EFFECT OF SPROUTING AND DIFFERENT PACKAGING MATERIALS ON THE QUALITY OF TOFU

by

Ashmita Koirala

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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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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Ashmita Koirala

Department of Food Technology

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Institute of Science and Technology

Tribhuvan University, Nepal

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Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Effect of Sprouting and Different packaging Materials on the Quality of Tofu*** presented by **Ashmita Koirala** has been accepted as the partial fulfilment of the requirement for the **B. Tech. degree** in **Food Technology.**

<u>Dissertation Committee</u>

1. **Head of Department** ____________________________________

   (Mr. Navin Gautam, Asst. Prof.)

2. **External Examiner** ____________________________________

   (Mr.Bijay Khanal, S.F.R.O, DFTQC)

3. **Supervisor** ____________________________________

   (Mrs. Mahalaxmi Pradhananga, Asst. Prof.)

4. **Internal Examiner** ____________________________________

   (Mr. Sabin Bdr. Khatri, Teaching Asst.)

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(Ashmita Koirala)

Abstract

The study was focused on the effect sprouting of soybean on yield, proximate composition, and sensory quality of tofu and different packaging materials (LDPE, PP, metalised plastic) on the chemical (moisture, PH, peroxide value, acid value), microbial quality (TPC, yeast and mold count) of sprouted tofu stored in refrigerated temperature (4°C). Tofu from unsprouted and sprouted soybean was prepared. The sensory analysis of sprouted tofu was carried out for consumer acceptability. The obtained data were statistically analysed using two-way ANOVA (no blocking) at 5% level of significance. The sprouted tofu was packed in LDPE, PP and metalised plastic and stored in refrigerated temperature (4ºC).

From the sensory evaluation, sprouted tofu got highest mean sensory score. The levels of crude protein and crude fiber were higher in the product made from sprouted soybean, whereas the levels of fat, ash, and carbohydrates were found to be decreased. The chemical and microbiological analysis of sprouted tofu packed in LDPE, PP and metalized plastic was carried out in interval of 3 days for 27 days of storage period but the sprouted tofu packed in LDPE was observed until 18th days only because it was observed that the sample in LDPE there was total off odor, yellowish in color, stickiness etc. On analysis of packaged product inside LDPE, PP and metalized plastic, PH and moisture content of the sample packed in metalised plastic decreased at a slower rate in comparison to sample packed in LDPE and PP. Peroxide value, acid value, TPC and yeast and mold count of the sample packed in LDPE increased at a higher rate in comparison to sample packed in PP and metalised plastic increased. While contrasting the yield % between the regular and sprouted tofu, the value of sprouted tofu was decreased slightly than that of regular tofu.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
TPC	Total plate count
SD	Standard deviation
LDPE	Low Density Polyethylene
PP	Polypropylene
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
CCT	Central Campus of Technology

Part I

Introduction

1.1 General Introduction

Soybean *(Glycine max L.)* is a leguminous crop that originated in China. Soybean is the most nutrient-dense natural vegetable food. The soybean is known as the "King of Legumes" because it has more protein (40%) and fat (19%) and less carbohydrate (33.3%) than practically all other pulses (Sharma, 1997). Soybean *(Glycine max),* a member of the Leguminosae family, is the world's most significant seed legume, accounting for 25% of worldwide edible oil and almost two-thirds of global protein concentrate for livestock feeding. Soybean includes a variety of nutraceutical substances, including isoflavones, tocopherol, and lecithin, in addition to 20% oil and 40% protein (Agarwal *et al.*, 2013).

Tofu is a curd manufactured from soybeans *(Glycine max)* that is made by curdling fresh hot soymilk with a coagulant. It can be eaten fresh, pickled, or smoked. Tofu is cholesterol-free, low in saturated fat, and high in protein (Ojha *et al.*, 2014). Coagulation of soymilk plays an important role in the tofu-making process. Commercial coagulants are classified into two types: salts and acids. It comes in both bulk and individual bags, both refrigerated (Pal *et al.*, 2019).

Sprouted tofu is a terrific alternative to ordinary tofu because it is more nutritious, easier to digest and includes more protein, calcium, iron, and omega-3 fats (Ojha *et al.*, 2014). Sprouting is a non-thermal, non-chemical technique that results in higher quality processed products such as milk and tofu. Similarly, sprouted bean tofu has a higher tofu index, good color and textural qualities (D.Agrahar, 2014). It is the practice of soaking and leaving seeds until they germinate and begin to sprout. This practice is reported to be associated with improvements in the nutritive value of seeds (Ojha *et al.*, 2014).

Tofu can be filled in bulk, in plastic bags, in heat sealed water-filled tubes or vacuum packed (William Shurtleff and Aoyagi, 2000). Tofu-based product's quality and shelf-life are impacted by the materials used for packaging and storage. However, their high moisture and protein content make them ideal for bacterial development, resulting in a short shelf life even in refrigerated conditions (Zheng *et al.*, 2020).

1.2 Statement of problem

Tofu is a nutritionally dense, protein-rich soybean food. It is usually made by coagulating heated soy milk with a coagulant, then moulding and pressing to achieve the desired texture (Tiande Cai and Chang, 1999). For moral, ecological, and health-related reasons, a great number of people have expressed a wish to consume fewer animal products. The great nutritional value of tofu and its incorporation into vegetarian and low-calorie diets increase its consumption.

Sprouted tofu is an excellent alternative to regular tofu since it is easier to digest and includes more protein, deity fiber, etc. It is known for decreasing beany and grassy tastes (Ojha *et al.*, 2014). Despite being highly nutritious, short shelf life of tofu is a major problem. Tofu is a highly perishable product and suffers from limited shelf life, largely because of its high moisture content, has a maximum shelf-life of 1-2 days even when refrigerated commercially. The ultimate quality and shelf life of products based on tofu are influenced by the choice of packaging materials and storage technologies (Tkaczewska *et al.*, 2023).

1.3 Objective

1.3.1 General objective

The general objectives of the dissertation work are to study the effect sprouting and different packaging materials on the quality of tofu made from sprouted soybean.

1.3.2 Specific objectives

1. To analyze the raw material i.e. raw soybean.
2. To study the effect of sprouting on the physio-chemical properties of soybean.
3. To prepare and analyze the regular and sprouted tofu.
4. To evaluate the sensory properties of regular and sprouted tofu.
5. To study the shelf life of sprouted tofu by using different packaging materials.

1.4 Significance of the study

This study will facilitate the possibility of employing sprouting as a non-chemical, non-thermal technique to improve tofu quality and also promotion of use of different packaging materials to extend the shelf life. This will appeal the consumers who tend to avoid the tofu due to its beany and gassy flavours. Nowadays, people generally focus on their health, so consumption of sprouted tofu will be very beneficial as it is a source of protein which is available in more digestible form, fiber and minerals. The presence of fiber in tofu made from sprouted soybean in significant levels aids in the treatment of gastrointestinal disorders. Since, it is perishable in nature there is a need of proper processing for its long-term storage including packaging which will add value to the compound and would also get a significant market value.

1.5 Limitations of the study

1. Only one variety of soybean (Brown variety) was taken.
2. Change in sensory and chemical parameters could not be studied on daily basis.
3. Only three types of packaging materials were used to study the shelf life of sprouted tofu.
4. Textural analysis of the product was not carried out due to the lack of an instrument.

Part II

Literature review

2.1 Historical Background

Soybean cultivation originated in China. During the first part of the twenty-first century, China was the world's top producer and exporter of soybeans (Qiu LiJuan and Chang RuZhen, 2010). 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).

Since the Chinese Han dynasty, tofu has been made in China for over 2,000 years. Tofu is a type of bean curd made from soybeans that is widely available across the country (Zhang et al., 2017). Tofu is first mentioned in Japan around 1185 A.D. Tofu is currently widely produced throughout East Asia, with over 29,000 manufacturers in Japan alone (W Shurtleff and Aoyagi, 1979). In January 1984, there were 191 commercial tofu manufacturers in the United States, 75 in Western Europe, and 33 in Canada (William Shurtleff and Aoyagi, 1985).

2.2 Soybean

2.2.1 Introduction

The soybean is a dicotyledon seed (two cotyledons) held together by the hull. The soybean seed is made up of three key components: the seed coat or hull, the cotyledons, and the germ or hypocotyl. The hilum, which is the point of attachment to the pod, is included within the seed coat. On removal of the hull, the cotyledons separate and the germ is dislodged. The commercial soybean contains about 8% hull, 90% cotyledons, and 2% hypocotyl (Perkins, 1995).

Soybean *(Glycine max)* is one of the most common crops. It is a diploidized tetraploid (2n=40) from the Leguminosae family, subfamily *Papilionoideae*, tribe *Phaseoleae*, genus *Glycine Willd*, and subgenus *Soja (Moench)*. 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," because it is largely used for seed production (CFIA, 1996).

2.2.2 Classification of Soybean

Table 2.1 Classification of soybean

Taxon	Scientific name and common name
Kingdom	*Plantae* (plants)
Subkingdom	*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)
Spices	*Glycine max* (L.) Merr. (soybean)

Source : CFIA (1996)

Despite the fact that only 10% of the world's soybean production is used for human consumption, the bean is a key ingredient in many other goods. Many of these soy products are made from soybeans, while others get their protein from isolated soy proteins, soy protein concentrate, soy flour, or soy milk. Fermented and non-fermented soy foods are the two categories that are most frequently used. Traditional fermented foods include natto, miso, tempeh, and fermented tofu. Traditional soy foods without fermentation include tofu, okara, and soynuts (Shrestha, 2017).

2.2.3 Physical properties of soybean

Different varieties, environmental circumstances, and agronomical conditions can affect the physiochemical attributes of soybean varieties. According to reports, the average bulk density and weight of 1000 kernels of Nepalese soybeans were 0.767 g/cc and 153.15 g, respectively. Soybean seeds have a weight range of 120 to 180 mg, of which 10% is hull (Katwal, 1984).

Size, hardness, density, and appearance are some of the physical factors that are frequently taken into account while selecting soybean for tofu production. The chemical contents of soybean, as well as their interactions with water, other ingredients, temperature, and pH, are important.

Table 2.2 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)

2.2.4 Chemical composition and nutrient of soybean

The average figures for the major nutrients are 40% protein, 20% lipid, 35% Carbohydrate and 5% ash on dry weight basis. The moisture content of mature soybean is 12-15% Snyder and Kwon, 1987. Soybean seeds contain a protein concentration of 35-40% by dry weight, making them a reasonably priced source of protein for human consumption (Derbyshire *et al.*, 1976). The majority of soy protein is globulin (a salt soluble portion), which can be extracted with water. These are moderately insoluble at pH near to their isoelectric pH (4.5), with solubilities peaking between 2.0 and 3.5 and above 7.0 pH. Soy proteins, which contain both polar and nonpolar amino acids, can bind to water and lipids and hence act as protective colloids and emulsion stabilizers. Protein is another important commercial component of soybeans. Soy protein has an amino acid composition that complements cereal protein, although it is limited in methionine and sulphur-containing amino acids for most animals, including humans (Snyder and Kwon, 1987).

Soybean protein contains proteinaceous substances known as trypsin inhibitors, which inhibit protein digestion, and hemagglutinins (lectins), which are important nutritionally but must be heat-inactivated because they have a negative impact on the nutritional quality of soybean protein. Goitrogens and the antivitamins D, E, and B12 can also be found in soybeans (Liener, 1981).

The small components of refined soy oil fraction, such as phospholipids and lecithin, can alter the colour and stability of the refined oil. Soy oil contains around 80% unsaturated fatty acids, with linoleic being the most prevalent unsaturated fatty acid (Weiss, 1983).

Soybean seeds contain a significant amount of carbohydrates, approximately 30% by dry weight. Soybean has both soluble and insoluble carbohydrates. Soybean seeds contain around 10% soluble carbohydrates, comprising 5% sucrose, 1% raffinose, and 4% stachyose. Humans do not digest or absorb the latter two nutrients. However, the human digestive tract's intestinal bacteria can metabolise these oligosaccharides, creating flatulence (Steggerda *et al.*, 1966).

Lipooxygenase, which has been utilised to bleach the carotenoids in wheat flour in place of bromides and other comparable chemical compounds, is abundant in soybeans. Raffinose, stachyose, and sucrose, which are complex carbohydrates, replace monosaccharides as the beans mature. These typically exist in mature beans at levels of 1, 4, and 5%, respectively (Snyder and Kwon, 1987). Chemical composition of raw soybean according to DFTQC is shown in table 2.3. Amino acid of soybean in terms of g/16g N is shown in Table 2.4. Mineral composition is shown in Table 2.5.

Table 2.3 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
Minerals	5 g
Fibre	4.2g
Energy	411 K Cal
Calcium	226 mg
Phosphorous	546 mg
Iron	8.5 mg
Carotene	10 µg
Thiamine	0.66 mg
Riboflavin	0.22 mg

Source: (DFTQC, 2012)

Table 2.4 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 2.5 Mineral composition per 100 g

Sample	mg/100g
Calcium	226
Phosphorous	546
Iron	8.5

Source:(DFTQC, 2012)

2.3 Tofu

Tofu is a versatile soybean food made by coagulating soymilk 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, inexpensive, and nourishing food, which is consumed by people of developed as well as developing nations. It is an excellent source of nonanimal protein in the diet of vegetarian (Pal *et al.*, 2019). The quality of the beans, the amount of stirring, the coagulants, and the pressing of the curd can all have a significant impact on the quality of tofu (C.-C. R. Wang and Chang, 1995). The texture of tofu ranges from soft to firm to extra-hard (Pal *et al.*, 2019).

2.3.1 Types of tofu

2.3.1.1 Firm Tofu

Making firm tofu, also referred to as "Cotton tofu," involves coagulating soymilk with a synthetic or natural coagulant and pressing the soymilk through cheesecloth. It is partially coagulated and pressed to remove excess water, and the tofu's moisture level ranges from 76 to 81% (DeMan *et al.*, 1986).

Natural coagulants like Phyllanthus acidus (gooseberry), Tamarindus indica (tamarind), Citrus limon (lemon), Garcinia indica (garcinia), and Passiflora edulis (passion fruit), as well as synthetic coagulants like $CaSO_4$ (calcium sulphate) and $MgCl_2$ (magnesium chloride), are used to achieve coagulation. Soaking and washing soybeans for 12 hours at room temperature produces soymilk. To create soybean slurry, ground soybean is mixed with water (1:8) and heated at 85°C for 45 minutes while stirring regularly. The heated slurry is clarified with cheese cloth to produce soymilk. Soymilk is heated to 95°C for 5 minutes, then stirred for 5 minutes and allowed to coagulate for 15 minutes with 0.2% artificial and/or 2% natural coagulant. The coagulated material is then squeezed (Rekha and Vijayalakshmi, 2010).

2.3.1.2 Silken Tofu

It has a very high water content and a creamy, smooth feel (Pal *et al.*, 2019). The moisture percentage of soft/ silky tofu is approximately 87-90% (H. Wang *et al.*, 1983).

Soft/silken tofu is made by coagulating soymilk, which is frequently done in the container in which the product is purchased by consumers. It is produced by coagulation of soymilk with salt ($CaSO_4$ or $MgCl_2$) or acid (glucono-δ-lactone). These coagulants are typically used either individually or in combination and impart textural and flavor properties to the tofu (Evans *et al.*, 1997). The raw soybean was ground and combined with 400g of hot water per (Evans *et al.*, 1997) dry technique. Soymilk was extracted manually using a centrifugal juice extractor for both methods. The coagulant concentration utilized ranged from 1.5 to 5.0 g per kg. Then, 220 ml of soymilk were placed into a plastic container, allowed to cool to room temperature, and then mixed quickly and completely with 7 ml of coagulant. To finish the curd formation, the container was sealed and indirectly heated for 35 minutes at 85°C; the lids used for sealing had holes to allow gases to escape, and the silken tofu was kept at 4°C.

2.3.1.3 Dried Tofu

Dry tofu is the firmest tofu and has a moisture level that is typically less than 76% (H. Wang and CW, 1982).

To manufacture dry tofu, raw soybeans are steeped in six times as much water at 21–22 °C for eight hours. After soaking, extra water is removed, hydrated soybeans are ground quickly, and the resulting soymilk is clarified by centrifugation. To manufacture dried tofu, soymilk was boiled for 20 minutes while containing 1 g of an antifoaming agent, and it then rested at 96°C for 3 minutes. Nigari (3%) ($MgCl_2$) is added and allowed to settle for 10 minutes in order to create curd. Before being coagulated once more, the curd is stirred for 30 seconds. The soy curd is then placed in a wooden mould that has been lined with fabric and is 25cm x 25cm x7cm, and it is squeezed for 30minutes at 34.8g per cm^2 (TD Cai and Chang, 1997)

2.3.1.4 Sufu

Sufu is a traditional and strongly flavored fermented tofu recipe in China that resembles a soft creamy-type cheese (Steinkraus, 1996). Chou and Hwan (1994) made sufu from *Aspergillus taiwanensis* (CCRC 31159) and *Aspergillus selegans* (CCRC 31342).

B. Z. Han *et al.* (2001) created red sufu by using salt to coagulate soymilk to create tofu. After that, pure *Actinomucor elegans* as 3.227 culture was sprayed over the diced tofu, and it was cultured for 48 hours at 28°C and 90% relative humidity. The salt concentration increased by 16% after 5 days of continuous salting. It was then preserved for 2 months in a sealed bottle filled with a mixture of sugar, chiang (a miso made from wheat), spices, alcohol, and kojic red rice (Japanese red yeast rice).

2.3.1.5 Low Fat Tofu

A byproduct of soy oil production, defatted soy flour, can be used to make low-fat tofu. Low-fat tofu can be made from supercritical CO_2 extracted soy flour with the same consistency as full-fat tofu. Soymilk viscosity decreases from 50 to 40 cp as a result of the flour's reduction in fat content from 19.5% to 7.1%. However, low-fat tofu has a greater overall yield (69.7%) than full-fat tofu (60.8%). The decrease in fat content has no impact on the texture or sensory properties of tofu (Shin *et al.*, 2014).

2.3.1.6 Sprouted Tofu

Tofu made from sprouted soyabean is called sprouted tofu. It is understood that during the sprouting process, a series of metabolic changes are activated, enhancing the nutritional value of sprouted legumes and lowering the levels of antinutritional elements like trypsin inhibitor and phytic acid (Murugkar and Jha, 2009).

2.3.2 Factor influencing the quality of Tofu

2.3.2.1 Varieties of soybean seeds

According to Bhardwaj *et al.* (2003) tofu yield was substantially connected with seed size, seed oil, the rate at which water was absorbed after 1 and 16 hours of soaking, and seed protein. Tofu strength was also observed to be influenced by the location of the farm where it was grown. A number of soybean genotypes from diverse regions were examined by Bhardwaj *et al.* (2003) to determine how they affected the quantities of fatty acids and oil in the tofu. The genotypes that were used were BARC-8, BARC-9, MD86-57788, Enrei, Hutcheson, Nakasennari, S90-1056, Suzuyutaka, Ware, York, V71-370, and V81-1603. Hutcheson contained more oil (24.0 g/100 g) and more total saturated fatty acids (3.80 g/100 g) than BARC-8 and BARC, which had lower oil contents of 15.8 and 11.3 g/100 g and 11.3 g/100 g, respectively. Depending on where the seeds were grown, tofu's total saturated fatty acid concentration changed.

2.3.2.2 Effect of coagulants

The coagulation process is the most important phase in the manufacture of tofu (Rui *et al.*, 2016). Different coagulants used to make tofu have an impact on its qualities, including yield, texture, colour, and sensory features. Using calcium sulphate, epsom salt, lemon juice, alum, and top water from fermented corn, Obatolu (2008) studied the coagulation of soymilk. Alum produced the least amount of tofu (442 g), while calcium sulphate produced the highest (565.7 g). The hardness, chewiness, and brittleness of tofu ranged from 525.6 to 1008 g, 2.3 to 4.5 kg, and 1035 to 1482 g, respectively. Tofu produced with GDL yielded about 404g (Jackson *et al.*, 2002).According to the Ezeama and Dobson (2019) fresh tofu was made using soybean flour and three coagulants: lime, Epson salt, and tamarind. The pH of the samples was found to be 7.20 for Epson salt, 5.00 for lime, and 5.50 for tamarind. Epson salt had the shortest coagulation time of 6 minutes. Tseng and Xiong (2009) examined the rheological properties of inulin-containing silken tofu coagulated with glucono-δ-lactone (GDL) upon heating. When inulin was added at a rate of 1 to 4%, hardness increased from 115.8 to 137.4 g, cohesiveness decreased from 0.86 to 0.82, percentage deformability ranged from 5.19 to 8.25, and rupture force increased from 424.3 to 487.9 g.

No and Meyers (2004) investigated the use of chitosan as a coagulant in the preparation of tofu with a long shelf life. Chitosans with six different molecular weights (1106, 746, 471, 224, 28, and 7 kDa) were employed in their studies with various processing settings to examine the potential of chitosan as a coagulant in tofu manufacturing. Chitosan had little impact on the features (yield and hardness) and whey (volume and turbidity) of tofu products, but it did have an effect on the sensory quality and shelf life of tofu. The best treatment for coagulating soy milk was 1% chitosan solution in 1% acetic acid at 80°C for 15 minutes.

2.3.2.3 Effect of enzymes

Yasir *et al.* (2007) studied the texture of tofu using the transglutaminase (tga) enzyme. The fracture force of the control tofu was greater than 30 N, but the fracture force of the tga-tofu was 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 more than 30 N. This demonstrated that the amount of enzyme in tofu has a significant effect on its texture.

Rizkaprilisa and Setiadi (2018) employed papain derived from papaya fruit as a novel coagulant to increase tofu yield. Soybean protein was extracted using the soybean:water ratio of 1:2. The soybean extract (200 g) was heated, and when the temperature reached 70 to 80

C, papain (3 and 6 g) and $CaSO_4$(1 and 2 g) were added. The maximum yield of tofu with 2 g of $CaSO_4$ was determined to be 66%. The greatest yield of tofu treated with 6 g of papain was 65%. They found that tofu coagulated with papain had a greater protein content (9.3%) than tofu coagulated with $CaSO_4$ (6.5%).

2.3.2.4 Effect of process parameter

The processing parameters such as solid content of milk, thermal treatment of soybeans with sodium bicarbonate, stirring time after adding coagulant, and tofu moulding of tofu have a considerable impact on its flavour, quality, and texture. Tofu softness (3.2 N) increased with blanching time, whereas control tofu had a firm texture (7.8 N). Smooth tofu was made from soymilk with a Brix solid concentration of 7° Brix after 10 minutes of thermal treatment with 1% sodium bicarbonate treated soybeans. The duration of stirring during coagulation and moulding parameters had a substantial effect on tofu yield. After adding the coagulant, stir the milk for 5 seconds before settling and pressing the tofu with a load of 1,000 g for 15 minutes, followed by 500 g for another 15 minutes yielded (22.6 g/100 ml of milk) soft textured firm tofu (Rekha and Vijayalakshmi, 2013).

2.3.3 Health Benefits of tofu

Tofu is a fantastic source of numerous nutrients, including protein, vitamins, and minerals, which are essential for maintaining good health. According to research, eating soy-based products may help reduce breast cancer, osteoporosis, and cardiovascular disease (Pal *et al.*, 2019). Additionally, soy protein consumption successfully decreased serum concentrations of triglycerides, LDL cholesterol, and total cholesterol as compared to animal proteins (Potter, 1998). Health benefits of tofu summarized by (Pal *et al.*, 2019) are given below

1. In postmenopausal women, it guards against endometrial cancer.
2. Kidney disorders are avoided.
3. It relieves the symptoms of menopause in women.
4. It enhances brain health.
5. It prevents liver damage.
6. It controls blood sugar and aids in the treatment of diabetes.

7. It increases immunity.
8. It lowers the risk of lung cancer.

2.4 Importance of sprouting

Sprouting is the process of soaking seeds and then waiting for them to germinate and sprout. Regular tofu is made from cooked soybeans, but sprouted tofu is made from sprouted soybeans. In comparison to conventional tofu, sprouted tofu contains more protein and calcium (Ojha *et al.*, 2014). Sprouting results in a sequence of metabolic changes that improve the nutritional content of sprouted legumes while decreasing anti-nutritional factors such as trypsin inhibitor and phytic acid, as well as flatulent factors (Murugkar and Jha, 2009). It is known for decreasing beany and grassy tastes. It can also increase protein content, vitamin concentration, dietary fibre, mineral bioavailability, and reduce tannin and phytic acid. Calcium levels, as well as copper, manganese, zinc, riboflavin, niacin, and ascorbic acid, may improve (Ojha *et al.*, 2014).

2.5 Preparation of regular and sprouted tofu

Soybean seeds were properly cleansed to remove dust, debris, stubbles, and foreign substances. At room temperature (RT), the seeds were soaked in tap water for 12 hours. After draining the extra water, the seeds were rinsed once more with tap water.

According to the D.Agrahar (2014), soymilk was prepared using the Soycow model SC 100 in the grinding cum blanching (GCB) unit. Seeds were arranged in a single layer on muslin cloth and placed in a RH chamber for 72 hours at a temperature of 25 °C, 90% RH. The minimal amount required for the Soy Cow model was 1 Kg of soybean per batch for both control and treatments. Dehulled soy splits were steeped in water at room temperature for 4 hours before being put into the GCB device to make control soymilk. To make the test soymilk, sprouted soybeans with 60% moisture were dehulled by hand, the hulls were discarded, and the seeds and cotyledons were fed into the unit. In the unit, water was added to the soybean in a 5:1 ratio, and a pressure of 1 kg/cm^2(15 psi) was formed by infusing culinary steam at pressures ranging from 1-3 kg/cm^2 (15 to 54 psi). The infused steam replaced the air in the unit, reducing soybean contact with oxygen. Grinding and blanching were begun and continued until the required temperature (listed below) was reached. The slurry was filtered and milk extracted once the unit's pressure was released. Using the traditional method, the temperature in the GCB unit reached 121°C, the temperature at which trypsin inhibitor is entirely destroyed. The milk obtained from the preceding steps was chilled to roughly 80°C before adding 2.5% citric acid (of the total milk obtained). The mixture was coagulated, and the tofu was separated from the whey using a muslin cloth before pressing it in a tofu press.

Soybean (1000g) was soaked overnight in warm water (60°C) adding 0.5% $NaHCO_3$. The water-to-soybean ratio was 2:1, and 250 g of 1000g soybean (T_0) was soaked overnight before discarding the water. According to Zinia *et al.* (2022), soybean (1000 g) was soaked overnight in warm water (60°C) adding 0.5% $NaHCO_3$. The water-to-soybean ratio was 2:1, and 250 g of 1000g soybean (T_0) was soaked overnight before discarding the water. Soybean was manually dehulled and cleaned three times with a continuous flow of potable water. The remaining soybean (750 g) was divided into three groups. Each group has 250 g of soybean and is labelled T_1, T_2, and T_3. All three samples were stored in the dark for two, four, and six days, respectively. During overnight soaking, water was drained and washed with potable water at 6 to 8 hour intervals at 28 C. After 2, 4, and 6 days of germination, soybean (T_1, T_2, and T_3) were manually dehulled and cleaned three times with a continuous flow of potable water. Soybeans, both sprouted and unsprouted, were blanched in a 0.5% sodium bicarbonate solution for 10 minutes at 80°C. The water-to-soybean ratio was 2:1. With the addition of hot water (100°C, soybean: water 14 1:4), the blanched soybean was ground using a super mass colloider and a basket centrifuge. After filtering through a double layer cheese cloth, soy milk was obtained from sprouted and nonsprouted soybeans. The soymilk was homogenised and pasteurised for 30 minutes at 65°C. Soymilk from the T_0, T_1, T_2, and T_3 samples was heated to 95 C for 5 min., cooled to 80°C by steady stirring at room temperature, and then re-weighed. $CaSO_4$ was added as a coagulant at a rate of 0.2% (w/v) in the milk, along with 2% lemon juice. Slowly adding the coagulant while stirring continuously and letting it sit at room temperature for 20 minutes were both successful. In order to press the tofu, weights of 1.0, 1.5, and 2.0 kg/cm^2 were placed on the tofu for 45 minutes. The coagulated milk was then transferred to a tofu mould (16 ×18 ×3.5 cm) lined with cheese cloth. Following pressing, the tofu was taken out of the mould and submerged in cold water (6˚C) for 30 minutes. The tofu was put in the cheesecloth to absorb the whey.

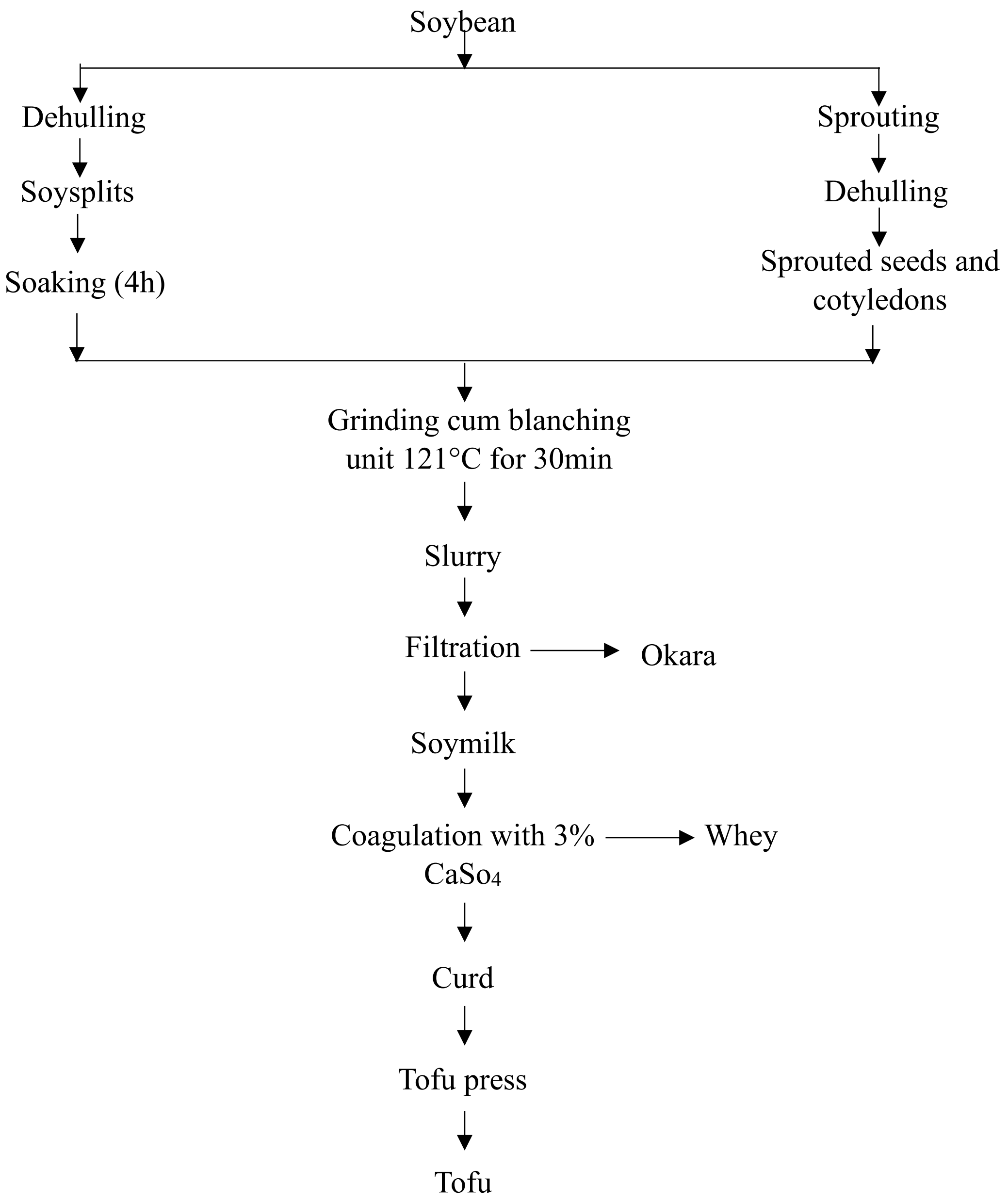


Fig 2.1: Flowchart of preparation of soymilk, tofu and okara from sprouted and unsprouted soybean

2.6 Effect of sprouting on the chemical aspect of soybean and tofu

Soybean products are a good source of protein, carbohydrates, low in fat and rich in minerals content (Murphy *et al.*, 1997).Tofu is a cheap source of highly digested protein, and its isoflavones have been shown to slow the development of arteriosclerosis by lowering plasma lipid levels (Fasoyiro, 2014; Huang *et al.*, 2003). Tofu has some disadvantages, such as a beany or grassy flavour that makes it less popular with consumers and a relatively high fat content. It is also perishable (Kim *et al.*, 2007). According to Rekha and Vijayalakshmi (2013) sprouting is known to reduce undesired flavours such as beany and grassy to a minimum and maximise total acceptance for soymilk manufactured from two-day-germinated soybeans, as well as in deteriorating nutty odor and taste . Chemical composition of raw and germinated soybean is shown in table 2.6.

Table 2.6 Chemical composition of raw and germinated soybean

Sample	Moisture%	Crude protein% (db)	Crude fat% (db)	Total Ash% (db)	Crude Fibre% (db)	Carbohydrate% (db)
Non germinate	10.33±0.37	37.72±3.73	20.53±3.54	4.56±0.45	5.75±0.62	31.41±0.74
Germinated	57.16±0.34	41.43±0.94	13.62±0.63	4.29±0.28	10.04±0.76	30.51±1.4

Source: (Ojha *et al.*, 2014)

Table 2.7 Chemical composition of tofu made from soybean and sprouted soybean

Tofu	Moisture %	Crude protein% (db)	Crude fat% (db)	Total Ash% (db)	Crude Fibre% (db)	Carbohydrate % (db)
Regular tofu	68.33±0.57	39.42±1.48	21.15±0.79	5.75±0.15	6.78±0.33	26.91±2.23
Sprouted tofu	67.88±0.12	44.73±1.34	15.81±1.72	3.93±0.24	11.98±0.71	23.56±2.17

Source : (Ojha *et al.*, 2014)

2.7 Chemical aspects tofu

Table 2.8 Proximate composition of tofu

Parameters	g/100g
Moisture	80.5
Protein	16.5
Fat	0.3
Carbohydrate	1.6
Fiber	0.03
Mineral	1.1

Source : (DFTQC, 2012)

PH, Moisture, peroxide value and acid value are the important chemical characteristics of tofu which help to monitor keeping quality of paneer.

2.7.1 PH

Anbarasu and Vijayalakshmi (2007) investigated that, the initial pH of control tofu and tulsi-treated tofu were 6.88 and 6.90, respectively, and after 24 hours, both control (6.20) and tulsi-treated tofu (5.89) showed a dramatic fall in pH. A logarithmic phase of bacterial growth between 24 and 48 hours would have resulted in effective nutrition utilisation, acid generation, and a significant fall in pH. pH gradually increased after 2 days to (6.92) and (6.73) at the end of 7 days in control and tulsi-treated tofu, respectively. During storage, the pH of tulsi-treated tofu was lower than that of control tofu.

The pH were found to be lower with the addition of 0.02% turmeric extract investigated by (K.-N. Park *et al.*, 2007) on tofu shelf life at 25°C for 12 days.

2.7.2 Moisture

Anbarasu and Vijayalakshmi (2007) reported that, the moisture content of control tofu fluctuated, with fresh tofu having a moisture content of 70.37% and showing a 5% increase after 7 days (75.22%). Protein disintegration and lipid globule oxidation would have loosened the gel structure of tofu, allowing more water to be absorbed. On the other hand, in the tulsi-treated tofu (76.49%), a decrease in moisture content was detected on the first day (75.21%), followed by a progressive increase until the conclusion of 7 days. However, after 7 days of storage, moisture content increased by only 1% (77.30%).

2.7.3 Acid value and peroxide value

Tkaczewska *et al.* (2023) study the possibility of using innovative single and double layer films with antioxidant properties as packaging materials for vegetarian products. It was found that films are not efficient in inhibiting the oxidation of tofu lipids. The acid and peroxide value of fresh tofu were 1.31KOH/g and 1.43(meq/kg) respectively. Lipid oxidation can be initiated and accelerated by different mechanisms, including the production of singlet oxygen, enzymatic and nonenzymatic generation of free radicals and active oxygen. (Rekha and Vijayalakshmi, 2013) lipid oxidation affects the shelf life of a product and its nutritional value by altering its vitamin and mineral content. At the same time, lipid oxidation may make the product susceptible to microbial deterioration.

2.8 Microbiology aspects

Tofu microbiological research is significant since its shelf life without preservatives is quite short. Tofu will normally spoil within 1 to 3 days if not refrigerated or preserved in any way (Lim, 1984). Lactic acid bacteria are typically the microorganisms that degrade tofu (Dotsom *et al.*, 1977). The bacterial infection Yersiniosis is caused by the genus *Yersinia*. The specific bacterium that causes yersiniosis in humans is Yersinia enterocolitica (Aulisio *et al.*, 1983). Other bacteria linked to tofu degeneration include *Clostridium botulinum, Salmonella typhimurium,* and *Staphylococcus aureus* (Kovats *et al.*, 1984).

2.8.1 Standard plate counts

Tofu preparation, which includes boiling the soymilk, has been shown to be successful in removing much of the initial vegetative microorganisms. However, contamination is possible due to the postboiling pressing of the curds to produce cakes and the handling of the cakes before packaging. This, along with the fact that tofu is frequently housed in the produce department of many grocery stores, where temperature mistreatment is conceivable, makes tofu a potential public health threat (Rehberger *et al.*, 1984). Rehberger *et al.* (1984) discovered that tofu held at 10°C until the manufacturer's pull date exhibited high aerobic plate counts (up to $5.6x10^{6}$ cfu/g) and psychrotroph counts (up to 7.9×10^{7}cfu/g), indicating possible microbial deterioration.

Anbarasu and Vijayalakshmi (2007) studied the influence of tulsi (Ocimum sanctum) extract on the shelf life of tofu at room temperature. Tulsi extract increased 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. After seven days of storage, the overall microbial count in non-treated tofu was 6.21×10^{8}cfu/g, while tulsi-treated samples contained 1.08×10^{9}cfu/g.

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

High hydrostatic pressure processing (HHPP) has also been used to investigate microbe inactivation in tofu. Prestamo *et al.* (2000) tested the benefits of high hydrostatic pressure therapy on tofu by applying a pressure of 400 MPa at 5°C for 5, 30, and 45 minutes at various temperatures. The microbial count dropped with time, falling from 5.54×10^{4}cfu/g to 0.31, 1.56, and 2.38 log units, respectively. They discovered a 2log unit decrease in psycrotrophs and a 1log unit decrease in mesophiles respectively.

K.-N. Park *et al.* (2007) investigated the effect of turmeric extract (Curcuma aromatica

Salab.) on tofu shelf life at 25°C for 12 days. The bacterial count was 1000 times lower in turmeric extracted tofu than in control tofu. Tofu is supposed to spoil when the viable count surpasses 10^7cfu/ml.

2.8.2 Yeast and mold

Protein-rich tofu has a very low shelf life. In an effort to extend shelf life, tulsi (Ocimum sanctum) extracts, which are widely available in rural regions, were used. In freshly prepared samples and 7-day-old control tofu samples, yeast and mould counts range from 1.0×10^2 to 3.37×10^4 CFU/g, respectively, whereas in tulsi-treated samples, an initial 2-log increase from 1.0×10^2 CFU/g was seen after 2 d, followed by a 2-log decrease after 4d, and again an increase to 2.33×10^5 CFU/g was observed at the end of 7d (Anbarasu and Vijayalakshmi, 2007).

Table 2.9 Tofu standard at 40°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)

2.9 Self Life of Tofu

Soy curd is a good source of high-quality protein. Tofu has a shelf life of roughly 1-3 days (depending on temperature) without any means of preservation or refrigeration (Pontecorvo and BOURNE, 1978). Tofu is well known for its great nutritional value, however because of its high protein, moisture content, and neutral pH, it is perishable by nature (Lee *et al.*, 2014).

2.9.1 Measures to improve shelf life of Tofu

Previously, many techniques of preserving tofu to lengthen its shelf life were documented. Some of them include making tofu from ozone-treated soybeans (I. Park *et al.*, 1994), use of electrolyzed water (EW) in the production of tofu (Tatsumi *et al.*, 2002), tofu packed in PE films coated with nisin incorporated MC/HPMC (methylcellulose—MC and hydroxypropyl methylcellulose—HPMC) solution (Cha *et al.*, 2003), tofu treated with high pressure (Prestamo *et al.*, 2000), in-package microwave treatment (Wu and Salunkhe, 1977), use of the biopolymer chitosan in tofu preparation (No and Meyers, 2004).

2.9.1.1 Low Temperature Storage

The storage temperature is most likely the most critical factor in maintaining the quality and increasing the shelf life of packaged foods. In most circumstances, increasing the storage temperature lowers the quality and acceptance of packaged foods. Biological reactions typically rise by a factor of two to three for every 10°C increase in temperature (J. H. Han, 2005). Because microbiological development is a main cause of spoilage of tofu, cooler temperature storage can considerably help to increase its shelf life.

2.9.1.2 Electrolyzed Water (EW)

Tofu products degrade quickly and have a short shelf life. To control microorganisms in tofu production, electrolyzed water (EW) was used during the soybean soaking phase of packed tofu manufacturing. The accessible chlorine in EW, which is a mixture of hypochlorous ions, hypochlorous acid, and chlorine, functions as a steriliser (Tatsumi *et al.*, 2002)

2.9.1.3 Nisin

NS has a high ability to suppress microorganism growth. In order to package tofu, Cha, Chen, Park, and Chinnan (2003) used a coated polyethylene (PE) film that contained Nisin methylcellulose and hydroxypropyl methylcellulose. To investigate the inhibitory effect on Listeria in tofu, the film made direct contact with the suspension. The outcomes shown that the use of Nisin films to package tofu can successfully prevent the growth and survival of L.monocytogenes Brie-1 (Cha *et al.*, 2003). Furthermore, Schillinger, Becker, Vignolo, and Holzapfel (2001) demonstrated that NS could totally suppress the growth of Listeria monocytogenes in homemade tofu products held at 10°C for a week (Schillinger *et al.*, 2001).

2.9.1.4 Hydrogen peroxide

Hydrogen peroxide therapy could be an effective preservative. Without hydrogen peroxide treatment, firm tofu has a one-day shelf life at room temperature. Firm tofu's room-temperature shelf-life was increased to 2 and 3 days when the hydrogen peroxide concentration was 95 and 395 ppm, respectively (Chang and Chen, 1999).

2.9.1.5 In-Package Microwave Treatments

Fresh soybean curds were microwave-heated in-package to extend shelf life. Storage at 4.5°C, soybean curds pretreatment with microwave heating to 65°, 80°, and 95°C have shelf-life of 16, 21, and 27 days, respectively, compared to 7 days in the control. Storage at higher temperatures significantly shortened the shelf-life of soybean curds, both control and microwave treated. Soybean curds treated with microwave heating to 65°, 80°, and 95°C had a shelf life of 5, 7, and 9 days when stored at 13°C. At 21°C, the shelf-life was only 1.5, 2, and 3 days (Wu and Salunkhe, 1977).

2.9.1.6 Irradiation technology

Ionising radiation is used to irradiate food in order to kill microorganisms (Zanardi *et al.*, 2018). Shurong *et al.* (2006) evaluated the effect of adopting irradiation technology on the microbiological safety and acceptance of the sensory and nutritional aspects of tofu products. Their findings suggested that irradiation doses less than 2 kGy had no significant effect on the crude fat, crude protein, and AA content of packaged tofu, and that the sensory quality of packaged tofu does not vary considerably over time. Maurya *et al.* (2018) evaluated the effects of gamma irradiation on tofu using three distinct packing materials: biaxially oriented polypropylene, high-density polyethylene, and low-density polyethylene. The samples were kept for up to 20 days. Tofu's colour ranged from light cream to light yellow, according to the results. This could be because radiation eliminates moisture, which alters the colour of the sample. The combination of low-density polyethylene and 1.25-kGy gamma radiation successfully increased the shelf life to 15 days without affecting the tofu's quality.

2.9.1.7 Modified atmosphere packaging

Tofu was preserved using modified atmosphere packing. Tofu was packaged in air (control) and 30% carbon dioxide (CO_2)/70% nitrogen (N_2), achieved either flushing or vacuum compensation, and stored in cold conditions. Microbial counts in air-packed tofu were 1 to

4 log cycles higher after 10 days of storage when compared to tofu packaged in modified environment. After 3 weeks of storage, the counts in modified atmosphere-packaged tofu reached the same level as those in air-packaged tofu (Van Campenhout et al., 2013).

2.9.1.8 Packaging of Tofu

Food packaging is one of the most significant operations in the food industry since it helps to retain food quality during storage, transit, and distribution. It prevents food from physical, chemical, and biological damage and keeps it fresh (Sarkar and Kuna, 2020).

Product packaging is the final step in the tofu production process. The packing materials and storage technology used will influence the ultimate flavour, quality, and shelf life of the tofu product. Tofu is sold in stores in bulk, in waterfilled tubs, in plastic bags or vacuum packed (William Shurtleff and Aoyagi, 2000). The high moisture and protein content (in tofu products) provides an excellent ambient medium for the growth of microorganisms, and so the shelf life is only a few days even under refrigerated conditions (Rossi *et al.*, 2016).

Some of the important packaging materials are shown below;

1. low-density polyethylene which is flexible, strong, resilient, easy to seal, and moisture resistant. Low-density polyethylene is used in products such as bread and frozen food bags, flexible covers, and squeezable food bottles (Sarkar and Kuna, 2020)

2. Polypropylene (PP) is denser, tougher, and more transparent than polyethylene. It is chemically resistant and serves as an efficient water vapour barrier (Sarkar and Kuna, 2020).

3. Metalized plastic: Metallized films are polymer films coated with a thin layer of metal, usually aluminium. The key reasons that metallized films are replacing aluminium foil are reduced aluminium utilisation and material cost. For metallized film the vaporized aluminum layer thickness is 50 nm (Ge *et al.*, 2021). Metallized polymer films are useful because of their very good barrier properties (Jamieson and Windle, 1983).

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials

Raw material collected for the preparation of regular and sprouted tofu are as follows

3.1.1.1 Soybean

Brown variety of soybean (*Glycine max*) was collected from the local farmer of Chakraghatti.

3.1.1.2 Packaging materials

LDPE, PP and metalised plastic were used for packaging of samples during shelf life determination.

3.1.2 Apparatus and chemical required

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

3.2 Methods

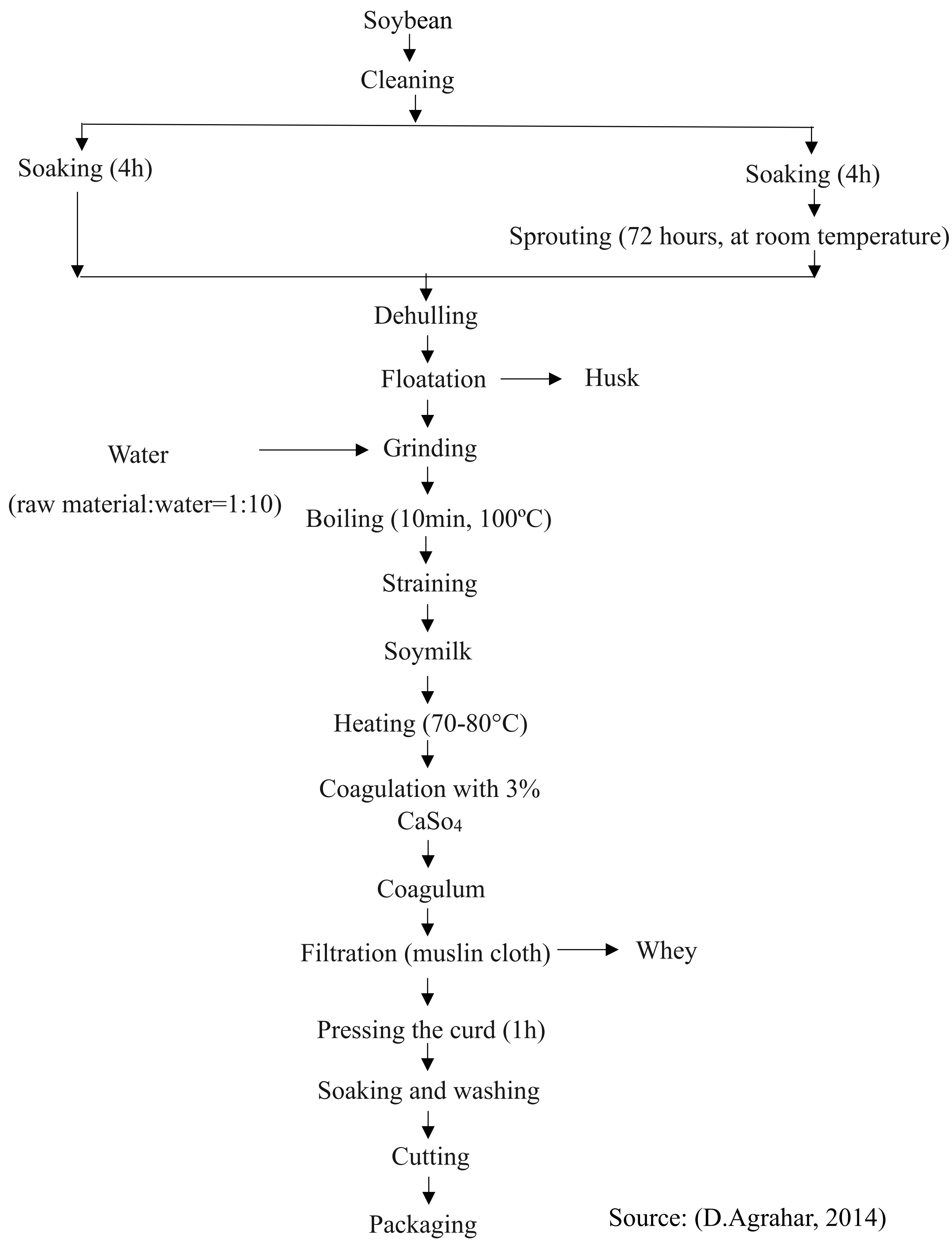
3.2.1 Preparation of regular tofu

500g of brown soybean, which was soaked individually for 4hours. The swollen beans were manually dehulled and ground in a grinder. The grinding was done with the addition of water on an intermittent basis. By adding water, the bean-to-water ratio was maintained at 1:10. The mash was heated and cooked for 10 minutes before being strained through muslin cloth.

Milk was cooled to around 80 °C subsequently 3% calcium sulphate was added (of the total milk obtained). 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 and vacuum packed in three different packaging materials

3.2.2 Preparation of sprouted tofu

Soybean seeds were completely washed and made free from dust, dirt, stubbles and foreign substances. At room temperature (RT), the seeds were soaked in tap water for 4 hours. After draining the excess water, the sample was washed with tap water. Seeds were put in a single layer on muslin cloth for 72 hours. The sprouted seeds which had around 60%-70% moisture dehulled manually and remaining process was similar as regular tofu.



Source: (D.Agrahar, 2014)

Fig 3.1: Flowchart for preparation tofu made form unsprouted and sprouted soybean

3.3 Analysis of raw materials and product

3.3.1 Physical analysis of raw material

3.3.1.1 1000 kernel weight

The 1000 kernels weight of soybean was estimated by measuring the weight of 1000 kernels of soybean and after selecting the suitable sample size by quartering method, as indicated by (Buffo *et al.*, 1998).

3.3.1.2 Bulk density

The bulk density was determined as described by (Clementson *et al.*, 2010) by pouring the grains into the funnel-shaped hopper, centering the hopper above the measuring bushel, quickly opening the hopper valve, and allowing the grains to flow freely into the measuring bushel. The extra material levelled off with gentle zigzag strokes using a standard Seedburo striking staff after the bushel was filled. After weighing the filled measuring bushel, the mass of grains in the bushel was calculated by subtracting the mass of the measuring bushel itself. The bulk density (ρ) of grain was calculated using the formula

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

3.3.1.3 Color

Color was determined by visual method. The soybean was spread on separate tray and color and surface was diligently examined.

3.3.2 Chemical analysis of raw materials and product

3.3.2.1 Determination of moisture content

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

3.3.2.2 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).

3.3.2.3 Determination of crude fat

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

3.3.2.4 Determination of crude fibre

The crude fibre content of the sample was determined by acid base hydrolysis as described by AOAC (2005).

3.3.2.5 Determination of crude protein

The crude protein content of the soybean 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).

3.3.2.6 Determination of carbohydrate

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

3.4 Yield

Percentage yield of tofu was calculated as per

$$\text{Yield of tofu solid\%} = \frac{\text{Weight of dry matter in tofu(g)}}{\text{Weight of dry matter } in\ soybean} \times 100$$

3.5 Analysis of tofu for chemical characteristics during storage

3.5.1 Moisture

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).

3.5.2 PH

PH was measured with a glass-electrode pH meter as described by AOAC (2005).

3.5.3 Acid value

Acid value was determined as described by KC and Rai (2007).

$$\text{Acid Value}= \frac{\text{ml of alkali} \times \text{N of alkali} \times 56.1}{\text{Weight of sample (g)}}$$

3.5.4 Peroxide value

Peroxide value was determined as described by KC and Rai (2007).

$$\text{Peroxide Value}= \frac{N \times (V_{s-}V_B) \times 1000}{wt \text{ of sample}}$$

N= Normality of sodium thiosulfate

V_S= Sodium thiosulphate consumed by sample(ml)

V_B= Sodium thiosulphate consumed by blank(ml)

3.6 Microbiological analysis

Total Plate Count (TPC) and yeast and mold count was determined by pour plate technique on Plate Count Agar (PCA) and Potatoes Dextrose Agar (PDA) medium (incubated at 30°C/48 h). The TPC and yeast and mold of sample was expressed in terms of log colony forming units (cfu) per gram.

3.7 Sensory Evaluation

Sensory of tofu prepared was performed for color, flavor, texture, taste, overall acceptance by 6 semi-trained panelists. 9 point hedonic rating test was used and a sensory evaluation card as shown in Appendix A was provided to the panelists.

3.8 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 significant.

Part IV

Result and discussion

Raw material (soybean) was collected from the local farmer at Chakraghatti and then subjected to proximate analysis. The best product among two variations was determined by carrying out sensory evaluation and shelf life of best product was determined by using three different packaging materials (LDPE, PP, metalised plastic).

4.1 Physical properties of soybean

Physical properties of soybean was determined and obtained results are shown in Table 4.1

Table 4.1 Physical properties of soybean

Physical properties	Soybean
1000 kernel wt. (g)	194.51±0.649
Bulk density (kg/HL)	82.667±0.577

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

Poudel (2022) reported the value of 1000 kernel wt. and bulk density as 195.146g, 83(kg/HL) which was more than the mean values of soybean of our study. This was due to the physiochemical qualities of different soybean cultivars varied depending on the strain, climatic conditions, and agronomical conditions.

4.2 Proximate composition of soybean

The proximate composition of raw and sprouted soybean was obtained as given in Table 4.2.

Table.4.2 Proximate composition of raw and sprouted soybean

Attributes	Raw Soybean	Sprouted Soybean
Moisture% (wet basis)	9.57^{a}±0.38	56.34^{b}±0.24
Ash % (db)	5.65^{a}±0.15	5.34^{b}±0.24
Crude fat % (db)	19.05^{a}±0.50	13.20^{b}±0.30
Crude fiber % (db)	4.43^{a}±0.15	9.45^{b}±0.32
Crude protein % (db)	36.51^{a}±0.40	41.22^{b}±0.33
Carbohydrate % (db)	34.36^{a}±0.95	30.78^{b}±0.30

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

4.3 Chemical composition of soybean

Proximate analysis of the raw soybean for various parameters like moisture content (%), Ash (%) crude fat (%), crude fiber (%) crude protein (%), and Carbohydrate (%) (in dry basis except moisture content) were found to be 9.57%, 5.65%,19.05%, 4.43%, 36.51% and 34.36% respectively as given in Table 4.1.

DFTQC (2012) reported respective proximate values in dry basis were 10.2, 5, 17.7, 4.2, 33.3 and 29.6 % respectively. The moisture content of raw soybean (9.57% in wet basis) was lower than value obtained by Ojha *et al.* (2014) i.e. 10.33%, DFTQC (2012) i.e.10.2% and Snyder and Kwon, (1987) i.e. 12-15%. Soybean moisture content varies depending on maturity stage, weather conditions, variety, storage conditions, and packaging material. The ash contain in soybean 5.65% (db) which was higher than the value obtained by DFTQC (2012) i.e.5, Ojha *et al.* (2014) i.e. 4.56% and Snyder and Kwon, (1987) i.e. 5%. The crude fat of soybean was found to be 19.05% higher than the value obtained by DFTQC (2012) i.e. 17.7% but lower than that obtained by Ojha *et al.* (2014) and Snyder and Kwon, (1987). Different factors, such as moisture level, location, climate, maturity, and soybean variety, may affect fat content. This demonstrated that the amount of fat depends on the type of soybean. Crude fiber of soybean was found to be 4.43% (db) higher than that obtained by

DFTQC (2012) but lower than that obtained by Ojha *et al.* (2014) i.e. 5.75%. Crude protein contain in soybean was found to be 36.51% (db) was lower than the value obtained by Ojha *et al.* (2014) i.e. 37.72% and Snyder and Kwon, (1987) i.e. 40% but higher DFTQC (2012) i.e. 33.3%. This could be as a result of nitrogenous material being lost during digestion, which evenly decreased the total protein amount. Carbohydrate content obtained in soybean was 34.36% (db) which is higher than that obtained by DFTQC (2012) i.e.29.6% ,Ojha *et al.* (2014) i.e. 31.41% but lower than that obtained by Snyder and Kwon, (1987). The cause of the variance in carbohydrate content could be due to environmental factors, soybean variety, or experimental error.

4.4 Effect of sprouting on the chemical composition soybean

Soybeans after soaking for 12hours were germinated for 72h. The samples were then subjected for analysis. The result obtained is presented in Table 4.2.

As shown in table, moisture content was significantly ($p < 0.05$) increased after sprouting from 9.57% to 56.34% which is similar to the results reported by Ojha *et al.* (2014). The increase of moisture contain is due the hydration of soybean after sprouting.

The ash contain was decreased significantly ($p < 0.05$) after sprouting of soybean from 5.65% to 5.34%. Ojha *et al.* (2014) also reported the decrease in ash contain of soybean. Soaking may have caused a decrease in total ash.

Crude fat content was decreased significantly ($p < 0.05$) after sprouting of soybean from 19.05% to 13.20%. This finding is similar to the results reported by Ojha *et al.* (2014). The decrease in fat may be due to the usage of fat as an energy source to initiate germination.

Crude fiber content was increased significantly ($p < 0.05$) after sprouting of soybean from 4.43% to 9.45%. Ojha *et al.* (2014) also reported the increase in fiber content after sprouting which may due to the due to the synthesis of structural carbohydrates such as cellulose and hemicelluloses, a major constituent of cell walls

Protein content was increased significantly ($p < 0.05$) after sprouting of soybean from 36.51% to 41.22%. Similarly, Ojha *et al.* (2014) reported increase in the percentage of protein in soybean during sprouting. Breakdown of complex protein molecules into simpler forms and breakdown of nutritionally undesirable substances may be the reason for the significant rise in crude protein content.

Carbohydrate content was decreased significantly ($p < 0.05$) after sprouting of soybean from 34.36% to 30.78%. Ojha *et al.* (2014) also reported the decrease in carbohydrate content in soybean after sprouting. The usage of carbohydrates as a source of energy for embryonic growth may be the cause of this.

4.5 Sensory properties

Statistical analysis of sensory scores obtained from 6 semi-trained panelist using 9-point hedonic rating scale (9= like extremely, 1= dislike extremely) for regular and sprouted tofu. Panelists are those who have tasted tofu. The ANOVA and LSD table for sensory evaluation are presented in Appendix B.

4.5.1 Color

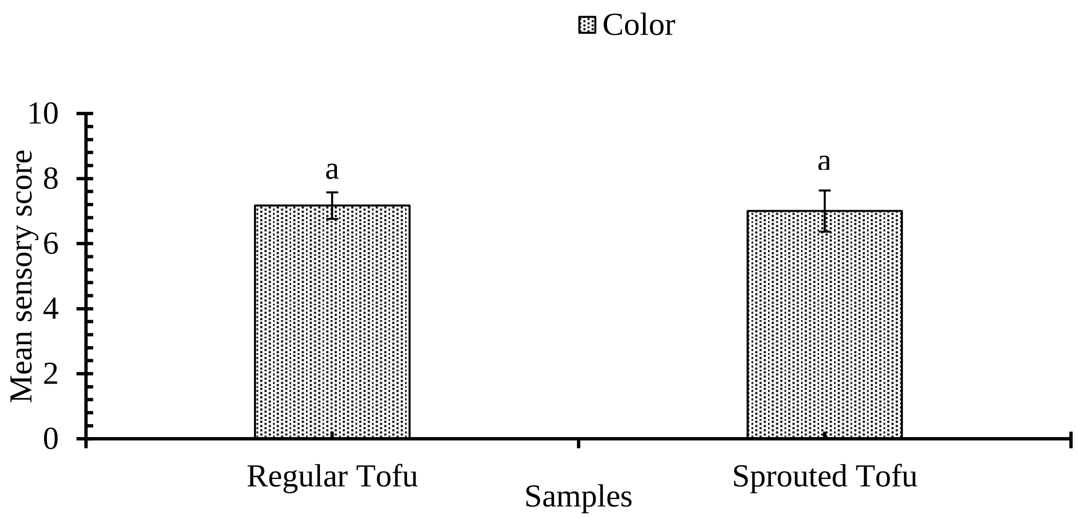


Fig 4.1. Mean sensory score of two samples in terms of color

The mean sensory score for color were found to be 7.16 and 7 for the regular and sprouted tofu respectively. The obtained mean values are presented in bar diagram in Figure 4.1. Statistical analysis showed that regular tofu was no significantly different ($p>0.05$) in color with sprouted tofu. This showed that the color of sprouted tofu and regular tofu closely resembled with each other.

4.5.2 Flavor

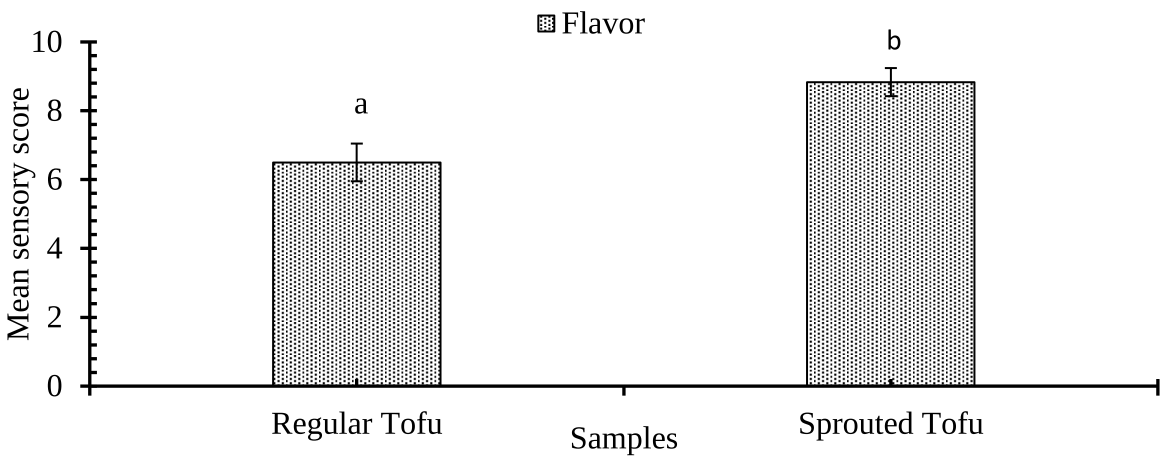


Fig 4.2: Mean sensory score of two samples in terms of flavor

The mean sensory score for flavor were found to be 6.5 and 8.83 for the regular and sprouted tofu respectively. The obtained mean values are presented in bar diagram in Figure 4.2. Statistical analysis showed that regular tofu was significantly different ($p<0.05$) in flavor with sprouted tofu. This is because unsprouted soybean seed have strong beany flavour than sprouted soybean and thus affected the overall flavour of regular tofu (Murugkar and Jha, 2009).

4.5.3 Texture

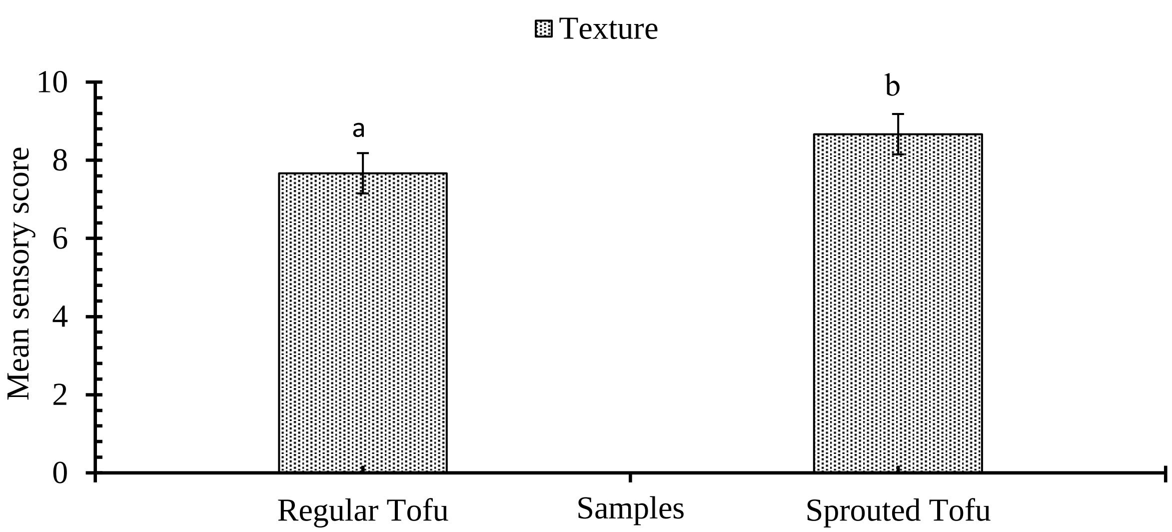


Fig 4.3: Mean sensory score of two samples in terms of texture

The mean sensory score for texture were found to be 7.667 and 8.667 for the regular and sprouted tofu respectively. The obtained mean values are presented in bar diagram in Figure 4.3. Statistical analysis showed that regular tofu was significantly different ($p<0.05$) in texture with sprouted tofu. Texture of sprouted tofu was highly accepted in comparison with regular tofu. Tofu becomes less firm during sprouting of seed due to a decrease in fat content and an increase in protein solubility (Ojha *et al.*, 2014).

4.5.4 Taste

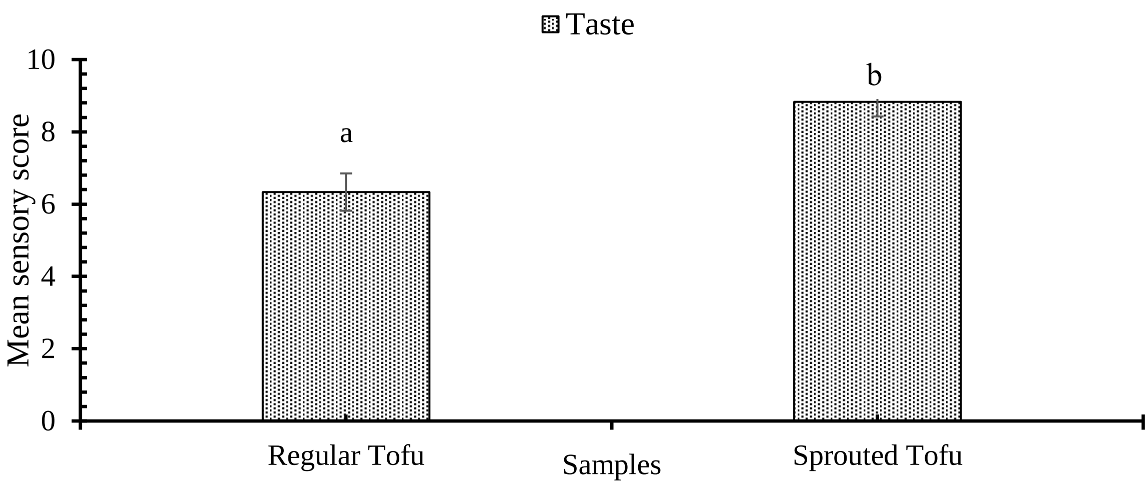


Fig 4.4: Mean sensory score of two samples in terms of taste

The mean sensory score for taste were found to be 6.33 and 8.833 for the regular and sprouted tofu respectively. The obtained mean values are presented in bar diagram in Figure 4.4. Statistical analysis showed that regular tofu was significantly different ($p<0.05$) in taste with sprouted tofu. The high value in tofu taste indicates a high savoury taste. This is a proportional result to the crude protein and crude fat levels. The greater the crude protein level, the more savoury the flavour. As protein content of sprouted tofu is greater than regular tofu (Ojha *et al.*, 2014).

4.5.4 Overall Acceptance

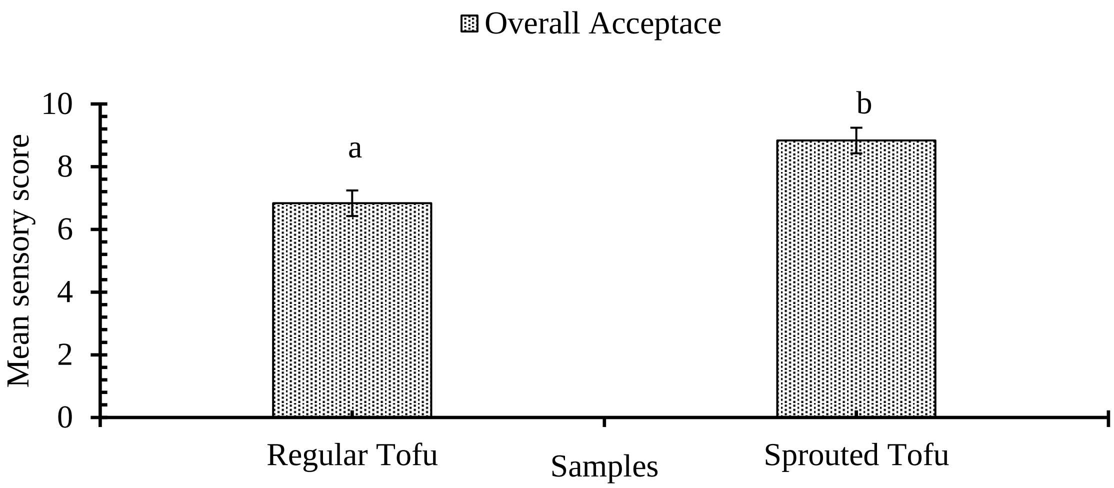


Fig 4.5: Mean sensory score of two samples in terms of overall acceptance

The mean sensory score for taste were found to be 6.83 and 8.83 for the regular and sprouted tofu respectively. The obtained mean values are presented in bar diagram in Figure 4.5. Statistical analysis showed that regular tofu was significantly different ($p<0.05$) with sprouted tofu.

The color, flavour, texture and taste of sprouted tofu were very much liked. Therefore, the overall acceptability of sprouted tofu was found to be significantly superior based on the sensory characteristics of tofu.

4.6 Yield of tofu

The percentage yield of regular tofu and sprouted tofu are given in Table.

Table 4.3 Yield of tofu prepared from 500g soybean

Tofu	%Yield of tofu
Regular tofu	$41.76^{a}\pm0.557$
Sprouted tofu	$39.173^{b}\pm0.835$

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

The yield of regular and sprouted tofu was found to be 41.76% and 39.173%. There was a significant decrease ($p<0.05$) in yield % of sprouted tofu. Dry matter loss during soaking and sprouting could be the cause of the decrease in sprouted tofu output.

The above outcomes are higher than 7.6-18.3% reported by Oboh (2006). However, Shokunbi *et al.* (2011) reported yield of tofu as 58.69% coagulated from $CaSO_4$. The variations in soybean varieties and processing techniques could be the cause of these yield differences.

4.7 Proximate composition of tofu

The proximate composition of regular and sprouted tofu was obtained as given in Table 4.

Table 4.4 Proximate composition of regular and sprouted

Attributes	Regular tofu	Sprouted tofu
Moisture % (wet basis)	$65.341^{a}\pm0.584$	$64.232^{b}\pm0.281$
Ash % (db)	$4.043^{a}\pm0.099$	$3.098^{b}\pm0.132$
Crude Fat % (db)	$22.390^{a}\pm0.386$	$16.041^{b}\pm0.230$
Crude Fiber % (db)	$5.741^{a}\pm0.174$	$10.597^{b}\pm0.366$
Crude Protein % (db)	$38.715^{a}\pm0.110$	$43.280^{b}\pm0.282$
Carbohydrate %	$29.103^{a}\pm0.354$	$26.994^{b}\pm0.809$

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

The moisture content, ash, crude fat, crude fiber, crude protein, carbohydrate of regular tofu was found to be 65.341%, 4.043%, 22.390%, 5.741%, 38.715%, 29.103% respectively and that of sprouted tofu was found to be 64.232%, 3.098%, 16.041%, 10.597%, 43.280%, 26.994% respectively.

Moisture content significantly ($p<0.05$) decreased in sprouted tofu as compared to the regular tofu. This finding is similar to the results reported Ojha *et al.* (2014).

Ash content significantly ($p<0.05$) decreased in sprouted tofu as compared to regular tofu. Ojha *et al.* (2014) also reported decrease in ash content of sprouted tofu as compared to regular tofu.

The fat content was significantly ($p<0.05$) decreased in sprouted tofu. The utilization of fat as an energy source to initiate germination may be responsible for the decline in fat. The decrease in fat may be caused by the loss of total solids.

Fiber content was significantly ($p<0.05$) increased in sprouted tofu. The loss of starch and the synthesis of structural carbohydrates like cellulose and hemicelluloses during the sprouting of soybean may be the cause of an increase in crude fibre of sprouted tofu.

Protein content was significantly ($p<0.05$) increased in sprouted tofu as compared to regular tofu, ranging from 38.715% to 43.280%. This may be due to the breakdown of complex protein molecules into simpler forms and breakdown of nutritionally undesirable substances.

Sprouted tofu showed a significant ($p<0.05$) decrease in carbohydrate. Decrease in carbohydrate content may be due to the synthesis of α-amylase during germination of soybean, which breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination.

4.8 Effect of different packaging material on the chemical characteristics of sprouted tofu during storage

4.8.1 Moisture

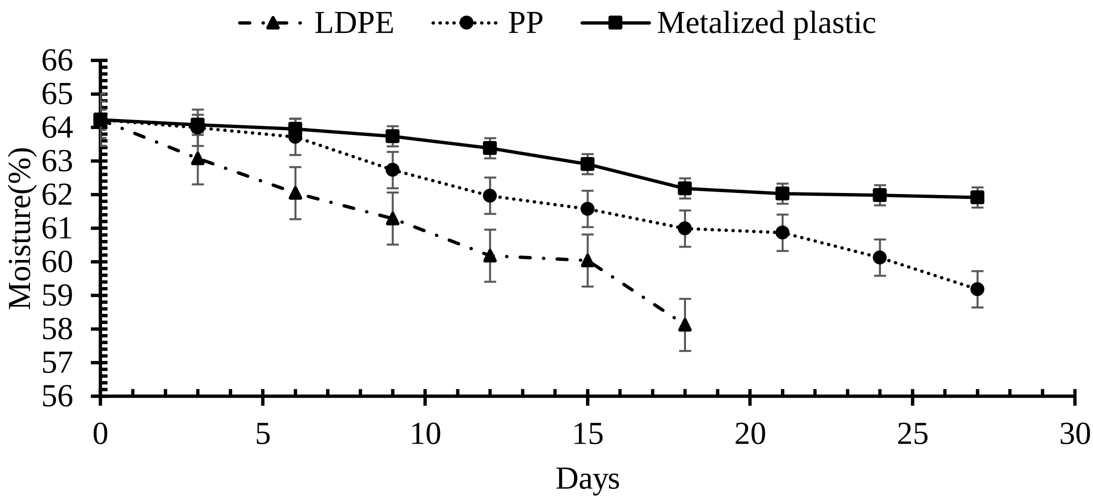


Fig 4.6: Change**s** in moisture of packaged sprouted tofu

The initial moisture content of sprouted tofu was found to be 64.232% which decreased considerably in three different packaging materials i.e., in case of LDPE it is decreased up to 58.123% in 18 days, it is decreased up to 59.183% and 61.916% in case of PP and metalized plastic in 27days respectively as shown in figure 4.6.

The loss in moisture was slightly higher in LDPE than PP because PP serves as an efficient water vapour barrier, denser and tougher than LDPE (Sarkar and Kuna, 2020). Moisture loss in metalized plastic is very low because of its very good barrier than LDPE and PP (Jamieson and Windle, 1983).

4.8.2 PH

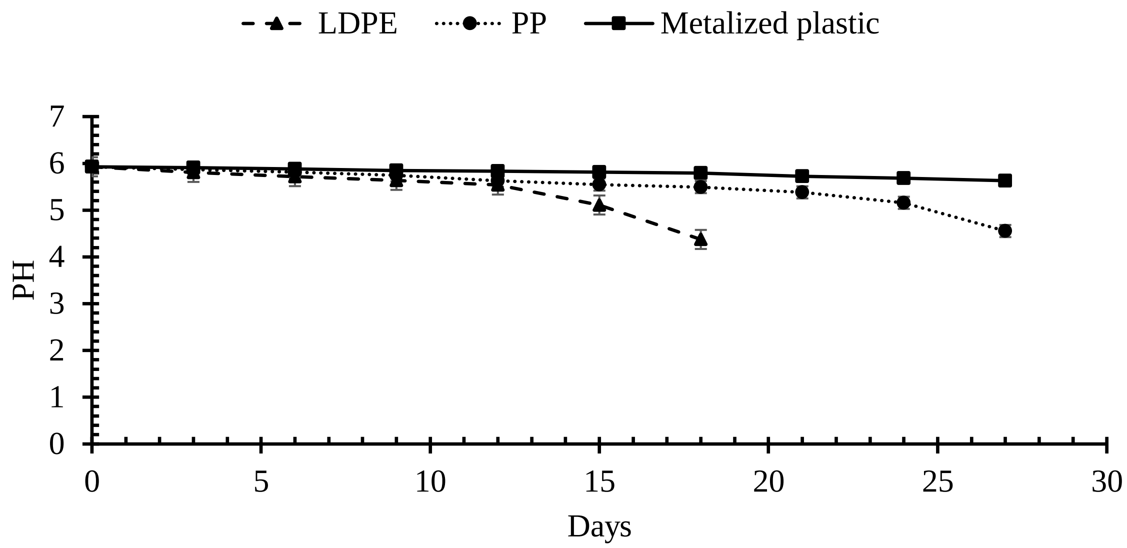


Fig 4.7: Changes in PH of packaged sprouted tofu

The initial PH of sprouted tofu was found to be 5.926 which slightly decreased in three different packaging materials i.e., in case of LDPE it decreased up to 4.373 in 18 days, it is decreased up to 4.553 and 5.63 in case of PP and metalized plastic in 27days respectively as in shown in figure 4.7. Dotsom *et al.* (1977) also found decrease in PH upon storage.

4.8.3 Acid Value

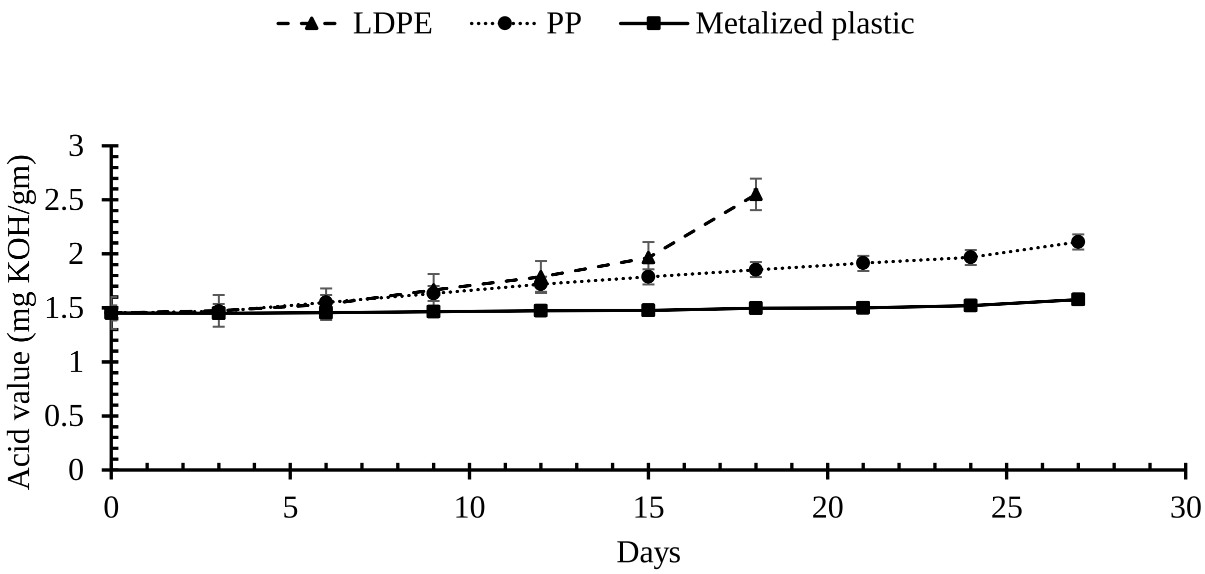


Fig 4.8: Changes of acid value of packaged sprouted tofu

The initial av of sprouted tofu was found to be 1.453 similar to av of fresh tofu obtained by (Tkaczewska *et al.*, 2023) which increased considerably in three different packaging materials i.e., in case of LDPE it is increased up to 4.373 mg KOH/g in 18 days, it is increased up to 2.11 mg KOH/g and 1.576 mg KOH/g in case of PP and metalized plastic in 27days respectively as shown in figure 4.8. Increases in acid values in LDPE higher than PP and metalized plastic during the period of storage because of low oxygen barrier properties of LDPE and PP than metalized plastic (Jamieson and Windle, 1983).

4.8.4 Peroxide Value

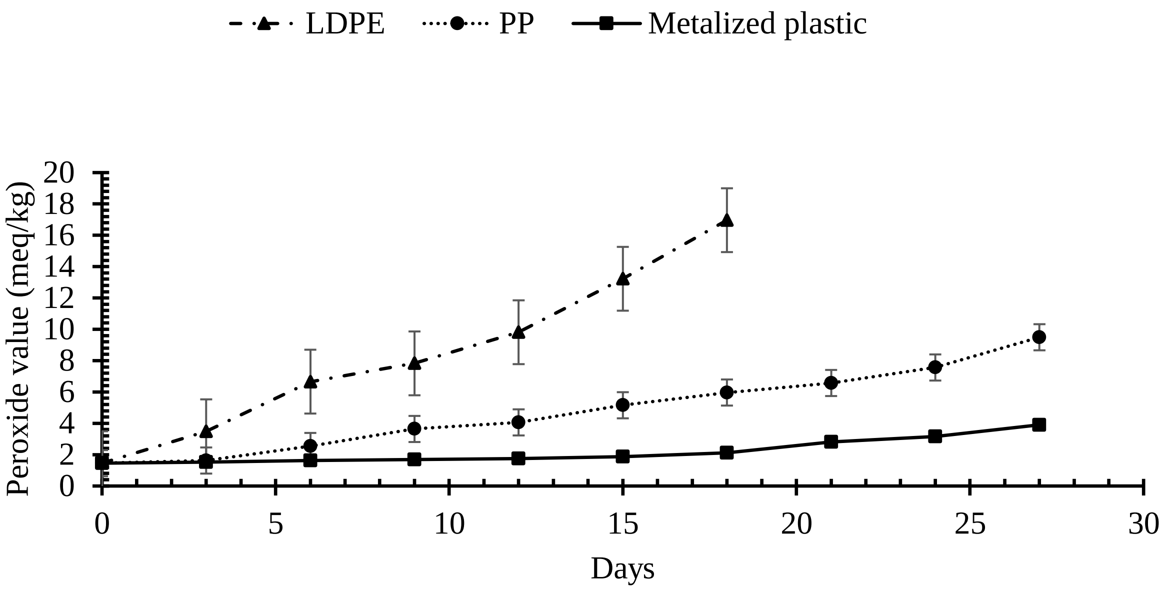


Fig 4.9: Changes of peroxide value of packaged sprouted tofu

The initial pv of sprouted tofu was found to be 1.456 (meq/kg) similar to the pv of fresh tofu obtained by Tkaczewska *et al.* (2023) i.e., 1.43 (meq/kg) which increased considerably in three different packaging materials i.e., in case of LDPE it is increased up to 16.963 (meq/kg) in 18 days, it is increased up to 9.493 (meq/kg) and 3.903 (meq/kg) in case of PP and metalized plastic in 27days respectively as shown in figure 4.9. Increases in peroxide values in LDPE higher than PP and metalized plastic during the period of storage because of low oxygen barrier properties of LDPE and PP than metalized plastic (Jamieson and Windle, 1983).

4.8.5 Total Plate Count

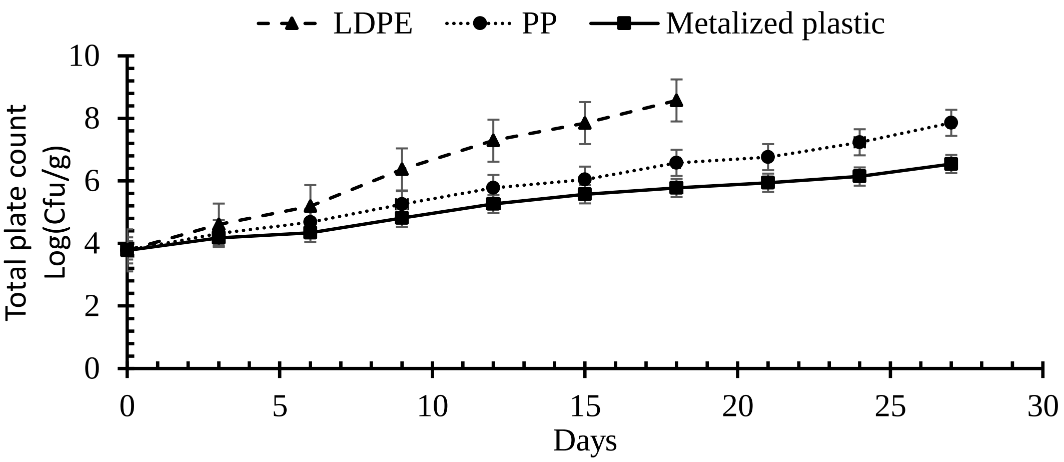


Fig 4.10: Changes of total plate count of packaged sprouted tofu

Sprouted tofu samples in this work were microbiologically tested for 27 days of storage and the results are presented in fig 4.10. The initial total plate count of sprouted tofu was found to be 3.778 log(cfu/g) which increased considerably in three different packaging materials i.e., in case of LDPE it is increased up to 8.574 log(cfu/g) in 18 days, it is increased up to 7.857 log(cfu/g) and 6.539 log(cfu/g) in case of PP and metalized plastic in 27days respectively as shown in figure 4.10.

Shelf life is alternatively defined as the time required to achieve 10^6cfu/g (Tkaczewska *et al.*, 2023). Thus, it may be concluded that the microbiological quality of the sprouted tofu packed in LDPE is acceptable until at least the 9th day of storage.

K.-N. Park *et al.* (2007) investigated the effect of turmeric extract (Curcuma aromatica Salab.) on tofu shelf life at 25°C for 12 days. Tofu is supposed to spoil when the viable count surpasses 10^7cfu/ml. Thus, it may be concluded that sample packed in LDPE supposed to spoil after 12^{th} days of storage. The sample packed in PP is supposed to spoil after 24^{th} days of storage. The sample packed in metalised plastic is acceptable until the 27^{th} days of storage.

4.8.6 Yeast and mold count

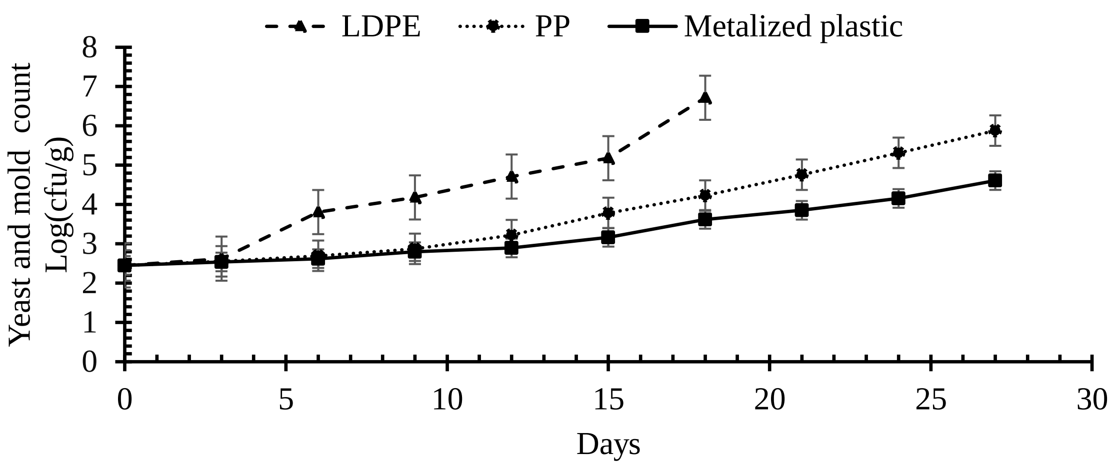


Fig 4.11: Changes of yeast and mold count of packaged sprouted tofu

Sprouted tofu samples in this work were microbiologically tested for 27 days of storage and the results are presented in fig 4.11. The initial yeast and mold count of sprouted tofu was found to be 2.450 log (cfu/g) which increased considerably in three different packaging materials i.e., in case of LDPE it is increased up to 6.715 log (cfu/g) in 18 days, it is increased up to 5.879 log (cfu/g) and 4.608 log (cfu/g) in case of PP and metalized plastic in 27days respectively as shown in figure. The sample in LDPE became unacceptable from 18th days of storage due to visible mold growth. The results of yeast and mold count of freshly prepared control sample reported by Anbarasu and Vijayalakshmi (2007) is similar to above outcomes of fresh sprouted tofu. The sample packed in metalized plastic showed slower increase in yeast and mold count in comparison to the sample packed in LDPE and PP. This may be due the its very good barrier properties (Jamieson and Windle, 1983).

Part V

Conclusion and recommendation

5.1 Conclusions

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

1. On the basis of following sensory score: color, flavour, texture, taste and overall acceptance, sprouted tofu scored the highest in flavour, texture, taste and overall acceptance.

2. After analysis, it was observed that there was a significant increase in the moisture content, crude fiber, crude protein in the sprouted soybean whereas, the opposite result was seen in case of ash, crude fat and carbohydrates.

3. On the basis of chemical composition there was significant increase in crude fiber and crude protein in sprouted tofu.

4. The final outcome obtained after chemical and microbiological analysis of the sample sprouted tofu packed in LDPE, PP and metalized plastic showed that storage stability of sprouted tofu on metalized plastic was best as compared to LDPE and PP.

5.2 Recommendation

Based on this study, following recommendations have been made

1. For longer shelf life of sprouted tofu, metalized plastic should be used which was consumable even after 27 days.

2. Antinutritional factors of sprouted tofu can be analysed.

Part IV

Summary

Tofu is a non-fermented soymilk curd produced by a non-fermented technique. It is a versatile and nutritious dish created from soybean curd. It has gained popularity due to its inclusion in vegetarian, vegan, and hypocaloric diets. It is one of the most important and widely consumed traditional foods in most East and Southern Asian countries. Tofu is low in saturated fat and high in protein, and it is devoid of cholesterol. It can be made from sprouted soybean. Tofu made from sprouted soybean associated with improvements in the nutritive value, increasing protein digestibility, increasing sensory properties and reducing the beany flavour.

A study is carried out to know the effect of sprouting and different packaging materials on the quality of tofu made from sprouted soybean. Sensory evaluation of regular and sprouted tofu were 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. Sprouted tofu got the highest mean score than regular tofu. Moisture content, ash, crude fat and carbohydrate were decreased in tofu made from sprouted soybean from 65.341% to 64.232%, from 4.043% to 3.098%, 22.390% to 16.041% and 29.103% to 26.994% but crude fiber and crude protein were increased in from 5.741% to 10.597% and 38.715% to 43.280%.

The 1000 kernel wt., and bulk density of soybean were 194.51g and 82.666 kg/HL. The % yield of regular and sprouted tofu from 500g soybean were 41.76% and 39.173%. The sprouted tofu was packed in three different packaging materials i.e., LDPE, PP and metalised plastic. The storage stability in three different packaging materials was study in every 3 days interval in refrigerated temperature (4°C) up to 27 days. Analysis of PH, moisture, peroxide value, acid value, total plate count and yeast and mold count was performed. Between the packaging materials metalised plastic was more preferred as there was no such increase in PH, moisture, peroxide value, acid value, TPC, yeast and mold count and no growth of mold.

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Appendices

Appendix A

Sensory analysis score card

Name of the panelist: Date:

Name of the product: Sprouted and non-sprouted soybean tofu

Observe the product by visual and testing. Use your appropriate scale to show your attitude by giving the point that best describes your feeling about the samples.

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

SN	Sensory Parameters	Sample
1	Flavor	
2	Color	
3	Texture	
4	Taste	
5	Overall Acceptance	

Comments:

Signature:

Appendix B

ANOVA results of sensory analysis

Table B.1: ANOVA (no interaction) for color of regular and sprouted tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Tofu	3	0.1667	0.0556	0.29	0.829	0.5348
Panelist	5	2.8333	0.5667	3.00	0.045	0.6550
Residual	15	2.8333	0.1889			
Total	23	5.8333				

Table B.2: ANOVA (no interaction) for flavor of regular and sprouted tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Tofu	3	32.66667	10.88889	122.50	<.001	0.3669
Panelist	5	3.33333	0.66667	7.50	0.001	0.4493
Residual	15	1.33333	0.08889			
Total	23	37.33333				

Table B.3: ANOVA (no interaction) for texture of regular and sprouted tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Tofu	3	6.0000	2.0000	15.00	<.001	0.4493
Panelist	5	3.3333	0.6667	5.00	0.007	0.5503
Residual	15	2.0000	0.1333			
Total	23	11.3333				

Table B.4: ANOVA (no interaction) for taste of regular and sprouted tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Tofu	3	37.5000	12.5000	125.00	<.001	0.3891
Panelist	5	2.8333	0.5667	5.67	0.004	0.4766
Residual	15	1.5000	0.1000			
Total	23	41.8333				

Table B.5: ANOVA (no interaction) for overall acceptance of regular and sprouted tofu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d.
Tofu	3	24.0000	8.0000	60.00	<.001	0.4493
Panelist	5	1.3333	0.2667	2.00	0.137	0.5503
Residual	15	2.0000	0.1333			
Total	23	27.3333				

Appendix C

Table C1: t-test (two- product assuming unequal variance) for moisture of the sprouted soybean with raw soybean

	Raw soybean	*Sprouted soybean*
Mean	19.05666667	56.34666667
Variance	0.259633333	0.058133333
Observations	3	3
Pearson Correlation	0.582258216	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-154.5277774	
P(T<=t) one-tail	2.09377E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	4.18755E-05	
t Critical two-tail	4.30265273	

Table C2: t-test (two- product assuming unequal variance) for ash of the sprouted soybean with raw soybean

	Raw soybean	*Sprouted soybean*
Mean	5.633333333	5.34
Variance	0.023333333	0.0588
Observations	3	3
Pearson Correlation	0.998906107	
Hypothesized Mean Difference	0	
Df	2	
t Stat	5.633622717	
P(T<=t) one-tail	0.015046614	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.030093229	
t Critical two-tail	4.30265273	

Table C3: t-test (two- product assuming unequal variance) for crude fat of the sprouted soybean with raw soybean

	Raw soybean	*Sprouted soybean*
Mean	19.05666667	13.20333333
Variance	0.259633333	0.092233333
Observations	3	3
Pearson Correlation	0.907391031	
Hypothesized Mean Difference	0	
Df	2	
t Stat	38.03932367	
P(T<=t) one-tail	0.000345187	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000690374	
t Critical two-tail	4.30265273	

Table C4: t-test (two- product assuming unequal variance) for crude fiber of the sprouted soybean with raw soybean

	Raw soybean	*Sprouted soybean*
Mean	4.43	9.453333333
Variance	0.0237	0.105833333
Observations	3	3
Pearson Correlation	-0.713823162	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-19.40522077	
P(T<=t) one-tail	0.001322535	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002645069	
t Critical two-tail	4.30265273	

Table C5: t-test (two- product assuming unequal variance) for crude protein of the sprouted soybean with raw soybean

	Raw soybean	*Sprouted soybean*
Mean	36.51333333	41.22333333
Variance	0.160133333	0.113633333
Observations	3	3
Pearson Correlation	0.971377509	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-75.42036044	
P(T<=t) one-tail	8.78776E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000175755	
t Critical two-tail	4.30265273	

Table C6: t-test (two- product assuming unequal variance) for carbohydrate of the sprouted soybean with raw soybean

	Raw soybean	*Sprouted soybean*
Mean	34.36666667	30.78
Variance	0.906533333	0.0931
Observations	3	3
Pearson Correlation	0.981709044	
Hypothesized Mean Difference	0	
Df	2	
t Stat	9.482108566	
P(T<=t) one-tail	0.005470003	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.010940005	
t Critical two-tail	4.30265273	

Table C7: t-test (two- product assuming unequal variance) for yield of the sprouted tofu with regular tofu

	Regular Tofu	*Sprouted Tofu*
Mean	41.76	39.17333333
Variance	0.3109	0.698533333
Observations	3	3
Pearson Correlation	-0.859406416	
Hypothesized Mean Difference	0	
Df	2	
t Stat	3.329736182	
P(T<=t) one-tail	0.039788565	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.079577131	
t Critical two-tail	4.30265273	

Table C8: t-test (two- product assuming unequal variance) for moisture of the sprouted tofu with regular tofu

	Regular tofu	*Sprouted tofu*
Mean	65.34166667	64.232
Variance	0.341382333	0.079077
Observations	3	3
Pearson Correlation	0.32790932	
Hypothesized Mean Difference	0	
Df	2	
t Stat	3.437041921	
P(T<=t) one-tail	0.037611755	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.07522351	
t Critical two-tail	4.30265273	

Table C9: t-test (two- product assuming unequal variance) for ash of the sprouted tofu with regular tofu

	Regular tofu	*Sprouted tofu*
Mean	4.043333333	3.098
Variance	0.009926333	0.017577
Observations	3	3
Pearson Correlation	-0.983200378	
Hypothesized Mean Difference	0	
Df	2	
t Stat	7.080447839	
P(T<=t) one-tail	0.009684695	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.01936939	
t Critical two-tail	4.30265273	

Table C10: t-test (two- product assuming unequal variance) for crude fat of the sprouted tofu with regular tofu

	Regular tofu	*Sprouted tofu*
Mean	22.39066667	16.04166667
Variance	0.149240333	0.053108333
Observations	3	3
Pearson Correlation	-0.815693158	
Hypothesized Mean Difference	0	
Df	2	
t Stat	18.6523773	
P(T<=t) one-tail	0.001430982	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002861964	
t Critical two-tail	4.30265273	

Table C11: t-test (two- product assuming unequal variance) for crude fiber of the sprouted tofu with regular tofu

	Regular tofu	*Sprouted tofu*
Mean	5.741	10.59766667
Variance	0.030441	0.134134333
Observations	3	3
Pearson Correlation	-0.199891109	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-19.29227744	
P(T<=t) one-tail	0.001338003	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002676006	
t Critical two-tail	4.30265273	

Table C12: t-test (two- product assuming unequal variance) for protein of the sprouted tofu with regular tofu

	Regular tofu	*Sprouted tofu*
Mean	38.71533333	43.28066667
Variance	0.012185333	0.079982333
Observations	3	3
Pearson Correlation	-0.670825134	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-21.59712446	
P(T<=t) one-tail	0.001068524	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002137048	
t Critical two-tail	4.30265273	

Table C13: t-test (two- product assuming unequal variance) for carbohydate of the sprouted tofu with regular tofu

	Regular tofu	*Sprouted tofu*
Mean	29.10933333	26.994
Variance	0.125346333	0.655381
Observations	3	3
Pearson Correlation	-0.810944831	
Hypothesized Mean Difference	0	
Df	2	
t Stat	3.282857263	
P(T<=t) one-tail	0.040796725	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.081593449	
t Critical two-tail	4.30265273	

Appendix D

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. Hot air oven
15. Filter paper

Chemical required

1. Petroleum ether
2. Acetone
3. Sulfuric acid
4. Sodium hydroxide
5. Oxalic acid
6. Hydrochloric acid
7. Boric acid
8. Catalyst mixture
9. Plate count agar
10. Alcohol
11. Phenolphthalein
12. Potato dextrose agar

13 Acetic acid

14 Chloroform

15 Starch

16 Distilled water

17 Potassium iodide

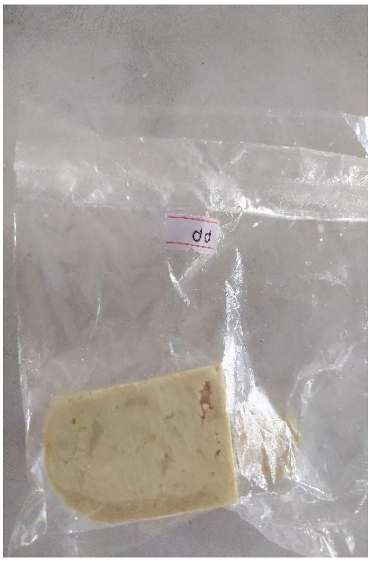
18 Sodium thiosulphate

Color plates

P1: Preparing of tofu

P2: Sprouted tofu packed in LDPE, PP and metalized plastic

P3: Microbiological analysis



P4: Sprouted tofu in LDPE on 18th days of storage

P5: Sprouted tofu in PP on 27th days of storage

P6: Sprouted tofu in metalised plastic on 27th days of storage