EFFECT OF CARDAMOM AND GINGER EXTRACT ON TOFU QUALITY AND STORAGE STABILITY

by

Pritam Khatri

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2024

Effect of Cardamom and Ginger Extract on Tofu Quality and Storage Stability

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

by

Pritam Khatri

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

December, 2024

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *Effect of Cardamom and Ginger Extract on Tofu Quality and Storage Stability* presented by Pritam Khatri has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

Dissertation Committee

1. Head of Department _____

(Mr. Navin Gautam, Asst. Prof.)

2. External Examiner _____

(Mr. Birendra Kumar Yadav, Assoc. Prof.)

3. Supervisor _____

(Mr. Om Prakash Panta, Asst. Prof.)

4. Internal Examiner _____

(Mrs. Babita Adhikari Dahal, Assoc. Prof.)

December 30, 2024

Acknowledgements

It is a true pleasure to reflect on the journey of dedication and hard work that has culminated in the completion of this thesis. This achievement would not have been possible without the support and guidance of many individuals, to whom I am deeply grateful.

First and foremost, I would like to extend my deepest gratitude to my esteemed guide, Prof. Om Prakash Panta, for his unwavering support, insightful guidance, and motivating words throughout this journey. His constructive recommendations have been invaluable.

I am also sincerely thankful to Asst. Prof. Navin Gautam, Head of the Department of Food Technology, and Assoc. Prof. Dr. Dil Kumar Limbu, Campus Chief of the Central Campus of Technology, along with Prof. Basanta Kumar Rai, Head of the Central Department of Food Technology, for providing me with the opportunity and the necessary resources to complete this dissertation successfully. A special note of appreciation goes to my teachers, as well as the library and laboratory staff, whose assistance, both direct and indirect, played a crucial role in the completion of this work. I must also express my heartfelt thanks to my classmates, whose companionship and shared experiences have been an irreplaceable part of this journey. I will forever cherish the memories we've created together. Special acknowledgment goes to my friends Gaurab Luitel, Prekshya Timsina, Chhiree Sherpa, Pratik Ghimire, Nischal Bhattarai and all my beloved juniors for their support and encouragement along the way.

Lastly, I am forever indebted to my parents and family members. No words can truly capture the depth of their love, encouragement, and unwavering moral support, especially during the most challenging times. This work would not have been possible without them.

Date of Submission: December 30, 2024

(Pritam Khatri)

Abstract

The study aimed to develop, evaluate, and assess the storage performance of flavor-enhanced herbal tofu. It investigated how different flavor additions and packaging materials (LDPE and metalized plastic) influenced the yield and sensory attributes of tofu, as well as its chemical properties (moisture, pH, peroxide value, acid value) and microbial quality (total plate count, yeast, and mold) when stored at refrigerated temperatures (4°C). Tofu was prepared by incorporating varying amounts (0.2–0.8%) of cardamom and ginger extracts separately into soymilk immediately after heat treatment. Consumer acceptability was evaluated through sensory analysis, and the resulting data were statistically analyzed using a two-way ANOVA without blocking at a 5% significance level.

The most favorable samples identified were sample C (0.6% cardamom extract) and sample F (0.4% ginger extract), along with a control sample. These selected samples were then packaged in both LDPE and metalized plastic and stored under refrigeration at 4°C for further evaluation. The study examined the chemical and microbiological stability of both herbal-treated tofu and normal tofu, packaged in LDPE and metalized plastic, over a 16-day storage period at intervals of 4 days. Key parameters analyzed included moisture, pH, peroxide value, total plate count, and yeast and mold count. Results revealed that tofu treated with herbal extracts and packaged in metalized plastic exhibited a slower reduction in moisture and pH, as well as a slower increase in peroxide value, total microbial count, and yeast and mold growth compared to tofu packaged in LDPE. This indicates that metalized plastic provided superior protection and better storage stability for the tofu samples, likely due to its enhanced barrier properties against moisture and oxygen, which helped slow down spoilage and quality degradation during refrigeration.

Contents

List of Tables

List of Figures

List of Plates

List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
BSI	Bureau of India Standards
CCT	Central Campus of Technology
Db	Dry Basis
FAO	Food and Agricultural Organization
GDL	Gluco-delta-lacton
HHPP	High Hydrostatic Pressure Processing
LDPE	Low Density Polyethylene
MAP	Modified Atmospheric Packaging
MP	Metalized Plastic
SD	Standard deviation
Tga	Transglutaminase
TPC	Total plate count

Part I

Introduction

1.1 General introduction

Soya bean (*Glycine max L.),* is a type of legume that is native to East Asia and is extensively cultivated for its edible beans, which are used to make fermented bean paste, soy milk, and soy sauce. An annual herbaceous plant in the Fabaceae family, soybeans are cultivated for their edible seeds. Soybean cultivation was originated in Asia about 5000 years ago, in China, then followed by Japan then to United State in about 19th century(Valliyodan *et al.*, 2016). More than 36% protein, 30% carbohydrates, and significant amounts of dietary fiber, vitamins, and minerals can all be found in its seeds. Additionally, about 20% of it is oil, making soybeans the most important crop for the production of edible oil (T. K. Lim, 2012).

Tofu is a curd obtained by the coagulation of hot soya milk with coagulant. It can be eaten fresh, pickled, or smoked. Tofu is rich in protein content, cholesterol free and low in saturated fat (Ojha *et al.*, 2014). Soymilk coagulation is a crucial step in the production of tofu. Coagulants such as $CaCl_2$ or $CaSO_4$ (Oboh, 2006), citric acid (D. A. Murugkar, 2015), Calcium sulphate (CS), Nigari ($MgCL_2$) and glucono-d-lactone (GDL) (Kohyama *et al.*, 1995) are majorly used in preparation of tofu. Tofu's high moisture and nutrient content make it highly vulnerable to microbial spoilage and subsequent biochemical degradation. Tofu spoils more quickly at room temperature because that's when microorganisms are most active. Tofu's shelf life has been extended through a number of methods, including the use of chemical preservatives, antimicrobial agents, paraffining, deep-fat frying, dipping the tofu in treated water, packaging in a modified atmosphere, low-temperature preservation, and hurdle technology (Y. S. Kim *et al.*, 2007).

Since ancient times, people have relied on herbs and spices to keep food fresh and safe to eat. By the end of the last century, scientists had discovered that the oils from these plants can stop food from spoiling and prevent browning. Now, there's a growing interest in using these natural compounds from plants to preserve food. On the other hand, the species and herbs give a good flavor and musk the undesirable flavors (Dorantes *et al.*, 2000). The use of naturally occurring antimicrobial compounds derived from plants, animals, and microbes to prevent food spoilage microorganisms, the growth of food-borne pathogens in food, and

other related issues is known as the "natural food preservation method" food items. Researchers are becoming more and more interested in the application of these natural antimicrobial agents as a safe substitute for physical and chemical food preservatives, which have numerous negative effects and put consumers' health at risk. Foods containing antimicrobial compounds can also increase the shelf life of processed or unprocessed foods by lowering the viability or rate of microbial growth (Beuchat and Golden, 1989). Similarly, spices and herbs are rich source of antioxidants. The antioxidant properties of herbs are due to presence of some vitamins, flavonoids, terpenoids, carotenoids and phytoestrogens (Shan *et al.*, 2011).

Large Cardamom (*Ammomum subulatum Roxburg*), also known as 'Black Cardamom' or 'Nepalese Cardamom' or 'Greater Indian cardamom' or 'Aalaichi' is an indigenous to moist deciduous and ever green forests of sub-himalayan tracts. It is a high value spice crop that is grown most commonly in the mid-hill districts of eastern Nepal (Bhattarai *et al.*, 2013). The extract derived from this species is known to possess multiple biological properties, including antioxidant, antimicrobial, analgesic, anti-inflammatory, anticancer, and flavor-enhancing activities (R. Joshi *et al.*, 2013).

Ginger (*Zingiber officinale Rosc*.) belongs to the Zingiberaceae family and is named "Zingiber" after the Greek "Zingiberi" and Sanskrit "Singabera," which both mean "horn," reflecting the resemblance of the ginger rhizome to a deer antler. The term "Officinale" is derived from the Latin "Officina," indicating its medicinal or pharmaceutical usage (Sozzi *et al.*, 2012). Phenolic compounds in ginger had positive effects on food and health. Ginger application in both fields is closely related. Ginger is a natural functional food that provides pharmacological contributions like antioxidants, antihyperglycemic, antimicrobial, anticarcinogenic, anti-inflammatory, antitumor, antilipidemic, antimutagenic, and others (Subroto and Indiarto, 2021).

1.2 Statement of problem

Creating high-quality tofu with excellent flavor and nutrition is a challenge due to the complex process involved in its production. This involves selecting the right soybeans, using suitable coagulants, and packaging the tofu correctly. Despite its popularity in vegetarian, vegan, and low-calorie diets globally, achieving consistent quality remains difficult,

requiring both commercial expertise and scientific research (Zheng *et al.*, 2020). Tofu spoils primarily because of the proliferation of microorganisms, which trigger various physical and chemical alterations, resulting in the emergence of undesirable flavors in the product. This deterioration occurs as a consequence of microbial activity, leading to changes in texture, taste, and overall quality of the tofu. Various methods for increasing the shelf life of tofu has been made. Food additives such as sorbic acid, potassium sorbate, solution of hydrogen peroxide has been tried successfully to increase the shelf-life of tofu. There has been increasing concern of the consumers about foods free of chemical preservatives because of their possible toxic effect in human beings. Thus, considering the above facts, the present research is designed to developed herbal tofu using cardamom and ginger and effect of their addition on tofu was noted.

1.3 Objectives

1.3.1 General objective

To study the effect of additional of large cardamom and ginger extract on tofu quality and its storage stability.

1.3.2 Specific objectives

The specific objectives whereas follow:

1. To carry out proximate analysis of ingredients used (soybean, cardamom, ginger).
2. To obtain the yield of ethanolic extract of cardamom and ginger and determine its total phenols and antioxidant activity.
3. To optimize the cardamom extract and ginger extract in tofu based on sensory evaluation and its storage stability.
4. To study the effects of different packaging material on storage stability of optimized herbal tofu.
5. To evaluate the cost of best herbal extract tofu.

1.4 Significance of study

The development, evaluation, and storage studies of flavor-enriched herbal tofu hold significant importance in enhancing the nutritional value of tofu, a widely consumed plant-based protein. By incorporating herbs, this study aims to create a more appealing and functional food product, offering health benefits beyond basic nutrition, such as antioxidants and vitamins. It aligns with the growing demand for natural alternatives to artificial flavors, promoting a cleaner and healthier product. The research also addresses the challenge of tofu's bland taste by improving its flavor, potentially increasing consumer acceptance and encouraging broader adoption of plant-based proteins. There are more than 20 volatile compounds which were found to be associated with beany flavor among them hexanal is the most significant compound contributing to make beany flavor in soy products (B. Wang *et al.*, 2021). Additionally, understanding the storage stability of herbal tofu will provide valuable insights into optimizing its shelf life for commercialization. This study supports local agriculture by reducing reliance on imported flavoring agents and tofu products, promoting sustainability and economic benefits. Furthermore, the introduction of herbs commonly used in traditional cuisine could make tofu more culturally relevant, adding culinary diversity and increasing its appeal in local markets.

Large cardamom and ginger are rich in phenols and thus act as antioxidants in our body and apart from this the antimicrobial property of the herbs work hand to hand to replace or reduce the synthetic preservation thus assuring food safety. Since cardamom and ginger are available locally in Nepal, on other hand the use of such indigenous herbs would be promoted.

1.5 Limitation of study

1. Only one variety of soybean (white variety) and two herbs (large black cardamom and ginger) was taken.
2. Change in sensory and chemical parameter could not be studied on daily basis.
3. Only two types of packaging material were used to study the shelf life of sample.
4. Textural analysis of the product was not carried out due to lack of an instrument in lab.

Part II

Literature review

2.1 Historical background

China's farmers grew soybeans as early as 5,000 years ago. A Yankee clipper ship arrived in the United States in 1804, carrying soybeans from China. And American farmers planted their first soybeans in 1829. For soy sauce, they raised a range of crops (Zheng *et al.*, 2020). The first mention of soybeans dates back to China, coinciding with the construction of the Egyptian pyramids. The books Pen Tsaokong Mu, published by Emperor Shang Nung, describe China's flora, including the soybean. Soybean cultivation as a food crop has grown significantly in China, Korea, Japan, and other Asian countries as a result of the Buddhist religion's ban on eating meat. As of right now, soybeans are mostly produced and exported from the United States (Beuchat and Golden, 1989).

For Nepal's hill farmers, soybean (*Glycine max L.*) is a vital legume crop. The majority of its growth occurs in the mid-hills, between three and five thousand feet above mean sea level. Soybean seeds are rich in protein (40–45%), and they also contain 20% of oil-rich along with minerals and the vitamins B, C, and E (Yofa *et al.,* 2021). Its seed is also used as green pods, soybean oil, roasted grain, green manure, animal feed, and industrial products. This plant is used both as a legume and an oil seed crop. The agricultural, nutritional, and economic values of soybean are well recognized so it has huge importance in the Nepalese economy (Shrestha *et al.*, 2021).

Tofu, a time-honored creation originating from China more than 2000 years ago, primarily consists of soybeans. The process typically involves soaking dried soybeans at a lower temperature of around 22ºC for 9-10 hours or to higher temperatures, roughly 32ºC, for a duration of 4-6 hours. Finally, the soaked soybeans undergo grinding to achieve the desired texture and consistency, completing the transformation into tofu (Purcell *et al.*, 2014). Soybeans can be prepared with sodium bicarbonate to reduce taste and improve tofu texture. Ground slurry is separated into two components: solid pulp (okara) and soymilk. Soy milk is boiled at 100°C to 110°C for 3-10 minutes. Cooking causes denaturation of the protein and removed the beany taste. Soymilk curd and whey are separated using salt coagulants including calcium and magnesium chlorides, as well as sulfates. Citric acid or

gluons delta-lactone can be used as acid coagulants. The resultant bean curd forms white blocks of different softness. It was then maintained for future use. Tofu's texture might be firm, tight, or softened (K.V *et al.*, 2021).

2.2 Soybean

2.2.1 Introduction

Soybeans are often called the “miracle crop.” They are the world’s foremost provider of vegetable protein and oil. The bushy, green soybean plant is a legume related to peas, groundnuts (peanuts) and alfalfa. Soybeans are included in the category of oilseed, which is a generic reference to crops with seeds that can produce edible and/or non-edible oil in economic quantities. The most versatile of the world’s major crops, soybeans can be grown in a wider variety of soil and climatic conditions than any other major world crop. Consequently, soybeans are the most widely grown oilseed in the world. The soybean is a dicotyledon seed (two cotyledons joined together by a hull). The soybean seed consists of three main components: the seed coat or hull, the cotyledons, and the germ or hypocotyl. The hilum, or point of attachment to the pod, is contained in the seed coat. When the hull is removed, the cotyledons separate, displacing the germ. The commercial soybean includes around 8% hull, 90% cotyledons, and 2% hypocotyl (Perkins, 1995).

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 (1986)

The world's leading soybean producers include the US, China, North and South Korea, Argentina, and Brazil. In Nepal, soybeans are known as 'Bhatmas.' Agricultural farms of Khumaltar, Kakani, and Rampur studied 138 soybean samples from several districts in Nepal, ranging in altitude from 500 to 1800 m. They found that white, brown, grey, and black soybean varieties are the most often grown. The seeds are referred to by numerous names according on their variety, color, and region, including Nepale, Hardi, Saathiya, Darmali, Maily, Kalo, and Seto (Lama, 2009).

Soybeans are utilized in a variety of goods, with just 10% being consumed by humans. Soy foods may contain soybeans, isolated soy proteins, protein concentrate, soy flour, or soy milk. Soy foods are often classified into two types: fermented and nonfermented. Traditional fermented foods include natto, miso, tempeh, and fermented tofu. Traditional soy foods without fermentation include soynuts, okara, and tofu, etc (Nagai and Tamang, 2010).

Table 2.2 Traditional and non-traditional soy products being are

Traditional products		Non-traditional Products
Fermented	Non-Fermented	
Soy yogurt	Cooking-oil	Lecithin
Tempeh	Hydrogenated fat	Protein concentrate
Sauce	Flours, flakes and grits	Protein isolate
Miso	Roasted/fried nuts	Protein hydrolysate
Natto	Sprouted beans	Liquid protein
Kinema	Cooked beans	Texturized soy-protein
Meju	Milk	
Sufu	Tofu	
Taosi	Bakery products	
	Splits (dal)	

Source: Nagai and Tamang (2010)

2.2.3 Physical properties of soybean

Soybean physiochemical properties vary according on variety, environment, and agronomical conditions. The average bulk density and weight of 1000 Nepalese soybean

kernels were 0.767 g/cc and 153.15 g, respectively. The weight of soybean seeds ranges from 120 to 180 mg, with the hull accounting for 10% (Jensen *et al.*, 1995).

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 2.3 Proximate composition of soybean and seed parts

Seed part	Whole seed weight (%)	Protein (%) N x 6.25	Lipid (%)	Carbohydrate (%) (incl. fiber)	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 composition of major nutrients in soybeans on a dry weight basis includes approximately 40% protein, 20% lipid, 35% carbohydrate, and 5% ash. The moisture content of mature soybeans ranges from 12% to 15%. Soybeans are valued as an economical source of protein for human consumption due to their protein concentration of 35% to 40% by dry weight (Derbyshire *et al.*, 1976). The predominant protein in soybeans is globulin, which is soluble in salt and can be extracted using water. These proteins are somewhat insoluble near their isoelectric pH of 4.5 but become more soluble at pH levels above 7.0. Soy proteins, containing both polar and nonpolar amino acids, have the ability to bind water and lipids, making them useful as emulsion stabilizers and protective colloids. While soy protein complements cereal protein in terms of amino acid composition, it is generally deficient in

methionine and other sulfur-containing amino acids for most animals, including humans (Rossi *et al.*, 2016).

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 minor components in refined soy oil, like phospholipids and lecithin, can influence both the color and stability of the oil. Soy oil is composed of approximately 80% unsaturated fatty acids, with linoleic acid being the most abundant among them (Chen *et al.*, 2011). Soybean seeds are rich in carbohydrates, making up about 30% of their dry weight. They contain both soluble and insoluble carbohydrates. Specifically, around 10% of soybean seeds consist of soluble carbohydrates, which include approximately 5% sucrose, 1% raffinose, and 4% stachyose. While humans cannot digest or absorb raffinose and stachyose, the bacteria in the human digestive tract can metabolize these oligosaccharides, often leading to flatulence (Liu, 2012).

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 2.4 Nutritional value of raw soybean per 100 gm on dry basis

Parameters	Values
Moisture	10.2 g
Carbohydrates	29.6 g
Fat	17.7 g
Protein	33.3 g
Minerals	5 g
Fiber	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 2.5 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: Perkins (1995)

Table 2.6 Mineral composition per 100 g

Sample	mg/100g
Calcium	18.4
Phosphorous	144
Iron	1

Source: Pal *et al.* (2019)

2.3 Tofu

Tofu is a versatile and nutritious food made from soybeans. It's created by coagulating the proteins in soymilk, then pressing the curds into a solid, sliceable form. The process of making tofu can vary, which leads to different flavors, textures, and culinary uses depending on where it's made. Tofu is a natural and affordable food option enjoyed by people all over the world, from developed to developing nations. It's an excellent source of plant-based protein, making it a popular choice for vegetarians and anyone looking to include more non-animal protein in their diet (K.-N. Park *et al.*, 2007).

Tofu, also known as bean curd, is a plant-based food renowned for its nutritional and health benefits. It was first discovered by a Chinese cook about 2000 years ago and later introduced to Japan in the 18th century. Today, tofu is popular in many countries, including the Americas, Australia, Cambodia, China, Europe, India, Indonesia, Japan, Korea, Malaysia, Myanmar, New Zealand, Philippines, Singapore, Thailand, and Vietnam. Tofu is made by curdling soymilk, causing its proteins to coagulate, and then pressing the curds into a sliceable cake. The production methods, flavors, textures, and uses of tofu vary regionally. It is a natural, inexpensive, and nutritious food consumed worldwide in both developed and developing countries, providing an excellent source of non-animal protein for vegetarians (Zhang *et al.*, 2018).

Tofu's texture ranges from soft to firm to extra-firm. Soft tofu, with a smoother texture, is suitable for sauces, salad dressings, and desserts, while firm and extra-firm tofu are ideal

for baking, stir-frying, and grilling. Commonly used in Asian cuisine, particularly in East and Southeast Asia, tofu's neutral flavor allows it to blend easily into various recipes. In India, tofu serves as a low-fat substitute for paneer. Tofu can be frozen for up to five months, which changes its texture to become more spongy and absorbent, and its color to a more yellowish hue. Typical tofu-making involves several steps: cleaning, soaking, grinding beans in water, filtering, boiling, coagulation, and pressing. The coagulation step is crucial, typically using salts or acids as coagulants. The texture of tofu can also be modified by freezing, puréeing, or cooking. Fresh tofu is made directly from soy milk, while processed tofu is made from fresh tofu. It is available in bulk or individual packages, both refrigerated (Mujoo *et al.*, 2003).

Tofu is widely consumed for its health benefits, available in various forms such as baked, smoked, roasted, or fried. It can be used in savory and sweet dishes and is a versatile substitute for meat and seafood. Overall, tofu is a highly beneficial plant-based food enjoyed by people of all ages and genders worldwide (Azhar, 2024).

2.4 Types of tofu

2.4.1 Firm tofu

Firm tofu, also known as "Cotton tofu," is made by coagulating soymilk using either synthetic or natural coagulants. The process begins by heating the soymilk and then adding the coagulant, which causes the proteins in the milk to curdle and form curds. Once the curds have formed, they are transferred into a mold lined with cheesecloth. The curds are then pressed to remove excess water. The pressing step is crucial because it helps achieve the desired firmness of the tofu by reducing its moisture content. The result is firm tofu with a moisture level typically ranging from 76% to 81%, giving it a denser texture compared to softer types of tofu. This process not only gives firm tofu its characteristic texture but also makes it more versatile for cooking, as it can hold its shape better when stir-fried, grilled, or baked (Deman *et al.*, 1986).

2.4.2 Silken Tofu

Soft or silken tofu is known for its high-water content and creamy, smooth texture, with a moisture percentage of around 87-90%. It's made by coagulating soymilk, often directly in the container in which it's sold. To create this type of tofu, soymilk is coagulated using coagulants like calcium sulfate ($CaSO_4$), magnesium chloride ($MgCl_2$), or glucono-δ-lactone. These coagulants help set the texture and flavor of the tofu and can be used alone or in combination (Pal *et al.*, 2019).

The process starts with grinding raw soybeans and mixing them with hot water. The soymilk is then extracted using a centrifugal juice extractor. Depending on the recipe, the concentration of coagulant used can range from 1.5 to 5.0 grams per kilogram of soymilk. About 220 milliliters of soymilk is poured into a plastic container, cooled to room temperature, and then mixed with 7 milliliters of the coagulant. The container is sealed with lids that have small holes to let gases escape and is then heated indirectly at 85°C (185°F) for 35 minutes to allow the tofu to set. Afterward, the silken tofu is stored at 4°C (39°F) to maintain its freshness and texture. This process results in soft tofu with a delicate texture, making it ideal for dishes where a smooth, creamy consistency is desired (Evans *et al.*, 1997).

2.4.3 Dried Tofu

Dried tofu is the firmest variety of tofu, characterized by a moisture content of less than 76%. This lower moisture level is achieved by vigorously breaking the soybean curd to remove excess water before pressing, resulting in a denser, more compact texture compared to other types of tofu.

To prepare dried tofu, raw soybeans are soaked in six times their volume of water at 21-22°C for 8 h. Once soaked, the excess water is drained, and the hydrated soybeans are finely ground using a high-speed grinder. The resulting mixture is clarified by centrifugation to extract soymilk. To make dried tofu, the soymilk is boiled for 20 min with 1 g of antifoaming agent and then allowed to rest for 3 min at 96°C. Next, 3% nigari (magnesium chloride) is added to the soymilk, which is left to sit for 10 min to allow the curd to form. The curd is gently stirred twice, for 30 sec each time. The soy curd is then transferred into a wooden mold lined with cloth, measuring 25cm x 25cm x 7cm, and pressed at 34.8 g per square centimeter for up to 30 min to achieve the desired firmness (Cha *et al.*, 2003).

2.4.4 Sufu (Fermented tofu)

Sufu, commonly known as fermented tofu, is made by fermenting tofu with various strains of microorganisms, such as *Actinomucor elegans*, *Mucor racemosus*, *Mucor sufu*, *Mucor dispersus, Mucor wutuongkiao,* and species of Aspergillus. The quality of the final product largely depends on the specific strains used during the fermentation process, which influence its flavor, texture, and overall characteristics (B.-Z. Han *et al.*, 2001).

Dried tofu is the type of tofu with the lowest moisture content (less than 76%), manufactured by aggressively breaking soybean curd to remove excess water before pressing. It is made by soaking soybeans in a 1:6 ratio of water at 21-22°C for 8 h. Excess water is removed, and the soaked grains are crushed in a high-speed grinder before being centrifuged to produce soymilk. Soymilk is heated with 1 g of antifoaming agent for 20 min and then allowed to stand for 3 min at 96°C. The coagulant is added and left to stand for 20 min until the curd forms. The curd is churned for a few seconds and then coagulated again. Furthermore, it is put to a wooden mold coated with cotton and pasteurized for 30 min (Anjum *et al.*, 2023).

2.4.5 Low fat Tofu

The consumers of the modern era are craving for health foods with low fat. Tofu being an excellent source of protein holds a good chance for being a health food substitute by minimizing fat consumption.

Defatted soy flour, a byproduct of soy oil production, can be used to create low-fat tofu with the same texture as regular, full-fat tofu. This low-fat version is made using soy flour that has undergone supercritical CO_2 extraction. As the fat content of the flour is reduced from 19.5% to 7.1%, the viscosity of the resulting soymilk also drops from 50 to 40 cp. Despite this fat reduction, low-fat tofu actually yields more, at 69.7%, compared to the 60.8% yield of full-fat tofu. Importantly, the lower fat content doesn’t affect the tofu's texture or taste (Shin *et al.*, 2014).

2.4.6 Specialized varieties of tofu

To make pea tofu, blend soybeans and peas with glucono-d-lactone (GDL) acting as a coagulant. After soaking for 17 h at 20°C, peas and soybeans are combined 1:1. In order to

solidify, the soybean and pea milk are heated to 95°C for 15 min, deducted from GDL and $CaSO_4$ at 0.3% and 0.1%, respectively, for 45 min (Depalma *et al.*, 2019).

White whole soybeans are used in the production of sufupehtze. The quality of Sufupehtze is influenced by factors like the soy slurry's particle size, total solid content, coagulant type, and coagulant concentration. Sufupehtze that is similar to the traditional product can be made by maintaining 12% TS and using a blend of coagulants of gypsum and brine (5:5) added at a rate of 3% and heating at 80°C (Ran and Kan, 2012)

Sprouting tofu is made from soy beans that have sprouted. It is known that a number of metabolic changes are triggered during the sprouting process, which increases the nutritional content of sprouted legumes and reduces the concentrations of antinutritional substances like phytic acid and trypsin inhibitor (Murugkar *et al.*, 2009).

2.5 Gelation mechanism of tofu

Over time, many scientists have proposed different theories to explain how soy gels form. While several popular ideas have emerged, the current understanding still faces significant limitations and unresolved issues.

Kohyama and Nishinari (1993) introduced a widely recognized theory about how soy gel forms, particularly in tofu production. According to their hypothesis, the process occurs in two stages: protein denaturation and hydrophobic coagulation. In the first stage, heat is applied to the protein molecules in their native state, exposing their hydrophobic regions and causing denaturation. This denaturation leads to the production of negatively charged ions. In the second stage, glucono-delta-lactone (GDL) and calcium ions generate protons that help neutralize the proteins' net charge. This neutralization enhances the hydrophobic interactions between protein molecules, causing them to clump together, ultimately forming soy curd. Figure 2.1 illustrates Kohyama and Nishinari (1993) proposed mechanism of tofu gel formation. Soybean proteins are made up of two major fractions 7s ate of gelation of 7s globulins is (â-conglycinin) and 11s (glycinin). The 7s subunit is soluble while ,11s exists in a particulate form. It is determined that the rate of gelation of 7s globulins is much more slower than that of 11s proteins (Peng and Guo, 2015). Kohyama and Nishinari (1993) also investigated the process of gel formation in soybean 11S protein when a coagulant is added. They studied how temperature and rate-order kinetics affect gelation, observing that the rate

of gel formation increases with higher gelling temperatures and is influenced by coagulants like glucono-delta-lactone (GDL). Interestingly, they noted that, unlike other protein-based gels, the time it takes for soy gel to form is not dependent on protein concentration. However, in a follow-up study in Kohyama and Nishinari (1993), found that adding more 7S proteins reduced gelation time in the presence of GDL. This suggests that while 7S proteins form gels more slowly on their own, the right coagulants can speed up the process. Moreover, 7S proteins require lower concentrations to coagulate compared to 11S proteins. In summary, 7S proteins primarily influence the speed of gelation, while 11S proteins play a key role in determining the firmness of the soy gel.

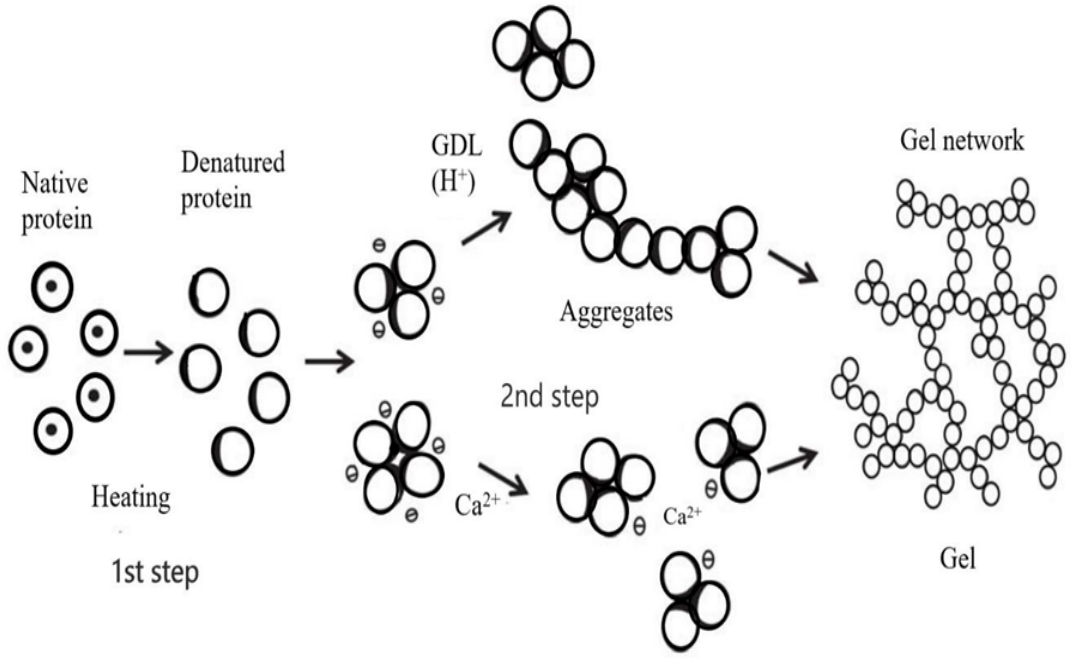

Fig. 2.1 Kohyama's mechanism of gel formation in tofu

Source: Kohyama and Nishinari (1993)

Another perspective on gel formation diverges from the particulate gel theory. This hypothesis suggests that intermolecular electrostatic repulsion plays a key role in promoting gel formation, which differs from the particulate gel model. The repulsion is thought to arise from surface charges and is influenced by factors such as the nature of the particles, pH levels, and the concentration of the coagulant. According to this theory, there is a need to

study how different components, like phytic acid, polyacid ions, proteins, and lipids, interact to better understand the gelation process (Deman *et al.*, 1986).

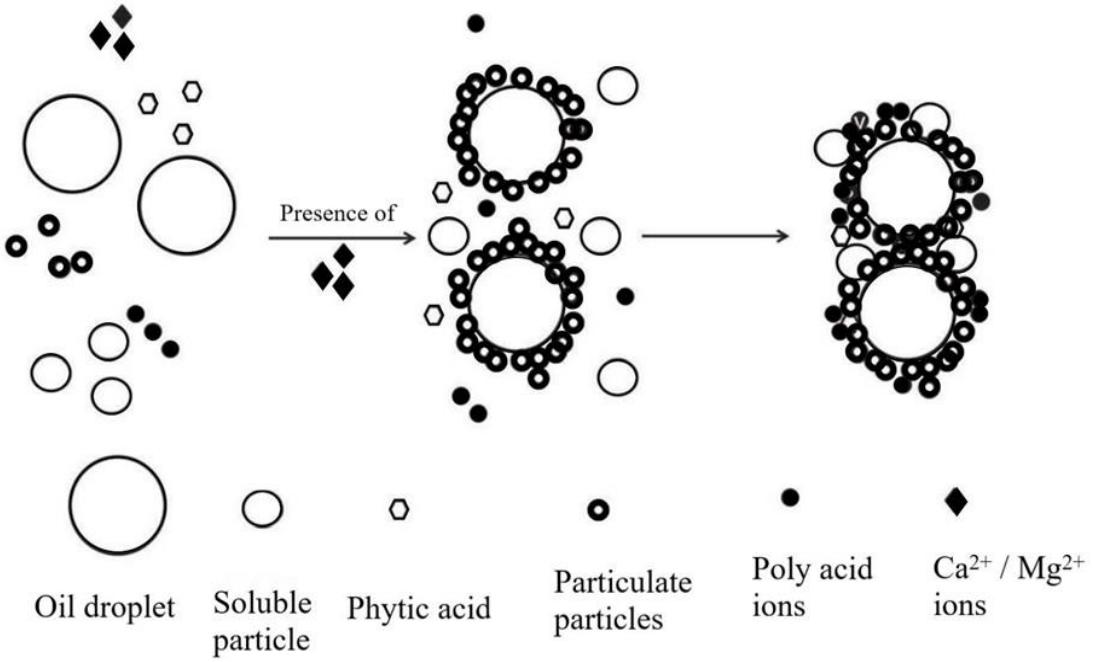

Fig.2.2 Formation of soy gel according to Shun-Tang's theory

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

Shun-Tang *et al.* (1999) introduced another model for soy gel formation, expanding on Kohyama's theory by incorporating the roles of soy protein, lipids, and small charged molecules like phytates and polyacid ions. Their model suggests that after 11S protein coagulates in the presence of calcium ions, small molecules prevent the coagulation of the protein-lipid interface as more coagulant is added. The lipid globules are then wrapped first by micelles rich in 11S protein and later by particles rich in 7S protein. As this process continues, the lipids form distinct oil bodies, which merge and become coated with oleosins, structural proteins that surround the lipids. This results in a layered, or "sandwiched," structure where the protein-lipid interfaces combine to create the soy milk gel matrix. This model is further replicated to understand yoghurt and cheese matrix (Grygorczyk *et al.*, 2014; Peng and Guo, 2015). Further improvement in the model is however thought to be necessary, as the soy gel matrix is thought to bear a particulate gel structure and the gelation occurs when protein-lipid complex coagulates due to intermolecular crosslinking. This complex appears as particle type proteins clustered together (Malaki Nik *et al.*, 2011).

2.6 Factor influencing the quality of tofu

In tofu production, manufacturers aim for high protein recovery and solid yield to maximize profits. The chemical makeup of tofu plays a crucial role in determining both its quality and quantity. This composition is influenced by the type of soybeans used and the specific production process. Over the past few decades, extensive research has explored the factors that affect tofu's quality and yield. Many studies indicate that the quality of the soymilk and the process of coagulation play significant roles in these outcomes. However, since tofu making involves a complex interplay of various factors, research findings often differ due to variations in preparation techniques (Zhang *et al.*, 2018). Some of the factors are given below

2.6.1 Varieties of soybean seeds

H. Bhardwaj *et al.* (2003) conducted a study to analyze how different soybean genotypes from various regions influenced the oil and fatty acid content in tofu. They tested several genotypes, including BARC-8, BARC-9, MD86-57788, Enrei, Hutcheson, Nakasennari, S90-1056, Suzuyutaka, Ware, York, V71-370, and V81-1603. Among them, the Hutcheson variety was found to have the highest oil content in tofu, with 24.0 g per 100 g. It also had a total saturated fatty acid content of 3.80 g per 100 g. In contrast, BARC-8 and BARC had much lower oil content, with values of 15.8 g/100 g and 11.3 g/100 g, respectively. Tofu total saturated fatty acid content varied depending on where the seed were cultivated. The composition of soybean seeds is influenced by both genetic factors and environmental conditions, though the extent of each factor's influence can change depending on the seed component being analyzed, the type of soybean, and the region where it's grown. Similarly, the environment where the soybeans are cultivated can impact the yield and quality of both soymilk and tofu (Poysa and Woodrow, 2002).

2.6.2 Effect of coagulants

Although making tofu is fairly straight forward, its texture is crucial to its quality and appeal due to its naturally mild flavor. The texture is largely determined by the choice and amount of coagulant used, with calcium and magnesium salts being the most common. Different coagulants are chosen depending on the type of tofu being made. Controlling the coagulation of soymilk is often the trickiest part of the process, as it involves the delicate balance of

several factors. For instance, raising the coagulation temperature or stirring more quickly after adding the coagulant can result in firmer tofu (Saio, 1979).

It was found that calcium chloride ($CaCl_2.2H_2O$) and magnesium chloride ($MgCl_2.6H_2O$) caused the soymilk to coagulate almost instantly, while calcium sulfate ($CaSO_4.½H_2O$), glucono delta-lactone (GDL), and magnesium sulfate ($MgSO_4.7H_2O$) worked at a slower pace. The texture of the tofu curd was significantly affected by both the type and concentration of the coagulant. Out of the five coagulants studied, 0.75% calcium sulfate and 0.4% fresh GDL solution were the most effective in producing tofu with a heavy bulk and smooth texture (Deman *et al.*, 1986). It was found that using calcium sulfate and modified nigari as coagulants, along with different stirring speeds and times, affected soft tofu's texture and yield. High stirring speeds produced firmer tofu but lower yields, while tofu made with modified nigari was softer than that made with calcium sulfate. Stirring for less than 25 seconds was ideal for making soft tofu, as longer times reduced yield (Joo and Cavender, 2020). Tseng and Xiong (2009) studied the texture of silken tofu containing inulin and coagulated with glucono-δ-lactone (GDL) during heating. As inulin was added in amounts from 1% to 4%, the tofu’s hardness increased from 115.8 to 137.4 g, while cohesiveness slightly decreased from 0.86 to 0.82. Deformability ranged between 5.19% and 8.25%, and the force required to rupture the tofu rose from 424.3 to 487.9 g.

No and Meyers (2004) also explored the use of chitosan as a coagulant in producing tofu with an extended shelf life. They tested chitosan with six different molecular weights (1106, 746, 471, 224, 28, and 7 kDa) under various conditions to see how effective it was in tofu production. While the chitosan didn't significantly affect the tofu's yield, hardness, or the properties of the whey, it did influence the sensory qualities and shelf life of the tofu. The most effective method for coagulating soy milk was found to be using a 1% chitosan solution in 1% acetic acid at 80°C for 15 minutes.

2.6.3 Effect of enzymes

The use of the enzyme Viscozyme L to break down carbohydrates in soy slurry. The enzyme worked best at 55°C, leading to increased levels of glucose and galactose in the tofu (1.36 and 0.19 g/100 g, respectively), confirming its activity compared to the control. Tofu treated with Viscozyme had higher levels of total phenolics (173 mg vs. 161 mg gallic acid

equivalents/100 g) and showed stronger antioxidant properties in specific radical tests. Additionally, the treated tofu had about four times more reducing sugars than the control under optimal conditions (30 Fungal Beta-Glucanase units/10 g solids, 55°C, for 30 min) (Rosset *et al.*, 2012).

Rizkaprilisa and Setiadi (2018) experimented with papain, an enzyme from papaya, as a new coagulant to boost tofu production. To extract the soybean protein, they used a 1:2 soybean-to-water ratio. They heated 200 g of soybean extract, and when it reached 70 to 80°C, they added papain in amounts of 3 or 6 g, along with calcium sulfate ($CaSO_4$) at 1 or 2 g. The highest tofu yield, 66%, was achieved using 2 g of $CaSO_4$, while 6 grams of papain produced a slightly lower yield of 65%. However, tofu made with papain had a higher protein content (9.3%) compared to tofu coagulated with $CaSO_4$, which had 6.5%.

Yasir *et al.* (2007) studied the effects of the transglutaminase (TGA) enzyme on tofu texture. They found that control tofu had a fracture force greater than 30 N, while tofu treated with TGA had lower fracture forces. Specifically, tofu with 1000 ppm of TGA had a fracture force of 25 N, whereas tofu with 5000 ppm exceeded 30 N. This showed that the enzyme concentration significantly influences tofu's texture.

2.6.4 Effect of processing parameter

Various processing parameters, such as the milk's solid content, heating the soybeans with sodium bicarbonate, stirring time after adding the coagulant, and tofu molding, greatly influence tofu's flavor, quality, and texture. Softer tofu (with a force of 3.2 N) was produced with longer blanching times, while the control tofu remained firm (with a force of 7.8 N). A smooth tofu texture was achieved from soy milk with a solid concentration of 7° Brix after a 10-minute thermal treatment of soybeans with 1% sodium bicarbonate. Additionally, stirring and molding times during coagulation played a key role in determining tofu yield. Stirring the milk for 5 seconds after adding the coagulant, allowing it to settle, and then pressing the tofu with 1,000 g of weight for 15 minutes, followed by 500 g for another 15 minutes, resulted in a yield of 22.6 g of soft, firm tofu per 100 ml of soy milk (Rekha and Vijayalakshmi, 2010).

2.7 Chemical aspects of tofu

2.7.1 Chemical composition of tofu

Soybean products are an excellent source of protein, carbohydrates, and minerals, while being low in fat. They are a staple in many Eastern countries' diets. Tofu is created by coagulating soy milk with either salt or acid, forming a soy protein gel that retains water, soy lipids, and other nutrients within its structure. The texture and flavor of tofu are influenced by the type of coagulant used during this process (Obatolu, 2008). Proximate composition and mineral composition of tofu is shown in Table 2.1 and 2.2 respectively.

Table 2.7 Proximate composition of tofu

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

Source: DFTQC (2012)

Table 2.8 Mineral composition of tofu

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

Source: DFTQC (2012)

2.7.2 Chemical characteristics of tofu

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

2.7.2.1 pH

Anbarasu and Vijayalakshmi (2007) studied the pH changes in control tofu and tulsi-treated tofu. Initially, the pH values were 6.88 for control tofu and 6.90 for tulsi-treated tofu. After 24 h, both types showed a significant drop in pH, with control tofu reaching 6.20 and tulsi-treated tofu dropping to 5.89. This decrease was likely due to a logarithmic phase of bacterial growth between 24 and 48 hours, leading to nutrient consumption, acid production, and a marked pH reduction. Over time, the pH began to rise, reaching 6.92 for control tofu and 6.73 for tulsi-treated tofu after 7 days. Throughout storage, tulsi-treated tofu maintained a consistently lower pH than control tofu.

2.7.2.2 Moisture

Anbarasu and Vijayalakshmi (2007) observed that the moisture content of control tofu varied, starting at 70.37% when fresh and rising by 5% to 75.22% after 7 days. This was likely due to protein breakdown and fat oxidation, which weakened the tofu's gel structure, allowing it to absorb more water. In contrast, tulsi-treated tofu, which began with 76.49% moisture, saw an initial drop to 75.21% on the first day, followed by a gradual increase over the next 7 days, reaching only a 1% increase to 77.30%.

Tofu made with 30% sesame had a lower moisture content compared to regular tofu, which can be an important factor in extending its shelf life. Moisture promotes microbial growth and spoilage, so with less water present, sesame-incorporated tofu is less prone to spoilage and might stay fresh longer. The reduced moisture also helps maintain the tofu's texture and quality over time, making it more stable for storage compared to tofu without sesame. This makes sesame-enriched tofu potentially more durable and appealing for long-term use (Poudel, 2022).

2.7.2.3 Peroxide value

Tkaczewska *et al.* (2023) investigated the use of innovative single and double-layer films with antioxidant properties as packaging for vegetarian products, including tofu. However, the study found that these films were not effective in preventing the oxidation of tofu's fats. Lipid oxidation, a process where fats break down, can be triggered by various factors like singlet oxygen production or the generation of free radicals through enzymatic and non-enzymatic reactions. Lipid oxidation negatively impacts the shelf life of tofu by deteriorating its quality and nutritional value. As fats oxidize, they alter the levels of essential vitamins and minerals, reducing the product's health benefits. Moreover, lipid oxidation makes the tofu more vulnerable to spoilage by promoting microbial growth, further limiting its freshness and overall safety. Controlling this oxidation is essential for preserving the tofu's nutritional integrity and extending its shelf life.

2.8 Microbiology aspects of tofu

The microbiological quality of tofu is influenced by several factors, including the quality of the soymilk used, the heat treatment applied, the tofu's moisture content, the level of contamination, and the storage conditions. If any of these factors are compromised, microorganisms can easily enter the tofu, leading to contamination. Microbes may come from multiple sources, such as the air, water used in the process, utensils, cutting knives, and cloths used for filtering. Even the person handling the tofu can introduce bacteria or other pathogens. Under substandard conditions, these factors increase the risk of microbial growth, which can spoil the tofu and make it unsafe for consumption. Proper hygiene and controlled environments are essential to maintaining the tofu's microbiological safety (Tuitemwong and Fung, 1991).

2.8.1 Standard plate counts

The process of making tofu, particularly boiling the soy milk, effectively eliminates most of the initial microorganism's present. However, contamination can occur after boiling, especially during the pressing of the curds to form tofu cakes and through handling before packaging. Additionally, tofu is often stored in the produce section of grocery stores, where improper temperature control is possible. This combination of post-boiling contamination risks and potential temperature mishandling makes tofu a possible public health concern, as

it can provide an environment for bacteria to grow if not stored or handled properly (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 5.6xl06 CFU/g) and psychrotroph counts (up to 7.9 X 107 CFU/g), indicating possible microbial deterioration.

Anbarasu and Vijayalakshmi (2007) investigated the effect of tulsi (*Ocimum sanctum*) extract on extending tofu's shelf life at room temperature. The tulsi extract was found to increase tofu's shelf stability. Initially, untreated tofu had an aerobic bacterial count of 5.96 x 10^2 cfu/g, while the tulsi-treated tofu had a slightly higher count of 2.37 x 10^3 CFU/g. After seven days of storage, the microbial count in untreated tofu rose to 6.21 x 10^8 CFU/g, whereas the tulsi-treated tofu reached 1.08 x 10^9 CFU/g. Although both samples saw an increase in bacteria, the tulsi extract helped control the growth, indicating its effectiveness in preserving tofu.

High hydrostatic pressure processing (HHPP) has been explored as a method to reduce microbial contamination in tofu. Préstamo *et al.* (2000) applied a pressure of 400 MPa at 5°C for different time durations—5, 30, and 45 minutes—to study its effect on microbial inactivation. The results showed that microbial counts significantly decreased over time. Initially, the microbial load was 5.54 x 10^4 CFU/g, but after the treatments, it dropped to 0.31, 1.56, and 2.38 log units, respectively, depending on the treatment time. Specifically, the study found a reduction of 2 log units in psychrotrophic bacteria (which thrive at low temperatures) and a 1 log unit decrease in mesophilic bacteria (which grow at moderate temperatures). This indicates that HHPP can effectively reduce bacterial populations in tofu, making it a useful method for improving its microbiological safety and extending shelf life without compromising quality.

Min *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 10^7 CFU/ml.

2.8.2 Yeast and mold

Tofu, being rich in protein, has a very short shelf life. To address this, Anbarasu and Vijayalakshmi (2007) used tulsi (*Ocimum sanctum*) extract, commonly available in rural

areas, to help extend tofu's shelf life. In their study, yeast and mold counts in freshly made and 7-day-old untreated tofu ranged from 1.0×10^2 to 3.37×10^4 CFU/g, respectively. In tulsi-treated tofu, there was an initial 2-log increase in yeast and mold counts, rising from 1.0×10^2 CFU/g after 2 days. This was followed by a 2-log decrease after 4 days. However, by the end of 7 days, the yeast and mold count increased again, reaching 2.33×10^5 CFU/g. This indicates that while tulsi extract initially slowed the growth of yeast and mold, the counts eventually increased over time.

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 (2007)

2.9 Shelf life of tofu

The shelf life of food products is an important feature for both manufacturers and consumers. The most important factor for shelf-life evaluation of food is safety, followed by quality including physical, chemical, and sensorial properties. Shelf-life studies can provide important information to manufacturers and consumers to ensure a high-quality product during the storage period.

Soy curd, commonly known as tofu, is an excellent source of high-quality protein. However, without preservation or refrigeration, tofu typically has a short shelf life of about 1 to 3 days, depending on the storage temperature. While tofu is celebrated for its rich nutritional benefits, its high protein content, moisture level, and neutral pH make it highly

perishable. These factors create an ideal environment for spoilage, which is why tofu requires proper storage to maintain freshness (Lee *et al.*, 2014).

2.9.1 Measures to improve shelf life of tofu

In the past, various techniques have been developed and documented to preserve tofu and extend its shelf life. These include traditional methods like coagulated by fruit juice (J.-Y. Kim and Park, 2006), chitosan (No and Meyers, 2004), making tofu from ozone treated soybean (I. Park *et al.*, 1994), tofu packed in PE films coated with nisin incorporated MC/HPMC (methylcellulose—MC and hydroxypropyl methylcellulose—HPMC) solution (Cha *et al.*, 2003).

2.9.1.1 Low Temperature Storage

Storage temperature is perhaps the most crucial factor in preserving the quality and extending the shelf life of packaged foods. Generally, as the storage temperature rises, the quality and acceptability of these foods decline. Biological reactions, including spoilage, tend to accelerate, often doubling or tripling with every 10°C increase in temperature (J. H. Han, 2005). Since microbial growth is a key cause of tofu spoilage, storing it at cooler temperatures can significantly prolong its shelf life by slowing down these biological processes.

2.9.1.2 Electrolyzed water

Tofu products spoil quickly and have a limited shelf life. To manage microbial growth during tofu production, electrolyzed water (EW) was used in the soybean soaking phase of manufacturing. The chlorine present in EW, which consists of hypochlorous ions, hypochlorous acid, and chlorine, acts as a disinfectant to sterilize and reduce contamination (Zhao Zhaohui *et al.*, 2002).

2.9.1.3 Nisin

The use of a polyethylene (PE) film coated with Nisin, methylcellulose, and hydroxypropyl methylcellulose to package tofu. Nisin (NS) is a natural preservative with strong antimicrobial properties. To test its effectiveness, the film was placed in direct contact with a suspension of Listeria monocytogenes (L. monocytogenes), a harmful bacteria that can

contaminate tofu. The results showed that the Nisin-coated film effectively inhibited the growth and survival of the L. monocytogenes strain Brie-1, demonstrating its potential to prevent microbial contamination and extend tofu's shelf life (Cha *et al.*, 2003).

2.9.1.4 Hydrogen peroxide

Hydrogen peroxide treatment has shown potential as an effective preservative for tofu. Normally, firm tofu has a shelf life of just one day at room temperature without any treatment. However, when treated with hydrogen peroxide at a concentration of 95 ppm, the shelf life extended to 2 days. Increasing the hydrogen peroxide concentration to 395 ppm further extended the shelf life to 3 days. This demonstrates that hydrogen peroxide can significantly help preserve tofu at room temperature by slowing down spoilage (H.-W. Chang and Chen, 1999).

2.9.1.5 Irradiation technology

Maurya *et al.* (2018) explored how gamma irradiation affected tofu when using three different packaging materials: biaxially oriented polypropylene, high-density polyethylene, and low-density polyethylene. The tofu samples were stored for up to 20 days. The study found that the tofu’s color shifted from light cream to light yellow, likely due to radiation removing moisture, which impacted the color of the samples. The combination of low-density polyethylene packaging and 1.25-kGy gamma irradiation was particularly effective, extending the tofu's shelf life to 15 days while preserving its quality, showing that this method can successfully enhance tofu's longevity without compromising its properties.

2.9.1.6 Packaging of tofu

Food packaging plays a crucial role in the food industry as it helps maintain the quality of food during storage, transport, and distribution. It serves as a protective barrier, shielding the food from physical damage, such as impacts or pressure, as well as chemical changes, like oxidation or moisture loss. Packaging also acts as a defense against biological threats, such as bacteria or mold, ensuring the food remains fresh and safe for consumption. By preserving the food's quality and extending its shelf life, effective packaging is essential in delivering products in optimal condition to consumers (Sarkar and Aparna, 2020).

Packaging is the final stage in tofu production, and the materials and storage methods used play a key role in determining the product's flavor, quality, and shelf life. Tofu is typically sold in bulk, in water-filled tubs, plastic bags, or vacuum-sealed packaging. Due to its high moisture and protein content, tofu provides an ideal environment for microbial growth, which limits its shelf life to just a few days, even when stored in refrigerated conditions. Proper packaging is crucial to minimize spoilage and maintain the tofu's freshness (Rahman and Asman, 2024).

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 Aparna, 2020)

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

3. Metallized films are polymer films that have a thin layer of metal, typically aluminum, applied to them. These films are becoming a popular alternative to aluminum foil because they use less aluminum and are more cost-effective. In metallized films, the aluminum coating is only about 50 nanometers thick. Metallized polymer films are useful because of their very good barrier properties (Ge *et al.*, 2021).

2.10 Health benefits of tofu

Tofu is an excellent source of essential nutrients like protein, vitamins, and minerals that support overall health. Studies suggest that incorporating soy-based products, like tofu, into one's diet may help reduce the risk of several diseases, including breast cancer, osteoporosis, and cardiovascular disease. Soy protein has been shown to have a positive effect on cholesterol levels, as research indicates that consuming soy protein can significantly lower serum levels of triglycerides, LDL cholesterol (often referred to as "bad" cholesterol), and total cholesterol when compared to animal proteins. This makes tofu a valuable addition to

a heart-healthy and balanced diet (Pal *et al.*, 2019). 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.

2.11 Trends towards use of natural ingredients

Consumers are increasingly worried about the potential toxic effects of chemical preservatives in food, driving demand for products that are free from synthetic additives. As a result, there is a growing call for limited use of these chemicals in food production. At the same time, people are seeking foods with a long shelf life that do not pose the risk of foodborne illnesses. This trend has placed pressure on the food industry to reduce or eliminate chemical preservatives and to adopt natural alternatives that ensure microbial safety while meeting consumer expectations for healthier, safer foods (Arora and Kaur, 1999).

In recent years, there has been a growing focus on finding natural antimicrobial substances to meet the needs of both food manufacturers and consumers. Many herbs and spices have long been known for their antimicrobial properties and have been used as natural alternatives for food preservation. Research has shown that these herbs and their derivatives can effectively inhibit the growth of bacteria, fungi, and microbial toxins, making them valuable in extending the shelf life of food products and enhancing food safety without the use of synthetic chemicals (Zaika, 1988).

2.12 Herbs and spices as preservatives

Botanically, herbs are soft-stemmed plants whose main stems die back to ground level. Depending on their life cycle, they can be classified as annuals (which do not regrow), biennials (which regrow once the next year), or perennials (which regrow each year). Herbs can be used fresh or dried, and they typically have a light, distinctive aroma. Despite their fragrant qualities, herbs generally contain only small amounts of essential oils (D. Bhardwaj *et al.*).

A spice is a plant-derived substance, typically dried, that comes from various parts of the plant such as seeds, fruits, roots, bark, or flowers. It is used in small amounts to enhance the flavor, add color, or act as a preservative in food. Unlike herbs, which are usually the fresh or dried leaves of a plant, spices are more concentrated and potent, offering distinct aromas and tastes that can transform a dish. Additionally, some spices have natural preservative properties, helping to extend the shelf life of food (Kunnumakkara *et al.*, 2009).

Plants and spices are rich sources of biologically active compounds, many of which exhibit strong antimicrobial properties. Essential oils, which are secondary metabolites produced by plants, are particularly effective at inhibiting the growth of a variety of food-spoilage and food-borne microorganisms, including bacteria, yeasts, and molds. Chemically, essential oils consist of aromatic and volatile compounds that are not only responsible for their fragrance but also play a crucial role in protecting the plant from pathogens. These compounds have the ability to disrupt the cell membranes of microbes, thereby preventing their growth and spread, making essential oils valuable for both plant defense and as natural antimicrobial agents in food preservation (Hyldgaard *et al.*, 2012). Only little can be said in a general way about their composition. Some of the main active constituents in herbs are as follows:

1. Acids: These are sour-tasting substances that often have antiseptic and cleansing properties, helping to disinfect and purify.

2. Alkaloids: Bitter compounds, usually derived from nitrogen, which can have strong effects on the central nervous system. Some are toxic and addictive, making them dangerous in large amounts.

3. Anthraquinones: Known for their bitter taste and ability to irritate, these compounds act as natural laxatives. They are also used as dyes.

4. Bitters: These compounds, often from irridoides and sesquiterpenes, have a strong bitter taste. They are known for boosting digestion and improving overall digestive health.

5. Coumarins: Antibacterial compounds that act as anticoagulants (preventing blood clotting) and give off a scent similar to freshly cut hay.

6. Flavones: These can taste bitter or sweet and are known for their diuretic, antiseptic, antispasmodic (relieving spasms), and anti-inflammatory effects. They are usually yellow and found in many plants.

7. Gums and Mucilage: These are bland, sticky, or slimy substances that have soothing and softening properties, often used for healing.

8. Resins: Known for their strong, acrid taste, resins are antiseptic and astringent, promoting healing. They are often found mixed with oils in substances like oleoresins or oleogum resins.

9. Saponins: These sweet compounds have hormonal, stimulant effects, and are often anti-inflammatory or diuretic. In water, they create a soapy texture.

10. Tannins: Astringent substances that often have antiseptic properties. They are useful in stopping bleeding and reducing bodily discharges.

11. Volatile Oils: These are aromatic and have antiseptic, fungicidal, irritant, and stimulant properties. They are found in many essential oils.

12. Glycosides: These compounds come in several types:

    - Cardiac glycosides: Affect the heart's contractions.
    - Synogenic glycosides: Bitter, antispasmodic, and sedative, affecting breathing and heart rate.
    - Mustard oil glycosides: Extremely irritant and acrid.
    - Sulfur glycosides: Acrid, with stimulant and antibiotic properties.

Many plants essential oils, especially those derived from herbs, have been proven effective in combating food-borne bacteria and molds. Beyond their antimicrobial activity, herbs and spices have long been recognized for their medicinal, preservative, and antioxidant properties. According to Souza *et al.* (2005), these natural compounds could be utilized as primary or supplementary antimicrobial agents in food preservation. Their ability to inhibit the growth of harmful microorganisms makes them valuable in extending the shelf life of food products while maintaining safety and quality, offering a natural alternative to synthetic preservatives.

2.13 Herb and spices in tofu

Tofu treated with tulsi extract had a moisture content of 76.4% and was softer than the control. While there was no significant difference in mesophilic bacterial count between the control and treated samples during storage, the tulsi-treated tofu maintained good sensory qualities throughout the study. It also showed reduced lipid peroxidation and 50% lower protease activity (4.7 units compared to 9.6 units in the control) after 7 days. The use of tulsi extract effectively extended the tofu's shelf life from 3-4 days to 7-8 days without refrigeration (Anbarasu and Vijayalakshmi, 2007).

The impact of turmeric (*Curcuma aromatica*) on the quality and shelf life of tofu. Tofu samples with varying concentrations of turmeric (0%, 0.005%, 0.010%, and 0.015%) were stored at 10°C for 12 days. As storage time increased, pH decreased, with 0.010% and 0.015% turmeric tofu having lower pH than the control (0%). Acidity and water content also decreased over time. Turbidity increased, especially after 6 days, and microbial counts were lowest in the 0.015% Texture analysis showed declines in hardness, gumminess, and brittleness after 12 days, while cohesiveness and springiness increased. Sensory evaluation ranked the 0.010% turmeric tofu highest for smoothness, aftertaste, and overall taste, with the 0.010% tofu being the most preferred overall (Min *et al.*, 2007).

The tofu yield increased with the addition of BNP, but pH decreased, and total acidity increased. In terms of texture, hardness, chewiness, and brittleness rose, while springiness and cohesiveness declined with higher BNP levels. Sensory evaluation showed that tofu with 0.6% BNP had the highest overall preference. The findings suggest that BNP enhances the

sensory qualities of tofu, with 0.6% being the optimal amount for improved texture and taste (Y.-M. Park *et al.*, 2014).

2.14 Large cardamom (*Ammomum subulatum Roxburg*)

Large cardamom, also known as Nepal cardamom (*Amomum subulatum Roxb*.), is a perennial spice crop that grows in the swampy regions of northeastern and central Himalayas in India. It has a long history of use in Ayurvedic medicine, with references as early as the 6th century BC, according to the ancient physician Susrata. The name "large cardamom" comes from both its larger-scale cultivation and its significant role in the spice trade. It is referred to by various names across different languages: "greater cardamom" in English, "Sthulaila" or "Bhadraila" in Sanskrit, "Bara Ilachi" in Bangla, "Badi Ilayachi" in Hindi, and "Peralam" or "Periyaelam" in Tamil, among others (Khatiwada *et al.*, 2019).

Large cardamom, a high-value spice, was introduced to Ilam, Nepal, in 1865 AD by Nepalese laborers, but its cultivation didn’t immediately take off commercially. It wasn’t until 1953 AD that the spice began to be grown on a larger, more organized scale. This shift marked the beginning of commercial cultivation in the region, with farmers starting to recognize the economic benefits of growing cardamom. Further development of cardamom farming occurred after the establishment of the Cardamom Development Centre at Fikkal in Ilam district in 1975. This center played a crucial role in promoting large-scale cultivation by providing farmers with the necessary technical support, research, and resources to enhance production and quality. The center's efforts helped transform large cardamom into one of the region's most important cash crops, boosting the economy of Ilam and surrounding area (Khatiwada *et al.*, 2019).

Table 2.10 Classification of large cardamom

Taxon	Scientific name
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*liliopsida*
Subclass	*Zingiberidae*
Order	*Zingiberales*
Family	*Zingiberaceae*
Genus	*Amomum*
Spices	*A.Subulatum*

Source: Gautam *et al.* (2016)

2.14.1 Chemical compounds isolated from large cardamom

Large cardamom contains essential oils, which are responsible for its distinctive, pungent aroma. The primary component of this essential oil is 1,8-cineole (77.4% of total essential oil) also known as eucalyptol**.** The fruit also contained β-myrcene (5%), α-terpineol (4.9%), hexanoic acid (3.0 %), toluene (2.4%),t-caryophyllene (2.3%) and terpinen-4-ol (2.3%) (Ali, 2007). Kikuzaki *et al.* (2001) isolated protocatechualdehyde, 1,7-bis (3,4-dihydroxyphenyl) hepta-4E, 6E-dien-3-one, protocatechuic acid, and 2,3,7-trihydroxy-5-(3,4-dihydroxy-E-styryl)-6,7,8,9-tetrahydro-5H-benzocycloheptane from the cardamom and reported that these aforementioned compounds were responsible for its unique pungency and fragrance.

The study highlights significant variations in the chemical composition of essential oil from large cardamom seeds grown at different altitudes in Himachal Pradesh, India. The analysis, using gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry, identified 55 compounds, representing 98% of the total oil, with major components such as 1,8-cineole, α-terpineol, DL-limonene, and β-

myrcene. The discovery of new compounds, including 4-terpineol, δ-3-carene, and α-terpinene, sets the chemical profile of large cardamom oil from Himachal Pradesh apart from that of Sikkim. Furthermore, the identification of 35 aroma-impact compounds, with five being particularly intense (DL-limonene, 1,8-cineole, β-myrcene, α-pinene, α-bisabolol), makes this the first study to characterize the odour-active components of large cardamom oil. These findings suggest that large cardamom oil from Himachal Pradesh has a unique aroma profile, offering potential for use as a new food flavor (Robin *et al.*, 2013).

2.14.2 Antimicrobial and antioxidant activity of Cardamom

Bano *et al.* (2016) has studies on the phytochemical screening and evaluation of antibacterial and antioxidant activities of the crude methanol extract derived from the seeds of cardamom. The crude methanol extract was evaluated against Enteropathogenic *E. coli, Listeria monocytogenes, Bacillus pumilus,* and *Escherichia coli for* antibacterial activity. Among the four bacteria, EPEC showed a maximum zone of inhibition, 20.3 mm. While it had a slightly lower antibacterial potential against normal E. coli, with a zone of inhibition at 19 mm, moderate activity was recorded against L. monocytogenes and B. pumilus.

Cardamom essential oil contained 71 compounds, with major components being α-terpinyl acetate (44.3%), 1,8-cineole (10.7%), α-terpineol (9.8%), and linalool (8.6%). In oleoresins, chloroform and methanol extracts contained α-terpinyl acetate as the major component (21.8%, 25.9%), while ethanol oleoresin contained 5hydroxymethylfurfural (28.9%). Very few components were present in diethyl ether oleoresin (0.61%). Antioxidant activity, screened in mustard oil, was comparable to synthetic antioxidants BHA and BHT at 0.02%. The extracted essential oil exhibited strong antibacterial activity at 3000 ppm against Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Salmonella typhi. Maximum antifungal activity was expressed with methanol and ethanol oleoresins against Aspergillus terreus at the rate of 3000 ppm (G. Singh *et al.*, 2008).

Cardamom, has been reported to be rich in antioxidant phytochemicals capable of scavenging free radicals and preventing oxidative damage. All studies point to the fact that the seeds and pods retain the highest amounts of these antioxidant phytoconstituents. Among these, phenolic compounds, including gallic acid equivalents, have been shown to exhibit

major antioxidant properties through their prevention of lipid peroxidation in the linoleic acid system, hence reflecting strong antioxidant potency (Saeed *et al.*, 2014).

2.15 Ginger (*Zingiber officinale Rosc*.)

Ginger (*Zingiber officinale*), a plant from the Zingiberaceae family, has been widely used as a spice and flavoring agent in food and beverages. It contains a variety of nutrients and bioactive compounds, including proteins, carbohydrates, fiber, ash, and antioxidants like beta-carotene. Additionally, ginger is rich in terpenoids, ascorbic acid (vitamin C), alkaloids, and polyphenols such as flavonoids, flavones, glycosides, and rutin. These compounds contribute to ginger's health benefits, including its use in herbal supplements (Aruoma *et al.*, 1997).

Ginger originated in southern China and later spread to India, the Maluku Islands (Spice Islands), other parts of Asia, and West Africa. It was introduced to Europe by ancient Romans during the 1st century through trade with India. After the fall of Rome, ginger was forgotten in Europe until it was reintroduced by Marco Polo (Moghaddasi and Kashani, 2012). Cultivation of ginger requires a tropical, subtropical, or humid climate. It can be grown at altitudes of up to 1500 meters with well-distributed rainfall and high humidity throughout its growth cycle. Ginger thrives in slightly acidic soil that is rich in humus, light, loose, friable, and well-drained, with a depth of at least 30 cm to support the proper growth of its rhizomes (Langner *et al.*, 1998).

Table 2.11 Taxonomic classification of ginger

Taxon	Scientific name
Kingdom	*Plantae*
Class	*Monocotyledon*
Order	*Zingiberales*
Family	*Zingiberaceae*
Genus	*Zingiber*
Spices	Zingiber officinale

Source: Kanadea and Bhatkhandeb (2016)

2.15.1 Chemical compounds isolated from Ginger

Ginger is rich in a variety of bioactive compounds, primarily phenolic and terpene constituents. Among its phenolic compounds, the most prominent are gingerols, shogaols, and paradols. In fresh ginger, the dominant polyphenols include 6-gingerol, 8-gingerol, and 10-gingerol. When ginger is exposed to heat or stored for extended periods, these gingerols convert into shogaols. Further, through hydrogenation, shogaols can be transformed into paradols (Mao *et al.*, 2019). In addition to these, ginger contains other phenolic substances like quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione. Ginger also has several significant terpene compounds, such as β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene, which are the primary components of ginger's essential oils. Apart from phenolics and terpenes, ginger is composed of polysaccharides, lipids, organic acids, and raw fibers, adding to its complex chemical profile and numerous health benefits (Vernin and Parkanyi, 2016). The volatile oil contains a mix of sesquiterpenes and monoterpenes. Zingiberene (36%), curcumene (18%), and farnesene (10%) dominate, giving the oil its warm, woody, and earthy aroma. Smaller amounts of monoterpenes, like cineole (1.3%), linalool (1.3%), borneol (2.2%), geranial (1.4%), and neral (0.8%), add fresh, floral, and citrusy notes. This blend creates a rich, balanced scent with stable and aromatic properties (Kiyama, 2020).

2.15.2 Antimicrobial and antioxidant activity of Ginger

Ginger (*Zingiber officinale*) is widely used in traditional medicine for a variety of purposes, such as antimicrobial, antioxidant, anti-inflammatory, and anticoagulant properties. It is generally recognized as safe by the FDA. Bioactive compounds, including monoterpenoids, sesquiterpenoids, phenolic compounds, aldehydes, ketones, alcohols, and esters, have been identified and possess broad-spectrum antimicrobial activities. Such unique characteristics make ginger an excellent natural alternative to synthetic antimicrobials. However, studies on its application in food preservation are limited. This review provides insights into ginger's bioactive compounds, potential food applications, and toxicity considerations as an antimicrobial agent (Beristain-Bauza *et al.*, 2019).

It has been reported that ginger acts against a large number of Gram-positive and Gram-negative bacteria, such as *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Enterococcus faecalis,Salmonella typhi, Salmonella typhimurium, Pseudomonas aeruginosa, Proteus species*, and several Bacillus species (*B. cereus, B. subtilis, B. megaterium) and Streptococcus faecalis (*Aleem *et al.,* 2020*).* (Riaz *et al.*, 2015) studied that gingerenone-A and shogaol have a potential *St.aureus* encodes a unique enzyme, 6- hydroxymethyl-7,8-dihydropterin pyrophosphokinase inhibitors.

Ginger is known for its high antioxidant action, which is mainly ascribed to its bioactive ingredients, including phenolic fractions like gingerols, shogaols, paradols, oleoresins, and essential oils. Antioxidants neutralize free radicals and, therefore, protect cells from damage by diminishing oxidative stress. The antioxidant properties of ginger delay the process of lipid peroxidation and increase the enzymatic antioxidants, reducing the risk for degenerative diseases caused by oxidative stress. These properties make ginger valuable not only in traditional medicine but also as a natural preservative in food systems (Chumroenphat *et al.*, 2011). Antioxidants are substances that help retard, regulate, or completely impede oxidation and deterioration of food quality. Antioxidants reduce risks of degenerative diseases associated with oxidative stress in the body. The compounds responsible for antioxidant activity in ginger include phenolic compounds, oleoresins, and essential oils (Yang *et al.*, 2020).

Part III

Materials and methods

This chapter provides information on the production of tofu and the incorporation rates of cardamom and ginger extracts. It outlines the storage conditions and analysis schedule for tofu, including the methods employed to monitor chemical changes, the procedures for microbiological analysis, and the sensory evaluation conducted during storage. Additionally, the statistical design used for data analysis is detailed.

3.1 Materials

3.1.1 Raw materials

Raw material collected for the preparation of herbal tofu areas follows

3.1.1.1 Soybean

White variety of soybean was bought from local market of Dharan.

3.1.1.2 Ginger and cardamom

Ginger and cardamom were bought from local market of Dharan.

3.1.1.3 Packaging material

LDPE and metalized plastic were used for packaging of samples during shelf-life determination.

3.1.1.4 Apparatus and chemicals required

All the chemicals, laboratory glassware and equipment utilized in the study were obtained from the laboratory of Central Campus of Technology. The major chemical and apparatus used are given in Appendix F.

3.2 Methods

3.2.1 Experimental design

The impact of adding extracts from two herbs, Ginger and cardamom to tofu, to observe changes in its physicochemical properties (such as texture, moisture content, and pH) and sensory properties (like taste, smell, and appearance) during storage where studied.

For cardamom, previous studies indicated that adding up to 0.6% cardamom extract to tofu and other food products is generally considered acceptable in terms of taste and safety. Based on these findings, the researchers set 0.6% as the maximum concentration of cardamom extract to use in their experiments (Garg *et al.*, 2016; R. Singh *et al.*, 2018). This threshold was chosen to ensure that the tofu remained palatable and maintained desirable qualities while still benefiting from the potential preservative and flavor-enhancing effects of the cardamom extract.

Previous studies have shown that adding up to 0.6% ginger extract to paneer and other food products is generally well-received in terms of taste and safety. Based on these findings, researchers established 0.6% as the maximum concentration of ginger extract for their experiments. This limit was set to ensure that the tofu would be both enjoyable to eat and maintain its desirable qualities, while also taking advantage of the preservative and flavor-enhancing properties of the ginger extract.

Table 3.1 Different formulations of cardamom extracts added to tofu.

Sample	Formulation
Control (I)	0% cardamom
A	0.2% cardamom
B	0.4% cardamom
C	0.6% cardamom
D	0.8% cardamom

Table 3.2 Different formulations of ginger extracts added to tofu.

Sample	Formulation
Control (I)	0% Ginger
E	0.2% Ginger
F	0.4% Ginger
G	0.6% Ginger
H	0.8% Ginger

Control (I) represents the tofu sample where no herbs extract where added. Sample A, B, C D represent the tofu sample where different concentration of cardamom extract 0.2%,0.4%,0.6%,0.8% respectively is added. Sample E, F, G, H represent the tofu sample where different concentration of ginger extract 0.2%,0.4%,0.6%,0.8% respectively is added.

3.2.2 Preparation of tofu

Tofu was prepared in the laboratory using a method describe by Jiang (2014) with slight modifications. Soybean seeds were soaked for 8 h at room temperature. After soaking, the beans were steamed at 121°C for 15 min. The beans were then bleached using a 0.5% sodium bicarbonate solution at 80°C for 20 minutes and subsequently dehulled. The treated beans were washed and ground with added water to form a milky slurry using a steel mill. This slurry was heated to boiling temperature and filtered through muslin cloth to separate the soymilk. Protein coagulation was carried out at 70-80°C by adding a 3% calcium sulfate ($CaSO_4$) solution, along with various concentrations of cardamom and ginger extracts. After adding the coagulant, the mixture was agitated and then set aside for 10-15 min to complete the coagulation process. The resulting precipitate was collected in a muslin cloth and pressed using iron blocks (an improvised mechanized press) for about an hour. This process yielded a soft, cake-like tofu, which was then cut into desired rectangular pieces. The flowsheet of the herbal extract incorporated tofu is shown in Fig. 3.2.

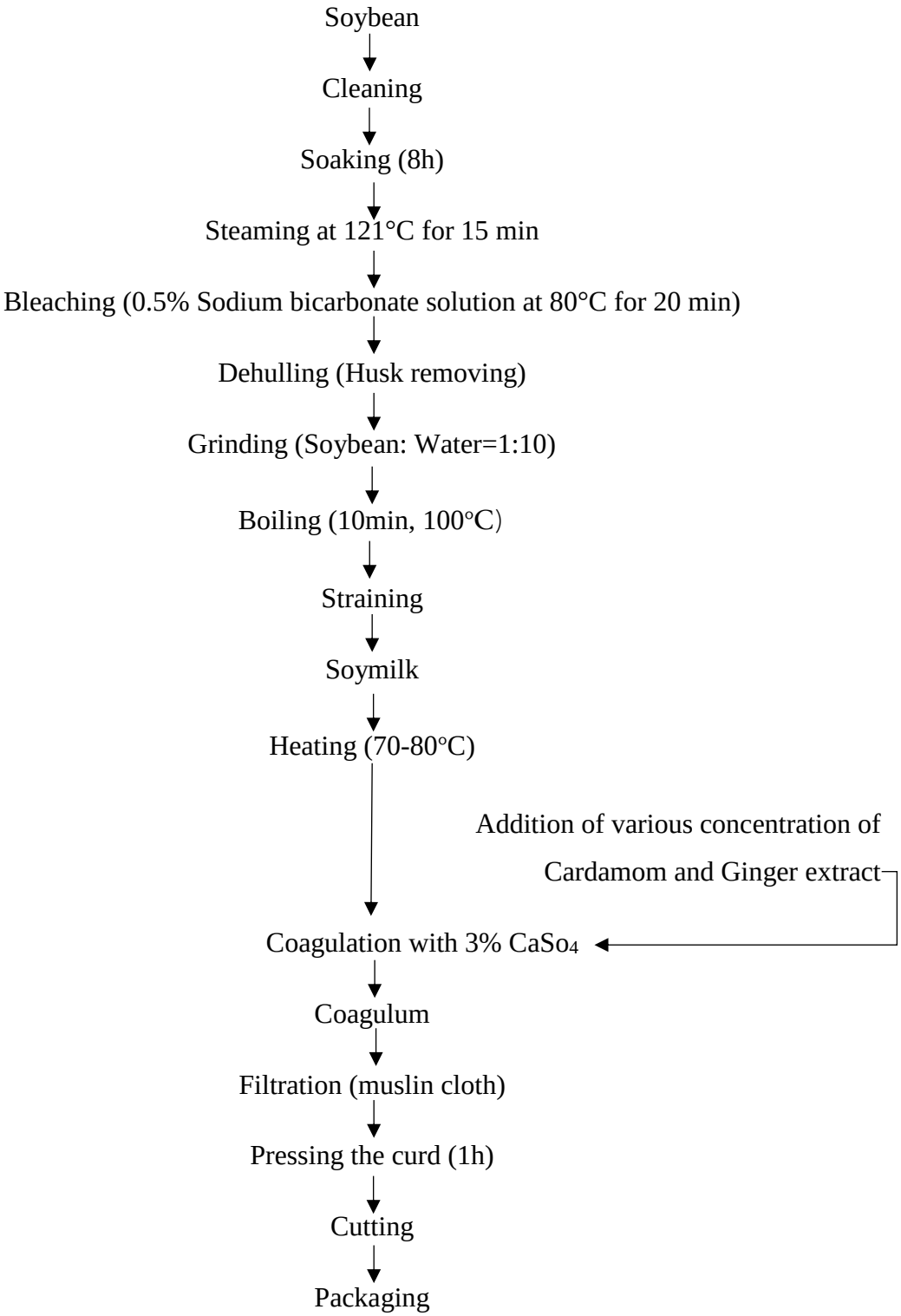

Fig. 3.1 Flowchart for preparation tofu with and without herbal treatment (cardamom and ginger).

Source: Jiang (2014)

3.2.3 Preparation of herbs extract

The herb extract was prepared following the method described by Baljeet *et al.* (2015). Cardamom and ginger were first cleaned, then dried at 50°C for 3-4 hours, and finally ground into a powder. The detailed process for preparing the extract is outlined in Fig. 3.1.

3.2.4 Analysis of spice extract

3.2.4.1 Determination of extraction yield and spice extract concentration.

To determine the extraction yield of the spices, the process involved filtering the extract through filter paper. After filtering, the leftover residue was dried thoroughly to ensure all the solvent used in the extraction was removed. The amount of spice extracted was then calculated by subtracting the weight of the dry residue left after filtration from the initial weight of the spice sample used for extraction. This difference gave the quantity of spice that was extracted along with the solvent.

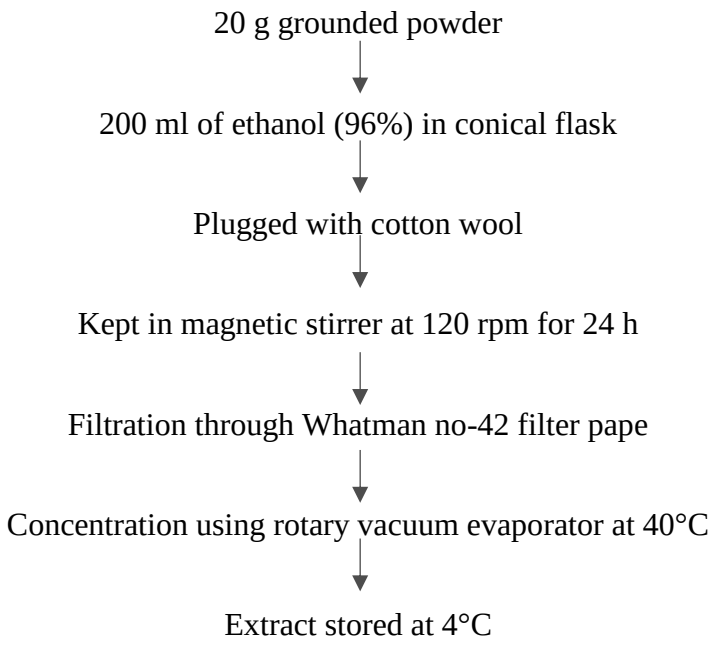

Fig.3.2 Preparation of herbs extract

Source: Baljeet *et al.* (2015)

For concentration of herb extracts, rotary vacuum evaporator was used. During concentration, it was assumed that the solvent is only evaporated while the portion of herb

in the extract remains unchanged. Thus, the concentration of herb extract concentrate was determined.

$$\text{Amount of herb extracted in solvent} = \text{Wt. of dried herb taken for extraction} - \text{Wt. of dried residue after extraction}$$

$$\text{Concentration of concentrated herb extract} = \frac{\text{Amount of herb extracted in solvent}}{\text{Final weight of concentrated extract}}$$

3.2.4.2 Determination of total phenolic content

The total phenolic content of the Cardamom and ginger extracts was measured using the method described by Genwali *et al.* (2013). To create a standard curve, different concentrations of gallic acid solutions (10, 25, 50, and 75 µg/ml) were prepared in methanol. For each concentration, 1 ml of the gallic acid solution was placed in a 20 ml test tube, to which 5 ml of 10% Folin-Ciocalteau reagent and 4 ml of 7% sodium carbonate (Na_2CO_3) were added, making a total volume of 10 ml. The mixture, which turned blue, was then mixed thoroughly and incubated at 40°C in a water bath for 30 minutes. The absorbance of the mixture was measured at 760 nm against a blank.

Similarly, various concentrations of the herb extracts (25, 50, 100, and 200 µg/ml) were prepared, and their absorbance was recorded using the same procedure as for the standard. The total phenolic content of the extracts was then expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight sample (mg/100 g).

3.2.4.3 Determination of antioxidant activity by DPPH assay

The free radical scavenging ability of the extracts was evaluated using the DPPH radical scavenging assay, based on the method described by Blois (1958). To start, a 0.1 mM stock solution of DPPH was prepared and diluted to reach an absorbance of approximately 0.9 at a wavelength of 517 nm. This absorbance measurement served as the control (A_0).

For the assay, three different concentrations of each extract were prepared through serial dilution of a 10 mg/ml stock solution in methanol. To test the extracts, 0.5 ml of each extract solution (the sample) was mixed with 2.5 ml of the 0.1 mM DPPH solution. The mixtures were then shaken well and left in the dark at room temperature for 30 minutes. After this

incubation period, the ab3.2.5.1sorbance of each sample was measured at 517 nm against the control solution.

The radical scavenging activity, which indicates the extract's ability to neutralize free radicals, was expressed as a percentage of the radical scavenging activity. This percentage was calculated using the following formula:

Inhibition % = 100 $(A_0-A_s)/A_s$

Where A_0 is the absorbance of the control (DPPH solution without extract) and as is the absorbance of the sample (DPPH solution with extract).

3.2.5 Analytical procedure

3.2.5.1 1000 kernel weight

To determine the weight of 1000 soybean kernels, the quartering method was used to select a representative sample. The quartering method involves dividing a larger sample of soybeans into smaller, equal portions until a manageable and representative subset is obtained. From this subset, exactly 1000 kernels were counted and weighed (Buffo *et al.*, 1998).

3.2.5.2 Bulk density

By pouring the grains into the funnel-shaped hopper, centering the hopper above the measuring bushel, swiftly opening the hopper valve, and allowing the grains to flow freely into the measuring bushel, the bulk density was ascertained as per the methodology outlined by Clementson *et al.* (2010). Once the bushel was full, the excess material was leveled off with gentle zigzag strokes using a standard seed buro striking staff. The mass of the grains within the measuring bushel was determined by deducting the mass of the measuring bushel after it had been filled and weighed. Using the formula, the bulk density (ρ) of grain was determined.

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

3.2.5.3 Color

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

3.2.5.4 Determination of moisture content

The moisture content of the sample was measured by the weight loss observed during heating in a thermostatically controlled oven set at either 100°C or 105°C, following the hot air oven method as specified by AOAC (2005).

3.2.5.5 Determination of ash content

The total ash content can be easily determined by incinerating all the organic matter in the food sample at 550°C using the dry ashing method, as described by AOAC (2005).

3.2.5.6 Determination of crude fat

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

3.2.5.7 Determination of crude fiber

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

3.2.5.8 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).

3.2.5.9 Determination of carbohydrate

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

% carbohydrate = 100 – (moisture + protein + crude fat + crude fiber + ash)

3.2.5.10 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) (K. Chang and Hou, 2003).

Percentage yield of tofu was calculated as per

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

3.2.6 Analysis of tofu for chemical characteristics during storage

3.2.6.1 Moisture

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

3.2.6.2 pH

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

3.2.6.3 Peroxide value

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

$$\text{Peroxide Value} = \frac{N \times (V_S - V_B)}{\text{Wt of sample (g)}} \times 1000$$

N= Normality of sodium thiosulfate

V_S= Sodium thiosulphate consumed by sample(ml)

V_B= Sodium thiosulphate consumed by blank(ml)

3.2.6.4 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.

3.2.7 Sensory analysis

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.

3.2.8 Statistical methods

Data were analyzed using ANOVA at 95% confident level using IBM SPSS Statistics (Version 27). Means of the data were compared by using Tukey's honestly significant difference (HSD) method at 5% level of significance.

Part IV

Result and discussion

In this study, tofu was prepared by incorporating cardamom and ginger extract at different percentage. 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, metalized plastic).

4.1 Physical properties of soybean

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

Table 4.1 Physical properties of soybean

Physical properties	Soybean
1000 kernel wt. (g)	196.51±0.649
Bulk density (kg/HL)	84.667±0.577

(Values are the means of three determinations ± standard deviations. Figures in the parenthesis are standard deviations)

Koirala (2024) reported the weight of 1000 soybean kernels was found to be 194.51 g, and the bulk density was 82.667 kg/HL. These values were lower than the averages we found in our research. The differences can be explained by the varying physical and chemical properties of different soybean varieties, which are influenced by factors like the specific soybean strain, the climate in which they are grown, and the agricultural methods used.

4.2 Proximate composition of soybean, cardamom and ginger

Table 4.2 Proximate composition of soybean was obtained as given in table

Attributes	Raw Soybean
Moisture% (wet basis)	9.37±0.59
Ash % (db)	5.42±0.32
Crude fat % (db)	19.43±0.32
Crude fiber % (db)	6.04±0.79
Crude protein % (db)	36.83±0.09
Carbohydrate % (db)	32.33±0.91

Table 4.3 Proximate composition of cardamom

Attributes	Cardamom seed
Moisture% (wet basis)	10.37±0.9
Ash % (db)	8.42±0.3
Crude fat % (db)	12.43±0.2
Crude fiber % (db)	12.04±0.7
Crude protein % (db)	6.83±0.3
Carbohydrate % (db)	42.33±0.9

Table 4.4 Proximate composition of ginger

Attributes	Ginger
Moisture% (wet basis)	83.18±0.98
Ash % (db)	3.17±0.12
Crude fat % (db)	3.4±0.23
Crude fiber % (db)	6.13±0.18
Crude protein % (db)	5.98±0.67
Carbohydrate %(db)	80.34±0.12

4.3 Yield of spice extract

The yield of cardamom extract was found to be 41.73%and ginger extract was found to be 47.53%. Similar observation was reported for yield of cardamom by Prasath *et al.* (2009) and by Uwadiae *et al.* (2019) for ginger.

4.4 Yield of tofu

The % yield of tofu obtained ranged from 139.87% to 152.23% with an average of 145.85% and the % of tofu solids ranged from 46.68% to 54.12% with an average of 46.05%.

Among the different samples, sample A-D represent the samples where the cardamom extract was treated from 0.2%-0.8% respectively on soya-milk before coagulation and sample E-H represent the samples where the ginger extract was treated from 0.2%-0.8% respectively on soya-milk before coagulation. Sample I represent the control sample where no herbs extract was added on soya-milk before coagulation. Maximum samples showed higher yield than the control samples.

Similar results were seen in addition of alfalfa (*Medicago sativa L.*) extracts in tofu yield. With increase in extract % there is increase in yield of tofu and tofu solids (S.-E. Kim *et al.*, 2012). The increase in tofu yield with the addition of herbal extracts can be attributed to their

ability to enhance water retention and improve protein coagulation. Herbal extracts like cardamom and ginger may have water-binding properties, allowing the tofu to hold more moisture, which increases the yield. Additionally, these extracts can interact with soy proteins, promoting better curd formation during the tofu-making process. Their antioxidant properties may also stabilize the tofu structure, reducing water loss and contributing to a higher yield overall (Anbarasu and Vijayalakshmi, 2007).

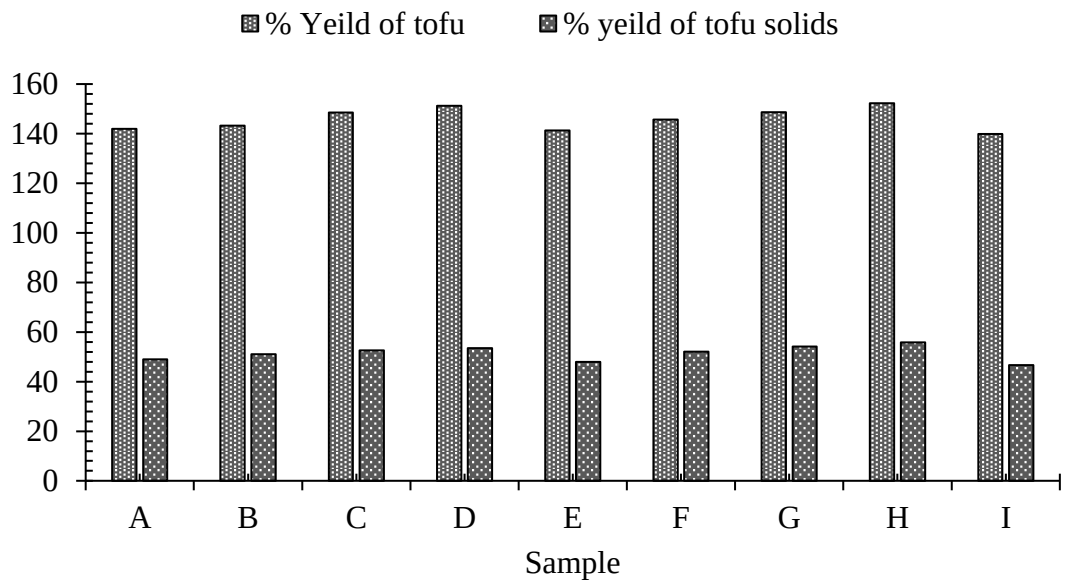

Fig. 4.1 %Yield of tofu sample and tofu solids

*Sample A-D represent tofu sample with cardamom extract treated in ratio of 0.2%, 0.4%, 0.6% and 0.8% respectively and sample E-H represent tofu sample with ginger extract treated in ratio of 0.2%, 0.4%, 0.6% and 0.8% respectively. Sample I represented control tofu

4.5 Optimization of cardamom extract and ginger extract in tofu by sensory

The sensory quality of a product plays a crucial role in determining whether it will be accepted or rejected for consumption. Key sensory attributes include flavor, texture and body, color and appearance, as well as overall acceptability. The tofu samples were assessed based on these sensory qualities. The results, which include the evaluation of tofu samples without herbal preservatives and those with herbal preservatives added are discussed in below.

4.5.1 Color and appearance

The effect of color and appearance of tofu samples treated with different proportion of cardamom and ginger extract added directly to the milk after heat treatment is depicted in figure below.

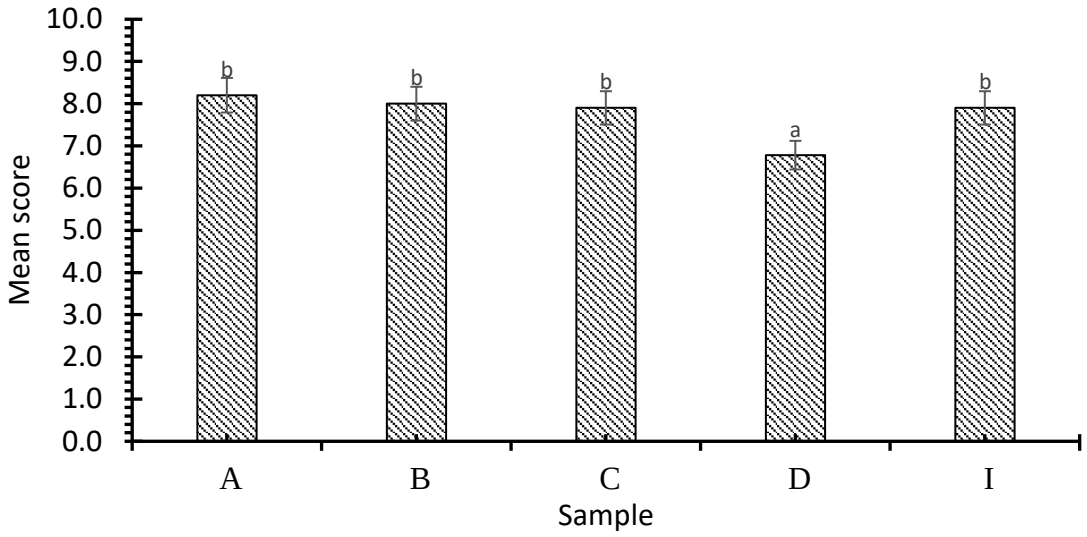

Fig.4.2 Effect of cardamom extract on color and appearance of tofu.

Note: A:0.2% CE, B:0.4% CE, C:0.6% CE, D:0.8% CE and I: Control. CE represent cardamom extract. Value on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists

The bar graph above illustrates the mean sensory score for the color and appearance which were found to be 8.242, 8.000, 7.931, 6.784 and 7.973 for sample A, B, C, D and I respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM). Sample A stands out with the highest mean score follows with B, I, C and with less mean score by Sample D.

The ANOVA showed that the mean sensory score for color and appearance of all sample were significantly different ($p<0.05$). From post hoc analysis, the mean color and appearance score of sample D was found to be significantly different ($p<0.05$) from rest of Sample A,

B, C and I. The mean score of Sample A, B, C and I were not significantly different (p>0.05) from each other.

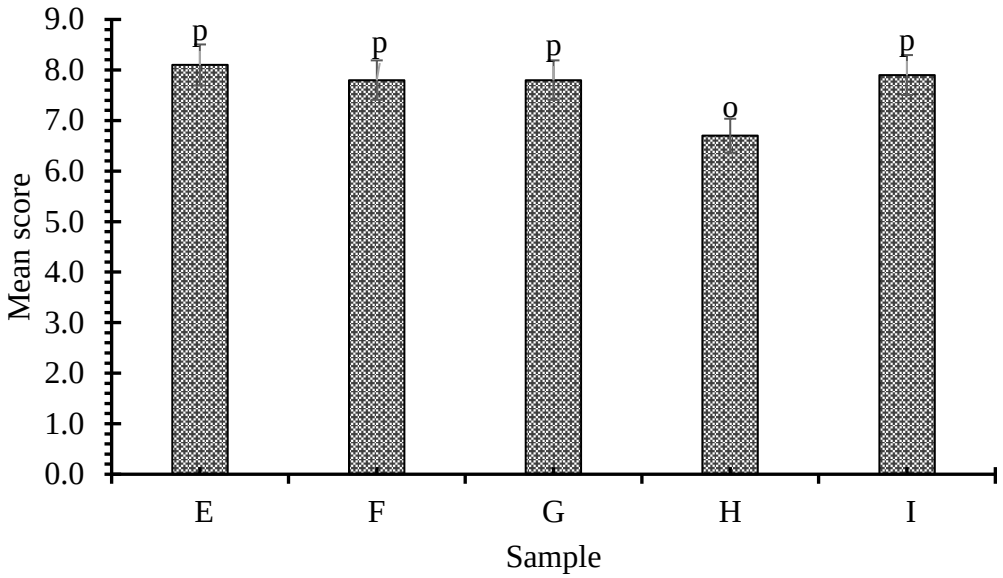

Fig.4.3 Effect of Ginger extract on color and appearance of tofu.

Note: A:0.2% GE, B:0.4% GE, C:0.6% GE, D:0.8% GE and I: Control. GE represent ginger extract. Value on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists.

The bar graph above illustrates the mean sensory score for the color and appearance which were found to be 8.090, 7.777, 7.800, 6.700and 7.973 for sample E, F, G, Hand I respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM). Sample E stands out with the highest mean score follows with I, G, F and with less mean score by Sample H.

The ANOVA showed that the mean sensory score for color and appearance of all sample were significantly different (p<0.05). From post hoc analysis, the mean color and appearance score of sample H was found to be significantly different (p<0.05) from rest of Sample E, F, G and I. The mean score of Sample E, F, G and I were not significantly different (p>0.05) from each other.

Our finding are consistent with previous research. S.-E. Kim *et al.* (2012) found that with addition of alfalfa (*Medicago sativa L*) extract increased the color of tofu will be changed darker. Similar observation was seen by red ginseng extract on tofu by Choi *et al.* (2010).

4.5.2 Flavor

The effect of flavor of tofu samples treated with different proportion of cardamom and ginger extract added directly to the milk after heat treatment is depicted in figure below.

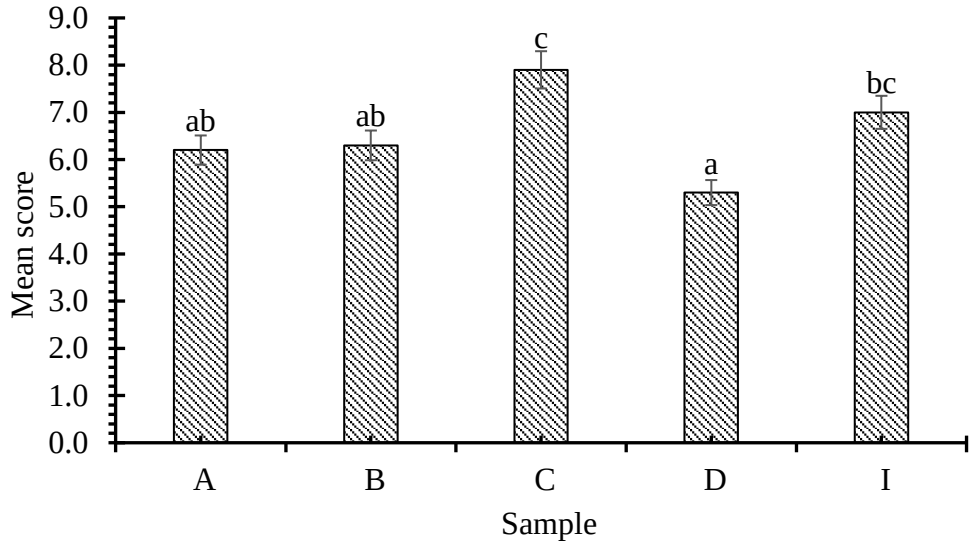

Fig.4.4 Effect of cardamom extract on flavor of tofu.

Note: A:0.2% CE, B:0.4% CE, C:0.6% CE, D:0.8% CE and I: Control. CE represent cardamom extract. Value on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists

The bar graph above show, the mean sensory score for the flavor which were found to be 6.278, 6.352, 7.950, 5.382 and 7.816 for sample A, B, C, D and I respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM). Sample D has highest mean score followed by sample I. Sample A and B show quit similar mean score and sample D show lowest mean score as compared to all samples.

The ANOVA showed that the mean sensory score for flavor of all sample were significantly different ($p<0.05$). From post hoc analysis, the sample A, B and D were not found to be significantly different ($p>0.05$) from each other but there is significantly different ($p<0.05$) from sample C and I. The sample A, B and I were not found to be significantly different ($p>0.05$) from each other but there is significantly different ($p<0.05$) from sample C and D.

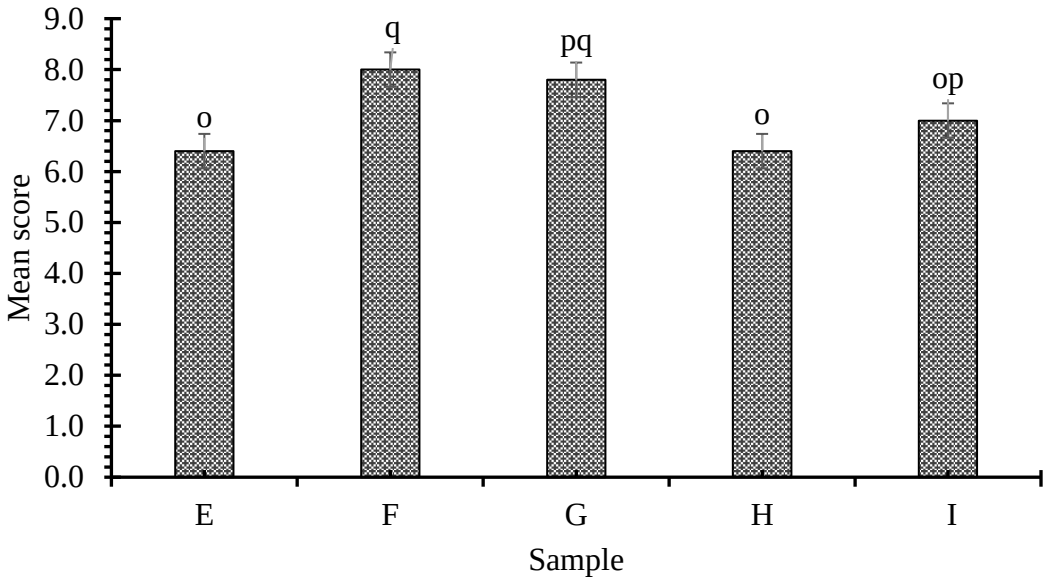

Fig.4.5 Effect of Ginger extract on flavor of tofu.

Note: A:0.2% GE, B:0.4% GE, C:0.6% GE, D:0.8% GE and I: Control. GE represent ginger extract. Value on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists.

The bar graph above illustrates the mean sensory score for the flavor which were found to be 6.263, 8.471, 7.078, 6.469 and 7.816 for sample E, F, G, Hand I respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM). Sample F stands out with the highest mean score follows with I, G, H and with less mean score by Sample E.

The ANOVA showed that the mean sensory score for flavor of all sample were significantly different ($p<0.05$). From post hoc analysis, the mean flavor score of sample E,

H and I were not found to be significantly different ($p>0.05$) from each other but were significantly different ($p<0.05$) from sample F and G. Also, the sample F, G and sample G, I were not found to be significantly different ($p>0.05$) from each other.

The sample C having cardamom extract 0.6% and sample F having ginger extract 0.4% has the highest score among all sample including control sample I. Tofu sample with 0.6% cardamom extract and 0.4% ginger extract is more acceptable due to harmony of flavor, with the increasing ratio of extract, taste become stronger and overpowering into tofu giving pungent taste. This is because extract have very strong flavor.

4.5.3 Body and texture

The effect of texture of tofu samples treated with different proportion of cardamom and ginger extract added directly to the milk after heat treatment is depicted in figure below.

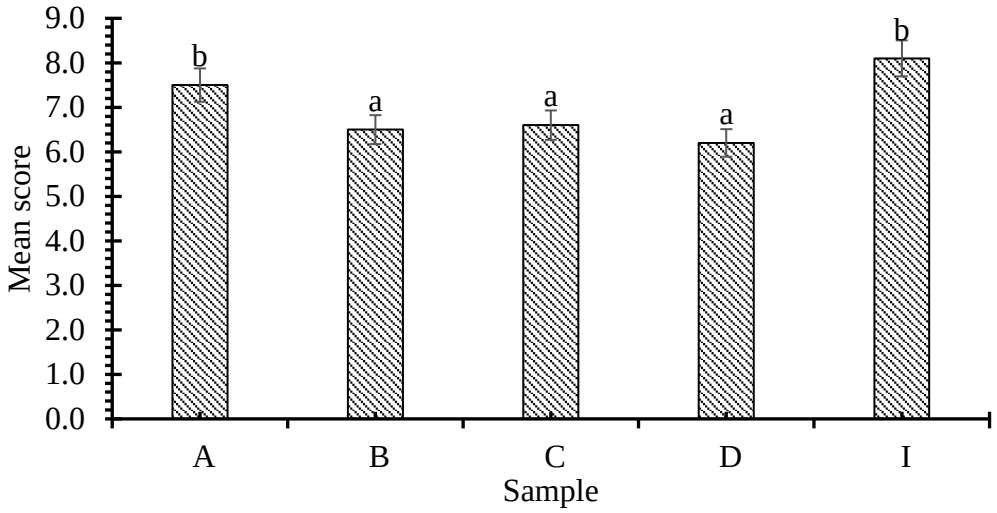

Fig.4.6 Effect of cardamom extract on body and texture of tofu

Note: A:0.2% CE, B:0.4% CE, C:0.6% CE, D:0.8% CE and I: Control. CE represent cardamom extract. Value on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists

The bar graph above illustrates, the mean sensory score for the body and texture which were found to be 7.552, 6.552, 6.651, 6.291 and 8.156 for sample A, B, C, D and I

respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM).

The ANOVA showed that the mean sensory score for body and texture of all sample were significantly different ($p<0.05$). From post hoc analysis, the mean flavor score of sample A and I were not found to be significantly different ($p>0.05$) from each other but were significantly different ($p<0.05$) from rest of sample B, C and D.

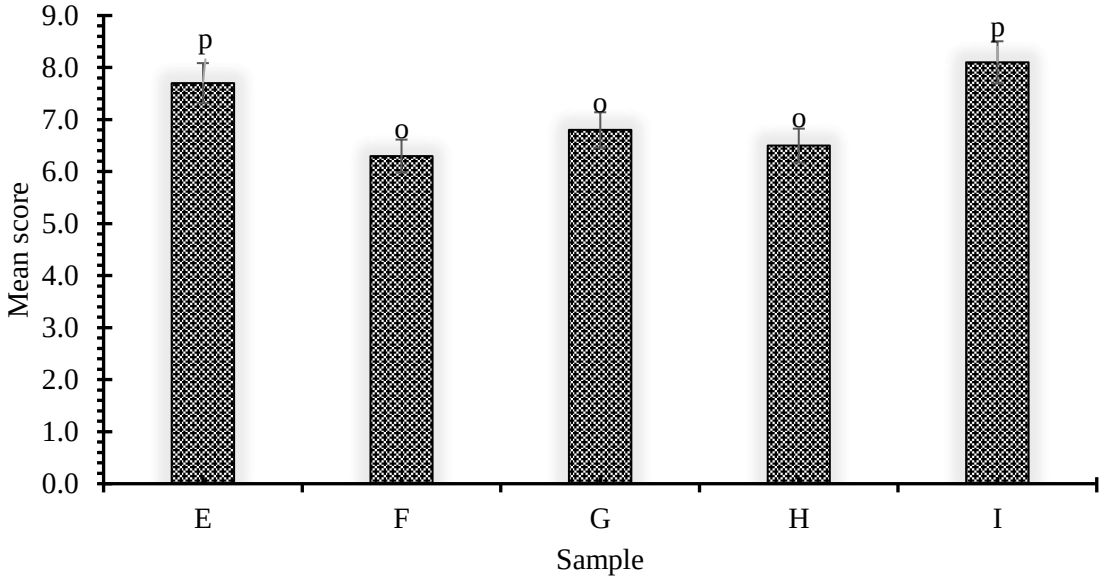

Fig.4.7 Effect of Ginger extract on body and texture of tofu

Note: A:0.2% GE, B:0.4% GE, C:0.6% GE, D:0.8% GE and I: Control. GE represent ginger extract. Value on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists.

The bar graph above illustrates, the mean sensory score for the body and texture which were found to be 7.748, 6.348, 6.840, 6.570 and 8.156 for sample E, F, G, H and I respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM).

The ANOVA showed that the mean sensory score for body and texture of all sample were significantly different ($p<0.05$). From post hoc analysis, the mean flavor score of sample E and I were not found to be significantly different ($p>0.05$) from each other but were

significantly different ($p<0.05$) from rest of sample F, G and H. Also the sample F, G and H were not found to be significantly different ($p>0.05$) from each other.

The findings of the study, however, indicated that the addition of herbal extracts, cardamom, and ginger, brings about significant differences in body and texture among the samples of tofu studied, with a p-value less than 0.05. This may be due to two main factors: water retention and interference by particles from the herbal extracts. The texture and body scores of the tofu samples with cardamom and ginger extracts were a bit lower compared with the control, possibly due to the increased moisture content. Herbal extracts often have interactions with soy proteins, which affect water-holding capacity and moisture distribution in soy foods. Indeed, this increase in moisture gives softer and less firm textures, probably outside of the specification for the desired firmness of tofu (Dżugan *et al.*, 2024).

4.5.4 Overall acceptability

The overall acceptability of tofu samples treated with different proportion of cardamom and ginger extract added directly to the milk after heat treatment is depicted in figure below.

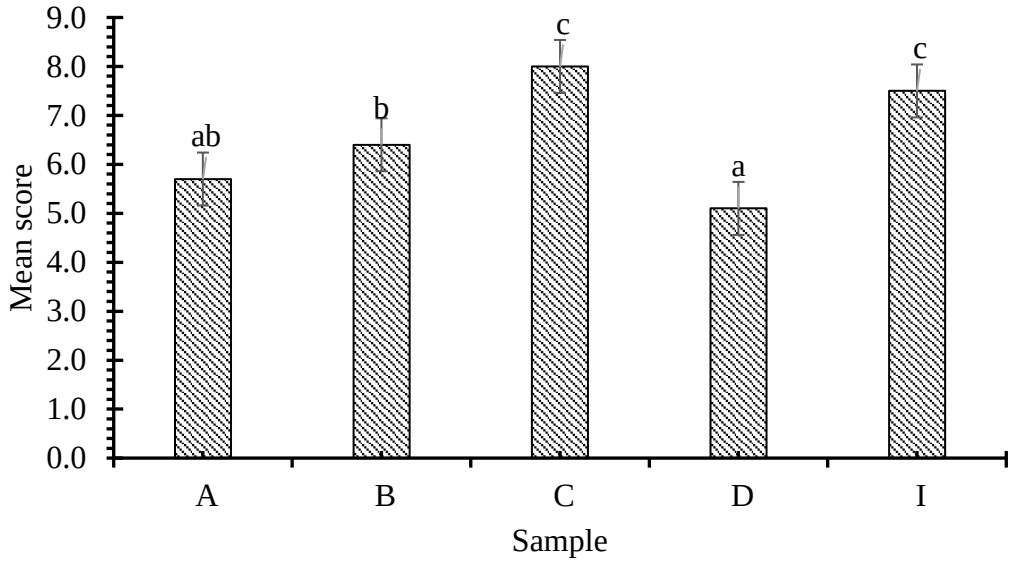

Fig.4.8 Effect of cardamom extract on overall acceptability of tofu

Note: A:0.2% CE, B:0.4% CE, C:0.6% CE, D:0.8% CE and I: Control. CE represent cardamom extract. Value on top of the bars bearing similar superscript are not significantly

different at 5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists

The bar graph above illustrates, the mean sensory score for the body and texture which were found to be 5.748, 6.496, 8.066, 5.156 and 7.552 for sample A, B, C, D and I respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM). Among all samples, sample C has highest overall acceptability and sample D was least.

The ANOVA showed that the mean sensory score for overall acceptability of all sample were significantly different ($p<0.05$). From post hoc analysis, the mean flavor score of sample A and D as well as sample C and I were not significantly different ($p>0.05$) from each other. The color, Flavor, texture and taste of sample C were very much liked by panelist. Concerning the control i.e., sample I, sample C got high score in terms of overall acceptability as shown in fig 4.9.

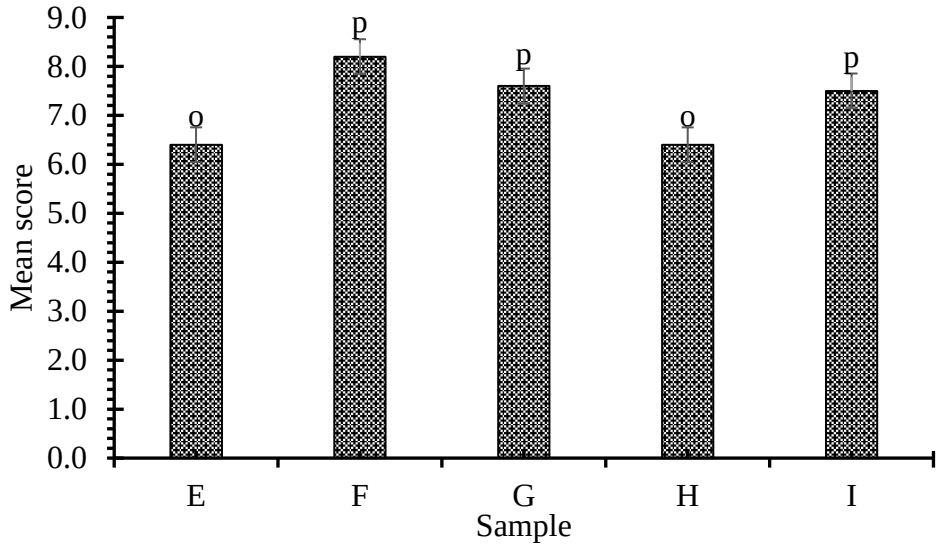

Fig.4.9 Effect of Ginger extract on overall acceptability of tofu.

Note: A:0.2% GE, B:0.4% GE, C:0.6% GE, D:0.8% GE and I: Control. GE represent ginger extract. Value on top of the bars bearing similar superscript are not significantly different at

5% level of significance. Vertical error bars represent the standard deviation of scores given by panelists.

The bar graph above illustrates, the mean sensory score for the body and texture which were found to be 6.263, 8.242, 7.651, 6.469 and 7.552 for sample E, F, G, H and I respectively. Each bar not only represents a sample mean score but also includes elegant error bars indicating standard error of the mean (SEM). Among all samples, sample F has highest overall acceptability and sample H was least.

The ANOVA showed that the mean sensory score for overall acceptability of all sample were significantly different ($p<0.05$). From post hoc analysis, the mean flavor score of sample E and H were not significantly different ($p>0.05$) from each other but were significantly different ($p<0.05$) from Sample F, G and I. Similarly, the sample F, G and I were not significantly different ($p>0.05$) from each other. The color, Flavor, texture and taste of sample F were very much liked by panelist. Concerning the control i.e., sample I, sample F got high score in terms of overall acceptability as shown in fig 4.10. Therefore, the overall acceptability of tofu with 0.4% of ginger extract sample F was found to be significantly superior based on the sensory characteristics of tofu.

4.6 Effect of cardamom extract and ginger extract in optimized tofu along with control

4.6.1 Proximate composition of cardamom extract and ginger extract in optimized tofu along with control tofu

The proximate composition of cardamom extract and ginger extract in optimized tofu along with control tofu.

Table 4.5 Proximate composition of cardamom extract and ginger extract in optimized tofu along with control tofu.

Attributes	Control tofu	Cardamom tofu (0.6%)	Ginger tofu (0.4%)
Moisture	64.23^{a}±0.13	64.98^{a}±0.32	65.04^{a}±0.17
Crude protein (db)	36.87^{a}±0.30	37.35^{a}±0.71	36.46^{a}±0.37
Crude fat (db)	20.90^{a}±0.84	21.48^{a}±0.43	21.19^{a}±0.32
Crude fiber (db)	5.70^{a}±0.03	5.69^{a}±0.12	5.73^{a}±0.18
Total ash (db)	4.08^{a}±0.40	4.12^{a}±0.61	4.05^{a}±0.31
Carbohydrates (db)	31.45^{a}±0.98	31.12^{a}±0.12	30.56^{a}±0.64

* Values are represented as mean ± standard deviation of triplicate determinations.

Moisture content, crude protein, crude fat, total ash and carbohydrates was not significantly ($p>0.05$) change in herbal extract tofu as compared to control tofu. This may be due to incorporating very less amount of herbal extract on tofu during processing. Although no literature has clearly mentioned the change in proximate composition of tofu while directly added to soya-milk before coagulation.

4.6.2 Estimation of total radical scavenging activity of optimized tofu along with control

The total phenolic content of the ethanolic extracts were calculated by using calibration curve (fig E1) obtained from the gallic acid solution ranging from 0 to 400 ppm. Based on the equation obtained, total phenolic content was calculated and expressed in mg GAE/g of dried extract. The phenolic content is shown below in Table 4.5.

Table 4.6 Total phenolic content of samples.

Sample	Total phenolic content (mg GAE/g of dried extract)
Control tofu	151.94±2.72
Cardamom powder	1143.99±1.26
Cardamom tofu (0.6%)	351.48±2.47
Ginger powder	1321.57±1.35
Ginger tofu (0.4%)	394.68±2.51

* Values are represented as mean ± standard deviation of triplicate determinations.

The above data obtained for total phenolic content of Cardamom and Ginger powder was similar to the data obtained by Amma *et al.* (2010) and T.-S. Lim *et al.* (2007). Similar data was also found in control tofu by Adhikari and Karki (2022). Total phenolic content for cardamom extract tofu (0.6%) was found to be 351.48±2.47 and for ginger extract tofu was found to be 394.68±2.51. From above table 4.5, it shows that the increased in total phenolic content in 0.6% cardamom extract incorporated tofu was increased up to 56.98% and ginger extract incorporated tofu was 61.67%. These results show that both cardamom and ginger are good source of phenolic content.

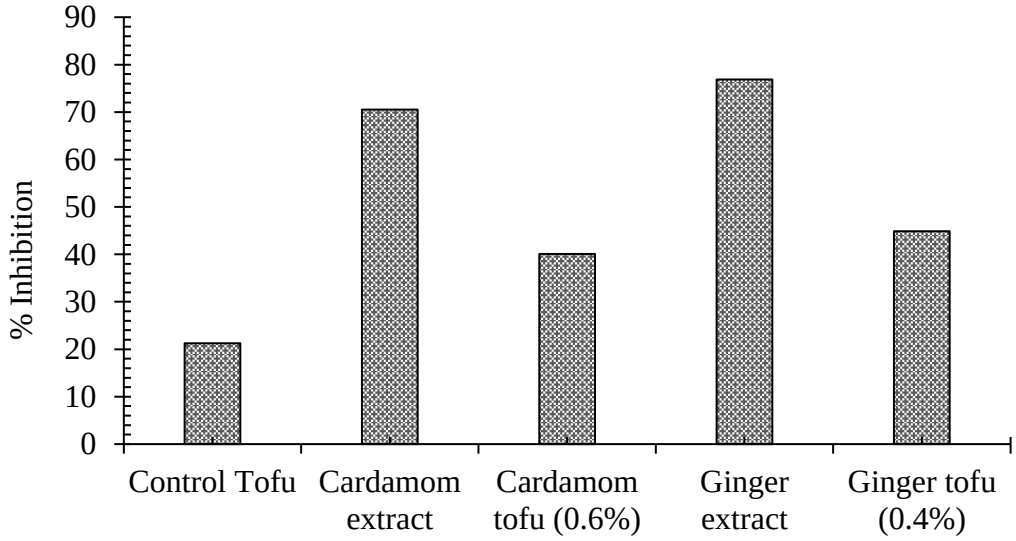

Fig. 4.10 DPPH radical scavenging activity% inhibition of Samples.

The DPPH assay measures the ability of the extract to donate hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution. It is a method for determination of antioxidant activity. Here, control tofu also show antioxidants activity due to present of isoflavones, phenolic compounds, clorogenic acid isomers, caffic acid, phytic acid and other compounds which are present in soybean seed (T. Wang, 2008). Among both optimized tofu, tofu having 0.4% ginger extract show highest % of inhibition of 44.89% where 0.6% cardamom extract tofu show 40.12% inhibition. Both cardamom and ginger extract showed a high antioxidant activity expressed in the form of % inhibition.

4.7 Effect of different packaging material on storage stability of optimized herbal tofu

4.7.1 Moisture

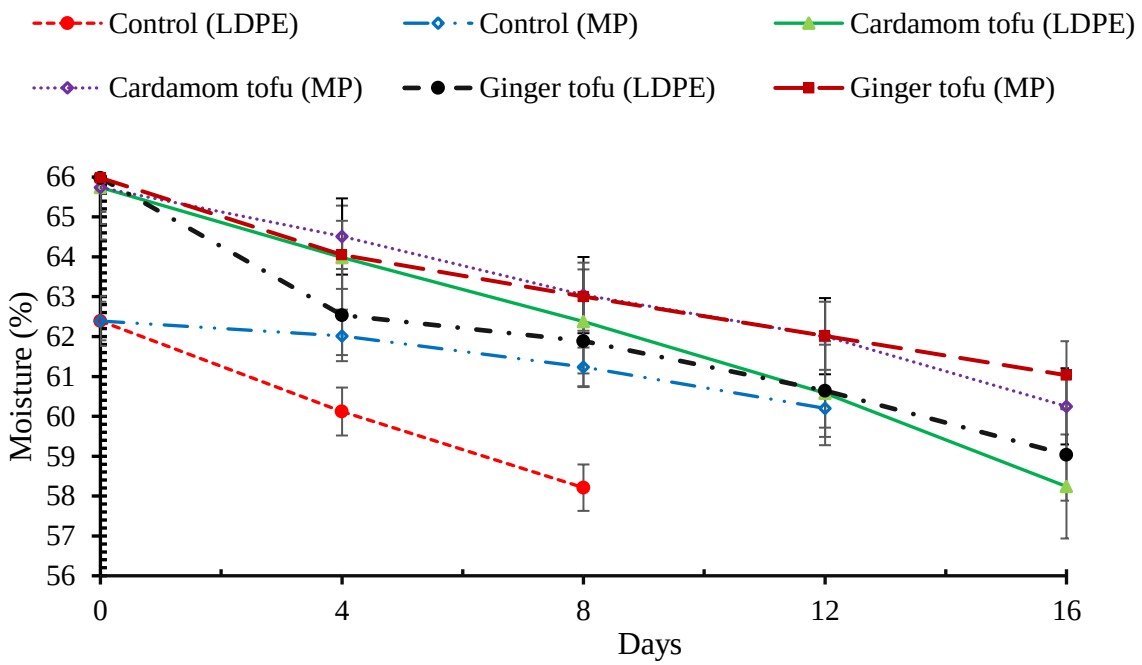

Fig.4.11 Change in moisture of packaged control and optimized herbal tofu during storage.

The control tofu had an initial moisture content of 62.39%, which progressively decreased within a period in two different packaging materials. On LDPE packaging, it reduced to 58.12% in 8th days, while in metalized plastic, it reduced to 60.20% in 12th days. Similarly, for tofu containing 0.6% cardamom extract, with an initial moisture content of 65.74%, the decrease after 16th days of storage was to 58.24% in LDPE and 60.25% in metalized plastic.

For tofu containing 0.4% ginger extract, the initial moisture content was 63.98%, and over the same period the decrease was to 59.04% in LDPE and 61.48% in metalized plastic. This also strongly consolidates the role of packaging materials on the moisture retention of tofu during storage.

From the graph, we can conclude that tofu treated with 0.4% ginger extract and packed in metalized plastic exhibited the least decrease in moisture content. The loss in moisture was slightly higher in LDPE than metalized plastic. This show that metalized plastic is very good barrier than LEDP (Zheng *et al.*, 2020).

4.7.2 pH

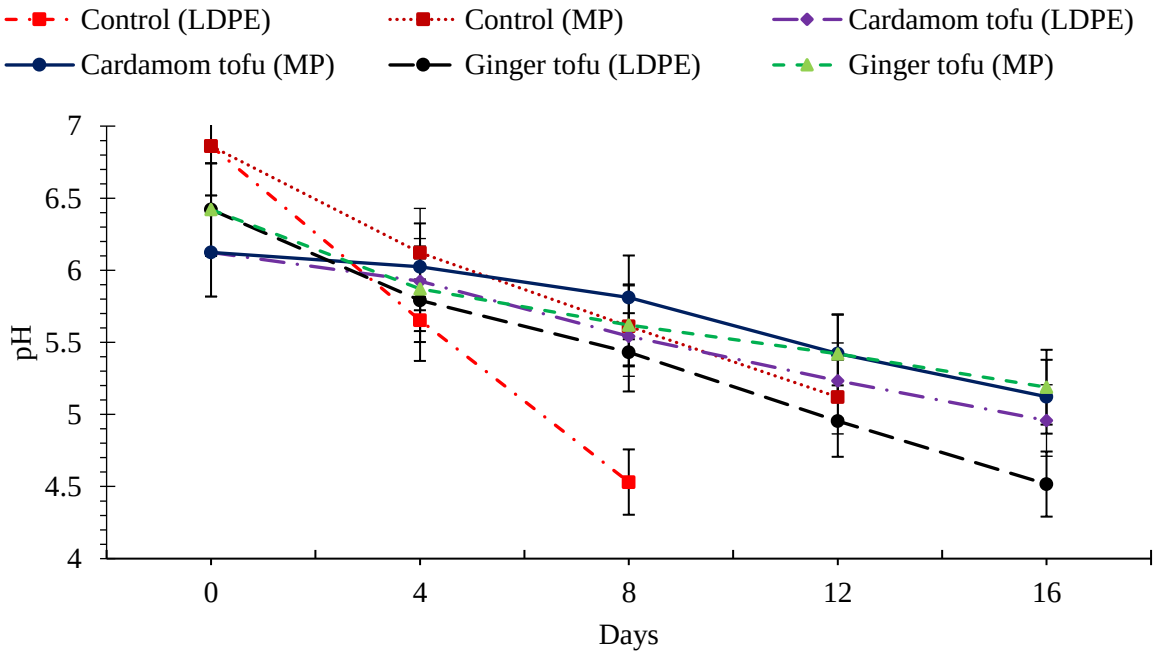

Fig.4.12 Change in pH of packaged control and optimized herbal tofu during storage.

The control tofu had an initial pH 6.862 which progressively decreased within a period in two different packaging materials. On LDPE packaging, it reduced to 4.531 in 8th days, while in metalized plastic, it reduced to 5.121 in 12th days. Similarly, for tofu containing 0.6% cardamom extract, with an initial PH of 6.124, the decrease after 16th days of storage was to 4.958 in LDPE and 5.123 in metalized plastic. For tofu containing 0.4% ginger extract, the initial PH was 6.421, and over the same period the decrease was to 4.517 in LDPE and 5.189 in metalized plastic.

This decrease in pH tofu samples during storage is generally from microbial fermentation, in which microorganisms such as lactic acid bacteria produce acidic metabolic by-products like lactic acid. The above-cited phenomena will be influenced by packaging material type, since those of higher oxygen permeability-for example, LDPE-will allow microbial development at a higher rate compared to the more protective ones, which include metallized plastic. Beyond this, the natural enzymatic activity of tofu and a higher moisture and nutrient content, especially in tofu, contribute to acid formation with time and result in a more considerable pH drop during extended storage. Although no literature has clearly mentioned the change in pH of herbal tofu while directly added to soya-milk before coagulation but Dostom *et al.* (1977) found similar change in pH of regular tofu.

4.7.3 Peroxide value

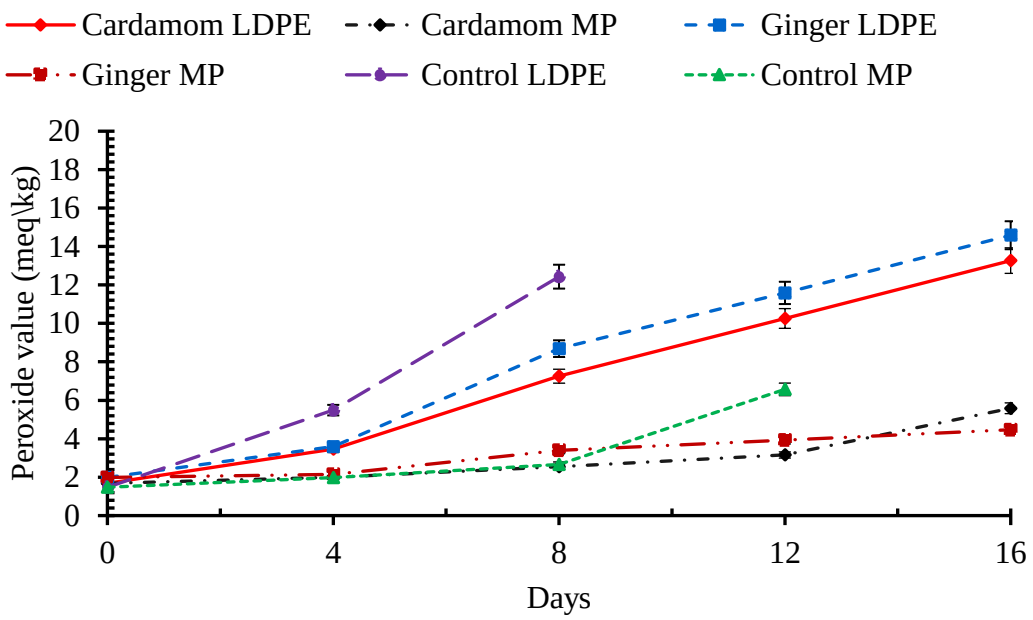

Fig.4.13 Change in peroxide value of packaged control and optimized herbal tofu during storage.

The control tofu had an initial pv 1.475 (meq/kg) which progressively increased within a period between two different packaging materials. On LDPE packaging, it increased up to 12.432 (meq/kg) in 8th days, while in metalized plastic, it increased up to 6.568 (meq/kg) in 12th days. Similarly, for tofu containing 0.6% cardamom extract, with an initial pv was 1.678(meq/kg), the increased after 16 days of storage was to 13.267 (meq/kg) in LDPE and

5.584 (meq/kg) in metalized plastic. For tofu containing 0.4% ginger extract, the initial pv was 1.985 (meq/kg), and over the same period the it was increased up to 14.587 (meq/kg) in LDPE and 4.468 (meq/kg) in metalized plastic.

These active principles of ginger including gingerol, shogaol and zingerone which possess high antioxidant activities. Gingerol, the fresh spice fraction, acts by scavenging free radicals, while shogal formed during drying or he is much more potent oxidation inhibitor. Zingerone is also produced when it is heated, which acts against oxidative deterioration. These combined fractions thereby retard the oxidation of lipids and formation of injurious peroxides (Hasan *et al.*, 2012). Cardamom is rich in flavonoids, phenolic compounds, and essential oils like cineole and terpinene, which act as antioxidants. These compounds neutralize free radicals, prevent oxidative stress, and protect fats from degradation by stabilizing reactive molecules. Tofu packaged in LDPE is more prone to a rapid increase in pv due to higher oxygen permeability, while metallized plastic offers better protection against oxidative degradation, resulting in a slower rise in pv as shown from above graph.

4.7.4 TPC

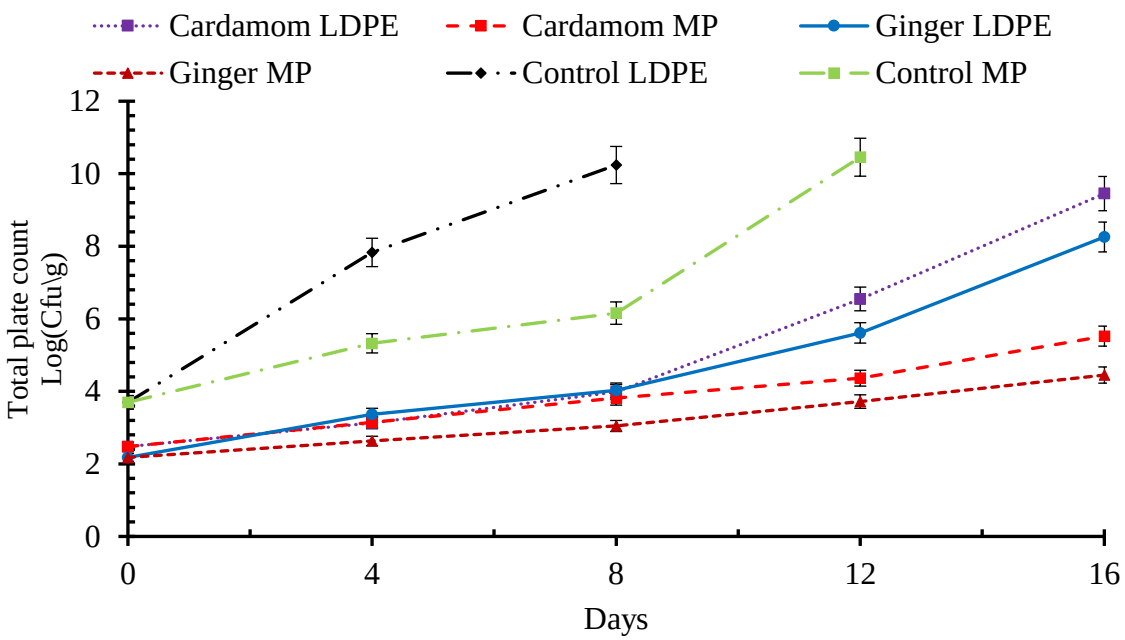

Fig.4.14 Change in total plate count of packaged control and optimized herbal tofu during storage

The control tofu had an initial total plate count was found to be 3.698 log (CFU/g) which increased considerably within a period in two different packaging materials. On LDPE packaging, it increased up to 10.24 log (CFU/g) in 8^{th} day, while in metalized plastic, it increased up to 10.456 log (CFU/g) in 12^{th} day of storage and unfit for consumption. Similarly, for tofu containing 0.6% cardamom extract, with an initial total plate count of 2.477 log (CFU/g), the increased after 16 days of storage was to 9.452 log (CFU/g) in LDPE and 5.523 log (CFU/g) in metalized plastic. For tofu containing 0.4% ginger extract, the initial total plate count was 2.178 log (CFU/g), and over the same period the growth was to 8.256 log (CFU/g) in LDPE and 4.449 log (CFU/g) in metalized plastic. Microbial count on herbal tofu packed in LDPE until 16^{th} day of storage remained within acceptable limit and after 16^{th} day of storage slime development and undesirable odor was observed. The herbal tofu sample packed in metalized plastic is acceptable until 16^{th} days of storage. Among all the samples ginger tofu packed in metalized plastic show less increase in microbial growth followed by cardamom tofu packed in metalized plastic.

The tofu contain moisture, which favor the growth of microorganism. The major spoilage of tofu is only due to the growth of microbes. Plant extracts and their components are hydrophobic, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death of the microbes (B. Joshi *et al.*, 2009). The study of K.-N. Park *et al.* (2007) investigated the potential of turmeric extract to extend the stability of tofu during storage at ambient temperature (25°C). They monitored the bacterial proliferation in tofu over a 12-day period. Bacterial load is one of the most critical factors in tofu spoilage, and the authors decided that tofu was "spoiled" when the number of bacteria reached or exceeded 10 million colony-forming units per milliliter (10^7 CFU/ml).

4.7.5 Yeast and mold

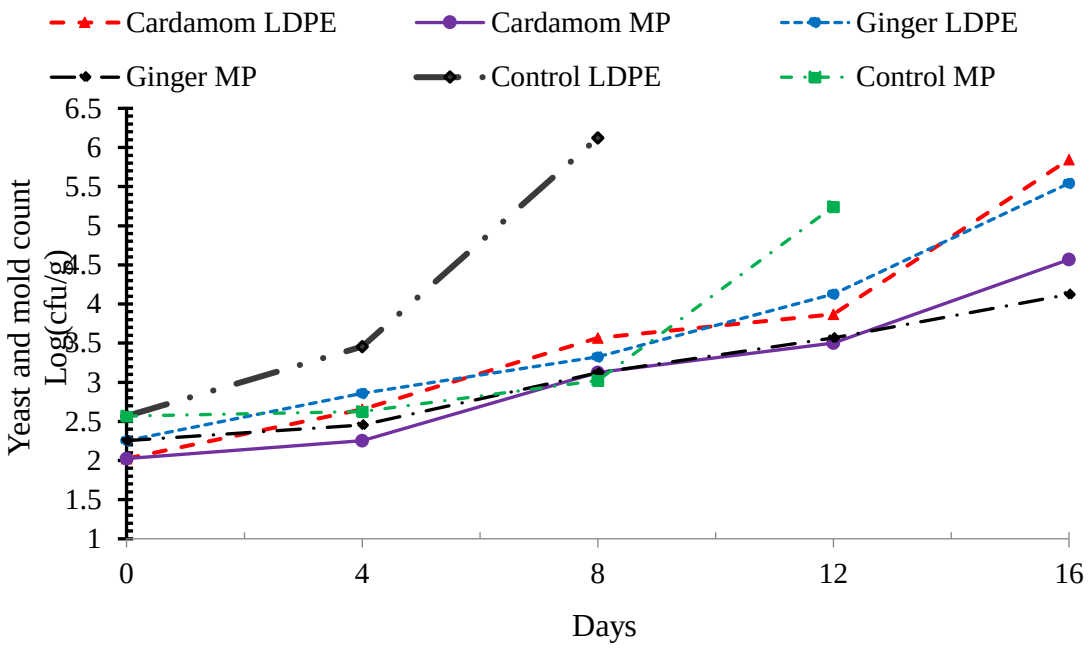

Fig.4.15 Change in yeast and mold count of packaged control and optimized herbal tofu during storage.

The control tofu had an initial yeast and mold count was found to be 2.568 log (CFU/g) which increased considerably within a period in two different packaging materials. On LDPE packaging, it increased up to 6.124 log (CFU/g) in 8th days, while in metalized plastic, it increased up to 5.245 log (CFU/g) in 12th days. Similarly, for tofu containing 0.6% cardamom extract, with an initial yeast and mold count of 2.025 log (CFU/g), the increased after 16th days of storage was to 5.846 log (CFU/g) in LDPE and 4.568 log (CFU/g) in metalized plastic. For tofu containing 0.4% ginger extract, the initial yeast and mold count was 2.254 log (CFU/g), and over the same period the growth was to 5.542 log (CFU/g) in LDPE and 4.123 log (CFU/g) in metalized plastic. The sample packed in metalized plastic showed slower increase in yeast and mold count in comparison to the sample packed in LDPE. This may be due the its very good barrier properties (Jamieson and Windle, 1983).

Part V

Conclusions and recommendations

5.1 Conclusions

As per objectives, methodologies as mentioned was followed and based on the result and discussions of research followed, following conclusions can be made:

1 Yield of ethanolic extract of cardamom and ginger was found to be 41.73% and 47.53%. Both extract showed a high phenolic content and high free radical scavenging activity.

2 The addition of cardamom and ginger extract significantly affected the sensory scores of the tofu samples.

3 On the basis of sensory score: color, flavor, texture and overall acceptance, 0.6% cardamom extract and 0.4% ginger extract tofu scored the highest score. The addition of cardamom and ginger extract and storage days significantly affected the moisture, pH, peroxide value, and microbial count of tofu samples. Among cardamom and ginger extract, ginger extract tofu shows best for storage stability of tofu.

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

5 Cost of tofu treated with cardamom extract tofu was estimated to be and ginger 153.22/kg extract tofu was estimated to be 146.0/kg.

5.2 Recommendations

The present work is preliminary study of flavor of selected herbs in tofu. Due to time constraints, limited work has been done in this research. From this study, following points can be recommended:

1. Antimicrobial potential of the herbal extract can be studied.

2. Other common herbs and spices can be tested for their potential to act as preservatives in tofu.
3. Cardamom and ginger extract can be used as preservative in other food item.
4. Antinutritional factors of tofu can be determined.

Part VI

Summary

Tofu is a major traditional product with a very short shelf life, thus storage and marketability in the organized sector is limited. To overcome this issue, the use of preservatives has been tried. The use of natural spices and herbs has also been tried that have established medicinal, preservative, and antioxidant properties. Particularly, ginger, with its highly effective antimicrobial and antioxidant activities, and cardamom, a medicinal herb from Nepal's Himalayan region, were examined in light of its high preservative potential. Both ginger and cardamom individual extracts were applied as preservatives to tofu.

This study was done to optimization of cardamom and ginger extract on tofu and its quality as well as optimized herbal tofu's storage stability. Ethanolic extracts of cardamom and ginger was done and analyzed for their total phenolic content and radical scavenging activity. By varying concentrations of extract (0–0.8%), were incorporated into tofu during its preparation before coagulation. The optimized herbal tofu samples were then compared to control sample to assess the impact of the extracts on shelf life and storage stability. The findings suggest that the antioxidant properties of the extracts may help extend tofu's storage life. This study is also carried out to know different packaging materials on the quality of herbal tofu. Sensory evaluation of tofu was carried out based on color, flavor, texture and overall acceptability. The data obtained were statistically analyzed using two-way ANOVA (no blocking) at 5% level of significance. Among all the sample C and F having 0.6% large cardamom extract and 0.4% ginger extract got highest mean score than control tofu.The study assessed the storage stability of herbal- incorporated and control tofu using two packaging materials: LDPE and metalized plastic, under refrigerated conditions (4°C) for 16^{th} days. Analyses of pH, moisture, peroxide value, acid value, TPC, and yeast and mold counts were conducted every four days. It showed that metalized plastic was more effective in preserving the tofu. Herbal-incorporated tofu, particularly when packed in metalized plastic, exhibited superior stability due to the antioxidant properties of the cardamom and ginger extracts. Additionally, the cost of production for herbal tofu was calculated at NRs153.22/kg for cardamom-treated tofu and NRs146.00/kg for ginger-treated tofu, reflecting the added value of incorporating these extracts. Metalized plastic, combined with herbal treatments, proved to be the best approach for extending tofu's shelf life.

References

Adhikari, S. and Karki, T. B. (2022). Quality Assessment of Tofu from Black Soybean (Glycine max.) by using Natural and Synthetic Coagulant. *J. Food Sci. Technol.* **13**, 54-61.

Aleem, M., Khan, M. I., Shakshaz, F. A., Akbari, N. and Anwar, D. (2020). Botany, phytochemistry and antimicrobial activity of ginger (Zingiber officinale): *Int. J. Herb Med.* **8** (6), 36-49. [doi:10.23880/ipcm-16000142].

Ali, S. (2007). Cardamom processing. Practical Action Technical brief. http://practicalaction.org/practicalanswers/product_info.php?manufacturers_id=17&products_id=76 (Accessed 10 Nov, 2018).

Amma, K., Rani, M. P., Sasidharan, I. and Nisha, V. N. P. (2010). Chemical composition, flavonoid-phenolic contents and radical scavenging activity of four major varieties of cardamom. *Int. J. Biol. Med. Res.* **1** (3), 20-24.

Anbarasu, K. and Vijayalakshmi, G. (2007). Improved shelf life of protein-rich tofu using Ocimum sanctum (tulsi) extracts to benefit Indian rural population. *J. of Food Sci.* **72** (8), 300-305.

Anjum, S., Agnihotri, V., Rana, S., Pandey, A. and Pande, V. (2023). The Impact of Processing Methods and Conditions on Nutritional Properties of Soybean-Based Tofu: *J.of Food Eng. and Technol.* **12** (1), 1-13. [doi:10.32732/jfet.2023.12.1.1].

AOAC. (2005). "Official methods of analysis of the Association of Official Analytical Chemists". Vol. 3. Association of Official Analytical Chemists. Arlington, Virgina, USA. . [[ISBN 978-0-935-58475-2]].

Arora, D. S. and Kaur, J. (1999). Antimicrobial activity of spices. *Int. J of Antimicrobial Agents* **12** (3), 257-262. [doi:10.1016/S0924-8579(99)00074-6].

Aruoma, O. I., Spencer, J. P., Warren, D., Jenner, P., Butler, J. and Halliwell, B. (1997). Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *J. Food Chem*. **60** (2), 149-156. [doi:10.1016/S0308-8146(95)00254-5].

Azhar, S. (2024). A Quantitative and Qualitative Study on University Students' Perceptions of Soy-based Food Products and the Aspects that Influence these Perceptions. Food Sci. Thesis. Kansas State Univ., America.

Baljeet, S., Simmy, G., Ritika, Y. and Roshanlal, Y. (2015). Antimicrobial activity of individual and combined extracts of selected spices against some pathogenic and food spoilage microorganisms. *Int. J. Food Res.* **22** (6), 2594.

Bano, S., Ahmad, N. and Sharma, A. (2016). Phytochemical screening and evaluation of anti-microbial and anti-oxidant activity of Elettaria cardamom (Cardamom). *J. of Appl. Nat. Sci.* **8** (4), 1966-1970. [doi.org/10.1016/j.anaerobe.2019.102089].

Beristain-Bauza, S. D. C., Hernández-Carranza, P., Cid-Pérez, T. S., Ávila-Sosa, R., Ruiz-López, I. I. and Ochoa-Velasco, C. E. (2019). Antimicrobial activity of ginger (Zingiber officinale) and its application in food products.*Int. J. Food Rev.* **35** (5), 407-426. [doi:10.1080/87559129.2019.1573829].

Beuchat, L. R. and Golden, D. A. (1989). Antimicrobials occurring naturally in foods. *Agric. and Food Sci.Chem.* **43**, 134-142.

Bhardwaj, D., Mann, B., Sharma, R., Kumar, R., Rashmi, H., Gandhi, K., Sukthija, M. and Sharma, M. Studies of Physicochemical, Microbiological and Sensory characteristics of Paneer (Indian Cottage Cheese) to check the efficacy of clove-oil nanoemulsion in enhancing its shelf-life. *Indian J. of Dairy Sci.***77** (4).

Bhardwaj, H., Hamama, A., Rangappa, M., Joshi, J. and Sapra, V. (2003). Effects of soybean genotype and growing location on oil and fatty acids in tofu. *J. Plant Foods for Human Nutr.* **58**, 197-205. [doi:10.1023/B:QUAL.0000040268.06587.2a].

Bhattarai, S., Nepali, N., Thaller, J., Thewarapperuma, A. and Webb, J. (2013). Promoting agribusiness innovation in Nepal, feasibility assessment for an agribusiness innovation center.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. **181** (4617), 1199-1200. [doi:10.1038/1811199a0].

Buffo, R. A., Weller, C. L. and Parkhurst, A. M. (1998). Relationships among grain sorghum quality factors. *Cereal Chem.* **75** (1), 100-104.

Cfia. (1986). "The Biology of Glycine Max (L.) Merr. (Soybean)". Canadian Food Inspection Agency. Retrieved from https://inspection.canada.ca/plant-varieties/plants-with-noveltraits/applicants/directive-94-08/biology-documents/glycine-max-l-merr/eng/1330975306785/1330975382668. [Accessed 30 August 2021].

Cha, D. S., Chen, J., Park, H. J. and Chinnan, M. S. (2003). Inhibition of Listeria monocytogenes in tofu by use of a polyethylene film coated with a cellulosic solution containing nisin. *Int. J. of Food Sci. Technol.* **38** (4). [doi:10.1046/j.1365-2621.2003.00684].

Chang, H.-W. and Chen, W.-L. (1999). Effects of the application of hydrogen peroxide on the preservation of firm tofu. *J. Food Drug Anal.* **7** (3), 8. [doi:10.38212/22246614.2869].

Chang, K. and Hou, H. (2003). Science and technology of tofu making. *In:* "Handbook of Vegetable Preservation and Processing".). pp. 530-572. CRC Press.

Chen, B., Mcclements, D. J. and Decker, E. A. (2011). Minor components in food oils: a critical review of their roles on lipid oxidation chemistry in bulk oils and emulsions. *Crit. Rev. in Food Sci. Nutr.* **51** (10), 901-916. [doi:10.1080/10408398.2011.606379].

Choi, G.-H., Kim, K.-C. and Lee, K.-H. (2010). Quality and antioxidant characteristics of soft tofu supplemented with red ginseng extract during storage. *J. of the Korean Soc. of Food Sci. Nutr*. **39** (3), 414-420. [doi:10.3746/jkfn.2010.39.3.414].

Chumroenphat, T., Khanprom, I. and Butkhup, L. (2011). Stability of phytochemicals and antioxidant properties in ginger (Zingiber officinale Roscoe) rhizome with different drying methods. *J of Herbs, Spices Med. Plants*. **17** (4), 361-374.

Clementson, C. L., Ileleji, K. and Rosentrater, K. (2010). Evaluation of measurement procedures used to determine the bulk density of distillers dried grains with solubles (DDGS). *American Soc. of Agric. and Bio. Eng.* **53** (2), 485-490. [doi:10.13031/2013.29557].

Deman, J., Deman, L. and Gupta, S. (1986). Texture and microstructure of soybean curd (tofu) as affected by different coagulants. *J. Food Struct.* **5** (1).

Depalma, K., Smith, B. and Mcdonald, A. G. (2019). Effect of processing conditions, biochemical properties, and microstructure on tofu production from yellow field peas (Pisum sativum). *J. of Food Sci.* **84** (12), 3463-3472. [doi:10.1111/1750-3841.14940].

Derbyshire, E., Wright, D. and Boulter, D. (1976). Legumin and vicilin, storage proteins of legume seeds. *J. Phytochem.* **15** (1), 3-24. [doi:10.1016/S0031-9422(00)89046-9].

DFTQC. (2012). "Food Composition Table for Nepal". Department of Food Technology and Quality Control ((Ministry of Agriculture Department)), Nepal. pp. 5-11. Retrieved from www.dftqc.gov.np. [Accessed 30 august, 2021].

Dorantes, L., Colmenero, R., Hernandez, H., Mota, L., Jaramillo, M. E., Fernandez, E. and Solano, C. (2000). Inhibition of growth of some foodborne pathogenic bacteria by Capsicum annum extracts. *Int. J. of Food Microbiol.* **57** (1-2), 125-128. [doi:10.1016/S0168-1605(00)00216-6].

Dostom, C. R., Frank, H. A. and Cavaletto, C. G. (1977). Indirect methods as criteria of spoilage in tofu (soybean curd). *J. Food Sci.* **42** (1), 273-274. [doi:10.1111/j.1365-2621.1977.tb01270.x].

Dżugan, M., Tomczyk, M., Szymański, P., Grabek-Lejko, D. and Miłek, M. (2024). The Influence of Selected Herb Additives on the Organoleptic and Antioxidant Properties and Storage Stability of Frozen Homemade Tofu. *J. Appl. Sci*. **14** (15).

Evans, D. E., Tsukamoto, C. and Nielsen, N. (1997). A small scale method for the production of soymilk and silken tofu. *Crop Sci.* **37** (5), 1463-1471. [doi:10.2135/cropsci1997.0011183X003700050008x].

Garg, G., Sharma, S., Dua, A. and Mahajan, R. (2016). Antibacterial potential of polyphenol rich methanol extract of Cardamom (Amomum subulatum). *J. of Innov. Biol*. **3** (1), 271-275.

Gautam, N., Bhattarai, R., Khanal,R., Sharma, B. and Oli, P. (2016). Technology, Chemistry and Bioactive Properties of Large Cardamom (Amomum Subulatum Roxb.): An Overview. *Int. J. of Appl. Sci. Biotechnol*. **4** (2), 139-149. [doi:10.3126/ijasbt.v4i2.15104].

Ge, C., Verma, S. S., Burruto, J., Ribalco, N., Ong, J. and Sudhahar, K. (2021). Effects of flexing, optical density, and lamination on barrier and mechanical properties of metallized films and aluminum foil centered laminates prepared with polyethylene terephthalate and linear low density polyethylene. *J. of Plast. Film Sheeting* **37** (2), 205-225. [doi:10.1177/8756087920963532].

Genwali, G. R., Acharya, P. P. and Rajbhandari, M. (2013). Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. *Nepal J. of Sci. Technol.* **14** (1), 95-102.

Grygorczyk, A., Duizer, L., Lesschaeve, I. and Corredig, M. (2014). Gelation of recombined soymilk and cow's milk gels: Effect of homogenization order and mode of gelation on microstructure and texture of the final matrix. *J Food Hydrocoll.* **35**, 69-77. [doi:10.1016/j.foodhyd.2013.04.011].

Han, B.-Z., Rombouts, F. M. and Nout, M. R. (2001). A Chinese fermented soybean food. *Int. J. of Food Microbiol*. **65** (1-2), 1-10.

Han, J. H. (2005). "Innovations in food packaging". Elsevier. [0080455174].

Hasan, H. A., Raauf, A. R., Razik, B. and Hassan, B. R. (2012). Chemical composition and antimicrobial activity of the crude extracts isolated from Zingiber officinale by different solvents. *J. Pharm* **3** (9), 1-5. [doi: 10.4172/2153-2435.1000184].

Hyldgaard, M., Mygind, T. and Meyer, R. L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *J. Frontiers in Microbiol*. **3**, 12.

Jamieson and Windle. (1983). Structure and oxygen-barrier properties of metallized

polymer film. *J. Mater. Sci***. 18**, 64-80. [doi:10.1007/BF00543811].

Jensen, S. K., Liu, Y.-G. and Eggum, B. (1995). The influence of seed size and hull content on the composition and digestibility of rapeseeds in rats. *J. Animal Feed Sci. Technol.* **54** (1-4), 9-19.

Jiang, L. (2014). Effect Of Pressing Treatment On The Freeze-Drying Process Of The Tofu. *J. of Food Sci. and Tech*. **52** (2015), 2886-2893. [doi:10.1007/s13197-014-1320-z].

Joo, K. H. and Cavender, G. A. (2020). Investigation of tofu products coagulated with trimagnesium citrate as a novel alternative to nigari and gypsum: Comparison of physical properties and consumer preference. **118**, 108819. [doi:10.1016/j.lwt.2019.108819].

Joshi, B., Lekhak, S. and Sharma, A. (2009). Antibacterial property of different medicinal plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. *J. of Sci. Eng. Tech*. **5** (1). [doi:10.70530/kuset.v5i1.248].

Joshi, R., Sharma, P., Sharma, V., Prasad, R., Sud, R. and Gulati, A. (2013). Analysis of the essential oil of large cardamom (Amomum subulatum Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. *J. of the Sci. of Food Agric.* **93** (6), 1303-1309.

K.V, S., Sanjitha.S, Santhiya.S, Kalaiselvisenthil and Vasuki.V. (2021). An overview of Tofu: History, Types, manufacturing process and Health benefits *J. of Uni. of Shanghai for Sci. and Technol*. **23** (10), 505-515.

Kanadea, R. and Bhatkhandeb, D. (2016). Extraction of ginger oil using different methods and effect of solvents, time, temperature to maximize yield. *Int. J. of Adv. in Sci. Eng. Technol.* **4** (2), 241-244.

Kelli. (2007). Tofu standard. Retrieved from http://www.cbpanet.com/ueditor/net/upload/2013-07-25/a8f6eea3-8457-49cc-a38310f9b1b65047.pdf [Accessed 25 April, 2022].

Khadka, N. (2015). Effect of addition of spices (garlic and ginger) on the quality of tofu. . B. Tech Dissertation. Tribhuvan Univ, Nepal.

Khatiwada, A., Subedi, A. and Dangol, R. (2019). A review on status of production of large cardamom in Nepal and its marketing in national and global scenario. *Malaysian J. of Halal Res.* **2** (1), 16-21.

Kikuzaki, H., Kawai, Y. and Nakatani, N. (2001). 1, 1-Diphenyl-2-picrylhydrazyl radical-scavenging active compounds from greater cardamom (Amomum subulatum Roxb.). *J. of Nutr. Sci. Vitaminol.* **47** (2), 167-171.

Kim, J.-Y. and Park, G.-S. (2006). Quality characteristics and shelf-life of tofu coagulated by furie juice of pomegranate. *J. of the Korean Soc. of Food Cult.* **21** (6), 644-652.

Kim, S.-E., Lee, S.-W., Yeum, D.-M. and Lee, M.-J. (2012). Quality characteristics of tofu with added alfalfa (Medicago sativa L.) extracts. *J. of the Korean Soc. of Food Sci. Nutr.* **41** (1), 123-128.

Kim, Y. S., Choi, Y. M., Noh, D. O., Cho, S. Y. and Suh, H. J. (2007). The effect of oyster shell powder on the extension of the shelf life of tofu. *J. Food Chem.* **103** (1), 155-160.

Kiyama, R. (2020). Nutritional implications of ginger: Chemistry, biological activities and signaling pathways. *J. of Nutr. Biochem.* **86**, 108486. [doi:10.1016/j.jnutbio.2020.108486].

Kohyama, K. and Nishinari, K. (1993). Rheological studies on the gelation process of soybean 7 S and 11 S proteins in the presence of glucono-. delta.-lactone. *J. of Agric. Food Chem.* **41** (1), 8-14. [doi:10.1021/jf00025a003].

Kohyama, K., Sano, Y. and Doi, E. (1995). Rheological characteristics and gelation mechanism of tofu (soybean curd). *J. of Agric. Food Chem.* **43** (7), 1808-1812. [doi:10.1021/jf00055a011].

Koirala, A. (2024). Effect of prouting and different packaging materials on the quality of tofu. B. Tech. Dissertation. Tribhuvan Univ, Nepal.

Kunnumakkara, A. B., Koca, C., Dey, S., Gehlot, P., Yodkeeree, S., Danda, D., Sung, B. and Aggarwal, B. B. (2009). Traditional uses of spices: an overview. *Molecular Targets Therapeutic uses of Spices Modern uses for Ancient Med*. 1-24.

Lama, S. (2009). Effect of blending of soymilk in the preparation of rasogolla. B.Tech. (Food) Dissertation. Tribhuvan University, Nepal.

Langner, E., Greifenberg, S. and Gruenwald, J. (1998). Ginger: history and use. *Adv. in Therapy.* **15** (1), 25-44.

Lee, S., Cho, H. and Lee, K.-G. (2014). Volatile compounds as markers of tofu (soybean curd) freshness during storage. *J. of Agric. Food Chem.* **62** (3), 772-779. [doi:10.1021/jf404847g].

Liener, I. (1981). Factors affecting the nutritional quality of soya products. *J. of the American Oil Chemists' Soc.* **58** (3Part2), 406-415. [doi:10.1007/BF02582390].

Lim, T.-S., Kwon, O.-J., Kwon, J.-H. and Kim, H.-K. (2007). Monitoring of extraction yields and functional properties of ginger (Zingiber officinale) extracts using response surface methodology. *J. of the Korean Soc. of Food Sci. Nutr*. **36** (3), 348-354.

Lim, T. K. (2012). "Edible medicinal and non-medicinal plants". Vol. 1. Springer.

Liu, K. (2012). "Soybeans: chemistry, technology, and utilization". Springer. Aspen Publishers: Gaithersburg, MD. . [146151763X].

Malaki Nik, A., Alexander, M., Poysa, V., Woodrow, L. and Corredig, M. (2011). Effect of soy protein subunit composition on the rheological properties of soymilk during acidification. *J. Food Biophysics.* **6**, 26-36.

Mao, Q.-Q., Xu, X.-Y., Cao, S.-Y., Gan, R.-Y., Corke, H., Beta, T. and Li, H.-B. (2019). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). *Food Sci.* **8** (6), 185. [doi.org/10.3390/foods8060185].

Maurya, S., Shukla, S. and Gour, L. (2018). Studies on physical and hunter colour of gamma irradiated tofu (soy paneer). *Int. J. Curr. Microbiol. Appl. Sci.* **7**, 2008-2018. [doi:10.20546/ijcmas.2018.703.236].

Min, Y.-H., Kim, J.-Y., Park, L.-Y., Lee, S.-H. and Park, G.-S. (2007). Physicochemical quality characteristics of tofu prepared with turmeric (*Curcuma aromatica Salab*.). *Korean J. of Food Cookery Sci*. **23** (4), 502-510.

Moghaddasi, M. S. and Kashani, H. H. (2012). Ginger (*Zingiber officinale*): A review. *J. of Med. Plants Res*. **6** (26), 4255-4258.

Mujoo, R., Trinh, D. T. and Ng, P. K. (2003). Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *J Food Chem*. **82** (2), 265-273. [doi:10.1016/S0308-8146(02)00547-2].

Murugkar, Agrahar, D. and Jha, K. (2009). Effect of sprouting on nutritional and functional characteristics of soybean. *J. Food Sci. Technol.* **46** (3), 240-243.

Murugkar, D. A. (2015). Effect of different process parameters on the quality of soymilk and tofu from sprouted soybean. *J. Food Sci. Technol.* **52**, 2886-2893. [doi:10.1007/s13197-014-1320-z].

Nagai, T. and Tamang, J. P. (2010). Fermented legumes: Soybean and non-soybean products. *J Ferment. Foods Bev. of the World*. 191-224.

No, H. K. and Meyers, S. P. (2004). Preparation of tofu using chitosan as a coagulant for improved shelf-life. *Int. J. of Food Sci. Technol.* **39** (2), 133-141. [doi:10.1046/j.0950-5423.2003.00772.x].

Obatolu, V. A. (2008). Effect of different coagulants on yield and quality of tofu from soymilk. *J. European Food Res. Technol.* **226**, 467-472.

Oboh, G. (2006). Coagulants modulate the hypocholesterolemic effect of tofu (coagulated soymilk). *African J. of Biotechnol.* **5** (3), 290-294. [doi:10.1089/jmf.2006.208].

Ojha, P., Karki, T. and Maharjan, S. (2014). Effect of sprouting on physic-chemical properties of tofu. *J. Nutr. Health Food Eng.* **1** (2), 1-8. [doi:10.15406/jnhfe.2014.01.00011].

Pal, M., Devrani, M. and Ayele, Y. (2019). Tofu: A popular food with high nutritional and health benefits. *Food Bev. Process*. **5** (4), 54-55.

Park, I., Kim, S. and Kim, S. (1994). Storage of soybean curd prepared with ozone treated soybean. *J. Food Sci*. **43**, 969-972.

Park, K.-N., Park, L.-Y., Kim, D.-G., Park, G.-S. and Lee, S.-H. (2007). Effect of turmeric (*Curcuma aromatica Salab*.) on shelf life of tofu. *Korean J. of Food Preserv*. **14** (2), 136-141.

Park, Y.-M., Cho, H.-S. and Park, B.-H. (2014). Quality characteristics of tofu prepared with Boehmeria nivea powder. *J. of the East Asian Soc. of Dietary Life.* **24** (4), 465-471.

Peng, X. and Guo, S. (2015). Texture characteristics of soymilk gels formed by lactic fermentation: A comparison of soymilk prepared by blanching soybeans under different temperatures. *J. Food Hydrocolloids.* **43**, 58-65. [doi:10.1016/j.foodhyd.2014.04.034].

Perkins, E. G. (1995). Composition of soybeans and soybean products. *In:* "Practical handbook of soybean processing and utilization".). pp. 9-28. Elsevier.

Poudel, B. (2022). Effect of Incorporation of Sesame Seed in the Quality of Tofu. B. Techh Thesis. Department of Food Technology Central Campus of Technology Institute, Nepal.

Poysa, V. and Woodrow, L. (2002). Stability of soybean seed composition and its effect on soymilk and tofu yield and quality. *J. Food Res. Int.* **35** (4), 337-345. [doi:10.1016/S0963-9969(01)00125-9].

Prasath, D., Venugopal, M., Senthilkumar, R. and Leela, N. (2009). Hybrid performance for yield and yield components in cardamom (Elettaria cardamom Maton). *J. Euphytica.* **168**, 49-60.

Préstamo, G., Lesmes, M., Otero, L. and Arroyo, G. (2000). Soybean vegetable protein (tofu) preserved with high pressure. *J. of Agric. Food Chem.* **48** (7), 2943-2947. [doi:10.1021/jf991251y].

Purcell, L. C., Salmeron, M. and Ashlock, L. (2014). Soybean growth and development. *Arkansas soybean production handbook.* **197**, 1-8.

Rahman, F. N. F. and Asman, S. (2024). Physicochemical Properties and Shelf-Life Evaluation of Nutrient Fortified Dehydrated Tofu for Satiety. *Enhanced Knowledge in Sci. Technol*. **4** (1), 425-434.

Rai, B. and Kc, J. J. M. K. A., Kathmandu. (2007). Basic food analysis handbook.

Ran, C. and Kan, J. (2012). Effect of process condition on the quality of white whole-soybean sufu pehtze. J. *of Food Sci*. **33** (24), 160-164.

Rehberger, T., Wilson, L. and Glatz, B. (1984). Microbiological quality of commercial tofu. *J. of Food Prot*. **47** (3), 177-181. [doi:10.4315/0362-028X-47.3.177].

Rekha, C. and Vijayalakshmi, G. (2010). Influence of natural coagulants on isoflavones and antioxidant activity of tofu. *J. of Food Sci. Technol.* **47**, 387-393. [doi:10.1007/s13197-011-0245z].

Riaz, H., Begum, A., Raza, S. A., Khan, Z. M.-U.-D., Yousaf, H. and Tariq, A. (2015). Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan. *Int. J. Current Pharm.* **4** (7), 405-409.

Rizkaprilisa, W. and Setiadi, S. (2018). Comparative study of CaSO4 and papain enzyme as coagulants in the tofu production. *Indonesian Food Nutr. Progress***. 15** (2), 79-84. [doi:10.22146/ifnp.33349].

Robin, J., Poonam, S., Virbala, S. and Ashu, G. (2013). Analysis of the essential oil of large cardamom (*Amomum subulatum Roxb*.) growing in different agro-climatic zones of Himachal Pradesh, India. *J. of The Sci. of Food and Agric*. **93** (6), 1303-1309. [doi:10.10002/jsfa.5886].

Rosset, M., Prudencio, S. H. and Beléia, A. D. P. (2012). Viscozyme L action on soy slurry affects carbohydrates and antioxidant properties of silken tofu. *Int. J. Food sci. Technol.* **18** (6), 531-538. [doi:10.1177/1082013211433076].

Rossi, F., Felis, G. E., Martinelli, A., Calcavecchia, B. and Torriani, S. (2016). Microbiological characteristics of fresh tofu produced in small industrial scale and identification of specific spoiling microorganisms (SSO). **70**, 280-285. [doi:10.1016/j.lwt.2016.02.057]

Saeed, A., Sultana, B., Anwar, F., Mushtaq, M., Alkharfy, K. M. and Gilani, A.-H. (2014). Antioxidant and antimutagenic potential of seeds and pods of green cardamom (Elettaria cardamomum). *Int. J. of Pharm*. **10** (8), 461-469.

Saio, K. (1979). Tofu-relationships between texture and fine structure. *J. Cereal Foods World*. **24**, 342-354.

Sarkar, S. and Aparna, K. (2020). Food Packaging and Storage. *Food Packaging and Storage.* **3**, 27-51.

Shan, B., Cai, Y.-Z., Brooks, J. D. and Corke, H. (2011). Potential application of spice and herb extracts as natural preservatives in cheese. *J. of Med. Food* **14** (3), 284-290.

Shin, W.-K., Kim, W. and Kim, Y. (2014). Physicochemical and sensory characteristics of a low-fat tofu produced using supercritical CO_2 extracted soy flour. *Food Sci. Biotechnol.* **23**, 43-48.

Shrestha, R., Das, S. and Neupane, R. (2021). Effect of spacing and plant density on yield performance of determinate soybean variety Tarkari Bhatmas-1 under mid hill condition.

Shun-Tang, G., Ono, T. and Mikami, M. (1999). Incorporation of soy milk lipid into protein coagulum by addition of calcium chloride. *J. of Agric. Food chem.* **47** (3), 901-905. [doi:10.1021/jf9805247].

Singh, G., Kiran, S., Marimuthu, P., Isidorov, V. and Vinogorova, V. (2008). Antioxidant and antimicrobial activities of essential oil and various oleoresins of Elettaria cardamomum (seeds and pods). *J. of the Sci. of Food Agric*. **88** (2), 280-289. [doi:10.1002/jsfa.3087].

Singh, R., Kaushik, R. and Jaglan, V. (2018). Antibacterial and antioxidant activity of green cardamom and rosemary extract in food products: A brief review. *Pharma. Innov. J.* **7** (6), 568-573.

Souza, E. L. D., Stamford, T. L. M., Lima, E. D. O., Trajano, V. N. and Barbosa Filho, J. M. (2005). Antimicrobial effectiveness of spices: an approach for use in food conservation systems. *Brazilian Archives of Bio. Technol*. **48**, 549-558. [doi:10.1590/S1516-89132005000500007].

Sozzi, G., Peter, K., Nirmal Babu, K. and Divakaran, M. (2012). Capers and caperberries, Second Edi. ed, Handbook of Herbs and Spices: Woodhead Publishing Limited.

Subroto, E. and Indiarto, R. (2021). Ginger rhizomes (Zingiber officinale) functionality in food and health perspective: a review. *Food Res.* **5**, 497-505. [doi:10.26656/fr.2017.5(1).361].

Tkaczewska, J., Jamróz, E., Zając, M., Guzik, P., Gedif, H. D., Turek, K. and Kopeć, M. (2023). Antioxidant edible double-layered film based on waste from soybean production as a vegan active packaging for perishable food products. J. *Food Chem.* **400**, 134009. [doi:10.1016/j.foodchem.2022.134009]

Tseng, Y.-C. and Xiong, Y. L. (2009). Effect of inulin on the rheological properties of silken tofu coagulated with glucono-δ-lactone. *J. of Food Eng.* **90** (4), 511-516. [doi:10.1016/j.jfoodeng.2008.07.017].

Tuitemwong, K. and Fung, D. Y. (1991). Microbiological study of tofu. *J. of food Prot.* **54** (3), 212-216. [doi:10.4315/0362-028X-54.3.212].

Uwadiae, S., Aifesume, H. and Ayodele, B. (2019). Effect of extraction temperature, time and volume of diluent on oil yield from ginger (Zingiber officinale) in a batch-mode process. *J. of Appl. Sci. Environ. Manag.* **23** (4), 611-615.

Valliyodan, B., Qiu, D., Patil, G., Zeng, P., Huang, J., Dai, L., Chen, C., Li, Y., Joshi, T. and Song, L. J. S. R. (2016). Landscape of genomic diversity and trait discovery in soybean. **6** (1), 23598.

Vernin, G. and Parkanyi, C. (2016). Chemistry of ginger. *In:* "Ginger".). pp. 107-200. CRC Press.

Wang, B., Zhang, Q., Zhang, N., Bak, K. H., Soladoye, O. P., Aluko, R. E., Fu, Y. and Zhang, Y. (2021). Insights into formation, detection and removal of the beany flavor in soybean protein. *J. Trends in Food Sci. Technol.* **112**, 336-347. [doi:10.1016/j.tifs.2021.04.018].

Wang, T. (2008). Minor constituents and phytochemicals of soybeans. *In:* "Soybeans".). pp. 297-329. Elsevier.

Yang, Q.-Q., Cheng, L.-Z., Zhang, T., Yaron, S., Jiang, H.-X., Sui, Z.-Q. and Corke, H. (2020). Phenolic profiles, antioxidant, and antiproliferative activities of turmeric (Curcuma longa). *J. Ind. rops Prod.* **152**, 112561.

Yasir, S. B. M., Sutton, K., Newberry, M., Andrews, N. and Gerrard, J. (2007). The impact of transglutaminase on soy proteins and tofu texture. *J. Food Chem.* **104** (4), 1491-1501. [doi:10.1016/j.foodchem.2007.02.026].

Yofa, R., Perdana, R., Aldillah, R., Muslim, C. and Agustian, A. (2021). Strategies to increase soybean production by increasing the distribution of new superior varieties. *IOP Conference Series: Earth and Environmental Science*. **892**, 012067.

Zaika, L. L. (1988). Spices and herbs: their antimicrobial activity and its determination. *J. of Food Safety***. 9** (2), 97-118.

Zhang, Q., Wang, C., Li, B., Li, L., Lin, D., Chen, H., Liu, Y., Li, S., Qin, W. and Liu, J. (2018). Research progress in tofu processing: From raw materials to processing conditions. *J. Crit. Rev. in Food Sci. Nutr.* **58** (9), 1448-1467. [doi:10.1080/10408398.2016.1263823].

Zhao Zhaohui, Z. Z., Saito, M., Yoshihashi, T., Nakahara, K. and Tatsumi, E. (2002). Microorganism control in packed tofu manufacture with electrolyzed water.

Zheng, L., Regenstein, J. M., Teng, F. and Li, Y. (2020). Tofu products: A review of their raw materials, processing conditions, and packaging. *Comprehensive Rev. in Food Sci. Food Safety* **19** (6), 3683-3714. [doi:10.1111/1541-4337.12640].

Appendices

Appendices A

Sensory analysis score card

Name of the panelist: Date:

Name of the product: Large cardamom and ginger extract incorporated tofu.

Dear panelist, you are provided with 9 samples of herbal extract incorporated tofu on each proportion with variation on extract concentration 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 slightly – 6 Dislike moderately – 3

Like very much – 8 Neither like nor dislike – 5 Dislike very much – 2

Like moderately – 7 Dislike slightly – 4 Dislike extremely – 1

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

Any comments:……

Signature

Appendix B

ANOVA table for different mean comparisons of tofu containing optimized large cardamom extract by varying proportion.

Table B.1 ANOVA table for mean comparisons of tofu containing optimized large cardamom extract by varying proportion.

		Sum of Squares	df	Mean Square	F	Sig.
Color and appearance	Between Groups	12.507	4	3.127	15.711	<0.001
	Within Groups	8.956	45	0.199		
	Total	21463	49			
Flavor	Between Groups	37.720	4	9.430	13.822	<0.001
	Within Groups	30.700	45	0.682		
	Total	68.420	49			
Body and texture	Between Groups	22.920	4	5.730	13.571	<0.001
	Within Groups	19.000	45	0.422		
	Total	41.920	49			
Overall acceptability	Between Groups	45.800	4	11.450	16.783	<0.001
	Within Groups	30.700	45	0.682		
	Total	76.500	49			

Tukey HSD analysis of tofu containing cardamom extract.

Table B.2 HSD test for color and appearance

Parameter		N	Subset for alpha=0.05	
			a	b
Tukey HSD[a]	D (0.8%)	10	6.7800	
	C (0.6%)	10		7.9000
	I	10		7.9000
	B (0.4%)	10		8.0000
	A (0.2%)	10		8.2000
	Sig.		1.000	0.566

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000

Table B.3 HSD test for flavor

Parameter		N	Subset for alpha=0.05		
			a	b	c
Tukey HSD[a]	D (0.8%)	10	5.3000		
	A (0.2%)	10	6.2000	6.2000	
	B (0.4%)	10	6.3000	6.3000	
	I	10		7.0000	7.0000
	C (0.6%)	10			7.9000
	Sig.		0.069	0.211	0.124

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table B.4 HSD test for Texture

Parameter		N	Subset for alpha=0.05	
			a	b
Tukey HSD[a]	D (0.8%)	10	6.2000	
	B (0.4%)	10	6.5000	
	C (0.6%)	10	6.6000	
	A (0.2%)	10		7.5000
	I	10		8.0000
	Sig.		0.646	0.432

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table B.5 HSD test for overall acceptability

Parameter		N	Subset for alpha =0.05			
			a	b	c	d
Tukey HSD[a]	D	10	5.3000			
	A	10	6.2000	6.2000		
	B	10		6.5000	6.5000	
	I	10			7.5000	7.5000
	C	10				8.0000
	Sig.		0.124	0.926	0.069	0.660

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix C

ANOVA table for different mean comparisons of tofu containing optimized ginger extract by varying proportion.

Table C.1 ANOVA table for mean comparisons of tofu containing optimized ginger extract by varying proportion.

		Sum of Squares	df	Mean Square	F	Sig.
Color and appearance	Between Groups	12.120	4	3..030	10.408	
	Within Groups	13.100	45	0.291		<0.001
	Total	25.220	49			
Flavor	Between Groups	26.080	4	6.520	13.583	
	Within Groups	21.600	45	0.480		<0.001
	Total	47.680	49			
Body and texture	Between Groups	24.480	4	6.120	20.864	
	Within Groups	13.200	45	0.293		<0.001
	Total	37.680	49			
Overall acceptability	Between Groups	28.880	4	7.220	22.407	
	Within Groups	14.500	45	0.322		<0.001
	Total	43.380	49			

Table C.2 HSD test for color and appearance

Parameter		N	Subset for alpha=0.05	
			a	b
Tukey HSD[a]	H (0.8%)	10	6.78000	
	F (0.4%)	10		7.8000
	G (0.6%)	10		7.8000
	I	10		7.9000
	E(0.2%)	10		8.1000
	Sig.		1.000	0.726

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table C.3 HSD test for flavor

Parameter		N	Subset for alpha=0.05		
			a	b	c
Tukey HSD[a]	E (0.8%)	10	6.2000		
	H (0.8%)	10	6.4000		
	I	10	7.0000	7.0000	
	G (0.6%)	10		7.8000	7.8000
	F (0.4%)	10			8.0000
	Sig.		0.091	0.091	0.967

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table C.4 HSD test for Texture

Parameter		N	Subset for alpha=0.05	
			a	b
Tukey HSD[a]	F (0.4%)	10	6.3000	
	H (0.8%)	10	6.5000	
	G (0.6%)	10	6.8000	
	E (0.2%)	10		7.7000
	I	10		8.1000
	Sig.		0.253	0.474

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table C.5 HSD test for overall acceptability

Parameter		N	Subset for alpha=0.05	
			a	b
Tukey HSD[a]	E (0.2%)	10	6.2000	
	H (0.8%)	10	6.4000	
	I	10		7.5000
	G (0.6%)	10		7.6000
	F (0.4%)	10		8.2000
	Sig.		0.933	0.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix D

Table for cost evaluation of herbal extract tofu

Table D.1 Cost evaluation of herbal extract tofu

Ingredients	Rate	Quantity	Cost (NRs) Cardamom tofu	Cost (NRs) Ginger tofu
Soybean	200/kg	200g	40	40
CaSo4	800/kg	6g	7.5	7.5
Large Cardamom	2200/kg	2g	4.4	
Ginger	140/kg	2g		0.28
Total cost			51.9	47.78
Final cost with 10% overhead			57.09	52.56
Product prepared			372.6g	360.4g
Cost per kg			153.22/kg	146.0/kg

Appendix E

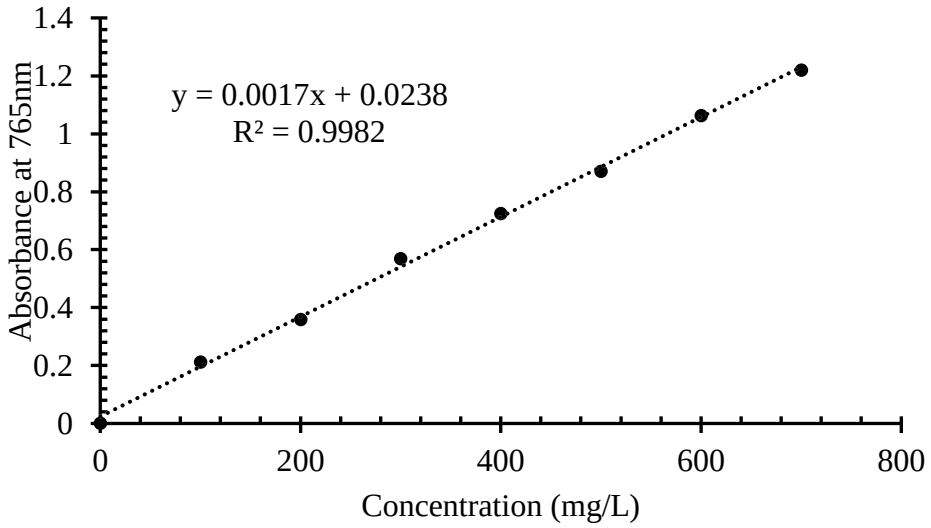

Fig E.1 Standard Gallic acid curve

Appendix F

A.1 Apparatus

- Pressing machine
- Muslin cloth
- Grinder
- Heating arrangement
- Thermometer
- Digital electronic balance
- Beaker
- Volumetric flask
- Measuring cylinder
- Conical flask, funnel, test tube
- Soxhlet assembly
- Bushner filter assembly
- Petriplate
- Hot air oven
- Filter paper
- Vacuum sealer
- Refrigerator
- Kjeldhal digestion and distillation set

- pH meter
- Bacterial incubator
- Rotary vacuum evaporator
- Colony counter
- Autoclave
- Water bath
- Petri plates
- Spectrophotometer
- Magnetic stirrer
- Muffle furnace

A.1 List of Chemical

- Petroleum ether
- Acetone
- Sulfuric acid
- Oxalic acid
- Hydrochloric acid
- Boric acid
- Catalyst mixture
- Plate count agar
- Phenolphthalein
- Acetic acid

- Ethanol
- Potato dextrose agar
- Starch
- Sodium thiosulphate
- Gallic acid
- DPPH
- Sodium carbonate
- Sodium hydroxide
- Disodium Hydrogen Phosphate
- Folin-Ciocalteau reagent
- Petroleum ether
- Methanol

Color plates

Plate 1 Tofu samples in LDPE

Plate 2 Tofu sample for sensory score

Plate 2 Kjeldhal distillation set

Plate 3 Concentration of herbs extract

Plate 5 Plating for microbial analysis.

Plate 6 Microbial counting by colony counter

Plate 7 Samples after 16 days.

Plate 8 Analysis of herbs extract.