A COMPARATIVE STUDY ON THE NUTRITIONAL CHARACTERIZATION OF COAGULANT ASSISTED TOFU

A thesis submitted in partial fulfillment of the requirement for the award of the degree

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Submitted by

Monika Konsam

(Exam Roll No: M4FTB22003)

Under the guidance of

Prof. Prasanta Kumar Biswas

Head of the Department

Department of Food Technology and Biochemical Engineering

FACULTY OF ENGINEERING & TECHNOLOGY

JADAVPUR UNIVERSITY

KOLKATA-700032

FACULTY OF ENGINEERING AND TECHNOLOGY

JADAVPUR UNIVERSITY

KOLKATA-700032

CERTIFICATE

This is to certify that the thesis entitled **"A Comparative Study on the Nutritional Characterization of Coagulant Assisted Tofu" submitted by Monika Konsam** of Registration No-156624 of 2020-2021 is in partial fulfillment of the requirements for the award of the degree of **Master Of Technology in Food Technology and Biochemical Engineering of Jadavpur University.**

This is an authentic work carried by her under our supervision and guidance. To the best of our knowledge, the matter embodied in the thesis has not been submitted to any other university/institute for the award of any degree.

Countersigned

……………………………….	………………………………………..
Prof. Prasanta Kumar Biswas	Dean, Faculty Of Engineering &
Supervisor and Head,	Technology,
Department of Food Technology and Biochemical	Jadavpur University,
Engineering, Jadavpur University,	Kolkata-700032
Kolkata-700032	

FACULTY OF ENGINEERING AND TECHNOLOGY

JADAVPUR UNIVERSITY

KOLKATA-700032

The foregoing thesis is hereby approved as a creditable study in **Master Of Technology In Food Technology and Biochemical Engineering** and presented in a manner satisfactory to warrant is acceptance as prerequisite to the degree for which it has been submitted. It is understood that by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion therein but approve this thesis only for the purpose for which it is submitted.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work by me as part of my Master of Technology in Food Technology and Biochemical Engineering studies.

All information in this document has been obtained and presented in accordance with academic rule and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

NAME : MONIKA KONSAM

Exam Roll Number : M4FTB22003

Registration Number : 156624 of 2020-2021

Thesis Title : A Comparative Study on the Nutritional Characterization of Coagulant Assisted Tofu

Signature with Date :

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ABSTRACT

Soybeans are known as the golden grain among all the grains due to their unique characteristics. It has a protein content of approximately 35–40% on a dry weight basis, with high quality amino acids and a lipid content of approximately 20%. It has the potential to overcome malnutrition caused by the deficiency of protein in most developing countries. It is not only rich in proteins but also rich in micronutrients as well as phytochemicals, for which it is considered a functional food. In recent decades, the demand for soy products has been increasing on a daily basis owing to its health-benefit properties. Tofu is one of the most highly demanded soy products, which is used as a substitute for meat. In the present study, tofu was developed using different coagulants, namely, citric acid, magnesium chloride, and calcium sulphate, along with varying concentrations in separate batches. The physico-chemical and sensory analyses were performed for each tofu sample. The study showed that tofu coagulated from calcium sulphate of 0.2% (w/v) obtained the highest protein content of 50.38% and the antioxidant content of TPC and TFC were 1.487 GAE mg/g and 0.299 CE mg/g, respectively, on a dry weight basis. It was also found that tofu coagulated with 0.15% (w/v) of citric acid had a protein content of 48.36%, which was the highest overall acceptability in terms of aroma, taste, and texture according to sensory analysis.

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ABBREVIATION

$MgCl_2$ - Magnesium Chloride

$MgSO_4$- Magnesium Sulphate

$CaSO_4$- Calcium Sulphate

$CaCl_2$- Calcium Chloride

CH_3COOCa- Calcium Acetate

TPC- Total phenolic Content

TFC- Total Flavonoid Content

GAE- Gallic Acid Equivalent

CE- Catechin Equivalent

NIN- National Institute of Nutrition

TVC- Total Viable Count

YMC- Yeast and Mould Count

PUFA- Polyunsaturated Fatty Acid

DB- Dry Weight Basis

WB- Wet Weight Basis

HCL-Hydrochloric Acid

$AlCl_3$- Aluminium Chloride

$NaNO_2$- Sodium Nitrite

H_2SO_4- Sulphuric acid

Na_2CO_3-Sodium Carbonate

CONTENTS

CHAPTER 1

INTRODUCTION

Soybean (*Glycine max*) is a dicotyledonous plant that belongs to the Fabacea family. It is believed that it originated from East Asia, particularly Northern China. It is presently consumed all over the world because of its versatile properties. It is suitable to grow in moist and warm climates and also prefers to grow in sand or loam soil.

Soyabean has a high protein content of 35-40% (db) with good quality of amino acids, as an alternative to animal protein, which resulted in gaining more attention than other crops, and also has a significant amount of carbohydrates of 25-30% (db), followed by dietary fibres, vitamins, minerals, and phytochemicals. It is one of the most important pulse crops for the production of edible oil since it contains 20% lipids, which contributes to some extent to the Indian agricultural economy. Besides, soy oil is considered a healthy oil since it contains the maximum amount of unsaturated fatty acids like omega 3 fatty acids, linoleic acid and linolenic acid (Adelakaun et al.,2012).

Table1: Nutritional profile comparison: soyabeans vs other pulses on dry weight basis (Jithender et al.,2020)

Type of pulses	Carbohydrates (g/100)	Fats (g/100g)	Protein (g/100g)	Calcium (mg/100g)	Energy (Kcal)
Soyabeans	20.9	19.5	40.2	240	432
Green gram	59.9	1.2	24.5	76	348
Lentils	59.0	0.7	25.1	69	343
Bengal gram	59.8	5.6	20.8	56	372
Dry peas	56.5	1.1	19.7	75	315

According to World Health Organisation and US Food and Drug Administration, a new approach has been established for assessing protein quality known as Protein digestibility corrected amino acid score (PDCAAS) on the basis of amino acids. The quality of plant based proteins differed from plant to plant. Among all the plants, soyabean has the highest protein quality where protein corrected digested amino score is 1.00, which is very close to animal proteins like meat, eggs and dairy products (Qin et al,2022). It has significant potential to overcome malnutrition caused by a lack of protein in the ever-growing Indian population due to its high protein content.

1.1 Globally production of soyabean

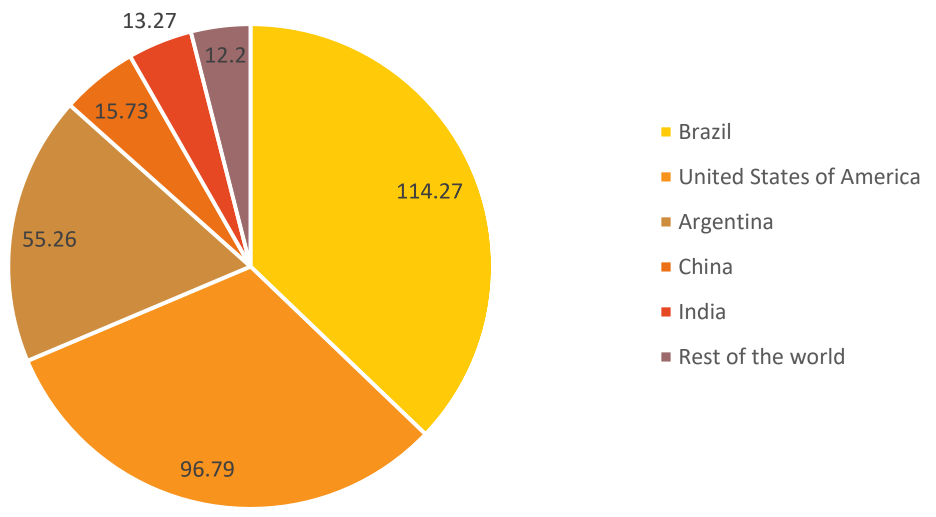

Fig 1: Pie chart showing global production of soyabean
(in million tonnes)

During 2019-2020 estimation, the global production of soyabeans has reached 333.67 million tonnes as compared with 2014-2015, which was estimated at 315 million tonnes. Brazil led the way with 34.25% of total production, followed by the United States with 29.01%, Argentina with 16.56%, China with 4%, India with 3.98%, and the rest of the world with the remaining However, despite being in fifth place in terms of production, India contributes 11.34 million hectares or 9.41% of the global area for the production of soyabean.

In India, Madhya Pradesh is the largest producer of soyabeans followed by Maharastra, Rajasthan ,Karnataka and Telangana.

1.2 Health benefits of soyabean

The most crucial part in today's hectic and pressured lifestyle is maintaining a healthy body and mind. So, having a proper balanced diet is very important to stay fit all the time. The food that we consume reflects the health status of our body. In India, being a developing country, there are difficulties in getting a proper balanced diet to all the people. According to NIN, 2/3 of the population is not getting a proper diet; 35% of people are living under the poverty line; 55% of children and 60–70% of women are suffering from malnutrition and anaemia respectively. Among all the pulses, soyabeans have the potential to solve some of this problem.

Soybeans, as a part of a regular diet, have earned worldwide recognition as a functional food that helps to protect against various diseases. Functional foods are those foods which contain naturally occurring bioactive substances. In addition to the typical nutritional value of food, they have the potential to promote good health, but their absence does not cause any deterioration in normal body metabolism. Soyabeans contain bioactive compounds such as saponin, phenolics, trypsin inhibitors, flavonoids etc. Isoflavones, a subclass of flavonoids, play a huge role in attracting people's attention towards soyabeans. The major isoflavones present in soyabeans are glycitein, genistein, and daidzein. Isoflavones are also considered as phytoestrogens because they resemble the structure and activity of estrogen, which helps to cure osteoporosis, which occurs after menopause by preventing bone reabsorption (Hossein Jooyandeh,2011). Many researchers claim that the isoflavones present in soyabeans have the potential to lower the risk of various types of cancer, such as lungs, prostate, breast, colon etc. Soyabean isoflavones have more diadzein than geneistein, but genistein has captured more attention for its anticancer effects in a variety of ways, including inhibiting the enzymes that promote cancer, interfering with hormone action, and blocking the chain that feeds tumors (Riaz et al., 1999; and Broihier et al., 1997).

According to epidemiological studies, Eastern cultures who consume soy on a daily basis have a lower chance of cardiovascular disease and chronic diseases, including low density lipoprotein and total cholesterol. It was reported that patients with hyperlipidemia who consumed soy protein with isoflavones were found to have a decreased blood cholesterol level (Ho et al.,2000:Nagata et al.,1998). As per the Food and Drug administration, person who consume a diet which contains soy proteins have the potential to reduce coronary heart diseases. However, in order to get the advantages of soy proteins, the FDA mentioned that a person should incorporate no less than 25 gm of soya proteins as their daily intake in their diets. Many researchers have reported that the consumption of soy protein around 50gm on their daily intake not only keep healthy but also help to lowers the risk of chronic diseases . Around 33% of Americans liked soy-based products, and 31% are very concerned about the health benefits of soy in their meals, which include controlling obesity (31%), lowering the number of heart attacks and strokes (27%), and certain types of cancer (20%).

1.3 Antinutritional factors present in soyabean

Soyabean are an excellent source of proteins and other nutrients, but they also contain antinutritional factors, which restricts the uptake of nutrients in human body leading to deficiencies of nutrients. Trypsin inhibitors is considered as the major antinutritional factors found in soyabeans, which inhibit the trypsin enzymes from breaking the peptides bonds between the amino acid lysine and arginine. They are the proteins that inhibit the activity of trypsin in the gut and also interfere with other certain proteins. Consumption of untreated soyabean can cause problems in our body, yet they are heat sensitive. Antinutritional factors can be removed by using traditional method of cooking like pressure cooking, fermentation, dehusking, milling or autoclaving at 120^0C for 15-30mins can completely destroy antinutritional factors(Anderson and wolf,1995).Saponins, tannins, phytic acids are also present in soyabeans as antinutritional factors. Tannins are polyphenolic compounds present in the seed coat of soyabeans.They hinder iron and B-vitamin absorption as well as bind to proteins, rendering them unavailable and interfering with the digestive proteins trypsin and α-amylose, resulting in indigestibility of protein trypsin and carbohydrates. Removing seed coat is the only method to eliminate tannins from the soyabean (Liener et al,1994). Saponins are glycosides compounds with a frothy texture that are sometimes referred to as natural detergents.It causes nausea and vomiting and even can lead to haemolysis of RBCs. However saponins being a antinutritional factors it posseses many health benefits, one of them are it helps to lower blood cholesterol by preventing from being reabsorbed and so boosting its excretion. Phytic acid is the important source of phosphorus in soyabeans. However, it can combine with certain metals like iron, zinc, calcium, and magnesium in the digestive tract which makes them unavailable, resulting in multimineral deficiencies in the body. On the contrary, they possess antioxidant as well as anticancer properties (Liener et al,1994).Modern technology advancement such as fractionation ,extraction ,ultrafiltration or extrusion has modulated this antinutritional factors modulated into functional foods and nutraceuticals.

1.4 Role of soyabeans in the Food Processing Sector

Soyabeans has occupied a prominent space in the food processing sector because of its versatility in terms of availability, low cost and nutritional quality. Soyabean has been used to make a variety of food products. Generally, soy products are classified as fermented and non-fermented products. Most commonly consumed fermented soyabean products are,miso,natto, soya sauces and tempeh. Fermentation helps to reduce the beany flavour of soyabean that mainly leads to an objection but not only that it also minimise the antinutritional factors which enhance its nutritional value up to the mark. Non fermented soyabean products including full fat soy flour, medium fat soy flour, soy nuts, soy noodles, fortified soy biscuits, soymilk, tofu(unfermented), soy based sweets etc.

Soy products has been used as an alternatives of traditional dairy products over the past few decades. It became one of the best option for those who are lactose intolerance as well as for vegans as they are lactose free as well as good sources of highly digestible proteins. Soymilk, soy cheese, soy yoghurt, soya cream, flavoured soymilk, fortified soymilk,soya panner(tofu), are easily available in the market. Soy protein is also used as an ingredients to stabilize the emulsification process in the bakery products like cakes, cookies, breads, pies etc. resulting in enhancement of overall quality of the product (Hossein.,2011). Soy products plays a vital role in substituting meat and meat products as it is used as low cost meat with good quality protein as an alternatives which also help to reduce the cardiovascular diseases which resulted from the long term consumption of red meat .In the meat industry, soy protein is used as hydrating the meat products as well as to bind and emulsifies which makes the meat products more juicy and also overall enhance the palatability of the meat products .Textured soy protein, which is prepared by removing the fats from the soy flour also known as by products for the extraction of soyabean oil followed by extrusion process resulting in variety of sizes and shapes is used as meat extenders. It has similar texture with ground or minced meat so it is used as a meat analogue in vegan’s foods like patties, sausages, urgers etc.(Gnansambandam and Zayas,1994; Hossein.,2011).

1.5 Soymilk

As health concerns among consumers increase, the demand for healthy and nutritious food products also increases. Soymilk is one of the healthiest beverages and is widely consumed all over the world as a substitute for animal milk. It is also rich in bioactive compounds, which help in the reduction of certain diseases, and also rich in vitamins, minerals, and healthy fats. It is a good option for those who have problems with lactose intolerance. Soymilk is mainly prepared by soaking the soyabean seeds overnight, blanching the soaked beans, grinding them with water, filtering using muslin cloth to remove insoluble residue called okara, heating the soymilk, fortification, and packaging. However, the technologies and processing parameters in each step have a greater impact on the nutritional quality, such as soy protein recovery and removal of antinutritional factors, of soymilk and its derived products (Giri & Mangaraj, 2012).

Table 2: Nutritional composition in one cup serving of soymilk (Bahareh Hajirostamloo,2009)

Protein	6.73g
Carbohydrates	4.43g
Fat	4.67g
Lactose	0
Phosphorous	120.1mg
Iron	1.4mg
Calcium	9.8mg
TSS	10.4 g
pH	6.74
Acidity	0.24%
Fatty acids	0.52g
Fibre	3.2g
Energy	79kcal

1.6 Tofu (Soy-paneer)

Tofu, also known as soy paneer, is one of the most popular soyabean products, which is derived from soymilk and widely consumed all over the world, including both developed and developing countries. It is known as "meat without bones," which is considered a great alternative to animal protein for vegan consumers. It has an excellent source of proteins with all the required amino acids, polyunsaturated fatty acids like omega 3 fatty acids, vitamins, including fat soluble and water soluble vitamins, minerals, etc. It is prepared by adding coagulants to hot soya milk and then pressing the resulting curd into solid white blocks. The final quality and yield of tofu products may be affected by the different types of coagulant and the processing parameters. Basically, there are three types of coagulant, namely, acid, salt, and enzyme coagulant, which induce gelation. It has a generally higher moisture content of 70 to 90% compared to milk paneer, which is 40 to 50%. Hence, it is also known as "hydrated gel food." However, due to its high nutrient content and relatively high pH, which typically ranges from 5-7, it is perishable owing to microbe infection. Tofu is classified according to the moisture content present in it as well as processing parameters (Shurtleff et al., 2013). Types of tofu are given as below:

Hard tofu (firm): The hard tofu is prepared by the addition of an appropriate coagulant with an appropriate concentration in hot soymilk and subjected to a temperature of 75^0C to 85 ^{0}C, which leads to gelation followed by the pressing of the unbroken soybean curd using suitable pressure. The hard tofu has around 70-80% of the moisture content. It has a compact 3-D network, which results in a hard texture and also imparts a strong beany flavour compared to other types. Firm tofu can be smoked and seasoned to enhance the flavour, stir-fried, or stewed in soup.

Soft tofu: It is prepared with the same procedure as hard tofu but with a slight change in the processing parameters. The soft tofu has a higher moisture content of around 90% and has a similar texture to soft cheese. However, it has the ability to hold its shape after cutting and has less beany flavour. It blends with smoothies, pudding, salad dressings, etc.

Packed tofu: The preparation of packed tofu is different from that of hard and soft tofu. After the addition of suitable coagulant with an appropriate concentration to hot soymilk, it is subjected to a low temperature of around 40 ^{0}C, which leads to gelation and transfer to a pouch pack for moulding without giving any pressure. It has a moisture content of around 90% and produces the highest yield because the chances of losing proteins that are soluble in water are minimal. It has a similar texture to soft cheese and also has a very light beany flavour.

AIM AND OBJECTIVES

The aim of the present study was to characterized the nutritional properties of tofu coagulated using different coagulants.

The study has been subdivided into following objectives:

- To prepare tofu using different coagulants with varying ratios.
- To analyse the physico-chemical properties of prepared tofu (using different coagulants).
- To identify which tofu coagulant has better nutritional retention quality along with higher sensory attributes and overall acceptability .

CHAPTER 2

REVIEW OF LITERATURE

Soyabean seeds are well-known for their high protein content (35–40%) as well as other nutritional properties. Soyabean protein is considered a complete protein with a good amino acid composition, and it is highly sought after in the food industry. This high protein content has resulted in the production of soymilk and its products.

2.2 Effect of processing factors on the quality of soymilk

- Ikya et al.,2013 analyzed the impacts of varying cooking temperature(80^{0}C, 90^{0}C,100^{0}C and 110^{0}C) on the quality of soymilk. The study revealed that with the increase in temperature, the nutritional composition of soy milk also increases except the fat and moisture content. The Protein, carbohydrates, fibre, and ash content of soymilk heated at temperature 110^{0}C were 3.5%,2.41%,1.58%, and 0.58% respectively whereas soymilk heated at 80^{0}C were 3.40%,1.3%.0.45% and 0.53%. In addition ,With the increase in temperature leads to decrease in microbial load .The results showed that soymilk heated at 80^{0}C had the highest microbial load of 2.3×10^3(TVC) and 1.2×10^2 (YMC) whereas soymilk heated 110^{0}C obtained the lowest microbial load of 1.4×10^3(TVC) and 0.3×10^2(YMC).However , most of panelist did not like the soymilk heated at very high temperature i.e. 110^{0}C because deeper colour intensity which occurred due to maillard reaction as well development of new off flavours. Among all the samples, soymilk heated at 100^{0}C secured the highest acceptability from the sensory analysis as well as in terms of nutritional composition.
- Tripathi et al.,(2015) investigated a study to determine the best extraction method of soymilk in terms of quality and reduction of beany flavour by using different methods, namely, traditional oriental method (TOM) or cold water grinding method, deodorization method (DOM), rapid hydration hydrothermal cooking method (RHHCM), airless grinding method (AGM), Illinoi method (IM) and hot water grinding method (HWGM). The findings revealed that the soymilk extracted through TOM obtained a higher solid content of 8% but less in conductivity at 3.03 Ms. The reason could be improper solubilization during grinding. The highest moisture content was obtained in soymilk extracted through HWGM and AGM, with the same value of 90.50%, but the lowest in TOM (89.20%). Furthermore, the highest pH was observed

in soymilk extracted through IM, which could be due to the presence of Na_2CO_3 during cooking. Soymilk Lipoxygenase is an enzyme that imparts a beany flavour to soymilk, which decreases its acceptability but susceptible to high temperatures and pH. The highest protein and fat recovery was found in IM and AGM with a value of 3.29%, 3.12%, 2.36%, and 2.39%, respectively. However, IM extracted soymilk recovered less carbohydrates content with 2.46% compared to other extraction methods due to prolonged soaking and cooking at various stages, which might be due to hydrolyzation of carbohydrates. The improper inactivation of lipoxygenase enzyme provides a conducive environment for the production of hydroperoxide in the presence of air, water, and PUFA, soymilk extracted using TOM had the strongest beany flavor compared to others, which simply means the higher the lipoxygenase enzyme content, the higher the hydroperoxide. DM and IM obtained the lowest hydroperoxide values. The highest overall acceptability was found in soymilk extracted through the deodourization method and IM because of their less beany flavour as well as the highest nutritional value.

- Proper heating of soymilk is necessary to improve the quality of soymilk as well as inactivate antinutritional factors. Okara is an insoluble residual material produced as a by-product during the extraction of soymilk. It is high in fibre and protein content due to the leaching of insoluble denatured protein fraction. Varghese and Pare, (2019) conducted a study to determine the efficiency of microwave assisted extraction (MAE) on the yield and protein quality of soymilk compared to the conventional method of heating using different processing parameters. MAE was designed in such a way that grinding and heating occurred in a continuous manner. The results of the findings showed that the yield of MAE soymilk obtained was 4.86kg from 1 kg of soyabean compared to that of 3.86kg from 1kg of soyabean conventional heating method (steam infusion), which resulted in a decrease in production of okara. The reason might be that solublisation of the cell wall of soyabean occurs due to microwave heating, which results in a decrease in the weight of okara. The protein solubility of MAE soymilk was higher at a rate of 39.1% than the conventional method because it took a long time to reach a certain temperature compared to microwave heating, which resulted in an increase in protein denaturation, which means more hydrophobicity amino acids were formed, which led to a decrease in protein solubility of soymilk. The temperature (70^0C), power level (540W) and stirring speed (140rpm) were found to have the highest protein solubility of 25.5%. Furthermore, trypsin inhibitory is an enzyme which interferes with the activity of trypsin. On the contrary to protein solubility, protein digestibility increases with an increase in temperature. The reason behind this protein digestibility is correlated with the activity of the trypsin inhibitor enzyme. The higher the temperature, the more the trypsin inhibitor. At 810W and 90^0C, trypsin inhibitor activity was found to be highest at 18% and lowest at 3% at 540W and 70^0C. The study concluded that the optimized process parameters were found to be 80, 674W, and 160 rpm in response to protein solubility (18.2%), protein digestibility (82.8%), and TIA (13.8%) led to maximum desirability of 0.95.

2.2 Okara

- Okara is an insoluble material produced as a by-product during the production of soymilk. As reported by Santos et al., (2019) 1.2kg of okara on a weight basis was produced from 1kg of soyabean during the production of soymilk, which will lead to an economic loss in the processing of soyabean industry. The nutritional composition of okara, such as protein, carbs, lipids, dietary fibre, insoluble dietary fibre, and soluble dietary fibre, was 15.2% to 33.4%, 3.8% to 5.3%, 8.3% to 10.9%, 40.2% to 58.1, 40.2% to 50.8%, and 4.2 to 14.6 respectively (Vong and Liu,2016). The nutritional composition may vary according to differences in processing parameters. Despite its high nutritional value, it is considered waste due to its higher moisture content, which causes perishability, the presence of insoluble dietary fibre, which causes indigestion, and the presence of PUFA, which causes off-odor compounds to be produced by the action of the lipoxygenase enzyme (Vong and Liu,2019).

- Many researchers have developed innovative methods for converting waste into value-added products that can be used as ingredients or additives in the food industry. One of the methods is bio-valourisation, which is the transformation of resulting foods into value-added products by microorganisms (bacteria, fungi, and yeast). Thereby reducing the production of waste, it leads to a decrease in environmental pollution. As reported by Liu et al.,(2016) the fermentation caused by Fungus on the okara has the capacity to convert the fibre content in okara into oligosascharides having a low molecular weight and release free antioxidants, which act as a synergist between the polysaccharides of fungi and components of bioactive compounds present in okara.

- Meitauza is a famous food in China, prepared from fermented okara. Xu, Liu, and Zhang, (2012) evaluated the quality of meitauza by inncoulation of pure culture of *A.mucor* and *Z.mobilis.* The findings revealed that fermentation caused by these above mentioned fungi leads to a reduction in moisture content as well as crude protein content because the nitrogen source was used up during fermentation. In addition, the amount of free amino acids was increased, which gave a strong as well as sweetening taste.
- Ostermann-Porcel et al., (2017) developed a gluten-free cookie using okara flour as an ingredient with a different concentration separately, whereas manioc flour was used as a control to compare its nutritional composition. It was obtained that the cookies developed from 50% of okara flour resulted in higher protein, fibre, and fat with a value of 12.84%, 3.93, and 22.10 than that of the control, which had 3.26% of protein, 15.40% of fat, and no fibre content. However, it was noticed that the spread factor decreases with an increase in okara content on account of the high protein content in okara. Moreover, the specific volume of cookies decreased with increases in the incorporation of okara, which might be changes in the structure due to its high fibre content as well as lower retention of gas in the dough. Although cookies developed from 15% of okara flour were deemed the most desirable compared to the others, according to the opinion given by the panelist from the sensory analysis.

2.3 Role of Coagulation in the production of tofu.

when soymilk is subjected to heat treatment, denaturation occurs which resulted in the repulsion of protein particles. It can be neutralised with the help of coagulant by lowering electrostatic repulsion between the particles of proteins molecules. Following that, aggregation occurs through hydrophobic interaction which resulted in the development of tofu (Guo and Ono et al.,2005). As a result, coagulation is the most important step in the processing of tofu where an appropriate coagulant with an appropriate concentration gives the proper effect of salt ions and H^+ions in the processing. Mainly there are three types of coagulant namely,acid coagulant,salt coagulant and enzymatic coagulant.

- Traditionally,tofu is mainly prepared from salt coagulant such as calcium and magnesium salts. Prabhakarn et al.,(2006) investigated a comparison study to check the physical properties and quality of tofu which were prepared from different types of salt coagulants such as salts of Ca^{2+} and Mg^{2+}. The study discovered that tofu prepared with magnesium sulphate had the highest water content, resulting in a decrease in textural hardness. The reason could be due to incomplete precipitation of soyabean, which results in a loose network structure of protein, but the amount of isoflavones obtained in this tofu was highest due to the release of less whey. Tofu prepared from calcium acetate obtained the highest textural properties such as hardness, chewiness and gumminess, which might be due to the development of strong hydrogen bond and calcium ion protein bridge. However, it was also reported that tofu prepared from calcium sulphate was better compared to the other mentioned coagulants in terms of yield, firm and smooth texture, as well as isoflavone content.

- Under the acid coagulants, all the organic acids are included, such as citric acid, tartaric acid, gluoconic, malic acid, etc. Glucono delta lactone has been commonly used for the preparation of packed tofu. Each acid coagulant has different pH values to induce gelation of thermally denatured proteins of soyabean. Grygorczyk and Corredig (2013) reported a comparison of gelation rates induced by lactic acid bacteria (LAB) and glucono delta lactone (GDL). It showed that gelation induced by LAB has a higher pH value of 6.3 and 5.9 than that of GDL acidification. Cao et al. (2017) investigated a study using acid coagulants such as citric acid, malic acid, and tartaric acid with a variant concentration of 0.12 g/100 ml to 0.18 g/100 ml and acidification with a pH value range of 5.25 to 5.65. It was found that higher concentrations of acid coagulant led to a decrease in hardness as well as water holding capacity. However, tofu prepared with 0.14% concentration acts as an optimum concentration of each acid coagulant, which showed the highest record in terms of texture and water holding capacity

- Karim et al. (1998) conducted a study in which carrageenan was used as an additive with different concentrations in tofu prepared with different coagulants such as $CaSO_4$, CH3COOCa, and GDL. Tofu coagulated with GDL obtained a higher moisture content than $CaSO_4$ and CH3COOCa. It was observed that the moisture content of $CaSO_4$ and CH_3COOCa coagulated tofu was increased when treated with carrageenan, which led to the increase in the yield, but there were no significant differences in GDL. The reason could be due to entrapment of water in the void space of the gel network formed by the interaction of protein and carrageenan with the calcium ions. However, this carrageenan and ionic effect were absent in GDL coagulated tofu. As a result, treatment with carrageenan helped in the reduction of syneresis and resulted in an increased yield.

Textural properties such as hardness, springiness, cohesiveness, and chewiness of $CaSO_4$ and CH3COOCa coagulated tofu were significantly reduced when treated with carrageenan, but there was no significant difference in GDL. All the tofu samples were yellow to light yellow in colour, but the tofu coagulated with CH_3COOCa and carrageenan were lighter, redder and yellower in colour, with a value of 84.47, 0.12, and 14.9 respectively.

- Different coagulants have different impacts on the recovery of nutritional composition from the tofu. Shokunbi et al. (2011) investigated a study using different coagulants such as $CaCl_2$, $MgCl_2$, CaSO4, $MgSO_4$, alum and Pap steep water to check the recovery percentage of nutritional and antinutritional composition present in tofu. They found that $MgSO_4$ induced tofu recovered the highest percentage of protein, fibre, ash, and moisture content on a dry basis of 67.31, 9.75, 8.80, and 77.93, respectively, whereas $CaSO_4$ and $CaCl_2$ induced tofu recovered the highest percentage of fat and carbohydrates, with 12.28% and 16.39%, respectively. In addition, $MgSO_4$ induced tofu recovered a lesser amount of antinutritional composition on a dry weight basis, such as phytate and oxalate with 0.66 g and 0.34 in 100 g, respectively, and trypsin inhibitor with 1.45 TIU per mg of protein. Shoukunbi et al. (2011) also reported that $MgSO_4$ coagulated tofu had a good amount of minerals as well as vitamins such as vitamin E, vitamin B1, vitamin B2, and carotene with 6.44, 3.02, 0.31, and 278.04 mg in 100 g of tofu, respectively. Fortunately, arsenic, cadmium, and lead were not detected in any of the coagulated tofu from the mentioned coagulants.
- Over the past decades, synthetic coagulants have been used for the preparation of tofu. However, synthetic coagulants are not always available everywhere, such as in rural areas and all the time in households. Many researchers have started to investigate natural coagulants. Sanjay et al.,(2008) conducted an experiment using natural coagulants to compare with synthetic coagulants to check their respective proximate values. They found that tofu prepared with *Garcinia inidica* recovered the highest protein and fat content of 61.7% and 26.3%, respectively, compared to synthetic coagulant with that of 56.3% and 23.3%. The study showed that natural coagulant can be used as a substitute for synthetic coagulant.

2.4 Effect of processing parameters on the quality of tofu

- Rekha and Vijayalakshmi,(2013) reported that processing parameters have a greater impact on the overall quality of tofu. They found that the duration of blanching of soy beans ,solid content of soymilk, stirring time, pressure and duration of pressure reflect the texture and yield of tofu. Blanching of soyabeans more than 15 minutes results in the soft texture but not firm and also help in the reduction of beany flavour. Soymilk with a solid content of 9^0 brix results in a result harder in texture (6.00 N) as compared to a solid content of 7^0 brix (4.39 N). The reason behind this could be that higher solid content results in the lower retention of water in tofu, resulting in a harder texture. Moreover, stirring times longer than 20s resulted in a reduction of yield, whereas applying the weight of 500g used for moulding for 30 mins results in a higher yield of 22.6g/100ml of soymilk with a smoother in texture than that of 1 kg, which results in a less yield of 22.3 with a hard texture.

- Among all the processing parameters, coagulation temperature plays a crucial role in determining its textural properties. Mathare et al.,(2009) conducted a study to find out the optimal temperature for coagulation by using $CaSO_4$ as a coagulant. Soymilk was heated at different temperatures, from 85 to 95^0C, separately. The findings revealed that the hardness of the tofu increased when the temperature reached 80 °C. However, when the temperature reached 90°C, the hardness decreased. The reason could be due to changes in the formation of networks between protein molecules. Furthermore, cohesiveness, adhesiveness, and chewiness were increased in temperature but decreased when the temperature reached 95 ^{0}C, but the springiness of tofu increased when the temperature reached 80 to 85^0C but remained constant at 90^0C. In addition, the study also claimed that an increase in temperature increases the yield. From the study, it was concluded that 85 to 90 ^{0}C was the optimal temperature for coagulation for the production of tofu.

- Soybeans are well known for their phytochemical content. Phenolics and flavonoids present in soyabeans mainly act as antioxidants. Many researchers have claimed that incorporating soyabean and its products into daily meals has the potential to reduce cholesterol levels, lower cardiovascular diseases, and types of cancer. Mujic et al. (2011) analyzed the total phenolic content and isoflavone content for five Croatian varieties of soyabean procured from different locations. The study discovered that the amount of TPC and isoflavones present in the soyabean differed from variety to variety. They obtained a TPC value that ranged from 0.87 to 2.16 mg GAE in 1 gram of soybean, whereas total isoflavones ranged from 0.81 to 2.13 mg in 1 gram of soybean. However, according to Kumar et al. (2010), the amount of TPC present in the varieties of Indian yellow soyabean ranged from 1.04 to 1.54 mg GAE in 1 gram of soyabean.

- Rekha and Vijayalakshmi ,(2009) conducted a study to check the effect of natural and synthetic coagulants on the recovery of isoflavones and antioxidant activity on tofu. They found that the tofu coagulated with natural coagulant (garcinia extract) obtained higher antioxidant activity (82.1%) compared to synthetic coagulant (63.1%).The difference in the antioxidant activity between natural coagulant and synthetic coagulant might be due to the higher polyphenol content in the natural coagulant. Furthermore, the compounds of isoflavones, namely, daidzein and geneistein (glucosides) and daidzin and genstin (aglycone) were identified and separated with the help of HPLC. It was observed that there were no significant differences in the recovery of glycosidic and aglyconic isoflavones in between tofu coagulated with synthetic and natural coagulants, with a range of 0.31 to 0.47 and 0.31 to 0.43 mg in 1 gram of tofu.

2.5 Sprouted and fermented tofu

- Sprouting is a germination process that occurs when the seeds are hydrated for hours until the sprouts begin to appear. Many researchers have claimed that sprouted seeds have better retention of nutrients and fewer antinutritional factors that create various hindrances in the metabolism of our bodies. Sprouted tofu is also widely consumed in many parts of the country. Ojha et al.,(2014) conducted a study to compare the nutritional value as well as yield of sprouted tofu (ST), regular tofu (RT), and tofu prepared from a combination of sprouted and unsprouted seed (CT). The findings showed variation in the physicochemical properties of all types of prepared tofu. The highest moisture content (67.9%) and crude protein (44.7%) were observed in sprouted tofu due to the breaking down of complex molecules of protein into simpler form. However, the fat and carbohydrate content in ST were less as compared to the RT and CT, which might be due to the use up of fat energy as well as to the -amylase enzyme breaking the complex carbs into simpler forms which are used as nutrients during germination. The crude fibre content was observed more in ST and CT as compared to

RT with a value of 11.98%, 9.78%, and 6.78%, respectively, because during sprouting, the synthesizing of structural carbohydrates might be increased. Furthermore, the highest yield was obtained in RT (42.33%) than in CT (37.69%) and (39.7%) on account of losing dry matter during sprouting.

- Fermentation is a bioconversion process in which sugar molecules are converted into C_2H_5OH and CO_2 in the presence of a microorganism but in an anaerobic condition. It has been used to preserve food since time immemorial. Fermentation helps to release the locked nutrients from the cell wall in food, thereby increasing nutritional properties. Wang et al.,(2020) examined a study to determine the overall quality of tofu using lactic acid bacteria (Lab) along with different coagulant of chlorides and sulphates of magnesium and calcium. The tofu was prepared by the inoculation of 4% culture of *Lactobacillus casei* in the soymilk and allowed to ferment for 5hrs at 37^0C followed by homogenisation at 750 rpm for one minute. The coagulants were then added separately and heated for 20 minutes to coagulate before being moulded with pressing.The findings revealed that yields and WHC were not much different among the *L.casei* – coagulants tofu but observed differences on textural properties of tofus. *L.casei* with $MgCl_2$ and $MgSO_4$ tofu has higher hardness with 410.87g and 394.72g respectively than salts of calcium with *L.casei.* The reason might be due to calcium salts has lower rate coagulation compared to magnesium salts. Fermentation leads to development of flavour in tofu, as a result fermented tofu are also known as flavoured tofu. The volatile compounds which are mainly responsible for imparting the flavours were dipentylfuran, hexanal, 1,3 octenol and 1-hexanol formed the degradation of PUFA. In the present study , the highest amount of hexanal,1.3 octenol and 1-hexanol were detected in *L.casei.*and $MgSO_4$ with 7.21%,13.05% and 20.90% respectively and 2-pentyl furan in *L.casei* and $CaCl_2$ with 16.63 % . The overall acceptability was higher in tofu prepared with *L.casei.*and $MgSO_4$ on account of its highest content main volatile flavouring compounds.

2.6 Shelf life of tofu

- Tofu is a highly perishable food and prone to microbial contamination due to its high water content and its highly nutritious nature. As a result, tofu has a very short shelf life. Tuitemwong and Fung,(1991) reported that tofu will spoil within 1-3 days only without refrigeration or any kind preservatives. Rahman et al.,(2013) analyzed the shelf life of tofu which were treated with chemical preservatives such as potassium sorbate ,potassium metabisulphite and sodium benzoate.Then the treated tofu samples were kept at room temperature as well in refrigeration condition in order to check the storage condition .It was observed that tofu samples kept at room temperature were able to store upto 2days only while the samples kept in refrigerated condition were able to store up to 14 days.
- Many researchers have postulated the use of natural preservatives in food to improve the nutritional quality and shelf life extension. No and Meyer,(2004) investigated the capability of chitosan as an coagulant in order to examined the shelf life of tofu.1% acetic acid was used as a dissolving solvent for 1% chitosan. Chitosan solution and soymilk were taken in the ratio 1:8 ,80 C^0 and 15 min was used as coagulating temperature and tide respectively for the preparation of tofu .The findings revealed that chitosan coagulated tofu extended the self life upto 3 days as compared to that of $CaCl_2$ coagulated tofu and also it had little effect on the characteristics (yield and hardness) and whey (volume and turbidity) of tofu.

CHAPTER 3

MATERIALS AND METHODS

3.1 PRODUCT DEVELOPMENT

3. 1.1 Raw materials:

Soyabeans were procured from the station market of Jadavpur,South- Kolkata,India.

3.1.2 Chemicals:

Citric acid, magnesium chloride, and calcium sulphate were used as coagulants in the production of tofu. All the chemicals used in the present study were analytical grade and purchased from Merck Life Science Private Ltd., Mumbai.

3.1.3 Preparation of soymilk and tofu:

Soyabeans were cleaned and soaked in water at room temperature for overnight. The hydrated beans were drained, rinsed and blanched at 85^0C for 10 minutes in order to reduce the beany flavour to some extent. The blanched soyabeans were grounded with water in a ratio of 1:9 by using a bajaj mixer grinder. The slurry was filtered through double-layered muslin cloth in order to separate the insoluble residue called okara. Soymilk was ready to be used for further steps.

For the preparation of tofu, the obtained soymilk was heated with constant stirring by using mantle heater. After reaching 85°C, different concentrations of citric acid (0.15% w/v and 0.2% w/v), magnesium chloride (0.2% w/v), and calcium sulphate (0.2% w/v) were added as coagulants in separate batches and stirred for 5 seconds. The coagulated soymilk was left to rest for about 15 minutes and then transferred into a muslin cloth lined wooden mould. The curd was pressed with 1000 g initially for 30 min, followed by 500 g for 30 min. After that, the tofu was removed from the mould and soaked in cold water for 30 min for further analysis.

Fig 2:Steps of production of tofu; a)overnight soaked beans

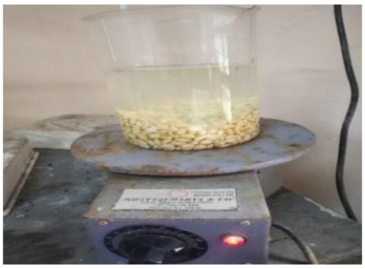

Fig 2: b)Blanching

Fig 2: c) Grinding

Fig 2: d) Filtration of soymilk

Fig 2: e) Coagulation of soymilk

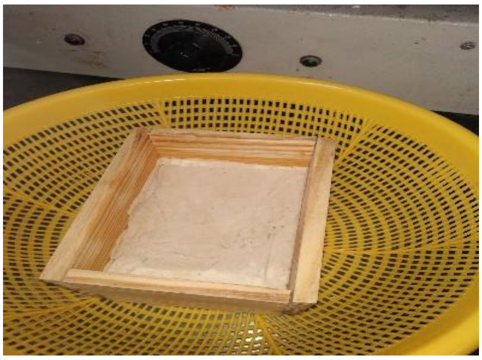

Fig 2: f) Moulding

Fig 2: g) Fresh tofu

Fig 2: h) Soaking in cold water for 30 min

Fig 2: i) slices of tofu

3.2.PHYSICO-CHEMICAL ANALYSIS

3.2.1 Moisture Content:

Moisture content of tofu samples were determined by using wensar moisture analayzer. 2g of each samples were taken in a pre weighed metallic dishes and kept in the analyzer at 105^{0}C. Samples were kept in the analyzer until constant weight and total moisture content was displayed on the screen.

Fig 3: Wensar Moisture Analyzer

3.2.2 pH measurement

pH of the of each samples were recorded by using systonic auto pHmeter. Commercially prepared buffer pH 4.01 and buffer pH 9.12 were used for calibration..

Fig 4: Systonic Auto pH meter

3.2.3 Texture analysis

Texture analysis of tofu was done according to the method followed by Zhan et al.,(2013) with slight modification using Instron (TA.XT Plus Texture Analyzer,USA).The samples were cut into cube of 1.5×1.5×1.5 cm by using sharp knife and compressed twice 50% deformation using p/5 probe with trigger force 5g, pretest speed 5.0mm/s, test speed1.0mm/s and post test speed 5 mm/s. Hardness, springiness, gumminess and chewiness were determined.

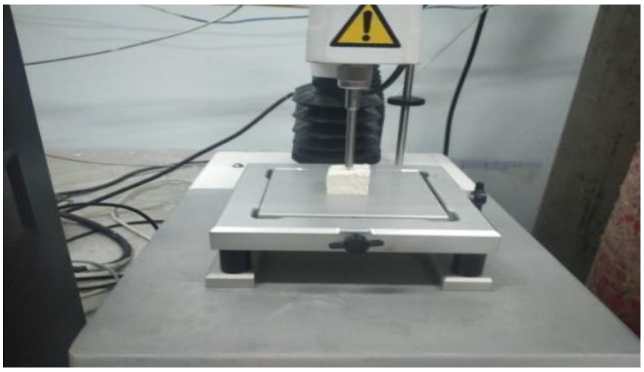

Fig 5 : Texture Analyzer

3.2.4 Colour

The color profile of the samples were analyzed by using Hunter Lab color measure system .A white standard plate of 3.5 cm (L*=93.49; a*=-1.07;b*=1.06) was used for calibration. The results were expressed in L* lightness (0 yields black and100 indicates white); a* redness (negative values indicate green and positive value indicates red) and b* yellowness (negative values indicate blue and positive value indicate yellow values).

Fig 6 : Hunter Lab Colorimeter

3.2.6 Total Carbohydrate estimation

3.2.6.1.Method: Phenol Sulphuric acid method (Tamboli *et al*.,2020)

3.2.6.2 Materials :

- 5% Phenol : 5 ml of phenol is diluted with 95 ml of distilled water
- Sulphuric acid 96% reagent grade
- Glucose was used as a standard

3.2.6.3 Procedure:

100 mg of sample was taken into a boiling tube and hydrolyzed by keeping it in a water bath for 3 hours with 5 ml of 2.5 N HCL and cooling it to room temperature. It was then neutralized by adding solid sodium carbonate until effervescence stopped. The volume was made up to 100 ml and filtered by using Whatman filter paper. The filtrate was collected and poured out

0.2 ml and made up the volume to 1 ml with distilled water. After that, 1ml of phenol and 5ml of sulphuric acid were added to the tubes and incubated for 30 min at room temperature. In a hot acidic medium, glucose was dehydrated to hydroxymethyl furfural and formed a yellow-orange product with phenol. This colour intensity was measured at 490 nm. From the standard graph, the amount of carbohydrate present in the sample was calculated.

Amount of total carbohydrates present in 100 mg pf sample= $\frac{mg\ of\ glucose}{volume\ of\ test\ sample}$ **×100**

Fig 7: Yellow- orange colour indicates the presence of carbohydrates in the samples

3.2.7 Crude fat estimation

3.2.7.1Method: Soxhlet method (AOAC, 1990)

3.2.7.2.2 Materials:

- Soxhlet apparatus
- Rotary evaporator
- Petroleum ether as solvent

3.2.7.2 Procedure:

To estimate the crude fat content, 3g of sample was taken in an extraction thimble by handling it with tongs and placing it in the extraction unit. The flask, which contained petroleum ether, was connected to the extractor. Then it was boiled at a 45-60^{0}C temperature and run for up to 6 hours. After finishing, the evaporated petroleum ether was collected through a rotary evaporator. The flask was cooled and then weighed.

Crude Fat % = $\frac{Weight\ of\ the\ flask\ with\ fat - Weight\ of\ the\ empty\ flask}{weight\ of\ the\ sample} \times 100$

Fig 8 : a) Soxhlet Apparatus

Fig 8:b) Flask with fat after distillation

3.2.8 Crude Protein Estimation

3.2.8.1: Method: Kjeldhal method (AOAC,1990)

3.2.8.2: Materials required

- Kjeldhal Apparatus
- Catalyst: Mixture of potassium sulphate, copper sulphate and selenium dioxide
- Con. H_2SO_4
- 4% Boric acid
- 40% NaOH
- 0.1N Hcl
- 0.01% Methyl red indicator

3.2.8.3 Procedure

Kjeldhal method is universally accepted and considered as the standard method for the estimation of crude protein present in the sample. It was performed in three steps namely, digestion, distillation and titration.

Digestion: 2 gm of sample and catalyst mixture were transferred into a 500 ml digestion flask simultaneously and 20 ml of sulphuric acid was added to the flask. After that, the digestion flask was placed in the digestion chamber and subjected to 100–200°C for about 2 hrs. The heating process was continued until the sample solution turned into a clear greenish-coloured solution, which determined the nitrogen present in the organic compounds was converted into ammonium sulphate. It also marked the end of the acid digestion process. After the digestion step was completed, the digested sample was allowed to cool at room temperature for about 1-2 hrs. The digested flask was then filled with 15-20 ml of distilled water and transferred to a volumetric flask to make up the volume to 100 ml.

Distillation is a combined process of both boiling and condensation. From the 100ml of prepared digested sample, 10ml was taken into the distillation flask followed by 50 ml of 40% NaOH and 50 ml of distilled water, which resulted in the liberation of NH_3 gas from the solution.The distillation flask was connected to a conical flask which acts as a receiving flask through a tube which contains 30ml of 4% boric acid. Through condensation, the NH_3 gas liberated in the solution comes out of the distillation flask and is condensed into the receiving flask. Due to the low pH of the solution in the receiving flask, the ammonia gas and boric acid were converted to the ammonium ion and borate ion, respectively.

Titration: Finally, in order to quantify the amount of nitrogen present in the sample, the distillated solution was mixed with a few drops of 0.01% methyl red indicator, which resulted in a yellow colour, and then titrated against 0.1N Hcl until the colour changed from yellow to slightly pink and noted the end point as the titrant value. 6.25 was used as the conversion factor to determine crude protein percent.

Nitrogen%$=\frac{\boldsymbol{V(titrant\ volume)\times N(normality\ of\ acid)\times F(acid\ factor)\times Mol.weight\ of\ niytrogen}}{\boldsymbol{weight\ of\ the\ sample\times 10}}$

Crude protein % $= \boldsymbol{N\% \times dilution\ factor \times 6.25}$

Fig 9 : a) Digestion of sample at 100-200^{0}C

Fig 9: b) Distillation of digested sample

Fig 9: c)Titration of distillate sample

3.2.9 Total ash estimation

3.2.9.1 Method: AOAC, 1990

3.2.9.2 Materials required: Muffle furnace

3.2.9.3 Procedure:

To estimate the ash content, 3g of sample was taken in a crucible and measured as an initial weight and **2**then placed in muffle furnace at 550^{0}C for 4 hours till the ash was formed. After the temperature has reduced, the final weight of the sample was measured.

$$\textbf{Total Ash\%} = \frac{\textit{Weight of crucible with ash} - \textit{weight of empty crucible}}{\textit{weight of the sample}} \times \mathbf{100}$$

3.3 ESTIMATION OF ANTIOXIDANT CONTENT

3.3.1Extraction of samples:

Samples were extracted for the determination of antioxidant content by mixing 1g of sample with 20 ml of 80% methanol and sonicated for 10 minutes with the help of a sonicator in order to uniformly homogenised the sample and followed by cold centrifuged at 4^{0}C for 10 minutes at 9000 rpm. Then,the supernatant was collected for further analysis.

Fig 10: Sonicator

3.3.2 Total Phenolic content

3.3.2.1 Method :Folin-Ciocalteu method (Sharma and Gujral ,2010)

3.3.2.2Materials required:

- 7% Na2CO_3
- Folin- Ciocalteu's reagent
- Spectrophotometer

3.3.2.3 Procedure:

0.2 ml of extracted sample was taken into test tubes followed by 1.8 ml of distilled water and 0.2 ml of Folin-Ciocalteu's reagent and mixed properly. After allowing it to stand for 5 min, 2 ml of 7% Na2CO_3 was added followed by 0.8 ml of distilled water and again allowed to incubate for 1 hr. 30 min. in the dark to stabilize the formed blue colour and the absorbance was recorded at 760 nm in a spectrophotomer. Gallic acid was used as a standard.

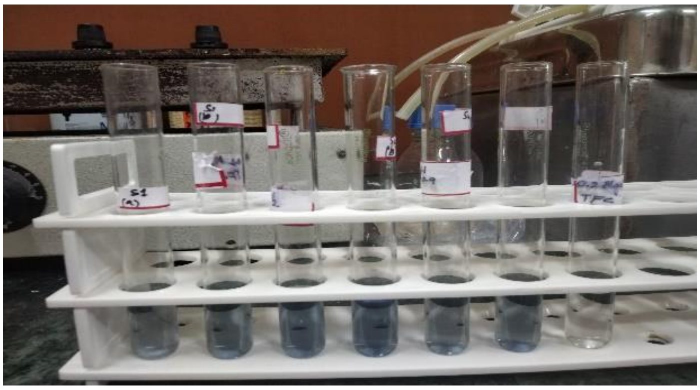

Fig 11: Blue colour indicates the presence of phenolic content in the samples

3.3.3 Total Flavonoid content

3.3.3.1 Method: $Alcl_3$ method (Xu and Chang,2007)

3.3.3.2 Materials required:

- 10% $Alcl_3$
- 5% $NaNO_2$
- 1M NaOH
- Spectrophotometer

3.3.3.3 Procedure:

1ml of extracted sample was taken into test tubes and mixed with 4ml of distilled water followed by 0.3ml of $NaNO_2$ and 0.3ml of $Alcl_3$. After allowed to stand for 5 minutes, 2ml of 1M NaOH was added to neutralized and allowed to incubate for 25 minutes in the room temperature to stabilized the tinted yellow colour formed. The absorbance was recorded at 510nm in spectrophotomter. Catechin was used as a standard.

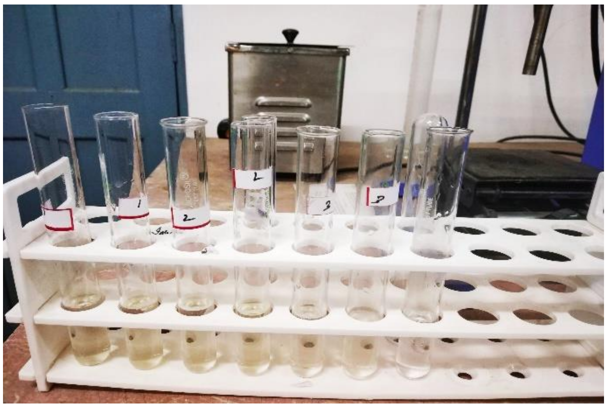

Fig 12: Tinted yellow colour indicates the presence of flavonoids in the samples

3.4 SENSORY ANALYSIS

Sensory analysis is one of the crucial step to determined the overall acceptability of developed product. Hedonic scale was used to measure the sensory score of the samples. Four samples were served to the panelist. Total 10 panelist took part in the analysis from the Department of Food Technology and Biochemical Engineering, Jadavpur University.

Each of panelists were given the score of samples for appearance , taste ,texture, colour and the overall acceptability of the product on a scale of 9 points, ranging from “like extremely” to “dislike extremely”.

Fig 13: a) Sample -1 (0.15% citric acid)

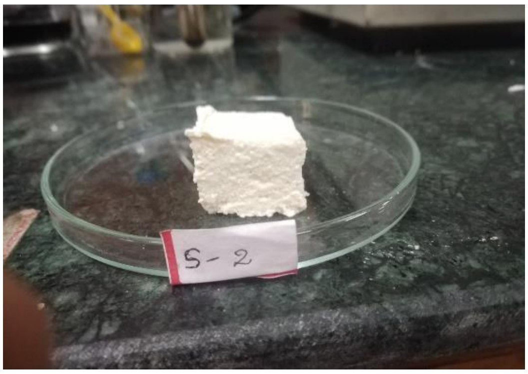

Fig 13: b) Sample-2 (0.2% citric acid)

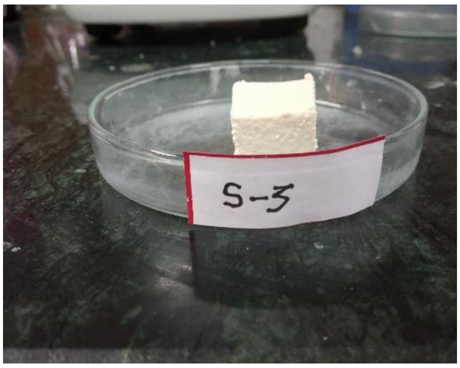

Fig 13: c) Sample-3 (0.2% $MgCl_2$)

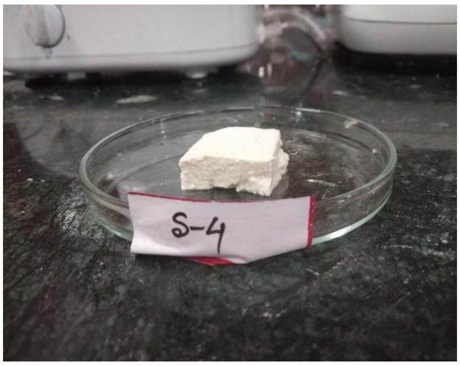

Fig 13:d)Sample-4 (0.2% $CaSO_4$)

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Moisture content and pH:

Moisture content is one of the most important characteristics to reflect the textural properties of tofu. The moisture content and pH of tofu samples prepared by using different coagulants were depicted in table1. In the present study, the moisture content varied from 69% ± 0.42 to 73.99% ± 0.86. The differences in gel network caused by different anion and its ionic strength towards the water retention capacity of soy protein gels lead to the variation in the moisture content of tofu prepared by using different coagulant (Dzikunoo et al.,2015). The pH plays an important role in the gelation of tofu. According to Zhang et al.,(2018) and Kanauchi et al.,(2015) due to the addition of acid or salt coagulant leads to release of hydrogen or metal ions resulted in lowering the pH to neutralize the isoelectric point which leads to precipitate the soy protein resulting in the formation of tofu. However ,the pH for the gelation differ from coagulant to coagulant as well as concentration to concentration. In the current study, the pH ranged from 4.75 ± 0.07 to 5.85 ± 0.07.The findings were correlated as reported by Cao et al., (2017) the pH ranges of citric acid coagulated tofu were 5.3 to 5.6.

Table 3: Moisture content and pH of tofu coagulated with different coagulant

Sample	Moisture content (%)	pH
S-1	69±0.42	5.05±0.21
S-2	72.01±2.50	4.75±0.07
S-3	73.99±1.16	5.6±0.25
S-4	73.15±0.86	5.85±0.07

Results were expressed as mean ± standard deviation. S-1: 0.15%(w/v) citric acid coagulated tofu,S-2: 0.2%(w/v) citric acid coagulated tofu,S-3: 0.2% (w/v) $MgCl_2$ coagulated tofu and S-4: 0.2%(w/v) $CaSO_4$ coagulated tofu

4.2 Texture Analysis

Table 4: Textural properties of tofu coagulated with different coagulant

Sample	Hardness (g)	Springiness	Cohesiveness	Gumminess
S-1	126.49±1.46	0.95±0.04	0.44±0.02	55.87±2.23
S-2	75.66±3.72	0.86±0.01	0.45±0.01	34.05±0.6
S-3	68.46±2.99	1.14±0.2	0.52±0.02	39.4±0.9
S-4	56.21±1.53	1.01±0.03	0.49±0.02	36.66±1.66

Results were expressed as mean ± standard deviation. S-1: 0.15% (w/v) citric acid coagulated tofu,S-2: 0.2%(w/v) citric acid coagulated tofu,S-3: 0.2%(w/v) $MgCl_2$ coagulated tofu and S-4: 0.2% (w/v)$CaSO_4$ coagulated tofu

Texture plays a crucial role in expressing the quality of tofu. The texture analysis, including hardness, springiness, gummines and cohesiveness of tofu prepared with different coagulants was observed and the results were depicted in table 4. Hardness denotes the ability to withstand destructive force. Tofu with a higher hardness value has a harder and firmer texture.The value of hardness of varied from 126.49±1.46g to 56.21±1.53g .It was observed that tofu prepared with acid coagulants have higher hardness value than salt coagulants. Dzukinoo et al.,(2015) reported that the high moisture content present in salt coagulated tofu resulted in a softer texture. In the present study,it was also showed that change in acid concentration leads to differences in the hardness. Tofu prepared with 0.15% citric acid has a higher value of hardness, with 126.49±1.46g than with 0.2% citric acid, which was 75.66±3.72g. These findings were correlated with the study investigated by Cao et al.,(2017), which reported that an increase in the organic acid concentration leads to a decrease in hardness. Springiness is the ability of the food samples to return to their original state after being compressed and destructive force is removed.It is related to the elasticity of food; the higher the springiness, the higher the elasticity. Cohesiveness is the energy required to break the internal bonding in the samples. Tofu prepared with 0.2% $MgCl_2$ has a higher springiness and cohesiveness value with 1.14±0.2

and 0.05±0.02 respectively, which means more energy was required to break the internal bond due to higher elasticity. Gumminess is the energy required to disintegrate the semi solid food until it can be swallowed.Tofu with 0.15% citric acid was obtained the highest value of gumminess with 55.87±2.23.

4.3 Colour

Colour is considered as an important physical characteristics for assessing the external quality of developed food products.The effect of different coagulants with different concentration on the colour of tofu samples were depicted in table 5.It was observed that acid coagulated tofu was lighter in colour than salt coagulated and also recorded that the increase in concentration of citric acid from 0.15% to 0.2% resulted to an increase in L*(whitness),a*(redness) and b*(yellowness) value with 91.2±1.18 to 90.2±0.22, 0.43±0.06 and 15.42±0.15 respectively. The results of the present study agreed with the findings reported by Dzikunoo et al.,(2015) where increase in concentration of citric acid from 2% to 3.5% leads to an increase in L^* (whitness),a*(redness) and b*(yellowness) value which were70.73 to 71.24,-0.18 to -0.81 and 15.00 to 17.80 respectively. However, tofu prepared with 0.2% $MgCl_2$ has more redness and 0.2% $CaSO_4$ has more yellowness compared to the citric acid coagulated tofu with with a value of 1.53±0.12 and 24.41±0.77 respectively.

Table 5: Colour profile of tofu coagulated with different coagulant

SAMPLE	L*(whiteness)	a*(redness)	b*(yellowness)
S-1	90.2±0.22	0.11±0.07	13.73±0.34
S-2	91.2±1.18	0.43±0.06	15.42±0.15
S-3	85.75±0.26	1.53±0.12	16.29±0.35
S-4	86.4±0.82	0.27±0.02	24.41±0.77

Results were expressed as mean ± standard deviation. S-1: 0.15%(w/v) citric acid coagulated tofu,S-2: 0.2%(w/v) citric acid coagulated tofu,S-3: 0.2% (w/v) $MgCl_2$ coagulated tofu and S-4: 0.2%(w/v) $CaSO_4$ coagulated tofu

Nutritional Analysis

Table 6: Nutritional profile of prepared tofu using different coagulants

Sample	Proteins %	Fats %	Total Carbohydrates %	Total Ash %
S-1	48.36±0.33	22.95±1.06	20.6±0.85	1.95±0.01
S-2	48.63±0.71	19.26±0.35	19.07±2.07	1.41±0.13
S-3	46.33±1.13	25.05±1.07	24.07±2.32	2.98±0.04
S-4	50.38±0.35	23.71±0.32	21.4±1.83	3.2±0.14

Results were expressed as mean ± standard deviation (dry weight basis). S-1: 0.15% (w/v) citric acid coagulated tofu,S-2: 0.2%(w/v) citric acid coagulated tofu,S-3: 0.2% (w/v) $MgCl_2$ coagulated tofu and S-4: 0.2% (w/v) $CaSO_4$ coagulated tofu.

Nutritional composition is the most important part of a developed food products .Consumers always want a food with good nutritional value .The nutritional analyses of tofu prepared with different coagulants were depicted in Table 6. In the present comparative study of tofu prepared with different coagulants showed that the percentage of protein, fats, total carbohydrates and total ash in tofu ranged from 46.33 to 50.38%, 19.26 to 25.05 %,19.07 to 24.07% and 1.41 to 3.42 % respectively. Furthermore, tofu prepared with 0.2% $CaSO_4$ i.e. S-4 has obtained the highest protein content with 50.38% as well as total ash content with 3.2%, followed by tofu prepared with 0.15% citric acid i.e. S-1 and 0.2% citric acid i.e. S-2 with 48.36% and 48.63% respectively, which also indicated that the change in concentration of the same coagulants does not show any visible differences in the protein content. $MgCl_2$ i.e. S-3 showed less protein content compared to the other coagulants with 46.33% but obtained a higher percentage of fats and total carbohydrates with 25.05% and 24.07% respectively. However, the findings of the present study for the protein content present in tofu prepared with $CaSO_4$ and $MgCl_2$ were slightly lower than the results reported by Sanjay et al.,(2008) which were 56.4% and 55.5%, respectively, but the protein content of tofu prepared with citric acid was very close to the findings of tofu with lemon juice reported by Gartaula et al., 2013, which was 51.58%.The differences in the nutritional value of tofu might be due to different processing method or different varities in soyabean.

4.5 Antioxidant Content

Phenolics and flavonoids are naturally occurring active antioxidants as well as an important phytochemicals present in soyabean. The antioxidants has the capacity to fight against free radicals,which results in the prevention of harmful diseases like cardiovascular diseases, cancer, menopausal symptoms etc. (Karimi et al.,2010). Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of tofu prepared with different coagulants were presented in table. It was observed that TPC and TFC were obtained in the range of tofu 1.111±0.02 to 1.487±0.01(GAE mg/g) and 0.126±0.01 to 0.299±0.02 (CE mg/g) respectively. Tofu prepared with 0.2% $CaSO_4$ showed highest TPC value 1.487±0.01 and TFC value 0.299±0.02 respectively. The total phenolic content of the current study was slightly lower than the findings reported by Rosset et al,(2014) ,where the tofu treated with viscoenzyme L showed higher TPC content 1.72.± 6.38 GAE mg/g in which the control was 1.61 ±8.68 (GAE mg/g).

Table 7: **Antioxidant content of prepared tofu using different coagulants**

SAMPLE	TPC (GAE mg/g)	TFC (CE mg/g)
S-1	1.281±0.02	0.157±0.02
S-2	1.111±0.02	0.126±0.01
S-3	1.465±0.00	0.152±0.01
S-4	1.487±0.01	0.299±0.02

Results were expressed as mean ± standard deviation (dry weight basis). S-1: 0.15%(w/v) citric acid coagulated tofu,S-2: 0.2%(w/v) citric acid coagulated tofu,S-3: 0.2%(w/v) $MgCl_2$ coagulated tofu and S-4: 0.2% (w/v) $CaSO_4$ coagulated tofu.

4.6 Sensory Analysis

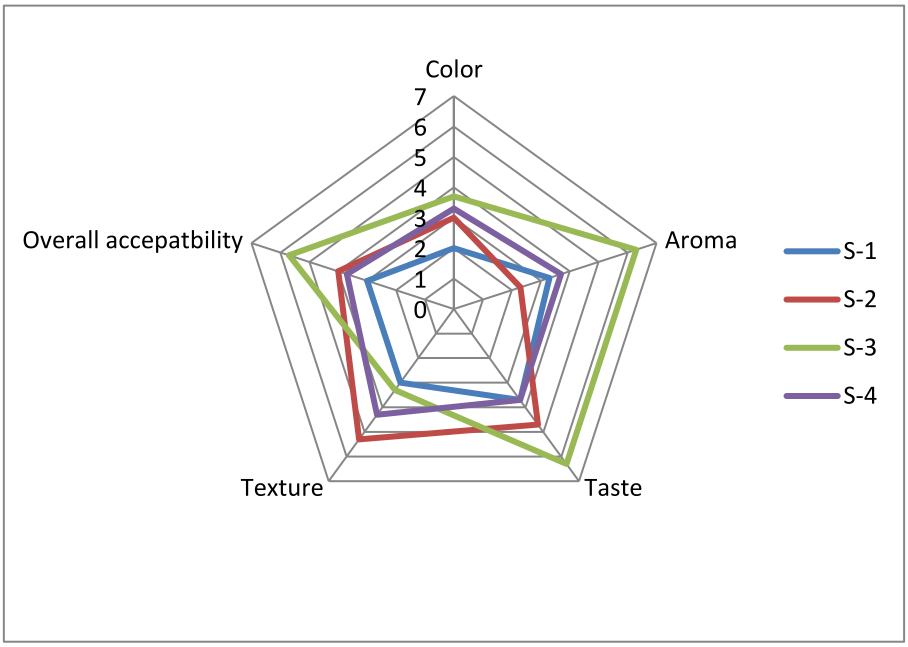

Fig 14: Sensory graph for the samples of tofu

The sensory analysis of all the four tofu samples prepared with different coagulants was subjected to sensory evaluation for the sensory attributes like colour, taste, texture, aroma, and overall acceptability. A total of ten panel members scored each attribute, and the results were averaged and shown in figure 14. It was observed that the colour or appearance of all the developed products was liked moderately to liked very much, as the average score for S-1, S-2, S-3, and S-4 was 2, 3, 3.7, and 3.3 respectively. The aroma of S-2 was liked very much by the panelists with a score of 2.3, followed by S-1 and S-3, which were slightly liked with a score of 3.3 and 3.7, respectively. However, most of the panelists did not like the aroma of S-3 with a score of 6.7. Taste is one of the most important attributes in deciding the consumer's acceptability of developed food products. The taste of S-1 and S-2 was slightly liked with the same score of 3.7, followed by S-2 with a score of 4.7, but the taste of S-3 was not liked by the panelists because of its bitterness, which resulted in lowering the score by 6.3. The texture of all the samples was slightly liked, except S-2, due to its less binding nature. The overall acceptability of tofu prepared with 0.15% citric acid, i.e. S-1, was highest because of its favourable taste, aroma, and texture, and tofu prepared with 0.2% $MgCl_2$, i.e. S-3, showed the lowest score with 5.7 in overall acceptability due to bitterness in taste and the aroma was not so preferable for the consumer.

CHAPTER 5

CONCLUSION

In this present research work, the study has been undertaken to develop soy products and assess their acceptability for consumer preference.Tofu was developed using different coagulants but with the same processing parameters on separate batches and their physico-chemical properties were analyzed. In separate batches, each soymilk sample was heated to 85°C and their respective coagulants, namely citric acid, magnesium chloride and calcium sulphate in varying concentration were added, followed by press moulding by applying a load of 1000 g for 30 minutes and later 500 g for 30 minutes. The findings showed that tofu prepared from $CaSO_4$ (0.2% w/v) obtained the highest protein content of 50.38% and the highest antioxidant content, such as TPC of 1.487 GAE mg/g and TFC of 0.299 CE mg/g on dry weight basis. However, it turned out to be the softest tofu among all the samples, as it had the lowest hardness value of 56.21g. Furthermore, tofu prepared from $MgCl_2$ (0.2%w/v) obtained the highest fats and carbohydrates of 25% and 24%, respectively, but the lowest protein content of 46.33%, but the texture was neither too hard nor too soft on account of higher values of springiness and cohesiveness, which were 1.14 and 0.52 respectively, resulting in strong binding of tofu. However, tofu prepared from citric acid at 0.15% and 0.2% was lighter in colour, in which their lightness values were 90.2 and 91.2, respectively. Tofu prepared from citric acid at 0.15% (w/v) had the highest overall acceptability in terms of taste, aroma, colour, and texture, as well as nutritional value, with protein, fats, total carbohydrates, and total ash of 48.36%, 22.95%, 20.6, and 1.95%, respectively.

CHAPTER 6

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