DEVELOPMENT AND QUALITY EVALUATION OF PLANT-BASED HIGH PROTEIN MEAT ALTERNATIVES

Nishat Tasnim Oishee

Roll No.: 0121/06

Registration No.: 988

Session: January-June, 2021

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

OCTOBER 2023

Authorization

I hereby assert that I am the sole author of the thesis which has been submitted to Chattogram Veterinary and Animal Sciences University (CVASU) as part of my academic pursuits. I willingly grant CVASU the authority to lend this thesis to other institutions or individuals for the exclusive purpose of scholarly research. Furthermore, I provide explicit permission to CVASU to photocopy or reproduce any part or the entirety of this thesis, should other organizations or individuals request it for scholarly research purposes.

To the best of my knowledge and to the extent permitted by the available technology, I affirm that the electronic copy of this thesis, which has been submitted to the CVASU Library, is a faithful representation of the original print version of the thesis.

Nishat Tasnim Oishee
October, 2023

DEVELOPMENT AND QUALITY EVALUATION OF PLANT-BASED HIGH PROTEIN MEAT ALTERNATIVES

Nishat Tasnim Oishee

Roll No.: 0121/06

Registration No.: 988

Session: January-June 2021

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

Supervisor

Taslima Ahmed

Assistant Professor

Department of Applied Food Science and Nutrition

Chairman of the Examination Committee

Mohammad Mozibul Haque

Assistant Professor & Head

Department of Applied Food Science and Nutrition

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

OCTOBER 2023

PLAGIARISM VERIFICATION

Title of Thesis: ***Development and Quality Evaluation of Plant-Based High Protein Meat Alternatives***

Name of the Student: Nishat Tasnim Oishee
Roll number: 0121/06
Registration No.: 988
Department: Applied Food Science and Nutrition
Faculty: Food Science and Technology
Supervisor: Taslima Ahmed

This is to report that as per the check 18% of the content of the above thesis is stated to be plagiarized and is covered as per plagiarism policy and institutions issued from CASR, Chattogram Veterinary and Animal Sciences University. The report has been sent to the coordinator, CASR via email.

The thesis may be considered for the evaluation.

Taslima Ahmed
Assistant Professor
Department of Applied Food Science and Nutrition
Faculty of Food Science and Technology

ACKNOWLEDGEMENTS

"In the Name of Allah, the Most Beneficent, the Most Merciful. All the praises and thanks be to Allah, the Lord of the 'Alamin (mankind, jinns and all that exists)."

I wish to begin by extending my heartfelt gratitude to The **Almighty Allah**, whose blessings and guidance have enabled me to successfully navigate the journey of my academic pursuits, culminating in the completion of my study and dissertation in the field of Applied Human Nutrition and Dietetics, and this thesis for the attainment of a Master of Science degree (MS).

I am grateful to Professor **Dr. Md. Ashraf Ali Biswas**, Dean of the Faculty of Food Science and Technology at CVASU, and Professor **Dr. A.S.M. Lutful Ahasan**, Vice Chancellor of CVASU, for their unwavering support and encouragement.

My supervisor, **Taslima Ahmed**, from the Department of Applied Food Science & Nutrition, provided invaluable guidance throughout my academic journey. I am also appreciative of **Mohammad Mozibul Haque**, the Head of my department, for providing the opportunity and his continuous support in my pursuit of academic endeavors. Their collective contributions have been instrumental in my academic success.

I would like to express my sincere gratitude for the financial support I received through the National Science and Technology (NST) fellowship program, graciously provided by the Minister of Science and Technology, Government of Bangladesh. Additionally, I extend my heartfelt thanks to the Research and Extension department of Chattogram Veterinary and Animal Sciences University for their generous financial assistance.

I am deeply grateful to my friend **Subrata Bhattacharjee**, Food Safety Officer of Bangladesh Food Safety Authority (BFSA) for his steadfast support and encouragement throughout the research process.

Lastly, I am profoundly grateful to my beloved family and friends for their unwavering cooperation, boundless cheerfulness, and constant inspiration throughout the course of my study. Their support has been an essential source of motivation and strength, and I am thankful for their presence in my journey.

The Author
October 2023

Table of Contents

List of Figures

List of Tables

List of Abbreviation

Abbreviations	Elaboration
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CHO	Carbohydrate
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	Gallic Acid Equivalent
TE	Trolox equivalent
QE	Quercetin equivalents
Kcal	kilocalorie
SPSS	Statistical Package for Social Science
Etc	Et cetera
Et al	Et alii/ et aliae/et alia
SD	Standard Deviation
PDCAAS	Protein Digestibility-Corrected Amino Acid Score
DIAAS	Digestible Indispensable Amino Acid Score

ABSTRACT

Plant-based high-protein meat alternatives are essential in Bangladesh to address nutritional deficiencies, promote sustainable and cost-effective food sources, and enhance public health. Research in this area is critical to develop locally relevant and affordable solutions that improve overall nutrition while considering cultural dietary preferences. The main objective of this study is to formulate plant-based meat alternatives using soybean and wheat gluten with a focus on achieving elevated protein content, evaluating nutritional attributes, assessing microbiological safety and bioactive compounds, and conducting sensory and cost analysis. When examining soy meat and gluten meat side by side, soy meat showed elevated levels of moisture (75.48%), crude fiber (0.08%), ash (0.93%), total phenolic content (2.41 mg GAE/100g), and antioxidants (3.12 mg TE/100g). In contrast, it exhibited lower protein (13.65%) and total flavonoid content (11.07 mg QE/100g) compared to gluten meat. ANOVA (Analysis of Variance) and Tukey's test was employed in this study, with a significance level of 5%, to compare and analyze all the collected data. The energy content per 100g was 124.56 kcal for soy meat and 112.48 kcal for gluten meat. During the 15-day storage period at refrigeration temperature, microbiological analysis yielded no detection of yeasts, molds or bacteria in any of the samples. Despite the texture and taste preference for gluten meat, soy meat was the overall preferred choice in sensory evaluation, excelling in appearance, aroma, and overall acceptability.

Keywords: Plant-based meat, Soy meat, Gluten meat, Nutritional profile, Sensory evaluation, Antioxidant activity

Chapter 01: Introduction

In recent years, the global food landscape has witnessed a paradigm shift in consumer preferences towards sustainable and health-conscious dietary choices. The exponential growth of the plant-based food industry stands as a testament to this transformation. The environmental, ethical, and health issues related to the production and consumption of traditional animal-based meat have been addressed in part by the development of plant-based meat substitutes. As resource depletion, climate change, and growing obesity rates continue to pose problems for the world, the development and evaluation of innovative high-protein plant-based meat substitutes have become a crucial area of research.

Plant-based meat substitutes are innovative products designed to seamlessly replace traditional meat in the human diet while closely replicating the texture, flavour, and visual appeal of meat products (Tziva et al., 2020).

Plant-based meat replacements are crafted to satisfy evolving consumer preferences and ensure future food security. With nutritional profiles closely mirroring traditional animal meats, they offer a sustainable solution that mitigates the adverse effects of livestock farming on the environment and human well-being. These innovations not only cater to our cravings but also contribute to a healthier planet and population (Ahmad et al., 2022). These plant-based meat substitutes stand out as excellent protein sources with the remarkable ability to replicate the texture, color, nutritional content, and taste of specific meats (Choudhury et al., 2020).

The culinary alchemy of mushrooms, rice, lentils, soy protein, and wheat gluten, combined with meat-like flavor additives, yields a finished product that remarkably mimics the texture and taste of meat (P. Kumar et al., 2017).

Studies have shown that the production of meat alternatives derived from soymeal and gluten is generally more environmentally sustainable compared to chicken, lab-grown, and mycoprotein-based meat substitutes (Smetana et al., 2015).

The market for meat substitutes is experiencing rapid growth on a global scale, extending beyond just catering to vegetarian consumers. It now appeals to meat enthusiasts who are motivated by health, ethical, cost, and sustainability considerations and are seeking to reduce their meat consumption (Dagevos et al., 2013).

The Bangladesh Standards and Testing Institution (BSTI) plays a crucial role in certifying food product standards in Bangladesh, but notably, there is currently no

established certification or grading system for meat and meat products within the country. A study in Khulna, Bangladesh, exposed rampant fraudulent practices in 16 slaughterhouses, with five involved in unhygienic processes and harmful chemical contamination of meat (Rahman & Sumon, 2018). This concerning revelation underscores the urgency of exploring and promoting plant-based meat alternatives as a safer and more sustainable option in the face of these food safety challenges.

In the pursuit of plant-based meat alternatives, it is imperative to recognize that while extensive research has been conducted on this subject globally, there remains a distinctive and uncharted path within the culinary landscape of Bangladesh. This research endeavors to embark on this unique journey by infusing Bangladeshi flavors and traditions into the development of plant-based products. Specifically, the focus is on creating soy meat and crafting gluten meat enriched with the aromatic spices that define our cuisine. This endeavor seeks not only to contribute to the ever-evolving field of plant-based foods but also to offer a culturally resonant and delectable option, tailored to the preferences and heritage of the Bangladeshi palate.

Aims and Objectives:

1) To formulate plant-based meat alternatives using soybean and wheat gluten as primary protein sources.
2) To assess product quality through proximate composition analysis, microbiological safety evaluation, and quantification of bioactive compounds.
3) To perform sensory evaluation tests and conduct cost analysis for a comprehensive assessment of the developed plant-based meat alternatives.

Chapter 02: Review of Literature

2.1 Plant-based Meat Alternatives

Humans have always seen meat as a necessary component of their diet. Meat eating has been essential to human evolution since it has been connected to Homo sapiens' ancient brain growth and development (Williams et al., 2017). The demand for meat has increased globally by 58% over the past two decades as a result of rising global population and strong economic expansion. The globe consumed 320 tonnes of beef in 2018, and by 2027, it is expected that market would grow by 15%. However, in recent years, concerns have grown over the inefficiencies of meat production compared to crop harvesting and the harmful effects of meat intake on human health. Food businesses are exploring for ways to deliver meat substitutes derived from nonanimal proteins to consumer markets that have comparable looks, mouthfeels, and scents to traditional meat as a result of these growing concerns (S. Kumar, 2016). The two main meat substitutes being studied at the moment in the field of food science are culture-based meats (also known as clean meat or in vitro meat) and plant-based meat, which is made from proteins extracted from plants using the proper structural techniques. In recent years, fungi-based meat substitutes like QuornTM products and insect-based meat analogs have also been commercialized. These include insect-based burgers from Coop (a Swiss food shop) and insect-fortified burgers from Bugfoundation (a German food firm) (He et al., 2020).

2.2 Protein

For adults, the recommended dietary allowance (RDA) for protein stands at approximately 0.8 grams of protein per kilogram of body weight per day. This translates to roughly 48 grams of protein for a person weighing 60 kilograms. Animal-based foods like meat are frequently advised for fulfilling protein requirements due to their ability to deliver dietary protein with relatively low calorie content. Moreover, they are generally recognized as superior sources of protein quality when compared to plant-based alternatives.

Two primary standards, the protein digestibility-corrected amino acid score (PDCAAS) and the digestible indispensable amino acid score (DIAAS), serve as crucial measures for assessing the quality of dietary protein sources. Notably, plant-based proteins often yield lower scores, typically ranging from 0.4 to 0.9, while animal proteins consistently

exceed 0.9. This disparity in PDCAAS/DIAAS values for plant sources can be attributed, in part, to the presence of "anti-nutrients" such as phytates and trypsin inhibitors, which hinder the efficient digestion and absorption of protein (Gilani et al., 2012).

Plant-based meat alternatives offer a notable advantage by incorporating concentrated or isolated forms of proteins derived from sources like soy and pea. These purified protein sources have fewer anti-nutritional factors, resulting in PDCAAS/DIAAS values that are comparable to many animal proteins, including meat. This enhancement in protein quality makes plant-based alternatives a compelling choice for those seeking nutritious and sustainable protein options (Hodgkinson et al., 2018).

2.3 Soybeans

Soybean, known as the "golden miracle bean," has long been the leading source of protein globally, serving as a foundation for nutritious foods and a crucial ingredient in diverse industrial applications (Verma et al., 2013).

Soybeans were first cultivated in China. Soy meals have been eaten for thousands of years in eastern nations including China, Korea, and Japan. In America, soybeans have been utilized to produce a range of soy meals since the 1980s. As a result, certain soybeans are referred to as vegetable legumes, and soy products are often consumed as vegetables. It's been well established for a long time that soy protein is nutritionally beneficial. Soybean is now recognized as a healthy diet due to the current understanding of its possible health advantages, which include lowering the danger of heart illnesses, avoiding certain malignancies, decreasing postmenopausal disorders, and boosting bone mass density. 'Consumption of 25 gm soy proteins per day along with a reduced cholesterol diet would minimize the risk of cardiovascular disease,' the US Food and Drug Administration has authorized a safety claim for processed foods containing soy proteins (K. C. Chang, 2005).

Figure 2.1: Soybean

2.4 Soy Meat

Soy meat is known as Tofu in the cuisines of China, Japan, Korea, and Southeast Asia. The nutritional value and health advantages of tofu make it an appealing food. Due to its relatively affordable price and elevated protein bioavailability, it has been employed more and more in a variety of culinary recipes in place of dairy products. Due to different soybean cultivars, coagulating agents, and preparation methods, tofu has a range of physical-chemical, functional, microbiological, and sensory attributes (Dey, 2017).

Due to its nutritional value and versatility in cooking, tofu is frequently used in many recipes. It is frequently referred to as "poor man's meat." Soymilk that has been compressed and formed into solid blocks is used to make tofu. It has a buttery flavor and a delicate texture. This cheese-like meal is produced by curdling fresh, hot soymilk with one or more coagulating agents (Shalini et al., 2021).

2.5 Health benefits of Soy Meat

The number of proteins, fats, vitamins, minerals, and isoflavones in soy meat is relatively significant. It also includes all nine of the necessary amino acids: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. It is a significant source of protein. It also contains copper, magnesium, zinc, and vitamin B1. Soy meat has been proven to have extremely few calories and is gluten-

free. It is suitable for usage by a wide range of people since it is cholesterol-free. Spices and flavors like onion and ginger can be added to soy meat (Zheng et al., 2020).

Also found for having soy isoflavones, soy meat can aid in reducing the inflammation of blood vessels and enhancing their flexibility (Beavers et al., 2012).

In addition, research revealed that giving individuals at risk for stroke 80 mg of isoflavones daily for 12 weeks increased blood flow by 68% (Chan et al., 2008)

Soy meat also includes the chemicals known as saponins are expected to have beneficial effects on blood cholesterol levels and the elimination of bile acids from the body, both of which can reduce the chance of developing heart disease (Eze et al., 2018).

Soy offers valuable functionalities owing to its high content of water-soluble dietary fiber, which includes galactose, arabinose, galacturonic acid, xylose, fructose, and rhamnose. Additionally, it contains oligosaccharides like raffinose and stachyose, along with the essential amino acid tryptophan (Nakata et al., 2017). These components contribute to the nutritional and dietary benefits of soy-based products.

2.6 Wheat gluten

Wheat stands out among the edible grains due to a remarkable feature: its flour contains a distinctive protein complex known as "gluten." This unique gluten protein complex possesses the extraordinary ability to be transformed into a dough with precisely the right rheological properties essential for crafting leavened bread (Uthayakumaran et al., 2002).

It possesses a remarkable natural capability to create thin protein films when subjected to stretching forces. These films can be further transformed into fibrous, proteinaceous materials. These distinctive properties arise from the molecular composition and subsequent mesoscopic behavior of wheat gluten (Don et al., 2003).

Indeed, the rheological properties of gluten extend far beyond bread production. They play a vital role in a broad spectrum of food items that are uniquely crafted from wheat. This includes staples such as noodles and pasta, as well as versatile creations like pocket breads, pastries, cookies, and a variety of other wheat-based products (Macritchie, 1992).

The dough-forming protein found in wheat, or gluten, is crucial for a variety of technical uses, from enhancing the baking abilities of items that are leavened to the creation of novel dietary constituents along with other biomaterials (Day, 2011).

Numerous related but separate proteins, mostly gliadin and glutenin, make up the complex combination known as gluten. Secalin in rye, hordein in barley, and avenins in oats are examples of related storage proteins that together make up gluten (Biesiekierski, 2017).

Glutenin and gliadins, the primary wheat storage proteins, hold a prominent position, comprising approximately 60-85% of the total protein content within the wheat grain. These proteins are notably abundant in amino acids such as asparagine, glutamine, arginine, or proline. However, it's worth noting that they exhibit relatively low levels of nutritionally critical amino acids such as lysine, tryptophan, and methionine (Zilic, 2013).

2.7 Bioactive Components

2.7.1 Flavonoid Compounds

A significant group of natural compounds are flavonoids. In particular, flavonoids are a group of polyphenolic-structured secondary plant compounds. Fruits, vegetables, cereals, bark, roots, stems, flowers, and tea are typical sources of them. It has been established that flavonoids contain anti-inflammatory and antioxidant characteristics and are crucial for a wide range of biological processes that take place in plants, animals, and microbes. Plants contain compounds called flavonoids that have a role in a number of biological processes, such as floral color and fragrance, fruit pollinator attraction, fruit dispersion, seed and spore germination, and the development and advancement of seedlings. Additionally, flavonoids contribute to the development of seedlings (Griesbach, 2005). Many different roles are played by flavonoids, such as those of molecule signaling, allopathic substances, phytoalexins, detoxifying intermediaries, and antimicrobial resistance agents (Takahashi & Ohnishi, 2004). Flavonoids could possibly function as distinctive filters against UV rays. Additionally, they protect plants from a variety of both abiotic and biotic stimuli' negative impacts (Samanta et al., 2011). Six structural subgroups of flavonoids may be differentiated chemically: flavones, flavonols, flavanones, flavanonols, flavan-3-ols (catechins), and

anthocyanidins. These molecules (aglycones) may also be alkoxylated or esterified in addition to being commonly glycosylated (at one or more sites with a variety of sugars). This has enabled the discovery of almost 5000 unique flavonoids in plant materials (Harborne & Williams, 2000). The synthesis of an aluminum chloride complex, that is used in the majority of reported techniques for measuring flavonoids, provides the basis for the processes of analysis used to quantify the number of flavonoid compounds in diverse plants (Grubesic et al., 2007).

2.7.2 Phenolic Compounds

The majority of the widely distributed secondary metabolites that exist in the plant kingdom are phenolic compounds, though the precise type of phenolic molecule that is present varies according to the phylum. The malonate/acetate framework, also referred to as the polyketide system or the shikimic acid route, produces phenolic chemicals biosynthetically and they make up approximately forty percent of the organic carbon that moves around in the environment (Chapman & Ragan, 1980).

Scientific research has shown that plant-derived phenolics help prevent a variety of chronic conditions linked to oxidative damage, such as heart disease, cancer, and neurological problems (Dai & Mumper, 2010).

2.7 Antioxidant Activity

Antioxidants are responsible for scavenging free radicals and protecting our bodies from a variety of diseases that are associated with free radicals. The oxidative process, which is regulated by free radicals, is connected to the beginning, growth, and end of the mechanism. The body can produce antioxidants, and many different foods naturally contain them (Alam et al., 2020).

Chapter 03: Materials and Methods

3.1 Study Area

The experiment was conducted in the lab of the Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, department of Applied Food Science and Nutrition, Applied Chemistry and Chemical Technology, Poultry Research and Training Center (PRTC), Department of Animal Science and Nutrition.

3.2 Study Duration

The experiment was conducted for a period of six months from April to September 2023.

3.3 Experimental Design

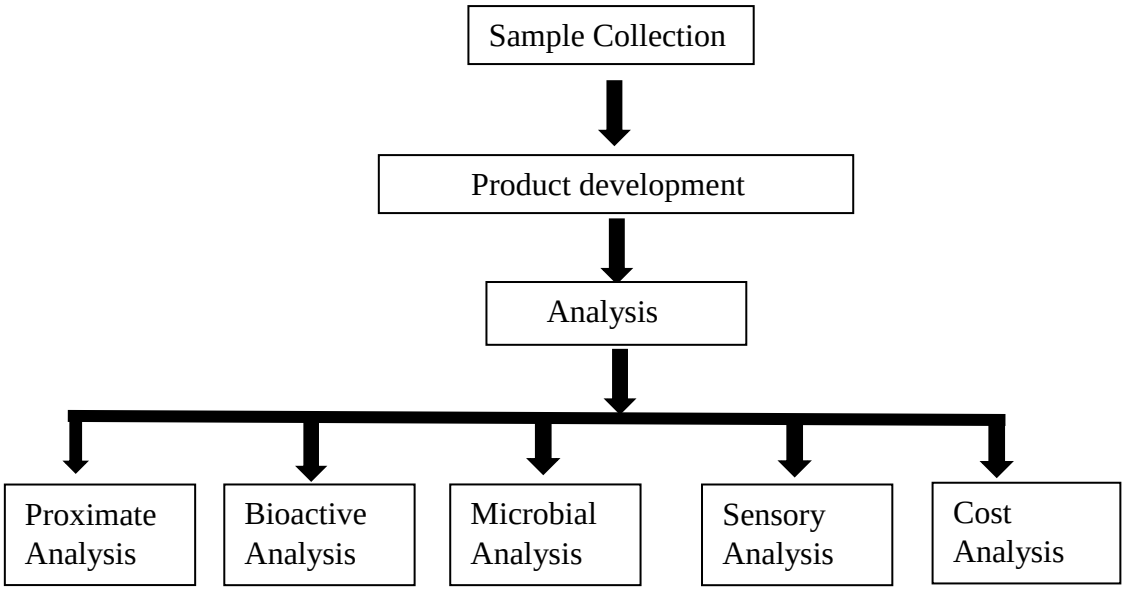

Figure 3.1: Experiment's step-by-step design

3.4 Sources of Ingredients

Soybean was collected from Riaz Uddin Bazar, a local market of Chattogram district. Other ingredients required for the creation, such as wheat flour, salt, baking soda, and vinegar, were purchased from the local market and superstore. The laboratory provided additional supplies that were required for the experiment.

3.5 Preparation

3.5.1 Preparation of Soy Meat

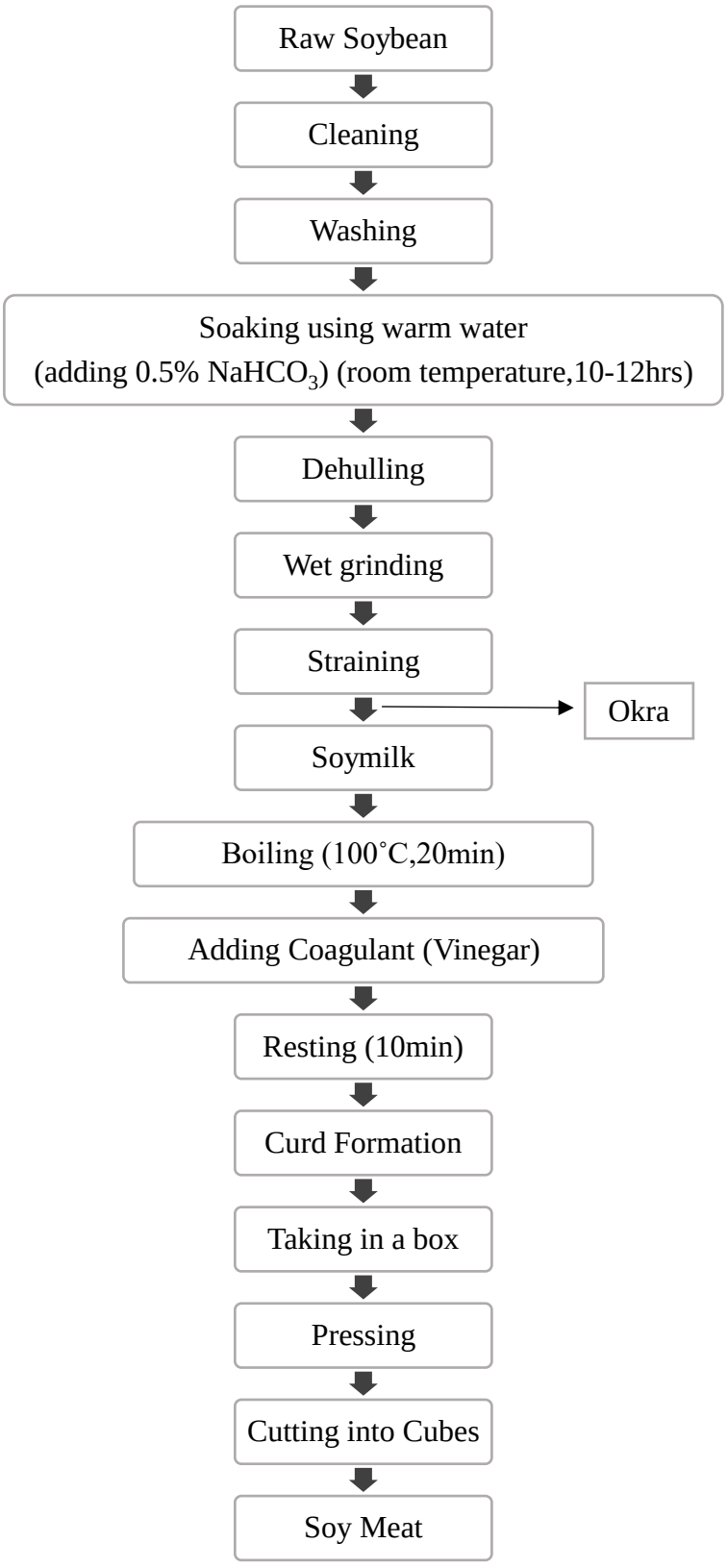

Figure 3.2: Processing steps of Soy Meat

3.5.2 Preparation of Gluten Meat

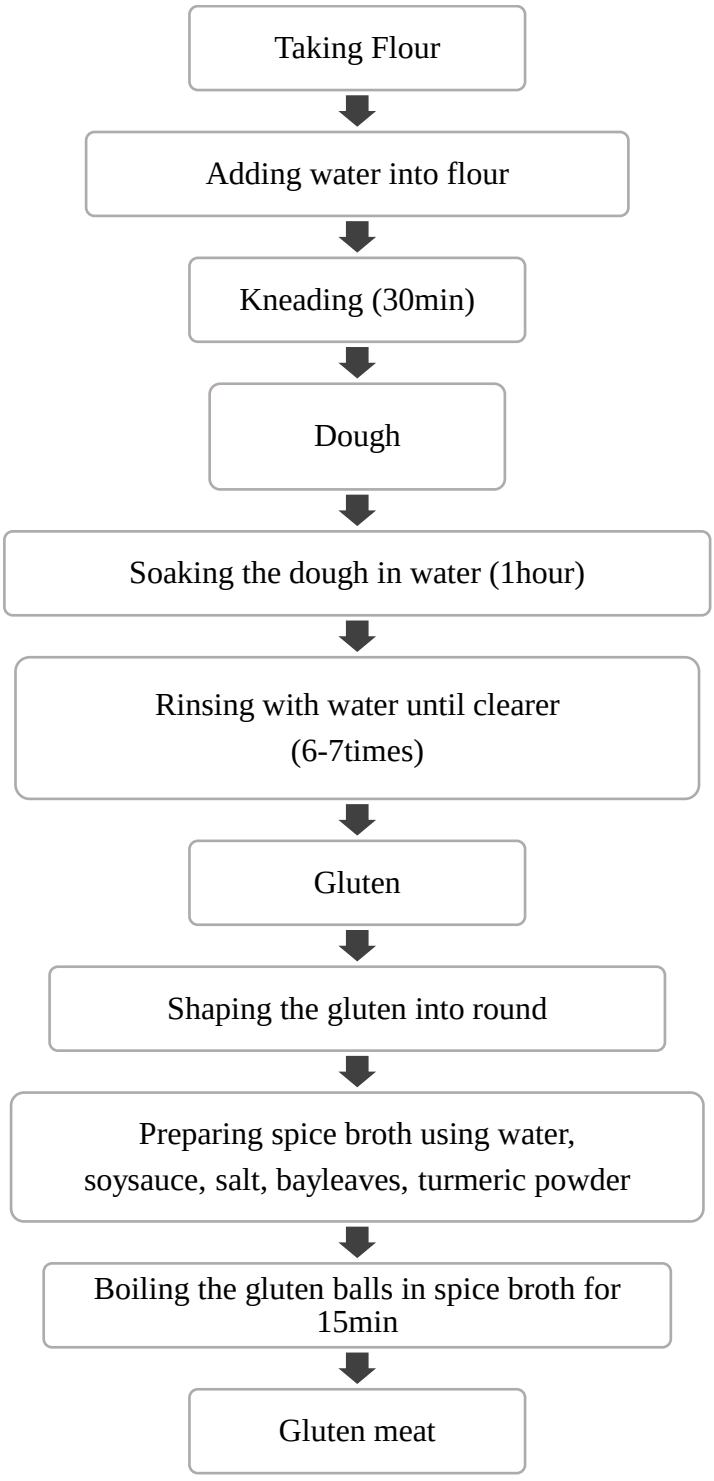

Figure 3.3: Processing steps of Gluten Meat

3.6 Proximate Analysis

In accordance with the Association of Official Analytical Chemists, the protein, fat, fiber, and ash content of products were evaluated on a dry weight basis in triplicate (AOAC International, 2016).

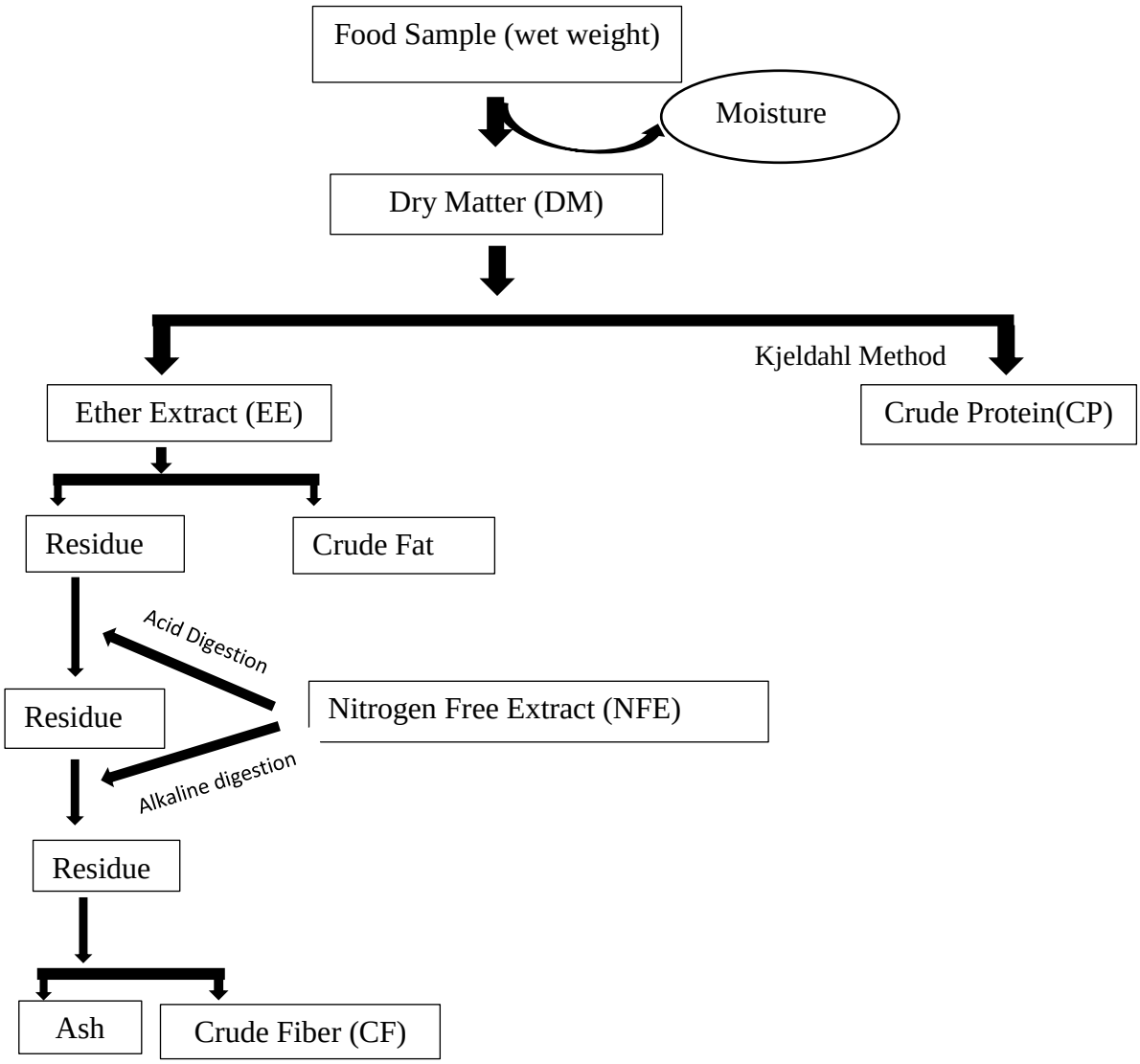

Figure 3.4: Proximate Analysis

3.6.1 Moisture content

Moisture monitoring holds paramount importance in the realm of meal preparation and testing, serving as a pivotal and extensively employed metric. The moisture levels within food carry significant economic implications for both those involved in processing and the end consumers. This stems from the fact that the quantity of moisture present in a food serving has an inverse relationship with its dry matter content. Moreover, moisture exerts a substantial influence on the texture, uniformity, and overall

quality of food products. The determination of moisture content was accomplished through a methodology recommended by the Association of Official Analytical Chemists, underlining its reliability and standardization (AOAC International, 2016).

Procedure

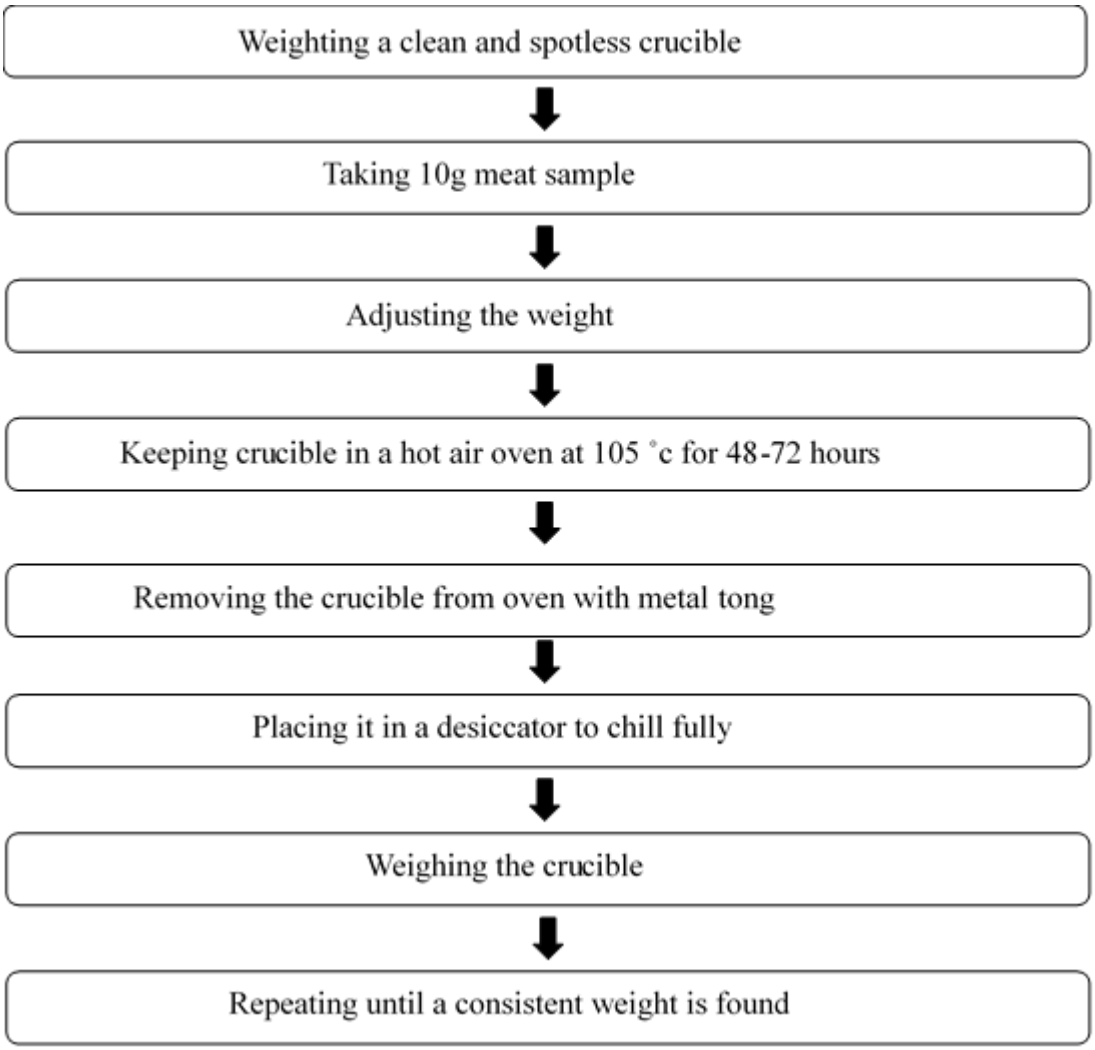

Figure 3.5: Moisture Content Determination

Calculation: The percent of moisture was calculated as follow

Moisture % = $\frac{W_i - W_f}{W_s} \times 100$

Here, W_i = Initial weight; W_f = Final weight; W_s = Sample Weight

3.6.2 Estimation of Dry matter (DM)

Dry matter encompasses the solid residue that persists once water has been extracted, underscoring the foundational components present in a substance. Conversely, water content offers insight into the volume of water contained within a particular item. Dry

matter constitutes the core constituents that endure following water removal, while moisture content quantifies the actual water volume within the examined entity. It's important to recognize that the dry matter fraction of food not only serves as a reservoir of essential elements necessary for sustenance, growth, reproduction, pregnancy, and lactation but also forms the basis for nutrient analysis and formulation.

Calculation: The determination of total solids was conducted following the established protocols set forth by AOAC (2016). By harnessing the data acquired during the moisture measurement process, it became feasible to ascertain the proportion constituting the entirety of solid content.

DM % = 100 – Moisture Content %

3.6.3 Estimation of Ash

The calculation of ash percentage was executed in accordance with the methodologies delineated by AOAC (2016). The concept of ash content pertains to the quantity of inorganic substances that persist subsequent to the incineration of all organic matter. This analytical approach underscores the determination of mineral components and non-combustible elements, offering insights into the material's mineral composition and potential nutritional attributes.

Procedure

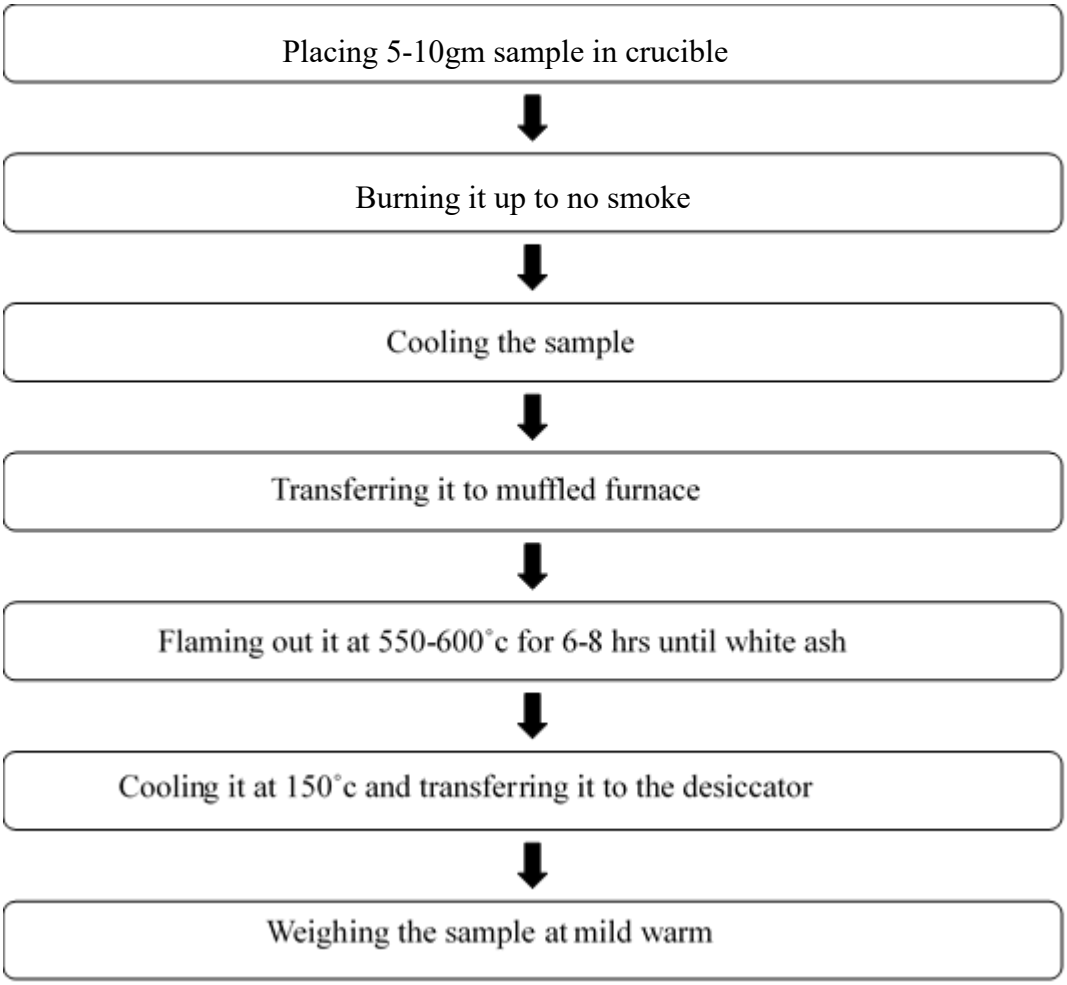

Figure 3.6: Ash Determination

Calculation: The following expression was employed to ascertain the ash content:

$$\text{Ash \%} = \frac{\text{Amount of ash supplied by sample}}{\text{sample weight}} \times 100$$

3.6.4 Estimation of Crude Fat

To analyze crude fat content in food samples using AOAC (2016) protocols, the samples are dissolved in organic solvents like methanol or chloroform. The dissolved mixture is filtered to separate the liquid part. This filtrate is divided and dried, leaving behind the fat extract. By weighing the extract, the fat content is determined using a Soxhlet instrument.

Procedure:

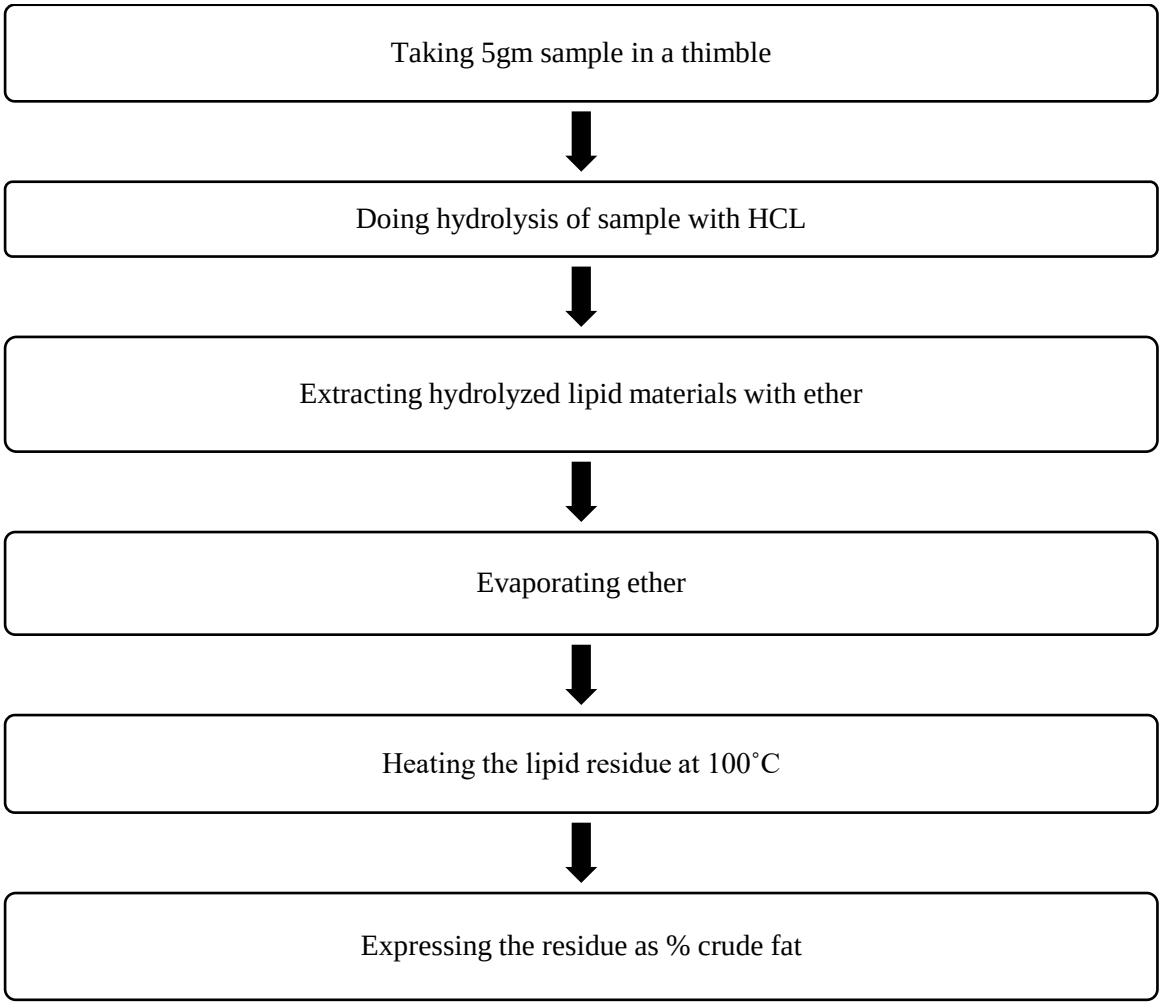

Figure 3.7: Crude Fat Determination

Calculation: The following expression was employed to ascertain the fat content:

$$\text{Fat \%} = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3.6.5 Estimation of Crude Fiber

Crude fiber, which comprises the indigestible carbohydrate portion, primarily consists of cellulose, hemicellulose, and lignin. To assess its content, a specific procedure is employed. For determining crude fiber content using the AOAC method (2016), this approach is adopted.

Procedure:

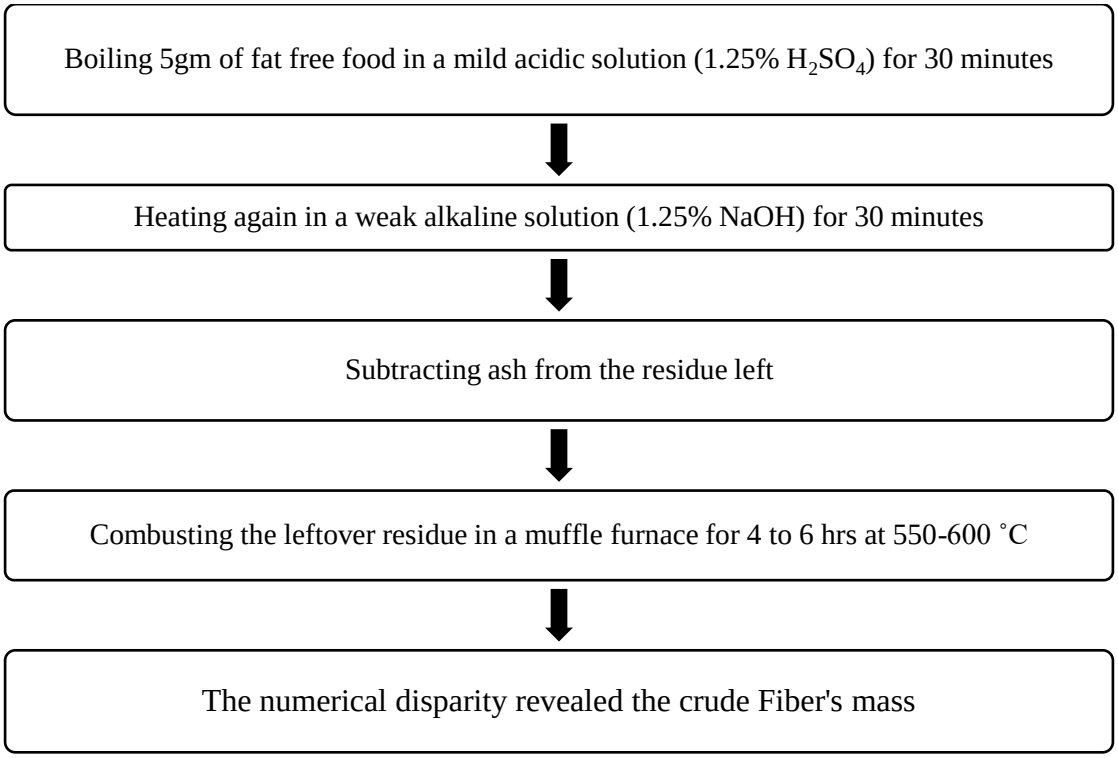

Figure 3.8: Crude Fiber Determination

Calculation: The following expression was employed to ascertain the fiber content

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

Here, W_1 = Weight of crucible, crude fiber and ash

W_2 =Weight of crucible and ash

W = Weight of sample

3.6.6 Estimation of Crude Protein

The Kjeldahl method finds application in quantifying nitrogen levels within both organic and inorganic specimens. This technique holds significance in assessing Kjeldahl nitrogen across various domains, including foods, beverages, meat, feeds, cereals, facilitating the computation of protein content. Beyond this, the Kjeldahl method extends its utility to nitrogen analysis in diverse contexts such as wastewaters, soils, and other sample types. By offering a reliable means to measure nitrogen content, the Kjeldahl method plays a pivotal role in diverse scientific and analytical endeavors.

Procedure

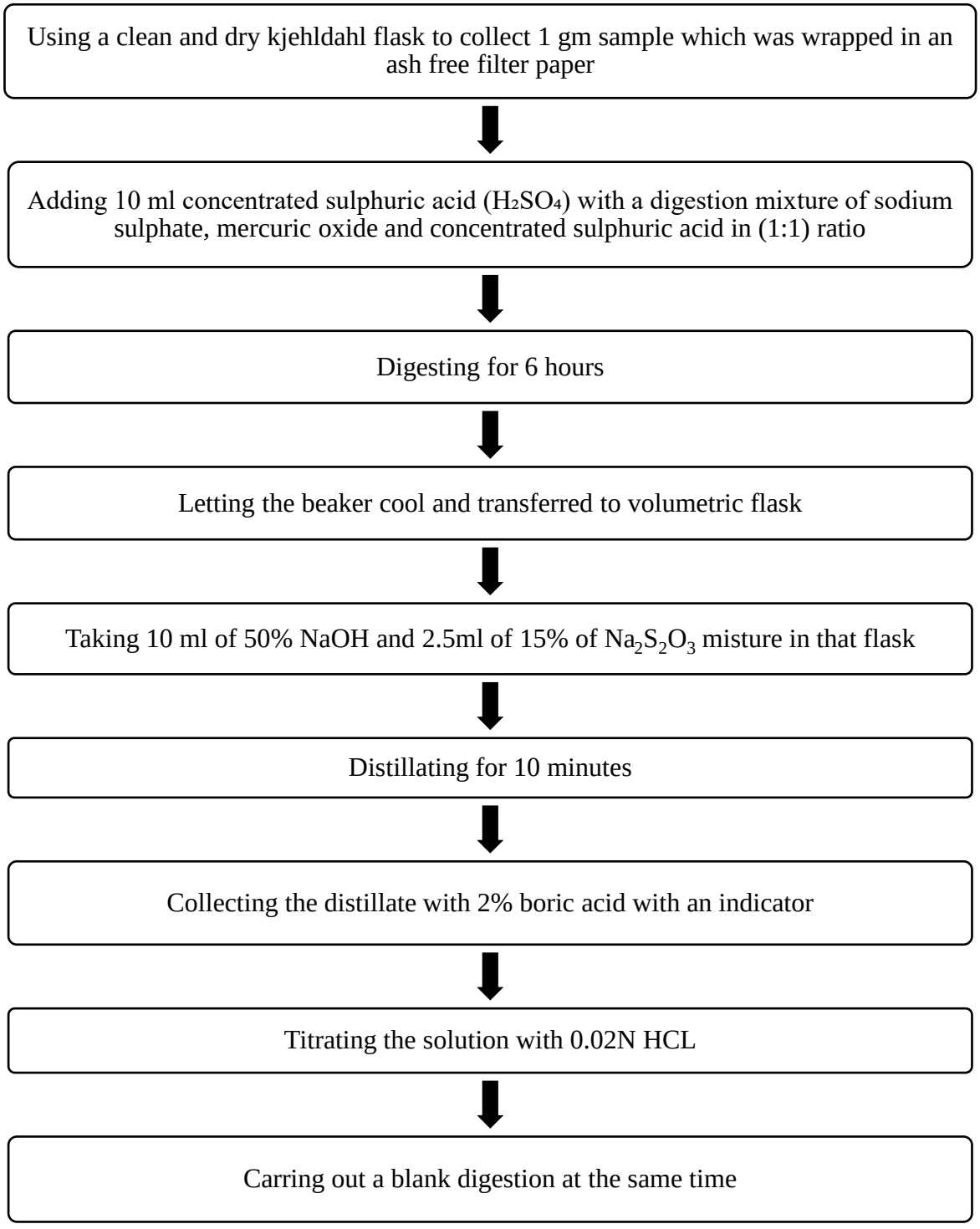

Figure 3.9: Crude Protein Determination

Calculation: The following expression was employed to ascertain the protein content

$$\text{Nitrogen \%} = \frac{(\text{ml of standard acid} - \text{ml of blank}) \times \text{N of acid} \times 1.4007}{\text{Sample Weight in gram}}$$

Protein % = Nitrogen % × 5.71 (for soy based meat)

3.6.7 Estimation of Total Carbohydrate

The carbohydrate content was established through a calculation involving the Nitrogen Free Extractive (NFE). This was achieved by taking 100 and subtracting the sum of the other proximate components. In essence, the NFE serves as a way to derive the carbohydrate content by accounting for the remaining constituents after accounting for other components.

Calculation: The following expression was employed to ascertain the carbohydrate content:

Carbohydrate % = 100% - (Protein + Fat + Fiber + Ash + Moisture content) %

3.7 Determination of Bioactive Compounds

Extract preparation

For the determination of Total Polyphenol Content (TPC) and Total Flavonoid Content (TFC), 1 gram of the sample was utilized. To extract the desirable compounds, a 10 ml volume of 100% ethanol was added to each of these material-filled tubes. These mixtures were left undisturbed for a duration of 72 hours, allowing the ethanol to interact with the sample and dissolve the target compounds. During this 72-hour period, the contents of the tubes underwent continuous straining at intervals of 4 hours. This process ensured that the extraction was thorough and efficient. Once the 72-hour extraction period had concluded, the resulting filtrate was carefully collected. This filtrate represented the ethanol extract, containing the compounds of interest that had been successfully extracted from the original material.

3.7.1 Total Phenolic Content (TPC)

The Folin-Ciocalteu (FC) reagent technique is adapted by Al-Owaisi et al., (2014) with some minor modifications to measure the Total Polyphenol Content (TPC) of the samples' extracts. The method was significantly adjusted which was initially described by Vergani et al., (2016) for assessing TPC in sample.

Here's a brief overview of their procedure:

1. **Sample Preparation**: 1 ml of ethanoic extract is taken from sample.
2. **Reagent Mixing**: The extract was mixed with 1.5 ml of the FC reagent in a falconer tube.

3. **Incubation:** The mixture was left at room temperature for three minutes to allow for the chemical reactions to occur.
4. **Addition of Na_2CO_3:** After the initial incubation, 1.5 ml of a 7.5% Na_2CO_3 solution is added to the tube.
5. **Settling Time:** The mixture was allowed to settle for 60 minutes, ensuring that all reactions were complete.
6. **Absorbance Measurement:** The absorbance of the solution was then measured at a wavelength of 765 nm using a UV-visible spectrophotometer (UV2600, Shimadzu Corporation, USA), with pure C_2H_5OH (ethyl alcohol) as the reference (blank).
7. **TPC Calculation:** Finally, Total Polyphenol Content (TPC) is calculated and expressed as milligrams of gallic acid equivalents (mg GAE/g) per gram of extracts.

3.7.2 Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the samples was evaluated with a slightly modified version of the aluminum chloride colorimetric method originally outlined by (C. C. Chang et al., 2002).

Here's a concise description of the procedure:

1. **Sample Preparation:** A stock solution of the extracts was prepared at a concentration of 1 mg/ml.
2. **Dilution:** Aliquots of 0.5 ml of the diluted extract were mixed with 1.5 ml of 95% C_2H_5OH in a cuvette.
3. **Reagent Addition:** To this mixture in the cuvette, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 mole/L potassium acetate, and 2.8 ml of distilled water were added.
4. **Incubation:** The cuvette was allowed to sit at room temperature for 30 minutes, permitting the chemical reactions to take place.
5. **Absorbance Measurement:** The absorbance of the mixture was measured using a UV-visible spectrometer. The cuvette containing 10% aluminum chloride was replaced with the equivalent volume of distilled water in the blank reference.

6. **Standard Curve:** To determine the total quantity of flavonoids in the sample, the absorbance of the extract was compared to a standard curve generated using quercetin as the reference standard.
7. **TFC Calculation:** The Total Flavonoid Content (TFC) was then calculated and reported as milligrams of quercetin equivalents (mg QE/g).

3.8 Determination of antioxidant capacity by DPPH scavenging method

Extract preparation

A Felcon tube was loaded with a 1-gram sample, to which 10 mL of absolute methanol was introduced. Allowing this blend to sit for 72 hours, regular straining every 4 hours was conducted. At the end of this period, the mixture was filtered, producing a methanoic extract showcasing the outcome of the experiment.

Procedure

Taking inspiration from a methodology slightly modified from (Rahim, 2010), the antioxidant capacity of the extracts was assessed through the DPPH test. In this technique, 6 mg of DPPH was dissolved in 100 mL of 100% methanol, forming a methanoic DPPH solution. The same solution was prepared by dissolving 6 mg of DPPH in 100 mL of 100% methanol.

To commence the test, the methanoic extract was mixed with 2 mL of the DPPH solution. After gently agitating the mixture, it was left to incubate in darkness for 30 minutes at room temperature. The absorbance was then gauged at 517 nm using a UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA).

For establishing a control reference, 1 mL of methanol was combined with 2 mL of the DPPH solution, with the methanol serving as a blank. The comparison of sample absorbance to the absorbance of the DPPH reference solution enabled the evaluation of scavenging capability.

The quantification of the antioxidant potential was executed using the subsequent formula:

$$\text{Inhibition \%} = \frac{(\text{Blank absorbance} - \text{Sample absorbance})}{\text{Blank absorbance}} \times 100$$

In this context, the standard used was Trolox, while the TEAC composite served as the calibration standard curve for Trolox equivalent antioxidant capacity. The outcomes of the analysis were presented in milligrams (mg) per 100 grams of powder on a dry

weight (DW) basis. This approach allowed for the determination of the antioxidant mobility of the examined extracts, providing insights into their potential health-promoting attributes.

3.9 Microbiological Analysis

3.9.1 Aerobic Plate Count (Bacterial Plate Count)

The Aerobic Plate Count (APC) serves as a vital indicator for assessing bacterial populations within a given sample. This metric is also known by various names, including Aerobic Colony Count (ACC), Standard Plate Count (SPC), Mesophilic Count, and Total Plate Count (TPC), all referring to the same concept.

The determination of Total Viable Bacterial Count (TVC) is achieved through the Standard Plate Count (SPC) technique, which is a subset of APC. The underlying principle of this test is based on an assumption that each bacterial cell will yield a visible colony when combined with agar enriched with appropriate nutrients. Importantly, APC is designed to target organisms that thrive in aerobic conditions within the mesophilic temperature range (approximately 25 to 40°C). It is essential to note that APC doesn't encompass the entirety of the bacterial population present in a sample.

While APC lacks the ability to differentiate between various types of bacteria, it plays a crucial role in several areas. It serves as a valuable tool for assessing organoleptic acceptability, ensuring sanitary quality, and monitoring adherence to good manufacturing practices. Furthermore, APC functions as an indicator of food safety, shedding light on the microbial activity within a product.

Additionally, APC can provide critical insights into the shelf-life of a food item and offer early indications of impending organoleptic changes. This multifaceted test is an essential component of quality control in the food industry, aiding in the maintenance of product integrity and safety (Banwart, 2012).

Sample preparation

The accuracy and reliability of analyzing and interpreting results depend significantly on the correct sampling method. It is crucial that the sample chosen truly represents the entire product mass. To achieve this, the samples were thoroughly mixed to ensure homogeneity. Subsequently, 25 grams of this well-mixed samples were measured into a 250 ml flask.

For sample dilution, Phosphate Buffer Saline (0.6 M KH_2PO_4, pH 7.2) was employed. Approximately 100 ml of this buffer saline was added to the flask and mixed thoroughly using a to-and-fro motion. The volume was then adjusted to the desired level using the same buffer water.

Maintaining sterility throughout the process is paramount. Therefore, all equipment, solutions, and tools utilized underwent sterilization by subjecting them to a temperature of 121°C for a duration of 15 minutes.

The resulting prepared sample was further diluted by a factor of 10, making it a 1×10-1 dilution or a stock solution for subsequent analysis. This meticulous sample preparation is fundamental in ensuring the accuracy and validity of the analytical process (Andrews, 1992).

Dilution

A series of dilutions was carried out using 9 ml blank solutions in the following manner:

- Initial Dilution (10^{-1}): 1 ml of the sample was mixed with 9 ml of blank solution (a 1:10 ratio), resulting in the first dilution (labeled as 10^{-1}).
- Mixing: After the initial dilution, the mixture was thoroughly mixed using a vortex mixer (labeled as 'c') to ensure uniformity.
- Subsequent Dilutions: From the 10^{-1} dilution, 1 ml of the mixture was transferred to the next tube and mixed effectively. This step created the 10^{-2} dilution.
- Repeat for Further Dilutions: The same process was repeated successively until reaching a 10^{-6} dilution, with each step involving the transfer of 1 ml from the previous dilution into the next tube, followed by thorough mixing.

Standard plate counts

A Standard Plate Count (SPC) was employed to assess the microbial levels in the prepared and stored samples. This data serves as a valuable indicator of food quality and can predict the product's shelf life.

To perform the SPC, the following steps were taken:

1. Using a sterile pipette, 1 ml of the diluted sample was carefully dispensed into each sterile empty petri-dish containing nutrient agar media (Plate count agar) maintained at a temperature of 45°C.

2. The plates were gently swirled on a flat surface to ensure even distribution of the sample within the agar.
3. After the media solidified, the plates were inverted and placed in an incubator set at 37°C for a duration of 24 hours.

This incubation period allows the microbes present in the samples to grow and form visible colonies on the agar plates. Subsequently, these colonies can be counted, providing valuable information about the microbial load in the samples and, by extension, their quality and potential shelf life (AOAC, 1990).

Calculating and Logging

Following the 24-hour incubation period, the plates were carefully examined for bacterial colonies. To ensure accuracy and ease of counting, plates with colonies that were well-separated, distinct, and easy to distinguish were chosen for analysis. Plates containing colonies that were segregated, overlapping, or confusing were excluded from the count.

The selection criteria aimed at plates with a colony count ranging from 30 to 250. Colonies falling within this range were considered bright, clear, and countable. These selected plates provided a representative sample for further analysis.

To calculate the number of colony forming units (CFU) per gram or milliliter, the following formula was applied:

Number of CFU/g or mL = Average CFU on selected plates × Dilution factor

The process of determining the viable bacterial count involved multiple steps, including sample preparation, sample dilution, performing standard plate counts, and finally, counting and recording the results. The incubation of plates occurred at 37°C for a duration of 24 hours, allowing the bacterial colonies to grow and become visible for accurate quantification (AOAC, 1990)

3.9.2 Fungal analysis

Media Preparation

Sabouraud Dextrose Agar (SDA) is a specialized growth medium. It's selective, favoring the growth of yeasts, dermatophytes, and various fungi, including filamentous bacteria like Nocardia. This selectivity arises from its mildly acidic pH (around 5.0), which inhibits bacterial growth. For enhanced effectiveness, antibacterial agents can be added.

The medium itself is nutrient-rich, comprising 10g Mycological peptone (an enzymatic digest of casein and animal tissues), 40g Dextrose, and 15g Agar per liter, with a pH of 5.6 at 25°C. It provides fungi and yeasts with the necessary amino acids and nitrogenous compounds for growth.

To maintain sterility, all media are autoclaved at 121°C for 15 minutes following the manufacturer's guidelines. Notably, unlike many selective agars for mold and yeast cultures, SDA places rigorous nutritional requirements for growth.

In summary, Sabouraud Dextrose Agar is a versatile medium supporting a wide range of fungal strains, including Nocardia and dermatophytes. It's defined by its selective properties, nutrient-rich composition, and adherence to established protocols for consistent results (Raton et al., 1998); (Safety et al., 2012).

Procedure of media preparation

To prepare Sabouraud Dextrose Agar (SDA) for culturing, 65 grams of the medium were first suspended in one liter of purified water. This mixture was then diligently dissolved by heating, all while being stirred continuously for approximately one minute.

Afterward, the prepared medium underwent autoclaving at a high temperature of 121°C for a duration of 15 minutes. This sterilization process ensured that the medium was free from contaminants.

Once sterilized, the mixture was allowed to cool to a temperature range of 45°C to 50°C. It was only after reaching this specific temperature range that the medium was dispensed into petri dishes.

To achieve the isolation of colonies, a sterile inoculating loop was used to streak the material onto the SDA medium. This step is crucial for obtaining well-defined and isolated colonies of the target organisms.

The petri dishes were then carefully placed in an incubator, positioned upside down (agar side up), and maintained at a temperature between 25°C and 30°C. High humidity conditions were maintained to create an optimal environment for the growth of fungi and yeasts.

Monitoring for the development of fungal cultures was conducted on a weekly basis. It's important to note that cultures were stored for a duration of 4 to 6 weeks before being declared as negative. This storage period allows sufficient time to observe and confirm the absence of fungal growth in the culture.

In summary, this method outlines the steps involved in preparing and culturing samples using Sabouraud Dextrose Agar, emphasizing the importance of proper sterilization, isolation techniques, and regular monitoring during the incubation period.

Interpretation

SDA plates should be examined for:

- Isolated Colonies: Distinct fungal colonies in streaked areas should be looked.
- Confluent Growth: Dense fungal growth in heavily inoculated regions should be observed.
- Color and Morphology: The color (creamy to white for yeast, multicolored for molds) and overall appearance of colonies should be noted.
- Additional Confirmation: Additional tests like microscopy or biochemical assays should be considered for conclusive results.

3.10 Energy Estimation

The energy content of the samples was calculated based on the protein, fat, and carbohydrate content of the food item. This calculation was performed using the formula described in a study by (Baer et al., 1997).

$$\text{Energy Content} = (\text{Protein} \times 4.1) + (\text{Fat} \times 9.2) + (\text{Carbohydrate} \times 4.1)$$

3.11 Cost Analysis

The price of the product was determined by considering the overall cost of the ingredients required for its manufacturing. The total cost was expressed in the local currency, taka. To make it more standardized and informative, the price per 100 grams of the product was also calculated. This allows for a consistent and easily comparable measure of the product's cost.

3.12 Sensory Evaluation

Sensory evaluation played a pivotal role in the quest to create a finished product that would resonate with consumers. This crucial step involved subjecting the creation to

the discerning palates of a tasting panel, carefully selected from the academic community of CVASU, comprising both esteemed teachers and eager students. The product, soy meat and gluten meat underwent rigorous scrutiny by a panel of 15 individuals. Each panelist was presented with two products, discreetly labeled as samples A and sample B. Their mission was to assess and rate these formulations across a spectrum of sensory attributes, including appearance, aroma, texture, taste, and overall acceptability. Each rating was a reflection of the product's performance in these critical dimensions. While it is acknowledged that this panel's evaluation may not perfectly mirror consumer sentiment, it undeniably shed light on the essential qualities that a superior product must possess. By scrutinizing these elements through the eyes, taste buds, and judgments of the panelists, the aim was to craft a product that stands at the pinnacle of consumer satisfaction (Drake, 2021)**.**

3.12.1 Affective test

In the realm of food product success, consumer preference reigns supreme. To discern this preference effectively, consumer panels serve as reliable barometers, tasked with the crucial mission of singling out the favored choice among competing samples. Each panelist articulates their preference by assigning ratings based on specific quality criteria outlined on a score sheet. Employing hedonic rating scales adds a quantitative layer, allowing us to measure the sheer pleasure derived from each sample, providing valuable insights into sensory delight. Beyond preference, we delve into the realm of acceptability, gauging the frequency with which panelists would choose to savor a particular sample—a key indicator of a product's potential to captivate consumer palates and thrive in the marketplace.

The tasters tried two samples and shared their thoughts by rating them. They used a scoring system to evaluate different aspects like taste, appearance, flavor, texture, sweetness, and overall likability of the two samples. This evaluation was done using a five-point Hedonic scale, which helps measure how much they liked or disliked each aspect of the samples (Drake, 2021). The scale was created in a way that:

Table 3.1: Rating scale for sensory evaluation

Ranks	Scores
Like very much	5
Like moderately	4
Neutral	3
Dislike moderately	2
Dislike very much	1

3.13 Statistical Analysis

The data was initially stored in Microsoft Excel 2016 and later analyzed using IBM SPSS Statistics Version 26. The results are presented as Mean ± SD (Standard Deviation). A one-way ANOVA was conducted to assess overall group differences, and Tukey's pairwise comparison analysis determined which specific groups were significantly different. A significance level of $P<0.05$ was used, indicating that differences observed are statistically significant.

Chapter 04: Result

4.1 Nutritional Attributes

Nutritive value of Soy meat and Gluten meat is shown in Table 4.1. Soy meat contains highest percentage of crude fiber (0.08±0.03%), ash (0.930±0.04%) and crude fat (5.50±0.03%). On the other hand, gluten meat contains highest percentage of crude protein (16.39±0.04%).

Table 4.1: Proximate analysis report showing nutritional composition

Sample name	**Soy Meat**	**Gluten Meat**
Dry matter (%)	24.54 ± 0.06^{b}	27.64 ± 0.04^{a}
Moisture (%)	75.48 ± 0.03^{a}	72.41 ± 0.06^{b}
Fiber (%)	0.08 ± 0.03^{a}	0.04 ± 0.03^{b}
Ash (%)	0.93 ± 0.04^{a}	0.30 ± 0.02^{b}
Fat (%)	5.50 ± 0.03^{a}	0.12 ± 0.01^{b}
Protein (%)	13.64 ± 0.04^{b}	16.39 ± 0.04^{a}

Values are means ± SD of triplicate determination. Values in the same column with the different superscripts differ significantly ($P<0.05$).

4.2 Bioactive Components

Several tests were performed to identify bioactive components value difference between soy meat and gluten meat and this result has been presented in table 4.2. This table shows that soy meat sample showed highest Total Phenolic Content (2.40 mg GAE /100gm) and Total Antioxidant (3.11 mg TE/100gm). On the other hand, gluten meat sample showed highest Total Flavonoid Content (14.00 mg QE/100gm). Each of the parameters has shown significantly different value in soy meat and gluten meat sample.

Table 4.2 Bioactive compound analysis of Soy meat and Gluten meat

Sample	Total Phenolic Content (mg GAE/ 100gm)	Total Flavonoid Content (mg QE/100gm)	Total Antioxidant (mg TE/100gm)
Soy meat	2.40 ± 0.02^{a}	11.06 ± 0.07^{b}	3.11 ± 0.00^{a}
Gluten meat	1.16 ± 0.01^{b}	14.00 ± 0.07^{a}	2.59 ± 0.02^{b}

Values are means ± SD of triplicate determination. Values in the same column with the different superscripts differ significantly ($P<0.05$).

4.3 Microbial Analysis

In Table 4.3, the assessment of total viable count and fungal count in soy meat and gluten meat samples stored at 4°C for 15 days showed consistently negligible results. Notably, no yeast or mold presence was detected both at the time of production and after the 15-day storage period, with a total viable count registering at zero.

Table 4.3: Microbiological evaluation of Soy meat and Gluten meat

Sample	TVC (CFU/ml)		Yeast and Mold	
	1^{st} day	15^{th} day	1^{st} day	15^{th} day
Soy Meat	No growth	No growth	No growth	No growth
Gluten Meat	No growth	No growth	No growth	No growth

4.4 Energy Estimation

From the figure 4.1, Energy content in Soy Meat was calculated in highest amount (124.56 kcal/100g) and lowest (112.481 kcal/100g) in Gluten Meat.

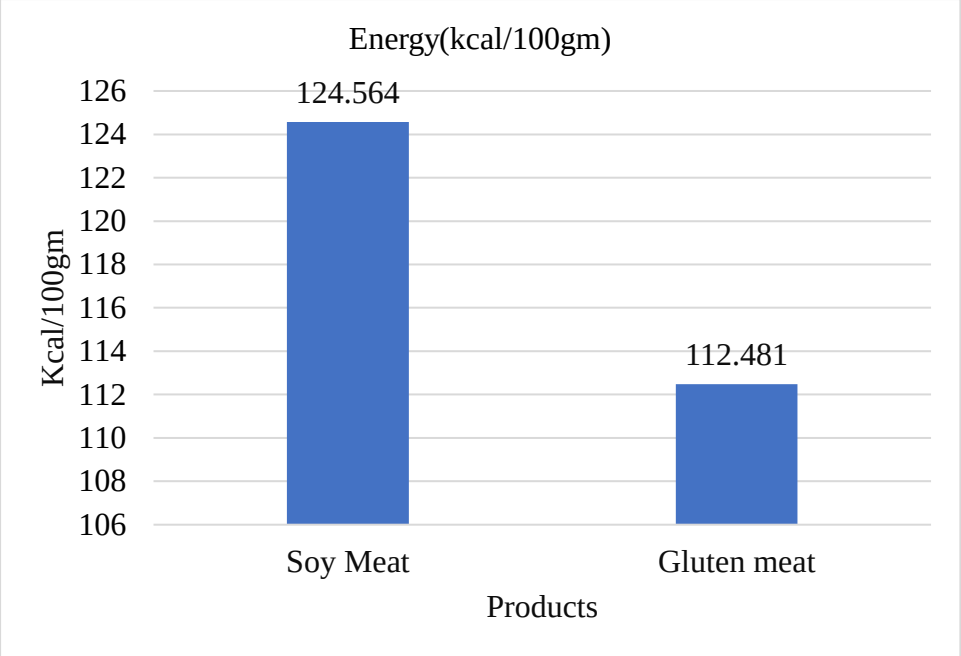

Figure 4.1: Comparison of energy content between Soy meat and Gluten Meat

4.5 Cost Analysis

Table 4.4 Production cost of Soy Meat

Heads	**Tk/kg**	**Quantity used (kg/1kg soy meat)**	**Total Tk**
1. Expenditure of raw material			
Soybean	130	0.715	92.95
Baking Soda	1334	0.007	9.34
Vinegar	132	0.043	5.68
Subtotal			107.97
2. Processing cost (15% of raw material)			16.20
3. Packaging Cost			40
Total production cost of 1kg Soy Meat			164.17

So, Price of 100gm Soy meat = 164.17/10 taka

= 16.4 taka

Table 4.5 Production cost of Gluten Meat

Heads	**Tk/kg**	**Quantity used (kg/1kg gluten meat)**	**Total Tk**
1. Expenditure of raw Material			
Flour	70	2.4	168
Soy-sauce	280	0.1	28
Salt	50	0.1	5
Bay leaves	600	0.02	12
Turmeric Powder	450	0.05	22.5
Subtotal			235.5
2. Processing cost (15% of raw material)			35.33
3. Packaging Cost			40
Total production cost of Gluten Meat			310.83

So, Price of 100gm Gluten Meat = 310.83/10 taka

= 31.08 taka

4.6 Sensory Evaluation

Soy meat received the most positive feedback overall. Gluten meat (except taste and texture) had the lowest level of acceptability in table 4.6 when compared to the other samples.

Table 4.6: Hedonic rating test for sensory evaluation of Soy meat and Gluten Meat

Sample	**Appearance**	**Aroma**	**Taste**	**Texture**	**Overall Acceptability**
Soy Meat	4.30 ± 0.78^{a}	4.64 ± 0.51^{a}	3.33 ± 0.65^{b}	3.66 ± 0.69^{b}	4.10 ± 0.63^{a}
Gluten Meat	3.26 ± 0.64^{b}	3.70 ± 0.54^{b}	3.78 ± 0.74^{a}	4.01 ± 0.81^{a}	3.43 ± 0.52^{b}

Values are means ± SD of triplicate determination. Values in the same column with the different superscripts differ significantly ($P<0.05$).

Chapter 05: Discussion

5.1 Nutritional Attributes

Table 4.1 shows the approximate nutritional composition of soy meat and gluten meat. According to the data of proximate analysis moisture content of soy meat (75.48%) is higher than gluten meat (72.41%). Beef meat from prior research showed 76.14%, and chicken had 75.13% (Karakok et al., 2010). Previous study done by researchers showed that moisture content in soy meat was 79.9% which is larger value than found in this study (Obatolu, 2007). Soy meat starts with soybeans, which have a natural water content, and retains some of this moisture during processing due to its high water-holding capacity. In contrast, gluten meat is derived from wheat gluten, which is processed to remove moisture, resulting in a lower moisture content in the final product.

The amount of crude fiber found in the soy meat (0.08%) is almost two times higher than the gluten meat (0.04%). During the measurement of ash content, it was found that the soy meat contains more ash percentage (0.93%) than the gluten meat (0.30%). That is almost three times the gluten meat. It is a reasonable presumption that, the divergence in ash content between soy meat and gluten meat is primarily rooted in their ingredient compositions and processing methods. Soy meat, originating from mineral-rich soybeans and the use of coagulants, naturally yields a higher ash content, while gluten meat, made from low-mineral wheat gluten with thorough starch removal, maintains a lower ash content.

One of the crucial criteria for quality control in many food items is fat content (Guthausen et al., 2004). According to this study, soy meat exhibits a significant fat content of 5.50%, a marked contrast to gluten meat, which contains only 0.12% fat. In previous research, beef meat had a fat content of 1.27%, while chicken meat had a fat content of 1.82% (Karakok et al., 2010). The process of making gluten meat involves washing wheat flour dough to remove starch, leaving behind the gluten. Since gluten is derived from wheat flour, which has very little fat, gluten meat has a low-fat content as well.

Crude protein content in soy meat was found 13.64% in a previous study which is lower than the value found in this investigation (Obatolu, 2007). In an earlier study, the protein of beef meat and chicken meat was found to be 22.36% and 22.33% respectively (Karakok et al., 2010). It could be assumed that, in making gluten meat, wheat flour

dough is prepared, and the starch is washed away, leaving behind the concentrated gluten. This process effectively isolates the protein, resulting in a product that is almost pure protein.

5.2 Bioactive Compounds

Bioactive chemicals are critical for supporting the immune system and avoiding chronic diseases in humans, in addition to supplying vital nutrition. (Liu & Hotchkiss, 1995). Hence, the precise measurement and analysis of these compounds hold significant importance. The data illustrating the content of bioactive compounds in products can be found in Table 4.2.

Studies have demonstrated a strong association between diets rich in polyphenols and a lower risk of several cancers, cardiovascular disorders, diabetes, and neurological conditions (Cory et al., 2018). The polyphenol content is almost double (2.40±0.02 mg GAE/100gm) in soy meat rather than gluten meat in this study. Soybeans, the main ingredient in soy meat, contain phenolic compounds, especially isoflavones (Kalaiselvan et al., 2010) which contribute to its higher phenolic content. Wheat flour, the main ingredient in gluten meat, does not naturally contain significant levels of phenolics, and the washing process during gluten meat production is assumed to be further reduced the phenolic content.

Flavonoids have been the subject of extensive research in the fields of human and animal health, revealing a diverse array of biological and pharmacological activities. These compounds have demonstrated significant anti-inflammatory, antioxidant, antimicrobial, and anti-cancer properties, showcasing their potential therapeutic benefits (Mouradov & Spangenberg, 2014). In our study, total flavonoid content of gluten meat had the highest value (14.00±0.07 mg QE/100gm) and soy meat showed the least value.

5.3 Antioxidant Capacity

It's evident that there is a notable variance in antioxidant capacity among all the samples presented in Table 4.2. The use of DPPH as a substrate for assessing antioxidant activity is widespread, particularly in the study of free radical scavenging abilities of both biological and chemical compounds. In this study, soy meat, has the maximum (3.11 ±0.00 mg TE/100 g) antioxidant capacity than gluten meat (2.59±0.02 mg TE/100g).

5.4 Microbial Analysis

Obtained data in Table 4.3 showed that yeasts and molds, bacteria were not detected in all the samples throughout the storage period. So, when stored at a temperature of 4˚C, the prepared samples maintained their safety for consumption for a period of up to 15 days. Mold, as noted by (Muck, 2010), thrives in aerobic conditions and struggles in oxygen-depleted environments. In contrast, yeast exhibits versatility, flourishing in both aerobic and anaerobic settings. Moreover, the pH requirements for yeast and mold vary significantly, spanning from pH 2 to well beyond pH 9 across different food products. Heat treatment plays a pivotal role in diminishing the presence of potentially harmful microorganisms while simultaneously extending the shelf life of soy-based products (El-Boraey et al., 2015). The same principle applies to gluten-based meat substitutes, where boiling during processing steps serves a similar purpose.

5.5 Sensory Evaluation

Data in Table 4.6 show that the sensory test of soy meat and gluten meat proved the superiority of soy meat compared to gluten meat sample for appearance, aroma and overall acceptability. The sensory panel test corroborated a pattern consistent with (El-Boraey et al., 2015) findings regarding the presence of a beany taste and flavor. These attributes, as highlighted by (Murugkar, 2014), undeniably the presence of a beany flavor play a pivotal role in shaping consumers' preferences for soy-based products, influencing their likelihood of liking or disliking these products.

Chapter 06: Conclusion

Plant-based foods made from grains and pulses have long served as important sources of protein. Due to their high protein content, soy meat and gluten meat make great meat alternatives. An assortment of innovative food items made from a variety of raw materials, reflecting a global shift towards sustainable and plant-centric diets, support this emerging trend. In summary, this research project aimed to develop and evaluate plant-based high-protein meat alternatives with a focus on creating Bangladeshi-style soy and gluten meats mirroring the sensory characteristics of traditional meat dishes. Proximate analysis unveiled key differences in nutritional composition, with soy meat having higher moisture and fat content, and gluten meat boasting more protein due to distinct processing methods. The assessment of bioactive components revealed that soy meat had a greater polyphenol content, while gluten meat displayed higher flavonoid levels. Antioxidant capacity tests showed soy meat's superior antioxidant properties. Microbial analysis confirmed the safety of both products during storage with no detection of yeasts, molds, or bacteria, underscoring the importance of heat treatment during processing. Sensory evaluations favored soy meat for appearance, aroma, and overall acceptability, despite a beany taste inherent to soy-based products. This research contributes to plant-based food science, offering a culturally resonant alternative and highlighting the health potential of bioactive compounds. The findings underscore the importance of sensory appeal in consumer acceptance, promoting the adoption of these plant-based high-protein meat alternatives tailored to local preferences.

Chapter 07: Recommendations and Future Perspectives

Plant-based meat alternatives were not as widely available as traditional meat products in Bangladesh. They were primarily found in select urban areas and specialty stores. Some local entrepreneurs and startups had started to explore plant-based meat production and distribution, although on a relatively small scale. The adaptation of plant-based alternatives to fit into the diverse and culturally rich Bangladeshi cuisine was seen as a crucial factor for success.

The following recommendations and perspective are offered for the continued research effort in light of the findings of the current investigation:

1. The experimental results may be supported by further research.
2. The microbiological testing duration can be extended for the plant-based high-protein meat alternatives to a range of 30 to 90 days for more comprehensive evaluation of the products' shelf life.
3. Further research can be focused on improving their taste, texture, and sensory attributes to enhance the quality and consumer appeal of soy and gluten meat products.
4. Comprehensive studies can be conducted on the nutritional impact of soy and gluten meat consumption, including long-term health effects.
5. Mineral content and amino acid profile of soy meat and gluten meat can be analyzed.
6. Functional ingredients that can be incorporated into soy and gluten meat products to enhance their nutritional value can be researched. This may include fortifying these products with vitamins, minerals, or bioactive compounds to meet specific health goals.
7. The use of locally available plant-based ingredients, such as lentils, chickpeas, and rice can be investigated to develop cost-effective and culturally relevant plant-based meat alternatives
8. Consumer acceptance studies in Bangladesh can be conducted to understand the factors influencing the adoption of plant-based meat alternatives within the local context.

REFERENCE

Ahmad, M., Qureshi, S., Akbar, M. H., Siddiqui, S. A., Gani, A., Mushtaq, M., Hassan, I., & Dhull, S. B. (2022). Plant-based meat alternatives: Compositional analysis, current development and challenges. *Applied Food Research*, *2*(2), 100154.

Al-Owaisi, M., Al-Hadiwi, N., & Khan, S. A. (2014). GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves. *Asian Pacific Journal of Tropical Biomedicine*, *4*(12), 964–970.

Alam, M., Biozid, S., Faruk, M., Abeden, M., Ferdous, K. U., Nitul, I., & Islam, M. (2020). *Anti-oxidant activity of methanolic extract of aquatic flowering plant Nymphaea capensis leaf*.

Andrews, W. (1992). Manual of food quality control. 4. Rev. 1. Microbiological analysis. Food and Drug Administration. *FAO Food and Nutrition Paper*, *14*(4 Revis 1), 1–338.

AOAC 1990. (1990). No More Riffling With a. *75 Years of Reporting Analytical Science 1915-1990*, *73*(1), 1–192.

AOAC International. (2016). Official methods of analysis of AOAC International. *Association of Official Analysis Chemists International*, 3172. https://www.techstreet.com/standards/official-methods-of-analysis-of-aoac-international-20th-edition-2016?product_id=1937367

Baer, D. J., Rumpler, W. V, Miles, C. W., & Fahey, G. C. (1997). Human and Clinical Nutrition Dietary Fiber Decreases the Metabolizable Energy Content and Nutrient Digestibility of Mixed Diets Fed to Humans 1. *Journal of Nutrition*, *127*(4), 579–586.

Banwart, G. (2012). *Basic Food Microbiology*. Springer US.

Beavers, D. P., Beavers, K. M., Miller, M., Stamey, J., & Messina, M. J. (2012). Exposure to isoflavone-containing soy products and endothelial function: A Bayesian meta-analysis of randomized controlled trials. *Nutrition, Metabolism, and Cardiovascular Diseases : NMCD*, *22*, 182–191.

Biesiekierski, J. R. (2017). What is gluten? *Journal of Gastroenterology and Hepatology*,

32(S1), 78–81.

Chan, Y.-H., Lau, G., Yiu, K.-H., Li, L., Chan, H.-T., Fong, D., Tam, S., Lau, C.-P., & Tse, H.-F. (2008). Reduction of C-reactive protein with isoflavone supplement reverses endothelial dysfunction in patients with ischaemic stroke. *European Heart Journal*, *29*, 2800–2807.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, *10*(3), 178–182.

Chang, K. C. (2005). Chemistry and technology of tofu making. *Handbook of Food Science, Technology, and Engineering - 4 Volume Set*, 2969–2992.

Chapman, D. J., & Ragan, M. A. (1980). Evolution of Biochemical Pathways: Evidence From Comparative Biochemistry. *Annual Review of Plant Physiology*, *31*(1), 639–678.

Choudhury, D., Singh, S., Seah, J. S. H., Yeo, D. C. L., & Tan, L. P. (2020). Commercialization of Plant-Based Meat Alternatives. *Trends in Plant Science*, *25*(11), 1055–1058.

Consultation, J. F. W. (2007). Protein and amino acid requirements in human nutrition. *World Health Organization Technical Report Series*, *935*, 1–265, back cover.

Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Frontiers in Nutrition*, *5*.

Dagevos, H., & Voordouw, J. (2013). Sustainability and meat consumption: is reduction realistic? *Sustainability: Science, Practice and Policy*, *9*(2), 60–69.

Dai, J., & Mumper, R. (2010). Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules (Basel, Switzerland)*, *15*, 7313–7352.

Day, L. (2011). Wheat gluten: production, properties and application. In *Handbook of Food Proteins*. Woodhead Publishing Limited.

Dey, A. (2017). Tofu: Technological and Nutritional Potential. *Indian Food Industry Magazine*, *36*(3), 8–24.

Don, C., Lichtendonk, W. J., Plijter, J., & Hamer, R. (2003). Glutenin Macropolymer: a Gel Formed by Glutenin Particles. *Journal of Cereal Science*, *37*, 1–7.

Drake, M. A. (2021). Sensory evaluation. *Encyclopedia of Dairy Sciences: Third Edition*, *2*, 572–576.

El-Boraey, N., Elashrey, H., & Ismail, M. (2015). Chemical Composition, Sensory Evaluation, Rheological Properties and Starter Activity of Admixtures of Buffalo's, Cow's and Soymilk. *American Journal of Food Science and Nutrition Research*, *2*, 119–127.

Eze, N., Okwume, G., Eseadi, C., Udenta, E., Onyeke, N., Ugwu, E., Akubue, B., Njoku, H., & Ezeanwu, A. (2018). Acceptability and consumption of tofu as a meat alternative among secondary school boarders in Enugu State, Nigeria: Implications for nutritional counseling and education. *Medicine*, *97*, e13155.

Gilani, G., Xiao, C.-W., & Cockell, K. (2012). Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on Protein Quality. *The British Journal of Nutrition*, *108*, S315–S332.

Griesbach, R. (2005). *Biochemistry and genetics of flower color* (Vol. 25, pp. 25:89-114.).

Grubešić, R., Vuković, J., Kremer, D., & Vladimir-Knežević, S. (2007). Flavonoid content assay: Prevalidation and application on Plantago L. Species. *Acta Chimica Slovenica*, *54*, 397–406.

Guthausen, A., Guthausen, G., Kamlowski, A., Todt, H., Burk, W., & Schmalbein, D. (2004). Measurement of fat content of food with single-sided NMR. *Journal of the American Oil Chemists' Society*, *81*, 727–731.

Harborne, J., & Williams, C. (2000). Harborne JB, Williams CAAdvances in flavonoid research since 1992. Phytochemistry 55:481-504. *Phytochemistry*, *55*, 481–504.

He, J., Evans, N., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. *Comprehensive Reviews in Food Science and Food Safety*, *19*.

Hodgkinson, S., Montoya, C., Scholten, P., Rutherfurd, S., & Moughan, P. (2018). Cooking Conditions Affect the True Ileal Digestible Amino Acid Content and Digestible Indispensable Amino Acid Score (DIAAS) of Bovine Meat as

Determined in Pigs. *The Journal of Nutrition*, *148*.

Kalaiselvan, V., Kalaivani, M., Vijayakumar, A., Sureshkumar, K., & Venkateskumar, K. (2010). Current knowledge and future direction of research on soy isoflavones as a therapeutic agents. *Pharmacognosy Reviews*, *4*(8), 111–117.

Karakok, S. G., Ozogul, Y., Saler, M., & Ozogul, F. (2010). Proximate analysis. Fatty acid profiles and mineral contents of meats: A comparative study. *Journal of Muscle Foods*, *21*(2), 210–223.

Kumar, P., Chatli, M. K., Mehta, N., Singh, P., Malav, O. P., & Verma, A. K. (2017). Meat analogues: Health promising sustainable meat substitutes. *Critical Reviews in Food Science and Nutrition*, *57*(5), 923–932.

Kumar, S. (2016). Meat Analogs "Plant based alternatives to meat products: Their production technology and applications." *Critical Reviews in Food Science and Nutrition*, 0.

Liu, R. H., & Hotchkiss, J. H. (1995). Potential genotoxicity of chronically elevated nitric oxide: A review. *Mutation Research/Reviews in Genetic Toxicology*, *339*(2), 73–89.

Macritchie, F. (1992). *Physicochemical Properties of Wheat Proteins in Relation to Functionality* (J. E. B. T.-A. in F. and N. R. Kinsella (Ed.); Vol. 36, pp. 1–87). Academic Press.

Mouradov, A., & Spangenberg, G. (2014). Flavonoids: A metabolic network mediating plants adaptation to their real estate. *Frontiers in Plant Science*, *5*(November), 1–16.

Muck, R. (2010). Silage microbiology and its control through additives. *Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science - REV BRAS ZOOTECN*, *39*.

Murugkar, D. (2014). Effect of sprouting of soybean on the chemical composition and quality of soymilk and tofu. *Journal of Food Science and Technology*, *51*.

Nakata, T., Kyoui, D., Takahashi, H., Kimura, B., & Kuda, T. (2017). Inhibitory effects of soybean oligosaccharides and water-soluble soybean fibre on formation of putrefactive compounds from soy protein by gut microbiota. *International Journal of Biological Macromolecules*, *97*, 173–180.

Obatolu, V. (2007). Effect of different coagulants on yield and quality of tofu from soymilk. *European Food Research and Technology*, *226*, 467–472.

OECD-FAO Agricultural Outlook 2018-2027 Middle East and North Africa. (2018).

Rahim, K. (2010). *Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves 1*. *1084*, 1077–1083.

Rahman, M., & Sumon, H. H. (2018). Meat Adulteration at Khulna City of Bangladesh: A Criminological Analysis. *International Journal of Humanities, Social Sciences and Education*, *5*(4), 109–115.

Raton, B., Microbiology, M. F., Examiner, P., Witz, J. C., Examiner, A., & Hanley, S. (1998). *United States Patent (19). 19*.

Safety, F., Authority, S., Health, M. O. F., Welfare, F., & Delhi, N. E. W. (2012). *Manual of methods of analysis of foods microbiological testing*.

Samanta, A., Das, G., & Das, S. (2011). Roles of flavonoids in Plants. *International Journal of Pharmaceutical Science and Technology*, *6*, 12–35.

Shalini, K. V., Sanjitha, S., Santhiya, S., Vasuki, V., & Kalai, S. S. (2021). *An overview of Tofu: History, Types, manufacturing process and Health benefits*. *23*(10), 505.

Smetana, S., Mathys, A., Knoch, A., & Heinz, V. (2015). Meat Alternatives: Life Cycle Assessment of Most Known Meat Substitutes. *The International Journal of Life Cycle Assessment*, *20*.

Takahashi, A., & Ohnishi, T. (2004). The significance of the study about the biological effects of solar ultraviolet radiation using the Exposed Facility on the International Space Station. *Biological Sciences in Space = Uchū Seibutsu Kagaku*, *18*(4), 255–260.

Tziva, M., Negro, S. O., Kalfagianni, A., & Hekkert, M. P. (2020). Understanding the protein transition: The rise of plant-based meat substitutes. *Environmental Innovation and Societal Transitions*, *35*(July 2019), 217–231.

Uthayakumaran, S., Newberry, M., Phan-Thien, N., & Tanner, R. (2002). Small and large strain rheology of wheat gluten. *Rheologica Acta*, *41*, 162–172.

Vergani, L., Vecchione, G., Baldini, F., Voci, A., Ferrari, P. F., Aliakbarian, B., Casazza,

A. A., & Perego, P. (2016). Antioxidant and hepatoprotective potentials of phenolic compounds from olive pomace. *Chemical Engineering Transactions*, *49*, 475–480.

Verma, S., Upadhyay, S., Chandra, R., & Paul, A. (2013). PREPARATION OF PROCESSED CHEESE SPREAD USING TOFU, MOZZARELLA AND CHEDDAR CHEESE. *INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES*, *Volume 2*, 19–23.

Williams, A., & Hill, L. (2017). Meat and Nicotinamide: their causal role in our evolution, history and demographics. *International Journal of Tryptophan Research*, *10*.

Zheng, L., Regenstein, J. M., Teng, F., & Li, Y. (2020). Tofu products: A review of their raw materials, processing conditions, and packaging. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 3683–3714.

Zilic, S. (2013). Wheat gluten: Composition and health effects. *Gluten: Sources, Composition and Health Effects*, *April 2013*, 71–86.

Appendices

Appendix A: Preparation of Soy meat

Raw Soybean

Soaking using 0.5% $NaHCO_3$

Wet Grinding

Straining

Soymilk

Okra (by product)

Curd Formation

Pressing

Soy Meat

Appendix B: Preparation of Soy meat

Weighing flour

Dough

Soaking dough in water

Shaping gluten into round

Cooking in spice broth

Gluten meat

Brief Biography

Nishat Tasnim Oishee passed the Secondary School Certificate Examination in 2013 with a grade point average (GPA) of 5.00 from Bangladesh Mahila Samiti Girls' High School, Chattogram, and then Higher Secondary Certificate Examination in 2015 with a grade point average (GPA) of 4.83 from Chattogram College, Chattogram. She obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh in 2019 (held in 2020). Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition (CVASU). Her passion lies in fostering better health by offering tailored guidance and recommendations while also elevating public awareness about nutrition and food safety. She is deeply dedicated to empowering individuals with the information and insights needed to make informed choices for their well-being.