EFFECT OF INCORPORATION OF SESAME SEED IN THE QUALITY OF TOFU

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

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Approval Letter

This *dissertation* entitled ***Effect of Incorporation of Sesame Seed in the Quality of Tofu*** presented by **Bhawana Poudel** has been accepted as the partial fulfilment of the requirement for the **B. Tech. degree in Food Technology.**

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Abstract

The study was carried out in order to prepare sesame seed incorporated tofu and evaluate its sensory and physiochemical properties. Raw material (soybean and sesame seed) was collected from the local market of Dharan. Response Surface Methods was used for the formulation of recipe for this, DOE (Design Expert) 13.0.1.0 software was used. Sesame seed incorporated tofu was prepared in laboratory in different proportion of sesame seed namely A, B, C, D and E with 0%, 7.5%, 15%, 22.5% and 30 % sesame seed. The sensory analysis of sesame seed incorporated tofu of different concentration was carried out for consumer acceptability. The obtained data were statistically analysed using two-way ANOVA (no blocking) at 5% level of significance.

From sensory analysis sample E i.e. the tofu with the incorporation of 30% of sesame seed was best formulation and subjected for further physiochemical analysis. The moisture content, crude protein, fat, fibre, total ash and total carbohydrate of sesame seed incorporated tofu were found to be 58.86%, 44.12%, 41.21%, 0.677%, 4.37% and 9.3% (db) respectively whereas the same parameters for soybean tofu were found to be 64.596%, 61.67%, 29.793%, 1.489% 3.167% and 3.92% respectively. These findings suggest that sesame seed can be successfully incorporated in soybean up to the 30% without any adverse effect on sensory attributes. The iron content and calcium content were increased after addition of sesame seed from 9.28 to 19.67mg and 187.296 to 273.373mg respectively. The calcium, iron, fat, carbohydrate and ash content were found to be higher in sesame seed incorporated tofu in comparison to normal soybean tofu. Methionine content was also increased from 1.175 to 2.47 g/16g N in sesame incorporated tofu.

Table of Contents

List of Tables

List of Figures

List of Plates

List of Abbreviations

Abbreviation	**Full form**
ANOVA	Analysis of variance
TPC	Total plate count
SD	Standard deviation
HDPE	High Density Polyethylene
Db	Dry Basis
FAO	Food and Agricultural Organization
GDL	gluco-delta-lacton
tga	transglutaminase
GCB	Grinding Cum Blanching
HHPP	High Hydrostatic Pressure Processing
MAP	Modified atmospheric packaging
BBSM	Bhat-Bhateni Super Market
CCT	Central Campus of Technology

Part I

Introduction

1.1 General Introduction

Tofu has been produced in China for over 2,000 years (since the Chinese Han period). Tofu is a form of bean curd prepared from soybeans that can be found throughout the country (Zhang *et al.*, 2017). During the Song Dynasty, it became a popular dish in China (960 to 1279) (Needham and Yates, 1976; Wilkinson, 2018). During the Edo period (1603-1868), tofu manufacture was popular among Japanese residents, and it was eventually exported to the rest of Southeast Asia (Shrtleff and Aoyagi, 2010).

Soybeans, among other things, are regarded as a complete protein source for vegetarians and those who desire to minimize their meat consumption (Kulkarni, 2004). Traditional soy foods prepared from whole soybeans include soymilk and tofu. Soy foods are strong in protein and vitamins, but low in calories, carbohydrates, fats, and omega-3 fatty acids. They are also cholesterol-free, readily digested, and incredibly adaptable in the kitchen, making them tempting new food mainstays for a wide range of healthy diets. Soybeans are high in phytoestrogen-like isoflavones and genistein that have been implicated in the prevention of certain cancers. Isoflavones are closely related to antioxidant flavonoids (Anderson and Wolf, 1995).

Sesame seed is an underappreciated legume that is high in oil (48-58%), protein (16.96%), and carbohydrate (26.04%). The amount of dietary fibre is likewise considerable (16.9%). Sesame seeds are high in iron, magnesium, manganese, copper, and calcium, as well as thiamine and tocopherol (Bedigian, 2003). Lignans, which include sesamin, are found in sesame seeds and have antioxidant and anticancer characteristics. Sesame seeds also contain phytosterols, which have been shown to lower blood cholesterol levels (Obiajunwa *et al.*, 2005).

Tofu is a highly nutritious, protein-rich, cheese-like delicacy derived from soybean milk that is popular in Asian cuisines. When compared to animal protein, it is a cheap form of protein that is readily available and inexpensive to the average person. Several workers have reported the creation of tofu from soybeans (Lim *et al.*, 1990; Moizuddin *et al.*, 2006; Kong *et al.*, 2008b). It is a global challenge to supply a source of protein other than meat for the world's population. Sesame seed is low in lysine but high in sulphur amino acids like methionine and cystine, making it a good supplement for diets that include soybean, which is low in sulphur amino acids (Elfaki *et al.*, 1991). This work is aimed at enhancing the utilization and diversification of underutilized legumes. Therefore, the study was planned to prepare tofu from a blend of soybean and sesame seeds and study its proximate composition, antinutrient constituent and acceptability of the product.

1.2 Statement of the problem

Soybeans are good sources of protein, lipid, and other nutrients. Soybeans contain all the essential amino acids except sulphur-containing amino acid (methionine), which must be supplied in the diet because they cannot be synthesized by the human body (Lokuruka, 2010). Sesame seed is deficient in lysine but rich in sulphur-containing amino acids such as methionine and cystine. Soybean sesame seed combination makes it an appropriate supplement to diets that is deficient in methionine and lysine (Elfaki *et al.*, 1991).

Many people have shown a strong desire to reduce the amount of animal products in their diet for ethical, environmental, and health reasons. Consumption of tofu is motivated by its high nutritional value and inclusion in vegetarian and hypocaloric diets. In this regard, there is need to develop an alternative way to fulfill the nutrition requirement of people in low cost.

There have been many experiments on tofu using a different kind of substitution for improvement of nutrient content like spiced tofu, smoked tofu, seasoned tofu etc. However, sesame seed was not used in any experiment. Sesame has been used for the production of baked goods (such as bagels, bread, breadsticks, hamburger buns and rolls), bread crumbs, cereals (such as granola and muesli), chips crackers, tofu etc. in different parts of country but industrial scale production and utilization of sesame has not been practiced yet in Nepal.

Thus, incorporation of sesame seed for tofu production can be milestone to overcome the nutritional shortcomes mentioned above.

1.3 Objectives

1.3.1 General objective

The general objective of the dissertation work is to study the effect of the incorporation of sesame seed on the quality of tofu.

1.3.2 Specific objectives

1. To analyse the raw materials i.e., soybean and sesame seed.
2. To prepare the tofu using soybean and sesame seed in different proportions using DOE 13.0.1.0 experiment
3. To evaluate the sensory properties of prepared tofu.
4. To analyse the physiochemical properties of tofu.
5. To improve the level of acceptance (shelf life).
6. To perform cost evaluation of prepared tofu.

1.4 Significance of the study

Due to the high protein content, low in cost and availability of soybean, the soya product can be the solution. Among the many products of the soybean, tofu is well known one. Sesame seed is easily produced, and combining it with soybean in the preparation of tofu will help to reduce the country's excessive reliance on imported soybean while also conserving foreign exchange.

Anti-nutrients present in the seed hulls of raw sesame and soybean seeds, such as phytate and oxalate, can affect mineral bioavailability in humans. It improves iron, magnesium, manganese, copper and calcium availability. However, previous research have shown that processing methods such as soaking, cooking, fermentation, and germination can drastically reduce the anti-nutrients (Brandon *et al.*, 1991; Ene-Obong and Obizoba, 1996). Cooking or dry heating also eliminates the trypsin inhibitor found in soybeans. Heat treatment improves the nutritional quality of soybeans by increasing protein digestibility. Incorporation of sesame seed also helps to improve amino acid availability, particularly methionine that is lacking in soybean and lysine that is lacking in sesame seed. Furthermore, it increases vitamin E. The antioxidative agents (sesamin, sesamolin, sesamol, their glycosylated forms sesaminol glucosides and tocopherol make the oil very stable and therefore it has a long shelf life (Suja *et al.*, 2004).

Therefore, incorporation of sesame seed with soybean to make tofu provides a good opportunity to improve nutritional quality of vitamin, mineral and polyunsaturated fatty acid consumed by many people.

1.5 Limitations of the study

1. Textural analysis of the product was not carried out due to the lack of an instrument.

Part II

Literature review

2.1 Historical background

Due to a lack of documents, most of the early history of soybeans is unknown; however, historians have put together a colourful history of the soybean. Evidence suggests that the soybean was domesticated in the eastern half of northern China during the Zhou period. Bronze inscriptions and early texts from the 11th century are the oldest records. Domestication is a trial-and-error process that does not have a set timeline; thus, it most likely took place throughout the Shang dynasty (ca 1500-1100 B.C.). Soybeans were probably distributed throughout China by trade missions by the first century A.D., and eventually to other Asian countries. The Kojiki (Records of Ancient Matters), which was completed in 712 A.D., has the oldest Japanese reference to the soybean. Diaries of European visitors to China and Japan from the 16th and 17th centuries contain various allusions to native soy cuisine. They said that Asians were highly inventive in converting soybeans into a variety of stable foods, including tofu, soy milk, miso, and soy sauce. These foods were unknown to the early traders and explorers. Soybeans were originally recorded in China around the same time as Egyptian pyramids were being built. Emperor Shang Nung issued the books pen tsaokong mu and pen tsaokong mu, which explain the flora of China, including the soybean. The Buddhist religion, which forbade people from eating meat, had a significant impact on the growth of soybean as a food crop in China, Korea, Japan, and other Asian countries. Currently, the United States is the major producer and exporter of soybeans (Liu, 1999).

Soybeans are a significant source of high-quality protein. High-protein soybean cultivars enable the manufacture of foods with higher nutritional value and yield, such as soy milk and tofu. (Liu, 1999). Low polyunsaturated linoleic and linolenic fatty acid concentrations and a higher oleic acid content improve oil stability and avoid oxidation and off-flavor generation during food preparation (Fehr, 2007).

Isoflavones are estrogen like, antifungal, antioxidant, and anticancer chemicals found in soybeans. Isoflavones in soybean grains come in a variety of forms, including glucosides, acetylglucosides, malonylglucosides, and aglycones. The biological activity of the latter form, which is made up of daidzein and genistein, is higher. As a result, soybean cultivars with high isoflavone aglycone concentration can improve the functional qualities of soy meals. (Aldin *et al.*, 2006).

Tofu, like many other foods, originated in China. Tofu is credited to Liu An, King of Huai-nan, who reigned from 179 to 122 B.C., according to popular Chinese legend (first documented in 1587 by Li Shih-chen in the Pen-ts'aokang-mu). Shinoda (1971) and others conducted extensive searches of early Chinese literature and found that the oldest known written reference to tofu was in 950 A.D (Shurtleff and Aoyagi, 1983). The first known mention to tofu in Japan is from 1185 A.D. Tofu is now widely manufactured throughout East Asia, with over 29,000 factories in Japan alone (Shurtleff and Aoyagi, 1979). Tofu is also becoming more popular in Western countries. In January 1984, the United States had 191 commercial tofu producers, Western Europe had 75, and Canada had 33. (Shurtleff and Aoyagi, 1984).

2.2 Tofu

Tofu is a typical Asian soybean product that resembles a protein gel (Saio, 1979). Tofu is a multipurpose soybean product created by curdling soymilk to coagulate the proteins and pressing them into a sliceable cake. Tofu is made in a variety of ways, with different flavor, texture, and applications depending on where it is made. Tofu is a natural, low-cost, and nutrient-dense food that is consumed by people in both industrialized and underdeveloped countries. In a vegetarian's diet, it is a good source of non-animal protein. Tofu comes in a variety of textures, ranging from soft to firm to extra-firm (Pal *et al.*, 2019). Tofu is low in saturated fat, cholesterol-free, and high in protein. Soybean varieties, quality, and processing conditions all influence tofu yield and quality (Cai and Chang, 1998).

2.3 Gelation mechanism of tofu

Several scientists have presented various gel formation theories in succession. The following are some prevalent hypotheses of soy gel production; nevertheless, the currently accepted ideas have several flaws. A common soy gel formation theory was given by Kohyama *et al.* (1995). They suggested that the tofu gelation mechanism consists of two steps: protein denaturation and hydrophobic coagulation. The first step is to heat the protein molecules in their original state, which contain hydrophobic areas, in order to denature them. Negatively charged ions are produced when soybean protein is denatured. In the second step, protons' genes are activated by glucono-delta-lactone (GDL), and calcium ions aid to neutralize the protein's net charge. This leads to a greater hydrophobic contact between the molecules of neutral proteins, which causes protein aggregation and the development of soy curd. Figure 2.1 depicts the Kohyama gel formation method in tofu. In conclusion, the coagulant neutralizes the net charge on the denatured proteins, and the hydrophobic interactions cause the denatured soy proteins to aggregate. As a result, a tofu gel matrix is formed. (DeMan *et al.*, 1986). However, the process of denaturation is still ongoing and will continue throughout storage, resulting in tofu syneresis. Mu-rekatete *et al.* (2014) investigated the effect of coagulants on tofu gelation behaviour. The salt-induced gels had a high storage modulus (G') and a shorter gelation period, according to the researchers.

7S (â-conglycinin) and 11S (soybean protein) are the two primary portions (glycinin). 7S is a soluble protein, whereas 11S is a particulate protein(Peng *et al.*, 2016). The rate of gelation of 7S globulins is found to be significantly slower than that of 11S proteins. (Kohyama and Nishinari, 1993). 11S globulin fractions are the most important protein in determining the hardness of soy gel (tofu) (Saio and Watanabe, 1978).

Kohyama *et al.* (1992) investigated the rheology of soybean 11S protein gel formation in the presence of a coagulant. The gelation curve was studied in terms of temperature and rate order kinetics. They discovered that the rate of gelation is affected by the coagulant glucano-ä-lactone and increases with gelling temperature. They also discovered that, unlike other protein-based gels, the soy gel's latent time is unaffected by protein concentration. However, in a separate investigation on 11S and 7S proteins conducted by Kohyama and Nishinari (1993), the gelation duration was observed to be reduced as the number of 7S globulins increased in the presence of GDL. As a result, even though 7S proteins take longer to gel, the rate of gelation by 7S globulins can be accelerated in the presence of the right coagulants. Furthermore, when compared to 11S fractions, 7S proteins can coagulate at lower concentrations. As a result, it is clear that 7S proteins are primarily responsible for gelation rate, but the hardness of soy protein is regulated by the 11S protein fraction.

Furthermore, Nakamura *et al.* (1985) investigated the 11S fraction of soy protein in minor fractions such as A1, A2, A3, A4, and A5. The hardness of the gel was found to be related to the percentage of A3 subunit present in the soy cultivar, implying that the hardness of the soy gel is proportional to the amount of A3 subunit present in the soy cultivar. In addition, Nishinari *et al.* (1991) looked into the effect of the A5 subunit of soy globulins on tofu gel properties. They concluded that soy gels made with A5 negative soybean cultivars produced harder tofu gels than positive cultivars.

However, Kohyama *et al.* (1992)'s model of soy gelation has a critical flaw: it ignores the lipids present in the soybean during the gelation process. Lipids are poorly distributed in the gel interspaces when compared to proteins. The lipid is integrated in the gel matrix as the coagulant is introduced (Yamano *et al.*, 1981).

Clark *et al.* (2001) presented a different perspective on gel formation that differed from the particulate gel theory. They theorized that intermolecular electrostatic repulsion has a big role in encouraging the creation of a gel that isn't as particulate as a particulate gel. The repulsion is thought to be caused by surface charges and is influenced by the particle's composition, pH, and coagulant concentration. This hypothesis suggested that particulate matter such as phytic acid, polyacid ions, proteins, and lipids must be studied in combination.

Concomitantly, Shun-Tang *et al.* (1999) suggested a new model for soy gel formation that incorporates soy protein, lipids, and tiny charged molecules, primarily phytates and polyacid ions. In addition to Kohyama's hypothesis, it posits that after the 11S protein has coagulated in the presence of Ca^{2+} ions, smaller molecules inhibit the protein-lipid interface from coagulating as the coagulant is added. Particulate 11S rich proteins micelles are now wrapped around the lipid globules, followed by soluble 7S rich particles. During this process, lipids agglomerate and become coated with oleosins, forming particular oil bodies (structural proteins). This micelle appears sandwiched, many such sandwiched protein-lipid interface forms soy-milk gel matrix. Figure 2.2. shows the formation of soy gel according to Shun-Tang's theory. This model is further replicated to understand yogurt and cheese matrix. This micelle looks to be sandwiched, and a soy-milk gel matrix is formed by several such sandwiched protein-lipid interfaces.

According to Shun- Tang's idea, Figure 2.2 shows the formation of soy gel. This model is then replicated in order to better understand the yogurt and cheese matrix (Ringgenberg *et al.*, 2013; Grygorczyk *et al.*, 2014; Peng and Guo, 2015). However, because the soy gel matrix is anticipated to have a particulate gel structure and the gelation occurs when the protein-lipid complex coagulates due to intermolecular crosslinking, further development in the model is thought to be necessary. This complex appears as clumps of particle-type proteins (Nik *et al.*, 2011).

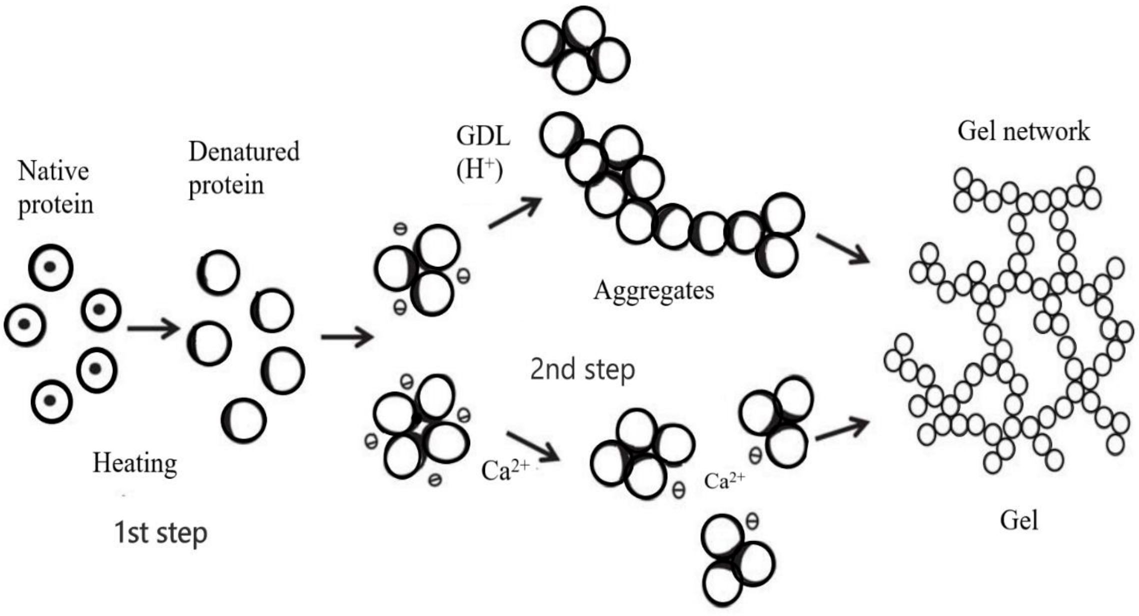

Fig. 2.1: Kohyama's mechanism of gel formation in tofu

Source: Kohyama *et al.* (1995)

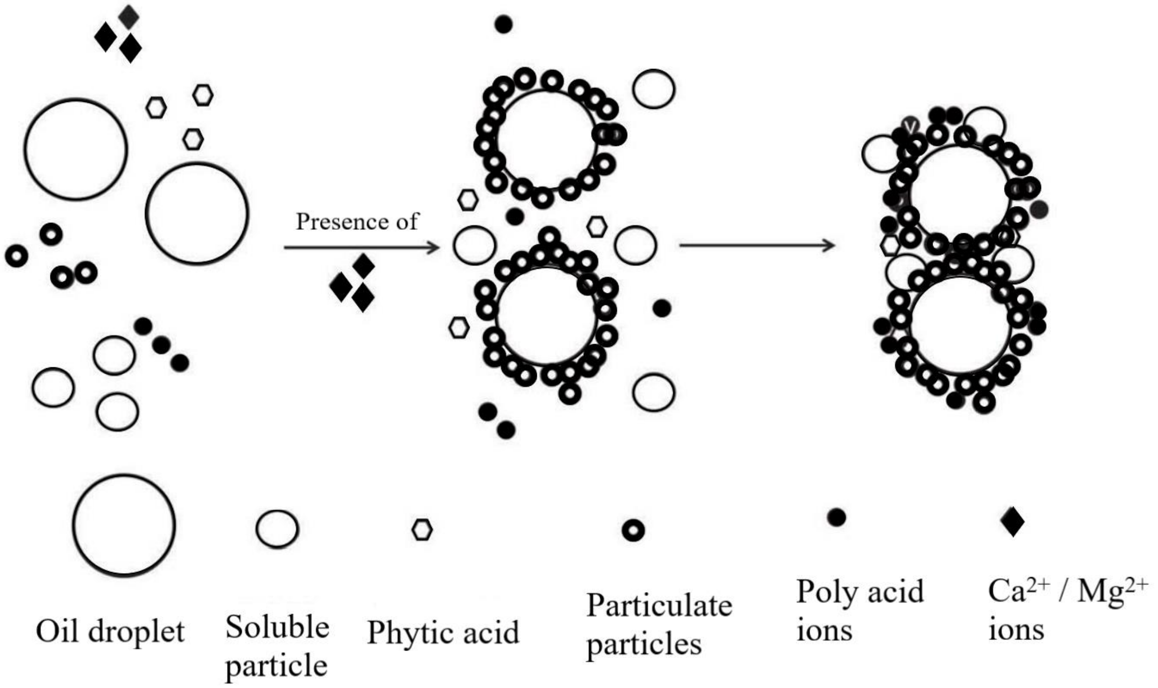

Fig. 2.2: Formation of soy gel according to Shun-Tang's theory

Source: Shun-Tang *et al.* (1999)

2.4 Types of Tofu

In last few decades efforts have been made to introduce numerous varieties of tofu such as silken tofu, firm tofu, dried tofu, fermented tofu and low-fat tofu. A brief description is given below.

2.4.1 Firm Tofu

Firm tofu, often known as "Cotton tofu," is made by coagulating soymilk with a synthetic or natural coagulant and pressing it through cheese cloth. To eliminate extra water, it is partially coagulated and pressed, and the moisture content of the tofu ranges from 76 to 81 % (DeMan *et al.*, 1986). It is firm because excess of moisture is squeezed using heavy weights.

Coagulation is accomplished using natural coagulants such as Phyllanthus acidus (gooseberry), Tamarindus indica (tamarind), Citrus limon (lemon), Garcinia indica (garcinia), and Passiflora edulis (Passion fruit), as well as synthetic coagulants such as $CaSO_4$ (Calcium sulphate) and $MgCl_2$ (Magnesium chloride). Soymilk is made by soaking and cleaning soybeans at room temperature for 12 h. To avoid charring, soybean slurry is made by mixing ground soybean with water (1:8) and heating it at 85°C for 45 min while stirring constantly. Soymilk is made from the heated slurry that has been clarified using cheese cloth. Soymilk is heated to 95°C for 5 min, then swirled for 5 min and allowed to coagulate for 15 min with 0.2% synthetic and/or 2% natural coagulant. The coagulated mass is then pressed for 20 min in a tofu mold lined with double cheese cloth at 1 kg pressure for the first 20 min and 0.5 kg pressure for the remaining 20 min. Finally, tofu is kept at a temperature of 4˚C (Rekha and Vijayalakshmi, 2010).

2.4.2 Silken Tofu

The bean curd used to make soy milk is coarse and uneven in texture, but silky (Kinugoshi) tofu is fine and smooth in texture, as the name suggests (Beddows and Wong, 1987). Silken tofu is a type of tofu that is made by hardening all of the soymilk, giving it a soft and delicate texture. Soft/ silky tofu has an approximate moisture content of 87-90 % (H. L. Wang *et al.*, 1983).

Yang and James (2013) used the soaking method to make silky tofu, which involved soaking 55 g of soybeans in 200 g of deionized water at 15°C for 16- 17 h. For blending and getting soy slurry, drained soybeans were rinsed and 400 g of deionized water was added.

The slurry was cooked at 98°C for 15 min. In the dry method used by Evan *et al.* (1997) raw soybean of 60 g was ground and blended with 400 g of boiled water. Extraction of soymilk from both the method was by manual centrifugal juice extractor. Concentration of coagulant used ranged from 1.5 to 5.0 g per kg. Then 220 ml of soymilk was poured in a plastic container and cooled to room temperature, 7 ml of coagulant was added and stirred vigorously and thoroughly. The container was sealed and indirectly heated at 85°C for 35 min to complete the curd formation, lids used for sealing was provided with holes to escape the gases and silken tofu was stored at 4°C.

2.4.3 Dried Tofu

Dried tofu, which has a moisture level of less than 76 %, is the firmest of all tofu types. Because the soybean curd is vigorously broken to remove surplus water before pressing, dry tofu has a low moisture level (Cai and Chang, 1997).

Raw soybeans are steeped in 6-folds of water at 21-22°C for 8 h to make dried tofu. Excess water is drained after soaking, and hydrated soybeans are crushed in a high-speed grinder, then clarified by centrifugation to produce soymilk. Soymilk was heated for 20 min with 1 g of antifoaming agent and then rested for 3 min at 96°C to make dried tofu. For the formation of curd, Nigari (3%) ($MgCl_2$) is added and left to sit for 10 min. The curd is agitated for 30 s before being coagulated again. Soy curd is then put to a wooden mold lined with cloth (25 cm x 25 cm x 7 cm) and pressured for 30 min at 34.8 g per cm^2 (Cai *et al.*, 1997).

2.4.4 Sufu (Fermented Tofu)

Sufu is also known as tofu that has been fermented. *Actinomucor elegans*, *Mucor racemosus*, *Mucor sufu*, *Mucor dispersus*, *Mucor racemosus*, *Mucor wutuongkiao*, and *Aspergillus* sp. ferment the tofu. The quality of the finished product is determined by the fermentation strains utilized. Chou and Hwan (1994) developed sufu using *Aspergillus taiwanensis* (CCRC 31159) and *Aspergillus selegans* (CCRC 31342).

Tofu was sliced into pieces and weighed 25 g with 70% moisture content, immersed at 95°C for 6 min to avoid putrefactive organisms, and dried for 2 h in a laminar air flow hood. Spraying 0-2 ml of the culture on the surface of each piece was used to inoculate the test microorganisms. The pieces were kept at 97 % relative humidity and incubated for 48 h at

30°C. Finally, the sufu was aged for 75 days at 25°C while being combined with brine solution. Han *et al.* (2001) made red sufu by coagulating soymilk with salt to make tofu. The diced tofu was then sprayed with pure *Actinomucor elegans* as 3.227 culture and cultured for 48 h at 28°C with a relative humidity of 90%. Continuous salting for 5 days resulted in a 16% rise in salt concentration, which was then preserved for 2 months in an enclosed bottle with a mixture of kojic red rice (Japanese red yeast rice), sugar, chiang (wheat-based miso), an alcoholic beverage, and spices.

2.4.5 Low Fat Tofu

Consumers in the present day are looking for low-fat, healthy meals. Tofu, as a good source of protein, has a good potential of being a healthy food substitute because it reduces fat consumption. Soy flour is a by-product made from soy oil cake, which has a low fat level and a high protein content with strong biological value. (Kellor, 1974). According to Shin *et al.* (2014), supercritical CO_2 extracted soy flour has the ability to produce low-fat tofu with the same texture as full-fat tofu. The fat content of the flour is lowered from 19.5 to 7.1%, resulting in a drop in soymilk viscosity from 50 to 40 cp. However, the overall yield of low-fat tofu (69.7%) is higher than full fat tofu (60.8 %). Tofu's textural and sensory qualities are unaffected by the fall in fat content. Low fat tofu, on the other hand, received a lower grade than full fat tofu because of its roasted appearance and nutty flavour.

2.5 Factor influencing the quality of Tofu

2.5.1 Varieties of soybean seeds

Bhardwaj *et al.* (2004) evaluated a variety of soybean genotypes from various places to see how they affected the tofu's oil and fatty acid levels. BARC-8, BARC-9, MD86-57788, Enrei, Hutcheson, Nakasennari, S90-1056, Suzuyutaka, Ware, York, V71-370, and V81-1603 were the genotypes utilized. Hutcheson had a greater oil content (24.0 g/100 g) in tofu and its total saturated fatty acid content was 3.80 g/100 g, whereas BARC-8 and BARC showed lower oil content of 15.8 and 11.3 g/100 g respectively. Tofu's total saturated fatty acid content varied depending on where the seeds were cultivated. Jayasena *et al.* (2010) reported decrease in the yield when lupin was incorporated into tofu.

2.5.2 Effect of coagulants

The properties such as yield, texture, color and sensory attributes are influenced by different coagulants used to manufacture tofu. Obatolu (2008) investigated the coagulation of soymilk using calcium sulfate, epsom salt, lemon juice, alum, and top water from fermented maize. Calcium sulphate (565.7 g) yielded the most tofu, whereas alum yielded the least (442 g). Tofu's textural qualities, such as hardness, chewiness, and brittleness, ranged from 525.6 to 1008 g, chewiness from 2.3 to 4.5 kg, and brittleness from 1035 to 1482 g. Using GDL, the yield of tofu was roughly 404 g (Jackson *et al.*, 2002). Tseng and Xiong (2009) the rheological properties of silken tofu with inulin and GDL coagulation. Inulin was added at a rate of 1 to 4%, resulting in an increase in hardness from 115.8 to 137.4 g, a drop in cohesiveness from 0.86 to 0.82, a range of percentage deformability from 5.19 to 8.25, and an increase in rupture force from 424.3 to 487.9 g. Coagulants such acids (lactic acid, acetic acid, and rice vinegar) and salts ($CaSO_4$ and $MgSO_4$) were utilized at industrial scale (18 L coagulant in 45 L soymilk) with yields ranging from 178.85 to 186.94% in acid and 204.58 to 221.68% in salt, respectively (Sidar *et al.*, 2011).

In recent years, the use of chitosan in numerous industries has led to the creation of a variety of applications, including edible packaging made from chitosan, which has shown to be quite successful in the modern period. Six different varieties of chitosan were selected based on molecular weight (1106, 746, 471, 224, 28, and 7 kDa) and various treatments were used to extend the shelf life of tofu using chitosan as a coagulant. 1% chitosan solution in 1% acetic acid at 80°C for 15 min was the best treatment for coagulating soy milk. At 7°C for 7 days, a comparative storage study of $CaCl_2$ coagulated tofu and chitosan tofu found that chitosan tofu has a shelf life of 3 days longer than $CaCl_2$ coagulated tofu (No and Meyers, 2004).

2.5.3 Effect of enzymes

Yasir *et al.* (2007) investigated the texture of tofu using the transglutaminase (tga) enzyme. The control tofu had a fracture force of over 30N, while the tga-tofu had a fracture force of less than 25 N. Tofu treated with 1000 ppm transglutaminase had a facture force of 25 N, while tofu treated with 5000 ppm transglutaminase had a force of over 30 N. This proved that the amount of enzyme in tofu has a substantial impact on its texture.

Commercial transglutaminase was employed to induce gel formation in tofu in another investigation, and the addition of transglutaminase had a substantial effect on the textural profile of tofu as a function of pH and NaCl. Tofu's hardness and springiness were maximum at pH 6.4, whereas cohesiveness did not alter much over the pH range. At an ideal temperature of 37°C, adding sodium chloride (NaCl) solely influences the hardness of transglutaminase produced tofu, with no effect on springiness, gumminess, or cohesiveness (Tang *et al.*, 2007).

2.5.4 Effect of process parameter

The texture and quality of tofu are influenced by process parameters such as sodium bicarbonate ($NaHCO_3$) thermal treatment, total solid content of soymilk, stirring time, and moulding. Tofu softness (3.2 N) improved as blanching time increased, whereas control tofu had a hard texture (7.8 N). Smooth tofu was formed from soymilk with a Brix solid concentration of 7° Brix after 10 min of thermal treatment with 1% sodium bicarbonate treated soybeans. The yield of tofu is affected by both stirring duration and moulding. After adding the coagulant, 5 s of stirring resulted in a higher yield of 22.5 g/100 ml than 20 s of stirring (19.16 g/100 ml). In moulding, 1000 g weight for 15 min followed by 500 g yields 22.6 g/100 ml yield with regular texture and 4.2 N hardness, whereas 1000 g weight for 30 min yields 22.3 and 7.8 N yield and hardness, respectively (Rekha and Vijayalakshmi, 2013).

2.6 Chemical composition of Tofu

Soybean products are a good source of protein, carbohydrates, low in fat and rich in minerals content. They are part of principal meals in eastern countries (Murphy *et al.*, 1997). It's formed by coagulating soymilk with salt or acid, resulting in a soy protein gel that traps water, soy lipids, and other elements in the matrix. Tofu's texture and flavor are determined by the coagulants used (Shurtleff and Aoyagi, 1990). Jayasena *et al.* (2010) found that from their research incorporation of a lupin replacer decreased the protein content of tofu. Proximate composition and mineral composition of tofu is shown in Table 2.1 and 2.2 respectively.

Table 2.1 Proximate composition of tofu

Parameter	g/100 g
Moisture	80.5
Protein	16.5
Fat	0.3
Carbohydrate	1.6
fibre	0.03
Mineral	1.1

Source : DFTQC (2012)

Table 2.2 Mineral composition of tofu

Minerals components	mg/100 g
Calcium	18.4
Phosphorous	144
Iron	1

Source : DFTQC (2012)

2.7 Health benefits of tofu

Tofu has been linked to a variety of health benefits, according to numerous studies. Soy food consumption has been shown to reduce the risk of chronic diseases such as cancer (hormone-dependent), cardiovascular disease, and osteoporosis. Soybean protein has been shown to be hypocholesterolemic, and soy isoflavones have anti-atherogenic effects. (Carroll, 1991). In addition, as compared to animal proteins, soy protein intake successfully reduced serum concentrations of total cholesterol, LDL cholesterol, and triglycerides (Potter, 1998). Tofu contains antioxidants such as isoflavones, aglycones, and proteins that protect lipids from oxidation. Even though turning soybeans into tofu reduces the quantity of isoflavones present from 1267 mg per gram in soybean to 455 mg per gram in tofu, the amount present is sufficient to provide possible health benefit (Jackson *et al.*, 2002).

2.8 Functional tofu

Probiotic bacteria are live microorganisms that, when administered in sufficient proportions, can provide health advantages to the host. (FAO, 2001). Probiotic bacteria have a variety of health benefits, including gut microflora stabilization, indigestible saccharide decomposition, and the production of organic acid. Using *Lactobacillus casei* at a rate of 9.26 log cfu/g and a coagulant of $CaSO_4$, Zielinska *et al.* (2015) generated probiotic tofu at a temperature of 37°C for 2 h. The *Bifidobacterium animalis*, *Lactobacillus*, and other lactic acid bacteria were kept at 10^3, 10^8-10^9, and 10^9 -10^{10} cfu/ g for 15 days at 4°C, respectively, and sensory acceptability was assessed, which was highly acceptable with a high count of probiotic bacteria.

Keat-Hui *et al.* (2008) investigated the growth and bioactivity of probiotics in tofu using *Lactobacillus acidophilus* FTCC 0291, *Lactobacillus bulgaricus* FTCC 0411, *Lactobacillus fermentum* FTD 13, *Lactobacillus casei* FTCC 0442, and *Bifidobacterium bifidum* BB12 (2008). Over the course of 24 h, all of these microorganisms were tested for their á-galactosidase activity, with *L. bulgaricus* and *L. fermentum* performing best. *L. bulgaricus* and *L. fermentum* were inoculated on tofu and stored for 9 days at 4°C and 25°C, respectively, to produce probiotic tofu. The concentrations of both probiotic bacteria were kept at 10^6 cfu/g during the storage period.

Tofu is a perishable food that can spoil due to physical, chemical, and microbiological factors. To retain the quality, freshness, customer appeal, and sales of tofu, it requires packaging that protects it from deterioration. Cha *et al.* (2003) investigated the effect of nisin-coated HDPE films on *Listeria monocytogenes* inhibition. Tofu containing L. monocytogenes was tested for shelf life for 30 days at 5°C. Brie-1 was packed in both standard polyethylene pouches and nisin-coated polyethylene films. After 30 days, the polyethylene film with 1000 IU/g concentration had a lower count of 2.74 x10^4 to >101 than the other concentrations of 0, 100, and 500 IU/g, according to the study. The study found that packaging tofu with nisin film can help solve the problem of *L. monocytogenes* survival.

For a total of 28 days, a comparison of air and modified atmosphere packaged (MAP) tofu was conducted. CO_2 and N_2 were employed in the gas combination. The packaged samples were tested for headspace gas. After 10 days of storage, vacuum compensation package samples had lower oxygen levels than flushed samples, whereas air packed tofu samples had no change in O_2 levels. The CO_2 level reduced by 15% and 20% in vacuum compensation and flushed samples, respectively, and increased by 4% in air packed samples, according to short-term study. Oxygen content decreased for air packed tofu in a long-term storage trial, while there was no apparent increase for vacuum compensation and flushed packaged tofu. At a particular point in time, the decline and rise of all packing materials became equal in terms of carbon dioxide concentration (Campenhout *et al.,* 2013).

2.9 Preparation of tofu

Tofu is prepared by different method. Raw soybeans (Proto) were soaked in sixfold tap water by weight at room temperature (21-22˚C) for 8 h. The hydrated beans were drained and weighed. The beans were ground with tap water in the amount of weight difference between 5.5 kg and the weight of water absorbed in hydrated beans in a high-speed grinder (Chan Shen Machinery Co., Taoyuan, Taiwan) equipped with an automatic centrifugal filter to separate soymilk from the residue. After adding 1 g of the antifoaming agent, 4.6 kg of soymilk was heated to boiling in 20 min with gentle stirring and kept for 3 min at approximately 96˚C. When cooled to 9O˚C, the soymilk was poured within 20 s into a 20-L bucket containing 90-mL of water suspended with nigari at 3% of the dry bean weight. The milk-coagulant slurry was transferred immediately to an 8-L stainless pot, covered with a lid, and allowed to stand for 10 min to form bean curd. The bean curd was stirred manually for 30 s and covered again to coagulate for 5, 10, or 15 min. The coagulated soymilk was transferred into a cloth-lined wooden mold (25 x 25 x 7 cm) and pressed sequentially at 11.6 g/cm^2 for 10 min, 23.2 g/cm^2 for 10 min, and 34.8 g/cm^2 for 30 min. At the end of pressing, the cloth was removed, and the tofu weight was recorded.

According to Murugkar (2015) Soymilk was prepared in the grinding cum blanching (GCB) unit using the Soycow model SC 100 (Gupta and Gupta 1990) method. Around 1 kg of soybean per batch was taken both for control and treatments which is the minimum amount required for the Soy Cow model. For control soymilk, dehulled soy splits were soaked for 4 h in water at RT and then fed into the GCB unit. For the preparation of test soymilk, sprouted soybeans containing 60% moisture were dehulled manually, the hulls discarded and the seeds and cotyledons fed into the unit. Water was added to the soybean in the ratio 5:1 in the unit and a pressure of 1 kg/ cm^2 (15 psi) was generated by infusing culinary steam at a pressure of 1–3 kg /cm^2 (15 to 54 psi). The infused steam replaced the air in the unit, minimizing contact of soybean with oxygen. Grinding cum blanching was started and continued till the required temperature (given below) was achieved. After the pressure from unit was released, the slurry was filtered and milk extracted. The temperature in the GCB unit using the conventional method was 121 °C at which temperature trypsin inhibitor is completely destroyed. Milk obtained from the above processes was cooled to around 80°C subsequently 2.5% citric acid was added (of the total milk obtained). The mixture was coagulated and the tofu was separated from the whey using a muslin cloth after which it was pressed in a tofu press.

According to Kong *et al.* (2008a) Soybeans (431 g) were soaked in room temperature for overnight (10 to 11 hrs), then ground in water (water: bean = 7:1, w/w) using a soybean grinding machine (Chang-Seng Mechanical Co., Taoyuan, Taiwan) equipped with a centrifugal separator (120-mesh screen) that separated solid residue (Okara) and soymilk. Total volume of soymilk was measured and a small portion of the soymilk sample was freeze-dried for chemical analysis. A portion of the soymilk corresponding to 130 g soybean was taken and heated to boiling on an electric oven. After 5 min boiling, the soymilk was quickly cooled to 87˚C. A coagulant suspension, containing 2.6 g (approximately 2% of the soybean weight) of calcium sulfate dissolved in 20 mL water, was poured into soymilk rapidly; meanwhile the soymilk was stirred at 150 rpm using a stirrer (Model RZR1, Caframo LTD, Wiarton, Ontario, Canada) equipped with a rectangle paddle (14 cm length × 1.5 cm width). After the addition of coagulant, stirring continued for several seconds equal to the optimum stirring time (Aldin *et al.*) to produce the highest tofu yield. The tm was recorded. Then the mixture was poured immediately into a muslin cloth-lined wooden mold (13 × 13 × 7.5 cm), which was lined with a plastic film. The curd was left to incubate for 8 min. The plastic film was then removed. The tofu curd was packed tightly with the muslin cloth and pressed with iron blocks to remove whey. The iron blocks were used to first produce a pressure of 64.4 g/cm^2 on tofu gel for 15 min, followed by 96.7 g/cm^2 for another 15 min. Subsequently, the tofu was removed from the cloth, stored overnight, in water at 4 to 5˚C and used for further analysis. The tofu weight was recorded. The tofu yield was calculated and expressed as g tofu/100 g raw soybeans.

Soymilk and tofu were prepared using the method of Kong *et al.* (2008a) with slight modifications. Due variation in climatic condition, raw material, inappropriate condition and equipment, certain modification is done. Soybeans and sesame seeds of selected quality were blended at various proportions viz. soybean:sesame – 100:0, 70:30, 92.5:7.5, 77.5:22.5, 85:15. Soybean and sesame seeds were soaked overnight and dehulled. The treated beans/seeds were washed and ground with added water to a milky slurry in a steel mill. The slurry was then heated to boiling temperature and filtered through muslin cloth to separate soymilk. The coagulation of protein was done at 70-80°C by adding 9.75 g of potash alum dissolved in 10 ml of water. This was poured into soymilk, sesame-milk and soy-sesame milk respectively. After adding the coagulant, the mixture was agitated and then set aside for 10-15 min to complete the coagulation. The precipitate was collected in a muslin cloth and subsequently pressed using iron blocks (improvised mechanized press) for about an hour and the final moisture content was 70-76%. Finally, a soft, cake-like tofu resulted which was then cut into desired sizes (rectangular pieces). The flowsheet sesame incorporated tofu is shown in Fig. 2.3

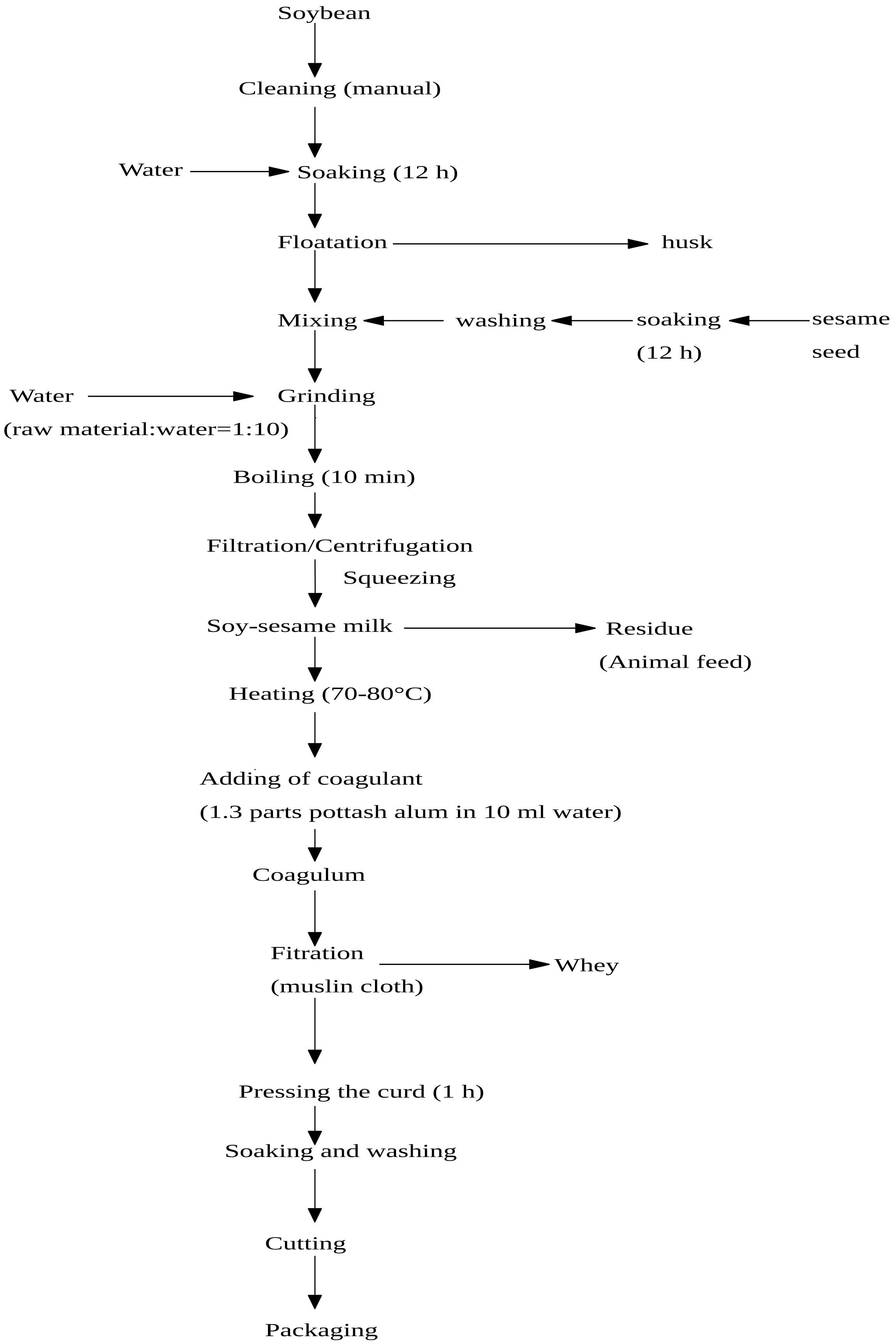

Fig. 2.3: Flowsheet of sesame seed incorporated tofu

2.10 Microbiology and shelf life of tofu

To proliferate, microorganisms require a nutrient-rich substrate, which tofu readily provides (Lee *et al.*, 2018). Yersiniosis is a bacterial infection caused by the genus *Yersinia*. *Yersinia enterocolitica* is the specific bacterium that causes yersiniosis in humans. Aulisio *et al.* (1983) reported a yersiniosis outbreak associated to tofu intake, which resulted in 87 illnesses. *Clostridium botulinum*, *Salmonella typhimurium*, and *Staphylococcus aureus* are some of the other bacteria associated to tofu deterioration (Kovats *et al.*, 1984).

Han *et al.* (2001) found the presence of microorganisms such as *Bacillus cereus* (10^3-10^5 cfu/g), *Clostridium perfringens* (10^5 cfu/g), out of which 85% were gram-positive bacteria, and lactic acid bacteria at 10^5-10^7 cfu/g in a commercial sufu experiment. *Listeria monocytogenes*, *Enterobacteriaceae*, and fungus were found to be absent in white and grey tofu samples, indicating that commercial sufu is safe to eat. The effect of tulsi (*Ocimum sanctum*) extract on the shelf life of tofu at room temperature was investigated by Anbarasu and Vijayalakshmi (2007). Tulsi extract extended the shelf life of tofu, with aerobic counts of 5.96×10^2 and 2.37×10^3 cfu/g in non-treated fresh tofu and tulsi treated tofu samples, respectively. The overall microbial count in non-treated tofu was 6.21×10^8 cfu/g, while tulsi treated samples had 1.08×10^9 cfu/g after seven days of storage. After 7 days, the number of yeasts and molds in fresh and tulsi-treated tofu remained the same ($< 1.0 \times 10^2$). The tofu that had been treated with tulsi extract may be kept for more than 7 days without refrigeration (Dey *et al.*, 2017).

Tofu's microbial count can be maintained using modified environment packing. When compared to modified atmosphere packaged tofu, air packaged tofu had a higher microbial count (1-4 log cycles), inhibited microbe development, and was safe to eat for up to 14 days (Campenhout *et al.*, 2013).

Microbe inactivation in tofu has also been explored using high hydrostatic pressure processing (HHPP). Several factors influence food HPP, including the kind of microbe, processing temperature, high-pressure treatment duration, and food composition. For the inactivation of vegetative cells, pathogens, and spoilage-causing bacteria in food, appropriate pressure treatment is provided based on the parameters. Prestamo *et al.* (2000) investigated the effectiveness of high hydrostatic pressure therapy on tofu by applying a pressure of 400 MPa at 5°C for 5, 30, and 45 min at different temperatures. With time, the microbial count decreased from 5.54 x 10^4 cfu/g to 0.31, 1.56, and 2.38 log units, respectively. They found a 2 log unit drop in psycrotrophs and a 1 log unit reduction in mesophiles, respectively. Microorganisms like *Salmonella*, *Pseudomonas*, and Gram-negative bacteria were also undetectable after treatment (Dey *et al.*, 2017).

Tofu has a shorter storage life than its basic material, which has proven to be a major barrier to large-scale commercial tofu manufacturing. In tropical conditions, it has a shelf life of roughly a day. Park et al. (2007) examined the impact of turmeric (*Curcuma aromatica* Salab.) extract on tofu shelf life at 25°C for 12 days. With the addition of 0.02% turmeric extract, the bacterial count and pH were determined to be lower. The bacterial count in turmeric extracted tofu was 1000 times lower than in control tofu. It is thought that tofu spoiling occurs when the viable count exceeds 10^7 cfu/ml. Oyster shell has been examined in relation to tofu, and it has been found that incorporating 0.2% oyster shell into tofu can extend the shelf life of tofu to up to 11 days at 10°C while maintaining an appropriate amount of viable microorganisms (Kim *et al.*, 2007). Standard of tofu at 40˚F (4.4˚C) is given in Table 2.3.

Table 2.3 Tofu standard at 40˚F (4.4˚C)

	Coliforms per gram	Standard plate count /grams
Excellent product with no sourness	Less than 10	Less than 100000
Acceptable product	11 to 500	100001 to 1 million
Marginal product	501 to 1000	Above 1 million to 5 million
Unacceptable product, sour and probably contaminated	Above 1000	Above 5 million

Source: Kelli (1986)

The high moisture content in tofu (maximum of 90%) with excessively high pH (ranging from 6.0 to 7.0) makes it an appropriate environment for spoilage and pathogenic microorganisms, which would require thermal treatment to enhance or retain the safety and prolong shelf life. The thermal process and freezing process are two of the most essential stages of tofu-making and negatively affect the quality of the final product, especially its texture and nutritional value. However, ozone, electrolyzed water, chitosan, acetic acid, plant extracts, and nisin have been comprehensively evaluated as alternative techniques for enhancing microbiological quality; they remarkably reduced nutritional value and sensory quality and added extra costs (Frias, Iglesias, Alvarez-Ordones, & Prieto, 2020). Non-thermal processing techniques have attracted much attention in the last decade for producing better quality and properties (Ali *et al.*, 2021).

On the other hand, lipid oxidation is a considerable challenge for tofu producers and the tofu industry due to its effect on shelf life and quality characteristics of final soy products (Rekha & Vijayalakshmi, 2013). Texture, color, flavor and odor are considered manufacturing defects in fatty products due to lipid oxidation. In addition, lipid oxidation minimizes the nutritional value of stored products by affecting some vitamins and amino acids.

2.11 Sensory analysis

In order to know about product quality, question relating to discrimination, description, or preference sensory analysis is done. Sensory analysis is done by different method i.e., difference test, rating test, sensitivity test, descriptive flavour profile method and dilution flavour profile method. Different test is done in rating test i.e., ranking test, single sample, two sample, multiple sample hedonic rating, numerical scoring and composite. The sensory analysis of tofu sample was conducted using nine-point hedonic scale, where; 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely. Eleven semi trained panelist composed of faculties and students who were familiar with tofu were evaluated the samples in terms of color, flavor, taste, texture, and overall acceptability. Panelist were asked to record their liking of color by looking at the control tofu samples.

2.12 Soybean

2.12.1 Introduction

In many countries, soybean (*Glycine max*) is one of the most widely grown crops. It belongs to the Leguminosae family, subfamily *Papilionoideae*, tribe *Phaseoleae*, genus *Glycine Willd*, and subgenus *Soja* (*Moench*), and is a diploidized tetraploid (2n=40). It's an erect, bushy herbaceous annual that can grow up to 1.5 meters tall. It is also known as the king of legumes, and it is primarily produced for seed production. It has a wide range of applications in the 15 food and industrial sectors, and it is one of the most important sources of edible vegetable oil and protein for livestock feed (CFIA, 1996). Classification of soyabean is shown in Table 2.4.

Table 2.4 Classification of soybean

Taxon	Scientific name and common name
Kingdom	*Plantae* (plants)
Sub Kingdom	*Tracheobionta* (vascular plants)
Super division	*Spermatophyta* (seed plants)
Division	*Magnoliophyta* (flowering plants)
Class	*Magnoliopsida* (dicotyledons)
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae / Leguminosae* (legume and pea family)
Tribe	*Phaseoleae*
Genus	*Glycine Willd.* (soybean)
Species	*Glycine max* (L.) Merr. (soybean)

Source : CFIA (1996)

The United States, China, North and South Korea, Argentina, and Brazil are the world's top soybean producers. In Nepal, soybeans are known as 'Bhatmas.' The agricultural farms of

Khumaltar, Kakani, and Rampur collected 138 samples of soybeans from several districts ranging in altitude from 500 to 1800 m, and concluded that the most dominant soybean kinds in Nepal are white, brown, grey, and black in hue. It goes by various names according on the variety, color, and location of the seeds, such as Nepale, Hardi, Saathiya, Darmali, Maily, Kalo, Seto, and so on (Lama, 2009).

Although just 10% of the world's soybean output is used for human consumption, the bean is used to make a wide range of products. Many of these soy foods use soybeans, while others use isolated soy proteins, soy protein concentrate, soy flour, or soy milk as sources of protein. Soy foods are commonly split into two types: Fermented and non-fermented. Natto, miso, tempeh, and fermented tofu are all traditional fermented foods. Soynuts, okara, and tofu are traditional no-fermented soy foods (Shrestha, 2017).

2.12.2 History, Origin and Evolution

Soybean is a crop that is grown all over the world, and its history has been carefully documented by numerous researchers (Hymowitz, 1970; W. T. Guo, 1993; Singh and Hymowitz, 1999; J. Guo *et al.*, 2010). Evidence suggests that soybean was domesticated during the Shang dynasty, which ruled in the eastern half of northern China between 1700 and 100 BC (Singh and Hymowitz, 1999). It is one of the oldest cultivated food legumes, having been known to man for almost 5,000 years, and hence suggests it as a possible domestication site (Hymowitz, 1970). Molecular diversity research on soybean populations from the north and south regions revealed that this crop was domesticated in South China as well (Ding *et al.*, 2008). Thus, evidences suggest that both North and South China regions were involved in domestication of soybean since ancient times.

The earliest evidence of soybean farming can be found in bronze inscriptions and early literature dating back to around 1100 BC. Soybean trade extended to South China, Korea, Japan, and Southeast Asia as the Shang dynasty expanded. Soybeans were presumably distributed throughout China by trade missions by the first century AD, and eventually to other Asian countries. The Kojiki (recorded of ancient matters) was completed in 712 ad and contains the oldest Japanese reference to this crop. Several references to native soy foods can be found in the literature of European visitors to China and Japan in the sixteenth and seventeenth centuries. Samuel Bowen, a seaman working by the East India Company, introduced the first soybeans to the United States in 1765 and planted them in "Greenwich," a few miles east of Savannah, Georgia. Mr. Bowen utilized soybeans to make soy sauce and soybean noodles, which he exported to England.

The late type soybean from South China was found to be closer to the wild type, implying that wild soybean is a common ancestor for the cultivated type of South China, from which early cultivated types emerged during the dissemination process to North China. (Gai *et al.*, 2000) . The origin of soybean in South China was also supported by the increased genetic diversity of the South China population compared to the North China population. (Ding *et al.*, 2008).

2.12.3 Physical properties of soybean

There are different varieties of soybean varying in physiochemical properties according to the strain, climatic conditions and agronomical condition. The example of common physical criteria considered are color, bulk density, 1000 kernel weight, hectolitre weight and average seed weight. The average weight of 1000 kernel and average bulk density of Nepalese soybean were reported as 153.15 g and 0.767 g/cc respectively. The range of seed weight of soybean remains within 120 to 180 mg of which 10% is hull (Katwal, 1984).

Size, hardness, density and appearance are some of the physical criteria usually considered when selecting soybean for tofu preparation. Chemical constituents of soybean and their behaviour with water, other ingredients, temperature and pH are of importance (Dawadi, 1985). Proximate composition of soybean and seed parts is shown in Table 2.5.

Table 2.5 Proximate composition of soybean and seed parts

Seed part	Whole seed weight (%)	Protein (%) N x 6.25	Lipid (%)	Carbohydrate (%) (incl. fibre)	Ash (%)
Cotyledon	90	43	23	43	5.0
Hull	8	9	1	86	4.3
Hypocotyl	2	41	11	43	4.4
Whole seed	100	40	20	35	4.9

Source: Khadka (2015)

2.12.4 Major nutrient of soybean

The major composition of the soybean varies with the variety, growing season and location (Smith and Circle, 1978). However, reasonable average figures for the major nutrients are 40% protein, 20% lipid, 35% Carbohydrate and 5% ash on dry weight basis. To ensure stability of the mature soybeans, a moisture content of 12-15 is desirable (Snyder and Kwon, 1987). For their subsequent handling, the harvest's moisture content is essential. If too moist, considerable energy will be expended during drying, while if they are too dry, they may be brittle during harvest, leading to potential deterioration of oil quality and increased refining costs (Smith and Circle, 1978). Also, moisture content at harvest greater than 13% may lead to mold growth and due to the metabolism and growth of mold, moisture and temperature will both increase in the storage space leading to accelerated deterioration of soybean food value (H. L. Wang *et al.*, 1979).

Refined soy oil fraction is not 100% triglyceride, and the minor components which include phospholipids and lecithin, can influence the color and stability of the refined oil. Soy oil has about 80% of its fatty acids being unsaturated, with the predominant unsaturated fatty acid being linoleic (Weiss, 1983). The relative reactivity of oleic, linoleic and linolenic is 1:10:20 (Sonntag, 1979). As the linolenic acid in soybean oil has been blamed for the relative instability of the oil oxidation, efforts have been expended into breeding low linolenic acid varieties by genetic modification (O'Brien, 2004).

Protein is another major component of the soybean which has commercial value. Soy protein has an amino acid composition that complements that of cereals, although it is limiting in the especially methionine, sulphur-containing amino acids, for most species including humans (Snyder and Kwon, 1987).

Lipid extraction of soybeans with hexane at 60-70^{0}c for 30-40 min does not adversely affect soy protein solubility, the enzymatic activity of the defatted flour, or its trypsin inhibitor activity. In contrast to other phytohaemaglutenins, soy haemagglutenins do not seem to exert much effect on the nutritive value of soybeans (Snyder and Kwon, 1987). Soybeans are an excellent source of lipooxygenase which has been used to bleach the carotenoids of wheat flour instead of the use of bromides and related chemical compounds. As the beans mature, the content of monosaccharides declines and the complex carbohydrates-raffinose, stachyose and sucrose increase. These are normally present in the mature beans at levels of 1, 4 and 5%, respectively (Snyder and Kwon, 1987). Although stachyose and raffinose are not digested and absorbed as nutrients by humans, the intestinal microflora in the human gastrointestinal tract metabolizes these oligosaccharides, resulting in gas production causing flatulence in humans. The insoluble carbohydrates mainly: cellulose, hemicellulose and pectins are the equivalent of the insoluble dietary fibre. Soybeans contain nutritionally significant amounts of calcium and iron (Miller, 2008). Due to phytic acid , the bioavailability of some minerals such as zinc may be affected adversely (Navert *et al.*, 1985). Chemical composition of raw soybean according to USDA and DFTQC is shown in table 2.6 and 2.7. Amino acid of soybean in terms of g/16g N is shown in Table 2.8. Mineral composition is shown in Table 2.9.

Table 2.6 Chemical composition of raw soybean per 100 g

Parameters	Values
Energy	1,866 KJ (446 Kcal)
Carbohydrates	30.16 g
Sugar	7.33 g
Dietary fibres	9.3 g
Fat	19.94 g
Lysine	2.706 g
Methionine	0.547 g

Source: Koirala (2013)

Table 2.7 Nutritional value of raw soybean on dry basis

Parameters	Values
Moisture	10.2 g
Carbohydrates	29.6 g
Fat	17.7 g
Protein	33.3 g
Mineral	5 g
Fibre	4.2 g
Energy	411 K Cal
Calcium	226 mg
Phosphorous	546 mg
Iron	8.5 mg
Carotene	10 µg
Thiamin	0.66 mg
Riboflavin	0.22 mg

Source: DFTQC (2012)

Table 2.8 Amino acid of soybean in terms of g/16g N

Amino-acid	g/16g N
Arginine	6.79
Cystine	1.57
Histidine	2.58
Isoleucine	4.24
Leucine	8.21
Lysine	6.49
Methionine	1.50
Phenylalanine	4.93
Threonine	3.99
Tryptophan	1.05
Valine	5.22

Source: (Banaszkiewicz, 2011)

Table 2.9 Mineral composition per 100 g

Sample	mg/100g
Calcium	226
Phosphorous	546
Iron	8.5

Source: DFTQC (2012)

2.13 Sesame seed

2.13.1 Introduction

Sesame (*Sesamum indicum*), often known as benne, is a flowering plant belonging to the Sesamum genus. Sesame (*Sesamum indicum L.*), sometimes known as the "Queen of Oilseeds," is a ancient oil-producing crop. Sesame is a member of the Pedaliaceae family and Tubiflorae order. *Sesamum indicum L.* is the most widely farmed of the 37 species in the genus Sesamum. The majority of species have distributions in three areas: Africa, India, and the Far East (Pusadkar *et al.*, 2015). In Africa, there are a lot more wild relatives than in India, where there are fewer. It has spread rapidly over tropical regions of the world and is cultivated for its edible, which are produced in pods. In 2018, global output totaled 6 million tonnes, with Sudan, Myanmar, and India producing the most (FAOSTAT, 2020).

Sesame (*Sesamum indicum L.*; Pedaliaceae) is a dicotyledonous diploid (2n = 26) oil seed crop that is widely farmed in tropical and subtropical regions for its edible oil, proteins, vitamins, and amino acids. Sesame is a valuable cover crop that is grown for food (dry seeds), feed (seed, leaves, and young branches), and other uses. For example, the flowers are used to treat cancer, alopecia, and constipation, the roots have antifungal activity, and the leaves are used to treat infant cholera, diarrhoea, dysentery, and urinary infections. As a result, efforts are being made to raise knowledge about sesame oil and its other applications. Natural antioxidants found in sesame oil include sesamin, sesamolin, and sesamol, which are among the most stable vegetable oils with a long shelf life. Omega 6 fatty acids abound in sesame seed oil, whereas Omega 3 fatty acids are deficient. In order to improve the quality of sesame oil as a healthy oil, more Omega 3 fatty acids, such as alpha linolenic acids, must be produced via various desaturase enzyme pathways (Pusadkar *et al.*, 2015).

Sesame is also known in English as Gingelly and Sesame, in Ayurveda as Tila and Snehphala, and in Unani as Til and Kunjad. Sesame is an erect branch annual (sometimes perennial) that grows to a height of 0.5-2 m and has a well-developed root system. It has several flowers and produces a capsule with a number of little oleaginous (oily) seeds inside. Sesame seeds are tiny, measuring 4 mm long, 2 mm wide, and 1mm thick. Pearl-shaped, ovate, little, slightly flattened, and thinner at the hilum, they are pearl-shaped, ovate, and small. Size, form, growth, flower color, seed size, color, and content vary greatly between varieties and strains (Pusadkar *et al.*, 2015).

2.13.2 Major nutrient of sesame seed

The chemical composition of sesame seed from different reports is listed in Table 2.11 (DFTQC, 2012). Sesame seed is rich in oil and protein. The seeds contain 4.69% moisture, 49.7% fat, 17.7% protein, 4.45% ash, 11.8% fibre and 23.4% carbohydrate (DFTQC, 2012) which is shown in Table 2.11. The sesame seeds composition is influenced by genetics, environmental factors, variety, cultivation, climate, ripening stage, seed harvesting time, and analytical procedure (Onsaard, 2012).

Many health benefits have been linked to sesame seed, some of which have been related to a group of compounds known as lignans (sesamin, sesamolin, sesaminol and sesamolinol). Lignan aglycones and lignan glucosides are also found in sesame seed oil. Sesame seed oil is high in unsaturated fatty acids (83-90%, primarily linoleic acid (37-47%), oleic acid (35-43%), palmitic (9-11%), and stearic acid (5-10%), with a trace amount of linolenic acid.

Sesame cake is a high-protein, high-carbohydrate, and high-mineral-nutrient food. Because of its high level of sulfur amino acids i.e methionine and phytosterols, sesame seeds are particularly important for human nutrition. An aliphatic, sulfur-containing, essential amino acid called methionine also functions as a precursor of homocysteine, cysteine, creatine, and carnitine. Methionine can control the innate immune system, digestive function, and metabolic functions in mammals, according to recent studies. To counteract oxidative stress, it also affects lipid metabolism, the activation of endogenous antioxidant enzymes such methionine sulfoxide reductase A, and glutathione biosynthesis. Additionally, methionine restriction inhibits altered methionine/transmethylation metabolism, reducing DNA damage and carcinogenic processes as well as perhaps delaying the development of neurodegenerative, neuropsychiatric, and arterial diseases. This review focuses on the role of methionine in metabolism, oxidative stress, and related diseases (Martínez *et al.*, 2017).

Sesame (Sesamum indicum L.) is herbaceous annual plant. Sesame seed is also known as benniseed (Africa), benne (Southern United States), gingelly (India), gengelin (Brazil), sim-sim, semsem (Hebrew) and tila (Sanskrit). The average values of 1000 seed weight ranged from (2.74 - 3.16 g) (Zebib *et al.*, 2015). The bulk density (Bd) of sesame is 0.640 kg/m^3 (Arafa, 2007). Sesame's fruit is a capsule with 50-100 seeds within. Sesame seeds are pear-shaped, flattened, and overate. Sesame seed measures 2.80 mm in length, 1.69 nm width, and 0.82 mm thick. 1.56 mm, 7.80 mm^2, and 1224 kg/m^3, respectively, are the geometric mean diameter sphericity, surface area, and density. The coefficient of friction varies between 0.39 and 0.54 on glass and 0.54 on plywood, with a 32.0 degree angle of response (Tunde-Akintunde and Akintunde, 2004).

The antioxidative agents sesamin, sesamolin, sesamol, and their glucosylated derivatives sesaminol glucosides, as well as tocopherol, make the oil exceptionally stable, allowing it to last a long time. The inclusion of vitamin E among the vitamins in sesame seed is particularly intriguing in terms of sesame seed's usefulness as a health food (Pathak *et al.*, 2014). Chemical composition of sesame seed per 100 g and mineral content is shown in Table 2.10 and 2.12. Aminoacid content of sesame seed is shown in table 2.13.

Table 2.10 Chemical composition of sesame seed per 100 g

Parameters	Values
Water	4.69g
Energy	2400 kJ (573 kcal)
Carbohydrates	23.4 g
Sugar total including NLEA	0.3 g
Dietary fibres	11.8 g
Fat	49.7 g
Ash	4.45 g
Protein	17.7 g
Lysine	0.569 g
Methionine	0.586 g
Cystine	0.358 g

Source: USDA (2019)

Table 2.11 Nutritional composition of sesame seed according to DFTQC

Parameters	values
Moisture	4.28 g
Protein	25.9 g
Fat	49.57 g
Carbohydrate	12.4 g
Mineral	5.34 g
Fibre	2.53 g
Energy	599 K cal
Phosphorous	442 mg
Iron	4.59 mg

Source: DFTQC (2012)

Table 2.12 Mineral content in sesame seed

Parameter	Value (mg)/100g
Calcium, Ca	975
Iron, Fe	14.6

Source: USDA (2019)

Table 2.13 Amino acid composition of sesame seed

Amino acid	g/16 g N
Arginine	12.0-13.0
Histidine	2.4-2.8
Isoleucine	3.3-3.6
Leucine	6.5-7.0
Lysine	2.5-3.0
Methionine	2.5-4.0
Methionine + Cystine	3.8-5.5
Phenylalanine	4.2-4.5
Threonine	3.4-3.8
Tryptophan	2.0-2.4
Valine	4.2-4.4

Source: Yasothai (2014)

2.13.3 Antioxidant properties of sesame fractions

Plant-based foods are known to include a rich blend of natural chemicals that have antioxidative properties. Antioxidant activity seems to be linked to the protection of degenerative diseases like cancer, cardiovascular disease, atherosclerosis, and the aging process. Lignans, a category of phenylpropanoid chemicals found in sesame seeds, are an innate non-enzymatic antioxidant defence mechanism against reactive oxygen species that is important for health. Sesame lignans have antioxidant, antiproliferative, and antihypertensive activities, and are responsible for raising vitamin E antioxidant activity in lipid peroxidation systems, lowering cholesterol levels, increasing hepatic fatty acid oxidation enzymes, and showing antihypertensive effects. In addition to sesame lignans, sesame seed and oil contain other biologically active chemicals such as vitamin E (tocopherol homologues), particularly -tocopherol (Pathak *et al.*, 2014).

2.13.4 Pharmacological benefits of sesame seed and oil

Sesame seed and oil have a wide range of pharmacological applications and have long been used in Chinese and Indian medicine. Sesame oil contains burn healing properties, and when applied to the skin, it relieves a mild burn or sunburn while also aiding in the healing process. Because of its high emollient characteristics, sesame oil is an excellent massage oil. It aids in the healing of chronic skin problems when applied topically. It's been used as an antibacterial mouthwash in India, as well as to cure anxiety and insomnia, as well as impaired vision, dizziness, and headache. For common skin pathogens like *Staphylococcus* and *Streptococcus*, as well as common skin fungi like athlete's foot fungus, sesame oil is naturally antimicrobial (Pathak *et al.*, 2014).

2.13.5 Traditional uses

Sesame seeds have been used for traditional purposes from ancient times. Sesame seeds are utilized as a "symbol of immortality" in Hindu culture, and sesame oil is extensively used in prayers and rituals performed during a person's death. Tahini, sometimes known as "Butter of the Middle East," is a smooth, creamy paste produced from toasted ground hulled sesame seeds. It is a typical Middle Eastern staple. A portion of the nutrient-dense seed cake is fed to livestock, while the rest is crushed into sesame flour and utilized in healthy foods. Sesame oil is used extensively in southern Indian cuisine, and until recently, it was China's only cooking oil. Sesame seed is beneficial to the entire body, particularly the liver, kidney, spleen, and stomach. Its high oil content not only lubricates the intestines, but also feeds and nourishes the entire internal viscera. It is also known to blacken hair, particularly black sesame hair. As a result, it's used to treat white hair, constipation, and poor lactation. Sesame oil can also be used to cure intestinal worms including ascaris, tapeworm, and others (Pathak *et al.*, 2014).

2.13.6 Sesame industrial uses

Sesame has a variety of industrial applications. Sesame flowers are used to make fragrances and cologne in Africa. Cosmetics contain myristic acid as an ingredient. Sesamin possesses bactericidal and insecticidal properties, as well as antioxidant properties that help prevent cholesterol absorption and synthesis in the liver. It's employed in pyrethrum insecticides as a synergist. Sesame oil is used as a solvent, as an oleaginous carrier for pharmaceuticals, and as a skin softener, as well as in the production of margarine and soap. Margarine, soap, medicines, paints, and lubricants are all made from sesame oil. Chlorosesamone, derived from sesame roots, has antifungal properties. Ahmad *et al.* (2010) reported biodiesel production from sesame oil via transesterification with methanol in the presence of NaOH as a catalyst. They also discovered that sesame biodiesel outperformed mineral diesel in terms of environmental performance. This research backs biodiesel made from sesame seed oil as a viable alternative to diesel fuel (Pathak *et al.*, 2014).

PART II

Materials and methods

3.1 Materials

3.1.1 Raw materials

Raw material collected for the formulation of sesame incorporated tofu are as follows

3.1.1.1 Soybean

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

3.1.1.2 Sesame seed

White variety of sesame (*Sesame indicum*) named 'BBSM' was collected from the local market of Dharan.

3.1.2 Apparatus and chemical required

Apparatus and chemicals required are utilized from CCT laboratory. The apparatus and chemical used are in Appendix E.

3.2 Methods of experiment

3.2.1 Methodology

The independent variable of the experiment is sesame seed. The determination of threshold for sesame seed was carried out with the help of trial experiment. After trial experiment above 30% of sesame seed incorporation, tofu was not formed. Therefore, threshold for sesame seed incorporated tofu is set between 0-30% and finally design expert was used for recipe formulation. Design expert 13[Mixed design (simple lattice design))] was used to formulate the recipe. The independent variable for the experiment is concentration of sesame seed.

3.2.2 Formulation of recipe

The recipe formulation for the sesame seed incorporated tofu was carried out as given in Table 3.1. The amount given is on parts basis.

Table 3.1 Recipe formulation of tofu

Sample	Soybean	Sesame seed
A	100	0
B	92.5	7.5
C	85	15
D	77.5	22.5
E	70	30

The Tofu was made as per the recipe formulation and coded named A, B, C, D and E were given to each recipe.

3.3 Preparation of sesame seed incorporated tofu

Tofu was produced using the modified method of Kong *et al.* (2008). Soybeans and sesame seeds of selected quality were blended at various proportions viz. soybean: sesame – 100:0, 70:30, 92.5:7.5, 77.5:22.5, 85:15. Soybean and sesame seeds were soaked overnight and dehulled. The treated beans/seeds were washed and ground with added water to a milky slurry in a steel mill. The slurry was then heated to boiling temperature and filtered through muslin cloth to separate soymilk. The coagulation of protein was done at 70-80°C by adding 1.3 parts of potash alum dissolved in 10 ml of water. This was poured into soymilk, sesame-milk and soy-sesame milk respectively. After adding the coagulant, the mixture was agitated and then set aside for 10-15 min to complete the coagulation. The precipitate was collected in a muslin cloth and subsequently pressed using iron blocks (improvised mechanized press) for about an hour and the final moisture content was 70-76%. Finally, a soft, cake-like tofu resulted which was then cut into desired sizes (rectangular pieces) The flowsheet sesame incorporated tofu is shown in Fig. 3.1.

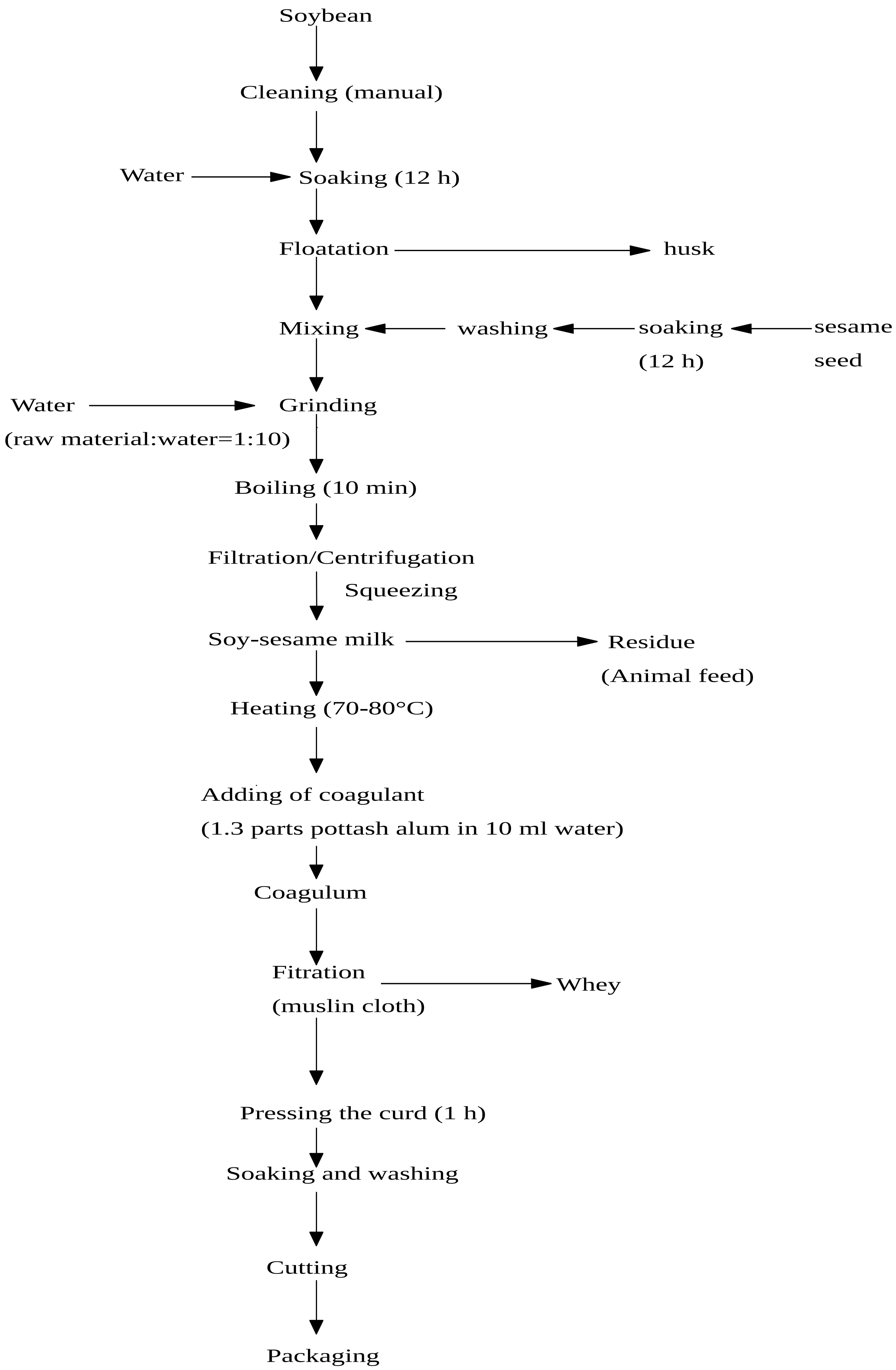

Fig. 3.1: Flowsheet of sesame incorporated tofu

3.4 Analysis of raw materials and product

3.4.1 Physical analysis of raw material and product

3.4.1.1 1000 kernel weight

The 1000 kernel weight of soybean and sesame seeds were determined by measuring the weight of 1000 kernels of soybean and sesame seeds after selecting the appropriate sample size by quartering method as stated in Buffo *et al.* (1998).

3.4.1.2 Bulk density

The bulk density was measured as mentioned in Clementson *et al.* (2010) by pouring the grains into the funnel-shaped hopper, the hopper was centred over the measuring bushel, the hopper valve was opened quickly, and the grains were allowed to flow freely into the measuring bushel. After the bushel was filled, the excess material was levelled off with gentle zigzag strokes using the standard Seedburo striking stick. The filled measuring bushel was then weighed, and the mass of grains in the bushel was determined by subtracting the mass of the measuring bushel itself. The bulk density (ρ) of grain was then calculated using the following expression

$$\text{Bulk density} = \frac{\text{Mass of grain}}{\text{Volume of bushel}}$$

3.4.1.3 Color

Color was determined by visual method. The soybean and sesame seed were spread on separate tray and color and surface was diligently examined. Similarly, the color of the products was analyzed. Appearance property i.e., the color of the tofu is affected by types of raw material i.e., soybean and sesame seed.

3.4.1.4 Density

Density of tofu was obtained by the ratio of mass to the volume of the tofu as per AOAC (2005)

$$\text{Density (g/cm}^3\text{)} = \frac{\text{Mass (g)}}{\text{Volume (cm}^3\text{)}}$$

3.4.2 Chemical Analysis

3.4.2.1 Determination of moisture content

Moisture content of the sample was determined by weight loss during heating in a thermostatically controlled oven at 100ºC or 105ºC by hot air oven method as given by AOAC (2005).

$$\text{Moisture content \%} = \frac{\text{Initial weight - final weight}}{\text{initial weight}} \times 100\%$$

3.4.2.2 Determination of Ash content

The determination of total ash can be conveniently carried out by incinerating all the organic matter of the food sample at 550°C by dry ashing method (AOAC, 2005).

$$\text{Ash \% (wet basis)} = \frac{\text{Ash}}{\text{sample}} \times 100$$

3.4.2.3 Determination of crude fat

The crude fat content of the sample was determined by solvent extraction method as described by AOAC (2005).

$$\text{Crude fat} = \frac{W_2 - W_1}{W} \times 100\%$$

Where, W_1 = weight of sample before drying

W_2 = weight of oil extracted + beaker

W = weight of sample

3.4.2.4 Determination of crude fibre

The crude fibre content of the soybean, sesame seed and tofu was determined by acid base hydrolysis as described by AOAC (2005).

$$\text{Crude fiber (\%, wb)} = \frac{(\text{Residue} - \text{Ash})\text{g} \times (100 - \text{F})}{\text{sample (g)}}$$

3.4.2.5 Determination of crude protein

The crude protein content of the soybean, sesame seed and the tofu sample were calculated indirectly by measuring total nitrogen content by micro Kjeldahl method. Factor 6.25 was used to convert the nitrogen content to crude protein as described by AOAC (2005).

$$\text{Protein content} = \frac{(\text{sample} - \text{blank}) \times \text{N of HCL} \times 14 \times 100 \times 100}{\text{Aliquot (ml)} \times \text{wt of sample (g)} \times 1000}$$

3.4.2.6 Determination of carbohydrate

Carbohydrate content was determined by the difference methods (AOAC, 2005) .

Carbohydrate (%) = 100 - (protein + fat + ash + crude fibre).

3.4.2.7 Determination of mineral

Iron in foods is determined by converting all the iron into ferric form using oxidizing agents like potassium persulfate ($K_2S_2O_8$) or hydrogen peroxide (H_2O_2) and treating thereafter with potassium thiocyanate (KSCN) to form the red ferric thiocyanate which is measured calorimetrically at 480 nm (AOAC, 2005). Similarly Calcium content was determined by titration method as described in AOAC (2005).

3.4.2.8 Determination of methionine

0.5 g of defatted sample was weighted into 50 ml conical flask. 6 ml of 2N HCL was added and autoclaved at 15lb pressure for an hour. A pinch of activated charcoal was added to the autoclaved sample and heated to boil. When hot it was filtered and washed the charcoal with hot water. After this, the filtrate was neutralized with 10 N NaOH to pH 6.5. The volume was made up to 50 ml with water after cooling at ambient temperature. 25 ml of made-up solution was transferred into a 100 ml conical flask. For standard curve; 0, 1, 2, 3, 4 and 5 ml of standard methionine solution was pipette out and made up to 25 ml with water. 3ml of 10 % NaOH was added which was followed by 0.15 ml sodium nitroprusside. After 10 min 1 ml of glycine solution was added. After another 10 min 2 ml orthophosphoric acid was added and shaken vigorously. After 10 min at 520 nm intensity of red color was red against a blank prepared in the same way but without nitroprusside. Standard curve of methionine content is shown in appendix F. From standard curve an equation was derived for the methionine content of the sample (Pieniazek *et al.*, 1975).

$$Y= 2.014x-0.0056$$

Methionine content in the sample = (Methionine content from the graph x 4) mg per g Methionine is usually expressed as percentage of protein or g per 16g N.

$$\text{Methionine content of sample} = \frac{\text{Methionine content from the graph}}{\text{Percent N in the sample}} \times 6.4 \text{ g per 16g N}$$

3.5 Yield

The tofu yield was calculated on the basis of the weight of pressed tofu obtained from 500 g beans and expressed as weight of tofu (g/100 g raw bean) (Jayasena *et al.*, 2010).

3.6 Microbiological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48 h). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37°C/48 h) (AOAC, 2005).

3.7 Statistical Analysis

Data will be statistically processed by GenStat (12th edition) developed by VSN International Limited for Analysis of Variance (ANOVA). Means of the data will be separated whether they are significant or not by using Least Significant Difference (LSD) method at 5% level of significance.

Part IV

Results and discussion

Tofu contains all essential amino acids except sulphur-containing amino acids i.e., methionine and cysteine. After incorporation of sesame seed, tofu contains complete amino acid as sesame seed is rich in methionine and cysteine. So, this work was carried out for making tofu as a complete food. The soybean and the sesame seed were collected to formulate sesame seed incorporated tofu of 0%, 7.5%, 15%, 22.5%, and 30% of sesame seed incorporation. At first raw materials (soybeans and sesame seeds) were brought from the market and these materials were subjected to proximate analysis. The best product among the five variations was determined by carrying out sensory evaluation and the detailed nutritional value of the best product was analysed.

4.1 Proximate composition of soybean and sesame seed

The proximate composition of soybean and sesame seed was obtained as given in Table 4.1.

Table 4.1 Proximate composition of soybean and sesame seed

Attributes	Soybean	Sesame seed
Moisture % (wet basis)	10.46±0.16	4.53±0.34
Ash % (db)	5.82±0.17	5.23±0.07
Crude fat % (db)	19.75±0.18	53.45±0.07
Crude fibre %(db)	4.66±0.21	2.46±0.03
Crude protein % (db)	37.93±0.065	26.08±0.02
Carbohydrate % (db)	31.82±0.596	12.74±0.18

[Values are the means of three determinations ± standard deviations. Figures in the parenthesis are standard deviations.]

4.1.1 Chemical composition of soybean

Proximate analysis of the soybean for various parameters like moisture content (%), Ash (%) crude fat (%), crude fibre (%) crude protein (%), and Carbohydrate (%) (in dry basis except moisture content) were found to be 10.46%, 5.82%,19.75%, 4.66%, 37.93% and 31.82% respectively as given in Table 4.1. The value in the wet basis of given parameter is 10.8%, 5.2%, 17.6%, 4.16%, 33.84% and 28.39%.

DFTQC (2012) reported respective proximate values in wet basis were 10.2, 5, 17.7, 4.2, 33.3 and 29.6 % respectively. The moisture content of soybean (10.46% wet basis) was lower than that suggested by Snyder and Kwon (1987). i.e. 12-15%. , higher than the value obtained by DFTQC (2012). The moisture content of the soybean may vary according to the maturity stage, climatic condition, variety, storage condition and packaging material. The crude protein content in soybean i.e. 37.93% (db) was lower than that obtained by (Snyder and Kwon, 1987) and higher than DFTQC (2012) i.e. 37.12% . This might be due to the loss of nitrogenous material during digestion which evenly reduced the final protein content. Crude Fibre content in soybean was found to be 4.66%(db) which was in accordance to the findings of DFTQC (2012) i.e. 4.67%. Ash content in soybean 5.82% (db) which was higher than that obtained by DFTQC (2012) i.e. 5.56% and Snyder and Kwon (1987). Fat content was found to be 19.75% slightly higher than that obtained by DFTQC (2012) i.e. 19.71% and Snyder and Kwon (1987). Fat content may depend on different factors like moisture content, geographical condition, climatic condition, maturity and variety of soybean. This showed that the variety of soybean affect the fat content. Carbohydrate content obtained in soybean was 31.82% (db) which is lower than that obtained by DFTQC (2012) i.e. 32.96%. Environmental conditions, variety of soybean, the experimental error might be the cause of variation in carbohydrate content.

4.1.2 Chemical composition of sesame

Proximate analysis of the sesame seed for various parameters like moisture content (%), Ash (%), crude fat (%), crude fibre (%), crude protein (%), and Carbohydrate (%) (in dry basis except for moisture content) were found to be 4.53%, 5.23%, 53.45%, 2.46%, 26.08% and 12.74% respectively as given in Table 4.1. The value in wet basis of given parameter is 4.533%, 5%, 51.03%, 2.36%, 24.9% and 12.17%.

DFTQC (2012) reported respective proximate values in wet basis were 4.28%, 5.34%, 49.57%, 2.53%, 25.9% and 12.4% % respectively. The moisture content obtained in sesame seed was higher than obtained by DFTQC (2012). The moisture content of the sesame seed may vary according to the maturity stage, climatic condition, variety, storage condition and packaging material. Crude protein content in sesame seed was lower than that obtained by DFTQC (2012). This might be due to the loss of nitrogenous material during digestion which evenly reduced the final protein content. Crude fat content was higher than that obtained by DFTQC (2012). Fat content may depend on different factors like moisture content, geographical condition, climatic condition, maturity and variety of sesame seed. This showed that the variety of sesame seeds affects the fat content. Ash content was lower than that obtained by DFTQC (2012). The lower value may be due to differences in variety and some experimental errors. The high ash content of sesame seed signifies higher mineral content and vice versa. Crude fibre content was lower than that obtained by DFTQC (2012). The amount of bran content in sesame seed, types of soil, maturity and varieties of sesame seed might be the reason for variation in crude fibre. Carbohydrate content was lower than that obtained by DFTQC (2012). Environmental condition, variety of sesame seeds, experimental error might be the cause of variation in carbohydrate content.

The sesame seeds composition is influenced by genetics, environmental factors, variety, cultivation, climate, ripening stage, seed harvesting time, and analytical procedure (Onsaard, 2012).

4.2 Physical properties of soybean and sesame seed

The physical properties of soybean and sesame seed were determined. The results obtained are presented in Table 4.2

Table 4.2 Physical properties of soybean and sesame seed

Physical properties	Soybean	Sesame seed
1000 kernel wt. (g)	195.146±0.44	3.19±0.03
Bulk density (kg/HL)	83±0.81	60.5±0.16

[Values are the means of three determinations ± standard deviations. Figures in the parenthesis are standard deviations.]

(Katwal, 1984) reported the value of 1000 kernel wt. and bulk density as 153.15kg/HL, 0.767 g/cm^3 which was less than the mean values of soybean of our study. This was due to different varieties of soybean varying in physiochemical properties according to the strain, climatic conditions and agronomical conditions.

Thousand kernel weight was found to be 3.19 which is within the range obtained by Zebib *et al.* (2015). Bulk density of our study was 60.5 kg/HL which was similar to the value obtained by Arafa (2007).

4.3 Sensory properties

Statistical analysis of sensory scores obtained from 11 semi-trained panelist using 9-point hedonic rating scale (9= like extremely, 1= dislike extremely) for sesame seed tofu formulation. Panelists are those who have tasted tofu. The ANOVA and LSD table for sensory evaluation are presented in Appendix B.

Here A (100% soybean + 0% sesame seed), B (92.5% soybean + 7.5% sesame seed), C (85% soybean + 15% sesame seed), D (77.5% soybean + 22.5% sesame seed), E (70% soybean + 30% sesame seed) as per design expert.

4.3.1 Color

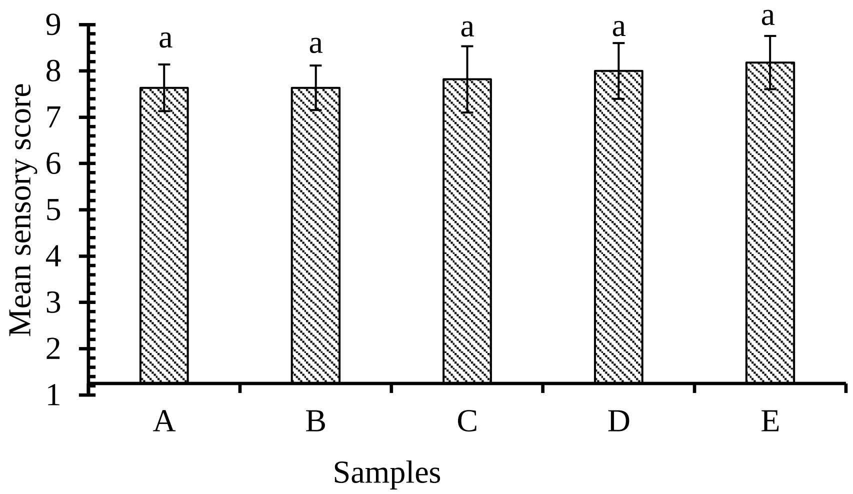

Fig. 4.1 The mean sensory score of different samples in terms of color

Here A, B, C, D and E denotes 0%, 7.5% 15%, 22.5%, and 30% sesame seed respectively. Vertical errors bar represents ± standard deviation of scores given by 11 panelist.]

The mean sensory score for color were found to be 7.636, 7.636, 7.818, 8.000 and 8.182 for the tofu formulation A, B, C, D, and E respectively. Statistical analysis showed that partial substitution of soybean with sesame seed had no significant effect ($p<0.05$) on the color of the different tofu formulation. Product E got the highest score from other formulation which is shown in Fig. 4.1. Similar trend was seen in the research of sesame seed incorporation in tofu (Ifesan *et al.*, 2012). The color of soy tofu and sesame incorporated tofu was same due to the use of white sesame seed in tofu preparation.

4.3.2 Flavour

The mean sensory score for flavour was found to be 7.273,7.364, 7.455, 8.091 and 8.636 for the Tofu formulation A, B, C, D and E respectively. Statistical analysis showed that the partial substitution of soybean with sesame seed had significant effect ($p<0.05$) on the flavour of the different tofu formulations. Product A, C and D were not significantly different to each other but significantly different to other which can be seen in Fig. 4.5. Similarly, product B and E were also not significantly different. Product E had a high mean score. The flavor of E (70% soybean flour and 30% sesame seed) and A (100% soybean) were found to be significantly superior.

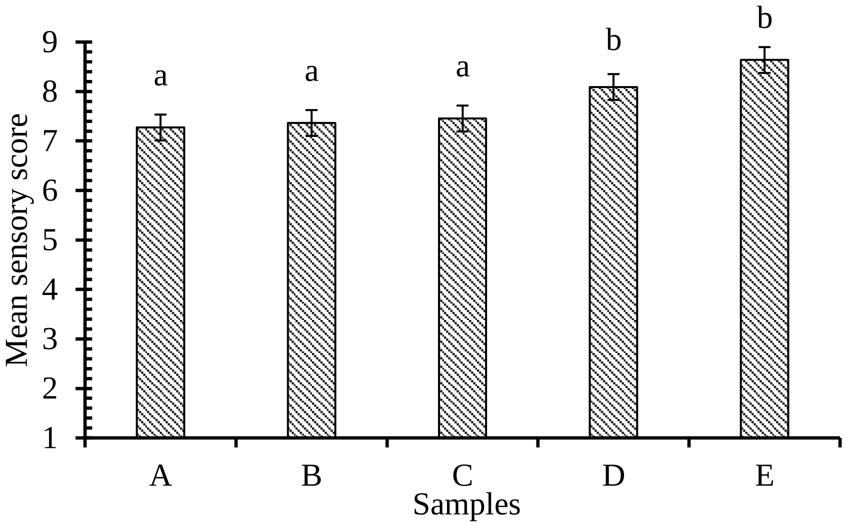

Fig. 4.2 The mean sensory score of different samples in terms of flavor

Here A, B, C, D and E denotes 0%, 7.5% 15%, 22.5%, and 30% sesame seed respectively. Vertical errors bar represents ± standard deviation of scores given by 11 panelist.]

Product E had the highest score compared to product A. Sesame incorporated tofu significantly affect the panelist acceptance concerning the flavour. This is because sesame seeds have a strong flavour and thus affect much overall flavour of tofu.

4.3.3 Texture

The mean sensory score for texture were found to be 7.545, 7.727, 7.909 and 8.273 for the tofu formulations A, B, C, D and E respectively. Statistical analysis showed that partial substitution of soybean with sesame seed had significant effect ($p < 0.05$) on the texture of different tofu formulations. Products A and E were significantly different from each other but the rest of the samples were not significantly different from other, which is shown graphically in Fig. 4.3. Product E got the highest score while product A recorded the lowest score.

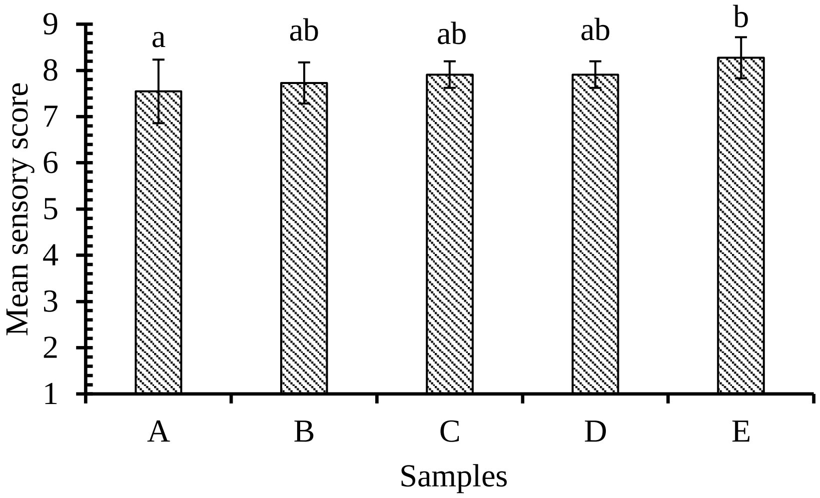

Fig. 4.3 The mean sensory score of different samples in terms of texture

Here A, B, C, D and E denotes 0%, 7.5% 15%, 22.5%, and 30% sesame seed respectively. Vertical errors bar represents ± standard deviation of scores given by 11 panelist.]

Product E was found to have a firmer texture as compared to other products. Generally, in processed foods hardness decreases with increasing fat content, thus, the product E which has a combination of 30% sesame and 70% soya was found superior to other products.

4.3.4 Taste

The mean sensory score for taste were found to be 7.273, 7.636, 7.636, 7.920 and 8.727 for the tofu formulations A, B, C, D and E respectively. Statistical analysis showed that partial substitution of soybean with sesame seed had significant effect ($p < 0.05$) on the taste of the different tofu formulations. Products A and E were significantly different to each other but rest of the sample were not significantly different from other, which is shown graphically in Fig. 4.4. Product E got highest score while product A recorded lowest score.

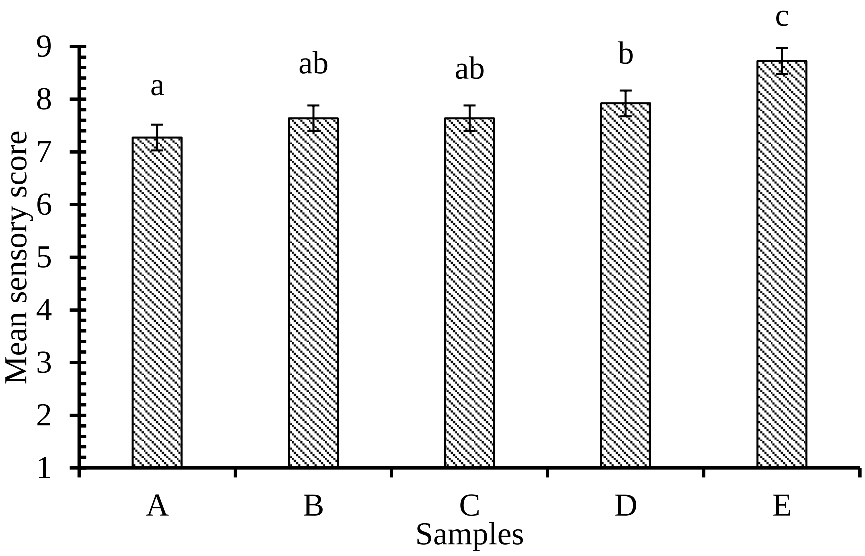

Fig. 4.4 The mean sensory score of different samples in terms of taste

Here A, B, C, D and E denotes 0%, 7.5% 15%, 22.5%, and 30% sesame seed respectively. Vertical errors bar represents ± standard deviation of scores given by 11 panelist]

The high value in tofu taste means that the savoury taste is high. This is a result that is proportional to the crude protein and crude fat content. In particular, the higher the crude fat content, the higher the savoury taste. As fat content of sesame is greater than soybean, optimum product (product E) was richer in fat content than that of control product (product A).

4.3.5 Overall acceptability

The overall score for different tofu products were obtained as 7.455, 7.727, 7.909, 8.091 and 8.455 respectively. Statistical analysis showed that partial substitution of soybean with sesame seed had significant effect ($p < 0.05$) on the overall acceptability of the different tofu formulations. Products A and E and products B and E were significantly different from each other but the rest of the products were not significantly different from other, which is shown graphically in Fig 4.5. Product E was the most while A was the least accepted by panelist.

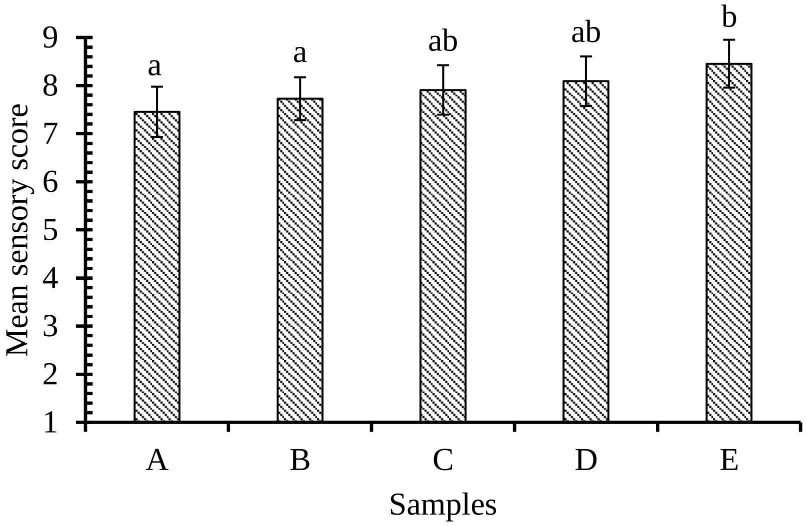

Fig. 4.5 The mean sensory score of different samples in terms of over acceptability

Here A, B, C, D and E denotes 0%, 7.5% 15%, 22.5%, and 30% sesame seed respectively. Vertical errors bar represents ± standard deviation of scores given by 11 panelist]

The color, flavour, texture and taste of sample E were very much liked. Concerning the control i.e., sample A, sample E got a high score in terms of overall acceptability as shown in Fig. 4.5. Therefore, the overall acceptability of the tofu up to 30 parts substitution by sesame seed sample E was found to be significantly superior based on the sensory characteristics of tofu.

4.4 Yield of products

Yield obtained from 500 g of total raw material of product A (100% soybean) and product E (30% soybean and 30% sesame seed) was 546.6 g and 499.6 g respectively. Sesame incorporation had a negative effect on tofu yield. Decrease in fresh tofu yield was recorded with the increase in sesame substitution.

Jayasena *et al.* (2010) reported a similar decrease in the yield when lupin was incorporated into tofu. There could be two main reasons for lower yield of sesame incorporated tofu. One could be the lower moisture contents of sesame-incorporated tofu samples. Tofu samples prepared by incorporating 30% sesame seed had significantly lower moisture contents than the control. This could be due to the differences in moisture-holding capacities of sesame and soy proteins. It was supported by the fact that the moisture-free yield of tofu showed a lesser rate of yield reduction than that of fresh yield of tofu. Another reason for the decrease in tofu yield with increasing sesame substitution might be the high pH of sesame milk (maintained at 8.0) that affects coagulation.

4.5 Physical analysis of products

4.5.1 Density of the product

The density of tofu (sample A) and sesame incorporated tofu (sample E) were determined. The result is shown in Table 4.3 below:

Table 4.3 Density of control and optimized sample.

Physical properties	Density (g/cm^3)
Sample A	1.12±0.09
Sample E	1.13±0.03

[Values are the means of three determinations ± standard deviations. Figures in the parenthesis are standard deviations.]

The density of the control sample was found to be 1.12 g/cm^3 whereas the density of best tofu was 1.13 g/cm^3. There was no change in the density of tofu when sesame seed was incorporated.

4.6 Proximate composition of products

The composition of the best product and the control tofu from chemical analysis was carried out. The result of the analysis is given in the Table 4.4.

Table 4.4 Proximate composition of products

Parameters	Product A (Control)	Product E (Best)
Moisture % (wet basis)	$64.956^{a}\pm0.11$	$58.86^{b}\pm0.59$
Crude Protein % (dry basis)	$61.67^{a}\pm0.327$	$44.12^{b}\pm0.51$
Crude Fat % (dry basis)	$29.793^{a}\pm0.97$	$41.21^{b}\pm0.83$
Crude Fibre % (dry basis)	$1.489^{a}\pm0.065$	$0.677^{b}\pm0.011$
Total ash % (dry basis)	$3.167^{a}\pm0.255$	$4.37^{b}\pm0.139$
Carbohydrate % (dry basis)	$3.92^{a}\pm0.552$	$9.3^{b}\pm0.257$
Calcium (mg)	$187.49^{a}\pm0.06$	$273.373^{b}\pm0.81$
Iron (mg)	$9.28\ ^{a}\pm0.13$	$19.67^{b}\pm0.19$
Methionine (g/16g N)	$1.175^{a}\pm0.01$	$2.47^{b}\pm0.01$

*Values are the means of triplicates and figures in the parenthesis are standard deviation of the triplicates. Values in the column having different superscripts are significantly different at a 5% level of significant.

The moisture content, protein, fat, crude fibre, ash and carbohydrate of product A were found to be 64.596%, 61.67%, 29.793%, 1.489%, 3.167% and 3.92% respectively and that of product E was found to be 58.86%, 44.12%, 41.21%, 0.677%, 4.37% and 9.3% respectively. The LSD shows that these proximate values are significantly different from product A. It is observed that there is a significant difference in the moisture content of product A and product E with the moisture content of 65.596% and 58.86% respectively. Moisture content decreased significantly ($p < 0.05$) in tofu incorporating sesame as compared to control. Previous studies confirmed a similar trend in which incorporation of sesame seed decreased the moisture content of tofu either significantly or insignificantly (Ifesan *et al.*, 2012). The low moisture content of tofu prepared with sesame incorporated may provide them with superior shelf life as compared to control.

Protein content in tofu showed an insignificant decreased ($p > 0.05$) with the incorporation of sesame seed, ranging from 61.67.% to 44.12%. This is due to the greater protein content of soybean. Previous studies confirmed a similar trend in which incorporation of a lupin replacer decreased the protein content of tofu, either significantly or insignificantly (Jayasena *et al.*, 2010). We can increase protein content by removing oil from sesame seeds.

The ash content of tofu increased in sesame seed incorporated tofu. The increase in ash content may be due to the high mineral content in the sesame seed incorporated in tofu i.e., calcium and iron. The high ash content in the sesame seed supplemented food would be of nutritional importance in most developing countries like Nepal. Sesame seed contains a lot of minerals, confirmed by its higher ash content.

After the incorporation of sesame seed, there was a significant increment of fat content from 29.793% to 41.21% at 5% level of significant. The higher fat content was due to the incorporation of sesame seed which contains a high percentage of fat.

Sesame seed incorporated tofu showed a decrease in fibre content. The crude fibre ranged from 4.66% in tofu (Product A) to 2.46% in the sesame seed incorporated tofu (Product E). This is due to the lower crude fibre present in the sesame compared to soybean.

Carbohydrate content significantly increased ($p < 0.05$) following the increment of sesame seed incorporation, from 3.92% (Product A) to 9.3 (Product E). Sesame seed incorporation contributed to the increase in carbohydrate content in tofu formulation since other ingredients were kept at a constant amount.

Methionine content is lowest in soybean and highest in sesame seeds. After incorporation of sesame seed, methionine content was increased from 1.175 g/16g N to 2.47 g/16g N in sesame-incorporated tofu. The high content of methionine in diet could be beneficial as it regulates metabolic processes, the innate immune system, and digestive functioning in mammals. It also intervenes in lipid metabolism, activation of endogenous antioxidant enzymes such as methionine sulfoxide reductase A, and the biosynthesis of glutathione to counteract oxidative stress. In addition, methionine restriction prevents altered methionine/transmethylation metabolism, thereby decreasing DNA damage and carcinogenic processes and possibly preventing arterial, neuropsychiatric, and neurodegenerative diseases (Martínez *et al.*, 2017). After this, there is a complete essential amino acid.

4.7 Shelf life evaluation of products

The shelf life of the tofu was studied for 6 days with triplicate products. The products were stored in ambient temperature and refrigerated conditions. The total plate count, coliform test, yeast and mold, and moisture content of the product were evaluated from the date of manufacture up to 6 days. Figure 4.6 shows change in total plate count during storage at a refrigerated temperature of product A and product E.

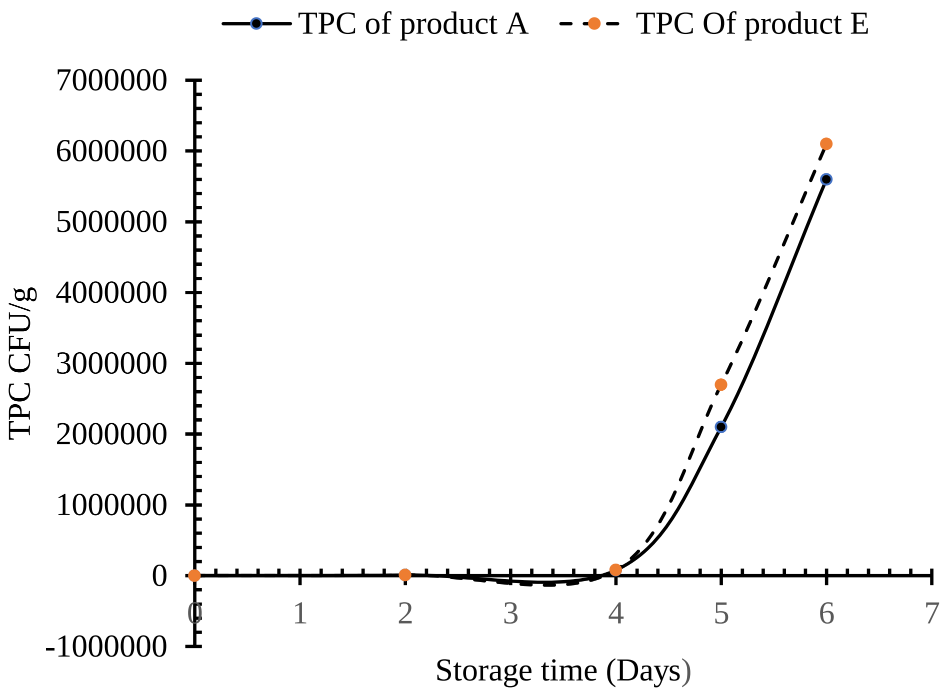

Fig. 4.6: Change in total plate count during storage at a refrigerated temperature of product A and product E

At ambient temperature, on 1st day total plate count of product A and product E was 1.6×10^3 and 1.8×10^3. At refrigerated temperature total plate count of product, A was observed to be 9.4×10^3, 7.8×10^4, 2.1×10^6 and 5.6×10^6 within 2, 4, 5 and 6 days respectively.

Similarly, for the product E the total plate count was observed to be 9.7×10^3, 8.18×10^4, 2.7×10^6 and 6.1×10^6 respectively. The change in total plate count of product A and product E at refrigerated temperature is shown in Fig. 4.6. Here, products A and B has crossed the permissible level till the 6th day of analysis. The coliform in the MacConkey agar was nil till the last day of analysis. The increase in the total plate count of product A and product E is as shown in Fig. 4.6.

The high moisture content in tofu (maximum of 90%) with excessively high pH (ranging from 6.0 to 7.0) makes it an appropriate environment for spoilage and pathogenic microorganisms, which would require thermal treatment to enhance or retain the safety and prolong shelf life (Ali *et al.*, 2021). The thermal process and freezing process are two of the most essential stages of tofu-making and negatively affect the quality of the final product, especially its texture and nutritional value (Ali *et al.*, 2021). So, product A and product E was kept at refrigerated temperature to observe the storage life of tofu.

At refrigerated temperature product A and product E was fit for consumption for 5 days. On the 5th day product undergoes toward marginal. After the 6th day product was unacceptable, sour and probably contaminated. Deviation from the original texture, color, flavor, and odour of the finished product were considered manufacturing defects in fatty product, which might be due to lipid oxidation. Lipid oxidation reduces the shelf life of product and nutritive value of stored product by affecting its vitamins and mineral contents. At the same time, lipid oxidation might also result in the susceptibility of the product to microbial spoilage (Rekha & Vijayalakshmi, 2013). As compared to soy tofu, sesame seed incorporated tofu had a high total plate count. This might be due to the high nutritional content of sesame-incorporated tofu which made it likely to microbial spoilage (Lee *et al.*, 2018). There were no colonies of coliform. No colonies of yeast and mold was found in day 6 for product A and product E at refrigerated temperature respectively.

4.8 Cost evaluation of products

The total cost associated with the control and best product was calculated and the cost of soy tofu (sample A) and sesame seed incorporated tofu (sample E) was NRs. 99.159 and NRs. 144.534 per 500 g tofu respectively including overhead cost and profit of 10%. The cost calculation is given in appendix D at Table D.1 and D.2.

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of the work done, following conclusions can be drawn.

1. Tofu formulation containing 30 parts (w/w) sesame seed was found to be appropriate formulation on the basis of sensory score.
2. There was significant difference in taste of tofu up to 30% substitution of sesame seed.
3. Analysis of soybean and sesame seed showed the significant increase in case of fat, fibre, carbohydrate and minerals in the latter as a result of which the nutritional quality of the tofu also seemed to be enhanced in the case of the sesame.
4. The chemical and microbiological analysis of product showed acceptability of sesame seed incorporated tofu was up to five days at refrigerated temperature without any artificial preservatives used.
5. Cost of optimized tofu per 500 g was found to be Rs. 144.534 which include the processing, manpower cost and profit margin.

5.2 Recommendations

The experiment can be further continued with the following recommendations:

1. Entrepreneur can utilize sesame seed up to 30 parts to enrich nutritional value of general tofu without hampering consumer acceptance.
2. Texture of the prepared tofu can be analysed using texture meter.
3. Av and PV value of tofu can be analysed.

Part VI

Summary

Tofu, a highly nutritious gel-like food product manufactured from soybean, is commonly consumed as a traditional food in many Asian countries. It has become globally popular because of its inclusion in vegetarian, vegan, and hypocaloric diets. Further value of Tofu can be added by incorporating sesame seed. Incorporation of soybean with sesame seed to make tofu provides a good opportunity to improve the nutritional quality of the fat, minerals like calcium, iron and magnesium, vitamin E and methionine content consumed by many people due to which helps to rise the nutritional status of the population. However, it has several uses it is not utilized in commercial way in context of Nepal.

A study was carried out to know about the effects of incorporation of sesame seed on tofu quality. Design expert (Response surface methodology) was used to design the experiment for formulating the recipe of tofu. Five different tofu formulation namely A (0 % sesame seed), B (7.5% sesame seed), C (15% sesame seed), D (22.5% sesame seed) and E (30% sesame seed) were prepared by tofu mixing process. Sensory evaluation was carried out based on color, flavour, taste, texture and overall acceptability. The data obtained were statistically analysed using two-way ANOVA (no blocking) at 5% level of significance. Product E (30% sesame seed and 70% soybean) got the highest mean sensory score after the product A (100% soybean). Moisture content, protein content and crude fibre were decreased after addition of sesame from 64.596% to 58.86%, 61.67% to 44.12% and 1.489% to 0.677%.

The 1000 kernel wt., and bulk density of soybean were 195.093g and 3.19g and sesame were 84kg/HL and 60.5kg/HL. Yield obtained from 500 of total raw material of product A (100% soybean) and product E (30% soybean and 30% sesame seed) was 546.6g and 499.6g respectively. Calcium and iron content was increased from 187.496 to 273.373 and 9.28 to 19.67 respectively. Methionine content is also increased from 1.175 to 2.47. The product was analysed for prediction of shelf life based on TPC (bacteria, yeast and mold). No colony of coliform were found from day 0 to day 5.

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Appendices

Appendix A

Sensory analysis score card

Name of the panelist: Date:

Name of the product: sesame seed incorporated tofu

Dear panelist, you are provided with 8 samples of sesame seed incorporated tofu on each proportion with variation on sesame seed content. Please test the following samples of tofu and check how much you prefer for each of the samples. Give the point for your degree of preference for each sample as shown below.

Judge the characteristics on the 1-9 scale as below:

Like extremely – 9
Like very much – 8
Like moderately – 7
Like slightly – 6
Neither like nor dislike – 5
Dislike slightly – 4
Dislike moderately – 3
Dislike very much – 2
Dislike extremely – 1

Parameters	Sample code							
	A	B	C	D	E	F	G	H
Color								
Flavor								
Taste								
Texture								
Overall acceptance								

Any comments:…………………………………………………………………………

Signature

Appendix B

ANOVA results of sensory analysis

Table B. 1 ANOVA (no interaction) for color of sesame seed incorporated Tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tofu type	4	2.4727	0.6182	1.73	0.163
Panelist	10	4.0364	0.4036	1.13	0.367
Residual	40	14.3273	0.3582		
Total	54	20.8364			

Table B. 2 ANOVA (no interaction) for flavour of sesame seed incorporated Tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tofu type	4	15.0182	3.7545	14.75	<.001
Panelist	10	4.7273	0.4727	1.86	0.081
Residual	40	10.1818	0.2545		
Total	54	29.9273			

Table B. 3 ANOVA (no interaction) for texture of sesame seed incorporated tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tofu type	4	3.2000	0.8000	3.48	0.016
Panelist	10	1.7091	0.1709	0.74	0.680
Residual	40	9.2000	0.2300		
Total	54	14.1091			

Table B. 4 ANOVA (no interaction) for taste of sesame seed incorporated tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tofu type	4	13.7455	3.4364	11.22	<.001
Panelist	10	2.1091	0.2109	0.69	0.729
Residual	40	12.2545	0.3064		
Total	54	28.1091			

Table B. 5 ANOVA (no interaction) for overall acceptability of sesame seed incorporated tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tofu type	4	6.2545	1.5636	6.99	<.001
Panelist	10	4.5091	0.4509	2.02	0.057
Residual	40	8.9455	0.2236		
Total	54	19.7091			

Appendix C

Table C.1 t-test (two- product assuming unequal variance) for moisture of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	64.95666667	58.86666667
Variance	0.018233333	0.531733333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	14.22360673	
P(T<=t) one-tail	0.002453268	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.004906536	
t Critical two-tail	4.30265273	

Table C.2 t-test (two- product assuming unequal variance) for protein of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	29.73	41.21333333
Variance	0.0372	1.039033333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-19.17235136	
P(T<=t) one-tail	0.001354726	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002709452	
t Critical two-tail	4.30265273	

Table C.3 t-test (two- product assuming unequal variance) for crude fat of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	29.73	41.21333333
Variance	0.0372	1.039033333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-19.17235136	
P(T<=t) one-tail	0.001354726	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002709452	
t Critical two-tail	4.30265273	

Table C.4 t-test (two- product assuming unequal variance) for crude fibre of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	1.489666667	0.677966667
Variance	0.006362333	0.000258503
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	17.27824931	
P(T<=t) one-tail	0.001666461	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.003332922	
t Critical two-tail	4.30265273	

Table C.5 t-test (two- product assuming unequal variance) for total ash of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	3.167333333	4.37
Variance	0.097729333	0.0292
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.846889713	
P(T<=t) one-tail	0.004985667	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.009971333	
t Critical two-tail	3.182446305	

Table C.6 t-test (two- product assuming unequal variance) for carbohydrate of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	3.926666667	9.3
Variance	0.458633333	0.0997
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-12.45539864	
P(T<=t) one-tail	0.000557674	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.001115349	
t Critical two-tail	3.182446305	

Table C.7 t-test (two- product assuming unequal variance) for calcium of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	187.4966667	273.3733333
Variance	0.009233333	0.996133333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-148.3452228	
P(T<=t) one-tail	2.27192E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	4.54384E-05	
t Critical two-tail	4.30265273	

Table C.8 t-test (two- product assuming unequal variance) for iron of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	9.28	19.66666667
Variance	0.0283	0.055433333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-62.1709683	
P(T<=t) one-tail	2.00457E-07	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	4.00915E-07	
t Critical two-tail	2.776445105	

Table C.9 t-test (two- product assuming unequal variance) for methionine of the optimum product (product E) with control (product A)

	Product A	*Product E*
Mean	1.175	2.47
Variance	0.000002	0.0002
Observations	2	2
Hypothesized Mean Difference	0	
df	1	
t Stat	-143.889	
P(T<=t) one-tail	0.002212	
t Critical one-tail	6.313752	
P(T<=t) two-tail	0.004424	
t Critical two-tail	12.7062	

Appendix D

Table D.1 Cost calculation of the control (sample A)

Particulars	Cost (Rs/kg)	Weight (g)	Cost (Rs
Soybean	160	500	80
Potash alum	200	9.75	1.95
Raw material cost			81.95
Processing and labor			8.195
(10% of raw material cost)			
Profit (10%)			9.014
Grand total cost (Rs/500g)			99.159

Table D.2 Cost calculation of the control (sample E)

Particulars	Cost (Rs/kg)	Weight (g)	Cost (Rs)
Soybean	160	350	56
Sesame seed	410	150	61.5
Potash alum	200	9.75	1.95
Raw material cost			119.45
Processing and labor			11.945
(10% of raw material cost)			
Profit (10%)			13.139
Grand total cost (Rs/500g)			144.534

Appendix E

Apparatus

1. Pressing machine
2. Muslin cloth
3. Grinder
4. Heating arrangement
5. Thermometer
6. Digital electronic balance
7. Beaker
8. Volumetric flask
9. Measuring cylinder
10. Conical flask, funnel, test tube
11. Soxhlet assembly
12. Bushner filter assembly
13. Petriplate
14. Dean and stark apparatus
15. Hot air oven
16. Filter paper
17. Screw press for water holding capacity

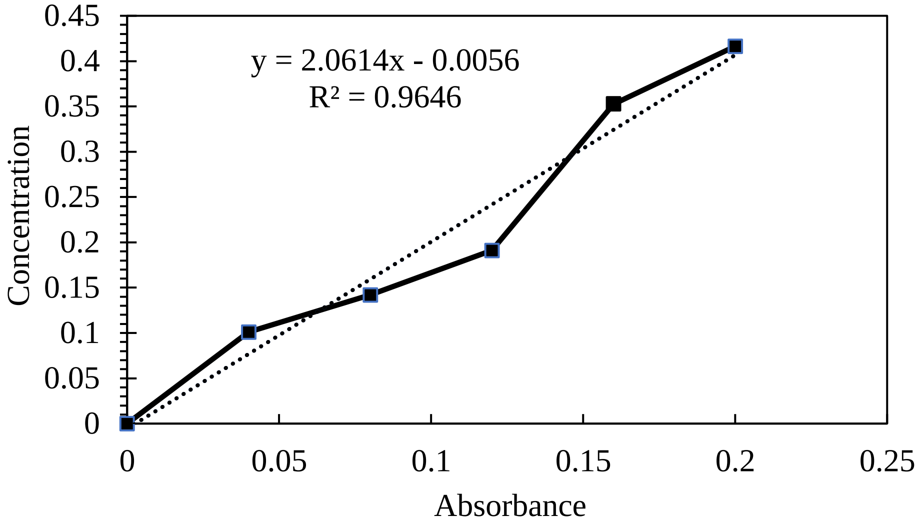
Chemical required

1. Potassium aluminium sulphate
2. Petroleum ether
3. Acetone
4. Sulfuric acid
5. Sodium hydroxide
6. Oxalic acid
7. Hydrochloric acid
8. Boric acid
9. Catalyst mixture
10. MacConkey agar
11. Plate count agar

12 Alcohol
13 Phenolphthalein
14 Sodium nitroprusside
15 Glycine
16 Orthophosphoric acid
17 Dl-methionine

Appendix F

Standard curve of methionine content



Color plates

P1: Sesame incorporated tofu

P2: Sesame incorporated fried tofu

P3: Microbilogical analaysis

P4: Vortex

P5: Crude fibre determination