A DISSERTATION ON

Development and physicochemical characterization of Powder-core beads of *Spinacia Oleracea*

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

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

BY
Ambreen Fatima Safvi
M. Tech Food Technology (IV Semester)
Roll No: 2101207003

UNDER THE SUPERVISION OF
Dr. Alvina Farooqi
(Professor and Head)
Department of Bioengineering

INTEGRAL UNIVERSITY, DASAULI, KURSI ROAD
LUCKNOW- 226026

DECLARATION FORM

I, **Ambreen Fatima Safvi**, a student of **M. Tech Food Technology** (II Year/IV Semester), Integral University have completed my six months dissertation work entitled **"Development and physicochemical characterization of Powder-core beads of *Spinacia Oleracea*"** successfully from **Integral University** under the able guidance of **Dr. Alvina Farooqi.**

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.

Ambreen Fatima Safvi
Student

Dr. Rahul Singh
Course Coordinator

CERTIFICATE

This is to certify that Miss **Ambreen Fatima Safvi** (Enrollment Number **1700100313**) has carried out the research work presented in this thesis entitled **"Development and physicochemical characterization of Powder-core beads of *Spinacia Oleracea*"** for the award of **M. Tech Food Technology** degree from Integral University, Lucknow under our supervision. The thesis 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. The dissertation was a compulsory part of her **M. Tech Food Technology** degree.

We wish her good luck and bright future.

Dr. Alvina Farooqui
(Supervisor)
Professor and Head
Department of Bioengineering
Integral University, Dasauli, Kursi Road,
Lucknow- 226026

INTEGRAL UNIVERSITY

CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **Ambreen Fatima Safvi,** a student of **M. Tech Food Technology** (II Year/IV Semester), Integral University has completed her six months dissertation work entitled **"Development and physicochemical characterization of Powder-core beads of *Spinacia Oleracea*"** successfully. She has completed this work from Integral University under the guidance of **Dr. Alvina Farooqui**, Professor and Head, Department of bioengineering. The dissertation was a compulsory part of her **M. Tech Food Technology** degree.

I wish her good luck and bright future.

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

INTEGRAL UNIVERSITY

TO WHOM IT MAY CONCERN

This is to certify that **Ambreen Fatima Safvi** a student of **M. Tech Food Technology** (II Year/IV Semester), Integral University has completed her six months dissertation work entitled **"Development and physicochemical characterization of Powder-core beads of *Spinacia Oleracea*"** successfully. She has completed this work from Integral University under the guidance of **Dr. Alvina Farooqui**. The dissertation was a compulsory part of her **M. Tech Food Technology** degree.

I wish her good luck and bright future.

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

ACKNOWLEDGEMENT

First of all, I bow in reverence to the Almighty Allah for blessing me with strong will power, patience and confidence which helped me in completing the present work and making me a proud muslim. I would like to thank Almighty Allah for blessing me with the best family and friends one could ever have.

I feel deeply obliged and indebted to my supervisor, **Dr. Alvina Farooqui**, (Professor and head, Department of Bioengineering, Faculty of Engineering) for her kindness and constant encouragement throughout the course of work. I am blessed to have her as my supervisor. She has helped me a lot during my dissertation work in many ways possible. Also, I would like to thank her for giving me such an opportunity to join the department laboratory and providing all the necessary facilities ever since I started my work.

Firstly, I feel ecstatic and blessed to thank my dear and near ones **Aisha Fatima**, **Sarah Sajid**, **Miss Hina Siddiqui**, and **Mrs. Gazala Anjum** for being my constant support during all these challenging years of my academics in Integral University. Thank you for boosting my self-esteem whenever I felt low and giving up on my dreams. You people are my great source of energy and pure happiness. Coming this far without you all would have been a real difficult task to survive in constant pressure caused by many people came in my life. You people are the pillars of my leading happy life. I assure that this friendship will continue and the roots of friendship will grow deep into our hearts even after the story of Integral University ends.

Secondly, I would like to thank some of the fabulous souls I had by my side through all these years in Integral University, **Faraz Ahmad Khan**, **Kalim Ahmad**, **Sheikh Rashid**, **Askari Raza**, **Amna Tamimi** and **Mantasha Khan**. They were not just my friends but also brother/sisters from another mother. Thank you for contributing beautiful part in my life and spending wonderful times that I would always cherish in my life. The moments we spent together especially on our Kashmir trip, will always be close to my heart.

I am elated with delight to avail this wonderful opportunity to express my sincere thanks to all the faculty members specially **Dr. Poonam Sharma** ma'am, **Dr. Arshied Manzoor** sir, and **Dr. Rahul Singh** sir for helping me in pursuing my dream goals and enlightening my path to success. A sincere thanks to the lab staff **Miss Priyanka Dubey** ma'am, **Miss Sufiya Aziz** ma'am, **Mr. Gyanendra Tripathi** sir and **Mr. Suhail Ahmad** sir of bioengineering department for their co-operation, affection, encouragement during my project pursuit.

My acknowledgement will be incomplete without mentioning my parents, my siblings and family members. It was their constant blessings that I was able to achieve my goal successfully. There are no sufficient words to express my feelings toward them. My stunning father **Mr. Najmul Haq Safvi** is the role model of my life in achieving my aim and working really hard for the sake of my ambitions and comfort. My loving mother **Mrs. Bushra Najam** for nourishing me and easing my discomfort, she always prayed for my success and worked hard in setting an example of beauty with brain. My beloved grandmother **Mrs. Anis Fatima** for her love and support which kept me motivated throughout all these years of my life, your endless affection towards me for being the eldest granddaughter in the Safvi family gave me inspiration to do well in my life. I would like to thank my beloved Brother and Sister **Ahmad Bilal Safvi** and **Alisha Fatima Safvi** for being an irritating yet helping and loving siblings I got as a present by Almighty Allah. I silently acknowledge my debt to all of them.

I would like to thank some of my batchmates for sharing an amazing journey with me specially **Zayeema Anjum**, **Alweera Ashfaq**, **Nazia Khursheed**, **Soumya Diwedi**, and **Madhulika Charan Di**. Thank you for calming my nerves whenever cortisol hit my brain and heart. You people held my hand to let me know I can do the best and have the ability to face all the problems. The craziness we enjoyed together, shouting on each other, ranting songs without any rhythm and allowing me to be myself are few moments which will live in my heart and brain rent free. Your existence brought me ample amount of peace and mental stability.

I would like to express my special thanks to **Dr. Owais Yousuf**, (Assistant Professor, Department of Food Technology, Islamic University of Science and Technology, Awantipora- 192122) whose continuous support, and invaluable guidance helped me to attain the excellence in my project work. He has not only guided me in my work but also gave me many valuable life lessons.

Date:- **Ambreen Fatima Safvi**

ABSTRACT

Nutraceuticals are products derived from food sources that are purported to provide extra health benefits, in addition to the basic nutritional value found in foods. The study was done to make a powder code hydrogel bead as a neutraceutical using spinach powder and sodium alginate by applying reverse spherification technique. Nutritional quality of spinach juice was increased in the form of encapsulated beads and to check physiochemical analysis of developed beads. Spherification is basically a process that seals a product in a jelly like membrane. The moisture content ranges from 8.63±0.04 to 12.34±0.02 of the bead samples with highest in sample 1 and lowest in sample 5. Ash content of the beads ranges from 32.42±0.01 to 36.81±0.01. bead with 4% sodium alginate has the highest ash content. Protein content of all the total samples was ranges from 31.45±0.03 to 32.33±0.0 where highest was of Sample 5. fat content was observed in the beads range from 3.97±0.01 to 4.35±0.01 where lowest was in sample 1 and highest was in sample 5. Fibre content of the beads ranges from 26.33±0.02 to 27.27±0.02, where sample 1 has the lowest and sample 5 has the highest range of fibre content. Carbohydrate content was much higher in the dried spinach powder (Control 1) as compared to that of fresh spinach juice (Control 2). No perfect pattern was observed in the energy content of the beads in fact beads samples have low energy content than that of spinach powder. Diameter of the beads was ranges from 16.32±0.02 to 17.85±0.01 with sample 5 having largest diameter. Weight of the beads was ranges from 1.45±0.02 to 1.8±0.02 where sample 5 has more weight than that of sample 1. Lastly thickness of the beads ranges from 1.11±0.02 to 1.34±0.02 with sample 5 having thicker membrane than that of all other bead samples. Further it can be concluded that sample 5 in all the bead sample showed good result comparatively to other bead samples.

TABLE OF CONTENT

LIST OF ABBREVIATIONS

Serial No.	Abbreviations	Full Forms
1.	ORAC	Oxygen Radical Absorbance Capacity
2.	DPPH	2,2-diphenyl-1-picrylhydrazyl
3.	GRAS	Generally Recognized as Safe
4.	CNS	Central Nervous System
5.	$NaC_6H_7O_6$	Sodium Alginate
6.	$C_6H_{10}CaO_6$	Calcium Lactate
7.	IMF	Intermediate Moisture Foods
8.	CSIR	Council of Scientific & Industrial Research
9.	AOAC	Association of Official Analytical Collaboration
10.	FSSAI	Food Safety and Standards Authority of India
11.	H_2SO_4	Sulfuric acid
12.	$CuSO_4$	Copper Sulfate
13.	K_2SO_4	Potassium Sulfate
14.	CO_2	Carbon dioxide
15.	H_2O	Water
16.	NaOH	Sodium Hydroxide
17.	HCl	Hydrochloric acid
18.	MSO	Methanolic extract of *Spinacia Oleracea*
19.	NAO	Natural Antioxidant
20.	RTE	Ready To Eat
21.	RDA	Recommended Dietary Allowance
22.	LCD	Liquid Crystal Display
23.	USD	United State Dollars

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER- 1
INTRODUCTION

Nowadays, the whole world is turning toward natural drugs and excipients. Natural materials do hold advantages over synthetic materials, because they are non-toxic, less expensive, and freely available. Furthermore, they can be modified to obtain tailor-made materials for the drug delivery system and they can compete with the synthetic agents available in the market. Plants are one of the most important sources of medicines. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. Medicinal plants have been used since prehistoric period for the cure of various diseases. Since these are in common use by the local people and are of great importance that is why a lot of people are engaged in the trade of important medicinal herbs throughout the world. Especially, people living in villages have been using indigenous plants as medicines since ages because this knowledge transfers from generation to generation and is based on lifelong experiences. Besides, the villages are far away from cities and mostly lack proper health facilities.

People in the modern world are busier and busy, leaving them little time to cook a nutritious dinner. A simple procedure using useful, healthful dietary ingredients is becoming more popular as a result. Again, as a result of globalization, people's preferences for novel food textures and appearances are changing at such a rapid rate that food producers occasionally struggle to keep up with the demand. Utilizing a colloidal particle system in the food sector is one of the promising techniques.

Hydrogels are three-dimensional colloidal systems made up of linear or branched polymer chains that are physically or chemically linked together and include a significant amount of water or biological fluid. Homogeneous or heterogeneous materials can be used to create hydrogels, which can be engineered to have certain mechanical and physicochemical properties. Because of its bioavailability, biocompatibility, customizable chemical or physical features, non-toxic nature, and flexible construction, hydrogels have shown promise in biomedical and food applications **(Maitra & Shukla, 2014).**

Nature is dominated by spherical or circular shapes. People find curved curves more attractive **(Bar & Neta, 2007)**. It also holds true for the way we eat. Fruits, meals, desserts,

chocolate, even dinnerware, are all rounded. All of these are subconscious influences, yet they all have a big impact on how people perceive food. meals with a rounded form and sweet meals are instantly associated by our brain with safe, amiable, pleasant, graceful, dreamy, and even beautiful shapes that promote quiet, peace, and relaxation. The circle's unavoidable beauty seems to have a strong biological foundation **(Saqib, Khaled, et al., 2022)**.

Considerable evidence exists for the role of antioxidative constituents of fruits and vegetables in the maintenance of health and disease prevention **(Ames et al., 1993)**. Spinach *(Spinacia oleracea)* is one of the most important antioxidative vegetables, usually consumed after boiling either fresh or frozen leaves. Freshly cut spinach leaves contain approximately 1,000 mg of total flavonoids per kilogram. The possible presence of flavonoid-like compounds in spinach was first reported in 1943 **(Panche et al., 2016)**, but nearly 20yr elapsed before the structure of the flavanol isolated from spinach leaves was established as patuletin (3,5,7,3′,4′-penthahydroxy-6-methoxyflavone) and the presence of spinacetin was confirmed **(Zane & Wender, 1961)**. In addition, the existence of several flavanol glycosides in a methanolic extract of spinach leaves was reported **(Aritomi & Kawasaki, 1984)**. The occurrence of at least 10 flavonoid glycosides has now been reported in spinach. These are glucuronides and acylated di-and triglycosides of methylated and methylene dioxide derivatives of 6-oxygenated flavanols **(Aritomi & Kawasaki, 1984)(Ferreres et al., 1997)**. Glucuronides are more water-soluble than glycosides and acylated compounds that remain in the tissue after cooking in boiling water.

Flavonoids and other phenolic constituents act as antioxidants by the free-radical scavenging properties of their hydroxyl groups. Extensive conjugation across the flavonoid structure and numerous hydroxyl groups enhances their antioxidative properties, allowing them to act as reducing agents, hydrogen-or electron-donating agents, or single t-oxygen scavengers **(Salah et al., 1995)**. Results from the in vitro oxygen radical absorbance capacity (ORAC) assay have shown that, among various fruit and vegetable extracts, foods with the highest ORAC activity include spinach, strawberries **(H. Wang et al., 1996)**, and blueberries **(Prior et al., 1998)**. The antioxidant capacity of spinach flavonoids has been determined by the free-radical scavenging assay using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical **(Gil et al., 1999)** and was compared with that of Trolox, a synthetic analogue of vitamin E. The most active products were those derived from patuletin with a 3′,4′-dihydroxyl group. The incorporation of a feruloyl residue increased the free-radical scavenging activity. During storage of spinach

leaves, a decrease in the total antioxidant activity was observed. Boiling of fresh-cut spinach leaves extracted approximately 50% of the total flavonoids and 60% of the vitamin C in cooking water; however, flavonoid glucuronides were extracted more than other glycosides **(Gil et al., 1999)**.

The purpose of the current review is to summarize previous reports of the beneficial effects of consumption of spinach leaves or spinach extracts on human health. The review provides epidemiological and preclinical data supporting the efficacy and safety of spinach consumption.

1.1 Objectives

The main objective of this study was:

1. To develop spinach beads using reverse spherification technique.
2. To increase the nutritional quality of spinach juice in the form of encapsulated beads.
3. Physiochemical analysis of developed beads.

CHAPTER- 2

REVIEW OF LITERATURE

A food hydrogel bead is a spherical, complex, three-dimensional colloidal system in which active ingredients may be disseminated or incorporated in the core and encased in a continuous sheath of protection. Depending on their use, gel beads may have a variety of internal or exterior structures. Gel beads hold great promise for the modern food system because they have the potential to successfully address a wide range of issues, including their ability to be incorporated into the food matrix without compromising quality, their ability to protect functional compounds from chemical, physical, or biological degradation, their ability to mask off-flavors, their ability to deliver them to a specific site-of-action where they exhibit activity, their ability to improve storage, handling, and utilization, and their ability to extend shelf life. There have already been reports of several chemicals being carried and trapped by gel beads used in foods. In addition to their functioning, they can create mouthfeels with enticing textures **(H. Li et al., 2022)** (G. Maleki et al., 2022). It has also been claimed that gel beads can absorb dangerous chemical substances **(Saqib, Liu, et al., 2022)**. Additionally, to overcome the weak mechanical qualities of the biomaterial, mechanically robust beads can be made using cutting-edge materials and techniques (such as coating, composite materials, etc) **(Lai et al., 2020)**. As a result, spherical gel beads have been developed with significant potential for the food design industry.

Food now satisfies hunger in more ways than one. Food-related emotional connections go deep, are intricate, and are still unravelling. It might involve the combination of physiology, psychology, and food science. Human’s eating habits have changed as a result of socioeconomic and cultural changes in society. A psychology-based healthy eating approach was born out of the need for firms to concentrate on cutting-edge food and drink formulations that consider mental and emotional wellbeing in order to adapt to these constantly changing human behaviors. The ability to access knowledge, particularly online, motivates and directs people to work towards living better lives. Rapid changes in people's eating habits are foreseen, especially in the wake of the COVID-19 pandemic. Food producers will find it difficult to reconnect with changing customer demand **(Saqib, Khaled, et al., 2022)**. As usual, taste and health are highly individualized. A hyper-individualized strategy to meeting customer demand can be built with the use of technology. The best way to give folks a meal

experience worth sharing is through texture. There is much more happening in our tongues than what we think we are tasting, whether it be an unusual or altered texture. It turns out that our taste senses are deceived by our minds. According to studies, we would pay twice as much for the same meal if it was more aesthetically pleasing and environmentally friendly.

Compounds including flavor, enzymes, antimicrobials, antioxidants, bioactive nutrients, minerals, probiotics, etc. can be encapsulated, transported, protected, and released using a colloidal system **(D. Li et al., 2021)(H. Li et al., 2022)**. The food system is heavily focusing on hydrocolloids because they can safely transport a variety of bioactive substances, are simple to tailor, and, most importantly, may alter the physicochemical and sensory qualities of food **(Goff & Guo, 2019)**.

Regarding the encapsulation of various chemicals in the food system, there has been a rise in scientific development and industrial production since the turn of the twenty-first century, both in terms of quantity and diversity **(Timilsena et al., 2020)**. The probiotics industry was estimated to be worth USD 58.17 billion and was projected to expand 7.5% between 2021 and 2030. While forecasts for the next ten years predict increases in Omega-3, vitamin supplements, herbal supplements, seaweed, pre-biotics, and nutraceuticals, respectively, of 8.2%, 6.2%, 9.1%, 10.8%, 6.6%, and 10.1%. Surprisingly, insect protein was predicted to grow by 27.4% between 2021 and 2028, outpacing all other popular health products. The food colloids market was nearly 10 million in 2021, with forecasted growth of 5.1% by 2030 **(*Food Hydrocolloids Market Size, Share, Growth Report,* 2030)**. This indicated the upcoming changes in global consumer consciousness about healthy lifestyles and food habits.

Apart from folk medicine, aromatic herbs have always been used for other things, like food preservation, which has gradually spread throughout the world **(Potortì et al., 2020)** with growing interest in the food industry because uncooked extracts of herbs, spices, and other plant materials rich in phenolics can raise acceptability, postpone the oxidative breakdown of lipids, and even improve the nutraceutical value of food products **(El-Sayed & Youssef, 2019)**. One of the main reasons why food quality deteriorates is due to oxidative lipid breakdown. Numerous aromatic herbs have demonstrated potential as natural antioxidants, with the potential for use in a variety of ways, including whole, milled, in the form of extracts, or as essential oils **(Giannenas et al., 2020)**.

Microencapsulation can be used as an exciting alternative to stabilize phenolics from natural extracts **(Lee & Chang, 2020)**, and as a result, it has attracted significant interest from the

food, pharmaceutical, nutraceutical, and cosmetic industries in recent years. It also has a wide range of applications in the design of functional products, such as food or food ingredients **(Teng et al., 2019)**.

The active ingredient is encased in a carrier (matrix) as part of the microencapsulation process to shield it from harmful outside influences like volatile losses, additional interactions with molecules like proteins, and various time-dependent temperature, oxygen, light, humidity, and chemical conditions **(Gheonea (Dima) et al., 2020)**. The construction of a protective shell to surround the delicate compound and encourage its regulated release is the process used there **(Tarone et al., 2020)**.

2.1 Why hydrogel beads?

The concept of encapsulation has evolved in food science since many food components cannot be used as a direct additive due to poor stability or adverse sensory impact. Again, some functional components also need to reach the targeted site, overcoming the harsh digestive environment. At the same time, some components needed to be modified for prolonged storage or transport convenience. Colloidal particulates lead the concept of encapsulation to an advanced level. Gel beads can be preferential over some conventional systems considering the following issues:

2.1.1 Safety: The first and foremost condition of a food system is that it must be safe. Hydrogel beads are generally prepared from natural food colloids. Thus, they are safe and usually do not interfere with the trapped component. Again, a reactive or incompatible component can be easily removed. With only a few exceptions, most of the biomaterials that are used for hydrogel beads are biocompatible, non-toxic, and certified as GRAS (generally recognized as safe) **(Goff & Guo, 2019)(Matricardi et al., 2015)**.

2.1.2 Attractive: After ensuring safety, the next things that come to the mind of a consumer are the attractive texture and taste. In this regard, hydrogel beads are an odds-on-favorite. It can be customized to any desired texture, size, shape, or color. It has all the potential to draw the attention of consumers of all ages **(Stribiţcaia et al., 2020)(Zegler, 2018)**.

2.1.3 Specific requirements: Since food production must be considered globally now, a specific requirement like "Halal" or "Kosher" needs to be fulfilled. Most hydrogel beads are made from a wide range of natural biopolymers that can easily meet these regulations. In

addition, dietary or safety regulations are also not a major concern since most of these materials are already approved globally **(Seisun & Zalesny, 2021)(Thies, 2012)**.

2.1.4 Targeted delivery: Hydrogels can easily be designed for a targeted delivery path like the mouth, stomach, intestine, or colon. Beads can be fabricated to sustain different digestive environments **(Q. Li et al., 2021)(Wong et al., 2021)**.

2.1.5 Easy fabrication: Although some bead manufacturing requires specific and sophisticated instrumentation, most gel beads are made without the use of expensive or high-tech equipment or processes. Regular food processing technologies can be applied to make beads with versatile encapsulants **(Q. Li et al., 2021)**.

2.1.6 Diversified: The same gel beads can be designed to carry diversified materials. The system can efficiently encapsulate both small and large molecules **(Wong et al., 2021)**.

2.1.7 Controllable delivery: The release of materials from gel beads can easily be controlled. Delivery like intestinal absorption requires slow, prolonged delivery, while flavor requires quick release in the mouth **(Mackie, 2012)**.

2.1.8 Survivability: Beads can protect active materials during processing, cooking, or in a harsh stomach environment. Bioactive compounds can be protected from the harsh external environment by improving the bioavailability of orally administered molecules within the hydrogel matrix **(Corstens et al., 2017)(T. Guo et al., 2017)**.

2.1.9 Transport and storage: Gel beads are simple to transport and store because they are mechanically sound enough to keep their shape and are no longer distinguishable from regular foods.

2.2 Types of gel beads

Gel beads can be classified based on their structure, properties, and applications. Nano, Micro, Macro, and millilitres sizes are all possible. Beads can be divided into the following categories based on their structure. Depending on the intended use, these types may be of various sizes.

2.2.1 Solid gel beads: These are the most common and popular kinds of beads. These beads can easily be manufactured from a variety of materials. It can be made from a single material or a combination of different colloidal materials. A wide range of active ingredients has been

reported to be trapped inside solid gel beads. Colloidal materials act as a retarding matrix in hydrogel beads. The active component may or may not interact with the gel matrix.

2.2.2 Liquid-core gel beads: This type of bead has an aqueous core covered by a seamless shell or membrane and is often referred to as a reservoir system. The membrane can function as a barrier or as a control for diffusion. This type of bead is generally produced by the ionotropic gelation method. A liquid droplet can be shaped into an insoluble cavity by a controlled ionic interaction between an anionic and cationic polymer within the boundary condition. These beads have recently gained popularity as directly edible beads with an exciting bursting mouthfeel **(Bremond et al., 2010)(Saqib, Ahammed, et al., 2022)**.

2.2.3 Emulsion core gel beads: This is comparable to the liquid-core beads mentioned above, with the exception that the core material could be an emulsion, nanoemulsion, liposome, or emulsion gel. This type of bead is commonly used with hydrophobic materials. Since the demand for functional foods is growing, food manufacturers and researchers are increasingly interested in developing hydrophobic delivery materials. Numerous scientific papers describe the encapsulation of versatile, functional components in emulsion beads and their successful delivery to the target site with high efficiency **(Feng et al., 2018)(Lin et al., 2020)**.

2.2.4 Double encapsulated beads: Compared to the other types of beads, this one is quite intriguing and intricate. This may also be described as the combination of beads described previously. In this type of bead, microbeads are typically re-encapsulated within relatively larger beads. In one system, two materials with opposing or incompatible properties can be encapsulated. Probiotics and antibiotics, for example, were encapsulated in an alginate matrix **(Z. Li et al., 2018)**.

2.3 Health and sensory aspects

In view of the powerful, robust, and multifunctional functions of hydrogels, hydrogel beads undoubtedly play a crucial role in modern food science, such as building foods with the required exciting texture, maintaining metastable food structure, and extending shelf life, designing food packaging, nutrition, delivery and bioavailability, calorie control, and food safety monitoring.

It is tough to predict the long run of gel science accurately, and it is also challenging to accurately predict the application potential of hydrogels in food science. Both colloidal gel

science and food science are developing rapidly. The current review discusses the application method of hydrogel beads in food science. Hydrogels are good candidates for use in delivery systems because they can take in and hold a large amount of water or biological fluids in a 3D network shape **(Liu et al., 2019) (McClements, 2017)**.

Other properties of hydrogel beads, such as response to stimuli to heat, pH, and light, are beneficial for controlled release into food delivery systems **(Cooper & Yang, 2019)**. Hydrogel beads can also play an essential role in reducing calorie intake, improving satiety, or reducing intake **(Cao & Mezzenga, 2020)**. **Wu et al., (2014)** created hydrogel granules from protein and fiber in fine soft substances that can be a healthier replacement for starch granules. **Thompson et al., (2017)** reported using a temperature-insensitive food-grade methylcellulose hydrogel blend to reduce the heat density of pancakes cooked at temperatures well above the boiling point of water. **Chung et al., (2013)** reported that emulsion hydrogels in food products reduce the fat content by replacing animal oils with vegetable oils.

The hydrogel beads have important structural properties (elasticity, stiffness, impact strength, and fracture toughness) as soft materials. Therefore, in addition to being used as a replacement to reduce calories, hydrogels can also improve texture or mouthfeel. For example, emulsion hydrogels can affect the textural properties of foods **(Dickinson, 2012)**.

Interestingly, replacing meat or starch with hydrogels with excellent textural characteristics or low oil content is also an effective way to reduce the calorie content of foods. Because the hydrogel has a high-water retention capacity in its structure, the three-dimensional hydrophilic polymer network can allow many molecules or ions to diffuse through the system freely.

In addition, the high adsorbent with high porosity, large surface area, and reusability makes the hydrogel beads an effective platform for adsorption or removal activity **(Gonçalves et al., 2017); (Janaína Oliveira Gonçalves et al., 2019); (X. Guo et al., 2019); (He et al., 2020); (M. Wang et al., 2019)**. For example, **Janaína Oliveira Gonçalves et al., (2020)** developed a promising adsorbent based on chitosan hydrogel beads substrates modified with carbon nanotubes to remove food dyes in simple, binary aqueous systems.

In addition, there are many research in the environmental sciences on using hydrogels to remove heavy metals and other contaminants that could also be considered for applications in food science.

2.4 Gelling mechanism of hydrogels

In polymer chemistry, crosslinking is a stabilization process whereby polymer chains are linked with each other by an ionic or covalent bond. Crosslinking restricts the mobility of polymer chains, transforming a liquid state into a solid or gel state. As a result, the individual properties of free-moving polymer chains are altered. Crosslinking enhances the mechanical, chemical, and thermal properties of any polymer. On the other hand, hydrogels are polymer structures that can contain a large amount of water or biological fluid. So, applications in food science, especially as beads, crosslinked hydrogels are widely utilized as carriers or shell materials for the delivery of functional materials.

The water status in a hydrogel is another important consideration. Water accommodated in a gel network can be classified into four groups, such as free water, interstitial water, bound water, and moderately bound or semi-bound water **(Redaelli et al., 2017)**. The final gel properties (mechanical, physical, or release properties), stability, and performance largely depend on the amount and type of water.

The changes in properties due to crosslinking are crucial and advantageous while designing beads for a particular purpose. A hydrogel polymer can be synthetic or natural, formed by homopolymers or co-polymers. Hydrogels can form three-dimensional netlike imperfect macromolecular network structures by linking multiple chains at specific binding sites. Thus, huge amounts of water are trapped in the gel network by hydrogen bonds. Polysaccharides, proteins, or interoperating these two are the most common types of hydrogels that have been utilized so far. There are two approaches to making crosslinked hydrogels.

1 Chemical crosslinking.

2 Physical crosslinking.

In chemical crosslinking, generally, a crosslinker (e.g., glutaraldehyde, transglutaminase, formaldehyde, dialdehyde) is used to form a covalent bond **(Ahammed et al., 2021); (Maitra & Shukla, 2014); (Zhu, 2010)**. Chemically crosslinked gels are mechanically strong and chemically stable, but for food applications, sometimes these crosslinkers are associated with health hazards. Physical crosslinking does not require any chemical agent to form a gel. Generally, these are ionically crosslinked, and there is weak interaction between polymer chains, for example, sodium alginate. Physical crosslinking is inhomogeneous and free chain ends or chain loops are available in the gel network. So, these gels are not permanent and

often reversible **(M. Wang et al., 2019)**. The properties of physically crosslinked gels are largely dependent on temperature, material properties, monomeric compositions, pH, concentration, etc. Physical and chemical crosslinking are often used in tandem. Physically formed protein-based gels, for example, can be chemically crosslinked to improve their mechanical properties.

Thermal gelation, or heat-induced gelation, is another very common and significant process of gelation. The molecules unfold or dissociate due to the absorption of energy produced by heat, then aggregate by the association of helices. After cooling down, they become rigid. The aggregation of helices produced stable gels, and the presence of salt increased the aggregation. In some cases, just heating will cause co-polymerization **(Redaelli et al., 2017)**.

Crosslinked polymer chains bring about significant changes in physical and chemical properties depending on crosslinking degree, crystallinity, crosslinkers, and polymer properties. Among the changes: decrease or increase of elasticity, decrease in flow behaviour, increase in polymer strength and toughness, increase in hydrophobicity, changes in glass transition temperature change to thermoset from thermoplastic are most notable where most of the properties are irreversible **(Maitra & Shukla, 2014)**. Crosslinking is a fundamental process to produce hydrogel beads. So far, it has been the most widely utilized approach. A regulated gelling technique is a must to develop the desired attributes of the final beads. Ionotropic gelation of sodium alginate or heat set gelation of agar or carrageenan can be mentioned as an example in this regard.

2.5 Designing hydrogel beads for active delivery

Before designing a hydrogel-beads-based delivery system, the prerequisite question will be why do we need to use a functionalized delivery system for foods? There are many food components that cannot be used simply in foods in their regular form (e.g., some essential lipids, vitamins, flavors, antimicrobials, etc.). A special delivery system is required to successfully incorporate them into the food matrix where they can withstand the adverse effects of physical–chemical degradation. Another reason is that some active compounds need to be delivered to a particular site of action. To transport these components up to the target site while protecting them from a hostile environment, carrier or coating materials are used. Among other uses, improving handling and storage, masking off-flavor, extending product shelf life, and enhancing foods' sensory attributes can be mentioned. So, it is clearly delineated that the design of hydrogel beads will be extremely divergent and pertinent to the

specific intention. For example, protein is digested in the stomach while fiber is indigestible and can resist degradation up to the colon. Therefore, in designing a delivery system for any location of the human GI tract, an in-depth understanding of the mechanisms and interactions of the carrier matrix and active materials is a prerequisite. At the same time, it is also necessary to make sure the active component remains uninterrupted or does not produce any undesired secondary component throughout the whole process. For example, milk protein was reported to interact with some polyphenolic compounds **(Serafini et al., 1996)** and some bioactive peptides, and was encapsulated to mask their undesirable bitter taste **(Sun et al., 2021)**. A hydrogel bead may not always be designed only to be transported through the GI tract. It can also be fabricated to increase a food's shelf life, esthetic value, or sensory value.

In order to develop hydrogel beads for active delivery following attributes may need to consider **(McClements, 2017)**

• Food grade

• Economic production

• Food matrix compatibility

• Protection against chemical degradation

• Loading capacity and retention

• Delivery mechanism

• Bioavailability/bioactivity

2.6 Fundamental considerations for fabricating beads

2.6.1 Gel bead composition: The most preeminent parameter that needs to be considered before designing hydrogel beads is the gel beads' composition. How a delivery system reacts, how efficiently it will deliver, how the intermediate reactants will react, the active compound and delivery matrix stability and sensitivity, how the matrix disassociates and reacts in different triggering conditions (e.g., temperature, pH, enzyme); these are all the essential properties **(Augustin & Sanguansri, 2012)**. Along with these regulatory and safety concerns, environmental issues, labelling, certification, or a special requirement like halal, kosher, or vegetarian needed to be addressed.

2.6.2 Release mechanisms: Gel beads may have many different release profiles depending on the specific purpose, such as burst release, sustained release, triggered release, and targeted release Structure and dimension: Hydrogel beads are generally spherical in shape. But other shapes, like pears, cylindrical, or irregular shapes, can also be formed **(Cao & Mezzenga, 2020)**. Depending on the indented use, the beads can range in size from micrometres to several millimetres. Direct edible gel beads specially designed for the esthetical purpose or texture modifier shape are very important. But a carrier with other systems in a matrix shape may affect release and stability since they can hardly be seen by the naked eye.

2.6.3 Gels bead Biocompatibility: The biocompatibility and bioavailability of gel beads during transport and dissolution are very complex and significant. Hundreds of factors may be involved in the human digestion track. Based on gel bead formulations, the GI environment can be influenced by ionic strength, pH, surface charge, electric potentials, enzymes, flow behaviours, internal forces, binding to the biological surface, etc **(Kharkar et al., 2013) (Redaelli et al., 2017)**.

2.6.4 Gel bead stability: The fabricated gel beads must be stable up to the target site of action unless every triumph will be meaningless. The active site may be anywhere from the mouth to the colon **(Đorđević et al., 2015)**. For example, if a bead is designed for a textural profile, it must uphold its texture during oral processing from storage, freezing, or osmotic environment; if it is designed for the colon, it must survive from oral processing, gastric environment, or intestinal digestion **(Shahidi et al., 2020)**.

2.7 Challenges and future trends

There has been a significant amount of research on active delivery using biomaterials, and currently, many encapsulation technologies have already been introduced in pharmaceuticals, cosmetics, and health care products, but the food industries are still lagging. There are very limited and classified hydrogel-based food products currently available on the market. The main challenge for the food industry is to make sure the ingredients and delivery system are food grade and relatively inexpensive. People will not buy food with the same money they are ready to spend on cosmetics, medicine, or health care products. Thus, the fabrication methods for hydrogel beads must be in accordance with food safety, economical, robust, and reproducible. The taste and smell of the food itself is still a major challenge for active delivery via food. It will not matter how efficient, effective, or essential a compound it is,

unless the food becomes attractive and tasty. Consumers' perception of food is very complex and personalized. In our present food system, foods remain common for all people. But personalized food production is in demand, and industries are focusing on that. Personalized foods mean foods that meet a specific person's demands or choices. We can only find infant or baby foods while there is little or no work for elderly people. But elderly people require special attention in terms of food choice as their digestive systems change with age. Again, any specific group of people may have food sensitivity (for example, food allergen) or special needs (for example, specific vitamin-rich food). We eat food for basically two reasons. There is a difference between food preferences and demand among countries. The first is to satisfy our hunger, and the second is to meet our nutritional and sensory requirements **(Mackie, 2012)**. In the least developed and developing countries, people can hardly meet their nutritional demands while striving for a full-filled stomach, while in developed countries, the sensory and functional attributes are very demanding, and people are suffering from obesity due to overeating. Therefore, a country-specific demand also needed to be addressed. Formulation of hydrogel-based foods with bioactive compounds (e.g., hydrogel beads) is a promising solution to meet the highly personalized and country-wise demand for food soon.

Now the research trend is moving towards developing new food grade biomaterials and emerging processes with more efficiency, keeping in mind current limitations. For example, some protein-based hydrogel systems can be allergic or sensitive to a certain group of people. Furthermore, modified proteins or starch may have less consumer appeal due to a lack of natural labelling. Sensory perception of biomaterials is still not widely popular. Scientists are considering all these issues and trying to develop cost-effective bio-materials since cost and taste are among the major challenges for developing a hydrogel-based active delivery system. With the evolution of the concept of molecular gastronomy, liquid core or acquis core hydrogel beads have attracted significant attention from consumers as they have exciting explorable textures. The size of the gel beads was transformed to millimetre size from the conventional micro-sized. This type of bead would be expected to be seen more abundantly in combination with other foods or drinks.

Another significant hindrance to food application is that most biomaterials cannot withstand high temperatures and shear during processing. As the materials for hydrogels are generally protein or polysaccharides, during food processing, they undergo physical and chemical changes. Market food has scarcely been imagined without processing. This problem

significantly affects the flourishing of hydrogel-based products in the food sector. So, in the future, a temperature-stable, biocompatible material will be highly predictable.

At present, the application of gel beads or hydrogel-based products is on a laboratory scale, as very few products on the market can be seen. And the research is published based on a simple delivery system (e.g., fasting condition). But the human GI tract and nutrient absorption are more complex. In addition, the human digestive system functions significantly differently from an infant to an adult and elderly person. To understand the actual absorption phenomenon, trials needed to be performed in a complex system mimicking the real absorption phenomenon **(Saqib, Khaled, et al., 2022)**.

However, developing feasible, sustainable, and successful hydrogel beads or any other hydrogel-based food products for consumers with scientifically proven outcomes requires collaboration and partnership between experts from nutraceutical ingredients, capsulation, and colloidal system technology and food manufacturers. The food market is likely to be driven by the need and desire for highly customized hydrogel-based food products.

2.8 Medicinal herb *spinacia oleraceae*

Spinacia oleracea is an edible flowering plant in the family of Amaranthaceous. *S. oleracea*, was long considered to be in the family Chenopodiaceae, but in 2003, that family was merged into the family Amaranthaceae in the order Caryophyllales. In Hindi it is known as “Paalak” and in English as “Spinach.” It is native to central and South Western Asia. It is cultivated for the sake of its succulent leaves. It has the largest consumption as favourite food in winter season of India **(Kirtikar, 1935)**. It is a rich source of vit-A, vit-C, vit-E, vit-K, vit-B6, vit-B2, magnesium, manganese, folate, betaine, iron, calcium, potassium, folic acid, copper, protein, phosphorous, zinc, niacin, selenium, and omega-3 fatty acids. Spinach cultivars are poor source of fat that make them good food for obese and diabetic people. Spinach also packed with several anti-oxidants like polyphenols, flavonoids and carotenoids which are shown to possess anti-inflammatory effects, antimutagenic potential, antineoplastic effects as well as chemo-preventive activates **(Ergene et al., 2006) (Verma, 2018)**. Various pharmacological activities of Spinacia oleracea such as, anti-oxidant, antiproliferative, anti-inflammatory, antihistaminic, CNS depressant, protection against gamma radiation, hepatoprotective have been reported. Various secondary metabolites like flavonoids, carotenoids, phenolic compounds have been reported from this plant 8. Spinach leaves are cooling, emollient, wholesome, antipyretic, diuretic, maturate, laxative, digestible,

anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leukoderma, scalding urine, arrest vomiting, biliousness, flatulence. And have been used in the treatment of febrile conditions **(Chopra et al., 1992)**.

2.8.1 CLASSIFICATION

Kingdom: Plantae

Super division: Spermatophyta

Division: Magnoliophyte

Class: Magnoliopsida

Order: Caryophyllales

Family: Amaranthaceae

Subfamily: Chenopodioideae

Genus: Spinacia

Species: Spinacia oleraceae

2.8.2 Nutrient contents

Spinach is a mineral-rich vegetable. An earlier study on the edible portion (87%) of spinach records (in %): moisture, 94.3; protein, 2.2; fat,0.7; fiber,0.6; mineral matter,1.7; carbohydrate, 2.9; and oxalic acid, 658 (mg/100g). Mineral composition includes (mg/100g): calcium,73; magnesium, 84; potassium, 206; iron, 10.9; phosphorus, 21; sodium, 58.5; copper, 0.01; sulphur, 30; nickel, 0.42; manganese, 9.61; molybdenum, 0.08; zinc, 13.53; and strontium, 0.077. Spinach is a good source of the vitamin B complex, ascorbic acid, vitamin A and carotene. It is also a natural source of vitamin K **(CSIR, 1949)**. Spinach shows presence of different carotenoids like lutein, β-carotene, violaxanthin and 9'- (Z)-neoxanthin **(Das & Guha, 2008)**.

2.8.3 Traditional uses

The plant is sweet, cooling, carminative, laxative, alexipharmic; useful in diseases of blood and brain, asthma, leprosy, biliousness; causes "kapha" (Ayurveda). It has been used in the treatment of urinary calculi. In experiments it has been shown to have hypoglycaemic

properties. The leaves are cooling, emollient, wholesome, antipyretic, diuretic, maturate, laxative, digestible, anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm scabies, leukoderma, scalding urine, arrest vomiting, biliousness, flatulence. They have been used in the treatment of febrile conditions. The seeds are useful in fevers, leucorrhoea, urinary discharges, lumbago, diseases of the brain and of the heart (Yunani). Seeds are laxative and cooling. They have been used in the treatment of difficulty in breathing, inflammation of the liver and jaundice. The green plant is given for the urinary calculi **(Kirtikar, 1935)**.

2.8.4 Pharmacological activity

2.8.4.1Anticancer Activity- This concluded that the spinach glycoglycerolipid fraction can inhibit mammalian pol activity, human cultured cancer cell growth, and in vivo solid tumor proliferation with oral administration. This fraction could help to prevent cancer and be a functional food with anticancer activity **(Maeda et al., 2008)**.

2.8.4.2 Anthelmintic Activity- Kredy, (2020) evaluated the anthelmintic activity of crude extract of Spinacia oleracea Linn. and different extract namely fresh juice extract and methanolic extract using Pheretima posthuman as test worms. Different concentrations 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, and 50 mg/ml of fresh juice extract and methanolic extract of Spinacia oleracea Linn (MSO) were studied to determine the time of paralysis and time of death of worms. Both the extract performed in-vitro anthelmintic activity **(Verma, 2018)**.

2.8.4.3 Antioxidant property- The chemical fraction of natural antioxidant (NAO) components in Spinacia oleracea was reported by Grossman. The study demonstrated the presence of both flavonoids and coumaric acid derivatives as antioxidant components of the aqueous extract of spinach leaves **(Kancherla et al., 2019)**.

2.8.4.4 CNS Depressant Effect- Treatment with Spinacia oleracea extract decreased the locomotor activity, grip strength, increased pentobarbitone induced sleeping time and markedly altered pentylenetetrazol induced seizure status in Holtzman strain adult male albino rats. S. oleracea increased serotonin level and decreased both norepinephrine and dopamine levels in cerebral cortex, cerebellum, caudate nucleus, midbrain and pons and medulla **(Das & Guha, 2008)**.

CHAPTER- 3

MATERIAL AND METHOD

This chapter comprises of all the details related to materials and methods which were used during the development of liquid core hydrogel beads for the preservation of *Spinacia Oleracea* juice, and their different testing.

3.1 Materials used

Fresh spinach leaves, Sodium Alginate, Calcium Lactate, clean petri dishes, Glass rod, Beakers, Conical flask, Dry crucibles, Spatula, Spherification spoon, Induction, Funnel and Chemicals/Reagents as per test's requirement.

3.2 Equipment used

3.2.1 Tray Dryer

Tray dryer of Gentek India Pvt. Ltd. (model AI 7781) was used for drying the spinach leaves before making its powder shown in the **fig 3.1**. The principle of a tray dryer is based on the process of convection drying. Convection drying involves the use of hot air to remove moisture from the material being dried. The hot air is circulated throughout the drying chamber, and as it passes over the trays, it removes moisture from the materials on the trays.

Fig 3.1 Tray dryer

3.2.2 Analytical Balance

Analytical balance of Wenser company (model PGB 6000, ranging to 6000 grams) was used for measuring the weight of all the materials and used for measuring the weight of samples

during different analysis shown in **fig 3.2.** It is based on the mass conservation principle. It is used to measure small mass in the sub-milligram range. It uses an electromagnet to generate a force to counter the sample being measured.

Fig 3.2 Analytical balance

3.2.3 Magnetic Stirrer

Magnetic stirrer of Remi Elektrotechnik Ltd. (model no. 2-MLH) was used for making several solutions used during the whole research work shown in the **fig 3.3**. It uses a rotating magnetic field to stir a non-magnetic liquid in a container. The rotating field is created by a magnet mounted on the stirrer underneath the container. As the magnet rotates, it creates a rotating magnetic field that extends into the liquid.

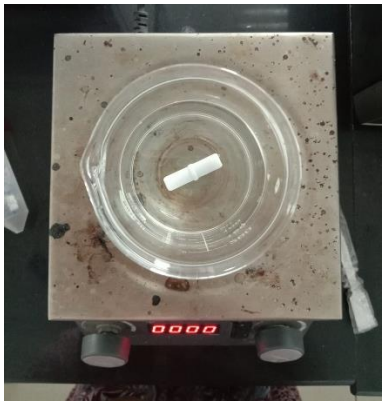


Fig 3.3 Magnetic Stirrer

3.2.4 Grinder

A simple grinder of Bajaj Appliances (model no. G×3 dix) was used during the experiment to crush the dried spinach leaves to make its powder shown in the **fig 3.4**. Grinding is the process of breaking up particles. Grinding is used to turn the solid blend into a granular form.

In chemical processes, crushing is usually followed by grinding to produce a fine-sized powder. Grinding is the process of breaking up particles.

Fig 3.4 Grinder

3.2.5 Hot air oven

Hot air oven of science tech company (temperature ranges 170°C for 30 mins, 160°C for 60 mins & 150°C for 50 mins) was used for moisture content analysis shown in **fig 3.5**. It is used for removing all the moisture from the product. It is a standardized conventional method and it attains desired temperature more rapidly.

Fig 3.5 Hot air oven

3.2.6 Heating mantle

Heating mantle of ambassador company was used for preparation of many reagents used for different tests shown in **fig 3.6**. Heating mantle produce energy by converting AC voltage. Intense energy is consumed to generate a high degree of required heat.

Fig 3.6 Heating mantle

3.2.7 Digital Calliper

Digital vernier calliper of precision measuring was used for measuring the diameter of the beads prepared and the thickness of the alginate coating on the beads shown in **fig 3.7**. The jaws move to and fro motion, due to the connection and disconnections between the sensors the signals will pass to the chip then the chip will send the data and allows the LCD to display the value. The whole process works with the help of a Battery.

Fig 3.7 Digital vernier calliper

3.2.8 Muffle furnace

Muffle furnace of Ambassador Company (maximum temperature 1200°C) was used of ashing shown in **fig 3.8**. It separates the object to be heated from all the product of combustion from the heat source. Heat is applied to a chamber through induction or convection by a high temperature heating coil inside an insulated material.

Fig 3.8 Muffle furnace

3.2.9 Soxhlet apparatus

Soxhlet apparatus was used to determine the total Fat content shown in **fig 3.9**. The fat extractor uses the solvent reflux and siphon principle to continuously extract the solid matter by pure solvent, which saves the solvent extraction efficiency and high efficiency. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

Fig 3.9 Soxhlet apparatus

3.2.10 Kjeldahl apparatus

Protein estimation of the prepared bars were analysed by kjeldahl method shown in **fig 3.10**. Food sample to be analysed is weighed into flask name digestion flask and then heated in presence of sulfuric acid with catalyst $CuSO_4$ and K_2SO_4 this process is called digestion. This process converts nitrogen present in food into ammonia and other organic matter to CO_2 and H_2O. After digestion the flask is connected to another flask named receiving flask by a tube. The solution in digestion flask is made alkaline by adding NaOH and convert ammonia sulphate into ammonia gas. The gas that formed get liberated from the solution and moves out of the digestion flask into another flask called receiving flask. It contains excess of boric acid. The ammonia gas converts into ammonium ion and boric acid convert to borate ions. Then nitrogen content is estimated by titration of the ammonium borate formed with standard hydrochloric acid using methyl red indicator for determining the end point. Once the nitrogen content has been determined it is converted to a protein content using conversion factors.

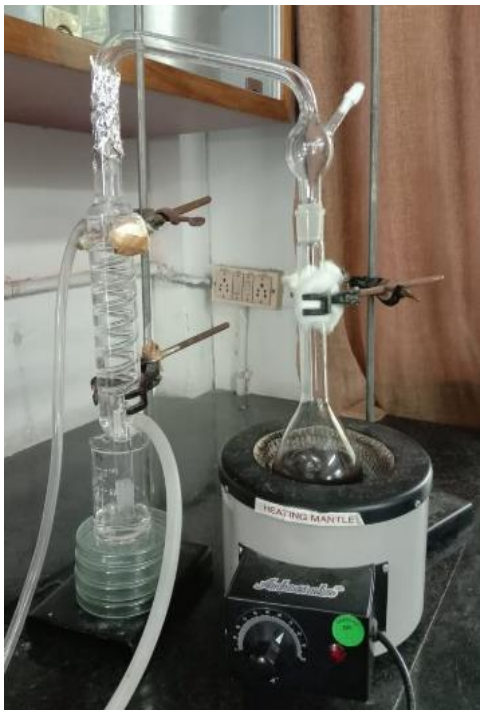


Fig 3.10 Kjeldahl apparatus

3.2.11 Induction cook-top

An induction stove of Bajaj Appliances (Majesty ICX 7 1300Watt) was used for blanching the spinach leaves. A copper coil under the cooktop creates electromagnetic energy. This magnetic energy interacts directly with induction-compatible cookware to make it hot. Because induction skips the step of heating the cooktop, it is a fast and even cooking method.

Induction cooking allows for fast cooking because the energy transfers directly to the cookware, so little to no heat or energy is lost between the cooking surface and your food. This means you can boil water or sear food quickly. Heat adjustments on an induction cooktop or range happen instantly, so you can heat or cool down your pot or pan quickly. Induction cooktops transfer energy to create heat directly within a pot or pan.

Fig 3.11 Induction Cook-top

3.3 Preparation of Powder-core beads

3.3.1 Selection of materials

Spinach *(Spinacia Oleracea)* leaves were collected from the local vendors and farmers near Integral University, Lucknow and then each leaf was selected carefully based on their physical properties like colour, external damage and pit feeding. Sodium Alginate powder (HIMEDIA), Calcium Lactate powder (HIMEDIA), and distilled water were obtained from the Food Analysis laboratories of Integral University. Five bead samples dipped in different composition of Sodium Alginate solution were prepared. For comparison two control samples were taken where Control 1 was pure spinach powder beads with no alginate coating and Control 2 was fresh spinach juice. **Table 3.1** shows the experimental plan of preparing five different samples of *Spinacia Oleracea* beads.

Table 3.1 *Spinacia Oleracea* beads sample prepared

Sample	Sodium Alginate	Calcium Lactate	Inversion time
Sample 1	2%	20%	1 hour
Sample 2	2.5%	20%	1 hour
Sample 3	3%	20%	1 hour
Sample 4	3.5%	20%	1 hour
Sample 5	4%	20%	1 hour
Control 1 (Spinach powder)	-	-	-
Control 2 (Spinach juice)	-	-	-

3.3.2 Preparation of Spinach Powder

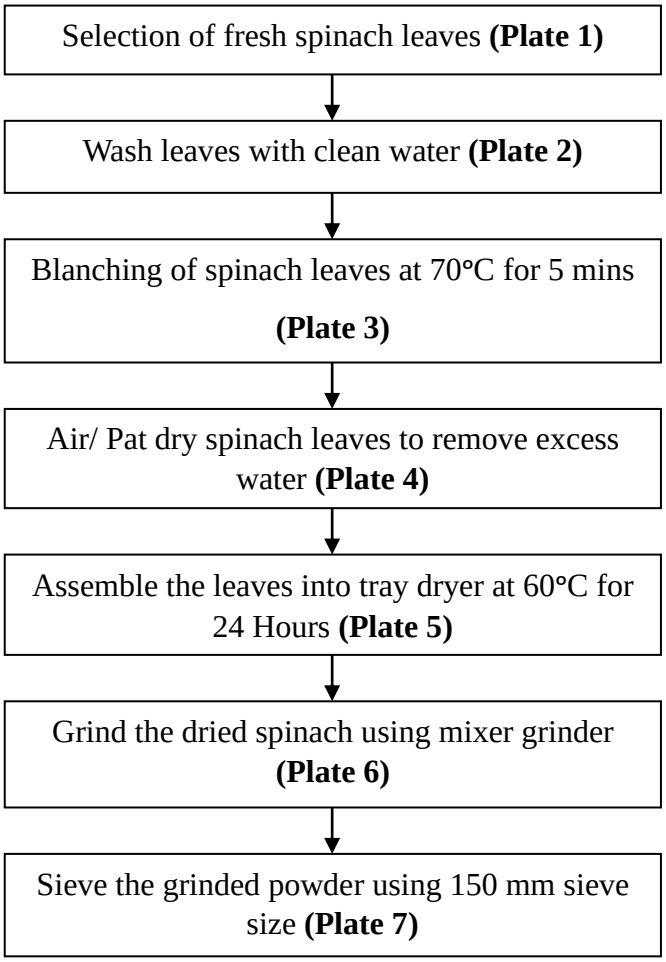


Fig 3.12 Flow diagram showing preparation of spinach powder

3.3.3 Preparation of beads

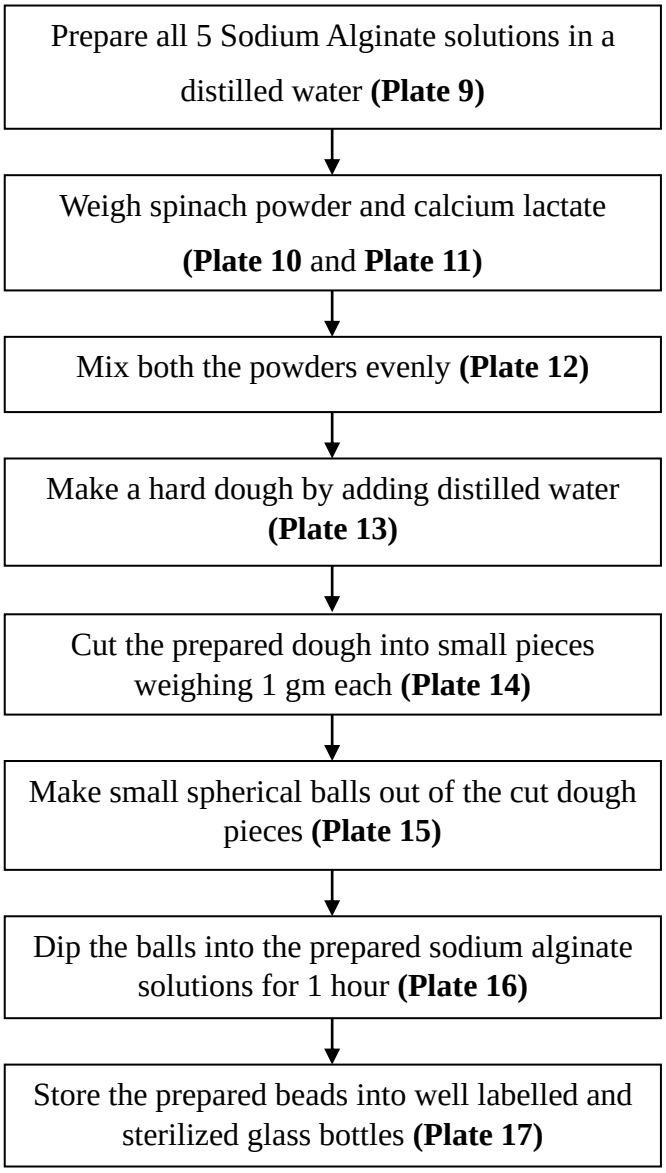


Fig 3.13 Flow diagram showing preparation of beads

Plate 1 Slection of fresh spinach leaves

Plate 2 Washing with clean water

Plate 3 Blanching at 70°C for 5 min

Plate 4 Air/Pat drying of spinach

Plate 5 Tray dried at 60°C for 24 Hours

Plate 6 Grinding of dried spinach leaves

Plate 7 Sieve the grinded powder from 150mm sieve size

Plate 8 Fresh spinach juice

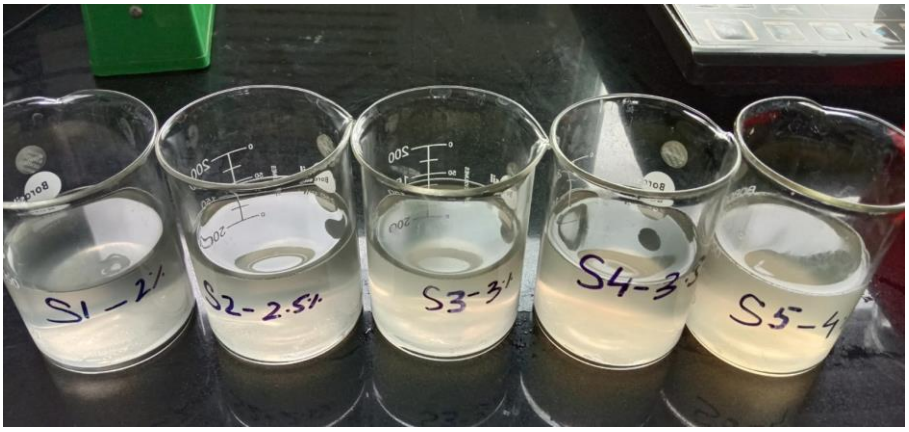


Plate 9 Different solution of Sodium Alginate with proper labelling

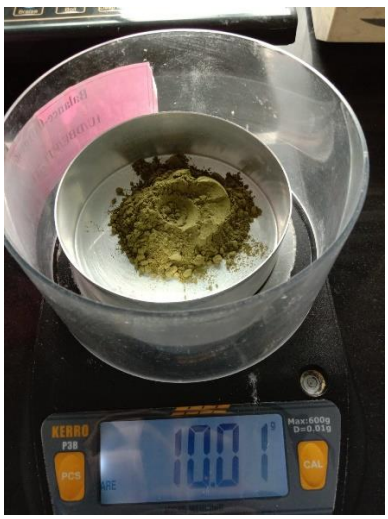


Plate 10 Weighing of Spinach Powder

Plate 11 Weighing of Calcium Lactate

Plate 12 Mixing of dry powders

Plate 13 Make hard dough using water

Plate 14 Cut small pieces of the hard dough

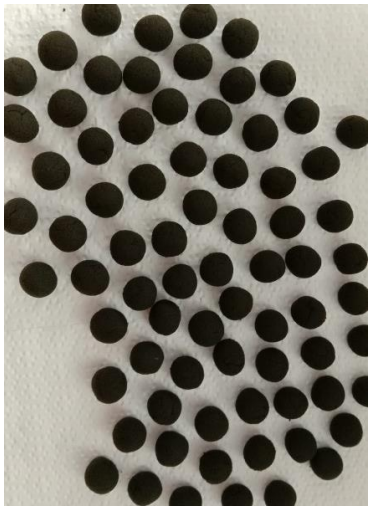
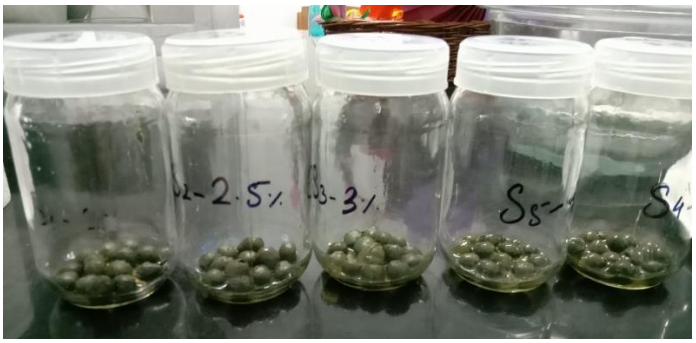
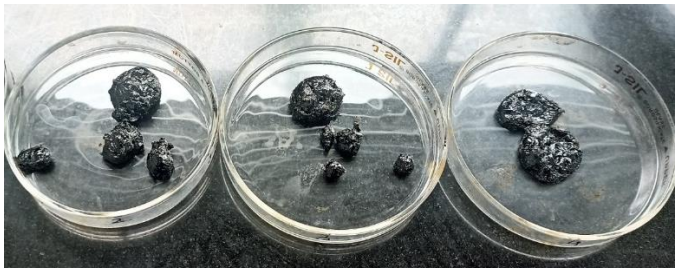


Plate 15 Small sphere ball of cut pieces

Plate 16 Dip the ball into Alginate Solution



Place 17 Store the prepared bead into clean bottles

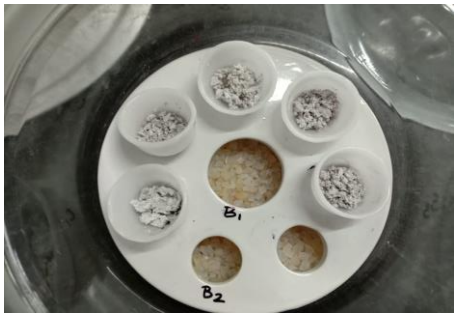


a) **b)**

Plate 18 a) b) Moisture content analysis

Plate 19 Weighing of beads

a) **b)**



c)

Plate 20 a) b) c) Ash content analysis




a) **b)** **c)** **d)**

Plate 21 a) b) c) d) Protein content analysis

Plate 22 Fat content analysis

Plate 23 Fiber content analysis

Plate 24 Diameter of beads

Plate 25 Measurement of membrane thickness

3.4 Physico-chemical analysis

3.4.1 Moisture content

For the determination of moisture content, small portions from each energy bar were taken and crushed into very small pieces using mortar pestle, then weighed 5 grams equally using electrical weighing balance. After weighing each sample was placed into clean and sanitized petri dishes and kept in hot air oven at 105ºC for 3 hours. Determination of moisture content was done following the method of AOAC, (2000). Moisture content analysis is show in **Plate 18**. Moisture content was calculated using the equation given below:

$$Moisture\ content(\%) = \frac{W_1 - W_2}{W_1} \times 100 \qquad \text{eq…1}$$

Where,

W_1= weight of sample, and

W_2= weight of sample after drying.

3.4.2 Ash content

For the determination of ash content, small portions from each energy bar samples were taken and crushed into very small pieces using mortar pestle, then weighed 5 grams equally using electrical weighing balance. After weighing each sample was placed into clean and sanitized crucibles and kept in a pre-heated muffle furnace at 550ºC for 5 hours. Before putting the crucible into the muffle furnace, it is kept on stove to get burn to avoid the production of fumes. Ash content was determined following the method of AOAC, 2000. Steps for ash content analysis are shown in **Plate 20**. Ash content was calculated using the equation given below:

$$Ash\ content\ (\%) = \frac{S_1 - S_2}{S_3} \times 100 \qquad \text{eq…2}$$

Where,

S_1= weight of crucible before ashing,

S_2= weight of crucible after ashing, and

S_3= weight of sample.

3.4.3 Fat content

For the determination of fat content, small portion from each energy bar samples were taken and crushed into small pieces using mortar pestle, then weighed 5 grams from each crushed sample. After weighing put the samples into thimble and then into the Soxhlet and then start the process of fat extraction. Petroleum ether was poured into the Soxhlet and used as a solvent. Then the solvent was evaporated/ heated with the sample in thimble at 80-90ºC. The process was runed for 16 hours to get the accurate amount of fat. Determination of fat content was done following AOAC, 2000. Fat content analysis is shown in **Plate 22**. Fat content was calculated using the equation given below:

$$Fat\ content\ (\%) = \frac{V_1 - V_2}{V_1} \times 100 \qquad \text{eq…3}$$

Where,

V_1= weight of empty round bottom flask; and

V_2= weight of round bottom flask after fat extraction

3.4.4 Protein content

Protein content was determined following AOAC, 2000 standards using kjeldahl apparatus. Kjeldahl method is done in three steps namely digestion, distillation, and titration. For digestion 2 grams crushed energy bars sample was taken into a digestion flask with 10 parts of K_2SO_4 (potassium sulfate) with 1 part of $CuSO_4$ (copper sulfate) and 20 ml of H_2SO_4 (sulfuric acid). The digestion flask was kept on heating mantle at 100ºC till the solution become crystal clear green colour and then kept for cooling. Upon cooling the colour of the solution changed to blue. 200 ml of distilled water was added to the blue solution with 4-5 drops of phenolphthalein indicator and approximately 50 ml of 40% NaOH (sodium hydroxide) solution. The digested solution was transferred into digestion bulb of kjeldahl unit with 50ml of 2% boric acid beaker on the other side of the unit called as condenser. Digestion bulb was boiled at 80ºC till the volume of the boric acid attached to the condenser increases up to 200ml in volume. This procedure is called as distillation. After distillation, the boric acid solution was titrated. 4-5 drops of methyl red indicator were added and 0.1% HCl (hydrochloric acid) was added into it drop by drop until it reaches its end point and colour changes to pink. Steps for protein content analysis are show in **Plate 21**. Protein content was calculated using the equation given below:

$$Protein\ content(\%) = \frac{(A - B) \times N \times 14.007 \times 6.25}{W} \quad \text{eq…4}$$

Where,

A= volume of HCl used for sample titration,

B= volume of HCl used for blank titration,

N= normality of HCl,

W= weight of sample,

14.007= atomic weight of nitrogen, and

6.25= conversion factor for food product.

3.4.5 Crude Fiber Content

Crude Fiber is a measure of the quality of indigestible cellulose, pentosans, lignin and other components of this type present in foods. 2g of sample was taken and transformed in 200 ml of 1.25% H_2SO_4 in spoutless beaker. It was boiled for 30 minutes. After that the flask was removed, and the solution was filtered through Whatman No. 54 filter paper. The residue was washed with hot distilled water. The left residue was the boiled in 1.25% NaOH solution for exactly 30 minutes. After 30 mins of boiling, the content was filtered through Whatman No. 54 filter paper and washed with hot distilled water using buchner funnel to apply gentle suction. The filter paper with the residue was dried in oven at 105 ºC for 3-4 hours till the content is fully dried and it's constant weighed is obtained. It was cooled in a desiccator and weighed. The loss in weight represented the crude fiber content. Crude Fiber content was calculated using AOAC 1995 standards. **Plate 23** shows the Fiber analysis test of beads. It was calculated using the equation given below

$$Crude\ fibre\ content\ (\%) = \frac{W_1 - W_2}{W} \times 100 \quad \text{eq…5}$$

Where,

W_1 = Weight of filter paper (g)

W_2 = Weight of residue + Filter paper (g)

W = Weight of sample (g)

3.4.6 Carbohydrates Content

Net carbs refer to the total amount of fully digestible carbohydrates contained within a product or meal. People can calculate net carbs by subtracting the whole amount of fiber and half the amount of sugar alcohols from the amount of total carbs on a product's nutrition label. The moisture, protein, fat, and ash content of a food are determined and then subtracted from the total weight of the food and the remainder, or "difference," is total carbohydrate. More recently, "net carbohydrates" has been determined by subtracting dietary fiber from the total carbohydrates value. It is calculated using the formula given below

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

eq…6

3.4.7 Total Energy Content

Calories refers to the total number of calories, or "energy" you get from all sources (carbohydrate, fat, protein, and alcohol) in a serving of a food or beverage. doing so allows a person to measure how much energy they are consuming per day. If a person takes in more than their body uses, they generally begin to gain weight. If a person takes in less than their body requires, they generally start to lose weight. The total calories in a food is calculated using the formula given below

$$Total\ energy\ content\ (kcal) = (4 \times \%\ protein) + (4 \times \%\ carbohydrates) + (9\ \times \%fat)$$

eq…7

3.5 Physical parameter analysis

3.5.1 Diameter of beads and thickness of alginate membrane

Vernier calliper calculates the straight linear distance between two points in layman's terms. The tips of the calliper are first fixed to fit across the spots to be recorded, then the calliper is removed and the distance between the tips is evaluated with a ruler. Open the jaws, position the bead/ membrane between jaw, and set the jaw so that it comfortably grips the body without exerting excessive pressure. Tighten the screw which holds the vernier scale in position and note the main scale reading. **Plate 24** and **Plate 25** shows the measurement of diameter of beads and membrane thickness respectively.

3.5.2 Final weight of the beads

Weight of the final beads with alginate coating were measured by using the analytical balance. 1 grams of sphere were dipped into different alginate solutions for 1 hours which gave them a slimy transparent membrane like structure as a covering. This membrane leads to gain in the weight of the beads. Weight of beads dipped in each solution are measured with the help of analytical balance in triplets so an average weight gain can be calculated easily. Weight of each bead were taken manually. **Plate 19** shows the measurement of beads weight.

CHAPTER- 4

RESULTS AND DISCUSSIONS

Total five bead samples coated with different $NaC_6H_7O_6$ (Sodium Alginate) solutions and 2 control samples, where; Control 1 was pure spinach powder and Control 2 was fresh spinach juice were prepared. All the energy bar samples were analysed for the physico chemical analysis namely Moisture content, Ash content, Protein content, Fat content, Fiber content, Carbohydrate content and Total Energy content as well as some physical parameter analysis of the prepared bead samples namely Diameter of beads, Final Weight of beads and Thickness of alginate coating.

4.1 Moisture analysis

Moisture content influences some physical properties of the food product including weight, density, viscosity, conductivity, and water activity. It is generally determined by the weight loss upon drying the food product. Moisture content of different samples prepared in laboratory was analysed and is shown in the **Table 4.1** and **Fig 4.1**. Moisture content of all the total samples was ranges from 6.32±0.03 to 89.06±0.26, where the lowest range was of Control 1 (spinach powder) and highest was of Control 2 (fresh spinach juice). Whereas the bead sample moisture content ranges from 8.63±0.04 to 12.34±0.02 from highest in Sample 1 to lowest in Sample 5. This change may occur due to the viscosity of the sodium alginate solutions, the higher the percentage of sodium alginate the viscous the solution is and the lower the moisture content. The moisture content of spinach powder produced in labs was nearly equal to that of **Koç & Dirim, (2018)** spinach powder.

Table 4.1 Moisture content analysis of all the samples

SAMPLE	Moisture content (%)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	12.34±0.02
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	11.58±0.06
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	9.86±0.02
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	9.14±0.09
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	8.63±0.04
Control 1 (Spinach Powder)	6.32±0.03
Control 2 (Spinach Juice)	89.06±0.26

Values are written as mean ± standard deviation.

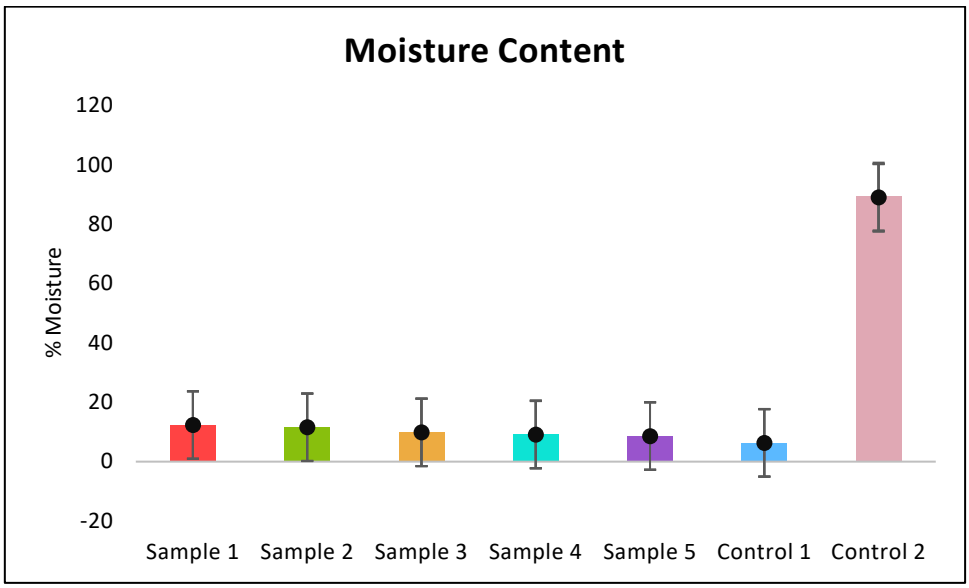


Fig 4.1 Moisture content of the all samples

4.2 Ash Analysis

Ash is the inorganic residue remaining after the removal of moisture and other organic matter in presence of oxidizing agents by providing heat. Ash content gives us the measure of total amount of minerals present in the food. Ash content include both, essential minerals, and toxic materials. In general, natural food will be containing less than 5% ash and many processed foods may have more than 10% ash content **(Michael Baker, 2018)**. Ash content of different samples prepared in laboratory was analysed and is shown in the **Table 4.2** and **Fig 4.2**. Ash content of all the total samples was ranges from 1.97±0.03 to 36.81±0.01, where the lowest range was of Control 2 (fresh spinach juice) and highest was of Sample 5. Ash content was increasing with the increase of sodium alginate solution dipping. Ash content of raw spinach powder was lower than that of the beads because of the addition of calcium lactate in them as well as sodium alginate solution. Ash content of the beads ranges from 32.42±0.01 to 36.81±0.01. bead with 4% sodium alginate has the highest ash content. Fresh spinach juice has the lowest ash content as compared to all other samples which indicates that it has low mineral content as compared to that of spinach powder. Increase of mineral content due to the alginate coating can be observed in **(Flamminii et al., 2020)** paper.

Table 4.2 Ash content analysis of all the samples

SAMPLE	Ash content (%)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	32.42±0.01
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	33.28±0.02
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	34.62±0.01
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	35.22±0.03
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	36.81±0.01
Control 1 (Spinach Powder)	17.94±0.03
Control 2 (Spinach Juice)	1.97±0.03

Values are written as mean ± standard deviation.

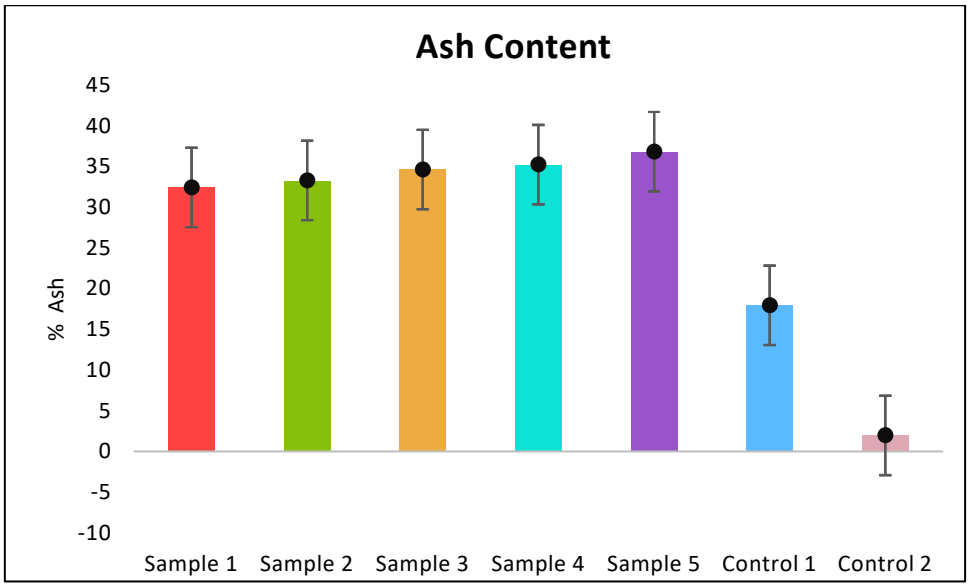


Fig 4.2 Ash content of the all samples

4.3 Protein Analysis

Protein is one of the three major nutrients needed for human body to grow. The only major component which contain nitrogen in most of the food is protein. Proteins are the essential biomolecules, consisting of one or more long chains of amino acid residues. They are large macromolecules, which play a vital role in growth and development, therefore it is called as the building blocks of the body. Protein content of different samples prepared in laboratory was analysed and is shown in the **Table 4.3** and **Fig 4.3**. Protein content of all the total samples was ranges from 2.97±0.07 to 32.33±0.03, where the lowest range was of Control 2 (fresh spinach juice) and highest was of Sample 5. Protein content of spinach juice was much lesser than that of spinach powder. Beads made up of spinach powder and calcium lactate with sodium alginate shows very slight increase in their protein content. The increase in the

protein content was affected by the coating of sodium alginate. Same increase of protein content was observed the healthy brown rice milk with sodium alginate addition from brown algae *Sargassum Binderi* as emulsifier **(Latifah & Warganegara, 2018)**.

Table 4.3 Protein content analysis of all the samples

SAMPLE	Protein Content (%)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	31.45±0.03
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	31.64±0.02
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	31.79±0.02
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	31.96±0.02
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	32.33±0.03
Control 1 (Spinach Powder)	31.1±0.05
Control 2 (Spinach Juice)	2.97±0.07

Values are written as mean ± standard deviation.

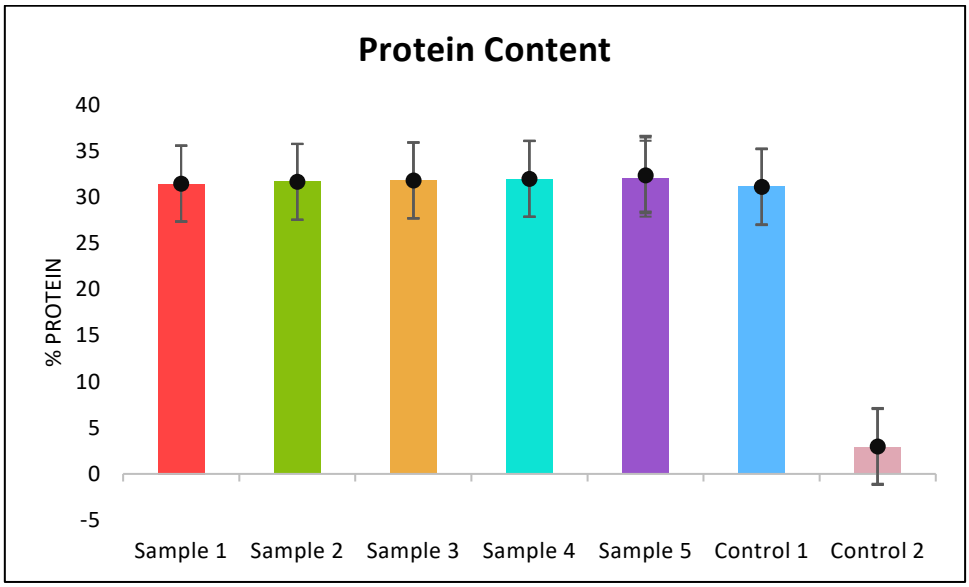


Fig 4.3 Protein content of the all samples

4.4 Fat analysis

Fats are one of the three main macronutrients along with carbohydrate and protein. Fats are saturated and unsaturated depending on how much of each fatty acid they contain. Fats and oils are esters of glycerol and three fatty acids. They are important in the diet as energy sources and as sources of essential fatty acids and fat-soluble vitamins, which tend to associate with fats. They also contribute satiety, flavor, and palatability to the diet. Fat content of different samples prepared in laboratory was analysed and is shown in the **Table 4.4** and

Fig 4.4. Fat content of all the total samples was ranges from 0.36±0.05 to 4.35±0.01, where the lowest range was of Control 2 (fresh spinach juice) and highest was of Sample 5. Very slight increase of fat content can be observed in the beads range from 3.97±0.01 to 4.35±0.01. In a study by **Bennacef et al., (2021)** tells that sodium alginate contain slight amount of fat in it. So, by the results given below it can be observed that slight increase of fats can be seen int the beads samples.

Table 4.4 Fat content analysis of all the samples

SAMPLE	Fat Content (%)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	3.97±0.01
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	4.01±0.01
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	4.21±0.02
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	4.27±0.02
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	4.35±0.01
Control 1 (Spinach Powder)	3.85±0.02
Control 2 (Spinach Juice)	0.36±0.05

Values are written as mean ± standard deviation.

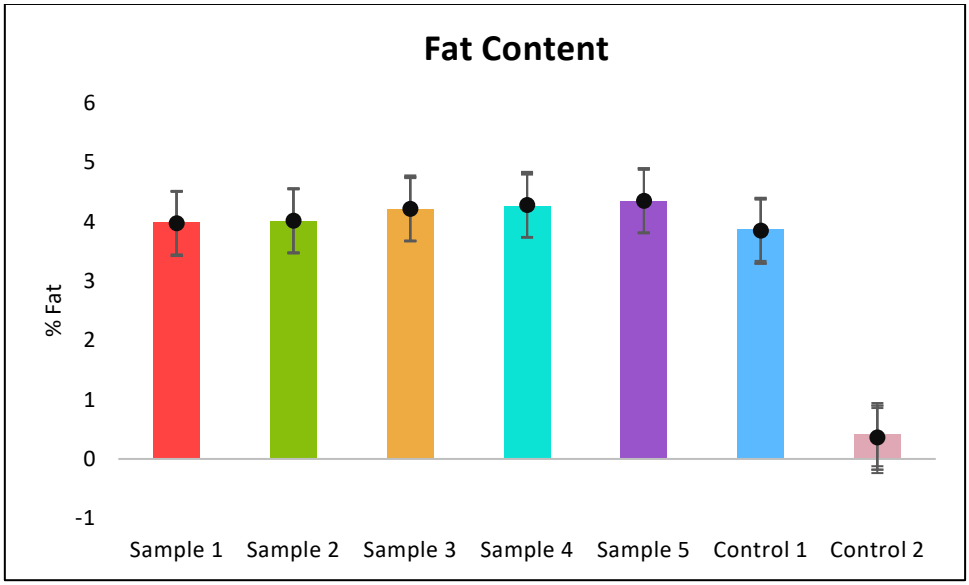


Fig 4.4 Fat content of the all samples

4.5 Fiber analysis

Dietary fiber, also known as roughage or bulk, refers to the indigestible carbohydrates found in plant-based foods. These fibers are mainly polysaccharides, which are complex carbohydrates that cannot be broken down by the human digestive enzymes. Fiber content of

different samples prepared in laboratory was analysed and is shown in the **Table 4.5** and **Fig 4.5**. Fibre content of all the total samples was ranges from 1.62±0.02 to 27.27±0.02, where the lowest range was of Control 2 (fresh spinach juice) and highest was of Sample 5. Fibre content of the beads ranges from 26.33±0.02 to 27.27±0.02, where sample 1 has the lowest and sample 5 has the highest range of fibre content. Calcium lactate added to the spinach powder also increased some amount of fibre in the beads where very little increase was affected by the sodium alginate solution. This slight increase due to the addition of sodium alginate and calcium lactate was also observed in the jelly produced by mango and pineapple using reverse spherification technique **(Low & Pui, 2020)**.

Table 4.5 Fiber content analysis of all the samples

SAMPLE	Fiber Content (%)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	26.33±0.02
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	26.55±0.01
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	26.83±0.02
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	26.98±0.04
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	27.27±0.02
Control 1 (Spinach Powder)	23.92±0.55
Control 2 (Spinach Juice)	1.62±0.02

Values are written as mean ± standard deviation.

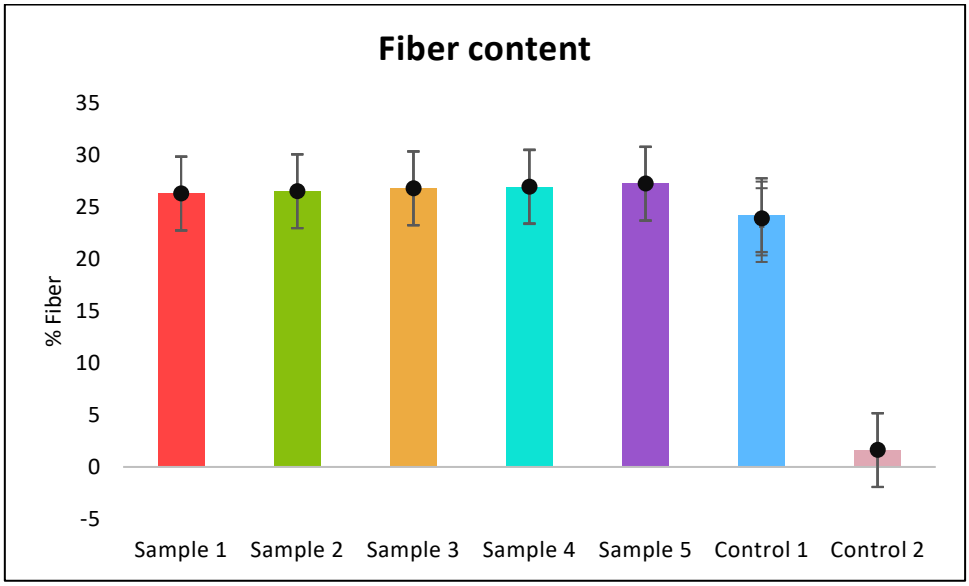


Fig 4.5 Fiber content of all the samples

4.6 Carbohydrate analysis

Carbohydrates are one of the most important components in many foods. Carbohydrates may be present as isolated molecules or they may be physically associated or chemically bound to other molecules. Individual molecules can be classified according to the number of monomers that they contain as monosaccharides, oligosaccharides, or polysaccharides. Molecules in which the carbohydrates are covalently attached to proteins are known as glycoproteins, whereas those in which the carbohydrates are covalently attached to lipids are known as glycolipids. Carbohydrate content of different samples prepared in laboratory was analysed and is shown in the **Table 4.6** and **Fig 4.6**. Carbohydrate content of all the total samples was ranges from 5.5±0.51 to 40.79±0.13, where the lowest range was of Control 2 (fresh spinach juice) and highest was of Control 2 (Spinach powder). Carbohydrate content of the beads was decreasing with the increase of sodium alginate membrane solution. Carbohydrate was calculated manually using the formula **eq…6**. Triplets of each sample were put in the equation of carbohydrate and then its mean and standard deviation was calculated using M.S. Excel. The results which came out were little different as sample 3 has higher carbohydrate content as compared to the sample 2 which was not in proper pattern. This error may occur due to inappropriate conditions or may be due to the incorrect order of calculation. Carbohydrate content was much higher in the dried spinach powder as compared to that of fresh spinach juice. The increase of carbohydrate content was observed in nutritional characterization and food value addition properties of dehydrated spinach **powder (Waseem et al., 2021)**.

Table 4.6 Carbohydrate content analysis of all the samples

SAMPLE	Carbohydrate content (%)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	20.16±0.58
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	19.49±0.06
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	19.52±0.03
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	19.4±0.08
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	17.89±0.29
Control 1 (Spinach Powder)	40.79±0.13
Control 2 (Spinach Juice)	5.5±0.51

Values are written as mean ± standard deviation.

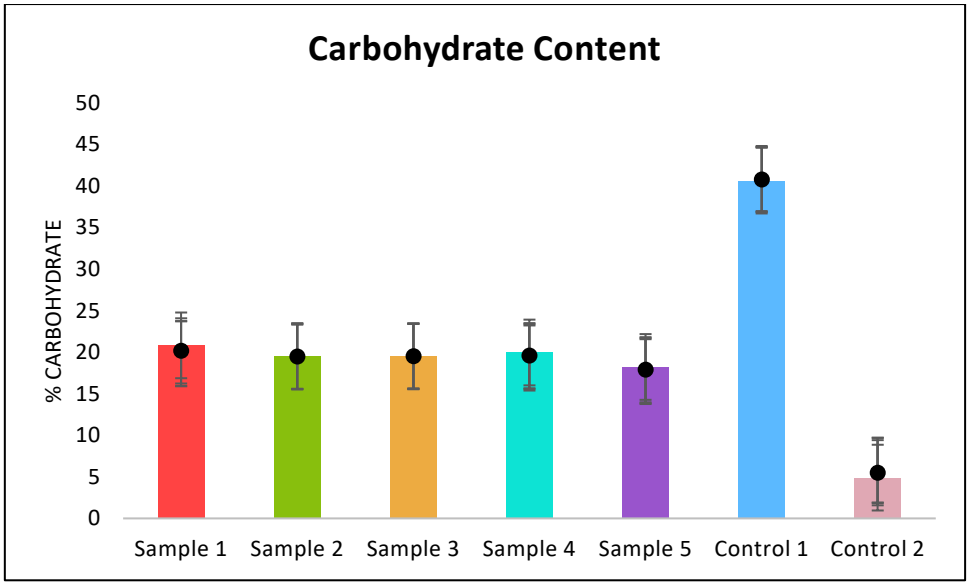


Fig 4.6 Carbohydrate content of all the samples

4.7 Total Calorie analysis

Calorie is the unit most used to express food energy, namely the specific energy (energy per mass) of metabolizing different types of food. For example, fat (lipids) contains 9 kilocalories per gram (kcal/g), while carbohydrates (sugar and starch) and protein contain approximately 4 kcal/g. This unit is also used to express recommended nutritional intake or consumption, as in calories per day. Calorie content of different samples prepared in laboratory was analysed and is shown in the **Table 4.7** and **Fig 4.7**. Spinach powder has high calorific value than that of spinach juice. Calories were calculated using the formula of total energy content i.e., **eq…7**. No perfect pattern was observed in the energy content of the beads in fact beads have low energy content than that of spinach powder.

Table 4.7 Total calorie content of all the samples

SAMPLE	Total Calorie Content (Kcal)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	240.84±0.04
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	240.64±0.31
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	243.17±0.11
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	243.94±0.36
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	240.05±0.27
Control 1 (Spinach Powder)	322.18±0.15
Control 2 (Spinach Juice)	37.13±1.87

Values are written as mean ± standard deviation.

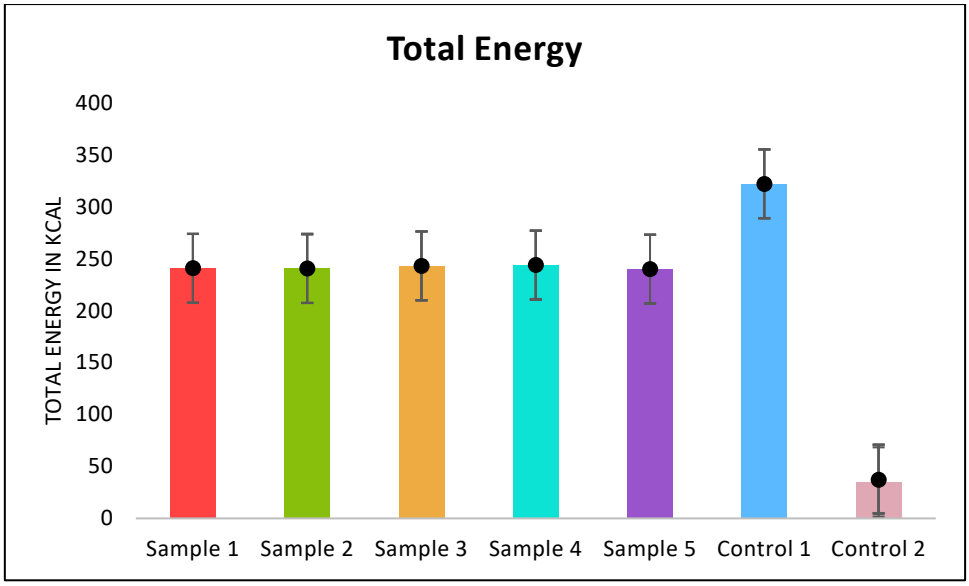


Fig 4.7 Total Calorie content of all the samples

4.8 Diameter of beads

Diameter of different bead samples prepared in laboratory was analysed and is shown in the **Table 4.8** and **Fig 4.8**. Diameter of the beads was ranges from 16.32±0.02 to 17.85±0.01. Diameter of the beads were calculated using digital vernier callipers. The beads were taken one by one of each samples and kept between the two jaws of the callipers and then calculated. The diameter of the beads was increasing with increase in the sodium alginate solution. Sample 5 has the largest diameter because of the thickness of the sodium alginate membrane. Similar results were seen in the beads prepared by cocoa extract to make a functional food **(Lupo et al., 2015)**. As the alginate content was increasing in the beads the samples were increasing in their size, and hence sample 5 with highest content of sodium alginate solution have much bigger diameter than that of all other samples.

Table 4.8 Diameter of the prepared bead samples

SAMPLE	Diameter of Beads (mm)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	16.32±0.02
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	16.65±0.04
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	17.23±0.02
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	17.53±0.01
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	17.85±0.01

Values are written as mean ± standard deviation.

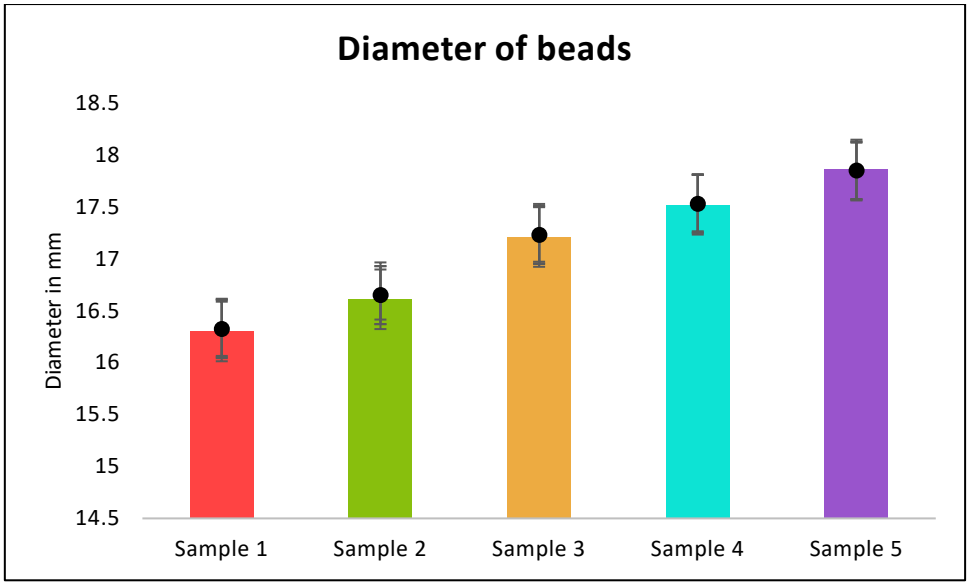


Fig 4.8 Diameter of the bead samples

4.9 Weight of beads

Weight of different bead samples prepared in laboratory was analysed and is shown in the **Table 4.9** and **Fig 4.9**. With the increase in the diameter of the beads, they were gaining slight weight in them. The weights of the beads were increasing with the increase of sodium alginate solution. Triplets from each beads samples were taken and put one by one on the weighing balance manually. Mean and standard deviation was then calculated using M.S. Excel. Weight of the beads was ranges from 1.45±0.02 to 1.8±0.02. Very slight increase was observed in the weights of the beads which was basically due to the increase of sodium alginate solution. Sample 5 with highest sodium alginate solution coating i.e., 4% was having more viscous nature and so it also formed a thick coating which ultimately leads to gain in weights of the beads samples. Same pattern of increased weights in the beads were also observed encapsulation of omega-3 fatty acids in nano emulsions and microgels **(Chen et al., 2017)**.

Table 4.9 Final weight of the prepared bead sample

SAMPLE	Final Wight of Beads (gm)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.45±0.02
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.5±0.02
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.64±0.02
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.7±0.02
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.8±0.02

Values are written as mean ± standard deviation.

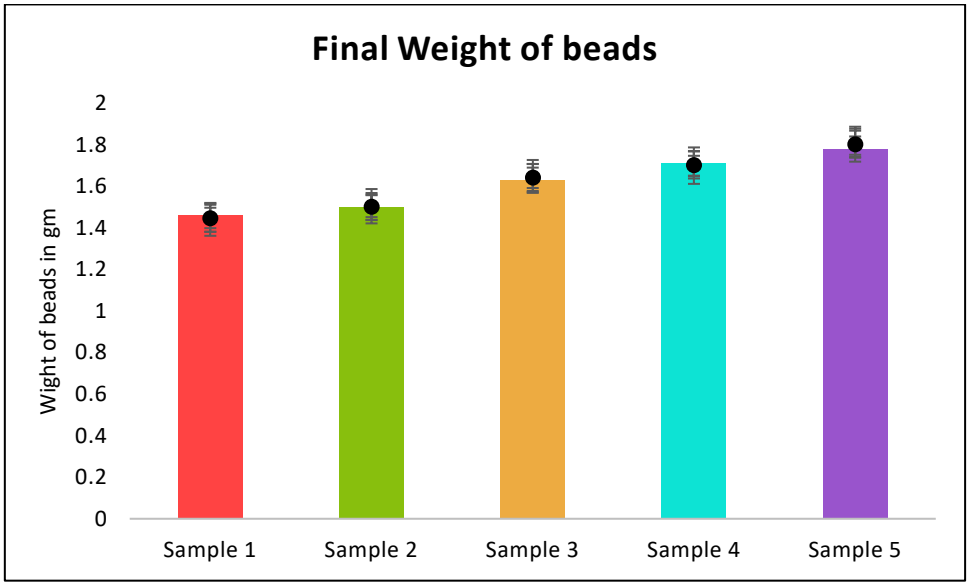


Fig 4.9 Final weight of the bead samples

4.10 Thickness of coating

Thickness of coated membrane of different bead samples prepared in laboratory was analysed and is shown in the **Table 4.10** and **Fig 4.10**. Thickness of the beads ranges from 1.11±0.02 to 1.34±0.02. The thickness of the beads were calculated using digital vernier callipers. The layer of the coating was taken off from the beads and then washed carefully, then kept between the two jaws of the callipers for taking the measurement. The thickness of the coating of the beads was increasing with the increase of sodium alginate percentage. Sample 5 has the thickest membrane as compared to other coatings. Almost similar results are found of the beads produced barberry based on calcium and alginate **(M. Maleki et al., 2020)**. Sample 5 with highest sodium alginate solution coating i.e., 4% was having more viscous nature and so it also formed a thick coating.

Table 4.10 Final thickness of alginate coating of the prepared bead samples

SAMPLE	Membrane Thickness (mm)
Sample 1 (2% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.11±0.02
Sample 2 (2.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.18±0.02
Sample 3 (3% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.2±0.01
Sample 4 (3.5% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.27±0.01
Sample 5 (4% $NaC_6H_7O_6$ and 20% $C_6H_{10}CaO_6$)	1.34±0.02

Values are written as mean ± standard deviation.

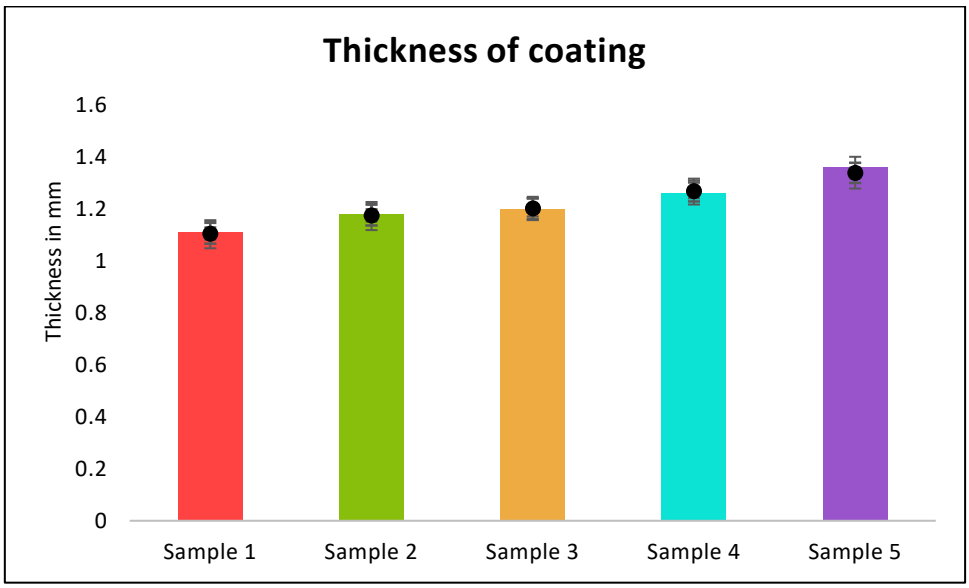


Fig 4.10 Thickness of alginate coating on the bead samples

CHAPTER 5

SUMMARY AND CONCLUSION

The main objective of this study was to make a powder code hydrogel bead using spinach powder and sodium alginate by applying reverse spherification technique. Nutritional quality of spinach juice was increased in the form of encapsulated beads and to check physiochemical analysis of developed beads. For this study fresh spinach leaves were obtained from the local market near Integral University. The leaves were physically checked and cleaned by washing them under running water. The cleaned leaves were then blanched so that their natural colour does not get extracted from the leaves. Blanching was done by following the correct steps i.e., at 70°C for 5 mins and then quickly dipped into the icy chilled water to retrain its natural green colour and stop the enzymatic reaction during the bead making procedure. Blanched leaves were then air dried to extract the extra water from them and then arranged in the tray drier to make them fully dried. Leaves were arranged in such manner that drying process can occur easily without harming any physical or nutritional loss to them. Spinach leaves were kept under tray drier for 24 hours at 60°C with fan on. When the leaves were fully dried, they were grinded to make fine powder from them. Grinded spinach powder was taken through sieve using 150 mm sieve size to make a proper dough of that spinach powder. Bigger particle size may cause little difficulty in making a fine dough from that spinach powder such as breaking or shabbiness in the beads shapes. Fine spinach powder was then mixed with calcium lactate and a smooth dough was prepared. Calcium lactate was added as it helps in the process of spherification. Spherification is basically a process that seals a product in a jelly like membrane. The dough was then cut into small sphere and dipped into sodium alginate solutions of different concentrations. After the beads were fully prepared, they were then taken for different physico-chemical and physical analysis.

The beads were compared with fresh spinach juice and raw spinach powder to understand the effect on the nutritional properties of spinach powder weather is affected or not by the addition of calcium lactate and sodium alginate. The beads sample showed very different result for the nutritional aspects as they have high mineral content and low carbohydrate content which also affected their calorific value as compared to that od spinach powder. But a very vast affect as compared to that of spinach juice. It can be concluded that sample 5 in all the bead sample showed good result comparatively to other bead samples. Sample 5 with 4%

of sodium alginate membrane also has a thick membrane layer which will not allow the spinach powder to get deteriorated easily. Sample 5 also has much higher nutritional composition than that of other bead samples. The above test can also help in further study to make a proper nutraceutical using sodium alginate as a membrane and can also help in storing important nutrient as it acts as a perfect barrier to external microbes. Nutraceuticals are products derived from food sources that are purported to provide extra health benefits, in addition to the basic nutritional value found in foods. Sodium Alginate can be used as an encapsulating material for beads preparation.

5.1 Future recommendations

- Flavouring agents can be used to increase the demand of the product.
- External additives like plasticizers can be added to increase the strength of the membrane.
- Increase of nutrient as an additives can be added to increase it nutritional value and prepare the beads as a proper nutraceuticals.

REFERENCES

Ahammed, S., Liu, F., Wu, J., Khin, M. N., Yokoyama, W. H., & Zhong, F. (2021). Effect of transglutaminase crosslinking on solubility property and mechanical strength of gelatin-zein composite films. *Food Hydrocolloids*, *116*, 106649. https://doi.org/https://doi.org/10.1016/j.foodhyd.2021.106649

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(17), 7915–7922. https://doi.org/10.1073/pnas.90.17.7915

Aritomi, M., & Kawasaki, T. (1984). Three highly oxygenated flavone glucuronides in leaves of Spinacia oleracea. *Phytochemistry*, *23*, 2043–2047. https://api.semanticscholar.org/CorpusID:84574743

Augustin, M. A., & Sanguansri, L. (2012). 2 - Challenges in developing delivery systems for food additives, nutraceuticals and dietary supplements. In N. Garti & D. J. McClements (Eds.), *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals* (pp. 19–48). Woodhead Publishing. https://doi.org/https://doi.org/10.1533/9780857095909.1.19

Bar, M., & Neta, M. (2007). Visual elements of subjective preference modulate amygdala activation. *Neuropsychologia*, *45*(10), 2191–2200. https://doi.org/https://doi.org/10.1016/j.neuropsychologia.2007.03.008

Bennacef, C., Desobry-Banon, S., Probst, L., & Desobry, S. (2021). Advances on alginate use for spherification to encapsulate biomolecules. *Food Hydrocolloids*, *118*(March). https://doi.org/10.1016/j.foodhyd.2021.106782

Bremond, N., Santanach-Carreras, E., Chu, L.-Y., & Bibette, J. (2010). Formation of liquid-core capsules having a thin hydrogel membrane: liquid pearls. *Soft Matter*, *6*(11), 2484–2488. https://doi.org/10.1039/B923783F

Cao, Y., & Mezzenga, R. (2020). Design principles of food gels. *Nature Food*, *1*(2), 106–118. https://doi.org/10.1038/s43016-019-0009-x

Chen, F., Fan, G. Q., Zhang, Z., Zhang, R., Deng, Z. Y., & McClements, D. J. (2017). Encapsulation of omega-3 fatty acids in nanoemulsions and microgels: Impact of delivery system type and protein addition on gastrointestinal fate. *Food Research*

International, *100*, 387–395. https://doi.org/10.1016/j.foodres.2017.07.039

Chopra, R. N., Nayar, S. L., Chopra, I. C., Asolkar, L. V, Kakkar, K. K., Chakre, O. J., Varma, B. S., & (India), C. of S. & I. R. (1992). Glossary of Indian medicinal plants ; [with] Supplement. In *TA - TT -*. Council of Scientific & Industrial Research New Delhi. https://doi.org/LK - https://worldcat.org/title/499428255

Chung, C., Degner, B., Decker, E. A., & McClements, D. J. (2013). Oil-filled hydrogel particles for reduced-fat food applications: Fabrication, characterization, and properties. *Innovative Food Science & Emerging Technologies*, *20*, 324–334. https://doi.org/https://doi.org/10.1016/j.ifset.2013.08.006

Cooper, R. C., & Yang, H. (2019). Hydrogel-based ocular drug delivery systems: Emerging fabrication strategies, applications, and bench-to-bedside manufacturing considerations. *Journal of Controlled Release*, *306*, 29–39. https://doi.org/https://doi.org/10.1016/j.jconrel.2019.05.034

Corstens, M. N., Berton-Carabin, C. C., Elichiry-Ortiz, P. T., Hol, K., Troost, F. J., Masclee, A. A. M., & Schroën, K. (2017). Emulsion-alginate beads designed to control in vitro intestinal lipolysis: Towards appetite control. *Journal of Functional Foods*, *34*, 319–328. https://doi.org/https://doi.org/10.1016/j.jff.2017.05.003

CSIR. (1949). The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products (Industrial Products—Part I). In *The Indian Medical Gazette* (Vol. 84, Issue 10, pp. 476–477).

Das, S., & Guha, D. (2008). CNS depressive role of aqueous extract of Spinacia oleracea L. leaves in adult male albino rats. *Indian Journal of Experimental Biology*, *46*(3), 185–190.

Dickinson, E. (2012). Emulsion gels: The structuring of soft solids with protein-stabilized oil droplets. *Food Hydrocolloids*, *28*(1), 224–241. https://doi.org/https://doi.org/10.1016/j.foodhyd.2011.12.017

Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., Kostić, I., Komes, D., Bugarski, B., & Nedović, V. (2015). Trends in Encapsulation Technologies for Delivery of Food Bioactive Compounds. *Food Engineering Reviews*, *7*(4), 452–490. https://doi.org/10.1007/s12393-014-9106-7

El-Sayed, S. M., & Youssef, A. M. (2019). Potential application of herbs and spices and their effects in functional dairy products. *Heliyon*, *5*(6). https://doi.org/10.1016/j.heliyon.2019.e01989

Ergene, A., Guler, P., Tan, S., Mirici, S., Hamzaoglu, E., & Duran, A. (2006). Antibacterial and antifungal activity of Heracleum sphondylium subsp. artvinense. *African Journal of Biotechnology*, *5*(11), 1087–1089.

Feng, W., Yue, C., Wusigale, Ni, Y., & Liang, L. (2018). Preparation and characterization of emulsion-filled gel beads for the encapsulation and protection of resveratrol and α-tocopherol. *Food Research International*, *108*, 161–171. https://doi.org/https://doi.org/10.1016/j.foodres.2018.03.035

Ferreres, F., Castañer, M., & Tomás-Barberán, F. A. (1997). Acylated flavonol glycosides from spinach leaves (Spinacia oleracea). *Phytochemistry*, *45*(8), 1701–1705. https://doi.org/https://doi.org/10.1016/S0031-9422(97)00244-6

Flamminii, F., Di Mattia, C. D., Nardella, M., Chiarini, M., Valbonetti, L., Neri, L., Difonzo, G., & Pittia, P. (2020). Structuring alginate beads with different biopolymers for the development of functional ingredients loaded with olive leaves phenolic extract. *Food Hydrocolloids*, *108*(October 2019), 105849. https://doi.org/10.1016/j.foodhyd.2020.105849

Food Hydrocolloids Market Size, Share, Growth Report. (2030). https://www.zionmarketresearch.com/report/cellular-concrete-market

Gheonea (Dima), I., Aprodu, I., Cîrciumaru, A., Râpeanu, G., Bahrim, G. E., & Stănciuc, N. (2020). Microencapsulation of lycopene from tomatoes peels by complex coacervation and freeze-drying: Evidences on phytochemical profile, stability and food applications. *Journal of Food Engineering*, *288*(March 2020). https://doi.org/10.1016/j.jfoodeng.2020.110166

Giannenas, I., Sidiropoulou, E., Bonos, E., Christaki, E., & Florou-Paneri, P. (2020). Chapter 1 - The history of herbs, medicinal and aromatic plants, and their extracts: Past, current situation and future perspectives. In P. Florou-Paneri, E. Christaki, & I. Giannenas (Eds.), *Feed Additives* (pp. 1–18). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-814700-9.00001-7

Gil, M. I., Ferreres, F., & Tomás-Barberán, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry*, *47*(6), 2213–2217. https://doi.org/10.1021/jf981200l

Goff, H. D., & Guo, Q. (2019). The Role of Hydrocolloids in the Development of Food Structure. In *Handbook of Food Structure Development*. The Royal Society of Chemistry. https://doi.org/10.1039/9781788016155-00001

Gonçalves, J O, Santos, J. P., Rios, E. C., Crispim, M. M., Dotto, G. L., & Pinto, L. A. A. (2017). Development of chitosan based hybrid hydrogels for dyes removal from aqueous binary system. *Journal of Molecular Liquids*, *225*, 265–270. https://doi.org/https://doi.org/10.1016/j.molliq.2016.11.067

Gonçalves, Janaína Oliveira, da Silva, K. A., Rios, E. C., Crispim, M. M., Dotto, G. L., & de Almeida Pinto, L. A. (2019). Single and Binary Adsorption of Food Dyes on Chitosan/Activated Carbon Hydrogels. *Chemical Engineering \& Technology*, *42*(2), 454–464. https://doi.org/https://doi.org/10.1002/ceat.201800367

Gonçalves, Janaína Oliveira, da Silva, K. A., Rios, E. C., Crispim, M. M., Dotto, G. L., & de Almeida Pinto, L. A. (2020). Chitosan hydrogel scaffold modified with carbon nanotubes and its application for food dyes removal in single and binary aqueous systems. *International Journal of Biological Macromolecules*, *142*, 85–93. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.09.074

Guo, T., Pei, Y., Tang, K., He, X., Huang, J., & Wang, F. (2017). Mechanical and drug release properties of alginate beads reinforced with cellulose. *Journal of Applied Polymer Science*, *134*(8). https://doi.org/https://doi.org/10.1002/app.44495

Guo, X., Xu, D., Yuan, H., Luo, Q., Tang, S., Liu, L., & Wu, Y. (2019). A novel fluorescent nanocellulosic hydrogel based on carbon dots for efficient adsorption and sensitive sensing in heavy metals. *J. Mater. Chem. A*, *7*(47), 27081–27088. https://doi.org/10.1039/C9TA11502A

He, J., Ni, F., Cui, A., Chen, X., Deng, S., Shen, F., Huang, C., Yang, G., Song, C., Zhang, J., Tian, D., Long, L., Zhu, Y., & Luo, L. (2020). New insight into adsorption and co-adsorption of arsenic and tetracycline using a Y-immobilized graphene oxide-alginate hydrogel: Adsorption behaviours and mechanisms. *Science of The Total Environment*,

701, 134363. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.134363

Kancherla, N., Dhakshinamoothi, A., Chitra, K., & Komaram, R. B. (2019). Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). In *Maedica* (Vol. 14, Issue 4, pp. 350–356). https://doi.org/10.26574/maedica.2019.14.4.350

Kharkar, P. M., Kiick, K. L., & Kloxin, A. M. (2013). Designing degradable hydrogels for orthogonal control of cell microenvironments. *Chem. Soc. Rev.*, *42*(17), 7335–7372. https://doi.org/10.1039/C3CS60040H

Kirtikar, K. R. (1935). Indian Medicinal Plants : By K.R. Kirtikar, B.D. Basu, and An I.C.S. In 4 volumes. In *TA - TT -* (2nd ed. Ed). Lalit Mohan Basu Allahabad. https://doi.org/LK - https://worldcat.org/title/645288579

Koç, G. Ç., & Dirim, S. N. (2018). Spray dried spinach juice: powder properties. *Journal of Food Measurement and Characterization*, *12*(3), 1654–1668. https://doi.org/10.1007/s11694-018-9781-9

Kredy, H. M. (2020). *Journal of Global Pharma Technology Derivatives*. *February*, 103–106.

Lai, W.-F., Wong, E., & Wong, W.-T. (2020). Multilayered composite-coated ionically crosslinked food-grade hydrogel beads generated from algal alginate for controlled and sustained release of bioactive compounds. *RSC Adv.*, *10*(72), 44522–44532. https://doi.org/10.1039/D0RA07827A

Latifah, R. N., & Warganegara, F. M. (2018). Production and characterization of the healthy brown rice milk with sodium alginate addition from brown algae Sargassum binderi as emulsifier. *IOP Conference Series: Earth and Environmental Science*, *139*(1). https://doi.org/10.1088/1755-1315/139/1/012046

Lee, Y.-K., & Chang, Y. H. (2020). Microencapsulation of a maca leaf polyphenol extract in mixture of maltodextrin and neutral polysaccharides extracted from maca roots. *International Journal of Biological Macromolecules*, *150*, 546–558. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.02.091

Li, D., Wei, Z., & Xue, C. (2021). Alginate-based delivery systems for food bioactive ingredients: An overview of recent advances and future trends. *Comprehensive Reviews*

in Food Science and Food Safety, *20*(6), 5345–5369. https://doi.org/https://doi.org/10.1111/1541-4337.12840

Li, H., Wang, T., Hu, Y., Wu, J., & Van der Meeren, P. (2022). Designing delivery systems for functional ingredients by protein/polysaccharide interactions. *Trends in Food Science & Technology*, *119*, 272–287. https://doi.org/https://doi.org/10.1016/j.tifs.2021.12.007

Li, Q., Duan, M., Hou, D., Chen, X., Shi, J., & Zhou, W. (2021). Fabrication and characterization of Ca(II)-alginate-based beads combined with different polysaccharides as vehicles for delivery, release and storage of tea polyphenols. *Food Hydrocolloids*, *112*, 106274. https://doi.org/https://doi.org/10.1016/j.foodhyd.2020.106274

Li, Z., Behrens, A. M., Ginat, N., Tzeng, S. Y., Lu, X., Sivan, S., Langer, R., & Jaklenec, A. (2018). Biofilm-Inspired Encapsulation of Probiotics for the Treatment of Complex Infections. *Advanced Materials*, *30*(51), 1803925. https://doi.org/https://doi.org/10.1002/adma.201803925

Lin, D., Kelly, A. L., & Miao, S. (2020). Preparation, structure-property relationships and applications of different emulsion gels: Bulk emulsion gels, emulsion gel particles, and fluid emulsion gels. *Trends in Food Science & Technology*, *102*, 123–137. https://doi.org/https://doi.org/10.1016/j.tifs.2020.05.024

Liu, Q., Huang, H., Chen, H., Lin, J., & Wang, Q. (2019). Food-Grade Nanoemulsions: Preparation, Stability and Application in Encapsulation of Bioactive Compounds. *Molecules*, *24*(23). https://doi.org/10.3390/molecules24234242

Low, Y. L., & Pui, L. P. (2020). Optimization of mango-pineapple jelly sphere production by frozen reverse spherification using a full factorial design. *Acta Scientiarum Polonorum, Technologia Alimentaria*, *19*(2), 207–218. https://doi.org/10.17306/J.AFS.0752

Lupo, B., Maestro, A., Gutiérrez, J. M., & González, C. (2015). Characterization of alginate beads with encapsulated cocoa extract toprepare functional food: Comparison of two gelation mechanisms. *Food Hydrocolloids*, *49*, 25–34. https://doi.org/10.1016/j.foodhyd.2015.02.023

Mackie, A. (2012). 3 - Interaction of food ingredient and nutraceutical delivery systems with the human gastrointestinal tract. In N. Garti & D. J. B. T.-E. T. and D. S. for F. I. and N.

McClements (Eds.), *Woodhead Publishing Series in Food Science, Technology and Nutrition* (pp. 49–70). Woodhead Publishing. https://doi.org/https://doi.org/10.1533/9780857095909.1.49

Maeda, N., Kokai, Y., Ohtani, S., Sahara, H., Kumamoto-Yonezawa, Y., Kuriyama, I., Hada, T., Sato, N., Yoshida, H., & Mizushina, Y. (2008). Anti-tumor effect of orally administered spinach glycolipid fraction on implanted cancer cells, colon-26, in mice. *Lipids*, *43*(8), 741–748. https://doi.org/10.1007/s11745-008-3202-5

Maitra, J., & Shukla, V. K. (2014). Cross-linking in Hydrogels - A Review. *American Journal of Polymer Science*, *4*(2), 25–31. https://doi.org/10.5923/j.ajps.20140402.01

Maleki, G., Woltering, E. J., & Mozafari, M. R. (2022). Applications of chitosan-based carrier as an encapsulating agent in food industry. *Trends in Food Science & Technology*, *120*, 88–99. https://doi.org/https://doi.org/10.1016/j.tifs.2022.01.001

Maleki, M., Mortazavi, S. A., Yeganehzad, S., & Pedram Nia, A. (2020). Study on liquid core barberry (Berberis vulgaris) hydrogel beads based on calcium alginate: Effect of storage on physical and chemical characterizations. *Journal of Food Processing and Preservation*, *44*(5), 1–12. https://doi.org/10.1111/jfpp.14426

Matricardi, P., Alhaique, F., & Coviello, T. (2015). *Polysaccharide Hydrogels: Characterization and Biomedical Applications*. Pan Stanford. https://books.google.co.in/books?id=BrHwrQEACAAJ

McClements, D. J. (2017). Recent progress in hydrogel delivery systems for improving nutraceutical bioavailability. *Food Hydrocolloids*, *68*, 238–245. https://doi.org/https://doi.org/10.1016/j.foodhyd.2016.05.037

Michael Baker. (2018). *Ash in Core*.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, e47. https://doi.org/10.1017/jns.2016.41

Potortì, A. G., Bua, G. D., Lo Turco, V., Ben Tekaya, A., Beltifa, A., Ben Mansour, H., Dugo, G., & Di Bella, G. (2020). Major, minor and trace element concentrations in spices and aromatic herbs from Sicily (Italy) and Mahdia (Tunisia) by ICP-MS and multivariate analysis. *Food Chemistry*, *313*, 126094. https://doi.org/https://doi.org/10.1016/j.foodchem.2019.126094

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., & Mainland, C. M. (1998). Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of Vaccinium Species. *Journal of Agricultural and Food Chemistry*, *46*(7), 2686–2693. https://doi.org/10.1021/jf980145d

Redaelli, F., Sorbona, M., & Rossi, F. (2017). *10 - Synthesis and processing of hydrogels for medical applications* (G. Perale & J. B. T.-B. P. for B. A. Hilborn (Eds.); pp. 205–228). Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-08-100262-9.00010-0

Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P., & Rice-Evans, C. (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics*, *322*(2), 339–346. https://doi.org/10.1006/abbi.1995.1473

Saqib, M. N., Ahammed, S., Liu, F., & Zhong, F. (2022). Customization of liquid-core sodium alginate beads by molecular engineering. *Carbohydrate Polymers*, *284*, 119047. https://doi.org/https://doi.org/10.1016/j.carbpol.2021.119047

Saqib, M. N., Khaled, B. M., Liu, F., & Zhong, F. (2022). Hydrogel beads for designing future foods: Structures, mechanisms, applications, and challenges. *Food Hydrocolloids for Health*, *2*(May), 100073. https://doi.org/10.1016/j.fhfh.2022.100073

Saqib, M. N., Liu, F., Chen, M., Ahammed, S., Liu, X., & Zhong, F. (2022). Thermo-mechanical response of liquid-core beads as affected by alginate molecular structure. *Food Hydrocolloids*, *131*, 107777. https://doi.org/https://doi.org/10.1016/j.foodhyd.2022.107777

Seisun, D., & Zalesny, N. (2021). Strides in food texture and hydrocolloids. *Food Hydrocolloids*, *117*, 106575. https://doi.org/https://doi.org/10.1016/j.foodhyd.2020.106575

Serafini, M., Ghiselli, A., & Ferro-Luzzi, A. (1996). In vivo antioxidant effect of green and black tea in man. *European Journal of Clinical Nutrition*, *50*(1), 28–32.

Shahidi, F., Ambigaipalan, P., Abad, A., & Pegg, R. B. (2020). Food and Bioactive Encapsulation. In *Handbook of Food Preservation*.

https://doi.org/10.1201/9780429091483-38

Stribiţcaia, E., Krop, E. M., Lewin, R., Holmes, M., & Sarkar, A. (2020). Tribology and rheology of bead-layered hydrogels: Influence of bead size on sensory perception. *Food Hydrocolloids*, *104*(October 2019). https://doi.org/10.1016/j.foodhyd.2020.105692

Sun, X., Okagu, O. D., & Udenigwe, C. C. (2021). Chapter 15 - Encapsulation technology for protection and delivery of bioactive peptides. In F. Toldrá & J. Wu (Eds.), *Biologically Active Peptides* (pp. 331–356). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-821389-6.00028-5

Tarone, A. G., Cazarin, C. B. B., & Marostica Junior, M. R. (2020). Anthocyanins: New techniques and challenges in microencapsulation. *Food Research International*, *133*, 109092. https://doi.org/https://doi.org/10.1016/j.foodres.2020.109092

Teng, X., Zhang, M., & Devahastin, S. (2019). New developments on ultrasound-assisted processing and flavor detection of spices: A review. *Ultrasonics Sonochemistry*, *55*, 297–307. https://doi.org/https://doi.org/10.1016/j.ultsonch.2019.01.014

Thies, C. (2012). 8 - Microencapsulation methods based on biopolymer phase separation and gelation phenomena in aqueous media. In N. Garti & D. J. McClements (Eds.), *Encapsulation Technologies and Delivery Systems for Food Ingredients and Nutraceuticals* (pp. 177–207). Woodhead Publishing. https://doi.org/https://doi.org/10.1533/9780857095909.2.177

Thompson, B. R., Horozov, T. S., Stoyanov, S. D., & Paunov, V. N. (2017). Structuring and calorie control of bakery products by templating batter with ultra melt-resistant food-grade hydrogel beads. *Food Funct.*, *8*(8), 2967–2973. https://doi.org/10.1039/C7FO00867H

Timilsena, Y. P., Haque, M. A., & Adhikari, B. (2020). Encapsulation in the Food Industry: A Brief Historical Overview to Recent Developments. *Food and Nutrition Sciences*, *11*(06), 481–508. https://doi.org/10.4236/fns.2020.116035

Verma, S. (2018). a Study on Medicinal Herb Spinacia Oleraceae Linn: Amaranthaceae. *Journal of Drug Delivery and Therapeutics*, *8*(4), 59–61. https://doi.org/10.22270/jddt.v8i4.1767

Wang, H., Cao, G., & Prior, R. L. (1996). Total Antioxidant Capacity of Fruits. *Journal of*

Agricultural and Food Chemistry, *44*, 701–705. https://api.semanticscholar.org/CorpusID:96904905

Wang, M., Guo, L., & Sun, H. (2019). Manufacture of Biomaterials. In R. Narayan (Ed.), *Encyclopedia of Biomedical Engineering* (pp. 116–134). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-801238-3.11027-X

Waseem, M., Akhtar, S., Manzoor, M. F., Mirani, A. A., Ali, Z., Ismail, T., Ahmad, N., & Karrar, E. (2021). Nutritional characterization and food value addition properties of dehydrated spinach powder. *Food Science and Nutrition*, *9*(2), 1213–1221. https://doi.org/10.1002/fsn3.2110

Wong, S. K., Lawrencia, D., Supramaniam, J., Goh, B. H., Manickam, S., Wong, T. W., Pang, C. H., & Tang, S. Y. (2021). In vitro Digestion and Swelling Kinetics of Thymoquinone-Loaded Pickering Emulsions Incorporated in Alginate-Chitosan Hydrogel Beads. *Frontiers in Nutrition*, *8*. https://doi.org/10.3389/fnut.2021.752207

Wu, B., Degner, B., & McClements, D. J. (2014). Soft matter strategies for controlling food texture: formation of hydrogel particles by biopolymer complex coacervation. *Journal of Physics: Condensed Matter*, *26*(46), 464104. https://doi.org/10.1088/0953-8984/26/46/464104

Zane, A., & Wender, S. H. (1961). Flavonols in Spinach Leaves. *The Journal of Organic Chemistry*, *26*(11), 4718–4719. https://doi.org/10.1021/jo01069a531

Zegler, J. (2018). Mintel Global Food & Drink Trends 2018. *Mintel Group Limited*, 41. https://gastronomiaycia.republica.com/wp-content/uploads/2017/10/informe_mintel_tendencias_2018.pdf

Zhu, J. (2010). Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials*, *31*(17), 4639–4656. https://doi.org/https://doi.org/10.1016/j.biomaterials.2010.02.044