Development and Characterization of Gluten-free Functional Cookies Enriched with Unripe Papaya Powder

Thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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November 2024

Dedicated

To

my beloved family

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CERTIFICATE

It is certified that the work contained in this thesis entitled **"Development and Characterization of Gluten-free Functional Cookies Enriched with Unripe Papaya Powder"** submitted by **Mrs. Paushali Mukherjee** for the award of the degree of Doctor of Philosophy has been carried out in the Department of Chemical Engineering, Indian Institute of Technology Guwahati under our supervision. To the best of our knowledge, this work is not submitted elsewhere for the award of any other degree or diploma.

Date: 11/25/2024

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DISCLAIMER

The experimental, formulation and characterization related data presented in this Ph.D. thesis was carried out by me and is reported after due verification. To the best of my knowledge, the work summarized in this Ph.D. thesis is not submitted elsewhere for the award for any other degree or diploma.

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Ph.D. Thesis Abstract

This Ph.D. thesis addresses the development and characterization of novel gluten-free and gluten-reduced functional cookies. Such novel and optimal formulations were achieved through the incorporation of various gluten-free grain flours, and through the optional substitution of optimally oven dried papaya peel (PPE) and pulp (PPU) powders. Subsequently, the thesis also delved into the further functionalization of the cookies through the fortification with either encapsulated catechin or papaya leaf extract. The utilized non-gluten flours refer to the flours/ powders of rice, roasted chickpea, Bengal gram, soy, finger millet, oats, dried unripe papaya pulp and peel. These ingredients are well known indigenous yet abundantly agricultural produces of the North-east India.

Subsequent to the assessment of the physicochemical, functional and nutritional properties and other characterization such as the FESEM, FTIR, XRD, particle size, DSC, and color of the mentioned flours/powders, the cookie formulational research was targeted based on sensory analysis. This was followed with the characterization of optimally formulated cookies for the assessment of their morphological and nutritional properties. The ultimately achieved cookie formulations can be categorized into (a) gluten-reduced functional cookies with enriched constitution of bioactives and sensory parameters (b) millet and non-millet based functional cookies with and without substitution of the PPU and PPE flours and (c) gluten-free functional cookies further enriched in terms of encapsulated fortificants (such as catechin extract infused encapsulant or papaya leaf extract infused encapsulant). In summary, all cookies were assessed in terms of physico-chemical properties, functional properties, sensory properties, rheological

behavior, bio-accessibility, and storage characteristics. These achievements enabled to critically address highly subjective knowledge gaps in the field of study and through the creation of innovative cookie solutions that addressed good combinations of healthier, nutritional, sensory and storage parameters.

The fulfilled objectives of the Ph.D. thesis have been listed as follows:

1. Preparation and characterization of the north-east India based gluten-free grain flours and unripe papaya pulp and peel powder.
    a. Evaluation of physico-chemical properties of the gluten-free grain flours which are abundantly produced in the North-east India.
    b. Optimality of drying process parameters for the retention of maximum bioactive components for unripe papaya pulp and peel powder production and physico-chemical properties of the produced powders.
2. Preparation and sensory characterization based ingredient compositional optimization of gluten-free cookies being formulated with grain based flours along with in-vitro bioactivity, storage and acrylamide studies.
    a. Sensory evaluation based on 9-point and fuzzy logic methodologies.
    b. Evaluation of other characteristics of grain flours based optimal gluten-free cookie formulations.
3. Preparation and sensory characterization based ingredient compositional optimization of unripe papaya peel and pulp powder substituted gluten-free cookies along with in-vitro bioactivity, storage and acrylamide studies.
    a. Sensory evaluation based on 9-point and fuzzy logic based sensory characteristics

b. Evaluation of other characteristics of grain flours and unripe papaya pulp and peel powder based optimal gluten-free cookie formulations

4. Inclusion complexation and ion gelation-based encapsulation of catechin extracts from commercial green tea leaves and papaya leaf extract respectively and subsequent characterization of the gluten-free cookies prior to and after fortification with the mentioned encapsulants.

The thesis first objective ascertained the optimal drying of the sliced unripe papaya pulp and grated unripe papaya peel at 105°C for 45 minutes duration. Thereby, excellent nutritional content, and flavor profiles were achieved for the PPU and PPE in terms of the maximum retention of total phenolic content, total flavonoid content, antioxidant activity, and vitamin C content. Both PPU and PPE exhibited high levels of ash and crude fiber content and their compatibility with various starch samples. Also, similar characterization in terms of mineral content and functional properties were addressed for the mentioned commercially procured grain flours. Among the alternate eight distinct flours, the green gram flour was analyzed with the highest moisture content at 12.59%. In all grain/legume flours and PPU/PPE powder samples, the water activity altered from 0.45 to 0.62. Also, while the PPE has been analyzed with the highest ash content of 8.34 g/100g, the soy flour was analyzed to possess lowest ash content value of 5.51 g/100g. The PPE also had the highest crude fiber content of 11.95g/100g. This is followed by PPU (9.05g/100g) and soy flour (6.39g/100g). Further, the green gram flour had the highest concentration of carbohydrates at 88.71g/100g. This is followed by wheat (86.56g/100g) and rice flours (78.68g/100g). Soy flour had the highest protein level at 42.7g/100g. This is followed by Bengal gram flour (22.57g/100g) and roasted chickpea flour (18.84g/100g). While oats flour had the highest fat content at 7.77g/100g, the wheat flour had the lowest fat content value of 1.03g/100g. Similar alterations in

the mineral content as well conveyed the need for composite powders based cookie formulation. Through such efforts, best cookie formulations can be achieved through the alteration of ingredients compositional alterations.

For the fulfillment of the second objective of the Ph.D. thesis, the formulational research utilized various functional ingredients such as the grain flours, wholesome shortenings, and alternate sweeteners. Accordingly, the thesis enabled the realization of cookies with enhanced combinations of bio-active constituents, mineral content, and sensory parameters. Among the 106 alternate cookie formulations, only six formulations qualified as the best formulations with optimal sensory profiles. Among these six best formulations being achieved with only grain/legume flours and not the PPU and PPE, the MS2 formulation possessed highest overall acceptability score (8.78). This is followed by the WS2 formulation (8.56). Based on the fuzzy logic scale, the MS2 formulation (GM-Sugar-Butter) achieved the highest score of 0.82 ("excellent" category). Also, MS2 exhibited the lowest melting point at 190°C, and a comparatively weaker structure. This is supported by its lower ΔCp value (1.014 J/(gK)). MS2 also contained Mg (7.7 ppm). For the millet based cookie formulation RaRJ5, good mineral content in terms of Ca (3.36 ppm) was ascertained. For the formulation, promising post in-vitro AA value of 17.25% was achieved. Also, RaOaJ5 exhibited good mineral content in terms of Mg (9.94 ppm); Mn (0.68 ppm) content. The sample also had the highest ΔCp value (2.316).

For the fulfillment of the third Ph.D. thesis objective, among 96 alternate PPU and PPE substituted cookie formulations, very few formulations have been opined to the best in terms of the assessed sensory parameters. The sensory score was assessed with two robust methodologies, namely the 9-point hedonic scale and fuzzy logic scale. Also, nutritional analyses including total carbohydrate, total protein, total fat, fiber content, and mineral composition were conducted for the PPU/PPE substituted cookies. Among the sensory analysis based best rated PPU/PPE based cookie samples, the UMS2 and UMS5 formulations have been the best with the 9-point hedonic scale based overall acceptability scores of 8.21 and 7.94, respectively. These scores are supplemented with high ratings for taste, breakability, and texture. Also, URJ2 received commendable ratings for overall acceptability scores of 7.89. Additionally, according to the fuzzy logic scale, the UMS2 (GM-Sugar-Butter) sample achieved the highest score of 0.49 ("excellent" rating). Similarly, URJ2 attained a fuzzy score of 0.38 ("excellent" rating). Nutritional analyses including total carbohydrate, total protein, total fat, fiber content, and mineral composition were conducted for the PPU/PPE substituted cookies. The obtained values were impressive and affirming of the greater functionalization of the PPU/PPE based cookie formulations. The rheological analysis of the dough of alternate cookie sample formulations with and without PPE/PPU powders affirmed useful insights into the stiffness or softness of the dough.

The fourth and final thesis objective involved the assessment of the fortified cookie formulations with either ion gelation based papaya leaf extract encapsulant (Calcium alginate-pectin as a wall material) or ion complexation based catechins extract encapsulant (maltodextrin wall material). Accordingly, a comparative assessment of the IC and IG methods was first addressed. Among IG and IC methods, the IC method ascertained better encapsulation yield (90.53% vs. 86.86% for IG) and TpC efficiency (88.4% vs. 2.35% for IG). The encapsulation process ensured superior retention of bioactive compounds, and even after high-temperature baking and in-vitro digestion. The fortified cookies for the former case (1% ALEXPB cookies) affirmed remarkable nutritional parameters in terms of 5.21g/100g soluble protein; 49.48 mg GAE/g total phenolic content (TpC); 217.22 mg QE/g total flavonoid content (TfC); 31.86% antioxidant activity (AA) assessed after in-vitro digestion. The fortified baked cookie formulation MS2 and UMS2 samples with either 100 mg dried commercial green tea extracts (CE) or 100 mg inclusion complexation based encapsulated dried commercial green tea extracts (IC) affirmed (a) no influence of fortification on the sensory characteristics of the cookies; (b) antioxidants activity of 43.04% for MS2 fortified with 100 mg of CE, 85.04% for MS2 fortified with 100 mg of IC, 51.7% for UMS2 fortified with 100 mg of CE and 86.61% for UMS2 fortified with 100 mg IC; and (c) in-vitro digestion based antioxidants activity of 3.08% for MS2 fortified with 100 mg of CE, 16.19% for MS2 fortified with 100 mg of IC, 4.42% for UMS2 fortified with 100 mg of CE and 19.3% for UMS2 fortified with 100 mg IC. Other characterization methods targeting storage characteristics and acrylamide content assessment yielded promising findings.

In summary, the Ph.D. thesis ascertained the generation of gluten-free novel functional cookies with the unripe papaya pulp and peel powders and other gluten-free grain flours. These findings will further strengthen the noble cause of the cost effective value added product development with the underutilized horticultural produces of the world.

Novelty Statement

The research presented in this thesis is notable for its multifaceted approach to food science, and for a strong emphasis on both innovation and sustainability. The key elements that highlight the novelty of the conducted Ph.D. thesis work can be enlisted as follows:

1. The study successfully identified the optimal drying temperature and time for unripe papaya pulp slices and peel, and for the significant retention of bioactive components (total phenolic content, total flavonoid content, and total antioxidant activity) within a very short drying period of 45 minutes at 105°C oven drying temperature. Such a rapid drying process is highly promising as it balances efficiency with the preservation of nutritional quality. This is an important aspect for food processing industries and for the desired target to affirm healthy characteristics in the dried fruit or vegetable products.
2. The thesis explores a wide range of cookie formulations (almost 106) using grain flours that are indigenous to Northeast India. This aspect of the research is particularly novel as it leverages regional agricultural biodiversity to create gluten-free and gluten-reduced options with diverse flavors and substantial nutritional profiles, including mineral content. This not only contributes to the development of healthier baked goods but also supports the greater utility of local resources. Thereby, the thesis promotes regional agricultural practices and biodiversity.
3. The research incorporates unripe papaya pulp powder and peel, which are typically underutilized or considered waste products, into cookie formulations. This approach exemplifies innovative waste reduction and value addition strategies. Thereby, the

conducted research work can transform low-value agricultural by-products into nutritious food ingredients. Such an aspect underscores the sustainability and eco-friendliness of the research and addresses both food waste and nutritional enrichment.

4. The demonstrated method of catechin extraction from green tea using hot water and without utilizing chemical solvents is a significant innovation. This eco-friendly extraction method ensures that the final product is free from harmful residues. The product aligns with the growing consumer demand for natural and safe food additives. It also demonstrates the feasibility of sustainable practices in food processing.
5. The thesis delves into the fortification of cookies and through the convenient usage of the green extraction and encapsulation technologies for both catechin and papaya leaf extracts. The encapsulation techniques enhance the stability and bioavailability of these bioactive compounds and thereby ascertain upon the associated health benefits being affirmed through the retention of bioactives in the baking process. This is particularly novel as it combines advanced food technology with traditional ingredients. Such an approach is for the generic methodology based functional food product development.
6. Novel cookie formulations have been created with wheat, rice, soy, green gram, oats, finger millet Bengal gram, chickpea flour and unripe papaya pulp and peel powder ingredients. These can be categorized as gluten-reduced, gluten-free, millet based cookies and unripe papaya cookies.

Overall, the thesis has the highest subjective and generic methodology due to its comprehensive methodology that integrates optimal processing techniques, innovative product development, and sustainable practices. The thesis research work not only advances scientific knowledge in food technology but also provides practical solutions for the enhancement of the nutritional value of food products.

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Nomenclature

Abbreviations

AA	Antioxidant activity
AcA	Acrylamide
ANOVA	Analysis of variance
BD	Bulk Density
DSC	Differential Scanning Calorimetry
FESEM	Field emission scanning electron microscopy
HPLC	High pressure liquid chromatography
FTIR	Fourier transform infrared radiation
NMR	Nuclear magnetic resonance
TC	Total Plate Count
TpC	Total polyphenolic content
TfC	Total flavonoid content
PdI	Polydispersity index
ISO	International Organization for Standardization
YMC	Yeast and mold count
RDA	Recommended dietary allowances
DPPH	2,2- Diphenyl-1-picrylhydrazyl
DLS	Dynamic light scattering

FCR	Folin ciocalteu reagent
GAE	Gallic acid equivalent
VC	Vitamin C
QE	Quercetin equivalent
OAC	Oil absorption capacity
WAC	Water absorption capacity
XRD	X ray diffraction
Aw	Water activity

Nomenclature

ALEXPB	Papaya leaf extract calcium alginate beads
CE	Catechin extracts
EU	Enzymatic unit
HWE	Hot water extraction
IC	Inclusion complexation
IG	Ion gelation
PLE	Papaya leaf extract
PPE	Papaya peel powder
PPEc	Papaya peel coarse powder
PPEf	Papaya peel fine powder
PPU	Papaya pulp powder
PPUc	Papaya pulp coarse powder

PPUf	Papaya pulp fine powder
RC	Relative crystallinity
Mc	Moisture content
MS2	Green gram-sugar-butter cookie formulation
RS2	Rice-sugar-butter cookie formulation
WS1	Wheat-sugar-ghee cookie formulation
WS2	Wheat-sugar-butter cookie formulation
RaRJ5	Finger millet- rice-jaggery-shortening cookie formulation
RaOaJ5	Finger millet- oats-jaggery-shortening cookie formulation
UMS2	PPU-PPE-green gram-sugar-butter cookie formulation
UMS5	PPU-PPE-green gram-sugar-shortening cookie formulation
URJ2	PPU-PPE-rice-jaggery-butter cookie formulation

Chapter 1:

Introduction and Literature Review

Chapter 1

Introduction and Literature Review

The chapter addresses research strategies that aim towards the development of gluten-free cookies enriched with functional constituents from grains, and with enhanced their bioavailability and bioactivity traits. Section 1.1 details upon the significance of gluten-free functional cookies from a nutritional and medicinal perspective. Accordingly, the section emphasizes upon the need to address nutritional enrichment through the incorporation of abundant horticultural produce such as the unripe papaya vegetable from Northeast India. The primary goal of such investigations is to fully explore the application potential of gluten-free flours. Thereafter, the section also elucidates upon the enhanced bioavailability of thermo-sensitive bioactive compounds in functionalized cookies. Accordingly, the exploration of micro-encapsulation technique for the bioactive components has been detailed for the green tea extracted catechins and papaya leaf extracted bioactives.

Section 1.2 details upon the targeted perspectives of the Ph.D. thesis. These were based on few important issues that shall be targeted in the available prior art. The section first devotes to the attributes of gluten-free powders. Additionally, the section explores the potential of alternate vegetable powder sources, such as unripe papaya pulp and peel powder, as a viable option for gluten-free functional powder in baked cookie formulations. Consequently, the research efforts converge upon the formulation and sensory optimization of cookies with various types of shortening and sweetening agents for their optimal constitution. Thus, such formulations could be potentially enriched with fiber and can be conveniently substituted with unripe papaya powder to generate a gluten-free product alternative. Such cookies were also assessed with other characterization techniques. Thereafter, the existing prior art in the field *of encapsulation techniques for catechins found in green tea and bio-actives in papaya leaf has been delineated. Further, in-vitro bio-activity assessment, the effect of high temperature processing on the storage, bio-actives constitution in cookie formulations, sensory characteristics, and acrylamide level alteration due to the temperature effect have been addressed in terms of process and product optimality characteristics.*

A detailed account of the prior art in the above mentioned targeted perspectives has been addressed in section 1.3 of the thesis. These refer to research schemes associated to gluten-free powder production and characterization in terms of nutritional profiling, functional properties etc.; formulation of gluten-free functional cookies which constitute sensory analysis based optimal weight ratios of flours, powders, sugars, shortening agents and other additives; catechins and bio-actives extraction followed by encapsulation and fortification in cookie products; and in-vitro, storage characteristics and acrylamide content analysis of the formulated cookies. Subsequently, section 1.4 delineates upon the extent of subjective and objective gaps in the chosen research themes of the Ph.D. thesis. Thereby, the objectives of the thesis and organizational details of the thesis have been respectively detailed in sections 1.5 and 1.6 of the chapter.

1.1 Orientation and Motivation to the Field of Study

Cookies mostly constitute richer proportions of flour, sugar and shortening agents. Being classified as a category of baked goods, the cookies are often produced with the soft wheat flour that possesses lower moisture content (Delcour & Hoseney, 2010). Among various cookie products, the sugar-snap cookie is widely recognized due to its elevated levels of sugars and fats, and relatively lower moisture content. Alongside bread products, chemically leavened gluten-free products such as cookies, biscuits and crackers have also gained enhanced consumer attention due to their unique flavor, acceptable texture profiles and convenient characteristics such as portability. According to the Cookies Market Size, Share & Trends The global cookies market has experienced notable growth in recent years. As of 2024, the market size is expected to reach approximately USD 12.14 billion, with projections indicating it will grow to USD 18.77 billion by 2029, at a compound annual growth rate (CAGR) of 9.10% during the forecast period from 2024 to 2029 (https://www.grandviewresearch.com/industry-analysis/cookies-market, 2024). Thereby, the cookies market has been projected to exhibit about 5.3% compound annual growth rate upto the year 2025.

Ongoing research trends in food products emphasizes upon the custom designing of the products for those with the celiac disease disorder. On a worldwide basis, approximately 1% of the population have been estimated to be with celiac disease disorder (Kurppa et al., 2024). Celiac disease is a well-known autoimmune system disorder and inflammatory disease. Celiac disease is a chronic autoimmune ailment in which the consumption of gluten, a protein present in wheat, barley, and rye, causes an immune reaction that damages the lining of the small intestine. This damage reduces nutrient absorption and can cause a variety of symptoms and consequences. It is triggered due to the gluten's non-desired functionality in the upper small intestine (Shan et al., 2002). Those who suffer with celiac disease exhibit symptoms such as malnutrition, diarrhea, growth retardation, anemia, and fatigue (Green & Jabri, 2003). Wheat, rye, and barley are cereals that contain significant proportions of gluten. Gluten is a group of proteins naturally present in specific types of cereal grains, namely wheat, barley, and rye. It is accountable for the elastic consistency of dough, facilitating its rapid expansion and preservation of its form, and imparting baked items with their resilient texture. Henceforth, such produces are not to be consumed by the patients with the celiac disease. In celiac disease or who exhibit gluten sensitivity or who prioritize their health parameters in this regard.

Functional foods refer to a meal (solid or liquid) providing specific nutritional benefits for human body and therefore regarded as a healthy food. Such foods ascertain nutritional benefits from the naturally occurring micro or macro nutrients added through fortification and enrichment methods. Food bioactives exist in smaller constitution in fruits, vegetables or animal foods and were proven with medicinal benefits for the human body. Thereby, adopting fortification methods, they can be incorporated as an ingredient in functional foods or drinks. Accordingly, conventional foods can be functionalized such as cookies fortified with vitamin C, rice fortified with iron, salt fortified with iodine etc. In the north-east region of India, tea is the most consumed beverage and is also readily available due to wider cultivation. In Assam, over 800 tea estates cultivated tea. Fresh tea leaves contain catechin, a bioactive substance and flavan-3-ol compound. Catechins are flavan-3-ol compounds, a subgroup of flavonoids present in green tea or fresh tea leaves. They constitute between 20-35% of the green tea dry weight and which varies based on tea cultivar type, location of cultivation, and climate. The five major catechins present in tea leaves are (-) - Epigallocatechin gallate (EGCG), (-) – Epigallocatechin (EGC), (-) - Epicatechin gallate (ECG), (-) - Epicatechin (EC) and (+) - Catechin (C) (Baruah et al., 2023; Wei et al., 2011).

Tea catechins have strong antioxidant activity and provide numerous health benefits which include antibacterial, anti-inflammatory, antimicrobial, prevention of cancer and cardiovascular diseases. Catechins can inhibit the absorption of cholesterol by interacting with the micellar absorption of cholesterol in the small intestine (Baruah et al., 2024; Ogawa et al., 2016). Catechins may reduce food intake by affecting appetite-regulating neuroendocrine metabolic regulators. They also reduce calorie intake by slowing the emulsion process and gastrointestinal fat and protein absorption. Additionally, catechins affect the gut microbiota, particularly lacto- and bifidobacteria, which aid in food digestion. They create short fatty acids that accelerate lipid metabolism. They also inhibit preadipocyte growth and proliferation as stated in previous literature. They reduce lipid production, increase lipid breakdown, and improve lipid metabolism. Catechins boost white adipose tissue oxidation, thermogenesis, and lipid excretion through feces (Basu et al., 2023). The catechins are responsible for the medicinal benefits of fresh, green, and white tea. In this Ph.D. thesis an endeavor is made to formulate catechins enriched cookie formulations. The key features targeted in the Ph.D. thesis aims to overcome the limitation associated to direct consumption of catechin, which is detrimental for derived functionality as more than 95% of the catechin consumed is lost in the complex digestion environment of the human body. Accordingly, the thesis aims to adopt encapsulated catechin extracts for the cookies functionalization. Thereby, enhanced bioavailability and nutritional benefits can be achieved.

This Ph.D. thesis targets to develop a range of gluten-free functional cookies by focusing on the physico-chemical properties and formulation of gluten-free composite powders, dough, and the resulting cookie products. The research will utilize various functional ingredients, including flours and powders, dried vegetable powders, wholesome shortenings, and alternate sweeteners, to create cookies with enhanced nutritional content, bioactives, mineral content, and sensory parameters. In summary, the thesis aims to address the challenges associated with the development of gluten-free baked cookie products. Such challenges include technological, nutritional quality, and sensory properties related limitations and issues. The further functionalization of the gluten-free cookies with the bio-actives and catechin also pose a stiffer challenge in the cookie product development and especially in terms of the high temperature baking process and highly complex digestive human body environment. Such conditions are detrimental towards the sustainable exploration of encapsulated catechins and bioactives from leafy extracts in terms of the enhanced bioavailability and bioactivity of the cookie products. Accordingly, the thesis aims to achieve enriched nutritional characteristics of the functional gluten-free cookie products.

1.2 Targeted Perspectives

Based on the aforementioned prominent characteristics, features and issues, the intended focus of the Ph.D. thesis will be delineated in this section and in the subsequent sub-sections. The

Ph.D. thesis work aims to characterize and utilize grain based gluten-free flours and unripe papaya powder in the optimal formulation of various types of cookies. The formulation research is to be targeted through the alteration of the proportions of various types of grain flours, dried vegetable powders, shortening agents, sweeteners and other additives. The organoleptic properties of a drink or food product is important to ascertain upon the formal consumer acceptance. Henceforth, it shall be given equal priority along with the aspect of the nutritional benefits. In this Ph.D. thesis, among alternate formulations, the best formulation will be chosen based on the sensory characteristics.

Further, the encapsulation process of the bio-active compounds within food grade materials can serve as a protective environment for the bio-actives. Thereby, the potential health benefits of such cookies can be sustained. Additionally, the acrylamide content and storage characteristics of the selected formulated cookies can be also evaluated along with the evaluation of the bioactive constitution. The sustainable exploration of the constituent bio-actives has been in terms of their capacity to endure the process of digestion and thereby maintain their nutritional functionality due to the minimal degradation within the gastrointestinal tract of the human kind. Briefly, the targeted perspectives of the Ph.D. thesis have been stated as follows:

a. Assessment of nutritional, physical, thermal and morphological characteristics of grain-based gluten-free flours for the preparation of nutritionally enriched baked cookies.

b. Evaluation of unripe papaya pulp and peel powders as alternate sources of gluten-free ingredients for the preparation of functionally enriched gluten-free baked cookies.

c. Assessment of dough rheology characteristics of the best performing cookies in terms of the sensory properties.

d. Encapsulation of catechins enriched green tea and bio-actives enriched papaya leaf extracts in the maltodextrin and sodium alginate-pectin system respectively and fortification of sensory optimization based best cookie formulations with the encapsulated catechins/bio-actives infused materials (maltodextrin/sodium alginate-pectin).

e. In-vitro, storage and acrylamide characteristics of the sensory analysis based optimally inferred cookie formulations.

An overview of the prior art has been elucidated in the following sub-sections and for the above mentioned targeted thesis perspectives.

1.2.1 Functional Significance of Grain-based Gluten-free Flours and Cookies

The development of gluten network in the cookie system is relatively limited and is primarily dependent upon the type of desired final product. The hard texture of sweet and semi-sweet cookie system requires appropriate gluten network development (Cairano et al., 2018). On the other hand, cookies prepared with the short dough (constituting higher proportions of fat and sugar) primarily relies on the starch gelatinization principle and gluten network development is not applicable for such a case (Torbica et al., 2013). Thus, the sugar-snap cookies require very limited gluten network development and accordingly soft wheat flour constitution is very high in such cookie formulations. Further, the lower constitution of gluten protein in the short dough reduces the cookie spread parameter (HadiNezhad & Butler, 2009; Pareyt et al., 2008). Such reduced cookie spread parameter shall be also investigated in the formulational research investigations. Accordingly, the utilization of gluten-free flours in the cookie formulation shall be analyzed from the perspective of desired texture and cookie spread parameters.

According to the prior research being conducted in the field of gluten-free cookies, biscuits, and crackers, the key gluten-free flours being deployed were (a) those derived from cereals such as rice, maize, sorghum, millet (Cairano et al., 2018); (b) those derived from legumes such as chickpea, Bengal gram, green gram, and their combinations (Xu et al., 2020; Öksüz & Karakaş, 2016).

Among these, the rice flour emerged as a very good choice and has been frequently employed in conjunction with other flours. These refer to the flours derived from maize starch and pea protein (Mancebo et al., 2016). Furthermore, oats flour was also utilized in the development of gluten free cookies (Duta & Culetu, 2015). However, the conducted investigations did not address a comparative research framework for the selection of alternate flours based on their functional properties and the characteristics of the prepared cookies.

1.2.2 Functional Significance of Unripe Papaya based Gluten-free Powders and Cookies

Among major nutrients such as fats, proteins and carbohydrates, the carbohydrates constitution in the grains is significant. Henceforth, the grains are constituted as a favorite source of energy in many food products. Grains are indeed a significant source of carbohydrates, making them a vital component in many food products. They serve as a primary source of energy in various items such as bread, pasta, cereals, rice, and baked goods. However, emerging evidence suggests that the long-term consumption of the high carbohydrate diet leads to the enhanced risk of hyperglycemia and diabetes. With a high prevalence of diabetes, the current dietary guideline recommends low-carbohydrate consumption in the daily food intake of the humankind. Thereby, glycemic control can be effectively addressed and achieved through sustainable improvement in the quality food consumption patterns (Dyson et al., 2018). For this purpose, dried vegetable powders can be explored as an alternate ingredient in the food product.

Among vegetables and fruits produced in the tropical countries, the unripe papaya vegetable has several promising features such as the richer constitution of beneficial nutritional ingredients, affordability based on the price and abundance of the produce. All these prompt upon its choice as a viable and alternate gluten-free flour. Presently, natural food sources are widely used as a healthier food choice for glycemic control due to their enriched nutritional content such as resistant starch, dietary fiber and phytochemical compounds. Such substitutions have been already demonstrated in few baked products. For example, the partial replacement of wheat with unripe banana flour and black rice flour has been proven to enhance total phenolic compounds and antioxidant capacity in the cookie product (Agama-Acevedo et al., 2012).

The ripened papaya fruit is consumed as a fresh fruit and also as a processed food ingredient in products such as dried papaya, candy, and ice cream (Verma et al., 2020). However, even though the unripe papaya is well known for its nutritional and functional characteristics, its applications are limited in both domestic and as well as industrial sectors. Among various processing schemes, unripe papaya can be explored for the development of alternate gluten-free flours for subsequent application in the food product development and research.

Also, the papaya is a well-known richer source of natural antioxidants that quench the free radicals (Mukherjee et al., 2022). The papaya fruit possesses richer constitution of phytochemicals, which being natural antioxidants are often desired in the food pallete due to the concerns associated to the synthetic antioxidants. The papaya fruit possesses richer constitution of phytochemicals such as phenolic compounds, glucosinolates, flavonoids, vitamins and minerals which being natural antioxidants are often desired in the food pallete due to the concerns associated to the synthetic antioxidants. Apart from free radical scavenging activity, the antioxidants found in such plant resources possess antibacterial, antiviral, antimetastasis activity, antiulcer activity, antimutagenic, antiallergic and anticarciogenicity (Koul et al., 2022). Papaya is a rich source of three powerful anti-oxidants namely vitamin C, vitamin A and vitamin E. Henceforth, papaya belonging to the *Caricaceae* family, produced in large quantities in India (India being world's largest producer), has been used for medication against a variety of diseases such as cancer and cardiac diseases (Milind et al, 2011). Various parts of the papaya plant, such as the leaves, fruit, seed, flower, and root, are used to prepare medicines (Pavithra et al., 2017). The ripened papaya is consumed both as a fruit and as a processed product. The un-ripe fruit/vegetable is consumed as a cooked vegetable, and as an ingredient in papaya salad and cooked dishes. The papaya fruit is also an excellent source of calcium and many other minerals, which are recommended in a balanced human diet and nutrition. The fruit contains both macro and micronutrients such as Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn. It also contains a digestive enzyme namely papain which is effectively used for the treatment of trauma, allergies and spot injuries.

The by-products of papaya mainly constitute peels and seeds. These make upto 20 to 25% by fruit weight (Pavithra et al., 2017). Papaya peels have been found to be rich in fibre with total, soluble and insoluble dietary fibre (g/100) content of 59.8 ± 0.5, 19.93 ± 0.01 and 39.9 ± 0.5, respectively (Calvache et al., 2016). Besides being rich in nutrients such as protein, carbohydrates, ash, fat and minerals (phosphorous and potassium), they are also a potential source of antioxidants (Wall, 2006). The papaya peel has been suggested to possess various wound healing properties. Often, the papaya fruit/vegetable pulp portion is extensively utilized and the peel is disposed as a waste in the environment. Such a useful resource, being considered as a waste, can be effectively utilized to simultaneously reduce environmental deterioration and target economically viable value added product development (Yusof et al., 2019). Today, consumer food products are targeted to constitute richer dietary fiber content due to associated health benefits. Also, consumers prefer natural constituents in these products as synthetic ingredients are opined to be toxic to the human health. Considering these aspects, the papaya peel with a generous amount of fiber content can be utilized in the development of innovative food and other products (Calvache et al., 2016).

An example of such innovative products is the papaya peel jam product which has been documented to have enhanced fiber content and sensory properties (Pathak et al., 2019). While unripe papaya peel has not been explored till date for its inclusion in the biscuits/cookies formulation, similar investigations have been addressed to target enhanced nutritional properties of the biscuits through the inclusion of pumpkin seeds (Abdelgadir et al., 2019), papaya peel and seeds (Jiang et al., 2022) and papaya pulp (Parni and Verma, 2014).

1.2.3 Dough Rheology of the Cookies

Food rheology studies often target the flow properties of single food components. These flow properties may exhibit a complex rheological response function during the flow analysis of a composite food matrix. Further, the effect of processing on the food structure and its flow behaviour can be as well analyzed. In due course of the production of processed foods, their major constitution and other added ingredients do affect the food quality and product performance in terms of the rheological analysis. Henceforth, rheological investigations shall be targeted for each ingredient in the context of their relation to food processing, and their final discernment (Fischer and Windhab, 2011).

Rheology, as a valuable tool, provides a quantitative measure for the amount of stress in the dough. Such a property is closely related to the extent and quality of the constituents in the molecular gluten network. The rheological measurements of the dough are primarily targeted to fulfill the following objectives: (a) to obtain a quantitative description of the mechanical properties of the material (b) to gain information related to the molecular structure and composition of the material (c) to characterize and guess the material's performance during processing and for quality control.

Rheological measurements significantly aid in the studies associated to process control and design. This is due to the reason that they elucidate upon the behavior of the dough under a given set of conditions that often alter during the inevitable processing schemes (Richardson, 1998), such as mixing, sheeting, proofing and dough baking. In order to examine process conditions and achieve acceptable product performance and consumer acceptance, rheological instrumentation and measurements have become essential tools in analytical laboratories (Amjid et al., 2013). The authors inferred that the prediction of storage and stability parameters along with an understanding in the texture design, and associated knowledge of the rheological and mechanical properties of various food systems is very important to resolve all issues that may arise as stiffer challenges in the large scale processing of a food product.

Rheological properties are independent of size, shape and how they are measured. In other words, they are universal and just like the speed of light or density of water, they remain constant and independent of the adopted methodologies, working principle and associated instrumentation. A mature understanding of the rheological behavior of flour dough is of greater significance from the practical view point. Dough rheology directly affects the baking performance of flours. Henceforth, rheological analyses often as well converge and infer upon the optimality of dough formulation in terms of the effective processing and baking competence. Significant research thrust has been given for the rheological studies and for a longer time frame. However, the associated complexity of biological system-based resources poses stiffer challenges for a mature understanding in the rheological properties of the composite powders based dough systems (Usman et al., 2020).

Cookies as baked products primarily constitute flour, sugar, fat and a little water. Along with other minor ingredients, these constituents are thoroughly mixed to achieve the cookie dough (Yağcı, 2019). Thus, the cookie dough characteristics do significantly influence dough preparation and handling parameters, cookie baking process parameters and the quality of the final product (Pareyt et al., 2009). Cookie spread, i.e. the extent to which the dough piece spreads during baking represents a major parameter for the assurance of the quality product preparation (HadiNezhad and Butler, 2009). Generally, cookie spread is associated to sugar, fat and protein content. Due to higher fat and sugar content in the cookie recipes, its gluten network development is restricted. However, the inclusion of gluten-free proteins in the wheat flour cookies has not been investigated in the cookie dough system and especially during the baking process (Donelson and Gaines, 1998). Also, according to Pareyt et al. (2009), higher protein content in the cookies results in a reduced cookie spread. Further, cookie spread is also controlled by the dough viscosity (HadiNezhad and Butler, 2009). Due to lower water content, cookie dough is generally more elastic and less extensible in comparison to the bread dough. Since cookie dough rheology characterization is related to dough-handling properties, the tendency of the cookie dough to get contracted is a very important parameter in the evaluation of the cookie quality (Pedersen et al., 2004).

Despite its prominence, works published till date did not delineate upon the rheological behavior of gluten-free cookie dough. To the best of the knowledge of the author, few published articles in the field of cookie or gluten-free dough rheology are those that addressed either wheat-containing cookie dough (Pedersen et al., 2004) or gluten-free bread dough (Lazaridou, & Biliaderis, 2009). Such investigations are important as they prompt upon the successful production of nutritionally improved gluten-free cookies for automated industrial processing based product realization.

In general, dough rheology affecting factors and the influence of various additives on the rheological properties of flour doughs are often addressed in the dough rheology studies. Accordingly, sensitive component interactions are often identified and through this, possible stiffer challenges during baking can be overcome. Among the most commonly used methods for the rheological testing of doughs, the shear oscillation based dynamic rheological study being generally deployed under deformation condition is inappropriate for the preparation of dough and demonstrates little relationship with the final product characteristics.

1.2.4 Encapsulation of Heat Sensitive Bioactive Components and Cookie Fortification

Catechins are the most constituent polyphenols in the tea and are vital bioactive substances. However, due to the low bioavailability and low permeability of catechins, their utility has not been explored yet to the full potential. This is due to the degradation of catechins during their passage through the gastrointestinal tract. Catechins are highly sensitive to low pH and undergo structural changes during oxidation. During their passage through the human intestinal system after consumption, they do get exposed to the high pH environment (approximately pH 6.1 to 7.5) (Cai et al., 2018; Manach et al., 1999; Prakash et al., 2019). Further, the prevalent reactive oxygen species in the intestine system cause higher degradation to the catechins (Neilson et al., 2007). In-vitro studies did convey that the bioavailability of tea catechins has been severely affected by the gastrointestinal conditions. Accordingly, a net retention of 5.3% was reported for the catechins. Further, the Epigallocatechin (EGC) and Epigallocatechin Gallate (EGCG) exhibited an even more significant decline of 4.6% and 6.1% respectively (Cai et al., 2018).

To resolve this issue, bioactives encapsulation has been targeted as an effective solution. Encapsulation facilitates the coating of bioactive substance with a layer of a protective material. Such a material protects the coated substance during the ordeals of storage, processing, and human digestion process.

Limited research on in vivo pathways for encapsulated catechins: There are limited research carried out on the pathways of absorption of catechins post-encapsulation in vivo. In vitro studies are available but in vivo studies and human trials need to be conducted to better understand the nutritional benefits of catechin's encapsulation.

The advantages of encapsulating catechins are:

1. Enhances bioavailability: Catechins lose their functionality during the gastro-intestinal digestion process due to complex digestion environments and structural changes due to polymerization, epimerization, and oxidation reactions. Encapsulation protects catechin during the digestion process and enhance their bioaccessibility.

2. Enhances stability and shelf life: Encapsulated catechins have better shelf life and stability.

3. Masks undesired flavors: Catechins have a bitter and astringent taste and encapsulation can mask these flavors in products where its taste or flavour is considered unacceptable.

Disadvantages or limitations of encapsulating catechins:

Limited research in green encapsulation technologies: Various research works have been conducted to enhance the encapsulation efficiency of tea catechins while very less have focused in green and low chemical based encapsulation strategies. This is the major reason why encapsulated tea catechins are not a popular formulation in food products.

Microencapsulation and nanoencapsulation are two well-known approaches being suggested for the encapsulation of polyphenols from tea. These two encapsulation methods differ fundamentally in terms of the size of the deposited or coated particles. The microencapsulation affirms a final particle size of about several hundred micrometers (µm). On the other hand, nanoparticle substances being entrapped in a protective coating with a radius below 500 nm is targeted in the nanoencapsulation process (Joye et al., 2014). Both micro and nano encapsulation methods are highly effective to enhance the bioavailability of the tea catechins. However, among these two methods, nanoencapsulation has a comparative edge. Compared to the microencapsulated products, the smaller size of nano-encased particles allows them to get more easily absorbed into the body cells. For such encapsulation purpose, most commonly deployed protective coating materials for the encapsulation refer to starch and starch derivatives, proteins, and liposomes. Deployed methods for encapsulation refer to inclusion complexation, spray drying, coacervation, antisolvent precipitation, supercritical precipitation, freeze drying, infusion and binding, gel electrophoresis, extrusion, mixing, and adsorption (Puligundla et al., 2017).

1.2.5 In-vitro, Storage and Acrylamide Studies of Gluten-free Cookies

1.2.5.1 In-vitro Studies

As a relevant processing method, the baking process has been reported to enhance the resistant starch content in the baked product. However, structural alterations that are responsible for the reduction of the starch digestibility depend upon the baking conditions such as time and temperature (Marrelli et al., 2013). In this regard, limited information exists in the research theme of the baking conditions influence upon the starch digestibility profile of gluten-free rice-based cookies.

Giuberti & Gallo, (2020) studied the total and individual short chain fatty acid profiles and related kinetics of four different retrograded resistant starch (RS3)-rich ingredients using an in vitro approach based on enzymatic digestion followed by in vitro large intestine fermentation. For comparison purpose, native high amylose maize starch (i.e., RS2) was included, being one of the most common commercial type of RS used in food product formulation. Further, cereal food researchers need an in-vitro method so as to reach international consensus for a deeper introspection into starch hydrolysis issues. Thereby, discriminating upon the alterations in the product structure, the presence or absence of lipids, dietary fiber, gluten or specific proteins, and even the processing technique can sensitively influence the functional cookie product characteristics. Accordingly, such studies shall be addressed along with a comparative assessment strategy for the identification of key factors that affect glucose metabolism.

1.2.5.2 Storage Studies

Cookies usually have a long storage period of about three months. During product storage, different chemical reactions occur within the cookie and thereby cause product deterioration. Koczoń et al., (2016) proposed a scheme for the chemical reactions that are responsible for fat oxidation in biscuits during the storage period. In the conducted study, the primary factors considered to influence oxidative stability were initial water content, initial fat content, and storage period duration. Fat being considered as a primary component in a cookie recipe includes both native lipids and those additionally used for the appropriate formulation of the dough (Caponio et al., 2009). In this regard, it shall be noted that margarine, shortening, vegetable, and hydrogenated fats are widely used in the cookie recipes. The selection of a particular fat in a cookie formulation is often based on technological and economical parameters, nutritional aspects, sensory profile and product shelf life factors (Caponio et al., 2009).

A higher degree of variability in fatty acid composition and high constitution of trans-isomers of unsaturated fatty acids has been found in the cookies and biscuits products (Caponio et al., 2009). Manufacturing conditions, storage conditions, degree of saturation, content of unsaturated fatty acids have been reported by the authors to ascertain sensitive alteration in the storage characteristics. Also, the number and position of double bonds in the molecules have been as well opined to be primary factors that are responsible for the sensitive alteration of the rate of oxidation of such baked products (Wsowicz et al., 2004).

The storage stability of cookies, as for many baked products, could be defined as the proper maintenance of sensory and physicochemical properties (appearance, freshness or moistness, colour, firmness etc.) and through the prevention of undesired changes associated with staling or some other process such as lipid oxidation during storage (Fiszman et al., 2013). Also, it is known that, at times, the physical changes coupled with subsequent chemical reactions do limit the product shelf life. Accordingly, it was found that most biochemical and microbiological reactions are controlled and regulated with the water activity of the cookie. Henceforth, the water activity can be considered as a useful indicator for the assessment of product stability. Moreover, colour can be used as an indicator for the onset of various undesired biochemical reactions and associated changes that alter the sustainability of the cookie product (Savenkova et al., 2019).

1.2.5.3 Acrylamide Analysis

Acrylamide is a neurotoxic compound and a probable carcinogen, with the chemical formula C_3H_5NO. It is produced during high-temperature cooking processes, particularly in starchy foods through the Maillard reaction involving the amino acid asparagine. This chemical has been shown to significantly reduce cell viability and induce oxidative stress in human cells, primarily by generating radical oxygen species upon metabolism (Huchthausen et al., 2023; Celik et al., 2018). The genotoxic effects of acrylamide are also notable, as it can cause DNA damage by forming reactive intermediates like glycidamide, leading to mutations (Celik et al., 2018). Chronic exposure to acrylamide has been linked to reproductive toxicity and increased cancer risk in animal studies, raising concerns about its impact on human health (Acrylamide Britanica, 1980). Acrylamide (AcA), a toxic and carcinogenic compound, is naturally formed in the high-temperature processing environment and at thermal operations above 120℃. Cookies greatly contribute to the dietary AcA intake. This is especially true for the case of children. Food stuffs contributes approximately 27% and 56% of the AcA intake for children lower than and greater than two years of age, respectively (Abt et al., 2019). Thus, typical modifications in the ingredients and processing conditions should be addressed to mitigate the AcA formation.

Cookies are traditionally baked with the soft wheat flour. The texture of cookies primarily depends upon sugar and fat that respectively impart and ensure crispness and friability. Cookies are easy to produce without gluten, as gluten only plays a secondary role in the baking based end-product quality of cookies. As a key ingredient, rice is a good choice to achieve baked gluten-free butter cookies.

The selection of appropriate raw food materials for the minimization of AcA levels is beneficial for the butter cookie industry. For almost 20 years, significant research efforts devoted to the mitigation of the formation of AcA (a probable carcinogen) during the processing of starch-rich food. In an effort to reduce the amount of acrylamide formed, several strategies have been proposed such as the reduction of the processing temperature, an earlier processing scheme endpoint, and/or the modification of recipes. However, there is broad agreement that the AcA gets preferably formed from Asparagine and reducing sugars and through the Maillard reaction. Among these, the latter is responsible for the generation of the brown color and many other active components of baked food product that impart desirable sensory characteristics such as taste, smell etc.

1.3 Prior Art

Based on the targeted research perspectives summarized in section 1.2, the available prior art in the chosen research theme can be classified into the following themes:

a. Investigation of properties of powders derived from various grains and unripe papaya peel and pulp systems.
b. Formulation and characterization of cookies being prepared with the grain-based flours.
c. Development and characterization of cookies prepared with the unripe papaya pulp and peel powders.
d. Evaluation of the rheological properties of the dough containing gluten-free flours.
e. Cookie fortification with various encapsulated bioactive components.
f. Storage, acrylamide, and in-vitro characteristics of the optimally formulated gluten-free cookies.

A brief overview of the available literature in the above-mentioned themes has been presented in the following sub-sections.

1.3.1 Alternate Gluten-Free Flours and their Characteristics

Legumes constitute an important source of dietary protein in large segments of the world's population. This is especially evident in those countries in which the consumption of animal protein is limited due to their non-availability or due to self-imposed religious or cultural habits. In a relevant prior art study, the physicochemical, functional, and morphological characteristics of flours obtained from two types of kidney bean (Shalimar Rajmah-1 and Shalimar Rajmah 132) and two green gram cultivars (Shalimar Moong-1 and Shalimar Moong-2 (Wani et al., 2011) have been addressed. The authors investigated moisture, protein, fat, ash, and carbohydrate contents of flours and found them to vary as 13.00 -14.00, 14.78 - 25.02, 1.00 - 2.00, 3.00 - 4.00, and 56.98 - 66.22 g/100 g, respectively. For the flours, Hunter color parameters namely "L," "a," and "b" values varied in the range of 71.22 - 82.65, −1.80 to −0.12, and 15.20–22.85, respectively.

Rice (*Oryza sativa*, L.), one of the most important crops in the world and in especially Asia, is a principal staple food in many regions of the world's human population (Kaur et al., 2016). Today, 4000 identified rice varieties exist with diverse physicochemical and functional properties (Rathna Priya et al., 2019). Apart from its consumption as a cooked granular form, rice flour is popularly used as a key ingredient in novel food products. This is due to its bland taste, higher digestibility, hypoallergenic properties, and lower price (Qiao et al., 2011). The diversity in rice flour could be desirably achieved and these are attributed to the alterations in the granular size, amylose, lipid-complexed amylose gelatinization, and starch composition. All these affect the quality of rice flour in a complex framework and henceforth eventually affect the quality of the produced product. Henceforth, the selection of appropriate rice flour for the baked cookies production is very important.

1.3.1.2 Utilization of Unripe Papaya Pulp and Peel Powder as Gluten-free Substitutes

In a relevant investigation (Akintunde et al., 2022), the harvested fresh ripe papaya peels were first dried in an oven at 45°C for 48 h. Thereafter, they were grinded for subsequent analysis and characterization according to standard procedures. The proximate analysis of the peel conveyed higher constitution of proteins (11.67 ± 0.04%), crude fiber (32.51 ± 0.03%), carbohydrate (47.33 ± 0.08%), ash (5.98 ± 0.03%) and fat content (2.51 ± 0.13%).

Table 1.1: Literature data summary of the properties of alternate gluten-free flours and powders

S. No.	Flour varieties studied	Targeted Parameters	Best data	Reference
1.	Chickpea flour; Dried green pea flour	Proximate; Functional; Pasting properties	Dried green pea flour: Pasting temperature 77.65°C, peak viscosity (729 cp), trough (690.34 cp), final viscosity (935 cp), and setback (244.67 cp) WAC: dried green pea flour is 1.95g/g; Oil absorption capacity (g/g) for dried green pea:1.79±0.63 Crude fat:1.94±0.53 Crude fiber: 4.96±0.32	Wani & Kumar, 2014
2.	Kidney bean flour; green gram flour	Physico-chemical; Light transmittance and syneresis; Pasting properties; Functional properties; Rheology; Thermal properties; Morphology; Composition	Green gram flour: Protein (g/100 g) :25.02 ± 0.2 (Shal moong 1); 23.90 ± 0.2 (Shaal moong 2); Fat (g/100 g) 1.43 ± 0.001 (Shal moong 1): 1.55 ± 0.001(Shaal moong 2); Amylose (g/100 g): 12.90 ± 0.33(Shaal moong 1) 10.59 ± 0.40 (Shaal moong 2); Bulk density (g/mL): 0.62 ± 0.02(Shal moong 1); 0.62 ± 0.02 (Shaal moong 2)	Wani et al., 2019
3.	Bengal gram flour; black gram flour; Green gram flour; Lentil	Proximate; Functional; Sensory;	Green gram (moisture):1.3g/100g; Protein:25.6g/100g; Fat:1.3g/100g; Total ash:3.3g/100g; Bulk density (g/mL): 0.66; Water absorption capacity: 186 mL/100g; Bengal gram: Crude fiber: 1.3g/100g ; fat absorption capacity: 298 mL/100g	Nagmani & Prakash, 1997
4.	Rice flour	Physicochemical Properties; Morphology; In Vitro Starch Digestion; Bile binding and cholesterol micellization	RD43 rice flour: irregular and polyhedral shape with a volume mean diameter of 103±0.15μm. Amylose content of RD43 rice:19.04% and Hom Mali rice flour: 16.38%, % Cholesterol Micellization Inhibition:12.95% The degree of crystallinity in RD43 rice flour (24.55%) lower than Hom Mali rice flour (26.8%).	Sukleau et al., 2020

The mineral analysis affirmed that the papaya (*Carica papaya* L. var solo 8) peels are especially rich in potassium and phosphorus. Among these, potassium was found to be the most abundant mineral in the papaya peel (516.33 ± 0.82 mg/100g). The phytochemical analysis affirmed higher levels of total phenolic (65.48 ± 0.39 mg (GAE)/100 g DW), flavonoid (5.58 ± 0.83 mg (QE)/100 g DW) and tannin content (10.51 ± 0.93 mg (TAE)/100 g DW). The methanolic extracts of the papaya (*Carica papaya* L. var solo 8) peels exhibited higher DPPH radical scavenging activity (81.89 ± 0.14%) (Didier et al., 2017).

On the other hand, according to another investigation (Joymak et al., 2021), the papaya fruit processing industries generate a large volume of unripe papaya waste and by-products. To reduce the papaya waste, the unripe papaya pulp powder (PPU) was manufactured and was incorporated into the formulation of a pancake. For the PPU, the authors reported an average particle size of 140.8±2.1 µm. Further, the PPU has been analyzed for richer constitution of polyphenolic compounds, dietary fiber and ferric reducing antioxidant power (FRAP). Also, in comparison to the wheat flour, the PPU had higher values of water absorption index, water solubility index and swelling index and lower level of amylose. For the targeted goal of cholesterol reduction, the PPU has been confirmed to reduce the extent of cholesterol micellization and also binded with the bile acids. A summary of the best findings in the mentioned prior art in the field of PPU and PPE characterization for eventual gluten free product development has been presented in Table 1.2.

Table 1.2: A data summary of unripe papaya powder characteristics

S. No.	Flour varieties studied	Parameters investigated	Best Findings	Reference
1.	Ripe papaya peel powder	Proximate, mineral content, phytochemical content	Proximate analysis showed high level of proteins (11.67 ± 0.04%), crude fibre (32.51 ± 0.03%), carbohydrate (47.33 ± 0.08%), ash (5.98 ± 0.03%) 0.03%) and fat (2.51 PPU 85.67±1.620.13%). Phytochemical composition showed high level of total phenolic (65.48 ± 0.39 mg (GAE)/100 g DW), flavonoids (5.58 ± 0.83 mg (QE)/100 g DW) and tannins (10.51 ± 0.93 mg (TAE)/100 g DW).	Didier et al., 2017
2.	PPU	Physico-chemical, functional properties, nutritional properties	PPU: 85.67±1.62 (TpC (mg GAE/100 g); 411.58±38.0 (FRAP (mmol $FeSO_4$/100 g); 37.87±3.69 (DPPH Activity (mg Ascorbic Acid/100 g); 13.53±0.11: WAI (g/g); 11.40± 0.18: WSI (g/100 g); 0.58±0.01: Bulk Density (g/mL); 15.28±0.1: SP; 1.44±0.33: Amylose content; 77.16%-carbohydrate, 56.14% of dietary fiber and 21.02% of available carbohydrates. 11.61% moisture, 5.22% ash, 4.65% protein, and total fat was the least components (1.36%).	Joymak et al., 2021

1.3.2 Characterization of Grain based Gluten-Free Cookies

Till date, several attempts targeted an enhancement in the nutritional properties of the cookies through the inclusion of various gluten-free flours. Thereby, a protein rich formulation was sought. In one such attempt, the researchers developed a functional gluten-free cookies by substituting soy flour with the whole wheat flour (Ndife et al., 2014). The authors targeted enriched cookies with soy-flour substitution (0 – 50 wt %) to the wheat flour. Thereby, it was assessed that the prepared cookies were nutritionally superior (in terms of higher protein, fat, fibre energy) to the whole wheat cookies. Also, the soy flour enriched cookies possessed higher mineral content in terms of potassium (460.82 mg/100g); magnesium (42.65 mg/100g) and iron (3.19 mg/100g). Further, the ash content increased significantly (2.95%). Finally, the sensory evaluation studies conveyed that the best formulations were those achieved with 30 wt% (sample C with 8.65 overall acceptability ratings) and 50 wt% (sample D with 8.10 overall acceptability ratings) substitutions of the soy flour.

To ensure upon the protein enriched food product requirements, legume supplementation can be conveniently targeted. This is due to the fact that legumes (especially green gram flour) have higher protein and complex hydrocarbon content. Further, they are also enriched with an appreciable quantity of bioactive ingredients and minerals. Such custom designed products can therefore provide adequate protein needs for the vegetarian population of India. The biological nutritional benefits of the green gram flour are further enhanced for the case in which or rice flour is combined with the flour. This is due to the complementary relationship of the existent essential amino acids. In a relevant prior art, to enhance the quality of wheat based biscuits, the supplementation with the high protein content based green gram flour was targeted (Shukla et al., 2016). The authors adopted the incorporation of green gram dhal flour at 30, 40, 50 and 60 weight % into the wheat flour. Subsequently, the protein enriched cookies were subjected to storage studies upto 60 days in two alternate packaging pouches (HDPE, LDPE). Accordingly, the samples were assessed for alterations in moisture, protein, fat and ash content. The sensory analysis further revealed that the 50 wt % green gram flour substitution affirmed highest sensory score in terms of colour, aroma, taste, texture, appearance and enhanced overall organoleptic properties of the formulation. The formulations also exhibited enhanced protein content and ash constitution of 14.25 %, and 2.62 % respectively.

In another relevant study, researchers formulated multigrain cookies based on ragi, whole wheat, barley, soy bean, jowar, bajra, Bengal gram and oats. For the alternate formulations, the authors also conducted sensory analysis for the optimization of the organoleptic properties. Along with these, the authors also addressed the relevance of the associated nutritional benefits.

Table 1.3: Literature data summary of cookies formulated with grain-based gluten-free flours

S. No.	Flour varieties studied	Parameters investigated	Best sample	Reference
1.	Wheat flour and soy flour composite flour; Substitution of soy flour 0 – 50%	Composite flour: functional and proximate Enriched cookies: physical, chemical. Mineral, microbial and sensory	Cookies substituted with 50% soy flour with highest Sodium- 8.56 mg/100g; Calcium- 65.26 mg/100g; Potassium- 460.82 mg/100g; Magnesium- 42.65 mg/100g; Iron- 3.19 mg/100g; Protein- 24.65%; Crude fiber- 5.73%; Food energy value-578.21kcal; Taste-8.35; Aroma-8.33; Overall acceptability-8.1	Ndife et al., 2014
2.	Green gram dhal flour (Substitution: 30, 40. 50 and 60 %); Wheat flour;	Physico-chemical and sensory evaluation of green gram dhal substituted cookies	Cookies with 50% substitution has the highest sensory score (overall acceptability: 8.6); protein content- 14.30 (60% substitution); upto 60 days both HDPE and LDPE pouches are good for storage	Shukla et al., 2016
3.	Wheat grass powder, ragi, whole wheat, barley, soy bean, jowar, bajra, Bengal gram and oats.	Wheat grass powder substitution :0 – 30% Proximate, organoleptic and cost analysis of multigrain cookies	30% Wheat grass powder highest protein content 21.99%; 5% wheat grass powder substituted: highest sensory score	Shashank et al., 2020

The control sample being prepared with multigrain *atta* (ragi, whole wheat, barley, soy bean, jowar, bajra, Bengal gram and oats) achieved very good sensory scores in comparison to the wheat grass substituted cookies. Significant alterations in their organoleptic properties were attributed to the incorporated wheatgrass powder.

Table 1.3 summarizes the best available prior art data in the field of the gluten-free flour (grain flour) based functional cookie formulation. In these studies, the grain flours refer to rice, soy, green gram, Bengal gram, finger millet, and oats flours/powders.

1.3.3 Formulation and Characterization of Cookies Enriched with Papaya Powders

Multiple investigations have been conducted to assess upon the improved characteristics of the cookies being supplemented with papaya fruit, peel and seed powder. In one study, researchers studied the characterization parameters of papaya peel flour (PPE) and in terms of its chemical constitution. Thereafter, the flour was used to formulate nutritionally enriched value added cookies. Accordingly, assessments were addressed for nutritional properties, physicochemical characteristics and organoleptic attributes. The experimental findings clearly conveyed the promising features of 5% formulated papaya peel flour based cookies in terms of nutritional constitution and antioxidants content in comparison to other formulated cookies (92.5% wheat flour: 7.5% PPE), (90% wheat flour:10% PPE) and control (100% wheat flour:0% PPE). The significantly higher dietary fiber content of 5% formulated papaya peel flour based cookies contributed to their higher digestibility and consumers acceptance indices and are henceforth were concluded to ascertain upon associated health benefits (Bokaria & Ray, 2016).

To analyze the utility of RSM technique as a statistical and methodological approach for the enhancement and assurance of the quality characteristics of food products, a relevant prior art addressed the development of mathematical models that serve as useful tools for the prediction of the sensitive influence of various factors on the important characteristics of cookies (such as texture, color and moisture content). The findings of the conducted investigations conveyed the need to utilize and explore various properties of papaya peel and seed powder. These powders are often a waste product in the papaya processing industry and can be used for value addition in terms of nutrients, functional properties and dietary fiber. The optimization of functional cookies formulation was performed with the central composite rotatable design (CCRD) of the response surface methodology (RSM). The prepared functional cookies contained higher amount of protein content (24.36%) in comparison to that of the control cookies (9.90%). Also, higher crude fiber content (7.94%) has been assessed with respect to the wheat based control cookies (0.83%). The researchers also explored the effect of papaya seed powder (PS) and papaya peel powder (PPE) supplementation (in the range of 2 to 10%) on the physicochemical properties, bioactive compounds, antioxidant activities, sensory attributes, in-vitro absorption capacities and starch digestibility. On the basis of the quality evaluation in terms of the sensory properties of the developed products, it was ascertained that the acceptable levels of protein and dietary fiber content can be achieved through the formulation suggested as 3.82 g papaya peel powder, 1.24 g papaya seed powder and 96.63 g wheat flour (Bhosale & Iranna, 2018).

In another study, the authors conducted a detailed analysis to explore the underutilized or neglected but highly nutrients enriched papaya pulp powder in the cookie products. The fresh cookies possessed 4.18% moisture, 12.80% protein, 24.25% fat, 2.10 mg/100 g crude fiber, 58.15% carbohydrates, 410.25 ųg/100 g β-carotene and 163.51 mg/100 g potassium. The sensory evaluation of cookies was carried out regularly at an interval of one month. The mean score for color and appearance, texture, flavor, taste and overall acceptability were 8.28, 8.33, 8.31, 8.03 and 8.24 respectively on a 9 point hedonic scale (Jadhav et al., 2023).

Rheological investigations target studies associated to the deformation and flow of materials. Thereby, the studies enable a useful understanding into how a given material behaves towards the applied stress or strain. In general, the dough exhibits a linear behavior upon the minimal strain, otherwise it exhibits a non-linear trend. The extent to which the dough exhibits linearity is a function of the type of the dough and adopted methods for mixing and testing. Pareyt et al. (2010) inferred that the higher fat percentage in the cookies considerably diminished their dough elasticity due to the physical obstruction towards gluten development. The addition of non-wheat flour for baked goods can facilitate even greater negative influence on the gluten network. This leads to weakened dough property and degradation in the cookie dough quality characteristics. The reported studies till date did not delineate significantly upon the gluten free flours influence on the dough rheology. So is the case for the substitution of wheat flour with PPU and PPE flours in gluten free cookie formulations. Henceforth, further discussion in the prior art is not possible.

Table 1.4 summarizes the available best literature data in the field of the formulation and characterization of papaya powder based gluten-free flour substituted cookies. The reported data in the table refers to improved characteristics in terms of physico-chemical, organoleptic and nutritional attributes.

1.3.4 Extraction and Microencapsulation of Catechin and Papaya Leaf Extract for the Fortification of Cookies

The green tea catechin extraction method varies from conventional hot water method to the enzymatic extraction method. Catechins are sensitive to prolonged exposure to the high temperature environment. Henceforth, enzymatic extraction serves as an alternate and pragmatic approach for catechin extraction. Considering these aspects, the prior art has been presented for both conventional (hot water extraction) and enzymatic extraction processes and in the following sub-sections.

Table 1.4: Characterization of papaya powder substituted gluten-free cookies

S. No.	Flour varieties studied	Parameters investigated	Best sample	Reference
1.	Wheat flour and ripe papaya peel flour	Papaya peel flour and peel flour substituted cookies: (100:0; 95:5; 92.5:7.5; 90:10) Physico-chemical; nutritional; proximate; sensory	Best overall acceptability (7.6) for 95:5 substituted cookie formulation compared to other substituted cookies with ash (4.5%); protein (9.3 %);total fiber (0.017 %); soluble solid (15); antioxidants (9.0 g/100g)	Bokaria & Ray, 2016
2.	Matured papaya peel and seed powder; wheat flour	Physical, chemical, textural and sensory evaluation of ripe papaya peel and seed powder substituted cookies	Cookies with 96.63 g wheat flour; 3.82 g papaya peel powder and 1.24 g papaya seed powder scored highest sensory score; crude fiber was found 7.94% significantly higher from control (0.83) cookies, Ash: 3.32%; with less amount of fat (16.54%) and carbohydrate (44.48%)	Bhosale & Iranna, 2018
3.	Ripe papaya peel and papaya seed powder	Papaya peel powder (PP) and seed powder (PS) substitution upto 0- 10% (0, 2, 4, 6, 8 and 10%)	PP showed higher quantities of phenolic compounds compared to PS; Chlorogenic acid: PP (1116.67 µg/g) and PS (79.14 µg/g); 4 % PP substituted cookies highest overall acceptability 5.34	Jiang et al., 2022
4.	Ripe papaya pulp powder, Maida	Papaya pulp powder substitution :0 – 25 % Proximate, organoleptic and storage studies of cookies	15% powder highest sensory score 8.75 (Overall acceptability); color & appearance (8.5); Texture (9.0); Flavor (9.0); taste (8.5) with protein content 12.76%; crude fiber 2.07%; carbohydrates 58.1%; β-carotene 410.27µg/100g	Jadhav et al., 2023

1.3.4.1 Hot Water Extraction

Conventionally, the extraction of polyphenols including catechin compounds from fresh tea leaves and green tea is carried out with the hot water extraction processes. Various other organic solvents such as methanol, acetonitrile, ethyl acetate, chloroform could be also explored for the high quality extraction of polyphenols (Choung et al., 2014). However, in the tea industry for consumer acceptance, hot water extraction method is more commonly deployed for catechin and polyphenols extraction.

In the hot water extraction process, drying temperature, drying time and solid to water ratio are the most significant independent variables. Several studies considered these three variables and accordingly optimized the hot water extraction process performance. In one such study, the authors optimized the hot water extraction conditions for the highest yield of total phenolic and flavonoid content. Accordingly, the best conditions have been reported as 90°C, 40 min, and 1:50 solid to water ratio (Chandini et al., 2011). In another prior art, the authors studied the effect of extraction time and temperature on the extraction yield of individual catechin concentrations. It was analyzed that at higher temperatures, gallated catechins such as Epigallocatechin Gallete (EGCG) and Epicatechin Gallate (ECG) deteriorated significantly in comparison to other catechins. Such losses primarily occurred due to oxidation, polymerization, and epimerization reactions. The optimal yield was achieved at the extraction time, temperature, and tea to water ratio of 30 min, 80°C, and 1:50 respectively (Vuong et al., 2010). Thus, it can be inferred that the hot water extraction process needs further optimization targets in terms of reduced extraction temperature and time for enhanced catechin recovery.

1.3.4.2 Microencapsulation of Catechins

The encapsulation of bioactives in wall materials such as β cyclodextrin, starch, and amylose can be conveniently achieved through the inclusion complexation, a bottom-up approach. In a relevant prior art, the authors used β cyclodextrin for the catechin encapsulation with the molecular inclusion method. To do so, catechin and β cyclodextrin were mixed in 1:1 ratio and thereby an average particle size of 518 nm was achieved in the prepared powder. Also, the study reported enhanced thermal properties in the encapsulated system. An increase in the melting temperature to 250.4°C were reported for the system along with the relevant changes in the crystalline pattern. However, the conducted investigation has been the first to provide insights into this kind of encapsulation. It furthered research activities that targeted the application of inclusion complex interactions with starch systems (Krishnaswamy et al., 2012). In another study, the authors conducted a detailed analysis on the preparation and application of the nano-encapsulated inclusion complex of β cyclodextrin and catechin in a variety of food systems such as cheese, yogurt, and milk. To do so, firstly, catechin and β cyclodextrin were mixed in equimolar ratio and the mixture was dried in a vacuum dryer. Subsequently, the dried encapsulates were reported with a yield and encapsulation efficiency of 84 and 94% respectively. Morphological studies conveyed that the original structure of catechin and β cyclodextrin was completely lost and was replaced by irregular aggregates in the size range of 5-100 µm. The authors also reported an enhancement in the thermal properties (increased melting temperature of 202°C). The encapsulated complex sustained 94% retention of antioxidant capacity despite being exposed to the high temperature environment at 100°C for 60 min (Ho et al., 2017). The authors in their extended work applied encapsulated catechins in various food systems. The findings conveyed higher stability of the encapsulated catechins in solid food system i.e. cheese in comparison to solid-liquid and liquid food systems (Ho et al., 2019).

Another research group also explored the possibility of V type carbohydrates such as amylose as a wall material for the encapsulation of catechins with the inclusion complexation method. In a relevant prior art, the authors used potato amylose for catechin hydrate encapsulation. Thereby, the authors studied the in-vitro digestion profile after encapsulation. Prior to the encapsulation, the catechin hydrate was lyophilized into the hexadecyl catechin. Consequently, the thermal properties and photostability improved after encapsulation. Also, it was analysed that the helical V shaped amylose structure has been able to successfully entrap the hexadecyl catechin hydrates and thereby ascertained an encapsulation efficiency of 86.5% (Wang et al., 2021). In a recent research conducted by the authors (Wani & Uppaluri, 2023), calcium alginate-pectin beads were prepared with the precursors as wall materials and papaya leaf extract was used as an active agent for encapsulation with the ion gelation method. The optimality of process variables (sodium alginate concentration (1–3%w/v), calcium chloride concentration (3–9% w/v) and syringe pump flow rate (0.5–3.5 mL/min) for the maximization of output response (encapsulation efficiency that varied in the range of 32.52–85.6%) was targeted by the authors. In summary, such findings paved the pathway for a possible application of sodium alginate-pectin and maltodextrin as efficient wall materials for the encapsulation of bioactive components and catechins respectively. Table 1.5 summarizes the best findings data in the research theme of the extraction and micro-encapsulation of catechins and bioactives.

1.3.4.3 Bioactives Fortification of the Cookies

Cookies are traditional non-perishable snacks that can be subjected to easy fortification with polyphenols from selected resources as well as byproducts. For this purpose, Catechin, the potential antioxidant component in the green tea and flavonols derived from flavonoid polyphenol molecules, can be conveniently explored as they are abundantly available. The sensitive influence of the addition of green tea powder on the chemical, physical, and hedonic characteristics of oat cookies has been addressed in a relevant prior art (Susanti et al., 2021). The addition of green tea powder as an oatmeal fortificant up to 6% in the dough has been addressed by the authors. Accordingly, green tea fortified oatmeal cookies (GTOC) were produced with high fibre (5.76 % for 2 %) and appropriate ash (1.8 % for 2 %) content but with lower fat and carbohydrate content, and energy. Sensory studies conveyed that the fortification of oatmeal cookies up to 2% fortification with the green tea powder has been the best.

Table 1.6 summarizes the best reported data in the field of the bioactives fortification of the cookie products.

Table 1.5: Literature data summary in the research theme of the extraction and micro-encapsulation of catechins

S. No.	Bioactive molecule	Wall material	Method	Encapsulation efficiency (%)	Size(nm)	Melting point, °C	Best sample	Reference
1.	Catechin	β-cyclodextrin	Inclusion complexation	-	518.78	242.69	Catechin:β-cyclodextrin 1:1	Krishnaswamy et al., 2012
2.	Catechin	• β-cyclodextrin (β-CD) • Hydroxypropyl β-cyclodextrin(HPβ-CD) • Methyl β-cyclodextrin(Mβ-CD)	Inclusion complexation	• 86 - β-CD • 89 - HPβ-CD • 94 - Mβ-CD	5-100 µm	• 202 - β-CD • 196- HPβ-CD • 197- Mβ-CD	Cat – Mβ-CD	Ho et al., 2017
3.	Catechin; Hexadecyl catechin	Amylose from potato	Inclusion complexation	86.5% - amylose HC IC*	-	-	Hexadecyl catechin amylose complex	Wang et al., 2021
4.	Bioactives from aqueous papaya leaf (PL) extracts	Sodium alginate-pectin	Ion gelation	PL extract: (72.62 ± 0.23%)	Encapsulated dried alginate-pectin 3.66 ± 0.03 µm	Beads-pectin and PL extract (ALEXPB), one endothermic and one exothermic peak at 200°C and 295°C	Optimum conditions for maximum encapsulations efficiency of beads loaded with pectin correspond to 2% w/v sodium alginate concentration, 6.61% w/v $CaCl_2$ concentration and 3.50 mL/min syringe pump flow rate	Wani & Uppaluri, 2023

1.3.5 In-vitro, Storage and Acrylamide Studies of Optimally Formulated Cookies

1.3.5.1 In-vitro Studies

In one relevant prior art, the authors aimed to produce new functional cookies with high nutritional properties and low calorie content. The authors investigated the effects of incorporating wheat germ flour (WGF) at levels of 10–30% as a substitute for whole wheat flour (WWF), and with coffee silver skin (CSS) in the same proportion. Thereby, the CSS served as a natural functional additive to substitute for fat in cookie formulations. The potential of CSS and wheat germ flour (WGF) has been explored for their integration into the low-fat and high dietary fiber cookies. The altered formulations were explored in terms of physical, nutritional, functional (mineral content, antioxidant properties and in vitro bio-accessibility) and sensory properties. In this conducted study, the authors analyzed that the total phenol content of the cookies for the added WGF-CSS case ranged from 1813.72 to 1838.45 mg gallic acid equivalent (GAE) per kilogram of dry weight (mg GAE kg^{-1}). However, the total phenolic bioaccessibility values ranged between 53.39 and 56.84% (Büyük & Dulger, 2023).

Agricultural by-products such as olive and grape seeds, cereals such as barley and legumes such as lupine and chickpea are rich in bioactive compounds. Henceforth, the flours of these

Table 1.6: Literature data summary in the research theme of bioactives based fortification of cookies

S. No.	Fortified item	Fortified with	Fortification level	Best sample	Reference
1.	Oatmeal cookies	Green tea powder	0% 2 % 4 % 6% 8 %	2% GTP scored highest sensory value; with high Protein: 14.45% Crude fibre:5.76% With lower amount of Carbohydrates:53.01% Fat: 21.45 %	Susanti et al., 2021

resources could provide better options for the fortification of the baked cookies. Accordingly, wheat crackers with 10 – 30% olive seed, 10 – 30% grape seed, 10 – 40% lupine, 10 – 30% barley and 20 – 60% and 80% chickpea flours were prepared. Thereby, the conducted study targeted the total phenolic content and antioxidant activity content of the wheat crackers prior to and after baking of the wheat crackers. It was analyzed that the in-vitro digestion analysis ascertained upon very high bioavailability characteristics of the fortified cookies. Following in-vitro gastrointestinal digestion, the total phenolic content of all cracker formulations were reduced for the case of the enhanced grape seed flour constitution (20 g to 60 g G1– G3) in comparison to the only wheat (W) formulation ($p < 0.05$). More than half of the total polyphenols (53.89%) were preserved after digestion in the G1 (20 g: Grape seed) crackers. For the case highest reduction was confirmed in this study (Chatziharalambous et al., 2023).

1.3.5.2 Storage Studies

Only one research investigation addressed the storage study of gluten free baked cookie products. In a relevant prior art (Mishra et al., 2015), the gluten-free biscuits which are also suitable as a fasting supplement were addressed. The product was developed through the varied constitution of the powder of the fruits (water chestnut and makhana) and starchy vegetable (potato). The biscuits being developed through the creamery method were evaluated in terms of the physical properties, proximate composition, sensory characteristics and storage characteristics for 90 days at room temperature. For a variation in the constitution of potato and makhana powder, the biscuits were found to have enhanced diameters that varied 1.026 to 1.059 cm and 1.046 to 1.059 cm, respectively. Incidentally, the diameter of the biscuits was 1.075 cm for the biscuits prepared with only water chestnut powder. After 90 days’ storage period, the moisture content of control (CW0M0), WM0 and WM2 biscuit samples were 4.31, 4.26 and 4.29 % (w.b.) (Mishra et al., 2015).

Table 1.7: Literature data summary in the research theme of cookies in-vitro studies

S. No.	Cookies varieties studied	Best findings	Reference
1.	Wheat crackers enriched with 10–30% olive seed, 10–30% grape seed, 10–40% lupine, 10–30% barley and 20–60% and 80% chickpea flours	Total polyphenols (53.89%) preserved after digestion in the G1 crackers, whereas the decrease was observed in the G2 (47.71%) and G3 (46.23%) samples; O3 crackers preserved the majority of their antioxidants among the enriched crackers (AA% = 86.90%)	Chatziharalambous et al., 2023
2.	Low-fat functional cookies substituted with wheat germ flour (WGF) and coffee silverskin (CSS)	Total phenol content of the cookies with added WGF-CSS ranged from 1813.72 to 1838.45 (mg GAE kg^{-1}); phenolic bioaccessibility values ranged between 53.39 and 56.84%; Cupric reducing antioxidant capacity (CUPRAC) method gave higher bioaccessibility values (44.55–51.19%): general acceptability scores of the cookies varied between 5.66 and −7.08, and the 10% WGF cookie (F2) (6.48) sample received the score that was closest to that of the control	Büyüka & Altiner,2023

respectively. This referred to an increment of 0.02, 0.06 and 0.08% for the respective cases. Despite affirming of the fatty acid constitution increment from 0.02 to 0.04%, the three biscuit formulations have been opined to be bereft of any rancid odor or off flavor by the panelists during the sensory evaluation process (Mishra et al., 2015).

A summary of the best findings of the above mentioned literature in the research theme of the storage characteristics of the cookies has been presented in Table 1.8.

1.3.5.3 Acrylamide Studies

Till date, no maximum permitted constitution of the acrylamide content in the foods has been established. However, the European Commission has recently published a directive that aims to control acrylamide levels in the food products produced by its member states (CE, 2007). This could serve as a starting point for the future regulation activities by the commission. Once

Table 1.8: Literature data summary in the research theme of the storage characteristics in the cookies

S. No.	Reference	Cookies varieties studied	Best findings
1	Mishra et al., 2015	Water chestnut; Makhana; Potato powder	Moisture content of control (CW0M0), WM0 and WM2 biscuit samples after 90 days' storage was 4.31, 4.26 and 4.29% (w.b.), respectively with an increment of 0.02, 0.06 and 0.08 %, respectively; storage brought an increment of 0.02 to 0.04%in FFA content of all the studied three biscuit samples; The bacterial load in CW0M0, WM0 and WM2 biscuit samples was 1.3, 1.48 and 1.3 log cfu/g while no yeast and mould counts observed in all three biscuit samples.

a critical level of acrylamide is adopted by the food industry, the segmentation algorithm described in the regulation can be used as a tool for the online safety evaluation purpose. Being an objective, rapid and non-contact tool, the computer vision approach can be regarded as a powerful technique for the acrylamide inspection and evaluation purposes. For the evaluation of color, two alternate approaches exist in the field of computer vision based techniques. These are average color (overall feature) and color segmentation (distribution feature) approaches. Among these, the distribution feature has an advantage due to the fact that the information from every class has been considered in comparison to the global description of the image. Accordingly, the authors made an attempt to develop a simple on-line procedure for the categorization of foods based on the acrylamide content being analyzed through the vision based inspection.

Further, in a related prior art, it is interesting to note that both the browning and acrylamide formation effects followed the same kinetic pattern during the baking process of the cookies. Thus, based on the correlation, a browning ratio lower than 8% can be inferred to ascertain the lowest possible limit of the acrylamide content in the cookies. Thereby, the correlation was used to predict acrylamide concentrations of the tested cookies. Regardless of the recipe formulation and baking conditions, the browning ratio was found to be a reliable indicator of the acrylamide concentration (Gokmen et al., 2008).

In another study (Sung & Chen, 2017), the researchers examined the effects of cookie ingredients and cookie formulation on the acrylamide constitution of the cookies. The research group prepared cookies without adding leavening agents, sodium bicarbonate and ammonium bicarbonate, sucrose, glucose, fructose and with chitosan. The authors also studied the pH, water activity, browning index, and reducing sugar of cookies. In comparison to the control cookies samples that had a high browning index value of 218, the model cookies possessed a lower value of 33 after being prepared with cake flour and water and after 15 minutes baking duration. Also, the cookies prepared with cake flour, water and shortening agents did not ascertain upon a high acrylamide concentration. The highest mitigation (55.2%) of acrylamide formation was for the case in which ammonium bicarbonate was omitted in the control cookie formulation. A positive correlation existed between acrylamide formation and baking time. Also, baking time significantly influenced the physicochemical properties of cookies. Among all ingredients, ammonium bicarbonate was the most effective ingredient to have caused the formation of acrylamide in the prepared cookies. Further, chitosan addition was analyzed to have mitigated the formation of acrylamide during the baking of the cookies.

Table 1.9 summarizes the best reported data in the field of the acrylamide content analysis of the cookie products.

1.4 Possible Scope for Further Research

1.4.1 Study of Grain based Flours Available in North-East India and Unripe Papaya based Powders

Till date, very few researchers conducted studies with respect to the nutritional benefits and physico-chemical properties of the grain based flours such as green gram, soy, rice, roasted

Table 1.9 Literature data summary in the research theme of acrylamide analysis in the cookies formulation

S. No.	Cookies varieties studied	Best findings	Reference
1.	Wheat flour; Different sugars; Leavening agents	Recipe 4: 35.0 g glucose and 1.2 g ammonium bicarbonate Baking conditions- 200 °C × 10 min- 38.6 ± 2.7 (browning ratio); 127.3 ± 6.0 (acrylamide content) Baking conditions- 200 °C × 20 min - 94.3 ± 3.4 (browning ratio) 335.7 ± 7.4 (acrylamide content)	Gokmen et al., 2008
2.	Reducing sugar, sodium bicarbonate, ammonium bicarbonate	Sodium bicarbonate, ammonium bicarbonate and both leavening agents contained 26.6%, 55.2% and 81.1% acrylamide Model cookies containing leavening agents, cake flour and water with sodium bicarbonate and ammonium bicarbonate, contained 458.6 ng/g and 163.4 ng/g acrylamide after 15 min baking time	Sung & Chen, 2017

chickpea flour and Bengal gram flour. All these flours shall be targeted in this Ph.D. thesis due to their abundant availability in the North-east India. The conducted studies did confirm the nutritional and pasting properties of chickpea flour but not the Bengal gram flour (Wani & Kumar, 2014). Also, physico-chemical, light transmittance and syneresis, pasting properties, functional properties, rheology, thermal properties morphology and composition of green gram flour was investigated in another literature (Wani et al., 2019). However, to the best of the knowledge of the author, the proximate, functional, morphological, compositional, crystallinity, thermal, and color indices of green gram, Bengal gram, chickpea, soy, rice, finger millet, and oats have not been thoroughly studied in comparison to wheat. Thus, based on the limitations of the available prior studies, the evaluation of physico-chemical profile of the selected grain based gluten free flours shall be targeted in this Ph.D. thesis and as a part of the thesis first objective.

Vegetable flours or dried powders can be also considered as a good option for the alternate gluten free source. Among several such resources, unripe papaya pulp is rich in various nutritional and bioactive constituents and is an abundant horticultural produce of Northeast India. Further, the unripe papaya peel is also a nutritionally dense by-product of the vegetable or fruit. In a relevant literature (Joymak et al., 2021), the proximate analysis of the ripe papaya peel conveyed appropriately high level of proteins, crude fiber, carbohydrate, ash and fat content. The mineral analysis affirmed that the papaya (*Carica papaya* L. var solo 8) peels are especially rich in potassium and phosphorus. Also, unripe papaya powder (UPP) in comparison to the wheat flour had higher values of water absorption index, water solubility index and swelling index and lower level of amylose. Further, the combinatorial influence of drying parameters on the nutritional and physico-chemical characteristics of unripe papaya pulp and peel powder have not been explored. Considering the mentioned lacunae in the mentioned literature, the optimal conditions for the drying of unripe papaya pulp and peel powder was not studied in the context of physico-chemical profile and shall be considered as the second part of the first objective of this Ph.D. thesis.

1.4.2 Grain Flour based Cookie Formulations and its Characterization

A reported study intended to produce whole meal enriched cookies with high energy, protein and fibre content characteristics. To do so, flours obtained from whole wheat grain and roasted full fat soya bean were targeted for the evaluation of the nutritional, microbial quality and sensory characteristics (Ndife et al., 2014). The study targeted the development of a composite flour program whose objective was to seek ways for the substitution of wheat with flours, starches and proteins from indigenous crops. The formulation of foods from low-lysine staples such as grains fortified with legumes has been proposed as a practical and sustainable approach to improve the protein nutritional value of foods for vulnerable people in developing countries. The mentioned prior art simplified the method adopted for the easy production of quality cookies in terms of their constitution with excellent carriers of a blend of different and varied functional ingredients, good sensory qualities and shelf-life stability. Despite such an affirmation, the conducted investigation had several lacunae. Firstly, combinational varieties of the gluten-free flours for cookies formulation were not investigated to infer upon the sensitive influence of the constitution of various alternate gluten-free flours on the desired characteristics of the cookies. Secondly, sensory based optimization was not addressed to choose the best formulation among several combinations of flours, fats and sweetening indices. Thirdly, only one primary grain based flour was altered and all other flours constitution was kept constant. Such an approach for cookies formulation is not mature. Fourthly, in addition to the flour constitution alteration, other constituents such as fats, oils and sweetening agents such as sugar, jaggery and honey were not altered. Thus, the formulational research had limitations towards the realization of cost effective and scalable formulations. Considering the above-mentioned research gaps, the preparation of various grain based gluten free cookies formulation shall be addressed along with the alteration of the constitution of flours, fats and sweetening agents as the second objective of the Ph.D. thesis.

1.4.3 Supplementation of Unripe Papaya Powder into Gluten-free Cookies

Till date, mature research emphasis has been laid upon the substitution of wheat with ripe papaya powder in the cookie formulations. Various formulations have been explored for the effective utility of ripe papaya pulp and peel powder in the functionally enriched cookies development studies. The emphasis in these investigations was upon the nutritional properties, physicochemical characteristics and organoleptic attributes (Bokaria & Ray, 2016; Bhosale & Iranna, 2018; Jiang et al., 2022; Jadhav et al., 2023).

Also, a research investigation reported the improved nutritive value of high protein cookies that were realized with a composite flour prepared as a blend of soybean and unripe banana flour soybean (Shrestha & Noomhorm 2002). Thereby, wheat flour substitution was explored. The conducted studies had the following lacunae. Firstly, only the middle part of unripe papaya is usually chosen for the preparation of dried papaya powder based products. Thereby, the by-products generated during processing such as the upper and lower parts of the unripe papaya and its peel are discarded as a waste. Consider the enriched nutritional and functional properties of the unripe papaya, the alternate fiber enriched powders of unripe papaya and peel shall be effectively explored. Thereby, the underutilized or neglected portions of the papaya fruit/vegetable can be explored for their utility in the value added functional food product development. In other words, the application of papaya and its by-products as fiber enriched food resources in the development of dietary fiber enriched functional cookies in the gluten free cookies shall be explored in terms of the formulational research and associated desired palette of cookies properties. This has not been studied till date and shall be set as the 3rd objective of the thesis.

1.4.4 Fortification of Gluten-free Cookies with Encapsulated Catechin and Papaya Leaf Extract

Encapsulation is well known to be an expensive process as it involves the utilization of expensive chemicals and drying systems. Very few literatures addressed cost effective chemical free/minimal chemical-based processes for the encapsulation of catechin extracts. In a relevant prior art, the authors prepared catechin encapsulate with β-cyclodextrin as the wall material and with inclusion complexation method (Ho et al., 2019). Till date, no literature reported encapsulation of catechin extracts from fresh tea leaves and with the inclusion complexation method. Another investigation targeted the influence of dried apple pomace powder on the nutritional characteristics of gluten free cookies (Kruczek et al., 2016). The authors considered the evaluation of antioxidant/antiradical characteristics of the cookies with three different methods. The authors as well addressed the physical characteristics such as texture, volume and colour of the cookies and the role of formulational alterations on these properties. However, no literature addressed the cookies that can be prepared through the encapsulation of either catechin extract from commercial green tea or bioactives from papaya leaf extracts. The effect of dual methods (inclusion complexation and ion gelation) on the encapsulation of catechin extracts and papaya leaf extracts shall be studied in detail. Moreover, the sensory and other characteristics of encapsulated papaya leaf and catechin extracts based cookies shall be also addressed as there is a significant limitation in terms of the further functionalization of the gluten-free cookies. Accordingly, the sensitive influence of encapsulated catechin and papaya leaf extracts on the effective and desired fortification characteristics of the gluten-free cookies shall be addressed and set as the fourth objective of the Ph.D. thesis.

1.4.5 In-vitro, Storage and Acrylamide Studies of Optimal Gluten-free Formulations

In-vitro studies were conducted by a research group (in terms of the protein digestibility) for the cookies enriched with *Moringa oleifera* leaf powder (MOLP) decolourised (D-MOLP)and non-decolourised MOLP (ND-MOLP) (Agba et al., 2024). The cookies containing D-MOLP or ND-MOLP both exhibited significantly ($p < 0.05$) higher protein digestibility (58.82–76.43%) compared to the control wheat cookies (52.54%). This improvement aligns with previous studies that reported enhanced protein digestibility of Moringa-enriched cookies. Thus, very few studies were addressed till date to evaluate upon the bioaccessibility of the antioxidants or polyphenols of the gluten free cookies. Further, there has not been any literature that addressed such studies for the case of gluten free cookies being enriched with PPU and PPE. A research group (Olowookere & Malomo, 2024) aimed to investigate the in vitro antioxidant and antidiabetic activities of the cookies produced from wheat and kidney bean composite flours.

Similarly, storage studies for gluten-free baked cookie products were also addressed in a limited content. According to the authors (Mishra & Priyanka, 2015), among six treatments i.e., (100:0, 97.5:2.5, 95:5, 92.5:7.5, 90:10) depending upon different sensory attributes like color, taste, texture and overall acceptability during shelf life study, sample T1 (97.5:2.5) was found satisfactory to store for a period of up to 60 days. However, detailed investigations targeting the influence of constitution of the replacing gluten free flours, shortening agents and sweeteners have not been addressed to ascertain upon the shelf life characteristics of the cookies products. Also, very few studies were conducted for the effective utility of PPU and PPE in the gluten free cookies development and for their storage study characteristics.

In another study (Açar & Pollio, 2012), to confirm upon the interactions of nonfat dry milk, sodium chlorine, high-fructose corn syrup, sugar and shortening agent in the cookie formulation, a model cookie recipe without sodium bicarbonate or ammonium bicarbonate addition was examined to assess upon the role of leavening agents in the acrylamide formation. The cookies preparation through the inclusion of chitosan has been inferred to mitigate the acrylamide formation. Accordingly, the role of formulations for the onset of acrylamide in cookies and models cookies prepared with flour and water were analyzed. Also, in another study, the physicochemical characteristics of the cookies were evaluated to develop better strategies for the reduction of acrylamide content of the baked products (Sung & Chen, 2017). Considering the above cited literature, it can be ascertained that, firstly, no literature till date addressed the acrylamide study of functional gluten free cookies with various grains and unripe papaya powder.

In summary, in-vitro studies targeting the enhanced bioavailability characteristics of cookies prepared with the gluten free grain flours and unripe papaya flours shall be targeted and addressed as the first sub-part of the fifth objective of the Ph.D. thesis. Similarly, the antioxidants/polyphenols stability during storage period shall be studied for the mentioned cookies as a second sub-part of the fifth objective of the Ph.D. thesis. Finally, the acrylamide studies for the prepared cookies shall be addressed to evaluate upon the existence or negation of rancidity in the products prepared. This shall be regarded as the third sub-part of the fifth objective of the Ph.D. thesis.

In summary the targeted objectives of the Ph.D. thesis are justified with the following rationale. Firstly, the enhanced content of gluten-free grain flours shall be first targeted so as to achieve the gluten-free reduced cookies. To do so, the flours based on abundant produce of North-east India shall be deployed. Secondly, gluten-free cookies with diverse sensory profiles based on alternate additives (fats and oils such as butter, ghee, coconut oil, sugar, sweetening agents etc.) shall be achieved to provide ample scope for rigorous product development based on ongoing trends in the consumer ratings. Thirdly, dried papaya powders being achieved with quicker drying process shall be infused to enhance the nutritional profile of functional cookies should be targeted. In this regard, the ultimate goal of such endeavor is to achieve vegetable flour infused gluten-free cookie products. Also, the optimum usage of papaya peel shall be demonstrated. Finally, the further infusion of such products shall be customized in terms of maltodextrin encapsulated catechins and sodium alginate encapsulated papaya leaf extract. For all these rationale, relevant gaps in the literature must be addressed through a comprehensive characterization strategy.

1.5 Objectives

Considering the production of optimal gluten free functional cookies based on dried papaya peel and pulp powder ingredient as the ultimate objective of the Ph.D. thesis, the following objectives have been set based on the mentioned lacunae in various sub-themes that relate to the ultimate objective:

1. Preparation and characterization of the north-east India based gluten-free grain flours and unripe papaya pulp and peel powder.
    a. Evaluation of physico-chemical properties of the gluten-free grain flours which are abundantly produced in the North-east India.
    b. Optimality of drying process parameters for the retention of maximum bioactive components for unripe papaya pulp and peel powder production and physico-chemical properties of the produced powders.
2. Preparation and sensory characterization based ingredient compositional optimization of gluten-free cookies being formulated with grain based flours along with in-vitro bioactivity, storage and acrylamide studies.
    a. Sensory evaluation based on 9-point and fuzzy logic methodologies.
    b. Evaluation of other characteristics such as nutritional composition, physicochemical properties of grain flours based optimal gluten-free cookie formulations.
3. Preparation and sensory characterization based ingredient compositional optimization of unripe papaya peel and pulp powder substituted gluten-free cookies along with in-vitro bioactivity, storage and acrylamide studies.
    a. Sensory evaluation based on 9-point and fuzzy logic based sensory characteristics

b. Evaluation of other characteristics of grain flours and unripe papaya pulp and peel powder based optimal gluten-free cookie formulations

4. Inclusion complexation and ion gelation-based encapsulation of catechin extracts from commercial green tea leaves and papaya leaf extract respectively and subsequent characterization of the gluten-free cookies prior to and after fortification with the mentioned encapsulants.

1.6 Organization of the Thesis

The Ph.D. thesis targeted the formulation and characterization of functional gluten-free grain flour based cookies enriched with unripe papaya pulp and peel powder and further fortified with encapsulated papaya leaf extract and green tea extracted catechins into sodium-alginate-pectin and maltodextrin respectively. This goal is based on identified research gaps, the scope of further research, and established objectives. To achieve these objectives, the Ph.D. thesis has been organized into 7 chapters.

The PhD thesis has been structured into seven chapters and as follows:

Chapter 1 outlines research strategies for developing gluten-free cookies enriched with grain flours. It highlights the nutritional and medicinal significance of incorporating horticultural produce like unripe papaya pulp and peel from Northeast India. The chapter explores the potential of gluten-free flours and details the micro-encapsulation of bioactive components from green tea (catechins) and papaya leaf extracts. It reviews prior studies on characterizing gluten-free flours, dried unripe papaya powders, and grain flour-based cookies enriched with these powders and further fortified with encapsulated bioactives. The chapter concludes by finalizing the objectives of characterization of functional gluten-free sugar-snap cookie formulations.

Chapter 2 details the materials and procedures used to achieve the thesis objectives. Methods for each objective are meticulously outlined. The sugar-snap cookie formulation, cookie characterization (including sensory analysis), and procedures for evaluating the dough's rheological behaviour are described. Additionally, it covers the methodologies for inclusion complexation and ion gelation-based encapsulation of catechin extracts in maltodextrin and sodium alginate-pectin systems. Finally, it outlines the in-vitro digestion, storage, and acrylamide analysis of sensory-optimized cookie formulations

Chapter 3 explores the use of rice, roasted chickpea, Bengal gram, soy, finger millet, and oats, along with dried unripe papaya pulp and peel powders, as gluten-free alternatives to wheat flour in Northeast India. These substitutes offer diverse flavors, enhanced nutrition, and support regional agricultural sustainability. It details the optimization of key parameters for maximum retention of bioactive components and examines the functional characteristics and mineral content of the unripe papaya powders. Characterization techniques such as FESEM, FTIR, XRD, color, particle size distribution, and DSC analyses were used. The chapter also compares the impact of oven drying temperature on the moisture and nutritional content of unripe papaya powders and assesses water and oil absorption capacities, which are crucial for food processing and sensory qualities.

Chapter 4 examines gluten-free cookies formulated with rice, soy, green gram, finger millet, oats, Bengal gram, and roasted chickpea powders, the gluten-reduced cookies incorporated wheat, Bengal gram, and roasted chickpea flours. The study aims to assess upon the sensitive influence of variant powder compositions on the sensory and nutritional properties of the cookies. Characterization targeted were towards the assessment of texture, color, nutritional composition, and sensory attributes.

Chapter 5 investigates the potential of the utility of unripe papaya pulp (PPU) and peel powder (PPE) as substitutes for grain-based powders in cookie formulations. The primary aim of such efforts is to enhance the cookies' nutritional profile. Accordingly, the grain-based powders being typically used in cookie recipes was replaced with variant proportions of PPU and PPE for the nutritional enrichment of the cookies. The sensory evaluation scores for various cookie samples affirmed notable differences in the sensory parameters.

Chapter 6 elaborates the hot water extraction of the catechins from the commercial green tea and bioactives from papaya leaves. Following this, encapsulation of this catechin extract by inclusion complexation (IC) and ion gelation (IG) have been addressed. Thereafter, encapsulated IC based encapsulated catechin and IG encapsulated papaya leaf extract (PLE) were introduced to the best performing gluten-free cookies for the enhancement of antioxidant activity of the cookies.

Chapter 7 outlines the major conclusions for the obtained research findings of the Ph.D. thesis. Thereby, the chapter enlists the possible research pathways in the field of the formulation and characterization of gluten-free flours and correspondingly enriched and fortified cookie formulations.

Chapter 2:

Materials and Methods

Materials and Methods

The chapter elucidates upon the adopted materials and followed methods for the fulfillment of the Ph.D. thesis objectives. Accordingly, the chapter is organized into the following sections – (a) materials required for the thesis work (b) procedures adopted for the preparation and characterization of grain powder and dried unripe papaya pulp and peel powder (c) sugar snap cookie formulation and methodology adopted for the characterization of cookies (including sensory analysis) (d) procedures followed for the evaluation of the rheological behavior of the dough of the cookie formulations (e) inclusion complexation and ion gelation based encapsulation of hot water based catechin extracts in maltodextrin and sodium alginate –pectin system respectively, and their subsequent characterization (f) procedures adopted to study the influence of catechin extracts (prior to and after encapsulation) on the bio-active fortification efficacy in the formulated cookies and (g) procedures followed for the in-vitro digestion, storage and acrylamide analysis of the optimized cookie formulations.

2.1 Materials

Unripe papaya fruits (in immature condition) were procured from Shingimari area, Kamrup, Assam, India (26.2205°N and 91.6241°E). The unripe papaya fruit samples were carried in a tightened polyethylene pouch so as to avoid external contamination during transport. For the carried out investigations, fully green matured unripe papaya fruit with green peels were selected.

Sodium hydroxide pellets, oxalic acid dehydrate, sulfuric acid (98%), dextrose, absolute methanol and petroleum ether were procured from Merck India; Anthrone extrapure, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) extrapure, Folin ciocalteu reagent (FCR) and 2,6-dichlorophenol indophenol sodiumsalt (DCPIP) were bought from SRL Pvt. Ltd., India; sodium bicarbonate, Bovine Serum Albumin and Bradford Reagent were purchased from

Rankem, SRL and Sigma Aldrich, India respectively. Quercetin and Gallic acid (98 % pure) were obtained from Sigma-Aldrich Ltd, India. All solutions were duly prepared with the deionized water (DI) being procured from a laboratory scale commercial unit (Make: M/s Millipore, Model: Elix-3, Milli-Q, USA).

Rice powder, refined flour (Rajdhani, Select), chickpea flour (Besan, Rajdhani- Gram flour Grade-1), roasted chickpea flour (Bengal Gram flour, Rajdhani), soy flour (Urban Platter Soya Bean Flour), green gram (Tata Sampann) unpolished green moong dal (Whole), ragi flour (Pure & Sure Organic Ragi Flour), oats (Kellogg's), soybean oil (Fortune soyabean Oil), coconut oil (Pure & Sure Organic Coconut Oil Cold Pressed), butter (AMUL Butter), ghee (Gowardhan Cow), cookie shortening agent (CCDS Bakers Shortening), groundnut oil (Pure & Sure Organic Groundnut Oil), sugar (Uttam Sugar Sulphurless Sugar), honey (Zandu Pure Honey, 100% Purity, No Added Sugar), artificial sweetener (Sugar Free Natura), jaggery powder (Organic India) were purchased from the market complex, IIT Guwahati and online web portal of Amazon, India.

2.2 Preparation and Characterization of Grain Flours and Dried Unripe Papaya Pulp and Peel Powder

2.2.1 Drying of Unripe Papaya Pulp and Peel

Prior to the conduct of all experiments, the unripe fresh papaya vegetable was procured from the market-complex of IIT Guwahati, Assam, India. After peeling and washing, the seeds were removed in the green pulp. Thereby, the samples for the drying studies were prepared by cutting the green peel and pulp portions of the vegetable into rectangular slices ($3.5 \times 2 \times 0.05$ cm^3). The samples were then placed on trays and were kept in laboratory scale hot-air-oven (Make: REICO with two same sized trays of 2400 x 27.800 inch^2). With an overall dimension (inch^3) of 2400 × 2400 × 2400 and an insulation thickness of 200, the hot air oven hosts 2 trays (dimensions of 1800 × 1800 × 200). The drying of the samples was conducted for various temperature time combinations which were duly that altered in the range of 65- 105 ºC and 45 - 180 min respectively. In this regard it should be noted the milled products procured commercially have been termed as flours in the Ph.D. thesis. Also, the term refers to the produce in the lab and through the processes of drying, grinding and sieving.

2.2.2 Analytical Characterization of Grain Flours and Unripe Papaya Powders

2.2.2.1 Bioactive Compounds

For the dried samples, total phenolics (TpC) and flavonoids (TfC), antioxidant-activity (AA) vitamin C (VC) and proximate characteristics such as moisture-content (Mc), ash-content, soluble-protein-content, total carbohydrate-content, fat-content etc. were evaluated. The aqueous extract of dried unripe papaya powder (PPU) and grain flour was investigated for the TpC, TfC, % AA and VC context. The TpC was estimated with the FCR method (Tharasena & Lawan, 2014). Using a calibration curve that was prepared with the gallic acid, the TpC content of the sample was determined with the prior art reported method (Wani and Uppaluri, 2022) and was expressed in terms of mg of gallic acid equivalents (GAE)/g dry weight of PPU/grain flour sample (mg/g sample). $AlCl_3$ method was adopted to determine the TfC of the aqueous extract (PPU and grain powders) (Tharasena & Lawan, 2014). The TfC of the sample was determined in terms of mg of QE/g dry weight of powder. The AA of the PPU methanolic extract was determined with the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay method (Mukherjee et al., 2022). For the TfC and AA analyses, the processed samples were analyzed for their absorbance at 750 and 517 nm respectively. For the VC evaluation, 2,6-Dichlorophenol Indophenol (DCPIP) titration assay was deployed to determine the ascorbic acid content of the powder extract (Anjali et al., 2012). All investigations were carried out in triplicate, and the findings were reported as the mean ± standard deviation ($p < 0.05$ considered to be significant).

2.2.2.2 Nutritional Properties

Proximate characteristics such as yield, moisture (Mc), carbohydrate (Clegg anthrone method), protein content (using Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09), fat content (using extraction method that adopted petroleum ether under reflux conditions in a Soxhlet apparatus (AOAC Method 963.15), ash content (using gravimetric method that involved burning at 550°C in a furnace (AOAC Method 923.03) (AOAC, 2005), soluble-protein content (using Bradford method) (Bradford 1976), crude-fiber content (using hot sulfuric acid-sodium hydroxide treatment method) and mineral content were conducted. For such assessments, procedures summarized in former publications were duly followed (Mondal et al., 2019).

2.2.2.3 Functional Properties

The water absorption capacity (WAC) and index (WAI) are prominent functional properties. The WAC and WAI were determined for the wheat flour and were assessed by following the method of (Godswill, 2019). The WAI was determined by first placing 1g (M_o) of the sample in a screw capped tube. Thereafter, 12 mL of distilled water was added and the mixture was agitated for 30 min at room temperature. Subsequently, the sample was centrifuged at 3000 rpm for 30 min. Thereby, the supernatant liquid layer was collected and the recovered volume was measured in a graduated cylinder. Using the measured wet pellet weight (M_1), the WAI was expressed in terms of g per g of sample dry weight and with the expression as:

$$WAI = M_o - M_1 \tag{2.1a}$$

The WAC being expressed in terms of mL/g was determined with the expression:

$$WAC = V_0 - V_f \tag{2.1b}$$

Following the WAI and WAC, oil absorption capacity (OAC) was analyzed with the method of Batham et al. (2013). For this purpose, firstly, one gram (OM_0) of the sample was dispersed in 5 mL of refined sunflower oil, and the slurry was centrifuged at 3200 rpm for 25 min at room temperature. Thereafter, the supernatant was decanted and the centrifuge tube was inverted for 5 min on a paper towel. Thereby, the obtained residue was weighed (OM_1). Using OM_0 and OM_1 the OAC (%) was determined with the expression:

$$\% \, OAC = \frac{(OM_1 - OM_0)}{OM_0} \times 100 \quad (2.2)$$

The method of Lee et al. (2012) was followed for the assessment of the SP and solubility. To do so, firstly, about 0.5 g of the sample was dispersed in 15 mL of distilled water in a screw-cap tube. Thereafter, the dispersion was placed in a water bath at 90°C for 30 min. Thereby, the mixture was shaken thoroughly every 5 min. Following this, the dispersion was immediately cooled and centrifuged at 3000 rpm for 20 min. duration. Thereafter, the amount of solids in the supernatant was measured after drying the wet sample in a hot air oven at 120 °C for 6 h. Using the weight of the wet precipitate, the solubility (%) and swelling percentage (g/g) were determined with the following expressions:

$$\% \, Solubility = \frac{Weight\ of\ dry\ solids\ in\ supernatant}{Weight\ of\ the\ flour} \times 100 \quad (2.3a)$$

$$\% SP = \frac{Weight\ of\ the\ precipitate}{Weight\ of\ the\ flour} \times (100 - Solubility) \quad (2.3b)$$

The bulk density of the sample was obtained with the gravimetric method. To do so, the method presented by Okezie & Bello (1988) was followed. This involved the weighing of 100 g of the sample and its loading in a calibrated 250 mL measuring cylinder. Thereby, the volume was recorded for the bulk density (BD). The BD was assessed with the following expressions:

$$BD = \frac{m}{Vo} \quad (2.4a)$$

where, m is the mass of the sample, Vo is the unsettled apparent volume.

For the evaluation of dispersibility, about 10 g of the sample was first stirred vigorously with 100 mL of distilled water and was allowed to settle for 3 h. Thereafter, the volume of the settled particles was recorded and subtracted from 100. This difference was termed as the percent Dispersibility (Kulkarni et al., 1991).

2.2.2.4 Thermal Properties

The thermal properties of the powders (grain flours, PPU and PPE) and cookies were analyzed with a differential scanning calorimeter (make: NETZSCH model: DSC 3500 Sirious), differential scanning calorimeter. To do so, 7 mg of sample was placed in a 40 µL concave aluminum crucible with a lid. A hole was pierced in the lid and thereafter, the system was placed inside the DSC pan for the thermal analysis in the temperature range and heating rate of 30 - 400°C and 10°C/min respectively. In the DSC experiment, the inert environment was maintained at a flow rate of 60 mL/min of N_2 (protective gas).

2.2.2.5 Morphology

The morphology of the powder (grain flour, PPU and PPE) and cookies was assessed with the Gemini 300 FESEM instrument (make – Zeiss, model - Gemini) (Rajisha et al., 2014). The powders were first drop casted with a piece of glass and aluminium foil. Thereafter, 0.1 g of the sample was mixed with 10 mL of water and the mixture was subjected to sonication for 10 min duration. Subsequently, the mixture was subjected to the casting on a glass piece covered with an aluminum foil. Eventually, the drop casted sample was attached to the stub with the double sided carbon tape. Finally, the samples were gold coated under vacuum to achieve the pre-treated sample for analysis with Gemini FESEM instrument.

2.2.2.6 Determination of Average Particle Size, PdI and Yield

The average particle size and polydispersity index (PdI) of the dried powders (grain flours, PPU, PPE) and cookies were evaluated with the Delsa Nano C particle analyzer (dynamic light scattering method) (DLS, Litesizer™ 500, Anton Paar, USA) (Ge et al., 2017). Thereafter, 0.1

g of sample was mixed with 20 mL water and was sonicated for 10 mins. Thereafter transferred into a cuvette for analysis at 25°C and 170° scattering angle. For the assessment, the deployed diluent water possessed a refractive index and viscosity of 1.3328 and 0.8818 cP respectively. The yield of the unripe papaya pulp and peel powder were evaluated as a ratio of the weight of the quantity of powder produced after drying to the weight of the fresh initial sample (prior to drying).

2.2.2.7 XRD and FTIR Analysis

The PPU, PPE, grain powders and cookie samples were analyzed with an X ray diffractometer (make: Rigaku, model: Micromax-007HF). The obtained diffractograms were analyzed with Origin pro software (version 9.0) for the evaluation of the crystallinity of the sample. The XRD measurements were conducted at 40 kV and 100 mA conditions of the instrument and in the 2θ range of 3 - 40°C. The structural analysis of the PPU, PPE and grain powders along with cookie samples were conducted with the FTIR instrument (FTIR; Japan; Shimadzu; IR Affinity1). The FTIR spectra were measured in the wavelength range of 400 – 4000 cm^{-1}.

2.2.2.8 Color

The colour indices of both types of the dried powder samples (PPU, PPE, grain flours and cookies) were determined with a colorimeter (Make: Data color, Model: 550, USA) set up. Thereby, L*, a* and b* parameters were assessed by the instrument (Caparino et al., 2012). In these, 'L*' value represents lightness (0 for black color and 100 for white color) and the 'a*' scale represents the red/green dimension (positive value for the red colour and negative values for the green colour). Further, the 'b*' scale represents the yellow/blue dimension, (assigned positive value for the yellow colour and assigned negative values for the blue colour). For each case, the measurements for L*, a* and b* were conducted for three alternate regions of the assessed powders and cookie samples. Finally, the color data were reported as the mean of these three measurements.

2.2.2.9 Statistical Analysis

Each sample was analyzed in triplicate for its functional property and proximate properties assessment. The obtained data was further assessed with the standard deviation approach. The statistical analysis was conducted with the SPSS 16 software.

2.3 Formulation and Characterization of Grain and Unripe Papaya Powder Substituted Gluten-free Cookies

2.3.1 Preparation of Cookies

The cookies dough formula constituted various gluten-free flours (rice, green gram, soy, roasted chickpea, Bengal gram flours) along with the emphasis to replace some of these with the varied constitution of the PPU, PPE (0 wt% for control and upto 33 wt% into the various grain based formulations). The other ingredients were: shortening agent (26.6 wt%), sugar or jaggery (21.6 wt%), sodium bicarbonate (0.3 wt%) and liquid milk (5-10 mL). Such specifications were based on few trials also utilized. This aspect further complicated the rigorous production of consistent, high-quality cookies. Further, details of these would be addressed in chapter 4. Appropriate fat and sweetening ingredients (sugar and jaggery) were creamed with a hand mixed blender and for 5 to 10 min. duration. Thereafter, sieved flours were blended severely for the proper entrapment of air which effects the texture of cookies. Thereby, baking powder was added and the mixture was matured to form the dough for another 5 min. duration of the kneading process. The dough was divided thereafter into small balls of 30 g each and was then flattened into the cookie cutter (1 cm thickness: 1 cm). The flattened dough was kept in the refrigerator for 10 min for better hydration of the cookie. The cookies were then transferred to a baking tray and were baked at 150°C for 20 min in an OTG equipment (Prestige, Verona, Italy). After baking, the cookies were cooled at room temperature (20°C) and were packed in sealed bags prior to the analysis.

2.3.2 Cookies Characterization

2.3.2.1 Color and Textural Analysis

The cookies prepared being with proportional alterations of flours, fats and sweetening agents were packed and stored in pouches at room temperature. The physical characteristics of cookies such as width, thickness and spread factor were measured on a fortnight basis for the samples stored up to 60 days. For this purpose, methods detailed in AACC (3) were duly followed.

The width (w) of cookies was measured by placing six cookies horizontally and on an edge to edge basis. Thereafter, the cookies were rotated at 90° angle for duplicate measurements. Similarly, the thickness (T) of cookies was measured. Also, the spread factor (SF) was determined with the expression

$$SF = \frac{W}{T} \times CF \times 10 \qquad (2.5)$$

where CF is the correction factor at the constant atmospheric pressure (10.0 in this case)

The colour parameters of the grinded cookies were measured with a colorimeter (Make: Data color, Model: 550, USA). Also, prior to the analysis, black and white calibration tiles were used to standardize the instrument. The determined color parameters were: L* (lightness factor, 0 = black, 100 = white), a* (−a = green, +a = red) and b* (−b = blue, +b = yellow). The colour coordinates a* and b* were used to calculate chroma, the hue angle between the control and the gluten free different cookies formulations. Both control and alternate cookies formulations were considered for the color assessment. For each sample, 15 measurements were conducted and the average color value was thereby evaluated.

The texture properties of the cookies were measured through a compression test being conducted on an Instron Texture Analyzer (TA.XT. plusC, Stable Microsystems, UK). The equipment is equipped with a cylinder probe of 3 mm diameter and a load cell of 500 N. The tests were conducted at a test speed of 1 mm/s and a distance of 4 mm. The maximum force (hardness) required to break the cookies was recorded and the average of six replicates has been reported in the thesis.

2.3.2.2 Sensory Analysis

The sensory attributes of the sugar snap gluten free cookies, namely, color, taste, crispness, odor, appearance and overall acceptability, were determined by a panel of thirty consumers (15-70 years of age; equal proportions of males and females). The candidates in the panel were screened for their frequency of cookie snack consumption, which is accepted to be atleast once a month. Each panelist evaluated various formulations of sugar snap cookies (such as grain based and unripe papaya pulp and peel flour based cookies) with the 9-point hedonic scale. In this scale, the lowest value (1) has been assigned for extreme disliking case and the highest value (9) has been assigned for the extreme liking case. During the sensory evaluation, gluten free cookies of each experimental type were placed separately on plastic plates that were labelled randomly with three-digit codes. Prior to the tasting activity of a sample, lemon water was served to the panelists to neutralize their mouth feel. Thereby, the testing quality can be maintained for a sequence of samples. Also, freshwater was served to the panelists for the cleaning of the mouth in the intermittent duration between two consecutive samples. In the fuzzy modelling alternate approach, linguistic variables were used to develop a relationship between independent and dependent variables. The adopted method utilized linguistic data from both subjective and objective evaluations (Mukherjee et al., 2023; Jaya & Das, 2003). The linguistic variables used were excellent, good, not satisfactory etc. For the analysis, the independent variables were color, aroma, taste, mouth-feel, convenience etc. The corresponding dependent variables were ranking, rejection, acceptance, strong and weak attributes of cookie samples.

2.3.2.3 Rheological Testing of Cookie Dough

The rheological behaviour of the cookie dough was determined immediately after the sheeting process and with a (Physica MCR 301, Anton Paar) by adopting PP50 (cone and plate geometry). The rheometer was equipped with a 50-mm parallel plate measuring geometry. The plates were serrated in order to prevent the slippage of the dough. During such studies, a dough sample was placed on the lower plate, and the upper plate was lowered until the gap of 3.0 mm was reached. Thereafter, the excess dough was trimmed and the edges were sealed with the paraffin oil so as to prevent of the dough drying during measurements. Also, prior to 10 min duration of the measurements, the sample was left to rest. For the relaxation of residual stresses such measurements were conducted with triplicates and at 30 ºC. The frequency sweep test was carried out from 0.1 to 100 Hz, and under a 5-Pa strain level. The test was within the linear viscoelastic region for all cookie dough samples. Thereafter, the frequency sweeps were plotted in terms of G'(f) and G"(f) and in a double logarithmic diagram. The experimental data of G' versus frequency were subjected to fitness data analysis. This is represented with the following equations where G' is the storage (elastic) modulus (Peressini et al., 2000). The values of tan δ, which represent the ratio of energy lost or dissipated (G") to the energy stored in the material and recovered from the material it per cycle of sinusoidal deformation (G') were also reported. Since frequency sweep test is a small deformation test which does not destroy the dough structure, creep recovery test was conducted immediately after conducting frequency sweep measurements on the same dough sample. Few the tested initial experiments confirmed that the tested cookie dough reached a steady viscous flow in this time range.

2.3.2.4 Analytical Characterization

The sensory analysis based optimal gluten-free cookie formulations were subjected to various characterization studies such as the assessment of bioactive compounds, nutritional and functional characteristics, thermal properties, morphology, yield, XRD and FTIR analysis. A detailed account of the adopted procedures has been presented in sections 2.2.2.1 - 2.2.2.7 of the Ph.D. thesis.

2.4. Hot Water Extraction and Encapsulation of Catechin and Papaya Leaf Extracts

Catechins were extracted from Tetley commercial green tea leaves and with a modified hot water extraction method (Chandini et al., 2011). To do so, firstly, 10 g of dried tea leaves were solubilized in 500 mL of water and were subjected to the extraction in a water bath (Equitron S7514) at 90°C for 40 minutes. The extract solution was then filtered with Whatman filter paper. The Whatman filter papers are extensively utilized for various applications. These include but not limited to gravimetric analysis, sample preparation, and general laboratory filtration. Their ability to support diverse analytical techniques render them to be indispensable in laboratories belonging to multiple scientific fields. Thereafter, the extract solution was concentrated in a rotary evaporator dryer system (Make: Buchi Rotavapor Model: R 300) at 60°C. The concentrated solution was eventually dried in an oven dryer for 12 h at 60°C. The dried catechin extracts were stored at -19°C for a maximum duration of two weeks to facilitate further characterization.

The papaya leaves were dried at ambient temperature (25°C) in the laboratory environment. The moisture content (AOAC, 2010) of the dried papaya leaf powder was about 2.5 ± 0.045%. For this, a known quantity of papaya leaf powder was dispersed in a fixed quantity of DI water. Thereafter, the mixture was stirred on a magnetic stirrer for a few minutes. Subsequently, the mixture was subjected to pulsed mode of sonication as per the optimum conditions obtained for the case. The optimum conditions for pulsed ultrasound-assisted extraction (PUSAE) were 58.59°C extraction temperature, 18.82 min sonication time, and for a fixed choice of the loading ratio. Eventually, the mixture was filtered with a filter paper (Whatman #1, GE

Healthcare Life Sciences, India) and the supernatant was collected and stored in a refrigerator for further analysis.

The encapsulation of papaya leaf extract (PLE) was conducted with the ion gelation method. A two-step procedure delineated by the researchers (Zhang et al., 2021) was followed along with minor modifications. Firstly, calcium alginate-pectin beads were prepared by contacting sodium alginate-pectin mixture with 100 mL of the optimized PLE. Thereby, the mixture was heated at 50°C on a magnetic stirrer for 5 min at 600 rpm to achieve a uniform mixture. Thereafter, the mixture was kept aside for 20 min to allow the removal of air bubbles in the solution. Eventually, using a syringe pump (22G stainless steel needle, 0.5–3.5 mL/min flow rate), the solution was added drop wise to 3–9% w/v $CaCl_2$ solution. The height between the needle and the collecting solution surface was kept constant and at 10 cm. The subsequently obtained beads were allowed to solidify in a stirred gelling bath (3–9% w/v, $CaCl_2$) for 30 min. Finally, the mixture was filtered with a filter paper (Whatman #1, GE Healthcare Life Sciences, India) and the beads were washed thrice with DI water. Thereby, the obtained solidified wet beads were divided in two portions. One portion of the beads was dried at 40°C for 24 h and were termed as dry beads. They were stored in a desiccator until further use.

A modified inclusion complex method was followed to encapsulate the catechin extract (Krishnaswamy et al., 2012; Wani & Uppaluri, 2022). The literature reported methods adopted a 1:1 ratio (on a molecular weight basis) for the wall material and bioactive compound at 0.006 mol/L. Nevertheless, considering the fact that the catechin extract consists of a combination of various catechin compounds, including EGCG, ECG, EGC, EC, C, and other polyphenols, a fixed weight basis was established in this study for the catechin extracts. Thus, a precise molecular weight basis was not considered and the molecular weight of the catechin being reported in previous studies was presumed. Accordingly, 0.87 g of catechin extracts were mixed in 500 mL distilled water. Consequently, 1.51 g of maltodextrin was added to the aqueous catechin extract. For effective dissolution, the solution was stirred on a magnetic stirrer at 500 rpm for 12 h in a dark environment. Thereafter, the solution was concentrated in a rotary evaporator at 60°C, and the concentrated samples were subjected to drying in a vacuum oven for 12 h at 50°C and 760 mm Hg vacuum pressure. The dried micro-encapsulated particles were stored at -19°C for a storage period of 2 weeks to facilitate further characterization.

2.4.1 Fortification and Characterization of Cookies with Inclusion Complex and Ion Gelation Encapsulated Bioactive Extracts

Among 9 alternate cookie formulations that were identified to be best with the best sensory data, only two green gram flour based cookies were chosen for the fortification purpose. While one formulation was prepared with only green gram flour (MS2), the other formulation was prepared with the PPU powder substitution (UMS2). Both cookies samples were subjected towards further characterization such as bioactives constitution, in-vitro study, thermal properties, XRD and FTIR analyses. For the fortification of gluten free cookies, another formulation based on rice flour with 0.4% and 1% encapsulated papaya leaf extracts was considered. Further, catechin extract, papaya leaf extract, encapsulated catechin and papaya leaf extract samples were subjected to FTIR, morphology, thermal properties, and crystallinity analyses. The methods adopted for thermal properties (DSC), morphology, and crystallinity (XRD) have been explained in sub-sections 2.2.2.4, 2.2.2.5 and 2.2.2.6 respectively in the Ph.D. thesis.

2.5 In-vitro Digestion, Storage and Acrylamide Study of Sensory based Optimized Cookie Formulations

2.5.1 In-Vitro Digestion Study

The in-vitro digestion studies for the sensory optimization based grain and PPU-PPE based gluten-free cookies were conducted as per the procedure reported in a relevant prior art

(Cairano et al., 2021) and with few modifications. Accordingly, the bioaccessibility of the grain based and PPU based sensory optimized cookies with and without fortification approach were assessed. Adopting digestive enzymes, pH adjustments, digestion times, and salt concentrations, simulated environments have been achieved for oral, gastric and intestinal phases of digestion. A brief account of these phases are as follows.

a) Gastric phase: After oral phase, the solution was mixed with 30 mL solution of 140 Mm NaCl and 5 Mm KCl. Subsequently, 0.5 mL of 11000 U/mL Himedia pepsin 1: 1000, EC 232-629-3 solution being prepared in SGF electrolyte stock solution was added to the sample mixture. After thorough mixing, the pH of the mixture was reduced to the value of 5 and with 6M HCl. Thereafter, the vessel containing final mixture was agitated in a water bath at 100 rpm for two hours at a temperature of 37°C.

b) Intestinal phase: After the gastric phase, the solution was well mixed with 2.5 mL of pancreatin (Pancreatin from Porcine Pancreas, P7545-25G, Sigma Aldrich) solution being prepared in the SIF electrolyte stock solution. To this system, 40 µL of 0.3M $CaCl_2$ was added and the pH of the mixture was adjusted to 7 through the addition of 0.5 cc of 1M NaOH. Finally, the agitation procedure was similar to that mentioned for the gastric phase was conducted.

After completing enzymatic reaction process, the sample solution vessel was submerged in a cold bath for 10 minutes. Eventually, the sample solution was then centrifuged for 40 minutes at 5000 rpm. The resultant supernatant was then tested for total phenolic content and radical antioxidant activity (DPPH scavenging method).

The bioaccessibility index (BI) was used to assess upon the sensitive influence of impact of in-vitro gastrointestinal digestion on the content of RSA and was determined with the following equation (c).

$$Bioaccesibility = \frac{A}{B} \times 100 \quad (2.6)$$

where *A* and *B* are radical antioxidant activities (DPPH) of the samples prior to and after digestion (in-vitro) respectively.

2.5.2 Storage Study

The storage study of the cookies was conducted as follows. Firstly, the cookies were packed in biaxial oriented polypropylene pouches and were kept at the ambient condition. Thereafter, the cookies were evaluated for shelf life and through the assessment of moisture content, water activity, free fatty acids, microbiological quality and sensory evaluation at the regular time duration of 0, 30 and 60 days. The moisture content was determined with the oven drying method. The water activity (a_w) of freshly powdered cookie samples was measured at 25±0.5 °C and with a water activity meter (BIOBASE WA-60 A). The a_w data was collected from the probe itself.

The fat extracted from the cookie (0.2 g) was diluted with 0.8 mL of $CDCl_3$.The mixture was agitated thoroughly and was thereafter transferred to 5 mm NMR tubes. Thereby, ^{1}H-NMR spectra were recorded with a Bruker Advance III 400 Spectrometer (ASCEND 600, Bruker, Germany). The instrument was operated at 9.4 Tesla, which corresponds to the resonance frequency of 400.13 MHz for the ^{1}H nucleus. Also, the system was and equipped with a direct detection probe head with four nuclei and field gradients on the z-axis. The typical parameters for ^{1}H-NMR spectra were as follows: 45° pulse, 2.05 s acquisition time, 6.4 kHz spectral window, 16 scans, and 26 K data points. The average acquisition time of the ^{1}H-NMR spectra was approximately 2 min. The spectral acquisition and processing were facilitated with the TopSpin 3.2 software (Bruker BioSpin).

The total plate count (TC), yeast and mould count (YMC) for the existence of *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in the aqueous extracts of the stored cookie samples were analyzed. The total plate count (TC), yeast and mould count (YMC) for the existence of *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in the aqueous extracts of the stored cookie samples were analyzed. Plate Count Agar should be utilized under aerobic or microaerophilic conditions at a temperature of 35°C to achieve the best results for total plate count. Potato Dextrose Agar is the chosen medium for the YMC. The plates should be incubated at temperatures of 25 ± 1°C for 3 to 5 days to ensure sufficient growth of yeasts and molds. To do so, the standard methods described in the manual of the Food and Agriculture Organization, United Nations (Culetu et al., 2020) were adopted.

2.5.3 Acrylamide Study

Acrylamide is a suspected carcinogen required to be listed on food labels in California and products commercially traded within the European Union. Acrylamide may exist in trace levels in food products such as potato products, coffee, crackers, etc., Acrylamide is produced due to the high temperature based reaction of few amino acids, such as Asn, Arg and Lys, with the reducing sugars. Current methods for the quantitative detection of acrylamide in the food sample are costly, time consuming, and are dependent on expensive scientific instrumentation. The Liquid Chromatography-Mass Spectrometry (LC-MS) is a classic example for the same. In this work, the qualitative assessment of acrylamide in the cookie sample was followed. To do so, ATR (Attenuated total reflectance) - FTIR (Fourier Transform Infrared) spectroscopy was followed which is inexpensive, fast, and easy to use.

The Fourier transform infrared (FTIR) analysis is a powerful tool that is often used for the identification of the alternate types of chemical bonds in a molecule. The infrared absorption spectrum serves as a 'molecular finger-print' and thereby generates a spectra that facilitates the identification of functional groups in the analyzed organic sample. For the detection of acrylamide, the ATR- FTIR spectrometer (Perkin Elmer- Spectrum 1) was used to obtained the processed infrared spectra. The instrument is equipped with a three-reflection diamond ATR accessory along with a deuterated triglycine sulfate (DGTS) detector and uses a Michaelson interferometer to disperse the light. The sampling solids facility in the instrument was used to apply a uniform pressure to the sample. Using four background scans and four sample scans that facilitate improved signal to noise ratio, the FT-IR spectra were collected for the cookie sample which was subjected to preparation procedures that were mentioned in section 2.5.3 contains critical information that has been highlighted in the context of the relevant discussions of the Ph.D. thesis. With a spectral resolution of 4 cm^{-1} and an air background, the spectra were collected in the frequency range of 4000 - 400 cm^{-1}. Duplicate spectra were obtained for the same cookie sample so as to ascertain upon the respective spectral peaks.

The existence of acrylamide in the ATR-FTIR spectra is confirmed through the existence of peaks at the range of (3350 and 3160), (1670-1612), (1400-1100), (1420 and 1350) and (1138 and 980) (Murugan et al., 1998). Thus, the absence of these peaks quantifies the non-formation of the acrylamide during the high temperature based baking of the gluten free cookie products.

Chapter 3:

Exploring the Nutritional and Functional Potential of Northeast India's Gluten-free Grains Combined with Unripe Papaya Pulp and Peel Powder

Chapter 3

Exploring the Nutritional and Functional Potential of Northeast India's Gluten-free Grains Combined with Unripe Papaya Pulp and Peel Powder

In this chapter, the characteristic profile of gluten-free flours of different sources based on grain flour indigenous to Northeast India and unripe papaya pulp and peel powder has been presented. Following this, optimality condition of drying parameters i.e. temperature-time has been addressed. A concise introduction has been presented in section 3.1 of the chapter. Thereafter, the results obtained for the for the drying parameter optimality study of unripe papaya pulp and peel has been summarized in section 3.2. Section 3.3 entails the results and discussion of functional characteristic profile of the eight types of grain flour and unripe papaya pulp and peel powder. Accordingly, section 3.4 delineate upon the experimental results obtained the mineral content of grain based flours and PPU-PPE powders. Section 3.5 encompasses the nutritional parameter analysis for grain based flours and unripe papaya powder (pulp and peel). Section 3.6 presents different characteristics such as FESEM, FTIR, XRD, Particle size, DSC, color etc. of grain flours and PPU and PPE powders. Thereafter, in section 3.7, a brief literature comparison has been addressed to clearly mention upon the novelty of the current investigation. Finally, the significant findings of the conducted investigations have been summarized in section 3.8 of the chapter.

Overview

This overview explores the utilization of rice, roasted chickpea, Bengal gram, soy, finger millet, oats, along with unripe papaya pulp and peel powder in dried form, as indigenous gluten-free alternatives to wheat flour in Northeast India. These nutritious substitutes offer diverse flavor *profiles and enhanced nutritional content, catering to dietary preferences and promoting regional agricultural sustainability. This chapter elaborates upon the detailed intricate process of sensitivity-driven optimization of key parameters to maximize the retention of bioactive components. Consequently, the chapter entails upon the functional characters of grain flours and PPU-PPE powders. Furthermore, the mineral constituent and other proximate study has been addressed for both grain flours and unripe papaya pulp and peel powders. Grain based flours and PPU-PPE powders were further characterized by FESEM, FTIR, XRD, color, Particle size distribution and DSC. A comparative analysis with existing literature studies is also presented in section 3.7, followed by a detailed summary and conclusions in section 3.8.*

3.1 Introduction

The Northeast India region, rich in biodiversity, offers a variety of gluten-free grains like millets (oats, finger millet), rice, soybean, green gram, chickpea, and Bengal gram. These ingredients not only form staples of the local diet but also provide essential nutrients contributing to the region's food security. Additionally, unripe papaya, abundant in tropical and subtropical regions, presents health benefits due to its high papain enzyme content, fiber, vitamins (especially vitamin C), and minerals. Efforts to utilize unripe papaya in value-added products alongside regional gluten-free grains and pulses can address nutritional needs and promote sustainability in food processing. Scientific research on the nutritional analysis of these ingredients is limited, emphasizing the need for inexpensive processing techniques to unlock their potential. Drying methods like hot-air-oven and cabinet drying offer scalable solutions to preserve the nutritional integrity of unripe papaya while extending its shelf life.

Integration of dried unripe papaya pulp and peel powder with regional gluten-free grains can lead to the development of novel, nutritionally rich baked products. Studies focusing on optimizing drying parameters and understanding the bioactive components of unripe papaya are crucial for sustainable production. Comprehensive characterization of these ingredients, including nutritional, functional, and morphological analyses, can guide the formulation of affordable, ready-to-consume baked goods tailored to the region's needs. By leveraging insights from existing literature and assessing the efficacy of chosen flours for desired baked product characteristics, such efforts aim to provide valuable guidelines for developing nutritious and accessible baked goods utilizing unripe papaya and regional gluten-free flours in Northeast India. This approach aligns with the goal of promoting food security, sustainability, and nutritional well-being in the region.

3.2 Sensitivity Driven Drying Processes to Preserve Bioactive Constituents in PPU and PPE

The study investigated the drying of unripe papaya pulp (PPU) and peel (PPE) at various time and temperature combinations to determine optimal conditions. Different forms of PPU (sliced - SL, grated - GR) were analyzed alongside PPE extract (PPE). Samples underwent drying and grinding, then were divided into coarse (PPUc, PPEc) and fine (PPUf, PPEf) portions to preserve bioactive compounds. Results showed that higher temperature (105°C) for shorter duration (45 min) yielded the lowest moisture content and highest antioxidant activity (AA). PPU in PE form at 65°C had the highest vitamin C (VC) concentration, while at 95°C, GR had the lowest VC. Operating at 105°C produced the highest total phenolic content (TpC) and total flavonoid content (TfC). Lowest total flavonoid content (TfC) was at 65°C for PE. GR at 75°C had the lowest TpC. Both GR and SL at 65°C showed the lowest AA content. Overall, decreasing temperature and increasing exposure time resulted in a decline in TpC, TfC, and AA. Fig. 3.2 (a – c) depict the bioactives alterations of SL form of PPU, GR form of PPU and PE form of PPE.

Fig. 3.3 (a – d) illustrate the nutritional characteristics of PPUc, PPUf, PPEc, and PPEf samples, respectively. At 105°C, coarse PPU (PPUc) displayed the highest levels of total phenolic content (TpC) (0.83 mg GAE/g), total flavonoid content (TfC) (0.65 mg Quercetin/g), and

(a) (b) (c) (d)

(e) (f) (g) (h)

Fig. 3.1: Fresh and dried powder samples of (a) unripe fresh sliced papaya pulp; (b) oven dried unripe papaya pulp; (c) unripe papaya coarse pulp powder; (d) unripe papaya fine pulp powder and (e) unripe papaya peel fresh; (f) oven dried unripe papaya peel; (g) unripe papaya coarse peel powder; (h) unripe papaya fine peel powder

antioxidant activity (AA) (96.12%). Conversely, fine PPU powder (PPUf) at 95°C exhibited the highest vitamin C content (VC) (342.85 mg/100g). However, both coarse and fine PPE samples at 65°C showed the lowest VC values (71.65 mg/100g), with the lowest AA observed for fine PPE (PPEf) (82.50%). Additionally, TfC levels were negligible for both PPEc and PPEf at 65°C. The TpC range for PPUc altered from (0.83 to 0.42 mg GAE/g) across the temperature range of 105 to 65°C. PPUc consistently outperformed PPUf in terms of TpC, TfC, and AA characteristics, while PPEc exhibited higher levels of TpC, TfC, and AA compared to PPEf. Moisture content decreased from (14.8% to 11.92%) for PPU with a temperature increase from 65 to 105°C. Across all combinations of time and temperature, PPUc consistently maintained lower moisture content compared to PPUf, PPEc, and PPEf samples, with a consistent trend observed in TpC, TfC, and AA for all samples.

3.3 Functional Properties of the Grain-based Flours and Unripe Papaya Powders

Water absorption capacity (WAC) and oil absorption capacity (OAC) are vital factors in food processing, influencing yield and sensory qualities. For instance, wheat flour has a WAC of 1.9 g/g, higher than rice powder at 1.18 g/g, while chickpea flour boasts a WAC of 2.21 g/g compared to black gram flour's 2.14 g/g. Meanwhile, optimal OAC contributes to mouthfeel and flavor retention, with chickpea flour at 0.67 g/g and black gram flour at 0.56 g/g. Water soluble impurities (WSI) in chickpea and black gram flours are measured at 26.75% and 29.79%, respectively. Flour density, ranging from 0.66 to 0.79 g/mL, has no significant impact on bulk density (BD). Notably, PPU and PPE powders show favorable swelling index, lower BD, and moderate least gelation concentration (LGC).

3.4 Analysis of the Mineral Composition of Grain based Flours and Unripe Papaya Powders

Various minerals play crucial roles in maintaining optimal bodily functions. Sodium (Na) is essential for fluid balance and nerve-muscle function, with roasted chickpea flour containing 57.02 ppm and green gram flour having the lowest at 3.9 ppm. Potassium (K) is vital for cardiovascular health, with roasted chickpea flour highest at 1175.18 ppm and oats lowest at 61 ppm. Magnesium (Mg) supports energy generation and muscle function, with soy flour highest at 206.64 ppm and rice lowest at 21.82 ppm. Calcium (Ca) is crucial for bone health,

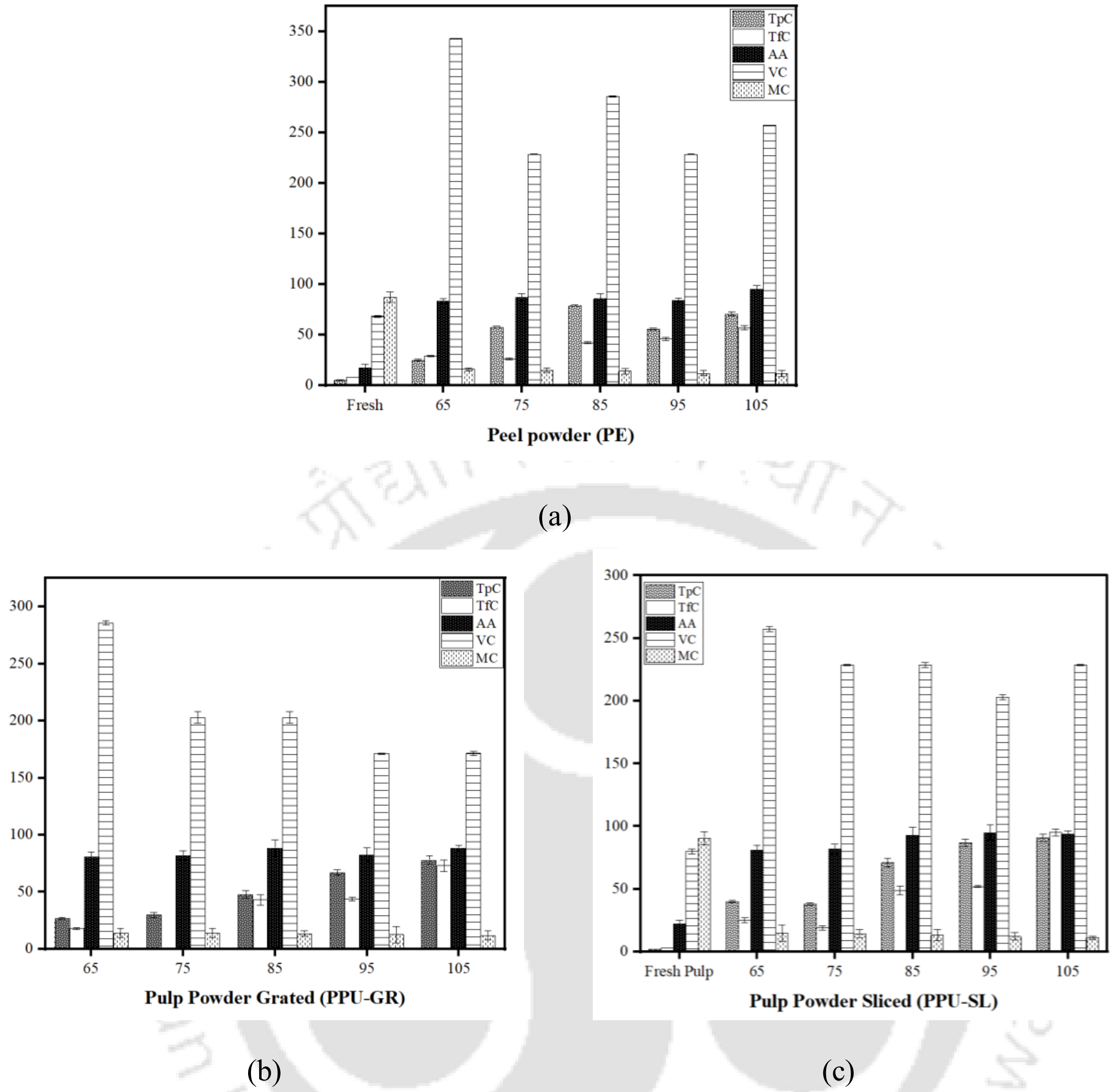
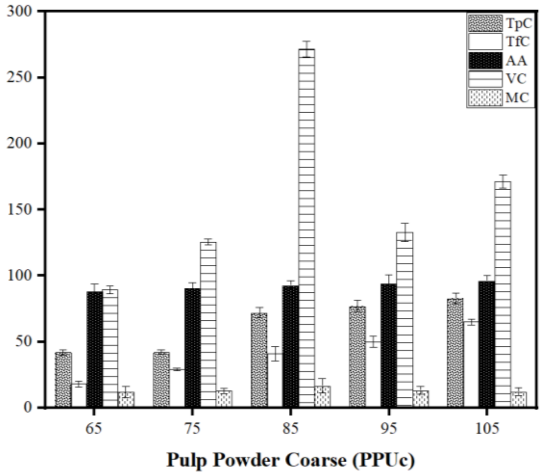
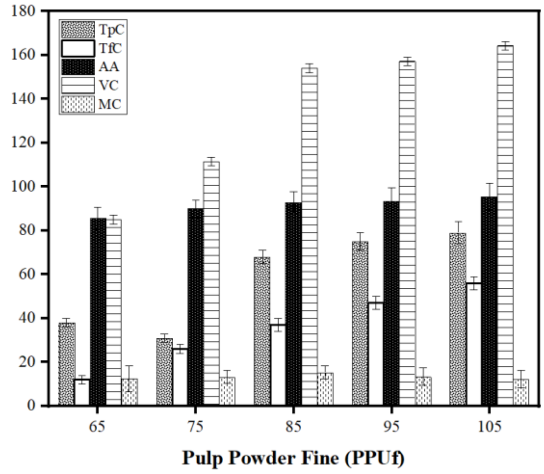


Fig. 3.2: Bar charts depicting the influence of oven drying temperature on the bioactives and moisture content characteristics of (a) unripe papaya peel (PE form of PPE), (b) grated unripe papaya pulp (GR form of PPU) and (c) sliced unripe papaya pulp (SL form of PPU) samples

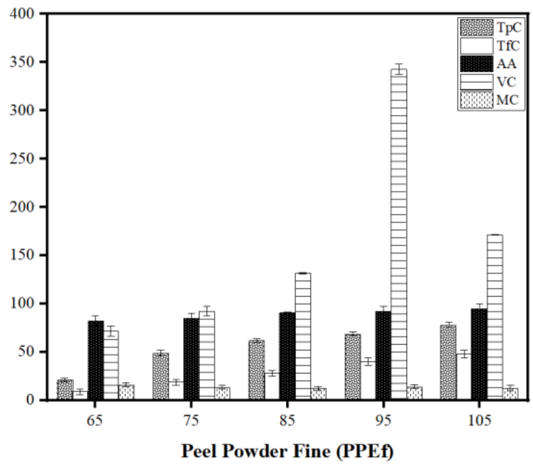
with finger millet flour highest at 364.76 ppm. Manganese (Mn), found significantly in oats at 5.03 ppm, supports metabolism and bone production. Iron (Fe), highest in roasted chickpea flour at 7.78 ppm, aids oxygenation and metabolism, while zinc (Zn), highest in roasted chickpea flour at 4.61 ppm, impacts immunity and wound healing. Copper (Cu), highest in Bengal gram flour at 5.46 ppm, is vital for energy production and brain function. Additionally, an assessment of unripe papaya pulp and peel powder (PPU and PPE) revealed high potassium levels (1456 ppm in PPE, 1291.6 ppm in PPU), emphasizing their cardiovascular and



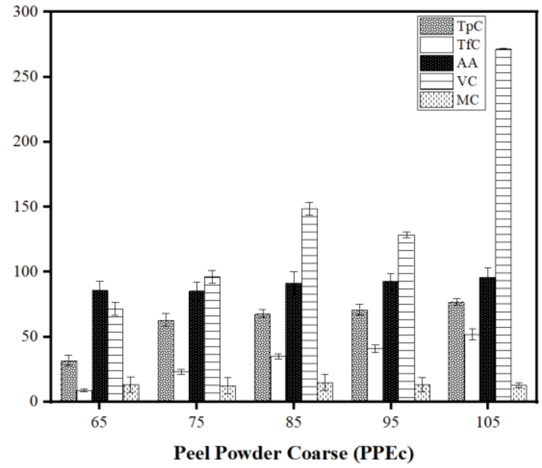
(a)



(b)



(c)



(d)

Fig. 3.3: Bar charts depicting the influence of oven drying temperature on the bioactives and moisture content characteristics of (a) Pulp powder Coarse (PPUc) (b) Pulp Powder fine (PPUf) (c) Peel Powder Fine (PPEf) (d) Peel Powder Coarse (PPEc)

electrolyte balance benefits. PPE contained notable calcium (110.04 ppm), sodium (13.12 ppm), and magnesium (151.61 ppm) levels, whereas PPU had lower concentrations. Both PPE and PPU contained iron and zinc, crucial for immunity and metabolism, with PPE exhibiting higher levels. Copper was detected in PPE (0.42 ppm) but not PPU, supporting energy production and brain function. Combining PPU, PPE, and selected flours ensures a balanced mineral composition, enhancing overall health and well-being.

Table 3.1: Data summary of the functional properties of PPU, PPE and other grain-based flours

S. No.	Sample	Swelling Index (g/g)	Solubility Index (%)	Water Absorption Capacity (g/g)	Oil Absorption Capacity (g/g)	Bulk Density (g/mL)	Dispersibility	Least Gelatinization Conc. (LGC) (% m/v)
1.	Wheat	6.55±0.04	8.09±0.05	1.9±0.07	1.16±0.02	0.79±0.00	70±2.9	9±1.35
2.	Rice	8.99±0.21	3.55±0.01	1.18±0.05	1.27±0.07	0.69±0.02	54±3.7	20±1.69
3.	Roasted Chickpea	5.86±0.92	4.44±0.23	2.75±0.01	1.04±0.02	0.74±0.02	68±3.5	20±1.45
4.	Bengal Gram	6.69±0.43	2.68±0.07	2.23±0.04	0.89±0.03	0.71±0.03	75±3.6	1±0.46
5.	Soy	7.84±0.36	3.96±0.45	3.04±0.27	0.99±0.04	0.66±0.4	68±3.7	7±0.81
6.	Green Gram	6.09±0.35	2.41±0.01	1.21±0.83	1.18±0.05	0.72±0.02	79±4.2	13±0.22
7.	Finger Millet	6.01±0.28	6.42±0.08	1.52±0.31	1.03±0.5	0.56±0.05	78±5.0	8±1.46
8.	Oats	6.18±0.08	5.49±0.42	3.22±0.07	1.07±0.03	0.68±0.16	69±2.3	9±0.94
9.	PPU	10.02±0.2	2.46±0.31	4.45±0.8	1.05±0.4	0.62±0.4	56±2.5	10±1.72
10.	PPE	9.49±0.16	2.79±0.17	4.65±.0.51	0.98±0.3	0.58±0.18	52±1.9	12±2.61

Table 3.2: A summary of the mineral content data of PPU, PPE and other grain-based flours

S. No.	Sample (Flour)	Na (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
1.	Wheat	4.29±0.12	162.87±0.43	26.2±0.57	2.92±0.88	0.63±0.32	1.74±0.71	-	0.98±0.10
2.	Rice	10.25± 1.3	105.02± 1.08	21.82± 0.55	14.88± 1.64	0.78± 0.25	2.25±0.92	0.51± 0.08	2.76±0.04
3.	Roasted Chickpea	57.02± 1.6	1175.18± 1.2	104.18±2.07	11.93± 1.11	1.58±0.27	7.78±0.16	1.17±0.05	4.61±0.88
4.	Bengal Gram	24.01±1.4	696.84±2.33	72.55±1.95	5.92±2.22	0.99±0.07	4.65±0.67	5.46±0.52	1.89±0.9
5.	Soy	4.71±0.34	608.54±0.98	206.64±2.89	36.59±1.17	1.87±0.35	6.67±0.74	0.67±0.04	2.02±0.06
6.	Green Gram	3.90±0.86	854.31±1.65	117.83±2.03	10.82±0.94	0.61±0.05	3.31±0.08	0.56±0.06	1.16±0.14
7.	Finger Millet	11.07±1.04	408.21±2.07	145.77±2.19	364.76±2.48	3.84±0.73	4.69±0.42	3.85±0.02	2.45± 0.51
8.	Oat	19.83±0.87	61.08±0.53	144.08±3.06	80.23±1.49	5.03±0.38	4.03±0.71	0.57±0.20	1.98±0.05
9.	PPE	13.12±1.43	1456.19±4.66	151.61±2.23	2.82±0.31	110.04±2.61	3.47±0.54	4.94±0.28	0.42±0.05
10	PPU	11.85±1.29	123.67±2.84	123.67±2.84	0.54±0.07	15.89±1.45	0.79±0.08	1.59±0.59	-

3.5 Additional Nutritional Parameters

Table 3.3 summarizes the nutritional parameters for eight distinct grain flours, as well as PPU and PPE powder samples. Green gram flour exhibits the highest moisture content at 12.59%. The water activity ranges from 0.45 to 0.62 across all flours and PPU/PPE samples. PPE has the highest ash content at 8.34 g/100 g, while soy flour has the lowest at 5.51 g/100 g. PPE also has the highest crude fiber content at 11.95 g/100 g, followed by PPU at 9.05 g/100 g and soy flour at 6.39 g/100 g. Green gram flour has the highest concentration of carbohydrates at 88.71 g/100 g, followed by wheat flour (86.56 g/100 g) and rice flour (78.68 g/100 g). Soy flour has the highest protein level at 42.7 g/100 g, followed by Bengal gram flour (22.57 g/100 g) and roasted chickpea flour (18.84 g/100 g). Oats have the highest fat content at 7.77 g/100 g while wheat flour has the lowest at 1.03 g/100 g. These parameters highlight the nutritional diversity of the flours and underscore the potential for developing beneficial food products using composite flour combinations, including PPU and PPE.

3.6 Different Characteristics of Grain Flour, PPU and PPE

3.6.1 Morphology Study

The morphological characteristics of various flours including wheat, rice, green gram, Bengal gram, chickpea, soy, finger millet, oats, unripe papaya pulp (PPU), and unripe papaya peel powder (PPE) were examined using FESEM micrographs. Wheat, green gram, Bengal gram, and roasted chickpea exhibited a granular structure with round and oval shapes, with grain sizes ranging from 2.75 to 15.14 µm. Finger millet and oats showed regular 3D round shapes with surface cracks. Soy flour granules were predominantly triangular with some irregularities, while rice flour granules were small, ranging from 2.34 to 3.45 µm, and displayed 3D square

Table 3.3: Nutritional parametric data summary of grain based flours, PPU and PPE

S. No.	Sample type	Moisture content (%)	Water activity	Ash (g/100g)	Crude fiber (g/100g)	Total carbohydrate (g/100g)	Total protein (g/100g)	Total fat (g/100g)
1.	Rice	12.46±0.05	0.55± 0.08	0.71±0.45	3.42 ± 1.05	78.68± 0.01	7.55±0.57	6.21±0.19
2.	Wheat	12.23±0.30	0.62±0.04	1.27±0.38	0.65±0.93	86.56± 2.55	9.87±0.88	1.03±0.07
3.	Roasted Chickpea flour (*Sattu*)	5.27±0.26	0.4±0.05	3.15±0.09	4.63±1.61	33.39± 0.93	18.84±1.07	3.86±0.09
4.	Bengal Gram (*Besan*)	7.93±0.09	0.55±0.07	2.48±0.21	2.39±0.33	43.21± 1.82	22.57±1.23	4.76±0.86
5.	Finger Millet (*Ragi*)	8.85±0.09	0.62±0.23	1.74±0.12	3.4±0.94	45.35± 2.10	7.9± 2.06	1.15±0.53
6.	Green Gram	12.59±0.34	0.62±0.11	3.49±1.06	5.14±0.7	88.71± 1.08	18.66±0.95	1.07±0.04
7.	Soy	8.96±1.49	0.61±0.09	5.51±0.04	6.39±0.19	17.90±0.11	42.7±2.49	2.05±0.56
8.	Oat	6.96±0.08	0.53±0.04	1.67±0.12	4.27±0.48	28.35±0.47	13.02± 0.07	7.77±0.09
9.	PPE (105°C)	2.07±0.4	0.46±0.01	8.34±0.85	11.95±1.67	62.77±1.23	4.81±0.54	2.54±1.09
10.	PPU (105°C)	2.92±0.31	0.32±0.01	6.11±0.33	9.05±0.98	59.01±2.35	3.56±0.62	2.07±0.04

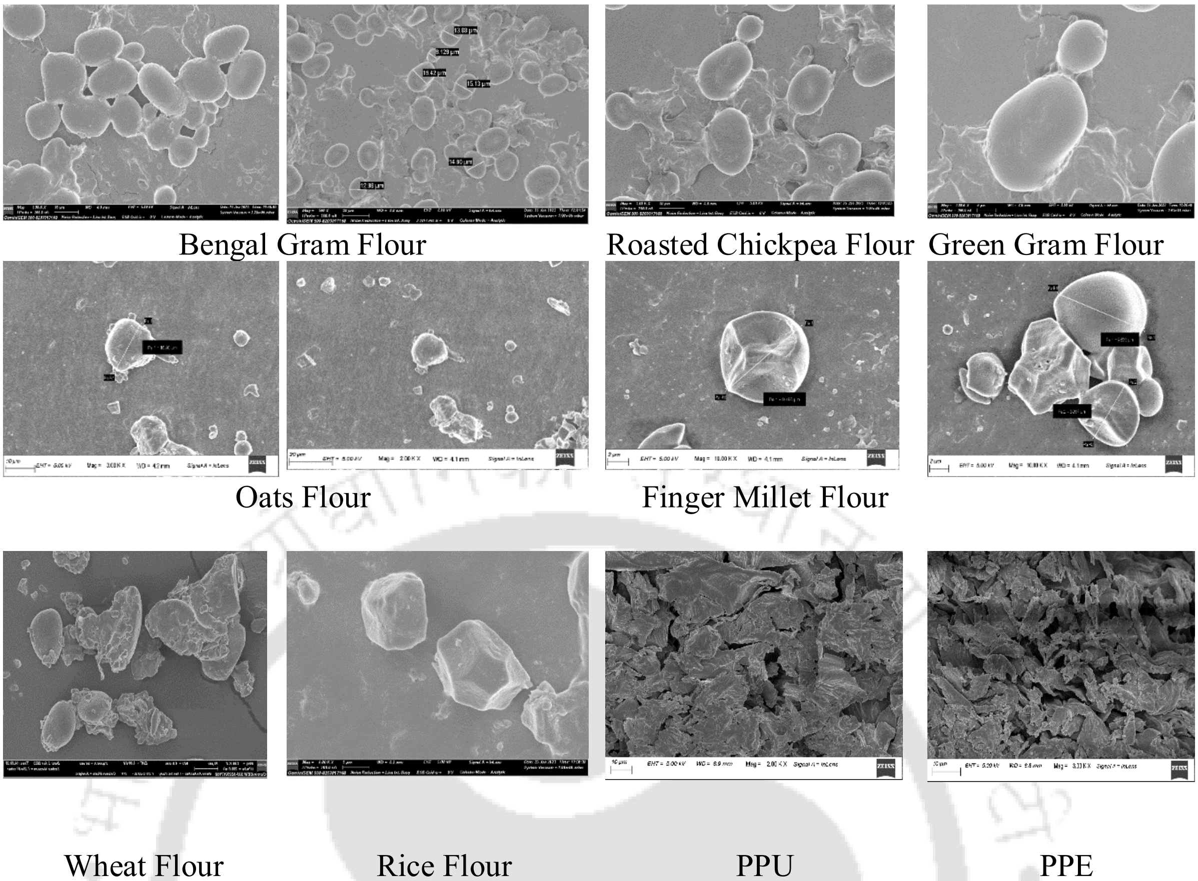
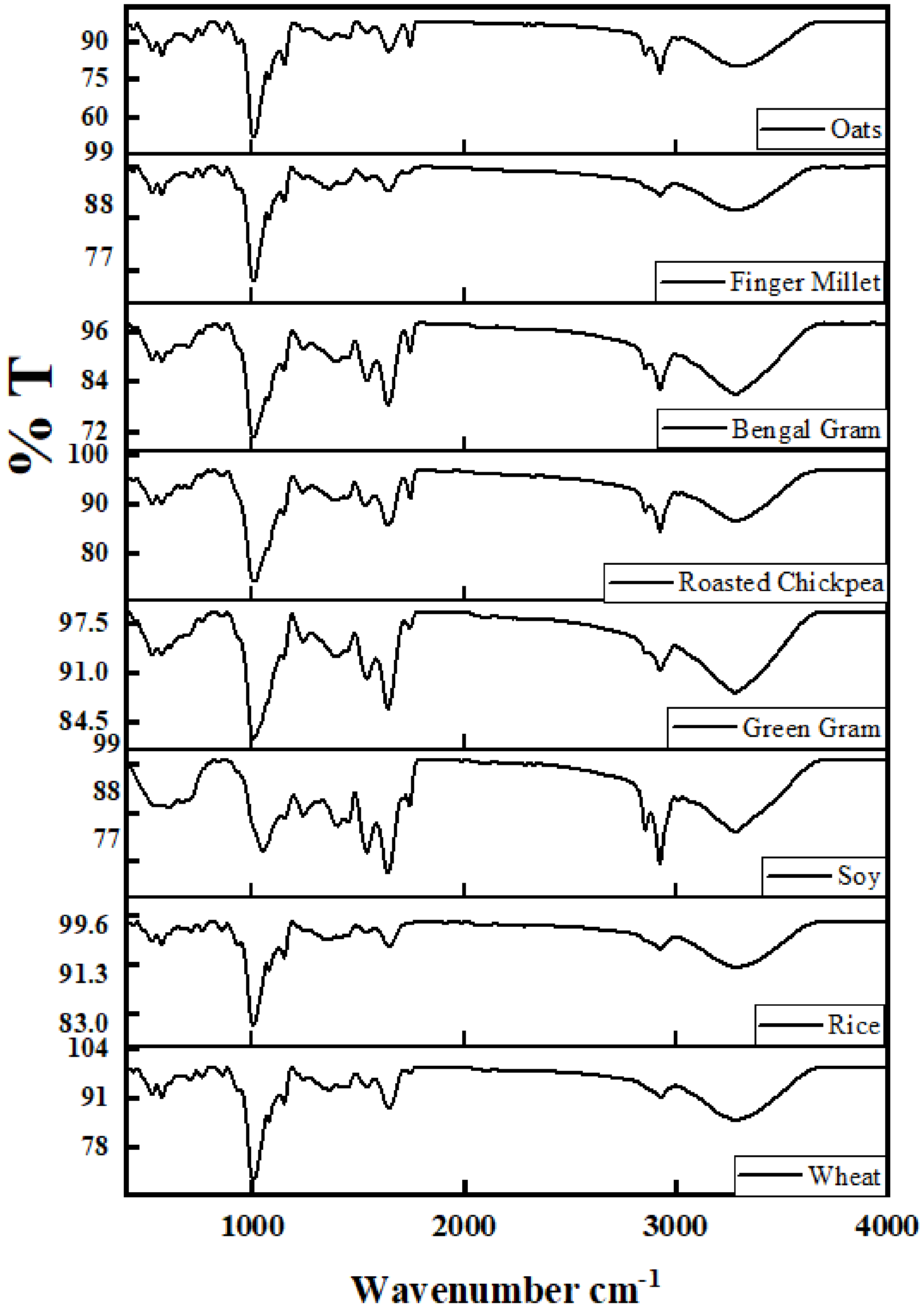


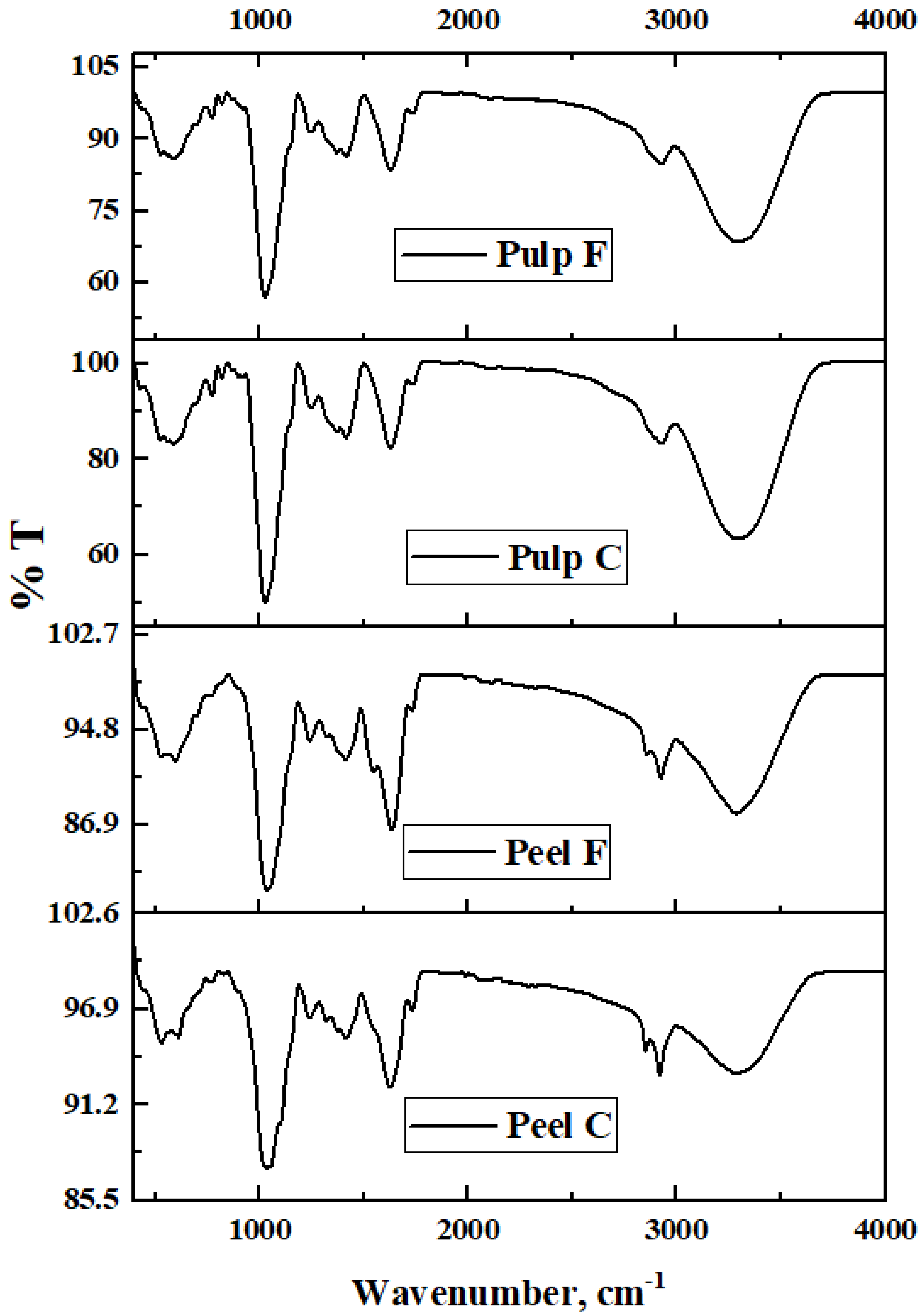
Fig. 3.4: FESEM micrographs of eight alternate grain-based flours, PPU and PPE powders or rectangular shapes. PPU and PPE micrographs revealed triangular shapes with irregularities, with average granule lengths ranging from 7.45 to 10.14 µm.

3.6.2 FTIR Study

Figure 3.5 illustrates peaks from FTIR data, indicating functional groups in PPU, PPE (Fig. 3.5 a), and grain-based flours (Fig. 3.5 b). Peaks at 3270-3290 cm^{-1} suggest O-H stretching, common in carboxylic acids and alcohols. Peaks at 2904 cm^{-1} (PPUc), 2923 cm^{-1} (PPUf, PPEf, oats, roasted chickpea, Bengal and green gram), and 2914 cm^{-1} (PPEc, finger millet, rice) suggest O-H and C-H stretching, representing alkene compounds. Additionally, peaks at 1724 cm^{-1} (PPEc, Bengal gram, soy), 1625 cm^{-1} (all grain-based varieties, PPE, PPU), and 1232 cm^{-1} (PPU, PPE) indicate aromatic compounds. A peak at 1020 cm^{-1} (PPU, PPE, all grain-based flours) suggests aliphatic fluoro and aromatic CH stretch.


% T
90
75
60
Oats
99
88
77
Finger Millet
96
84
72
Bengal Gram
100
90
80
Roasted Chickpea
97.5
91.0
84.5
Green Gram
99
88
77
Soy
99.6
91.3
83.0
Rice
104
91
78
Wheat
1000
2000
3000
4000
Wavenumber cm-1

(a)



(b)

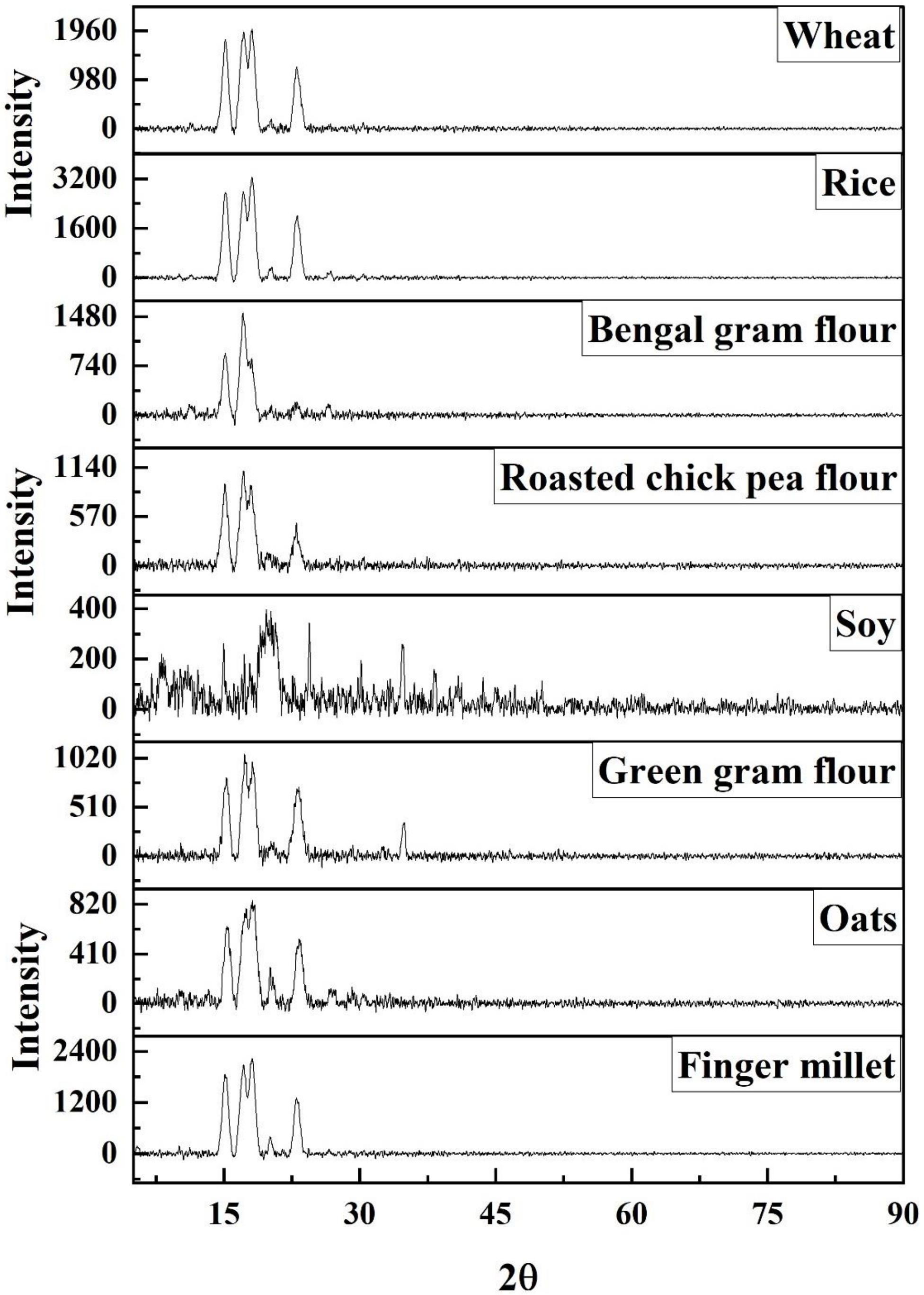
Fig. 3.5: FTIR Spectra of (a) grain based flours and (b) PPU and PPE powders

3.6.3 Crystalline Properties of Grain based Flours and Unripe Papaya Powders

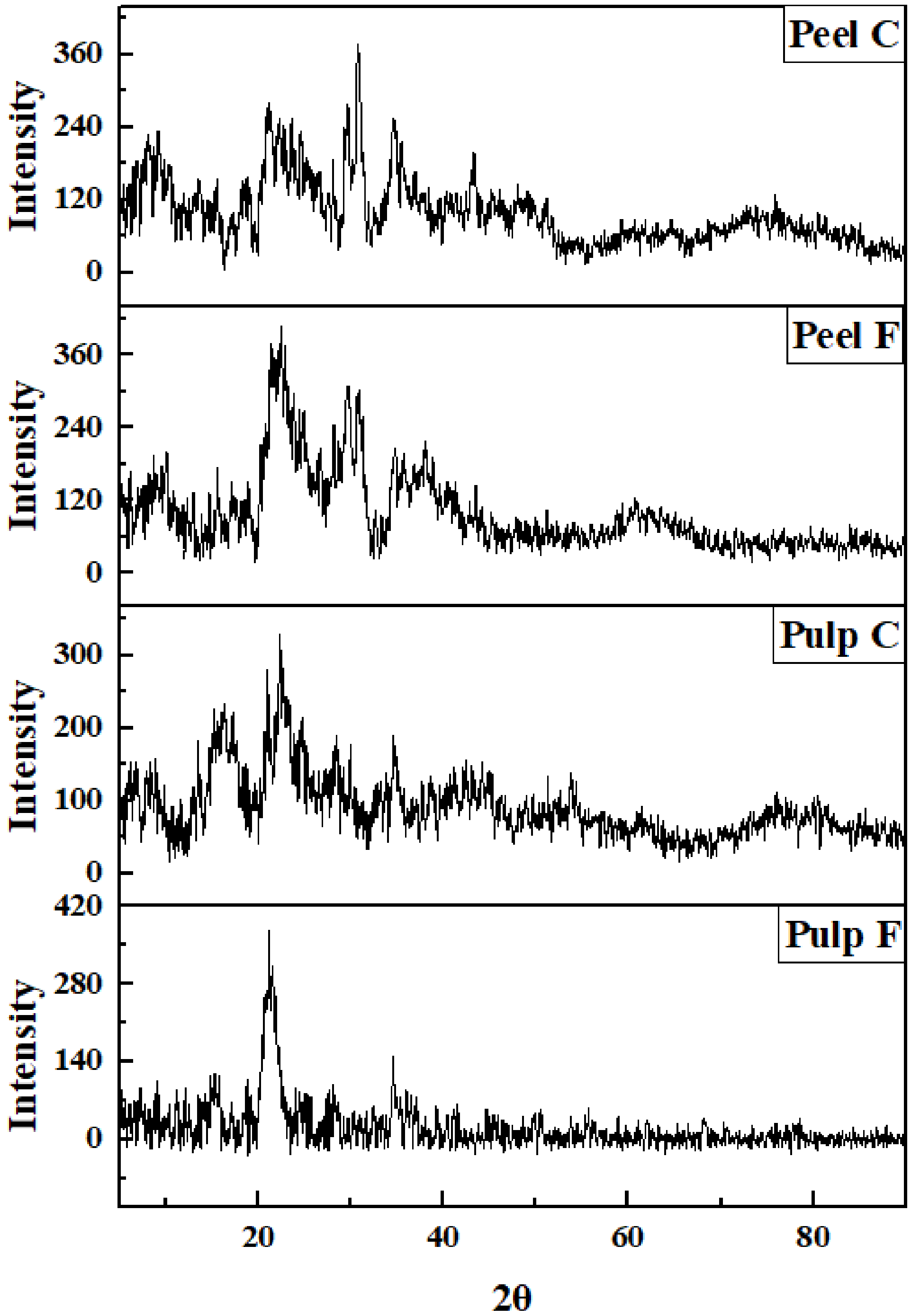
Figure 3.6 (a) illustrates XRD-based crystallinity analysis of eight grain-based flour samples. Starch from cereals, tubers, peas, and beans exhibit distinct peaks classified as A, B, and C classes. Peaks at 2θ diffraction angles of 17.7°, 19.9°, 26.4°, and 30.4° affirm a crystallinity pattern of A and B mixtures in all grain-based flours. Oats demonstrate the highest relative crystallinity (50.49%), followed by green gram (49.94%) and wheat (42.01%). Bengal gram flour has the lowest relative crystallinity (18.87%). Soy flour has relatively higher relative crystallinity (29.98%) compared to rice (39.28%), finger millet (37.49%), and roasted chickpea (31.29%). PPE exhibits lower relative crystallinity (11.42%) compared to PPU (16.23%) (Fig.3.6 (b)). Variations in crystallinity are influenced by flour chemical composition, including ash, lipid, protein, and fiber content.

Table 3.4: Particle size distribution data of eight alternate flours, PPU and PPE powders

S. No.	Sample name	PdI	Average diameter (μm)
1..	Finger Millet	0.97±0.2	1.25±0.36
2.	Soy	1.00±0.6	1.50±0.05
3.	Green Gram	0.17±0.05	4.78±0.24
4.	Bengal Gram	1.00±0.71	9.62±0.87
5.	Rice	0.22±0.07	2.46±0.14
6.	Oats	1.00±0.08	5.68±0.35
7.	Roasted Chickpea	1.00±0.63	8.16±0.62
9.	Wheat	1.00±0.52	1.42±0.05
10.	Pulp C	0.39±0.06	19.81±0.51
11.	Pulp F	0.23±0.07	18.6±1.99
12.	Peel C	0.17±0.05	18.72±1.44
13.	Peel F	0.43±0.01	16.50±0.28


Wheat
Rice
Bengal gram flour
Roasted chick pea flour
Soy
Green gram flour
Oats
Finger millet
Intensity
Intensity
Intensity
1960
980
0
3200
1600
0
1480
740
0
1140
570
0
400
200
0
1020
510
0
820
410
0
2400
1200
0
15
30
45
60
75
90
2θ

(a)



(b)

Fig. 3.6: XRD plots of (a) grain based flours and (b) PPU and PPE flours

3.6.4 Particle Size Distribution

At 105°C, PPE shows distinct characteristics across sample forms. Coarse and fine powders have average diameters of 18.72 µm and 20.50 µm, with corresponding PdI values of 0.17 and 0.43. PPUc and PPUf have PdIs of 0.39 and 0.23, with diameters of 19.81 µm and 18.6 µm, respectively. Wheat, roasted chickpea, oats, Bengal gram, and soy have uniform PdIs of 1.00, while rice flour has a PdI of 0.22 and an average diameter of 2.46 µm. Finger millet has the smallest average diameter at 1.25 µm, followed by wheat (1.42 µm) and soy (1.5 µm). Green gram boasts an average diameter of 4.78 µm with a PdI of 0.17. Bengal gram and oats have average diameters of 9.62 µm and 5.68 µm, respectively, while roasted chickpea has an average diameter of 8.16 µm. These variations will significantly impact the structural integrity and crispness of baked cookies made with composite powders.

3.6.5 Thermal Properties

The thermal characteristics of grain-based flours vary significantly. Bengal gram flour has a wide melting range (54.3°C to 124.4°C) with a peak temperature at 81.6°C, while roasted chickpea flour has a broader range (53.6°C to 144.2°C) and a lower peak temperature (69.2°C). Oats and finger millet flour exhibit higher peak temperatures (90.5°C and 88.9°C, respectively), indicating their ability to withstand higher temperatures before melting. Finger millet flour also shows a significantly higher delta Cp value (2.42 J/(gK)), suggesting a substantial change in heat capacity during melting. These insights influence their applications in food processing and formulation research for functional baked food product development.

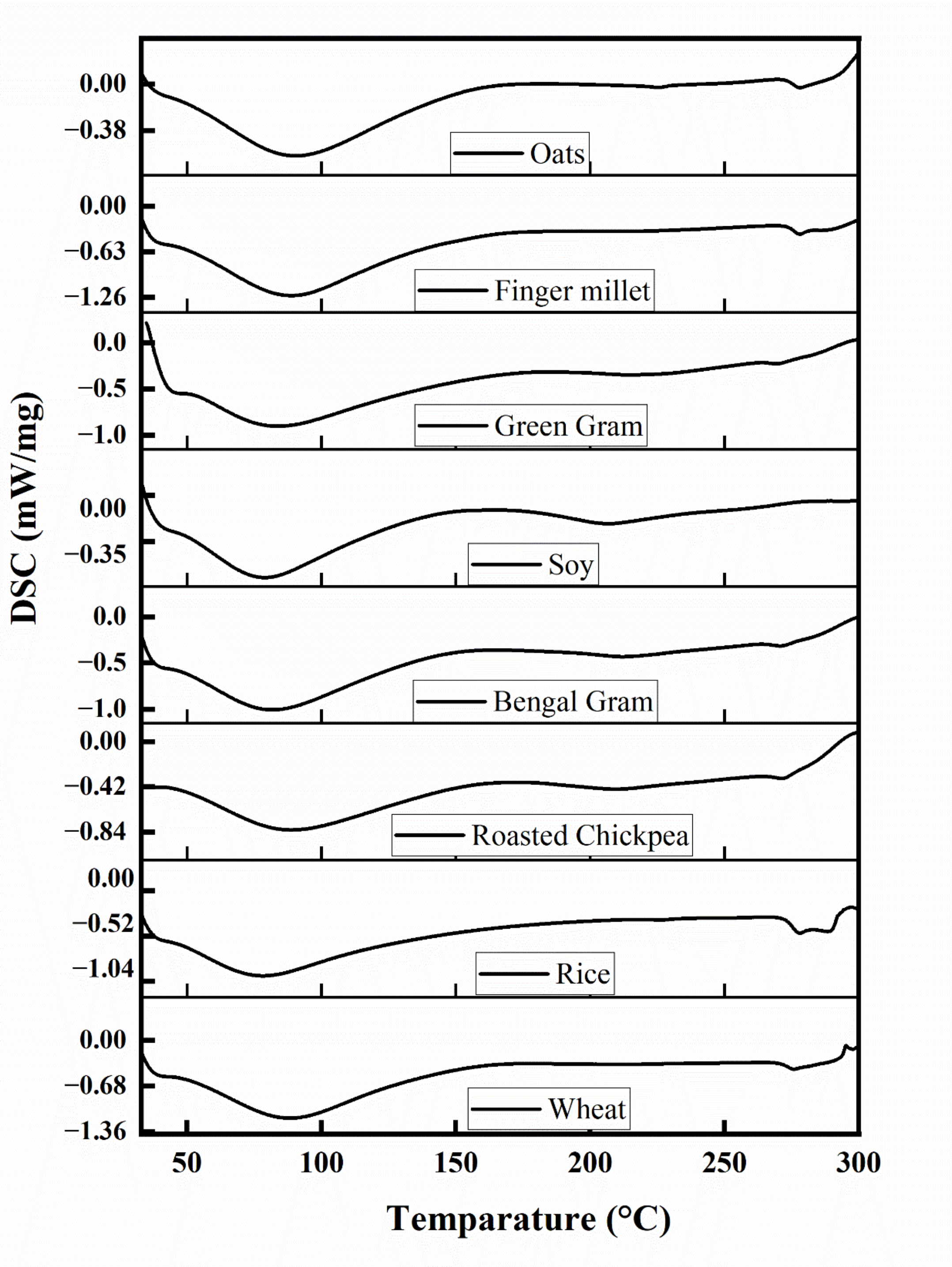
3.6.6 Color Analysis

In terms of hue angle, finger millet exhibits the lowest value at 72.78. However, wheat had marginally higher value at 76.09. This was followed by roasted chickpea at 77.99. Conversely, green gram confirmed the highest hue angle at 87.00. This was closely trailed by rice at 86.12 and oats at 85.41. Notably, PPE demonstrates a higher hue angle of 83.19 in comparison to the

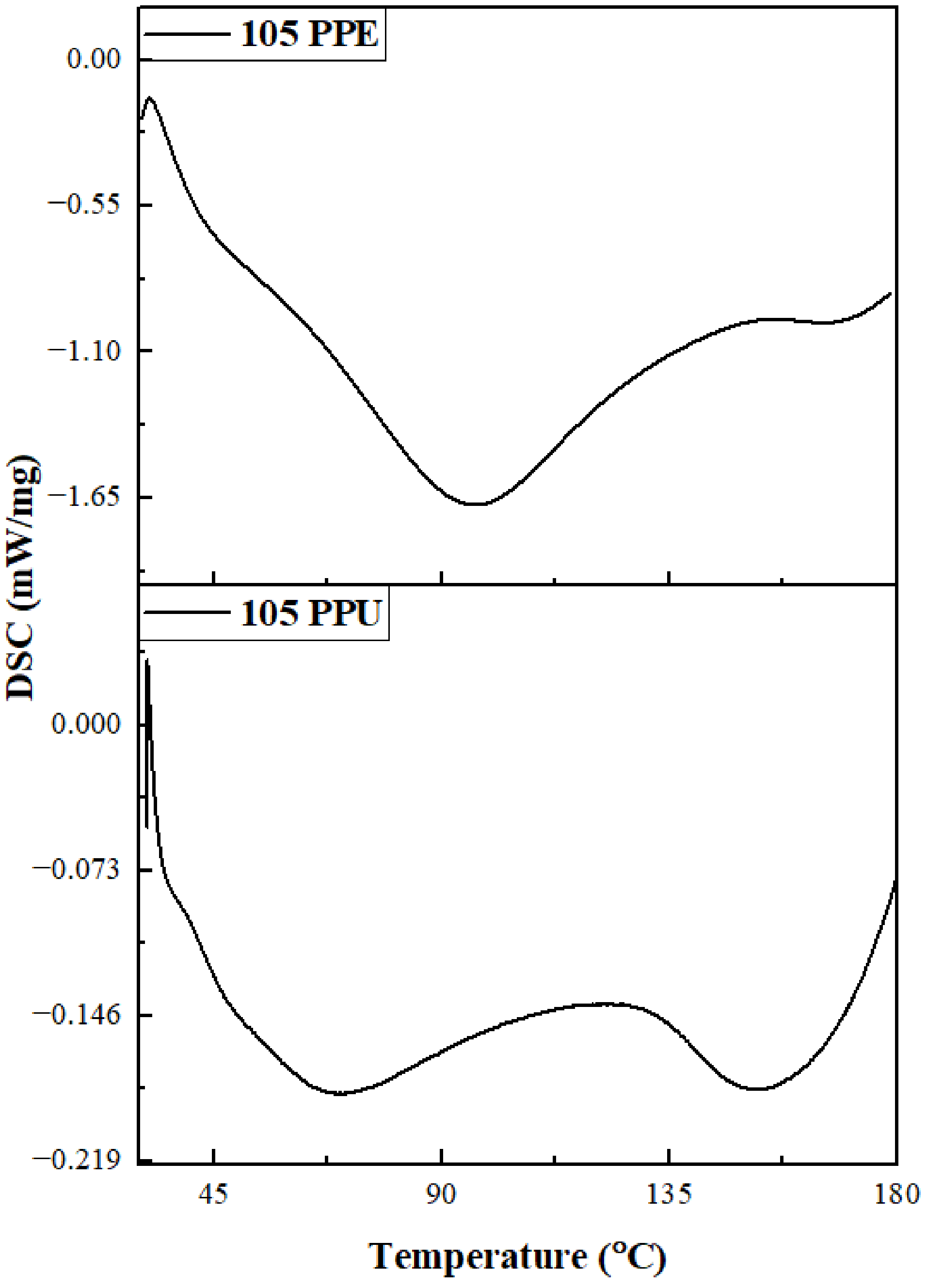
PPU (80.85). Soy had a hue angle of 83.82 alongside a chroma value of 22.85. Among the grains, rice has the lowest chroma at 7.1, and oats and wheat had a marginally higher value at 9 and 9.74, respectively. PPE exhibits the highest chroma at 29.10. This was followed by PPU at 24.41 and Bengal gram at 23.11. Finger millet and green gram affirmed chroma values of 13 and 17.95, respectively.

3.7 Literature comparison

The nutritional analysis reveals that unripe papaya pulp (PPU) and peel powder (PPE) possess notable levels of protein, with PPU containing 3.56 g/100 g and PPE containing 4.81 g/100g, indicating their significance as protein sources in diets. Additionally, both PPU and PPE exhibit substantial crude fiber content, with PPE having the highest value at 11.95 g/100 g, highlighting their potential in promoting digestive health. Moreover, PPU and PPE demonstrate exceptionally high vitamin C content, with PPU containing 228.57 mg/100 g and PPE containing 257.14 mg/100 g, indicating their efficacy in meeting daily vitamin C requirements for consumers. The phenolics and flavonoids content of PPU and PPE further accentuate their nutritional value, with PPU containing 91 mg and 95 mg, and PPE containing 71 mg and 56 mg, respectively, suggesting their potential antioxidant properties. Notably, PPE exhibits a higher antioxidant capacity (6.09 mmol) compared to PPU (3.16 mmol), underscoring its superior antioxidative potential. Additionally, the moderate fat content in both PPU (2.07 g/100 g) and PPE (2.54 g/100 g) aligns with balanced dietary recommendations, while their carbohydrate content, with PPU at 59.01 g/100 g and PPE at 62.77 g/100 g, indicates their suitability for inclusion in varied diets. Comparatively, when juxtaposed with other vegetable flours such as pumpkin seed flour, jackfruit seed flour, and moringa leaf flour, PPU and PPE demonstrate competitive nutritional profiles, emphasizing their versatility and nutritional benefits for consumers.


0.00
−0.38
Oats
0.00
−0.63
−1.26
Finger millet
0.0
−0.5
−1.0
Green Gram
0.00
−0.35
Soy
0.0
−0.5
−1.0
Bengal Gram
0.00
−0.42
−0.84
Roasted Chickpea
0.00
−0.52
−1.04
Rice
0.00
−0.68
−1.36
Wheat
DSC (mW/mg)
50
100
150
200
250
300
Temparature (°C)

(a)



(b)

Fig. 3.7: DSC plots of (a) grain-based flours and (b) PPU and PPE powders

Table 3.5: Color analysis data summary of eight grain flours, PPU and PPE powders

S. No.	Sample	b*	a*	Hue Angle	Chroma
1.	PPU	24.1±0.2	3.88±0.03	80.85±1.03	24.41±0.19
2.	PPE	28.89±0.4	3.45±0.03	83.19±0.92	29.10±0.52
3.	Soy	22.72±0.5	2.46±0.02	83.82±0.96	22.85±0.23
4.	Rice	7.08±0.34	0.48±0.07	86.12±0.84	7.10±0.41
5.	Finger Millet	12.42±0.9	3.85±0.02	72.78±0.95	13.00±0.37
6.	Bengal Gram	22.87±0.8	3.35±0.03	81.67±0.55	23.11±0.28
7.	Wheat	9.45±0.93	2.34±0.03	76.09±0.46	9.74±0.65
8.	Roasted Chickpea	21.06±0.6	4.48±0.02	77.99±0.88	21.53±0.34
9.	Green Gram	17.93±1.0	0.94±0.04	87.00±0.18	17.95±0.89
10.	Oats	8.97±0.91	0.72±0.05	85.41±0.44	9.00±0.54

The comparative analysis of various grain-based flours reveals significant variations in their nutritional compositions. Finger millet and oats, as indicated by our current study, exhibit promising levels of protein, with finger millet containing 7.9 g/100 g and oats containing 13.02 g/100 g, suggesting their potential as protein-rich alternatives. In terms of fiber content, finger millet demonstrates a moderate level of 3.4 g/100 g, while oats exhibit a higher fiber content of 4.27 g/100 g, indicating their potential in promoting digestive health. However, specific nutritional parameters such as vitamin C, phenolics, and flavonoids are not available for these flours in our current study. Comparatively, literature sources provide additional insights into the nutritional attributes of finger millet and oats, with Kim et al. (2019) reporting varying levels of these parameters. For instance, finger millet reported by Kim et al., (2019) contains 7.3 g/100 g protein and 13.7 g/100 g fiber, while oats contain 16.9 g/100 g protein and 10.6 g/100 g fiber. Additionally, other grain-based flours such as sorghum, pearl millet, amaranth, quinoa, and buckwheat, as reported by Chen et al., (2020) and Anberbir et al., (2024) exhibit diverse nutritional profiles with varying protein, fiber, fat, and carbohydrate contents, highlighting their potential as nutritious alternatives in diverse dietary plans.

3.8 Summary

The exploration of potential combinations for papaya pulp flour (PPU) and papaya peel powder (PPE) in cookie formulations reveals promising avenues for enhancing nutritional content, flavor profiles, and overall sensory attributes. Incorporating PPU and PPE with whole grain flours like sorghum, millet, and buckwheat can enrich cookies with dietary fibre, protein, and essential minerals. Additionally, adding ingredients like oats, known for their high fibre and antioxidant properties, further boosts the nutritional value. Flavour synergies can be achieved by incorporating spices, nuts, and dried fruits, offering complexity and depth to taste profiles. Alternative sweeteners like honey or maple syrup provide healthier options, aligning with consumer preferences for natural ingredients. Plant-based fats like coconut oil contribute rich flavors and textures, while functional ingredients such as chia seeds add omega-3 fatty acids and antioxidants. Overall, strategic combinations of PPU and PPE with complementary ingredients enable the development of innovative, nutritionally balanced cookies, catering to diverse consumer preferences and promoting health and wellness.

Table 3.6: Literature reported nutritional data of papaya pulp flour (PPU), papaya peel powder (PPE), and other vegetable flours

S. No	Flours	Protein (g/ 100g)	Crude Fiber (g/100 g)	Vitamin C (mg/100g)	Total Phenolic Content (mg GAE/ 100g)	Total Flavonoid Content (mg QE/100g)	Antioxidants (mmol)	Fat (g/ 100g)	Total Carbohydrate (g/100g)	References
1.	PPU	3.56	9.05	228.57	91	95	3.16	2.07	59.01	Current work
2.	PPE	4.81	11.95	257.14	71	56	6.09	2.54	62.77	Current work
3.	PPU	8.2	5.4	103	120	45	8.7	2.3	68	Pathak et al. (2022)
4.	PPE	7.6	6.2	90	110	40	7.5	2.6	65	Rather et al. (2023)
5.	Bamboo shoot peel flour	4.5	3.2	8	80	30	5	3.5	72	Garcia et al. (2020)
6.	Jackfruit seed flour	5.0	3.8	12	90	35	6	2.8	70	Prasad & Gupta (2018)
7.	Moringa leaf flour	2.6	4.3	17	95	25	7.2	4.7	63	Kadke et al. (2018)
8.	Pumpkin seed flour	32	11	0.8	55	20	4.5	49	11	Kumari & Sindhu (2019)
9.	Bottle gourd flour	3.9	1.5	14	70	28	6.5	0.2	6.8	Gajera, et al. (2017)

Table 3.7: Comparative nutritional analysis of PPU, PPE and selected gluten-free grain flours

S. No	Flours	Protein (g/ 100g)	Crude Fiber (g/100 g)	Vitamin C (mg)	Total Phenolic Content (mg GAE/100g)	Total Flavonoid Content (mg QE/100g)	Fat (g/100g)	Carbohydrate (g/100g)	References
1.	Finger millet	7.9	3.4	-	-	-	1.15	45.35	Current work
2.	Oats	13.02	4.27	-	-	-	7.77	28.35	Current work
3.	Finger millet	7.3	13.7	0.3	4.5	2.8	1.2	68	Kim et al. (2019)
4.	Oats	16.9	10.6	0.7	8.1	5.3	6.8	62	Kim et al. (2019)
5.	Sorghum (Jowar)	9.7	6.3	0.5	5.2	-	3.3	72	Chen et al. (2020)
6.	Pearl millet (Bajra)	10.4	8.5	0.8	6.8	-	5.2	67	Chen et al. (2020)
7.	Amaranth	13.6	7	1.1	7.5	4.7	5.9	65	Chen et al. (2020)
8.	Quinoa	14.1	7	1.2	8.3	4.8	6.1	62	Anberbir et al. (2021)
9.	Buckwheat	13.3	10	0.4	6.5	4.8	3.4	71	Anberbir et al. (2021)

The conducted investigations affirmed several significant insights. Firstly, unripe papaya in grated form (GR) contains lower levels of bioactive components and higher moisture content in comparison to the sliced unripe papaya (SL). Secondly, among all unripe papaya pulp and peel samples and across diverse time (45 -120 min) and temperature (65 -105°C) combinations, the PE at 105°C demonstrated the lowest moisture content and highest antioxidant activity. Thirdly, at 105°C, the PPUc exhibited remarkable levels of total phenolic content (TpC), total flavonoid content (TfC) and AA and thereby affirmed significant potential for value addition during the oven drying processes. Other key conclusions derived from this study are multifaceted. Fourthly, akin to grain based flours, both PPU and PPE contain essential minerals, and thereby ascertain their nutritional importance. Fifthly, PPU, PPE and other grain based flours (excluding wheat) are enriched with ash, crude fiber, and protein content. All these enhance their nutritional profile. Sixthly, analysis via FESEM, FTIR and particle size distribution revealed similarities in average diameter and composition among all grain based flours and powder variants. Seventhly, DSC thermograms and XRD plots corroborate the stable structure of these entities and even high-temperature processing after exposure to the environment. Consequently, the Northeast India region holds promising potential for the transformation of such entities into cost effective, nutritionally enriched products such as soups, biscuits, and chips. Thereby, the mentioned produces can profusely contribute to both economic and nutritional advancements in the region.

Chapter 4:

Development of Gluten-free Cookies with Indigenous Grain Flours of Northeast India: Formulation and Characterization

Development of Gluten-free Cookies with Indigenous Grain Flours of Northeast India: Formulation and Characterization

In this chapter, gluten-free cookies being prepared with the powders of the indigenous grains of Northeast India have been characterized for their sensory attributes, proximate, functional, polydispersity index (PdI), morphology, thermal, and crystalline properties. Section 4.1 of the chapter presents a brief introduction on the preparation of grain based gluten-free cookies. Thereafter, section 4.2 elucidates upon the adopted procedure and sensory optimization findings of the prepared cookies. Section 4.3 presents the dough rheology findings of the best scored cookie formulations. Section 4.4 and 4.5 respectively address a detailed analysis of the proximate, in-vitro and physical properties of the best rated cookie formulations respectively. Sections 4.6 – 4.10 detail upon the thermal, compositional, relative crystallinity findings of the six best rated cookies. Thereafter, the significant findings of the conducted investigations have been compared with the best reported literature findings in section 4.11. Finally, a summary of the reported findings in the chapter has been presented in the final section (section 4.12).

Overview

Gluten-free grain flour based sugar snap cookies are one of the most commonly targeted bakery products for their beneficial health applications. Staple cereal, legume and millet based flours are preferred for the formulation of a diverse group of cookies. This is in lieu of their modifiable variegated nature, cheaper cost, and easy availability. The sensory attributes, physico-chemical, thermal and crystalline properties of the products were few key targets in *the conducted recent studies. Also, in comparison to high gluten content based conventional wheat formulation, the green gram flour based cookie formulation scored the best in this work (in terms of sensory attributes) and thereby affirmed associated nutritional benefits.*

In this chapter, altogether hundred and six gluten-reduced and gluten-free formulations were prepared with a combination of eight types of grain flours, six types of fats/oils and four types of sweetening agents. The details of such formulations have been presented in Appendix A of the Ph.D. thesis. All such prepared cookie formulations were optimized for their sensory scores being obtained with the 9-point hedonic scale and fuzzy logic methods. Further characterizations were assessed for the six sensory analysis based optimal cookie formulations that scored an overall acceptability score (9-point hedonic scale) of over and above a value of 7.85.

4.1 Introduction

Sugar snap cookies, renowned for their indulgent sweetness and texture, hold a significant place in the global snack market. Accordingly, a steady growth trajectory exists in the consumer demand for classic treats. By 2030, the cookies market industry is projected to grow from USD 27.56 Billion in 2024 to USD 45.4 Billion. This amounts to a compound annual growth rate (CAGR) of 6.43% during the forecast period (2024-2030) (Source: https://www.marketresearchfuture.com/reports/cookies-market-1924).

Wheat flour is the primary ingredient in the cookie dough. It primarily consists of starch, water and protein. Due to the minimal gluten development of sugar snap cookies, it is possible to produce gluten-free cookies with the gluten-free flours. This is due to these flours not possessing any gluten containing ingredients (Donelson, 1988). However, in comparison with the cookies prepared with the wheat flour, the gluten-free flours produce cookies with a variant characteristics of physico-chemical parameters. Such alterations depend upon the cereal origin and the milling process (Mancebo et al., 2015).

In this chapter, while gluten-free cookies were formulated with rice, soy, green gram, finger millet, oats, Bengal gram, and roasted chickpea powders, the gluten-reduced cookies incorporated wheat, Bengal gram, and roasted chickpea flours. The conducted study aims to assess upon the sensitive influence of variant powder compositions on the sensory and nutritional properties of the cookies. The characterization targeted were in terms of the assessment of texture, color, nutritional composition, and sensory attributes. Bengal gram and roasted chickpea powder proportions remained constant in all formulations and accordingly served as foundational components.

Considering the mentioned ingredients as indigenous produces of the North-east India, the chapter aims to achieve cookies with very good sensory scores. Thereby, it is envisaged to compare the chosen gluten-free flours suitability for the cookies' preparation in terms of nutritional, morphological, thermal, crystallinity, particle size distribution characteristics. Thereby, mature understanding into the relationships between various flour properties for the manufacture of sugar snap cookies were sought. Accordingly, relationships can be affirmed between various flour properties and the best performing cookies' quality parameters.

4.2 Development of Grain Flours based Cookie Formulations

In the cookie formulation experimental investigations, the focus was towards the creation of a range of gluten-free cookies. Accordingly, meticulous attention was towards the balanced proportions of ingredients and for the realization of best combinations of flavor, texture and nutritional values in the prepared cookies. The cookies formulation base constituted wheat flour (W), Bengal gram flour and roasted chickpea flour. These together imparted a harmonious blend of flavors and textures. Each batch of cookies constituted four cookies being specified as a standardized 30g dough ball. The total dry mix for four cookies was measured about 122g. Other ingredients such as fats/oils ghee (1), butter (2), soybean oil (3), groundnut oil (4), cookie shortening (5), or coconut oil (6) were selected and tailored as per specific recipe requirements.

Accordingly, cookies with variegated flavor and moisture content were realized with good sensory scores. The cookie preparation process began with the storage of raw materials in containers being kept either at room temperature (26°C) or under refrigeration (4°C) conditions. These conditions are as per the specific ingredient requirements and storage recommendations. In a bowl, all necessary ingredients with their respective proportions were combined. Accordingly, powdered sugar or alternative sweetening agents such as jaggery, honey, or artificial sweeteners, were blended with the required amount of fat or oil and flours. Thereby, a dough with a fluffy, aerated, and smooth creamy texture was always achieved. To ensure uniformity, different flours and powders were meticulously sieved. This prevented the formation of lumps and as well promoted effective mixing. Using a spatula, the ingredients are slowly and softly mixed to achieve a cohesive dough. Thereafter, final touches of liquid double-toned milk was allowed to enhance flavor and moisture content. Finally, the cookies were baked (150°C for 20 min), cooled and packaged for sensory and other addressed characterization. Appendix A summarizes the ingredients composition in the 106 alternate gluten-reduced and gluten-free cookie formulations. Among these, only six formulations were selected based on their overall acceptability score being equal to or greater than 7.85.

4.2.1 Sensory Score Driven Optimization of the Formulated Cookies

Hedonic sensory evaluation of the cookies was conducted with 28 male and female candidates and with age ranging from 18 to 70 years. The subjects were of various socioeconomic backgrounds and were habitual cookie consumers. All cookie samples were analyzed one day after baking. For sensory evaluation, samples were presented as whole pieces on white plastic dishes. The dishes were coded with four-digit random numbers and were served in random order. The cookies were evaluated on the basis of acceptability of their appearance, odor,

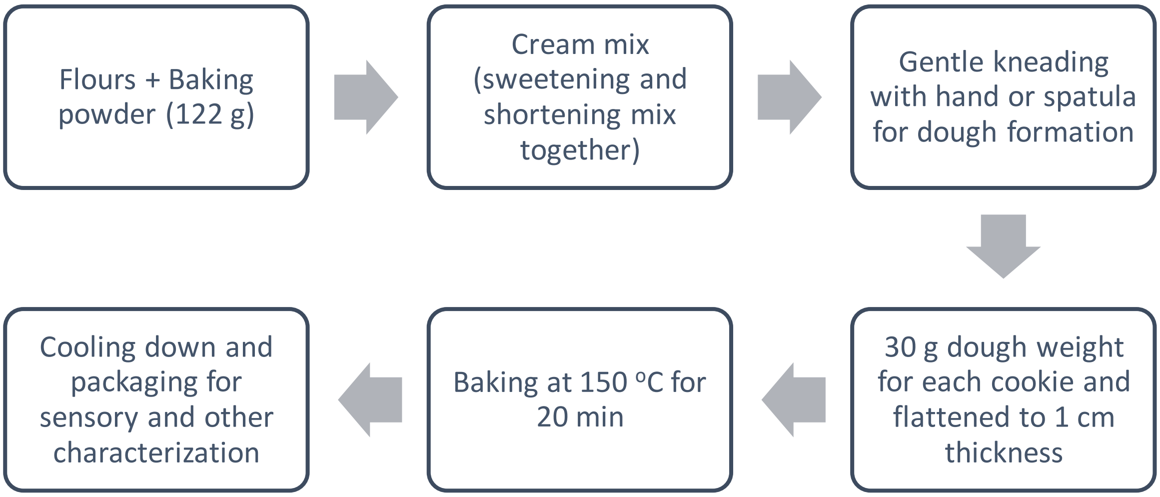


Fig. 4.1: Flowchart depicting the preparation procedure for alternate cookie formulations

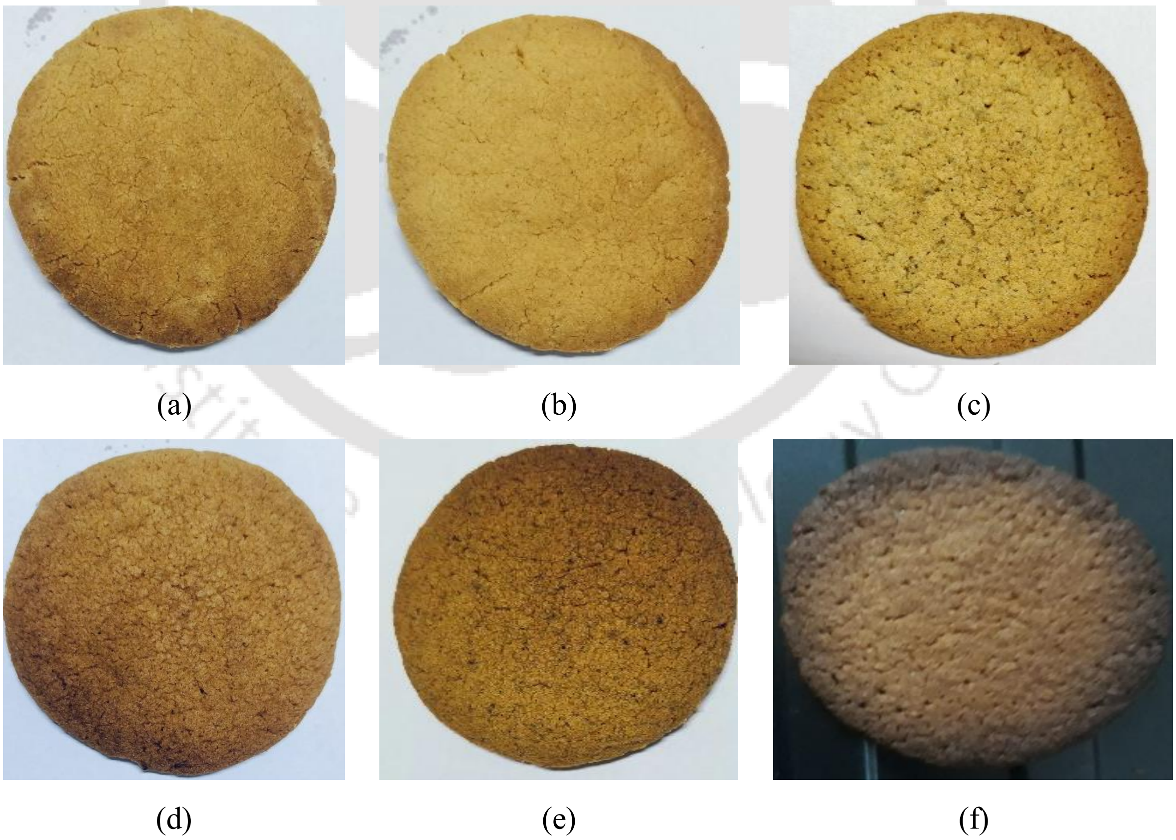


Fig. 4.2: Photographs depicting grain flour based alternate cookie formulations namely (a) WS1, (b) WS2, (c) MS2. (d) RS2, (e) RaRJ5, (f) RaOaJ5 samples

texture, taste and overall appreciation and as per the nine-point hedonic scale. The scale of values ranged from “like extremely” to “dislike extremely”. These respectively correspond to the highest and lowest scores of “9” and “1” respectively. The panelists also provided ratings in the form of NS (Not satisfactory), F (Fair), M (Medium), G (Good) and E (Excellent) for samples, and NI (Not Important), SI (Somewhat Important), I (Important), HI (Highly Important), and EI (Extremely Important) for the cookie product and as per the fuzzy logic analysis based sensory evaluation method. Such data were recorded with a fuzzy linguistic score sheet. The sensory evaluation scores being achieved for different cookie samples reveal variations across all attributes (Table 4.1). The gluten-reduced WS2 (Wheat-Sugar-Butter) sample exhibited high scores in most sensory attributes and has been henceforth affirmed for its best favorable sensory profile. The sample demonstrated excellent combinations of flavor, color, texture, breakability, taste, aftertaste, crispiness, and overall acceptability. In the wheat sugar butter (WS2) cookie formulation, the gluten proteins play a crucial role for the enhancement of sensory attributes such as texture, mouthfeel, and flavor release. For the case of hydrated wheat flour, the gluten proteins interact to form a network that provides the necessary structural integrity for the highly desired crispy yet tender texture of the resultant cookies. Through the effective trapping of the air in the dough, the gluten network ascertains the creation of a light and airy product and simultaneously prevents the excessive spread. Thereby, cookie’s shape can be maintained. Additionally, the gluten binding with the water does influence the softness and chewiness of the cookies and fosters a balanced mouthfeel property. Gluten also interacts with butter and sugar and accordingly controls the dough's spread and texture. While the butter-gluten interaction limits the cookie development in terms of a tender crumb, the sugar-gluten interaction competes for water. The latter case inhibits gluten formation and balances the tenderness and structure of the cookies. Furthermore, gluten's ability to hold fats and aromatic compounds facilitates a gradual release of the flavour during the chewing phase. This feature enriches the sensory experience and associated factors. Together, all above mentioned factors illustrate the interaction of gluten proteins with other mentioned ingredients to be highly responsible for the appealing sensory characteristics of the WS2 formulation. Similarly, sample WS1 also scored well in the respective sensory scores. The RS2 also achieved good overall acceptability (8.24) score. The MS2 (GM-Sugar-Butter), RaRJ5 (Finger millet-Rice-Jaggery-Shortening) and RaOaJ5 (Finger millet-Oats-Jaggery-Shortening) formulations received commendable sensory ratings among all attributes and especially in the scores of taste, aftertaste, and overall acceptability. This conveyed their potential as preferred options among the evaluated samples. The findings underscore the significance of ingredient combinations and formulations in sensitively altering the sensory characteristics of cookies. In other words, product development and optimization efforts were successful. Soybean oil also exhibited promising attributes to certain formulational alterations. Groundnut oil and coconut oil ascertained typical aroma attribute and was henceforth very much liked by few panelists. Based on the fuzzy logic scale, MS2 (GM-Sugar-Butter) formulation achieved the highest score of 0.82 ("excellent" category). Following closely, the RS2 (Rice-Sugar-Butter) formulation attained a fuzzy score of 0.78 ("excellent" category). Subsequently, WS2 (Wheat-Sugar-Butter) and WS1 (Wheat-Sugar-Ghee) formulations received scores of 0.75 and 0.72, respectively ("excellent" category). These findings highlight the favorable sensory attributes of the mentioned samples and especially in terms of the scores of the flavor, color, texture, breakability, taste, aftertaste, crispiness, and overall acceptability scores and as per the fuzzy logic sensory methodology. According to the fuzzy logic scale, the RaOaJ5 (Finger millet-Oats-Jaggery-Shortening) formulation received the highest score of 0.86 ("very good" category). Subsequently, the RaRJ5 (Finger millet- Rice-Jaggery-Shortening) formulation obtained a score of 0.74 (very good category). In the conducted studies, taste and overall acceptability are of prime importance. This is followed by flavor, color, and breakability. These as well conveyed their higher sensory importance. The aftertaste and texture can be considered to be important but to a lesser extent. Thus, the results emphasized upon the significance of taste and overall acceptability in determining the perceived quality of the cookie samples. Henceforth, they highlight the greater and prominent role of flavor, color, and breakability in the assessed sensory scores of the subjects.

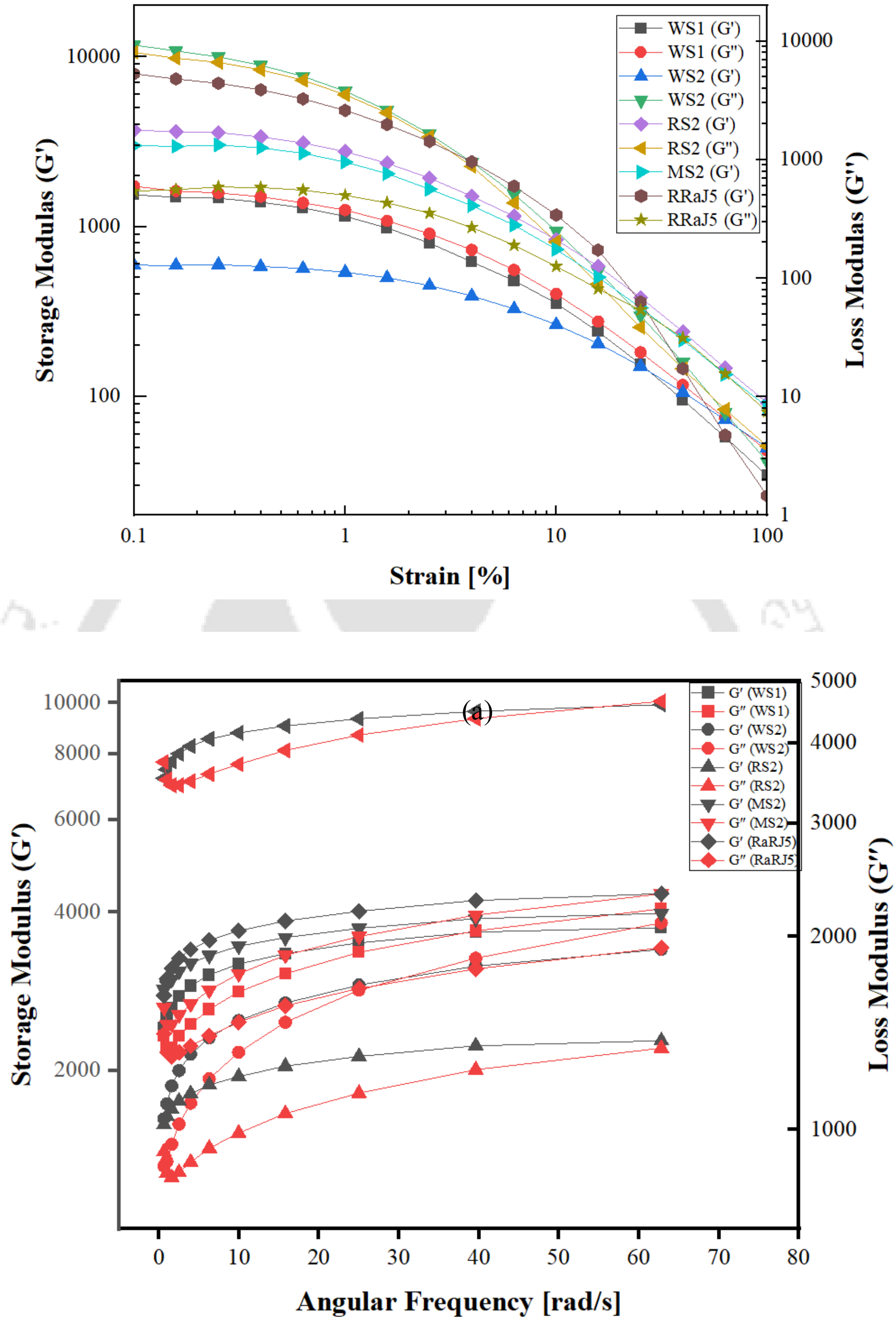
4.3 Dough Rheology of the Optimal Cookie Formulations

The assessed amplitude and frequency sweep data offer a detailed insight into the rheological characteristics of various dough samples. The data conveys varied value range for the best performing compositions being inferred from sensory analysis as wheat-sugar-ghee (WS1), wheat-sugar-butter (WS2), rice-sugar-butter (RS2), green moong-sugar-butter (MS2), rice-finger millet-jaggery-shortening (RaRJ5), finger millet-oats-jaggery-shortening (RaOaJ5) formulations. The rheological behavior of each sample was evaluated in terms of strain, storage modulus, and loss modulus. The strain, represented as a percentage, serves as the independent variable (plotted on the x-axis) and the storage and loss moduli (both measured in Pascals) are depicted on the y-axis. The data analysis reveals distinct trends in the mechanical response that conveys heightened stiffness and viscosity under greater deformation condition. However, the specific nuances in the behavior of each dough composition underscore the significance of the unique rheological properties of mentioned formulations. Such insights are invaluable for the optimization of processing parameters and for the development of tailored food products with desired textural attributes.

Table 4.1 9-point hedonic scale based sensory analysis data summary of gluten-free and gluten-reduced grain based cookies

S. No.	Sample Description	Sample ID	Flavor	Color	Texture	Breakability	Taste	After taste	Crispiness	Overall acceptability
1.	Wheat-sugar-ghee	WS1	8.29±0.	8.14±0.69	8.5±0.76	8.35±1.1	8.5±1.25	8.42±1.2	8.33±1.09	8.44±0.94
2.	Wheat-Sugar-butter	WS2	8.29±0.5	8.4±0.81	8.64±0.89	8.28±1.11	8.07±0.83	8.07±0.83	8.21±0.91	8.56±1.11
3.	GM-Sugar-Butter	MS2	8.21±0.9	8.35±1.07	8.47±1.34	8.16±0.89	8.71±1.11	8.57±0.97	8.36±0.95	8.78±1.04
4.	Rice-Sugar-Butter	RS2	8.14±1.1	8.14±1.1	8.48±1.62	8.59±1.28	8.45±1.43	8.27±1.4	8.13±1.28	8.24±1.31
5.	Finger millet-Rice-Jaggery-Shortening	RaRJ5	8.07±0.92	8.05± 1.43	7.91±1.34	7.89±1.27	7.99±1.11	8.29±1.15	7.98±1.26	8.09±1.02
6.	Finger millet-Oats-Jaggery-Shortening	RaOaJ5	8.28±1.25	8.08± 1.11	8.17±1.27	7.85±1.07	8.64±1.11	7.85±1.10	8.00±1.41	8.21±0.99

The rheological behavior of various dough samples, including wheat-sugar-ghee (WS1), wheat-sugar-butter (WS2), rice-sugar-butter (RS2), green moong-sugar-butter (MS2), finger millet- rice-jaggery-shortening (RaRJ5), was investigated at different angular frequencies. For all samples, the storage modulus, representing the dough's stiffness, enhanced with increasing angular frequency. This confirmed upon the greater ability to resist deformation. Additionally, the loss modulus, reflecting the dough's viscous behavior and energy dissipation, exhibited varying trends across samples. These findings provide valuable insights into the rheological properties of dough and for altered processing conditions. Fig. 4.3 (a) depicts the rheological behavior of various cookie dough formulations, in terms of the storage modulus (G') and loss modulus (G") as functions of strain percentage. The graph illustrates that at lower strain percentages (around 0.1%), formulations such as the WS3 exhibit the highest G' values (of approximately 10,000 Pa and G" values of around 1,000 Pa). This conveys greater rigidity and elastic properties in the formulations. G' values reduced to (approximately 100 Pa) and G" values reduced (to around 10 Pa) for formulations such as the WS2. This conveys their transition to a more fluid-like state. Different formulations are represented by distinct symbols, with some maintaining higher modulus values in the considered strain range. Such a trend infers upon their stronger structural integrity. In summary, the comparative analysis underscores the variability in rheological properties among different cookie dough formulations and provides insights into their textural and mechanical characteristics.



(b)

Fig. 4.3: Dough rheology characteristics of optimally formulated cookies - (a) amplitude sweep data, (b) frequency sweep data

The rheological analysis of rice sugar butter cookie (RS2) formulations, incorporating alternate fats and oils such as cookie shortening (CS), peanut butter (PB), soybean oil (SB), and coconut oil (CN), elucidates distinct characteristics in the storage and loss modulus for each variant (Fig. 4.4). At lower angular frequencies (around 0 rad/s), the storage modulus (G') values altered from approximately 2000 Pa to 8000 Pa. As the angular frequency increases to around 60 rad/s, the G' values for these formulations reached upto approximately 10,000 Pa. The loss modulus (G"), which indicates viscous behaviour, starts at around 1000 Pa to 2000 Pa and at lower angular frequencies for most formulations. The general trend observed is that an increase in both G' and G" is apparent with increasing angular frequency for all formulations. This conveys a typical behavior indicating that the respective doughs become more elastic and viscous at higher frequencies. Ghee-based doughs demonstrate notably higher storage modulus values in comparison to butter, SB, and CN oil-based doughs (Fig. 4.4). This conveys a more solid-like behavior due to ghee's higher saturated fat content and crystalline structure. Fig. 4.4 illustrates the variation in storage modulus (G') and loss modulus (G") of different dough formulations and for the wider range of angular frequencies (0 to 70 rad/s). The filled symbols represent G' (elastic properties), and the open symbols represent G" (viscous properties). In contrast, butter doughs exhibit relatively lower storage modulus values and thereby signify a softer consistency. SB doughs exhibit intermediate storage modulus values and thereby reflect their semi-solid nature. Additionally, the CN oil-based doughs display the lowest storage modulus, and accordingly convey a more fluid-like behavior. Regarding loss modulus, ghee doughs exhibited higher values in comparison to other formulations. This suggests greater energy dissipation during deformation. Such a comparative analysis underscores the significant influence of fat and oil composition on the rheological properties of rice sugar cookie dough. Accordingly, valuable insights can be gained for the optimization of altered formulations and for the realization of desired product attributes.

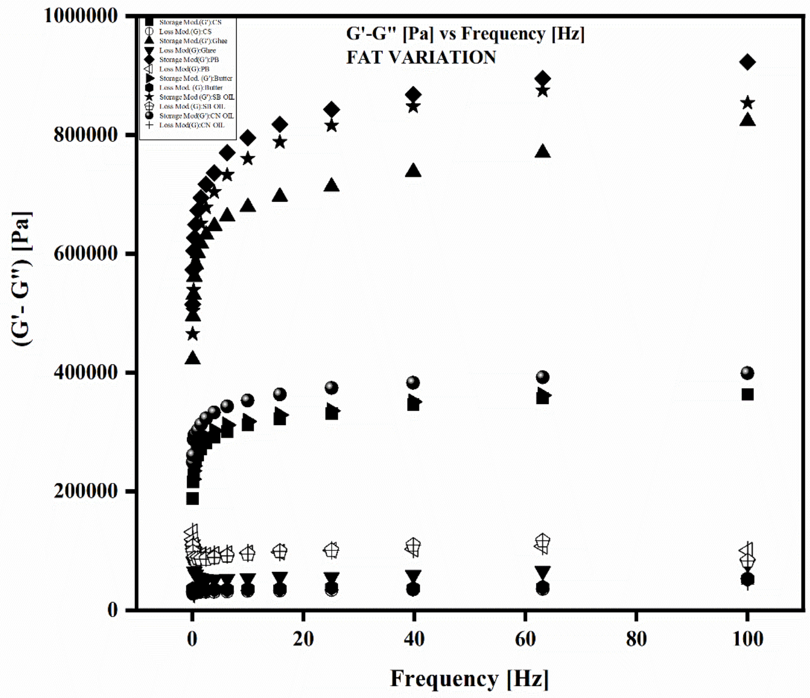


Fig. 4.4: Dough rheology characteristics of RS2 cookie formulation and for alternate choices of fats and oils

At lower frequencies (around 0 Hz), the storage modulus (G') values range from approximately 10,000 Pa to 1,000,000 Pa. For instance, formulations with ghee (G' for Ghee) exhibited higher G' values, and around 800,000 Pa. As the frequency increases to around 100 Hz, these formulations reached higher G' values (close to 1,000,000 Pa for ghee). In comparison to the G', the loss modulus (G"), indicating viscous behaviour, starts at lower values. For ghee, G" values are approximately 400,000 Pa and 300,000 Pa at lower frequencies and increase marginally with frequency enhancement. Other formulations such those with the soybean oil (G' for soybean oil based sample) and coconut oil (G' for coconut oil based sample) exhibit lower G' values (around 200,000 Pa to 400,000 Pa). Their corresponding G" values are also lower and indicate weaker structural properties. In summary, the graph highlights that the formulations with ghee and palm butter have superior elastic and viscous properties across a wide frequency range. Thus, they are more robust in comparison to other formulations achieved with the soybean oil and coconut oil, (with lower modulus values). Hence, such later formulations have weaker structural properties.

4.4 Nutritional Characteristics of Best Rated Grain Flour Cookies

4.4.1 Mineral Content

The assessed data offers useful insight into the mineral content of various cookie samples, and for the respective assessment of elements such as magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) (all measured in parts per million (ppm)) (Table 4.2). The rice-based cookie (RS2) possess lower concentrations of most elements (potassium at 17.97 ppm) and thereby suggest a comparatively lower mineral content. MS2 contains a good amount of Mg (7.7 ppm). The findings corroborate with the mineral content assessed for the green gram, roasted chickpea and Bengal gram flours. Similarly, WS2, another variant consisting of wheat-sugar-ghee, displays substantial concentrations of potassium (22.22 ppm), calcium (0.56 ppm), and manganese (0.04 ppm). However, its levels of magnesium (2.85 ppm) and copper (0.02 ppm) are marginally lower in comparison to the MS2 formulation. Comparatively, RaRJ5 and RaOaJ5, millet-based gluten-free formulations, exhibit notable concentrations of calcium (3.36 ppm and 3.49 ppm, respectively), and substantial levels of magnesium (8.85 ppm and 9.94 ppm, respectively) and manganese (0.56 ppm and 0.68 ppm, respectively). These findings underscore the significance of ingredient choice in determining the nutritional quality of cookies. Accordingly, the choice has implications for dietary diversity and health related factors.

4.4.2 Other Proximate Characteristics

Table 4.3 summarizes the nutritional composition of various samples labeled from WS1 to RaOaJ5. The sample WS1 contained 23.58 g/100 g of total carbohydrates, 2.56 g/100 g of total protein, 1.7 g/100 g of soluble protein, 11.14 g/100 g of total fat, and 0.98 g/100 g of ash.

Table 4.2: Mineral content data of optimal grain flour cookie formulations

S. No.	Sample Code	Mg (ppm)	K (ppm)	Ca (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
1.	WS1	2.39±0.25	19.60±0.42	0.37±0.08	0.04±0.01	0.21±0.04	0.03±0.01	0.07±0.01
2.	WS2	2.85±0.55	22.22± 1.8	0.56±0.28	0.04±0.05	0.34±0.62	0.02± 0.08	0.076±0.04
3.	MS2	7.70±0.57	60.98± 3.9	1.03± 0.16	0.06±0.04	0.34±0.15	0.38±0.27	0.13±0.04
4.	RS2	2.55±0.95	17.97±2.33	0.38±0.27	0.03±0.07	0.18±0.67	0.015±0.02	0.089±0.09
5.	RaRJ5	8.85±1.06	54.13±0.53	3.36±0.49	0.56±0.38	0.29±0.07	0.03±0.20	0.08±0.05
6.	RaOaJ5	9.94±2.04	57.01±0.85	3.49±1.56	0.68±0.08	0.33±0.13	0.03±0.01	0.13±0.05

Among the formulations, MS2, a green gram-based gluten-free cookie, has been the best with the highest protein content (14.31g/100 g) and soluble protein (5.66 g/100 g). While RS2, a rice-sugar-butter-based formulation, exhibits the highest total carbohydrate content (30.5 g/100 g), the RaRJ5, a millet-rice-jaggery formulation, affirms relatively lower total carbohydrate content (17.77g/100 g). In terms of fat content, RaOaJ5, another millet-based gluten-free formulation, demonstrates a notable level of total fat (12.07g/100 g). Additionally, the ash content, indicating the mineral content of the cookies, varies across formulations. Thereby, RS2 exhibited the highest ash content (3.01g/100 g). Similarly, other samples exhibited variations in their nutritional content. These variations in nutritional composition provide insights into the dietary profile and potential health implications of the samples. Additionally, they inform dietary choices and formulations in food product development and can thereby cater to specific nutritional needs and preferences.

4.4.3 In-vitro Antioxidant Activity

The DPPH radical scavenging activity of baked samples WS1 and WS2 was assessed at 26.78% and 24.93% respectively. While RaRJ5 and RaOaJ5 displayed elevated levels of antioxidant activity at 34.42% and 37.81% respectively, the RS2 and MS2 exhibited antioxidant activities of 32.22% and 33.51% respectively. Following in-vitro digestion, a significant loss of

Table 4.3: Proximate characteristics of best rated grain based cookie formulations

S. No.	Sample	Total Carbohydrate (g/100 g)	Total Protein (g/100 g)	Soluble Protein (g/100 g)	Total Fat (g/100 g)	Ash (g/100 g)
1.	WS1	23.58±0.21	2.56±0.16	1.7±0.67	11.14±0.5	0.98±0.3
2.	WS2	23.58±0.36	2.56±0.54	1.05±0.34	9.11±0.82	1.44±0.6
3.	MS2	22.87±1.05	14.31±0.43	5.66±.0.48	11.43±0.97	2.08±0.5
4.	RS2	30.5±1.1	2.9±0.66	1.22±0.07	11.5±0.8	3.01±0.84
5.	RaRJ5	17.77±2.05	2.57±0.88	0.91±0.27	11.78±0.73	1.26±.0.41
6.	RaOaJ5	19.86±1.84	3.49±0.75	1.21±0.68	12.07±1.92	2.36±1.01

antioxidant activity was assessed in all samples. While WS1 and WS2 exhibited the lowest retention of antioxidant activity at 4.06% and 4.47% respectively, the RS2 and MS2 retained higher levels at 9.13% and 10.38% respectively. Notably, RaRJ5 and RaOaJ5 exhibited the highest retention of antioxidant activity after in-vitro digestion and with values of 17.25% and 19.82% respectively.

4.5 Physical Properties of the Optimally Formulated Cookies with Grain Flours

Table 4.4 summarizes the physical characteristics and hardness data of various samples being labelled from WS1 to RaOaJ5. The sample WS1 possessed a weight of 27.59 g, a diameter of 79.34 mm, and a thickness of 8.03 mm and spread ratio of 9.88. The color parameters were a hue angle of 68.17°, a chroma value of 39.51, and color coordinates (b*, a*) of (14.69, 36.68). Its hardness was measured at 11.81 N. Similarly, other samples exhibited relative variations in weight, diameter, thickness, spread ratio, color parameters (hue angle, chroma, and color coordinates), and hardness. Such an analysis of the cookie samples reveals significant variations in their physical characteristics and hardness. Conversely, despite affirming similar spread ratios, the cookies with finger millet and oats (RaRJ5, RaOaJ5) tend to have lower

Table 4.4: Physical properties of best rated grain based cookie formulations

S. No.	Sample Code	Wt. (g)	Dia (mm)	Thickness (mm)	Spread Ratio	Color				Hardness
						b*	a*	Hue Angle	Chroma	(N)
1.	WS1	27.59±1	79.34±0.2	8.03±0.04	9.88±0.2	36.68±0.9	14.69±0.2	68.17±0.09	39.51 ±0.34	11.81±0.51
2.	WS2	26.96±0.9	76.27±0.3	8.2±0.05	9.3±0.5	39.15±0.3	14.31±0.52	69.92±0.4	41.68 ±0.16	15.27±22.08
3.	MS2	27.93±0.8	81.27±0.5	8.1±0.06	10.03±0.3	38.05±0.5	14.58±0.64	69.03±0.57	40.75 ±0.09	8.07±2.8
4.	RS2	26.55±0.7	83.26±0.6	7.48±0.07	11.13±0.61	39.65±0.5	15.88±0.55	68.17±0.43	42.71 ±0.54	5.62±1.6
5.	RaRJ5	28.27±1.3	76.3±0.8	8.9±0.06	8.57±0.25	19.47±0.71	10.89±0.82	60.78±0.69	22.31 ±0.54	13.36±2.55
6.	RaOaJ5	26.71±1.2	69.93±0.7	5.1±0.04	13.71±0.19	24.14±0.5	13.25±0.4	61.24±0.03	27.54 ±0.06	16.05±5.32

hardness values. This is mostly due to the fibrous nature of the ingredients and for their contribution to a softer texture. The findings underscore the influence of ingredient composition on cookie texture and hardness. Accordingly, the conducted research prompts upon the need to achieve the tailored formulations and for the realization of desired sensory attributes and quality.

4.6 Thermal Properties of the Optimally Formulated Cookies with Grain Flours

Fig. 4.5 depicts the DSC curves for all optimal cookie formulations. The differential scanning calorimetry (DSC) analysis revealed distinct thermal characteristics among various cookie formulations. Conversely, MS2 exhibited the lowest melting point at 190°C. This suggests a comparatively weaker structure (also well supported with its lower ΔCp value of 1.014 J/ gK). Remarkably, RaOaJ5 displayed the highest ΔCp value of 2.316. This is indicative of the samples' significant energy absorption during the melting process. WS1, with a melting peak at 220°C and ΔCp of 0.762, exhibited relatively lower thermal stability. WS2 affirmed a marginally lower melting peak at 217.9°C but a higher ΔCp of 1.422. This suggests enhanced thermal stability in comparison to the WS1.

4.7 FTIR Analysis of the Optimally Formulated Cookies with Grain Flours

Fig. 4.6 depicts the FTIR spectra of alternate best performing cookie formulations. In all cookie samples, broadband absorption spectra being observed between 3600 and 3000 cm^{-1} affirmed significant water absorption. This is consistent with literature reported trend (Santos et al., 2021). The peaks at 3360 and 3180 cm^{-1} correspond to N-H valence stretching vibrations from cross-linking bridges and hydroxyl [OH] groups. The trend thereby suggests the presence of proteinaceous and polysaccharide components (Cavalcanti et al. 2023). In all formulations, the presence of the C=O functional group, typically associated with lipids, is evident in the spectral region between 1746 and 1720 cm^{-1} and for all formulations. Bands corresponding to peptide linkages such as amide I (1690 - 1600 cm^{-1}), amide II (1575 - 1480 cm^{-1}), and amide III (1301 - 1229 cm^{-1}) further support the presence of proteins within the cookies (Kong & Yu, 2007). Additionally, bands located at 1200 - 900 cm^{-1} are attributed to C-O and C-C stretching vibrations, and to C-O-H and C-O-C deformations of carbohydrates. This affirmed richer carbohydrate content within the cookie formulations. In summary, the spectral features collectively provide insights into the chemical composition and structural characteristics of the cookies. Thereby, they highlight the presence of proteins, lipids, and carbohydrates and their obvious contribution to the texture and flavor of the cookies.

4.7.1 Qualitative Detection of Acrylamide

Acrylamide, an unsaturated amide, is found at times in various thermally processed foods. It is generated in food products containing higher content of reducing sugars such as glucose and proteins. Such constituents are especially rich in amino acids in their structure. The acrylamide

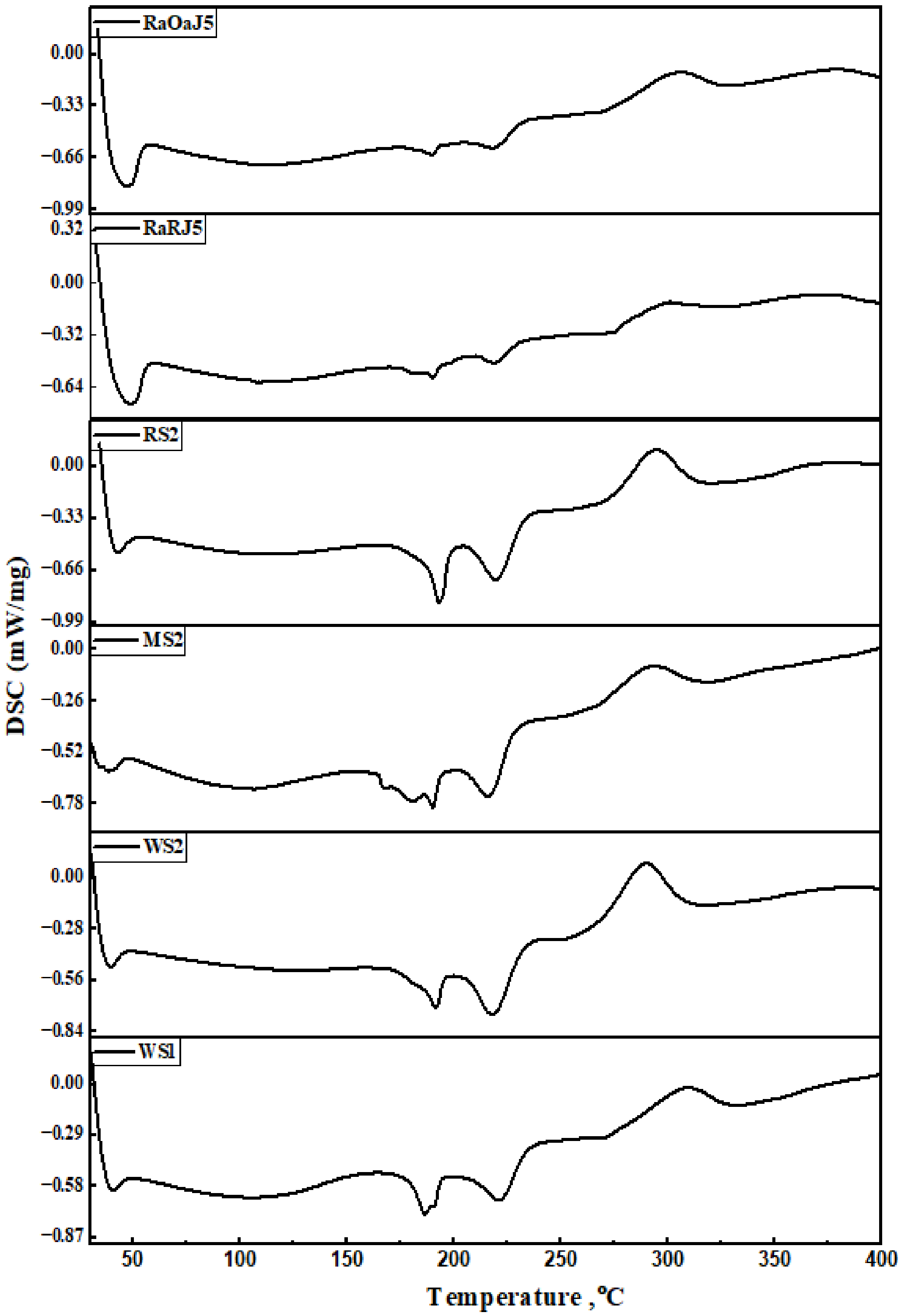


Fig. 4.5: DSC plots of best rated grain based cookie formulations

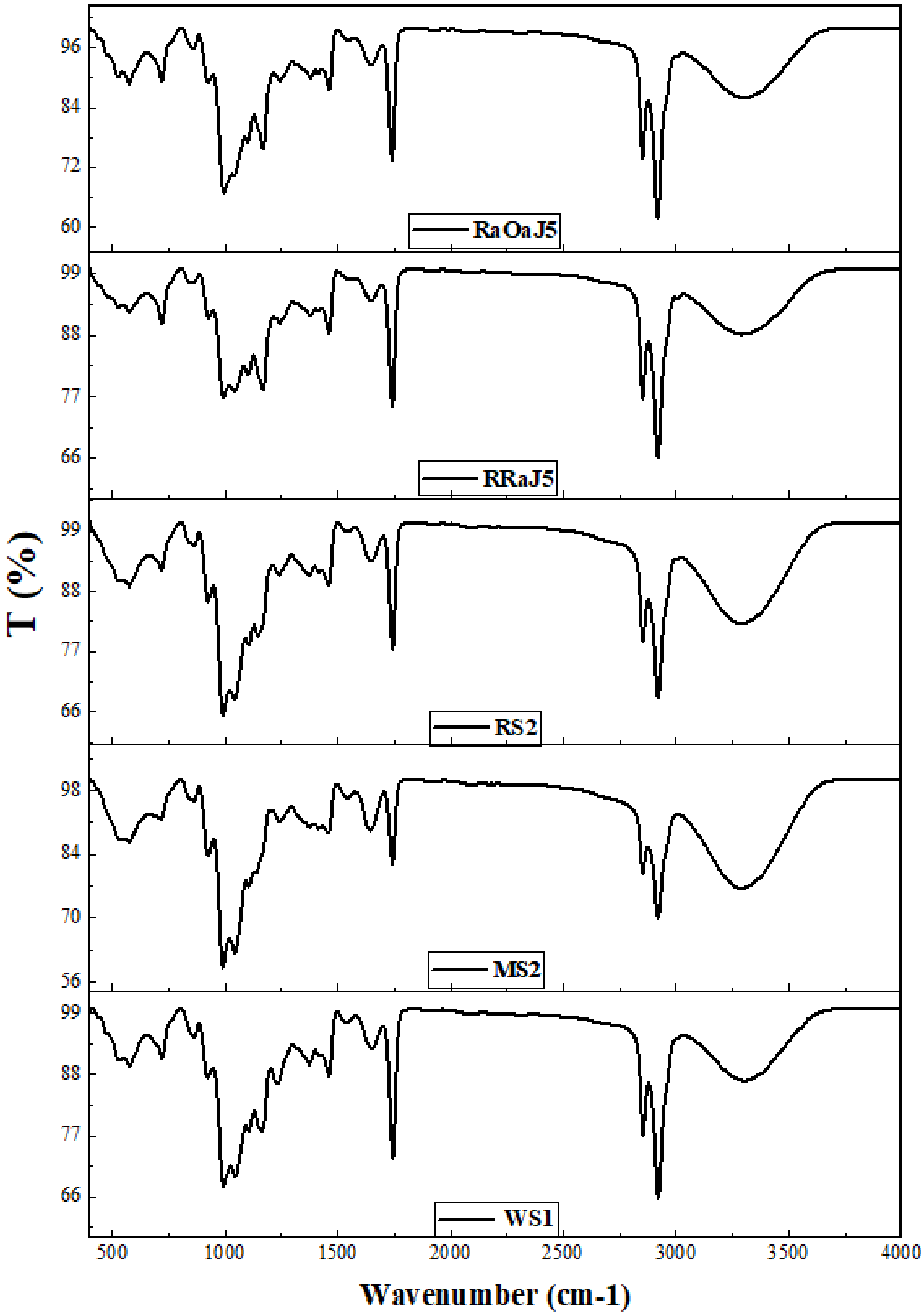


Fig. 4.6: FTIR spectral plots of best rated grain based cookie formulations

is produced due to constituent amino acids and in the high temperature heating environment (> 170ºC). Fourier transform infrared (FT-IR) is a powerful tool for the identification of various types of chemical bands of a molecule. These bands are produced as an infrared absorption spectrum, (also dubbed as molecular "finger-print"). FT-IR is also the most useful method for the identification of chemicals present in organic samples. The presence of all mentioned peaks associated with acrylamide in a food sample would strongly suggest the presence of acrylamide. However, the absence of one or more peaks does not necessarily mean that acrylamide is not present.

The acrylamide formation in food samples is a complex chemical process. It is influenced by various factors such as temperature, moisture content, pH, and the composition of the food matrix. Therefore, depending on these factors, the presence and intensity of peaks in the FTIR spectrum may vary sensitively.

In some cases, acrylamide may form in trace amounts. This leads to weaker or lesser prominent peaks in the FTIR spectrum. Additionally, the detection limit of the FTIR instrument and the sensitivity of the method may as well influence the ability to detect lower levels of acrylamide. While the presence of characteristic peaks associated with acrylamide is a strong indicator of its presence, it's essential to consider other analytical methods and techniques for confirmation. This is valid especially for cases dealing with lower concentrations or complex food matrices. For such cases, successfully proven assessment methods refer to liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), or enzyme-linked immunosorbent assays (ELISA). These methods ascertain quantitative analysis of the acrylamide content in the cookie sample.

Based on the achieved FTIR peaks, the presence of peaks at 1644 cm^{-1} and 3330 cm^{-1} suggests that acrylamide may indeed be present in WS1 and WS2 formulations. Based on the achieved peaks, the peaks at 1630 cm^{-1}, 3322 cm^{-1}, and 3325 cm^{-1} suggests that the acrylamide may indeed be present in the MS2 sample. Also, the presence of peaks at 1625 cm^{-1} and 3290 cm^{-1} suggest that acrylamide may be present in RS2 sample. Further, the presence of a peak at 1632 cm^{-1} suggests that the acrylamide may be present in the RaRJ5 sample.

4.8 Relative Crystallinity of the Optimally Formulated Cookies with Grain Flours

The relative crystallinity percentages of various dough samples reveal notable differences in their structural organization. Wheat-Sugar-Ghee (WS1) and Finger millet-Oats-Jaggery-Shortening (RaOaJ5) formulations exhibit the highest crystallinity percentages (41.8% and 33.03%, respectively). This conveys a relatively ordered structure. In this regard, it can be noted that the presence of ghee in WS1 and shortening in RaOaJ5 may contribute to the formation of crystalline structures. Ghee and shortening contain saturated fats. These tend to solidify and form crystalline arrangements under appropriate conditions such as cooling. Additionally, the combination of wheat flour and sugar provides a framework for crystallization to occur, as these two ingredients have molecular structures that are conducive to form ordered arrangements. Conversely, Rice-Sugar-Butter (RS2) formulation possessed the lowest crystallinity at 7.2%. This suggests a more disordered or amorphous composition. The lower relative crystallinity being apparent in Rice-Sugar-Butter (RS2) dough sample in comparison to other samples can be attributed to several factors. The ingredients themselves could contribute to the lower crystallinity. The rice flour contains starch, which tends to form amorphous structures upon mixing with other ingredients and water. Additionally, the presence of sugar and butter in these dough formulations could inhibit the formation of crystalline structures. This is due to their interference with molecular arrangements during dough formation and baking. Cookie formulations such as Wheat-Sugar-Butter (WS2), and Finger millet-Rice-Jaggery-Shortening (RaRJ5), exhibit intermediate levels of crystallinity (ranging from 25.66% to 32.86%). These variations are likely to arise from differences in ingredient composition, processing methods, and formulation techniques. Thereby, all these findings highlight the diverse structural characteristics that are inherent in each alternate dough sample.

4.9 Morphological Studies of the Optimally Formulated Cookies with Grain Flours

4.9.1 FESEM Image Analysis

The FESEM (Field Emission Scanning Electron Microscopy) morphology analysis reveals distinct features among different cookie samples, and accordingly provides insights into their structural characteristics (Fig. 4.7). The regular circular structures observed in the FESEM images of WS1 and WS2 suggest a homogenous distribution of ingredients within the cookie matrix. This contributes to their uniform appearance. In contrast, the triangular-like structure of the FESEM image of the MS2 resembles the original morphology of green gram flour. Thereby, it indicates the presence of intact particulates within the cookie samples. RS2 sample displays an elliptical regular structure in the samples' FESEM image. Thereby, it indicates a different composition in comparison to other samples. Finally, the comparable structures observed in the FESEM images of RaRJ5 and RaOaJ5 convey similarities in ingredient composition or processing techniques. These findings underscore the relationship between cookie structure and formulation and highlight the potential influence of the samples' structure sensory attributes and consumer acceptance indices of the formulations.

4.9.2 Particle Size Distribution

The results from the dynamic light scattering (DLS) analysis reveal variations in the particle size distribution and average diameter in various cookie formulations (Table 4.5). Among alternate samples, the RaOaJ5 sample exhibits the largest average diameter of 2557 nm. This is followed by RS2 sample (average diameter of 2270 nm). Conversely, RaRJ5 has the smallest average diameter of 1097 nm. These differences in particle size can be attributed to the

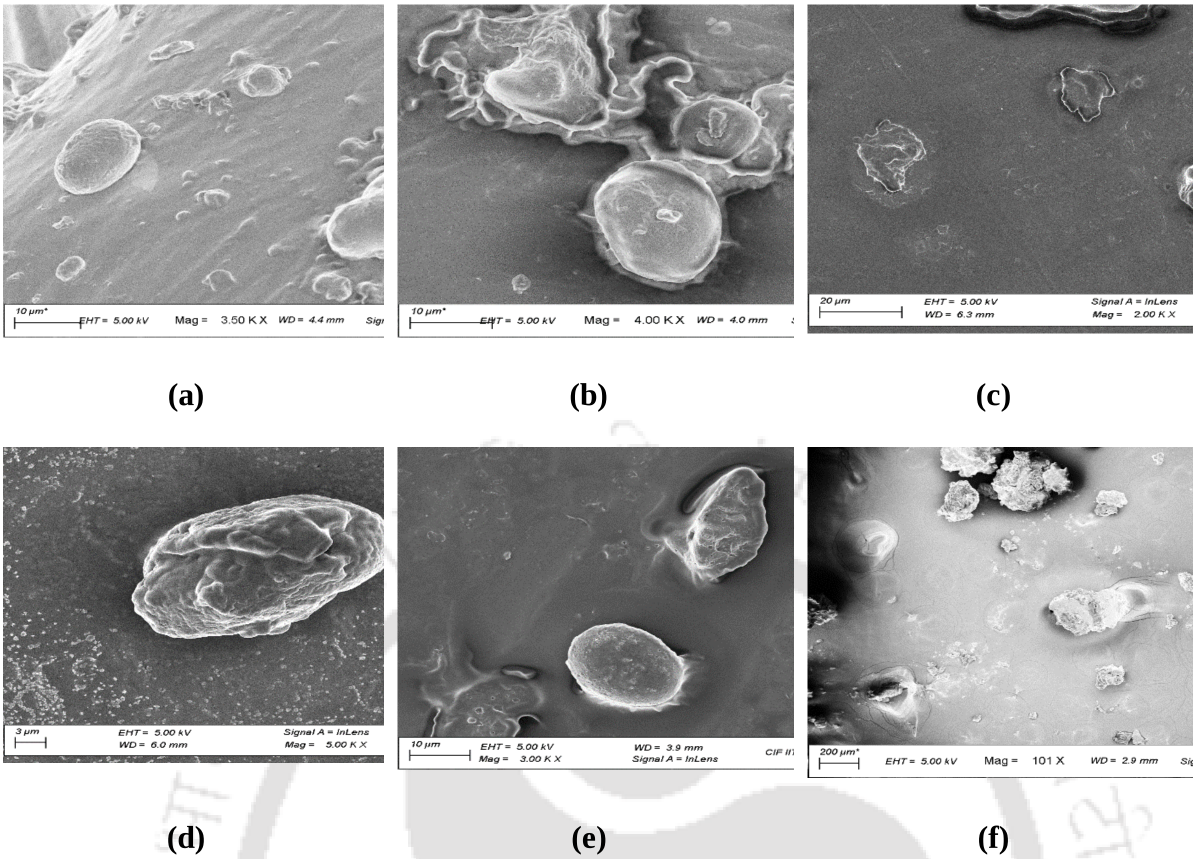


Fig. 4.7: FESEM images of best rated gluten-free and gluten-reduced cookies: (a) WS1, (b) WS2, (c) MS2, (d) RS2, (e) RaOaJ5 and (f) RaRJ5 formulations.

variations in ingredient composition and processing methods. Formulations with larger average diameters convey the presence of larger aggregates or particles. Such aggregates result from the interaction between ingredients during mixing or baking. Conversely, smaller average diameters suggest finer particle sizes or more uniform dispersion of ingredients. The polydispersity index (PdI) provides an insight into the uniformity of particle sizes within each sample (values closer to zero indicating a narrower size distribution). The RS2 and RaOaJ5 had lower PdI 0.332 and 0.485 and the highest PdI 0.485 was achieved for the RaRJ5 sample. Overall, the findings highlight the importance of particle size characterization in analyzing the obtained physical properties and potential sensory attributes of food products such as cookies.

4.10 Storage Study

Moisture and water activity play an important role in the storage of cookies. A minor increase in the moisture content of cookies from 0th day to 60th day has been apparent (Table 4.6). Such an increase in moisture could be related to the general hygroscopic nature of the packed cookies. The nature of packaging material and its porosity plays an important role in the moisture uptake of a sample. The authors (Nagi et al., 2012) reported that biscuits being packed and stored in metalized polyester or biaxially oriented poly propylene exhibit higher moisture content than those being packed and stored in paper-aluminum foil polyethylene laminate pouches. Cookies packed in laminate pouches absorb lesser moisture during storage. This is due to the impervious nature of aluminum foil in the laminate to air and water vapor. Similar trends with increased moisture content of cereal bran being incorporated biscuits after 60 days storage period were noted by Nagi et al., (2012). A corresponding rise in Aw value in the cookie samples was also observed. Sixty days of storage in controlled conditions induced minor alteration in the sensory changes in cookies. The panelists reported reduced cookie hardness. This is also confirmed by the instrumental analysis (Table 4.6). The reduced breaking strength, being measured with the three-point peak force or hardness tests, has been apparent in stored cookies being prepared with or without fat replacement (Forker et al., 2012).

Table 4.5: Particle size distribution data of best rated grain based cookie formulations

S. No.	Sample name	PdI	Average diameter (nm)
1.	WS1	0.59±0.06	1822 ± 19.36
2.	WS2	0.57±0.04	1743 ± 31.98
3.	RaOaJ5	0.48±0.02	2557 ± 10.50
4.	RaRJ5	0.93±0.09	1097 ± 7.83
5.	RS2	0.32±0.05	2270 ± 23.49
6.	MS2	0.68±0.03	1626 ± 18.02

In all cookie formulations, no rancid off-flavour has been reported by the panelists and for a storage period of 60 days.

Food-borne illness is caused by microorganisms. Henceforth, human consumption necessitates upon their absence from food products. The microbial count for TC, yeast and mold (YMC), *E. coli, S. aureus, B. cereus* and *salmonella* affirmed their non-existence after preparing them (Table 4.7). This suggests that microbial quality of cookies during storage of 60 days remained good. All six best rated cookie formulations had nil plate counts (CFU/mL) and yeast and mould counts after processing (Table 4.7). Agu and Okoli (2014) found similar results for beniseed and unripe plantin enriched wheat biscuit. After 30 days, WS1, WS2 had 0.4 and 0.1 CFU/mL TC. Both types of cookies had no yeast or mould growth after 30 days. The millet based cookies such as RaRJ5 and RaOaJ5 cookies had total plate count (TC) that altered from 0.5 – 0.8 CFU/mL TC. However, no yeast and mold count (YMC) growth was detected. RS2 and MS2 had TC lower than 0.2 CFU/mL and for 60 days' storage period. After 60 days, the wheat based cookies WS1 and WS2 had a TC of 1.0 and 1.4 CFU/mL respectively. Millet based cookies i.e. RaRJ5 and RaOaJ5 had maximum number of total plate count.

4.10.1 ^{1}H-NMR Spectra based Fatty Acid Profile of Cookies during Storage

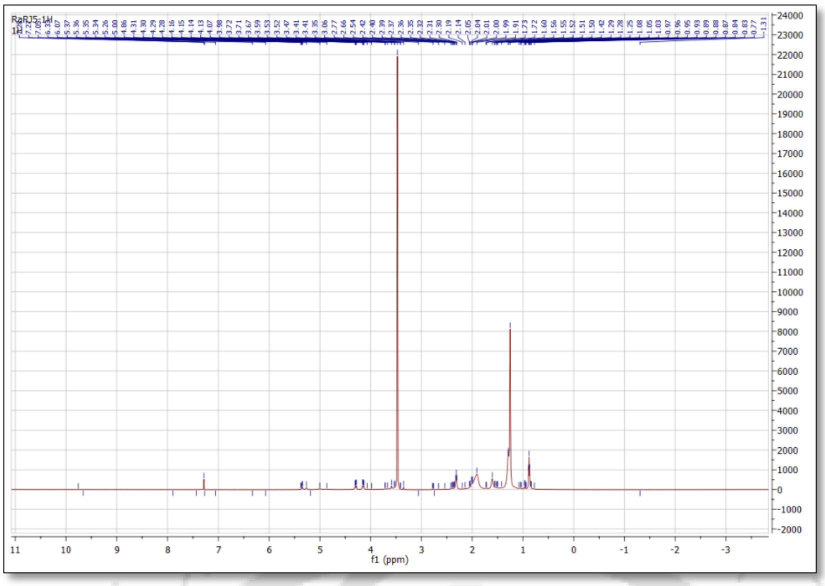
The ^{1}H-NMR spectra of the fat extracted from the grain-based gluten-free cookies were used to determine their fatty acid profiles. Figure 4.9 illustrates the ^{1}H-NMR spectra of the fat extracted from the millet grain based optimal cookie formulation RaRJ5 being assessed in the storage study for the 0th and 60th day cases of the prepared cookies. For MS2, WS2, WS1, RS2 and RaOaJ5 cookie formulations, the stability of fatty acids in cookies during storage were also assessed with the proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The graphical representations have been presented in Appendix C of the Ph.D. thesis. In Fig. 4.8, the peaks at 0.81 ppm, 1.19 ppm, 1.51 ppm, 2.20 ppm, 2.35 ppm, 2.77 ppm, 3.73 ppm, 4.04 ppm, 4.29 ppm, 5.09 ppm, 5.53 ppm, 5.74 ppm, 6.06 ppm, 6.49 ppm, 7.28 ppm, 7.59 ppm, 9.14 ppm, 9.41

ppm, and 9.97 ppm in the NMR spectra correspond to various proton environments that are typically found in fatty acids. The peaks at lower chemical shifts (0.81-3 ppm) are indicative of alkyl chain protons such as methyl and methylene groups. Peaks around 4-6 ppm suggest the presence of olefinic protons and thereby indicate unsaturated fatty acids. The higher chemical shift regions (around 7-10 ppm) are often associated with protons in more complex environments. These possibly include aromatic compounds or oxidized fatty acids. The consistent presence of these peaks suggests that the fatty acid profile includes a mix of saturated and unsaturated fatty acids, and with desired stability in the chemical structure for a period of 60 days. Similar findings can be analyzed from the graphical representations presented in Appendix C for all other optimally reported grain flours based cookie formulations.

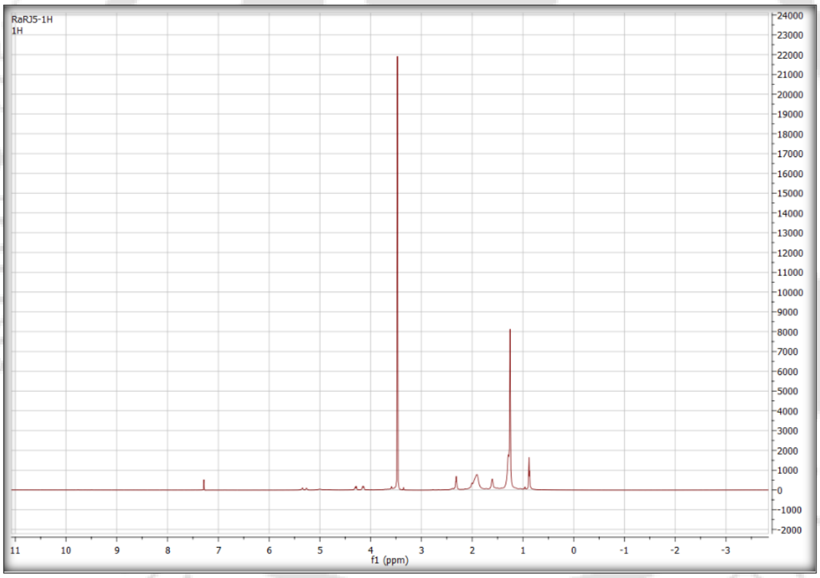
In summary, the conducted research investigations have been able to identify relevant changes that would occur in the fatty acid profile with storage time. Accordingly, insights could be gained into the optimal practices that shall be followed to maintain cookie quality and extend shelf life. The ^{1}H NMR spectrum of the cookies conveys a mixture of saturated and unsaturated fatty acids. No significant changes occurred in the NMR spectra of the cookie formulation RaRJ5 in the 60-day storage period. The fatty acid profile, as indicated by the positions and intensities of the peaks in the NMR spectra, remained stable. This affirmed that the fatty acids in the cookies did not undergo substantial chemical alteration during the storage period.

4.11 Comparative Assessment with Commercially Available Gluten-free Cookies

Most grain flours based cookies have been designed to serve as a gluten-free alternative. Such formulations mimic traditional sugar cookies. The deployment of a diverse gluten-free flour blend facilitates the similar cookies' texture and flavor to that being assessed in the conventional cookies. However, in such conventional cookies, the nutritional profile is dominated with simple carbohydrates and sugars. Thus, the cookies with little protein or fiber



(a)



(b)

Fig. 4.8: ^{1}H-NMR Spectra of fat extracted from the RaRJ5 cookie formulation (a) for 0th day and (b) 60th day storage period cases

have lower nutritional density in comparison to other gluten-free snacks being constituted with ingredients such as legumes or whole grains. The absence of fat may be appealing for some dietary preferences. However, such cookies lack the satiety and potential health benefits of healthy fats. The commercial gluten-free sugar cookies from "Gluten-Free Heaven" offer a convenient option for those needing to avoid gluten. Each serving (1/24 of the package) contains 90 calories, a value being recommended as a relatively low-calorie snack. The cookies have 0 g of fat, including no saturated or trans fats. This appeals to individuals desiring a low-fat diet. They contain 45 mg of sodium. In the total carbohydrate content of 15 g, 9 g corresponds to that of the sugars. In other words, a significant portion of the calories come from sugar. The cookies do not have any dietary fiber and henceforth do not offer much desired satiety or digestive benefits. Lastly, without protein content, the commercial cookies infer that they are a simple source of carbohydrates and sugars and are not a balanced snack. The RS2 gluten-free cookies (serving size: 30g), containing 30.5±1.1% moisture, 2.9±0.66% ash, 1.22±0.07% fat, 11.5±0.8% protein, and 3.01±0.84% fiber, offer a more nutritionally balanced option in comparison to the commercially available "Gluten-Free Heaven" sugar cookies. The RS2 cookies are rich in protein and fiber, which contribute to better satiety, muscle maintenance, and digestive health. This renders them to be a healthier choice. In contrast, the commercial cookies are fat-free and have a higher sugar content. Thus, they are sweeter but nutritionally less dense due to no protein or fiber content. Thus, the comparative analysis highlights that while "Gluten-Free Heaven" sugar cookies may appeal to those seeking a low-fat sweet treat, the RS2 cookies provides significant health benefits and a more balanced nutritional profile.

4.12 Literature Comparison

The literature based best data comparative analysis provided insights into the nutritional and bioactive properties of various cookie compositions (Table 4.8). The study by Kishorgoliya et al. (2015) reports a multi-grain cookie formulation. It was achieved with wheat flour, chickpea flour, barley, maize flour, pearl millet flour, and finger millet flour. Such multi-grain composition based cookies were characterized with a carbohydrate content of 66.9%, crude fiber with a 1.12% - 1.55%, and ash content of 1.29% - 1.76%. These values reflect a balanced nutritional profile. However, it shall be noted that the specific bioactive components were not detailed in the reported data.

In contrast, the present study focuses on the MS2 cookie formulation, which combines green gram flour, sugar, and butter. The MS2 formulation exhibits a significantly lower carbohydrate content of 22.87 g/100g, and thus a distinct nutritional composition being tailored for different dietary needs. The MS2 cookies are also notable for their protein content (14.31g/100g) and fat content (11.43g/100g). This suggests a more protein-rich and higher-fat profile in the cookies in comparison to the multi-grain cookies analysed by Kishorgoliya et al. (2015) . Additionally, the MS2 formulation demonstrates substantial soluble protein content at 5.66g/100g, highlighting its potential benefits for protein intake.

From a bioactives content perspective, the MS2 cookies possessed promising antioxidant activity of 33.51% pre-digestion and 10.38% post-in-vitro digestion. These values, while impressive, are marginally lower than those achieved for RaRJ5 and RaOaJ5 formulations (17.25% and 19.82% of antioxidant activity post-digestion, respectively). The higher retention rates in RaRJ5 and RaOaJ5 underscore the effectiveness of these formulations in preserving bioactive components during digestion. The findings of Thongram et al. (2016) on the chickpea flour, pigeon pea flour, moong bean flour, cowpea flour, and wheat flour based composite flour cookies, highlight similarities with respect to the formulations studied in the present study and for protein and fat content. The multigrain cookies formulated by Kamran et al. (2023) incorporating chickpea flour, wheat flour, barley, maize, pearl millet, and finger millet flour, also exhibit comparable nutritional profiles. Accordingly, they emphasize upon the potential of multigrain formulations in enhancing protein and fat content for an acceptable level of moisture, ash, and carbohydrates. Furthermore, the cookies formulated by Kamran et al. (2023) with finger millet, oats, and brown rice align closely with the nutritional composition being assessed in the present study. This reinforces the versatility of alternative grain-based formulations in producing cookies with desirable nutritional attributes. In summary, the studies collectively support the feasibility and effectiveness of the incorporation of diverse flour blends in cookie formulations for the desired enhanced nutritional value and antioxidant properties. Thus, they provide valuable insights for further exploration and optimization in cookie product development.

4.13 Summary

The sensory evaluation scores of the cookie samples reveal notable differences in their sensory attributes. WS2 exhibits consistently high scores in various attributes and confirms its favorable sensory profile in terms of flavor, color, texture, and overall acceptability. Samples MS2 and RaOaJ5 achieved commendable ratings for all attributes and these excelled in taste, aftertaste, and overall acceptability. The fuzzy logic analysis further supports the findings, with samples MS2, RS2, WS1, and WS2 being classified in the "excellent" category, and RaOaJ5 and RaRJ5 categorized to be "very good." The findings convey the significant role of ingredient combinations and proportions in achieving the best sensory characteristics of cookies. The rheological analysis offers valuable insights into the mechanical behavior of dough samples and under variant conditions. The ghee-based doughs demonstrated higher stiffness in comparison to butter, soybean oil, and coconut oil-based doughs and henceforth a more solid-like behavior. Conversely, butter doughs exhibit relatively softer consistency, the soybean oil doughs demonstrate intermediate stiffness, and coconut oil-based doughs exhibit the lowest stiffness. These distinctions underscore the influence of fat and oil composition on dough rheology, and their prominence to optimize processing parameters for the realization of desired product attributes. The analysis of mineral content highlights nutritional differences among the cookie samples. While rice-based cookies affirmed lower mineral concentrations, and thereby conveyed comparatively lower nutritional content, other cookies convey the choice of ingredients and their proportions for dietary product desired diversity in terms of optimal health related factors.

The detection of peaks associated with acrylamide formation provides further insights into the potential presence of this compound in selected cookie formulations. However, considering the complex nature of acrylamide formation and detection limitations, the absence of characteristic peaks does not rule out its presence.

Moreover, the rheological analysis and FESEM morphology reveal structural differences among dough and cookie samples. This conveys greater influence of ingredient composition and processing methods. While the dynamic light scattering (DLS) analysis further elucidates variations in particle size distribution, the moisture content and sensory changes during storage underscore the importance of formulation and packaging for the achievement of good storage period cookie quality. Microbial analysis affirmed satisfactory microbial quality during storage. This highlights the safety of the evaluated cookie formulations. Overall, these comprehensive analyses provide valuable insights into the chemical, structural, and quality attributes of the cookie formulations. They henceforth convey possible future optimization efforts and for enhanced their nutritional and sensory profiles. The sensory evaluation scores being obtained for various gluten reduced and gluten free grain flour based cookie samples reveal profound variations in all attributes. Among all the formulations, the MS2 (8.78) was found to have higher overall acceptability score. This is followed by WS2 (8.56). Based on the fuzzy logic scale, the MS2 formulation (GM-Sugar-Butter) achieved the highest score of 0.82 ("excellent" category). Also, MS2 exhibited the lowest melting point at 190°C, and a comparatively weaker structure. This is supported by its lower ΔCp value (1.014 J/(gK)). MS2 also contains Mg (7.7 ppm). For the millet based cookie formulation RaRJ5, good mineral content in terms of Ca (3.36 ppm) was ascertained.

Table 4.6: Storage parameters of best rated grain based gluten-reduced and gluten-free cookie formulations

S. No.	Cookie formulation	Storage parameters								
		0th			30th			60th		
		Mc	Aw	Hardness	Mc	Aw	Hardness	Mc	Aw	Hardness
1.	WS1	3.88±0.12	0.44±0.3	11.81±0.51	4.04±0.37	0.48±0.5	12.5±2.03	4.52±0.4	0.49±0.24	12.07±1.33
2.	WS2	4.05±0.11	0.46±0.14	15.27±22.08	4.46±0.51	0.47±0.5	15.89±2.76	4.79±0.6	0.48±0.27	15.49±5.06
3.	MS2	3.95±0.7	0.45±0.09	15.07±2.8	4.00±0.08	0.47±0.2	17.2±1.36	4.06±0.43	0.48±0.07	16.9±1.81
4.	RS2	4.13±0.34	0.44±0.15	12.62±1.6	4.27±0.59	0.45±0.06	13.45±2.7	4.31±1.04	0.47±0.54	13.04±2.55
5.	RaRJ5	4.59±0.34	0.47±0.09	13.36±2.55	4.65±1.06	0.49±0.06	14.28±5.03	4.82±0.86	0.5±0.08	14.09±7.89
6.	RaOaJ5	4.42±0.51	0.46±0.03	16.05±5.32	4.7±0.93	0.47±0.02	17.6±7.44	4.8±0.61	0.48±0.15	17.5±8.04

Table 4.7: Microbial analysis data summary of gluten-free and gluten-reduced best rated cookie formulations

S. No.	Cookie formulation	Microbial count					
		0th		30th		60th	
		TC*	YMC**	TC*	YMC**	TC*	YMC**
1.	WS1	ND	ND	0.4±0.02	ND	1.0±0.5	ND
2.	WS2	ND	ND	0.1±0.03	ND	1.4±0.6	ND
3.	MS2	ND	ND	0.1±0.01	ND	0.13±0.02	ND
4.	RS2	ND	ND	0.12±0.02	ND	0.17±0.04	ND
5.	RaRJ5	ND	ND	0.65±0.06	ND	2.82±0.47	ND
6.	RaOaJ5	ND	ND	0.7±0.09	ND	3.3±0.45	ND

TC* means Total Plate Count; YMC** means Yeast and Mold Count expressed in CFU/mL units

Table 4.8: A summary of best data achieved in this work and in the literature for the grain based cookie formulations.

S. No.	Cookie Composition	Proximate Composition	Bioactive Components	References
1.	• C3: 50% Wheat flour • 10% Chickpea flour • 10% Barley • 10%Maize flour • 10%Barley • 10%Pearl millet flour • 10% Finger millet flour	• Carbohydrate content: 66.9% • Crude fiber range:1.12-1.55% • Ash:1.29-1.76%	-	Kishorgoliya et al., 2015
2.	• MS2: 60% Green gram flour • 20% Bengal gram flour • 20% Chickpea flour	• Total carbohydrate content :22.87g/100g • Total protein :14.31g/100g • Soluble protein content : 5.66g/100g • Fat :11.43g/100g	• MS2- AA:33.51% After in-vitro • AA: 10.38%	This work

For the cookies, promising post *in-vitro* AA value of 53.42% was achieved. Also, RaOaJ5 exhibited good mineral content in terms of Mg (9.94 ppm); Mn (0.68 ppm) content. The sample also had the highest ΔCp value (2.316). The NMR study ascertained no alteration in the fatty acid profile for the millet based cookies (formulations RaOaJ5, RaRJ5) and for all the other sensory analysis based optimal cookie formulations.

In conclusion, the comprehensive analysis of sensory attributes, rheological behavior, and nutritional composition provided valuable insights into the quality and characteristics of various cookie formulations. These insights can inform product development efforts and can guide the formulation of cookies with improved sensory profiles, nutritional content, and textural attributes. Accordingly, consumer preferences can be met in terms of sensory qualities and dietary needs.

Chapter 5:

Exploring the Viability of Unripe Papaya Pulp and Peel Powder as Substitutes in Grain based Gluten-free Flour Cookies: Characterization and Nutritional Analysis

Chapter 5

Exploring the Viability of Unripe Papaya Pulp and Peel Powder as Substitutes in Grain based Gluten-free Flour Cookies: Characterization and Nutritional Analysis

The chapter details upon the enrichment of prior achieved best cookie formulations with the unripe papaya pulp and peel powder and the subsequent characterization of optimally formulated cookies in terms of sensory analysis, morphological analysis. Section 5.1 provides a concise introduction of the chapter. Section 5.2 discusses the sensory evaluation based findings. Section 5.3 – 5.10 provide a detailed explanation of the proximate, dough rheology, morphological, FTIR, thermal, and crystallinity properties of the unripe papaya peel and pulp powder substituted optimal cookie formulations. Subsequently, section 5.11 details upon the comparative assessment of the findings with those being reported as the best in the literature. Finally, section 5.12 provides a concise summary of the chapter.

Overview

The Ph.D. thesis research work investigates the potential of the unripe papaya pulp (PPU) and peel powder (PPE) as substitutes for the optimal formulation of alternate grain flours based cookie formulations. The primary aim of such efforts is to enhance the cookies' nutritional profile. Accordingly, the grain-based powders being optimally constituted in cookie formulation was replaced with variant proportions of PPU and PPE and for the nutritional enrichment of the cookies. The sensory evaluation scores for various cookie samples affirmed notable differences in the sensory parameters. Also, detailed characterization of the cookies was addressed for the sensory analysis based optimal formulation of the cookies and with the *optimal substitution of the unripe papaya pulp and peel powder. The findings convey that both PPU and PPE possess valuable nutritional attributes and functional properties and accordingly enhance the nutritional value and sensory characteristics of the cookies. Thus, the conducted research contributes to the exploration of alternative ingredients for baked goods and thereby offers a sustainable approach for the betterment of dietary intake and though the appropriate promotion of the underutilized food resources.*

5.1 Introduction

Among the myriad of products undergoing reformulation, cookies emerge as a focal point, and reflect a global affection for this classic baked indulgence. The cookie is an unleavened crusty baked, sweet edible item being prepared from wheat flour, shortening (hydrogenated fat) and sugar. They are normally produced as lighter products and through the appropriate mixing of ingredients with the baking powder (cereal flour mixed with sodium carbonate and sodium bi-phosphate) (Lakshmi M et al., 2018). In the cookie formulation, wheat flour acts as a basic and major ingredient. This is due to the greater role of gluten proteins that do not exist in the flours of other cereals (Ortolan & Steel., 2017). Gluten proteins ensure dough elasticity in the baking process and as well provide higher organoleptic attributes to the end product (Wang et al., 2017). However, the ever increasing emphasis upon the gluten-free trends and the pursuit for healthier alternatives have spurred innovation in cookie formulations. Such an endeavor entails strategic modifications in their composition, and with an emphasis upon the augmentation of protein content for enhanced cookie quality in terms of the nutritional parameters.

Papaya (*Carica papaya* L.) belongs to the family Caricaceae. Papaya (*C. papaya* L.) is the fourth most important tropical fruit in the globe (Ogawa et al., 2016). However, product exploration of unripe papaya has not been addressed till date for the cookie product. Given that the North-east India has abundant produce of the papaya, unripe papaya pulp and peel powder can be conveniently explored for their supplementation in the cookies. An important aspect in this regard is the shorter drying duration of the sliced unripe papaya pulp and grated peel samples that renders them to ascertain easy and sustainable production of the pulp and peel powders. Such explorations strengthen and meet nutritional requirements, support local agricultural economic sustainable growth, enhance functional food product development and design efforts etc. Considering this critical knowledge gap, the chapter aims to utilize unripe papaya pulp (PPU) and peel (PPE) powder as substitutes for enhanced quality characteristics of the innovative formulation based cookie product. The research activity aims to further propel achieved research outcomes of best rated gluten-free and gluten-reduced cookies that were reported in the previous chapter of the Ph.D. thesis. To do so, PPU and PPE substitution was targeted to enhance sensory and nutritional properties of the cookie products. Accordingly, healthier wholesome snacks can contribute to the overall well-being of the consumers.

5.2 Cookie Formulation and Sensory Score Driven Evaluation for the Optimal Cookie Formulations

5.2.1 Development of Cookie Formulations

The conducted studies investigated the potential of unripe papaya pulp (PPU) and peel powder (PPE) as substitutes for grain-based powders in cookie formulations. The primary aim of such efforts is to enhance the cookies' nutritional profile. Accordingly, the grain-based powders being typically used in cookie recipes was replaced with variant proportions of PPU and PPE for the nutritional enrichment of the cookies. Initially, altogether 96 formulations were prepared with strategy being similar to the grain based cookie formulations being reported in the chapter 4 of the Ph.D. thesis. However, PPU and PPE had not been incorporated in the millet based cookie formulations. For the development of functional and gluten-free cookies, the grain-based cookie formulations being prepared with wheat, rice, soybean, and green gram were fortified with unripe papaya pulp (PPU) and peel powder (PPE). However, the fewer millet-based formulations were not subjected to the PPU and PPE incorporation as satisfactory organoleptic properties were not achieved for such formulations. In such cases, challenges existed in achieving acceptable texture, taste, and overall sensory appeal. Additionally, the dough formation with PPU and PPE incorporated millet flour proved to be difficult. Thus, only grain flour proportion was reduced for a proportional and constant proportional inclusion of the PPU and PPE. Appendix B presents the alternate cookie formulations that were subjected sensory analysis for the identification of PPU-PPE based optimal cookie formulations. Among these, only few qualified for further investigations. The sensory evaluation scores being assessed for various optimal alternate cookie formulations reveal significant alterations among all attributes. In the papaya powders' enriched formulations, the respective proportions of 12.5g of unripe papaya pulp (PPU) and 2.5 g of unripe papaya peel powder (PPE) were arrived on a trial basis and with the consideration of organoleptic and functional properties. Higher levels of PPU (above 12.5 g) in the formulations led to excessive sogginess in the cookies. This features detrimentally affected both texture and taste. Additionally, the dough consistency and baking suitability decreased with increased PPU, and rendered it to be highly challenging for the achievement of a proper cookie structure. For the PPE, amounts beyond 2.5 g introduced bitterness and undesirable darkening of the cookie color. Even though lower levels of PPU and PPE ascertained satisfactory sensory scores of the formulations, the mentioned respective proportions of the PPU and PPE optimized the cookies' performance in terms of the sensory qualities, texture, or dough handling properties. In other words, further enhancement in the functionalization with the PPU and PPE has been detrimental for the mentioned responses. With the 9-point hedonic scale, both UMS2 and UMS5 formulations obtained an average overall acceptability of 8.21 and 7.94 respectively. The formulations also received the highest indices for taste, breakability and texture profile. The URJ2 and UMS1 formulations received good acceptability scores of 7.89 and 7.45 respectively. These results underscore the significance of ingredient combinations and their proportions in the alteration and optimization of the sensory characteristics of cookies. Soybean oil was also analyzed to affirm promising attributes to their optimal formulations. Also, few sensory panelists opined that the groundnut oil and coconut oil were able to impart aroma based organoleptic attributes. Based on the fuzzy logic scale, the sample UMS2 (GM-Sugar-Butter) sample achieved the highest score of 0.49, ("excellent" category). Following closely, sample URJ2 attained a score of 0.38, ("excellent" category). Subsequently, UMS5 and UMS3 samples received scores of 0.35 and 0.28, respectively ("excellent" category). These findings highlight the favorable sensory attributes of these samples and especially in terms of the fuzzy logic method based flavor, color, texture, breakability, taste, aftertaste, crispiness, and overall acceptability. In such sensory evaluation, taste and overall acceptability have been inferred to be of extreme importance. This is followed by flavor, color, and breakability. These as well had higher significance in the subject's interest. However, the aftertaste and texture have been considered to be important but to a lesser extent. These results emphasize upon the significance of taste and overall acceptability in determining the perceived quality of the cookie samples. Simultaneously, they also highlight the importance of flavor, color, and breakability for enhanced consumer scores.

Table 5.1: Sensory analysis data summary of unripe papaya pulp and peel powder enriched alternate optimal cookie formulations

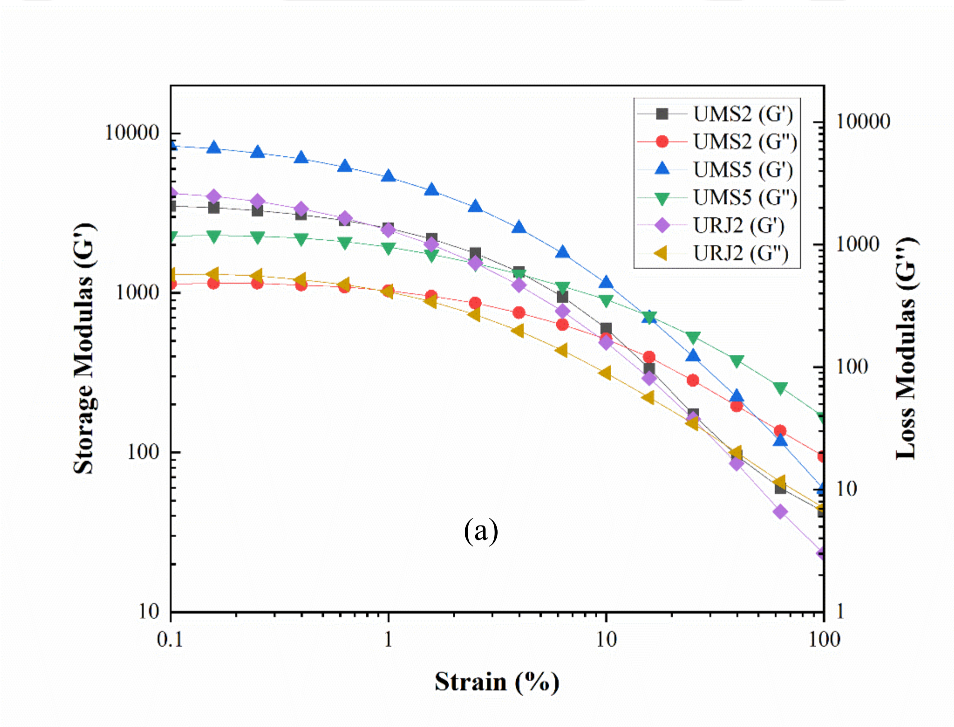
S. No.	Sample Description	Sample ID	Flavor	Color	Texture	Breakability	Taste	After taste	Crispiness	Overall acceptability
1.	PPU-PPE-GM-Sugar-Butter	UMS2	8.05±1.84	8.1±1.27	7.95±1.29	8.02±0.95	7.96±1.67	7.97±1.79	8.04±1.31	8.21±1.79
2.	PPU-PPE-GM-Sugar-Shortening	UMS5	8.61±2.06	8.14±1.34	8.06±1.57	8.00±1.27	7.57±2.22	7.96±2.41	7.87±1.72	7.94±1.93
3.	PPU-PPE-Rice-Jaggery-Shortening	URJ2	8.02±0.91	8.03± 1.27	7.87±1.29	7.97±1.17	7.96±1.11	8.28±1.07	7.98±1.11	7.89±0.69
4.	Green gram-Sugar-Butter	MS2	8.21±0.9	8.35±1.07	8.47±1.34	8.16±0.89	8.71±1.11	8.57±0.97	8.36±0.95	8.78±1.04

5.3 Dough Rheology of Optimal PPU-PPE Cookies

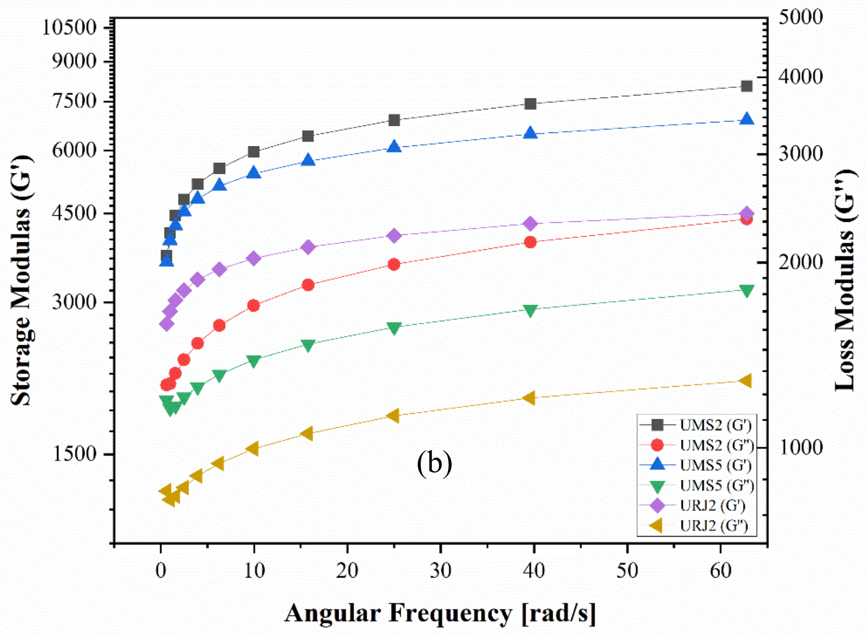
The dough rheology characteristics in terms of amplitude sweep and frequency sweep data offer a detailed insight into the rheological characteristics of various dough samples for the PPU-PPE-green moong-sugar-butter (UMS2), PPU-PPE-green moong-sugar-shortening (UMS5), and PPU-PPE-rice-jaggery-butter dough (URJ2) formulations. For each sample, rheological attributes were evaluated in terms of strain, storage modulus, and loss modulus. The strain, represented as a percentage, serves as the independent variable plotted on the x-axis. With frequency, the elastic modulus (G') increases more rapidly than the viscous modulus (G"). The mentioned dough samples exhibited appropriate differences in the dynamic mechanical behavior. The UMS2 and UMS5 samples exhibited higher storage moduli in comparison to URJ2, and affirmed greater elastic behavior. For the UMS2, at a strain of 0.1%, the storage modulus (G') is approximately 8000 Pa and the loss modulus (G") is around 2000 Pa. For an increase in the strain value to 1%, the G' reduced to about 4000 Pa and G" reduced to 1000 Pa. At 10% strain, G' further reduced to 2000 Pa. However, the G" measured roughly 800 Pa. Finally, at 100% strain, G' and G" reduced approximately to 500 Pa and 300 Pa, respectively. For the UMS5, at 0.1% strain, G' is roughly 9000 Pa and G" is around 1000 Pa. For a strain value of 1%, the G' decreases to about 7000 Pa, and G" reduced to 900 Pa. At 10% strain, G' reduced to approximately 3000 Pa, and G" was around 500 Pa. At the highest strain of 100%, the G' and G" reduce to about 1000 Pa and 200 Pa, respectively. For the case of URJ2, the initial 0.1% strain results in a G' of about 5000 Pa and a G" of approximately 3000 Pa. At 1% strain, the G' reduced to around 3000 Pa, and G" reduced to about 2000 Pa. At 10% strain, both G' and G" measured approximately 1000 Pa. At 100% strain, both G' and G" further reduced to around 300 Pa. All samples exhibited a reduced modulus with increasing strain values, an observation that consistently conveys a transition from elastic to viscous behavior at higher strain values. At lower strains (around 0.1% to 1%), the storage modulus (G') is much higher than the loss modulus (G"). This conveys that the material is predominantly elastic. With further increase in the strain (1% to 10%), both G' and G" reduce and convey that the material lost its elasticity and became more viscous.

(a) (b) (c)

Fig. 5.1: Photographs depicting unripe papaya pulp (PPU), peel (PPE) and grain flours based alternate optimal cookie formulations namely (a) UMS2, (b) UMS5, (c) URJ2 samples



(a)



(b)

Fig. 5.2: Dough rheology characteristics of unripe papaya powder based optimally formulated cookies - (a) Amplitude sweep; (b) Frequency sweep characteristics

In the graphical representation, the storage and loss moduli, both measured in Pascals, have been depicted on the y-axis. Examining the data reveals distinct trends in the mechanical response of the dough samples for variant strain levels. Generally, for most samples, an increase in strain correlates with elevated storage and loss moduli. Such a trend is indicative of heightened stiffness and viscosity under greater deformation condition. However, the specific nuances in the behaviour of each dough composition underscore the relevance and uniqueness of the rheological properties of alternate formulations. Such insights are invaluable for the optimization of processing parameters and for the development tailored food products with desired textural attributes.

Through the analysis of the frequency sweep curve, one can fine tune and customize the formulation and processing conditions for the realization of the desired mechanical performance for specific applications. For all samples, both G' and G" increased with increasing frequency. This affirmed that the materials became stiffer and more elastic at higher frequencies. In other words, the trend is that of the viscoelastic materials. Both storage modulus (G') and loss modulus (G") increased with increasing angular frequency for all samples. UMS2 has the highest moduli (both G' and G"), and conveyed that it is the stiffest sample among the three. UMS5 had intermediate moduli and moderate stiffness. The URJ2 had the lowest moduli and least stiffness. Thus, it is the most compliant sample. Understanding the frequency sweep behavior of these materials can guide their application in the processing schemes of the food industry. Accordingly, the influence of stiffness and viscoelastic properties on the texture and mouthfeel of cookies can be conceptualized and corroborated.

5.4 Nutritional Characteristics of the PPU-PPE based Optimal Cookies

5.4.1 Mineral Content

The obtained mineral content data offers insight into the mineral content of various cookie samples, and for the concentrations of elements such as magnesium (Mg), potassium (K), marginal levels of the calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) in parts per million (ppm). Comparing alternate samples, the unripe papaya-based cookies (UMS2 and UMS5) have been the best with notably higher levels of potassium (64.22 ppm and 67.37 ppm, respectively) and calcium (1.37 ppm and 1.89 ppm, respectively). This conveys the potential and promising nutritional advantage of the combination of the PPU, PPE and green moong powder. The URJ2 contains highest combinations of Mg (9.24 ppm), K (76.25 ppm), Ca (3.38 ppm). Accordingly, the significance of jaggery and rice incorporation in the formulation for enhanced mineral content can be assessed.

5.4.2 Other Proximate Attributes

Table 5.3 presents the nutritional composition of various cookie samples. The UMS2 formulation had a lower total carbohydrate content of 21.08 g/100 g but a higher total fat content of 13.65 g/100 g. The UMS5 sample exhibited similar trends and had a total carbohydrate content of 21.56 g/100 g and a total fat content of 22.81 g/100 g. Maximum soluble protein (3.09 g/100 g) and total protein content (10.44 g/100g) was found in UMS5 formulations.

Such a result corroborates to the inclusion of cookie shortening ingredient and its contribution to the protein content enhancement in the cookies. Thus, the mentioned variations in nutritional composition provide insights into the dietary profile and the associated potential health implications of the samples. Additionally, they provide directions for newer formulations in food product development and cater to specific nutritional needs and preferences.

5.5 Physical Properties of the PPU-PPE based Optimal Cookies

Table 5.4 presents the physical characteristics and hardness values of various samples. The UMS5 sample had a diameter of 69.86 mm and a thickness of 6.1 mm. This resulted in a spread ratio of 11.45. The color parameters of the sample were a hue angle of 64.40°, a chroma value of 33.07, and color coordinates (b*, a*) of (14.29, 29.82). The hardness index of UMS5 was at 26.27 N. These physical characteristics and hardness indices provide valuable insights into the texture, color, and structural attributes of the samples. All of them are important for the assessment of the samples' quality and consumer acceptance.

The analysis of the cookie samples reveals significant variations in their physical characteristics and hardness indices. Notably, cookies containing unripe papaya powder (UMS2, UMS5, URJ2) exhibit lower spread ratios in comparison to the wheat-based alternate cookie formulation. Thus, they refer to a denser texture and potentially reduced moisture content. Additionally, the unripe papaya powder-based cookies generally exhibit higher hardness

Table 5.2: Mineral content data of unripe pulp and peel powder based optimal cookie formulations

S. No.	Sample Code	Mg (ppm)	K (ppm)	Ca (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
1.	UMS2	7.12±0.57	64.22±2.61	1.37±0.93	0.048±0.03	0.32±0.05	0.03±0.04	0.11±0.06
2.	UMS5	7.68±2.03	67.37±1.35	1.89±0.94	0.052±0.05	0.27±0.08	0.03±0.06	0.12±0.04
3.	URJ2	9.24±2.19	76.25±2.07	3.38±0.48	0.07±0.03	0.33±0.02	0.03±0.02	0.12± 0.01
4.	MS2	7.70±0.57	60.98± 3.99	1.03± 0.16	0.06±0.04	0.34±0.15	0.38±0.27	0.13±0.04

values, and henceforth a firmer texture. This is attributed to the higher moisture absorption capacity of unripe papaya powder, and its eventual influence on the dough consistency and final cookie texture. In summary, these findings underscore the influence of ingredient composition on cookie texture and hardness, and thereby highlight the potential for tailored cookie formulations in terms of the desired sensory attributes and quality.

5.6 Thermal Characteristics of the PPU-PPE based Optimal Cookies

Differential scanning calorimetry (DSC) analysis revealed distinct thermal characteristics among various cookie formulations. Fig. 5.3 illustrates the DSC curves for all optimally prepared cookie formulations. The highest melting peak was assessed in the UMS2 sample, (250.1°C) and affirmed its superior thermal stability in comparison to other cookie formulations. Such a high melting point value corroborates with its high heat capacity change (ΔCp) value of 1.397 J/(gK). These indices signify strong intermolecular forces within the sample structure. Overall, the DSC results convey diverse thermal properties of the cookie formulations. This is due to the deployed ingredients, their proportions and processing conditions. Among all samples, UMS2 formulation possessed a very good thermal stability and strong intermolecular interactions.

5.7 FTIR Analysis of the PPU-PPE based Optimal Cookies

Fig. 4 depicts the FTIR spectra for various broadband absorption bands being assessed between 3600 and 3000 cm^{-1} and in all cookie formulations samples. The study conveys significant water absorption in the samples. This is consistent with the findings of the prior art (Santos et al., 2021). Peaks at 3360 and 3180 cm^{-1} correspond to N-H valence stretching vibrations from

Table 5.3: Proximate parameter data of unripe papaya pulp and peel powder based alternate optimal cookie formulations

S. No.	Sample	Total Carbohydrate (g/100 g)	Total Protein (g/100 g)	Soluble Protein (g/100 g)	Total Fat (g/100 g)	Ash (g/100 g)	Antioxidant activity (%)	Antioxidant activity (Post in-vitro) (%)
1.	UMS2	21.08±2.4	9.02±0.47	2.3±0.61	13.65±2.64	2.77±0.25	40.99±3.96	13.85±1.44
2.	UMS5	21.56±3.06	10.44±1.88	3.09±.0.36	22.81±3.39	2.02±0.08	41.06±4.02	14.04±1.06
3.	URJ2	17.57±2.9	2.14±0.53	0.85±0.08	11.44±1.6	1.96±0.37	43.84±2.65	15.33±0.96
4.	MS2	22.87±1.05	14.31±0.43	5.66±.0.48	11.43±0.97	2.08±0.5	33.51±3.54	10.38±1.38

Table 5.4: Physical properties data of unripe pulp and peel powder based optimal cookie formulations

S. No.	Sample Code	Wt. (g)	Dia. (mm)	Thickness (mm)	Spread Ratio	Color				Hardness
						b*	a*	Hue Angle	Chroma	(N)
1.	UMS2	28.93 ±0.81	67.68±0.43	5.2±0.12	13.01±0.24	31.39±0.13	18.11±0.25	60.02±0.42	36.24±0.44	27.59±6.9
2.	UMS5	28.26 ±0.56	69.86±0.89	6.1±0.31	11.45±0.83	29.82±0.11	14.29±0.66	64.40±0.37	33.07±0.58	26.27±7.1
3.	URJ2	28.87 ±0.70	71.03±0.82	6.5±0.5	10.93±0.29	29.91±0.0.09	17.71±0.38	59.37±0.25	34.76±0.64	19.91±4.8

cross-linking bridges and hydroxyl [OH] groups. Thereby, they suggest the presence of proteinaceous and polysaccharide components (Cavalcanti et al., 2023). The presence of the C=O functional group, typically associated with lipids, is evident in the spectral region between 1746 and 1720 cm^{-1} and in all formulations. Bands corresponding to peptide linkages, such as amide I (1690 - 1600 cm^{-1}), amide II (1575 - 1480 cm^{-1}), and amide III (1301 - 1229 cm^{-1}), further support the presence of proteins within the cookies (Kong & Yu 2007).

Additionally, bands located at 1200 - 900 cm^{-1} have been attributed to C-O and C-C stretching vibrations, as well as C-O-H and C-O-C deformations of carbohydrates. All these convey good carbohydrate content within the cookie formulations. Thus, the spectral features collectively provide insights into the chemical composition and structural characteristics of the cookies. Accordingly, they highlight the presence of proteins, lipids, and carbohydrates, and their eventual contribution to the overall texture and flavour of the cookies.

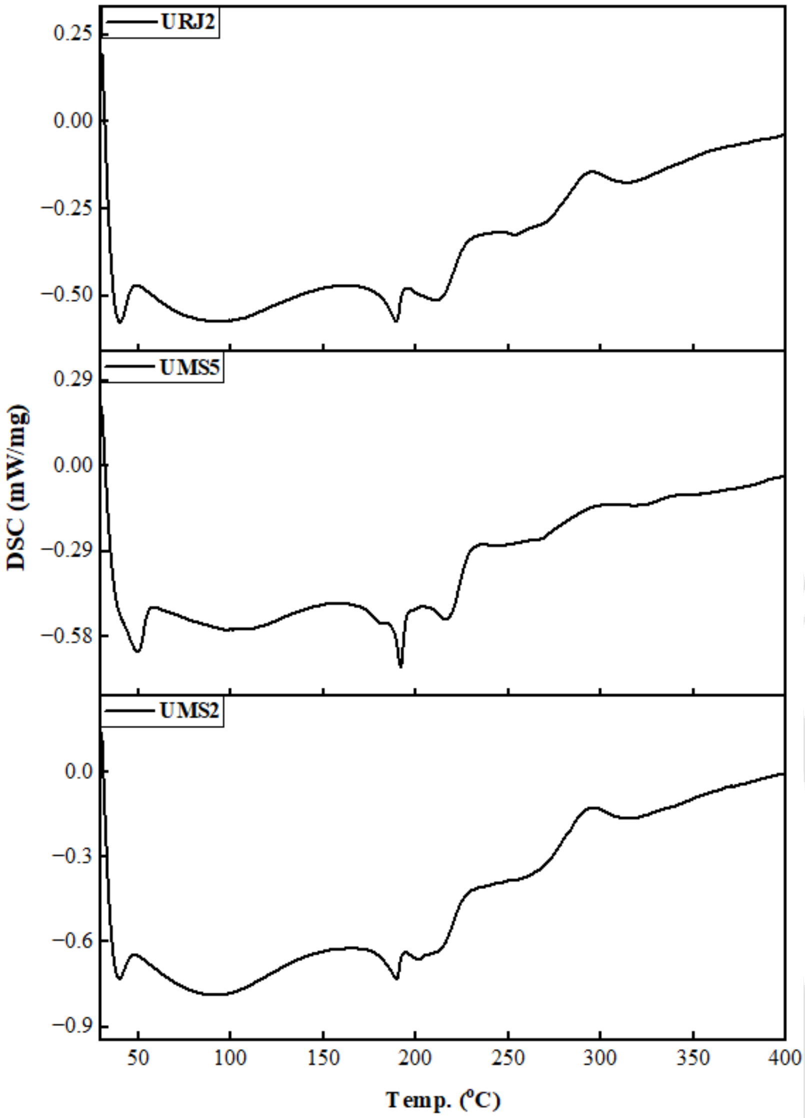


Fig. 5.3: DSC curves of the PPU-PPE-based optimal cookie formulations

5.8 Qualitative Detection of Acrylamide of the PPU-PPE based Optimal Cookies

Acrylamide, an unsaturated amide, is found in various thermally processed foods. It is generated in food products containing high content of reducing sugars such as glucose and proteins that are especially rich in amino acid containing asparagines. Such constituents arise due to the heating at higher temperature (> 170°C). Fourier transform infrared (FT-IR) is a powerful tool for the identification of various functional groups and associated corroboration

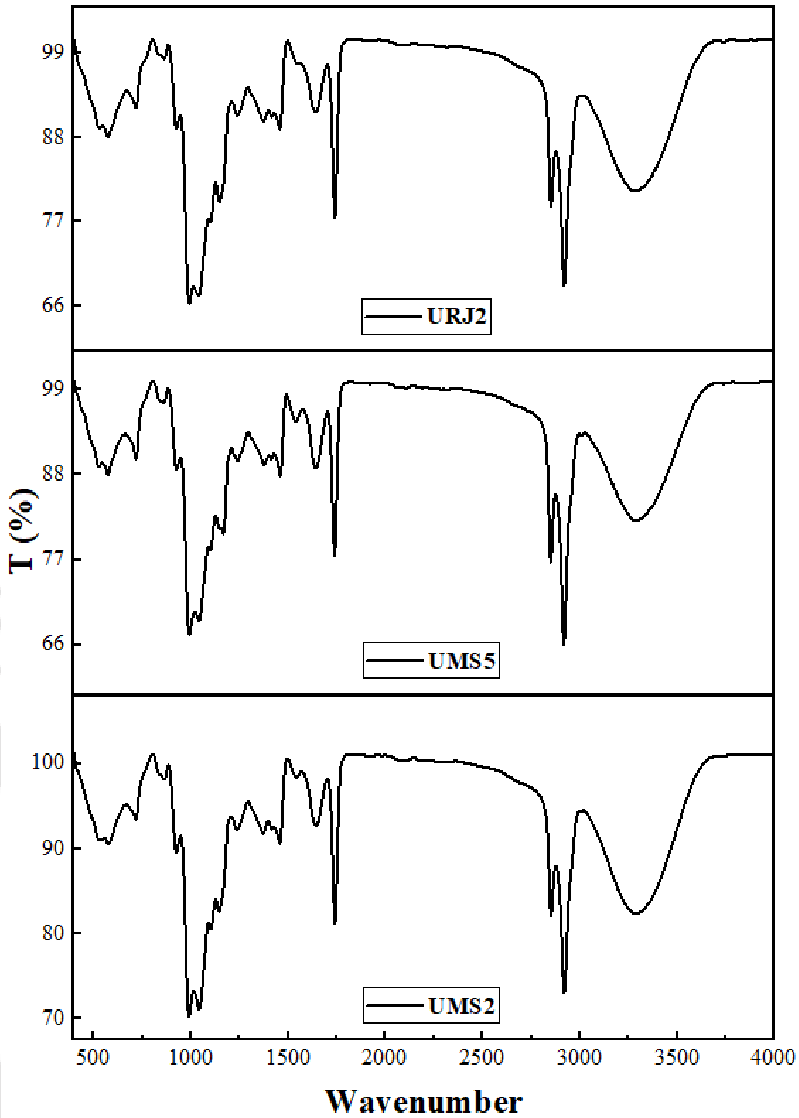


Fig. 5.4: FTIR spectra of the PPU-PPE-based optimal cookie formulations

with the types of chemical bands in a molecule. Thus, the bands can be correlated to the produced infrared absorption spectrum, a molecular "finger-print". Acrylamide formation in food is a complex chemical process. It is influenced with various factors such as temperature, moisture content, pH, and the composition of the food matrix. Therefore, depending upon these factors, the presence and intensity of the relevant peaks in the FTIR spectrum are likely to vary significantly.

In some cases, acrylamide may form in trace amounts. This leads to weaker or lesser prominent peaks in the FTIR spectrum. Additionally, the detection limit of the FTIR instrument and the sensitivity of the method may as well influence the ability to detect lower levels of the acrylamide.

In summary, the presence of peaks associated with the acrylamide in a food sample would strongly suggest its presence. However, the absence of one or more peaks does not necessarily mean that the acrylamide is not present.

While the presence of characteristic peaks associated with acrylamide is a strong indicator of its presence, it's essential to consider other analytical methods and techniques for confirmation. This is especially valid for the cases involving lower concentrations or complex food matrices. Such methods refer to liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), or enzyme-linked immunosorbent assays (ELISA). All these facilitate quantitative analysis and confirm upon the acrylamide presence.

Based on the obtained FTIR peaks, the presence of peaks at 1644 cm^{-1} and 3330 cm^{-1} in the URJ2 suggests that the acrylamide may be existent in the sample. Also, the presence of peaks at 1630 cm^{-1}, 3322 cm^{-1}, and 3325 cm^{-1} suggests that acrylamide may indeed be present in the URJ2 sample. Based on the obtained peaks, UMS2 and UMS5 samples affirm the non-existence of peaks. This conveys that the acrylamide carcinogenic products were not produced in these two formulations and thereby ascertain the promising health related factor of the mentioned samples.

5.9 Crystalline Properties of the PPU-PPE based Optimal Cookies

The relative crystallinity percentages for various dough samples reveal notable differences in their structural organization. The unripe papaya powder-rice-jaggery-butter (URJ2) sample exhibited the lowest crystallinity at 8.03%. This conveys a more disordered or amorphous constitution of the sample. Such a lower value is attributed to several factors. Firstly, the ingredients themselves could contribute to the lower crystallinity. Both rice and unripe papaya powder contain starches, which tend to form amorphous structures upon mixing with other ingredients and water. Additionally, the presence of sugar and butter in these dough formulations could inhibit the formation of crystalline structures. This is due to their interference with the molecular arrangements during dough formation and baking. The samples such as unripe papaya-green moong-sugar-shortening (UMS5) exhibit intermediate levels of crystallinity (32.86%). Such variations are likely to arise due to the differences in ingredient composition, processing methods, and formulation techniques. In summary, the mentioned findings highlight the diverse structural characteristics that are inherent in each dough sample.

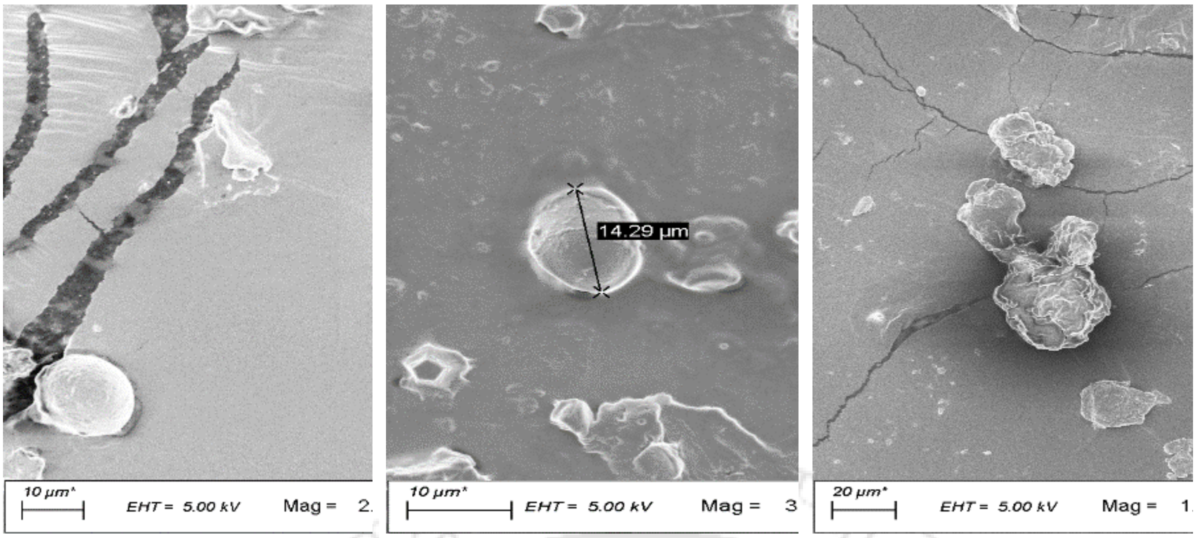
5.10 Morphology and Particle Size Distribution of the PPU-PPE based Optimal Cookies

5.10.1 FESEM Image Analysis

The FESEM (Field Emission Scanning Electron Microscopy) morphology analysis reveals distinct features among various cookie samples and henceforth provided insights into the altered structural characteristics of various samples. The micrographs of wheat based cookies, and unripe PPU and PPE based UMS2 cookies possessed a triangular shaped morphology. This is possibly due to the unique composition of unripe papaya and green moong. The UMS5 has shares similar structural characteristics to those assessed for the best analyzed UMS2 cookies.

5.10.2 Particle Size Distribution

The results from the dynamic light scattering (DLS) analysis reveal variations in the particle size distribution and average diameter of various cookie formulations. Such differences are attributed to the variations in ingredient composition and processing methods. While URJ2 has the lowest PdI of 0.33, the UMS5 has the highest PdI of 0.54. Further, while UMS2 has the lowest average particle size of 1256 nm, the UMS5 has the highest value of 1666 nm.



(a) (b) (c)

Fig. 5.5: FESEM micrographs of (a) UMS2, (b) UMS5 and (c) URJ2 samples

Formulations with larger average diameters may indicate the presence of larger aggregates or particles. These potentially result from the interaction between ingredients during mixing or baking. Conversely, smaller average diameters suggest finer particle sizes or more uniform dispersion of ingredients. The polydispersity index (PdI) provides an insight into the uniformity of particle sizes within each sample (values closer to zero indicating a narrower size distribution). Overall, these findings highlight the correlation of particle size characterization with the physical properties and potential sensory attributes of the cookies.

5.11 Storage Study of the PPU-PPE based Optimal Cookies

Moisture and water activity play an important role in the storage of cookies. From 0^{th} day to 60^{th} day, a minor enhancement in the moisture content of cookies has been apparent (Table 5.6). This could be due to the general hygroscopic nature of the packed cookies. This nature of packaging material and its porosity as well have an important role to decide upon the moisture uptake. Rao et al., (1995) reported that the stored biscuits packed in metalized polyester or biaxially oriented poly propylene exhibit higher moisture content than those packed in paper-aluminium foil polyethylene laminate pouches. Cookies packed in laminate pouches absorbed lesser moisture during storage. This could be due to the impervious nature of aluminium foil in the laminate to air and water vapour. Similar results affirming increased moisture content of cereal bran incorporated biscuits at the end of 60 days of storage were reported by (Nagi et al., 2012) .

A corresponding increase in Aw value in both cookie samples was also observed. Sixty days of storage in controlled conditions induced marginally altered sensory properties in the cookies. For the case, the panellists reported a reduced hardness of the cookies. This is confirmed as well with the instrumental analysis (Table 5.6). A reduced breaking strength, being measured with the three-point peak force or hardness tests, is usually reported for stored cookies, and for cases with or without fat replacement (Forker et al., 2012). In all cookie formulations, no rancid off-flavour was reported by the panellists and for up to 60 days of storage.

5.11.1 Microbial Analysis

Food-borne illness is caused by microorganisms. The human consumption necessitates upon their absence in the food products. The microbial count for total plate count, yeast and mould, *E. coli, S. aureus, B. cereus* and *salmonella* was undetected for all tested cookie formulations. This suggests that the microbial quality of cookies during a storage period of 60 days is good. All the best rated cookie formulations (such as UMS2, UMS5 and URJ2 formulations) had nil plate counts (CFU/mL) and yeast and mould counts after processing. Agu and Okoli (2014) found similar results for beniseed and unripe plantin enriched wheat biscuits. After 30 days, the PPU-PPE based cookies had TC count altering from 0.5 – 0.8 CFU/mL. However, total plate count affirmed no YMC growth. After 60 days, unripe papaya powder based cookies were detected with total plate count as 2.1 (UMS2), 1.8 (UMS5) and 2.6 (URJ2) CFU/mL.

5.11.2 Fatty Acid Profile During Storage by ^{1}H-NMR Spectra

The ^{1}H-NMR spectra of the fat extracted from the PPU-PPE-based gluten-free cookies were used to determine their fatty acid profiles. Figure 5.6 illustrates the ^{1}H-NMR spectra of the fat extracted from the PPU-PPE based optimal cookie formulation UMS2 being assessed in the storage study for the 0th and 60th day cases of the prepared cookies. For UMS2, UMS5 and URJ2 cookie formulations, the stability of fatty acids in cookies during storage were also assessed with the proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. The graphical representations have been presented in Appendix D of the Ph.D. thesis. Such research studies aim to identify changes in the fatty acid profile with storage conditions. Accordingly, insights could be gained into the optimal practices that shall be followed to maintain cookie quality and extend shelf life. The ^{1}H NMR spectrum of the cookies conveys a mixture of saturated and unsaturated fatty acids. Strong peaks in the range of 0.8-1.2 ppm suggest the significant presence of saturated fatty acids with terminal methyl groups. Peaks in the range of 1.2-2.5 ppm affirm various methylene environments within the fatty acid chains.

The presence of peaks in the followed region (2.5-3.5 ppm) suggests methylene groups that are adjacent to unsaturated bonds or other functional groups. The presence of peaks in 4-6 ppm range affirmed unsaturated fatty acids in the sample. Peaks in the region of 9-10 ppm, are small but convey oxidative degradation that lead to aldehyde formation in the UMS2 sample. These peaks were absent in the other two formulations. The spectrum indicates both saturated and unsaturated fatty acids in the cookies. While saturated fatty acids are more stable, the

Table 5.5: Particle size distribution data of unripe pulp and peel powder based optimal cookie formulations

S. No.	Sample name	PdI	Average diameter (nm)
1.	URJ2	0.33±0.16	1480 ± 20.45
2.	UMS2	0.40±0.20	1256 ± 23.32
3.	UMS5	0.54±0.18	1666 ± 8.99

unsaturated fatty acids are prone to oxidation. The presence of peaks in the 0.8-1.2 ppm and 1.2-2.5 ppm range suggests saturated fatty acids. However, peaks in 4-6 ppm range confirm unsaturated fatty acids. Even though UMS2 exhibited marginal aldehyde formation at the 0^{th} day, it didn't alter after 60^{th} day of storage. The consistent presence of these peaks suggests that the fatty acid profile includes a mix of saturated and unsaturated fatty acids, and with desired stability in the chemical structure for a period of 60 days. Similar findings can be analyzed from the graphical representations presented in Appendix D for all other optimally reported PPU-PPE based cookie formulations.

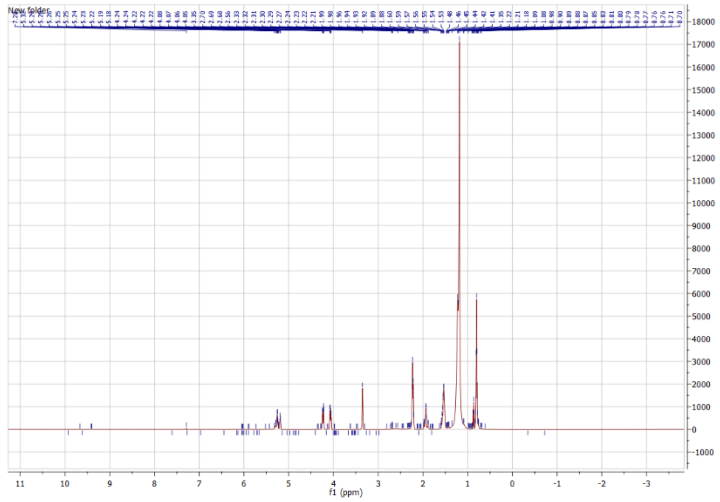
5.12 Comparison of PPU-PPE Best Rated Cookies with Commercially Available Papaya Cookies

In a comparative study of papaya cookies from the "Green Care" brand and the UMS2 cookie formulation, significant differences in their nutritional and mineral profiles were observed. The papaya cookies, containing ingredients such as naturally grown papaya, whole wheat flour, raw sugar, and A-2 ghee, provide 477.55 calories per 100 g. They are rich in carbohydrates (74.33%), and had a notable sugar content (24.68%), and also contained 4.67% protein, 1.87% moisture, 1.18% ash, 1.21% crude fiber, and 17.95% fat. In contrast, the UMS2 cookies offer a more balanced nutritional profile with 21.08 g/100 g total carbohydrates, 9.02 g/100 g total protein, 2.3 g/100 g soluble protein, 13.65 g/100 g total fat, 2.77 g/100 g ash, 40.99% antioxidant activity, and 13.85% antioxidant activity (post in-vitro). The mineral content of UMS2 cookies affirms 7.12±0.57 ppm Mg, 64.22±2.61 ppm K, 1.37±0.93 ppm Ca, 0.05±0.03 ppm Mn, 0.32±0.05 ppm Fe, 0.03±0.04 ppm Cu, and 0.11±0.06 ppm Zn. While the commercial papaya cookies are higher in carbohydrates and fats and thereby provided substantial energy, the UMS2 cookies deliver a significant amount of protein and essential minerals and notable antioxidant constituents. Accordingly, they are more fruitful and potential healthier alternative. The choice between these cookies can be influenced by specific dietary goals, such as the need for higher protein versus higher carbohydrate intake, with UMS2 cookies also offering enhanced health benefits through their mineral content and antioxidant activity. Considering the recommended daily allowances (RDA), the UMS2 cookies align better with dietary recommendations and offer a higher contribution to protein and essential minerals and without excessive calories or sugars. Therefore, based on the RDA, the UMS2 cookies can be considered as a healthier and enriched nutrient diet alternative.

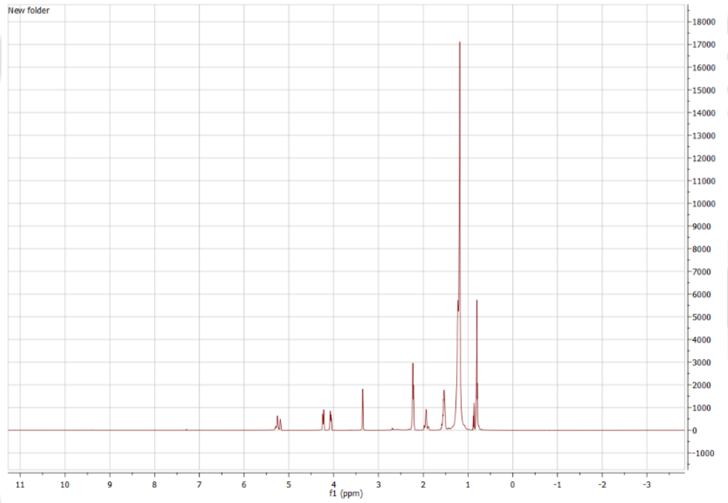
5.13 Literature Comparison

Table 5.8 presents a comparative analysis of various cookie formulations that incorporated ripened papaya pulp flour (PPuF). Considering proximate composition, bioactive components, and antioxidant activity being reported in various studies, the best formulation UMS2 has been comparatively assessed with the literature reported findings. These have been summarized in Table 5.6. A research group (Varastegani et al., 2015) explored cookies made with wheat flour and with 15%, 30%, and 50% ripened PPuF. The cookies with 30% ripened PPuF had an ash content of 3.3%, protein at 3.48%, and crude fiber at 2.48%. However, those with 50% PPuF had a crude fiber content of 3.13%, a carbohydrate content at 59.98%, and a fat content at 22.78%. The DPPH method based antioxidant activity for the 50% PPuF formulation was 9.12%. In comparison, the UMS2 cookies, which incorporated unripe papaya powder along with green moong, sugar, and butter, had a total carbohydrate content of 21.08±2.4 g/100 g, a higher protein content at 9.02±0.47 g/100 g, and fat at 13.65 g/100 g.

The UMS2 formulation exhibited a significantly higher antioxidant activity of 40.99%, and a notable 13.85% post in-vitro digestion. Pathak et al., (2018) studied cookies containing 10% ripened PPuF. These cookies exhibited a higher protein content of 6.96 g/100 g and significant sodium levels of 351.42 mg/100 g.Their bioactives content was assessed as the total phenolic content (TpC) of 1.705 µg GAE/mg sample and a total flavonoid content (TfC) of 1.017 µg



(a)



(b)

Fig. 5.6: [1]H-NMR Spectra of fat extracted from the UMS2 cookie formulation (a) for 0th day and (b) 60th day storage period cases

QE/mg sample. Comparatively, UMS2 cookies had a higher protein content of 9.02±0.47 g/100 g and showcased a more substantial antioxidant activity of 40.99%. All these assessments highlighted their superior bioactive potential with respect to the 10% ripened PPuF based cookie formulations.

Cookies with variant ratios of wheat to papaya peel flour (90:10, 92.5:7.5, and 95:5) were examined, and were reported by another research group (Bokaria & Ray, 2016). While the

90:10 ratio cookies had an ash content of 3.8%, protein at 8.14%, and total carbohydrate content of 63.5%, the 95:5 ratio had an ash content of 4.5%. The protein content for all formulations altered from 7.8 to 9 g/100 g. In comparison to the UMS5 formulation, the UMS2 cookies had a comparable protein content of 9.02±0.47 g/100 g but lower total carbohydrate content of 21.08±2.4 g/100 g. Additionally, the antioxidant activity of UMS2 cookies was significantly higher at 40.99%. This demonstrated the enhanced bioactive benefits of the UMS2 formulation. The UMS2 cookies in the Ph.D. thesis being formulated with unripe papaya powder, green moong, sugar, and butter, exhibited a comprehensive nutritional profile with 21.08±2.4 g/100 g of total carbohydrates, 9.02±0.47 g/100 g of protein, and 13.65 g/100 g of fat. The samples' antioxidant activity was notably high at 40.99% and was significant with 13.85% after in-vitro digestion. These characteristics indicate that the UMS2 cookies not only provide a balanced nutritional composition but also offer superior bioactive benefits in comparison to the other best formulations being summarized in the table.

5.14 Summary

Among the unripe papaya pulp and peel powder based cookie formulations, the sample UMS2 achieved the highest sensory analysis based fuzzy score of 0.49 ("excellent" category). The sample also achieved best sensory score as per the Hedonic scale based sensory analysis methodology. The powders used in UMS2 also affirmed the lowest average diameter of 1256 nm. Fourier-transform infrared spectroscopy (FTIR) analysis confirmed that there were no peaks related to acrylamide content in the UMS2 sample. This ensured its safety and quality. Additionally, UMS2 cookies demonstrated a balanced nutritional profile with significant antioxidant activity. This rendered them to be a superior choice for health-conscious consumers.

Table 5.6: A summary of storage study parameters data of unripe pulp and peel powder based optimal cookie formulations

S. No.	Cookie formulation	Storage parameters								
		0th			30th			60th		
		Mc	**Aw**	**Hardness**	**Mc**	**Aw**	**Hardness**	**Mc**	**Aw**	**Hardness**
1.	UMS2	4.8±0.58	0.48±0.18	27.59±6.93	4.92±0.61	0.50±0.2	28.58±3.95	5.25±0.96	0.49±0.24	27.07±1.33
2.	UMS5	4.74±0.62	0.48±0.09	26.27±7.04	4.8±1.06	0.49±0.07	27.63±3.77	5.16±2.69	0.48±0.27	25.49±5.06
3.	URJ2	4.38±0.57	0.47±0.08	19.91±54.08	4.71±1.9	0.48±0.08	21.03±10.43	5.06±0.66	0.48±0.07	20.9±1.81

Table 5.7: Microbial analysis data of unripe pulp and peel powder based optimal cookie formulations

S. No.	Cookie formulation	Microbial count					
		0th		30th		60th	
		TC	**YMC**	**TC**	**YMC**	**TC**	**YMC**
1.	UMS2	ND	ND	0.5±0.06	ND	2.1±0.06	ND
2.	UMS5	ND	ND	0.8±0.06	ND	1.8±0.09	ND
3.	URJ2	ND	ND	0.71±0.09	ND	2.6±0.66	ND

Table 5.8: A summary of best reported data of unripe papaya powder based cookies formulations

S. No.	Cookie Formulation	Proximate Composition	Bioactive Components	References
1.	UMS2	Total Carbohydrate (g/100 g:21.08±2.4; Total Protein(g/100 g):9.02±0.47; Total Fat (g/100 g): 13.65	AA:40.99%; 13.85% (post in-vitro)	This work
2.	Wheat flour; 15%, 30% and 50 % Ripe papaya pulp flour(PPuF)	30PPuF Ash:3.3%; Protein:3.48%; Crude fiber:2.48% 50PPuF-Crude fiber:3.13%, Carbohydrate: 59.98%; Fat: 22.78%	50PPuF: Antioxidant activity-9.12% (DPPH)	Varastegani et al., 2015
3.	10% Ripe Papaya Pulp Flour	Protein:6.96 g/100g Sodium (Na): 351.42mg/100gm	TpC:1.705μg GAE/mg sample TfC:1.017μg CE/ mg sample	Pathak et al., 2018
4.	90:10; 92.5:7. 5; 95:5: wheat: papaya peel flour	90:10; Ash-3.8%; Protein- 8.14%; Total carbohydrate-63.5%;95:5: Ash-4.5%;	90:10-7.8(gm/100 gm); 92.5:7. 5-8.4 (gm/100 gm); 95:5-9gm/100 gm	Bokaria & Ray 2018

In contrast, the UMS5 sample demonstrated the good retention of antioxidant activity (AA) at 14.04%. This affirmed its high potential for the preservation of vitamin C content. UMS5 also had the highest potassium (K) content at 67.37 ppm. This is beneficial for the maintenance of electrolyte balance. The sample had a moderate calcium (Ca) content of 1.89 ppm. Furthermore, UMS5 exhibited the highest polydispersity index (PdI) of 0.54. This confirmed a wider distribution of particle sizes, and an average diameter of 1666 nm. All these corroborate and reflect upon its unique textural properties. The URJ2 sample, meanwhile, exhibited the lowest PdI of 0.33 and accordingly suggested a more uniform particle size distribution. The URJ2 also contained significant mineral content, in terms of Mg (9.24 ppm), K (76.25 ppm), and Ca (3.38 ppm).

While UMS2 has been the best, UMS5 and URJ2 highlighted varied strengths in nutrient retention and mineral content. Thus, the UMS5 excelled in vitamin retention and potassium content, and URJ2 provides a rich source of essential minerals. Collectively, these findings underscore the potential of unripe papaya pulp and peel powder in substitution to achieve nutritious and appealing functional foods.

Chapter 6:

Enhancing Functional Attributes of Gluten-free Cookies through Innovative Encapsulation of Catechin and Papaya Leaf Extracts

Chapter 6

Enhancing Functional Attributes of Gluten-free Cookies through Innovative Encapsulation of Catechin and Papaya Leaf Extracts

In this chapter, the fortification of cookies with two alternate encapsulants has been targeted and assessed. Thereby, two alternate types of fortified cookie products were considered. These were (a) rice cookies fortified with calcium alginate-pectin encapsulated papaya leaf extract encapsulant being achieved with the ion gelation method), and (b) best gluten-free cookies fortified with maltodextrin encapsulated catechins extract of the green tea (encapsulant achieved through the inclusion complexation method and after conducting a comparative assessment with the ion gelation method). Thereby, the fortification efficacy was assessed in terms of the bioactives constitution. Along with this important characteristic parameter, other characterization was as well considered and assessed. Accordingly, the chapter has been arranged as follows. After a brief introduction in section 6.1, section 6.2 delineates upon the fortification efficacy of rice cookies with sodium alginate encapsulated papaya leaf extract. The section addresses characterization findings in terms of sensory attributes, proximate characteristics, morphological characteristics, thermal properties, and bio-actives constitution of the cookies. Among these, the bio-actives constitution was considered prior to and after baking condition of the cookies and in terms of the in-vitro digestion based assessment of the TpC, TfC and AA. Thereafter, in section 6.3, the efficacy of green tea extract based catechins encapsulation in maltodextrin wall material through the inclusion complexation and ion gelation methods has been discussed in terms of the relevant findings *summarized as encapsulation efficiency, yield, particle size and morphology, FTIR analyses, thermal properties and XRD analyses. Subsequently, in section 6.4, the fortification of two alternate cookies being identified as the best in the previous chapters of the PhD thesis was carried out with the IC based encapsulated catechin extract of the green tea leaves. Thereby, characterization of the cookies was considered in terms of the bio-accessible total antioxidants activity content. Finally, section 6.5 presents a summary of the findings of the chapter.*

Overview

Considering the fact that the catechins extracted from green tea have been not studied for their encapsulation efficacy with ion gelation and inclusion complexation methods, the chapter initially delineated towards the identification of the best encapsulation method in the considered methods. Thereby, the cookies fortification was addressed and assessed for two alternate type of fortifications (hot water extract of the papaya leaf and catechins extract) in the prepared cookies. Also, diverse characteristic profiles and enhanced nutritional content for the cookies were targeted and assessed to meet the variegated dietary preferences. Accordingly, the detailed bioactivity and bioaccessibility of the gluten free rice and legume based cookies was primarily targeted. Henceforth, MS2 and UMS2 cookie formulations being identified in the previous chapters as the best cookies were fortified with encapsulated catechins extract for their subsequent in-vitro digestion based bio-accessible content constitution. These findings were as well corroborated with the findings from other characterization findings such as FESEM, FTIR, XRD, particle size distribution and DSC analyses. The literature data comparison was as well addressed in a dedicated sub-section for the affirming of the subjective and objective novelty aspects of the conducted thesis research works.

6.1 Introduction

To meet the ever increasing consumer demand for healthier products, a contemporary issue being faced by the food industry is with respect to the inclusion of sustainable and functionally beneficial bioactive constituents in the conventional foods. Among many such viable compounds, natural polyphenols constitute an important class. This is due to their abundant existence in all plant parts. Thereby, they became an essential element of human diet for the prevention of wide variety of adequate nutrition-based diseases. These bioactive compounds possess anti-inflammatory, antitumor, anticancer, antibacterial and antidiabetic characteristics.

Catechins, abundant in green tea, have emerged as promising compounds in the realm of flavonoids. Thereby, they contribute contributing to human health and through the primary aspect of disease prevention. The notable catechins found in green tea, such as (-)-epigallocatechin-3-gallate (EGCG) and (-)-epicatechin-3-gallate (ECG), exhibit remarkable biological and chemical characteristics. Among the leafy category, aqueous papaya leaf (PL) extracts have been reported to contribute towards the prevention and treatment of diabetes, malaria, cardiovascular and many other chronic diseases. The utilization of catechins, particularly from green tea, has garnered significant attention. This is primarily due to their potent antioxidant properties. While green tea has historically been appreciated for its beverage application, recent endeavours aim to integrate catechin-rich extracts into a diverse array of food products, extending from bread (Goh et al., 2015) to yoghurt (Agarwal et al., 2022) and with enhanced shelf life and product quality.

The incorporation of bioactive extracts into polymer matrices is often targeted for their stability and bioavailability. Thereby, it is a well-known and proven method to custom serve the health benefits associated to the bioactives. However, given their limited availability, the challenge lies in harnessing the full potential of catechins. Encapsulation is well known to be an expensive process as it involves the utilization of expensive chemicals and drying systems. Very few literatures addressed cost effective chemical free/minimal chemical-based processes for the encapsulation of catechin extracts. Encapsulation emerges as a pivotal solution and thereby facilitates the sustainable and controlled release of bioactives into desired environments. Methods such as inclusion complexation (IC) and ion gelation (IG) offer efficient means to encapsulate catechins, and as well ensure their stability and efficacy in food formulations. Among many wall materials, maltodextrins, amylose, amylopectin, dextrins, polydextrose, Na-alginate are common starch sources and starch derivative compounds for the needful. In the food industry, maltodextrin is one of the most widely utilized wall materials for the encapsulation of functional food constituents. Due to its non-toxicity and biocompatibility, alginate, a representative natural polysaccharide that forms strong gel complexes in the presence of Ca^{2+} ions, has been claimed to be acceptable for food applications (Narsaiah et al., 2019).

Prior to the conduct of the mentioned research activity, the following shall be noted with respect to the extent of the subjective findings mentioned in the prior art. In a detailed study conducted by our research group, calcium alginate-pectin beads were prepared with sodium alginate and pectin as wall materials and papaya leaf extract (ALEXPB) as an active agent was successfully encapsulated with the ion gelation method (Wani & Uppaluri, 2023). The authors addressed process parametric optimization in terms of the bio-actives encapsulation efficiency. In other words, further research shall not be devoted towards the ascertaining of the encapsulation efficiency of the bio-actives of the papaya leaf extract system. On the contrary, research efforts to encapsulate green tea extract based catechins in the maltodextrin wall material have not been addressed in a comparative framework for the alternate well known methods namely ion gelation and inclusion complexation method. Henceforth, the Ph.D. thesis shall at first ascertain upon the efficacy of these two methods and thereby identify the best method for the cookie fortification studies. Since encapsulation, characterization including thermal, physical, crystalline properties were addressed for the papaya leaf extract in the mentioned publication, these will not be addressed in the Ph.D. thesis. However, this is not the case for the catechins encapsulation. The green tea extract based catechins encapsulation will be as follows (mentioned in section 2.4 of the Ph.D. thesis). Firstly, green tea being available in the dried form in the market is first subjected to hot water extraction for the realization of the aqueous catechin extract. Thereafter, the aqueous green tea extraction was concentrated with rotary evaporator and then with the oven drying for the realization of the dried form of the extract. Using the dried catechins extract sample, inclusion complexation method was followed to encapsulate catechins in maltodextrin.

The definitive utility of the mentioned wall materials for the retention of bioactive constituents even after baking is the subject of primary interest of this chapter. Accordingly, the sustainable and controlled released of bioactives in the human digestion environment is sought. As a supplementary cause, the morphological, thermal, and crystalline characteristics of the fortified cookies is as addressed.

Ongoing research trends convey the successful fortification of catechins extracts in food products such as bread, cereals, cakes, biscuits, dairy goods, noodles, instant noodles, candy, ice cream, and fried snacks (Bora et al., 2018; Vuong et al., 2011). Compared to other bakery products, cookies are always preferred as a wholesome food. This is due to their promising attributes such as portability, variety in formulation, and low moisture content. Cookies primary consist sugar, fat, and flour. *Triticum aestivum*, the crop being used to prepare wheat flour has a long human consumption history and is a main crop in temperate regions. Essential amino acids, minerals, healthy phytochemicals, and dietary fiber are all provided by wheat. Further, whole wheat meal flour is especially enriched with these nutrients. The gluten-protein fraction of wheat is primarily responsible for the realization of a viscoelastic dough for the preparation of high quality bread, pasta, and other food products (Shewry, 2009). Celiac disease patients could not tolerate the protein fraction due to health restrictions. As an alternative, they wish to consume products with minimal or no gluten protein content. Due to their high nutritional value and the dietary requirements of a sizable portion of the human population, alternate grains are becoming more and more popular for their utility in the production of cereal-based foods for celiac disease patients. Rice flour (*Oryza sativa*) is a very important gluten-free flour for such applications. Thus, the gluten-free flour in cookies formulations aim to replace wheat with rice and thereby achieve products with good and acceptable sensory characters. In this regard, a variety of factors influence the physicochemical characteristics of rice flour. Grain legumes play an important role in human nutrition, especially in the dietary pattern of low-income groups in developing and underdeveloped countries. Hence, supplementation with legumes is an excellent vehicle for the ascertaining of adequate protein content in baked foods such as biscuits, cookies, and cakes. These are widely consumed due to their long shelf life and for their good eating quality (Akubor, 2003; Hooda & Jood, 2005). Legumes are rich in proteins, carbohydrates, many water-soluble vitamins, especially vitamin B complex, and minerals such as calcium and iron (Sreerama et al., 2012). *Cicer arietinum* (Chickpea), also known as Bengal gram, is one of the earliest cultivated legumes of the family *Fabaceae* and is an excellent source of the essential nutrients, viz. proteins, iron, folate, phosphorous, and dietary fiber (Kumari et al., 2017). *V. radiate* (Moong bean) is native to the Indian subcontinent. Further, to either enrich or sustain losses incurred during constitution, processing and handling, foods are often fortified to enhance their nutritional value or makeup for the losses. For such a purpose, polyphenols serve as excellent food additives to promptly satisfy dietary requirements.

Drawing inspiration from previous research findings, the encapsulated extracts are integrated into rice-based gluten-free cookies, and thereby augment their functional properties.

Furthermore, referring to the reported findings in the earlier chapters of the Ph.D. thesis, formulations such as MS2 (chapter 4) and UMS2 (chapter 5) being optimized for sensory attributes can serve as ideal candidates for the fortification with encapsulated catechins. The encapsulation process not only enhances the stability and bioavailability of catechins but also opens avenues for the development of nutritionally rich baked goods. Through such cookies fortification with the encapsulated catechins, the conducted study in the Ph.D. thesis aims to evaluate the retention efficacy of bioactive constituents after baking and in terms of in-vitro digestion based bio-accessible content. These endeavours underscore the potential of encapsulation in enhancing antioxidant activity and bioavailability and thereby ascertains the generation of healthier and further functionalized food products.

6.2 Cookies Fortified with Na-Alginate Encapsulated Papaya Leaf Extract

6.2.1 Background

To enhance the nutritional profile of cookies and mitigate nutrient losses during processing, fortification becomes imperative. Polyphenols, known for their nutritional benefits, are utilized as additives. However, their susceptibility to degradation during baking necessitates encapsulation. Papaya leaf extract (PLE), rich in diverse bioactive compounds, offers promising health benefits. However, its utilization is limited due to sensitivity to light, heat, and enzymes. Encapsulation, particularly ion gelation-based encapsulation onto sodium alginate pectin beads, offers a solution to these challenges. This is highlighted in a previous study based our research group. In the previously conducted research work (Wani & Uppaluri, 2023), the ion gelation technique employing FCD-based Response Surface Methodology (RSM) was effectively utilized to encapsulate TpC (total polyphenol) molecules from papaya leaf (PLE) extract within Ca-Al beads loaded with pectin. Optimal conditions for the realization of maximum encapsulation efficiency were determined as 2% w/v sodium alginate concentration, 6.61% w/v $CaCl_2$ concentration, and a syringe pump flow rate of 3.50 mL/min.

Under these optimized conditions, the encapsulation efficiency of pectin-loaded beads reached 86.7%.

The primary objective of the conducted work is to investigate the efficacy of encapsulation in preserving the bioactive integrity of PLE and to affirm upon the nutritional quality of cookies. Through comprehensive analyses of antioxidant properties, sensory attributes, and characterization via differential scanning calorimetry (DSC) and field emission scanning electron microscopy (FESEM), the study aims to elucidate upon the sensitive influence of encapsulation on the cookies' properties.

6.2.2 Rice Cookies Formulation and Fortification by ALEXPB

Table 6.1 outlines the basic formulation of the cookie dough. This was established based on previously conducted studies. Other ingredients constituents is similar to that presented in 6.1 of the Ph.D. thesis. ALEXPB powder was incorporated into the rice flour and at variant concentrations (0%, 0.4%, and 1% by weight). Subsequently, the dough was gently kneaded for approximately 15 minutes. This ensured uniform consistency. The dough was then flattened to form round-shaped cookies with a thickness of 1 cm and a diameter of 60 mm. Baking was carried out in an electric oven at 150°C for 25 minutes (Fig. 6.1). Prior to baking, the cookies were chilled in the refrigerator at 6°C for 10 minutes. Replicated samples were prepared with various batches of the dough. Freshly baked cookies were tested and analyzed on the same day.

6.2.3 Characterization of ALEXPB Fortified Rice Cookies

6.2.3.1 Sensory Attributes of Cookies

The sensory evaluation by the respective panel convey that excepting overall acceptability, no differences were found in all other sensory attributes for all three types of cookies (control, 0.4%, and 1% ALEXPB) fortified cookies. Flour blends containing 0.4% and 1% ALEXPB

Control Cookies 0.4% ALEXPB Cookies 1% ALEXPB Cookies

Fig. 6.1: Images of (a) Control Cookies, (b) 0.4% ALEXPB and (c) 1% fortified cookies

Table 6.1: Constituents used for the preparation of cookies

Constituents	**Control**	**B_1**	**B_2**
Rice flour (g)	20	19.6	19
Bengal gram flour (g)	10	10	10
Sugar (g)	12.5	12.5	12.5
Shortening (g)	22.5	22.5	22.5
Baking powder (g)	1	1	1
Vanilla essence (mL)	1	1	1
Milk (mL)	5		

**B1 and B2 correspond for 0.4% and 1% respectively of the ALEXPB-fortified cookies*

powder produced cookies with significantly lower scores for all sensory attributes. Consumer panel sensory scores of cookie color, crunchiness, and breakability were not significantly different from those obtained for the control case (Table 6.2). Consumer acceptance regarding appearance and overall acceptability were significantly ($p < 0.05$) higher for cookies prepared with 1% ALEXPB fortification. Apart from the appropriate raw material being used for the cookies preparation, such data provides information with respect to the formulation and quality of the products. In summary, in comparison with the control cookies, the highest sensory score was achieved for cookies prepared with ALEXPB fortification.

6.2.3.2 Proximate Composition of Control and ALEXPB Fortified Cookies

The proximate parameters (for both control and ALEXPB-fortified cookie samples) have been presented in Table 6.3. The control samples exhibited a protein content of 15.93 g/100g and an ash content of 1.27g/100g. Upon fortification with the ALEXPB, the flour blend in the cookies ascertained enhanced levels of ash and fiber protein. Such an increase in the properties can be attributed to the higher concentration of these nutrients in the ALEXPB powder. Further, while the control sample had a comparable fat content, it exhibited a marginally higher protein content.

Table 6.2: Sensory attributes of cookies by 9-point Hedonic scale

Sample type	Taste	Flavour	Crunchiness	Breakability	Color	Appearance	Aftertaste	Overall acceptability
Control cookie	7.5±0.60	7.6±0.75	7.9±0.46	8.06±0.76	7.4±0.67	7.5±0.5	7.8±0.86	7.9±0.46
0.4% ALEXPB	7.7±0.73	7.8±0.66	8±0.51	8.1±0.48	7.6±0.81	7.7±0.73	7.6±0.42	8.2±1.05
1% ALEXPB	7.8±0.88	7.7±0.25	7.9±1.04	8.2±1.4	7.6±0.39	7.9±0.64	7.5±0.30	8.3±0.93

Table 6.3: Proximate composition of control and ALEXPB fortified cookies

Samples	Moisture (g/100g)	Ash (g/100g)	Fat (g/100g)	Total protein content (g/100g)	Soluble protein
Control cookies	3.48±0.88	1.27±0.11	25.21±0.33	15.93±0.31	5.85±1.31
0.4% cookies	3.29±0.59	1.31±0.43	25.26±0.29	15.48±0.34	5.78±0.89
1% cookies	2.82±0.91	1.39±0.55	25.54±0.48	15.18±0.37	5.21±2.07

6.2.3.3 Bioactive Constituents in the Cookies

In terms of bioactive constituents, dried ALEXPB affirmed good TpC, TfC, and AA values of 302.58 mg/g GAE, 280 mg/g GAE and respectively (Table 6.4). Since the dough contains moisture content for all three alternate samples, the TpC and AA values are quite similar for control, 0.4%, and 1% fortified cookies. The TpC values for cookies (control, 0.4%, and 1%) have been assessed as 21.65, 24.74, and 28.54 mg/ g GAE respectively. The dough for control cookies possesses antioxidant activity of 6.04%. However, for the 0.4% and 1% fortified dough samples, the AA enhanced to 11.42 and 16.37% respectively. While TfC value was 155 mg/g Quercetin for the control dough, 0.4% fortified dough possessed even higher value of 185 mg/g Quercetin. Further, 1% fortified dough affirmed a TpC value of 217 mg/g Quercetin. Similarly, for the control cookies case, 11.65 mg/ g GAE and 15.71% of AA has been apparent. For the case, the TfC reduced to 49 mg/g Quercetin after baking. However, the incorporation of ALEXPB enhanced the TpC to 30.62 mg/ g GAE and 49.48 mg/ g GAE for the 0.4 and 1% fortified cookies respectively. Despite achieving reduced TpC after baking in the control case, 0.4 and 1% ALEXPB-fortified cookies respectively exhibited higher values such as 72 mg/g Quercetin and 92 mg/g Quercetin. Antioxidants do significantly exist in the 1% cookies (31.86%) in comparison to the control cookies (15.71%) and the 0.4% (18.52%) cookies case. Imeneo et al. (2020) prepared biscuits with lemon by-products and achieved a TpC of 0.59 mg GAE g^{-1} d.w. in the lemon pomace extract; 12 and 15 mg GAE g^{-1} d.w., respectively in the dough and in the biscuits control sample (without lemon by-products); 28 and 30 mg GAE g^{-1} d.w. respectively in the dough and biscuits prepared with fresh lemon peel; 29 and 32 mg GAE g^{-1} d.w. respectively in the dough and biscuits prepared with fresh lemon peel and lemon pomace extracts; 28 and 34 mg GAE g^{-1} d.w., respectively, for dough and biscuits prepared with lemon pomace extracts. In this article, it was also reported that the antioxidant activity (measured with the DPPH assay) was influenced with various lemon by-products. Henceforth, the DPPH values were higher for the control cookies in comparison to the values obtained for the fortified cookies. After in-vitro digestion, the TpC of all cookies reduced significantly and were achieved as 2.25 mg/g GAE, 4.21, and 6.55 mg/ g GAE for control, 0.4%, and 1% fortified cookies respectively. However, the TfC values were stable and remained similar for all cookie samples. The corresponding values are 18.36 mg/g Quercetin (control cookies), 29.88 mg/g Quercetin (0.4% cookies) and 33 mg/g Quercetin (1% cookies). Similarly, the antioxidant values are 11.77% for control, 14.55% for 0.4%, and 28.45% for 1% cookies. Lagana et al., (2022) studied cookies fortified with orange peel powder (OPP). The authors reported the following systematic enhancement in the TfC: 1.15 mg GAE g^{-1} for control without OPP, 5.54 mg GAE g^{-1} for 5% OPP sample; 6.10 mg GAE g^{-1} 10% OPP sample, 8.31 mg GAE g^{-1} for 15% OPP sample and 9.12 mg GAE g^{-1} for 20% OPP sample. Thereby, the literature findings corroborated inference that the cookies with higher OPP (pectin) possessed higher TpC content. The bio accessibility parameter was also evaluated in terms of the total antioxidant activity (prior to and after in-vitro digestion). From the experimental analysis, it was found that the control cookies had the lowest quantity of bio-accessible antioxidant activity (74.92%). With the inclusion of the ALEXPB, the bio accessible activity of the antioxidants enhanced significantly. For the 0.4% ALEXPB case, the total antioxidant activity was found to be 78.56%. This is significantly lower than that obtained for the 1% fortified cookies (89.29%). In other words, the inclusion of enhanced ALEXPB content further increased total antioxidant activity.

6.2.3.4 Physical Properties

Table 6.5 summarizes various physical properties of the cookies in terms of diameter, thickness, and weight of control, and ALEXPB-fortified cookies. The data conveyed that the incorporation of ALEXPB reduced the spread ratio in comparison to the control case. However,

Table 6.4: Availability of bioactive components of cookies before and after in-vitro digestion

Sample type	Total Phenolic Content (TpC) mg GAE /g	Total Flavonoids Content (TfC) mg QE /g	Total Antioxidants Activity (%)
ALEXPB	302.58±1.09	280±1.41	-
Dough: Control cookies	21.65±0.73	155±3.54	6.04±0.39
Dough: 0.4% cookies	24.74±1.46	185±3.60	11.42±2.64
Dough: 1.0% cookies	28.54±0.68	217±2.22	16.37±0.86
Before in-vitro digestion			
Control cookies	11.65±0.42	49±0.71	15.71±0.86
0.4% cookies	30.62±1.04	72±0.69	18.52±0.12
1% cookies	49.48±0.95	92±1.67	31.86±0.63
After in-vitro digestion			
Control cookies	8.25±0.34	18.36±0.58	11.77±1.66
0.4% cookies	14.21±0.76	29.88±0.23	14.55±0.70
1% cookies	26.55±1.13	33±0.40	28.45±0.91

Table 6.5: Physical properties of cookies

Sample type	Diameter (cm)	Thickness (cm)	Weight (g)	Spread ratio
Control cookies	7.3±0.44	0.7±0.02	26.60±0.22	10.43±0.18
0.4% cookies	7.1±0.58	0.75±0.05	27.92±0.26	9.46±0.29
1% cookies	7.0±0.61	0.77±0.04	28.04±0.35	9.09±0.34

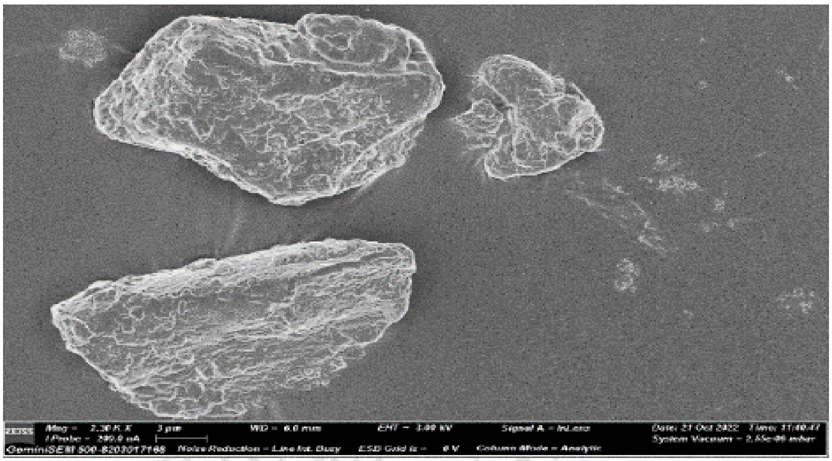
the cookies weight reduced with the inclusion of ALEXPB. The reduced spread ratio is due to the presence of greater number of hydrophilic sites. The incorporation of sodium alginate facilitated this aspect. Also, the gain in the weight affirms upon the presence of water molecules.

6.2.3.5 Morphology

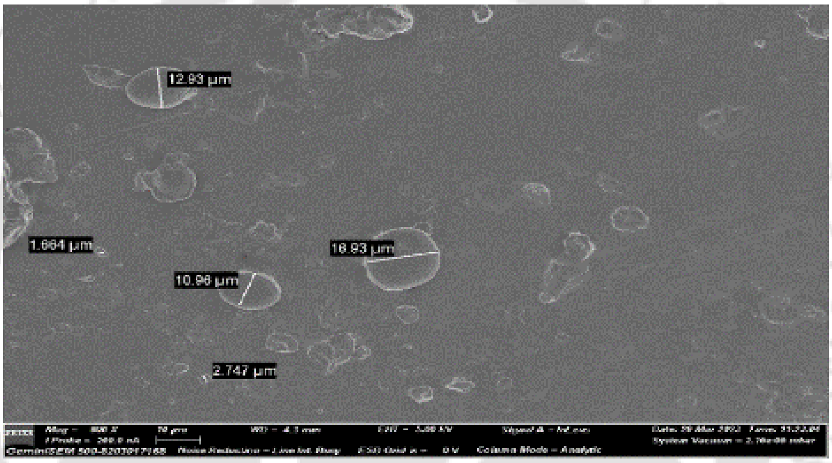
The FESEM image analysis was conducted at various magnifications for the analysis of the morphology of the ALEXPB powder, control cookies, and 1% fortified cookie samples (Fig. 6.2). For the case of control cookies, the FESEM images clearly depicts the presence of circular-shaped particles and with an average diameter ranging from 1.66 to 16.93 µm. Interestingly, upon fortification with 1% ALEXPB, the morphology of the cookie samples exhibited a notable alteration and with triangular-shaped particles. The particles possessed a particle size range of 400 to 700 nm and as well affirmed the successful incorporation of ALEXPB into the cookie matrix at the nanoscale level.

6.2.3.6 Thermal Properties

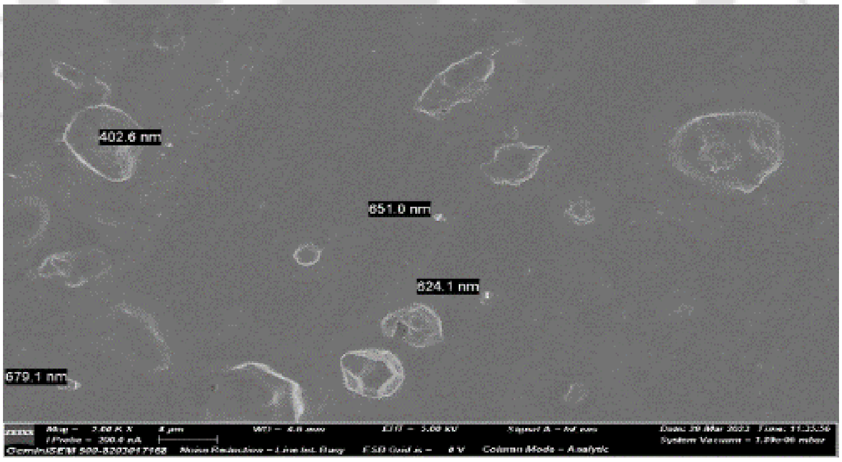
The thermal characteristics of the encapsulated PLE, unfortified cookies, and ALEXPB-fortified cookies were investigated with the differential scanning calorimetry (DSC) method (Fig. 6.3). This figure illustrated a comparative analysis of the melting behavior, peak temperature, and ΔCp of alternate cookie formulations. It can be observed that the melting peak significantly improved for the ALEXPB-fortified cookies in comparison to the control cookies. While the control cookies exhibited a melting point range of 274.5 - 293.1°C (onset-end temperature), the fortified cookies convey a narrower melting point range of 248.1- 268.6°C, respectively. These considerable variations observed after encapsulation can be attributed to alterations in the properties of the wall material. Specifically, calcium alginate, being the primary wall material, exhibited a lower melting temperature. This leads to reduced peak temperature in the fortified cookies. Additionally, the ΔCp value was found to reduce in ALEXPB-fortified cookies in comparison to the control sample. Thereby, the observation conveys alterations in thermal properties due to encapsulation.



(a)



(b)



(c)

Fig. 6.2: FESEM images of (a) ALEXPB, (b) Control Cookies and (c) 1% fortified cookies

6.2.4 Literature Comparison

The literature findings convey that the incorporation of lemon by-products in biscuit formulations (Imeneo et al., 2020) did alter total phenolic content (TpC) values. Thus, for the control biscuits, the TpC values altered from 12 to 15 mg GAE g^{-1} d.w. The TpC values increased with the addition of lemon pomace extracts and fresh lemon peel. Eventually, the TpC reached 34 mg GAE g^{-1} d.w. in biscuits prepared with lemon pomace extracts. Similarly, the antioxidant activity (measured with the DPPH assay) was influenced with the presence of lemon by-products. Thereby, control cookies exhibited higher DPPH values in comparison to the fortified biscuit samples. Also, the work of (Lagana et al., 2022) conveyed cookies fortification with orange peel powder (OPP). Thereby, the authors inferred increased TpC values for the cookies with higher OPP content. Thereby, the TpC values altered as 1.15 mg GAE g^{-1} for the control and thereafter reached 9.12 mg GAE g^{-1} for cookies fortified with 20% OPP. These findings are consistent with the trends mentioned in the Ph.D. thesis that conveyed that the incorporation of ALEXPB led to higher TpC values in comparison to control cookies. In summary, the potential of plant by-products in enhancing the antioxidant profile of baked goods has been demonstrated in the Ph.D. thesis.

6.3 Green Tea Extract Encapsulation by Inclusion Complexation and Ion Gelation

6.3.1 Background

Among various catechins, the green tea catechins that belong to the polyphenols category possess promising combinations of biological and chemical characteristics and are found abundantly in green tea. Organic methanol and acetonitrile solvents are often deployed for catechins extraction from tea leaves. Their extractive quantification refers to an equi-volume

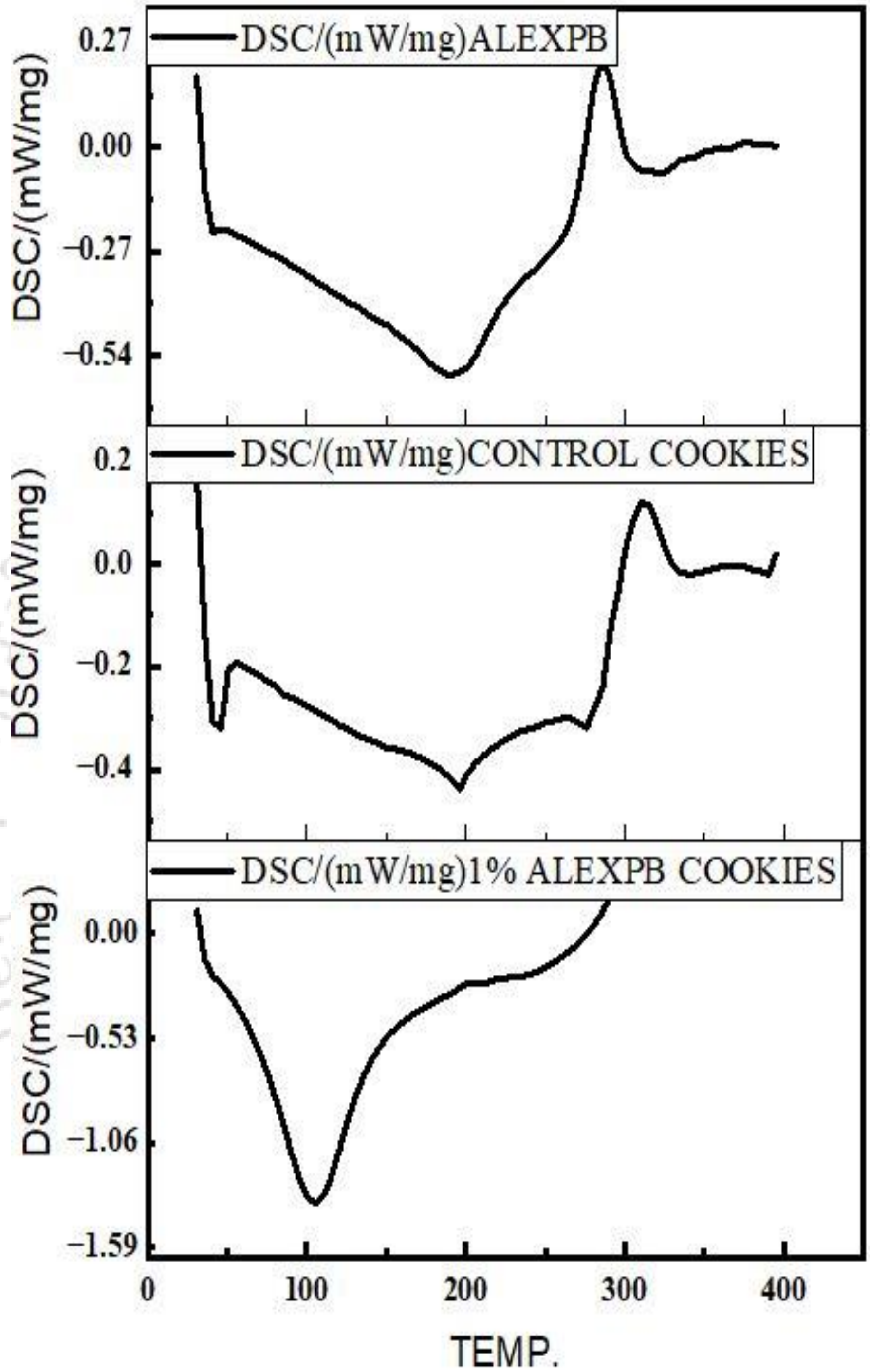


Fig. 6.3: DSC stacked plots for ALEXPB, Control cookies and 1% ALEXPB fortified cookie

ratio (Goto et al., 1996; Wei et al., 2009). However, such research methodology does not accurately represent and ensure sustained catechin levels in tea beverage products. For Korean green tea leaves, hot water extraction conducted respectively in the temperature and time range of 50 - 100°C and 15 - 4 h and subsequent re-extraction with 50% aqueous ethyl acetate solution affirmed that the best yield could be obtained at 80°C and for 45 min time duration (Row & Jin, 2006). The sustainable and controlled release of bioactive into desired environment is often achieved through an encapsulation or protective covering. Catechins encapsulation into starch or its derivative sources can be easily achieved through either inclusion complexation (IC) or ion gelation (IG) methods. In the IC method, the carrier molecule (such as starch) interacts and forms coordination complexes with the guest molecule. This is accomplished through a combination of van der walls forces, hydrogen bonding, hydrophobic interactions, and electrostatic forces (Song et al., 2011). In comparison to many other methods, the IC method serves as a green alternative to the encapsulation of molecules in micro to nano ranged sizes and thereby omits the utilization of hazardous chemicals and dangerous residues in the final product. Often, in lieu of their complex formation capabilities, waxy maize starch, cyclodextrin, maltodextrin etc. are the deployed encapsulation materials.

6.3.2 Encapsulation Techniques

Catechin extract was encapsulated with either a modified ion gelation or an inclusion complexation method (Abreu et al., 2020). In the ion gelation-based encapsulation method, firstly, catechin extract and sodium alginate were mixed in a 1:1 ratio and were solubilized together in distilled water (2% w/v). Thereafter, using a syringe pump (0.5 mL/min), the catechin-alginate extract was added dropwise to 5% w/v $CaCl_2$ solution. This resulted in the formation of the beads. The beads were subsequently washed on a repeated basis with distilled water. This ensured surface contaminants' removal. Thereafter, the beads were dried in a vacuum oven for 12 h at 50°C and 25 mm Hg pressure. The dried microencapsulated beads were stored at -19°C for further characterization.

A modified inclusion complex method was followed to encapsulate the catechin extract. To do so, 0.87 g of catechins were mixed in 500 mL of distilled water and subsequently with 1.51 g of maltodextrin. For effective dissolution, the solution was stirred on a magnetic stirrer at 500 rpm for 12 h in a dark environment. Thereafter, the solution was concentrated in a rotary evaporator at 60°C and the concentrated samples were subjected to drying in a vacuum oven for 12 h at 50°C and 760 mm Hg vacuum pressure. The dried micro encapsulated particles were stored at -19°C and for a storage period of 2 weeks. Such efforts facilitated further characterization.

6.3.3 Characterization of IC and IG based Encapsulates

6.3.3.1 Encapsulation Efficiency (% EE) and Yield

For both IC and IG methods, the encapsulation efficiency was evaluated either in terms on the total phenolic content (TpC) or total flavonoid content (TfC). For the case of IG based catechin encapsulation, the TpC and TfC based % EE were found to be very low (2.35 and 2.56% respectively) in comparison with the IC based catechin encapsulation (88.4 and 9.7 % for TpC and TfC respectively). The lower % EE for the IG case was due to the inability to release the entrapped catechin after extraction. However, for the IC case, better release of encapsulated polyphenols and flavonoids was apparent.

The encapsulation yield was found to be greater for the IC method (90.53%) in comparison to that obtained with the ion gelation method (86.86%) (Fig. 6.4). For the IC method, the modified method involved a scale up of the initial concentration and 12 h incubation period at a much lower temperature (60°C) in the rotary evaporator in comparison with the literature reported value (100°C). Also, the samples were dried in a vacuum dryer at 50°C and under anaerobic conditions which might have enhanced the total yield of encapsulated catechins. It is well

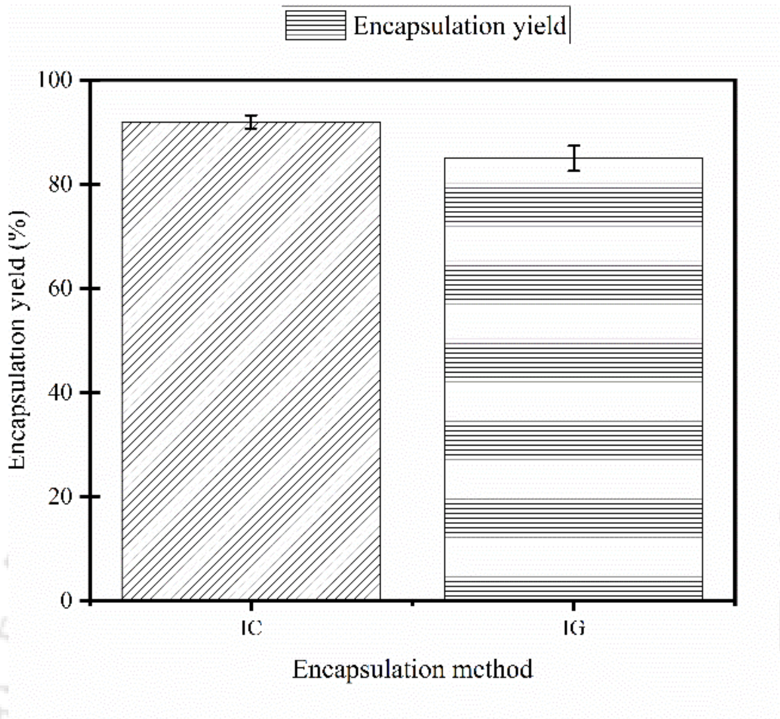


Fig. 6.4: Encapsulation yield of IC and IG method of encapsulation

known that the exposure to high temperature incurs loss of catechins and their functionality. For the IG case, the initial concentrations of the raw materials were scaled up to achieve higher yield characteristics.

6.3.3.2 Particle Size and Morphology

For both IC and IG cases, the catechin extract encapsulation increased the average particle size of the system (Fig. 6.5). In the Ph.D. thesis, the average particle size of catechin extract varied from 1399 -1581 nm and 1399 - 2034 nm respectively for the samples prepared with IC and IG methods. The particle size increment was higher for the ion gelation case. This may be due to the reason that for the IG case, substantial encapsulation could have occurred due to the entrapping of catechins in a bead type structure. However, FESEM image analysis needs to corroborate upon this hypothesis. For the IC case, due to the usage of water as a solvent, a marginally different encapsulation could have occurred. However, this may not be as

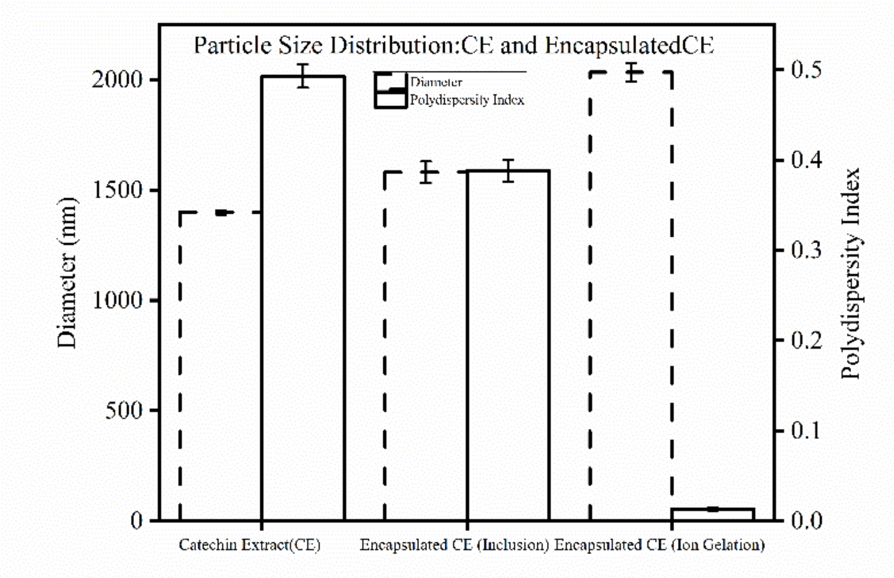


Fig. 6.5: Particle size distribution study of IC and IG method of encapsulation

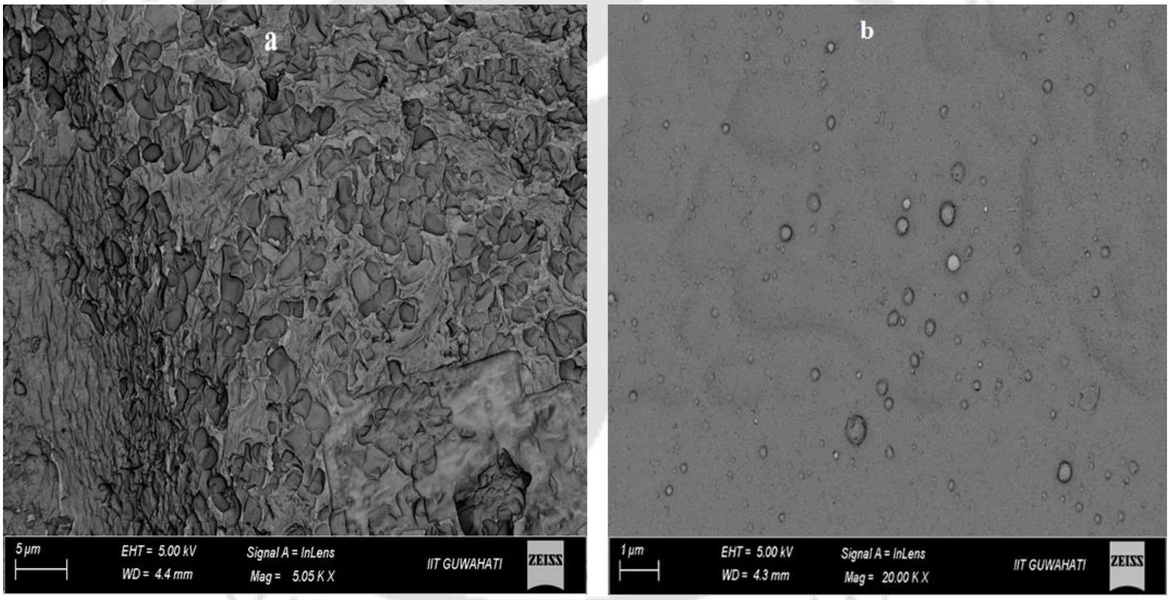


Fig. 6.6: FESEM images of encapsulated catechin extract using a - IC method b - IG method

substantial as that of the IG case. The polydispersity index of the raw catechins and encapsulated catechin extracts were also determined. Among both methods, the catechin extract using IG method affirmed a lower PdI of 0.011 and henceforth more uniformity of the particles. The same needs to be corroborated with the FESEM analysis. On the contrary, for the IC case, a higher PdI of 0.378 was obtained and this affirmed upon the existence of particles with a wider size range.

6.3.3.3 FTIR Analysis

The FTIR spectra of catechin, IC and IG method based encapsulated catechins have been depicted in Fig. 6.7. The spectral illustrations demonstrated possible interaction between catechin and maltodextrin or sodium alginate matrix. The presence of a broad spectrum in the wavelength range of 3250 to 3650 cm^{-1} confirmed water content and hydroxyl groups. Spectral peaks could be found for raw catechin extract, encapsulated IC and IG catechin extracts and in the regions of 1600 – 1300, 1200 – 1000 and 800 – 600 cm^{-1}. These peaks correspond to the hydroxyl compounds. Only for the IC encapsulated catechin case, at 1238 cm^{-1} and at 1144 cm^{-1}, peaks were found. These correspond to protein amide III and aliphatic secondary alcohol groups respectively. Double bond region was observed only for the IC encapsulated catechin case. For both raw catechin and IC encapsulated catechin samples, at 2930 cm^{-1}, spectral peaks were detected, and this confirmed upon the presence of aliphatic compounds. At 1022 cm^{-1}, cyclohexane ring (vibrational) related peak was observed for both raw and IG encapsulated catechins. Catechin-maltodextrin exhibited the characteristic peaks of catechin and maltodextrin. Almost similar broad range peaks were observed for both IC and IG based encapsulated catechin samples.

6.3.3.4 Thermal Properties

The thermal characteristics of the encapsulated catechins and their comparison with the raw catechin were targeted with the differential scanning calorimetry method (Fig. 6.8). Thereby, the melting behavior, peak temperature, ΔC_p were measured and compared. It was observed that, in conjunction with the raw catechins, the melting range improved significantly for both encapsulation methods. While the catechin extracts possessed a melting point range of 122.66 - 181.33°C (onset - end temperature), the maltodextrin encapsulated catechins (IC method) and calcium alginate encapsulated catechins (IG method) possessed a melting point range of 118.33

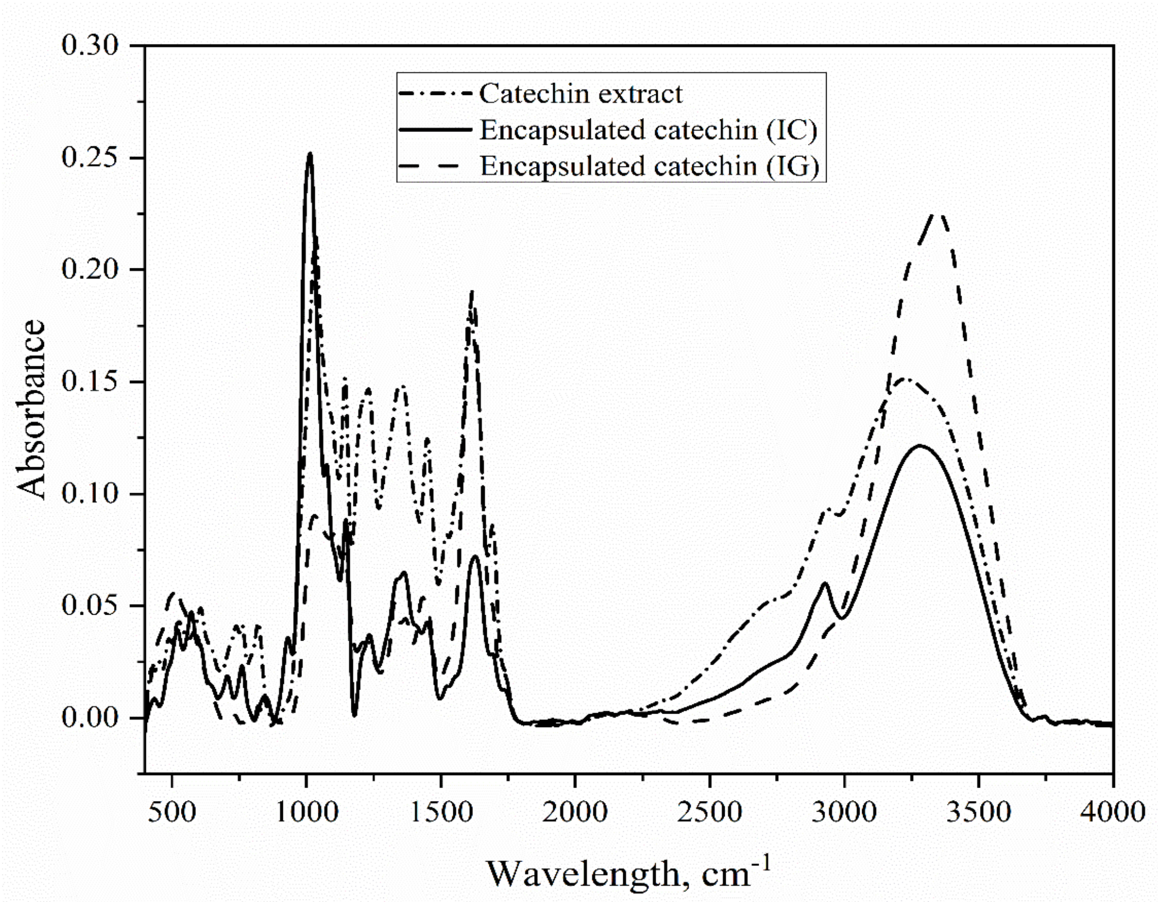


Fig. 6.7: FTIR plots of catechin extracts, IC encapsulated CE, IG encapsulated CE

- 214 and 118.66 - 225.33°C respectively. These substantial variations after encapsulation occurred due to the alteration in the wall material properties. An initial endothermic peak was observed in the temperature range of 0 - 50°C. This is due to water depletion. The second endothermic peak at peak temperatures of 131.46, 108.9, 118.1°C for CE, IG encapsulated CE samples and IC encapsulated CE respectively, confirmed upon the initiation of melting of the compounds. The ΔC_P values for the catechin extract was found to be lower than the values obtained for the encapsulated catechins. Precisely, it has been analysed that the calcium alginate encapsulated catechins (being prepared with IG method) possessed the highest ΔC_p value (15.56 J/g/K) in comparison with the catechin extracts case (1.29 and 1.84 J/g/k) and maltodextrin encapsulated catechins case (1.82 J/g/k). Thus, the calcium alginate based catechin encapsulation ascertained higher thermal stability.

6.3.3.5 XRD Results

The XRD results of the Tetley catechin extract confirmed large crystalline peaks at 2θ values of 25.12° and minor peaks at 8.22° (Fig. 6.8). The major crystalline peak at 25.12° had a moderately high intensity of 942 and thereby confirmed upon the semi crystalline nature of the catechin extract. Using origin software (version origin pro 2022b), the relative crystallinity was assessed to be 50.52 for the Tetley tea extract. This had been corroborated to the basic nature of flavan-3-ols and other compounds such as cellulose, carotenoids etc., that contributed to the crystalline structure of the sample.

For the calcium alginate encapsulated catechin extracts, newer peaks existed at 2θ values of 8.86, 24.52 and 32°. The peaks at 8.86° and 24.52° were similar to the peaks observed in the catechin extract. The new peaks that existed in the calcium alginate encapsulated catechin extracts were wider and reflect upon the wall material characteristics (calcium alginate). For this case, in comparison with the catechin extracts, the intensity of the major peak (8.86°) reduced from 942 to 489. The encapsulated catechin extracts thus had a relative crystallinity of 53.33° and this confirmed upon of the reduced peak intensity and with the existence of new and wider peaks.

The X ray diffractogram of maltodextrin encapsulated catechin extracts was quite similar to that of the catechin extract diffractogram. However, the peak at 8° was absent and also the major peak at 21° had a significantly higher intensity (1769) in comparison with the XRD of the catechin extracts. The resultant relative crystallinity of encapsulated CE with the maltodextrin wall material was found to be 79.12. A high relative crystallinity of the maltodextrin encapsulated catechin extract (CE) conveyed better thermal properties and stability of the catechin. This feature can be promising for the materials' effective function in the gastro-intestinal digestion process.

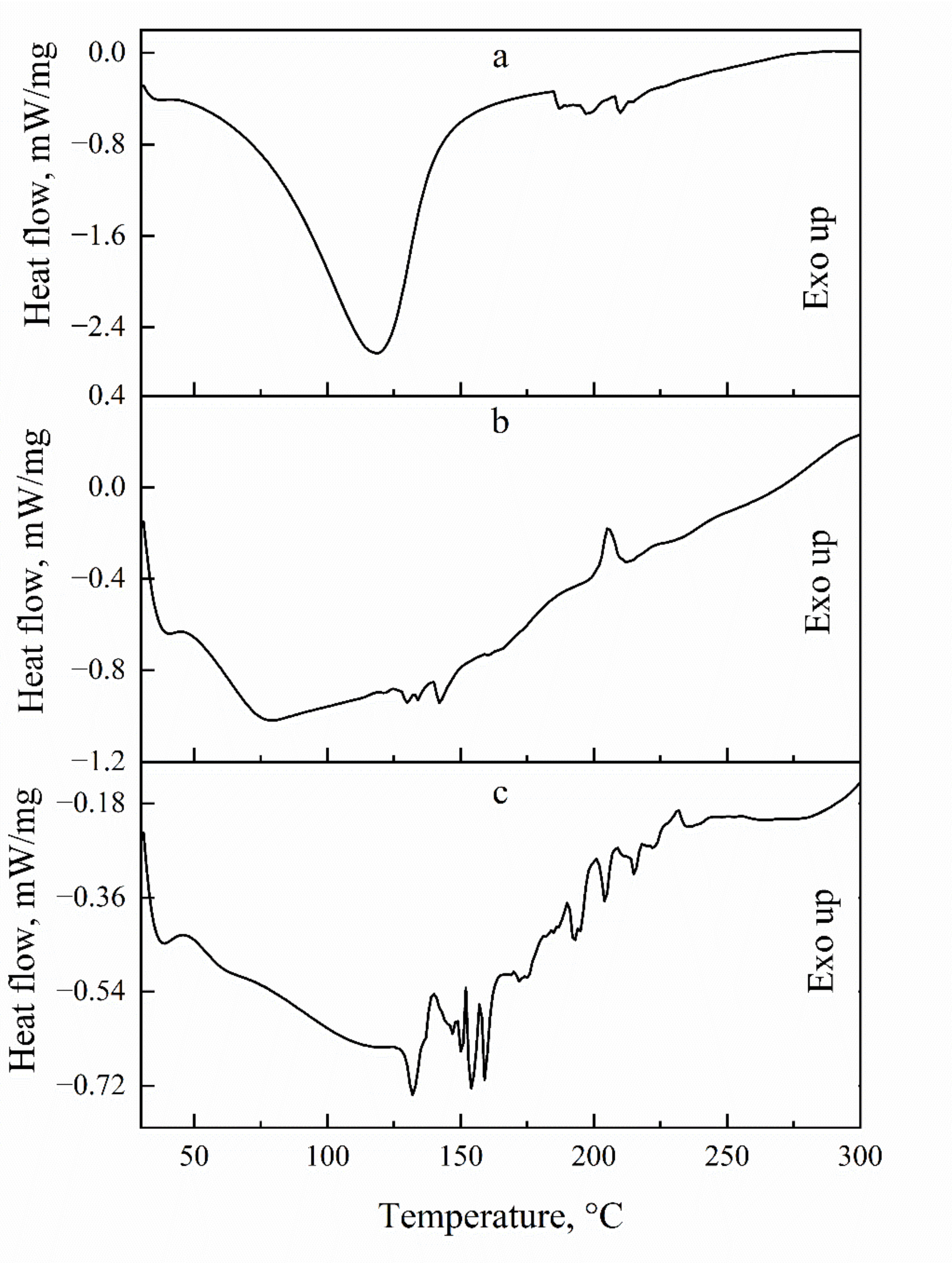


Fig. 6.8: DSC curves of a - Calcium Alginate encapsulated catechin b - Maltodextrin encapsulated catechin, c - Catechin extract

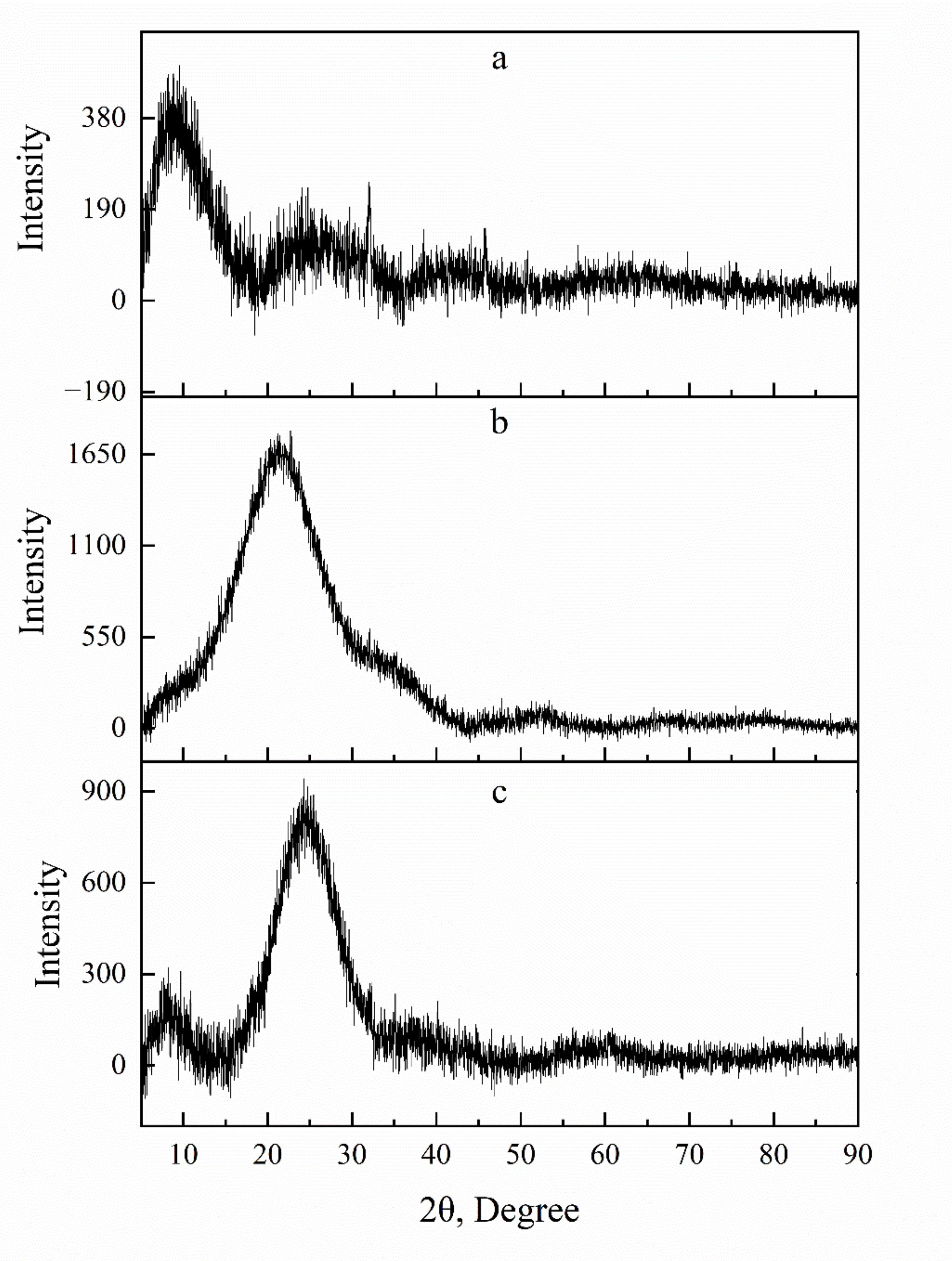


Fig. 6.9: XRD plots for a - Calcium alginate encapsulated catechin – IG, b - Maltodextrin encapsulated catechin extract – IC, c - Catechin extract

6.3.4 Literature Comparison

The encapsulation efficiency (EE), encapsulation yield, particle size, and thermal characteristics of catechins encapsulated with different methods have been compared in Table 6.6. Encapsulation methods include inclusion complexation (IC) with maltodextrin, ion gelation (IG) with sodium alginate, direct catechin extract, IC with β-cyclodextrin, and IG with sodium alginate (Kim et al., 2016; Ho et al., 2018).

In this study, the IC with maltodextrin exhibited a high encapsulation yield of 90.53%, with an EE of 9.7% for total flavonoid content (TfC) and an 88.4% for total phenolic content (TpC). The resultant particles had a size of 1.592 µm, and a polydispersity index (PdI) of 0.378. The thermal analysis revealed an onset temperature of 118.3°C, an end/melting temperature of 214°C, a peak temperature of 118.1°C, and a specific heat capacity (Cp) of 15.56 J/g/K.

Comparatively, the IG with sodium alginate resulted in an encapsulation yield of 86.86%, and an EE of 2.56% for TfC and 2.35% for TpC. The particle size was marginally larger at 2.029 µm, and for a lower PdI of 0.011. Thermal analysis affirmed an onset temperature of 118.6°C, an end/melting temperature of 225.3°C, a peak temperature of 108.9°C, and a Cp of 1.82 J/g/K.

In contrast, direct catechin extract exhibited an encapsulation yield (EE) of 50.52%. The thermal characteristics exhibited an onset temperature of 122.6°C, an end/melting temperature of 181.3°C, a peak temperature of 131.4°C, and a Cp of 1.29 J/g/K.

Literature data analysis also considered best data reported by Ho et al., (2018) and Kim et al., (2016) . As per the authors, IC with β-cyclodextrin achieved an encapsulation yield of 94% and an EE of 84% for antioxidant activity. Additionally, IG with sodium alginate resulted in an EE of 9.14% for the catechin content (Kim et al., 2016). These findings provide valuable insights into the efficacy and performance of alternate encapsulation methods for catechins.

6.4 Fortification of Cookies with IC based Encapsulated Catechin Extract

6.4.1 Background

The sustainable and controlled release of bioactives into desired environment is often achieved through an encapsulation or protective covering. Among many such materials, maltodextrins, amylose, amylopectin, dextrins, polydextrose, Na-alginate are common starch sources and starch derivative compounds. In the food industry, maltodextrin is one of the most widely utilized wall materials for the encapsulation of functional food constituents.

Due to its non-toxicity and biocompatibility, alginate, a representative natural polysaccharide that forms strong gel complexes in the presence of Ca^{2+} ions, has been claimed to be acceptable for food applications. Catechins encapsulation into starch or its derivative sources can be easily achieved through either inclusion complexation (IC) or ion gelation (IG) methods. In the IC method, the carrier molecule (such as starch) interacts and forms coordination complexes with the guest molecule. This is accomplished through a combination of van der walls forces, hydrogen bonding, hydrophobic interactions, and electrostatic forces. Compared to many other methods, the IC method serves as a green alternative to the encapsulation of molecules in micro to nano ranged sizes and thereby omits the utilization of hazardous chemicals and dangerous residues in the final product. Often, in lieu of their complex formation capabilities waxy maize starch, cyclodextrin, maltodextrin etc. are the deployed encapsulation materials. With reference to inclusion complex (IC), the literature findings indicated improved combinations of yield, microencapsulation efficiency, and antioxidant characteristics through the compositional optimality of the IC. Further, the literature comparison table 6.6 of this chapter conveyed superior characteristics of IC based maltodextrin encapsulation. Henceforth, this approach was followed for fortification studies.

6.4.2 Cookie Formulation and Fortification with IC Based Catechin

The green gram-based gluten free cookie formulation with diverse taste and dietary preference (MS2) constituted composition similar to that of the wheat based cookies mentioned in chapter 4 but with 45 g green gram flour substituting wheat flour (other ingredients being sugar- 45 g; Bengal gram flour- 15 g; roasted chickpea flour- 15 g; baking powder- 2 g and butter of 45 g in a total formulation weight of 122 g). Thereby, the cookie sample exhibited impressive 9-point hedonic scale based overall acceptability score of 8.78. Similarly, the fiber content and mineral content enriched gluten-free cookie formulation UMS2 being prepared with unripe papaya pulp powder (PPU) and unripe papaya peel powder (PPE) formulation (specified as in chapter 5 of green gram flour based cookie formulation MS2 and with 12.g of PPU, 2.5 g of PPE and 45 g of butter) affirmed promising mineral content as Na (41.24 ppm), K (64.22 ppm), Fe (0.32 ppm). The formulation also affirmed taste-texture-overall satisfaction ascertaining 9-point hedonic scale based overall acceptability score of 8.21.

For the fortification purpose, fortification sources were considered (a) only dried catechin extract (CE) and (b) IC based encapsulated catechin extract and at a concentration of 50 mg and 100 mg. The fortification was addressed for both MS2 and UMS2 formulation based cookies. The fortified cookie preparation procedure has been similar to that mentioned in respective sections 2.4.1 in the previous chapters.

6.4.3 Bioaccessibility of Total Antioxidants Activity

As best assessed findings, the fortified baked cookie formulations of MS2 (as mentioned in chapter 4) and UMS2 (as mentioned in chapter 5) with either 100 mg dried commercial green tea extracts (CE) or 100 mg inclusion complexation based encapsulated dried commercial green tea extracts (IC) affirmed (a) no influence of fortification on the sensory characteristics of the

Table 6.6: Literature comparison table for catechin encapsulated by IC and IG method

S No.	Encapsulation methods	Encapsulation yield (%)	EE (%)	RC (%)	Particle size (μm)	PDI	Onset Temp. (°C)	End/melting Temp. (°C)	Peak Temp. (°C)	C_p, J/g/K	Reference
1	IC – Maltodextrin	90.53±1.28	9.7±0.45 (TfC) 88.4± 0.91 (TpC)	79.12±1.06	1.592±10.14	0.378±0.013	118.3±4.16	214±2.0	118.1±2.19	15.56±0.54	This work
2	IG – Sodium Alginate	86.86±2.41	2.56±0.12 (TfC) 2.35±0.05 (TpC)	53.33±0.68	2.029±48.69	0.011±0.0015	118.6±3.51	225.3±2.51	108.9±2.15	1.82±0.056	This work
3	Catechin extract	-		50.52±0.75	1.394±40.73		122.6±4.50	181.3±2.51	131.4±2.90	1.29±0.025	This work
4	IC – β cyclodextrin	94	84 (Antioxidant activity)	-	-	-	160	202	-	-	Ho et al., 2018
5	IG – Sodium alginate	-	9.14 (Catechin content)	-	131.3	-	-	-	-	-	Kim et al., 2016

Cookies; (b) antioxidants activity of 43.04% for MS2 fortified with 100 mg of CE, 85.04% for MS2 fortified with 100 mg of IC, 51.7% for UMS2 fortified with 100 mg of CE and 86.61% for UMS2 fortified with 100 mg IC; and (c) in-vitro digestion based antioxidants activity of 3.08% for MS2 fortified with 100 mg of CE, 6.19% for MS2 fortified with 100 mg of IC, 4.42% for UMS2 fortified with 100 mg of CE and 9.3% for UMS2 fortified with 100 mg IC.

Further, 100 mg CE and 100 mg IC based fortification were considered to evaluate the baked cookies antioxidants activity values. While 100 mg CE case conveyed about 51.7% antioxidant activity, 100 mg IC case conveyed 86.61% antioxidant activity for the UMS2 case. Similarly, MS2 formulation affirmed 43.04% antioxidants activity for 100 mg CE case and 85.04% for 100 mg IC case. Post in-vitro digestion studies affirmed that for MS2, CE 100 possessed antioxidants activity as 3.08%. Corresponding value for IC 100 case has been 16.19%. The corresponding values for UMS2 were 4.42 % and 19.3% for 100 mg CE and 100 mg IC cases respectively.

6.4.4 Literature Comparison

The supplementation of biscuits with green tea extract (GTE) or grounded tea leaves has been explored in the literature and for various purposes (Gośliński et al., 2009; Sharma & Zhou, 2011). In a relevant study (Donsì et al., 2017) a green tea extract (GTE) was encapsulated within electrosprayed protein micro particles with gelatin and zein. The protective ability of both systems on the green tea catechins was evaluated. These micro particles exhibited higher encapsulation efficiencies of approximately 90 g per 100 g. They were found to be highly effective in stabilizing the catechins and even after thermal treatment at 180°C for 12 minutes. Thereby, about 85% to 90% of the initial catechins content was preserved. In comparison, free GTE experienced a significant loss of almost 40% of its catechins content after baking at the similar process conditions. These findings are in good agreement with the trends reported in this work, despite the fact that the conducted work in the Ph.D. thesis provides newer and diversified bio-actives data in the encapsulation based fortified cookie samples.

6.5 Summary

The best-case scenario, represented by 1% ALEXPB-fortified cookies, affirmed remarkable parameters such as 5.21g/100 g soluble protein, 49.48 mg GAE/g total phenolic content (TpC), 217.22 mg QE/g total flavonoid content (TfC), and 31.86% in-vitro digestion based AA. This highlights the superior retention of bioactive compounds, particularly phenolic content and antioxidant activity and even after exposure to harsh enzymatic digestion and high-temperature processing environment. The successful encapsulation demonstrated promising outcomes in terms of nutritional, physical, physicochemical, and sensory attributes. Therefore, the addition of ALEXPB to other high-temperature based baked food products is strongly recommended. Furthermore, the encapsulation of various essential nutritional extracts within a similar edible coating is highly advisable for enhanced nutritional benefits and stability.

The carried-out investigations provided numerous insights and inferences for the catechins green tea based catechin extracts encapsulation with inclusion and gelation methodologies. Firstly, both encapsulation techniques exhibited enhanced thermal stability in terms of melting end temperature (214℃ for IC; 225.33℃ for IG), relative crystallinity characteristics (79.12 % and 53.33 % for IC and IG respectively), and the capacity to withstand the harsh digestive environment and thereby support the sustained and controlled release of these beneficial polyphenols. These findings affirmed that the encapsulation technique facilitated greater stability against thermal effects in comparison with the raw dried catechin extract. Secondly, in comparison with the IG method, the IC method exhibited better encapsulation yield (90.53% for IC; 86.86% for IG) and TpC based efficiency (88.4%; 2.35% respectively). However, the IG based beads have been robust i.e., regular in shape and difficult to break the shape and could not release significantly higher polyphenolic compounds in comparison with the insertion method-based beads. Thus, it is important to evaluate further whether the robust beads could function effectively the stringent environment of the human digestive system. Thirdly, the IG based encapsulated catechins are very likely to withstand higher processing temperatures than the IC method-based encapsulates. Fourthly, from a morphological perspective, the IC method conveyed the achievement of particles with wider size range (0.378) in conjunction with those being achieved with the IG method (0.011). However, the bio-accessibility of such encapsulates needs to be evaluated from in-vitro and in-vivo analysis. Finally, the IG method needed further investigation from the perspectives of process parametric optimization and their influence on the improved efficiency.

The catechin extract system based fortified cookies affirmed antioxidants activity of 43.04% for MS2 fortified with 100 mg of CE, 85.04% for MS2 fortified with 100 mg of IC, 51.7% for UMS2 fortified with 100 mg of CE and 86.61% for UMS2 fortified with 100 mg IC. Further, in-vitro digestion based antioxidants activity values were 3.08% for MS2 fortified with 100 mg of CE, 16.19% for MS2 fortified with 100 mg of IC, 4.42% for UMS2 fortified with 100 mg of CE and 19.3% for UMS2 fortified with 100 mg IC. These findings further instil confidence in the beneficial exploration of catechins and papaya leaf extract encapsulants for enhanced cookies fortification.

Chapter 7:

Conclusions and Future Work

Chapter 7

Conclusions and Future Work

In section 7.1, the chapter summarizes the significant conclusions being deduced from the work conducted in the Ph.D. thesis. Subsequently, section 7.2 entails upon the possible scope for future work in the chosen research themes.

7.1 Conclusions

The Ph.D. thesis fulfilled the ultimate objective of various functional grain based gluten-free cookie formulations that were further enriched with dried unripe papaya pulp and peel powders and were further subjected to fortification with encapsulated catechin and papaya leaf extracts. The conducted research work has been able to address the pertinent gaps in the field of grain based gluten-free functional cookie formulation. Important conclusive findings of the conducted research work have been presented in the following sub-sections.

7.1.1 Exploring the Nutritional and Functional Potential of Northeast India's Gluten-Free Grains Combined with Unripe Papaya Pulp and Peel Powder

This chapter addressed the potential of various grain flours (wheat, rice, soy, green gram, chickpea, Bengal gram, oats and finger millet) and unripe papaya pulp and peel powders as gluten-free alternatives to the conventionally used wheat flour. The emphasis of the conducted work was especially in the context of the grains indigenously produced in Northeast India. The conducted study involved the assessment of the nutritional, sensory, and functional properties of few selected flours and their compatibility with the A-type starch. The research emphasis was upon dietary diversity promotion and regional agricultural sustainability. Comprehensive analyses of the physical, chemical, and thermal properties of the mentioned flours provided useful insights into their applications in food product development. The findings highlight the potential of the selected indigenous resources for the creation of cost-effective, nutritionally enriched food products. Thereby, both economic and nutritional advancements in the region's food industry can be achieved. The following conclusions can be enlisted from the conducted detailed assessments:

- The PPU and PPE powders being prepared at oven drying conditions of 105°C drying temperature and 45 minutes duration ascertained good combinations of nutritional content and flavor profiles. For these optimal process conditions that are very easy to meet from even processing cost perspective, the unripe papaya pulp flour and peel flour retained maximum levels of bioactive components (TpC, TfC, AA, VC). The PPU powder deduced from the sliced form exhibited superior bioactives characteristics (TpC: 91 mg GAE/100 g; TfC: 95 mg Quercetin/100 g; AA: 93.75%; VC: 228.57 mg/100 g) in comparison to the grated form. While the PPE possessed the highest ash (8.34 g/100 g) and crude fiber (11.95 g/100 g) content, the PPU possessed 6.11 g/100 g ash and 9.05 g/100 g crude fiber content. Both PPU and PPE were compatible with A-type starch or cereal starch samples.
- Grain flours emerged as significant sources of essential minerals. These are exemplified in the roasted chickpea flour with notable levels of potassium (1176 ppm), sodium (57.02 ppm), iron (7.78 ppm), and zinc (4.61 ppm). Among other flours, the finger millet flour is the best in terms of its high calcium content (364.76 ppm). All other properties highlight the diverse mineral profiles being offered by various mentioned grain flours.
- Known for their diverse nutritional profiles, the grain flours revealed promising functional properties such as the highest protein content of the soy flour (42.7 g/100 g), exceptional water absorption capacity of the oats flour (3.22 g/g) etc., Thus, soy flour can be targeted for protein-rich dietary formulations and oats flour can enhance moisture retention in baked goods that aim to achieve good product quality and freshness in the product. Finger millet

flour's low bulk density (BD) of 0.56 g/mL ascertains practical benefits in terms of reduced packaging requirements and contributions to environmental sustainability.

- Additionally, the broad melting range of Bengal gram flour (54.3°C to 124.4°C) can promisingly influence cooking processes in terms of the versatile applications in the culinary endeavours. Furthermore, PPE's high chroma value (29.10) suggests its significant role in altering the color of baked food products. Such a property enhances their visual appeal and consumer acceptance indices.

7.1.2 Developing Nutritious Gluten-Reduced and Gluten-free Cookies with Indigenous Grains of Northeast India: Formulation and Characterization

The following notable conclusions have been enlisted for the research work conducted in the field of the grain based gluten-free cookie formulations:

- The conducted study scrutinized the sensory and nutritional attributes of gluten-free and gluten-reduced cookies formulated with diverse grain flours. The conducted work was targeted to gauge upon the influence of different powder/flour compositions and other ingredients on the product characteristics. The WS2 formulation consistently impressed with high scores across sensory attributes. This inferred upon its favourable profile in flavor, color, texture, and overall acceptability indices. Among all formulations, the MS2 garnered the highest overall acceptability score and was closely followed by WS2 for the inferred strong consumer appeal. Despite its lower melting point and weaker structure, the MS2 attained an "excellent" rating on the fuzzy logic scale. Thus the formation with notable levels of magnesium is a nutritionally valuable option.
- Millet-based cookie formulations, particularly RaRJ5 and RaOaJ5, exhibited promising mineral content and post in-vitro antioxidant activity. RaOaJ5 exhibited higher combinations of magnesium (9.94 ppm) and manganese (0.68 ppm) contents and a good ΔCp value (2.316).

- The rheological analysis ascertained valuable insights into the mechanical behavior of dough samples. The ghee-based doughs demonstrated higher stiffness in comparison to the butter, soybean oil, and coconut oil-based doughs. Thereby, the dough had a solid-like behavior. Conversely, butter doughs exhibited relatively softer consistency, and the soybean oil doughs demonstrated intermediate stiffness. The coconut oil-based doughs exhibited the lowest stiffness.
- The MS2 formulation exhibited the lowest melting point at 190°C, and a comparatively weaker structure. This is supported by its lower ΔCp value (1.014 J/gK). The RaOaJ5 sample also had the highest ΔCp value (2.316). The NMR study ascertained no alteration in the fatty acid profile for the millet based cookies (RaOaJ5, RaRJ5 formulations).

  A summary of the achieved best formulations and their characteristics has been presented in Table 7.1.

7.1.3 Exploring the Viability of Unripe Papaya Pulp and Peel Powder as Substitutes in Grain-Based Gluten-free Flour Cookies: Characterization and Nutritional Analysis

Notable findings in the research theme of the enrichment of grain flour based gluten-free cookie formulation with unripe papaya pulp and peel powder have been enlisted as follows:

- Both UMS2 and UMS5 formulations obtained an average overall acceptability of 8.21 and 7.94 respectively. The formulations also received the highest indices for taste, breakability and texture profile. The URJ2 and UMS1 formulations received good acceptability scores of 7.89 and 7.45 respectively. These results underscore the significance of ingredient combinations and their proportions in the alteration and optimization of the sensory characteristics of cookies.

Table:7.1 A summary of optimally rated cookie formulations and their characteristics

S. No.	Best Cookie Formulation	Composition (for 4 cookies)	Sensory Score	Other Characteristics
1.	Green-gram - Sugar-Butter (MS2) (Gluten-free)	• Green gram: 45 g • Butter: 45 g • Sugar: 45 g • Roasted Chickpea flour (*Sattu*):15g • Bengal gram (*Besan*) (15 g) • Baking powder(2g) • Milk (double toned)- 7 mL	• 8.74 (Overall acceptability)- 9-point Hedonic scale • 0.82 ("excellent" category)- Fuzzy scale	• Total Carbohydrate (g/100 g): 22.87 • Total Protein (g/100 g): 14.31 • Soluble Protein (g/100 g): 5.66 • Total Fat (g/100 g): 11.43 • Ash (g/100 g): 2.08 • K (ppm): 60.98 • Fe (ppm): 0.34 • Cu (ppm): 0.38 • Zn (ppm):0.13 • MS2- AA:33.51% • After in-vitro AA: 10.38%
2.	Wheat- Sugar-Butter (WS2) (gluten-reduced)	• Wheat: 45 g • Butter: 45 g • Sugar: 45 g • Roasted Chickpea flour (*Sattu*):15g • Bengal gram (*Besan*) (15 g) • Baking powder(2g) • Milk (double toned)- 7 mL	• 8.56 (Overall acceptability)- 9-point Hedonic scale • 0.75 ("excellent" category))- Fuzzy scale	• Total Carbohydrate (g/100 g): 23.58 • Total Protein (g/100 g): 2.56 • Soluble Protein (g/100 g): 1.05 • Total Fat (g/100 g): 9.11 • Ash (g/100 g): 1.44 • K (ppm): 22.22 • Fe (ppm): 0.34
3.	Finger millet-Oats-Jaggery-Shortening (Millet based)	• Finger millet: 22.5 g • Oats: 22.5 g • Butter: 45 g • Sugar: 45 g • Roasted Chickpea flour (*Sattu*):15g • Bengal gram (*Besan*) (15 g) • Baking powder(2g) • Milk (double toned)- 7 mL	• 8.21 (Overall acceptability)- 9-point Hedonic scale • 0.86 ("very good" category))- Fuzzy scale	• Total Carbohydrate (g/100 g): 19.86 • Total Protein (g/100 g): 3.49 • Soluble Protein (g/100 g): 1.21 • Total Fat (g/100 g): 12.07 • Ash (g/100 g): 2.36 • K (ppm): 57.01 • Ca (ppm):3.49 • Mn (ppm): 0.68
4.	PPU-PPE-Green-gram-Sugar-Butter (UMS2) (Unripe papaya pulp and peel powder based)	• PPU:12.5g • PPE:2.5g Green gram: 30 g • Butter: 45 g • Sugar: 45 g • Roasted Chickpea flour (*Sattu*):15g • Bengal gram (*Besan*) (15 g) • Baking powder(2g) • Milk (double toned)- 7 mL	• 8.21 (Overall acceptability)- 9-point Hedonic scale • 0.49 ("excellent" category)	• Total Carbohydrate (g/100 g): 21.08 • Total Protein (g/100 g): 9.02 • Soluble Protein (g/100 g): 2.3 • Total Fat (g/100 g): 13.65 • Ash (g/100 g): 2.77 • K (ppm): 64.22 • Ca (ppm):1.37

Among the unripe papaya pulp and peel-based cookie formulations, the sample UMS2 achieved the highest fuzzy sensory analysis based score of 0.49 ("excellent" category). The powders used in UMS2 also exhibited the lowest average diameter of 1256 nm. Fourier-transform infrared spectroscopy (FTIR) analysis confirmed that no peaks exist with respect to the acrylamide content in the UMS2 sample. This ensured their safety and quality. Additionally, UMS2 cookies demonstrated a balanced nutritional profile with significant antioxidant activity. This renders them to be a superior choice for health-conscious consumers.

- On the other hand, the second best UMS5 formulation demonstrated the good retention of antioxidant activity (AA) at 14.04%. This affirmed its high potential for the preservation of vitamin C content. The UMS5 formulation also had the highest potassium (K) content at 67.37 ppm. This property is beneficial for the ascertaining of electrolyte balance in the body. However, the formulation had a moderate calcium (Ca) content of 1.89 ppm. Henceforth, calcium fortification may be further necessary to ascertain bone health affirming quality of the cookies. Furthermore, UMS5 exhibited the highest polydispersity index (PdI) of 0.54. This affirmed a wider distribution of particle sizes for an average diameter of 1666 nm and the formulation's unique textural properties.
- The URJ2 sample, exhibited the lowest PdI of 0.33, and thereby affirmed a more uniform particle size distribution. The URJ2 also contained significant mineral content in terms of Mg (9.24 ppm), K (at 76.25 ppm), and Ca (3.38 ppm).

7.1.4 Enhancing Functional Attributes of Gluten-Free Cookies through Innovative Encapsulation of Catechin and Papaya Leaf Extracts

Notable findings in the research theme refer to the following enlisted conclusions.

- The best-case scenario, represented by 1% ALEXPB-fortified cookies, affirmed remarkable parameters such as 5.21% soluble protein, 49.48 mg GAE/g total phenolic content (TpC), 217.22 mg QE/g total flavonoid content (TfC), and 31.86% in-vitro digestion based AA. This highlights the superior retention of bioactive compounds, particularly phenolic content and antioxidant activity and even after exposure to harsh enzymatic digestion and high-temperature processing environment. The successful encapsulation demonstrated promising outcomes in terms of nutritional, physical, physicochemical, and sensory attributes.

- Both encapsulation techniques exhibited enhanced thermal stability in terms of end point of the melting temperature (214°C for IC; 225.33°C for IG), relative crystallinity characteristics (79.12% and 53.33% for IC and IG respectively), and the capacity to withstand the harsh digestive environment and thereby support the sustained and controlled release of these beneficial polyphenols. These findings affirmed that the encapsulation technique facilitated greater stability against thermal effects in comparison with the raw dried catechin extract.
- In comparison with the IG method, the IC method exhibited better encapsulation yield (90.53% for IC; 86.86% for IG) and TpC based efficiency (88.4%; 2.35% respectively). However, the IG based beads have been robust i.e., regular in shape and difficult to break the shape and could not release significantly higher polyphenolic compounds in comparison with the insertion method-based beads. Thus, it is important to evaluate further whether the robust beads could function effectively in the stringent environment of the human digestive system.
- The IG based encapsulated catechins are very likely to withstand higher processing temperatures than the IC method-based catechin encapsulates. From a morphological perspective, the IC method conveyed the achievement of particles with wider size range (0.378) in conjunction with those being achieved with the IG method (0.011). However, the bio-accessibility of such encapsulates needs to be evaluated from in-vivo analysis.

- The catechin extract system based fortified cookies affirmed antioxidants activity of 43.04% for MS2 fortified with 100 mg of CE, 85.04% for MS2 fortified with 100 mg of IC, 51.7% for UMS2 fortified with 100 mg of CE and 86.61% for UMS2 fortified with 100 mg IC. Further, in-vitro digestion based antioxidants activity values were 3.08% for MS2 fortified with 100 mg of CE, 16.19% for MS2 fortified with 100 mg of IC, 4.42% for UMS2 fortified with 100 mg of CE and 19.3% for UMS2 fortified with 100 mg IC. These findings further instill confidence in the beneficial exploration of catechins extract based encapsulants for enhanced cookies fortification.

7.2 Future work

The following list elucidates upon the possible scope for further research in the addressed research themes of the Ph.D. thesis:

- Further exploration of ingredient combinations to enhance sensory attributes, rheological properties, and nutritional content of cookies shall be targeted. This could involve experimentation with different types and proportions of underutilized millet flours that are native to Northeast India (such as barnyard and kodo millet) in the existing formulation.
- Investigate the sensitive influence of processing parameters such as mixing techniques (the alternate methods of mixing and realizing a dough), baking temperature and time (these were not altered in the Ph.D. thesis work), baking method (such as air frying), and cooling methods (such as in refrigerator or cooled air) on the sensory characteristics of the prepared cookies. These parametric influences shall be assessed for their sensitive influence on the sensory attributes, rheological behavior, and nutritional content. Such studies will lead to improved production efficiency and consistency.
- Continued research into strategies shall be addressed for acrylamide formation reduction in cookies but with the ascertaining of good sensory qualities and shelf life of the cookies. The

conducted study involved a qualitative assessment of acrylamide in optimal cookie formulations and with the FTIR analysis of the baked cookies. However, given the rising public awareness and health concerns related to acrylamide, a quantitative analysis would provide a comprehensive understanding of its levels in the final product. Future research could prioritize such quantitative measurements and offer detailed insights into the sensitive influence of the baking conditions and ingredient interactions on acrylamide formation. This may involve an exploration into alternative ingredients such as tubers with lower starchy content and other flours not constituting amino acid and asparagine. Thereby, acrylamide formation during baking can be reduced and if possible mitigated.

- The primary focus of the Ph.D. thesis was upon the formulation optimality and associated initial characterization of the enriched and fortified cookies. Thereby, basic enhancement in their nutritional profile for sensory attributes based optimality of the composition was sought. While bioavailability is indeed an important factor in determining the ultimate nutritional benefit in terms of the assessed nutrient absorption and metabolism, it requires extensive investigations through either specific in-vitro or in-vivo methods. Such studies often demand additional resources, equipment, and protocols. These are beyond the scope of the formulation research and initial product evaluation. Therefore, while bioavailability studies could certainly add value, they are not immediately essential for the validation of the quality and acceptability of the formulated cookies as nutritionally-enriched products. However, future research must address at least in-vitro bioaccessibility and bioavailability characterization. Thereby, an enhanced comprehensive benchmarking of the nutritional profile can be established to compliment the findings of the study.
- In the conducted study, the target was to optimize the cookie formulations and thereby achieve desired sensory and nutritional attributes. The associated characterization based findings was conducted to supplement the mentioned target. Henceforth, an exhaustive

analysis of all lipid byproducts in the achieved formulations was not considered. While trans fatty acids may form under certain high baking temperature based condition and for specific fat types, it is very likely that their formation is dependent upon the nature of the oils or used fats. Further, it shall be noted that the selected fats in the formulations were chosen for their stability under the baking conditions. Thereby, it was presumed that the trans-fat formation could be minimized under those circumstances. Furthermore, the baking process parameters were carefully controlled to limit excessive exposure of the cookies and thereby avoid the elevation of the constitution of the trans fatty acids in the resultant cookies. Thus, while quantitative trans-fat analysis would provide additional data, the choice of formulations and baking conditions (such as baking temperature and time) are very likely to minimize trans-fat formation and thereby support the nutritional quality and stability of the prepared cookies. Nonetheless, future investigations could address the quantitative assessment of the trans-fatty acids in the baked cookies.

- Further examination of the structural differences observed among dough and cookie samples shall be targeted with the advanced imaging techniques such as 3D imaging techniques. This could provide deeper insights into the effects of ingredient composition and processing methods on the texture, appearance, shelf life and stability of the baked cookie products.
- Conduct investigations to ascertain upon the sensitive influence of various packaging materials (such as HDPE, LDPE) and storage conditions (driving accelerated storage study) on the time based alteration of cookie quality parameters. A deeper understanding into these aspects can provide useful insights into the better shelf life of the cookies and assessed quality parameters such as the moisture content, texture, sensory attributes and product freshness.
- Future research shall explore various ways through which cookies fortification can be ascertained with additional nutrients such as protein, phenolic content, vitamins (such as

vitamin D, vitamin B complex) or bioactive compounds (such as Omega-3-fatty acids). Such customized product oriented research can diversify and enhance the nutritional value of the prepared cookies.

Through comprehensive analyses, the research underscores the proximate, thermal, constitutional and functional properties of grain based flours, highlighting their compatibility with A-type starches and their potential to promote dietary diversity and regional agricultural sustainability. The findings reveal significant promise in creating cost-effective, nutritionally enriched grain flour based gluten-reduced and gluten-free cookie formulations. Thereby, the findings support both economic growth and nutritional advancements in the region's bakery food industry. Furthermore, the research elucidates upon the crucial role of incorporation of indigenous ingredients such as unripe papaya pulp and peel powder in addressing dietary preferences. Also, fortification of cookies with encapsulated of catechin extracts from green tea and bioactives of papaya leaf extracts using green and efficient inclusion complexation and ion gelation procedures underscore the potential for these ingredients to serve as versatile and sustainable alternatives in food formulation.

Moreover, the identified avenues for future research highlight the ongoing commitment to innovation and advancement in the field of functional food product development. Through continued exploration of ingredient combinations, processing techniques, and fortification strategies, researchers aim to further optimize product quality, shelf life, and nutritional content. This collective effort holds promise for the enhanced economic growth, promotion of public health, and fostering of cultural sustainability in Northeast India's food industry. In essence, the conclusions drawn from this chapter pave the way for a holistic approach to food product development that prioritizes nutritional enrichment, sensory appeal, and regional agricultural resilience. The Ph.D. thesis investigated the sensitive influence of encapsulation on enhanced bioaccessibility of nutrients and with the in-vitro digestion study. Future research can be undertaken using in-vivo animal models for the assessed interaction of encapsulated catechin extracts inside the pathways of the animal body.

In summary, the Ph.D. thesis proposed variegated functional gluten-free cookie formulation with the principle of greater utilization of horticultural produces of Northeast India. Also, the concept of fortification of cookies can be widely utilized to enclose a variety of other biologically active substances and for diversified functional food applications. Accordingly, thorough investigations will enhance the development of gluten free functional food products for wider consumer acceptance. Such research directions being generic in nature can further revolutionize the baked food product development to eventually meet the customized needs of the consumers.

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Publications

List of Publications

A) Publications

1. **Paushali Mukherjee,** Kamal Narayan Baruah, Ramagopal V.S. Uppaluri (2023). Encapsulation and Characterization of Commercial Green Tea Extracts Using Green Methods: A Comparative Study of Inclusion Complexation and Ion Gelation. Journal of Food Measurement and Characterization. DOI: https://doi.org/10.1007/s11694-023-02250-7
2. Kamal Narayan Baruah, Siddhartha Singha, **Paushali Mukherjee**, Ramagopal V.S. Uppaluri (2023). Optimization of the enzymatic extraction of catechins from Assam tea leaves. Biomass conversion and biorefinery, https://doi.org/10.1007/s13399-023-04684-x
3. **Paushali Mukherjee**, Rushikesh Rajendra Kudave, Ramagopal Uppaluri (2022). Formulation and Characterization of Squash Enriched Cookies. Agro and Food Processing Technologies. NERC 2022. Springer, Singapore. https://doi.org/10.1007/978-981-19-9704-4_13

B) Article submitted/under review

1. **Paushali Mukherjee**, Khalid Mehmood Wani, Ramagopal V.S. Uppaluri. Synergistic Synthesis and Comprehensive Characterization of Papaya Leaf Extract-Infused Functional Cookies. Journal: Food and Humanity (Submitted).
2. **Paushali Mukherjee**, Ramagopal V.S. Uppaluri (2024). A Comprehensive Exploration of the Nutritional and Functional Potential of North East India's Gluten-Free Grain and Unripe Papaya Pulp and Peel Powder.

3. **Paushali Mukherjee**, Ramagopal V.S. Uppaluri (2024). Developing Nutritious Gluten-Reduced Cookies with Indigenous Grains of Northeast India: Formulation and Characterization.
4. **Paushali Mukherjee**, Ramagopal V.S. Uppaluri (2024). Exploring the Viability of Unripe Papaya Pulp and Peel Powder as Substitutes in Grain-Based Gluten-free Flour Cookies: Characterization and Nutritional Analysis.

D) Indian Patent to be submitted

1. **Paushali Mukherjee**, Ramagopal V.S. Uppaluri (2024). Formulation and fortification of grain-based functional cookies enriched with unripe papaya pulp and peel powder.
2. Omkar Bal**, Paushali Mukherjee**, Omkar S. Deshmukh (2024). Formulation and Rheological Study of Sugar-free Biscuit Cream using Unripe Banana Flour.

Appendices

Appendix A: Alternate Cookie Formulations Investigated for Sensory Analysis based Identification of Best Grain Flours based Recipes

In the Appendix, Tables A1-A6 summarizes all grain flours based cookie formulations (and their ingredient proportions) that were assessed for the sensory analysis based identification of best cookie formulations. Among these, Tables A1, A2, A3, A4, A5, A6 respectively address wheat flour based gluten-reduced, rice flour based gluten-free, green gram flour based gluten-free, soy flour based gluten-free, finger millet-rice based gluten-free and finger millet-oats gluten-free formulations. For all cases, the provided proportions are for a batch of 4 cookies (122g dry mix).

Table A1: Alternate formulation data in terms of the ingredients composition of wheat based gluten-reduced cookie

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	Wheat-Sugar-Ghee	WS1	Ghee:45g	Sugar: 45g	• Wheat flour: 45g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	Wheat-Sugar-Butter	WS2	Butter:45g		
3.	Wheat-Sugar-Soybean oil	WS3	Soybean oil:22.5mL		
4.	Wheat-Sugar-Groundnut oil	WS4	Groundnut oil:22.5mL		
5.	Wheat-Sugar-Cookie Shortening	WS5	Cookie Shortening:45g		
6.	Wheat-Sugar-Coconut oil	WS6	Coconut oil:22.5mL		
7.	Wheat-Jaggery-Ghee	WJ1	Ghee:45g	Jaggery:33.75g	
8.	Wheat-Jaggery-Butter	WJ2	Butter:45g		
9.	Wheat-Jaggery-Soybean oil	WJ3	Soybean oil:22.5mL		
10.	Wheat-Jaggery-Groundnut oil	WJ4	Groundnut oil:22.5mL		
11.	Wheat-Jaggery-Cookie Shortening	WJ5	Cookie Shortening:45g		
12.	Wheat-Jaggery-Coconut oil	WJ6	Coconut oil:22.5mL		
13.	Wheat-Honey-Ghee	WH1	Ghee:45g	Honey:22.5mL	
14.	Wheat-Honey-Butter	WH2	Butter:45g		
15.	Wheat-Honey-Soybean oil	WH3	Soybean oil:22.5mL		
16.	Wheat-Honey-Groundnut oil	WH4	Groundnut oil:22.5mL		
17.	Wheat-Honey-Cookie Shortening	WH5	Cookie Shortening:45g		
18.	Wheat-Honey-Coconut oil	WH6	Coconut oil:22.5mL		
19.	Wheat-Artificial sweeteners-Ghee	WA1	Ghee:45g	Artificial sweetener: 4.16 g	
20.	Wheat- Artificial sweeteners -Butter	WA2	Butter:45g		
21.	Wheat- Artificial sweeteners-Soybean oil	WA3	Soybean oil:22.5mL		
22.	Wheat- Artificial sweeteners-Groundnut oil	WA4	Groundnut oil:22.5mL		
23.	Wheat- Artificial sweeteners -Cookie Shortening	WA5	Cookie Shortening:45g		
24.	Wheat-Artificial sweeteners-Coconut oil	WA6	Coconut oil:22.5mL		

Table A2: Alternate formulation data in terms of the ingredients composition of rice based gluten-free cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	Rice-Sugar-Ghee	RS1	Ghee:45g	Sugar: 45g	• Rice flour: 45g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	Rice-Sugar-Butter	RS2	Butter:45g		
3.	Rice-Sugar-Soybean oil	RS3	Soybean oil:22.5mL		
4.	Rice-Sugar-Groundnut oil	RS4	Groundnut oil:22.5mL		
5.	Rice-Sugar-Cookie Shortening	RS5	Cookie Shortening:45g		
6.	Rice-Sugar-Coconut oil	RS6	Coconut oil:22.5mL		
7.	Rice-Jaggery-Ghee	RJ1	Ghee:45g	Jaggery:33.75g	
8.	Rice-Jaggery-Butter	RJ2	Butter:45g		
9.	Rice-Jaggery-Soybean oil	RJ3	Soybean oil:22.5mL		
10.	Rice-Jaggery-Groundnut oil	RJ4	Groundnut oil:22.5mL		
11.	Rice-Jaggery-Cookie Shortening	RJ5	Cookie Shortening:45g		
12.	Rice-Jaggery-Coconut oil	RJ6	Coconut oil:22.5mL		
13.	Rice-Honey-Ghee	RH1	Ghee:45g	Honey:22.5mL	
14.	Rice-Honey-Butter	RH2	Butter:45g		
15.	Rice-Honey-Soybean oil	RH3	Soybean oil:22.5mL		
16.	Rice-Honey-Groundnut oil	RH4	Groundnut oil:22.5mL		
17.	Rice-Honey-Cookie Shortening	RH5	Cookie Shortening:45g		
18.	Rice-Honey-Coconut oil	RH6	Coconut oil:22.5mL		
19.	Rice-Artificial sweeteners-Ghee	RA1	Ghee:45g	Artificial sweetener: 4.16 g	
20.	Rice- Artificial sweeteners -Butter	RA2	Butter:45g		
21.	Rice-Artificial sweeteners-Soybean oil	RA3	Soybean oil:22.5mL		
22.	Rice- Artificial sweeteners-Groundnut oil	RA4	Groundnut oil:22.5mL		
23.	Rice-Artificial sweeteners -Cookie Shortening	RA5	Cookie Shortening:45g		
24.	Rice-Artificial sweeteners-Coconut oil	RA6	Coconut oil:22.5mL		

Table A3: Alternate formulation data in terms of the ingredients composition of green gram based gluten-free cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	Green gram-Sugar-Ghee	MS1	Ghee:45g	Sugar: 45g	• Green gram flour: 45g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	Green gram-Sugar-Butter	MS2	Butter:45g		
3.	Green gram -Sugar-Soybean oil	MS3	Soybean oil:22.5mL		
4.	Green gram -Sugar-Groundnut oil	MS4	Groundnutoil:22.5mL		
5.	Green gram -Sugar-Cookie Shortening	MS5	Cookie Shortening:45g		
6.	Green gram -Sugar-Coconut oil	MS6	Coconut oil:22.5mL		
7.	Green gram -Jaggery-Ghee	MJ1	Ghee:45g	Jaggery:33.75g	
8.	Green gram -Jaggery-Butter	MJ2	Butter:45g		
9.	Green gram -Jaggery-Soybean oil	MJ3	Soybean oil:22.5mL		
10.	Green gram -Jaggery-Groundnut oil	MJ4	Groundnut oil:22.5mL		
11.	Green gram -Jaggery-Cookie Shortening	MJ5	Cookie Shortening:45g		
12.	Green gram -Jaggery-Coconut oil	MJ6	Coconut oil:22.5mL		
13.	Green gram -Honey-Ghee	MH1	Ghee:45g	Honey:22.5mL	
14.	Green gram -Honey-Butter	MH2	Butter:45g		
15.	Green gram-Honey-Soybean oil	MH3	Soybean oil:22.5mL		
16.	Green gram -Honey-Groundnut oil	MH4	Groundnut oil:22.5mL		
17.	Green gram -Honey-Cookie Shortening	MH5	Cookie Shortening:45g		
18.	Green gram -Honey-Coconut oil	MH6	Coconut oil:22.5mL		
19.	Green gram -Artificial sweeteners-Ghee	MA1	Ghee:45g	Artificial sweetener: 4.16 g	
20.	Green gram - Artificial sweeteners - Butter	MA2	Butter:45g		
21.	Green gram -Artificial sweeteners-Soybean oil	MA3	Soybean oil:22.5mL		
22.	Green gram - Artificial sweeteners-Groundnut oil	MA4	Groundnut oil:22.5mL		
23.	Green gram -Artificial sweeteners - Cookie Shortening	MA5	Cookie Shortening:45g		
24.	Green gram -Artificial sweeteners-Coconut oil	MA6	Coconut oil:22.5mL		

Table A4: Alternate formulation data in terms of the ingredients composition of soy based gluten-free cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	Soy-Sugar-Ghee	SoS1	Ghee:45g	Sugar: 45g	• Soy flour: 45g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	Soy-Sugar-Butter	SoS2	Butter:45g		
3.	Soy -Sugar-Soybean oil	SoS3	Soybean oil:22.5mL		
4.	Soy -Sugar-Groundnut oil	SoS4	Groundnutoil:22.5mL		
5.	Soy -Sugar-Cookie Shortening	SoS5	Cookie Shortening:45g		
6.	Soy -Sugar-Coconut oil	SoS6	Coconut oil:22.5mL		
7.	Soy -Jaggery-Ghee	SoJ1	Ghee:45g	Jaggery:33.75g	
8.	Soy -Jaggery-Butter	SoJ2	Butter:45g		
9.	Soy -Jaggery-Soybean oil	SoJ3	Soybean oil:22.5mL		
10.	Soy -Jaggery-Groundnut oil	SoJ4	Groundnut oil:22.5mL		
11.	Soy -Jaggery-Cookie Shortening	SoJ5	Cookie Shortening:45g		
12.	Soy -Jaggery-Coconut oil	SoJ6	Coconut oil:22.5mL		
13.	Soy -Honey-Ghee	SoH1	Ghee:45g	Honey:22.5mL	
14.	Soy -Honey-Butter	SoH2	Butter:45g		
15.	Soy -Honey-Soybean oil	SoH3	Soybean oil:22.5mL		
16.	Soy -Honey-Groundnut oil	SoH4	Groundnut oil:22.5mL		
17.	Soy -Honey-Cookie Shortening	SoH5	Cookie Shortening:45g		
18.	Soy -Honey-Coconut oil	SoH6	Coconut oil:22.5mL		
19.	Soy -Artificial sweeteners-Ghee	SoA1	Ghee:45g	Artificial sweetener: 4.16 g	
20.	Soy - Artificial sweeteners -Butter	SoA2	Butter:45g		
21.	Soy -Artificial sweeteners-Soybean oil	SoA3	Soybean oil:22.5mL		
22.	Soy - Artificial sweeteners-Groundnut oil	SoA4	Groundnut oil:22.5mL		
23.	Soy -Artificial sweeteners -Cookie Shortening	SoA5	Cookie Shortening:45g		
24.	Soy -Artificial sweeteners-Coconut oil	SoA6	Coconut oil:22.5mL		

Table A5: Alternate formulation data in terms of the ingredients composition of finger millet-rice based gluten-free cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	Finger millet-Rice-Jaggery-Ghee	RaRJ1	Ghee:45g	Jaggery:33.75g	• Finger millet flour: 22.5g • Rice flour:22.5g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	Finger millet-Rice-Jaggery-Butter	RaRJ2	Butter:45g		
3.	Finger millet-Rice-Jaggery-Soybean oil	RaRJ3	Soybean oil:22.5mL		
4.	Finger millet-Rice-Jaggery-Groundnut oil	RaRJ4	Groundnutoil:22.5mL		
5.	Finger millet-Rice-Jaggery-Cookie Shortening	RaRJ5	Cookie Shortening:45g		

Table A6: Alternate formulation data in terms of the ingredients composition of finger millet-oats based gluten-free cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	Finger millet-Oats-Jaggery-Ghee	RaOaJ1	Ghee:45g	Jaggery:33.75g	• Finger millet flour: 22.5g • Oats flour:22.5g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	Finger millet-Oats-Jaggery-Butter	RaOaJ2	Butter:45g		
3.	Finger millet-Oats-Jaggery-Soybean oil	RaOaJ3	Soybean oil:22.5mL		
4.	Finger millet-Oats-Jaggery-Groundnut oil	RaOaJ4	Groundnutoil:22.5mL		
5.	Finger millet-Oats-Jaggery-Cookie Shortening	RaOaJ5	Cookie Shortening:45g		

Appendix B: Detailed Composition along with their proportions of Unripe Papaya Pulp and Peel Powder based Cookie Formulations

In the Appendix, Tables B1-B4 summarizes all grain flours based cookie formulations (and their ingredient proportions) that were assessed for the sensory analysis based identification of best cookie formulations. Among these, Tables B1, B2, B3, B4 respectively address wheat flour based gluten-reduced, rice flour based gluten-free, green gram flour based gluten-free and soy flour based gluten-free formulations. For all cases, the provided proportions are for a batch of 4 cookies (122g dry mix).

Table B1: Alternate formulation data in terms of the ingredients composition of wheat, unripe papaya pulp powder (PPU) and unripe papaya peel powder (PPE) based gluten-reduced cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	PPU-PPE-Wheat-Sugar-Ghee	UWS1	Ghee:45g	Sugar: 45g	• Wheat flour: 30g • PPU:12.5g • PPE:2.5g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	PPU-PPE-Wheat-Sugar-Butter	UWS2	Butter:45g		
3.	PPU-PPE-Wheat-Sugar-Soybean oil	UWS3	Soybean oil:22.5mL		
4.	PPU-PPE-Wheat-Sugar-Groundnut oil	UWS4	Groundnut oil:22.5mL		
5.	PPU-PPE-Wheat-Sugar-Cookie Shortening	UWS5	Cookie Shortening:45g		
6.	PPU-PPE-Wheat-Sugar-Coconut oil	UWS6	Coconut oil:22.5mL		
7.	PPU-PPE-Wheat-Jaggery-Ghee	UWJ1	Ghee:45g	Jaggery:33.75g	
8.	PPU-PPE-Wheat-Jaggery-Butter	UWJ2	Butter:45g		
9.	PPU-PPE-Wheat-Jaggery-Soybean oil	UWJ3	Soybean oil:22.5mL		
10.	PPU-PPE-Wheat-Jaggery-Groundnut oil	UWJ4	Groundnut oil:22.5mL		
11.	PPU-PPE-Wheat-Jaggery-Cookie Shortening	UWJ5	Cookie Shortening:45g		
12.	PPU-PPE-Wheat-Jaggery-Coconut oil	UWJ6	Coconut oil:22.5mL		
13.	PPU-PPE-Wheat-Honey-Ghee	UWH1	Ghee:45g	Honey:22.5mL	
14.	PPU-PPE-Wheat-Honey-Butter	UWH2	Butter:45g		
15.	PPU-PPE-Wheat-Honey-Soybean oil	UWH3	Soybean oil:22.5mL		
16.	PPU-PPE-Wheat-Honey-Groundnut oil	UWH4	Groundnut oil:22.5mL		
17.	PPU-PPE-Wheat-Honey-Cookie Shortening	UWH5	Cookie Shortening:45g		
18.	PPU-PPE-Wheat-Honey-Coconut oil	UWH6	Coconut oil:22.5mL		
19.	PPU-PPE-Wheat-Artificial sweeteners-Ghee	UWA1	Ghee:45g	Artificial sweeteners: 4.16 g	
20.	PPU-PPE-Wheat- Artificial sweeteners - Butter	UWA2	Butter:45g		
21.	PPU-PPE-Wheat- Artificial sweeteners-Soybean oil	UWA3	Soybean oil:22.5mL		
22.	PPU-PPE-Wheat- Artificial sweeteners-Groundnut oil	UWA4	Groundnut oil:22.5mL		
23.	PPU-PPE-Wheat- Artificial sweeteners - Cookie Shortening	UWA5	Cookie Shortening:45g		
24.	PPU-PPE-Wheat-Artificial sweeteners-Coconut oil	UWA6	Coconut oil:22.5mL		

Table B2: Data summary of detailed ingredients composition of rice, unripe papaya pulp powder (PPU) and unripe papaya peel powder (PPE) based gluten-free cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	PPU-PPE-Rice-Sugar-Ghee	URS1	Ghee:45g	Sugar: 45g	• Rice flour: 30g • PPU:12.5g • PPE:2.5g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	PPU-PPE-Rice-Sugar-Butter	URS2	Butter:45g		
3.	PPU-PPE-Rice-Sugar-Soybean oil	URS3	Soybean oil:22.5mL		
4.	PPU-PPE-Rice-Sugar-Groundnut oil	URS4	Groundnut oil:22.5mL		
5.	PPU-PPE-Rice-Sugar-Cookie Shortening	URS5	Cookie Shortening:45g		
6.	PPU-PPE-Rice-Sugar-Coconut oil	URS6	Coconut oil:22.5mL		
7.	PPU-PPE-Rice-Jaggery-Ghee	URJ1	Ghee:45g	Jaggery:33.75g	
8.	PPU-PPE-Rice-Jaggery-Butter	URJ2	Butter:45g		
9.	PPU-PPE-Rice-Jaggery-Soybean oil	URJ3	Soybean oil:22.5mL		
10.	PPU-PPE-Rice-Jaggery-Groundnut oil	URJ4	Groundnut oil:22.5mL		
11.	PPU-PPE-Rice-Jaggery-Cookie Shortening	URJ5	Cookie Shortening:45g		
12.	PPU-PPE-Rice-Jaggery-Coconut oil	URJ6	Coconut oil:22.5mL		
13.	PPU-PPE-Rice-Honey-Ghee	URH1	Ghee:45g	Honey:22.5mL	
14.	PPU-PPE-Rice-Honey-Butter	URH2	Butter:45g		
15.	PPU-PPE Rice-Honey-Soybean oil	URH3	Soybean oil:22.5mL		
16.	PPU-PPE-Rice-Honey-Groundnut oil	URH4	Groundnut oil:22.5mL		
17.	PPU-PPE-Rice-Honey-Cookie Shortening	URH5	Cookie Shortening:45g		
18.	PPU-PPE-Rice-Honey-Coconut oil	URH6	Coconut oil:22.5mL		
19.	PPU-PPE-Rice-Artificial sweeteners-Ghee	URA1	Ghee:45g	Artificial sweeteners: 4.16 g	
20.	PPU-PPE-Rice- Artificial sweeteners -Butter	URA2	Butter:45g		
21.	PPU-PPE-Rice-Artificial sweeteners-Soybean oil	URA3	Soybean oil:22.5mL		
22.	PPU-PPE-Rice- Artificial sweeteners-Groundnut oil	URA4	Groundnut oil:22.5mL		
23.	PPU-PPE-Rice-Artificial sweeteners -Cookie Shortening	URA5	Cookie Shortening:45g		
24.	PPU-PPE-Rice-Artificial sweeteners-Coconut oil	URA6	Coconut oil:22.5mL		

Table B3: Data summary of detailed ingredients composition of green gram, unripe papaya pulp powder (PPU) and unripe papaya peel powder (PPE) based gluten-free cookies

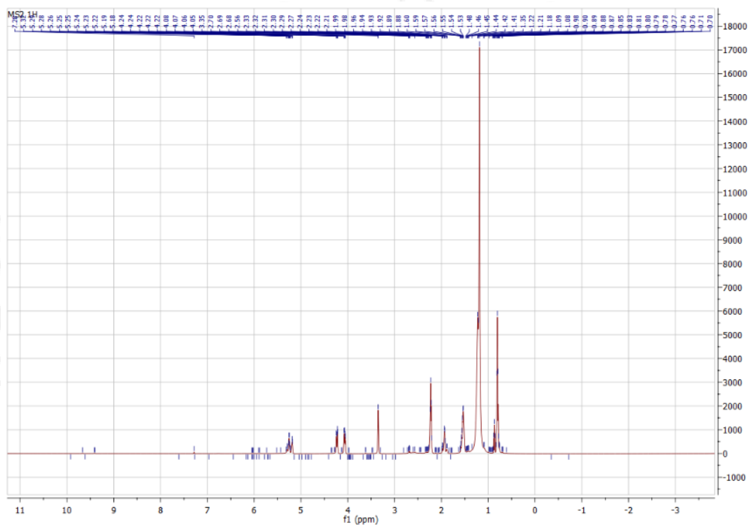
S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	PPU-PPE-Green gram-Sugar-Ghee	UMS1	Ghee:45g	Sugar: 45g	• Green gram flour: 30g • PPU:12.5g • PPE:2.5g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Rice flour: 45g • PPU:12.5g • PPE:2.5g • Roasted Chickpea Flour: 15g
2.	PPU-PPE-Green gram-Sugar-Butter	UMS2	Butter:45g		
3.	PPU-PPE-Green gram -Sugar-Soybean oil	UMS3	Soybean oil:22.5mL		
4.	PPU-PPE-Green gram-Sugar-Groundnut oil	UMS4	Groundnutoil:22.5mL		
5.	PPU-PPE-Green gram-Sugar-Cookie Shortening	UMS5	Cookie Shortening:45g		
6.	PPU-PPE-Green gram-Sugar-Coconut oil	UMS6	Coconut oil:22.5mL		
7.	PPU-PPE-Green gram-Jaggery-Ghee	UMJ1	Ghee:45g	Jaggery:33.75g	
8.	PPU-PPE-Green gram-Jaggery-Butter	UMJ2	Butter:45g		
9.	PPU-PPE Green gram -Jaggery-Soybean oil	UMJ3	Soybean oil:22.5mL		
10.	PPU-PPE Green gram -Jaggery-Groundnut oil	UMJ4	Groundnut oil:22.5mL		
11.	PPU-PPE-Green gram-Jaggery-Cookie Shortening	UMJ5	Cookie Shortening:45g		
12.	PPU-PPE-Green gram-Jaggery-Coconut oil	UMJ6	Coconut oil:22.5mL		
13.	PPU-PPE-Green gram -Honey-Ghee	UMH1	Ghee:45g	Honey:22.5mL	
14.	PPU-PPE Green gram -Honey-Butter	UMH2	Butter:45g		
15.	PPU-PPE-Green gram-Honey-Soybean oil	UMH3	Soybean oil:22.5mL		
16.	PPU-PPE-Green gram -Honey-Groundnut oil	UMH4	Groundnut oil:22.5mL		
17.	PPU-PPE-Green gram -Honey-Cookie Shortening	UMH5	Cookie Shortening:45g		
18.	PPU-PPE-Green gram -Honey-Coconut oil	UMH6	Coconut oil:22.5mL		
19.	PPU-PPE-Green gram -Artificial sweeteners-Ghee	UMA1	Ghee:45g	Artificial sweeteners: 4.16 g	
20.	PPU-PPE-Green gram - Artificial sweeteners -Butter	UMA2	Butter:45g		
21.	PPU-PPE-Green gram -Artificial sweeteners-Soybean oil	UMA3	Soybean oil:22.5mL		
22.	PPU-PPE-Green gram - Artificial sweeteners-Groundnut oil	UMA4	Groundnut oil:22.5mL		
23.	PPU-PPE-Green gram -Artificial sweeteners -Cookie Shortening	UMA5	Cookie Shortening:45g		
24.	PPU-PPE-Green gram -Artificial sweeteners-Coconut oil	UMA6	Coconut oil:22.5mL		

Table B4: Data summary of detailed ingredients composition of soy, unripe papaya pulp powder (PPU) and unripe papaya peel powder (PPE) based gluten-free cookies

S. No.	Cookie Formulation	Sample ID	Variable Fat Ingredient and their proportion	Variable Sweetening Agents	Fixed Ingredients and their Proportions
1.	PPU-PPE-Soy-Sugar-Ghee	USoS1	Ghee:45g	Sugar: 45g	• Soy flour: 30g • PPU:12.5g • PPE:2.5g • Roasted Chickpea Flour: 15g • Bengal gram Flour: 15g • Baking powder: 2g • Double toned milk: 7 mL
2.	PPU-PPE-Soy-Sugar-Butter	USoS2	Butter:45g		
3.	PPU-PPE-Soy -Sugar-Soybean oil	USoS3	Soybean oil:22.5mL		
4.	PPU-PPE-Soy -Sugar-Groundnut oil	USoS4	Groundnutoil:22.5mL		
5.	PPU-PPE-Soy -Sugar-Cookie Shortening	USoS5	Cookie Shortening:45g		
6.	PPU-PPE-Soy -Sugar-Coconut oil	USoS6	Coconut oil:22.5mL		
7.	PPU-PPE-Soy -Jaggery-Ghee	USoJ1	Ghee:45g	Jaggery:33.75g	
8.	PPU-PPE-Soy -Jaggery-Butter	USoJ2	Butter:45g		
9.	PPU-PPE-Soy -Jaggery-Soybean oil	USoJ3	Soybean oil:22.5mL		
10.	PPU-PPE-Soy -Jaggery-Groundnut oil	USoJ4	Groundnut oil:22.5mL		
11.	PPU-PPE-Soy -Jaggery-Cookie Shortening	USoJ5	Cookie Shortening:45g		
12.	PPU-PPE-Soy -Jaggery-Coconut oil	USoJ6	Coconut oil:22.5mL		
13.	PPU-PPE-Soy -Honey-Ghee	USoH1	Ghee:45g	Honey:22.5mL	
14.	PPU-PPE-Soy -Honey-Butter	USoH2	Butter:45g		
15.	PPU-PPE-Soy -Honey-Soybean oil	USoH3	Soybean oil:22.5mL		
16.	PPU-PPE-Soy -Honey-Groundnut oil	USoH4	Groundnut oil:22.5mL		
17.	PPU-PPE-Soy -Honey-Cookie Shortening	USoH5	Cookie Shortening:45g		
18.	PPU-PPE-Soy -Honey-Coconut oil	USoH6	Coconut oil:22.5mL		
19.	PPU-PPE-Soy -Artificial sweeteners-Ghee	USoA1	Ghee:45g	Artificial sweeteners: 4.16 g	
20.	PPU-PPE-Soy-Artificial sweeteners -Butter	USoA2	Butter:45g		
21.	PPU-PPE-Soy-Artificial sweeteners-Soybean oil	USoA3	Soybean oil:22.5mL		
22.	PPU-PPE-Soy-Artificial sweeteners-Groundnut oil	USoA4	Groundnut oil:22.5mL		
23.	PPU-PPE-Soy-Artificial sweeteners -Cookie Shortening	USoA5	Cookie Shortening:45g		
24.	PPU-PPE-Soy-Artificial sweeteners-Coconut oil	USoA6	Coconut oil:22.5mL		

Appendix C: ¹H-NMR Spectra of Grain-based Gluten-free Cookies During Storage Period

In this section, the ¹H-NMR spectra of fat extracted from the grain-based gluten-free cookies during storage period has been illustrated for an analysis of the fatty acid profile.



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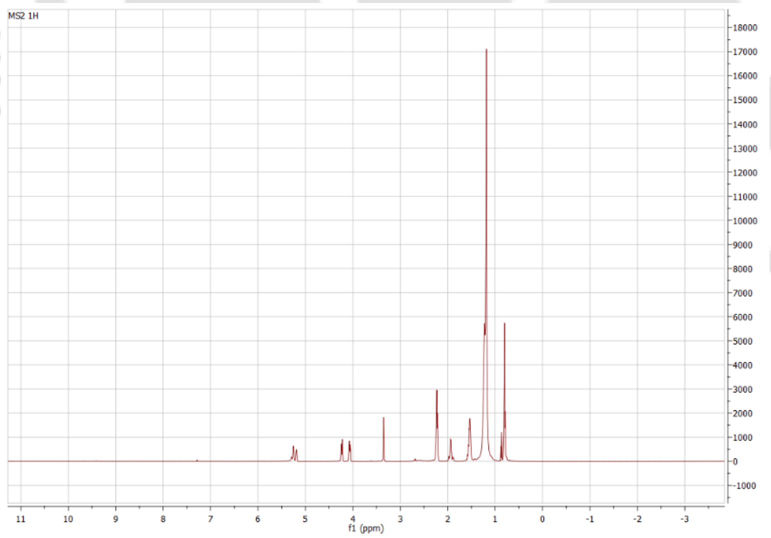
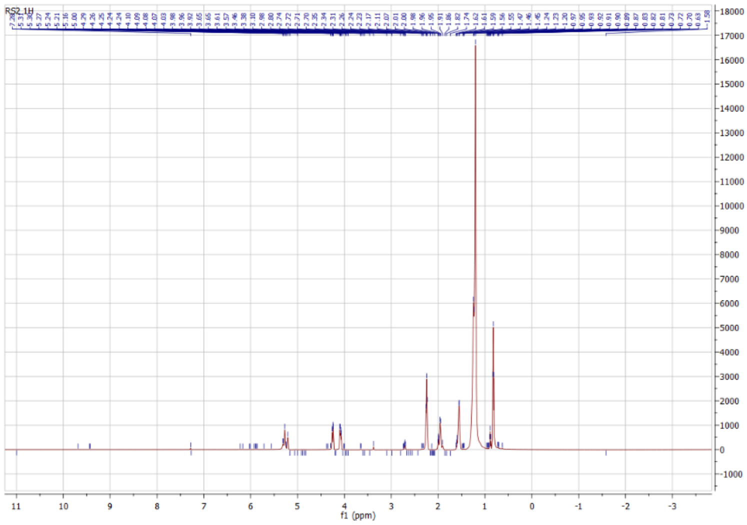


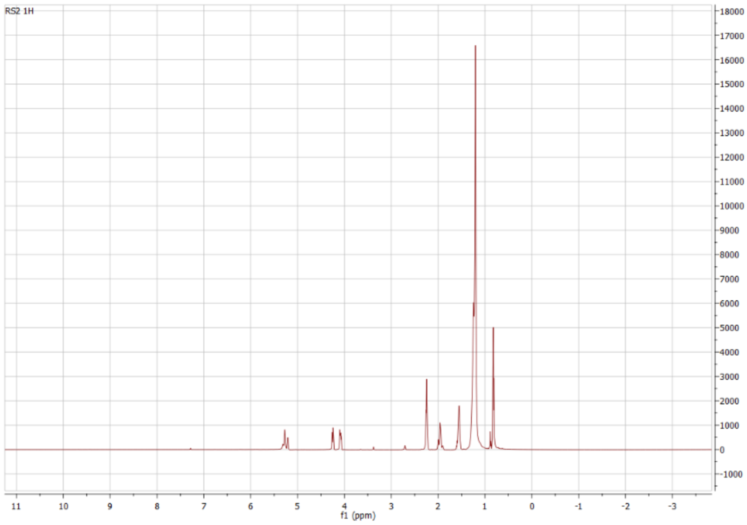
(b)

Figure C1: ¹H NMR graphs of fat extracted from the MS2 cookie sample a) 0th day; b) 60th day

Figure C1, C2, C3, C4 and C5 respectively depict the spectral profiles for zeroth day ('a' figure) and 60th day ('b' figure) for the MS2, RS2, WS1, WS2 and RaOaJ5 cookie formulations.

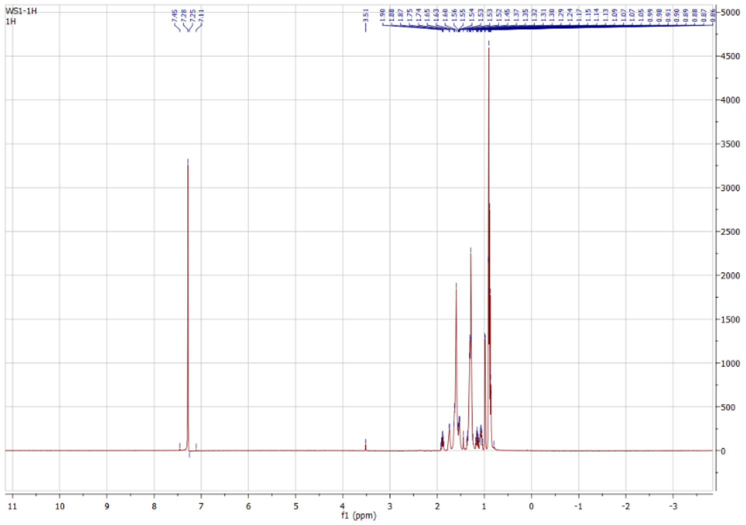


(a)



(b)

Figure C2: ^{1}H NMR graphs of fat extracted from the RS2 cookie sample a) 0th day; b) 60th day



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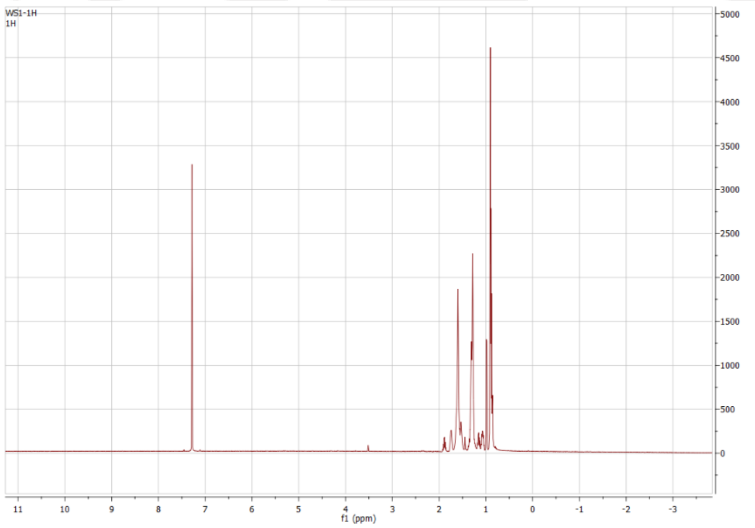
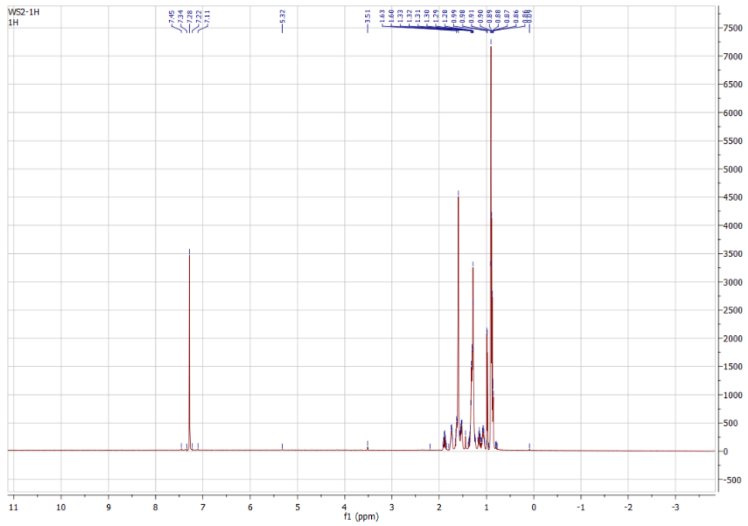


(b)

Figure C3: ^{1}H NMR graphs of fat extracted from the WS1 cookie sample a) 0th day; b) 60th day



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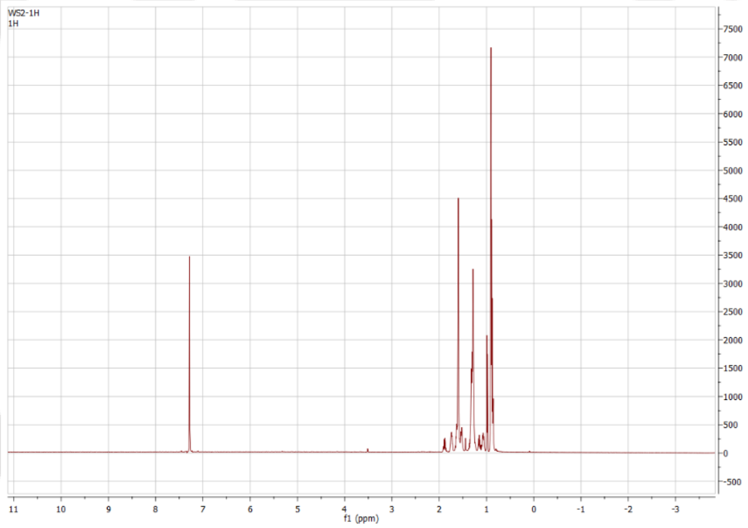
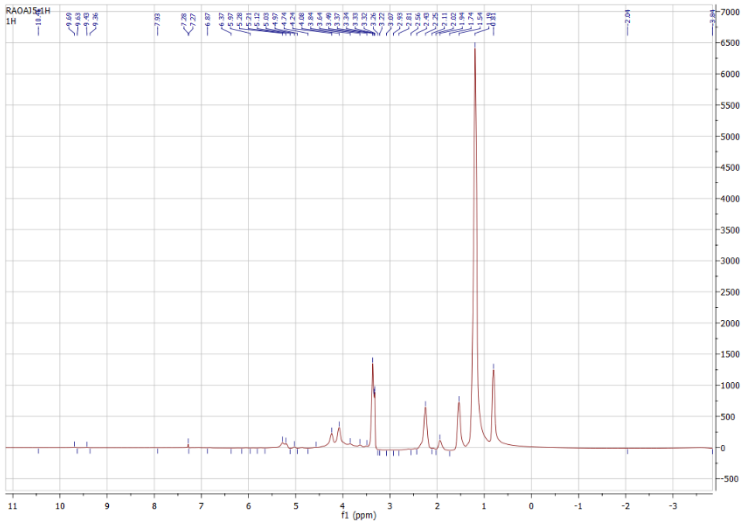
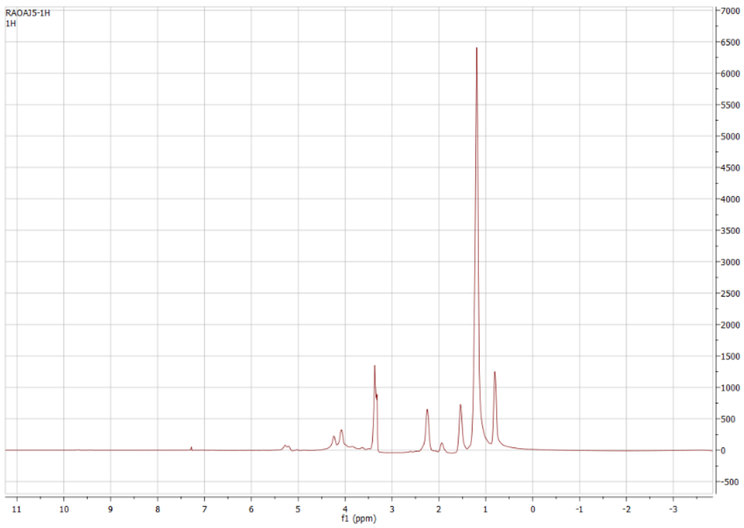


(b)

Figure C4: ^{1}H NMR graphs of fat extracted from the WS2 cookie sample a) 0^{th} day; b) 60^{th} day



(a)

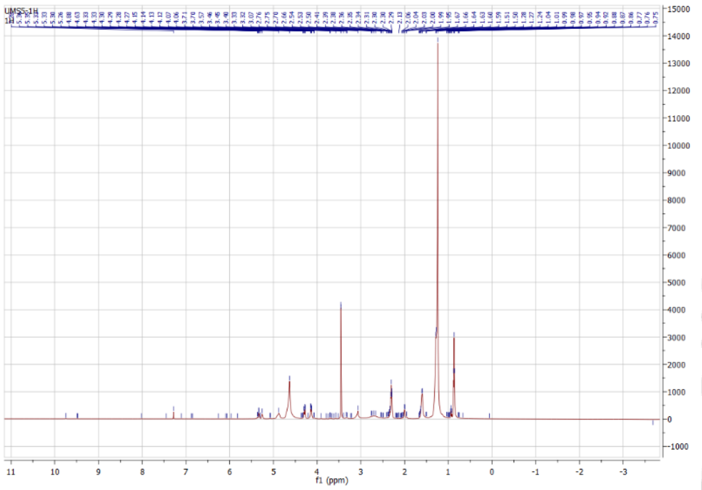


(b)

Figure C5: [1]H NMR graphs of fat extracted from the RaOaJ5 cookie sample a) 0th day; b) 60th day

Appendix D: ^{1}H-NMR Spectra of Unripe Papaya Pulp and Peel Powder based Gluten-free Cookie Formulations

In this section, the ^{1}H-NMR spectra of fat extracted from the unripe papaya peel and pulp



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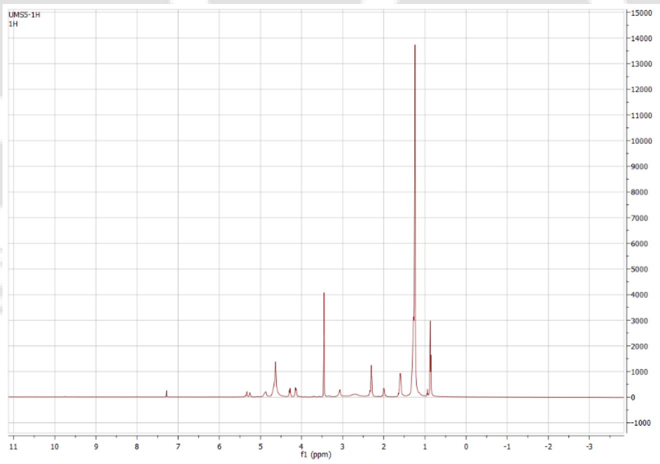
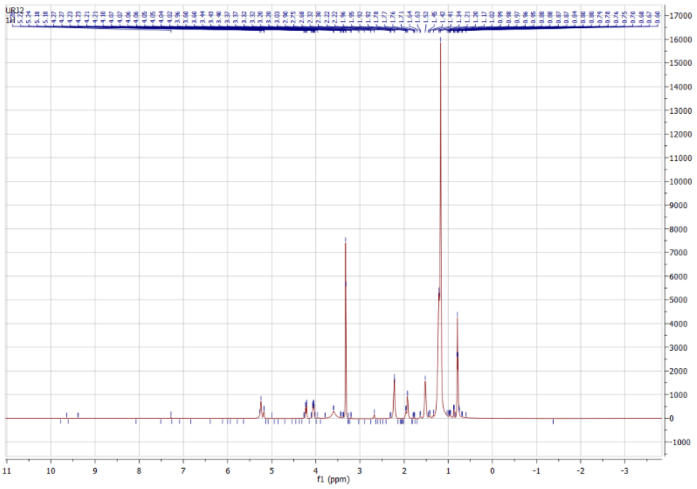


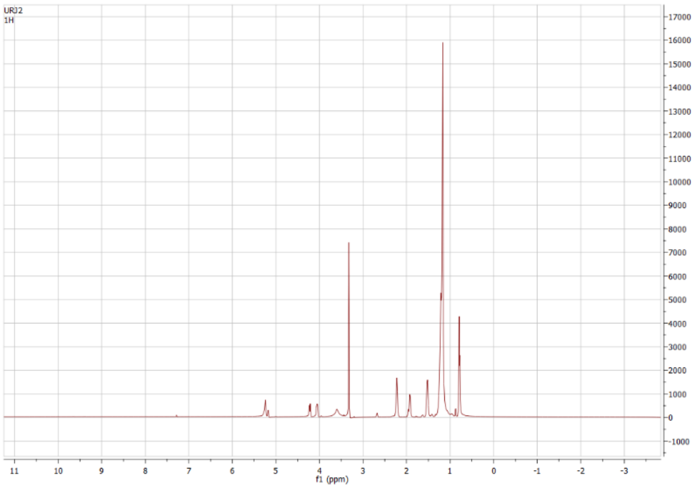
(b)

Figure D1: ^{1}H-NMR Spectra of fat extracted from the UMS5 cookie formulation for (a) 0th day and (b) 60th day storage period cases powder and grain-based gluten-free cookies during storage period has been illustrated for an analysis of the fatty acid profile.

Figure D1 and D2 respectively depict the spectral profiles for zeroth day ('a' figure) and 60th day ('b' figure) for the UMS5 and URJ2 cookie formulations.



(a)



(b)

Figure D2: ^{1}H-NMR Spectra of fat extracted from the URJ2 cookie formulation for (a) 0th day and (b) 60th day storage period cases.