"NUTRIENT PACKED DIGESTIVE CRACKERS: HARNESSING THE POWER OF *Cannabis sativa L.* AND *Euryale ferox*"

Dissertation submitted to Mahatma Gandhi University in partial fulfilment of the requirements for the award of degree of

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B. Voc. Food Processing Technology

By

ASIN PADUVA (Reg. No: VB22FPT005)

NEHA JOSE BABY (Reg. No: VB22FPT017)

PV FATHIMA NIBA (Reg. No: VB22FPT018)

Under the guidance of

Ms. PINKU MARIA

Assistant Professor

ST. TERESA'S COLLEGE (AUTONOMOUS), ERNAKULAM

COLLEGE WITH POTENTIAL FOR EXCELLENCE

Nationally Re-Accredited at 'A++ Level (4th cycle).

Affiliated with Mahatma Gandhi University

Kottayam-686560

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DECLARATION

We, Asin Paduva (Reg. No: VB22FPT005), Neha Jose Baby (Reg. No: VB22FPT017), and PV Fathima Niba (Reg. No: VB22FPT018) hereby declare that the project entitled "NUTRIENT PACKED DIGESTIVE CRACKERS: HARNESSING THE POWER OF *Cannabis sativa L.* AND *Euryale ferox* " is a bonafide record of the project work done by us during the course of study and that the report has not previously formed on the basis for the award to us for any degree, diploma, fellowship or another title of any other University or Society.

Asin Paduva

Neha Jose Baby

PV Fathima Niba

Date:

Place:

CERTIFICATE

This is to certify that the project entitled " NUTRIENT PACKED DIGESTIVE CRACKERS: HARNESSING THE POWER OF *Cannabis sativa L.* AND *Euryale ferox* " for the Development of cookie submitted in partial fulfilment of the requirements for the Award of the degree of B. Voc Food Processing Technology to St. Teresa's College. Ernakulam is a record of bonafide research work carried by Ms. Asin Paduva, Ms. Neha Jose Baby and Ms. P.V. Fathima Niba under my guidance and supervision and that no part of the project has been submitted for the award of any other degree, diploma. fellowship or other similar titles or prize and that the work has not been published in part or full in any scientific or popular journal or magazine.

Ms. Sherin Mary Simon

Head of The Department

Food Processing Technology

St. Teresa's College (Autonomous)

Ernakulam

Ms. Pinku Maria

Project Guide

Assistant Professor

Food Processing Technology

St. Teresa's College (Autonomous)

Ernakulam

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Asin Paduva

Neha Jose Baby

PV Fathima Niba

ABSTRACT

With the global rise in life style diseases, especially diabetes and related health issues, there is a pressing need for innovative dietary solutions that combine nutritional value with functional benefits. This study presents the development of a nutrient-rich digestive cracker specifically designed for diabetes management, utilizing *Euryale ferox* (Fox nut) and *Cannabis sativa L* (Hemp hearts) as primary ingredients. The research objectives included the formulation of the crackers, sensory evaluation, nutritional composition analysis, and assessment of storage stability. The crackers were prepared using a conventional baking method, followed by rigorous sensory and proximate analysis to evaluate their acceptability and nutritional profile.

The findings demonstrate that the developed product is a powerhouse of nutrients, containing high levels of protein, fibre, and essential minerals. Sensory evaluation confirmed its palatability and consumer acceptability, making it a desirable functional food. The results highlight the potential of these crackers not only to cater to the dietary needs but also to address the broader demand for nutrient-dense, health-oriented snacks. Moreover, the study emphasizes the importance of incorporating ready to eat functional foods into daily diets to enhance health outcomes and foster improved lifestyle management.

This research serves as a step forward in the development of functional food products, paving the way for future innovations in cookies, crackers, and other convenient snack formats. By addressing both nutritional and functional requirements, this work contributes to the growing field of health-focused food technology and provides a valuable dietary option for health-conscious individuals and those managing chronic conditions.

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DM	Diabetes mellitus
HSP	Hemp Seed protein
GLA	Gamma-linolenic acid
ADHD	Attention Deficit Hyperactivity Disorder
HDL	High Density Lipo-protein
LDL	Low Density Lipo-protein
HPLC	High Performance Liquid Chromatography
EAAI	Essential Amino-acid Index
GI	Glycaemic Index
NCD	Non-Communicable Diseases
CVD	Cardio Vascular Diseases
mm	milli-metre
g	Gram

CHAPTER 1 INTRODUCTION

Life Style Diseases is promptly becoming more prevalent in our society, characterized by persistently high blood sugar levels which can lead to severe complications, including heart damage. We are in the midst of an epidemic; the epidemic of diabetes and its micro-vascular complications; without aggressive intervention, healthcare costs will be devastating to millions of patients predicted to be affected by diabetes over the next decades. However, this condition can be managed and potentially mitigated by consuming foods rich in antioxidants and possessing anti-diabetic properties. Nutritional therapy has emerged as a preferred method for managing metabolic diseases like diabetes mellitus (DM), due to its holistic approach and fewer side effects compared to synthetic drugs, which often have limited impact on extra-pancreatic degenerations caused by diabetes (Turner *et al.*,1998).

The growing body of scientific evidence and increased health awareness highlight the strong correlation between diet and overall health. This awareness has spurred researchers to innovate and promote functional foods that cater specifically to the dietary needs of individuals, particularly those with chronic conditions such as diabetes. With the surge in snack consumption, heightened health consciousness, and the demand for nutritious food options, researchers are now exploring the development of composite cookies. These cookies aim to provide a convenient yet healthy snack option that meets the dietary requirements of diabetic patients while also appealing to general consumers looking for nutritious food choices (Tharun, 2024).

Hemp (*Cannabis sativa* L.) is a plant cultivated for its fibre and seed oil. The seeds of hemp are not only nutritious but also offer health benefits such as reducing cholesterol and high blood pressure. Traditionally, hemp seeds have been consumed in foods and folk medicines, and also used as animal feed. Hemp contains 20–25% protein, 20–30% carbohydrates, 25–35% oil, and 10–15% insoluble fibre, along with a wide array of minerals. The highly polyunsaturated oil derived from hemp seeds has found uses in printer's ink, wood preservatives, detergents, and soaps. Hemp seed oil is considered to have a perfectly balanced ratio (3:1) of the two essential polyunsaturated fatty acids—linoleic and linolenic acids—making it ideal for human nutrition. Due to this balanced ratio and the presence of γ-linolenic acid, hemp seed oil is also perfect for use in light body oils and lipid-enriched creams, which are known for their high skin penetration (Oomah *et al*., 2002).

Hempseed seems to be underutilized as a nutritious food for human consumption. The essential amino acid content of hemp seed protein (HSP) surpasses that of soybeans, although lysine and tryptophan are the main limiting amino acids in hempseed. The seedcake that remains after oil extraction has the potential to be used as a high-protein flour for humans. Furthermore, hempseed protein contains a significant amount of the amino acid arginine, a metabolic precursor for the production of nitric oxide, which is important for the normal regulation of blood pressure. The cotyledon-containing fractions of hempseed are significantly richer in protein (>41.2%), lipid (>15.1%), and sugar content (>3.5%) compared to the hull-containing fractions, which are significantly richer in crude fibre content (>21.3%). Antinutrients, including trypsin inhibitors, phytic acid, glucosinolates, and condensed tannins, are mostly found in the cotyledon fractions.

The nutritional quality of HSP, based on amino acid composition and protein digestibility analysis, is influenced by variety/genetics and agronomic conditions such as soil type, fertilizer application, rainfall, and temperature, which can modify seed components, including protein structure, and the presence of antinutritional compounds. The concentration of antinutritional compounds, such as phytic acid, condensed tannins, and trypsin inhibitors, is usually very low in hempseeds. Antinutritional compounds are more concentrated in monoecious varieties compared to dioecious varieties, with phytic acid content being 63 and 75.4 g/kg in hempseed meal from dioecious and monoecious varieties, respectively (Xu *et al*, 2020).

Hemp seeds are rich in gamma-linolenic acid (GLA), which is an essential building block for prostaglandins—hormone-like chemicals that help smooth muscles, control inflammation, and regulate body temperature. GLA is crucial for various body functions, and research published in the European Journal of Pharmacology shows that GLA-supplemented diets can reduce inflammatory responses. It helps with conditions such as ADHD, breast pain, diabetes, diabetic neuropathy, heart disease, high blood pressure, multiple sclerosis, obesity, premenstrual syndrome, rheumatoid arthritis, and skin allergies. Additionally, studies have shown that hemp hearts and hemp seed oil can be significantly helpful in relieving rheumatoid arthritis symptoms. It is also aid in weight loss by acting as a natural appetite suppressant due to their high fibre content, which promotes satiety and helps curb excess hunger. Hemp seeds improve digestive health as well, with a study published in the American Journal of Gastroenterology indicating that hemp seed pill treatment effectively relieves functional constipation. The high insoluble and soluble fibre content in hemp hearts provides enough bulk to keep the gastrointestinal system regular. It benefits hair, skin, and nail health by improving dry, red, flaking skin. Hemp

oil is often included in high-end cosmetic products like lip balms, lotions, and soaps. Researchers have found that using hemp seed oil improves symptoms of atopic dermatitis (eczema), psoriasis, and other skin disorders. Furthermore, hemp seeds help reduce inflammation levels and strengthen the immune system due to their perfect fatty acid profile of omega-3 fats and GLA.

In terms of heart health, hemp seeds contribute to a healthy heart by providing fibre, plant-based protein, healthy fats, and reducing sugar intake. Studies on animals and humans strongly suggest that hemp seeds can improve cardiovascular health and high blood pressure. Consuming one to two tablespoons of hemp seeds in a morning smoothie can help lower blood pressure, reduce LDL cholesterol, raise HDL cholesterol, and improve triglycerides (Chauhan, 2020).

The lotus flower's seeds, known as "makhana" in the Indian subcontinent, are fox nuts (*Euryale ferox*). In addition to minerals like calcium, magnesium, iron, and phosphorus, they are high in protein and fibre. Fox nuts are shown to have strong antioxidant activity and to support cardiac, digestive, blood sugar, and body weight management, according to studies. Because fox nuts are low in calories and fatty acids, they are a healthy snack option. They provide a good supply of carbs, plant-based protein, and fibre. They also include important minerals that are essential for many biological functions, including zinc, phosphorus, potassium, and magnesium. When included in a balanced diet, the magnesium and potassium content of fox nuts may promote cardiovascular health by preserving proper cardiac rhythm and blood pressure management, so aiding in the maintenance of a healthy heart. Among other antioxidants, flavonoids and phenolic compounds have also been discovered to be more prevalent. Fox nuts' antioxidant properties may help reduce a person's vulnerability to conditions like cancer and heart disease.

Due to its low glycaemic index, fox nuts can be a healthy snack choice for diabetics or those trying to control their blood sugar levels. By delaying the absorption of glucose, the fibre content of fox nuts can help improve blood sugar regulation. A healthy digestive tract is also promoted by the high fibre content. It improves general gut health, encourages regular bowel movements, and lessens constipation. Because fox nuts are strong in fibre and low in calories, they offer a high satiety value that may help with weight control. The secondary goal was to evaluate the product's antioxidant activity, phytochemicals, and nutrients (Joseph, 2023).

The fox nut's extremely nutritional profile places it in the category of foods of outstanding grade. For vegetarian diets, it is also a rich source of protein. However, compared to other nuts,

its bland flavour and soggy texture make it less popular. Roasting and seasoning were employed in this study to increase consumer approval. Because of their black exterior seed coat, some scientists have called Makhana seeds "Black Diamonds" and "Black Gems of Wetlands." *Euryale ferox* plant has edible seeds that are processed into white puffed nuts and are the main reason for the appeal of the plant. Because of their high Essential Amino Acid Index (EAAI) of 89–93%, these nuts are considered a great food item in the dry fruit category and are very nutritious. Because of this, they are a fantastic source of vital amino acids that the body cannot produce on its own and must be obtained through diet. Low GI diets have been shown to maintain a healthy body weight, enhance insulin sensitivity and fasting triglyceride levels, and significantly lower health risk factors. Due to their low GI, fox nuts are a wonderful fit for the list of foods that are recommended for various non-communicable diseases. These nuts' low GI is caused by their complex carbohydrate content, which also makes them a fantastic snack for people with non-communicable diseases. The Canadian Diabetes Association has also recommended low-glycaemic-index foods as part of nutrition therapy for diabetes patients (Liaquat *et al*., Food Production, Processing and Nutrition, 2022).

The significant amounts of carbohydrates, protein, and dietary fibre found in *E. ferox* seeds have a stronger impact on nutritional quality. The essential oils found in the fox nut seed coat are rich in polyphenols, which gives them strong antibacterial and/or antioxidant properties. Although there are more bioactive chemicals in fox nut seeds, their processing retention has not yet been maximized (Biswas *et al*., 2016)

The study mainly emphasizes the development of nutrient rich digestive cracker by both hemp hearts and fox nuts in order to incorporate the health the benefits of the same in the product.

OBJECTIVES

- Development of Digestive Cracker utilizing *Euryale ferox* (Fox nut) and *Cannabis sativa L* (Hemp Hearts).
- Sensory Evaluation and Proximate Analysis of Nutritional composition.
- Study the Storage life of developed product.

SCOPE OF THE PROJECT

The purpose of this initiative is to develop a Digestive Cracker by:

- Utilization of underutilized functional food products.
- Encouraging the use of local cultivars.
- Promote a healthy and nutritious lifestyle.

CHAPTER 2 REVIEW OF LITERATURE

2.1 Lifestyle Diseases: A Closer Look

Non-communicable diseases (NCDs) account for approximately 40 million deaths annually, making up nearly 70% of global fatalities. These chronic conditions cannot be transmitted between individuals and arise from a combination of genetic, physiological, environmental, and behavioural factors. The primary types of NCDs include cardiovascular diseases, chronic respiratory illnesses, and cancer. Many of these, such as cardiovascular diseases (CVD), stroke, diabetes, and certain cancers, are closely tied to lifestyle choices and are thus often referred to as lifestyle diseases. Four key behavioural risk factors significantly contribute to the prevalence of NCDs: tobacco use, unhealthy diets, insufficient physical activity, and harmful alcohol consumption (Kalansooriya, 2023). According to WHO, low- and middle-income countries, as well as economically disadvantaged populations within all nations, bear the brunt of NCD-related deaths (Reddy, 2003). This creates a vicious cycle where poverty exposes individuals to these risk factors, and the resulting diseases push families deeper into financial hardship. For instance, in 2015, India faced economic losses exceeding $236 million due to unhealthy lifestyles and poor dietary habits (Bloom *et al.*, 2014). To mitigate the global burden of NCDs, it is essential to focus on addressing these diseases in the most affected regions and communities.

Non-communicable diseases (NCDs) are influenced by seemingly unrelated factors such as rapid, unplanned urbanization, the globalization of unhealthy lifestyles, and an ageing population. Observable issues like high blood pressure, elevated blood glucose, increased blood lipids, and obesity often reflect deeper, ingrained lifestyle habits. Additionally, NCDs typically have a lengthy latency period, spanning several years or even decades (Tabish, 2017).

Healthy, protein-rich functional foods, such as nutritional cookies, are emerging as an ideal solution for modern lifestyle demands while addressing critical health concerns like diabetes (Alkhatib *et al.,* 2017). In today's fast-paced world, these cookies provide a convenient yet nutritious snacking alternative, packed with essential nutrients that support overall health. By incorporating low-glycaemic index ingredients like whole grains, nuts and seeds, they help regulate blood sugar levels and prevent harmful spikes. Their high protein content enhances satiety, aiding in weight management, which is crucial for preventing and managing diabetes (Singh *et al*., 2018).

Functional cookies often feature ingredients like seeds such as Hemp (*Cannabis sativa*) and foxnuts (*Euryale ferox)*, which promote better metabolism, improve lipid profiles, and reduce inflammation—key factors in diabetes control (Kaur *et al*., 2023). Moreover, their presence in the market drives a positive change in eating habits by replacing unhealthy, high-sugar, high-fat snacks with nutrient-rich options. Fortified with bioactive compounds such as antioxidants, omega-3 fatty acids, and probiotics, these cookies also help mitigate complications related to diabetes (Nilgün Ertaş *et al.,* 2020). By including these healthier choices in daily diets, individuals can balance flavour with nutrition, making functional foods an essential part of combating lifestyle diseases like diabetes (Enkhmaa *et al.,* 2018).

2.2 *Euryale ferox* (Foxnut)

Figure 2.1: Foxnut

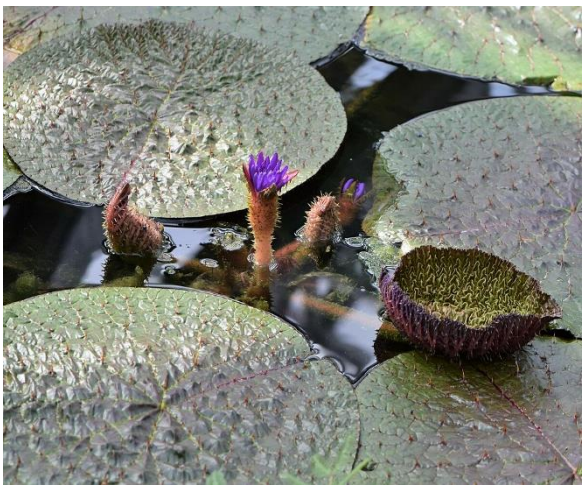


Figure 2.2 *Euryale ferox*

(Figure courtesy- Shutterstock)

SCIENTIFIC CLASSIFICATION OF FOXNUT

Table 2.1: Scientific classification of Foxnut

Kingdom	Plantae
Phylum	Tracheophytes
Class	Angiosperms
Order	Nymphaeales
Family	Nymphaeaceae
Genus	*Euryale*

In the Indian subcontinent, the freshwater crop known as "fox nut" (*Euryale ferox*), which is a member of the Nymphaeaceae family, is frequently called "Makhana." People in the Centre and Northeastern parts of the nation frequently eat the "black diamond" seeds as they are popped. Fox nuts, sometimes referred to as lotus seeds and gorgon nuts, are widely consumed in India and other Southeast Asian nations (Das *et al*., 2012). Bihar accounts for 80% of the production and cultivation of fox nuts in India (Kumari *et al.,* 2023). Because of their nutritional advantages, fox nuts are being consumed in much greater quantities. In addition to having a high mineral content, fox nuts are low in calories and fat. Micronutrient deficits can be less common if nutrient-dense Fox nuts are regularly substituted in a diet plan. Because of their high nutrient content, Fox nuts can be eaten popped or powdered and added to a variety of foods. Fox nut popping is thought to improve the phytochemical profile and other bioactive substances. The phytochemical action of Fox nuts is attributed to kaempferol, a flavonoid that is also found in almond skin. Research has indicated that because of its high amino acid index, it contributes significantly to cell metabolism by facilitating muscle recovery following physical activity (Liaquat *et al.,* 2022). It has the ability to prevent aging because it has been demonstrated to enhance the restoration of artery and vein flexibility and support the preservation of healthy tissues (Joseph 2023).

2.2.1 Nutritional composition

Euryale ferox is composed of carbohydrates, proteins, moisture, minerals, fat, phosphorus, calcium, and iron, with smaller amounts of ascorbic acid, phenol, and sugar, according to nutritional research done to identify its contents. Carbohydrates are the most abundant of these components, they are found in varying proportions. *Euryale ferox,* a developed starch-protein source, is widely found in tropical and subtropical climates with humid to sub humid conditions (Shukla *et al.*, 2023). The low glycaemic index of fox nuts makes them a healthy snack choice for diabetics or those trying to control their blood sugar. Because it slows down the absorption of glucose, the fibre component of fox nuts can help improve blood sugar control. Additionally, a healthy digestive tract is promoted by the high fibre content. Constipation is lessened, regular bowel movements are encouraged, and general gut health is improved. Being high in fibre and low in calories, fox nuts have a high satiety value that can help with weight control. Fiber reduces the tendency to overeat since it keeps one feeling satiated for longer during meals (Joseph, 2023).

Nuts have the third-highest phytochemical content, behind fruits and spices. Foods' overall nutritional value and antioxidant activity are significantly influenced by phenolic and flavonoid components. They have been shown to be effective in managing human diseases in a number of studies. In comparison to other nutrients, phenolic compounds have extremely strong antioxidant qualities. One serving of nuts each day helps prevent the development of chronic diseases such as cancer, high blood pressure, cardiovascular problems, type II diabetes, and neurodegenerative diseases. Nuts are undoubtedly tasty and easy snacks. Along with having a profile rich in bioactive compounds, fox nuts also include higher concentrations of macro- (Ca, P, K, Na, and Mg) and micro-minerals (Liaquat *et al*., 2022)

NUTRITIONAL VALUE OF FOXNUT

Table 2.2: Nutritional value of Foxnut (makhana or lotus seeds) per 100 g,

(Liaquat *et al*., 2022).

Calories	**347 kcal**
Protein	**9.7 g**
Carbohydrates	**77 g**
Dietary fibre	14g
Sugars	0 g
Fat	**1.2 g**
Sodium	**1 mg**
Calcium	**56 mg**
Iron	**2.6 mg**
Magnesium	**98 mg**
Phosphorous	**386 mg**
Potassium	**350 mg**
Zinc	**1.3 mg**

Foxnut typically grows up to four to six feet deep in shallow, persistent water bodies including lakes, ponds, depressions, marshes, and ditches. Temperatures between 20 and 35°C and relative humidity levels between 50 and 90% are ideal for growth and development. Several Indian states, including Bihar, West Bengal, Assam, Manipur, Tripura, Madhya Pradesh, Rajasthan, Eastern Odisha, and Eastern Uttar Pradesh, produce *Euryale ferox*. Bihar is responsible for 90% of this crop's cultivation in India (Islam *et al*., 2023).

To make ayurvedic remedies, *Euryale ferox* seeds are utilized. Paushtik Churana is one of the ayurvedic preparations. Vata and Pitta doshas are soothed by fox nuts. It might, however, raise Kapha dosha. It lessens chronic weariness and gives the body more power. It provides nourishment to the heart, ovaries, testicles, and uterus. Furthermore, its Pitta-relieving qualities lessen the burning feeling connected to all illnesses. It is advised that a normal adult take 10–20 grams of its powder daily.

Makhana kernel strengthens the kidneys and prevents diarrhoea. It is the primary ingredient in the Chinese medicinal formulas "Chien-Shih" and "Su-Shin," which are essential tonics for children's development (Nehal et al., 2015). It is a great stimulator of the immune system. It controls blood pressure as well. Additionally, it eases knee and lower back pain. It is used to treat infertility and early aging. This crop possesses antioxidant qualities. It is employed to control blood sugar levels. It boosts humoral defences. It's a healthy way to manage your weight. It possesses anti-diabetic and anti-inflammatory qualities. The alimentary canal is kept in good condition by its dietary fibres. Its potassium and magnesium content help to regulate blood pressure. It's a functional food and nutraceutical (Shukla *et al.*, 2023).

Fox nuts are also a relatively decent option because they have a much lower fat content than other nuts. However, there are questions over whether Fox nuts can be advised for those with diabetes and other metabolic diseases due to their high carbohydrate content. Its limited consumption can also be attributed to its soggy texture and dull taste. The aim of this research was to emphasize the nutritional value of fox nuts as a High Fibre-Low GI snack with extra advantages. Numerous medicinal benefits of fox nuts have also been documented in ancient Chinese and Indian medical literature, in addition to their nutritional value. Fox nuts have particular therapeutic qualities due to their phenolic components and antioxidant qualities, in addition to their macro and micronutrient makeup (Liaquat *et al*., 2022).

Significant amounts of carbohydrates, protein, and dietary fibre were found in E. ferox seeds, all of which have a stronger impact on nutritional quality. Essential oils that are high in polyphenols, which give them strong antioxidant and/or antibacterial properties, were found in the fox nut seed coat. The glucoside makeup of E. ferox seeds may also contribute to their antioxidant activity, however fox nuts' capacity to scavenge reactive oxygen species and activate TRP32 and Trx-1 proteins was connected to their cardioprotective qualities. According to recent studies, fox nut seeds have a remarkably high concentration of α-tocopherol acetate, which could be crucial for the plant's antioxidant capacity. Although fox nut seeds do contain

more beneficial chemicals, processing has not yet been improved to maximize their retention (Biswas *et al*., 2016).

2.3 HEMPSEED

Figure 2.3: Hemp hearts

Figure 2.4: *Cannabis sativa L*

SCIENTIFIC CLASSIFICATION OF HEMP

Table 2.3: Scientific classification of Hemp

Kingdom	**Plantae**
Phylum	**Magnoliophyta**
Class	**Magnoliopsida**
Subclass	**Hamamelidae**
Order	**Urticales**
Family	**Cannabaceae**
Genus	**Cannabis**
Species	**Sativa**

Hemp (*Cannabis sativa L*.) is an annual herbaceous plant from the Cannabinaceae family, believed to have originated in Eurasia. For over six millennia, cannabis has been cultivated for its applications in food, fibre, and medicine (Salentijn *et al*., 2015; Kerckhoffs *et al*., 2015; O'Brien & Arathi, 2019). It is categorized into two main groups—marijuana and hemp—based

on its usage. To qualify as hemp, plants must contain no more than 0.2% THC (Δ9-tetrahydrocannabinol) by dry weight in Europe and 0.3% in North America (Small, 2015; Fike, 2016; Leonard *et al*., 2020). These low THC levels are non-intoxicating (Cherney & Small, 2016).

The seeds of industrial hemp are commonly referred to as "hempseeds," while "hempseed oil" refers to the vegetable oil extracted from these seeds. Hempseed oil differs from aromatic essential oils, which are derived from the trichomes of female hemp flowers or foliage. In addition to being a rich source of oils and proteins, essential oils extracted from hemp flowers are also used as flavouring and fragrance agents (Bertoli *et al*., 2010).

Cannabis sativa is often found growing wild as a weed (Kronbergs *et al*., 2011; Mazian *et al*., 2018). Over time, wild hemp has been removed from its natural habitats and genetically modified to meet human needs (Fike, 2016). Historically, hemp was primarily grown for its fibres or as a dual-purpose crop for both fibres and seeds, with limited use as an oilseed crop. Cultivated for millennia in temperate Eurasia, hemp was introduced to North America in 1606 (Small, 2015). Recently, there has been a surge in global interest in industrial hemp as a multipurpose crop. Since 2015, for the first time, it has been cultivated on more than 20,000 hectares as a dual-purpose crop for seeds and fibres (Tang *et al*., 2016). Seed production is significantly influenced by plant genotype and growing conditions. (Amaducci *et al*., 2015).

Hemp plants adapt their growth patterns based on planting density. At high densities, they produce thinner stalks with fewer branches, whereas at low densities, suited for oilseed production, they develop highly branched structures with thicker stems. Hempseeds are smooth, nearly spherical achenes, varying in size from 2.5 to 4 mm in diameter and 3 to 6 mm in length, with colours ranging from light brown to dark grey (Sacilik *et al*., 2003).

(Werf *et al*., 1996; Johnson, 2013; Amaducci *et al*., 2015). Breeding programs aim to develop high-yielding, low-THC hemp varieties with desirable traits, including larger seeds for easier hulling, specific amino acid and fatty acid profiles, and other compounds beneficial for nutrition and medicine (Salentijn *et al.,* 2015; Fike, 2016).

2.3.1 Production of Hempseed

Hempseed has been utilized as a food source throughout recorded history, consumed raw, cooked, or roasted. It was recognized as one of the "five grains" of ancient China and has been used for over 3,000 years as both human food and animal feed (Cherney & Small, 2016).

Hempseed typically consists of around 30% oil, 25% carbohydrates, and 30% protein, along with significant amounts of dietary fibre, vitamins, and minerals (Callaway, 2004; Schluttenhofer & Yuan, 2017). Hemp cultivation for seed production began in China around 2800 BC, later spreading to India, Persia, and eventually Europe (Kolodziejczyk *et al*., 2012). In China, roasted hempseed is still sold as snacks by street vendors, and some traditional hempseed foods persist in parts of Europe. Historically, hempseed oil was also used for lighting, but by the 20th century, its primary use was in bird feed, with oilseed cultivation largely abandoned. Recently, however, Europe and Canada have made advancements in breeding oilseed cultivars. Today, major hempseed-producing countries include Canada, Australia, Austria, China, Great Britain, France, and Spain. In the U.S., states such as North Dakota and Kentucky have recently legalized hempseed production (Wang & Xiong, 2019).

While some local cultivars in China produce large hempseeds for snack food, domestication for large-scale human consumption remains limited. Hemp food products currently occupy a niche market, primarily in natural and specialty food outlets, but their exceptional taste and nutritional benefits suggest they may become more popular in Western diets. For example, blending extruded rice with hempseed powder has been found to improve nutritional quality, increase bulk density, and reduce water absorption index (Norajit *et al*., 2011).

Global hempseed production saw its peak in the 20th century, reaching 80,448 tons, but it has since declined. China, once the leading producer, reduced its output from 48,000 tons in 1970 to 26,000 tons in 2000, and only 14,931 tons in 2017. However, European countries, particularly France, have emerged as leading producers, with France achieving a peak output of 82,707 tons in 2017 (Schluttenhofer & Yuan, 2017).

2.3.2 Chemical composition of Hempseed

Hempseed is known to be highly nutritious, containing significant amounts of oil, protein, dietary fibre, vitamins, and minerals. Its composition includes 35.5% crude oil, 24.8% crude protein, 27.6% fibre, and 5.6% ash (Callaway, 2004). Another study (House *et al*., 2010) reported slightly lower oil, protein, and ash content in whole seeds but found much higher fibre content. Hempseed meal was shown to have a high protein content of 40.7% and 30.5% fibre.

Hempseed oil is rich in polyunsaturated fatty acids and contains an ideal ratio of omega-6 to omega-3 essential fatty acids (2:1 or 3:1), which is beneficial for human metabolism. Its health benefits, oil extraction methods, and their effects on yield and bioactive compounds.

Hempseed protein is considered highly nutritious and beneficial for health (Callaway, 2004). Different hemp protein products, such as hempseed meal, protein isolate, and protein concentrate, have been studied for their nutritional value and food applications. Aside from human food, hempseed and its by-products are excellent for animal feed.

NUTRITION AL VALUE OF HEMP

Table 2.4: Nutrition al value of Hemp per 100 g

Energy	**2,451 kJ (586 kcal)**
Carbohydrate	**4.67 g**
Fat	**48.75 g**
Saturated	4.600 g
Polyunsaturated	38.100 g
Protein	**31.56 g**
Glutamic acid	6.269 g
Arginine	4.550 g
Aspartic acid	3.662 g
Leucine	2.163 g
Sodium	**5 mg**
OMEGA3 (Alpha-Linolenic Acid)	**8.3g**
OMEGA6 (Linoleic Acid)	**28g**
OMEGA9 (Oleic Acid)	**6g**
GLA (Gamma-Linolenic Acid)	**1.4g**

2.4 Protein-Rich Nutritional Snacks

Protein-energy wasting is one of the primary causes of malnutrition among older adults. Adequate protein is essential for maintaining bone density, muscle mass, and muscle strength, as well as preventing unintended weight loss. However, Finnish studies have highlighted that protein intake among home-dwelling older adults is often insufficient. (Rautakallio *et al*., 2022)

Dietary interventions, particularly food-based protein fortification of standard diets, present a practical solution for addressing malnutrition in older adults. Such interventions have been shown to improve clinical outcomes associated with malnutrition (Carson *et al*., 2014). They are also cost-effective and require minimal implementation costs. Personalized dietary advice that promotes increased protein intake has demonstrated financial viability and measurable benefits. Optimized meal services, which enhance both the nutritional content of diets and overall quality of life, have been shown to reduce healthcare costs.

Protein-rich functional foods offer a nutritious and convenient solution to address key health concerns in today's fast-paced world. Their high protein content supports muscle recovery, making them especially beneficial for active individuals and older adults facing protein-energy wasting (Izadi *et al.,* 2018). With a low glycaemic index, foxnuts help regulate blood sugar levels, making these cookies an excellent choice for managing diabetes. Furthermore, their anti-inflammatory properties promote overall well-being, reducing the risk of chronic diseases. By blending traditional ingredients with modern food technology, these functional cookies exemplify the potential of innovative foods to tackle dietary challenges and support healthier lifestyles (Kashyap and Shukla, 2024).

Hemp protein-rich snacks represent a remarkable blend of nutrition, sustainability, and culinary innovation, catering to the rising demand for health-conscious snacking options. Made from the seeds of *Cannabis sativa*, hemp flour boasts an exceptional nutritional profile, offering high-quality plant-based protein with all nine essential amino acids, making it a complete protein source (Joaquín *et al.,* 2023). The robust protein content supports muscle repair and maintenance, making these cookies a perfect choice for athletes, fitness enthusiasts, and individuals seeking enhanced dietary protein intake. Rich in omega-3 and omega-6 fatty acids, hemp flour contributes to heart health and reduces inflammation, while its abundance of dietary fibre, essential minerals like magnesium, zinc, and iron, and antioxidants combats oxidative stress and promotes overall well-being (Krüger *et al.,* 2022).

These cookies are not only convenient for incorporating high-quality protein into daily diets but also play a crucial role in muscle recovery, weight management, and reducing the risk of chronic diseases (Xu *et al.,*2022). The presence of essential fatty acids further enhances cognitive function and supports long-term wellness. Beyond their health benefits, hemp protein-rich cookies align with sustainable practices, as hemp is an eco-friendly crop requiring minimal resources for cultivation. This makes it an ethical and environmentally conscious choice for health-focused consumers. Coupled with its rich nutty flavour, hemp flour adds a delightful taste, ensuring that these cookies appeal to a broad audience while meeting modern dietary needs (Cerino *et al*., 2021).

However, the cost of food can significantly impact diet quality, as nutrient-dense and high-quality foods are often more expensive. Functional foods play a crucial role in promoting health and wellness among the elderly population. As aging progresses, nutrient absorption and metabolic efficiency often decline, necessitating tailored dietary interventions. These foods, enriched with bioactive compounds, can support cognitive function, improve bone health, and strengthen immunity, addressing age-related challenges. Incorporating functional foods into diets not only enhances nutritional intake but also contributes to overall well-being and quality of life in elder individuals (Koskinen *et al.,* 2003). This underscores the importance of advancing research and development in functional food formulations for aging communities. Meal services have proven effective in boosting protein intake among older adults, particularly those living at home. Incorporating protein-rich meals and snacks into daily diets offers a practical and affordable approach to meeting protein requirements. Additionally, integrating protein-rich products into dietary advice and meal services can be an efficient strategy to combat malnutrition in older adults while promoting better overall health (Järvinen *et al.,* 2024).

CHAPTER 3 MATERIALS AND METHODOLOGY

3.1 INTRODUCTION

This chapter focuses on the materials and methods employed in developing cookies using foxnut and hemp seeds. The hemp seeds and foxnut were purchased online in their hulled form, packaged securely in high-quality, well-sealed package. Additional raw materials, including palm sugar and butter, were procured from a grocery store. The palm sugar was loosely packed in a Food grade polythene bag. All the ingredients obtained for the project were clean and of excellent quality.

3.2 RAW MATERIALS REQUIRED

1. Hemp seed (*Cannabis sativa*)
2. Foxnut (*Euryale ferox*)
3. Palm Sugar
4. Butter

Fig 3.1 Hemp seed

Fig 3.2 Foxnut

Fig 3.3 Palm sugar

3.3 EQUIPMENTS USED

Equipment's are a very essential part for making the development process of product easier, faster and precise. Below shown are the major equipment needed for this project of developing a Digestive cracker.

a) Weighing balance
b) Mixer grinder
c) Electric oven

Fig 3.4 Weighing balance **Fig 3.5 Mixer grinder** **Fig 3.6 Microwave Oven**

3.4 PROCESSING METHOD

Table 3.1 Four Different Proportions of Cookies

Sample	Hemp seed	Foxnut	All-purpose flour	Butter	Baking Powder	Palm Sugar
S1 (control)	-	-	75 g	8g	2g	15g
S2 (30%)	11.25 g	11.25 g	52.5 g	8g	2g	15g
S3 (40%)	15 g	15 g	45 g	8g	2g	15g
S4 (50%)	18.75 g	18.75 g	37.5 g	8g	2g	15g

The measurements of other ingredients were the same in all four proportions,

Cookies were prepared by combining the ingredients in 4 different ratios to find the appropriate combination. The ingredients for all the four samples [S1, S2, S3 and S4) were taken in different plates. They were kneaded thoroughly and shaped. The best binding and textural properties were exhibited by the Sample S4 and thus it was selected for further studies.

3.5 PROCESSING OF RAW MATERIALS

3.5.1 PREPARATION OF HEMP FLOUR

Good quality Hemp seeds purchased online was weighed using Radwag electronic balance and roasted in a heated pan for 5 minutes with continuous agitation. After cooling to room temperature, they were crushed in the mixer grinder.

Fig 3.7 Hemp flour

3.5.2 PREPARATION OF FOXNUT POWDER

Good quality Foxnuts purchased online, were weighed and roasted in a heated pan for 5 minutes with constant stirring. Once cooled to room temperature, they were powdered using a mixer grinder.

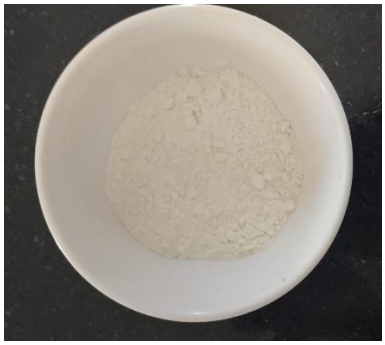


Fig 3.8 Foxnut powder

3.6 PREPARATION OF COOKIES

Cookies were prepared by using four different proportions listed in the **Table 3.1.**

After preparation finished product was further taken for physical and chemical analysis and sensory evaluation.

3.6.1 METHOD OF PREPARATION OF SAMPLE 1 (CONTROLLED)

The oven was preheated at 150°C.The baking tray was placed with parchment baking sheets. The wet ingredients were mixed until creamy texture was obtained. The dry ingredients were whisked and combined with wet mixture and dough is formed. The prepared baking sheets. cookies were shaped using a cookie cutter and was placed on prepared baking sheets. The cookies are then baked in the oven in 150°C for 10-12 minutes.

Preheat oven to 150°C

↓

Line baking sheets with parchment paper

↓

Mix wet ingredients: Butter (8g) + Palm Sugar (15 g) until creamy

↓

Whisk dry ingredients: All-Purpose Flour (75 g)

↓

Combine dry ingredients with wet mixture to form a dough

↓

Shape cookies and place on prepared baking sheets

↓

Bake cookies in oven (150°C) for 10–12 minutes

↓

Cool cookies to room temperature

3.6.2 METHOD OF PREPARATION OF SAMPLE 2 (30%)

The oven was preheated at 150°C.The baking tray was placed with parchment baking sheets. The wet ingredients were mixed until creamy texture was obtained. The dry ingredients were whisked and combined with wet mixture and dough is formed. The prepared baking sheets. cookies were shaped using a cookie cutter and was placed on prepared baking sheets. The cookies are then baked in the oven in 150°C for 10-12 minutes

Preheat oven to 150°C

↓

Line baking sheets with parchment paper

↓

Mix wet ingredients: Butter (8 g) + Palm Sugar (15 g) until creamy

↓

Whisk dry ingredients:

- All-Purpose Flour (52.5 g)
- Hemp Flour (11.25g)
- Foxnut Powder (11.25g)

↓

Combine dry ingredients with wet mixture to form a dough

↓

Shape cookies and place on prepared baking sheets

↓

Bake cookies in oven (150°C) for 10–12 minutes

↓

Cool cookies to room temperature

3.6.3 METHOD OF PREPARATION OF SAMPLE 3 (40%)

The oven was preheated at 150°C.The baking tray was placed with parchment baking sheets. The wet ingredients were mixed until creamy texture was obtained. The dry ingredients were whisked and combined with wet mixture and dough is formed. The prepared baking sheets. cookies were shaped using a cookie cutter and was placed on prepared baking sheets. The cookies are then baked in the oven in 150°C for 10-12 minutes

Preheat oven to 150°C

↓

Line baking sheets with parchment paper

↓

Mix wet ingredients: Butter (8 g) + Palm Sugar (15 g) until creamy texture

↓

Whisk dry ingredients:

- All-Purpose Flour (45g)

- Hemp Flour (15 g)

- Foxnut Powder (15 g)

↓

Combine dry ingredients with wet mixture to form a dough

↓

Shape cookies and place on prepared baking sheets

↓

Bake cookies in oven (150°C) for 10–12 minutes

↓

Cool cookies to room temperature

3.6.4 METHOD OF PREPARATION OF SAMPLE 4 (50%)

The oven was preheated at 150°C.The baking tray was placed with parchment baking sheets. The wet ingredients were mixed until creamy texture was obtained. The dry ingredients were whisked and combined with wet mixture and dough is formed. The prepared baking sheets. cookies were shaped using a cookie cutter and was placed on prepared baking sheets. The cookies are then baked in the oven in 150°C for 10-12 minutes

Preheat oven to 150°C

↓

Line baking sheets with parchment paper

↓

Mix wet ingredients: Butter (8 g) + Palm Sugar (15 g) until creamy

↓

Whisk dry ingredients:

All-Purpose Flour (37.5 g)/ Hemp Flour (18.75 g)/ Foxnut Powder (18.75 g)

↓

Combine dry ingredients with wet mixture to form a dough

↓

Shape cookies and place on prepared baking sheets

↓

Bake cookies in oven (150°C) for 10–12 minutes

↓

Cool cookies to room temperature

3.7 PHYSICO – CHEMICAL ANALYSIS

3.7.1 THICKNESS

The thickness of the cookies was measured in mm using Digital Vernier Calliper Skadio with 0.1 mm Resolution.

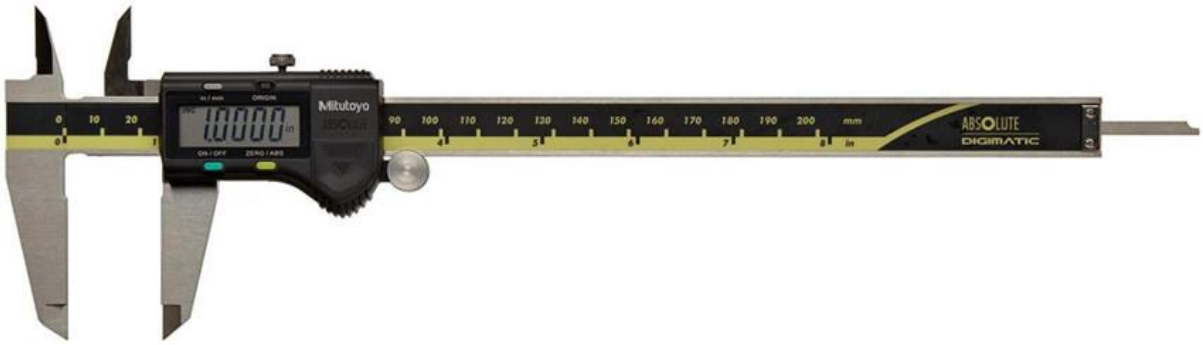


Fig 3.9 Digital Vernier Calliper

3.7.2 DIAMETER

The diameter of the cookies was measured in mm using Vernier Calliper Skadio with 0.1 mm Resolution.

3.7.3 VOLUME

Volume of cookie is defined as the area multiplied by thickness.

$$\text{Volume (cm}^3\text{)} = \frac{d^2\pi t}{4}$$

3.7.4 SPREAD RATIO

The spread ratio was determined by using this formula,

$$\text{Spread ratio} = \frac{Diameter}{Thickness}$$

3.7.5 DETERMINATION OF GLUTEN CONTENT BY WET METHOD

The wet method for determining the gluten content of flour or other food products is widely used due to its accuracy in isolating and quantifying gluten. The method involves the separation of gluten from other components in the sample using water, followed by the drying and weighing of the gluten fraction. A representative sample of the flour or food product (typically 10–20 g) was accurately weighed using an analytical balance. The sample was transferred into a 250 mL beaker or similar container. About 100 mL of distilled water was added to the beaker containing the sample. The sample was mixed thoroughly with a stirring rod or mechanical stirrer to form a uniform slurry. The mixture was allowed to stand for about 15-20 minutes to ensure that the starch and other non-gluten components hydrated and separated from the gluten. The mixture was then kneaded by hand or using a stirrer for a few minutes to allow the gluten to form a sticky, elastic mass. The slurry was rinsed with distilled water to remove soluble starches and other soluble materials. The rinsing process typically involved using a muslin cloth or sieve to collect the gluten mass, while washing away the non-gluten fraction. Excess water was used to ensure complete washing, and the washing process was repeated until the wash water ran clear. After thorough washing, the gluten mass was transferred into a clean beaker or bowl. The remaining non-gluten components (mostly starch) were discarded. The wet gluten mass was carefully removed from the container and placed in a pre-weighed porcelain dish or aluminium foil. The sample was dried in a hot air oven set to 105°C for 4-6 hours or until the gluten reached a constant weight. It was essential to ensure that the sample was completely dry, as any remaining moisture could lead to inaccurate results. After drying, the gluten was cooled in a desiccator to prevent moisture absorption from the air. The dried gluten was carefully weighed using an analytical balance to obtain the final weight. The weight of the gluten was recorded as the gluten content of the sample

3.8 SENSORY EVALUATION OF DEVELOPED COOKIES

For conducting the sensory evaluation, 5 point Hedonic scale was followed.

HEDONIC SCALE

Like extremely	5
Like slightly	4
Neither like nor dislike	3
Dislike slightly	2

Dislike extremely	1

3.9 PROXIMATE ANALYSIS

On the basis of highest score on the hedonic scale exhibited by S3, the sample was sent to the laboratory for proximate analysis. The procedure or analytical method conducted for the chemical analysis is based on the standard procedures conducted by Biogenic Labs, Nettoor.

3.9.1 DETERMINATION OF CARBOHYDRATE

The determination of total carbohydrates was carried out as per IS 1656:2007**,** using the difference method after the estimation of moisture, crude protein, ether extract, crude fibre, and total ash.

Total carbohydrates = 100 – (A+B+C)

Where

A= percent by mass of moisture

B = percent by mass of total protein

C = percent by mass of fat

D = percent by mass of Total ash

3.9.2 DETERMINATION OF MOISTURE CONTENT

Approximately 2–5 g of the sample was weighed into a pre-weighed dish. The sample was dried in a hot air oven maintained at 100–105°C until a constant weight was obtained. The moisture content (%) was calculated based on the weight loss.

Moisture Content (%) =Initial Weight / (Initial Weight−Final Weight) ×100

3.9.3 DETERMINATION OF TOTAL ASH

The determination of total ash content was carried out following the procedure outlined in IS 12711:1989. The method involved incineration of the organic matter at a high temperature to obtain the inorganic residue (ash).

A clean, dry crucible was accurately weighed and the weight was recorded. Approximately 2–5 g of the finely ground sample was placed into the crucible and was weighed accurately. The

crucible containing the sample was initially placed on a low flame (or in a hot air oven at approximately 250°C) until the material was completely charred, avoiding flaming. The crucible was then transferred into a preheated muffle furnace maintained at about 550°C.The sample was ignited for 4–6 hours or until a light grey or white ash was obtained, indicating complete combustion of organic matter. After ignition, the crucible was cooled in a desiccator to room temperature to prevent moisture absorption. The crucible with ash was accurately weighed. If any carbonaceous material was observed, the sample was reheated in the furnace until complete ashing was achieved.

Total Ash (%) = (Weight of Sample Taken / Weight of Ash) × 100

3.9.4 DETERMINATION OF PROTEIN CONTENT

The determination of protein content was performed following the Kjeldahl method as prescribed by IS 7219:1973 (Reaffirmed 2020), which specifies procedures for determination of protein in food and feed products.

About 0.5–2.0 g of the finely ground, well-mixed sample was accurately weighed into a Kjeldahl digestion flask.10–15 mL of concentrated sulfuric acid and a small amount of catalyst mixture were added to the flask. The sample was digested by heating gently at first and then more vigorously until the solution became clear, indicating complete digestion of organic matter. The digest was cooled to room temperature. The cooled digest was diluted with distilled water. The mixture was transferred to the distillation apparatus. Excess sodium hydroxide solution was added to make the solution strongly alkaline. The liberated ammonia was distilled and was absorbed into a known volume of standard boric acid solution containing an appropriate indicator. The ammonia collected in boric acid was titrated with standardized hydrochloric acid or sulfuric acid until the endpoint (colour change) was reached. A reagent blank was similarly prepared and titrated.

Nitrogen (%) =$W(V1-V2) \times N \times 1.4007$

Where:

V1 = Volume (mL) of acid used for sample titration

V2 = Volume (mL) of acid used for blank titration

N = Normality of standard acid

W = Weight of sample in grams

Then, protein content (%) was calculated by multiplying the nitrogen content (%) by a conventional factor, usually 6.25 for food/feed:

Crude Protein (%) =Nitrogen (%) ×6.25

3.9.5 DETERMINATION OF ENERGY VALUE

The energy content of the sample was determined using the FAO Food Energy Method, which calculates the total energy derived from the major macronutrients: carbohydrates, proteins, and fats, and includes alcohol when present. This method is based on the energy contributions of these components, using standardized physiological energy values for each nutrient.

The energy content of the sample was calculated by summing the caloric contributions from carbohydrates, proteins, fats, and alcohol (if applicable). The energy contributions were determined using the following conversion factors:

Carbohydrates: 4 kcal/g (17 kJ/g)

Proteins: 4 kcal/g (17 kJ/g)

Fats: 9 kcal/g (37 kJ/g)

Alcohol: 7 kcal/g (29 kJ/g)

The total energy content of the sample (in kcal or kJ) was calculated using the formula:

Total Energy (kcal or kJ) = (Carbohydrates (g)×4) +(Proteins (g)×4) +(Fats (g)×9) + (Alcohol (g)

3.9.6 DETERMINATION OF IRON BY AAS METHOD

The determination of iron (Fe) in food, feed, and other products was carried out according to the AOAC Official Method 999.11 (20th Edition), using Atomic Absorption Spectroscopy (AAS). This method enables the accurate and precise quantification of iron in a variety of matrices.

Approximately 1–5 g of the homogenized sample was accurately weighed using an analytical balance. The sample was subjected to acid digestion in order to break down organic matter and

release iron.10–20 mL of concentrated nitric acid (HNO_3) was added to the sample. The sample and acid mixture were heated on a hot plate or in a microwave digestion system until complete digestion was achieved, which was indicated by a clear solution. After digestion, the solution was cooled, and the volume was adjusted to 50–100 mL with deionized water in a volumetric flask. The digested sample was introduced into the AAS system using a liquid sample introduction method, such as a pneumatic nebulizer. The instrument measured the absorption of light at the iron-specific wavelength (248.3 nm), and the iron concentration in the sample was determined from the calibration curve. A blank solution (prepared without the sample, following the same digestion procedure) was analysed to account for any contamination or interference during the digestion process. The iron concentration in the sample was calculated using the calibration curve, which was constructed by plotting the known concentrations of the calibration standards against their corresponding absorbance values. The equation for calculating the iron concentration is as follows:

Iron Concentration (µg/g)=(Slope of Calibration Curve)/(Absorbance of Sample−Absorbance of Blank)

3.9.7 DETERMINATION OF MAGNESIUM BY AAS METHOD

The determination of magnesium in food, feed, and other products was carried out according to the method BL/MOA/CH/037, using Atomic Absorption Spectroscopy (AAS). A representative portion of the sample (approximately 1–5 g) was accurately weighed using an analytical balance. After digestion, the solution was allowed to cool, and the volume was adjusted to 50–100 mL with deionized water in a volumetric flask. The digested sample was introduced into the AAS system using a liquid sample introduction method, such as a pneumatic nebulizer. The AAS measured the absorption of light at the magnesium-specific wavelength (285.2 nm). The magnesium concentration in the sample was determined based on the absorbance value relative to the calibration curve. A blank solution, prepared using the same digestion procedure but without the sample, was analysed to account for any contamination during the digestion and analysis processes. The magnesium concentration in the sample was determined using the calibration curve, which was constructed by plotting the known concentrations of magnesium standards against their corresponding absorbance values.

Magnesium Concentration (µg/g) = (Absorbance of Sample−Absorbance of Blank)/ Slope of Calibration Curve

3.9.8 DETERMINATION OF MAGNESIUM BY AAS METHOD

The determination of zinc (Zn) in food, feed, and other products was carried out according to AOAC Official Method 999.11 (20th Edition) using Atomic Absorption Spectroscopy (AAS). A representative portion of the sample, typically 1–5 g, was accurately weighed using an analytical balance. The sample was subjected to an acid digestion process to break down organic matter and release the zinc. The digested sample was introduced into the AAS system using a liquid sample introduction method, such as a pneumatic nebulizer. The AAS measured the absorption of light at the zinc-specific wavelength (213.9 nm) and determined the zinc concentration in the sample based on the calibration curve. A blank sample (prepared without the sample but following the same digestion procedure) was analysed to account for any contamination during digestion and analysis. The blank's absorbance was subtracted from the sample's absorbance during calculation. The concentration of zinc in the sample was determined using the calibration curve.

Zinc Concentration (μg/g)=(Slope of Calibration Curve)/(Absorbance of Sample−Absorbanc e of Blank)

3.9.9 DETERMINATION OF DIETARY FIBRE

The determination of dietary fibre in food products was carried out following the guidelines provided in IS 11062: 1984. This method quantifies the total dietary fibre (TDF), which includes both soluble and insoluble fibre fractions. The method involves a series of extraction and filtration steps to separate the fibre components, followed by gravimetric determination of the residue.

A representative portion of the sample (5–10 g) was accurately weighed using an analytical balance. The sample was ground to a fine powder if necessary, using a suitable grinder to ensure uniformity. The sample was subjected to enzymatic treatment to remove starches and other non-fibre carbohydrates. This was done by adding 1% amylase solution and heating the mixture in a boiling flask at 60°C for 30 minutes with occasional stirring. After starch removal, the sample was treated with 1% protease solution and heated at 60°C for 30 minutes to digest the proteins. This ensures that only the fibre components remain. After the digestion, the solution was neutralized using 0.5% sodium hydroxide if necessary to maintain a neutral pH. The digested sample was washed with hot water to remove soluble sugars and other non-fibre substances.

The residue was then filtered through a Büchner funnel or other filtration apparatus. The filtrate was discarded, and the fibre residue was carefully washed with acetone and ethanol to remove any remaining soluble contaminants.

The fibre residue collected on the filter paper was then dried at 105°C in an oven until constant weight was achieved. The dried fibre residue was ashed at 550°C in a muffle furnace for 3–4 hours to burn off any organic material. The remaining ash represents the inorganic content of the fibre. After ashing, the sample was cooled in a desiccator and weighed. The total dietary fibre content was calculated as the difference between the initial weight of the sample and the weight of the residue after digestion, filtration, washing, and ashing.

Total Dietary Fiber (TDF) = (Initial Sample Weight−Weight of Ash Residue) / (Weight of the Sample) ×100

3.9.10 DETERMINATION OF TOTAL PLATE COUNT

The Total Plate Count (TPC), also known as Standard Plate Count (SPC), is a method used to estimate the number of viable microorganisms present in a sample. The procedure follows the guidelines provided in the Bacteriological Analytical Manual (BAM), 8th Edition, Chapter 3, which employs agar plate counting for the determination of bacterial populations in food and other samples.

A representative portion of the sample (typically 25 g or 25 mL) was weighed using an analytical balance. If the sample is solid, it was blended with a sterile diluent (such as peptone water or saline) to make a homogeneous suspension. The sample was then transferred into a sterile stomacher bag or a suitable container, and the required dilution (typically 1:10, 1:100, and 1:1000 dilutions) was prepared by adding sterile diluent. The sample was thoroughly mixed using a vortex mixer to ensure uniform distribution of microorganisms throughout the dilution. Serial dilutions of the sample were prepared in a series of sterile dilution tubes containing 9 mL of sterile diluent. Typically, three dilutions (e.g., 1:10, 1:100, and 1:1000) were made. Sterile pipettes were used to transfer 1 mL of each dilution onto the surface of sterile Plate Count Agar (PCA) or Tryptone Glucose Yeast Extract (TGYE) agar plates. The inoculum was evenly spread over the surface of the agar plate using a sterile spreader to achieve uniform distribution of the sample The inoculated plates were incubated at 35°C to 37°C for 24 to 48 hours in an aerobic environment to allow the growth of bacterial colonies. Plates were observed for colony growth at regular intervals. The incubation time may vary slightly depending on the type of sample and microbial load. After the incubation period, the number of colonies was

counted on the plates that showed a countable range (usually between 30–300 colonies). The number of colonies per plate was counted, and the colony-forming units (CFU) were calculated based on the dilution factor used.

TPC (CFU/g or CFU/mL) =Volume Plated (mL)Number of Colonies × Dilution Factor

3.9.11 SHELF-LIFE STUDIES

The shelf life refers to the period during which the food remains safe to eat. Various factors influence the shelf life of a product, including water activity, pH, oxygen levels, nutrients, natural microflora, and the use of preservatives. Additionally, factors like temperature and relative humidity also play a significant role. Shelf life is a critical aspect of the product that should be clearly stated on its label.

The cookies are made with hemp seed flour, foxnut powder, and other ingredients. They were packaged in an airtight container, wrapped in aluminium foil, and stored in a cool, dark, and dry place. Two samples, the controlled S1 and S3, were kept at room temperature for storage. Each sample was labelled with the date of production and it is inspected weekly.

CHAPTER 4 RESULT AND DISCUSSION

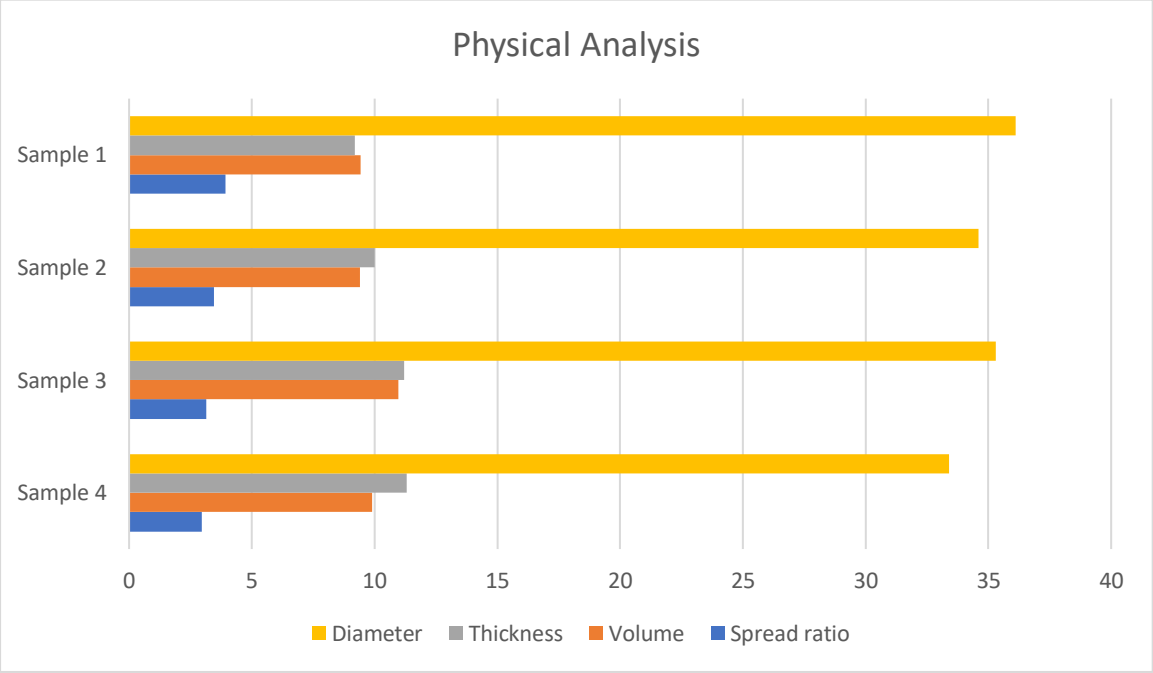
This chapter presents the findings and discussions derived from the observation conducted in the study title 'Development of Hemp seed based digestive cracker incorporated with foxnut'. The primary aim of this study was to create protein enriched digestive cracker. To achieve this goal, a cookie recipe contains Hempseed and Foxnut flour was developed.

4.1 PHYSICO - CHEMICAL ANALYSIS

Physico-chemical analysis of samples was carried out and all of the results were nearly similar for characteristics such as Diameter, Thickness, Volume and Spread ratio. For the physical analysis Vernier Caliper were used.

Table 4.1 Physical Analysis

Samples	Diameter (mm)	Thickness (mm)	Volume (cm^3)	Spread ratio
S1	33.4	11.3	9.9	2.95
S2	35.3	11.2	10.96	3.15
S3	34.6	10.0	9.4	3.46
S4	36.1	9.2	9.42	3.92



Graph 4.1 Physical Analysis

Fig 4.1 Sample 1

Fig 4.2 Sample 2

Fig 4.3 Sample 3

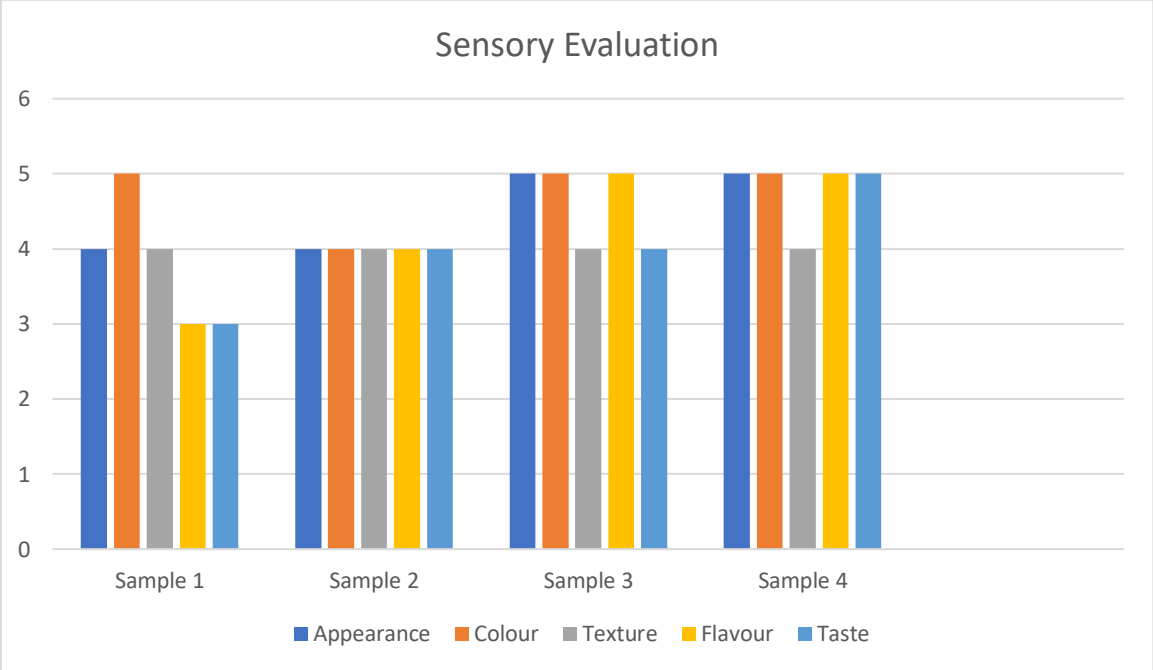
Fig 4.4. Sample 4

4.2 SENSORY EVALUATION

The sensory scores of cookies are depicted in **Table 4.2.** According to the results presented, there was a significant increase in appearance, taste and overall acceptability in S3 cookies. No significant change was observed in the colour, flavour, and texture of cookies prepared from hemp seed and foxnut flour.

Table 4.2 Sensory Analysis

Sample	Appearance	Colour	Texture	Flavour	Taste	Overall acceptance
S1	4	5	4	3	3	3
S2	4	4	4	4	4	4
S3	5	5	4	5	4	5
S4	5	5	4	5	5	5



Graph 4.2 Sensory Analysis

From the data obtained in Table 4.2, the best of the cracker samples of increased nutritional value in terms of organoleptic parameters is S3. In almost all indicators, the tasters gave it 5

points in each characteristic. It had a regular shape, the surface without cracks and slightly rough. The appearance is baked, the aroma is characteristic of this cracker, with no foreign tastes, and a slight hemp flour taste. There are no traces of non-molten material in the product.

4.3 PROXIMATE ANALYSIS

The proportion of the final developed product was Sample 3 (40%) was good of appearance and flavour. Hence was decided as the final product and sent to proximate analysis along with Sample 1 (controlled sample). Chemical analysis was done for the nutritional comparison such as Moisture, Total ash, Protein, Carbohydrate, Energy, Minerals, Dietary fibre and Total plate count in 500g of both the Sample. According to the results of the study of the physico-chemical quality indicators of the cracker samples of increased nutritional value and the control sample (Table 4.3), the differences in the obtained indicators are insignificant and all are within the limits specified in the standard. The result of the analysis is given below:

Table 4.3 Proximate Analysis

Sl. No	Parameter	Test Method	Unit	Test results	
				S 1	S 3
1	Moisture	IS 12711: 1989	g/100g	7.28	20.2
2	Total Ash	IS 12711: 1989	g/100g	0.94	1.41
3	Protein	IS 7219	g/100g	7.73	14.4
4	Carbohydrate	IS 1656 Annex C	g/100g	74.8	49.6
5	Energy	FAO (Food Energy Method)	Kcal/100g	414	386
6	Iron	AOAC Official Method 999.11 (20th Edition) (By AAS)	mg/100g	-	2.71
7	Magnesium	BL/MOA/CH/037 (By AAS)	mg/100g	-	7.05
8	Zinc	AOAC Official Method 999.11 (20th Edition) (By AAS)	mg/100g	-	0.88
9	Dietary Fiber	IS 11062: 1984	mg/100g	2.13	4.28
10	Total Plate Count	BAM 8th Edition Ch: 3	cfu/g	<10	<10

4.3.1 GLUTEN ANALYSIS

In the observation of gluten analysis, S1- controlled sample- contain **8.8%** wet gluten. While S3 (40%) contain **2.8%** of wet gluten.

- Sample 1 exhibited high elasticity and strength, typical of high-gluten doughs.

- Sample 3 showed reduced elasticity, aligning with its low-gluten composition and suitability for healthier, gluten-free cookie development. The dough washing method effectively differentiated the gluten levels between the two samples.

Sample 3's properties align with the objectives for developing healthier, gluten-free cookies. Further research on alternative ingredients and formulations is recommended.

4.3.2 SHELF LIFE

The shelf life refers to the period during which the food remains safe to eat. Various factors influence the shelf life of a product, including water activity, pH, oxygen levels, nutrients, natural microflora, and the use of preservatives. Additionally, factors like temperature and relative humidity also play a significant role. Shelf life is a critical aspect of the product that should be clearly stated on its label.

The cookies in question are made with hemp seed flour, foxnut powder, and other ingredients. They are packaged in an airtight container, wrapped in aluminium foil, and stored in a cool, dark, and dry place. Two samples, the controlled S1 and S3, are kept at room temperature for storage. Each sample is labelled with the date of production and it is inspected weekly. To date, regular inspections of the samples show no evidence of spoilage or Mold growth, no visible changes in texture over a period of four weeks.

Table 4.4 Shelf-Life Studies of Stored Cookies

Sl. No	Parameter	Test method	Unit	Test Result
1	Moisture content	IS 12711: 1989	g/100g	9.51
2	Total Plate Count	BAM 8th Edition Ch:3	Cfu/g	<10

CHAPTER 5 SUMMARY AND CONCLUSION

The research Thesis presented above focuses on the Nutrient packed digestive crackers by incorporating Hemp seed and foxnut. The study concluded that the digestive cracker prepared using Hemp seed flour and Foxnut powder had greater nutritional content.

Cookies baked with these ingredients were higher in Protein, Carbohydrate, Dietary Fibre, Vitamins and Minerals; it had the following contents per 100 grams: 14.4 g, 49.6g, 4.28 mg, 2.71mg, 7.05mg and 0.88 mg respectively.

A sensory study revealed that the samples such as **S2** Hemp seed flour (11.25 g) + Foxnut powder (11.25 g) + All-purpose flour (52.5 g) + Palm sugar (15 g) + Butter (8 g) i.e. **S3** i.e. Hemp seed flour (15 g) + Foxnut powder (15 g) + All-purpose flour (45 g) + Palm sugar (15 g) + Butter (8 g) had good acceptability among the panellists.

The proximate analysis carried out for the most important nutritional elements including Energy, Carbohydrate, Dietary Fiber, Protein, Ash, vitamins and minerals. Overall analysis indicated that cookies with acceptable physical and improved nutritional characteristics was produced by incorporating Hemp seed and Foxnut, thereby adding health benefit to baked products and also contributing to controlling several life style diseases like Diabetes. This has a higher content of protein and Dietary fibre along with potassium Zinc and Iron. These are a healthy substitute for typical snacks without sacrificing their distinctive flavour or nourishing properties. Better technologies can also be added to enhance the storage, processing, preservation of these cookies in the food industries.

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SCORE CARD

HEDONIC RATING SCALE

Name:

Product:

Date:

The samples are provided. Taste the sample and check how much you like or dislike each of the characters.

	Appearance	Colour	Texture	Flavour	Taste	Overall acceptance
Sample1						
Sample2						
Sample3						
Sample4						
Sample5						

5 point hedonic scale

5	Like a lot
4	Like a little
3	Neither like or dislike
2	Dislike a little
1	Dislike a lot

Comments: signature:

Biogenic Labs

16/338(23/600),
Mulakara Building,
Market Road, Nettoor P.O.,
Cochin,Kerala -682040
info@biogenicdabs.in
+91 85478 60385
+91 97476 08202

TEST REPORT

Kerala State Pollution control Board 'A' Grade Laboratory : PCB/LAB/C15/2018

Test Report No.	:BL/TR/2025/1447	Report issue date	: 01/04/2025

Name & address of the customer: Ms. Neha Jose Baby, St. Theresa's College, Ernakulam, Food Processing Department.		Sample Name Provided by the Customer	: Cookies
		Sample submitted by	: Customer
Sample ID	: BL/2025/1447	Sample quantity	: 500g
Sampling date (Provided by the customer)	: 24/03/2025	Customer Sample ID if any	:40% Sample
Sample receipt date	: 24/03/2025	Description & condition of the sample: Food stuff & received in good condition.	
Analysis performing date	: 24/03/2025– 01/04/2025		

CHEMICAL

Sl No	Parameters	Test Method	Unit	Test Result	Limit of Quantification (LOQ)
1	Moisture	IS 12711: 1989	g/100g	20.2	0.10
2	Total Ash	IS 12711: 1989	g/100g	1.41	0.10
3	Protein	IS 7219	g/100g	14.4	0.10
4	Fat	IS 12711	g/100g	14.4	0.10
5	Carbohydrate	IS 1656 Annex C	g/100g	49.6	-
6	Energy/Calorie	FAO (Food Energy Method)	Kcal /100g	386	-
7	Iron	AOAC Official Method 999.11 (20th Edition)(By AAS)	mg/100g	2.71	0.20
8	Magnesium	BL/MOA/CH/037 (By AAS)	mg/100g	7.05	5.00
9	Zinc	AOAC Official Method 999.11 (20th Edition)(By AAS)	mg/100g	0.88	2.00
10	Dietary Fibre	IS 11062: 1984	g/100g	4.28	0.10

❖ End of report ❖

For Biogenic Labs
Authorized signatory
SANGEETH K.S
LAB MANAGER

Biogenic Labs

16/338(23/600),
Mulakara Building,
Market Road, Nettoor P.O.,
Cochin,Kerala -682040
info@biogeniclabs.in
+91 85478 60385
+91 97476 08202

TEST REPORT

Kerala State Pollution control Board 'A' Grade Laboratory : PCB/LAB/C15/2018

Test Report No.	:BL/TR/2025/1447	Report issue date	: 01/04/2025

Name & address of the customer: Ms. Neha Jose Baby St. Theresa's College, Ernakulam Food Processing Department.	Sample Name Provided by the Customer : Cookies
Sample ID : BL/2025/1447	Sample submitted by : Customer
Sampling date (Provided by the customer) : 24/03/2025	Sample quantity : 500g
Sample receipt date : 24/03/2025	Customer Sample ID if any : 40% Sample
Analysis performing date : 24/03/2025 - 26/03/2025	Description & condition of the sample: Food stuff & received in good condition

BIOLOGICAL

Sl No	Parameter	Test Method	Unit	Test Result
1	Total Plate Count	BAM 8th Edition Ch:3	Cfu/g	<10

Note: <10 is equal to absent

❖ End of report ❖

For Biogenic Labs
Authorized signatory

CHINJU A.S
SENIOR MICROBIOLOGIST