PROCESSING AND STRUCTURING THE MEAT ANALOGUE BY USING PLANT-BASED DERIVED COMPOSITES

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

Astha Singh (181805) & Anirudh Joshi (181833)

Under the guidance of

Dr. Garlapati Vijay Kumar

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

DEPT. OF BIOTECHNOLOGY AND BIOINFORMATICS

MAY-2022

DECLARATION

I decree hereby that the work presented in this report entitled **"Processing and Structuring the meat analogue by using plant-based derived composites"** in partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted in the department of Biotechnology, Jaypee University of Information Technology, Waknaghat is a real record of my own work carried out over a period from August 2021 to May 2022 under the supervision of Dr Garlapati Vijay Kumar.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

(Astha Singh, 181805) **(Anirudh Joshi, 181833)**

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled **"Processing and Structuring the meat analogue by using plant-based derived composites"**, submitted by Astha Singh(181805) and Anirudh Joshi(181833) at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of their original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

(**Signature of Supervisor**)

Dr. Garlapati Vijay Kumar

Associate Professor

Department of Biotechnology and Bioinformatics

JUIT, HP-173234,India.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

We would like to take this moment to share our sincere appreciation and profound respects to our mentor Dr. Garlapati Vijay Kumar (Associate Professor, BT/BI) for his excellent direction, tracking and relentless encouragement over the course of this project.

From time to time, the blessing, support and encouragement provided by him will take me a long way on the path of life we are about to embark on.

We are also obligated to JUIT College and the staff members for the knowledge they provide in their respective fields during the time of our assignment. We are thankful for their cooperation.

Finally, We thank the Almighty for his relentless support, my parents and my classmates, without whom this victory would not have been possible.

(Astha Singh, 181805) **(Anirudh Joshi, 181833)**

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

RPI:	Rice protein Isolate
PPN:	Plant based Nuggets
IBD:	Inflammatory bowel disease
(LxWxH):	Length x Width x Height
BSA:	Bovine serum albumin
%:	Percentage
µl:	microlite
Na_2CO_3:	Sodium carbonate
NaOH:	Sodium hydroxide
NaK Tartrate:	Sodium potassium tartrate
$CuSO_4.5\ H_2O$:	copper sulfate
H_2SO_4:	Sulfuric acid
PBMA:	plant-based meat analogues
GHGs:	Greenhouse gases

ABSTRACT

As we know, veganism's popularity has spurred the need for meat substitutes, especially plant-based meat analogs (PBMA). Bioresources high in protein like algae, vegetables, and cereals have been investigated to determine whether they can replicate animal flesh's texture, flavor, olfactory attributes, and sensory. This study summarizes recent breakthroughs in functional food technology based on vegetable proteins and compares traditional and commercially accessible meat substitutes. The experiment performed is based on the production of meat analog by combining more than one protein source by using the freeze structuring technique. Soy protein concentrate and rice protein isolate were used for the study. Soy and brown rice protein source were taken in 5 different ratios to check the nutritious value of each analog and compare them. The following results were obtained: 100 percent soy-based meat nugget was found to have maximum protein concentration, and 100 % rice-based has minimum. While 100 percent rice-based meat nuggets were found to have maximum carbohydrate concentration and 100 percent soy-based has minimum. The samples' pH was in the range of 6 and is increasing, such as PP1 (100% soy) has minimum pH while PP5 (100% rice) has maximum pH.

Keywords: Plant-based ; Meat Analogues; Soy protein; Protein content; Brown rice

CHAPTER 1
Introduction

====================================

The worldwide community is looking for nutritious and ecologically sustainable resources as part of their dietary habits. The veganism's popularity, along with the rising worries concerning animal welfare, harmful impacts on human and environmental health, has spurred call for meat substitutes, especially plant-based meat analogues (PBMA). Bioresources high in protein like algae, vegetables, and cereals had been investigated for determining whether they can replicate the texture, flavor, olfactory attributes, and sensory of animal flesh. This study will summaries recent breakthroughs in functional food technology based on vegetable proteins and compare traditional and commercially accessible meat substitutes.

To increase the PBMA's structure and technological functionality, a short overview of numerous production techniques and their processing impacts is proposed for the goal of developing sustainable food. Various amalgamations of animal and plant proteins are used to examine the nutritive content, organoleptic character, and shelf-life of accessible food items. The good response fueled a boom in the global food business, which today includes plant proteins. The worldwide market trend of introducing well-known and auspicious food brands is discussed to explore the potential of PBMA. The world's population is quickly expanding, as is generally recognized. As a result, the world community has a significant problem in sustaining global food supply without jeopardizing the ecology.

Since the start of civilization, meat has been a significant source of proteins and other nutrients. Regardless of the fact that meat is an important part of our diet, it also contributes significantly to environmental change, GHGs (greenhouse gas emissions), animal suffering, and consumption of groundwater. Overuse of natural-resources and large-scale animal production produce GHGs, resulting in decreased consumption of meat and a desire for fresh and better meat substitutes. Meat substitute, often known as meat replacements, originated in reaction to the tremendous call for red meat. As a consequence of their less cost, low-risk ingestion, and meaty structure & feel, meat analogues are becoming more popular.

Meat replacements are mostly plant-based food preparations proteins obtained from pulses, cereals, microbes, flavoring additives, and other sources. Additionally, texturized vegetable protein, wheat gluten, mushrooms, and pulses are all regarded as good sources of animal protein substitutes. Furthermore, mycoprotein had an incredible profile that contained more protein, less fat, health-promoting chemicals, and a pleasing texture & taste. Though, there is a vacuum in research papers concentrating on the eating of meat alternatives on a regular basis. The current study aims to highlight numerous types of meat substitutes, various source of protein, procedures for production preparation, nutritional and functional features, as well as existing and future perspectives on meat substitutes.

CHAPTER 2
Review of Literature

==================================

2.1 Historical perspectives

2.1.1 Ancient Time

Traditional products such as tempeh, tofu, and seitan have been used as a substitute to meat protein source since antiquity The Han dynasty (206 BC–220 AD) in China invented tofu, a typical meat substitute. Around the Tang dynasty (618–907), tofu was widely consumed, and it was likely transmitted to Japan during the latter Tang or early Song dynasties *(Shurtleff & Aoyagi, 2013).*

2.1.2 Early 20th century

Products made from nut and cereal, like Protose and Nuttose, were developed in the early twentieth century by visionaries like John Harvey Kellogg to aid excellent health *(Shurtleff & Aoyagi, 2014).* Furthermore, in addition to conventional Asian products, soy protein concentrates, extruded defatted soy meal, or wheat gluten were used to make dry texturized vegetable protein *(King & Lawrence, 2019).*

2.1.3 Period from the mid-twentieth century to the late-twenties

Following World War 2, the production and packaging industries showed obvious evidence of growth, with considerable advancements in plant protein isolates, concentrates, and textured proteins. During a time when meat consumption was rising across the globe due to agricultural expansions and improved animal husbandry, these developments aided the development of soy-based meat replacements. Products like Tofurky, aimed at a specific vegetarian clientele, first appeared in the United States in 1980. *(Pullen, 2018).*

2.1.4 The early twenty-first century

Burger King was the first meat substitute in the United States, launching a standard plant-based burger in 2002. With the rise in demand for alternatives to traditional meat in the new millennium, users' awareness of health and sustainability indicators of their diets continued to rise *(Green, 2019)*. The modern era of goods like Impossible Burger and Beyond Burger has spawned a new age of analogues, effectively doubling the plant-based meat sector. Plant-based meat attempts to emulate the taste, smell, texture, look, and functioning of traditional burgers, sausages, and fillet. In addition, meat Plant proteins, lipids, and gums are used to create substitutes. Spices, as well as extruders or other distinctive processing technologies, have achieved widespread consumer acceptance *(Court E, 2018).*

2.2 Seafood and farmed meat Concerns and Considerations

In this section, we highlight some important public welfare and food system problems and considerations related to the production and consumption of farmed meat and seafood intended to guide the analyses of meat substitute that ostensibly seek to reduce few risks. Livestock production techniques have both positive and bad aspects (e.g., recycling of nutrient), (e.g., nutrient pollution).

2.2.1 Environmental

Although the land space utilised for livestock production has increased from 2.5 billion ha to 3.7 billion ha, it still only contributes for 18 percent of calories & 25 percent of protein in world food supply. According to some findings, well-managed grazing animals can trap carbon under certain soil, climatic, and animal density situations. According to other study, this effect is time-constrained, changeable, and could be negated by another GHGs produced by farmed animals.

Non-arable grasslands cover 1.3 billion hectares of the 2.6 billion ha dedicated to livestock in the world *(Mottet et al., 2017)*. This means that lowering beef consumption and production would not certainly save up enough space to feed people or other cattle. Beef provides 1.1 kg of protein in grassland-based systems in the UK, compared to 1.4 kg in milk and 0.5 kg in poultry and pork.

It is possible to produce up to 27 percent to 35 percent of the present beef supply entirely from pasture. Grassland production systems could also contribute to protein security. Ruminant meat, like pork and poultry, is today dependent on agriculture to the same extent *(Herrero et al., 2015)* - but this is expected to change over the next few decades. Grassland production systems are replacing the traditional cropland-fed model of livestock production in advanced countries, but they remain the predominant model in many developing nations.

Livestock production contributes more to biodiversity loss and disruptions to groundwater pollution than crop cultivation for human use. Surplus nutrient levels (mainly nitrogen & phosphorus) generate poisonous algae blooms that kill plants, fish, and other aquatic life, resulting in eutrophication. In general, well-handled pasture-based animal production systems can also provide important ecological benefits such as nutrient recycling and soil health, which can reduce reliance on synthetic fertilizers.

2.2.2 Public Health

According to *(Micha et al., 2012)*, the consumption of red and processed meat has been connected to an increasing risk of cardiovascular disease and type-2 diabetes. Some particular type of compounds in animal foods can encourage the establishment of intestinal microbiota, which produces metabolites directly linked to an increasing risk of heart-disease and IBD. Meats maintained with high quantities of salt or chemical supplement (such as: Hot dog, bacon, sausages) are classified as "red meat" and "processed meat." Above mentioned health hazards are not always recorded for the unprocessed categories of white meats like turkey and chicken. Red meat, especially for young children, could be a rich source of protein and minerals.

Consumption of Seafood, especially oily fish and particular mollusks high in omega-3 fatty acids, has been linked with a range of health benefits. There is not enough seafood available globally for everyone to reap the benefits of eating at recommended levels. Animal-borne pathogens can infiltrate the food supply through a variety of pathways, including if they are ingested by humans or if they contaminate water sources used for irrigation. In comparison to other foods, beef is particularly land-intensive, partly because cattle have a longer cycle and are less effective at turning feed into meat. *(Nijdam et al., 2012).*

2.2.3 Animal Welfare

Between 1961 and 2018, meat output (in tons) increased more than 4.5-fold, approximately twice the pace of growth in population. Industrialized food animal production is created to make large quantities of meat, egg, and milk quickly at a low cost. Many businesses maintain animals in congested spaces, frequently in restricted cages or case, with little outside access to show natural behaviors.

2.2.4 Economic

Traditional farms that were once diverse have been replaced in much of the industrialized world by businesses that specialize in growing certain crops or animals on a large scale throughout the last century. Large multinational firms have amalgamated local businesses and other organizations in order to dominate numerous stages of the food supply chain. These technologies have been attributed with increasing efficiency, lowering costs, and minimizing consumer prices, but they are also linked to a drop in worker wages (Oxfam America, 2015).

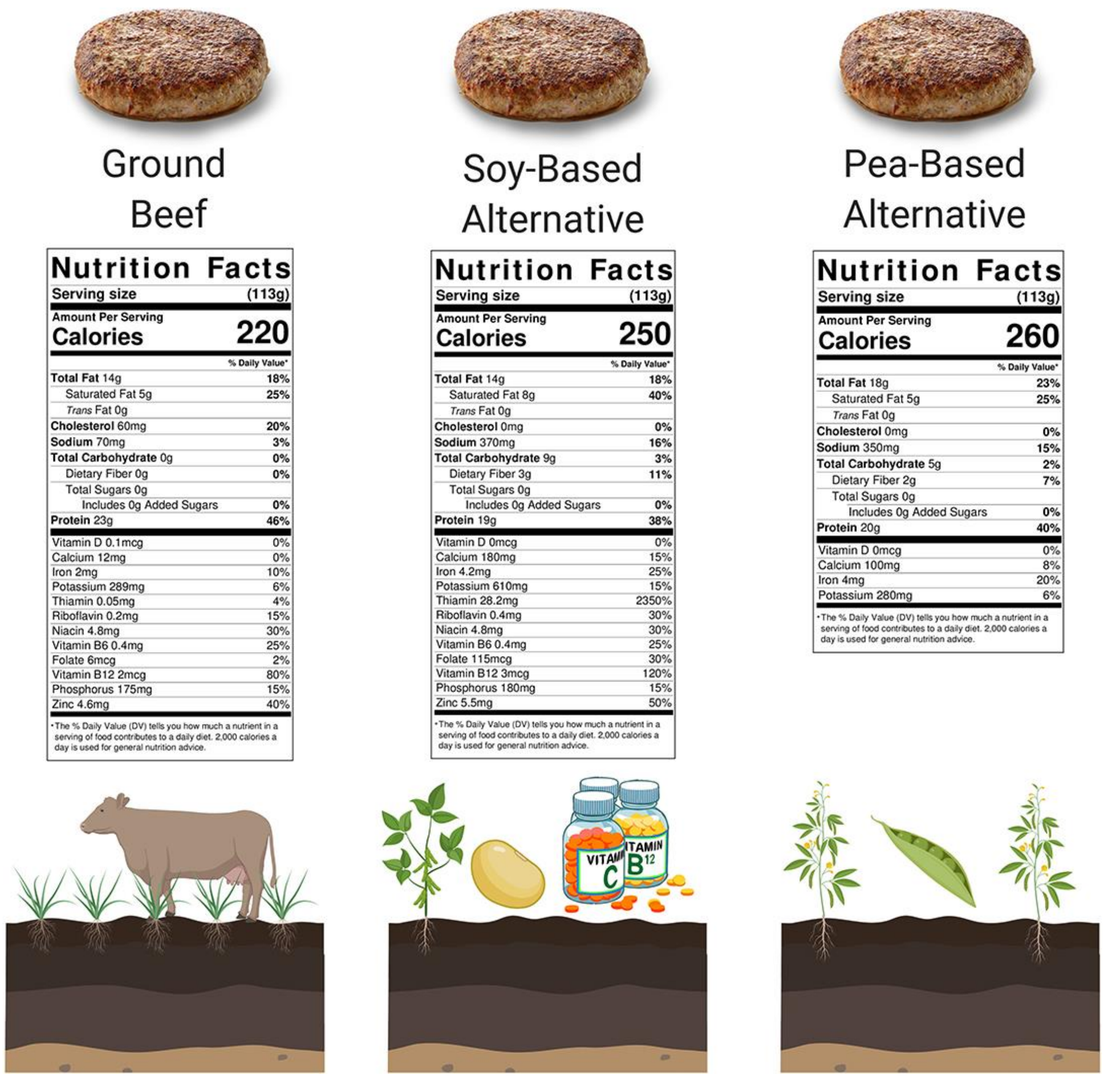

Figure 1 Comparison of Plant based meat vs farmed meat (Vliet et. al.,2020)

2.3 The meat alternative's Promises

Certain features of meat like: texture, flavoring, and nutritional profile can be approximated or even replicated using a variety of methods. Organic foods such as pulses, mushrooms, jackfruit imitate definite attributes of meat products. Products that aren't made to look like or designed to replicate meat but could be used in the same way (e.g., tempeh, bean burgers, tofu, seitan,), and highly processed meat-like burgers, fish fillets, and hot dog) are all examples of alternative source *(Lagally et al., 2017).* With recent technical breakthroughs aiming at reproducing certain properties of meat at molecular level, products in the last category have gained considerable traction during the last decade. To appeal to those who appreciate meat, certain items are meant to be "viscerally identical" to animal meat *(Stephens et al., 2018).* The majority of these plant-based alternatives employ soya, rice, wheat, chickpea, or pea protein concentrates or isolates as their major protein source, while there are other products made from fungi (such as mycoprotein) and lupin beans. Gardein Meatless Meatball, Impossible foods burgers, and Morningstar Farms Original Chik'n are two popular plant-based alternative brands and products.

Alternative meats are advocated for environment, animal well-being, and, in few situations, public health reasons. Impossible Foods' website proclaims, "Eat Meat. Save Earth," along with figures differentiating the water, land and GHG emissions related with an Impossible Burger with a normal cow burger *(Impossible Foods, 2020)*. The information regarding how “Fake Meat Will Save Us” are repeated in the popular press *(Egan, 2019)*. “Farmfree food” will allow us to hand back enormous expanses of land and sea to nature, allowing massive rewilding and carbon drawdown,” writes one journalist. It includes the eradication of animal exploitation, halting of deforestation, a substantial reducing use of fertilizer and pesticide, and the abolition of trawlers and longlines” *(Monbiot, 2020).* Meat made from cell is claimed to be “nutritious, safer, and disease-free" than farmed meat. *(Arshad et al., 2017).*

2.4 Implications for Public Health

2.4.1 Chronic Disease and Nutrition

According to study by *(Bohrer, 2019)* many plant-based alternatives include similar levels of nutritional value (protein, calories, and iron) as the meat they are meant to replace. Plant-based replacements, as highly-processed foods, have higher salt levels than unprocessed meats and possibly include chemicals and additives such as taste, colour, and different binding agents *(Bohrer, 2019; Curtain and Grafenauer, 2019)*. In spite the fact they may have quite likely macronutrient profiles, substituting plant-based meat for meat does not always indicate healthy eating habit *(Hu et al., 2019)*. Similarly, seafood replacements might be hypothetically enriched with omega-3 fatty acids, but it is unsure if this would deliver health benefit's equal to consuming full, unprocessed fish. In addition, extremely-processed food consumption is linked to increased calorie intake and weight gain, as well as a variety of negative lifelong health effects *(Lawrence and Baker, 2019).* More research is needed to determine whether plant-based alternatives are substituting unprocessed or processed foods in people's diets and, if so, whether they can contribute to long-term healthy eating habits. Food habits high in totally plant-based foods like vegies, whole grains, legumes, and nuts, on the other hand, have been linked to a lower risk of chronic diseases and negative health outcomes. Despite the fact that most plant-based alternatives are manufactured from legumes, it's still unclear whether plant protein isolates give the same nutritional advantages or reduce the risk of chronic illness as whole legumes. *(Hu et al., 2019).* Various plant-based meat substitute, for example, use soy protein isolates (having >90 percent soy protein), or concentrates (70–89 percent soy protein).

2.4.2 Food Hygiene

Many plant-based alternatives have at least minimum 1 significant food allergy, and the most frequent of them are of soy and wheat *(Food Drug Administration (FDA), 2004).*

Pea protein and lupin protein can trigger allergic reactions in the population which is already allergic to peanuts and soy, (albeit, it is uncommon) *(Lavine and Ben-Shoshan, 2019).* Gastrointestinal reactions / allergic reaction to mycoprotein-based plant-based alternatives (for example: Quorn) have been recorded; however, the prevalence of mycoprotein-related inauspicious reactions in the assorted population is debatable. Due to the abundance of certain dietary additives and gums in plant-based alternatives, people with intolerances to them should be cautious. Carrageenan, for example, is a seaweed-derived structural component that is extensively utilised to thicken, gel, or stabilize plant-based substitutes and other processed foods. Carrageenan's safety has long been questioned, with concerns about its ability to cause gastrointestinal inflammation, changes in intestinal microbiota, and other side effects like colon cancer and irritable bowel syndrome *(Bixler, 2017; David et al., 2018).* Furthermore, as Carrageenan grows in sea-water, it is likely to collect high levels of heavy metals, yet no studies have looked into arsenic, cadmium, lead, or mercury exposures from eating carrageenan-containing foods.

2.4.3 Occupational Safety and Health

The occupational exposure dangers experienced by workers in plant-based alternative manufacture are unknown, while they are presumably rarely dangerous than those posed by workers in animal meat processing. *(Vallaeys et al., 2010)*, A consumer union, has expressed concern over the usage of hexane in the manufacturing of soy protein isolates used in plant-based meat alternatives. Also, it might also be used to prepare pea protein isolates, albeit there isn't much information on that *(Tömösközi et al., 2001; Holt, 2018). (Environmental Protection Agency (EPA), 2000).* Hexane is toxic and extremely combustible solvent as well as a high-risk air pollutant. Overall, there is no precise data on the quantity of hexane used in the manufacturing of pea and soy protein isolates, as well as the extent of worker protection, environmental release prevention, or exposure monitoring is available.

2.4.4 Community Well-Being

Plant-based meat alternatives and (theoretically) cell-based meats both depend on existing agricultural crops, such as wheat, soybeans, and corn. Besides leading to fertilizer runoff, which can pollute localized groundwater supplies, the process of production of above-mentioned crops frequently uses pesticides that have been linked to long-term chronic health problems in farm workers and residents *(Harrison, 2011)*. Low-level herbicide uses in soybean cultivation, such as dicamba, 2,4-D, and glyphosate, has also been linked to multi-resistance in diseases, thereby jeopardizing the efficacy of numerous drugs. Furthermore, widespread usage of fungicides used in agriculture, e.g., for the production of soybeans & peas, has been linked to the increase of anti-fungal drug resistance, that has major ramifications for immunocompromised people *(Revie et al., 2018).* All of this being said, traditional meat frequently requires more pesticides to create in comparison to plant-based replacements because one traditional burger requires more animal feed than one plant-based burger requires soy.

2.5 Meat Analogues Composition

Development of meat-like products is the one trend, sometimes known as meat analogues, to provide a tasty alternative to meat. Developing the suitable texture and flavour for meat analogues are frequently the most difficult task for a food manufacturer *(Egbert and Borders, 2006).* A look of contemporary meat substitutes demonstrates that their distinctive characteristics, such as texture, flavour, and color, are dependent on the substances employed.

To understand how components affect the sensory properties of meat analogues, we must first look into their functions and purposes in a conventional recipe. A meat analogue, according to Egbert and Borders (2006) has textured vegetable proteins (10% to 25%), water (50% to 80%), nontextured proteins (4% to 20%), fat (0%-15%), flavorings (3% to 10%), binding agents (1% to 5%), and coloring agents (0.1% to 0.5%). In terms of sensory attribute, the combination of substances produces meat mimics that are acceptable. Texturized proteins can be used to replace meat in two different ways. The first technique involves merging texturized proteins with meat extension, while the second involves completely replacing meat with texturized proteins to create totally vegetarian goods *(Riaz, 2004).* Meat extenders do not have the same appearance, texture, or flavors as meat when cooked, but when blended with meat, they increase the foods in total functional characteristics. Meat analogues, on the other side, are intended to impersonate the flavor, aura, texture, color, feel of whole meat when properly moistened and cooked without the addition of any meat-based ingredients *(Riaz, 2004; Singh et al., 2008).* As a result, substances or chemicals can be utilized to enhance the endmost texture of the raw materials or aid in texturization.

2.5.1 Proteins

Plant-based proteins are in higher demand due to the growing desire for alternatives to animal-derived protein. The demand for new proteins is influenced by a variety of factors, including price, availability, suitability for incorporation into newer products, and, most significantly, their functional qualities *(Haque et al., 2016).* H2O and oil holding capabilities, solubility, foaming, emulsification, gelation characteristics, and other protein functions are required for the development of meat-analogue structures. However, the type of protein influences these functions (amino acid sequence, chemical composition, secondary and tertiary structure). Environmental elements, for example, Protein structure and function can be impacted by components such as temperature, pH, and ionic strength. Because of its unique qualities and inexpensive cost, soy protein is currently used in the majority of meat substitutes. Proteins produced by fermentation based on microorganisms and other substrates, as well as proteins from other oilseed crops, have already been included into the creation of meat analogues *(Kim et al., 2011).* Protein-rich sources such as rice, wheat, and maize as well as oil seeds, defatted flesh, meals, cereal & beany flours, and derivatives are currently used in the commercial manufacturing of meat analogues (e.g., defatted soy flour, soy protein concentrates, wheat flour) are being investigated *(Kumar et al., 2017).* There hasn't been any experimentation with new protein sources, such as leaves and algae in a meat-analogue formulation for textured protein.

2.5.2 Soy Protein

Soy is widely identified for its nutritional and functional benefits. It is often utilised for persons with cardiovascular illnesses and is regarded as substitute of red meat because of its proportional nutritious content *(Kumar et al., 2017).* On the Protein Digestibility Corrected

Amino Acid Score scale, soy protein received a score of 1.0, indicating that it is identical to animal protein *(Hoffman & Falvo, 2004)*. It helps to lower blood cholesterol, and minimize the risk of ischemic heart disease *(Golbitz & Jordan, 2006).* Chicken-style breast, sausage which are meat-free, chicken-style nuggets, and goods that bear a resemblance to sliced cooked meats all contain soy protein. As a result, textured vegetable protein is made from fibrous soy flour that has had the soluble carbohydrate removed and then textured by spinning or extrusion. As it allows for chewiness and fibrous features, it is thought to resemble meat muscle, delivering a distinct eating feel than other type of soy patterns *(Sadler, 2004)*. To save money without sacrificing nutrition, textured vegetable protein has been employed in a variety of meat-free comfort foods for example bean burger and pattie *(Penfield & Campbell,1990)*.

2.5.3 Cereal Protein

Cereals are considered as a very important constituent of food crops, and grain-derived products plays a notable role in the food processing industry. *(Malav et al., 2015)* study shows, cereal protein can be found in different form of flour, seeds, or flakes. Gluten is the main component of wheat protein, which has been processed and extruded to mimic meat in texture. Textured vegetable protein components found in wheat gluten-based meals can be used as meat extenders and substitutes. Gluten is also used as an extender and to bind bits for trimmings to create rearranged elements in in-ground meat pattie. Extruded, texturized, and converted into fibres, hydrated gluten might be used to make a variety of meat alternatives *(Malav et al., 2015)*.

2.5.4 Legume Proteins

Legume proteins from peas, lentils, lupines, chickpeas and various types of beans have also been studied for functional qualities including emulsification, foam stabilization, and gelatinization. Of these, pea proteins are the most capable for meat-analogue applications. Pea-based structures, on the other hand, are softer than soy-based products. As a result, it's being looked at how to improve gel strength by modifying protein hydrogen bonding, such as by including chaotropic ions to salts *(Sun and Arntfield, 2012)* or by improving processing variables such as pH, temperature, size of the protein particle, and so on *(Osen et al., 2014).* Chickpeas, lentils, and lupines studies have shown that they have good emulsion and foam stability abilities. With the exception of chickpeas, these proteins have lower gelling capabilities than soy.

2.5.5 Wheat Gluten

Wheat gluten is another extensively utilized protein. It's natural ability to create thin protein film that will be easily changed into fibrous proteinaceous materials when elongated. Thes distinguishing characteristics are the outcome of molecular properties and subsequent mesoscopic behavior *(Don et al., 2003)*. Gluten's disulfide protein linkage is a critical feature for the creation of a 3-D network *(Ooms et al., 2018)*, making it a major componet in the production of fibrous structures.

2.5.6 Mycoprotein

Long before 1985, the first mycoprotein product was introduced to the market. Mycoproteins are cholesterol-free, a balanced fatty acid profile and, have a low saturated fat level, and have a comparable fiber level to other vegetarian sources of protein. Due to its fibrous character, mycoprotein is able to considerably lower blood cholesterol level *(Denny et al., 2008)*. The fibrous arrangement of filamentous fungal mycelia is widely regarded as a meat substitute since it closely mimics the finished product. The binding agent formed from egg albumin is coupled with fungal biomass and flavouring chemicals, as well as the remaining components, depending on the final result, to make a product that mimics mycoprotein *(Denny et al., 2008)*. Protein binders form the gel as a result of the heating process, which adheres to the hyphae. The resultant product has alike textural-properties to meat items *(Rodger, 2001).*

2.5.7 Fat and Oil

The meat analogues that are currently available are low in fat. Defatted foods are commonly utilized to make meat analogues. Furthermore, fat/oil addition during processing has an effect on the fibrous structure creation. In previous research, it has been discovered that in the extrusion processing, those recipes having oil levels greater than 15 percent developed in material lubrication, that harmed macromolecule alignment *(Gwiazda et al., 1987).* The material can become greasy, *(Cheftel et al. 1992)*, found that this has a false impact on the shear forces applying during extrusion. On the other hand, the use of vegetable or fat oil to make meat analogue recipe, has benefits since it can help with tenderness and release of flavor *(Egbert and Borders, 2006)*, both of it are essential features for customers. Rapeseed, sunflower, palm, canola, soya, coconut, and corn oil, are among the fats/oils now used for plant-based meat substitutes. The use of oil/fat is deemed necessary as it can improve the meat analogues flavor by preserving volatile flavor components.

2.5.8 Coloring Agents

Color and color differences have a big impact on the quality of meat alternatives. Coloring ants are thus seen as a necessary component of meat supplements *(Kyriakopoulou et al., 2019)*. Turmin pigments, cumin, and carotene, are thermostable coloring additives that are popular with customers *(Vrljic et al., 2018).* Colorants that are not heat stable and reducing sugars that are similar to the final product are utilized in numerous combinations dependent on the color preparation *(Rolan et al., 2008)*. Furthermore, reducing sugar level can be used as a browning mediator; They can thus oppose the amine protein groups in a Maillard-type reaction, similar to meat browning *(Kyriakopoulou et al., 2019).* These are commonly used as coloring soln. before the process of extrusion in plant-based meat. The third method of combining colorants and proteins is to inject material into the extruder barrel or enter the extruder itself *(Orcutt et al., 2008).* Nonetheless, the availability of coloring agents in meat analogues is not of standard level; to remedy this issue, acidulants such as acetic acid, lactic acid, or citric acid, or combinations of these acids, are used *(Orcutt et al., 2008)*. Yet, the solution has a drawback in that, which is a change in pH causing protein to deteriorate and the final product's taste to vary. Color retention aids like hydrated alginate and maltodextrin are also employed in conjunction with the coloring agents to restrict or manage colour migration from the dyed structural meat-analogue *(Orcutt et al., 2008)*.

2.5.9 Flavors and other Ingredients

A meat analogue must have a good flavor and taste for the ordinary customer to accept it *(Kyriakopoulou et al., 2019)*. Their antecedents are presently used as flavor ingredients in meat analogues, together with iron complexes piquant spicing, meat, and salty scent. Sugar and Sulphur containing amino acids are vital for enhancing meat flavor. Furthermore, the mushroom concentrate can be utilized in lieu of monosodium glutamate or hydrolyzed vegetable protein to enhance flavor. When sodium, calcium, potassium, and magnesium were combined with plant proteins, the meat analogues' functional potentials enhanced *(Singh et al., 1997).*

2.5.10 Binding agents

Binding agent in the meat analogues either plant or animal-based substances that act as mutually a fat and water binder. Soy protein isolate and concentrate, xanthan gum, wheat gluten, carrageenan, eggs, milk proteins and other components are examples of such compounds. Water binding and protein network building are the primary functions of protein-rich ingredients, whereas flours and starches serve as fillers. The influence of varied concentrations of binding agents on the qualitative attributes and nutrient profile of plant-based substitute is being studied constantly. Wheat gluten is a propitious binder because of its cohesive and viscoelastic traits, which allows it to bind, form dough, and leaven. Mushroom-based sausage analogues with containing saturated fat of 5%, the effects of casein, xanthan gum, soy protein concentrate, and carrageenan were exhibited *(Arora et al., 2017).* Egg white oralbumen, which adds to binding, enhances the products' protein content, and provides additional physicochemical, is also employed in meat mimics.

2.6 Preparation and Processing of Meat Analog

Thermoplastic extrusion or fibre spinning are the two main technologies used to make meat analogue items at the moment. Thermoplastic extrusion entails adopting manufacturing methods that are frequently connected with a production of packed cereals. Extruders characterized straightforward and are thought to be efficient way to accommodate large-scale manufacturing. It as well creates the necessary settings for the development of good fibers. In a heated container, the wet mix is mixed at a temperature below the coagulation temperature of the proteins. The dough's viscosity decreases due to increased temperature and allows for the further homogeneous mixing procedure. It's important to avoid overmixing of dough because this has been shown to reduce the no. of fibers generated significantly *(Boyer, 1954).*

For maximum efficiency, set the extruders temperature at which the protein being utilized begins to solidify. Proteins in gluten coagulate at 75°C and soy at 68°C. Extruder inner walls temperature should be between 77 and 149 degrees Celsius while cooking the product.

During processing, turbulent circumstances generated by agitation and vigorous mixing should be avoided, as they contribute to the unwanted development of arbitrarily oriented, non-meaty fibres. Extrusion and stretching under non-turbulent or laminar circumstances is the only way to create unidirectional and parallel fibres. The laminar flow condition occurs when a fluid flows evenly with overlapping layers at low velocities, and it is often characterized by having a smooth surface. During the extrusion, the flesh analogue would be stretched at the same time. The quantity of linear expansion of the protein dough should ideally be around 50 percent in both directions *(Boyer, 1954)*. Because of its intricacy, fibre spinning method is not often utilised for the manufacturing of meat analogues, and it also undermines one of the key benefits of meat analogues. This technique of production raises the production cost, negating the benefit of developing a low-cost meat/protein alternative. In the textile business, fiber spinning processes were adapted from the spun fiber technique to generate synthetic fibers. Fibers are generated by forming filaments from the protein that serves as the beginning material. Proteins are dispersed in a dispersing medium, like alkaline aqueous solution, to start the process. Then, this dispersion is fed into a spinneret, which is a gadget for extruding polymer solutions into fibres and placed in an acidic salt soln. with a pH ranging from 4.0 to 5.0. Coagulation was improved by a factor of 5.6 to 6.4. The filaments would be having a diameter of roughly 0.003 inches after entering the spinneret's tiny die. These filaments are subsequently expanded or stretched until they are roughly 20 microns thick on average *(Boyer, 1954)*. Before further processing, the surplus salt soln. is separated from the fibers by centrifugation. Edible binders, like, starches, proteins, carboxymethylcellulose, dextrins, cereals, or their mixture, are added after the drying process to intact the fibers physically bound by acting as an adhesive or serving as a matrix in which the fibers imbedded. After that, the fibers are dipped in melted fat and squeezed altogether for making the end product. After that, the meat analogue is sliced into different shapes or length that is suitable for packaging and distribution or additional processing *(Boyer, 1954).*

Table 1. Known Meat-Analogue Companies and their Signature Products (Kyriakopoulou et al., 2019)

Country	Company	Product	Website
United states	Impossible Foods	Impossible Burger	https://www.possiblefoods.com
Canada	Gardein	Mandarin Crispy Chick'n	https://gardein.com/
United states	Beyond Meat	Beyond Burger	http://beyondmeat.com/
United states	Morning Star Farms	Grillers Original Burgers	https://www.morningstarfarms.com/
The Netherland	The Vegetarian Butcher	Vegan NOCHICKEN Chunks	http://www.thevegetarianbutcher.com/
Germany, Sweden, Netherlands	Tivall/Garden gourmet/Halsans KoK	Schnitzel	http://www.tivall.eu/

CHAPTER 3

Materials and Methods

==

Brown rice and Soy beans were purchased from the local market and rice protein isolate was produced in lab according to the *(Morita et. al., 1993)* with few modifications. The soy protein powder was produced in lab. Corn starch, vegetable oil (coconut oil), methylcellulose (MC) and baking powder were purchased from the market. The chicken powder flavoring was obtained from the market.

3.1 Preparation of Rice Protein isolate

To obtain rice protein isolate, brown rice was grinded for 3 min in a grinder. Then 500g rice flour was mixed with a solution of 0.6% Termamyl 120L- dissolved in water at a room temperature. The formed slurry was then heated at 98°C for 1.8 hours with stirring at different intervals. Gelatinisation and liquefaction occurred simultaneously. Rice Protein Isolate produced by washing 3 times with boiling water and 1 time with normal water and filtering through cheese cloth. Filtered protein isolate was then dried in a hot air oven overnight at 66^oC.

3.2Preparation of Soy Protein isolate

Soy beans were dried in a hot air oven overnight at 70^oC. Then, dried soy beans were grinded for 3 min until a fine powder is produced. Now, the powder was washed in boiling water until starch was washed off and filtered with cheese cloth. Filtered protein isolate was then again dried in a hot air oven overnight at 70^oC.

3.3 Preparation of plant-based nugget (PPN)

The PPNs were made using the composite mixtures listed in Table 1 with the total number of five distinct PPN ratios. Each of the composite ratio comprised of ice water 57%, 3.5% vegetable oil,0.3% salt, 11% corn starch, 2.51% baking powder, 1.5% Methyl Cellulose, 0.2% calcium chloride, in order to make 100 g of each composite protein analogue. The inclusion of baking powder and calcium chloride to the formulation was done to improve the analogous fibrous structure by increasing protein water binding capacity and generating air cells in the dough. Protein and MC emulsions were made independently to create the analogue. The protein emulsion was prepared by combining the ingredients in a food processor for 2 minutes on low speed (proteins, corn starch, salt, baking powder, calcium chloride solution, flavouring agent, and ice water). De- ionized water (DI) water was utilized in the whole experiment. This step was completed to completely hydrate the protein. A consistent MC emulsion was created by mixing MC powder, vegetable oil and ice water in a blender for 2 minutes at low speed. The formed protein and MC emulsions were mixed for 3 minutes. The PPN analogue batter was then moulded into a 5cm x 3cm x 2cm shape (LxWxH). At 98°C, the moulded product was steamed for 15 minutes. After that, it was frozen for 48 hours at -20°C. All formulations were prepared in triplicate.

For the testing purpose PPN were distorted and overnight dried in Hot air oven at 55 degree C. A fine powder of each dried samples was made for further testing.

Table 2. Formulation of PPN analogues with different protein ratios

Composites	Soy Protein%	Rice Protein%
PP1	100	0
PP2	75	25
PP3	50	50
PP4	25	75
PP5	0	100

3.4 pH

To determine the pH of each plant-based protein analogues, 2.5g sample is mixed in 100ml of distilled water and homogenized for 3 min. After the samples is homogenized, the pH was measured by using pH meter.

3.5 Protein Extraction

Protein extraction was carried out in accordance with *(Barbarino et. al, 2005)*. The samples were dissolved in 14 mL distilled water, after dissolving it is homogenized, and incubated at 25°C for 24 hours. Then samples were centrifuged for 15 minutes at 4000 g at 4 C. After the removal of supernatant, the pellet was re-dissolved in 14 mL of 0.1 M NaOH in 3.5 percent NaCl and incubated for 24 hours at 25 degrees Celsius. During both incubation periods, the samples were shaken constantly. After that, the samples were centrifuged for 15 minutes at 4000 g at 4 C. Protein estimation was performed on the final extracts after the two supernatants were mixed.

3.6 Protein Estimation

Estimation of Proteins by Lowry method

Lowry's method was used to determine the protein content.

PRINCIPLE: The Lowry method used for the determination of protein concentrations is grounded on the "peptide nitrogen[s]" reactivity with copper [II] ions under alkaline state, and the "copper-induced reduction of Folin-Ciocalteay phosphomolybdic phosphotungstic acid" to "heteropoly molybdenum blue" by the copper-induced reduction. The Lowry technique uses the "Folin–Ciocalteu reagent" to react Cu+ generated by peptide bond oxidation. This reaction yields heteropoly molybdenum Blue, a brilliant blue compound.

Reagents used:

"2% Na2CO3 in 0.1 N NaOH"

"1% NaK Tartrate in H2O"

"0.5% CuSO4.5 H2O in H2O"

Reagent A: 48 ml of i, 1 ml of ii, 1 ml iii.

Reagent B- 1-part Folin-Phenol [2 N]: 1 part H2O

Procedure:

- Fill 5 test tubes with 0.2 ml BSA working standard and 1 ml distilled water.
- The test tube filled with 1 mL distilled water is considered as blank.
- Incubate for 10 minutes with 4 mL of Reagent A.
- After 10 minutes of incubation, add 0.5 ml of reagent B and incubate it for 30 min in dark.
- Plot the standard graph with the absorbance at 660 nm.
- Estimate the protein amount present in all the samples.

Table 3. O.D. obtained from Lowry method for the estimation of protein

BSA (µl)	Conc. (mg)	Water (µl)	Reagent A(ml)		Reagent B (ml)		OD 660
0	0	1000	4	Incubate 10 mins	0.5	Incubate 30 mins	0.138
200	2	800	4		0.5		0.450
400	4	600	4		0.5		0.634
600	6	400	4		0.5		0.987
800	8	200	4		0.5		1.239
1000	10	0	4		0.5		2.355

3.7 Carbohydrate Estimation

Principle:

Conc.H2SO4 dehydrates carbohydrates to produce furfural. Anthranol an enol tautomer of anthrone, is the active form of the reagent, produces a green color in dilute solutions and a blue tint in concentrated solutions when combined with the carbohydrate furfural derivative. The blue-green solution's absorption maximum is 620 nm.

Reagents:

Test Solution: Glucose stock solution

Anthrone Reagent: 0.2 % anthrone in "concentrated H2SO4" (0.1gm in 100ml H2SO4)

Procedure:

- Pipette varied quantities of glucose solution from the supplied stock solution (200g/ml) into a series of test tubes and dilute to 1 mL with distilled water.
- Assume that tube 1 is blank and that tubes 2 through 6 are used to create a standard

curve. The unknown samples are in tubes 7-11.

- Add 4 mL of the anthrone reagent (provided) to each tube and vortex well to combine.
- Allow the tubes to cool.
- Place marbles/caps on top of the tubes and incubate for 17 minutes at 90o C.
- Allow it cool to ambient temperature before comparing the optical density to a blank at 620 nm.
- Create an absorbance versus. g glucose standard curve.

Table 4. O.D. obtained from Anthrone method for the estimation of carbohydrate

Volume of Glucose (µl)	**Conc. (mg)**	**Water (µl)**	**Anthrone (ml)**		**OD at 620**
0	0	1000	4		0.0
200	2	800	4	Incubate	0.21
400	4	600	4	8 mins	0.39
600	6	400	4		0.44
800	8	200	4		0.73
1000	10	0	4		0.91

CHAPTER 4
Results and Discussion

==

4.1 Product Preparation

Figure 2 Rice Protein Isolate

Figure 3 Soy Protein Isolate

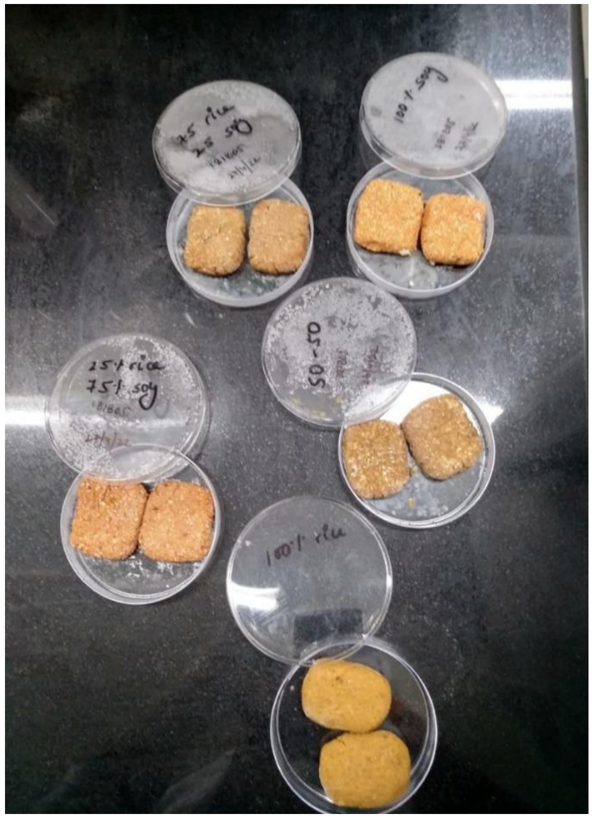

Figure 4 Prepared Plant based Nuggets

Figure 5 Distorted PPN for testing

4.2 pH

Table 5. pH of PPN analogues with different soy and rice protein ratios

Composite	pH
PP1	6.14
PP2	6.29
PP3	6.41
PP4	6.32
PP5	6.73

With an increasing amount of soy protein and a decreasing amount of rice protein, the pH of the trials showed a declining tendency. The formulation of PP5 had the highest pH (6.73) and PP1 had the lowest pH (6.14), while the pH of PP2 and PP4 are quite similar. The pH discrepancies across samples could be related to the protein sources' starting pH.

4.3 Protein Estimation

Table 6. Protein concentration in PPN analogues with different soy and rice protein ratios

Composite	Protein conc.(mg/ml)
PP1	0.15
PP2	0.09
PP3	0.14
PP4	0.13
PP5	0.07

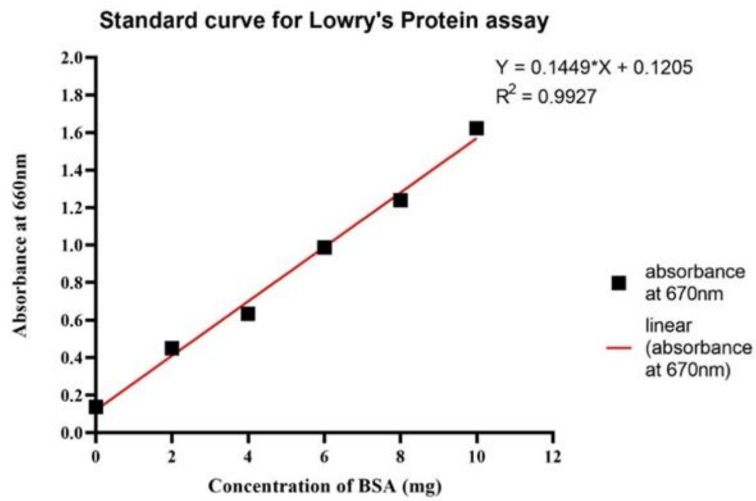

Figure 6 Standard Curve of Lowry's Assay for Protein Estimation

PPN made from 100% soy is found to have maximum protein concentration (0.15 mg/ml). While PPN made from 100% rice has minimum protein concentration (0.07 mg/ml).

The protein concentration was found to be in the order PP1>PP4>PP3>PP2>PP5

4.4 Carbohydrate Estimation

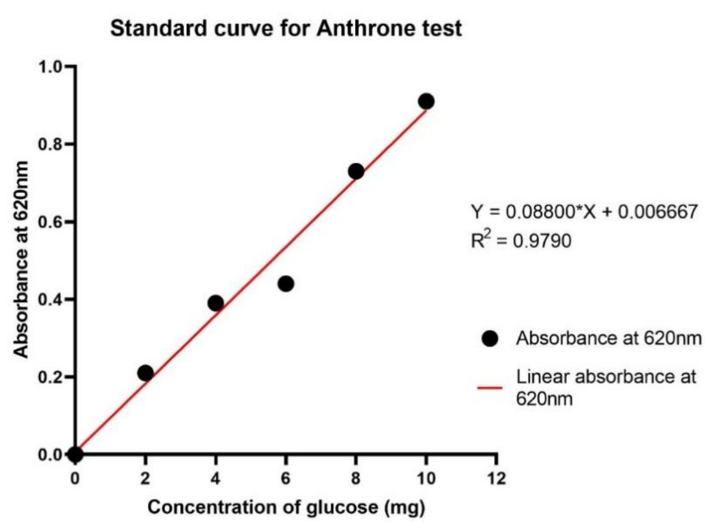

Figure 7 Standard Curve of Anthrone Test for Carbohydrate Estimation

Table 7. Carbohydrate concentration in PPN analogues with different soy and rice protein ratios

Composite	Carbohydrate conc. (mg/ml)
PP1	0.09
PP2	0.12
PP3	0.15
PP4	0.18
PP5	0.23

PPN made from 100% rice is found to have maximum carbohydrate concentration (0.23 mg/ml). While PPN made from 100% soy has minimum carbohydrate concentration (0.08 mg/ml). The carbohydrate concentration was found to be in the order PP5>PP4>PP3>PP2>PP1

CHAPTER 5
Conclusions

==

According to the literature, number of studies on different protein source for production of plant-based meat has been increased over the year. The reason behind the increase in studies is ethical, health and environmental issues. Also, as the number of studies increase food diversification increase. This study is based on the production of meat analogue from combining more than 1 protein source by using freeze structuring technique and checks the effect on nutritional profile of a product. For the study soy and brown rice protein source were taken in 5 different ratios to check the nutritious value of each analogue and compare them. Firstly, rice and soy protein isolates were produced and different analogs were made and then tests were performed. Protein test was performed by Lowry method which concluded: 100 percent soy-based meat nugget found to have maximum protein concentration and 100 percent rice based have minimum. On the other hand, Carbohydrate test was performed by Anthrone method which concluded: 100 percent rice-based nugget has maximum carbohydrate concentration and 100 soy have minimum. The pH of the samples was in range of 6 and is in increasing fashion. Such as PP1 (100% soy) has minimum pH while PP5 (100% rice) has maximum pH. Soy based nugget have maximum protein and minimum carbohydrate and soy-based nugget have maximum carbohydrate and minimum protein.

Table 8. Protein, pH, and carbohydrate values of PPN analogues with different ratios of soy and rice proteins

Composites	PP1	PP2	PP3	PP4	PP5
pH	6.14	6.29	6.41	6.32	6.74
Protein (mg/ml)	0.15	0.09	0.14	0.13	0.07
Carbohydrate (mg/ml)	0.09	0.12	0.15	0.18	0.23

REFERENCES

===

- Green E. 2019. Food discovery: “The Adventurous Consumer” tipped as top trend for by Innova Market Insights. https://www.foodingredientsfirst (2020.6.7)
- Pullen JP. 2018. Tofurky wants to save the world, one Thanksgiving roast at a time. It’s now sold 5 million. http://fortune.com/longform/tofurky-vegan-vegetarian thanksgiving-recipes-company-founding/ (2020.6.20)
- King T and Lawrence S. 2019. Meat the alternative-Australia's $3 billion opportunity. https://www.foodfrontier.org/wpcontent/uploads/2019/09/Meat_the_Alternative_FoodFrontier.pdf (2020.6.3)
- Shurtleff W and Aoyagi A. 2013. History of tofu and tofu products (965 CE to 2013). California: Soyinfo Centre, p.5. http://www.soyinfocenter.com/pdf/163/Tofu.pdf (2020.6.26)
- Court E. 2018. Beyond meat products out of stock at whole foods stores due to high demand. https://www.plantbasednews.org/ lifestyle/beyondmeat- (2020.6.2.)
- Egbert, R., Borders, C., 2006. Achieving success with meat analogs. Food Technology 60, 28-34.
- Riaz, M.N., 2004. Texturized soy protein as an ingredient. In: Proteins in Food Processing, Rolan, T., Mueller, I., Mertle, T.J., Swenson, K., Conley, C., Orcutt, Mac, W., Mease,
- Singh, P., Kumar, R., Sabapathy, S.N., Bawa, A.S., 2008. Functional and edible uses of soy protein products. In: Comprehensive Reviews in Food Science and Food

Safety, pp. 14-28.

- Haque, M.A., Timilsena, Y.P., Adhikari, B., 2016. Food Proteins, Structure, and Function, Reference Module in Food Science. Elsevier. https://doi.org/ 10.1016/B978-0-08-100596-5.03057-2.
- Kim, K., Choi, B., Lee, I., Lee, H., Kwon, S., Oh, K., Kim, A.Y., 2011. Bioproduction of mushroom mycelium of Agaricus bisporus by commercial submerged fermentation for the production of meat analogue. Journal of the Science of Food and Agriculture
- 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
- Lagally, C., Clayton, E. R., and Specht, L. (2017). Plant-Based Meat Mind Maps: An Exploration of Options, Ideas, and Industry: The Good Food Institute.
- Stephens, N., Di Silvio, L., Dunsford, I., Ellis, M., Glencross, A., and Sexton, A. (2018). Bringing cultured meat to market: technical, socio-political, and regulatory challenges in cellular agriculture. Trends Food Sci. Technology.
- Impossible Foods (2020). Mission.
- Egan, T. (2019, June 21). Fake meat will save us. The New York Times
- Monbiot, G. (2020). Lab-grown food will soon destroy farming – and save the planet. The Guardian
- Arshad, M. S., Javed, M., Sohaib, M., Saeed, F., Imran, A., and Amjad, Z. (2017). Tissue engineering approaches to develop cultured meat from cells: a mini review. Cogent Food Agric

- David, S., Shani Levi, C., Fahoum, L., Ungar, Y., Meyron-Holtz, E. G., Shpigelman, A., et al. (2018). Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods? Food Funct. 9, 1344–1352.
- Bohrer, B. M. (2019). An investigation of the formulation and nutritional composition of modern meat analogue products. Food Sci. Hum. Wellness 8, 320–329
- Lawrence, M. A., and Baker, P. I. (2019). Ultra-processed food and adverse health outcomes. BMJ 365:l2289
- Curtain, F., and Grafenauer, S. (2019). Plant-based meat substitutes in the flexitarian age: an audit of products on supermarket shelves. Nutrients 11:2603
- Lavine, E., and Ben-Shoshan, M. (2019). Anaphylaxis to hidden pea protein: a Canadian pediatric case series. J. Allergy Clin. Immunol. Pract. 7, 2070–2071
- Denny A, Aisbitt B and Lunn J. 2008. Mycoprotein and health. Nutr. Bull. 33: 298-310
- Rodger G. 2001. Production and properties of mycoprotein as a meat alternative. Food Technol. 55: 36-41.
- Malav, O.P., Talukder, S., Gokulakrishnan, P., Chand, S., 2015. Meat analog: a review. Critical Reviews in Food Science and Nutrition 55, 1241e1245.
- Sun, X.D., Arntfield, S.D., 2012. Molecular forces involved in heat-induced pea protein gelation: effects of various reagents on the rheological properties of salt-extracted pea protein gels. Food Hydrocolloids 28, 325e332.
- Osen, R., Toelstede, S., Wild, F., Eisner, P., Schweiggert-Weisz, U., 2014. High moisture extrusion cooking of pea protein isolates: raw material characteristics, extruder responses, and texture properties. Journal of Food Engineering 127, 67e74.

- Ooms, N., Jansens, K.J.A., Pareyt, B., Reyniers, S., Brijs, K., Delcour, J.A., 2018. The impact of disulfide bond dynamics in wheat gluten protein on the development of fermented pastry crumb. Food Chemistry 242, 68e74.
- Thiébaud, M., Dumay, E., Cheftel, J.C., 1996. Influence of process variables on the characteristics of a high moisture fish soy protein mix texturized by extrusion cooking. LWT-Food Science and Technology 29, 526e535.
- Egbert, R., Borders, C., 2006. Achieving success with meat analogs. Food Technology 60, 28e34.
- Rolan T, Mueller I, Mertle TJ, Swenson KJ, Conley C, Orcutt MW and Mease LE. 2008. Ground meat and meat analog compositions having improved nutritional properties. U.S. Patent 11,963,375
- Kyriakopoulou K, Dekkers B and Van Der Goot AJ. 2019. Plant-based meat analogues. In Sustainable meat production and processing. Galanakis CM. pp.103-126. ed. Elsevier Academic Press, Cambridge, Mass, USA
- Orcutt MW, Sandoval A, Mertle TJ, Mueller I, Altemueller PA and Downey J. 2008. Meat compositions comprising colored structured protein products. U.S. Patent 12,061,843.
- Arora, B., Kamal, S., Sharma, V.P., 2017. Effect of binding agents on quality characteristics of mushroom based sausage analogue. Journal of Food Processing and Preservation 41.
- Ryschawy, J., Dumont, B., Therond, O., Donnars, C, Hendrickson, J., Benoit, M., et al. (2019). An integrated graphical tool for analysing impacts and services provided by livestock farming. Animal 13, 1760–1772.

- Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C., and Gerber, P. (2017). Livestock: on our plates or eating at our table? A new analysis of the feed/food
- Herrero, M., Wirsenius, S., Henderson, B., Rigolot, C., Thornton, P., Havlík, P., et al. (2015). Livestock and the environment: what have we learned in the past decade? Annu. Rev. Environ. Resour. 40, 177–202.
- van Vliet S, Kronberg SL and Provenza FD (2020) Plant-Based Meats, Human Health, and Climate Change. Front. Sustain. Food Syst. 4:128. doi: 10.3389/fsufs.2020.00128
- Micha, R., Michas, G., and Mozaffarian, D. (2012). Unprocessed red and processed meats and risk of coronary artery disease and type 2 diabetes– an updated review of the evidence. Curr. Atheroscler. Rep. 14, 515–524.
- Oxfam America (2015). Lives on the Line: The Human Cost of Cheap Chicken.
- Nijdam, D., Rood, T., and Westhoek, H. (2012). The price of protein: review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. Food Policy 37, 760–770.
- Barbarino, E.; Lourenço, S.O. An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. J. Appl. Phycol. 2005, 17, 447–460.
- Morita, Tatsuya and Shuhachi Kiriyama. "Mass Production Method for Rice Protein Isolate and Nutritional Evaluation." Journal of Food Science 58 (1993): 1393-1396.