COMPARATIVE STUDY OF PLANT-BASED MEAT SUBSTITUTES

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

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By

Nandita (191813)

Under the guidance of

Dr. Garlapati Vijay Kumar

DEPT. OF BIOTECHNOLOGY AND BIOINFORMATICS

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY,

WAKNAGHAT, HP-173234, INDIA

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DECLARATION

I decree hereby that the work presented in this report entitled **"COMPARATIVE STUDY OF PLANT-BASED MEAT SUBSTITUTES** "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 2022 to May 2023 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.

(Nandita, 191813)

SUPERVISOR'S CERTIFICATE

This is to clarify that the work reported in B. Tech. thesis entitled "**COMPARATIVE STUDY OF PLANT-BASED MEAT SUBSTITUTES"** submitted by Nandita (191813) at Jaypee University of Information Technology, Waknaghat, India, is the 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)

Name of Supervisor -Dr. Garlapati Vijay Kumar
Designation -Associate Professor
Department of Biotechnology and Bioinformatics
Jaypee University of Information Technology
Waknaghat, Distt-Solan, H.P. – 173 234
E-mail -garlapati.vijaykumar@juit.ac.in
Date-

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(Nandita, 191813)

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LIST OF ABBREVIATIONS

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

ABSTRACT

The global community is looking for nutrient-dense, environmentally friendly materials for their diets. The increasing craze for a vegan diet, along with human health and animal protection, has increased the usage of meat substitutes such as plant-based meat. It has been examined if protein-rich bioresources, including algae, vegetables, and grains, may mimic animal meat's feel, taste, smell, and sensory qualities. This study evaluates conventional and readily available meat replacements and summarizes recent developments in food products depending on proteins derived from vegetables or green sources. Isolates from soya and brown rice were extracted to observe the pH, carbohydrates, and protein quantities in each substitute. PPN, with the highest protein concentration, is discovered to be made entirely of soy. PPN composed entirely of rice has low protein content, whereas PPN with high rice quantity has high carbohydrate content.

Keywords: Meat Substitutes; Protein isolates; Soy Protein; Rice Protein

CHAPTER 1
Introduction

Overview on several production methods along with their processing effects is suggested in order to enhance the PBMA's conventional structure and functional technology which aims at creating food that is healthy as well as sustainable. The nutritional value, organoleptic qualities, and shelf-life of readily available food items are examined using various combinations of animal and plant proteins. The positive response helped the global food industry, which now includes plant proteins, grow. To investigate the possibilities of PBMA, the global market trend of introducing well-known and auspicious food brands is discussed. The population of the world is growing swiftly, as is widely acknowledged. Due to which, creating a constant supply of food for the world along with preventing the decline of environment is one of the major difficulties faced by the international community.

Since the beginning of civilization, meat accounts for one of the main sources for protein and various nutrients. No one can deny the vital role meat plays in one's diet but it also impacts our environment, greenhouse gas emission, animal suffering and groundwater use negatively even deteriorating it. Theincreased use of natural resources and animal agricultureusing industrial processes leads to the emission GHGs, which is why one should reduce consumption of meat and uptake better and plant-based alternatives of meat. Meat substitutes or meat replacements are made in response to the enormous demand for red meat. Meat analogues are gaining popularity because to their lower cost, reduced risk of ingesting, and meaty texture and feel. Meat substitutes are mostly plant-based dietary dishes that contain proteins from grains, pulses, microorganisms, flavoring agents, and other sources. In addition, sources of quality animal protein alternatives include wheat gluten, mushrooms, lentils, and texturized vegetable protein. In addition, mycoprotein had a remarkable profile with more protein, less fat, health-improving substances, and a delectable texture and flavor. Presentstudy targets to provide variety of meat substitutes along with the techniques used for production and preparation. Functional characteristics and nutritional qualities are also one of the aims presented in current studies as well as current and future perspectives on meat substitutes.

CHAPTER 2
Literature Review

2.1HISTORY

2.1.1Ancient Time

The usage of meat as a prominent source of protein has been reduced and instead various conventional goods like tempeh, seitan and tofu has been utilised. From 206 BC- 220 AD during the Han Dynasty, China formulated their own meat substitute which is till date known by the name of Tofu. The consumption of Tofuwas observed during the Tang Dynasty (618 – 907) and it was the late Tang or early Song dynasty when Tofu was introduced in Japan. *(Shurtleff & Aoyagi, 2013).*

2.1.2 Early 20th century

Visionaries like John Harvey Kellogg created Protose and Nuttose, products made from nuts and cereal, in the early 20th century to promote optimum health *(Shurtleff & Aoyagi, 2014).* Additionally, soy protein concentrates, extruded defatted soy meal, or wheat gluten were employed to create dry texturized vegetable protein in addition to standard Asian goods *(King & Lawrence, 2019).*

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

Following World War II, there was clear evidence of expansion in the production and packaging sectors, as well as changes that are significant to plant protein isolates, concentrates, and textured proteins. These advancements helped the creation of soy-based meat substitutes at a duration when meat consumption was expanding globally as a result of agricultural expansions and improved animal husbandry. Around the United States, products like Tofurky that catered to a niche vegetarian market first emerged in 1980 (Pullen, 2018).

2.1.4The early twenty-first century

In the United States, Burger King introduced the first conventional plant-based burger in 2002. Users' awareness of health and sustainability indicators of their meals continued to improve as demand for meat substitutes increased in the new millennium (Green, 2019). Because of products like Impossible Burger and Beyond Burger, the market for plant-based meat has essentially doubled in the modern era.Plant-based meat makes an effort to resemble the flavor, aroma, texture, appearance, and functionality of classic burgers, sausages, and fillet. In addition, alternatives for meat are made using plant proteins, lipids, and gums. Extruders and other unique processing methods, along with spices, have gained considerable public popularity (*Court E,2018)*.

SEITAN

TOFU

Figure 1 – Ancient Plant-based meat examples

2.2 CONCERNS AND CONSIDERATIONS RELATING TO SEAFOOD AND FARMED MEAT

In this part, we outline several significant issues with public welfare and the food system as well as issues with the production and consumption of farmed meat and seafood in an effort to inform studies of meat alternatives that purport to minimize minimal dangers. Techniques used in livestock production have both good and harmful points (such as nutrient recycling) (e.g., nutrient pollution).

2.2.1 Environmental

There has been a significant increase in the land used for the raising and producing live stocks. Land usage has increased from 2.5 billion ha to 3.7 billion ha, only 18 percent of the world's calories and 25 percent of its protein come from cattle. According to some research, under specific animal density conditions, soil conditions and climatic conditions, good habitat grass eating animals can store carbon. Various other studies indicate that this effect can be variable, restrictive for certain period of time, and could be negated by other greenhouse gasses produced by livestock animals. Live stocks are grown on 2.6 billion ha out of which 1.3 billion ha of land is not suitable for growing crops (2017, Mottet et al.). Studies show that enough land can be released for food cultivation for human and animals if production of beef is reduced. Beef In grassland-based systems in the UK, beef supplies 1.1 kg of protein, compared to 1.4 kg in milk and 0.5 kg in poultry and pork.

Up to 27 to 35 percent of the current beef supply may be produced only from pasture. Systems for producing grassland could also help ensure the security of protein. Pork, poultry,and ruminant meat are all currently dependent on agriculture (2015, Herrero et al.), however this is anticipated to alter over the following few decades. In developed nations, grassland production systems are replacing the conventional cropland-fed model of livestock production, but in many developing countries, this paradigm still predominates. Contrary to crop cultivation for human use, livestock production has a greater impact on groundwater pollution and biodiversity loss. Eutrophication is the outcome of excess nutrient levels (mostly N & P), which cause toxic algae blooms that kill plants, fish, and other aquatic life. Generally speaking, properly managed pasture-based animal production systems can also offer significant ecological advantages like nutrient recycling and soil health, which can lessen dependence on synthetic fertilizers.

2.2.2Public Health

According to studies, eating red and processed meat increases the chance of developing type 2 diabetes and cardiovascular disease (Micha et al., 2012). Some ingredients in animal products may encourage the growth of microbes present in the intestine, which in turn creates compounds that are directly linked to an increased chances to cause heart disease and IBD. Meats kept with excessive salt or chemical augmentation are both referred to as processed meat and red meat (examples: hot dogs, bacon, and sausages). Unprocessed white meats like turkey and chicken are not often associated with the health hazards. Red meat may be a rich source of protein and minerals for young newborns in particular.

Consuming seafood has been connected to a number of health advantages, especially when it comes to fish that have oil content and certain mollusks that are high in omega3 fatty acids. The worldwide supply of seafood is insufficient for everyone to benefit from consuming it at the recommended amounts. Animal-borne viruses can enter the food system through a number of different routes, such as through human consumption or contaminating irrigation water sources. Because cattle have longer lifespans and are less effective at turning grain into meat, beef requires more acreage than other foods. (2012, *Nijdam et al)*.

2.2.3Animal Welfare

Meat production (measured in tones) increased more than 4.5 times between 1961 and 2018, roughly twice as fast as population growth. Industrialized food animal production was developed to swiftly and cheaply produce vast quantities of meat, eggs, and milk. Numerous establishments keep animals in crowded areas, typically in small cages or cases with minimal access to the outside for them to exhibit natural behaviors.

2.2.4Economic

Traditional farms that were historically diverse have been substantially replaced during the past century by corporations that are masters in cultivating particular crops or animals on a large scale. To exert control over different links in the food supply chain, large international corporations have merged local enterprises and other organizations. Although these technologies have been credited with boosting productivity, cutting expenses, and lowering consumer prices, they have also been connected to a decline in worker salaries (Oxfam America, 2015).

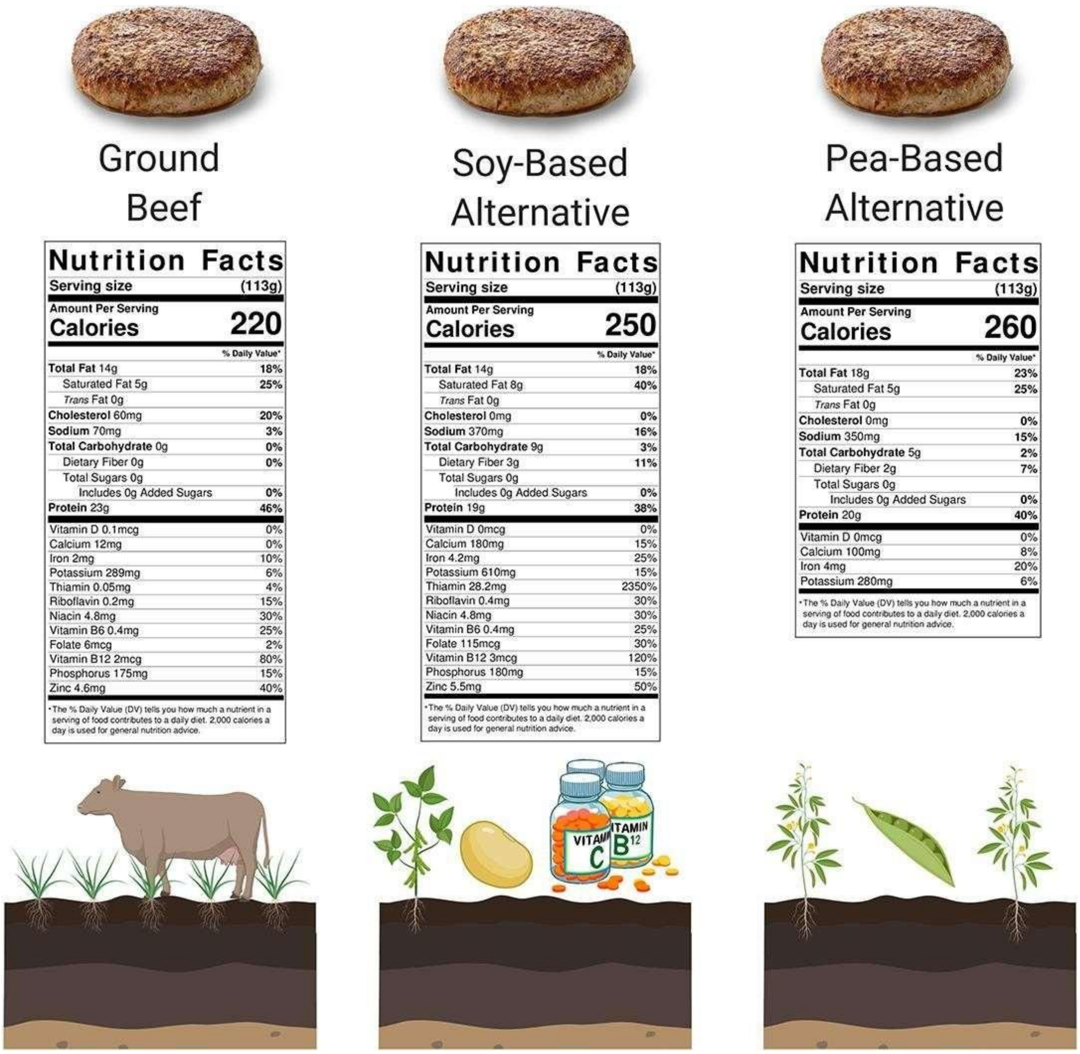

Figure 2 – Comparison of Plant-based meat and farm meat

2.3ADVANTAGES OF THE MEAT ALTERNATIVES

A multitude of techniques can be used to approximate or even replicate the texture, flavor, and nutritional profile of meat. Organic foods like jackfruit, mushrooms, and pulses mimic specific characteristics of meat products. Alternative sources include items like tempeh, bean burgers, tofu, seitan, and highly processed burgers, fish fillets, and hot dogs that are not intended to resemble or duplicate meat but can be utilised in the same way (2017, Lagally et al.). Due to recent technical developments that aim to molecularly mimic particular meat properties, products in the final category have witnessed tremendous increase over the past ten years. Some articles are meant to be "viscerally identical" to meat in order to be appealing to consumers who appreciate it (2018, Stephens et al.). However, the majority of these plant-based replacements employ pea, chickpea, wheat, soya, chickpea, or rice protein concentrates or isolates as their primary protein source. Products produced from fungus (such as mycoprotein) and lupin beans are present too. Adversely known plant-based alternatives are Gardein Meatless Meatball Morningstar Farms Original Chik'n, Impossible Foods burgers etc.

The promotion of alternative meats is done for reasons linked to the environment, animal welfare, and, in some circumstances, public health. The famous tagline on the Impossible Foods website is "Eat Meat Save Earth", which also includes comparisons of the water, land, and greenhouse gas emissions produced by an Impossible Burger and a standard cow burger (Impossible Foods, 2020). One journalist claimed that eating "farm free food" would allow us to rewild large areas of land and ocean, which would significantly reduce carbon emissions. In addition to a prohibition on trawlers and longlines, it demands an end to animal abuse, a halt to deforestation, a major reduction in the use of herbicides and fertilizers, and a halt to the use of insecticides.

2.4 IMPLICATIONS FOR PUBLIC HEALTH

2.4.1Chronic Disease and Nutrition

The nutritional value of many plant-based meat alternatives is comparable to that of meat (protein, calories, and iron), according to a study by (Bohrer, 2019). The salt content of plant-based substitutes is higher than that of unprocessed meats since they are highly processed foods, and they may also contain additional chemicals and additives for flavor, colour, and other combining agents (2019, Bohrer; 2019, Curtain and Grafenauer). Although switching from meat to plant-based meat may have fairly predictable macronutrient profiles, this does not imply that switching is a sign of a healthy eating pattern. (Hu et al., 2019). Similar to this, it is unknown whether adding omega-3 fatty acids to seafood substitutes will produce health benefits on par with eating whole, unprocessed fish. Additionally, eating foods that are highly processed is associated with consuming more calories, gaining weight, and having a number of detrimental health implications that could show its effects later in life (Lawrence and Baker, 2019). Further research is needed to determine whether plant-based substitutes for unprocessed or processed foods can support people's long-term healthy eating habits. Conversely, eating regimens high in entirely plant-based foods, such as vegetables, whole grains, legumes, and nuts, have been linked to a lower risk of chronic diseases and unfavorable health consequences. Despite the fact that the majority of plant-based alternatives are manufactured from legumes, it is still unclear if plant protein isolates offer the same nutritional advantages as whole beans or reduce the risk of chronic illness. 2019 (Hu et al). (Hu et al.). Various plant-based meat substitutes, for instance, use protein found in soya (soya protein percentage ranging more than 90 percent) or concentrates (soy protein consists in percentage ranging from 70 to 89).

2.4.2Food Hygiene

The most common food allergies among plant-based substitutes are to soy and wheat, which are included in many of them (Food Drug Administration (FDA), 2004).

In people who are already allergic to peanuts and soy, pea protein and lupin protein can cause allergic reactions, however this is unique (2019, Lavine and Ben-Shoshan). Mycoprotein-based PBA have been linked to gastrointestinal problems and allergic reactions; however, it is unclear how common these reactions are in the general population.

People with intolerances to them should be cautious because plant-based substitutes contain a lot of certain dietary additives and gums. For instance, carrageenan, a structural ingredient derived from seaweed, is often used to thicken, gel, or stabilize plant-based replacements and other processed meals. Concerns regarding carrageenan's potential to produce gastrointestinal inflammation, changes in intestinal flora, and other side effects like colon cancer and irritable bowel syndrome have long raised doubts about its safety (Bixler, 2017; David et al., 2018). Furthermore, because carrageenan grows in seawater, it is expected to accumulate significant quantities of heavy metals; however, no studies have examined the potential for exposure to arsenic, lead, mercury, and cadmium from consuming foods which include carrageenan.

2.4.3Occupational Safety and Health

Employees in plant-based alternative manufacturing may face occupational exposure risks, but these risks are probably less severe than those posed by workers in the production of animal meat (*Vallaeys et al,2010.),* A consumer organization has expressed worry over the use of hexane in the manufacture of soy protein isolates used in plant-based meat replacements. It could alsobe used to generate pea protein isolates, albeit there is not much information available on this. (*2018, Tömösközi et al., 2001; Holt*). (Environmental Protection Agency, 2000) Hexane is a highly hazardous air contaminant as well as a toxic and highly flammable solvent. Overall, no specific information regarding worker protection, environmental release prevention, or exposure monitoring, as well as the quantity of hexane utilized in the production of pea and soy protein isolates, is available.

2.4.4 Community Well-Being

Meat substitutes made from plants and (theoretically) meats made from cells both rely on currently produced agricultural goods like corn, soybeans, and wheat. The process of growing the crops frequently results in fertilizer runoff, which can contaminate nearby groundwater supplies, in addition to the use of pesticides that have been related to long-term chronic health issues in farm workers and communities (Harrison, 2011). Low-level herbicides used in soybean growing, such as dicabam, 2,4-D, and glyphosate, have been linked to multi-resistance in diseases, endangering the efficacy of several treatments. In addition, there is evidence that the widespread use of fungicides in agriculture, such as when cultivating peas and soybeans, has contributed to the development of anti-fungal medication resistance, which is dangerous for those with weakened immune systems (Revie et al., 2018). Traditional beef typically uses more pesticides to manufacture than its plant-based alternatives since one traditional burger requires more animal feed than one traditional burger doe.

2.5 MEAT ANALOGUES COMPOSITION

The one tendency to offer a tasty substitute for meat is the development of goods that resemble meat, also referred to as meat analogues. The most challenging aspect for a food manufacturer is typically creating the appropriate texture and flavor for meat substitutes (Egbert and Borders, 2006). An examination of modern meat replacements reveals that the ingredients used determine how they differ in terms of texture, flavor, and colour.

We must first examine each ingredient's roles and goals in a traditional recipe in order to comprehend how they affect the sensory characteristics of meat substitutes. As stated by Egbert and Borders (2006), meat alternatives comprise of 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%). It also contains textured vegetable proteins (10% to 25%), flavorings, and binding agents. The combination of ingredients creates acceptable meat imitators in terms of sensory quality.

There are two ways that textured proteins can be utilised in place of meat. The first method combines texturized proteins with meat extensions, whereas the second technique completely substitutes texturized proteins for meat to produce an alternative that is completely vegetarian (Riaz, 2004). Once this meat extender is cooked it does not mimic the flavour texture or look of meat but when it is mixed with meat, it helps to increase the overall food quality. The goal of meat analogues, on the other hand, is to replicate the flavor, aroma, texture, colour, and sensation of whole meat when properly hydrated and cooked without the use of any substances derived from meat (Riaz, 2004; Singh et al., 2008). As a result, chemicals or substances are proved to alter the texture of raw material used as an alternative of meat to help in texturization.

2.5.1 Proteins

The increased demand for plant-based proteins is a result of the growing interest in protein sources other than those derived from animals. Price, availability, eligibility for inclusion in novel goods, and, most importantly, their functional properties all have an impact on the demand for new proteins (Haque et al., 2016). For the construction of meat-analog structures, proteins withoil-holding capacities, solubility, foaming, emulsification, gelation properties, H_2O and other functions are needed. However, these actions are influenced by the type of protein (amino acid sequence, chemical composition, secondary and tertiary structure). Environmental factors, for instance, can have an effect on the structure and performance of proteins through factors like temperature, pH, and ionic strength. The majority of meat substitutes now use soy protein due to its distinct advantages and low cost. Proteins from other oilseed crops, as well as those produced by fermentation based on microbes and other substrates, have previously been included into the production of meat substitutes (Kimetal., 2011). Currently, the commercial production of meat analogues (such as defatted soy flour, soy protein concentrates, wheat flour) uses protein-rich sources such rice, wheat, and maize as well as oil seeds, defatted flesh, meals, cereal & bean flours, and derivatives (Kumar et al., 2017). New protein sources, such leaves and algae in a meat-analog formulation for textured protein, have not been tested.

2.5.2 Soy Protein

Soy is well known for its nutritional and practical advantages. Because of its comparable nutritive content, it is frequently used for those with cardiovascular diseases and is recognized as a red meat alternative (Kumar et al., 2017). Soy protein scored 1.0 on the Protein Digestibility Corrected Amino Acid Score scale, making it the same as animal protein (Hoffman & Falvo, 2004). It reduces the risk of ischemic heart disease and blood cholesterol levels (Golbitz & Jordan, 2006). Soy protein is present in chicken-style breast, vegetarian sausage, chicken-style nuggets, and products that resemble sliced cooked meats. Consequently, soluble carbohydrate is removed from fibrous soy flour before it is textured by spinning or extrusion to produce textured vegetable protein. It is said to imitate meat muscle due to its chewiness and fibrous characteristics, giving consumers a unique eating experience from other soy patterns (Sadler, 2004). A variety of comfort dishes without meat have employed textured vegetable protein., such as bean burger and pattie, to cut costs sans sacrificing nutrition (Penfield & Campbell,1990).

2.5.3 Cereal Protein

Cereals are regarded as a highly significant component of food crops, and items made from grains have a significant impact on the food processing sector. According to a study by Malav et al. (2015), cereal protein can be obtained in various forms of flour, seeds, or flakes. Wheat protein, which has undergone processing and extrusion to resemble meat in texture, primarily consists of gluten. Meals made with wheat gluten often contain vegetables used to make textured protein that can be utilised as meat extenders and substitutes. Additionally, gluten is employed as a binder and extender to bind trimming parts and generate rearranged parts of ground beef patties. Hydrated gluten can be used to extrude, texturize, and turn into fibres to create a variety of substitutes of meat (Malav et al., 2015).

2.5.4 Legume Proteins

It has also been investigated how legume proteins from peas, lentils, lupines, chickpeas, and different kinds of beans can be used for functional purposes such emulsification, foam stabilisation, and gelatinization. Pea proteins are the most useful for applications that simulate flesh. On the other hand, pea-based structures are softer than soy-based goods. As a result, researchers are examining ways to alter protein hydrogen bonding, such as by adding chaotropic ions to salts (Sun and Arntfield, 2012), or by optimisingprocessing variables including pH, temperature, protein particle size, and other factors (Osen et al., 2014). Studies on chickpeas, lentils, and lupines have revealed that they are effective at stabilising emulsions and foam. These proteins do not gel as well as other proteins, with the exception of chickpeas.

2.5.5 Wheat Gluten

Another protein that is widely used is wheat gluten. It has a built-in capacity to produce a slim protein film that can readily be stretched into fibrous proteinaceous materials. These distinctive qualities result from molecular features and subsequent mesoscopic behaviour (Don et al., 2003). Gluten is a significant component in the construction of fibrous structures because of its disulfide protein linkage, which is essential for the development of a 3-D network (Ooms et al., 2018).

2.5.6 Mycoprotein

The first mycoprotein product was released on the market well before 1985. Mycoproteins are comparable in fibre content to other vegetarian forms of protein, devoid of cholesterol, with a balanced fatty acid profile and low levels of saturated fat. Mycoprotein's fibrous nature allows it to significantly cut blood cholesterol levels (Denny et al., 2008). Since it closely resembles the final product, the fibrous arrangement of filamentous fungal mycelia is frequently used as a meat replacement. Depending on the end product, the remaining ingredients are coupled with the flavourings, fungal biomass, and egg albumin-derived binding agent to create a product that mimics mycoprotein (Denny et al., 2008). The gel is created by heating protein binders, which attaches to the hyphae. The finished product has similar textural characteristics as meat products (Rodger, 2001).

2.5.7 Fat and Oil

The latest meat substitutes on the market are low in fat. Meat substitutes are frequently made from defatted meals. Additionally, the formation of fibrous structures is impacted by the addition of fat or oil during processing. Previous studies have shown that recipes with oil levels greater than 15% evolved in material lubrication that damaged macromolecule alignment during the extrusion processes (Gwiazda et al., 1987). According to Cheftel et al. (1992), the material might become greasy, which has a misleading effect on the shear forces that are applied during extrusion. On the other hand, using vegetable or fat oil to prepare a meat-analogue recipe has advantages because it can aid in tenderness and flavour release, both of which are crucial qualities for clients (Egbert and Borders, 2006). These days, plant-based meat substitutes are made with a variety of fats and oils, including rapeseed, sunflower, palm, canola, soy, coconut, and corn oil. The addition of oil or fat is considered essential since it can enhance the flavour of meat substitutes by retaining volatile flavour components.

2.5.8 Coloring Agents

Color and colour variations have a big impact on the quality of meat alternatives. As a result, colouring ants are considered a vital ingredient in meat supplements (Kyriakopoulou et al., 2019). Customers favour the thermostable colouring additions turmin pigments, cumin, and carotene (Vrljic et al., 2018). Depending on the colour preparation, a variety of colourants that are not heat stable are combined with reducing sugars that are comparable to the finished product (Rolan et al., 2008). Additionally, Sugar reduction can be used as a browning mediator., opposing the amine protein groups in a Maillard-type process that resembles the browning of meat (Kyriakopoulou et al., 2019). These are frequently utilised as colouring solutions prior to the extrusion process in plant-based meat. The third way of blending proteins and colourants involves injecting material into the extruder barrel or directly inside the extruder (Orcutt et al., 2008). However, there are not enough colouring compounds available in meat analogues to meet industry standards. To address this problem, acidulants such acetic acid, lactic acid, or citric acid, or mixtures of these acids, are utilised (Orcutt et al., 2008). The disadvantage of the solution is that a change in pH causes protein to degrade and changes the flavour of the finished product. In order to control or limit colour migration from the coloured structural meat-analogue, colour retention aids like hydrated alginate and maltodextrin are also used.

2.5.9 Flavors and other Ingredients

For theaverage consumer willing to adopt a meat alternative, it must have a decent flavour and taste (Kyriakopoulou et al., 2019). Currently, iron complexes, piquant spicing, flesh, and salty aroma are used in meat mimics as flavouring elements. Amino acids that contain sugar and sulphur are essential for increasing meat flavour. In addition, the mushroom concentration can be used to improve flavour instead of hydrolyzed vegetable protein or monosodium glutamate. Plant proteins were coupled with salt, calcium, potassium, and magnesium, which increased the functional potential of the meat mimics (Singh et al., 1997).

2.5.10 Binding agents

The meat analogues' binding agents are either plant- or animal-based compounds that work as a fat and water binder in concert. Examples of such substances include soy protein isolate and concentrate, xanthan gum, wheat gluten, carrageenan, eggs, milk proteins, and other elements. The main purposes of components high in protein are to bind water and create protein networks, whereas flours and starches are used as fillers. It is continually being researched how different binding agent concentrations affect the nutritional profile and qualitative characteristics of plant-based substitutes. Due to its cohesive and viscoelastic properties, which enable it to bind, form dough, and leaven, wheat gluten is an advantageous binder. Analogues of sausage made from mushrooms that had 5% saturated fat and showed the effects of casein, xanthan gum, soy protein concentrate, and carrageenan (Arora et al., 2017). Meat imitators also include egg white or albumen, which improves binding, boosts the protein composition of the products, and adds extra physicochemical.

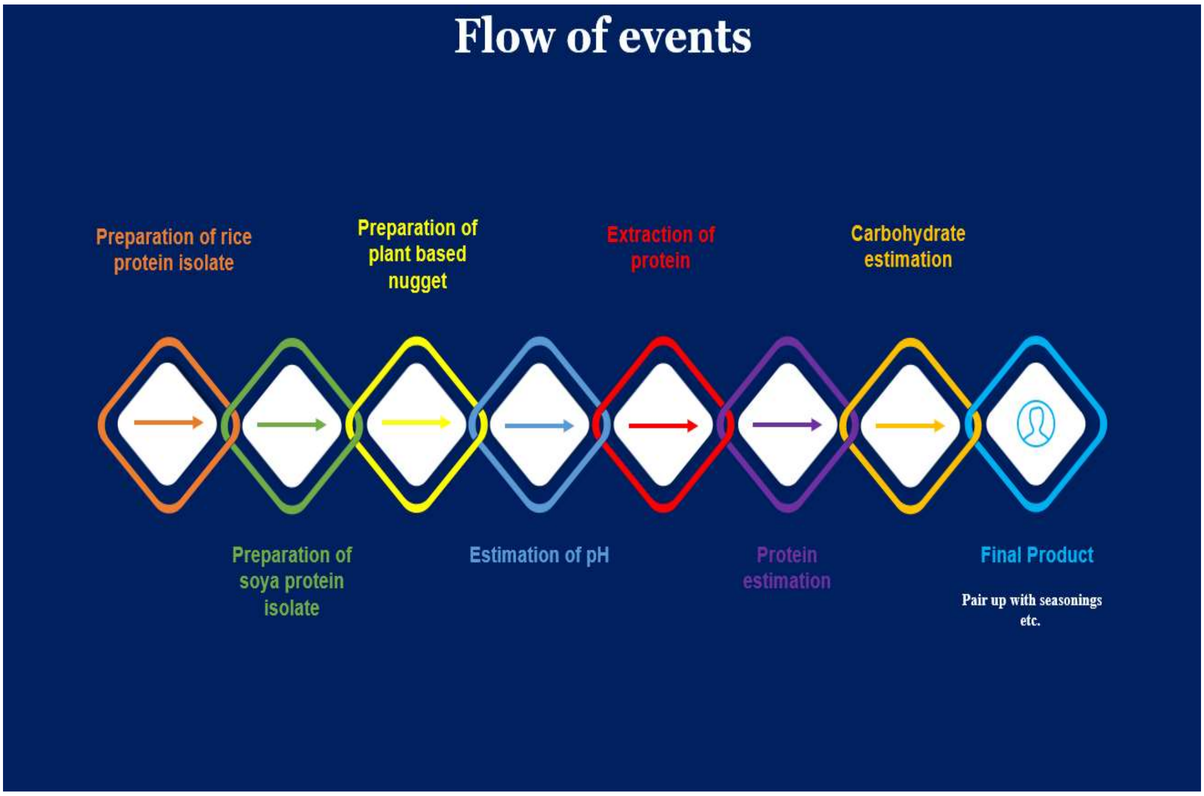

Figure 3 – Flow of events during experimentation

CHAPTER 3
Materials and Methods

3.1 PREPARATION OF ISOLATES

Brown rice and soy beans were bought from the neighborhood market, and rice protein isolate was created in the lab, mostly in accordance with the methodology described by Morita et al. in 1993. In a lab, soy protein powder was created. Market purchases included corn starch, methylcellulose (MC), baking powder, and vegetable oil (coconut oil). The flavour for the chicken powder was bought at the store.

3.1.1 Preparation of Rice Protein isolate

Brown rice was ground for three minutes in a grinder to create rice protein isolate. Then, 0.6% Termamyl 120L-dissolved in water at room temperature was added to 500g of rice flour. The created slurry was then heated for 1.8 hours at 98°C while being stirred occasionally. Concurrently, liquefaction and gelatinization were place. Rice protein isolate is made by boiling water three times, rinsing with regular water once, and filtering through cheesecloth. The dried protein isolate was then baked in a hot air oven at 66 degrees Celsius overnight.

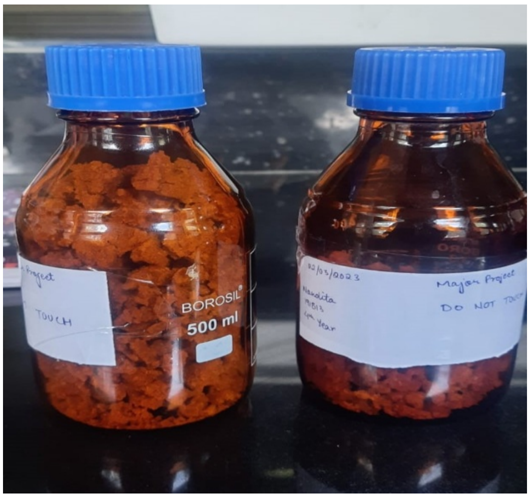

Figure 4 – Rice protein isolate

3.1.2 Preparation of Soy Protein isolate

Overnight, soy beans were dried at 70 degrees C in a hot air oven. Then, dry soy beans were ground for three minutes to create a fine powder. The powder was now rinsed in boiling water until the starch was removed, and then it was filtered through cheesecloth. Then, filtered protein isolate was once again dried in an overnight hot air oven at 70 degrees C.

Figure 5-Soya Protein Isolate

3.1.3 Preparation of plant-based nugget (PPN)

The composite mixtures shown in Table 1 were used to create the PPNs, which had a total of five different PPN ratios. To create 100 g of each composite protein analogue, the following ingredients were combined: 57% ice water, 3.5% vegetable oil, 0.3% salt, 11% maize starch, 2.51% baking powder, 1.5% methyl cellulose, and 0.2% calcium chloride. By boosting the protein's ability to bind water and creating air cells in the dough, the addition of baking powder and calcium chloride to the formulation improved the comparable fibrous structure. The equivalent was generated using separate protein and MC emulsions. 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). The ingredients were combined in a food processor and run at a low speed for two minutes to create the protein emulsion (proteins, corn starch, salt, baking powder, calcium chloride solution, flavouring agent, and ice water). The entire experiment used de-ionized water (DI) water. The protein was fully hydrated by completing this step. By blending MC powder, vegetable oil, and ice water for two minutes at a low speed, a uniform MC emulsion was produced. Three minutes were spent combining the created protein and MC emulsions. After that, the PPN analogue batter was shaped into a 5 cm by 3 cm by 2 cm size (LxWxH). The moulded item was steamed for 15 minutes at 98°C. It was then frozen at -20°C for 48 hours. It was then frozen at -20°C for 48 hours. Three duplicates of each formulation were created. PPN were distorted for testing purposes then overnight dried at 55 degrees in a hot air oven. Each dry sample was ground into a fine powder for testing purposes.

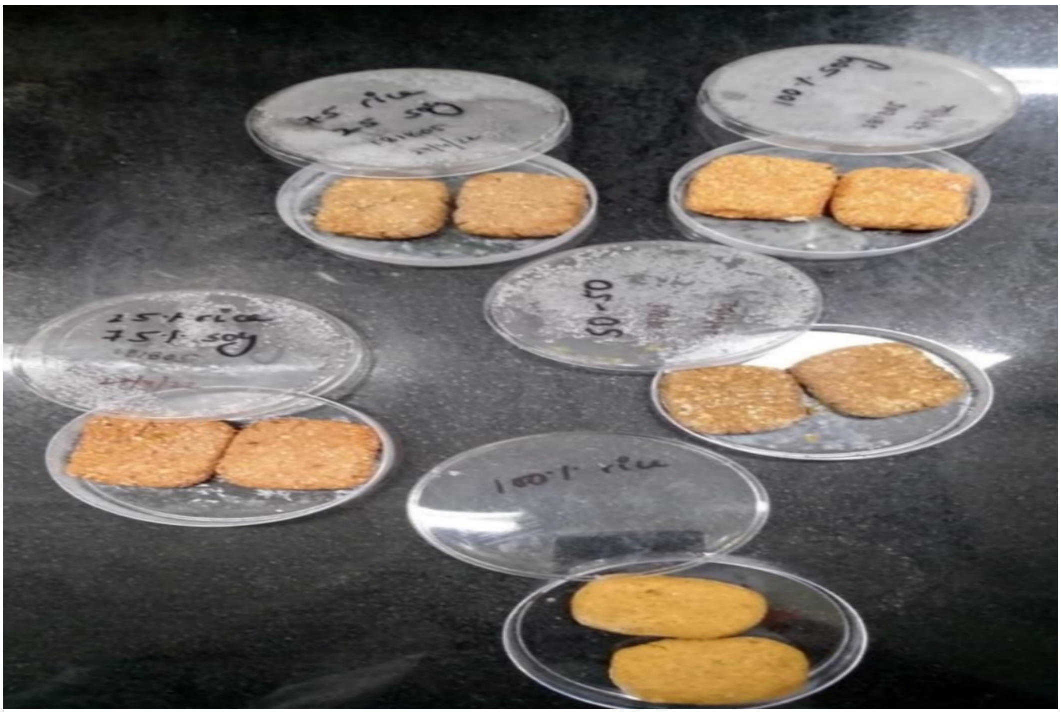

Figure 6 – Plant Based Nugget

Table 1 - Formulation of PPN analogues with different protein ratios

Composites	Soy Protein%	Rice Protein%
PP1	100	0
PP2	80	20
PP3	60	40
PP4	50	50
PP5	20	80
PP6	0	100

3.2 TESTING OF BIOLOGICAL AND CHEMICAL PARAMETERS

3.2.1 pH

2.5g of the sample is mixed with 100ml of distilled water and homogenized for three minutes in order to calculate the pH of each plant-based protein analogue. The pH of the samples was measured using a pH meter after they had been homogenized.

3.2.2 Protein Extraction

Protein extraction followed the guidelines of (Barbarino et. al, 2005). The samples were dissolved in 14 mL of distilled water, homogenized, and then incubated for 24 hours at 25°C. Following that, samples were centrifuged at 4000 g for 15 minutes at 4°C. The pellet was redissolved in 14 mL of 0.1 M NaOH in 3.5 percent NaCl after the supernatant had been removed, and it was then incubated for 24 hours at 25°C. The samples were vigorously shaken throughout both incubation times. The samples were then centrifuged for a further 15 minutes at 4000 g at 4°C. The final extracts were estimated for protein after combining the two supernatants.

3.2. 3 Protein Estimation

Protein estimation using the **Lowry method**

The protein content was ascertained using Lowry's technique.

The Lowry method, which measures protein concentrations, is based on two principles: the reactivity of peptide nitrogens with copper [II] ions in an alkaline state, and the copper-induced reduction of Folin-Ciocalteay phosphomolybdic phosphotungstic acid to heteropoly molybdenum blue. The "Folin-Ciocalteu reagent" is used in the Lowry method to react Cu+ produced by peptide bond oxidation. Heteropoly molybdenum Blue, a vivid blue chemical, is produced by this process.

Reagents used:

- "2% Na_2CO_3 in 0.1 N NaOH"
- "1% NaK Tartrate in H_2O"
- "0.5% $CuSO_4$.5 H_2O in H_2O"
- 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:

- Add 0.2 ml of BSA working standard and 1 ml of distilled water to 5 test tubes.
- A test tube containing 1 mL of distilled water is used as the blank.
- Place 4 mL of Reagent A in the incubator for 10 minutes.
- Add 0.5 ml of reagent B after 10 minutes of incubation, then let it sit for 30 minutes in the dark.
- Place the absorbance at 660 nm on the standard graph.
- Calculate the amount of protein in each sample.

3.2.4 Carbohydrate Estimation

Principle:

Furfural is created when Conc.H2SO4 dehydrates carbohydrates. The active form of the reagent, anthronol, an enol tautomer of anthrone, reacts with the carbohydrate derivative of furfural to create a green colour in diluted solutions and a blue tint in concentrated solutions. The maximum absorption of the blue-green solution is 620 nm.

Reagents:

Glucose stock solution is the test solution.

Anthrone Reagent: Concentrated H2SO4 with 0.2% Anthrone (0.1gm in 100ml H2SO4)

Procedure:

- Pipette various volumes of the given stock solution of glucose solution (200g/ml) into a series of test tubes, then diluted with distilled water to 1 mL.
- Assume that tubes 2 through 6 are utilised to construct a standard curve and that tube 1 is empty.
- The tubes 7–11 hold the unidentified materials.
- Each tube should receive 4 mL of the given anthrone reagent.
- Combine well by vortexing. Give the tubes time to cool.
- Place the caps or marbles on top of the tubes and incubate them at 90° C for 17 minutes. Before comparing the optical density to a blank at 620 nm, let it cool to room temperature.
- Construct a standard curve for absorbance against glucose.

CHAPTER 4

Results and Discussion

4.1 pH

The pH of the experiments shown a tendency to decline with increasing soy protein and decreasing rice protein. The pH of PP2 and PP4 are relatively comparable, while PP5's formulation has the greatest pH (6.73) and PP1's has the lowest pH (6.14). The beginning pH of the protein sources may be connected to the pH differences between samples.

Table 2 – pH of different meat substitutes

COMPOSITE	pH
PP1	6.17
PP2	6.28
PP3	6.46
PP4	6.35
PP5	6.72

4.2 Protein Estimation

The highest protein concentration was reported in PPN manufactured entirely from soy(0.15 mg/ml). While PPN made entirely of rice has the least amount of protein (0.07 mg/ml).

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

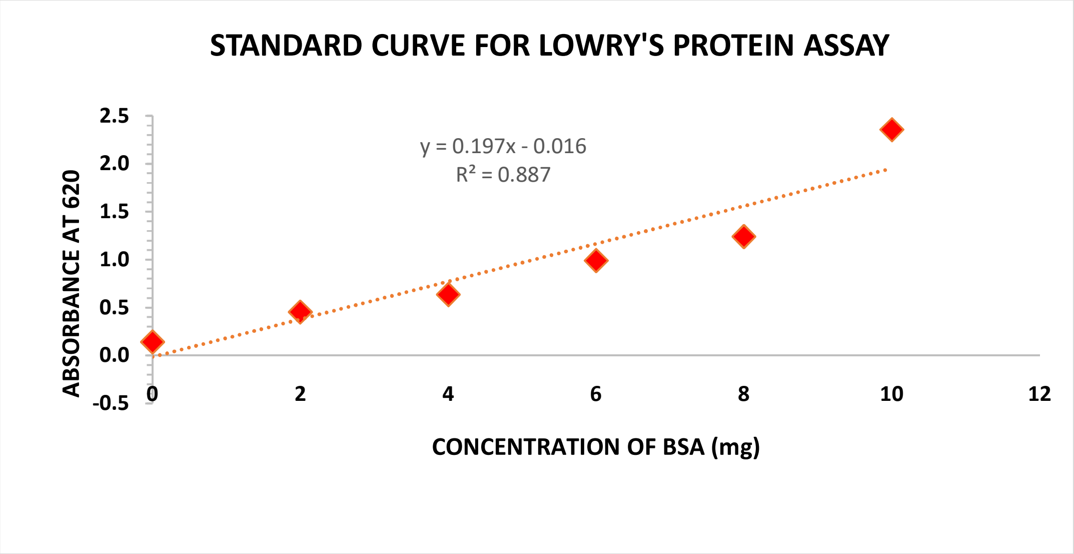

Figure – 7 Standard curve of Lowry's assay for protein estimation

Table 3 – Protein concentration in meat substitutes

Meat substitute	Protein Concentration
PP1	0.17
PP2	0.11
PP3	0.16
PP4	0.15
PP5	0.19

4.3 Carbohydrate Estimation

The highest carbohydrate concentration was obtained in PPN made entirely of rice (0.23 mg/ml). While PPN manufactured entirely from soy provides the least amount of carbohydrates (0.08 mg/ml),

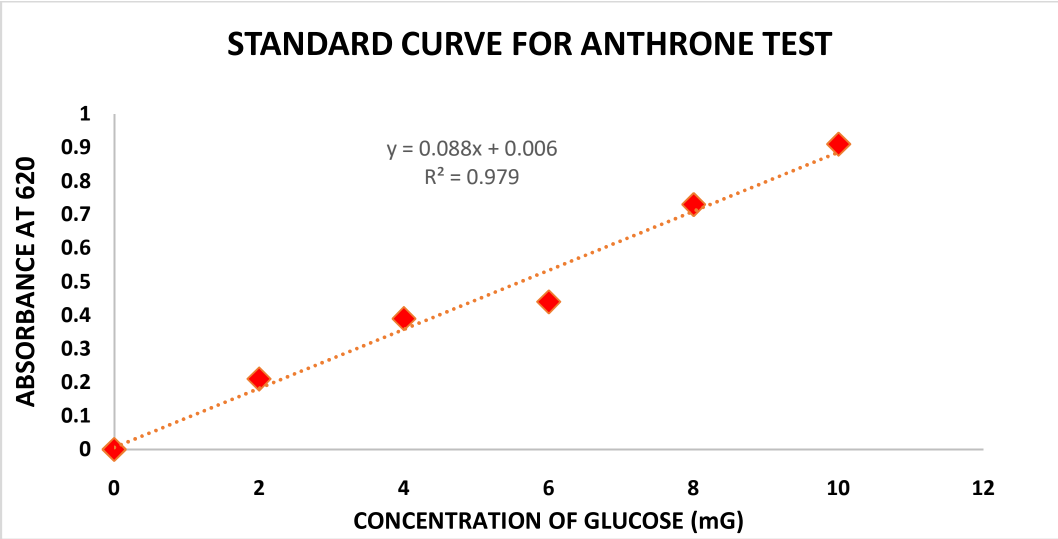

Figure 8 -Standard curve of Anthrone method for carbohydrate estimation

Table 4 – Carbohydrate concentration in meat substitutes

Meat substitutes	Carbohydrate Concentration
PP1	0.11
PP2	0.14
PP3	0.17
PP4	0.20
PP5	0.25

CHAPTER 5
Conclusion

According to the literature, a lot of study has been done in the past year on various protein sources for making plant-based meat. The rise in studies is being driven by worries about ethics, health, and the environment. As the number of research increases, so does the variety of foods. The production of meat analogues using the freeze structure procedure to mix several protein sources is the main topic of this study, which also examines the effects on a product's nutritional profile. Soy and brown rice protein sources were blended in the study in 5 different ratios to compare the nutritional content of each counterpart and look for any variations. Several analogues and rice and soy protein isolates were made before any experiments were started. A protein test using the Lowry method revealed that meat nuggets made entirely of soy had the highest protein concentration and those made entirely of rice had the lowest. However, the Anthrone method's carbohydrate test revealed that soy had the lowest carbohydrate concentration and rice-based nuggets had the highest. The samples' pH values ranged from 6 to 7, with 6 being the highest. For instance, PP1 (100% soy) has the lowest pH while PP5 (100% rice) has the highest pH. In soy-based nuggets, the amount of protein is at its highest and the amount of carbohydrates is at its lowest.

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