DEVELOPMENT AND STANDARDIZATION OF NUTRITIONALLY ENHANCED COMPOSITE FLOUR PRODUCTS: EVALUATION OF NUTRITIONAL, FUNCTIONAL, AND SENSORY QUALITIES

Dissertation submitted to

ST. TERESA'S COLLEGE (AUTONOMOUS), ERNAKULAM

Affiliated to

MAHATMA GANDHI UNIVERSITY

In partial fulfilment of requirement for the

AWARD OF THE DEGREE OF MASTER OF SCIENCE IN

HOME SCIENCE (BRANCH C)

FOOD SCIENCE AND NUTRITION

By

NIVA SOMARAJAN

Register No: AM23HFN013

DEPARTMENT OF HOMESCIENCE AND CENTRE FOR RESEARCH

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'Certified as bona fide research work'

DR. RASHMI H. POOJARA
Asst. Professor
Dept. Of Home Science
St. Teresa's College
Ernakulam

Signature of the Examiner

Signature of the Guide

28/04/25

Date

Signature of the Head of the Department

DEPARTMENT OF HOME SCIENCE
ST. TERESA'S COLLEGE
COCHIN-11

ST.TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

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Author Name	NIVA SOMARAJAN
Course of Study	M.Sc. Home Science Branch C- Food Science & Nutrition
Name of Guide	Dr. Rashmi H Poojara
Department	Home Science & Centre For Research
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Signature of Student

Signature of Guide

DR. RASHMI H. POOJARA
Asst. Professor
Dept. Of Home Science
St. Teresa's College
Ernakulam

Checked By
College Librarian

DECLARATION

I hereby declare that the thesis entitled 'DEVELOPMENT AND STANDARDIZATION OF NUTRITIONALLY ENHANCED COMPOSITE FLOUR PRODUCTS: EVALUATION OF NUTRITIONAL, FUNCTIONAL, AND SENSORY QUALITIES' is a Bonafide record work done by me during the study, under the supervision and guidance of Dr. Rashmi H Poojara, Associate Professor, Department of Home Science and Centre for Research, St. Teresa's College, Ernakulam.

NIVA SOMARAJAN

Place : Ernakulam

Date: 28/04/2025

DEPARTMENT OF HOME SCIENCE
AND CENTRE FOR RESEARCH
ST. TERESA'S COLLEGE AUTONOMOUS, ERNAKULAM

Dr. Rashmi H Poojara
Associate Professor
Department of Home Science & Center for Research

CERTIFICATE

This is to certify that the thesis entitled **'Development and Standardization of Nutritionally Enhanced Composite Flour Products: Evaluation of Nutritional, Functional, and Sensory qualities'** is an authentic record of the original research work carried out by **Ms. Niva Somarajan** with **Register No: AM23HFN013** under my supervision and guidance during the academic year 2023- 2025.

Ernakulam
28/04/2025

Dr. Rashmi H Poojara

DR. RASHMI H. POOJARA
Asst. Professor
Dept. Of Home Science
St. Teresa's College
Ernakulam

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Niva Somarajan

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CHAPTER 1

INTRODUCTION

Developing countries face a pressing global health problem of inadequate nutrition because of a lack of access to essential food and balanced meals. Multiple micronutrient deficiencies affect the population, lacking adequate intake of iron, protein, dietary fiber, and vital phytochemicals, with poor dietary diversity. It multiplies dietary deficiencies and lifestyle diseases, highlighting the need for cost-efficient, sustainable dietary solutions to prevent increasing health challenges.

Composite flours envisage a combination of different ingredients, including cereal, legumes, pseudocereals, tubers, and green leafy vegetables, to produce nutritious and functional food products that offer specific health advantages. The nutrient intake of individuals,specifically vulnerable groups, can be enhanced when composite flours are incorporated in traditional diets, offering greater dietary diversity, including proteins, vitamins, and minerals, while enhancing dietary fiber intake together with health-promoting components like flavonoids and phenolic acids. Composite flours can offer the benefits of functional foods in lieu of their antioxidant properties, along with their anti-inflammatory and metabolic regulatory effects (Olamiti *et al.,* 2024).

As individuals become more health-conscious due to busy lifestyles and schedules, significant changes will occur in the food commercial market as people now prioritize health due to time pressure amidst busy lives. Composite flours are well known to bring down various lifestyle disorders. The demand for health food is increasing, and the growing market for various types of health foods is prompting more advances in their formulation.The various ingredients that are used in the composite flour will improve the nutritional profile and also facilitate the incorporation of novel bioactive components. Nutrients such as proteins, vitamins, dietary fiber, minerals, and novel bioactive components such as flavonoids and phenolic acids are an integral part of composite flours. All of these improve the quality of diets consumed by individuals. Bioactive compounds are capable of preventing cellular damage and preventing the incidence of chronic diseases, specifically lifestyle diseases (Olamiti *et al.* 2024).

Elliot *et al*. (2022) suggested that traditional processing techniques like sprouting will enhance the bioavailability of iron by activating the enzyme phytase, which will break down dietary phytates. Due to this enzymatic process, the bioavailability of iron is improved and other micronutrients will also be absorbed more effectively in the body.Composite flours are mostly developed from locally available ingredients that are feasible and cost-effective. Some research has indicated that pre-treatments like soaking, sprouting, and controlled temperature drying will reduce antinutrients that inhibit iron absorption (such as tannins and phytates). Sprouting will promote the enzyme phytase, which will degrade phytate, enhancing the bioaccessibility and bioavailability of nutrients.

According to Tarffeeda *et al*. (2022), composite flour consumption can offer the possibility of managing lifestyle diseases. Combining different flours improves the sensory and functional qualities of food, which will enhance both dietary diversity and nutrient intake. The researchers indicated that the formulation of the composite flour they aim to create was a versatile product that can be used in various modern and traditional recipes like chapati, porridge, cookies, and energy bars and thus can be incorporated into daily diets. The formulation provides a cost-effective, sustainable substitute for traditional flours. This study shows that composite flour has the potential to develop a functional food innovation that will improve nutritional well-being and public health.

According to these findings, the study focuses on the '**Development and Standardization of Nutritionally Enhanced Composite Flour Products: Evaluation of Nutritional, Functional, and Sensory Qualities,**' which was undertaken with the following objectives:

- ❖ To formulate a composite flour using different combinations of nutrient-rich ingredients.
- ❖ To develop and standardize the products using formulated composite flour combinations and identify the most acceptable formulation based on sensory evaluation.
- ❖ To analyse the quality parameters of the composite flour- nutrient composition and functional attributes.
- ❖ To evaluate microbiological quality of the composite flour concerning microbial load and shelf life stability.

CHAPTER 2

REVIEW OF LITERATURE

The literature about the study 'Development and Standardization of Nutritionally Enhanced Composite Flour Products: Evaluation of Nutritional, Functional, and Sensory Qualities' is presented under the following headings;

2.1 Composite Flours and Potential ingredients

- 2.1.1 Wheat
- 2.1.2 Amaranth Seeds
- 2.1.3 Green Gram
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- 2.1.5 Amaranth Leaves
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2.5 Sensory Evaluation of Composite Flour Products

2.1 COMPOSITE FLOURS AND POTENTIAL INGREDIENTS

Olamati and Ramashia (2024) explored the incorporation of composite flour in pastry products. The enhancement of proteins, fiber, vitamins, and minerals, by blending the wheat flour and other flours that are derived from legumes, tubers, pseudocereals, and ancient grains, facilitated the increase of nutritional qualities of pastries. Moreover, mixing of these alternative flours will introduce the bioactive compounds, which are flavonoids, carotenoids, and phenolic acids, which contribute to anti-inflammatory and antioxidant properties, respectively. This study indicated that consumption of composite flour in the pastry formulations improves the overall quality of food products and offers potential health benefits.

Tangariya *et al.,* (2018) evaluated the nutritional composition of composite flour from chickpea, wheat, finger millet, and barley flours respectively. The researchers reported that composite flour has an increased content of total phenol at about 549.70 mg Gallic Acid Equivalents(GAE) /100g, compared to wheat flour is 379.0 mg GAE/100g and has a higher amount of calcium content of about 90.8 mg/100g as compared to wheat flour is 46.5 mg/100g content. Thus, the researchers suggested that the composite flour had enhanced potential health benefits.

In a study by Itagi and Singh (2011) multigrain composite mix formulation using a mix of cereals, legumes, millets, and nuts,it was reported that such formulations improve nutritional profiles, functional properties, and antioxidant properties of the flour. This suggests good potential to utilize these composite flours in the development of a variety of nutritious products. Kaur *et al.,* (2017) conducted a study focused on functional cookies development with the use of composite flour, which aims to enhance the isoflavone and β-glucan content. This research underscored the potential that composite flour will create health-promoting baked goods.Masri *et al.,* (2014) in their research study reported that composite flour utilization in various food products enhances the nutritional value, and cost is reduced by using the locally available raw materials for the composite flour formulations.

2.1.1 WHEAT

According to the research by Suman *et al.* (2019), Indian dietary culture primarily relies on homemade bread called chapati, which typically comes from wheat ingredients. 99.5% of rural Bihar individuals ate chapati every day, but only 78% ate rice daily.

Wheat (Triticum aestivum L.), from the Poaceae Family. Worldwide, wheat stands as the second fundamental food source after rice due to its connection with essential historical civilizations (Farook *et al.,* 2019). The largest nutritional origin, together with its status as the prime grain cultivation (Kumar *et al.*, 2015), allows wheat to supply 40% of total human energy and 20% of dietary protein requirements worldwide. The plant contains protein together with dietary fiber, along with lower levels of lipids and terpenoids, and essential vitamins, minerals, and phytochemicals (Adom *et al.,* 2003). Chaitanya *et al.,* (2020) confirmed that wheat contains essential minerals needed to preserve basic life functions of the body. Even though they exist in lower quantities than macronutrients, micronutrients cover essential human nutritional requirements, which stops deficiencies from causing related illnesses. Most deficient households lack iodine, iron, and selenium, which lead to chronic diseases among the leading causes of human mortality globally.

The paper by Guo *et al.,* (2013) demonstrates the emergence of wheat breeding research focused on different wheat colors such as purple, black, and blue varieties. Wheat possesses healthy antioxidant components, which include carotenoids, anthocyanins, and phenolic compounds that offer human health benefits. The colored wheat with high levels of anthocyanins would be suitable for product development because of its favorable characteristics and potential commercial outlook (Loskutov and Khlestkina, 2021; Garg *et al.,* 2022; Padhy *et al.,* 2022). Colored wheats serve as essential nutrient reserves that contain macroelements together with the essential microelements magnesium (Mg) and calcium (Ca), iron (Fe), zinc (Zn), and selenium (Se).The range of colored wheats comprises nourishing mineral components that exhibit diverse phenolic compounds that show potential value in fighting food-related malnutrition situations (Dangi *et al.,* 2023). Pigment wheat breeds contain 11.74 to 18.17 percent protein and an essential amino acid array covering 7.31 to 18.13 percent of the total content that exceeds white wheat levels (Tian *et al.,* 2018).

2.1.2 AMARANTH SEEDS

Amaranthus spp. Seeds serve as human food and are acclaimed due to their nutritional value, which makes them an appealing alternative dietary option. Amaranth seeds maintain a distinct shape as rounded lenses with a 1.5 mm diameter and a weight of 0.6 to 1.3 mg due to their premium protein content, together with fats, carbohydrates and dietary fibers, and minerals. Amaranth seeds show their prominent characteristic through their protein, which spans between 14.29% to 16.86%. Amaranth plants contain high quality, which contains all essential amino acids, together with lysine, but lacks these same nutritional features in wheat and corn (Maurya *et al.,* 2018) and (Singh *et al.* 2017).

According to Venskutonis and Kraujalis (2013), research on amaranth seeds and vegetables shows high protein content of about 13.6%, together with balanced amino acids and limited bioactive compounds found in other cereal seeds. Amaranth seeds have an amino acid composition that matches FAO/WHO nutritional standards, thus enhancing their protein content value.

Skwaryło-Bednarz *et al.,* (2020) establish that amaranth seeds contain between 5.80% to 8.0% fatty acids, which mainly consist of unsaturated oleic, linoleic, and linolenic acids. The antioxidant components of amaranth oil include the anti-cholesterol agent known as squalene alongside the antioxidants tocopherol and tocotrienols, which benefit skin health and lower cholesterol levels.

The carbohydrate component of seeds within amaranth range between 61.43% and 72.15%, with a bulk starch fraction that suits those who have allergies to gluten. The seed dietary fiber content amounts to 27.34%, with its makeup including both soluble and insoluble dietary fiber types. The abundant fiber in seeds improves digestive wellness and protects against various chronic diseases because of its function (Singh and Punia 2020).The study by Ogrodowska *et al.,* (2014) detailed the presence of bioactive compounds in amaranth seeds, where squalene and tocopherols are discussed among other bioactives along with their related health advantage points for cholesterol reduction and free radical defense system.

Studies suggest that eating amaranth seeds presents numerous health advantages to the human body. Scientific studies have demonstrated that amaranth seeds contribute to lowering plasma cholesterol while showing antitumor properties and blood glucose regulation that benefits patients with diabetes. The high-quality protein contained in amaranth, together with its essential amino acids, supports muscle development and produces positive metabolic outcomes for individuals as reported by Maurya and Arya (2018) and Khan *et al.,* (2022).

Soriano-García *et al.,* (2018) suggested that because the seeds are naturally gluten-free , amaranth remains viable for consumption by people who need gluten-free diets as well as individuals with celiac disease and gluten intolerance. The fiber content in amaranth helps digestion and extends satiety between meals, which might promote weight loss. The unsaturated fatty acids found in Amaranth, including omega-3 and omega-6 fatty acids, contribute to promoting heart health by reducing levels of bad cholesterol.

2.1.3 GREEN GRAM

Green gram exists as mung bean (*Vigna radiata*), which stands as a legume variety that people value for its nutritional advantages and numerous health benefits. As a vegetarian protein supply, green gram stands out because it contains vegetable proteins alongside carbohydrates and dietary fiber and vitamins, and minerals, along with a low fat content. Green gram delivers 22.87-27.76% protein, along with vital amino acids, such as lysine, that are absent from other cereal grains. The carbohydrate level in green gram exceeds 48.46-53.57%, which provides energy , yet its dietary fiber ranges from 11.83-15.79% and supports digestive health as it reduces the danger of gastrointestinal disorders. The mineral composition of green gram consists of phosphorus at 2716.66-4473.49 mg/kg, potassium at 3183.31-3597.61 mg/kg, magnesium approximately at 1506.51-1713.93 mg/kg, calcium at 166.38-340.62 mg/kg and iron at 40.16-348.79 mg/kg, these levels support bone health and muscle development and oxygen transportation (Sudhakaran *et al.,* 2024;Bhatty *et al.,* 2001).

Sudhakaran and Bukkan (2021). Suggested that green gram contains bioactive components, which, together with its nutritional value, provide multiple health advantages. Scientific studies demonstrate that green gram possesses antioxidant properties and anticancer effects, along with anti-inflammatory capabilities and hypolipidemic activities, which together make it beneficial for both disease prevention and management, especially for cardiovascular health and cancer cases. It contains galactooligosaccharides that can function as prebiotics, which stimulate beneficial gut microbiota growth to enhance digestive health. The numerous advantages of green gram led to its usage in developing functional food products, including mung bean milk along with non-dairy probiotic drinks, which incorporate green gram as their main component.

According to Bhatty *et al.,* (2001), the nutritional value of green gram changes based on both its preparation steps and what supplementary ingredients and cooking techniques are used during preparation. Protein digestibility improves alongside a reduction of antinutritional factors when using boiling or autoclaving cooking methods, since this increases the bioavailability of nutrients. The nutritional value of supplementary green gram is enhanced when combined with food components, allowing it to function as an ingredient for various culinary uses.

The biological components within green gram contain polyphenols and flavonoids, and peptides that manifest anti-inflammatory and antihypertensive, and antioxidant effects according to Singh *et al.,* (2021). Daily consumption of green gram produces wellness benefits through improved lipid metabolism, together with controlled fat levels and reduced susceptibility to multiple chronic diseases that include cardiovascular conditions and particular types of cancer. Green gram contains fiber that stimulates digestive health as it performs blood sugar level management similar to experiences of diabetic patients. Eating green gram leads to overall wellness benefits for human health because it provides various health advantages.

2.1.4 PEARL MILLET

The research by Ramya *et al.,* (2024) demonstrated that pearl millet (*Pennisetum glaucum*) contains higher nutritional value than the popular cultivated cereal crops. While there are two main limitations to using pearl millet for human consumption that include anti-nutritional factors (phytate, tannins, and polyphenols), reducing mineral absorption, and reduced storage quality because of elevated lipase activity.

As suggested by Jukanti *et al.,* (2016), Pearl millet functions as a primary cereal crop across the arid and semi-arid Asian and African regions. The cultivation of this crop serves mainly two purposes because farmers grow it for grain harvest and make use of its stover to produce dry fodder. Pearl millet demonstrates climate-resistance because it naturally adapts well to dry conditions and hot temperatures. The plant demonstrates tolerance to acidic, as well as saline conditions, and shows excellent adaptation in less productive marginal soil areas. Different yield attributes within pearl millet germplasm demonstrate wide genetic variation, together with diverse features for agricultural performance and both environmental and nutritional characteristics.

According to Satyavathi *et al.,* (2022), pearl millet has spread across 30 countries within tropical areas as a rainfed crop. Pearl millet cultivation exists in the largest quantities and produces the most output among all countries in the world in India. Open-pollinated varieties remain prevalent among Indian pearl millet cultivators as well as African farmers because they provide resistance against weather unpredictability, affordable seed costs, and better survival against various climatic threats. Due to its capability for quick growth, along with high photosynthetic capabilities and nutritional balance, and its ability to endure harsh environmental conditions, pearl millet achieves the status of 'super cereal'.

Pearl millet presents significant nutritional potential for use as a nutri-cereal according to Kaur *et al.,* (2021). The combination of climate change requirements and food diversity needs allows pearl millet to function as a secure food resource and nutritious dietary staple fit for future consumption.

2.1.5 AMARANTH LEAVES

According to Alegbejo *et al.,* (2014), the amaranth plant family includes sixty to seventy species, but forty of these species originated in the Americas. Amaranth plants flourish across temperate and tropical zones where people consume them as both a vegetable and a grain. Amaranth stems offer multiple nutritional values along with essential vitamins and minerals. Cooked amaranth in various forms, such as leaves, shoots, tender stems, and grains, serves as a pot herb in sauces along with soups or as an accompaniment to other vegetables and main dishes individually. Livestock use amaranth plants as their feed source. The traditional medicinal use includes roots and leaf preparations as laxatives, diuretics, anti-diabetic and antipyretic, and anti-snake venom, antileprotic, and anti-gonorrhea, and expectorant substances to relieve acute bronchitis symptoms. The leaves possess anti-inflammatory functions along with immunomodulatory activities, and they show anti-androgenic properties and anthelmintic properties.

Amaranthus spp. shows high nutritive value because it contains a variety of macronutrients and micronutrients with vitamins and minerals according to Coelho *et al.,* (2018). The nutritional value of Amaranth grain features essential amino acids, including lysine, because of its high-quality profile. Research investigations show that the essential nutrients from Amaranthus spp. Including phytochemicals offers useful health advantages.

Research by Sarker *et al.,*(2018) and Sarker *et al.,*(2022) demonstrates that amaranth leaves possess antioxidant compounds, including total polyphenols, flavonoids, and strong antioxidant properties. It indicates that amaranth leaf nutritional value may improve when plants face salinity stress, leading to enriched amounts of protein as well as ash, dietary fiber, minerals, β-carotene and ascorbic acid, and antioxidants. The genus species serves functionality in Europe and America as pseudo-cereals, yet the population of Africa primarily uses them for vegetable production. Until recently, Amaranthus gained recognition as a valuable food crop because it resists heat and drought and disease, pests, and both seeds and leaves offer high nutritional benefits. The entire plant functions as a medical treatment against multiple health problems in African medicine practices. This examines the leafy amaranthus vegetables, which have a traditional use in African cultures. This provides a conceptual overview of established information about taxonomy, ecology, and nutritional content, and the agricultural value of amaranths, while presenting findings on breeding along with reproductive biology of genetic resources. The scientists studied *A. blitum and A. caudatus in addition to A. cruentus, A. dubius, A. hypochondriacus, A. spinosus, A. thunbergii and A. tricolor, and A. viridis* respectively. Research explored opportunities for the development of amaranth with its nutritive and nutraceutical aspects and production systems and commercial potential, as well as taxonomic assessment and breeding techniques.

2.1.6 WHEAT GRASS

Scientific research indicates that wheatgrass derived from Triticum aestivum can deliver a wide variety of health advantages. Bar-Sela *et al.,* (2015) studied about the three basic chemical components found in wheatgrass are chlorophyll and flavonoids, along with vitamins C and E. Wheatgrass exists in the market as fresh juice and frozen juice as well as tablets and powders, but composition depends on their production methods as well as the wheatgrass cultivation environment. In laboratory experiments using fermented wheat germ extract, researchers have both established its anti-cancer properties and identified apoptosis through in vitro studies. Studies with animals show that wheatgrass shows preventive benefits against cancer and enhances both tumors' treatment along with antioxidant and immune system reactions. Wheatgrass produces therapeutic effects with chemotherapy, according to clinical studies, while simultaneously reducing chemotherapy-induced side effects, together with treating rheumatoid arthritis and ulcerative colitis in addition to helping patients with diabetes, obesity, hematological diseases, and oxidative stress.

Multiple nutrient compounds present in Wheatgrass make this plant highly important, according to Gunjal *et al.,* (2024). The production of wheatgrass occurs through indoor and outdoor cultivation techniques, which use various growing substrates as a plant cultivation medium. Various preservation approaches for wheatgrass have appeared recently to improve the bioactive content of wheatgrass. Multiple research studies establish that using wheatgrass and wheatgrass-based products enables diabetes management while addressing conditions such as atherosclerosis, kidney issues, colon diseases and anemia, and specific forms of cancer. Small wheatgrass dimensions enable people to absorb its advantageous compounds more effectively. To achieve maximum market potential for wheatgrass, consumers need to become aware of its nutritional benefits along with its therapeutic properties.

2.2 IMPACT OF PROCESSING METHODS ON NUTRITION

Processing methods sometimes manage to improve food nutritive properties. Modern technology, like high-pressure processing, enables the production of shelf-stable eating products that maintain their natural nutritional quality and taste attributes (Naik *et al.,* 2013). Food processing technologies produce different nutritional changes in foods due to several effects stemming from diverse techniques. Food preservation through thermal processes, which protects public safety, results in adverse changes to protein structure and nutritional content through the intricate reactions between food components (Zhang *et al.,* 2021).

Food processing methods determine how nutrition changes since they both depend on the chosen method and the specific food being handled. Processing methods either decrease nutritional value or enhance it according to the different techniques applied. Food quality improvement, along with enhanced nutrition and extended shelf-life, emerges from sous vide cooking as it simultaneously eliminates microorganisms (Onyeaka *et al.,* 2022). The extracting power of minerals and protein digestion capacity of biofortified bean flour increases after processing through malting and roasting, and extrusion cooking according to Nkundabombi *et al.,* (2015).

Food processing, such as fermentation or germination, improved the nutritional characteristics of millets while excessive dehulling, milling, or polishing diminished dietary content, including micronutrients, according to Gowda *et al.,* (2022). Research into the nutrient value transformations of millets during processing enables the food sector, as well as researchers and consumers, to choose appropriate processing methods that maximize nutritional worth and enhance nutrient accessibility while fighting food insecurity.

2.2.1 SOAKING

Serventi (2020) reported that soaking legumes is necessary for consumption, but nutritional losses occur during this process. A partial explanation for the altered nutritional profile of soaked legumes could be leaching in the processing water.Soaking and germination will increase the micronutrient content and decrease the antinutritional factors. It is suggested that there be a 16-hour soaking period at a temperature of 31 degrees Celsius, with a germination period of 48 hours (Tripathi *et al.,* 2013). The soaking water of haricot beans and green lentils softened the texture during storage, without affecting pasting properties and moisture content. Soluble fibre (beans) and phytochemicals (lentils) were speculated to enhance protein plasticity. The soaking water of legumes contain soluble and insoluble carbohydrates, protein, minerals, phenolic compounds, and saponins. These compounds are known to improve food texture and act as prebiotics.Soaking serves as an effective treatment that decreases cooking duration and strengthens texture features and nutrition content of food materials according to Amoah *et al.,* (2023). As a pre-treatment method, it provides unique benefits to legumes because it minimizes cooking duration and power usage.

2.2.2 SPROUTING

The lack of consensus regarding the definition of "sprouting" creates uncertainty about when grains should be considered sprouted. No established set of guidelines exists specifying the needed level of sprouted grain material in food products to generate health benefits. When grains undergo sprouting, they trigger hydrolytic enzymes to activate alongside their synthesis for nutrient accessibility in plant life cycles according to Lemmens *et al.,* (2018). Researchers have indicated that including sprouted grains in the diet delivers health benefits to people. Customer appreciation of sprouted cereals led to a surge of new food and beverage item releases to market. Without established regulations for sprouted foods, there is no framework for creating proper food labels. Supplementary bioactive compound production through sprouting requires either extended time (3 to 5 days) combined with high processing temperatures (25 to 35 °C). Sprouting leads to variations in nutrient composition, which generate health-positive effects.

Olawoye *et al.,* (2020) analyzed the results, which showed germination boosted amaranth flour's nutritional value and functional characteristics, and autoclaving upgraded its physical characteristics and flowability at a superior level than other processing methods.The nutritional value of composite flours becomes better through employing alternative processing approaches. The germination of millet grains during 48 hours improves protein content, along with protein digestibility and total sugar amounts, and reduces phytate concentration. The process of roasting millet grains at 140°C leads to improved protein digestibility yet reduces the amounts of protein as well as phytate and total sugars (Tumwine *et al.,* 2018).

2.2.3 DRYING

According to Olawoye *et al.,* (2020), drying methods, together with other processing techniques, control the functional properties in addition to the nutritional value of different grains and vegetables. Research shows that different processing techniques affect the nutritional features and functional behavior of amaranth seeds, specifically, although tray drying technology does not get direct mention. The study establishes that various treatments produce changes to protein solubility and color aspects, and overall functional capability.

Radojčin *et al.,* (2021) establish that drying process selection depends entirely on the targeted product format along with production requirements. Current methods of hot air drying maintain their popularity, but researchers study ultrasound and pulsed electric field, and high-pressure technology to develop superior product qualities and enhance process efficiency. Rani *et al.*, (2018) studied the impact of milling, malting, fermentation, blanching, and acid and heat treatment methods on mineral digestibility rates, with success in delaying flour storage problems such as bitterness and off-flavors, and rancidity.

2.2.4 MILLING

The influence of milling remains inconsistent concerning various nutrients and cereal types according to Nisha *et al.,* (2018). Cereal milling operations result in major dietary fiber, along with minerals and vitamins, as well as polyphenols and phytosterol losses, because these nutrients naturally reside in the bran layer. The amount of lost nutrients depends on how much grain processing happens, as well as which type of grain is being processed. Different technological methods combined with processing techniques were developed to enhance the functional characteristics and biovitamin availability in processed milled agricultural products.

Luithui *et al.,* (2018) indicate that milling by-products possess several health benefits because they include dietary fiber and phytosterols, together with vitamins and polyphenols, and minerals. These by-products receive low valuation, leading to their application in animal feed. Different processing techniques exist to develop higher functional and food value by enhancing nutritional characteristics together with sensory properties, and removing inhibitory components. Mechanical, as well as enzymatic and thermal techniques, make up the entire processing method. The technique targets to enrich functional characteristics as well as boost the extractability of valuable food elements while simplifying bran structures and increasing solubility while decreasing inhibitory components, and enhancing micronutrient bio-accessibility.

In-vitro availability of food material components and their nutritional values change based on the Bhati *et al.,* (2016) prior milling procedure selection. The combination of milling with malting, along with fermentation and blanching and acid, and heat treatments led to optimal mineral digestibility and minimized storage-related off-flavor and bitterness, and rancidity issues in flour according to S, R *et al.,* (2024).

2.3 FUNCTIONAL PROPERTIES OF COMPOSITE FLOURS

Studies on composite flour function have mostly taken place throughout developing regions that purchase large wheat flour imports to satisfy consumer demand for bakery items and pastries. A review was conducted on the functional properties of composite flour, which include water and oil absorption capability as well as foam ability and emulsion capability, and least gelation concentration and particle size distribution, to assess its development capability for food products. The functional properties of composite flour proved beneficial for improving different food products while maintaining their appearance quality and sensory perception, and nutritional value at lower costs to serve consumer needs. Mamat *et al.,* (2020)

Mamat *et al.,* (2020) conducted a review of functional properties needed to judge composite flour suitability for food product development. Composite flour possesses functional features, including emulsion formation and the capability to absorb oil and water, together with the distribution of granules and its minimum gel-forming concentration. Composite flour helps expand food product ranges because it enhances product aesthetics through improved taste and nutritional benefits, with reduced manufacturing costs to meet consumer needs.

The assessment and development process of millets and grains and nuts, and legume composite flour received analysis from Kumar *et al.,* (2021). The researchers studied composite flour nutrition together with functional characteristics, showing that flour contains a direct relationship between crude fiber and its composition, as well as its moisture content. This demonstrated that the product is suitable for multiple food production settings. Different combinations of composite flour produce dissimilar results according to Erukainure *et al.,* (2016). The addition of bambara flour to wheat composites caused a decrease in dough consistency and weakening of protein, alongside starch gelatinization effects and amylase activity, and retrograded behavior.

The functional properties of composite flours represent fundamental criteria that determine their feasibility for food production applications according to Awolu *et al.,* (2017). Selection and blending different flours through response surface methodology methods help optimize these functional properties of composites, according to studies. The functional capabilities of composite flours generate opportunities to develop various food products with better nutritional characteristics and desirable sensory features at reduced prices, according to Anberbir *et al.,* (2024).

2.4. DEVELOPMENT AND FORMULATION OF COMPOSITE FLOURS

According to Ayele *et al.,* (2017), the creation and development of composite flours creates numerous advantages by delivering better nutrition values and improved functional elements and broadening product options. Bread manufacturers must choose proper blending ratios because they determine the final characteristics of their products. It was determined that 49.0 - 71.0% wheat and 10.6 - 29.0% cassava with 18.2 - 22.0% soybean flours produced the best blend for bread manufacture regarding nutritional value, alongside sensory results.

The development process for composite flours shows promise because it enables the enhancement of nutrition with improved properties that result in new food products, according to Merlino *et al.,* (2022). The combination of pulsed, tubers, and non-traditional flours in food development produces nutritiously enriched products that retain desirable sensory features.

The combination of iron-rich ingredients in composite flours has led to malnutrition treatments and improved food product nutritional qualities according to Akande *et al.,* (2017) and Ali *et al.,* (2024). The fight against this menace has been boosted through the development of nutritious food formulations derived from local crops. Established that various ingredients blended lead to dramatic improvements in iron content and total nutritional factors of mixed flour products.

Sibian & Riar (2020) blending these flours increased the food products' functional properties by enhancing their swelling capacity and oil absorption and water retention abilities and emulsion activities, and foam stability. Research optimized cookie formulations composed of wheat flour with various combinations of germinated kidney bean, chickpea, and wheat flours, which led to increased protein content as well as fats and crude fiber over ungerminated wheat flour cookies.

2.5. SENSORY EVALUATION OF COMPOSITE FLOUR PRODUCTS

Carvalho & Conti‑Silva (2017) discovered that blended flours improved certain aspects that consumers perceived with their senses. The acceptability of banana peel flour inside cereal bars met testing expectations, even though sensory perceptions shifted based on the flour amount added to the product. The development of consumer-satisfactory products requires sensory analysis according to Starowicz *et al.,* (2017). The evaluation of functional buckwheat-containing traditional recipes requires tests of appearance, along with aroma, taste, and texture examinations and overall quality assessments performed by trained panels. Aroma plays a vital role in consumer preference, while its sensory assessment benefits from analytical workflows that measure food volatile components and how these compounds affect aroma intensity.

Gao Yang *et al.,* (2019) used appearance and odor/aroma and flavor/taste and texture/mouthfeel, and any aftertaste as the categories to study the attributes. Principal component analysis confirmed that blended teas became distinguishable through artificial fruit additives. The study f indicated that consumers react positively to blended tea products designed as mildly sweet with fruit flavor and weak in bitterness, astringency, pungency, strength, and fermentation tone. The main issue observed is astringency. The consumers selected teas that presented low bitterness together with reduced astringency, which did not deposit powder on the tongue, since these features negatively impacted their liking.

According to Symmank *et al.,* (2018), taste and price, together with label identification, remain well-understood product features, while sound appearance, packaging texture, and smell are poorly studied in existing research. An unequal distribution of research methods exists between consumer and sensory research fields. Surveys/questionnaires, along with acceptance tests, are commonly used, while other combinations of research methods appear uncommon in food industry studies. The process of measuring consumer food evaluation through food liking methods proved to be the most commonly used practice. In line with growing consumer dependence on product attributes, marketing professionals and product developers should simultaneously evaluate the effects of product attributes with extrinsic characteristics.

CHAPTER 3

MATERIALS AND METHODS

The study entitled 'Development and Standardization of Nutritionally Enhanced Composite Flour Products: Evaluation of Nutritional, Functional, and Sensory Qualities' was conducted, and the methodology adopted is discussed in detail in the following section.

3.1 Selection And Collection Of Raw Materials

- 3.1.1 Collection Of Raw Materials
- 3.1.2 Preparation of composite flour
- 3.1.3 Formulation of composite flour combination

3.2 Development of Recipes from Flour Combinations

3.3 Organoleptic Evaluation

3.4 Analysis Of Nutritional Quality Of Composite Flour

3.5 Analysis Of Functional Properties Of Composite Flour

3.6 Microbial Assessment Of The Composite Flour

3.1. SELECTION AND COLLECTION OF INGREDIENTS

The composite flour production contains Wheat (Triticum aestivum), Amaranth seeds (Amaranthus hypochondriacus), pearl millet (Pennisetum glaucum), green gram (Vigna radiata), wheatgrass (Triticum aestivum), and Amaranth leaves (Amaranthus viridis). All these ingredients are collected from local markets. Selected the raw materials because of their nutritional benefits.

3.1.1. COLLECTION OF RAW INGREDIENTS

Wheat, Amaranth Seeds, Green Gram, Pearl Millet, and Amaranth Leaves are collected from local markets. The wheat grass was collected after it had sprouted from the wheat grains.

PLATE 1: INGREDIENTS FOR THE DEVELOPMENT OF COMPOSITE FLOUR

3.1.2. PREPARATION OF COMPOSITE FLOUR

The preparation of composite flour involves different methods, they are soaking, sprouting, and drying. Each raw material has a different soaking and sprouting time for the selected ingredients. In addition to the preparation time, the soaking and sprouting procedures impact the seeds and grains' nutritional profile. The antinutritional factor compounds, like tannins and phytic acid, will be reduced by the extended soaking and sprouting. This otherwise makes it more challenging for mineral absorption(Pagar et al. 2021).

1. **CLEANING**: A water-based cleaning method removes dust, together with unwanted particles and dirt from the raw materials.

2. **SOAKING**: The soaking step plays a crucial role because it makes materials more pliable and improves their nutrition while strengthening enzyme processes for breaking down phytic acid and tannins.

3. **SPROUTING**: After soaking grains, receive a dampened clean cloth treatment followed by placement in a dark environment for promoting germination. Proper airflow through the sprouting area remains necessary throughout the germination stage. Sprouting transforms seeds into more nutrient-rich food that allows people to obtain higher levels of essential minerals, antioxidants as well and vitamins from legumes. During germination, specific enzymes become activated to make the food simpler to digest.

4. **DRYING**: A tray dryer operates to perform the drying procedures. A stainless steel tray with properly dispersed grains and legumes requires overnight drying under set temperature ranges of 55℃ - 60℃ to preserve nutritional values. By bringing down moisture levels, you can achieve longer shelf stability as well as stop microbial development.

5. **MILLING**: After drying, milling treatment at the grinder by running at low speed, following the drying process. The food retains the most nutrients when the machine operates at low speeds because it creates minimal heat during the process.

TABLE 1: PREPARATION OF RAW MATERIALS

RAW MATERIALS	SOAKING TIME IN HOURS	SPROUTING TIME IN HOURS	DRYING TIME IN HOURS
Wheat	12	24 - 48	6
Amaranth seeds	6	24	6
Green gram	6	24	6
Pearl millet	8	24 - 48	6
Wheatgrass	12	10-12 days	6
Amaranth leaves	-	-	6


Green gram

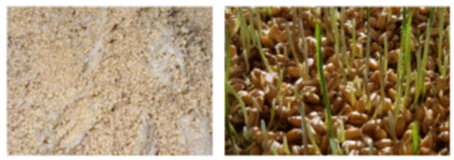

Pearl millet


Wheat


Amaranth seeds

PLATE 2: SOAKED GRAINS AND SEEDS FOR THE COMPOSITE FLOUR

Wheat Green gram Pearl millet



Amaranth seeds Wheatgrass

PLATE 3: SPROUTED GRAINS AND SEEDS FOR THE COMPOSITE FLOUR

Drying is facilitated by tray drying at 55℃ - 60℃ to ensure maximum nutrient retention. The thin layer drying kinetics of sprouted wheat utilizing a tray drying temperature between 50℃ and 80 ℃ revealed that lowered drying temperatures reduced moisture content and preserved the nutritional quality of the sprouts. This behavior under these circumstances was accurately predicted by the Wang and Singh model (Sharma *et al*.,2022).

PLATE 4: TRAY DRYER FOR DRYING

3.1.3. FORMULATION OF COMPOSITE FLOUR COMBINATION

Composite flour manufactured recently has not only focused on improving the functional properties of the final product but also on improving the nutritional composition. (Ubbor *et al.* 2014) Different combinations are necessary for the development of recipes.

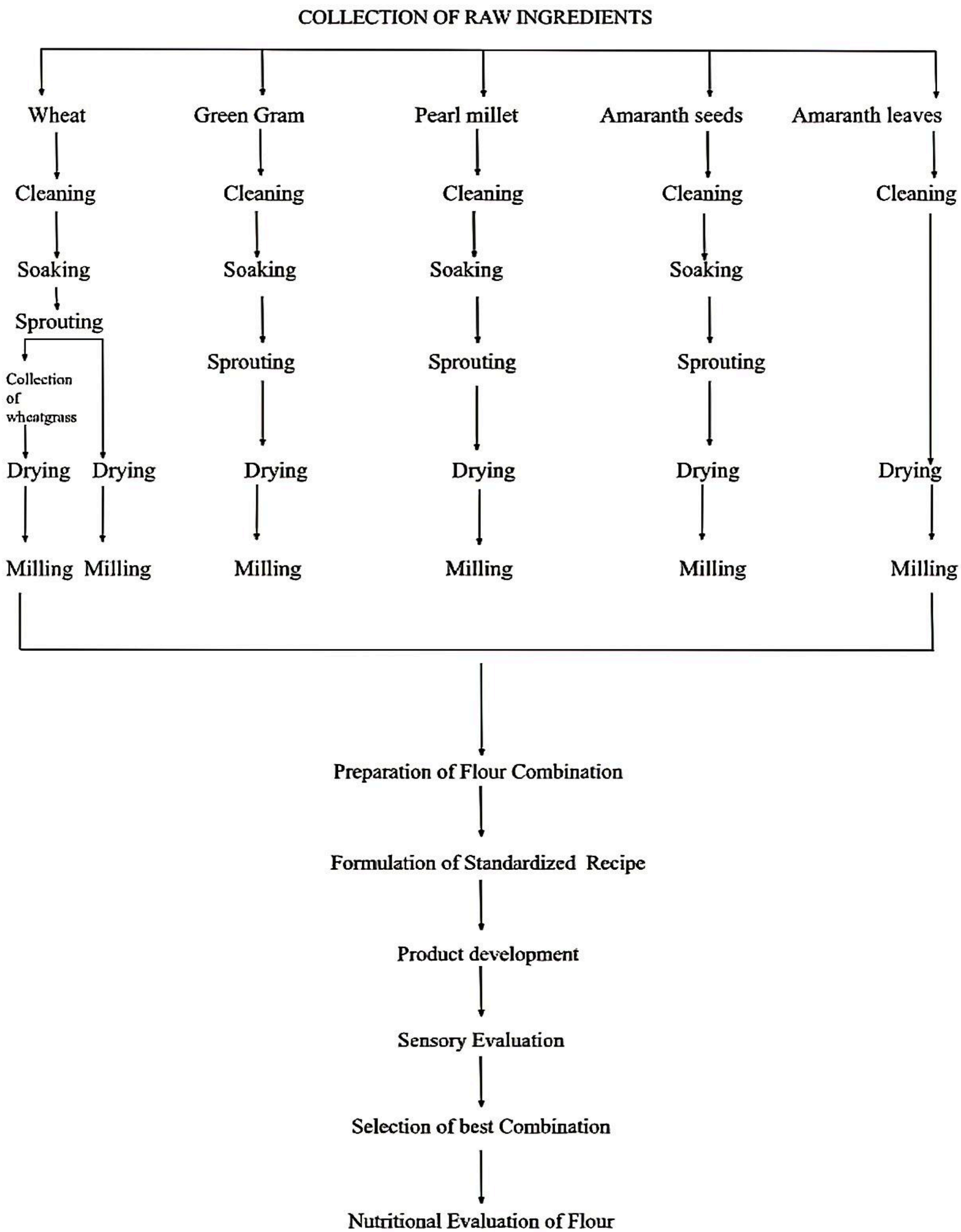


PLATE 5: FORMULATION OF COMPOSITE FLOUR

3.2. DEVELOPMENT OF RECIPES FROM THE COMBINATIONS

The formulated flour combinations of Wheat, Amaranth seeds, Green gram, Pearl millet (bajra), Amaranth leaves, and Wheatgrass powder under T1 (40:20:10:25:4:1), T2 (40:25:15:15:3:2), and T3 (40:10:25:20:2:3) were used to develop three different Energy balls, Cookies, and Chapati recipes. Each recipe was prepared with the use of different combinations of flour. A control is also included as T0, for adhering to the original recipes without the composite flour mix, which is the reference for the comparison. Multiple trials are essential for the systematic evaluation to develop composite flour recipes that are both nutritional and sensory appealing. (Deshpande *et al.* 2011)

3.2.1 RECIPES USING COMPOSITE FLOUR

The formulated flour combinations were used to develop three different recipes: Energy balls, Cookies, and Chapati, followed by three treatments T1 (40:20:10:25:4:1), T2 (40:25:15:15:3:2), and T3 (40:10:25:20:2:3), and a control is also included as T0, to adhere to the original recipes without the composite flour.

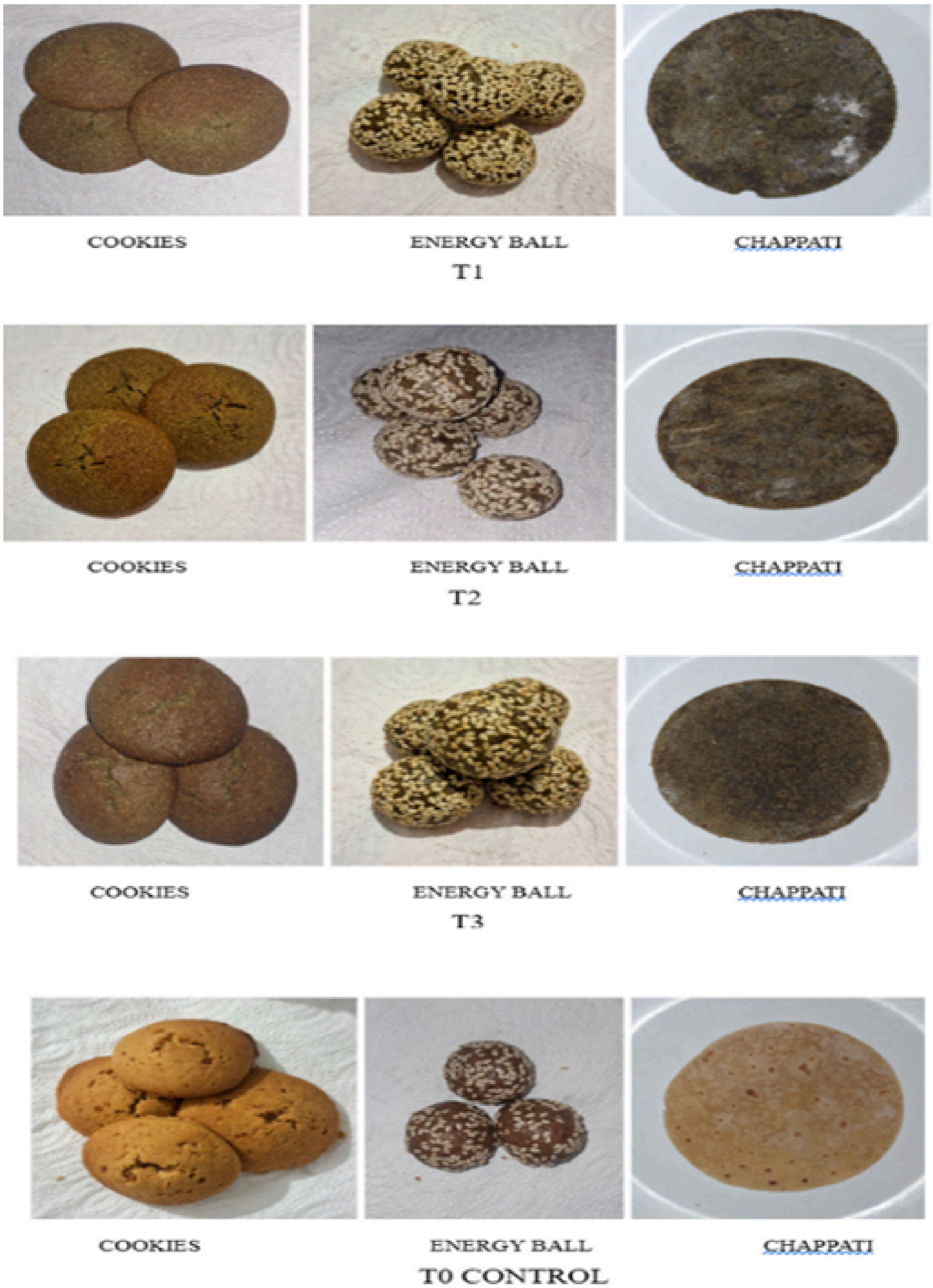


PLATE 6: DIFFERENT COMBINATIONS OF COMPOSITE FLOUR RECIPES

3.3. ORGANOLEPTIC EVALUATION

The sensory evaluation, also known as organoleptic evaluation, is essential for examining the quality and consumer appeal of food products developed from composite flour. Taste, texture, flavor, appearance, and overall acceptability are the general sensory attributes that are to be evaluated. Several studies have evaluated these sensory qualities in various composite flour-based products. (Pandit *et al.* 2020)

The evaluation of the composite flour's derived products was conducted using the hedonic scale method. The sensory attributes are taste, texture, flavor, appearance, and overall acceptability, were tested for the products by the selected panel judges.

3.3.1 SENSORY ATTRIBUTES

APPEARANCE: The appearance of any food is the most important feature, especially when it is linked to other aspects of food quality. Every raw food and manufactured product has an acceptable range of appearance that is determined by the factors associated with the consumer.

COLOUR: The eyes perceive the initial quality of food, and colour is one of the attributes to assess the desirability and acceptability of a food item, as well as the visual cues that can influence a person's decision. Simply changing the colour of a food can greatly improve its acceptability.

FLAVOUR: Humans can perceive, differentiate, and recognize odours. Stimulating odours and aromas additionally serve to quicken the appetite, even as off-aromas and odours assist purchasers to understand dangers, for example, in spoiled foods. The distinct stimuli (the totality of all gustatory, olfactory, haptic, and trigeminal stimuli) perceived through the tongue and within the oral hollow space for the duration of ingesting account for an enormously small part of the flavour.

TASTE: Our taste sensations start when taste stimuli connect with taste receptors located throughout the taste buds present on the tongue. Science shows that humans can identify at least five or six fundamental taste categories, which include sweet, sour, salty, bitter, and the distinctiveness of umami and fatty flavors. There exist twenty to thirty separate strength levels that define each taste perception.

TEXTURE: The texture is a primary attribute in the process of sensory evaluation. Evaluation of texture is a complex, dynamic method that contains visible notions of the product surface, product conduct in reaction to preceding handling, and integration of in-mouth sensations skilled for the duration of mastication and similarly swallowing. The human mind compiles all of these, and a unique sensation is constructed.

OVERALL ACCEPTABILITY: Overall acceptability of a food product can be evaluated through the appearance, colour, texture, and taste of the product.

The 9-point hedonic scale score was used, with 1 representing dislike and 9 representing like extremely. To rate each sensory attribute, the 10-panel judges were provided with the scorecards. Based on the scores of the panel judges, find the best formulation. The formulation with the highest score was selected for further nutritional analysis and shelf life studies.

PLATE 7: ORGANOLEPTIC EVALUATION

3.4. ANALYSIS OF NUTRITIONAL QUALITY AND OXIDATIVE STABILITY OF COMPOSITE FLOUR

The evaluation of both nutritional qualities and oxidative stability in the composite flour determined its market readiness and storage stability. The analytical study of nutritional elements in this flour included testing macronutrients, including carbohydrates and proteins, and crude fiber, while also determining micronutrient iron, because these essential nutrients address wellness deficiencies. The obtained values provide essential information about the complete dietary effects of the developed flour. The assessment of oxidative stability evaluated how strongly the flour resists lipid oxidation while undergoing storage, because this determines product freshness characteristics along with flavor and safety dimensions. The identification of peroxide value and free fatty acid content serves as assessment parameters to determine this measurement. A high-quality functional flour product needs both adequate nutritional content and stable oxidative properties for maintaining long-term value.

TABLE 2: METHODS ADOPTED FOR NUTRIENT ANALYSIS

PARAMETERS	UNIT	TEST METHOD
Moisture	%	AOAC 21st Edn. 2019; 925.10
Carbohydrate	g/100 g	AOAC 21st Edn. 2019; 986.25;ch.50
Protein	g/100 g	AOAC 21st Edn. 2019; 920.87;ch.32
Crude fiber	%	AOAC 21st Edn. 2019; 962.09
Peroxide value	meq/kg	AOAC 21st Edn. 2019; 965.33
Calcium	mg/100g	AOAC 21st Edn. 2019; 984.27
Iron	mg/100g	AOAC 21st Edn. 2019;944.02

3.5. ANALYSIS OF FUNCTIONAL PROPERTIES OF COMPOSITE FLOUR

The existing functional properties, including water and oil absorption and foam production, and emulsion handling, as well as least gelation level and particle size analysis, demonstrate how processed flour functions for food development. The functional attributes of food materials depend on their molecular clasp, along with physical design and constituents combined with their environmental stimuli processing, according to Hasmadi *et al.* (2019).The Functional properties, such as solubility, swelling power, water absorption capacity, oil absorption capacity, tapped density, and bulk density, were estimated.

3.5.1 SWELLING POWER

The swelling capacity examination for composite flour required the measurement of 10 grams of flour, followed by its addition to a calibrated cylinder with either 50 or 100 mL capacity. The mixture received 50 mL of distilled water in a cylinder filled with flour while gentle mixing ensured equal dispersion. A one-hour period of undisturbed setting at room temperature allowed the flour particles to achieve total hydration and swelling inside the cylinder. The research team measured the final volume that the swollen flour achieved after this time had passed. The researchers determined the swelling capacity in mL/g through this mathematical formula:

$$\text{Swelling power (\%)} = \frac{(\text{Final Volume of Swollen Flour} - \text{Initial Volume of Water})}{\text{Mass of Sample (g)}} \times 100$$

The measurement of swelling power determines the quantity of starch granules capable of absorbing water for swelling. The swelling power affects flour-based product textural properties, including thickening and gel formation. The gel stability and viscosity of puddings and porridges depend on these products, which makes a high swelling power beneficial for these foods. The swelling power depends on three factors: starch structure, as well as processing history, such as drying and sprouting, in addition to the amylose to amylopectin ratio.

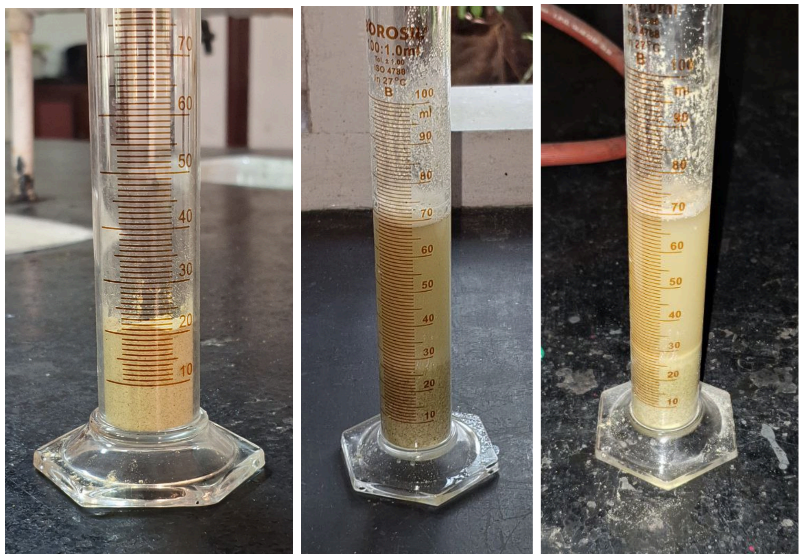


PLATE 8: SWELLING POWER OF COMPOSITE FLOUR

3.5.2. SOLUBILITY

The method for determining composite flour solubility required adding one gram of sample to a centrifuge test tube and mixing it with distilled water at 80 ℃ before performing centrifugation at 3000 rpm for 15 minutes. The research team moved supernatant material into a pre-donged Petri dish for weighing. The supernatant was evaporated for 2 hours at 130℃ to ensure that all moisture was removed. Then weighed again the dish and the remaining residue represented the amount of sample dissolved in water. The formula used for calculating solubility is

$$\text{Solubility (\%)} = \frac{\text{The weight of the dried sample in the supernatant}}{\text{Weight of original sample}} \times 100$$

The ability of flour particles to dissolve in water defines the solubility property. The measurement helps explain how starch molecules interact with water molecules to understand how flour particles distribute in solution. A desirable property for porridge and instant mixes is high solubility.

PLATE 9: SOLUBILITY OF COMPOSITE FLOUR

3.5.3. WATER ABSORPTION CAPACITY (WAC)

The analysis of composite flour water absorption required measuring 1 gram of sample before placing it into a test tube that had been weighed beforehand. The mixture received 10 mL of distilled water while the solution was stirred homogeneously to promote proper hydration. The specimen underwent one hour of sitting time without disturbance to reach full water uptake. The mixture underwent centrifugation at 3500 rpm for 30 minutes to obtain separation between unabsorbed water and the absorbed part. After centrifugation, the test tube was examined for excess water, which researchers carefully poured down the drain while the tube was positioned upside down over absorbent paper for additional drainage. The researchers determined the amount of water intake through a second weight measurement of the test tube. The weight measurement for absorbed water calculations started by deducting the test tube and sample initial weight from the final weight after water absorption. The mean water absorption values resulted from three completions of the procedure to confirm measurement precision.

$$\text{Water Absorption Capactity (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

Where:

- W_1 = Weight of sample

- W_2 = Weight of test tube + sample before hydration

- The weight of the test tube, together with the sample along absorbed water that drained off, would be represented by W_3.

A flour demonstrates its water absorption properties through its absorption capacity. The ability to absorb and retain water functions as a vital quality factor that shapes dough and batter consistency, together with viscosity. The product's better ability to absorb moisture increases its hydration properties, thus supporting its uses for porridge and weaning meals, and baked food products. The presence of hydrophilic elements, including proteins as well as starches and dietary fiber, can be detected via this property. The water absorption ability of composite flours having considerable legume and pseudo-cereals content stems from their proteins and fibers.

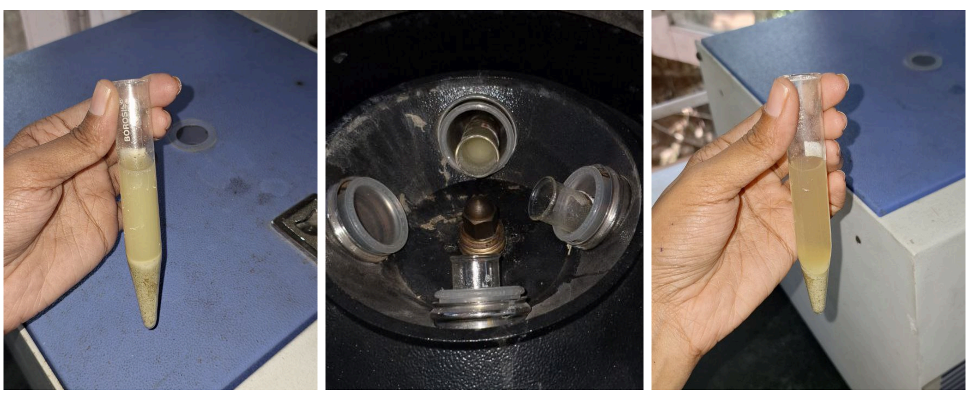


PLATE 10: WATER ABSORPTION CAPACITY OF COMPOSITE FLOUR

3.5.4. OIL ABSORPTION CAPACITY (OAC)

The method of centrifugation measured the oil absorption capacity of composite flour. A measurement of one gram of sample went into a prepared test tube, which had previously been weighed. The bucket received 10 mL of oil distribution following a mixed procedure for uniform distribution. One hour of standing time enabled the absorption process to complete effectively. The tube received 3500 rpm centrifugation treatment from a Spectra Scientific centrifuge for thirty minutes before unabsorbed oil separation. The test tube received a thorough initial weight measurement before receiving 10 mL of oil solution, which was mixed until complete distribution, after which separation of unabsorbed oil occurred through inversion above absorbent paper. The procedure required reading the final test tube weight to calculate the absorbed oil quantity through weight differences between the test tube, the sample, and the absorbate. The researchers experimented three times, which produced an average oil absorption capacity value for their evaluation.

$$\text{OAC (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

Where:

- W_1 = Weight of the sample
- W_2 = Weight of the test tube + sample before oil absorption
- The weights of the test tubes, samples, and absorbed oil (after draining) are calculated using value W_3.

This method determines how much flour can absorb lipids. The retention of flavor and mouthfeel depends on this property because fat is central to sensory experiences in cookies and energy balls. The food becomes denser in calories and develops its texture because of the starch interaction.

PLATE 11: OIL ABSORPTION CAPACITY OF COMPOSITE FLOUR

3.5.5. TAPPED DENSITY

To determine the tapped density of the composite flour, A 100 ml or 50 ml capacity graduated measuring cylinder was filled with the sample. Without tapping the record, the initial volume, then tap multiple times manually on a hard surface to find the tapped density of the sample. After tapping, the reduction is observed, and the final volume is recorded. The formula used for calculating the tapped density is

$$\text{Tapped density (g/ml)} = \frac{\text{Weight of sample (g)}}{\text{Tapped volume of sample (ml)}}$$

When we tap compacted flour, we measure its mass per unit volume under this measurement method, which is known as tapped density. The amount of storage space flour requires when settled down is determined by calculating tapped density for packaging purposes. Better storage efficiency indicates a higher tapping density.

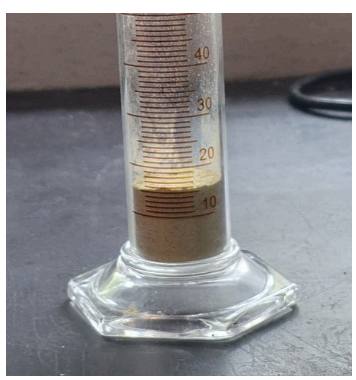


PLATE 12: TAPPED DENSITY OF COMPOSITE FLOUR

3.5.6. BULK DENSITY

A 100 ml or 50 ml capacity graduated measuring cylinder was filled with the sample. Without tapping the value was recorded and that is the bulk volume. The formula used for calculating bulk density was.

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of sample (g)}}{\text{Bulk volume of sample (ml)}}$$

Before tapping the test flour obtains its mass-to-volume ratio, which measures its bulk density. The proper use of this principle guides the development of packaging materials and processing systems, and transportation containers.A composite flour with low bulk density is ideal for the production of ready-to-eat snacks.

PLATE 13: BULK DENSITY OF COMPOSITE FLOUR

3.5.7. FOAMING CAPACITY

To determine the foaming capacity of the composite flour, a ten-gram sample was weighed and transferred to the calibrated 100 ml measuring cylinder, mixed with 50 ml of distilled water. Shake the cylinder for five minutes to create foam and incorporate air. The created foam volume is measured, and the initial form height. The formula used for calculating foaming capacity is

$$\text{Foaming capacity (\%)} = \frac{\text{Foam Volume}}{\text{Initial foam volume}} \times 100$$

A stable foam emerges from whipping or agitating flour proteins that possess the capability to trap air particles. This process is known as foaming capacity. Products that need aeration depend heavily on good foaming capacity, such as pancakes and cakes, and snack bars. Flour proteins that produce good foaming capacity help create both superior volume and lightness while building attractive texture.

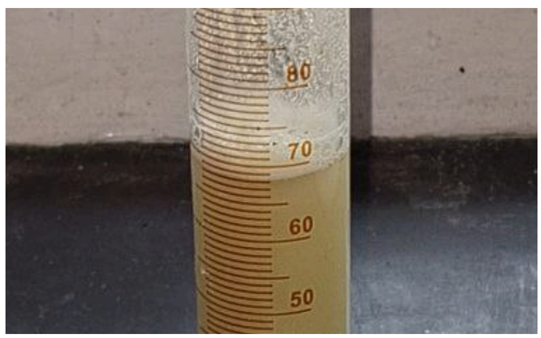


PLATE 14: FOAMING CAPACITY OF COMPOSITE FLOUR

3.6. MICROBIAL ASSESSMENT OF THE FLOUR

Food industry professionals need microbial enumeration methods as a primary tool to measure microbial populations in food products. The analysis enables food manufacturers to check both quality standards and product storage duration. Quality assessment of composite flour included microbial load examination during a 30-day storage period.

Serial dilution consists of progressively conducting dilution steps toward a substance or microbial sample through a series of liquid media or buffer solutions. The experiment requires this technique to lower substance concentrations, including microorganisms or solutions, into convenient quantities for experimental and analytical applications.

Microorganisms normally form multiple cell arrangements, such as chains, in addition, some of the bacteria may be clumped together. Therefore, when doing the plate count technique, we generally determine the number of Colony-Forming Units (CFUs) in that known dilution. The colony-forming unit may consist of a chain of bacteria rather than a single bacterium. For optimum accuracy of a count, the preferred range for total CFU/plate is between 30 to 300 colonies/plate.

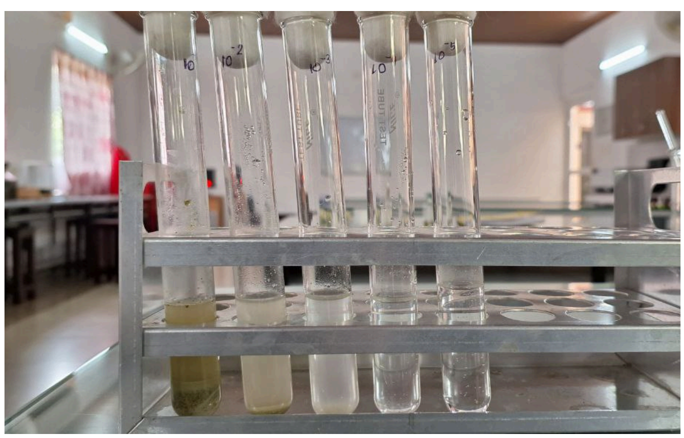


PLATE 15: SERIAL DILUTION

For the microbial assessment, agar plates were prepared according to the microbial type. Nutrient agar and nutrient broth were used for the culture media. The spread plate method was used to determine the microbial growth in the composite flour. 1 g of the composite flour sample is dissolved in 10 ml of distilled water. Prepare 9 ml of distilled water to fill five separate test tubes before covering them correctly with cotton plugs. The test tubes need autoclaving at 121℃ temperature with 15 lbw pressure for 15 to 20 minutes to accomplish their sterilization. At room temperature, allow the test tube with water to reach a stable temperature after the sterilization process. 1 ml of the sample is pipetted out (or I g sample) and transferred to a test tube with 9 ml of distilled water and labeled, and mixed well. Transfer 1 ml of sample from the first test tube to the second test tube, mix it well, label it as 10^-2, and continue the process. A 0.1 ml sample was pipetted out onto the agar surface and spread evenly with the use of a L-shaped glass rod to ensure uniform distribution. Incubate the plates at 37℃ for 24 hours. At the end of the incubation period, select all of the Petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated 100 many to count. Plates with fewer than 30 colonies are designated as too few to count. Count the colonies on each plate.

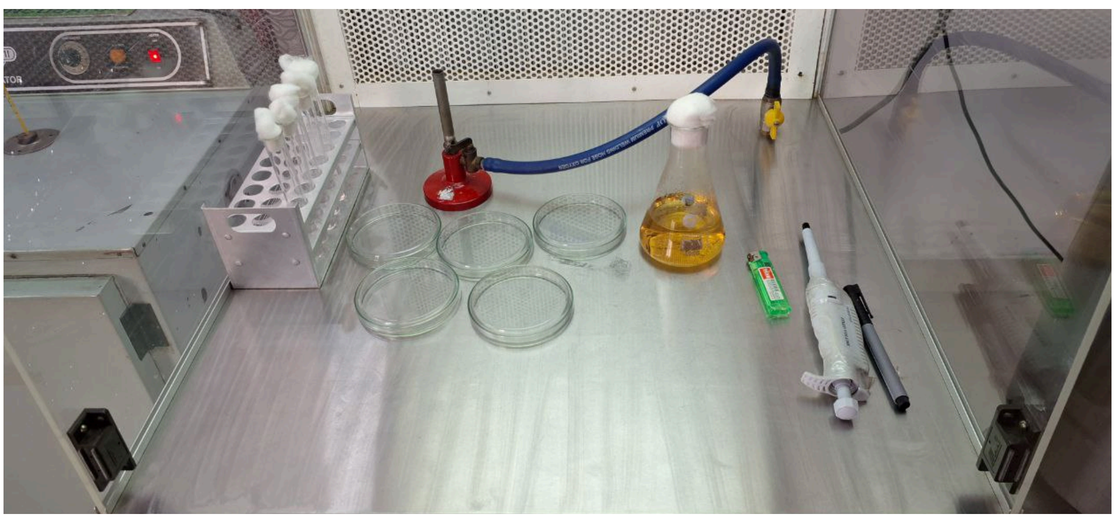


PLATE 16: LAMINAR AIRFLOW CHAMBER

PLATE 17: INCUBATION FOR MICROBIAL ANALYSIS OF COMPOSITE FLOUR

The microbial load was expressed as colony-forming units per gram (CFU/g). Using the formula:

$$\text{CFU/g} = \frac{\text{Number of the colonies x Dilution factor}}{\text{Volume plated (mL)}}$$

The standardized microbial assessment provides a reliable means to monitor the stability of microbes within the composite flour throughout storage duration. Standardized microbial assessment methodology generates vital information to measure shelf life duration, together with the detection of possible contaminant risks while guaranteeing safety and quality standards for foods meant for human consumption. Microbial enumeration results serve as a tool to assess the effectiveness of flour processing as well as storage methods evaluated in this study.

CHAPTER 4

RESULTS AND DISCUSSION

The present study, 'Development and Standardization of Nutritionally Enhanced Composite Flour Products: Evaluation of Nutritional, Functional, and Sensory Qualities,' demonstrates details about developing and standardizing nutritionally enriched composite flour products with nutritional and functional assessments and sensory evaluation. A nutritionally balanced composite flour contained wheat, along with amaranth seeds, green gram, pearl millet, amaranth leaves, as well as wheatgrass powder. Traditional and functional processing methods combined with soaking and sprouting, followed by drying and milling, were used to improve the nutritional content and accessibility of nutrients from the ingredients. This study implemented these processing methods to maximize both functional value and nutritional outcomes of the flour. The study examined the nutritional composition, functional attributes, microbial safety, and sensory characteristics of the developed products from the composite flour. The analysis, together with its interpretation of these findings, appears under different sub-sections of the discussion:

4.1 Formulation of Composite Flour Combinations

4.2 Nutritional and Functional Analysis of Composite Flour

4.2.1 Analysis of Macronutrient Content on Composite Flour

4.2.2 Analysis of Micronutrient Content on Composite Flour

4.2.3 Moisture Content And Oxidative Stability of Composite Flour

4.2.4 Functional Analysis Of Composite Flour

4.3 Quality Evaluation of Composite Flour

4.4 Total Microbial Content of Composite Flour

4.1. FORMULATION OF COMPOSITE FLOUR COMBINATIONS

The different composite flour combinations were used for the development of three different recipes: energy ball, cookies, and Chapati. Each recipe was prepared with the use of different combinations of flour, such as Wheat, Amaranth seeds, Green gram, Pearl millet (bajra), Amaranth leaves, and Wheatgrass powder under T1 (40:20:10:25:4:1), T2 (40:25:15:15:3:2), and T3 (40:10:25:20:2:3). Composite flour manufactured recently has not only focused on improving the functional properties of the final product but also on improving the nutritional composition. (Ubbor *et al.,* 2014) Different combinations are necessary for the development of recipes.Following the organoleptic evaluation, the best combination was found from the recipes with different combinations for further analysis.

TABLE 3: COMBINATIONS OF THE COMPOSITE FLOUR

INGREDIENTS	T1 (g)	T2 (g)	T3 (g)
Wheat	40	40	40
Amaranth seeds	20	25	10
Green gram	10	15	25
Pearl millet (bajra)	25	15	20
Amaranth leaves	4	3	2
Wheatgrass powder	1	2	3
Total (g)	**100**	**100**	**100**

Organoleptic evaluation of energy ball, cookies and chapati products indicated T2 as the best formulation choice. Evaluated samples from T2 received better results regarding sensory characteristics, which led to superior overall perception when compared to T1 and T3. The balanced nutritional considerations combined with acceptable sensory attributes of T2 formulation led to its selection for nutritional, functional, and microbial evaluations.

4.2. NUTRITIONAL AND FUNCTIONAL ANALYSIS OF COMPOSITE FLOUR

The nutrient analysis of composite flour was facilitated at Kerala Agriculture University, Trivandrum, and Food Quality Lab, Ernakulam.The analysis of macronutrients and micronutrients provides insights into the nutritional adequacy of the formulated composite flour. Evaluation will reveal the composite flour potential, which can assist body development and maintenance, helping fix dietary deficiencies and upgrade the basic food quality.

4.2.1. ANALYSIS OF MACRONUTRIENT CONTENT IN COMPOSITE FLOUR

The blending process of different flour types in composite flours creates significant changes in nutritional content when compared to traditionally processed single-source flours. The carbohydrate, protein, crude fiber content in composite flours are determined by both ingredient selection along the proportions in which these ingredients are combined.Study results demonstrated that the best combination of finger millet, orange-fleshed sweet potato, and soybean flour contained 64.27% carbohydrates with 16% protein and improved fiber concentration (Ali *et al.,* 2024). Research on germinated kidney bean, chickpea, and wheat composite flour revealed that optimized cookies contained 12.32% protein content and 5.64% crude fiber (Sibian & Riar, 2020). The baobab pulp flour incorporation into wheat flour led to elevated crude fiber levels of the composite flour without significant effects on protein distribution (Barakat, 2021).

Le Couteur *et al.,* (2015) in a previous study on diet-related aging effects has traditionally examined the effects of altering total calorie consumption along with individual changes to macronutrient content. The Geometric Framework serves as a nutritional geometric approach to explain age-related effects across different dietary conditions that consist of orthogonal macronutrient and total energy formulations. Scientific research involving free food access uses ad libitum protocols to analyze compensatory food intake behavior, which emerges naturally when animals are unconstrained. Geometric Framework research with insects and mice demonstrates that ad libitum feeding with protein-restricted diets enriched in carbohydrates produces the most extended mean lifespan in these animals, and caloric restriction through dietary dilution does not show significant effects.

TABLE 4: MACRONUTRIENT CONTENT OF COMPOSITE FLOUR (Per 100g)

PARAMETERS	NUTRITIONAL COMPOSITION
Carbohydrates (g)	70
Protein (g)	17
Crude fiber (g)	5.8

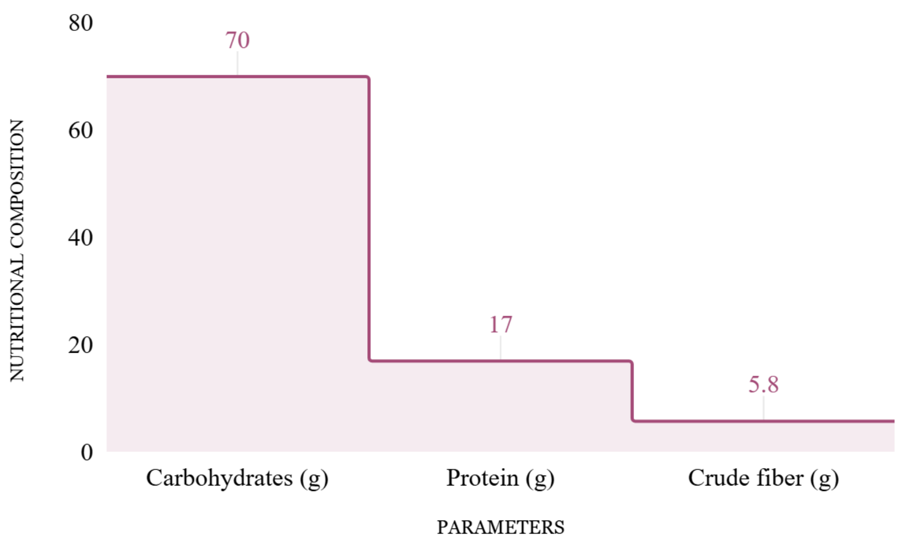


FIGURE 1: MACRONUTRIENT COMPOSITION OF COMPOSITE FLOUR (Per 100g)

70g of carbohydrate from the flour provides adequate energy.It has been suggested by Effiong *et al*. (2018); that the energy content of composite flours can be in the range of 358.50 to 364.53 Kcal/ 100 g. This implies that the present formulation of composite flour offers a well-balanced energy source that is suitable for daily consumption. The carbohydrate content of the developed composite flour depends heavily on wheat (*Triticum aestivum*) as well as both pearl millet

(*Pennisetum glaucum*) and green gram (*Vigna radiata*), which are staple grains and legumes that contain starch together with complex carbohydrates.

This combination of composite flour has a high concentration of protein (17g per 100g), which is indicated as an excellent plant-based protein source. Typically, wheat flour contains only 10.24% protein (Verem *et al.,* 2021). The composite flour increased its protein content by adding green gram and amaranth seeds (*Amaranthus hypochondriacus*) and legumes because these plant resources contain advanced amino acid compositions.

5.8g of crude fiber in 100g, which aids in reducing blood sugar and promotes digestive health. According to the studies that show crude fiber content of the composite flour ranges from 3.96 % to 7.43% (Dada *et al*., 2023). The indigestible plant substance found in crude fiber provides benefits to both digestion and feelings of fullness in addition to promoting regular bowel movements. This composite flour contains significant fiber content primarily because it includes both amaranth leaves (*Amaranthus spp.*) and wheatgrass (*Triticum aestivum* young shoots), which make excellent dietary sources and deliver functional bioactive compounds.

4.2.2. ANALYSIS OF MICRONUTRIENT CONTENT IN COMPOSITE FLOUR

Composite flours enhanced levels of micronutrients when compared to single-grain flours, particularly in terms of vitamins and minerals. Mohanty *et al.,* (2016) found that micronutrients, including vitamins and minerals, are necessary for health development and growth since they exist in small amounts. Over two billion people worldwide suffer from micronutrient deficiencies that create major health problems and pave the way for an array of lifestyle diseases.

TABLE 5: MINERAL COMPOSITION OF COMPOSITE FLOUR (Per 100g)

PARAMETERS	NUTRITIONAL COMPOSITION
Calcium (mg)	191.32
Iron (mg)	6.55

The calcium content was found to be 191.32 mg/100g, indicating a significant contribution to daily intake. In contrast, several composite flour calcium levels have been reported to range between 48.97 to 105.97 mg per 100g (Adebayo *et al*., 2024). The sources of calcium in this composite flour play crucial roles in muscle function and bone health, as well as enzyme activity. The evaluation indicated that the iron content in the flour amounted to 6.55 mg per 100g. The composite flour comprises different grains and legumes and offers a potential for an enhanced nutritional profile. According to previous studies, these types of composite flour blends can enhance the nutritional value and availability of minerals (Banua *et al.,* 2021).

The nutrient composition of the developed composite flour exceeded levels found in refined wheat flour (maida) or whole wheat flour. The micronutrient content of the composite flour revealed 6.55 mg of iron and 191.32 mg of calcium per 100g. Traditional mono flours like refined wheat flour contain between 2.51 to 3.35 mg of iron and 38.1 mg of calcium for each 100g serving according to Fernández-Canto *et al.,* (2023). Each 100 grams of whole wheat flour contains 2.95 to 4.15 mg of iron and 73.0 mg of calcium (Heshe *et al.,* 2016).

The composite flour receives its improved nutrient content through the incorporation of iron- and calcium-rich elements, including amaranth seeds and leaves and green gram and pearl millet, and wheatgrass powder. Evaluation results indicate that flour use serves as a protective factor against iron deficiency and calcium insufficiency in vulnerable individuals who lack essential nutrients in their diet. Standard flours fall short of composite flour, making it an advantageous staple food component and nutrient-rich dietary intervention since it delivers two outstanding health benefits at once.

4.2.3. ANALYSIS OF MOISTURE CONTENT AND OXIDATIVE STABILITY OF COMPOSITE FLOUR

The quality and shelf-life reliability of composite flours depend heavily on their moisture content and oxidative stability. The processing, along with storage operations of multiple flour blends, undergo significant changes based on their moisture content according to Carter *et al.,* (2015) and Nainggolan *et al.,* (2023). The production of wheat flour depends on water activity measurements rather than moisture content measurements because water activity provides better stability assessment through its effects on microbial growth and chemical stability, according to Carter *et al.,* (2015). The moisture content of cassava flour was within 6.22% and 11.07% due to significant effects of drying temperature combined with time duration (Nainggolan *et al.,* 2023). The evaluation of moistness and stability against oxidation within composite flours serves as an essential method to ensure product quality and extended shelf life. Different ingredients used in composite flour preparation, which include yellow pumpkin powder or baobab pulp flour, will impact both moisture levels and stability against oxidation (Aljahani, 2022; Barakat, 2021).

TABLE 6: MOISTURE CONTENT AND OXIDATIVE STABILITY OF COMPOSITE FLOUR

PARAMETERS	COMPOSITION
Moisture content	7.5 %
Oxidative stability	0.21 meq/kg

The shelf stability and susceptibility to rancidity are assessed by the moisture content and peroxide value. The moisture content is about 7.5%, the composite flour is within the acceptable range for storage. Moisture content below 10% helps to prevent microbial growth and inhibit enzyme reactions. Chandra *et al.* (2014) indicated that the moisture content in his product ranges considerably from 10.93% to 13.28% and is stable for storage. Hence, the moisture level of composite flour that is the range of 7.5% is within the acceptable range.

The peroxide value measurement of 0.21 meq/kg demonstrated minimal oxidation of lipids in the flour. According to experts, the peroxide value indicates how fresh a fat remains when values stay under 1.0 meq/kg, suggesting stable fat with minimal deterioration. The low peroxide value indicates that fat components found especially in seeds and legumes of the composite flour experience minimal oxidation, resulting in extended shelf life alongside nutritional preservation.Tangariya *et al.* (2018) found that increased peroxide value after about 60 days of storage will result in the development of rancidity. Storage at high temperatures, heat, and light are key factors for further acceleration in oxidative rancidity.

4.2.3. FUNCTIONAL ANALYSIS OF COMPOSITE FLOUR

Composition flours made from combining wheat flour with different ingredients show multiple functional characteristics, which determine their utility in food product creation. The functional properties of these combinations include water and oil capture potential, together with foaming abilities and emulsification power, in addition to their minimum gelation threshold and their distribution of particle sizes (Mamat *et al.,* 2020). It has been demonstrated through research that various flour combinations with wheat flour strengthen the functional traits of created composites.Non-wheat flours create improvements along with drawbacks, which affect functional properties when added to wheat flours. An increasing level of bambara flour within wheat flour led to the reduction of measurements for dough consistency and protein weakening, together with starch gelatinization and amylase activity, and retrogradation parameters (Erukainure *et al.,* 2016).

TABLE 7: FUNCTIONAL ANALYSIS OF COMPOSITE FLOUR

FUNCTIONAL PROPERTY	PROXIMATE VALUE
Water absorption capacity (%)	77
Oil absorption capacity (%)	77.9
Bulk density (g/ml)	21
Tapped density (g/ml)	0.94
Swelling property (%)	60
Foam capacity (%)	30
Foam stability (%)	66

The functional characteristics of the composite flour show excellent promise to enrich food applications across health-oriented compositions and baked goods. The developed composite flour demonstrated water absorption capacity of 77% and oil absorption capacity of 77.9% when measured. Research indicates that the functional values exceed 77.7% and 77.9% while surpassing traditional flour types, including wheat flour and rice flour, with normal ranges between 50-60%. The high-protein and high-fiber content of amaranth and green gram, and wheatgrass gives this flour mix its improved ability to bind moisture and fat. Research analysts studied different composite flour formulations through measurements of their oil absorption capacity (OAC). The standard wheat flour demonstrated the maximum oil absorption capacity at 2.47 g oil/g flour but composite flour variants displayed decreased capacities (Menon *et al.,* 2015)

The flour demonstrates good flow potential and handling qualities due to its compact density characteristics of 21 g/ml and tapped density level of 0.94 g/ml. The high bulk density measurement may contain a unit error (g/cm^3 or g/ml values remain between 0.5–1.0), yet it represents a denser product which can optimize packing space and boost storage capacity.The flour displays 60 % swelling capacity, which demonstrates its capability to absorb water before expansion. This feature contributes to better dough volume production and product consistency. Baked or steamed products benefit from this flour since its swelling capacity (60%) exceeds that of typical standard wheat flour (20–30%).

The foaming capacity level of 30% proves that the flour can excel in foods requiring air incorporation, such as muffins and fritters, and cake batter applications. The protein content in sprouted green gram and amaranth helps create and stabilize foam due to their composition.The solubility index measures 6.93%, showing that the flour displays good water-absorption properties yet shows limited solubility. Receiving structured items such as cookies or chapatis benefits from this characteristic since the flour maintains its structure while avoiding rapid breakdown, which preserves texture consistency.

4.3. QUALITY EVALUATION OF COMPOSITE FLOUR

The quality of a food product represents the sum of all properties and attributes of a food item that are acceptable to the customer, and it is an important factor in the successful development of a food product. The quality characteristics of food include external factors such as appearance (size, shape, color, gloss, and consistency), texture, and flavor; and internal factors (chemical, physical, and microbial). The best proportion of composite was selected through sensory evaluation and analyzed to learn the sensorial, nutritional, physicochemical, and microbial attributes.

To determine the acceptability, a sensory evaluation of composite flour products such as cookies, chapati, and nutriballs was carried out. The T0 is the control (original recipe) that scored highest on every attribute.

4.3.1 SENSORY EVALUATION OF DEVELOPED PRODUCTS FROM COMPOSITE FLOUR

Product development benefits significantly from sensory analysis because this method helps companies understand how their products perform in the market according to consumer acceptance. Sensory evaluation examines vital qualities which include appearance, together with taste and aroma, and texture characteristics, and overall acceptability, which facilitates the selection of the most ideal formulation for further development. Sensory evaluation scores are the mean rank scores of 10 judges who were selected to evaluate the developed products. The different parameters, like appearance, flavor, texture, taste, color, and overall acceptability for composite flour, were scored by a panel of judges using a 9-point hedonic rating scale. The maximum score that could be attained for each attribute was 9.

TABLE 8: ORGANOLEPTIC EVALUATION OF COOKIES DEVELOPED WITH COMPOSITE FLOUR

Formulation	Appearance ($\bar{X}$ ± SD)	Flavour ($\bar{X}$ ± SD)	Texture ($\bar{X}$ ± SD)	Taste ($\bar{X}$ ± SD)	Overall Acceptability ($\bar{X}$ ± SD)
T1	7.4 ± 1.6	7.8 ± 1.5	7.4 ± 1.5	7.8 ± 1.4	8.0 ± 1.2
T2	7.2 ± 1.7	7.2 ± 1.6	7.0 ± 1.7	7.2 ± 1.7	7.4 ± 1.6
T3	6.8 ± 1.8	6.6 ± 1.7	6.8 ± 1.8	6.8 ± 1.7	6.8 ± 1.6
T0	8.2 ± 1.2	8.2 ± 1.2	8.2 ± 1.2	8.2 ± 1.2	8.4 ± 1.1

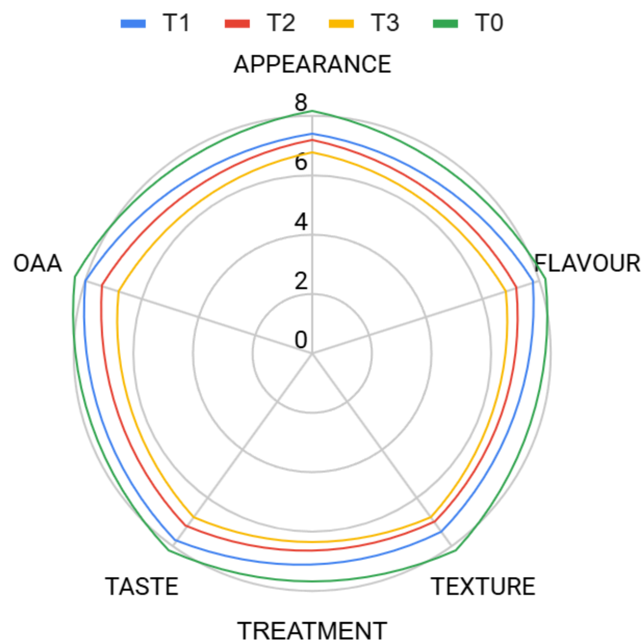


FIGURE 3: ORGANOLEPTIC EVALUATION OF COOKIES MADE WITH COMPOSITE FLOUR

The sensory evaluation used a 9-point hedonic rating scale to assess appearance, together with colour appearance and flavor, taste, texture, and overall acceptability of cookies made with composite flour mixtures (T1, T2, T3) and the standard control (T0). The evaluation is to understand consumers' desires and how products should appeal to them.

Appearance: People use appearance as their initial quality indicator to determine the evaluation of food products. The visual quality of cookies containing T1 (7.4 ± 1.6) surpassed that of T2 (7.2 ± 1.7) and T3 (6.8 ± 1.8), possibly due to better attractiveness. The control cookie located at T0 obtained the highest rating of 8.2 ± 1.2 because people found its wheat-based appearance familiar and uniform. Participants scored the appearance of T3 composite flour lower because of its darker and coarser quality.

Flavour:Sensory satisfaction mainly depends on flavour, with its two aspects of taste and aroma. T1 received the most favorable ratings (7.8 ± 1.5), followed by a combination of balanced taste elements. The flavor rating of T3 (6.6 ± 1.7) proved lowest since its composition primarily featured green gram and wheatgrass, which likely resulted in an undesirable earthy taste.

Taste:The ratings of flavor served as a close match with the way panelists perceived the taste of the formulations. The taste ratings showed T1 received the highest score with 7.8 ± 1.4 points, whereas T2 received 7.2 ± 1.7 points and T3 received 6.8 ± 1.7 points. Panelists evaluated the control (T0) at 8.2 ± 1.2 points because its conventional ingredients without green leafy elements provided an easier-to-gaze acceptability compared to other formulations.

Texture:The sensory experience, together with consumer satisfaction, depends heavily on texture attributes. Both T1 and T2 received similar ratings (7.4 ± 1.5 and 7.0 ± 1.7), which proves both products had satisfactory crunchiness levels and mouthfeel qualities. The texture score for T3 amounted to 6.8 ± 1.8 because of its coarse ingredients. Bakery texture standards were found in the control group (T0), which received a score of 8.2 ± 1.2.

Overall Acceptability:Overall acceptability emerges from the total perception of every sensory element. The desirability rating for T1 composite flour among subjects was scored at 8.0 ± 1.2, while samples T2 received 7.4 ± 1.6 and T3 received 6.8 ± 1.6 ratings. Because T2 exhibited superior nutritional characteristics, together with balanced sensory performance.

TABLE 9: ORGANOLEPTIC EVALUATION OF NUTRIBALLS DEVELOPED WITH COMPOSITE FLOUR

Formulation	Appearance ($\bar{X} \pm$ SD)	Flavour ($\bar{X} \pm$ SD)	Texture ($\bar{X} \pm$ SD)	Taste ($\bar{X} \pm$ SD)	Overall Acceptability ($\bar{X} \pm$ SD)
T1	8.2 ± 1.2	7.6 ± 1.3	7.8 ± 1.2	8.0 ± 1.2	7.8 ± 1.2
T2	8.0 ± 1.3	7.6 ± 1.3	7.8 ± 1.3	8.0 ± 1.3	8.0 ± 1.3
T3	7.8 ± 1.4	7.4 ± 1.4	7.4 ± 1.4	7.4 ± 1.4	7.4 ± 1.4
T0	7.0 ± 1.6	6.8 ± 1.6	6.8 ± 1.6	6.8 ± 1.6	6.8 ± 1.6

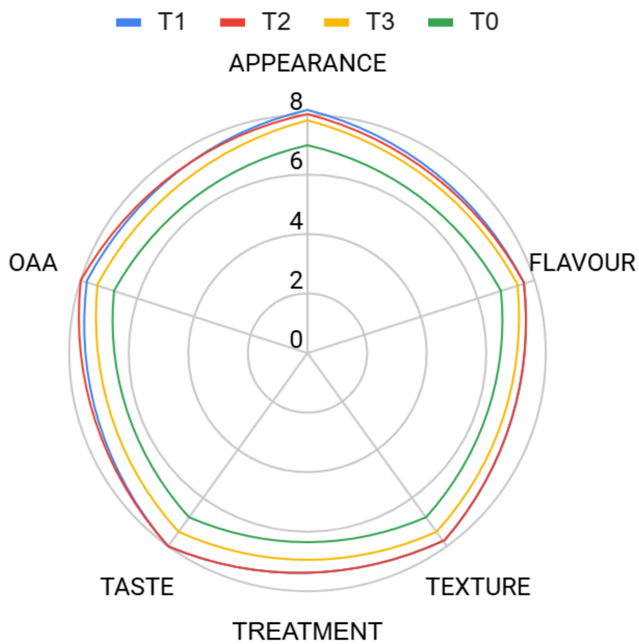


FIGURE 4: ORGANOLEPTIC EVALUATION OF ENERGY BALL DEVELOPED WITH COMPOSITE FLOUR

The sensory evaluation used a 9-point hedonic rating scale to assess appearance, together with colour appearance and flavor, taste, texture, and overall acceptability of energy ball made with composite flour mixtures (T1, T2, T3) and the standard control (T0). The evaluation is to understand consumers' desires and how products should appeal to them.

Appearance: Consumers show greater interest in ready-to-eat snacks when these snacks have attractive appearances. The ratings for T1 (8.2 ± 1.2) and T2 (8.0 ± 1.3) gave the highest scores, which indicates high attractiveness. T3 received an average rating of 7.8 ± 1.4, which pointed to positive reception, but the appearance of T0 stood in contrast with 7.0 ± 1.6 because of its lack of nutritional content in the composite mixtures.

Flavour: Panel members assessed the flavour of T1 and T2 to be 7.6 ± 1.3, which indicates the presence of a favorable balanced taste profile. The composite blends were preferred in terms of flavor as judged by the participants, who scored T3 at 7.4 ± 1.4 points while T0 scored the least at 6.8 ± 1.6 respectively..

Texture: The sensory experience of compact products such as energy ball gets significantly influenced by texture perception. Respondents who ate T1 and T2 rated the bites and mouthfeel as highly positive because each scored 7.8 ± 1.2 and 7.8 ± 1.3, respectively. T3 scored 7.4 ± 1.4, indicating moderate acceptance. Panel members rated T0 as 6.8 ± 1.6 , their least preferred choice due to its single-textured nature.

Taste:The evaluations of T1 and T2 matched each other (8.0 ± 1.2 and 8.0 ± 1.3), indicating relatively equal distribution of sweet and nutty flavors. The acceptability scores indicated T3 with 7.4 ± 1.4, while the control obtained 6.8 ± 1.6, indicating that blended flour products bring improved taste to food products.

Overall Acceptability:The sensory panel rated T2 highest for overall acceptability at 8.0 ± 1.3 while T1 occupied the second position at 7.8 ± 1.2, thus demonstrating their superior sensory qualities. The ratings for T3 (7.4 ± 1.4) revealed an acceptable level, so composite formulations received a better reception regarding nutritional content and sensory qualities than T0 (6.8 ± 1.6).

TABLE 10: ORGANOLEPTIC EVALUATION OF CHAPATI DEVELOPED WITH COMPOSITE FLOUR

Formulation	Appearance ($\bar{X}$ ± SD)	Flavour ($\bar{X}$ ± SD)	Texture ($\bar{X}$ ± SD)	Taste ($\bar{X}$ ± SD)	Overall Acceptability ($\bar{X}$ ± SD)
T1	5.6 ± 1.8	5.8 ± 1.7	5.2 ± 1.8	5.8 ± 1.7	5.8 ± 1.7
T2	7.0 ± 1.5	7.0 ± 1.5	6.8 ± 1.5	7.0 ± 1.5	7.0 ± 1.5
T3	6.4 ± 1.7	6.2 ± 1.7	6.0 ± 1.8	6.2 ± 1.7	6.2 ± 1.7
T0	7.4 ± 1.4	7.4 ± 1.4	7.4 ± 1.4	7.4 ± 1.4	7.4 ± 1.4

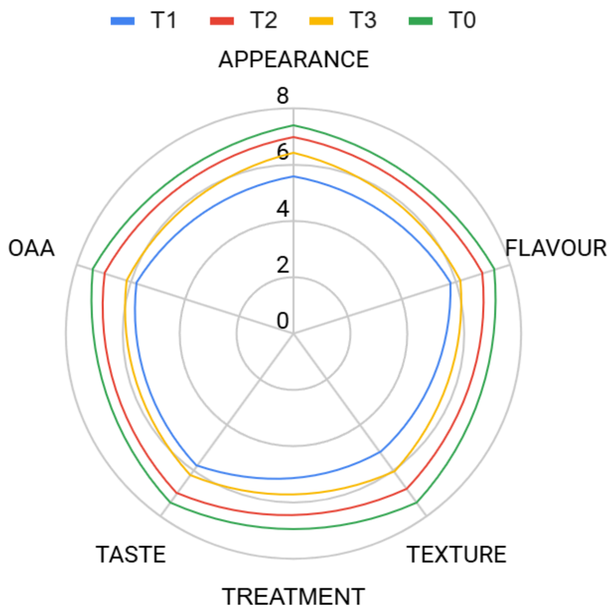


FIGURE 5: ORGANOLEPTIC EVALUATION OF CHAPATI MADE WITH COMPOSITE FLOUR

The sensory evaluation used a 9-point hedonic rating scale to assess appearance, together with colour appearance and flavor, taste, texture, and overall acceptability of chapati made with composite flour mixtures (T1, T2, T3) and the standard control (T0). Appearance:

The appearance rating of T0 (7.4 ± 1.4) reached its highest level, possibly because consumers were familiar with the uniform looks of refined wheat flour chapatis. The visual rating for 7.0 ± 1.5 scored T2 as the most appealing composite sample, while T3 achieved a score of 6.4 ± 1.7. Chapatis made with T1 received the lowest marks (5.6 ± 1.8), indicating the formulation resulted in a darker or less appealing appearance.

Flavour:Results for flavour scores matched those of appearance ratings. The control group (T0) achieved the highest mark (7.4 ± 1.4) because Panalists were already familiar with the refined wheat flour chapatis. The sensory results for T2 showed successful flavor enhancement because panelists rated it 7.0 ± 1.5. The Panalist rated T3 at 6.2 ± 1.7 but gave T1 the least acceptable flavour at 5.8 ± 1.7 since T1 probably contained higher amounts of millet and legumes that create earthy and beany tastes.

Texture:Chapatis demonstrate a significant role in determining their mouthfeel characteristics and pliability because of their texture. Panalist rated the samples based on texture, where T0 achieved the highest score (7.4 ± 1.4), followed closely by T2 (6.8 ± 1.5). This indicates that the developed products had respectable softness. Chapatis rated T3 received moderate scores, T1 obtained the lowest scores, which might be influenced by the increased fiber content, softening texture.

Taste:The ratings of T0 (7.4 ± 1.4) came out on top, while T2 (7.0 ± 1.5) followed closely in taste acceptance with well-balanced flavors. Test subjects scored T1 (5.8 ± 1.7) the least due to unappealing sensory interactions in its preparation formulas. Nonetheless, T3 (6.2 ± 1.7) received moderate acceptance.

Overall Acceptability:The traditional formulation T0 held the top position regarding acceptability, with mean ratings of 7.4 ± 1.4. The consumer acceptance of the enriched flour blend was high, as demonstrated by its 7.0 ± 1.5 score in T2. The participants approved T3 (6.2 ± 1.7) as moderately acceptable, but avoided T1 (5.8 ± 1.7) the most.

Ikade *et al.* (2024) studied cookies made using composite flours on several millets. Certain formulations were chosen by the sensory panels, especially those that had balanced amounts of various millet flours. This highlights the importance it is to improving composite flour mixtures to develop baked items with their best sensory qualities.

Among the other formulations, T2 proved to be the preferred choice because it maintained an ideal combination between various quality aspects, including taste, texture, flavor, and general liking scores. The panel members rated T2 with 7.2–7.4 points for cookies and 7.0 points for chapati, and 8.0 points for energy ball in all tested parameters. The panel members positively received these values, indicating that T2 delivers an enjoyable sensory experience throughout cookie and savory applications. The scores for T1 and T3 formulations were lower than those for other formulations because flour modification, along with processing methods, affected the finished product's organoleptic qualities, like texture and mouthfeel, and aroma.

For further analysis, T2 was selected, which ensures that it is suitable for product development.

4.4. TOTAL MICROBIAL CONTENT OF COMPOSITE FLOUR

On the 30th day of storage, the composite flour was evaluated for the microbial load to assess its shelf stability. This was examined by the spread plate method with serial dilutions, which ranged from about 10^{-1} to 10^{-5}. Too Numerous To Counts (TNTC) in the 10^{-1} dilution indicated high microbial growth, and the 10^{-5} dilution had less microbial growth, which was in the countable range, Too Less Counts (TLC).

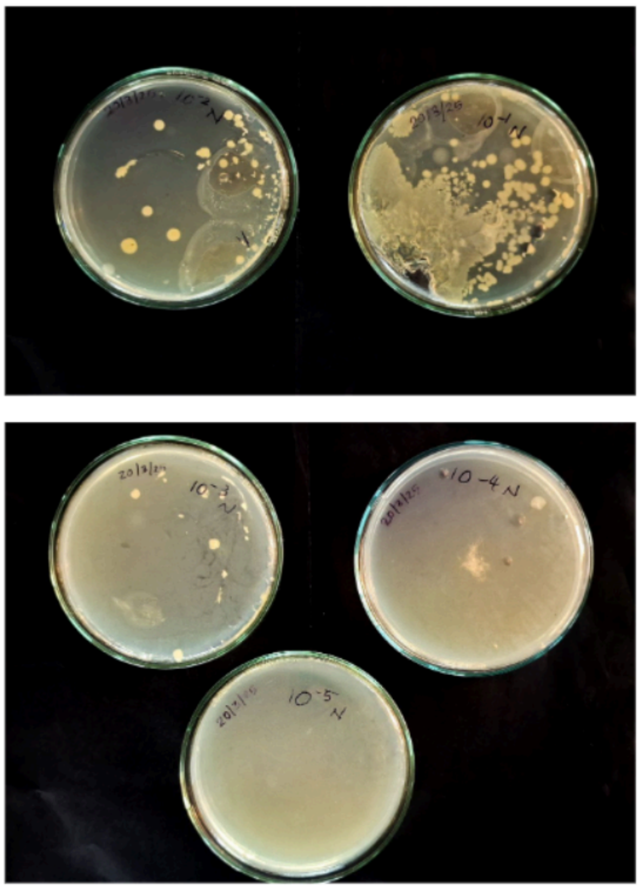


PLATE 18: MICROBIAL GROWTH ON THE 30TH DAY

After the incubation

- 10^{-1}, shows that Too Numerous To Count (TNTC) colonies indicate the excessive growth of microbial colonies.
- 10^{-2}, showed that the Too Few To Count (TFTC) colonies with a count of 24 colonies, which is in the acceptable range.
- 10^{-3}, showed that the Too Few To Count (TFTC) colonies with a count of eight colonies, which is below the standard countable range.
- 10^{-4}, showed that the Too Few To Count (TFTC) colonies with a count of two colonies.
- 10^{-5}, showed no visible microbial growth.

The composite flour underwent a total microbial assessment initial stage, which had zero microbial load during preparation and thus demonstrated proper hygiene conditions throughout processing and storage.The microbial analysis performed on day 30 showed substantial differences among the various dilution levels for colony formation. The highest dilution level at 10^{-1} yielded Too Numerous to Count (TNC) colonies because of the significant microbial abundance in those samples. The colony count progressively declined as the dilution volume increased. A countable number of microbial colonies existed at the 10^{-5} dilution, while some samples showed Too Less To Count (TLTC) levels.

Studies on the flour show that storage will affect the quality. Prolonged storage will degrade the quality, like color change, microbial growth, and oxidative rancidity (Berwal *et al*., 2017). Suitable packaging will reduce microbial growth (Awol *et al*., 2024). The findings show that modest microbial activity is present. Proper storage conditions and hygienic practices are essential to reduce microbial growth and maintain the quality of composite flour.

A nutritionally improved composite flour was successfully developed and standardized through the incorporation of wheat with amaranth seeds, green gram, pearl millet, amaranth leaves, and wheatgrass powder. The ingredients received nutritional enhancement through application of traditional and functional processing steps, which included soaking, sprouting, drying, and milling. A complete analysis assessed the nutritional value, along with functional properties and sensory quality, and microbial safety of the composite flour. The flour showed high content of carbohydrates and protein, and dietary fiber according to macronutrient analysis, and contained essential minerals, calcium, and iron, which confirmed its suitability for nutrient deficiency management through micronutrient profiling. Functional tests showed that the flour retained moisture and fat well while exhibiting favorable properties related to bulk and tapped density as well as swelling capacity and solubility, together with foaming capacity, which demonstrates food application potential. The microbial analysis demonstrated reduced moisture levels and exceptional oxidative protection against microbes through 30 days, which eliminated the need for preservatives during storage. The sensory testing of chapati, along with cookies and nutriballs produced using composite flour, reported excellent scores regarding taste, texture, and appearance quality. The study places composite flour as a functional, nutritious alternative to conventional mono-grain flours, which benefits consumers focusing on health while aiding individuals with nutritional requirements.

CHAPTER 5

SUMMARY AND CONCLUSION

The summary of the study entitled "Development and Standardization of Nutritionally Enhanced Composite Flour Products: Evaluation of Nutritional, Functional, and Sensory Qualities." The raw materials were selected based on their nutritional quality and collected from local markets in Menka, Ernakulam.

- For the development of composite flour, three different flour combinations T1 (40:20:10:25:4:1), T2 (40:25:15:15:3:2), and T3 (40:10:25:20:2:3) (Wheat, Amaranth seeds, Green gram, Pearl millet (bajra), Amaranth leaves, and Wheatgrass powder) were envisaged and used to develop cookies, chapati, and nutriballs respectively. With regards to organoleptic qualities, the most acceptable among the combinations, T2, is selected for further evaluation of attributes.
- A 100 gram composite flour portion provides 70 gram carbohydrates and 17 gram protein, with 5.8 gram crude fiber and 191.32 mg. calcium, and 6.55 mg. iron. This composite flour provides more protein benefits for individuals who aim to boost their protein consumption. Multiple grains and seeds combined in flour create a nutrient-dense diet because they provide essential nutrients absent in single-grain flours.
- The functional features of composite flour demonstrate essential functionality needed to perform in different food manufacturing applications. This flour shows a moisture-retention power of 20.2% along with a fat-retention ability of 19.8%, which assists in texture and mouthfeel formation throughout the final product production. The flour maintains a compactness level between low and high based on its 21 g/ml bulk density value and 0.94 g/ml tapped density result. When hydrated, the 50% swelling level provides a tendency to expand that influences the resulting texture of bread and cakes. These characteristics show that the flour can help create a light structure in baked products because its solubility stands at 6.93 percent, and foaming capacity reaches 47.61 milliliters.

- The composite flour shows excellent shelf stability because it contains a moisture content of 7.5%. A peroxide value of 0.21 meq/kg indicates that the flour does not easily oxidize or spoil because it remains fresh during storage time. This composite flour contains no added preservatives because the researchers monitored microbial growth, including yeast and mold counts, until the 30th day, demonstrating that artificial preservatives are unnecessary.
- The composition of this flour matches conventional flour properties, which creates excellent opportunities for diverse culinary creations. The flour provides exceptional nutrition that complements general wellness alongside its regular properties. The flour provides protein with fiber together with iron and calcium, which serve to enhance diets deficient in these nutrients. The nutritional strength of the flour exceeds functional abilities through its water retention and oil absorption, as well as foaming and swelling properties, which expand its culinary usability. The composite flour proves acceptable under sensory tests because it delivers pleasing results in taste tests, together with texture and visual aspects. The developed composite flour serves as a nutritious functional alternative to traditional mono flours because it benefits health-conscious populations and nutritionally vulnerable populations.
- The successful development of nutritionally improved composite flour through research studies contained significant limitations in their findings. The evaluation included only limited flour combinations despite the possibility that other nutritious ingredients could show better results. The research analyzed storage stability through a brief 30-day evaluation, thus providing first-level insights about shelf life duration. The study investigated proximate and functional properties but neglected to examine nutrient bioavailability because this essential knowledge is necessary for determining actual health benefits.
- Future investigative studies should expand this study by adding sorghum and buckwheat, and ragi as additional nutrient-rich ingredients because these foods possess distinct health benefits. Additional research must include longer-term product storage tests that take place under different environmental conditions to improve safety assessments and product durability predictions. The food safety evaluation process becomes more effective when additional screenings are performed to detect particular food pathogens.

The present study mainly examined nutritional aspects and functional properties, yet future research should study how consumers across various population groups perceive the products to advance commercialization purposes.

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APPENDIX 1
RECIPES

The formulated flour combinations were used to develop three different recipes: Energy balls, Cookies, and Chapati, followed by three treatments: T1 (40:20:10:25:4:1), T2 (40:25:15:15:3:2), and T3 (40:10:25:20:2:3)

RECIPE 1: COOKIES

Ingredients

- Composite flour - 1 cup
- Butter - ½ cup
- Jaggery powder - ½ cup
- Baking powder - ½ tsp
- Baking soda - ¼ tsp
- Vanilla essence - 1 tsp
- Salt - a pinch

Preparation

- Preheat the oven to 170℃
- In a bowl, mix the butter and jaggery until they turn creamy.
- Sift the composite flour with baking powder, baking soda, and salt.
- Mix the flour mixture with the butter mix.
- Allow to freeze for the consistency to roll into balls.
- Roll into small ball shapes and place in a baking tray
- Bake for 12 - 15 mins.

RECIPE 2: ENERGY BALL

Ingredients

- Composite flour - 1 cup
- Dates - ½ cup
- Almonds - ¼ cup
- Sesame seeds - 2 tbsp
- Jaggery - 1 tsp
- Ghee - 2 tbsp

Preparation

- Roast the composite flour on low heat until it turns aromatic.
- Add the ghee, and turn off the flame.
- In another pan, melt the jaggery with 1 tsp of water and make it into a syrup.
- Mix the roasted flour, nuts, and dates into the jaggery syrup and mix well.
- Then shape it into small balls.

RECIPE 3: CHAPATI

Ingredients

- Composite flour - 1 cup
- Warm water - as needed to knead
- Salt - ½ tsp
- Oil - 1 tbsp

Preparation

- Boil the water and salt
- In a mixing bowl, composite flour and gradually add the warm water.
- Knead it well into a soft dough with some oil and cover it, and rest it for some time
- Make into small balls, and roll out into thin chapatis.
- Cook it on a hot griddle on both sides.

APPENDIX 2

SENSORY EVALUATION

TITLE: Development And Standardization Of Iron-Rich Composite Flour Products: Evaluation Of Nutritional, Functional, And Sensory Qualities

HEDONIC SCALE FOR SENSORY EVALUATION

Recipes		Appearance	Aroma	Texture	Taste	Overall acceptability
Cookies	T1					
	T2					
	T3					
	T0					
Nutri ball	T1					
	T2					
	T3					
	T0					
Chapati	T1					
	T2					
	T3					
	T0					

***Kindly indicate your rating between 1-9**

9-POINT HEDONIC SCALE

SCORE	DESCRIPTION
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like Nor Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

APPENDIX 3
STORAGE STUDY - MICROBIAL ANALYSIS

SERIAL DILUTION AND STANDARD PLATE COUNT

The method of serial dilution involves successive stepwise reductions of a substance or microbial sample through a liquid medium or buffer system series. Laboratories use this method to decrease substance amounts, including microorganisms and solutions, because it enables better experimental control at lower analytical levels.

The number of microorganisms in samples with unknown concentration levels is determined using standard serial dilution procedures. The bacterial culture presents a billion bacteria throughout each milliliter of media, which surpasses human counting capability. The serial dilution technique starts by reducing a tiny bacterial culture portion until it reaches a suitable bacterial cell count for milliliter measurements.

Standard plate count is one of the most accurate means of enumeration of viable microbes because we get a visual indicator for every cell in the specimen. A small quantity of a sample after proper dilution is plated on culture media via spread plate techniques or pour plate technique, which, when incubated, develops into a visible colony through repeated fission.

Microorganisms normally form multiple cell arrangements, such as chains. In addition, some of the bacteria may be clumped together. Therefore, when doing the plate count technique, we generally determine the number of Colony-Forming Units (CFUs) in that known dilution. The colony-forming unit may consist of a chain of bacteria rather than a single bacterium. For optimum accuracy of a count, the preferred range for total CFU/plate is between 30 to 300 colonies/plate

MATERIALS

- Nutrient Agar
- Nutrient Broth
- Distilled water
- Conical flask
- Spatula
- Petri plate
- L rod glass
- Test tubes
- Pipette
- Cotton
- Autoclave
- Laminar airflow
- Incubator

PROCEDURE

1. Prepare 5 test tubes with 9 ml of distilled water and properly cover the test tubes with a cotton plug.

2. Sterilize the test tubes by autoclaving at 121℃ temperature and 15 lbw pressure for 15 to 20 minutes.

3. After sterilization, allow the test tube containing water to cool down properly.

4. 1 ml of the sample is pipetted out (or I g sample) and transferred to a test tube with 9 ml of distilled water and labeled, and mixed well.

5. Transfer 1 ml of sample from the first test tube to the second test tube, mix it well, and label it as 10^2. Continue the process.

6. 1 ml of diluted samples from each of the test tubes is plated to culture media via pour plate or to-spread plate technique.

7. Incubate the plates at 37℃ for 24 hours.

8. At the end of the incubation period, select all of the Petri plates containing between 30 and 300 colonies.

9. Plates with more than 300 colonies cannot be counted and are designated 100 many to count

10. Plates with fewer than 30 colonies are designated as too few to count.

11. Count the colonies on each plate

CALCULATION

$$\text{CFU/g} = \frac{\text{Number of the colonies x Dilution factor}}{\text{Volume plated (mL)}}$$

Where:

- Number of Colonies: The count of colonies on the agar plate
- Dilution Factor: The inverse of the dilution used (eg, if you plated from a 10^4 dilution, the dilution factor is 104.
- Volume Plated: The volume of the sample spread onto the plate (in mL).