A DISSERTATION ON

Development and shelf-life study of sensorially accepted microencapsulated *Spirulina* enriched cookies

SUBMITTED TO THE
DEPARTMENT OF BIOENGINEERING
FACULTY OF ENGINEERING
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT
FOR THE AWARD OF DEGREE OF
MASTER OF TECHNOLOGY
IN
FOOD TECHNOLOGY

BY

MADHULIKA CHARAN
M. Tech Food Technology (IV Semester)
Roll No: 2101207007

UNDER THE SUPERVISION OF

Dr. Alvina Farooqui
(Professor and Head)
Department of Bioengineering

INTEGRAL UNIVERSITY, DASAULI, KURSI ROAD
LUCKNOW- 226026

DECLARATION FORM

I, **Madhulika Charan**, a student of **M.Tech Food Technology** (2nd year/ 4th Semester), Integral University have completed my six months dissertation work entitled "**Development and shelf-life study of sensorially accepted microencapsulated *Spirulina* enriched cookies**" successfully from Integral University under the able guidance of **Prof. (Dr.) Alvina Farooqui.** I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Name and Signature of Student with Date

Ms. Madhulika Charan

Name and Signature of Course Coordinator with Date

CERTIFICATE

This is to certify that **Ms Madhulika Charan** (Enrollment Number 0900100809) has carried out the research work presented in this dissertation entitled "**Development and shelf-life study of sensorially accepted microencapsulated Spirulina enriched cookies**" for the award of M.Tech Food Technology from Integral University, Lucknow under our supervision. The dissertation embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of her **M.Tech Food Technology.**

I wish her good luck and bright future.

Prof. (Dr.) Alvina Farooqui
(Supervisor)
Professor and Head
Department of Bioengineering

CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Madhulika Charan**, a student of **M.Tech Food Technology** (2nd Year/ 4th Semester), Integral University has completed her six months dissertation work entitled "**Development and shelf-life study of sensorially accepted microencapsulated Spirulina enriched cookies**" successfully. She has completed this dissertation work from Integral University under the supervision of **Prof. (Dr.) Alvina Farooqui**, Department of Bioengineering, Integral University, Lucknow. The dissertation was a compulsory part of her **M.Tech. Food Technology.**

I wish her good luck and bright future.

Prof. (Dr.) Alvina Farooqui
Professor and Head
Department of Bioengineering
Faculty of Engineering

INTEGRAL UNIVERSITY

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TO WHOM IT MAY CONCERN

This is to certify that **Madhulika Charan**, a student of **M.Tech. Food Technology** (2nd Year/ 4th Semester), Integral University has completed her six months dissertation work entitled "**Development and shelf-life study of sensorially accepted microencapsulated Spirulina enriched cookies**" successfully. She has completed this work from Integral University under the guidance of **Prof. (Dr.) Alvina Farooqui**. The dissertation was a compulsory part of her M.Tech Food Technology.

I wish her good luck and bright future.

Prof. (Dr.) Alvina Farooqui
Professor and Head
Department of Bioengineering
Faculty of Engineering

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Place: Lucknow **Madhulika Charan**

Date:

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LIST OF ABBREVIATIONS

FAO	Food and Agricultural organisation
OSA	Octenyl Succinic Anhydride
SM	Spirulina Microencapsulation
SMA	Spirulina Microencapsulation Assisted by OSA
SeNPs	Selenium Nanoparticles
ABTS Radical	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid
DPPH Radical	2,2-diphenyl-1-picrylhydrazyl
FAME	Fatty Acid Methyl Ester
FID	Flame Ionisation Detector
PC	phycocyanin concentration
FTIR	Infrared Fourier Transform Spectroscopy
SEM	Scanning electron microscopy
OM	Optical microscopy
CC	Control cookies
SC	Spirulina Biomass Cookies
MSC	Microencapsulated Spirulina Cookies

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ABSTRACT

Our nutrition potential has finally caught up with our technology, ushering in a new era for the field of nutrition. More and more individuals are embracing organic foods and natural health. The organic food revolution is sweeping supermarket chains. We are nearing a critical mass of customers whose purchasing power is changing towards organic products. Functional foods or "Superfoods" are foods that have been intentionally modified or designed to provide specific health benefits beyond basic nutrition. These foods are typically fortified with additional nutrients or bioactive compounds, such as vitamins, minerals, fibre, probiotics, or antioxidants, which have been shown to promote health or reduce the risk of disease. Examples of functional foods include fortified cereals, yogurts with added probiotics, omega-3 fatty acids, and plant-based foods with phytochemicals. In this research we are identifying the benefits of one such superfood that is the *Spirulina plantensis*. Studies have confirmed the benefits of consuming Spirulina in measured quantities to suffice the requirement of daily proteins and vitamins for the human body. It is a good biosorbent for nutrients and therefore has numerous health benefits. In this research paper, we are trying to unlock the potential of Spirulina as a "Superfood" and prepare spirulina enriched cookies to enhance its Nutritional Value.

Keywords: *Spirulina Platensis*, Microencapsulated cookies, cyanobacteria, OSA, spray drying, sensorial acceptance

CHAPTER 1
INTRODUCTION

1.1 Overview

The Food and Agriculture Organization (FAO) defines sustainability as diets have low environmental impacts that contribute to food and nutrition security as well as a healthy lifestyle for current and future generations. Sustainable diets are both protective and beneficial. Respect for biodiversity and ecosystems, cultural acceptability, accessibility, and economic viability fair and affordable; nutritionally adequate, safe, and healthy; and maximizing natural and organic resources human capital *(Lonnie, M.,et al., 2019).*However, as industrialization and urbanization have increased, people have completely forgotten about their diet and daily nutrition, resulting in various macro and micro nutrient deficiencies. To overcome such deficiencies, they use a variety of synthetic drugs that can temporarily alleviate their symptoms but not permanently**.** *Spirulina platensis*, a species of microalgae is known for its high Calcium, vitamins and proteins level. *Spirulina* is a nutrient-rich superfood for admirable health. Superfoods are characterised as having additional health advantages and disease-preventing qualities to their natural nutritional factors.

Spirulina is the common name for human and animal food supplements produced primarily from two species of Cynobacteria, that is, *Spirulina platensis* and *Spirulina maxima*. *Spirulina* has many therapeutic properties such as hypocholestrolemic, immunological, antiviral and antiglutagenic effects. *Spirulina* is an excellent source of protein. *Spirulina* is the popular name for food supplements used by both humans and animals that are largely made from the cyanobacteria *Spirulina platensis* and *Spirulina maxima*. It contains 55-70 percent protein, 15-25 percent carbohydrates, 6-7 percent moisture and 8-13 percent minerals, 3-7 percent fat, and 8- 10 percent fibre.

Cyanobacteria are a diverse group of photosynthetic bacteria that are found in a variety of environments, ranging from freshwater and marine ecosystems to soil and even the skin of sloths! There are over 2,000 species of cyanobacteria, and they come in a wide range of shapes and sizes, from unicellular to multicellular forms.

Some strains of cyanobacteria are of particular interest due to their ability to produce unique secondary metabolites with potential applications in medicine, agriculture, and industry. For example, some strains of cyanobacteria produce toxins that can be harmful to humans and

animals, but other strains produce compounds that have anti-tumor, anti-inflammatory, and anti-microbial properties.

Cyanobacteria are also being investigated for their potential as a sustainable source of food and biofuels. They are capable of fixing atmospheric nitrogen and can be cultivated in a variety of environments, making them an attractive option for producing biomass for fuel and other applications.

Overall, cyanobacteria are a fascinating group of organisms with a wide range of potential applications in science and industry.

Unfortunately, food consumption and production have become more complex over time as food goes through intricate processes as well as longer and farther transportation. These unsustainable patterns have resulted in environmental degradation, accelerated climate change, and increased natural resource degradation, putting human health at risk *(Premanandh. 2011)*.

To address these issues, we hypothesized a new product development based on the fusion of *Spirulina* into multigrain cookies, which contains macro and micronutrients such as protein, iron, vitamins, carbohydrate, and so on.

Spirulina-based cookies are a unique and nutritious twist on traditional cookies, incorporating the vibrant blue-green microalgae known as *Spirulina*. *Spirulina* has gained popularity as a superfood due to its rich nutritional profile and potential health benefits. By incorporating *Spirulina* into cookie recipes, not only can you add an appealing color, but also enhance the nutritional value of the cookies. In this introduction, we will explore the key characteristics and potential benefits of *Spirulina*-based cookies, supported by relevant references.

Spirulina is a microscopic, spiral-shaped algae that grows in freshwater environments and has been consumed for centuries due to its nutritional value. It is rich in proteins, essential amino acids, vitamins, minerals, and antioxidants. The incorporation of *Spirulina* into cookies offers an opportunity to introduce these beneficial nutrients into a popular and convenient snack option (Gustiani, et al., 2019).

The inclusion of *Spirulina* in cookie recipes provides several potential health benefits. Firstly, *Spirulina* is a complete protein source, containing all essential amino acids required by the human body. This makes *Spirulina*-based cookies a suitable option for individuals seeking alternative plant-based protein sources (Miranda, et al., 2018). Additionally, *Spirulina* is known

for its antioxidant content, including compounds like phycocyanin and beta-carotene, which can help combat oxidative stress and promote overall health (Lau, et al., 2016).

Furthermore, *Spirulina* is a natural source of vitamins and minerals. It contains vitamins B, iron, magnesium, and other essential nutrients (*Gustiani, et al., 2019*). The incorporation of *Spirulina* into cookies allows for the introduction of these beneficial compounds, providing a convenient way to boost nutrient intake.

Spirulina-based cookies also cater to individuals with specific dietary preferences or restrictions. They can be suitable for those following vegan, vegetarian, or gluten-free diets, depending on the recipe formulation. The natural green color of *Spirulina* eliminates the need for artificial food dyes, appealing to those seeking more natural and wholesome food options (Ismail, et al., 2017).

In terms of flavor, *Spirulina* adds a unique earthy taste to cookies. This flavor can be complemented by other ingredients such as desiccated coconut, seeds, dried fruits, or chocolate, and or coconut oil allowing for diverse flavor combinations (Ismail, et al., 2017). The versatility of *Spirulina*-based cookies enables customization to suit different tastes and preferences.

1.2 Scope of this Research

This study is to explore the role of nutraceutical factors present in *Spirulina platensis* based multigrain cookies

1.3 Objective

1. Spray drying microencapsulation of *Spirulina platensis* biomass using octenyl succinic anhydride (OSA) and its incorporation in cookies for enhancing the taste.
2. Functional characterization of microencapsulated *Spirulina platensis* enriched cookies.
3. Replacement of fat with coconut oil to improve the shelf life and overall texture of the cookies.

CHAPTER 2

REVIEW OF LITERATURE

Traditional multigrain cookies are a popular baked treat that incorporates a variety of grains and seeds. These cookies offer a wholesome and nutritious alternative to traditional cookies by providing additional fibre, vitamins, minerals, and healthy fats. Microalgal biomass applications range from production of food and feed to high-value products for biotechnological applications (*Michele Greque de Morais et al., 2015*). Due to their enormous biodiversity as well as biochemical and molecular strategies for dealing with stress, microalgae can synthesize various bioactive chemicals (*R. Harun et al., 2010*). Among the various groups of microalgae, cyanobacteria are an exceptional source of bioactive compounds (*Wan-Loy Chu, 2012*). Over the last 30 years, microalgae biotechnology has developed and diversified significantly (*J.A.V. Costa et al., 2011*). For example, *Spirulina* has been used to feed indigenous people in Mexico and Chad (Africa) since ancient times. In Mexico, *Spirulina* was collected from Lake Texcoco and used to produce a cake referred to as Tecuitlatl, which means the stone's excrement. In Chad*, Spirulina* was harvested from the alkaline lake Kossorom and used for preparing a cake referred to as dihe (*Barsanti, L., et al., 2014; G. Abdulqader et al., 2000*). In addition to use as a consumable food product, *Spirulina* (*A. Vonshak, 1997*) is also useful as a functional ingredient because the biomass can be incorporated in various food products to enhance nutritional quality and for therapeutic action on chronic diseases (*Wan-Loy Chu, 2012; Iyer, U. M. Iyer et al., 2007*).

2.1 An overview of traditional multigrain cookies

Traditional multigrain cookies typically include a combination of various grains and seeds, along with traditional cookie ingredients. Common grains used in multigrain cookies include whole wheat flour, oats, barley, quinoa, and cornmeal. Seeds like flaxseeds, chia seeds, sesame seeds, and sunflower seeds are often added for their nutritional value. Other typical ingredients include sugar, butter or oil, eggs, vanilla extract, and leavening agents such as baking powder or baking soda.

Multigrain cookies offer several nutritional benefits compared to regular cookies. The inclusion of whole grains provides fibre, which aids in digestion, promotes satiety, and helps maintain stable blood sugar levels. These cookies are also rich in vitamins and minerals, including B vitamins, iron, magnesium, and zinc, which are essential for overall health. The addition of

seeds further enhances the nutritional profile by providing healthy fats, protein, and additional vitamins and minerals.

Traditional multigrain cookies often have a heartier texture compared to regular cookies due to the presence of whole grains and seeds. They can be slightly denser and have a more satisfying chew. The flavour of these cookies can vary depending on the grains and seeds used but is generally nutty and wholesome.

There are numerous recipe variations for multigrain cookies, allowing for customization based on personal preferences. Some recipes may include dried fruits, nuts, or spices to add more flavor and texture. Sweeteners can also be adjusted to suit individual taste preferences or dietary needs, with options like honey, maple syrup, or alternative natural sweeteners.

Multigrain cookies can be found in health food stores, specialty bakeries, or made at home. Making them from scratch allows for full control over ingredient choices and customization.

2.2. An overview on *Spirulina*

Spirulina is a type of blue-green algae that has gained popularity as a superfood due to its impressive nutritional profile. It has been consumed for centuries and is recognized for its potential health benefits. Here's an overview of *Spirulina,* supported by references:

Nutritional Composition: *Spirulina* is rich in various essential nutrients. It is an excellent source of complete protein, containing all essential amino acids needed by the body. It is also a good source of vitamins, including vitamin B12, provitamin A (beta-carotene), vitamin K, and minerals such as iron, magnesium, and potassium. *Spirulina* is known for its high content of phycocyanin, a potent antioxidant compound responsible for its vibrant blue-green color. Khan, Z., Bhadouria, P., & Bisen, P. S. (2005). Nutritional and therapeutic potential of *Spirulina*. Current Pharmaceutical Biotechnology, 6(5), 373-379.

Antioxidant and Anti-inflammatory Properties: *Spirulina* contains a range of antioxidants, including phycocyanin, chlorophyll, carotenoids, and vitamin E. These compounds help neutralize harmful free radicals and reduce oxidative stress in the body. Studies have also suggested that *Spirulina* exhibits anti-inflammatory effects, which may help alleviate chronic inflammation and related conditions. Belay, A. (2002). The potential application of *Spirulina (Spirulina*) as a nutritional and therapeutic supplement in health management. Journal of Applied Phycology, 14(2), 143-151.

Immune System Support: *Spirulina* has been shown to stimulate and modulate the immune system. It contains bioactive compounds that may enhance immune cell function, such as phycocyanin and polysaccharides. These compounds have demonstrated immune-boosting effects in various studies, potentially enhancing the body's defense against infections and diseases. Deng, R., & Chow, T. J. (2010). Hypolipidemic, antioxidant, and antinflammatory activities of microalgae *Spirulina*. Cardiovascular Therapeutics, 28(4), e33-e45.

Potential Anti-Cancer Properties: Some research suggests that *Spirulina* may have anti-cancer properties. Animal and test-tube studies have demonstrated that *Spirulina* extracts and specific compounds present in *Spirulina* exhibit potential anti-cancer effects. However, more research is needed to fully understand the mechanisms and effects of *Spirulina* on cancer prevention and treatment in humans.Pabon, M. M., et al. (2019). *Spirulina* as a source of phycocyanobilin—antioxidant with anticancer properties. Medicinal Chemistry Research, 28(6), 853-870.

Safety Considerations: *Spirulina* is generally considered safe for consumption. However, individuals with specific health conditions, such as phenylketonuria (PKU), or those on certain medications should exercise caution and consult a healthcare professional before taking *Spirulina* supplements. Additionally, it is crucial to source *Spirulina* from reputable manufacturers to ensure product quality and safety.

Torres-Duran, P. V., Ferreira-Hermosillo, A., & Juarez-Oropeza, M. A. (2007). Antihyperlipemic and antihypertensive effects of *Spirulina* maxima in an open sample of mexican population: a preliminary report. Lipids in Health and Disease, 6(1), 33.

It is worth noting that while *Spirulina* offers various potential health benefits, it should not replace a balanced diet or medical treatments.

Abdel-Moneim, A. M et al., (2022) examined the biological selenium nanoparticles (SeNPs) produced by Bacillus subtilis AL43 as well as three *Spirulina* extracts (methanol, acetone, and hexane) for their antibacterial and antioxidant properties. The outcomes demonstrated that tested pathogens were resistant to *Spirulina* extracts' antibacterial effects. Additionally, *Spirulina* extracts substantially and dose-dependently scavenged ABTS and DPPH radicals. In comparison to other extracts, the methanolic extract demonstrated higher total phenolic content, antibacterial activity, and antioxidant activity. Bacillus subtilis AL43 produced the selenium nanoparticles in an anaerobic environment, and they were identified as spherical, crystalline, 65.23 nm in size, with a net negative charge of 22.7. By testing SeNPs against three gram-positive, three gram- negative, and three strains each of Candida and Aspergillus spp.,

we demonstrated that SeNPs have significant antibacterial activity. Furthermore, SeNPs demonstrated dose-dependent scavenging of ABTS and DPPH radicals. There is a correlation between the biological activities of SeNPs and the total phenolic content of *Spirulina*. Our findings show that *Spirulina* and SeNPs have substantial antibacterial and antioxidant properties and appear to be viable options for dependable and safe medicinal uses.

Mullenix, G. J., et al., (2022) found that alternative sources of protein, besides soybean meal, are a long-term issue for commercial broiler producers. These substitute sources of protein must be high in protein, have a balanced amino acid profile, be very easily digestive, be safe for the bird's nutrition, and ideally have some other intrinsic benefit. Microalgae called *Spirulina* platensis is losing favor because of its high protein content, advantages for health, and negative effects on the environment. To ascertain the impact of *Spirulina* inclusion in reduced crude protein diets on broiler growth, carcass yields, breast fillet color, breast myopathy, and footpad quality, two trials (in female and male Ross 708 broilers) were carried out. The findings demonstrated that reducing crude protein decreased carcass yield in both studies, while negatively affecting male birds' growth performance more so than female birds. *Spirulina* supplementation at 10% in a diet with less protein enhanced male broiler footpad scores and raised meat and skin coloration across the board. Costs will always be a factor in determining whether *Spirulina* is used commercially, but this work helps advance basic research that will enable that.

Sankarapandian, V.,et al., (2022) stated that microalgae-based value-added goods are becoming more and more popular on the market as a result of their ability to reduce reliance on fossil fuels and expensive chemicals. This study aimed to create prebiotic compounds from the microalgae *Spirulina* sp. To achieve this. The microalgae were taken out of the fresh water and molecularly examined. The isolated isolates' dry biomass, chlorophyll content, phycocyanin, cytotoxicity, antibacterial, and antioxidant activities were examined. Additionally, because of their high nutritional content, value-added goods such as *Spirulina* cake, chocolate, tea, vermicelli, and *Spirulina* juice were created for a vulnerable population.

Koli, D. K et al., (2022)studied to enhance pasta made from semolina, locally grown *Spirulina* powder was used at percentages ranging from 2 to 15%. With the addition of *Spirulina*, green color pasta was created with nutritional and functional fortification, increasing the amount of protein, total phenols, flavonoids, iron, and calcium by up to 77.47 percent, 76.62 percent,

162.88 percent, and 57.27 percent, respectively, without negatively affecting the textural and sensory qualities. A FAME study found that enriched pasta had levels of –linolenic acid and docosahexaenoic acid that were 2 to 2.5 times higher. Additionally, phenolics, flavonoids, and antioxidant activity significantly improved when compared to control pasta. Proteins and other nutrients were not significantly lost during cooking, according to an analysis of theoretical and actual composition. Principal components analysis showed that *Spirulina*, especially at higher doses, significantly contributed to nutritional and functional aspects. Pasta with 12.5% added sugar *Spirulina* received a "loved very highly" rating, and a significant percentage of people planned to purchase it. As a preferable alternative to improve health and ward off disease, *Spirulina* enrichment at concentrations over 10 percent (12.5%) with considerable increases in nutritional and functional qualities without impacting textural or culinary quality and acceptable sensory evaluation may be used. Consuming green pasta with *Spirulina* may be a viable option to improve the livelihood and nutritional security of rural poor people as well as a good alternative for hidden hunger alleviation programs for mass nutrition, especially for infants and children. Green is a color that represents freshness, hope, renewal, and physical health.

Trotta, T et al., (2022) focuses on the function of *Spirulina* in the brain, emphasizing how it exerts its advantageous anti-inflammatory and antioxidant effects, acting on glial cell activation, and in the prevention and/or progression of neurodegenerative diseases, in particular Parkinson's disease, Alzheimer's disease, and Multiple Sclerosis. Because of these properties, *Spirulina* could be thought of as a potential natural drug. A small filamentous cyanobacterium called *Spirulina* thrives in alkaline water sources. Due to its high amounts of functional components, including phycocyanins, phenols, and polysaccharides, which have anti-inflammatory, antioxidant, and immunomodulating activities both in vivo and in vitro, it is widely used as a nutraceutical food supplement around the world. Numerous scientific articles have claimed that it has beneficial benefits on a variety of pathologies, including cancers, inflammatory illnesses, obesity, hypertension, hypercholesterolemia, and glycemia. Recent research has shown that *Spirulina* has neuroprotective effects on the neural system's growth senility, and several pathological illnesses, including neurological and neurodegenerative diseases.

Lafarga, T., et al., (2020) mentioned that *Spirulina* use by humans is not new; in the sixteenth century, it was gathered from Lake Texcoco and consumed in Tenochtitlan markets (today Mexico City). Microalgae are now used in a wide variety of food compositions. For their

marketing or as a coloring agent, the majority of these employ microalgae. *Spirulina* (and chemicals obtained from it) have the potential to be employed as ingredients in the creation of innovative foods, one of the biggest developments in the food industry. *Spirulina* has the potential to be utilized in the prevention or treatment of illnesses connected to metabolic syndrome, according to several human intervention studies. Reviewing the present and potential uses of these microalgae in the food and functional food industries were the goal of the current article. *Spirulina* consumption advantages and/or some of the most significant chemicals generated from *Spirulina* were also covered.

Marzieh Hosseini, Set.al.,(2013)founded that *Spirulina* and its derivatives have applications in agriculture, food processing, medicine, science, and cosmetics. It contains a lot of macro and micronutrients. *Spirulina* has been shown to have a number of pharmacological activities. This review article serves as an overview, introducing medical applications within each usage, providing a basic description of the involved disease, as well as the mechanism of action and application. *Spirulina*-infused foods also have improved stability, antioxidant rheological properties, and anti-staling properties. When the desired colour is green, S. platensis proved to be a good stable ingredient. All possible applications of *Spirulina* platensis in human food are discussed, including beverages, bakery products, candy, gel desserts, dairy, and confectionary.

There have been many studies in the literature aiming at developing food products nutritionally enriched by the addition of *Spirulina*, such as extruded snacks (Lucas, Morais, Santos, & Costa, 2018), snack bars (Lucas et al., 2020), yogurt (Da Silva et al., 2019), pasta (Ozyurt ¨ et al., 2015) and cookies (Batista et al., 2017; Bolanho, Egea, Campos, De Carvalho, & Danesi, 2014; Singh, Singh, Jha, Rasane, & Gautam, 2015). One of the limitations for *Spirulina* addition in food products reported by many of these studies is its undesirable impact in sensory quality. The biomass typically confers an unpleasant flavor and aroma, in addition to a green color appearance, which is also usually undesirable. Therefore, aiming at preserving the sensorial quality, a maximum of 10% of *Spirulina* biomass addition has been reported (Ozyurt ¨ et al., 2015), with most studies adding lower amounts (from 2% to 7%). In this context, spray-drying microencapsulation, one of the encapsulation techniques most used in the food industry, could be used to solve this problem by masking these undesirable sensorial attributes. Recently, Da Silva et al. (2019) reported the technological and sensorial advantages of incorporating yogurt with S. platensis microencapsulated with maltodextrin crosslinked with citric acid over its direct use. These authors, however, incorporated only 1% of *Spirulina* biomass, since their focus was on functional properties rather than nutritional enrichment. To the best of our

knowledge, the use of microencapsulation as a strategy to allow the addition of higher amounts of *Spirulina* biomass to food products, thus increasing their nutritional profile, while maintaining their sensorial quality, has not been investigated so far. Thus, the aim of this study was to develop nutritionally enriched and sensorially well-accepted cookies by incorporating spray-dried microencapsulated *S. platensis* biomass.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1 Growth and Maintenance of *Spirulina* Culture

Spirulina culture which was isolated from paddy field near agricultural farm Institute of Integral University in **Plant Tissue Culture (PTC)** lab, Algal Biotechnology and Stress Biochemistry, **Integral Information and Research Centre (IIRC)**, at **Integral University, Lucknow** and the strain no. is **IUAF001** submitted in NCBI. The culture was grown using Zarrouk's media.

Table 3.1 Lists of Glass ware/ Tools used

S.No.	Glass ware	Specification	Quantity	Company
1.	Petri plates	7.5cm Diameter	15	**Borosil**
		10 cm Diameter	2	
2.	Conical Flask	50 ml	6	**Borosil**
		100 ml	2	
		250 ml	4	
		500 ml	2	
		1000 ml	1	
		2000 ml	1	
3.	Beaker	50 ml	2	**Borosil**
		100ml	1	
		250ml	3	
		500ml	5	
4.	Knife	Stainless Steel	1	**Agaro**
5.	Chopping board	Wooden	1	**Floraware**
6.	Pointed Object	Needle	6	**Pony**
7.	Stainless steel utensils	Cook and serve big bowl	1	**Hawkin**
		Table spoons	3	
		Laddel	1	

Table 3.2 List of instruments used

S. No	Instrument	Model no./Version	Company
1.	Weighing balance	ALE-223	K- Roy
2.	Microwave	MC-7148MS	SAMSUNG
3.	Electronic Balance	MSW10A/VA	WENSAR
4.	Sieve	ASTM Standards	MAHEK INDUSTRIES
5.	Centrifuge	R8C Remi-frequency	J .S. enterprises
6.	Spectrophotometer	Alpha II (210966)	BRUKER
7.	Refractometer	HR-05	HM digital
8.	Magnetic Stirrer	KM057	BERXCO
9.	Digital Vernier Calipers	DIGE 150	z Hart
10.	OPUS Version	8.5 (Service Pack 1) Build 8.7.10	BRUKER

3.2 Zarrouk's media – It is a popular combination of salts and minerals used in *Spirulina* cultivation as the building blocks that help *Spirulina* grow and thrive.

Table 3.3- Lists of Chemicals used for the preparation of Zarrouk's media

1.	$NaHCO_3$(Sodium bicarbonate)	16.8gm/L	33.6gm/2L
2.	$NaNO_3$ (Sodium nitrate)	2.5gm/L	5.0gm/2L
3.	K_2SO_4 (potassium sulphate)	1.0gm/L	2.0gm/2L
4.	K_2HPO_4(dipotassium phosphate)	0.5gm/L	1.0gm/2L
5.	NaCl (sodium chloride)	1.00gm/L	2.0gm/2L
6.	$MgSO_4.7H_2O$(magnesium sulphate)	0.2gm/L	0.4gm/2L
7.	$CaCl_2.7H_2O$(Calcium Chloride)	0.04gm/L	0.08gm/2L
8.	$FeSO_4.7H_2O$(Ferrous Sulphate)	0.01gm/L	0.02gm/2L
9.	EDTA	0.08gm/L	0.16gm/2L

The media was prepared for 2 liters

3.3 *S. platensis* (*Spirulina*) Preparation

Zarrouk's Media was used for the growth of *Spirulina*. The optimal growth yield of the *Spirulina* were obtained at pH level of 9.5, while maintaining temperature of 20±5°C and light intensities of 2000 lux with time interval of 10 to 14 hour of lightness and darkness respectively.

3.3.1 Growth Study

The growth of the *Spirulina* culture was taken for 12 days using a spectrophotometer in which optical density was taken at 650nm. The wavelength was used to measure the amount of chlorophyll absorption in the *Spirulina*.

The growth curve of *Spirulina* platensis, like many microorganisms, typically follows a pattern of four main phases: lag phase, exponential phase, stationary phase, and death phase.

1. **Lag Phase**: This is the initial phase of the growth curve, where the cells are introduced into a new environment, and they are acclimating to the conditions. During this phase, the cells may not show significant growth as they adjust to the available nutrients and environmental conditions.

2. **Exponential Phase**: Once the cells have adapted, they enter the exponential phase, also known as the log phase. In this phase, the cells begin to reproduce rapidly, and the population size increases exponentially. *Spirulina* platensis, being a fast-growing microorganism, demonstrates a high rate of cell division during this phase.

3. **Stationary Phase**: As the exponential phase continues, the growth rate starts to slow down due to factors like depletion of nutrients, accumulation of waste products, and limited space in the growth medium. The growth rate eventually stabilizes, and the number of dividing cells equals the number of dying cells. The population reaches a state of equilibrium, and the cell density remains relatively constant.

4. **Death Phase**: In the death phase, the number of dying cells exceeds the number of dividing cells, and the population starts to decline. This phase may be triggered by adverse environmental conditions, nutrient depletion, or other stress factors.

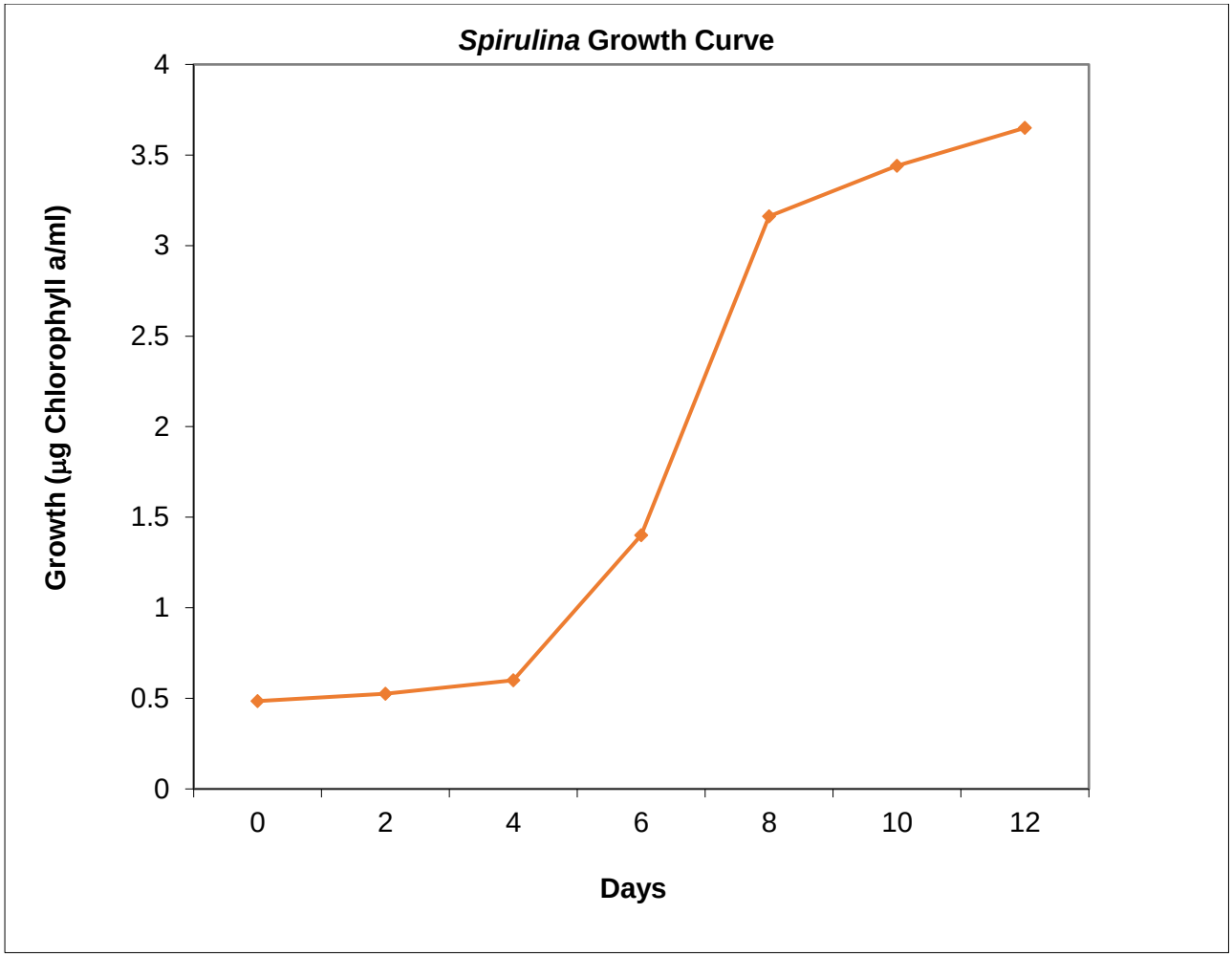


Fig. 3.1. *Spirulina Platensis* Growth Curve

The growth curve of *Spirulina platensis* is influenced by various factors, including temperature, light intensity, pH levels, nutrient availability, and culture conditions. To optimize the growth of *Spirulina* platensis, it is crucial to provide the right combination of these factors to ensure a healthy and productive culture.

Understanding the growth curve of *Spirulina* platensis as shown in Figure 4.7 is essential for its successful cultivation in various applications, including food production, nutritional supplementation, and wastewater treatment, among others. By carefully managing the growth conditions, researchers and cultivators can harness the full potential of *Spirulina platensis* as a valuable microorganism with numerous benefits.

3.4 Chemical characterization of *Spirulina* Biomass

Total protein content was determined according to Lowry, Rosebrough, Farr, & Randall (1951), modified by Mota, Souza, Bon, Rodrigues, & Freitas (2018). Total lipid content was determined according to Folch, Lees, & Stanley (1956). Ash content was determined according to the AOAC (2000). Total carbohydrates were determined by difference. Energy values were calculated from the contents of lipids, proteins and carbohydrates multiplied by the Atwater factors. Each sample was analyzed in triplicate. For minerals analysis the biomass (100 mg) was mineralized with HNO3 (Suprapur, Merck KGaA, Darmstadt, Germany) in a microwave oven. Sodium, potassium, phosphorous, calcium, magnesium, zinc, copper, iron, and manganese contents were determined by ICP-OES. For amino acids analysis, proteins were extracted according to Vasconcelos et al. (2005). For protein hydrolysis, samples were added with 7 M urea and 2 M thiourea, dried in a vacuum centrifuge for 15 min at 40 ◦C and added with 6 M HCl containing 0.1% phenol. The flasks atmosphere was modified to N2 and heated to 110 ◦C for 24 h. Amino acids profile was determined by LC-MS (Q-Exactive Plus, Thermo Scientific). Tryptophan could not be detected due its destruction during hydrolysis. Fatty acids profile was determined by analyzing the methyl esters (FAME) obtained after transesterification by GC-FID (GC-2010, Shimadzu®, Japan), as described by Lepage & Roy (1986). Identification was performed by comparison of retention times with that of a commercial FAME mix standard (Supelco® 37 Component FAME Mix, Sigma-Aldrich, Brazil)

3.5 Samples, standards and reagents

***Spirulina platensis* was acquired** from **Plant Tissue Culture (PTC)** lab, Algal Biotechnology and Stress Biochemistry, **Integral Information and Research Centre (IIRC)**, at **Integral University, Lucknow**.

3.6 Proximate analysis and chemical characterization of *Spirulina platensis*

3.6.1 Nutritional composition Proximate analysis (proteins, lipids, carbohydrates and ashes) was performed following official methods (AOAC, 2016): Protein content by macro-Kjeldahl method (N × 6.25); lipid content by Soxhlet extraction with petroleum ether; ash content by incineration at 550 ± 15 °C. Results were presented in g/100 g dw in all cases.

Carbohydrates were calculated as the difference of the sum of protein, lipid and ash content to 100 g dw. Energy was calculated according to the Atwater system following the equation: energy = 4 × (g protein + g carbohydrate) + 9 × (g fat), and expressed in kcal/100 g dw (Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004)

3.6.2. Chemical composition

3.6.2.1. Organic acids. Organic acids are compounds that were separated in a Sphere Clone (Phenomenex, Torrance, CA, USA) reverse phase C18 column (5 µm, 250 mm × 4.6 mm i.d) at 35 °C. After being eluted (3.6 mM sulphuric acid solution, at 0.8 mL/min) compounds were analysed by diode array detection at 215 nm (245 nm for ascorbic acid). Quantification was based in calibration curves obtained from commercial standards (ascorbic acid, citric acid, malic acid, oxalic acid, and quinic acid). Results were expressed in mg/ 100 g dw.

3.6.2.2. Fatty acids. After performing a transesterification step according to Barros et al. (2013), the resulting fatty acid methyl esters were profiled using a DANI 1000 gas chromatographer (GC) equipped with a split/splitless injector (1:40, 250 °C), a flame ionization detector (FID at 260°C), and a 50% cyanopropyl-methyl-50% phenylmethylpolysiloxane column (30 m × 0.32 mm i.d. × 0.25 µm df) (Macherey-Nagel, Düren, Germany). Hydrogen was used as the carrier gas (4.0 mL/min, 0.61 bar, 50 °C) and compounds were identified by comparing their relative retention times with those of a FAME standards mixture. Results are expressed as relative percentage.

3.6.2.3. Phycocyanin. Phycocyanins were extracted by the freezing and thawing method as described by Saran, Puri, Jasuja, and Sharma (2016), with some modifications. Different solvents were tested (distilled water, acetate buffer 0.1 M pH 6.0; phosphate buffer 0.1 M pH 7.0 and ethanol:water mixture 30:70 v/v). Three cycles were performed: freezing at −20 °C (1 h) and thawing at room temperature (first cycle: 15 min; second cycle: 30 min; last cycle: 45 min). After the thawing cycles, samples were centrifuged (6000 rpm, 15 min) and the phycocyanin content calculated as (results in mg/mL): PC = (Abs615 − 0.474Abs652/5.43) (Bennett & Bogorad, 1973). The purity of the extracted phycocyanin was determined by the Abs615/Abs280 ratio (Abs615: phycocyanin concentration; Abs280: total protein concentration). Extraction yield was calculated as: YP = (PC × V/S), where PC is the phycocyanin concentration, V the solvent volume and S the initial weight of *Spirulina*.

3.7 Microencapsulation of *Spirulina* platensis

Microencapsulation of *Spirulina* was carried out using octenyl succinic anhydride (OSA) starch as wall material in a ratio of 1:1 (w/w). A 5% (w/v) solution of *Spirulina* was progressively added to an OSA starch solution while stirring at 300 rpm for 30 min and dried in a Mini Spray Dryer with inlet temperature of 220 °C, nozzle diameter of 7 mm and pump flow of 6 mL/min.

3.7.1 Spray-drying microencapsulation process

The microencapsulation process of *Spirulina* was carried out using octenyl succinic anhydride (OSA) or octenyl succinic anhydride cross-linked with citric acid as encapsulating materials, named SM and SMA samples respectively. The crosslinking reaction involves the reaction of hydroxyl groups from OSA with carboxylic groups from citric acid (esterification reaction), and it was performed by spray drying process, according to the methodology developed by Francisco et al. (2018). A blank of SMA sample was also prepared (MA) consisting of OSA crosslinked with citric acid, but without S. platensis. The used OSA/S. platensis ratio was 1:1 (w/w). Briefly, for SM (Octenyl succinic anhydride/S. platensis), 15.75 g of *S. platensis* were dissolved in 150 mL of distilled water and added with 15.75 g of OSA. For SMA (OSA /S. platensis/citric acid), the previous mixture was added to 1.58 g of citric acid. These solutions were prepared just before the atomization. The used equipment was a Mini Spray Dryer B-290 Büchi (Flawil, Switzerland) set in the normal operation mode (nozzle diameter: 0.7 mm; atomized volume: 150 mL; solids content < 33%). The equipment conditions were: inlet temperature 170 °C, outlet temperature 95 °C, aspiration 90% and pump 20% (6 mL/min). The obtained samples were collected and kept in containers protected from light (4 °C) until further analysis. The overall yield was estimated as the ratio between the weight of recovered microspheres (dry basis) and the weight of atomized materials (dry basis) (OSA , *S. platensis* and citric acid, when applied).

3.7.2 Characterization of the microencapsulated samples

Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra of the base materials (S. platensis and octenyl succinic anhydride), and the microspheres (SM, SMA and MA), were acquired in an Alpha II (Bruker) apparatus in transmittance mode. Samples (1% w/w) were dispersed in KBr and pelletized prior to analysis. Due to the hygroscopic nature of citric acid, and the difficulty to produce suitable KBr pellets, ATR mode was used for this sample. The

following parameters were set: 32 scans/min, with a resolution of 16 cm−1 within the spectral range of 4000–5500 cm^{-1}. Spectra were obtained with OPUS software version 8.5 build 8.7.10.

3.8. Methodology

3.8.1 *Spirulina* Biomass Preparation-Culturing *Spirulina Platensis*

Culturing *Spirulina* platensis is a critical step in the process of incorporating it into food products. *Spirulina* is a photosynthetic microorganism belonging to the cyanobacteria family, and it can be cultivated in controlled environments such as open ponds or closed photobioreactors. The following steps outline the process of culturing *Spirulina* platensis:

- Maintenance and growth of *Spirulina platensis,* Optimization of growth phases present in PTC lab Integral Information and Research Centre (IIRC-1), Department of Bioengineering, Integral University.
- The biomass used in this study is harvested and collected from IIRC (PTC LAB-1). It is a homogenous mixture of *S. platensis* cultured After harvesting and hot air oven drying, the final product is a green powder.

a. **Selection of Strain:** The project selected a high-quality strain of *Spirulina* platensis known for its nutritional composition and suitability for food applications.

Fig.3.2. Strains of *Spirulina* was selected from the Mother Culture

b. **Preparation of Culture Medium:** *Spirulina* platensis requires a nutrient-rich medium to support its growth. Commonly used culture media include Zarrouk's medium, BG-11, and

modified BG-11. The medium is prepared by dissolving specific quantities of salts, trace elements, and a carbon source in distilled water.

Fig.3.3. Subculturing of *Spirulina* in Lab.

c. Inoculation: The culture medium is inoculated with a small amount of *Spirulina* culture to initiate the growth process. The inoculated culture is then incubated under controlled environmental conditions, including temperature, light intensity, and pH.

d. Growth and Harvesting: *Spirulina platensis* grows rapidly under optimal conditions, and periodic monitoring is conducted to assess its biomass density. Once the desired biomass is achieved, the *Spirulina* is harvested. Harvesting methods may include centrifugation, filtration, or a combination of techniques.

Fig. 3.4. The biomass growth of *Spirulina* is studied for about ten weeks.

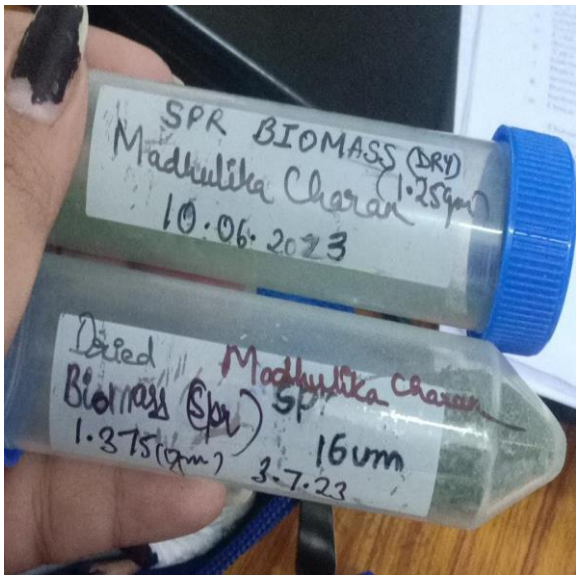
e. Post-Harvest Processing: After harvesting, the *Spirulina* biomass undergoes further processing steps, such as washing to remove impurities and drying to reduce moisture content. The dried *Spirulina* can be milled into powder or transformed into other forms suitable for incorporation into food products. After the biomass is dried and formed into a powder form, we will make the multigrain cookies based on different composition level of *Spirulina* ranging from 2% to 20% (w/w) by weight of the overall weight of the finish product.

3.8.2 *Spirulina* powder preparation

(a). Centrifugation of *S. platensis* Biomass

(b). Dried in hot air oven

(c). Crushing of dried *S. platensis* in powdered form



(d). Collection of Dried *S. platensis* Biomass

Fig. 3.5 *Spirulina* Powder Preparation

3.9. Cookies preparation

A set of vegan cookies samples were investigated in this study. For this, three different set of cookies were prepared: cookies produced without *Spirulina* biomass (control cookie, CC), with 20% (w/w) of non-encapsulated *Spirulina* biomass in substitution to multigrain flour (SC20%), and with 20% (w/w) of microencapsulated *Spirulina* biomass (microencapsulated *Spirulina* cookies, MSC20%) (Table 4.1). To prepare cookies, the ingredients were homogenized using a food processor and shaped by hand. Cookies were baked in an oven (170–180°C) for 15 min, cooled until room temperature and stored until analysis.

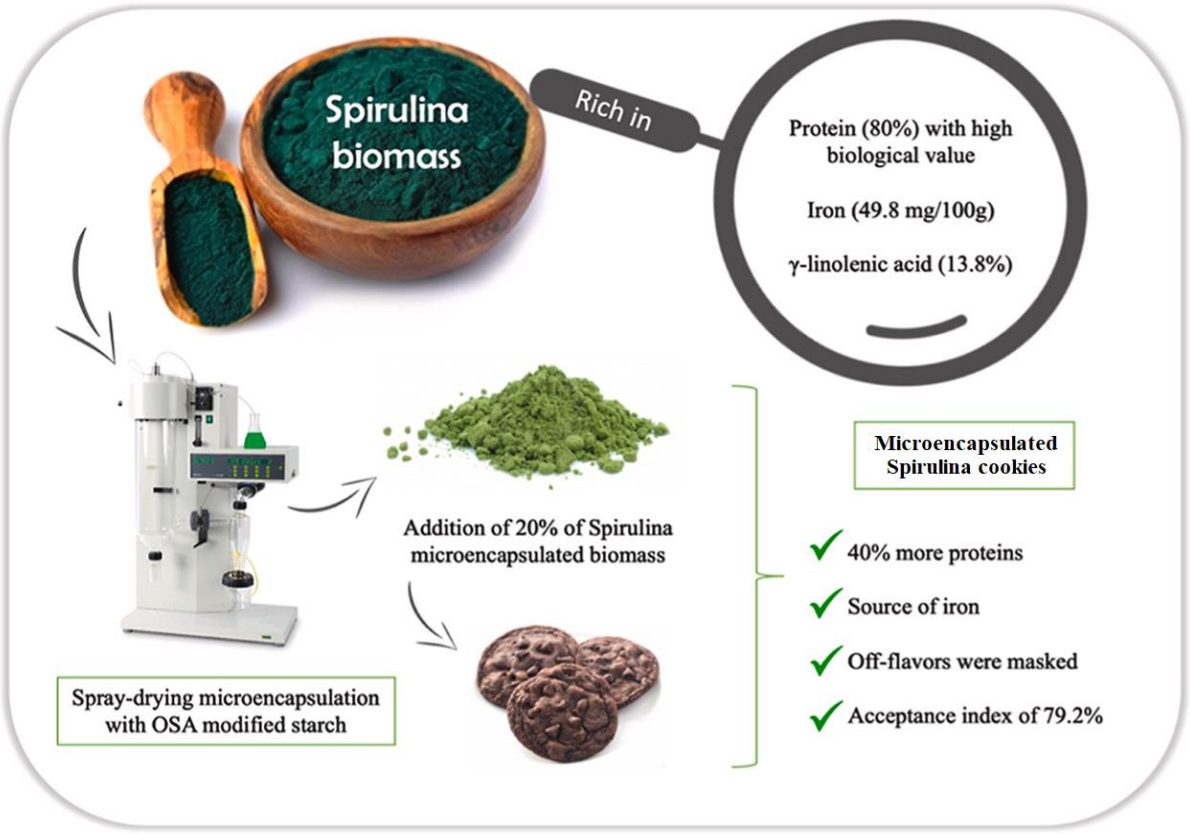


Fig.3.6 Process of Microencapsulated *Spirulina* enriched Multigrain Cookies (da Silva, S.P., do Valle, A.F. and Perrone, D., 2021. Microencapsulated *Spirulina* maxima biomass as an ingredient for the production of nutritionally enriched and sensorially well-accepted vegan biscuits. *Lwt*, *142*, p.110997)

Slight adaptations on the formulation of the third set of cookies samples were made to improve sensorial acceptance, based on the results of the sensorial analysis of the first sample set, and to maximize the nutritional benefits and apt for the taste buds of consumers, by using microencapsulated *Spirulina* platensis by spray drying of OSA. Cookies were produced according to the Good Manufacturing Practices to ensure their quality and safety.

Microbiological analysis of *Spirulina* biomass were performed at a certified laboratory (Food Analysis) which met the Indian legislation laid by the FSSAI. Thus, cookies were safe for the consumption by the volunteers who participated in the sensory analysis.

Table 3.4. Formulation of control cookies (CC), *Spirulina* cookies (SC20%) and microencapsulated *Spirulina* cookies (MSC20%)[1].

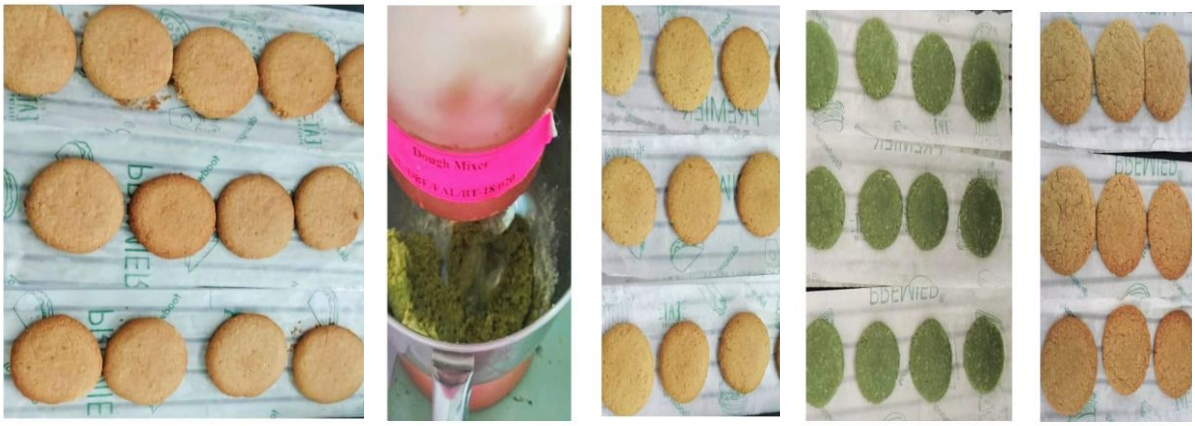
Ingredient	CC	*Spirulina* biomass	
		SC20%	MSC20%
Multigrain flour (g)	100.0	98.0	98.0
Honey or Stevia based sugar (g)	60	60	60
Coconut oil (g)	60	60	60
Desiccated Coconut (g)	30.0	30.0	30.0
Salt (g)	2.0	2.0	2.0
Baking powder (g)	5.0	5.0	5.0
Water (mL)	57.0	57.0	57.0
Vanilla extract (mL)	9.0	9.0	9.0
S. platensis biomass (g)	-	20.0	-
S. platensis microencapsulated biomass[2] (g)	-	-	20.0
Capsul (OSA[3]) (g)	-	-	20.0

[1]*Spirulina* cookies were produced partially substituting multigrain flour with 20% of either *Spirulina* biomass (SC20%) or encapsulated *Spirulina* biomass (MSC20%). [2]To ensure that the amount of biomass in the biscuits was 20%, double the mass of microencapsulated biomass was added considering the 1:1 proportion of *Spirulina* biomass:OSA used for spray-drying. [3]OSA = octenyl succinic anhydride.

3.10. Cooking Instructions or Recipe

- Preheat your oven to 350°F (175°C) and line a baking sheet with parchment paper.
- In a medium-sized bowl, combine the mutigrain flour, *Spirulina* powder, baking soda, and salt. Mix well and set aside.
- In a separate large bowl, whisk together the melted coconut oil, honey or stevia-based sugar substitute, and vanilla extract until well combined.
- Gradually add the dry ingredient mixture to the wet ingredients, stirring until a thick dough forms.
- Fold in the desiccated coconut, ensuring they are evenly distributed throughout the dough.

- Take tablespoonfuls of dough and roll them into balls. Place the dough balls onto the prepared baking sheet and flatten them slightly with the palm of your hand or a fork.
- Bake the cookies in the preheated oven for about 12-15 minutes or until they are lightly golden around the edges.
- Remove the cookies from the oven and allow them to cool on the baking sheet for a few minutes before transferring them to a wire rack to cool completely.
- Once cooled, store the cookies in an airtight container to maintain their freshness.
- These cookies will have the nutritional benefits of microencapsulated *Spirulina*, the sweetness of honey or a stevia-based sugar substitute, the tropical flavour of desiccated coconut, and the richness of coconut oil.



(a). Control Cookies (CC) (b). *Spirulina* Biomass Cookies (SC20%) (c). Microencapsulated *Spirulina* Cookies (MSC20%)

Fig.3.7 The finished product Control Cookie (CC), *Spirulina* Biomass Cookies (SC20%), and Microencapsulated *Spirulina* Cookies (MSC20%)

3.11. Physiochemical Analysis

3.11.1. Moisture Content

Initially, the weight of empty Petri plates was determined using an electronic balance. In flat bottom Petri plates, 2g of each sample were weighed. The dish and its contents were placed in a hot air oven that was thermostatistically controlled at 105ºC and heated for 1 hour and then the readings were calculated. Further samples were again kept at 30 minutes intervals until no further weight loss was observed. Finally, the dish was removed from the oven and placed in desiccators to cool before being weighed again (*Lomauro et al., 2004*). The following formula was used to calculate the moisture content of cookies sample.

$$Moisture\ Content\ (\%)\ = \frac{Loss\ in\ weight\ of\ sample\ (g)}{Initial\ Weight\ of\ sample\ (g)} \times 100$$

Fig.3.8 Analysis for Moisture Content in sample

3.11.2 Ash Content

The empty weight of the crucible was measured. In the crucible, 5g of each sample was weighed. Then it was ignited on the flame. For 4 hours, the crucible and its contents were placed in a muffle furnace at 550ºC. To cool the samples, the crucibles were removed from the furnace and placed in desiccators. Each sample's weight was determined (*M. R. Marshall, 2010*). The following formula was used to calculate the ash content of cookies samples.

$$Ash\ Content\ (\%) = \frac{Weight\ of\ Ash\ (g)}{Initial\ weight\ of\ sample\ (g)} \times 100$$

Fig.3.9 Analysis for Ash Content in sample

3.11.3 Fat Content

The Soxhlet method was used for extraction of fat of cookies sample which is described below: 5g of sample was taken and a thimble was made with the help of Whatman paper number one. The thimble was placed into the extracting tube and this tube was connected with the weighted flask and also the condenser. The heat vaporized the volatile solvent, which passed up the side arm and was condensed in the condenser. The condensed solvent is allowed to fall drop by drop onto the thimble. When a sufficient amount of solvent had been transferred to the extracting tube to fill the siphon arm, it was siphoned back into the weighed flask. This process was continued for a few hours until the extraction was completed. Then the bottom flask was removed, the volatile solvent was evaporated and the fat extracted was obtained as crude fat. Chemical Apparatus used in this method are Mortar and pestle (G.R. Dobush,,et al.,2015). The following formula was used to express the fat content of the sample:

$$Fat\ content\ (\%) = \frac{Weight\ of\ fat\ in\ sample(g)}{Initial\ weight\ of\ sample} \times 100$$

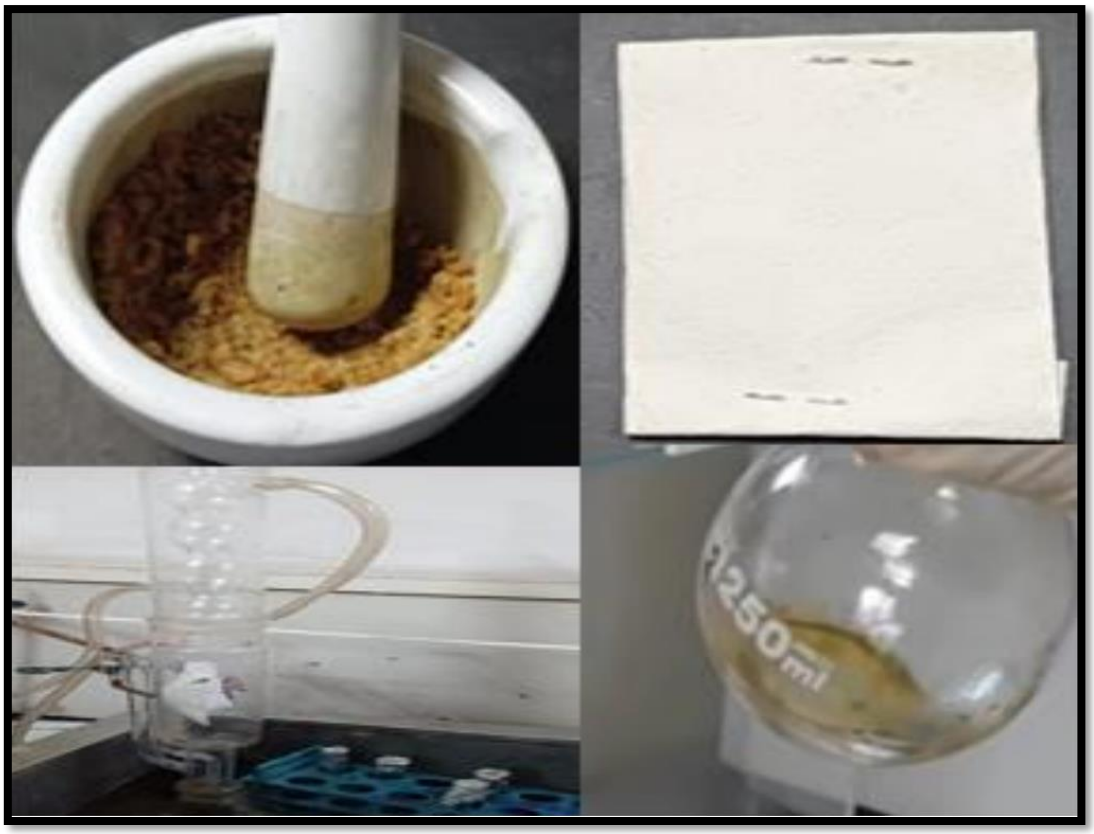


Fig.3.10. Analysis for Fat Content in the sample

3.11.4 Protein Content

For protein estimation in the cookie sample, the Kjeldahl method was used, as described below:

This method was being performed in 3 major steps:

3.11.4.1 Digestion

The food sample to be analyzed was weighed into a digestion flask and then digested by heating it in the presence of sulfuric acid (an oxidizing agent which digests the food), anhydrous sodium sulphate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, titanium, or mercury (to speed up the reaction). Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia and other organic matter to CO_2 and H_2O. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH_4^+) which binds to the sulfate ion (SO_4^{2-}) and thus remains in the solution:

N (food sample) $\longrightarrow$ **$(NH4)_2SO4$**

3.11.4.2 Neutralization

After the digestion has been completed the digestion flask was connected to a receiving flask by a tube. The solution in the digestion flask is then made alkaline by the addition of sodium hydroxide, which converts the ammonium sulphate into ammonia gas:

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + 2H_2O + Na_2SO_4$$

The ammonia gas that is formed was being liberated from the solution and moves out of the digestion flask and into the receiving flask - which contains an excess of boric acid. The low pH of the solution in the receiving flask converts the ammonia gas into the ammonium ion, and simultaneously converts the boric acid to the borate ion:

$$NH_3 + H_3BO_3 \text{ (boric acid)} \longrightarrow NH_4^+ + H_2BO_3^- \text{(borate ion)}$$

3.11.4.3 Titration

The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid, using a suitable indicator to determine the end- point of the reaction.

$$H_2BO_3^- + H^+ \longrightarrow H_3BO_3$$

The concentration of hydrogen ions(in moles)required to reach the end point is equivalent to the concentration of nitrogen that was in the original food. The following equation can be used to determine the nitrogen concentration of a sample that weigh grams using a x HCl acid solution for the titration:

$$\boldsymbol{N}\ (\%) = \frac{(\boldsymbol{Sample\ titration - blank\ titration}) \times (\boldsymbol{N\ of\ HCl}) \times (\mathbf{14})}{\boldsymbol{Weight\ of\ sample}\ (\boldsymbol{g}) \times \mathbf{1000}} \times \mathbf{100}$$

Where v_s and v_b are the titration volumes of the sample and blank, and 14g is the molecular weight of nitrogen N. A blank sample is usually ran at the same time as the material being analyzed to take into account any residual nitrogen which may be in the reagents used to carry out the analysis (A.G. Gornall, 2010). Once the nitrogen content has been determined it is converted to a protein content using the appropriate conversion factor:

$$\boldsymbol{Protein\ content}(\%) = (\%\boldsymbol{N} \times \mathbf{6.5})$$

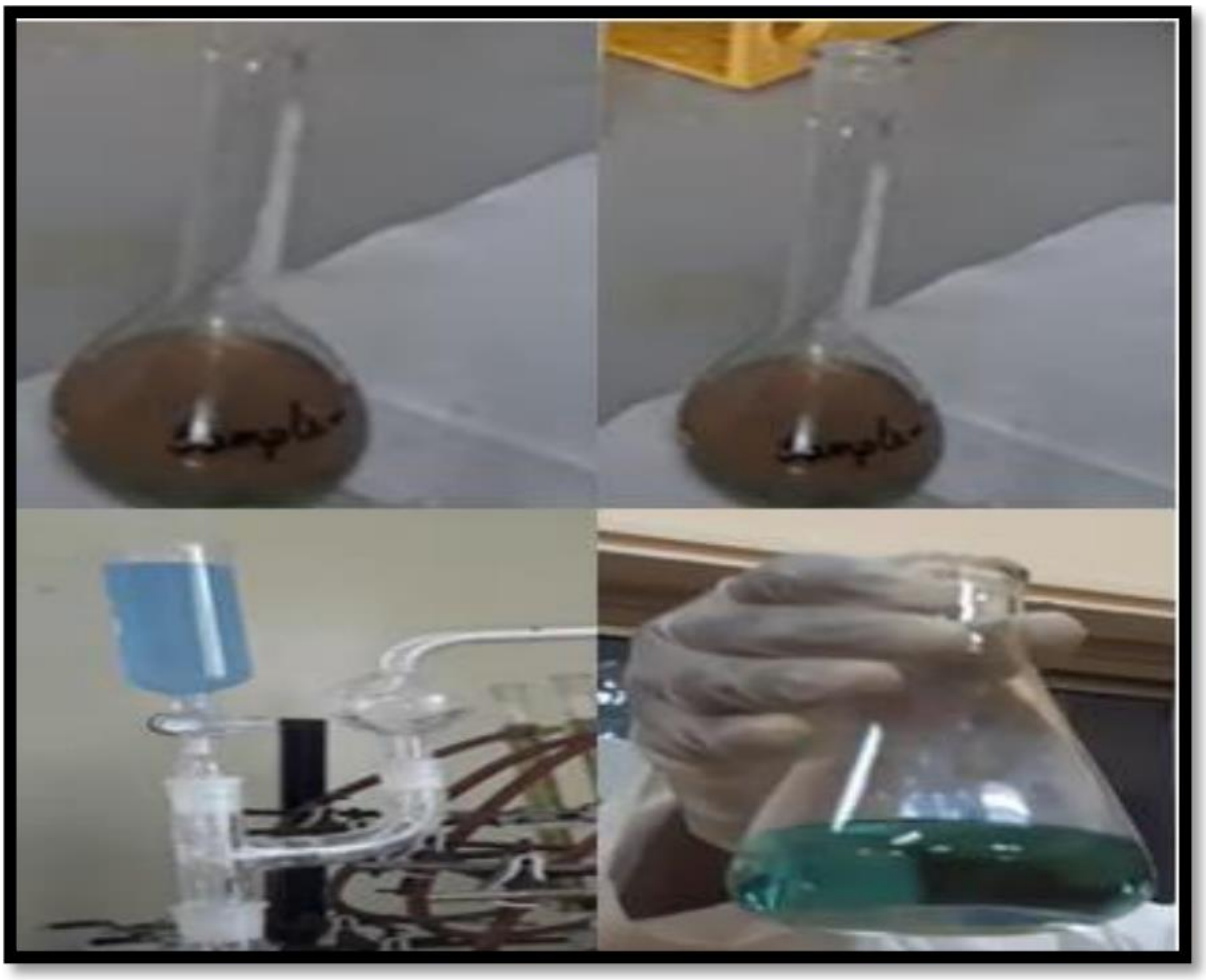


Fig.3.11. Analysis for Protein Content in the sample

3.11.5 Crude fibre Content determination

Two grams (2g) of the cookie sample from crude fat determination was weighed into a 750ml Erlenmeyer flask. Two hundred milliliters (200ml) of 1.25% H_2SO_4 was added and immediately flask was set on hot plate and connected to the condenser. The contents were boiled within 1 minute of contact with solution. At the end of 30 minutes, flask was removed and immediately filtered through linen cloth in funnel and washed with a large volume of water. Filtrate (containing sample from acid hydrolysis) was washed and returned into the flask with 200ml 1.25% NaOH solutions. Flask was connected to the condenser and was boiled for exactly 30 minutes. It was then filtered through Fischer's crucible and washed thoroughly with water and added 15ml 96% alcohol. Crucible and contents was dried for 2 hour at 105 °C and

cooled in desiccator and it was weighed. Crucible was ignited in a furnace for 30 minutes and after that it was cooled and reweighed.

$$\%\ Crude\ Fibre = \frac{weight\ of\ crude\ fibre}{weight\ of\ sample} \times 100$$

$$\%\ Crude\ Fibre = \frac{wt\ of\ crucible + sample(before - after)\ ashing}{weight\ of\ sample} \times 100$$

Fig.3.12. Analysis for Crude Fibre Content in the sample

3.11.6 Carbohydrate

The calculation of available carbohydrate (nitrogen-free extract-NFE) was made after completing the analysis for ash, crude fibre, ether extract and crude protein. The calculation was made by adding the percentage values on dry matter basis of these analysed contents and subtracting them from 100%.

Calculation:

$$Carbohydrate\ (\%)\ = 100\ -\ (\%\ moisture\ +\ \%\ fat\ +\ \%\ protein\ +\ \%\ ash)$$

3.12 Sensory Analysis

The sensory attributes for all three types of cookies including the control cookie were observed for 90 days at 30-day intervals. Hedonic Ratting Test was used to evaluate *Spirulina* enriched cookies for sensory attributes such as colour, flavour, texture, taste, size etc… The Hedonic Rating test was used to evaluate sensory characteristics. This test is used to assess consumer acceptance of a product. The methodology is presented in detail below.

A panel of ten expert judges of varying ages and eating habits were chosen, and the samples were served to them. The expert panelists were asked to rate the acceptability of the product based on their sense of organs on a scale of 9 points ranging from extremely like to extremely dislike. At the time of the evaluation, a test Performa was prepared and provided to them.

Table 3.5 Hedonic Scale table

Rating	Score
Like Extremely	9
Like very much	8
Like Moderately	7
Like Slightly	6
Neither like nor dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike very much	2
Dislike extremely	1

Table 3.6 The best accepted sample by the panellists was MSC20%

Product	**Colour**	**Texture**	**Taste**	**Aroma**	**Overall Acceptability**
Microencapsulated *Spirulina* Enriched Multigrain cookies	**Light greenish brown**	**8**	**9**	**9**	**9**

CHAPTER 4
RESULTS AND DISCUSSION

This chapter present the experimental results conducted to enhance the protein content of multigrain cookies with the incorporation of *Spirulina* powdered under ambient condition. During the analysis 3 different samples were taken and their comparative study is done in order to get the best results.

Sample 1 (S1) – Control multigrain cookies (CC)
Sample 2 (S2) – *Spirulina* Biomass Multigrain cookies prepared in lab (SC20%)
Sample 3 (S3) – Microencapsulated *Spirulina* Multigrain cookies prepared in Lab (MSC20%)

The experiments were carried out to determine various analysis of the Microencapsulated Multigrain cookies. The result of this investigation is discussed as under:

4.1 Physical characteristics of multigrain cookies

The physical characteristics like shape, color, average diameter and the average length of the *Spirulina* Multigrain cookies prepared in IIRC Laboratory of Integral University, Lucknow, Uttar Pradesh are presented in the table 4.1.

Table 4.1 Physical Characteristics of *Spirulina* Multigrain Cookies

S .No	Morphological Characters	Parameters of a multigrain cookie
1.	Shape	Round
2.	Color	Light Greenish brown in color
3.	Diameter	8 cm
4.	Length	8 cm
5.	Weight	15 g
6.	Shelf life	2-3 months

4.2 Effect on Physicochemical characteristics of *Spirulina* enriched Multigrain Cookies

The incorporation of *Spirulina* into multigrain cookies can have two significant effects on their physico-chemical characteristics. Firstly, the vibrant green pigments present in *Spirulina* may impart a pleasing color to the cookies, enhancing their visual appeal and attractiveness to consumers. Secondly, the addition of *Spirulina* may lead to changes in the cookies' nutritional profile, elevating their protein and mineral content due to *Spirulina*'s high protein and mineral content. Moreover, the combination of various grains in the recipe may contribute to improved texture and flavor complexity, resulting in a wholesome and nutritious cookie option for health-conscious individuals. These effects make *Spirulina* enriched multigrain cookies a promising choice for those seeking both aesthetic and nutritional benefits in a delightful treat.

Table 4.2 Proximate Analysis of different parameters of multigrain cookies

	Control Cookie	***Spirulina* Cookie SC (20%)**	**Microencapsulated *Spirulina* Cookie MSC (20%)**
Moisture	6.05±0.15	5.30±0.175	5.18±0.052
Protein	8.49±0.21	12.06±0.36	11.34±0.48
Fat	30.21±0.8	31.54±0.08	32.04±1.8
Ash	6.52±0.26	11.05±0.22	10.41±0.30
Carbohydrate	40.73±0.55	41.03±0.42	41.98±0.76
Crude Fibre	6.72±0.062	6.98±0.096	7.01±0.079

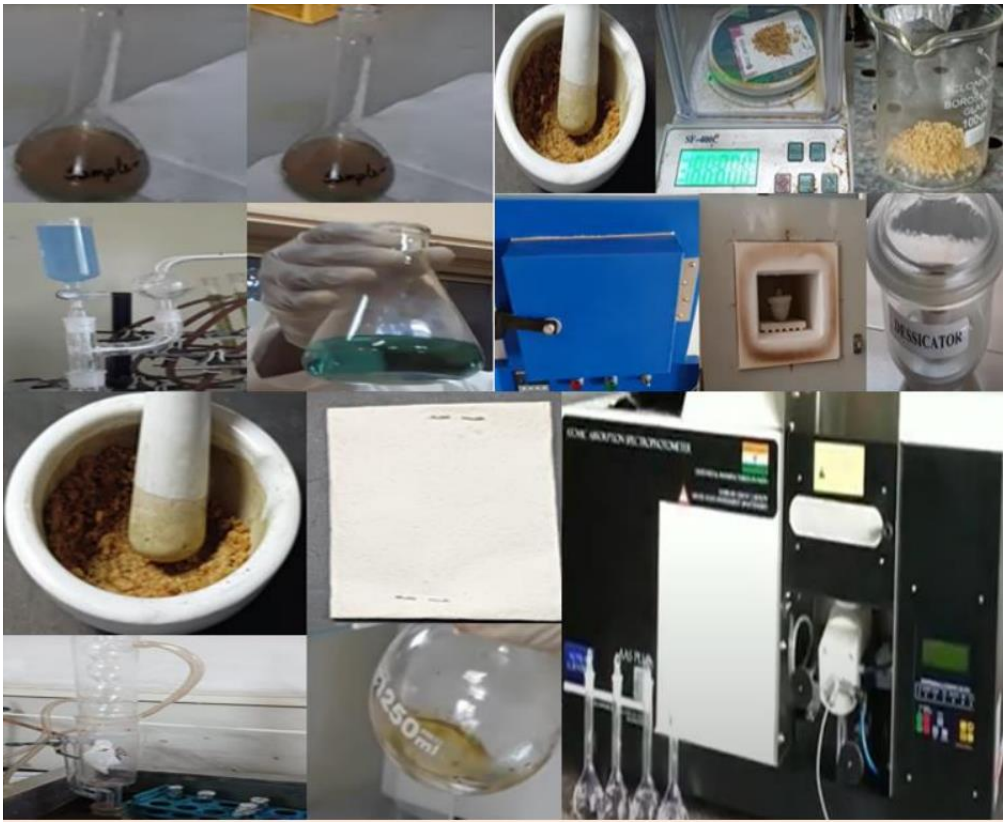


Fig. 4.1. Physiochemical Analysis of *Spirulina* Enriched Multigrain Cookies

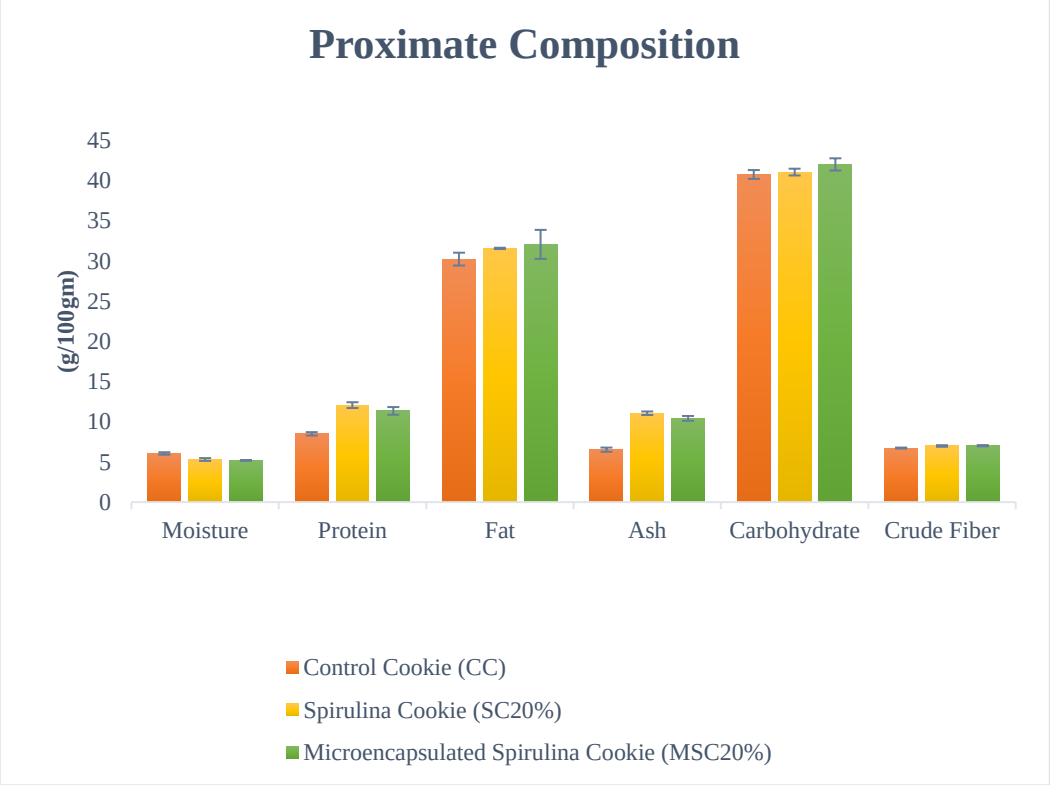


Fig 4.2. Proximate Analysis of *Spirulina* enriched Multigrain Cookies

4.2.1 Moisture Content

The storage period and *Spirulina* powder effected moisture content of packed cookies is presented in Figure 4.3. The moisture content decreased considerably due to *Spirulina* powder used. A reduction in moisture content can lead to improved shelf stability and a longer shelf life for the cookies. Lower moisture content helps prevent microbial growth and enzymatic reactions that could cause the cookies to spoil or become rancid.

A substantial decrease in moisture content may indicate that the cookies were adequately dried and processed before packaging, and the packaging itself is effective in maintaining the low moisture levels. This is especially important for products like cookies, where higher moisture content could lead to textural issues like softening or sogginess.

Table 4.3 Moisture Content (%) of cookies

Samples	0 day	30 days	60 days
S1	6.05	4.84	3.87
S2	5.30	4.24	3.39
S3	5.18	4.14	3.31

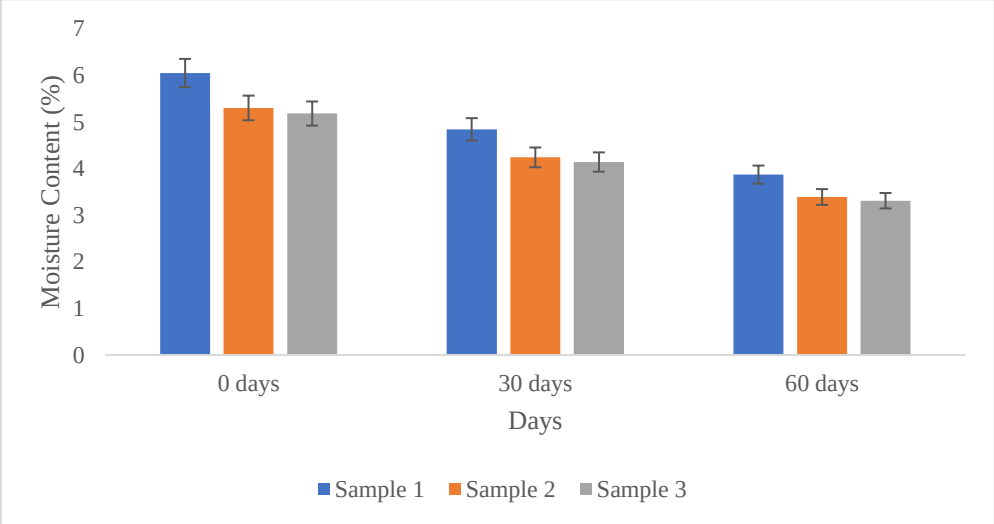


Fig 4.3. Moisture Content (%) in Multigrain Cookies

4.2.2 Ash Content

The effect of storage period and *Spirulina* powder on ash content of packed cookies is presented in Table 4.4

On critical evaluation of results, it was found that the ash content of cookies was considerably increased as shown in Figure 4.4. The increase in ash content in *Spirulina*-based cookies over

time is likely due to a process known as "ashing" or "mineralization." Ash content refers to the inorganic residue left behind after a sample is completely burned at high temperatures. In the case of cookies, the ash content primarily consists of minerals such as potassium, calcium, magnesium, phosphorus, and trace elements like iron, zinc, and copper.

Two major reasons resulting in the increase of the ash content in *Spirulina*-based cookies over time are as follows:

1. **Moisture Loss**: As the cookies age and lose moisture, the concentration of minerals in the remaining dry matter increases. This phenomenon is similar to evaporating water from a solution, where the solute (minerals) becomes more concentrated as the solvent (water) evaporates.
2. **Environmental Contamination**: If the cookies are exposed to external contaminants or environmental factors, they might pick up additional minerals or impurities, contributing to the increase in ash content.

Table 4.4 Ash Content (%) of cookies

Samples	0 day	30 days	60 days
S1	6.52	5.93	5.40
S2	11.05	9.83	8.94
S3	10.41	9.47	8.61

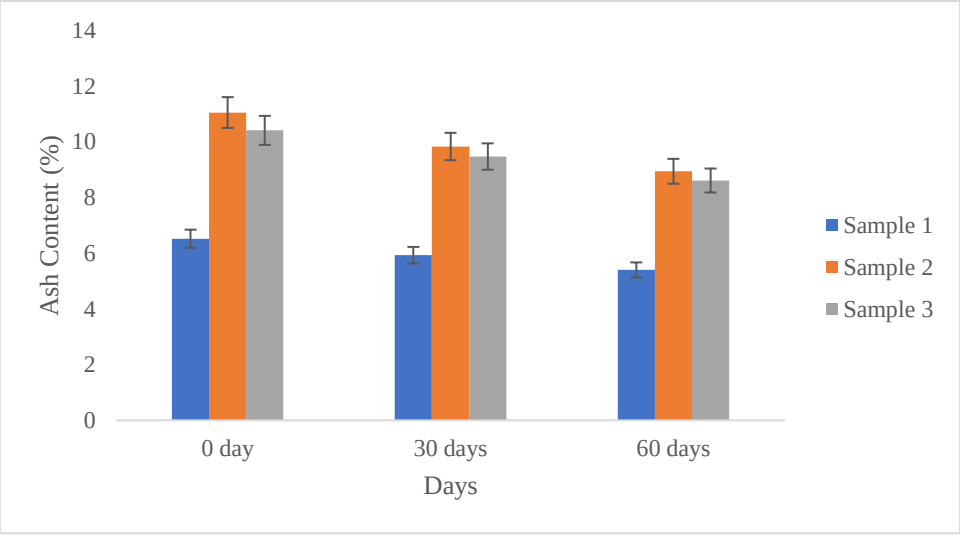


Fig 4.4. Ash Content (%) in Multigrain Cookies

4.2.3. Fat Content

The effect of storage period and *Spirulina* powder on fat content of packed cookies is presented in Table 4.5. The overall results clearly revealed that fat content of packed cookies decreased considerably with the increase in storage period as shown in Figure 4.5. The decrease in fat content in *Spirulina*-based cookies over time can be attributed to several factors that affect the stability of fats in food products:

1. **Oxidation**: Fats and oils are susceptible to oxidation, especially when exposed to air and light. Oxidation is a chemical reaction that breaks down the fats, leading to the formation of rancid and off-flavors. As the cookies age, the fats may undergo oxidation, resulting in a decrease in fat content.
2. **Migration**: In some cases, fats can migrate from the cookies to the surrounding packaging materials, especially if the packaging is not airtight. This migration can lead to a reduction in the fat content of the cookies.
3. **Absorption:** The cookies may absorb moisture from the environment, and as a result, the fats could leach out into the moisture, leading to a decrease in fat content.
4. **Ingredient Interaction**: Interactions between fats and other ingredients in the cookies, such as flour, sugar, and *Spirulina*, can influence the stability and retention of fats. Some ingredients might bind with fats and lead to their separation from the cookie matrix.

Table 4.5 Fat Content (%) of cookies

Samples	0 day	30 days	60 days
S1	30.21	25.07	20.05
S2	31.54	21.76	15.23
S3	32.04	20.54	13.15

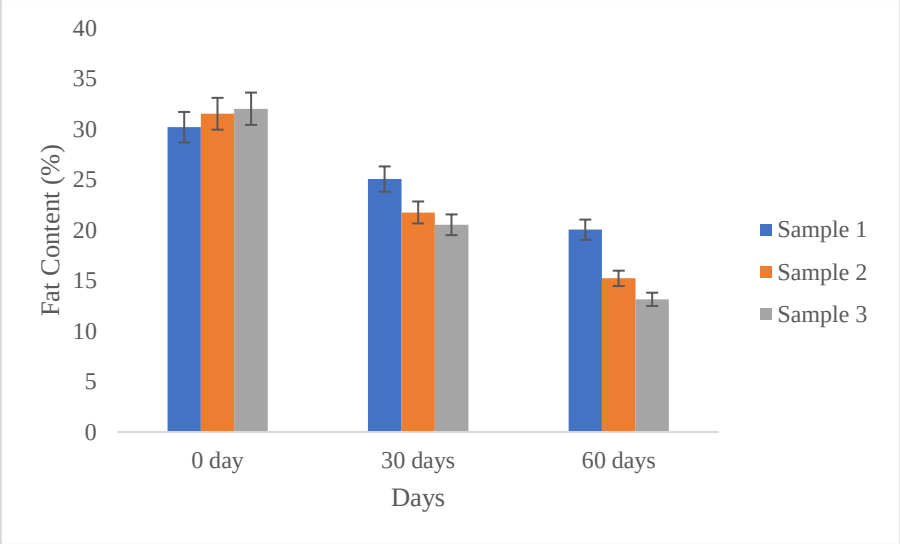


Fig 4.5. Fat Content (%) in Multigrain Cookies

4.2.4. Protein Content

The effect of storage period and *Spirulina* powder incorporated in packed cookies on protein content is presented in Table 4.6. The overall results revealed that the protein content increased considerably with the incorporation of *Spirulina* powder in cookies as shown in Figure 4.6. The increase in protein content in *Spirulina*-based cookies over time could be due to several reasons:

1. **Moisture Loss:** As the cookies age, they may lose moisture through evaporation. Since proteins are present in the dry matter of the cookies, their concentration would increase as the moisture content decreases.
2. **Protein Concentration in *Spirulina***: *Spirulina* is a rich source of protein, and it is one of the main ingredients in *Spirulina*-based cookies. Over time, some of the other components in the cookies, such as carbohydrates and fats, may degrade or decrease in concentration, while the protein content remains relatively stable, leading to a slight increase in the overall protein percentage.
3. **Microbial Activity**: In some cases, beneficial microbial activity can occur in the cookies, such as enzymatic reactions catalysed by certain microorganisms. This activity could contribute to minor changes in the protein content.

Table 4.6 Protein Content (%) of cookies

Samples	0 day	30 days	60 days
S1	8.49	8.49	8.49

S2	12.06	12.18	12.22
S3	11.34	11.66	11.68

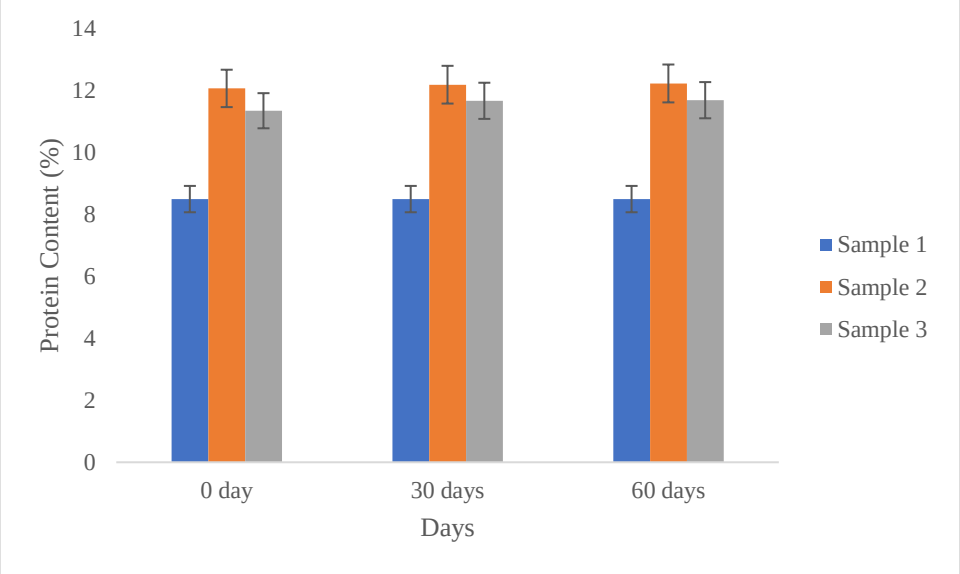


Fig 4.6. Protein Content (%) in Multigrain Cookies

4.2.5 Crude Fibre Content

The crude fibre content in *Spirulina*-based cookies is not expected to change significantly over the course of a shelf-life study as shown in Table 4.7, especially within a relatively short period like 30 days or 60 days. Crude fibre consists mainly of the indigestible plant material, such as cellulose and lignin, and it is generally stable in most food products. The overall results revealed that the crude fibre content remained the same during the 60 days shelf-life study of *Spirulina* powder incorporated cookies as shown in Figure 4.6.

During a shelf-life study, various factors can affect the nutritional composition of food products, including cookies. However, the changes in crude fibre content are not likely to be substantial for the following reasons:

1. **Stability of Fibre**: Crude fibre, being primarily composed of plant cell walls, is relatively stable and resistant to degradation by enzymes or microbial activity present in the cookies. Therefore, it is not significantly affected by the aging process or storage conditions.
2. **Minimal Leaching**: Unlike some other nutrients, such as vitamins and minerals, crude fibre does not readily leach out of the cookies into the surrounding environment or packaging. It remains predominantly bound within the cookie matrix.

3. **Low Water Solubility**: Crude fibre has low water solubility, which means it is not prone to being extracted or dissolved into the moisture present in the cookies during storage.
4. **Limited Enzymatic Activity**: Enzymes that could potentially break down the fibre are usually present in negligible amounts in baked goods like cookies. Therefore, enzymatic degradation of crude fibre is not a significant factor.

Table 4.7 Crude Fibre Content (%) of cookies

Samples	0 day	30 days	60 days
S1	6.72	6.72	6.72
S2	6.98	6.98	6.98
S3	7.01	7.01	7.01

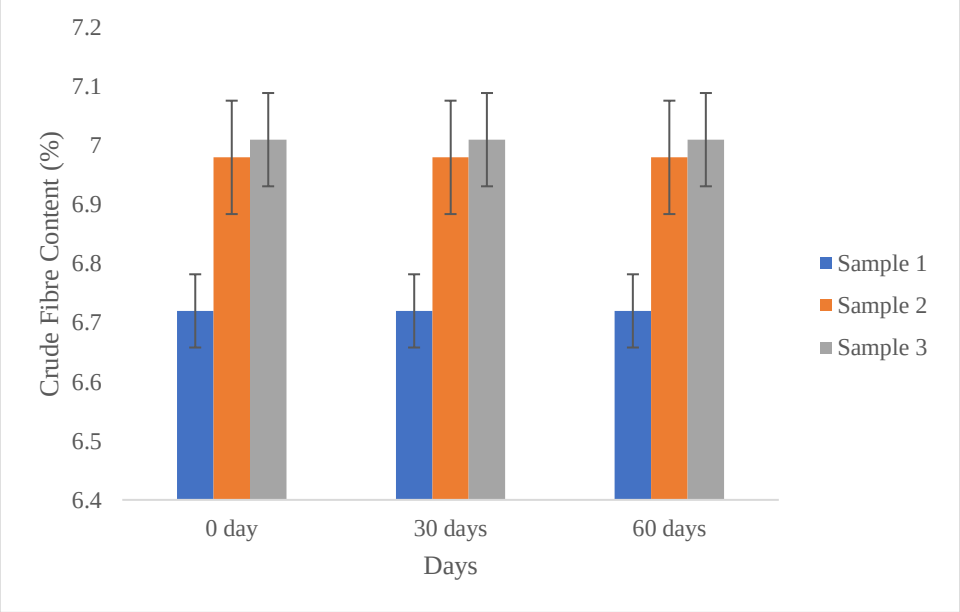


Fig 4.7. Crude Fibre Content (%) in Multigrain Cookies

4.2.6 Carbohydrate Content

In a shelf-life study of *Spirulina*-based cookies, the carbohydrate content is not expected to change significantly after 30 days or 60 days of storage as shown in Table 4.8. Carbohydrates are a fundamental component of cookies, primarily contributed by ingredients such as flour, sugars, and other carbohydrates present in the recipe. These carbohydrates are generally stable under normal storage conditions. The overall results revealed that the carbohydrate content

remained the same during the 60 days shelf-life study of *Spirulina* powder incorporated cookies as shown in Figure 4.8

Several factors contribute to the stability of carbohydrate content during shelf-life:

1. **Ingredient Stability:** The carbohydrate content in the cookies is determined by the types and quantities of carbohydrates used in the recipe. If the ingredients remain consistent throughout the study, the carbohydrate content should also remain stable.
2. **Low Water Solubility:** Carbohydrates, especially sugars and starches, have low water solubility, meaning they do not readily dissolve in the moisture present in the cookies during storage.
3. **Proper Packaging**: Adequate packaging can help protect the cookies from external factors that may lead to changes in carbohydrate content.
4. **Moisture and Temperature Control**: Proper storage conditions, including moisture and temperature control, can help preserve the carbohydrate content in the cookies.

Table 4.8 Carbohydrate Content (%) of cookies

Samples	0 day	30 days	60 days
S1	40.73	41.81	42.04
S2	41.03	41.45	41.95
S3	41.98	42.14	42.86

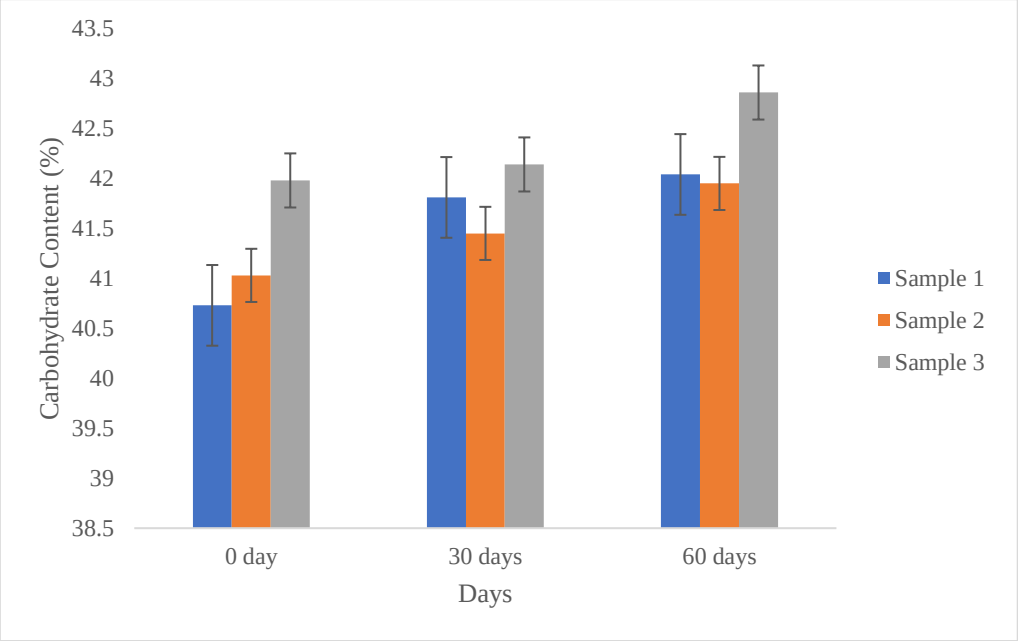


Fig 4.8. Carbohydrate Content (%) in Multigrain Cookies

4.3 Sensory attributes of *Spirulina* incorporated multigrain cookies as influenced by packaging materials, storage period and *Spirulina* powder.

Sensory attributes of *Spirulina* incorporated multigrain cookies were evaluated for fresh condition and at 30 days interval up to 3 months of storage. Nine-point Hedonic rating Different attribute selected were color, taste, aroma, flavor, texture, appearance, and overall acceptability.

4.3.1 Color

The packaging materials, storage period and *Spirulina* showed an effect on *Spirulina* incorporated cookies are presented in Fig 4.9. The color is an important sensory attribute for any new developed product. The color of cookies varied due to addition of *Spirulina* powder. The color of cookies decreased Slightly with storage period. Green colour was observed due to *Spirulina* powder, which was actually a new development as people were attracted towards it.

Table 4.9 Color of Cookies

Samples	0 day	30 days	60 days
S1	9	8.5	8
S2	8	7.5	7
S3	9	8.5	8

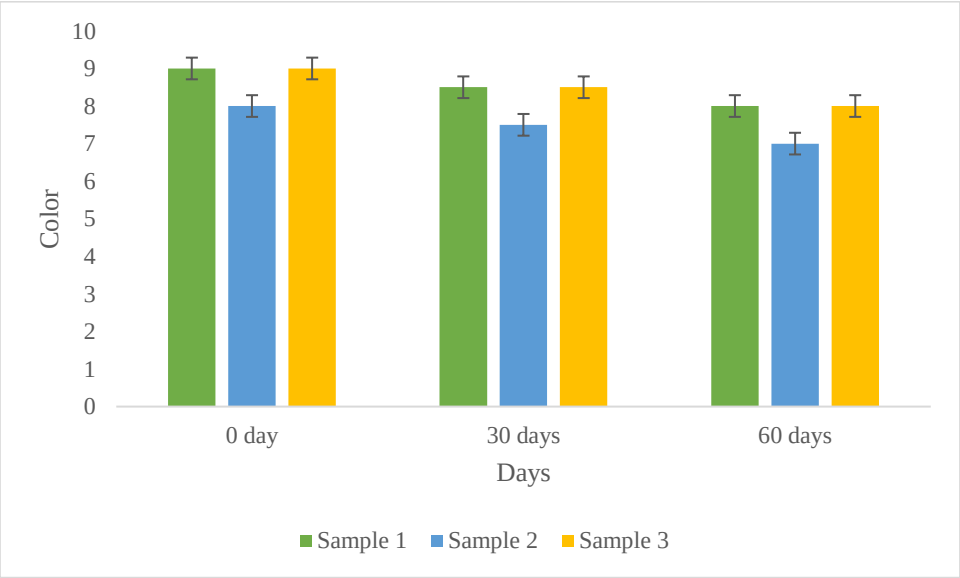


Fig 4.9. Color of Multigrain Cookies

4.3.2 Taste

The packaging material, storage period and *Spirulina* powder affected on taste of *Spirulina* incorporated cookies are presented in Fig 4.10. The taste of cookies varied due to incorporation of *Spirulina* powder. After sometime tastes change and it minor affected the sample, incorporated with *Spirulina* powder. The taste of the cookie was slightly reduced with increase in storage period.

Table 4.10 Taste of *Spirulina* enriched cookies over control cookies

Samples	0 day	30 days	60 days
S1	8.5	7.5	7.0
S2	8.0	7.0	6.5
S3	9.0	8.0	8.0

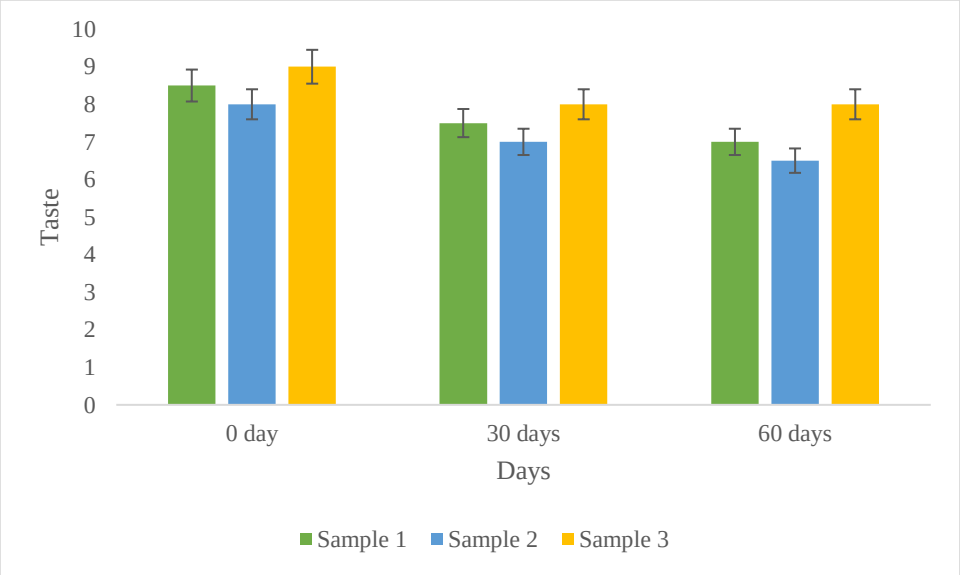


Fig 4.10. Taste of Multigrain Cookies

4.3.3 Aroma

The effect of packaging material, storage period and *Spirulina* powder on aroma of *Spirulina* incorporated cookie is presented in Fig 4.11. The aroma of cookies reduced with increase in storage period.

Table 4.11 Aroma of *Spirulina* enriched cookies

Samples	0 day	30 days	60 days
S1	7.0	7.0	6.5
S2	8.0	7.5	6.5
S3	9.0	8.5	7.5

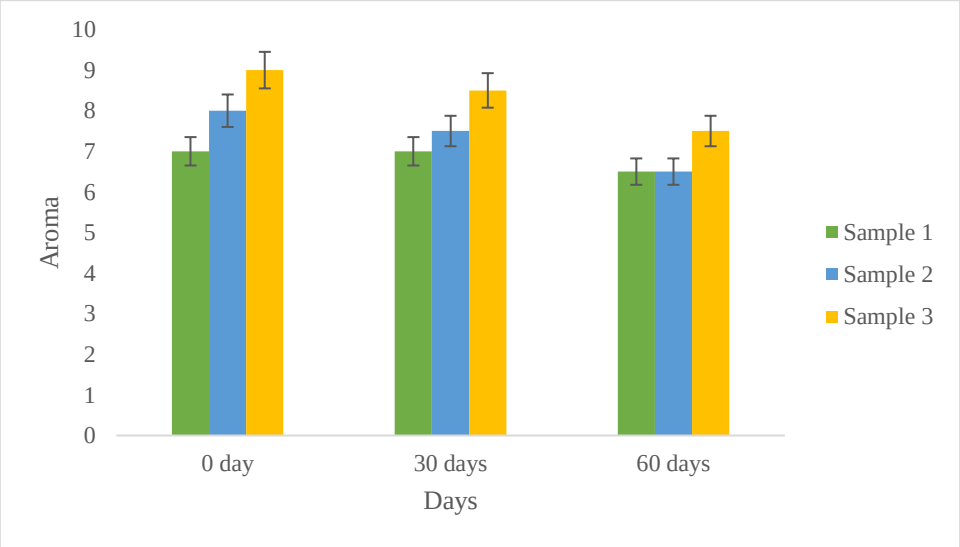


Fig 4.11. Aroma of Multigrain Cookies

4.3.4 Flavour

The effect of packaging material, storage time and *Spirulina* powder on flavour is presented in figure 4.12. The flavor of cookies decreased slightly with increases in storage period as shown in Table 4.12.

Table 4.12 Flavor of *Spirulina* enriched cookies during shelf-life study for two months

Samples	0 day	30 days	60 days
S1	8.5	8.0	8.0
S2	8.5	8.0	7.5
S3	9.0	8.5	8.5

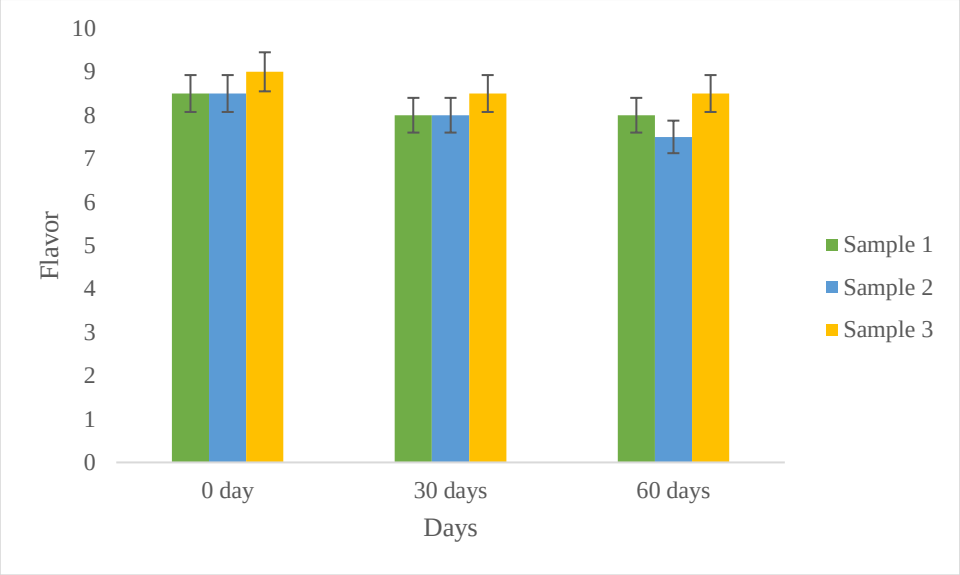


Fig 4.12. Flavour of Multigrain Cookies during the shelf life study for two months

4.3.5 Texture

The effect of packaging materials, storage period and *Spirulina* powder on texture of cookie is presented in Fig 4.13. There was a slight decrease in texture due to less moisture present in packed cookies. After 2 months of storage the score for texture of different samples is given below in Table 4.13.

Table 4.13 Texture of *Spirulina* enriched cookies during shelf-life study for two months

Samples	0 day	30 days	60 days
S1	9	7	5
S2	8	7.5	5.5
S3	9	8.5	6.5

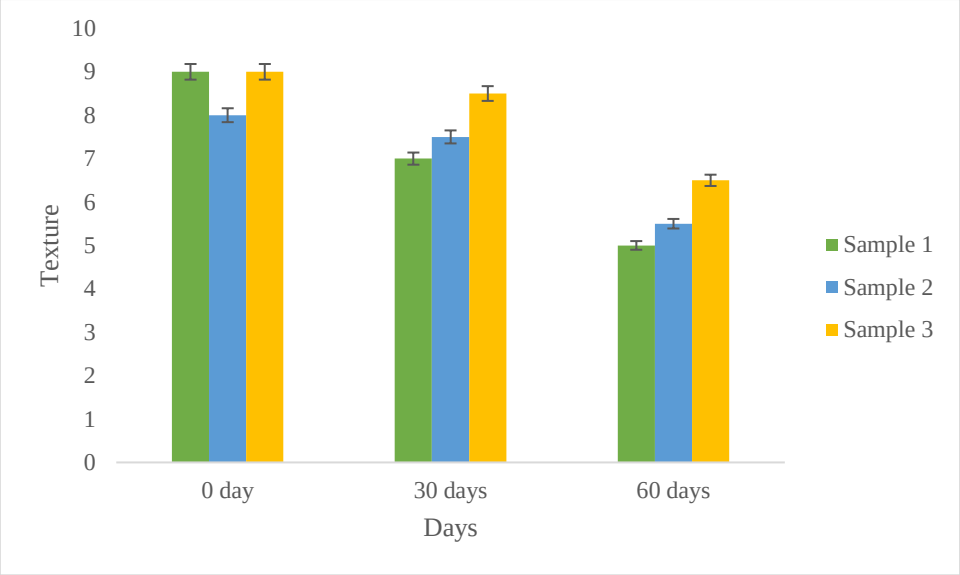


Fig 4.13. Texture of Multigrain Cookies during the shelf-life study for two months

4.3.6 Appearance

The effect packaging materials, storage period and *Spirulina* powder on appearance of cookie are presented in Fig 4.14. The highest score for appearance was obtained in Sample S3, i.e. the cookies containing microencapsulated *Spirulina* (MSC20%). There was a slight decrease in appearance score for storage of cookies.

Table 4.14 Appearance of *Spirulina* enriched cookies during shelf-life study for two months

Samples	0 day	30 days	60 days
S1	8.5	8	8
S2	8.5	8	7.5
S3	9	8.5	8

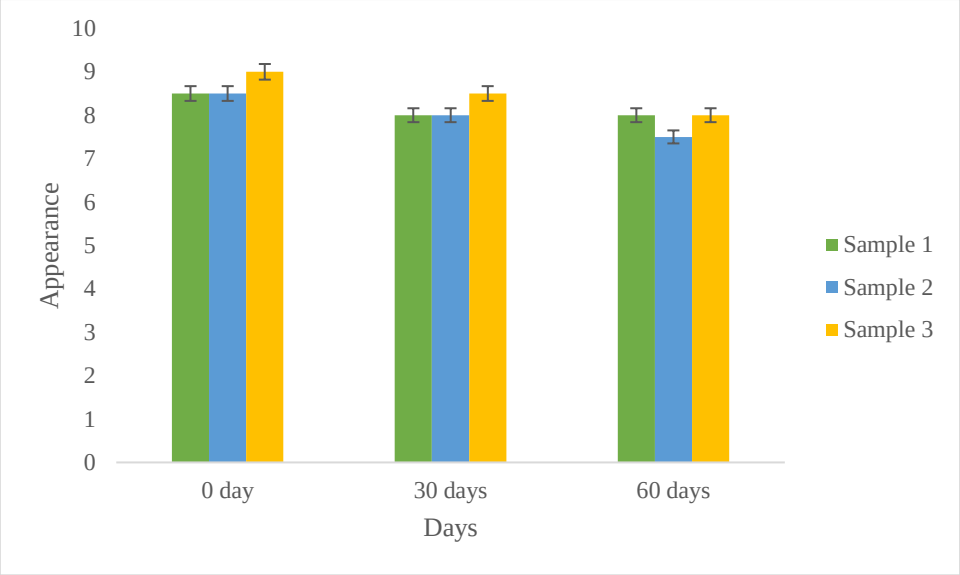


Fig 4.14. Appearance of Multigrain Cookies during the shelf-life study for two months

4.3.7 Overall Acceptability

The overall acceptability of a microencapsulated *Spirulina* based multigrain cookie in a shelf-life study refers to the extent to which consumers or sensory panellists find the cookie appealing, satisfying, and suitable for consumption over a specified period of time, in this case two months. It encompasses various sensory attributes such as taste, flavor, texture, appearance, and aroma, as well as any changes that might occur to these attributes during storage.

During the shelf-life study, cookies are periodically evaluated by sensory analysis to assess any changes in their sensory characteristics. If the overall acceptability of the cookie slightly reduces over the course of the study, it suggests that there may have been alterations in taste, flavor, texture, appearance, or other sensory attributes that have impacted how consumers perceive and enjoy the product. These changes might be due to ingredient interactions, degradation of certain components, moisture migration, oxidation, or other factors related to the storage conditions and the cookie's composition.

Understanding changes in overall acceptability is crucial for product development and quality assurance. It helps manufacturers identify potential issues affecting consumer preference and guides them in making improvements to ensure that the cookie maintains its desirable sensory qualities throughout its intended shelf life.

The effect of packaging materials and storage period of *Spirulina* based cookies on the overall acceptability of cookie is presented in Table 4.15. The overall acceptability of the

microencapsulated *Spirulina* based multigrain cookie decreased slightly with increase in storage period of two months as shown in Figure 4.15.

Table 4.15 Overall Acceptability of *Spirulina* enriched cookies during shelf-life study for two months

Samples	0 day	30 days	60 days
S1	8.5	7.5	7
S2	8.5	8	7.5
S3	9	8	8

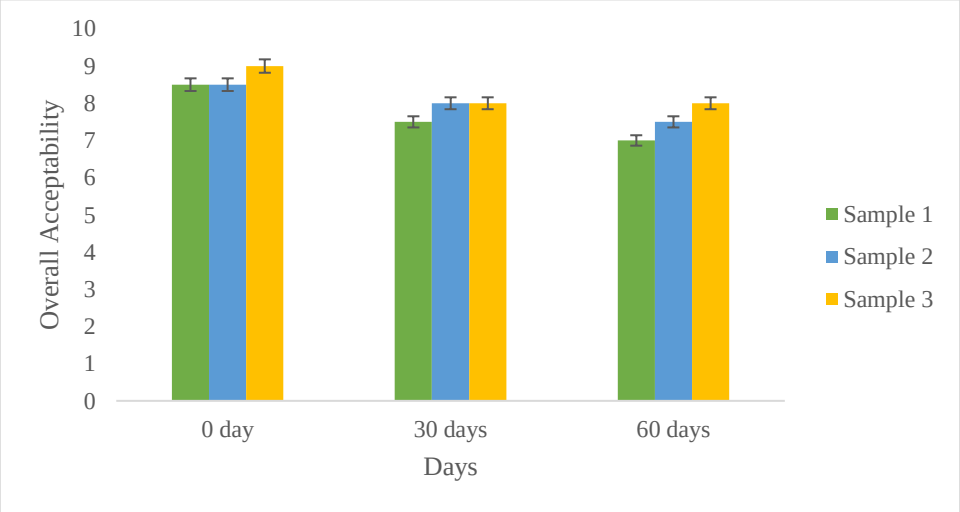


Fig 4.15. Overall Acceptability of Multigrain Cookies during the shelf-life study for two months

4.4 Fourier Transform Infrared Spectroscopy (FTIR) Fourier-Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique used to study the molecular composition and structure of a variety of materials, including biological samples like *Spirulina*. *Spirulina* is a type of blue-green microalgae that is often considered a superfood due to its high nutritional content. Enriching *Spirulina* through research involves enhancing its nutritional value, bioactive compounds, and other beneficial properties.

FTIR can play a crucial role in *Spirulina* enriched research in several ways:

Identification of Biomolecules: FTIR can identify various biomolecules present in *Spirulina*, such as proteins, lipids, carbohydrates, nucleic acids, and pigments. This helps researchers

understand the composition and potential changes in the bioactive compounds after enrichment processes.

Monitoring Chemical Changes: FTIR spectra can reveal changes in functional groups and chemical bonds within *Spirulina* samples. Researchers can monitor alterations in compounds that occur during enrichment procedures, such as changes in protein conformation, lipid oxidation, or carbohydrate modifications.

Quality Control: FTIR can be used for quality control purposes during the enrichment process. By analyzing FTIR spectra, researchers can assess the effectiveness of enrichment methods and ensure that the desired changes in the *Spirulina*'s composition are achieved.

Comparative Studies: FTIR spectra of enriched *Spirulina* can be compared to those of the unenriched counterpart. This comparison helps researchers understand the impact of enrichment on the molecular composition and structure of *Spirulina*, providing insights into its potential nutritional improvements.

Characterization of Bioactive Compounds: Enrichment often aims to increase the concentration of specific bioactive compounds in *Spirulina*. FTIR can help characterize these compounds and track their changes, providing valuable information about the enrichment process's success.

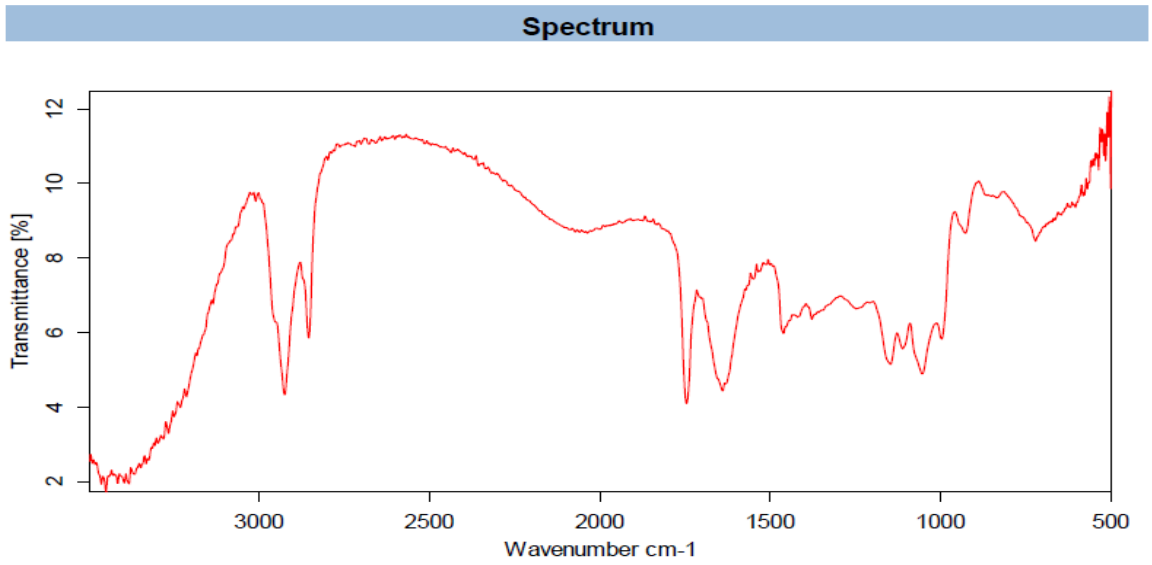
Quantitative Analysis: FTIR can be used for quantitative analysis of certain components in *Spirulina*. Researchers can establish calibration models to determine the concentration of specific compounds, aiding in assessing the success of enrichment strategies.

Microalgae Health and Stress Assessment: FTIR can be used to study the effects of stressors on *Spirulina*'s molecular composition. This is particularly useful in research aimed at understanding how enrichment processes affect the health, stress responses, and overall well-being of the microalgae.

Biocompatibility Studies: Enriched *Spirulina* might be intended for various applications, such as functional foods, nutraceuticals, or even pharmaceuticals. FTIR can assist in evaluating the compatibility of enriched *Spirulina* with other materials, potentially leading to innovative product development.

4.4.1 FTIR Method Analysis

Using the FTIR method, analysis was done of the functional groups present on the biosorbent and their effects on MNZ removal. In Figure 4.16, the FTIR spectra of fresh and used *S. platensis* are shown. Prior to the removal of the MNZ, the FTIR spectrum of the biosorbent showed the different main intense bands, around 2924.78 wave number corresponds to C-H stretching vibrations in alkanes, particularly in the CH2 groups, 2854.21 wave number is indicative of C-H stretching vibrations, typically associated with alkanes and aliphatic compounds, 1745.48 wave number is often attributed to C=O stretching vibrations, commonly found in carbonyl groups of aldehydes and ketones. 1639.32 wave number corresponds to C=C stretching vibrations in aromatic compounds, such as those present in conjugated systems like benzene rings, 1641 wave number is often indicative of C=C stretching vibrations in conjugated systems, such as those present in aromatic compounds like benzene rings,1461.28 wave number could be related to CH2 bending vibrations in alkanes or CH3 bending vibrations in substituted alkanes and 1157 wave number might correspond to C-N stretching vibrations in amines, particularly primary amines.



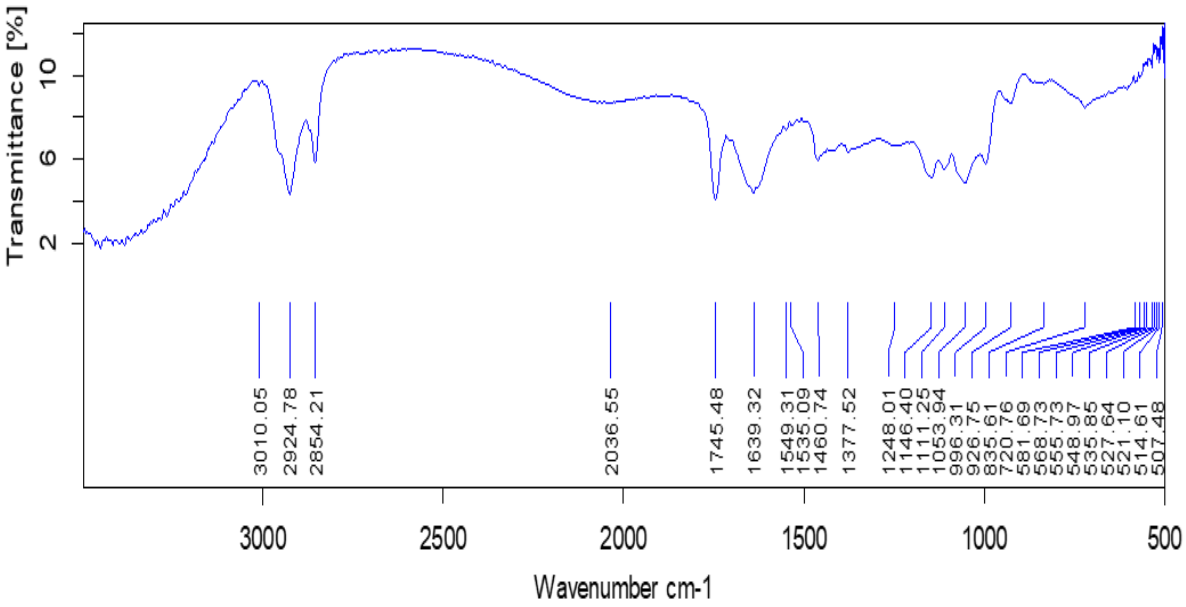
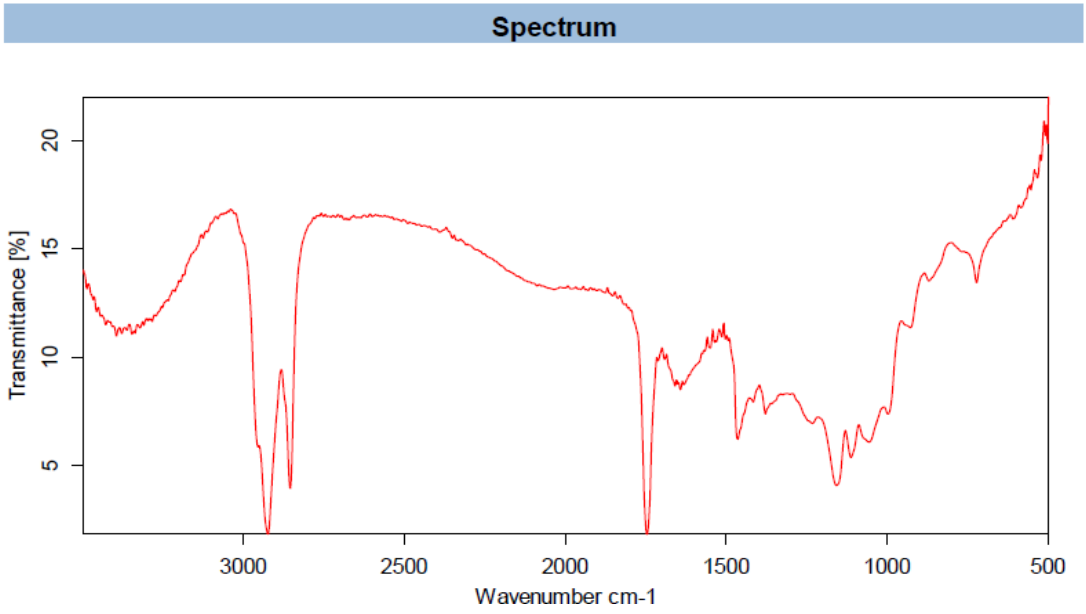


Fig. 4.16. FTIR analysis of *Spirulina* enriched cookies



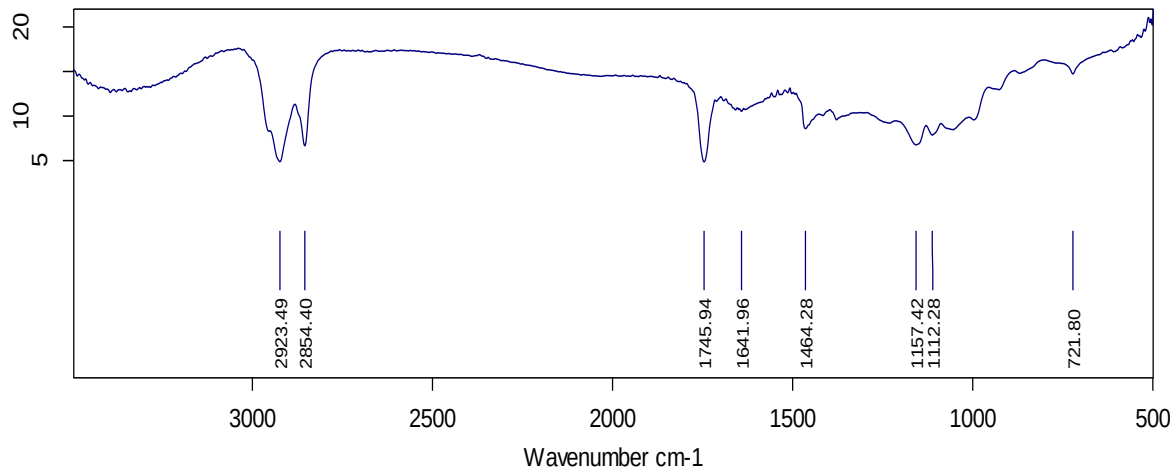


Fig. 4.17. FTIR analysis of Microencapsulated *Spirulina* cookies

CHAPTER 5
SUMMARY AND CONCLUSION

5.1 Summary

The combination of *Spirulina platensis* biomass and coconut oil addition in dough to the development of a novel *Spirulina*-based multigrain coconut cookies. The addition of *S. platensis* biomass as ingredient resulted in an innovative green colour, even more than the control without cyanobacterial biomass. This study suggests that *S. platensis* with antioxidant properties (also due to the coconut oil utilization) and containing alternative source of ingredients. This new product could become widely consumed by particular categories of people such as sportsmen, vegetarians, malnourished and the elderly, but also by consumers interested in a product with an innovative taste and colour.

5.2 Future aspects of the research

The coconut oil - enriched multigrain cookies incorporated with *Spirulina* can be further modified and made tastier by adding natural flavours and colours in a certain proportion. It could serve as the healthiest food for which can be consumed on daily basis and can also be stored for long term. *Spirulina* if grown in hygienic conditions can be used as space food.

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