DEVELOPMENT AND QUALITY EVALUATION OF ALLERGEN FREE VEGAN PROTEIN COMPOSITE FLOUR AND ITS APPLICATION IN FOOD PRODUCTS

DISSERTATION SUBMITTED

In partial fulfillment of the requirement for the award of the degree of

MASTER'S PROGRAMME IN
CLINICAL NUTRITION AND DIETETICS

By

ANSHIYA V P
(Register No: SM23MCN004)

DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

APRIL 2025

CERTIFIED AS BONAFIDE RESEARCH WORK

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Signature of Internal Examiner **Signature of External Examiner**

DECLARATION

I hereby declare that the project entitled **"DEVELOPMENT AND QUALITY EVALUATION OF ALLERGEN FREE VEGAN PROTEIN COMPOSITE FLOUR AND ITS APPLICATION IN FOOD PRODUCTS"** submitted in partial fulfillment of the requirement for the award of the degree of Master's Programme in Clinical Nutrition and Dietetics is a record of original research work done by me under the supervision and guidance of **Ms. Namitha Prastheena Joseph**, Assistant Professor, Department of Clinical Nutrition and Dietetics, Women's Study Centre, St. Teresa's College (Autonomous), Ernakulam and has not been submitted in part or full of any other degree/diploma/fellowship or the similar titles to any candidate of any other university.

Place: Ernakulam ANSHIYA V P

Date: 28/04/2025

CERTIFICATE

I here certify that the dissertation entitled **“DEVELOPMENT AND QUALITY EVALUATION OF ALLERGEN FREE VEGAN PROTEIN COMPOSITE FLOUR AND ITS APPLICATION IN FOOD PRODUCTS”** submitted in partial fulfillment of the requirement for the award of the degree of Master’s Programme in Clinical Nutrition and Dietetics is a record of original work done by **Ms. Anshiya V P** during the period of the study under my guidance and supervision.

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ABSTRACT

The growing demand for allergen-free and plant-based protein sources among vegetarians and individuals with food allergies. The present study aims to formulate allergen free vegan protein composite flour by combining millets, pulses and oil seeds in ratio 60:30:10 and evaluating its functional properties, nutritional profile, antinutritional factors, shelf life and invitro protein digestibility as well as diabetogenic activity. Kodo millet, chickpea green gram, and sunflower seed were chosen for the development of protein composite flour and these ingredients were combined in three proportions such as T1 (60:20:10:10), T2 (60:15:15:10) and T3 (60:20:10:10). Value added foods products were developed using the selected protein composite flour in three proportions T1 (70:30), T2 (60:40) and T3 (50:50) which were further evaluated for sensory acceptability by trained panel members using 9-point hedonic scale. The findings of the study indicated that T2 had the better functional properties, nutritional profile, protein digestibility, antidiabetic potential, shelf life and lower antinutritional factors. As of keeping quality, the product was stable till 47 days. Further, T2 was utilized for developing various value-added food products such as cheela, smoothie, pancake and thick soup; where T1 (70:30) had the highest organoleptic acceptability among all the value-added products. Thus, the study successfully developed allergen-free vegan protein composite flour by integrating millets, pulses and fatty seeds offering a promising dietary option for protein enhancement especially among vegetarians and individuals with food allergies.

Keywords: Protein composite flour, functional properties, nutritional profile, organoleptic evaluation, shelf-life analysis and value-added products.

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CHAPTER-I
INTRODUCTION

Over decades India has been following vegetarian diet and still there are people following this diet. Around 20 – 39% of Indian population is still following vegetarian diet (Corichi, 2021). Religious beliefs have an impact on the predominance of vegetarianism in India as well as the social norms, socioeconomic status and caste also have impacts on choosing vegetarian diet. Vegetarians mostly rely on grain and pulses for protein in their diet, but they are not complete protein and thus it is hard to gain complete protein from vegetarian diet.

Indian diet is mostly composed of grain, pulses and vegetables and people who consume non vegetarian foods as well as have high socioeconomic status have access to complete protein sources like egg, poultry, fish, etc. But people don't know how to use vegetarian sources the correct way for obtaining the right amount of protein. To reduce this gap of malnutrition, itis important to use underexploited protein rich crops because of major source of protein in the developing countries like India, where consumption of non-vegetarian food is still considered as religious and social taboo (Pratibha, 2021). In order to reduce the protein deficit in the diet of vegetarian population, inclusion of good protein sources is important. The protein sources can be pulses, millets or a combination of pulses and millets.

Atopic disease like food allergy, atopic dermatitis, asthma and allergic rhinitis affects about 20% of the population around the world (Dadha *et al.* 2024). Due to the development of allergic reaction individuals often avoid such food items and in the fear of developing those allergic reactions they even remove variety of food from their diet which leads to several nutrient deficiencies and disorders. All these happen due to lack of nutritional knowledge and proper education on food and nutrients. Once food allergy occurs the possible reaction the body shows are; itching, hives, redness, swelling, wheezing, trouble breathing, dizziness, etc. These allergic reactions occur due to defective immune system. Body's immune system has many antibody made up of immunoglobulins (Ig) such as immunoglobin A (IgA), immunoglobin D (IgD), immunoglobin M (IgM), immunoglobin E (IgE) and immunoglobin G (IgG). Allergic reaction occurs when common harmless substance comes in contact with body's immune system (IgE) the system reacts causing allergic reactions this is called hypersensitivity. Food allergy can be either IgE mediated or non-IgE mediated or a combination of both. A childhood allergy of egg or milk has been seen to be cured in few years in some cases while in other cases they persist. Most of the allergies are linked to protein present in the foods which generally are not allergic and body finds them allergic and causes allergic reactions. Protein present in milk, egg, peanut, tree nuts, fish, shellfish, soy, and wheat has been found to be causing allergic reactions (Wang *et al.* 2011). People allergic to milk are mostly allergic to lactose present in milk due to deficient lactase enzyme necessary for the breakdown of lactose sugar due to which they become intolerant to lactose sugar. In case of gluten sensitive enteropathy or celiac disease, people are allergic to gluten a protein present in wheat, rye and barley. On consumption of gluten or gluten containing food item allergic reactions occurs. In case of people with urticaria, when they get exposed to certain food they get allergic reactions or become sensitive to that particular food and develops symptoms such as itching, rashes, redness and swelling. They could be relieved on consumption of certain medications like antihistamines and by avoiding the allergens. People can also be allergic to certain nuts such as; peanuts, almonds, pistachios and other nuts which causes allergic reactions like hives, cramps, nausea or vomiting and swelling. Individuals can also be allergic to pulses and legumes like soybean, kidney bean and so on which on consumption causes allergic reaction.

Allergic reactions has various phases such as immediate hypersensitive reaction the first stage of allergic reaction occurs within few minutes or seconds of consumption, early reaction where reaction time differ from minutes to hours and late phase reaction which takes hours to cause reaction. Allergic reaction could acute which might be gone in few hours and reactions could be anaphylactic which is serious and potentially life threatening allergic reaction (Galli *et al.* 2008). Thus people try their best to stay away from allergens and avoid reactions but in the process of avoiding allergens they might avoid other foods which are not allergic which leads to nutrient deficit and many other health problems. To correct the deficiencies incorporation of several nutrients are important and most of the population in India are not aware of this and does not incorporate those essentials nutrients to their diet.

Protein being essential nutrient is very important for human beings for the development and growth of body and for the growth, synthesis and repair of tissues in the body. Proteins are required to provide support to immune system, for production of hormones and enzymes, provide structure and support to cells, for transport of nutrients in the body and at times serves as energy source. Protein is made up of 20 amino acid such as; alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Both animal and plant protein are made up of these 20 amino acids. Amino acids are of two types essential and non-essential amino acids, where essential amino acids cannot be synthesized by the body and dietary supplementation is necessary where as non-essential amino acids can be produced by human body. Out of 20 amino acids 9 amino acids are termed as essential amino acids they are; histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Deficiency of these amino acids can cause symptoms like stunted growth, poor development, lean muscles fragile skin and hair, impaired wound healing, tissue repair and other symptoms like poor mental health can occur. Thus dietary supplementation is necessary and healthy balanced diet includes them. In healthy diet protein could come from cereals, pulses, legumes, poultry, fish, egg, dairy products, nuts and seeds and in small amounts in fruits and vegetables. As per the Indian Council of Medical Research (ICMR) and the National Institute of Nutrition (NIN), the Recommended Dietary Allowance (RDA) for a normal individual should be consuming 0.83g/kg/day body weight per day protein which should include essential amino acids (ICMR-NIN, 2024). Non-vegetarian sources of protein are considered as complete protein due to their rich essential amino acid profile whereas vegetarian sources are considered incomplete as they lack certain essential amino acids. As people in India prefer vegetarian food over non-vegetarian due to several reasons such as cultural factors, economic status and religious beliefs they don't get complete protein leading to health issues and due to allergies to certain food they avoid most of the protein food items thus complications like stunted growth and delayed development occurs. But complete essential protein profile can be obtained from vegetarian sources by combining them like combining cereals and pulses can lead to complete essential amino acid profile otherwise known as complete protein. Combination of millets and pulses can result in producing complete protein requires for vegetarians and choosing hypoallergenic cereals and pulses can result in complete protein for allergic people. Millets like kodo millet, pulses like green gram and chick pea and oily seeds like sunflower seed are hypoallergenic in nature and have different essential amino acids contributing to complete protein. Thus the combination of these cereal, pulses and seed can give complete amino acid profile. In general vegetarians can get complete amino acid by combining different pulses and cereals.

Millets are cereals which belong to family *Poaceae* which are cultivated as grasses and they have small kernels. There are different types of millets such as; sorghum (jowar), proso (chena), pearl (bajra), foxtail (kakum), finger (ragi), browntop (korle), barnyard (sanwa), kodo (araka), buckwheat (kuttu), amaranth (rajgira) and little (moraiyo) millet. Millets are rich in macronutrients and micronutrients as well as rich in fiber which helps to provide good nutritional profile. Millets are widely produced in India and is one of the countries which produce millets in large amount. Millets can be grown in short period and withstand drought also have long storage period without insect damage (Adekunle, 2012). Millets are rich in protein, fiber, fat and mineral content. All millets are rich in calcium, iron, folic acid, niacin, potassium, magnesium and zinc (Parvathy and Thayumanavan, 1995). Millets are low in price and easily available but the consumption of millets is very low in different parts of country especially in southern India.

Kodo millet (*Paspalum scrobiculatum L.*) originated in Africa and later on started harvesting in India is a drought resistant type of millet grown in arid and semi-arid regions of Africa and Asian countries. Kodo millet is popularly known as *varagu or kodra* in India. It is rich in dietary fiber and minerals like iron, antioxidants and have lower phosphorus content compared to other millets and cereals (Dey *et al.* 2022). Different processing techniques can reduce the anti-nutrient content of kodo millet. The nutrient profile of kodo millet is better than that of other cereals like rice and wheat. Kodo millet is rich in vitamins, minerals, and phytochemicals containing sulfur, so it is called "nutria-cereals" It is also rich in essential amino acids, like lysine, threonine, valine, sulphur containing amino acids and the ratio of leucine to isoleucine is about 2.0 (Ravindran, 1992; Antony et al., 1996), but it is deficient in tryptophan amino acid. Kodo millet also has rich profile of vitamin B and minerals like calcium, potassium, magnesium and zinc. Kodo millet is beneficial for diabetic people due to its ability to lower blood glucose. As kodo millets are rich in antioxidants they helps in reversing or delaying aging process in individuals who consume this millet. Kodo millet also have anti cancerous property thus helps to fight or prevent cancer and also helps people with celiac disease who wants to avoid wheat, barley and rye to choose kodo millet as substitution (Dey *et al.* 2022). Due to the high antioxidant profile kodo millet helps to scavenge the free radicals causing cardio vascular diseases. Kodo millet can be incorporated in the preparation of variety of food items such as; roti, baked goods, dosa, ladoo, and so on.

Chickpea (*Cicer arietinum* L.) belongs to legume crop group commonly known as bengal gram or garbanzo bean. There are two distinctive market types of chickpea; desi and kabuli. India is the largest producer of chickpea in world. Chickpea is widely consumed across the country. The demand for chickpea is high due to its nutritional profile. Chickpea is the major source of protein in certain regions where non-vegetarian sources are expensive and chickpea is affordable. Chickpeas are good source of vegetarian protein and are hypoallergenic as well thus can be consumed by people who are allergic to non-vegetarian sources of protein. Also they are good source of dietary fiber, vitamins and minerals. Chickpeas are consumed in different forms across the world such as; grounded to form chickpea flour (besan), chickpea splits are used as dhal, chickpea is pasted to get milk, tofu is made out chickpea milk and in some regions especially in middle eastern countries chickpea is boiled and pasted to form hummus and so on. Due to the rich protein profile chickpea consumption can help prevent protein energy malnutrition in children and the bioavailability of protein is high compared to other forms of vegetarian protein. Application of different processing techniques can help remove anti-nutritional factors from chickpea such as; soaking, cooking/boiling, roasting, sprouting, fermentation and dehulling. Chickpea is inexpensive and locally available. The nutrient and antinutrient composition of chickpea help to prevent several chronic diseases and the oil present in chickpea have antiulcerative, antibacterial, antifungal, antitumoric and anti-inflammatory properties also they help in lowering cholesterol levels in human beings. The vitamin profile is also good in chickpea than other pulses.

Green gram (*Vigna radiate* L.) is one of the most important legumes grown and consumed in India. Green gram is less expensive and easily available and widely consumed by the population in our country. Green gram is excellent source of protein, high in dietary fiber, rich in vitamins and minerals. As it have low glycemic index and high folate content the consumption helps in lowering blood glucose level and neural tube defects in new born infants (GRDC, 1994; USDA Nutrient Database for Standard Reference, 2001). Green gram is commonly known as mung bean. The antinutrients present in green gram can be removed by different processing methods such as soaking, roasting, cooking, sprouting and fermentation. Green gram is grown in warm areas as well as they are drought resistant and is mostly cultivated in Asian countries. For people who cannot afford non-vegetarian protein or allergic to those protein sources can rely on protein from green gram. The protein present in green gram is easily digestible as compared to that of other legumes. This legume has several beneficial properties such as anticancer, antihypertensive, hypoglycemic, hypolipidemic, hepatoprotective properties. Thus consuming green gram along with cereal can improve the protein content and makes it complete protein.

Sunflower (*Helianthus annuus*) seeds are obtained from sunflower and are one of the nutritious seed consumed by human beings. They are consumed widely by humans for obtaining good oils and protein from the seed. The seed contains high amount of biologically available protein, fats, dietary fiber, vitamin E, vitamin B and minerals such as phosphorus, calcium, magnesium, iron, zinc, copper, manganese and selenium. On processing the seed sunflower oil can be obtained. They are also rich in antioxidants. There are various health benefits such as; antihypertensive, anti-inflammatory, antilipidemic and antidiabetic property (Vasudha and Sarla, 2021).

RELEVANCE OF THE STUDY

When it comes to protein intake, Indian diet lacks protein sufficiently because of reasons like religious beliefs, vegetarianism, food allergies and lack of knowledge about the importance of protein in the diet. Consumption of millets and pulses are common in many parts of the country but not in a combination. When combining millets, pulses and fatty seeds the nutrient profile is so rich that it provides complete protein, fiber, vitamin B, calcium, iron, zinc, magnesium and vitamin E. Thus it contributes to several health benefits and also makes up complete protein. Studying the nutrient profile, antinutrient factors, protein digestibility and the anti-diabetic potential of the composite flour developed by blending millets, pulses and fatty seeds helps to provide better insight about its health benefits and ensures a complete protein. Incorporation of millets, pulses and fatty seeds into daily diet provides strong basis for protein intake among vegetarian people. Development of food product from this type of composite flour might be very useful in improving the quantity and quality of protein intake as well as the protein digestibility in the body. Thus, an effort was made to develop a composite flour using kodo millet flour, chickpea flour, green gram flour and sunflower seed flour as an alternative protein source for vegetarians and individuals who are allergic to non-vegetarian protein, gluten and other allergies in order to combat the protein deficiency as well as to ensure confident and safe consumption of protein.

AIM

The current study entitled **"Development and Quality Evaluation of Allergen Free Vegan Protein Composite Flour and Its Application in Food Products"** aimed to formulate, standardize and evaluate the quality and functional properties of allergen free vegan protein composite flour developed from selected plant-based ingredients such as kodo millet flour, chickpea flour, green gram flour and sunflower seed flour and to assess its incorporation into the diet.

OBJECTIVES

The specific objectives of the study envisaged are as follows:

1. To formulate and standardize allergen free vegan protein composite flour using selected ingredients like chickpea flour, green gram flour, kodo millet flour and sunflower seed flour.
2. To analyze the nutrient and antinutrient composition of the developed composite flour.
3. To assess the functional properties of the developed composite flour.
4. To determine the protein digestibility and antidiabetic potential of the developed composite flour.
5. To ascertain shelf life of the developed composite flour.
6. To develop value added products from the developed composite flour.
7. To evaluate sensory acceptability of the value-added products.

CHAPTER-II

REVIEW OF LITERATURE

A thorough literature review has been conducted in accordance with the study's objective. Numerous researches on protein intake have been carried out and various strategies have been tried to enhance the availability of protein. However, this chapter lists studies that have been done both domestically and abroad under the following heads:

- Formulation of composite flour
- Nutritional evaluation of the composite flour
- Organoleptic evaluation of value added products from composite flour
- Effect of storage on physical, chemical and microbiological aspects of composite flour

2.1: FORMULATION OF PROTEIN COMPOSITE FLOUR

Jappe, U. (2023) conducted a study on *"Vegan diet – alternative protein sources as potential allergy risk"* this study emphasizes that allergenic proteins including storage proteins, oleosins, and lipid transfer proteins, which can cause severe allergic reactions, are present in a variety of popular vegan protein sources, including legumes, nuts, seeds, cereals, and pseudo-cereals. The study also advises that eating several allergenic foods may intensify allergy reactions and highlights the increased risk for people with pollen-related food allergies because of cross-reactivity. The results emphasize how important it is to be aware of allergy risks, particularly for those who have atopic diseases.

Kurek *et al.* (2022) evaluated a study on *"Novel Protein Sources for Applications in Meat-Alternative Products- Insights and Challenges"* which examines new protein sources for meat substitutes as well as the related sensory and technological difficulties. The study evaluates the nutritional profiles and functional characteristics of plant-based proteins (such as those found in legumes, cereals, and oilseeds) as well as non-traditional sources including algae, insects, and fungi that are pertinent to meat analogues. It talks about processing methods that simulate the textures of meat, like wet spinning and high-moisture extrusion. The authors also point out that it is difficult to replicate the nutritional value and sensory qualities of meat, highlighting the need for more study and consumer education to increase the acceptance of innovative protein-based meat substitutes.

Berue, S. (2012) performed a study titled *"Vegan Protein Supplements of High Biological Value"* the study emphasizes on the nutritional value of vegan protein powders, particularly for people with certain dietary requirements such kidney disease. It highlights how correctly mixed plant protein like rice, peas, and soy can offer a full amino acid profile and reach a high biological value that is on pace with animal proteins. According to the study, they are advantageous for those with chronic kidney disease since they can be included in diets low in potassium and phosphorus. All things considered, author comes to the conclusion that properly prepared vegan protein powders are a beneficial dietary choice for people following plant-based diets.

Quiñones *et al.* (2015) conducted a study on *"Development of Gluten Free Composite Flour Blend"* which focusses on developing composite mixes of non-wheat components to provide gluten-free flour substitutes. The research creates flours from a variety of regional and underutilized crops, including rice, cassava, and legumes, to meet the dietary requirements of those with coeliac disease or gluten sensitivity. To ascertain if these mixes are appropriate for baking and other culinary applications, the study assesses their functional, nutritional, and sensory qualities. According to the results, blends with the right proportions can have a texture, flavor, and nutritional content that are acceptable and on par with conventional wheat-based flours. The study emphasizes how these substitute flours can be used to enhance local agriculture and food security in addition to meeting health-related demands.

Umoh *et al.* (2023) conducted a study on *"Influence of extrusion process conditions on bulk density, water absorption capacity and oil absorption capacity of extruded aerial yam-soybean flour mixture"* in this study the researchers used a single-screw extruder and adjusted three important parameters: feed moisture (31–39%), screw speed (85–145 rpm), and barrel temperature (95–115°C). The functional characteristics of the extrudates were shown to be strongly impacted by each of these parameters. The ideal parameters for increasing bulk density, water absorption, and oil absorption were found to be 110°C for the barrel, 130 rpm for the screw, and 37% for the feed moisture content. In order to maximize the texture and functional characteristics of extruded goods derived from aerial yam and soybean flour combinations, the results emphasise the significance of modifying the extrusion parameters. This could have significant uses in the creation of food products.

Tharise *et al.* (2014) investigated a study titled *"Evaluation of physio-chemical and functional properties of composite flour from cassava, rice, potato, soybean and xanthan gum* *as alternative of wheat flour"* this study evaluated factors such swelling power, gelatinization temperature, water and oil absorption capabilities, moisture, protein, fat, ash, and carbohydrate content. According to the results, adding soybean boosted the protein content while xanthan gum enhanced the retention of water and oil. The composite flours were shown to be potential alternatives to wheat flour, particularly in gluten-free or allergy-sensitive food products, and they showed appropriate functional qualities for food processing.

Joye, I. (2019) performed a study on *"Protein Digestibility of Cereal Products"* this study examined the protein digestibility of different cereal-based products, emphasizing the ways in which digestibility is influenced by grain type, processing techniques, and protein interactions. According to the study, although cereals constitute a major protein source in many parts of the world, their proteins are typically less digestible than those of animals. By altering the structure of proteins and lowering anti-nutritional components, processing methods such as fermentation, extrusion, and enzymatic treatment can increase digestibility. However, other techniques, such as using too much heat, can cause proteins to aggregate, which could make them less digestible. In order to improve the nutritional value of cereal proteins, the research highlights the necessity of optimizing processing conditions, especially for populations that primarily consume plant-based diets.

Day, L. (2013) conducted study on *"Proteins from land plants – Potential resources for human nutrition and food security"* this study provides insights on the nutritional characteristics, role in food systems, and environmental sustainability of a variety of plant protein sources, such as legumes, grains, and oilseeds. It emphasizes that while some plant proteins may have limited amino acid profiles or is less digestible than animal proteins, they can nonetheless supply essential amino acids. The technological difficulties in extracting, processing, and enhancing the functional and sensory properties of plant proteins are also covered in the article. The study concludes by highlighting the growing significance of plant-based proteins in meeting the growing demand for wholesome, sustainable food worldwide, especially in light of dietary changes, environmental concerns, and population expansion.

Sim *et al.* (2021) performed a study on *"Plant Proteins for Future Foods: A Roadmap"* in this study the authors point out that although plant proteins have economic and environmental benefits, their use in food products is restricted because of their subpar functional qualities, which include solubility, foaming, emulsifying, and gelling. The article talks about developments in protein extraction, fractionation, and modification methods targeted at improving functioning in order to solve these issues. It also highlights how crucial it is to comprehend the interactions between proteins and polysaccharides and create innovative structural techniques in order to enhance the flavor and texture of plant-based diets. In order to promote global food security and sustainable diets, the authors support a multidisciplinary strategy that combines food science, nutrition, and technology to speed up the development of plant protein components.

Beena *et al.* (2022) conducted a study on ***"**Development of High Protein Composite flour pre-mix for Women Using Response Surface Methodology***"** the goal of the study was to blend several flours in order to preserve desired sensory qualities and an optimal protein level. In order to increase the pre-mix's protein, fibre, and vitamin content, pulses and grains were important additions. The optimized flour blend has the potential to be used in food products to address protein-energy deficiency, as the results showed that it satisfied the nutritional needs of women. The study emphasizes how RSM is used in food product development to create nutrient-dense, functional formulations.

2.2: NUTRITIONAL EVALUATION OF THE COMPOSITE FLOUR

Sudarsanan *et al.* (2023) conducted a study on topic *"Improved extraction and modification of plant protein from desi chickpea variety using green technology"* major goals of the study was optimizing protein production and improving functional qualities like solubility, emulsifying, and foaming capacity all crucial for food applications. To increase protein recovery and quality without the use of hazardous chemicals, the researchers used methods like enzymatic treatment and ultrasound-assisted extraction. According to their research, green technologies enhanced the chickpea protein's techno-functional qualities and extraction efficiency, making it more suited for use in plant-based food items. The study emphasizes how sustainable processing techniques can improve the usefulness and value of proteins derived from legumes.

Chavan *et al.* (1987) performed a study on "*Biochemistry and Technology of Chickpea (Cicer arietinum L.) Seeds"* the study highlighted the seeds' nutritional makeup, practical qualities, and processing methods. According to the study, chickpeas are an excellent source of critical minerals, B vitamins, proteins, and carbohydrates that are especially helpful for those living in underdeveloped nations. It covered a number of processing techniques to improve the nutritional value and digestibility of chickpeas, including soaking, boiling, and fermentation.

In order to increase the total nutritional content of dishes based on chickpeas, the review also discussed the existence of antinutritional variables and offered solutions to lessen their impacts. This study emphasizes how crucial chickpeas are to the world's food security and offers advice on how to best use them using the right processing methods.

Das *et al.* (2022) conducted study on title "*Conventional and emerging processing techniques for the post-harvest reduction of antinutrients in edible legumes*" the study emphasizes conventional techniques that have been successful in lowering levels of antinutrients such phytic acid, tannins, and protease inhibitors, including dehulling, soaking, boiling, pressure cooking, sprouting, and fermentation. The authors also go into cutting-edge strategies for maximizing the decrease of antinutrients in processing, such as response surface methodology, fermentation with helpful bacteria, and the utilization of enzymes. The research highlights how crucial it is to choose the right processing methods to improve the safety and nutritional value of meals based on legumes, which will benefit human health and food security.

Jimoh *et al.* (2023) conducted a study on *"Recent Advances in Drying Process of Grains"* in this study they evaluated current developments in grain drying technologies for oilseeds, legumes, and cereals emphasised the importance of reducing moisture content to avoid spoiling. The study contrasts established practices with cutting-edge approaches that provide higher efficiency and energy savings, such as hoover, infrared, microwave and hybrid drying systems. Additionally, it emphasizes the significance of real-time monitoring for consistent quality and the function of auxiliary treatments. In order to increase food security and sustainability, the evaluation recommends more research to optimize drying procedures with the goals of balancing cost-effectiveness, nutritional retention, and energy use.

Adeleke and Babalola (2020) performed evaluation on topic *"Oilseed crop sunflower (Helianthus annuus) as a source of food: Nutritional and health benefits"* according to the study, sunflower seeds and sunflower oil are abundant in proteins, vitamins (particularly vitamin E), minerals, antioxidants, and vital fatty acids. These nutrients have anti-inflammatory properties, enhance cardiovascular health, and may help avoid chronic illnesses, among other health advantages. The crop's economic significance, flexibility, and promise to improve food security particularly in poor nations were also covered by the writers. Overall, the study promotes broader use of sunflower in diets and food systems and highlights its potential as a functional food.

Dey *et al.* (2022) investigated on title *"Understanding the Antinutritional Factors and Bioactive Compounds of Kodo Millet (Paspalum scrobiculatum) and LittleMillet (Panicum sumatrense)"* according to the study, these millets contain antinutritional substances like phytates, tannins, and oxalates that may impair the absorption of minerals, even though they are high in nutrients and bioactives like phenolic acids, flavonoids, and dietary fibre that support antioxidant, antidiabetic, and anti-inflammatory qualities. In order to decrease these antinutrients and enhance the nutritional value and practical advantages of these underutilized grains, the authors stress the significance of processing techniques including soaking, fermentation, and germination. Overall, the study is in favor of promoting small millets and kodo as sustainable, healthful food sources.

Hridyani, H. (2014) conducted a study on *"Nutritional Composition of Chickpea (Cicerarietinum-L) and Value Added Products"* according to the study, chickpeas are an important meal in the fight against malnutrition, particularly in vegetarian diets, because they are a rich source of dietary fibre, high-quality protein, and vital vitamins and minerals. It also discusses their low glycaemic index and health advantages, such as lowering cholesterol and controlling diabetes. The analysis also highlights a number of contemporary and traditional chickpea-based value-added goods, including flour, snacks, and fermented meals, highlighting their contribution to food security and nutritional diversity.

Kakati *et al.* (2010) performed a study *on "Effect of Traditional Methods of Processing on the Nutrient Contents and Some Antinutritional Factors in Newly Developed Cultivars of Green Gram [Vigna radiata (L.) Wilezek] and Black Gram [Vigna mungo (L.) Hepper] of Assam, India"* according to the study, these techniques greatly decreased the quantities of antinutritional substances such phytates, tannins, and trypsin inhibitors while also improving protein digestibility and mineral availability, which improved the nutritional profile overall. The results back up the use of traditional processing to enhance the safety and nutritional value of legumes in diets, particularly in areas where pulses are a major source of protein.

2.3: ORGANOLEPTIC EVALUATION OF VALUE ADDED PRODUCTS FROM PROTEIN COMPOSITE FLOUR

Giacalone *et al.* (2022) performed a study on topic *"Understanding barriers to consumption of plant-based foods and beverages: insights from sensory and consumer science"* according to the study, a number of factors, including perceptions of sustainability, health, and ethical issues, as well as sensory qualities like flavour, texture, and appearance, affect consumers' acceptance of plant-based products. The authors point out that even while plant-based substitutes are becoming more and more popular; there are still issues with satisfying consumers' demands for convenience and flavour. According to the research, the adoption of plant-based diets may be increased if these sensory and psychological obstacles are addressed through better product development and focused marketing.

Fiorentini *et al.* (2020) conducted a study titled *"Role of Sensory Evaluation in Consumer Acceptance of Plant-Based Meat Analogs and Meat Extenders: A Scoping Review"* the study looks at how consumer preferences and adoption of plant-based meat substitutes are influenced by sensory qualities like flavour, texture, appearance, and scent. The authors talk about how difficult it is to replicate the sensory aspects of conventional meat products, pointing out that these characteristics are essential for plant-based alternatives to be successfully adopted. Enhancing sensory qualities may boost market acceptance of plant-based meat substitutes, they add, underscoring the need of sensory research in product development to satisfy customer expectations.

Kumara *et al.* (2024) performed a study on *"Sensory Evaluation of Plant-Based Meat: Bridging the Gap with Animal Meat, Challenges and Future Prospects"* the study emphasizes how difficult it is to replicate the texture, flavour, and juiciness of animal meat all of which are essential for satisfying customers. While noting that more study is required to completely bridge the sensory gap, the authors highlight numerous technical breakthroughs and additives used to increase the sensory appeal of plant-based meats. The study's conclusion highlights the value of sensory assessment in the creation of plant-based meat substitutes and contends that resolving these issues will be crucial to their eventual widespread use.

Tangariya *et al.* (2018) conducted a study titled *"Quality analysis of composite flour and its effectiveness for Chapatti formulation"* the study emphasizes that the texture, colour, and protein content were among the physical, chemical, and nutritional characteristics of the composite flour mixes that were examined in the study. Combining several flours improved the nutritional profile, especially in terms of micronutrients, fibre, and protein, the researchers discovered. Additionally, they assessed the sensory qualities of the chapattis created with the composite flour and came to the conclusion that the taste and texture of these blends were well-liked. According to the study, composite flour formulations have the potential to improve dietary diversity and provide a healthy and efficient substitute for making chapattis.

P. G. *et al.* (2013) conducted a study titled *"Sensory Evaluation of Wheat Cassava Soybean Composite Flour (WCS) Bread by the Mixture Experiment Design"* taste, texture, scent, and general acceptance were among the sensory qualities assessed in the study. It was discovered that adding soybean and cassava flour to wheat flour had a major effect on the bread's sensory qualities. According to the findings, a well-balanced combination of these flours improved the bread's nutritional profile without sacrificing its palatable texture. According to the study, wheat cassava soybean composite flour might be a good substitute for making wholesome bread with enhanced functional qualities.

Dada *et al.* (2017) conducted a study on "*Formulation, sensory evaluation, proximate composition and storage stability of cassava strips produced from the composite flour of cassava and cowpea"* according to the study, adding cowpea to the cassava strips enhanced their nutritional profile by raising their protein and fibre content while preserving palatable sensory attributes including flavour, texture, and scent. The strips are a good choice for long-term storage because the storage stability tests showed that they could hold up over time. According to the study's findings, mixing cassava and cowpea flour may result in a stable and nutrient-dense snack that could improve nutrition and food security.

Oluwalana *et al.* (2012) performed study on *"Quality assessment of flour and bread from sweet potato wheat composite flour blends"* the study evaluated the quality of bread produced using these mixes and concentrated on the physical and chemical characteristics of the composite flours, including their nutritional value. The findings demonstrated that while preserving a tolerable texture and flavour, the inclusion of sweet potato flour improved the bread's nutritional value, especially its fibre and micronutrient content. According to the study's findings, adding sweet potatoes to wheat flour blends may enhance bread's nutritional profile and offer a way to add more nutrient-dense items to everyday meals.

2.4: EFFECT OF STORAGE ON PHYSICAL, CHEMICAL AND MICROBIOLOGICAL ASPECTS OF COMPOSITE FLOUR

Forsido *et al.* (2021) conducted a study on topic *"Effects of storage temperature and packaging material on physio-chemical, microbial and sensory properties and shelf life of extruded composite baby food flour"* the study discovered that packing and storage conditions were important factors in preserving the baby food flour's quality. Reduced microbial growth, preservation of the flour's nutritional value, and preservation of its sensory qualities over time were all made possible by lower storage temperatures and the use of suitable packing materials, such as laminated films. According to the study's findings, extruded composite baby food flour may have a longer shelf life under ideal storage circumstances, which would make it a more practical and sustainable product for customers.

Kechkin *et al.* (2021) conducted a study titled *"Shelf life of composite flour mixtures"* the study focused on how variables like humidity, temperature, and packing materials affect how well composite flours retain their nutritional and sensory properties. The authors emphasised how important it is to regulate these factors in order to prolong the shelf life and preserve the quality of flour combinations, both of which are essential for the food business, particularly when creating more wholesome and sustainable food products. According to the results, using the best storage techniques might greatly extend the shelf life of composite flour combinations while maintaining their quality and safety both during use and storage.

Nasir *et al.* (2004) conducted a study on *"Effect Of Moisture and Packaging on the Shelf Life of Wheat Flour"* the study showed higher moisture content shortens shelf life and speeds up spoiling, moisture levels are important in determining how stable wheat flour is during storage. Additionally, it was discovered that the kind of packaging material had a big influence on how long the flour would last; certain materials provided stronger defense against oxygen and moisture, which helped to maintain the flour's quality. In order to extend the shelf life of wheat flour and guarantee its safety for human consumption, the study underlined the need of managing both moisture and packaging conditions.

Hu *et al.* (2020) conducted a study on *"Effect of Gaseous Ozone Treatment on the Microbial and Physicochemical Properties of Buckwheat-Based Composite Flour and Shelf-life Extension of Fresh Noodles"* according to the study, ozone treatment successfully decreased the microbial load in the composite flour, improving the noodles' safety and extending their shelf life. Furthermore, the ozone treatment improved several of the flour's physicochemical characteristics, which improved the quality of the noodles. Overall, the study indicates that gaseous ozone treatment is a viable technique for enhancing the fresh noodles' and buckwheat-based composite flour's safety, quality, and shelf life.

Violalita et al. (2021) performed a study on *"Shelf-life Prediction of Gluten-Free Dry Noodles Made from Composite Flour (Mocaf, Tapioca, Cornstarch, and Soybeans) Using Accelerated Shelf-life Testing (ASLT) Method with Arrhenius Equation Approach"* the study employed the Arrhenius equation in conjunction with the Accelerated Shelf-Life Testing (ASLT) method to track changes in moisture content and free fatty acid (FFA) levels at three different storage temperatures (25°C, 35°C, and 45°C). The results showed that the moisture content rose over time, with the biggest shifts occurring at higher temperatures. The noodles had an estimated shelf life of 161 days at 25°C, 194 days at 35°C, and 231 days at 45°C, according to the Arrhenius model. According to the study's findings, regulating the storage temperature is essential to preserving the quality and prolonging the shelf life of dry noodles free of gluten that are created using these composite flours.

CHAPTER-III

MATERIALS AND METHODS

The study titled "**Development and Quality Evaluation of Allergen Free Vegan Protein Composite Flour and Its Application in Food Products**" was conducted and the methodology adopted is discussed below under following headings:

3.1: Selection and Collection of raw materials

3.2: Preparation of raw materials

3.3: Formulation of protein composite flour

3.4: Analysis of functional properties of ingredients

3.5: Analysis of nutrient content of composite flour

3.6: Development and standardization of product

3.7: Organoleptic evaluation

3.8: Analysis of shelf life of the product

3.9: Statistical analysis

3.1: SELECTION AND COLLECTION OF RAW MATERIALS

Kodo millet, chick pea, green gram and sunflower seed were selected because of their good protein content and their hypoallergenic property. Good quality kodo millet (*Paspalum scrobiculatum*), chickpeas (*Cicer arietinum*), green gram (*Vigna radiate*) and sunflower seeds (*Helianthus annuus*) were selected and they were resourced from local market. Before the processing of raw materials they were washed and dried for better quality ingredients and to get rid of damaged ones. Then the ingredients were roasted to remove antinutrients and then they were powdered from the flour mill to form fine powder and then stored in airtight container.

3.2: PREPERATION OF RAW MATERIALS

Cleaned and washed ingredients were sundried each in separate batches. Then they were dry roasted in kadai till raw smell gone and then they were sent to flour mill for powdering the ingredients to fine powder.

3.3: FORMULATION OF PROTEIN COMPOSITE FLOUR

For clearing out all the debris from the ingredients they were washed then air-dried. Then the ingredients were sundried separately for two days in clean trays then they were dry roasted separately in kadai till raw smell was gone. Then the ingredients were packed separately and send to flour mill to powder the ingredients to fine powder. Then the powders were kept in airtight tubs and then they were mixed in three different proportions (T1, T2 and T3).

Sample 1: Protein composite flour (kodo millet flour – 60%, chickpea flour – 20%, green gram flour – 10%, sunflower seed flour – 10%) (T1)

Sample 2: Protein composite flour (kodo millet flour – 60%, chickpea flour – 10%, green gram flour – 20%, sunflower seed flour – 10%) (T2)

Sample 3: protein composite flour (kodo millet flour – 60%, chickpea flour – 15%, green gram flour – 15%, sunflower seed flour – 10%) (T3)

Table 1: Proportions used for formulation of the composite flour

Ingredients	T1	T2	T3
Kodo Millet Flour	60	60	60
Green Gram Flour	10	20	15
Chickpea Flour	20	10	15
Sunflower Seed Flour	10	10	10

Then these samples (T1, T2 and T3) were sent to laboratory for nutrient value analysis of each proportion and to select the best proportion out of three.

3.4: ANALYSIS OF FUNCTIONAL PROPERTIES

Functional properties are the fundamental physiochemical properties that reflect the complex interaction between composition, structure, molecular conformation and physiochemical properties of the food components (Kinsella, J.E., & Melachouris, 1976). Functional properties like water absorption capacity, oil absorption capacity and bulk density were observed.

3.4.1: Water absorption capacity

Water absorption capacity was determined by Sathe *et al*. (1981) method. Detailed method provided in APPENDIX I.

3.4.2: Oil absorption capacity

Oil capacity was determined by using Rosario & Flores, (1981) method which was slightly modified by Iyer & Singh, (1997). Detailed method is provided in APPENDIX-II.

3.4.3: Bulk density

Bulk density was analyzed; detailed method is given in APPENDIX-III

3.5: ANALYSIS OF NUTRIENT CONTENT OF THE COMPOSITE FLOUR

3.5.1: Proximate composition

Protein composite flour was analyzed for its proximate composition such as; ash, protein, fat, dietary fiber and carbohydrate.

Ash

The ash content of the composite flour was determined by SOP method by FSSAI (2023) provided in APPENDIX IV.

Protein

The protein content especially methionine and lysine was assessed in the composite flour using SOP method by FSSAI (2023) provided in APPENDIX VI.

Fat

The fat content including monounsaturated fatty acids and poly unsaturated fatty acids were assessed using SOP method by FSSAI (2023) method provided in APPENDIX VII.

Carbohydrate

Total carbohydrate content of the composite flour was analyzed using SOP method by FSSAI (2023) provided in APPENDIX IX.

Dietary fiber

Total dietary fiber content of the composite flour was analyzed using SOP method by FSSAI (2023) detailed in APPENDIX V.

3.5.2: Micronutrient analysis

Micronutrient analysis of the composite flour was conducted for micronutrient such as sodium, calcium, iron, zinc, magnesium, vitamin E and vitamin B complex.

Sodium

The sodium content of the composite flour was evaluated using SOP method by FSSAI (2023) provided in APPENDIX VIII.

Calcium

The calcium content of the composite flour was analyzed using SOP method by FSSAI (2023) provided in APPENDIX VIII.

Iron

The iron content of the composite flour was analyzed using SOP method by FSSAI (2023) provided in APPENDIX VIII.

Zinc

The zinc content of the composite flour was analyzed using SOP method by FSSAI (2023) provided in APPENDIX VIII.

Magnesium

The magnesium content of the composite flour was analyzed using SOP method by FSSAI (2023) provided in APPENDIX VIII.

Vitamin E

The vitamin E content of the composite flour was analyzed using SOP method by FSSAI (2023) provided in APPENDIX VIII.

Vitamin B complex

The vitamin B complex content of the composite flour was analyzed using SOP method by FSSAI (2023) provided in APPENDIX VIII.

3.5.3: Antinutrient analysis

Antinutrient profile of the formulated composite flour was analyzed for phytate, tannin and polyphenol.

Phytate

Total phytate content of the composite flour developed was assessed using SOP method by FSSAI (2023).

Tannin

Total tannin content of the formulated composite flour was analyzed using SOP method by FSSAI (2023).

Polyphenol

The polyphenol profile in the developed composite flour was evaluated using SOP method by FSSAI (2023).

3.5.4: Diabetogenic Activity

The Diabetogenic activity of the developed composite flour was assessed using SOP method by FSSAI (2023).

3.5.5: Protein Digestibility

Invitro protein digestibility of the composite flour was assessed using SOP method by FSSAI (2023).

3.6: DEVELOPMENT AND STANDARDIZATION OF THE PRODUCT

Four products were developed using the protein composite flour. They were; pancake, cheela, soup and smoothie.

3.6.1: Smoothie:-

Materials required;

Blender, banana, oats, protein composite flour, sweetener (dates), frozen black berry and milk.

Table 2: Standardization of smoothie

Ingredients	T0	T1	T2	T3
Banana	60	60	60	60
Oats	100	70	60	50
Protein Composite Flour	-	30	40	50
Sweetener (Dates)	5	5	5	5
Milk	100	100	100	100
Frozen Blackberries	10	10	10	10

Method;

T0 preparation:-

To a blender 60g banana was added to which 100ml milk was added. 10g frozen black berry was added to blender to which 100g oats was added and 5g dates was added as sweetener and blended together to get thick texture of smoothie.

T1 preparation:-

To a blender 60g banana was added to which 100ml milk was added. 10g frozen black berry was added to blender to which 70g oats was added with 30g protein composite flour and 5g dates was added as sweetener and blended together to get thick texture of smoothie.

T2 preparation:-

To a blender 60g banana was added to which 100ml milk was added. 10g frozen black berry was added to blender to which 60g oats was added with 40g protein composite flour and 5g dates was added as sweetener and blended together to get thick texture of smoothie.

T3 preparation:-

To a blender 60g banana was added to which 100ml milk was added. 10g frozen black berry was added to blender to which 50g oats was added with 50g protein composite flour and 5g dates was added as sweetener and blended together to get thick texture of smoothie.

3.6.2: Pancake:-

Materials required;

Blender, egg, milk, sweetener (sugar), salt, oat flour, protein composite flour, vanilla essence, baking powder, unsalted butter and pan.

Table 3: Standardization of pancake

Ingredients	**T0**	**T1**	**T2**	**T3**
Oat Flour	100	70	60	50

Protein Composite Flour	-	30	40	50
Egg	50	50	50	50
Milk	100	100	100	100
Sugar	10	10	10	10
Vanilla Essence	2	2	2	2
Butter	15	15	15	15
Salt	1	1	1	1
Baking Powder	5	5	5	5

Method;

T0 preparation:-

To a bender 100ml milk was added to which 50g egg, 15g butter, 2ml vanilla essence was added and blended. In a bowl all the dry ingredients such as 1g tsp salt, 100g oat flour, 5g baking powder, 10g sugar were mixed and then wet mix was added to dry ingredient and mixed till smooth pourable batter was formed and then after 10min pour the batter over heated pan greased with butter. Then pancake was cooked till golden brown.

T1 preparation:-

To a bender 100ml milk was added to which 50g egg, 15g butter, 2ml vanilla essence was added and blended. In a bowl all the dry ingredients such as 1g salt, 70g oat flour, 30g protein composite flour, 5g baking powder, 10g sugar were mixed and then wet mix was added to dry ingredient and mixed till smooth pourable batter was formed and then after 10min pour the batter over heated pan greased with butter. Then pancake was cooked till golden brown.

T2 preparation:-

To a bender 100ml milk was added to which 50g egg, 15g butter, 2ml vanilla essence was added and blended. In a bowl all the dry ingredients such as 1g salt, 60g oat flour, 40g protein composite flour, 5g baking powder, 10g sugar were mixed and then wet mix was added to dry ingredient and mixed till smooth pourable batter was formed and then after 10min pour the batter over heated pan greased with butter. Then pancake was cooked till golden brown.

T3 preparation:-

To a bender 100ml milk was added to which 50g egg, 15g butter, 2ml vanilla essence was added and blended. In a bowl all the dry ingredients such as 1g salt, 50g oat flour, 50g protein composite flour, 5g baking powder, 10g sugar were mixed and then wet mix was added to dry ingredient and mixed till smooth pourable batter was formed and then after 10min pour the batter over heated pan greased with butter. Then pancake was cooked till golden brown.

3.6.3: Cheela:-

Materials required;

Mixing bowl, oats flour, protein composite flour, yogurt, turmeric powder, red chili powder, cumin powder, salt, water, coriander leaves, onion, tomato, green chili, capsicum and ghee.

Table 4: Standardization of cheela

Ingredients	**T0**	**T1**	**T2**	**T3**
Oat Flour	100	70	60	50
Protein Composite Flour	-	30	40	50
Yogurt	20	20	20	20
Onion	15	15	15	15
Tomato	5	5	5	5

Capsicum	15	15	15	15
Coriander leaves	3	3	3	3
Water	70	70	70	70
Salt	2	2	2	2
Red Chili Powder	1	1	1	1
Ghee	1	1	1	1
Turmeric Powder	0.5	0.5	0.5	0.5
Cumin Powder	0.5	0.5	0.5	0.5
Green Chilies	3	3	3	3

Method;

T0 preparation:-

To a mixing bowl 100g oats flour was added to which 2g salt, 0.5g turmeric powder, 1g chili powder, 0.5g cumin powder, 20ml yogurt, 70ml water was added and mixed till smooth batter was formed. To the batter chopped vegetables such as; 15g onion, 5g tomato, 3g green chili, 15g capsicum and 3g coriander leaves were added. Then the batter was poured on heated and greased with 1g ghee pan and cheela was made.

T1 preparation:-

To a mixing bowl 70g oats flour was added to which 30g protein composite flour, 2g salt, 0.5g turmeric powder, 2g chili powder, 0.5g cumin powder, 20ml yogurt, 70ml water was added and mixed till smooth batter was formed. To the batter chopped vegetables such as;

15g onion, 5g tomato, 3g green chili, 15g capsicum and 3g coriander leaves were added. Then the batter was poured on heated and greased with 1g ghee pan and cheela was made.

T2 preparation:-

To a mixing bowl 60g oats flour was added to which 40g protein composite flour, 2g salt, 0.5g turmeric powder, 2g chili powder, 0.5g cumin powder, 20ml yogurt, 70ml water was added and mixed till smooth batter was formed. To the batter chopped vegetables such as; 15g onion, 5g tomato, 3g green chili, 15g capsicum and 3g coriander leaves were added. Then the batter was poured on heated and greased with 1g ghee pan and cheela was made.

T3 preparation:-

To a mixing bowl 50g oats flour was added to which 50g protein composite flour, 2g salt, 0.5g turmeric powder, 2g chili powder, 0.5g cumin powder, 20ml yogurt, 70ml water was added and mixed till smooth batter was formed. To the batter chopped vegetables such as; 15g onion, 5g tomato, 3g green chili, 15g capsicum and 3g coriander leaves were added. Then the batter was poured on heated and greased with 1g ghee pan and cheela was made.

3.6.4: Thick Soup:-

Materials required;

Sauce pan, olive oil, oats, onion, capsicum, tomato, garlic, carrot, green chili, coriander leaf, coconut milk, protein composite flour, salt, turmeric powder, cumin powder, oregano and pepper powder.

Table 5: Standardization of thick soup

Ingredients	T0	T1	T2	T3
Oats	100	70	60	50
Protein Composite Flour	-	30	40	50
Olive Oil	3	3	3	3

Onion	20	20	20	20
Capsicum	15	15	15	15
Tomato	15	15	15	15
Garlic	3	3	3	3
Carrot	15	15	15	15
Green Chili	3	3	3	3
Coriander Leaf	5	5	5	5
Coconut Milk	50	50	50	50
Salt	1	1	1	1
Turmeric Powder	0.5	0.5	0.5	0.5
Cumin Powder	0.5	0.5	0.5	0.5
Pepper Powder	1	1	1	1
Oregano	0.5	0.5	0.5	0.5
Water	200	200	200	200

Method;

T0 preparation:-

To a sauce pan 3ml olive oil was heated to which chopped 3g garlic was added and once it turned brown 20g onion was added and sautéed till soft then to that 15g tomato, 15g capsicum, 15g carrot, 3g green chili, 0.5g turmeric powder, 0.5g cumin powder, 1g pepper powder, 1g salt was added and sautéed for 2-3min. To this 100g oats was added with 200ml water and mixed and boiled for 1minute to this 0.5g oregano was added. To this 50ml coconut milk was added and boiled for 1 minute then 5g chopped coriander leaves were added then mixed and soup was obtained.

T1 preparation:-

To a sauce pan 3ml olive oil was heated to which chopped 3g garlic was added and once it turned brown 20g onion was added and sautéed till soft then to that 15g tomato, 15g capsicum, 15g carrot, 3g green chili, 0.5g turmeric powder, 0.5g cumin powder, 1g pepper powder, 1g salt was added and sautéed for 2-3min. To this 70g oats was added along with 30g protein composite flour and with 200ml water and mixed and boiled for 1minute to this 0.5g oregano was added. To this 50ml coconut milk was added and boiled for 1 minute then 5g chopped coriander leaves were added then mixed and soup was obtained.

T2 preparation:-

To a sauce pan 3ml olive oil was heated to which chopped 3g garlic was added and once it turned brown 20g onion was added and sautéed till soft then to that 15g tomato, 15g capsicum, 15g carrot, 3g green chili, 0.5g turmeric powder, 0.5g cumin powder, 1g pepper powder, 1g salt was added and sautéed for 2-3min. To this 60g oats was added along with 40g protein composite flour and with 200ml water and mixed and boiled for 1minute to this 0.5g oregano was added. To this 50ml coconut milk was added and boiled for 1 minute then 5g chopped coriander leaves were added then mixed and soup was obtained.

T3 preparation:-

To a sauce pan 3ml olive oil was heated to which chopped 3g garlic was added and once it turned brown 20g onion was added and sautéed till soft then to that 15g tomato, 15g capsicum, 15g carrot, 3g green chili, 0.5g turmeric powder, 0.5g cumin powder, 1g pepper powder, 1tsp salt was added and sautéed for 2-3min. To this 50g oats was added along with

50g protein composite flour and with 200ml water and mixed and boiled for 1minute to this 0.5g oregano was added. To this 50ml coconut milk was added and boiled for 1 minute then 3g chopped coriander leaves were added then mixed and soup was obtained.

3.7: ORGANOLEPTIC EVALUATION

Sensory evaluation has been defined as scientific field used to evoke, measure, analyze and interpret human reactions to meet sensory characteristics as perceived by sight, smell, taste, touch and hearing by the Institute of Food Technology(IFT).

From the standardized four products of different proportions best ones were chosen by trained panel of judges after tasting them. The sensory attributes tested in the product were appearance, color, flavor, taste, texture and overall acceptability.

The selected products and most acceptable ones were subjected to shelf life analysis. Detailed method is provided in APPENDIX X.

3.8: ANALYSIS OF SHELF LIFE OF THE PRODUCT

Shelf life of the most acceptable proportion was analyzed under different storage conditions. By determining the chemical, microbial, physical changes in the product. Various methods were used to determine different parameters of shelf life analysis. Methods such as STP, IS 3025, IS 5402, IS 5401, IS 5887and IS 5403 were used and details are provided in APPENDIX XI.

3.9: STATISTICAL ANALYSIS

Statistical analysis was conducted for nutrient analysis of protein composite flour and control (market product) and for the sensory evaluation results of value added products using SPSS version 20 details are provided in APPENDIX XII.

CHAPTER-IV

RESULT AND DISCUSSION

Formulated protein composite flour was evaluated for its nutritional quality, functional property, its application in development of value added products and for the keeping quality. Thus the results obtained during the evaluation process are given below under following titles.

4.1: Formulation of the protein composite flour

4.2: Nutrient analysis of the formulated composite flour

4.3: Functional property analysis of the developed composite flour

4.4: Formulation of value added products from the composite flour

4.5: Organoleptic evaluation of the value added products

4.6: Statistical analysis

4.7: Shelf life analysis of the composite flour

4.1: FORMULATION OF PROTEIN COMPOSITE FLOUR

For the development of the protein composite flour millets, pulses and fatty seeds were chosen such as kodo millet, chickpea, green gram and sunflower seed. They were cleaned, dried and ground to obtain fine flour and mixed together in different proportions (detailed information provided in methodology chapter table 1).

Plate 1: Protein Composite Flour T1

Plate 2: Protein Composite Flour T2

Plate 3: Protein Composite Flour T3

4.2: NUTRIENT ANALYSIS OF THE FORMULATED COMPOSITE FLOUR

The formulated composite flour in three different proportions such as T1, T2 and T3 were subjected to nutrient analysis for proximate composition, micronutrient composition, antinutrient composition, invitro protein digestibility and invitro diabetogenic activity.

Table 6 shows proximate composition, table 7 shows micronutrient content, table 8 shows antinutritional composition, table 9 shows invitro protein digestibility and table 10 shows diabetogenic activity.

Table 6: Proximate composition of protein composite flour per 100g:

PARAMETER	T1	T2	T3
Total Ash (%)	2.8	2.7	2.9
Total Calorie (Kcal)	265.9	257.7	262.8
Total Carbohydrate (g)	43	40	42
Total Fat (g)	2.3	2.1	2
Mono Unsaturated Fat (g)	0.7	0.6	0.9
Poly Unsaturated Fat (g)	0.4	0.3	0.4
Protein (g)	18.3	19.7	19.2
Lysine (mg)	70	79	75
Methionine (mg)	58	63	60

Dietary Fiber (g)	1.2	1.5	1.3

The proximate compositions of the protein composite flour in different proportions are given in table 6. In T1 (60:10:20:10-K:G:C:S) total ash is 2.1%, total calorie is 265.9kcal, total carbohydrate is 43g, total fat is 2.3g, monounsaturated fat is 0.7g, polyunsaturated fat is 0.4g, protein is 18.3g, lysine is 70mg, methionine is 58mg and dietary fiber is 1.2g per 100gram protein composite flour. In T2 (60:20:10:10-K:G:C:S) total ash is 2.7%, total calorie is 257.7kcal, total carbohydrate is 40g, total fat is 2.1g, monounsaturated fat is 0.6g, polyunsaturated fat is 0.3g, protein is 19.7g, lysine is 79mg, methionine is 63mg and dietary fiber is 1.5g per 100gram protein composite flour. In T3 (60:15:15:10-K:G:C:S) total ash is 2.9%, total calorie is 262.8kcal, total carbohydrate is 42g, total fat is 2g, monounsaturated fat is 0.9g, polyunsaturated fat is 0.4g, protein is 19.2g, lysine is 75mg, methionine is 60mg and dietary fiber is 1.3g per 100gram protein composite flour.

Table 7: Micronutrient content of protein composite flour per 100g:

PARAMETER	**T1**	**T2**	**T3**
Vitamin E (mg)	2	3	2.5
Vitamin B (mg)	1.5	1	0.7
Sodium (mg)	156	161	158
Iron (mg)	21	20	23

Calcium (mg)	40	52	49
Zinc (mg)	10	8	6
Magnesium (mg)	18	24	22

The micronutrient content of T1; vitamin E is 2mg, vitamin B complex is 1.5mg, sodium is 156mg, calcium is 40mg, iron is 21mg, zinc is 10mg and magnesium is 18mg per 100gram protein composite flour. The micronutrient content of T2; vitamin E is 3mg, vitamin B complex is 1mg, sodium is 161mg, calcium is 52mg, iron is 20mg, zinc is 8mg and magnesium is 24mg per 100gram protein composite flour. The micronutrient content of T3; vitamin E is 2.5mg, vitamin B complex is 0.7mg, sodium is 158mg, calcium is 49mg, iron is 23mg, zinc is 6mg and magnesium is 22mg per 100gram protein composite flour.

Table 8: Antinutrient composition of protein composite flour per 100g:

PARAMETER	T1	T2	T3
Tannin (mg)	190	200	182
Phytate (mg)	480	496	490
Polyphenol (mg)	160	176	168

The antinutrient profiles of T1 are; phytate is 480mg, tannin is 190mg and polyphenol is 160mg per 100gram protein composite flour. The antinutrient profiles of T2 are; phytate is 496mg, tannin is 200mg and polyphenol is 176mg per 100gram protein composite flour. The antinutrient profiles of T3 are; phytate is 490mg, tannin is 182mg and polyphenol is 168mg per 100gram protein composite flour.

Table 9: Invitro protein digestibility per 100g;

PARAMETER	T1	T2	T3
Protein Digestibility (%)	12	15	13

The invitro protein digestibility of T1 was 12% per 100g. The invitro protein digestibility of T2 was 15% per 100g. The invitro protein digestibility of T3 was 13% per 100g.

Table 10: Invitro diabetogenic activity per 100g;

PARAMETER	T1	T2	T3
Diabetogenic Activity (%)	14	16	15

The diabetogenic activity of T1 was 14% per 100gram protein composite flour. The diabetogenic activity of T2 was 16% per 100gram protein composite flour. The diabetogenic activity of T3 was 15% per 100gram protein composite flour. Thus from the nutrient analysis of three respective proportions of vegan protein composite flour T2 was identified as the best proportion due to its nutrient rich profile especially protein.

4.3: FUNCTIONAL PROPERTY ANALYSIS OF PROTEIN COMPOSITE FLOUR

The best proportion of composite flour was selected from nutrient analysis and that proportion of composite flour was subjected to various functional property analysis such as water absorption capacity, oil absorption capacity and bulk density of T2 (60:20:10:10 – K:G:C:S). Table 11 shows the values of functional property analysis.

Table 11: Functional property analysis of the protein composite flour (T2)

PARAMETER	UNIT	RESULT
Water Absorption Capacity	%	30.79
Oil Absorption Capacity	%	28.76
Bulk Density	g/L	772

From the obtained result it is understood that the water absorption capacity of the protein composite flour (T2) is 30.79%, oil absorption capacity is 28.76% and bulk density is 772g/L respectively. The mass of the powder per unit volume, including the space between the particles, is known as the bulk density of flour (or any powder). It's an important element for food processing, packaging, and formulation.

Bulk density = mass of the sample (g) / volume occupied (L)

The capacity of flour to absorb and hold onto water is known as water absorption capacity. The property of flour to bind or absorb oil is known as oil absorption capacity. It is crucial for mouthfeel, palatability, and flavor retention, particularly in meat and snack compositions.

4.4: FORMULATION OF VALUE ADDED PRODUCTS FROM THE COMPOSITE FLOUR

Out of the three proportions of protein composite flour the best one turned out to be T2 with proportion containing 60g kodo millet flour, 20g green gram flour, 10g chickpea flour and 10g sunflower seed flour respectively. This T2 was utilized for the production of value added products for incorporating the composite flour into daily life for better health and protein consumption. The products made from T2 was smoothie, cheela, pancake and soup (detailed explanation of proportions and method is provided in methodology chapter table 2, 3, 4 and 5).

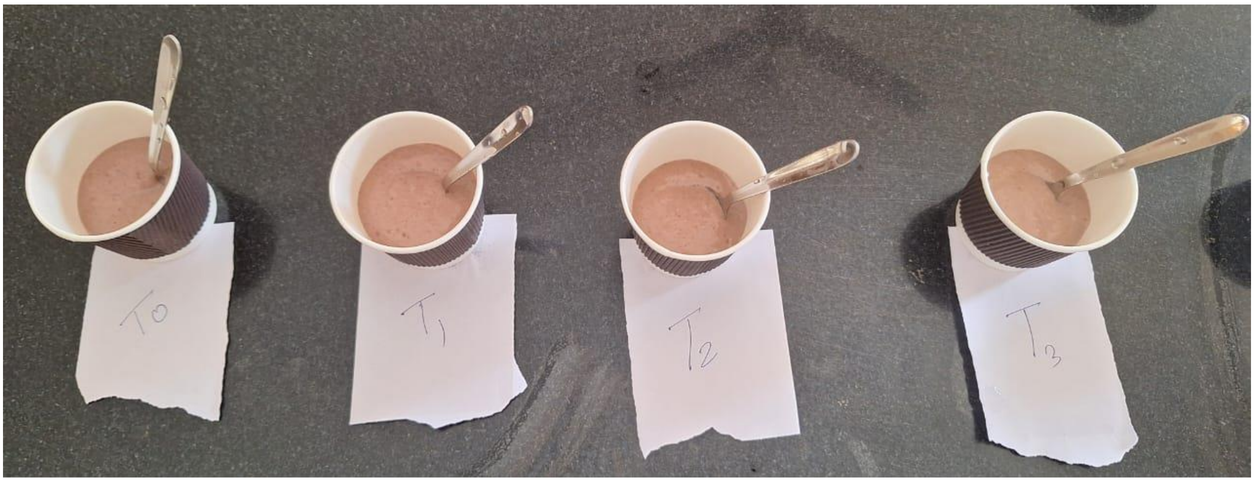

Plate 4: Smoothie in different proportion (T0, T1, T2 and T3 table 2)

Plate 5: Pancake in different proportions (T0, T1, T2 and T3 table 3)

Plate 6: Cheela in different proportions (T0, T1, T2 and T3 table 4)

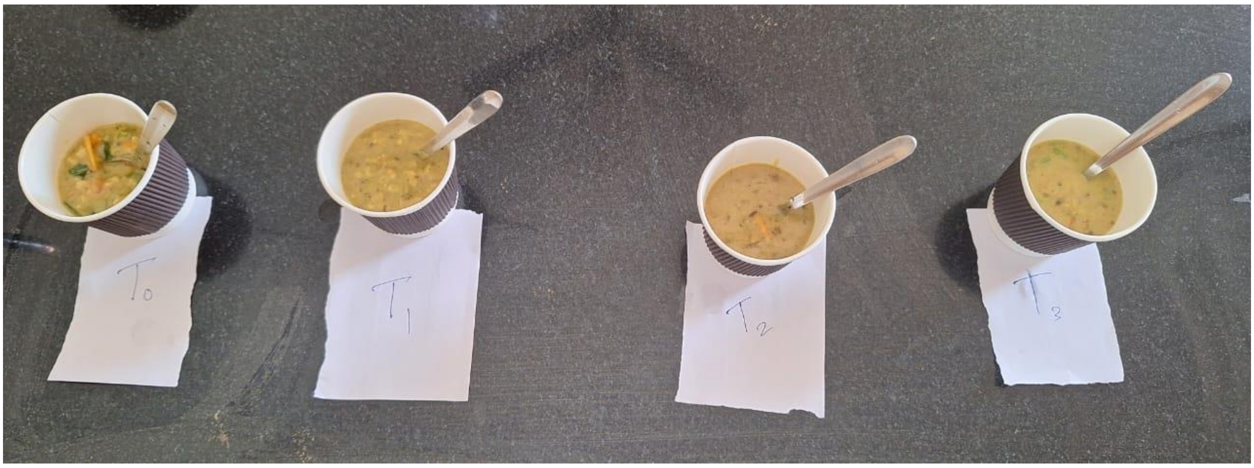

Plate 7: Thick soup in different proportions (T0, T1, T2 and T3 table 5)

These prepared value added products were then subjected to organoleptic evaluation to find out the best proportion and overall acceptability.

4.5: ORGANOLEPTIC EVALUATION OF THE VALUE ADDED PRODUCTS

Trained panel of 10 individuals were selected by 9point Hedonic scale (APPENDIX X). These panels tasted the products and rated them accordingly. This was done to select the best proportion out of three proportions and standard was kept as control to compare.


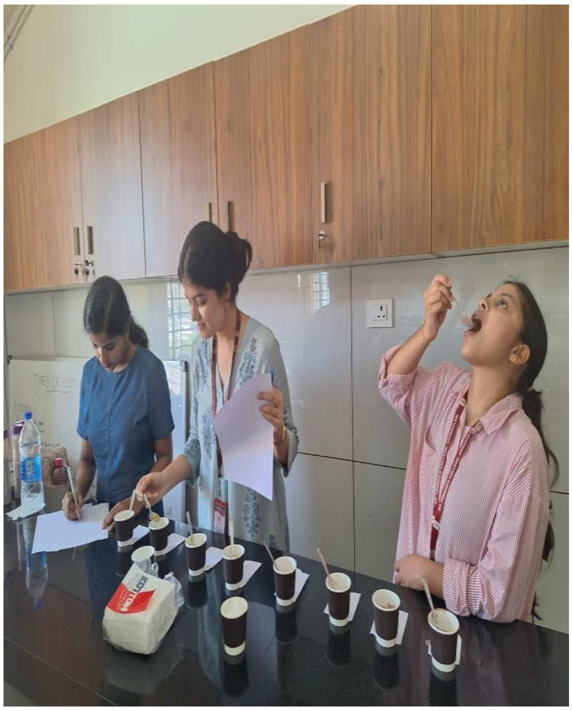

Plate 8: Organoleptic Evaluation by trained panel members

Sensory evaluation scores are shown in table 12, 13, 14 and 15. Panel members tasted the products such as smoothie, pancake, cheela and thick soup and graded them to different ranges which helped to select the best proportion out of three. T1, T2 and T3 were compared for taste, texture, color, flavor, appearance and overall acceptability.

Table 12: Sensory evaluation of cheela

Parameters	**T0**	**T1**	**T2**	**T3**
Appearance	8.00±0.66	8.00±0.66	7.90±0.87	6.8±1.81
Colour	8.00±0.66	8.00±0.66	7.7±0.48	6.9±1.96
Taste	7.70±0.47	8.10±0.87	6.5±1.26	6.6±1.50
Texture	7.70±1.05	7.90±0.73	7.3±0.82	6.7±0.94

Flavour	7.80±1.13	8.00±0.81	7.40±1.07	6.7±1.25
Overall acceptability	7.9±0.738	8.00±0.66	7.40±0.96	6.5±0.97

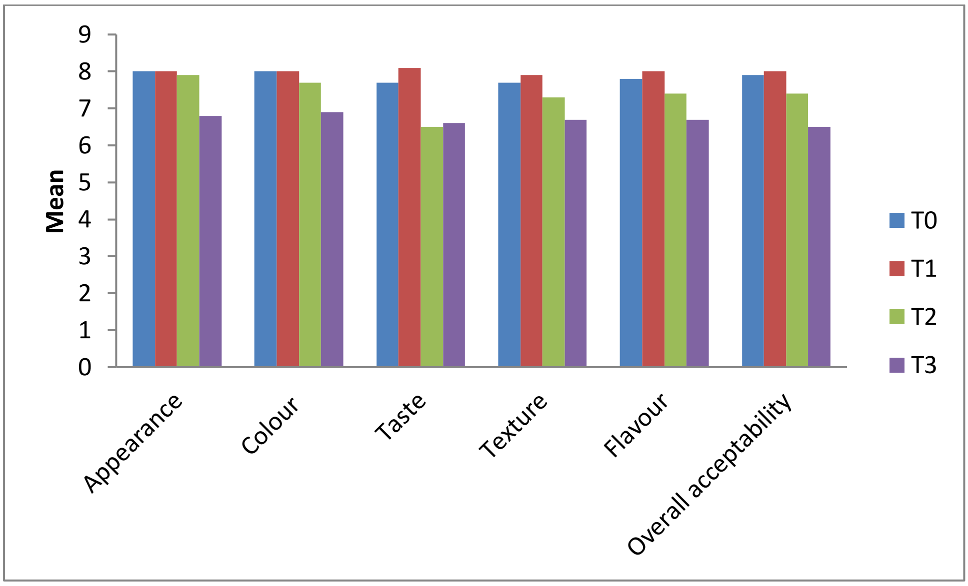

Figure 1: Sensory evaluation graph of Cheela

Panel members tasted cheela and graded according to different ranges (table 12) which helped to select the best proportion out of three. T1, T2 and T3 were compared for taste, texture, color, flavor, appearance and overall acceptability. From the statistical analysis conducted and the grading done by the panelists the overall acceptability is best for T1 (70:30).

Table 13: Sensory evaluation of Pancake

Parameter	T0	T1	T2	T3
Appearance	7.00±1.82	6.9±1.59	7.00±1.70	6.5±2.06
Colour	7.60±0.69	7.40±0.96	6.80±1.39	6.40±1.71

Taste	7.00±0.47	8.10±0.87	6.50±1.26	6.60±1.50
Texture	6.80±1.54	7.50±1.17	6.70±1.63	6.4±1.71
Flavour	6.9±1.10	7.8±1.03	7.10±1.10	6.50±1.71
Overall acceptability	7.30±0.48	8.00±0.81	6.6±1.26	6.50±1.78

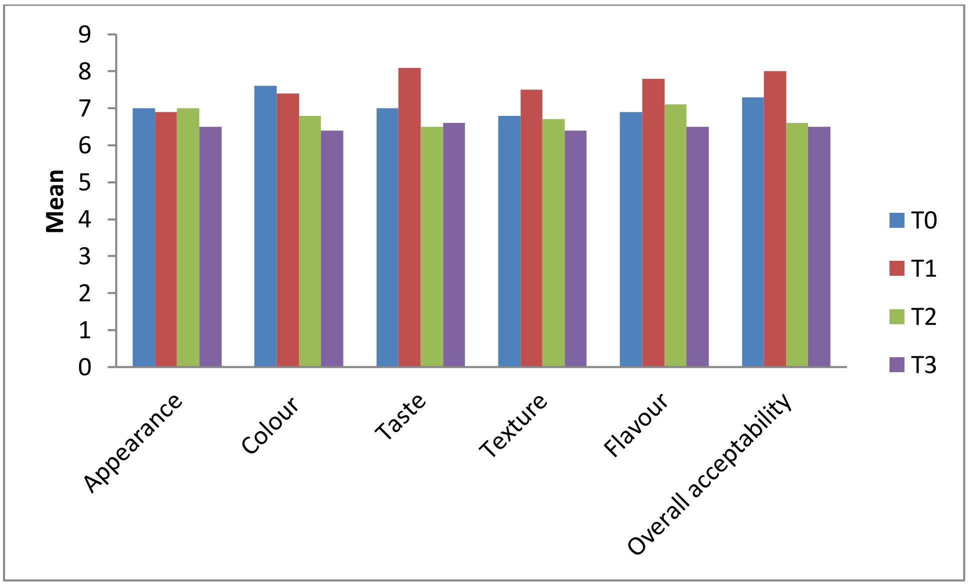

Figure 2: Sensory evaluation graph of Pancake

Panel members tasted pancake and graded it to different ranges which (table 13) helped to select the best proportion out of three. T1, T2 and T3 were compared for taste, texture, color, flavor, appearance and overall acceptability. From the statistical analysis conducted and the grading done by the panelists the overall acceptability is best for T1 (70:30).

Table 14: Sensory evaluation of Thick Soup

Parameter	T0	T1	T2	T3
Appearance	7.3±1.49	8.00±1.05	7.8±1.22	8.2±0.63
Colour	8.00±0.66	8.40±0.69	8.20±0.78	8.40±0.69
Taste	7.40±1.17	7.80±1.13	7.50±1.26	7.20±1.03
Texture	6.80±1.54	7.2±1.54	7.7±0.67	8.00±1.05
Flavour	7.40±1.17	7.90±0.99	7.70±1.05	7.40±0.84
Overall acceptability	7..60±1.07	8.10±0.73	7.90±0.99	7.80±0.78

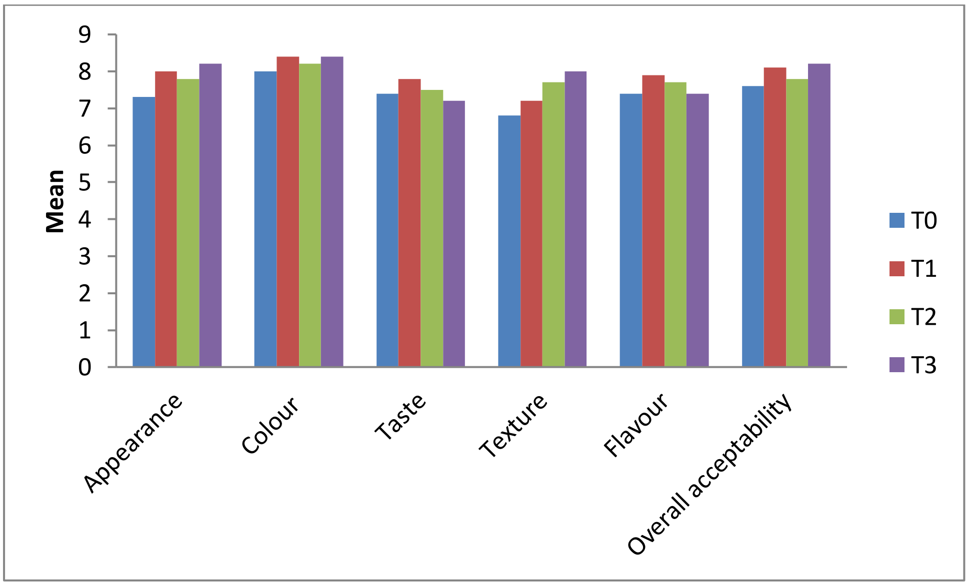

Figure 3: Sensory evaluation graph of Thick soup

Panel members tasted thick soup and graded it to different ranges which (table 14) helped to select the best proportion out of three. T1, T2 and T3 were compared for taste, texture, color, flavor, appearance and overall acceptability. From the statistical analysis conducted and the grading done by the panelists the overall acceptability is best for T1 (70:30).

Table 15: Sensory evaluation of Smoothie

Parameter	T0	T1	T2	T3
Appearance	8.20±0.78	8.2±0.78	8.30±0.67	8.10±0.73
Colour	8.00±0.66	8.2±0.63	8.20±0.42	8.20±0.63
Taste	7.90±0.73	7.90±0.99	7.60±1.07	7.5±0.85
Texture	7.8±0.91	7.80±1.03	7.90±0.73	7.90±0.87
Flavour	8.00±0.66	7.80±0.91	7.80±0.91	7.80±0.78
Overall acceptability	7.90±0.87	8.00±0.81	7.90±0.73	7.70±0.94

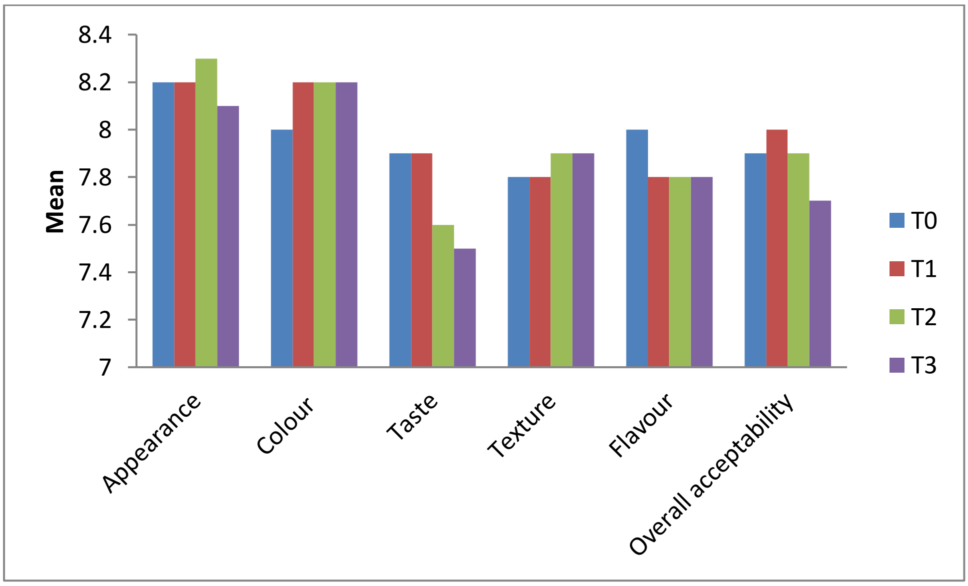

Figure 4: Sensory evaluation graph of Smoothie

Panel members tasted smoothie and graded it to different ranges which (table 15) helped to select the best proportion out of three. T1, T2 and T3 were compared for taste, texture, color, flavor, appearance and overall acceptability. From the statistical analysis conducted and the grading done by the panelists the overall acceptability is best for T1 (70:30).

From the result it is understood that T1 (70:30) is the most accepted proportion by the trained panel members in all value added products. T1 was accepted and also appreciated for its color, texture, taste, flavor and appearance.

4.6: STATISTICAL ANALYSIS

The nutrient analysis of the protein composite flour was compared to a nutrient composition of a market product. Market product was kept as control and compared against the nutrient analysis of protein composite flour. The result of comparison is shown in the table 16.

Table 16: Comparison of market product and protein composite flour

Parameter	T0	T1	T2	T3
Total Calorie	281±1.00	265.9±0.10	257.7±0.1	262.8±0.1
Total Carbohydrate	50.7±0.10	43.0±1.0	40.0±1.0	42.0±1.00
Dietary Fibre	10.2±0.1	1.2±0.1	1.5±0.1	1.3±0.1
Protein	16.3±0.1	18.3±0.05	19.7±0.1	19.2±0.1
Total fat	1.92±0.01	2.2±0.1	2.0±0.1	2.00±0.1
Sodium	19.45±0.01	156±1.00	161±1.00	158±1.00

Calcium	67.4±0.1	40±1.0	52±1.0	49±1.0
Iron	4.34±0.01	21±1.0	20±1.0	23±1.0
Magnesium	130.4±0.1	18±1.0	24±1.0	22±1.0

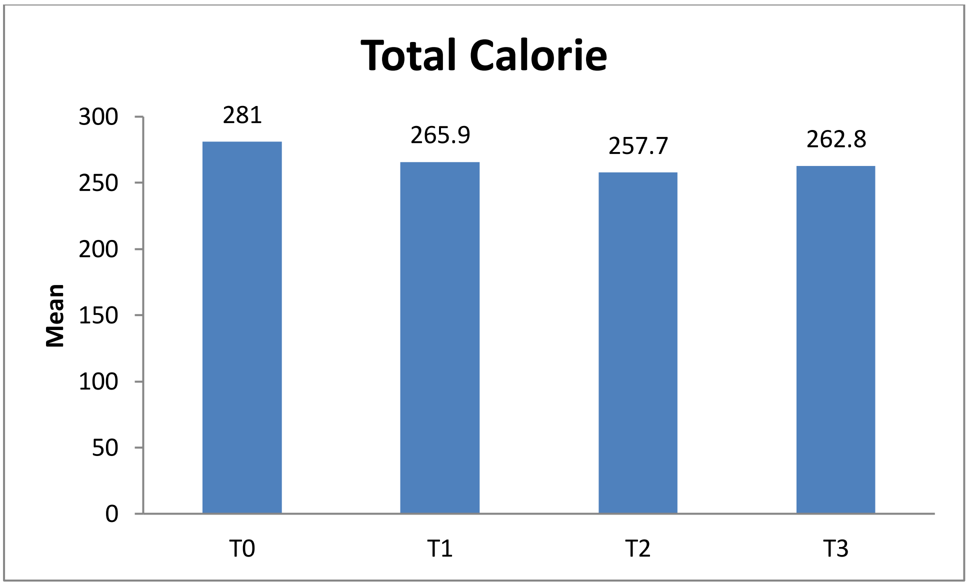

Figure 5: Comparison of total calorie

Total calorie was high in market product.

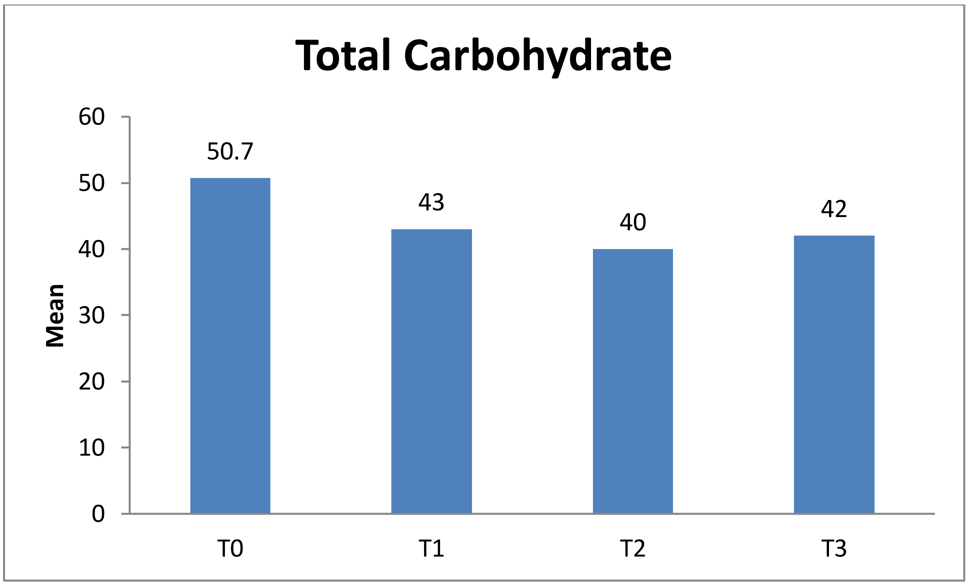

Figure 6: Comparison of total carbohydrate

Total carbohydrate was high in market product.

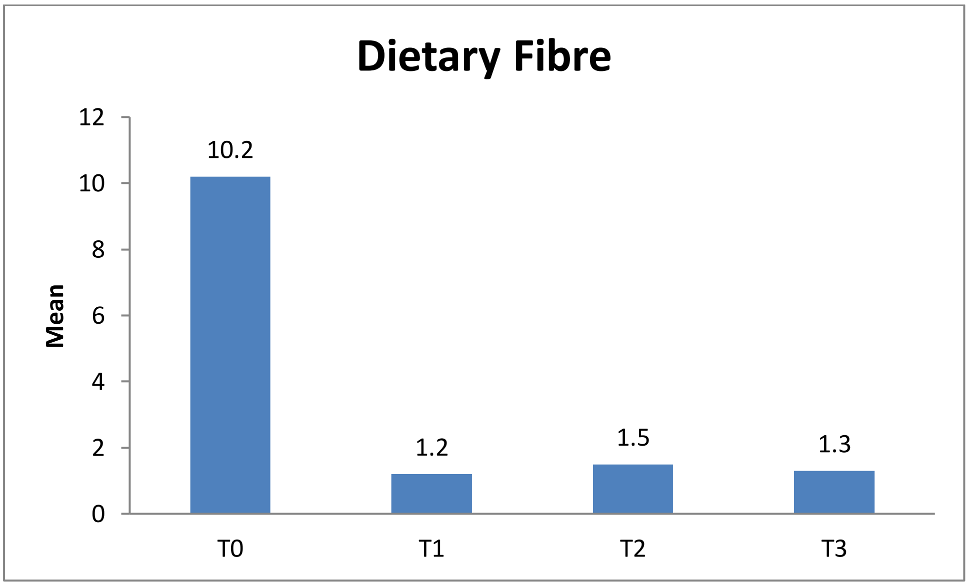

Figure 7: Comparison of dietary fiber

Total dietary fiber was high in market product.

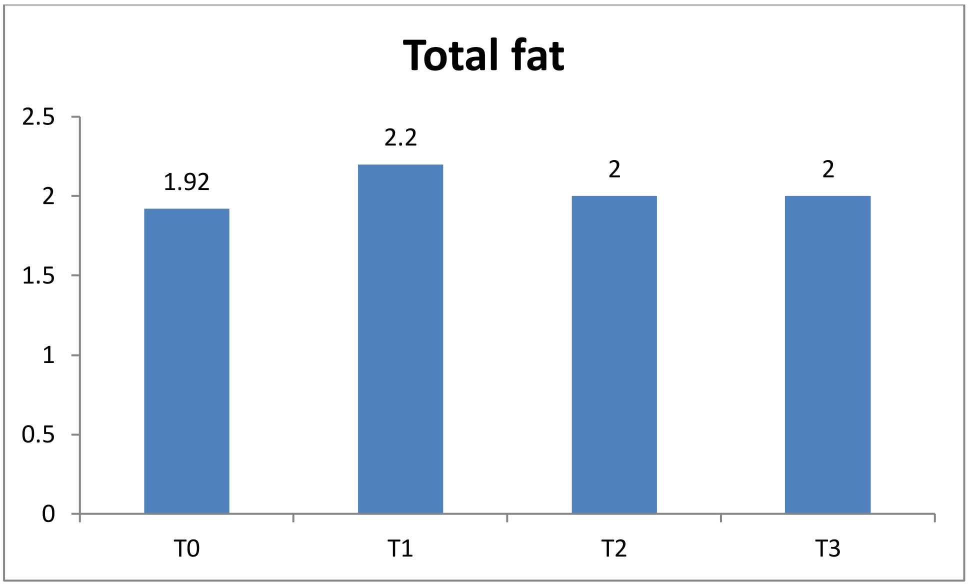

Figure 8: Comparison of total fat

Total fat content was high in T1 proportion of protein composite flour.

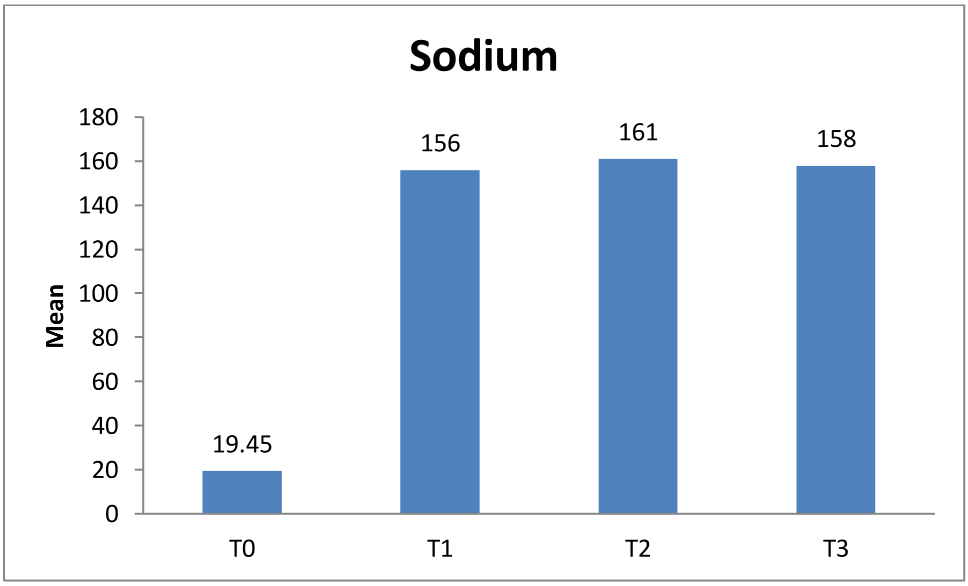

Figure 9: Comparison of sodium

Sodium was high in T2 proportion of composite flour.

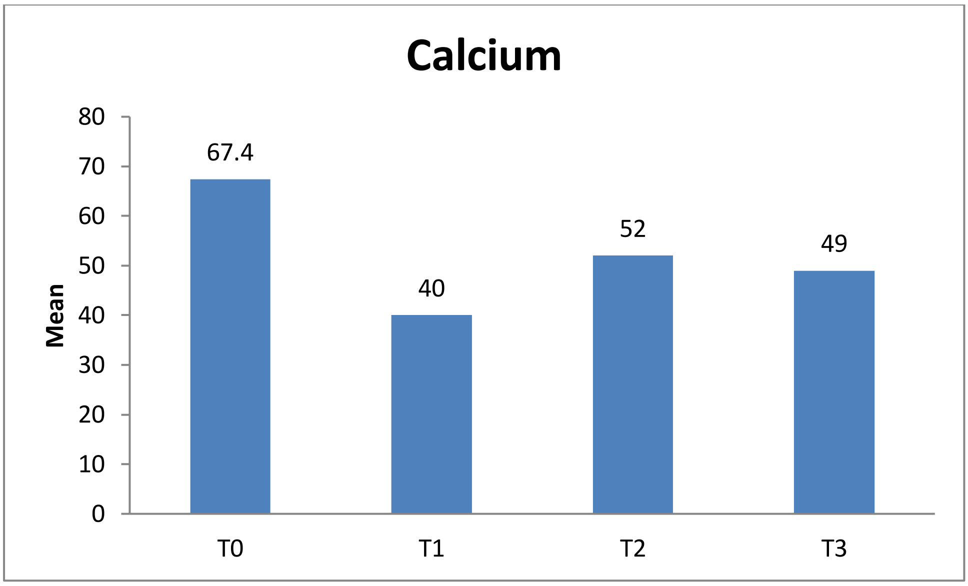

Figure 10: Comparison of calcium

Calcium was high in market product.

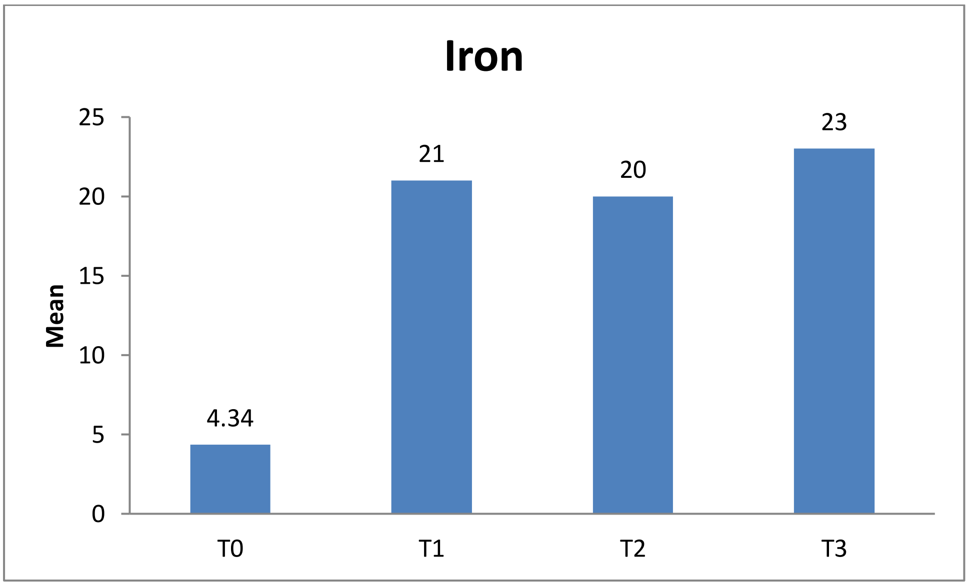

Figure 11: Comparison of iron

Iron was high in T3 proportion of composite flour.

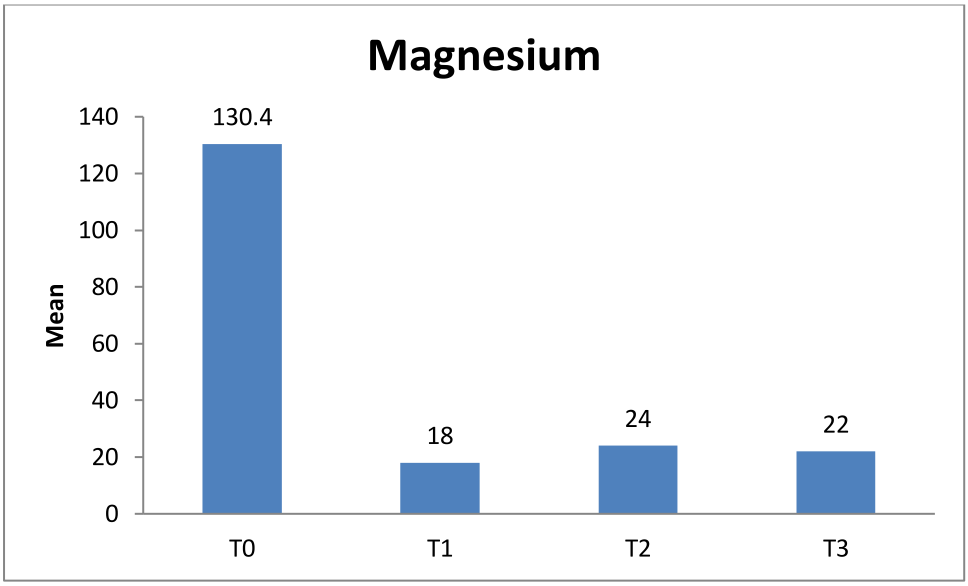

Figure 12: Comparison of magnesium

Magnesium was high in market product.

4.7: SHELF LIFE ANALYSIS OF THE COMPOSITE FLOUR

The result of shelf life analysis is provided in table 17.

Table 17: Shelf life analysis of composite flour

Parameter (Physical, chemical and microbiological)	Result	Permissible limit
Texture Profile Analysis	Failed after 47days	-
Colour	Off white	Acceptable if no discoloration
Appearance	Powdered	Should remain powdered
pH	6.9	5.5–7.0

Moisture	7.3	≤ 13–14
Rancidity	12	≤ 10
Alkalinity	80	≤ 100
Total Plate Count	16900	≤ 10^4 (10,000)
Coliform	14	≤ 10
E. Coli	Absent	Must be absent
Yeast and Mold	7	≤ 100
Aflatoxin	600	≤ 20

Shelf life or the keeping quality of the protein composite flour was studied under different conditions and the composite flour was stable for 47day under ambient storage condition. The color of the composite flour was off white, appearance of the composite flour was powdery, pH of the flour was 6.9, moisture content percentage of the composite flour was 7.3, rancidity percentage was 12, alkalinity was 80ppm and total plate count was 16900CFU/g. E.Coli was absent and aflatoxin was 14ppm. Yeast and mold presence was 7600CFU/g and coliforms count was 14mpn.

From the result conclusion is that the protein composite flour is not stable for more than 46days and after 46th day microbial load was high, rancidity of the composite flour was visible. The flour doesn't contain E. Coli, pH is stable, moisture content was stable, appearance was powdery and colour was great because it showed off white colour and no odd colors were developed.

CHAPTER-V

SUMMARY AND CONCLUSION

This study "Development and Quality Evaluation of Allergen Free Vegan Protein Composite Flour and Its Application in Food Products" was carried out aiming to develop vegan protein composite flour which can be used by allergic people without the thought of developing allergic reaction towards the protein in the food. The study was conducted based on certain objectives;

- To formulate and standardize allergen free vegan protein composite flour using selected ingredients like chickpea flour, green gram flour, kodo millet flour and sunflower seed flour.
- To analyze the nutrient and antinutrient composition of the developed composite flour.
- To assess the functional properties of the developed composite flour.
- To determine the antidiabetic potential of the developed composite flour.
- To ascertain shelf life of the developed composite flour.
- To develop value added products from the developed composite flour.
- To evaluate sensory acceptability of the value-added products.

Based on the objectives study was focused on the development of protein composite flour and to evaluate the functional properties, nutritional content and shelf stability. Also this study aimed in the application of the developed protein composite flour in to recipes in order to incorporate in daily life. Thus using the protein composite flour value added products was developed. For the developed products organoleptic evaluation was conducted by special trained panel members which helped in evaluating the physical properties of the value added products such as; appearance, taste, texture, color, flavor and over all acceptability.

Salient features of the study;

Millets and pulses when combined and consumed gives complete protein in the diet. Therefore combination of millets, pulses and fatty seeds for the mouthfeel were preferred for the development of composite flour. Kodo millet was chosen from millets, chickpea and green gram were chosen from pulses and sunflower seed was chosen from fatty seeds. These ingredients were purchased, washed and cleaned. The cleaned ingredients were sun dried in hygienic manner and then dry roasted in pan. Then the roasted ingredients were ground to fine powdery appearance. Thus separate flours were obtained such as kodo millets flour, chickpea flour, green gram flour and sunflower seed flour. These flours were mixed in three different proportions to form protein composite flour of three proportions. T1 comprised of 60g kodo millet flour, 20g chickpea flour, 10g green gram flour and 10g sunflower flour. T2 comprised of 60g kodo millet flour, 10g chickpea flour, 20g green gram flour and 10g sunflower seed flour. T3 comprised of 60g kodo millet flour, 15g chickpea flour, 15g green gram flour and 10g sunflower seed flour.

The formulated composite flours were analyzed for their nutrient quality. All three proportions were subjected to nutrient analysis for proximate analysis, micronutrient analysis, antinutrient analysis, *invitro* protein digestibility analysis and *invitro* diabetogenic activity. In T1 (60:10:20:10-K:G:C:S) total ash is 2.1%, total calorie is 265.9kcal, total carbohydrate is 43g, total fat is 2.3g, monounsaturated fat is 0.7g, polyunsaturated fat is 0.4g, protein is 18.3g, lysine is 70mg, methionine is 58mg and dietary fiber is 1.2g per 100gram protein composite flour. The micronutrient content of T1; vitamin E is 2mg, vitamin B complex is 1.5mg, sodium is 156mg, calcium is 40mg, iron is 21mg, zinc is 10mg and magnesium is 18mg per 100gram protein composite flour. The antinutrient profiles of T1 are; phytate is 480mg, tannin is 190mg and polyphenol is 160mg per 100gram protein composite flour. The invitro protein digestibility of T1 is 12% and diabetogenic activity of T1 is 14% respectively per 100gram protein composite flour. In T2 (60:20:10:10-K:G:C:S) total ash is 2.7%, total calorie is 257.7kcal, total carbohydrate is 40g, total fat is 2.1g, monounsaturated fat is 0.6g, polyunsaturated fat is 0.3g, protein is 19.7g, lysine is 79mg, methionine is 63mg and dietary fiber is 1.5g per 100gram protein composite flour. The micronutrient content of T2; vitamin E is 3mg, vitamin B complex is 1mg, sodium is 161mg, calcium is 52mg, iron is 20mg, zinc is 8mg and magnesium is 24mg per 100gram protein composite flour. The antinutrient profiles of T2 are; phytate is 496mg, tannin is 200mg and polyphenol is 176mg per 100gram protein composite flour. The invitro protein digestibility of T2 is 15% and diabetogenic activity of T2 is 16% respectively per 100gram protein composite flour. In T3 (60:15:15:10-K:G:C:S) total ash is 2.9%, total calorie is 262.8kcal, total carbohydrate is 42g, total fat is 2g, monounsaturated fat is 0.9g, polyunsaturated fat is 0.4g, protein is 19.2g, lysine is 75mg, methionine is 60mg and dietary fiber is 1.3g per 100gram protein composite flour. The micronutrient content of T3; vitamin E is 2.5mg, vitamin B complex is 0.7mg, sodium is

158mg, calcium is 49mg, iron is 23mg, zinc is 6mg and magnesium is 22mg per 100gram protein composite flour. The antinutrient profiles of T3 are; phytate is 490mg, tannin is 182mg and polyphenol is 168mg per 100gram protein composite flour. The invitro protein digestibility of T3 is 13% and diabetogenic activity of T3 is 15% respectively per 100gram protein composite flour. Thus from the nutrient analysis of three respective proportions of vegan protein composite flour T2 was identified as the best proportion due to its nutrient rich profile especially protein.

After the nutrient analysis and finding out the better proportion the selected proportion protein composite flour was subjected to functional property evaluation. The protein composite flour was evaluated for its water absorption capacity, oil absorption capacity and bulk density. The water absorption capacity of the protein composite flour (T2) was 30.79%, oil absorption capacity was 28.76% and bulk density was 772g/L respectively.

Once the functional evaluation was completed the protein composite flour was then used to develop value added products from it in order to enhance its application to daily diet and to enhance the health benefit. Total of four products were developed out of the protein composite flour. For each product it had three variations and a control was produced. The products were; smoothie, cheela, pancake and soup. All these four products were made with base ingredient oats either whole or flour. The control (T0) had 100% oats and other variations had different ratios of oats and protein composite flour. T1 had 70% oats and 30% protein composite flour while T2 had 60% oats and 40% protein composite flour where as T3 had 50% oats and 50% protein composite flour. All four products were in these proportions. Organoleptic evaluation was conducted by trained panel members who were selected by 9 point hedonic scale. During the organoleptic evaluation they assessed the products for their appearance, taste, texture, color, flavor and over all acceptability. The panel members tasted the products, noted the appearance, texture, flavor and then they graded them accordingly. Most acceptable combination was T1 (70:30) for its appearance, flavor, taste, texture, color and its overall acceptability.

The nutritional value of the protein composite flour was compared with nutritional properties of market product. By keeping the protein rich dosa flour as control (T0) and protein composite flour as different trails. The parameters which were compared were; total calorie, total carbohydrate, protein, total fat, dietary fiber, sodium, calcium, iron and magnesium. Iron and protein levels are increased in T1 to T3. T2 indicates a leaner, higher-protein choice because it has the highest protein content but the lowest calorie and carbohydrate values.

The protein composite flour was then analyzed for it keeping quality under various conditions. Its microbiological, chemical and physical stability were evaluated. The product is not shelf-stable under present storage conditions beyond 47days, despite the flour maintaining acceptable pH, moisture, and appearance, according to the combined chemical and microbiological deterioration indicators.

CONCLUSION

This study focuses on formulation and evaluation of vegan protein composite flour which is suitable allergic people. Value added products were developed from the protein composite flour and the nutritional profile of the composite flour was studied and compared with market product. The functional properties of the protein composite flour were evaluated. Keeping quality of the protein composite flour was analyzed and can be kept for less than 47days. Organoleptic evaluation of the value added products was conducted in three different proportions and best proportion was selected based on the evaluation done by trained panel members which meets the color, flavor, texture, taste and appearance as well as overall acceptability basis.

Future area of work

- Shelf life study can be conducted under different storage conditions.
- Study marketing of the protein composite flour.
- Study effect of this protein composite flour in diabetic people.
- Study on how the composite flour improves overall health.

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APPENDIX-I

WATER ABSORPTION CAPACITY

Method: Sathe *et al.* (1981) method

Scope: The method helps to analyze the water absorption capacity of the protein composite flour.

Procedure:

- 5g flour was taken and mixed with 20ml of distilled water.
- The contents were allowed to stand at 30ºC for 30min.
- Then centrifuged at 10,000rpm for 30 minutes.
- After centrifugation the volume of free liquid was recorded and retained volume was expressed as milliliter of water absorbed per gram of sample, on a dry basis.

Calculations:

Water Absorption Capacity (WAC) = V water−V supernatant / Weight of Sample

Where;

- V water is the initial volume of water (in Ml),
- V supernatant is the volume of the supernatant (in Ml),
- Weight of Sample is the amount of the sample (in grams).

APPENDIX-II

OIL ABSORPTION CAPACITY

Method: Rosario & Flores, (1981) modified by Iyer & Singh, (1997).

Scope: This helps to determine the oil absorption capacity of the composite flour.

Procedure:

- 1g of sample was mixed with 15ml of oil for 30minutes.
- The contents were allowed to stand in the water bath at 30ºC for 30minutes.
- The contents were then centrifuged at 3,000rpm for 20min and the volume of the supernatant was recorded.
- The oil absorption capacity was expressed as gram of oil bound per gram of the sample on a dry weight basis.

Calculation:

Oil Absorption Capacity (OAC) = V oil−V excess / Weight of Sample

Where:

- V oil is the initial volume of oil (in mL),
- V excess is the volume of excess oil that remains after the sample has absorbed the oil (in Ml),
- Weight of Sample is the amount of the sample used (in grams).

APPENDIX-III

BULK DENSITY

Scope: To determine the bulk density of the composite flour.

Procedure:

- 50g of flour sample was put into a 100 ml measuring cylinder.
- The cylinder was tapped several times on a laboratory bench to a constant volume.
- The volume of sample was recorded.

Calculation:

Bulk density (g/ml) = Weight of Sample (g)/Volume of sample after tapping (ml).

APPENDIX-IV

ANALYSIS OF NUTRIENT ANALYSIS OF THE PROTEIN COMPOSITE FLOUR

Estimation of Total Ash

Scope: The inorganic remnants that remain after organic matter has been completely oxidised or ignited are referred to as ash. A standard reference technique for figuring out how much total ash is in cereals and cereal products. It applies to all food grains and products, including barley, millet rye, oats, triticale, sorghum, kaffir, and wheat, durum wheat, rice (paddy, husked, and milled), as well as semolina or flour, biscuits, and other baked goods.

Principle: By burning the material to a consistent mass at a high temperature of 550 25 °C in a muffle furnace, all organic matter is eliminated.

Procedure:

Take a new sample for the total ash measurement. Weigh a dish that has been cleaned and dried (W1). About 5 g of the powdered sample should be precisely weighed and added to the plate. Put it in the oven for two hours at 130–133°C with the cover on. Once the items are in the oven, the time should be measured from the instant the oven reaches 130°C. After two hours, take out the dish, let it cool in the desiccator, and weigh it (W2). After determining the amount of moisture, light the dried material in the remaining dish with a hob flame until it is charred. A fume hood is required for this procedure. Move to a muffle furnace that is kept at 550 25 °C and keep burning till grey ash is produced. Weigh after cooling in a desiccator. Continue heating, cooling, and weighing every 30 minutes until the weight difference between two subsequent weigh-ins is less than 1 milligram. Take note of W2, the lowest weight. If ash still contains black particles add 2-3 drops of pre-heated water at 60 °C. Break up the ash and let it dry at 100 to 110 degrees Celsius. Re-ash at 550 degrees Celsius. Till the ash turns white or a little grey.

Calculation of ash,

Total ash on dry basis (% by weight) = $W2 - W \,/\, W1 - W \times 100$

Where,

W = Mass in g of empty dish

W1 = Mass in g of the dish with the dried material (moisture free) taken for test

W2 = Mass in g of the dish with the ash

APPENDIX-V

Estimation of Total Dietary Fiber

Principle: Crude fiber is lost upon burning the dry residue that remains after the sample is digested under particular conditions using 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH solutions. Filtration is used to separate the residue, which is then dried and ashed. The amount of crude fiber in the sample is reflected in the weight loss that occurs during ashing.

Reagents:

- Sulfuric acid, specific gravity 1.84 at 60 °F
- Sodium hydroxide pellets
- Ethyl alcohol, 95%, ACS grade
- Methylene chloride, anhydrous (dichloromethane), ACS grade
- Demineralized water
- Petroleum ether, initial boiling temperature, 35 °–38 °C; dry-flask end point, 52 °–60 °C; 95% distilling <54 °C, specific gravity at 60°F, 0.630–0.660
- Antifoam: Antifoam A compound diluted 1 + 4 with mineral spirits or petroleum ether, or H_2O-diluted antifoam B emulsion (1 + 4). Do not use antifoam spray.
- Blue litmus paper
- Bumping chips or granules: Broken Alundum crucibles or equivalent granules are satisfactory.

Procedure:

Accurately weigh a sample of 2.5–3 g, then move it to a Soxhlet extractor or other extraction equipment and use petroleum ether to extract it. The extracted material should be air dried before being transferred to a dry 1 L conical flask. Use a mixture of petroleum benzene and acetone to treat the product if the fat content is significant (>10%). If excess fat is not eliminated during the initial defatting process, it could impact the final outcome. Place the digestion flask on a hot plate or digestion rack that has been prepared and adjusted so that the acid will boil in about five minutes after adding 200 mL of the H_2SO_4 solution and connecting it to the condenser. To guarantee complete wetting and mixing of the sample, continue boiling rapidly for 30±1 minutes while rotating the flask often. Material that is not in touch with the solution should not be permitted to stay on the flask's surfaces. To control foaming, add one drop of diluted antifoam (using too much antifoam could produce unfavorable outcomes). Adding bumping52 chips or granules is another option. To provide precise timing, successive sample digestions should be initiated at roughly three-minute intervals. After 30 minutes of boiling, take out the flask and filter right away using the Buchner funnel or, for faster filtration, a filter cloth in a fluted funnel with a suction flask. Boil the water and rinse until the water is no longer acidic. Alkalinity can be checked using litmus paper. Quantitatively transfer the sample and ceramic fibre into the digestion flask while cleaning the Buchner filter or filter cloth with 200 milliliters of NaOH solution. It is convenient to use a wash bottle that holds 200 mL. Attach the flask to the reflux condenser, set it on the heating mantle, digesting rack, or hot plate that has been prepared, bring it to a boil in about five minutes, and then boil it for precisely twenty-eight minutes. To provide precise timing, successive sample digestions should be initiated at around 3-minute intervals. To ensure that the washings are no longer acid to litmus, remove the flask, filter through fine linen (about 18 threads per centimeter) that is held in a funnel, and then wash with boiling water. Crucible filters can be used in the filtration steps because unintentional linen tearing could pose a safety risk. Crucibles, such as the Porosity 2 filter crucible with a 50 mL volume, can also improve the accuracy of the results. To improve filtering and analyte recovery, filter aids (filter aid Celite (R) 545) can be applied. Bring a little amount of sodium hydroxide solution to a boil. Using 200 milliliters of boiling sodium hydroxide solution, wash the residue off the linen into the flask. Connect the flask to the reflux condenser right away and then boil it for precisely half an hour. Take out the flask and filter through the filtering cloth right away. Transfer the residue to a Gooch crucible that has been prepared with a thin, compact layer of ignited asbestos after thoroughly washing it with boiling water. Use around 15 mL of ethyl alcohol after thoroughly washing the residue with hot water. Use an air oven to dry the Gooch crucible and its contents at 105±2 °C until the weight remains constant. Allow to cool and weigh. Use a muffle furnace to burn the contents of the Gooch crucible until all of the carbonaceous material has been consumed. After cooling the Gooch crucible with ash in a desiccator, weigh it. Dry the crucible and its dregs for two hours at 130 °C. The product's limit of detection for crude fibre is about 0.2g/100g. When the crude fibre content is less than 10 g/100 g of the product, the repeatability limit is 0.3 g/100 g; when it is equal to or greater than 10 g/100 g of the product, the repeatability limit is 3% of the average. It is advised to use filter aid Celite® 545, 22140 Fluka instead of asbestos.

APPENDIX-VI

Estimation of Total Protein

Principle: The technique is predicated on the idea that food digestion is aided by concentrated sulphuric acid when a catalyst is present. Ammonium sulphate is produced from all of the nitrogen. By distillation in the presence of a base such as NaOH it is transformed into ammonia. The ammonia is titrated against 01N hydrochloric acid after being trapped in an acid, such as boric acid. The approach involves the following reactions with a conversion factor of 6.25; the nitrogen is multiplied to produce protein.

Procedure:

In the test part and pre-treatment, add 5–10 boiling aids, 15 g K2SO4, 1.0 mL of the copper sulfate46 solution, roughly 5 ± 0.1 g of the produced sample, weighed to the nearest 0.1 mg, and 25 mL of concentrated sulphuric acid to the clean and dry Kjeldahl flask. Additionally, rinse any copper sulphate solution, K2SO4, or sample that remains on the flask neck with the 25 mL acid. Mix the Kjeldahl flask's contents gently.

Digestion: After turning on the fume extraction system, the Kjeldahl flask containing the sample, sulphuric acid, and catalysts is gently heated to start the digesting process. To avoid foaming, the heating process is started on a low level and continued until white vapors start to form. The heat is then gradually raised until the solution turns clear and blue-green, signifying full digestion, first to a medium level for 15 minutes and then to the highest setting. It takes between 1.8 to 2.25 hours to complete this step. Within 25 minutes after digestion, the mixture is cooled to 25±2 °C. Accuracy may be impacted by crystallization, which must be prevented by proper cooling. To dilute the digest and make sure any crystals dissolve, 300–400 mL of water is added once it has cooled. The flask's neck should also be cleaned thoroughly. In order to prepare the mixture for the distillation process, boiling aids are added once more before letting it cool to 25±2 °C. If necessary, diluted samples can be kept in storage for a short time. However, undiluted digests should not be left overnight since crystallization can complicate further processing.

Distillation: The condenser water is turned on to start the distillation process. The diluted digest in the Kjeldahl flask is then gently mixed with 75 mL of 50% sodium hydroxide solution, creating a clear layer underneath the acid combination. An appropriate chemical reaction interface is guaranteed by this configuration. After that, the condenser outlet is inserted into a flask holding 50 mL of boric acid solution with indicator, and the flask is linked to the distillation apparatus. The Kjeldahl flask's contents are heated vigorously until boiling starts after being thoroughly swirled to blend the layers and remove any visible separation. Until uneven boiling (bumping) begins, distillation proceeds. At this stage, the water and heat flow are stopped, and the flask is detached. The final volume in the receiving flask should be approximately 200 mL, after roughly 150 mL of distillate has been collected. The distillate's temperature must not rise above 35 °C in order to guarantee effective condensation. Less distillate could be the result of inadequate dilution in the preceding stage.

Titration: Use a standard 0.1 N hydrochloric acid solution to titrate the boric acid receiving solution until the first pink tint appears. Take the burette reading to at least the closest 0.05 mL. The final point may be easier to see with a lit stir plate.

Alongside sample testing, a blank test is carried out using all the reagents except for the sample, which is substituted with 0.85 g sucrose and 5 mL water to mimic organic material. During digestion, sucrose aids in the consumption of sulphuric acid, simulating actual sample conditions. This helps identify contamination or irregularities and guarantees that the digesting circumstances are suitable (e.g., if the blank is pink before titration, the glassware may be filthy or contaminated).

Ammonium sulphate and amino acids (tryptophan or lysine) are used in assays to verify nitrogen recovery. These ensure the method's precision and assist pinpoint any nitrogen loss. For ammonium sulphate, a recovery of 99–100% is optimal. Higher recoveries can indicate titrant problems, whilst lower recoveries might indicate distillation, digestion, or equipment leakage. At least 98% amino acid recovery is required to confirm the effectiveness of digestion. In the end, proficiency testing that compares data from different laboratories is the most effective way to validate performance. In accordance with the manufacturer's instructions, fully automated Kjeldahl analyzers can also be utilized for increased convenience and uniformity.

Calculations:

Calculate the nitrogen content, expressed as % by mass, by following formula

$$W_n = 1.4007 \times (V_s - V_B) \times N / W$$

W_n=nitrogen content of sample, expressed as % by mass;

VS=volume in mL of the standard hydrochloric acid used for sample;

VB=volume in mL of the standard hydrochloric acid used for blank test;

N=Normality of the standard hydrochloric acid expressed to four decimal places;

W= mass of test portion in g, expressed to nearest 0.1 mg.

Express the nitrogen content to four decimal places.

The crude protein content, expressed as a % by mass, is obtained by multiplying the nitrogen content by 6.25. Express the crude protein results to three decimal places.

Protein = Wn x 6.25

Protein (dry weight basis) =Protein content ×100 / 100−Moisture (%)

APPENDIX-VII

Estimation of Total Fat

Principle: The ground sample is extracted with petroleum ether in a Butt-type extraction apparatus such as Soxhlet distiller or similar devices. The solvent is distilled off and the residue dried and weighed.

Procedure:

Weigh 2g of the ground, moisture-free sample, then wrap it in filter paper. Fill the butt tube device with the sample. Switch on the heating mantle and use petroleum ether to extract the sample for four to six hours at a rate of five to six drops per second. Use a water bath or a steam bath to evaporate the petroleum ether. Weigh the extracted oil's mass. To acquire accurate and dependable findings, it is crucial that the powder sample is fine enough as it has been observed that particle size of the ground soybean affects the extraction. After placing the thimble in the extraction cup, put the cup in the extractor and fill it with 150ml of petroleum ether.

Calculations:

$\%\ oil\ (moisture\ free\ basis) = W2\ /\ W1 \times 100$

Where:

W1= Mass of sample

W2= Mass of oil

APPENDIX-VIII

Estimation of Mineral content

Principle: The mineral (inorganic) is determined by dissolving the organic matter in Carbon tetrachloride.

Procedure:

The entire collected sample is put into a beaker filled with carbon tetrachloride in order to ascertain the mineral (inorganic) composition of foreign items. By letting the organic materials dissolve in the solution and the inorganic (mineral) matter sink to the bottom, this solvent aids in component separation. To separate the residue, the mixture is subsequently either filtered or decanted. After being dried at 100°C, the residue is weighed. This weight is then used to determine the proportion of mineral matter, which represents the sample's residual inorganic composition.

Calculation:

Inorganic matter (%) = Mass of inorganic residue / Mass of sample × 100

APPENDIX-IX

Estimation of Total Carbohydrates

Principle: This test is based on the reaction of glucose (a mononsachharide) with Barfoed's reagent. By means of the Barfoed's reaction, it is possible to differentiate reducing monosaccharaides from reducing disaccharides as monosaccharaides can reduce copper fast enough in comparison to disaccharides. The formation of green precipitate is a positive test for reducing monosaccharides.

Procedure:

Take 1 mL of solution in a test tube, add 2 mL Barfoed's reagent. For six minutes, keep the test tube in a boiling water bath. Use tap water to bring the test tube down to room temperature (25 ± 3 oC). Keep an eye out for color development.

APPENDIX-X

ORGANOLEPTIC EVALUATION OF PROTEIN COMPOSITE FLOUR

SENSORY EVALUATION SCORE CARD

9 Point Hedonic Rating Scale

NAME OF THE JUDGE:

SENSORY CHARACTERISTICS	SOUP				SMOOTHIE			
	T0 Control	**T1**	**T2**	**T3**	**T0** Control	**T1**	**T2**	**T3**
Appearance								
Color								
Taste								
Texture								
Flavor								
Overall acceptability								

1 - Dislike extremely

2 - Dislike very much

3 - Dislike moderately

4 - Dislike slightly

5 - Neither like nor dislike

6 - Like slightly

7 - Like moderately

8 - Like very extremely

9 - Like extremely

TASTE THRESHOLD TEST

SENSORY CHARACTERISTICS	SOUP				SMOOTHIE			
	T0 Control	**T1**	**T2**	**T3**	**T0** Control	**T1**	**T2**	**T3**
Appearance								
Color								
Taste								
Texture								
Flavor								
Overall acceptability								

NAME:

SIGNATURE:

CLASS:

MOBILE NO:

Procedure: For the sensory evaluation of the developed value added products using protein composite flour trained panels were selected using taste threshold test by placing 4 different flavors (such as salt, sweet, bitter and sour) in different proportions mixed in water. As of flavors salt was taken 2g salt mixed in 200ml water, sugar was taken for sweet flavor 2g sugar was mixed with 200ml water, coffee was taken for bitter flavor 0.2g was mixed with 200ml water and lime juice was taken for sour flavor 1ml was mixed with 200ml water. Four different proportions were taken and mixed with 100ml water and kept for taste threshold test. From the results people who qualified identified all the proportions correctly. From the tasting and correct grading panel of 10 trained members were selected. They participated in 9 point hedonic rating scale test. They were provided with the above form and asked to taste to products and in between rinsing mouth with water and rate the products according to the rating scale where 1 stands for dislike extremely and 9 stands for like extremely.

APPENDIX-XI

SHEL LIFE ANALYSIS OF THE PROTEIN COMPOSITE FLOUR

PHYSICAL PARAMETERS

pH and Alkalinity – IS 3025

The Bureau of Indian Standards (BIS) developed the IS 3025 (Part 11):1983 standard, which describes how to measure the pH of water and wastewater. The electrometric and colorimetric methods are the two main approaches it recommends. Using an indicator electrode (such a glass electrode that reacts to hydrogen ions) and a reference electrode (usually a saturated calomel electrode), this technique measures the electromotive force (EMF) of a cell. A high-impedance voltmeter that has been pH-calibrated is used to measure the EMF. Because of its dependability and simplicity of use, the glass electrode system is frequently employed.

The colorimetric method a pH indicator is added to the water sample in the colorimetric approach, which is appropriate for field measurements. The pH value is then estimated by comparing the resultant colour to standard colour solutions. This approach is helpful for rapid evaluations even though it is not as accurate as the electrometric approach.

MICROBIOLOGICAL PARAMETERS

Total Plate Count – IS 5402

A standardized procedure for figuring out the total viable aerobic microbial count in food and animal feed products is provided by Indian Standard IS 5402:2012, which is titled "Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Micro-Organisms — Colony-Count Technique at 30°C." This technique is essential for evaluating the safety and microbiological quality of consumables. Although the technique can be applied to a wide variety of goods meant for human consumption or animal nutrition, it might not work as well for some fermented foods or animal feeds that include complicated bacteria.

The sample should be homogenised, successive dilutions must be made, and these must be inoculated into solid agar media. Colony-forming units (CFU) per gram or milliliter of the sample are determined by counting the formed colonies following 72 ± 3 hours of aerobic incubation at 30°C. With counts deemed valid between 15 and 300 colonies per plate, the approach necessitates careful control of variables including incubation temperature and time. Emphasis is placed on quality control, which includes the use of blank and control samples and routine equipment and reagent validation.

Coliforms-IS 5401

The process for identifying and counting coliform bacteria in food and animal feed samples is described in Indian Standard IS 5401:2012, "Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Colony-count technique." Since coliforms are markers of potential fecal contamination and inadequate sanitation during processing, this approach is crucial for assessing hygienic quality. A selective agar medium, such as Violet Red Bile Agar (VRBA), is used to inoculate the test sample after it has been serially diluted. Colonies that acquire a precipitate zone and a red or pink colour after covering with the same media and incubating the plates at 30°C for 24 ± 2 hours are considered coliforms. Plates with 15–150 colonies are taken into account when making calculations. Colony-forming units (CFU) per gram or milliliter of the test sample are used to express the results. To guarantee the dependability of results, the standard also incorporates quality assurance procedures such the use of control strains and rigorous adherence to incubation conditions.

E.Coli – IS 5887

A standardized procedure for identifying and measuring Escherichia coli (E. coli) in food and water samples is provided by Indian Standard IS 5887 (Part 1):1976, which is titled "Methods for Detection of Bacteria Responsible for Food Poisoning – Part 1: Isolation, Identification, and Enumeration of Escherichia coli." Since E. coli is a sign of fecal contamination and the possible presence of harmful bacteria, this technique is essential for evaluating food safety. The sample must be homogenised and serial dilutions made before being put onto selective media like MacConkey agar. Colonies with distinctive morphology usually pink to red colonies because of lactose fermentation are counted following a 24- to 48-hour incubation period at 37°C. To verify the identity of E. coli isolates, further biochemical tests are carried out, such as the IMViC series (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization). Colony-forming units (CFU) per gram or milliliter of the sample are used to express the results. Microbiological testing laboratories all throughout India use and acknowledge this standard.

Yeast and Mold – IS 5403

A standardized process for counting live yeasts and molds in food items and animal feed is described in the Indian Standard IS 5403:1999 (Reaffirmed 2018), "Method for Yeast and Mold Count of Foodstuffs and Animal Feeds." Since the presence of yeasts and molds might suggest spoiling and possible health risks, this approach is essential for evaluating the microbiological quality and safety of consumables. The test sample is serially diluted and inoculated onto an appropriate solid agar medium as part of the procedure. To enable the growth of yeast and mold colonies, the inoculation plates are subsequently incubated at 25°C for a predetermined amount of time, usually five days. Colonies are counted following incubation, and the results are reported as colony-forming units (CFU) per milliliter or gram of the sample. A large variety of goods meant for human consumption or animal feed can be processed using this technique.

APPENDIX-XII

STATISTICAL ANALYSIS

Procedure: Statistical analysis was done using IBM SPSS version 20. Numerical variable overall acceptability of different variations was represented in mean and SD. To test the statistical significance of overall acceptability between each variations with control of each product, Independent sample t test was applied. A p value of less than 0.05 was considered to be statistically significant. A statistical technique for determining if there is a significant difference between the means of two independent groups is the Independent Samples t-test, sometimes referred to as the two-sample t-test. It is frequently used in experimental research, such as behavioral sciences, nutritional analysis, and sensory studies. Comparing the means of two unrelated groups in order to ascertain if any observed difference is statistically significant is the main goal of the independent t-test. It can be used, for instance, to compare a new food formulation's overall acceptance scores to a control. The following presumptions must be fulfilled in order to guarantee the validity of the t-test results;

- Observational independence: The samples need to be unrelated to one another.
- Normality: Each group's data should roughly follow a normal distribution; this is crucial for small sample numbers.
- Variance homogeneity: The two groups' variances need to be equal. The Levene's Test for Equality of Variances can be used to test this.

To establish statistical significance at a selected alpha level (usually 0.05), the resulting t-value is compared to a critical value from the t-distribution table. Interpretation $p < 0.05$: The two group means differ statistically significantly, so the null hypothesis should be rejected. $p \geq 0.05$: No statistically significant difference; the null hypothesis cannot be rejected. The Independent Samples t-test is used in food science, especially sensory evaluation studies, to compare:

- Acceptability ratings for experimental and control meal items.
- Differences in nutrient levels between modified and traditional formulations.
- Under various storage settings, shelf life varies over time.