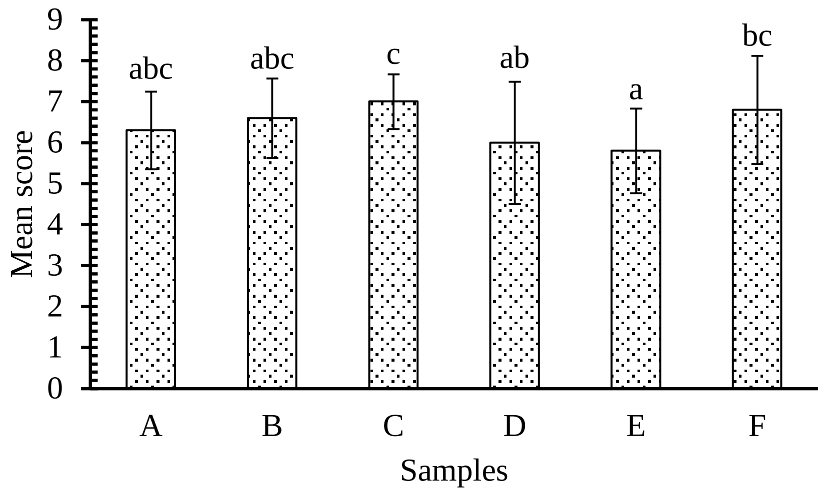

Fig. 4.3. Mean sensory score for texture of soy *paneer*

Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

The scores seemed to decrease with increasing soy milk except for 30 percent of soy concentration. This can be attributed to the fact that the beany texture was offensive to most of the people as dominated the overall texture of *paneer* (Jain and Mhatre, 2009). Sample C along with control F hence received highest sensory score.

4.2.4 Effect of formulation on flavor

The mean sensory score ± standard deviation of flavor of six samples A, B, C, D, E and F were found to be 5.8±1.13, 6.2±0.91, 7.1±0.56, 6±0.67, 6±0.94 and 6.6±1.26 respectively. The mean score was found to be highest for sample C which was significantly different from samples A, B, D, E but not from sample control F as shown in Fig. 4.4.

It was found that the incorporation of soymilk at 30% had flavor difference significantly ($p \leq 0.05$) than others. Further increase in the proportion of soymilk lowered the mean sensory score for flavor. According to (Chaudhary, 2014), the variation in flavor between the blends with 10 and 20% soymilk and 20 and 25% were non- significant. The blend consisting 75 and 100% proportion of soymilk were in acceptable range.

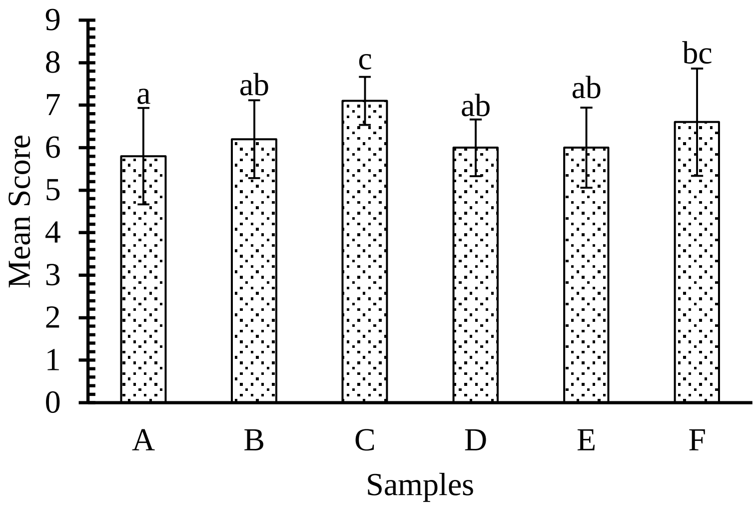

Fig. 4.4 Mean sensory score for flavor of soy *paneer*

Fig. 4.4 represents the mean sensory scores for flavor of soy *paneer*. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

4.2.5 Effect of formulation on overall acceptability

The mean sensory scores for overall acceptability of samples A, B, C, D, E and F were found to be 5.9±0.99, 6.6±0.84, 7.1±1.19, 6.3±1.05, 5.9±0.87 and 6.8±1.31 respectively which is shown in Fig. 4.5.

The mean sensory score was found to be highest for sample C followed by control F. Samples B and C, E and F were found to be significantly different from each other in terms of overall acceptability of soy *paneer*.

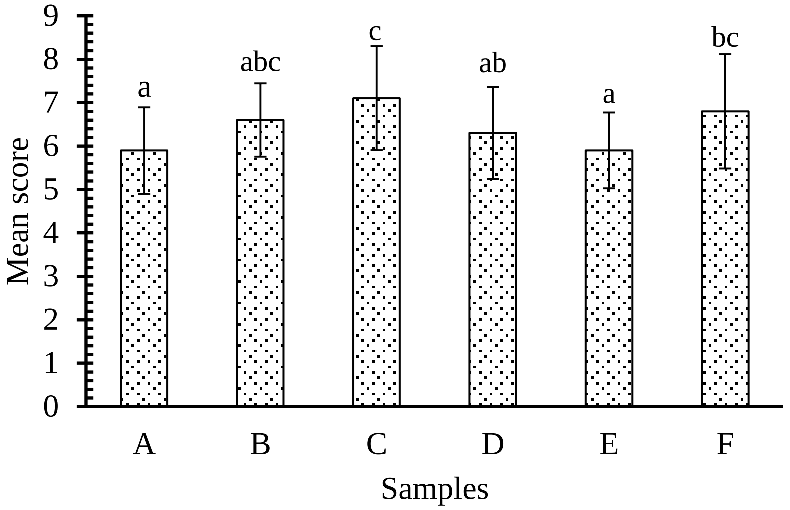

Fig. 4.5 Mean sensory scores for overall acceptance of soy *paneer*

Values on top of the bars bearing different superscript are significantly different from each other at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists. Samples A, B, C, D, E and F represent sample formulations as given in Table 4.2

Mean score of sample C was slightly greater than control F which indicates that sample C represents the highest or same overall acceptance as control F. Sample A had least score indicating that equal proportion of blending of soymilk and cow milk were not preferred by the panelists. Also samples B, D and E were significantly different from control and sample C.

Babaje *et al.* (1992) also observed lower scores for samples with high soy content in terms of acceptability of *paneer* and higher scores for samples with average soy content. The preference was in decreasing order with increasing proportion of soy milk.

Therefore, sample C was found to be the best in most of the parameters and overall acceptability as well. The formulation with 70% cow milk and 30% soymilk was chosen to be the best product by sensory evaluation and obtained data interpretation. This conclusion was derived based on sensory analysis of limited number of panelists and so the experimental results should be taken with some reservations as it may differ when subjected to other populations.

4.3 Chemical analysis of soy *paneer* and control

Sensory optimized *paneer* sample C and control sample was subjected to chemical analysis and the data obtained are as shown in Table 4.3. Protein content of sample C (23.83%) was found to be increased compared to control (19.93%) due to soy milk incorporation. Fat content and moisture was also found to be slightly increased. The soy *paneer* was found to be slightly acidic than control sample due to soybean content. The results were similar to results found by Chaudhary (2014).

Table 4.3 Proximate analysis of the best soy *paneer* sample 'C' and control

Parameter	Sample C	Control
Moisture content (%)	56.68±1.57	55.97±1.43
Total solids (%)	47.94±1.57	48.65±1.03
Fat (%)	19.04±0.06	18.98±0.05
Protein (%)	23.83±0.52	19.93±0.34
Ash content (%)	2.23±0.05	1.45±0.03
pH	5.350	6.52
Acidity (%)	0.507±0.01	0.41±0.005

4.4 Microbiological quality of soy *paneer*

Microbiological quality of sensory optimized *paneer* sample C were enumerated with respect to total plate count (TPC), yeast and mold count, and coliform count during storage at 5±1℃.

4.4.1 Total plate count (TPC)

The microbiological quality was determined by assessing its TPC which is presented in Table 4.3. Total plate count was found out to be 3.5×10^2. Lamdande *et al.* (2012) also noted similar changes in TPC count of *paneer* spread during storage for 0 day.

4.4.2 Yeast and mold count

Table 4.3 shows the yeast and mold count of *paneer* during storage at refrigeration temperature. Lamdande *et al.* (2012) noted similar changes in yeast and mold of *paneer* spread during storage for 0 day at 5±1ºC.

4.4.3 Coliform count

The changes in coliform count of *paneer* are presented in Table 4.2. Coliform counts reported in soy *paneer* was as according to Lamdande *et al.* (2012) Babaje *et al.* (1992).

Table 4.2 Microbiological analysis of soy *paneer*

Parameter	*Values
Total plate count TPC (cfu/g)	3.5×10^2
Yeast and mold count (cfu/g)	2.5×10^2
Coliform count (cfu/g)	N.D.

*Values are average of three determinations

*N.D= Not detected

4.5 Cost evaluation

The total cost associated with the best product was calculated and the cost of soy *paneer* per 30 g was NRs. 18.9, excluding labor cost, packaging cost and tax. The cost of market *paneer* per 30 g was NRs. 27 which was much higher than the cost of soy based *paneer*. Mass production further reduces this cost. From the cost calculation given in Appendix B, it can be seen that due to the low cost of soybeans to prepare soymilk, the cost of *paneer* has been decreased. If the byproduct can be utilized from the grinded soybean then the cost can be reduced even more which is suitable for all groups of families in society.

Part V

Conclusions and recommendations

5.1 Conclusions

The present work was carried out to study the acceptability of soy *paneer* and to observe the effect of blending of soy milk on cow milk on preparation of soy *paneer*. From the research, following conclusions were made

- Soy *paneer* with 70% cow milk and 30% soymilk was found best.
- Soy milk had significant effect on color, flavor, body, texture of the *paneer*. It had the highest effect on flavor of *paneer*.
- Production cost of the prepared soy *paneer* was reasonable i.e. NRs. 18.9 per 30 g within the reach of general population and much lower than dairy *paneer* so its commercialization could be done.

5.2 Recommendations

Based on the present study, the following recommendations have been made

- Shelf life of *paneer* samples can be studied using different preservation techniques.
- Effect of different of soybean varieties on the preparation of *paneer* by blending soy milk with cow milk can be studied.
- The shelf life of *paneer* prepared by blending soy milk with cow milk can be studied at different storage condition using different packaging materials.

Part VI

Summary

Paneer is a fresh cheese common in South Asia, especially in Indian, Pakistani, Afghan, Nepali, Sri Lanka, and Bangladeshi cuisines. It is an unaged, acid-set, non-melting farmer cheese made by curdling heated milk with lemon juice, vinegar, or any other food acids. *Paneer* is a desired dish and soymilk being lactose free as well as nutritious, is an ideal substitute for lactose intolerant and vegans. So the present work is conducted to study the consumer acceptance of soy *paneer*, its chemical and microbiological quality.

For the study, soybean and cow milk were purchased from local market of Dharan. Soymilk was prepared by soaking, autoclaving, and grinding the soybeans with water in 1:2 ratios. Design Expert ® 10 for two variables (soymilk and cow milk) at three levels was designed for experimental combinations. Using soymilk and cow milk, the mix was prepared as calculated in the formulation, heated, coagulated, pressed, whey separated, cut in desired sizes and dipped in chilled water at 4ºC.

The prepared soy *paneer* was analyzed chemically, microbiologically and by sensory analysis. From sensory analysis, the sample with 70% cow milk and 30% soymilk was found to be the best. It was found that soymilk and cow milk had significant effect on color, flavor, body, texture and overall acceptability of soy *paneer*. The chemical composition of the best soy *paneer* was analyzed. Moisture content, total solid, fat, protein, acidity, ash content, pH of best sample C were found out to be 56.68%, 47.94%, 19.04%, 23.83%, 0.507%, 2.23% and 5.350 respectively. Microbiological analysis for the final best *paneer* sample was done. Total plate count and yeast & mold count was found out to be 3.5×10^2 cfu/g and 2.5×10^2 cfu/g respectively at 0 day storage. Similarly, coliform count was not detected (N.D.) at 0 day storage.

It was concluded from the present study that soy *paneer* was nutritionally equivalent to dairy or plain *paneer*. It was found to be slightly yellowish in color and had a mild but not offensive soy flavor. Soy *paneer* was found moderately harder in texture than plain *paneer*.

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Appendices

Appendix A

Sensory evaluation card

Name of panelist: Date:

Name of the product:

Type of product:

You are provided different samples of soy *paneer* on each proportion of different varieties. Please conduct the sensory analysis based on the following parameter using the scale given. Panelists are requested to give ranks on their individual choice.

Sample	Color	Flavor	Shape	Texture	Overall
1					
2					
3					
4					
5					
6					

Comments:

Signature:

Appendix B

ANOVA for sensory analysis of soy *paneer*

Table E.1.1 Two way ANOVA (No blocking) for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	11.0000	2.2000	4.30	0.003
Panelist	9	13.0000	1.4444	2.83	0.010
Residual	45	23.0000	0.5111		
Total	59	47.0000			

Table E.1.2 Two way ANOVA (No blocking) for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	11.6833	2.3367	4.41	0.002
Panelist	9	24.6833	2.7426	5.18	<.001
Residual	45	23.8167	0.5293		
Total	59	60.1833			

Table E.1.3 Two way ANOVA (No blocking) for body

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	9.5333	1.9067	3.16	0.016
Panelist	9	28.0667	3.1185	5.17	<.001
Residual	45	27.1333	0.6030		
Total	59	64.7333			

Table E.1.4 Two way ANOVA (No blocking) for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	10.8833	2.1767	2.68	0.034
Panelist	9	29.0833	3.2315	3.97	<.001
Residual	45	36.6167	0.8137		
Total	59	76.5833			

Table E.1.5 Two way ANOVA (No blocking) for overall acceptance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	11.9333	2.3867	3.69	0.007
Panelist	9	31.7333	3.5259	5.46	<.001
Residual	45	29.0667	0.6459		
Total	59	72.7333			

Appendix C

Summary of the ANOVA of sensory evaluation of soy *paneer*

Sample code	Color	Flavor	Body	Texture	Overall
A	$6.3^{ab}\pm0.67$	$5.8^{a}\pm1.13$	$6.5^{ab}\pm0.84$	$6.3^{abc}\pm0.94$	$5.9^{a}\pm0.99$
B	$6.3^{ab}\pm0.48$	$6.2^{ab}\pm0.91$	$7.3^{c}\pm0.67$	$6.6^{abc}\pm0.96$	$6.6^{abc}\pm0.84$
C	$7.2^{c}\pm0.63$	$7.1^{c}\pm0.56$	$6.8^{bc}\pm1.13$	$7^{c}\pm0.67$	$7.1^{c}\pm1.19$
D	$5.9^{a}\pm0.73$	$6^{ab}\pm0.67$	$6.2^{ab}\pm1.31$	$6^{ab}\pm1.49$	$6.3^{ab}\pm1.05$
E	$6.4^{ab}\pm0.84$	$6^{ab}\pm0.94$	$6.1^{a}\pm0.99$	$5.8^{a}\pm1.03$	$5.9^{a}\pm0.87$
F	$6.9^{bc}\pm1.28$	$6.6^{bc}\pm1.26$	$6.5^{ab}\pm0.97$	$6.8^{bc}\pm1.31$	$6.8^{bc}\pm1.31$
LSD (5%)	0.6440	0.6553	0.699	0.813	0.724

Appendix D

Cost evaluation of the product

Ingredients	Quantity	Rate NRs	Quantity used	Rate NRs
Cow milk	1000g	70	70g	4.9
Soybean	1000g	100	30g	3
Citric acid	100g	550	2g	11
Total costing				18.9 per 30 g

Photo gallery

Plate 1 Pressing *paneer* in pressing arrangement

Plate 2 Different formulations of soy *paneer* samples

Plate 3 Sensory analysis of soy *paneer*

Plate 4 Microbial analysis of best soy *paneer*