A DISSERTATION ON

Preparation of Protein rich petha with the incorporation of *Spirulina* Powder

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
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M. Tech FoodTechnology (IV Semester)
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UNDER THE SUPERVISION OF

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DECLARATION FORM

I, **Tanzeel Hasan**, a student of **M.Tech Food Technology** (2^{nd} year/ 4^{th} Semester), Integral University have completed my six months dissertation work entitled **"Preparation of Protein rich petha with the incorporation of *Spirulina* powder"** successfully from **Integral University** under the able guidance of **Dr. Rahul Singh and Dr. Alvina Farooqui**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Name and Signature of Student with Date

Name and Signature of Course Coordinator with Date

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CERTIFICATE

Certificate that Mr **Tanzeel Hasan** 1600100618 has carried out the research work presented in this thesis entitled **"Preparation of protein rich petha with the incorporation of *Spirulina* powder"** for the award of **M.Tech Food Technology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself/herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of his **M.Tech Food Technology**

I wish him good luck and bright future.

Dr . Rahul Singh
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CERTIFICATE BY INTERNAL ADVISOR

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I wish him good luck and bright future.

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TO WHOM IT MAY CONCERN

This is to certify that **Tanzeel Hasan,** a student of **M.Tech. Food Technology** (2nd Year/ 4th Semester), Integral University has completed his six months dissertation work entitled **"Preparation of protein rich petha with the incorporation of *spirulina powder*"** successfully. He has completed this work from Integral University under the guidance of **Dr. Rahul Singh**. The dissertation was a compulsory part of his **M.Tech Food Technology**

I wish him good luck and bright future.

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Place: Lucknow Tanzeel Hasan

Date:

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ABSTRACT

The fast-growing life of the human population has demanded nutrient-enriched food products. In Uttar Pradesh traditionally almost 80% of the population intake sweets daily. Petha is one of the sweet products which is made up of *Ash gourd* and is in high demand with all classes of people. The pros and cons are not listed in many articles. Another thing is that the least literature of review is available about the value addition of Ash gourd. Therefore the present study was undertaken to study the Physico-chemical attributes of standard samples as well as processed petha in addition the quality attributes are also evaluated by determining the response variable consisting of sensory parameters for flavor, texture, color, and appearance, and overall acceptability. *Spirulina belongs* to the class of cyano bacteria that are also known as single-cell proteins. Incorporation of the *Spirulina* powder into petha was done. The present study aims to address the nutrient enrichment of the petha by incorporating Spirulina in it. The hypothesis was designed to give value addition to the traditionally cooked petha by making its protein, iron, and vitamin enriched. The study has shown that the value addition Ash Gourd can be made in the form of petha which is nutritionally rich and also can be commercially exploited. Attempts can be made by mixing *Spirulina* powder with natural additives and flavor.

Keywords: Ash Gourd, Petha, *Spirulina*, Process, Flavor

CHAPTER 1

INTRODUCTION

The Food and Agriculture Organization (FAO) defines sustainability as diets have low environmental impacts that contribute to food and nutrition security as well as a healthy lifestyle for current and future generations. Sustainable diets are both protective and beneficial. Respect for biodiversity and ecosystems, cultural acceptability, accessibility, and economic viability fair and affordable; nutritionally adequate, safe, and healthy; and maximizing natural and organic resources human capital (Lonnie, M.,*et al*.**,** 2019)**,**However, as industrialization and urbanization have increased, people have completely forgotten about their diet and daily nutrition, resulting in various macro and micro nutrient deficiencies. To overcome such deficiencies, they use a variety of synthetic drugs that can temporarily alleviate their symptoms but not permanently**.**
Unfortunately, food consumption and production have become more complex over time as food goes through intricate processes as well as longer and farther transportation. These unsustainable patterns have resulted in environmental degradation, accelerated climate change, and increased natural resource degradation, putting human health at risk (Premanandh**.** 2011**).**

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

The vegetable ash gourd (*Benincasa hispida*) is grown all over India, as well as in other South East Asian nations, Japan, China, and Australia. Various cuisines and traditional Indian medicine have both used it .Because of the thick wax content in the skin, which significantly extends the fruit's shelf life, the ash gourd is also known as the wax gourd. 15% of the fruit is made up of peel, which is typically discarded. Fruit's value is increased through peeling both domestically and commercially **(**Hache, E.**,***et al*..2019**).**

Ash gourd is a popular vegetable grown throughout India, particularly in Kerala. It is also known as "Winter Melon" or "Wax Gourd." It is grown for both its immature and mature fruits. Because of its medicinal properties, this vegetable/fruit is also used in ayurvedic medicinal preparations. This ash gourd is used to make the famous and delicious "Petha." This crop is grown in south India for vegetables and in north India for "Agra petha."Ash gourd is also known

as Winter melon (English), Petha, Pethakaddu (Hindi), Kohla (Marathi), Neerpoosanikai(Tamil), Kumbalanga (Malayalam), BoodidaGummadikaaya (Telegu), Budekumbalakayi, Boodugumbala (Kannada), Kumra, Chalkumra (Bengali) (Assamese).(http://www.niftem.ac.in)

The vegetable "petha," also known as ash gourd, is high in calcium, minerals, and carbohydrates**(**Javed, Z.,*et al*., 2014). Because of their high glucose and mineral content, all Petha products are highly recommended for growing children, lactating mothers, and people suffering from jaundice. It benefits the brain and nervous system by nourishing it. Because the Petha preparation does not use fat cooking oils, it has a low fat content and is cholesterol-free. It is filling and nutritive, acts as a blood coagulant, and is used to treat peptic ulcers and obesity. The delicious sweet preparations made from it are used to treat tuberculosis, heart weakness, and anaemia. Despite its high sugar content, this nourishing sweet has numerous nutritional and medical benefits and is a cheap source of instant energy for people as well as protecting them from high summer temperatures due to its cooling properties (Singh and Saini, 2014).

B. hispida is a monoecious, 5-angled, climbing or trailing herb with a sub orbicular stipulator bract at the petiole-root; simple, very hairy leaves on both surfaces, alternate, palmate or ovate blade in young plant, cordate root. Fruits are 30-45 cm long when young, succulent, densely hairy, and have a thick waxy deposit when ripe. The percentage of crude protein, ash, starch, lipid, crude fiber, alkaloid, flavonoid, tannin, and phytate may be accounted for by the multiple medicinal properties. The fruit's biochemical activity includes anti-oxidative, anti-inflammatory, anti-angiogenic, detoxifying, and curvative effects in the treatment of various conditions. Important minerals such as Ca, Mg, Fe, Cu, Zn, and Se are present (Doharey. V,*et al*., 2021**).**

Benincasa hispida is a vine grown for its very large fruit, which is eaten as a vegetable when mature. When young, the fruit is covered in a fuzzy coating of fine hairs. The flesh of the immature melon is thick and sweet. The fruit loses its hairs as it matures and develops a waxy coating, giving rise to the name wax gourd. The wax coating contributes to the fruit's long shelf life. The melon can grow to be up to 80 cm long. It has large leaves and yellow flowers. The peril is rather flat. Benincasa hispida, also known as wax gourd, is a vine grown for its very large fruit, which is eaten as a vegetable when mature. Benincasa is the only species in the genus. When young, the fruit is covered in a fuzzy coating of fine hairs. The flesh of the

immature melon is thick and sweet. The fruit loses its hairs as it matures and develops a waxy coating, giving rise to the name wax gourd. The wax coating contributes to the fruit's long shelf life. The melon can grow to be up to 80 cm long. It has large leaves and yellow flowers**(:** http://www.niftem.ac.in).

In India, ash gourds are commercially used to make candy and a variety of sweet delicacies known as murabba (petha, in northern India). India's Agra city (U.P.) has become a well-known business centre for the production and processing of murabba . Murabba is traditionally made from boiled white pumpkin or ash gourd peeling and deseeding It is then boiled in sugar syrup flavored with rose water or vanilla flavorings water Murabba is made from ash gourd through osmotic dehydration syrup made from sugar It is marketed as a sweet (i.e. petha), as well as in bakery and confectionery items (e.g. breads, cakes) (Food Safety and Standards Regulations, 2009).

The objective of this study is:-

1. Collection and Processing of Ash Gourd and *Spirulina* Culture

2. Preparation of petha

3. Incorporation of Spirulina into standard petha

4. Optimization of process parameters of standard petha

5. Physiochemical analysis of developed *Spirulina* petha

CHAPTER 2

REVIEW OF LITERATURE

2.1 An overview of Ash-gourd

According to Sew, *et al*., (2010), the seeds of the Ash gourd, a high-yielding fruit, and vegetable plant (***Benincasa hispid***). Nutritional characteristics (dietary fiber, crude protein, crude fat, crude fiber, ash, and energy) as well as the composition of oil fatty acids were examined for functional properties (particularly in medical therapy). The primary component of the seeds, according to close analysis, was the total dietary fiber.58.43 percent of the seed's composition. Crude protein and fat content were found to be 20.70 and 20.70 respectively percentages with 11.63 percent. Linoleic acid (C18:2) was the predominant component of the ash gourd seed oil that was extracted, which make up 67.37 percent of all fatty acids. Other significant fatty acids found were stearic (C18:0), palmitic (C16:0), and oleic (C18:1) acids contributing 17.11, 10.21, and correspondingly 4.83 percent.

Doherty. V, *et al.*, (2021) ***Benjamin Hispida*** (Ash gourd, Family: Cucurbitaceous), It was a well-liked vegetable crop that was extensively utilized for medical needs. The well-known plant ***B. hispida*** is grown throughout India's plains and on hills up to 1200 meters above sea level. It is a sizable climbing plant with high-speed stems that are slabby. The fruits are round, 30-45 cm long, and coated in wax. Fruits from ***B. hispida*** include a significant amount of volatile oils, glycosides, saccharides, proteins, carotenes, flavonoids, vitamins, minerals, ß-sitosterol, and uronic acid, according to phytochemical analysis. People typically referred to it as fruit or a vegetable. The fruit can be used in medicine in all of its components. Information about the herb's pharmaceutical properties is provided in the current review. Fruits are used to treat blood disorders, cardiotonic conditions, diuretics, dyspepsia, epilepsy, fever, etc.

Sharma, A.,(2021**)** mentioned that he values the addition of pears in the production of *petha*, which might become more popular across the nation and lengthen its shelf life, which is mostly unknown. It was decided that the response variable would be made up of sensory metrics for flavor, texture, color, and appearance as well as general acceptance. Consequently, the current study was undertaken to investigate the physicochemical characteristics of raw samples as well as processed *petha*. The ideal conditions for lime water concentration, lime water treatment duration, and sugar concentration for *petha* production were also predicted

using a central composite rotatable design (CCRD). Process standardization was done for sensory analysis and *petha* preparation. The study's findings revealed that pears, a seasonal fruit, offer significant nutritional, therapeutic, and economic benefits that can be used to standardize the process parameters and add value by making *petha*. It is also possible to conduct a storage study of prepared *petha* in which the goods may continue to be safe from a microbiological point of view. The ideal operating conditions were assessed and determined with success using the response surface methodology of CCRD design. The study has demonstrated that *petha*, which is rich in nutrients and may be used commercially, can be created from pears to increase their worth. It is possible to make attempts by combining ingredients and adding colors that might fascinate individuals.

Mandal, D., (2015) examines the current study on how pre-treatment with ultrasound alters the way osmotic dehydration of Ash gourds during Murabba processing. Revotek Ultrasonic Bath was used to apply ultrasonic waves to ash gourd cubes in a water bath for 10, 20, and 30 minutes. The cubes were then submerged in Brix sugar solution for a contact period of varying time and temperature of 40°, 50°, and 60°C to perform the osmotic dehydration process. The samples' solid gain and water loss were then calculated gravimetrically. Using a scanning electron microscope, the impact of ultrasonic on the ash gourd tissue was investigated (FE-SEM Supra 55[Carl Zeiss, Germany]). Additionally, Colorimeter (Hunter Color Lab), employing CIE Lab was used to track the cubes' color dynamics while they underwent osmotic dehydration. To forecast the rate at which water and other substances will move during osmotic dehydration, a mathematical model based on the fundamental law of diffusion was created concurrently. For both solid gain and water loss, the model was numerically solved utilizing developed code in the MATLAB environment. Additionally, apparent mass diffusivities are calculated and experimentally confirmed. The combined effects of ultra sonication, 70Brix osmotic solution concentration, and 60°C osmotic temperature have boosted solute gain and water loss. The developed model provides accurate predictions for solute uptake and water loss with a mean relative deviation modulus of less than 2.6 percent. In general, as the temperature of the osmotic solution rises, yellowness (b*) and chroma © values increased, whereas lightness (L*) and color intensity (E) values decreased. Additionally using Image software, ultra-sonication raised tissue porosity to 35%, perhaps as a result of the development of micro channels.

According to Sreenivas, K. M. *et al.,* (2011)**,** the vegetable ash gourd ***(Benincasa hispida***) is consumed in Asian nations, and the peel has a high concentration of edible waxy compounds. In

his study, strawberry (Fragaria ananassa) is used as a model system for the extraction, characterization, and application of ash gourd peel wax as an edible coating in fruits. The melting point of crude wax was 80 C, and its molecular weights by number (Mn) and weight (Mw) were 2,277 and 2,323, respectively. Strawberry was coated with a crude wax emulsion using the dipping process. The ideal values for wax quantity, sodium benzoate concentration, and dip time were 0.5 percent, 1 M, and 3 min, respectively. Carnauba wax coating produced results that were equivalent. The shelf life was extended by wax coating to 7 days at 25 °C, and the attributes such as texture, color, weight loss, titrable acidity, and microbiological counts were all satisfactory.

Kumari, D, *et al*., (2021) analyzed that to convert rice straw (RS) into ethanol and methane, the current work suggests a brand-new pretreatment process combining petha wastewater (PWW) and Mausam waste (MW). This method of pre-treatment is an illustration of waste-to-waste. The anaerobes in the microbial culture used have taken use of the organic materials in the RS as a source for the generation of biofuel (cow dung). Chemical and natural pretreatment techniques were examined for the solubilization of the lingo cellulosic content into reducible sugars to improve the generation of biofuel. Following PWW pretreatment (198 mg/L), RS pretreated with 2 percent NaOH yielded the highest glucose release (292 mg/L).methane. In addition to the pre-treatment techniques mentioned above, a further 5-minute microwave pre-treatment was also applied to improve the glucose release for ethanol and methane synthesis. Two batch runs were performed on each reactor. The greatest bioethanol yield for PWW and microwave pre-treated RS was 28.75 mg/L (1150 mg/kg rs), while the maximum methane yield for PWW pre-treatment RS was 11.86 percent of the total gas produced. This work is based on early research on the synthesis of ethanol and methane using RS with microwave-assisted PWW and MW pretreatment.

2.2 An overview of *Spirulina*

Abdel-Moneim, A. M *et al*., (2022) examined the biological selenium nanoparticles (SeNPs) produced by Bacillus subtilis AL43 as well as three Spirulina extracts (methanol, acetone, and hexane) for their antibacterial and antioxidant properties. The outcomes demonstrated that tested pathogens were resistant to Spirulina extracts' antibacterial effects. Additionally, Spirulina extracts substantially and dose-dependently scavenged ABTS and DPPH radicals. In comparison to other extracts, the methanolic extract demonstrated higher total phenolic content, antibacterial

activity, and antioxidant activity. Bacillus subtilis AL43 produced the selenium nanoparticles in an anaerobic environment, and they were identified as spherical, crystalline, 65.23 nm in size, with a net negative charge of 22.7. By testing SeNPs against three gram-positive, three gram-negative, and three strains each of Candida and Aspergillus spp., we demonstrated that SeNPs have significant antibacterial activity. Furthermore, SeNPs demonstrated dose-dependent scavenging of ABTS and DPPH radicals. There is a correlation between the biological activities of SeNPs and the total phenolic content of *Spirulina*. Our findings show that Spirulina and SeNPs have substantial antibacterial and antioxidant properties and appear to be viable options for dependable and safe medicinal uses.

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

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

Koli, D. K *et al.,* (2022**)**studied to enhance pasta made from semolina, locally grown *Spirulina* powder was used at percentages ranging from 2 to 15%. With the addition of *Spirulina,* green color pasta was created with nutritional and functional fortification, increasing the amount of protein, total phenols, flavonoids, iron, and calcium by up to 77.47 percent, 76.62 percent, 162.88 percent, and 57.27 percent, respectively, without negatively affecting the textural and sensory qualities. A FAME study found that enriched pasta had levels of –linolenic acid and docosahexaenoic acid that were 2 to 2.5 times higher. Additionally, phenolics, flavonoids, and antioxidant activity significantly improved when compared to control pasta. Proteins and other nutrients were not significantly lost during cooking, according to an analysis of theoretical and actual composition. Principal components analysis showed that *S*pirulina, especially at higher doses, significantly contributed to nutritional and functional aspects. Pasta with 12.5% added sugar Spirulina received a "loved very highly" rating, and a significant percentage of people planned to purchase it. As a preferable alternative to improve health and ward off disease, *Spirulina* enrichment at concentrations over 10 percent (12.5%) with considerable increases in nutritional and functional qualities without impacting textural or culinary quality and acceptable sensory evaluation may be used. Consuming green pasta with *Spirulina* may be a viable option to improve the livelihood and nutritional security of rural poor people as well as a good alternative for hidden hunger alleviation programs for mass nutrition, especially for infants and children. Green is a color that represents freshness, hope, renewal, and physical health.

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

senility, and several pathological illnesses, including neurological and neurodegenerative diseases.

Lafarga, T., *et al.*, (2020) mentioned that *Spirulina* use by humans is not new; in the sixteenth century, it was gathered from Lake Texcoco and consumed in Tenochtitlan markets (today Mexico City). Microalgae are now used in a wide variety of food compositions. For their marketing or as a coloring agent, the majority of these employ microalgae. Spirulina (and chemicals obtained from it) have the potential to be employed as ingredients in the creation of innovative foods, one of the biggest developments in the food industry. *Spirulina* has the potential to be utilized in the prevention or treatment of illnesses connected to metabolic syndrome, according to several human intervention studies. Reviewing the present and potential uses of these microalgae in the food and functional food industries were the goal of the current article. Spirulina consumption advantages and/or some of the most significant chemicals generated from *Spirulina* were also covered.

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

CHAPTER 3
MATERIAL

3.1 Collection of Fruit

The ash gourd was obtained from local vegetable market of Tedipulia, Lucknow, Uttar Pradesh. **(**26.8467° N, 80.9462° E)

3.2 Collection of *Spirulina* Culture

Spirulina culture was taken from the **Integral Information and Research Centre (IIRC)**, **Plant Tissue Culture**(PTC) lab at Integral University Lucknow. The culture was grown using Zarrouk's media.

3.3. Raw material

Table 3.1:- Lists of chemicals

S.No.	Chemicals	Chemical Formula	Company
1.	Sugar	$C_{12}H_{22}O_{11}$	Dhampur Sugar
2.	Lime	$Ca(OH)_2$	Indocal
3.	Alum	$KAl(SO_4)_2 \cdot 12H_2O$	Urban Platter
4.	Skimmed Milk	----------	Mother Dairy

Table 3.2:- Lists of Glass ware/ Tools used

S.No.	Glass ware	Specification	Quantity	Company
1.	Petri plates	7.5cm Diameter	15	Borosil
		10 cm Diameter	2	
2.	Conical Flask	50 ml	6	

		100 ml	2	
		250 ml	4	Borosil
		500 ml	2	
		1000 ml	1	
		2000 ml	1	
3.	Beaker	50 ml	2	
		100ml	1	
		250ml	3	Borosil
		500ml	5	
4.	Knife	Stainless Steel	1	Agaro
5.	Chopping board	Wooden	1	Floraware
6.	Pointed Object	Needle	6	Pony
7.	Stainless steel	Cook and serve	1	
	utensils	big bowl		Hawkin
		Table spoons	3	
		Laddel	1	

Table 3.3:- Lists of instrument used

S. No	Instrument	Model no.	Company
1.	Weighing balance	ALE-223	K- Roy
2.	Microwave	MC-7148MS	SAMSUNG
3.	Electronic Balance	MSW10A/VA	WENSAR
4.	Sieve	ASTM Standards	MAHEK INDUSTRIES
5.	Centrifuge	R8CRemi-frequency	J .S. enterprises
6.	Spectrophotometer	LI-2904	LASANAY
7.	Refractrometer	HR-05	HM digital
8.	Magnetic Stirrer	KM057	BERXCO

9.	Digital Vernier Callipers	DIGE 150	z Hart

3.4. Media used

3.4.1. Nutrient Agar media- It is a general-purpose, nutrient medium used for the cultivation of microbes supporting the growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for bacterial growth.

Table 3.4 – Composition of nutrient agar media

S No.	Constituents	Quantity(g/L)
1.	Peptone	5 gm
2.	Beef extract	3 gm
3.	Agar	15 gm
4.	NaCl	8 gm

3.4.2. Potato dextrose agar media - Potato Dextrose Agar (PDA) is used for the cultivation of fungi. Potato Dextrose Agar (PDA) is a general-purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products, and testing cosmetics. PDA can be used for growing clinically significant yeast and molds. The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.

Table3.5 – Composition of Potato dextrose agar media

S No.	Constituents	Quantity(g/L)
1.	Potato extract	4 gm
2.	Dextrose	20 gm
3.	Agar	15 gm

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

Table 3.6- Lists of Chemicals used for the preparation of Zarrouk's media

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

The media was prepared for 2 liters

METHODOLOGY

3.5. Preparation of standard petha

3.5.1. Processing of Ash gourd

Ash gourd was selected according to the required weight, shape, and size. Then it was washed, cutted and the seeds were separated. The pulp was sliced according to desired shape and size (3×3cm). The ash gourd pieces are soaked in lime water before being washed. Sugar syrup was prepared and mixed with lime water-treated pieces before being boiled . Boling until a sugar syrup concentration of 65-80° Brix is achieved. Pieces are cooked, packed, and stored.

3.6. Preparation of Ingredients

3.6.1Sugar Syrup Preparation - The refined sugar was used as a sweetening agent because of its ease storage due to least moisture content (≤1.5 %) and low Relative Humidity (20-25 %) The syrup was prepared by dissolving 980grams of sugar granules into 1.5liters of water and was continuously stirred until a homogenous solution was obtained.

3.6.2Preparation of lime water - It is a chemical compound that is also known as calcium carbonate (CaCO3). 500g lime was added into7 litres of water and was vigorously mixed for 2-3 hours until a homogenized solution is obtained. Then the un-dissolved lime was filtered out. Lime water was used for the preparation of petha to tighten or firm processed ash gourd. The washing and soaking times of processed ash gourd pieces vary depending on the type

3.6.3 Preparation of Alum – It is a chemical compound also known as potassium aluminium sulphate ($K_2SO_4.Al_2(SO4)_3. 24H_2O$) **.**45 grams of powdered alum was dissolved in 100ml of water and mixed until a homogenous solution is obtained. It is an optional additive used primarily at the time of syrup preparation from unrefined sugar to remove dirt and extraneous matter (husk, twigs, sack binder etc.).

3.6.4 Skimmed Milk - The cleaning of sugar syrup necessitates the use of numerous additives. The most important of these is skimmed milk. It is used to start the separation of impurities in the form of a scum top layer, which is then removed with large slotted ladles. The protein in the skimmed milk binds with impurities in the boiling syrup, causing them to float to the surface.

The reason for using skimmed milk is that the fat in the milk interferes with the binding process of the skimmed milk protein, reducing flocculation.

3.7. Product Preparation

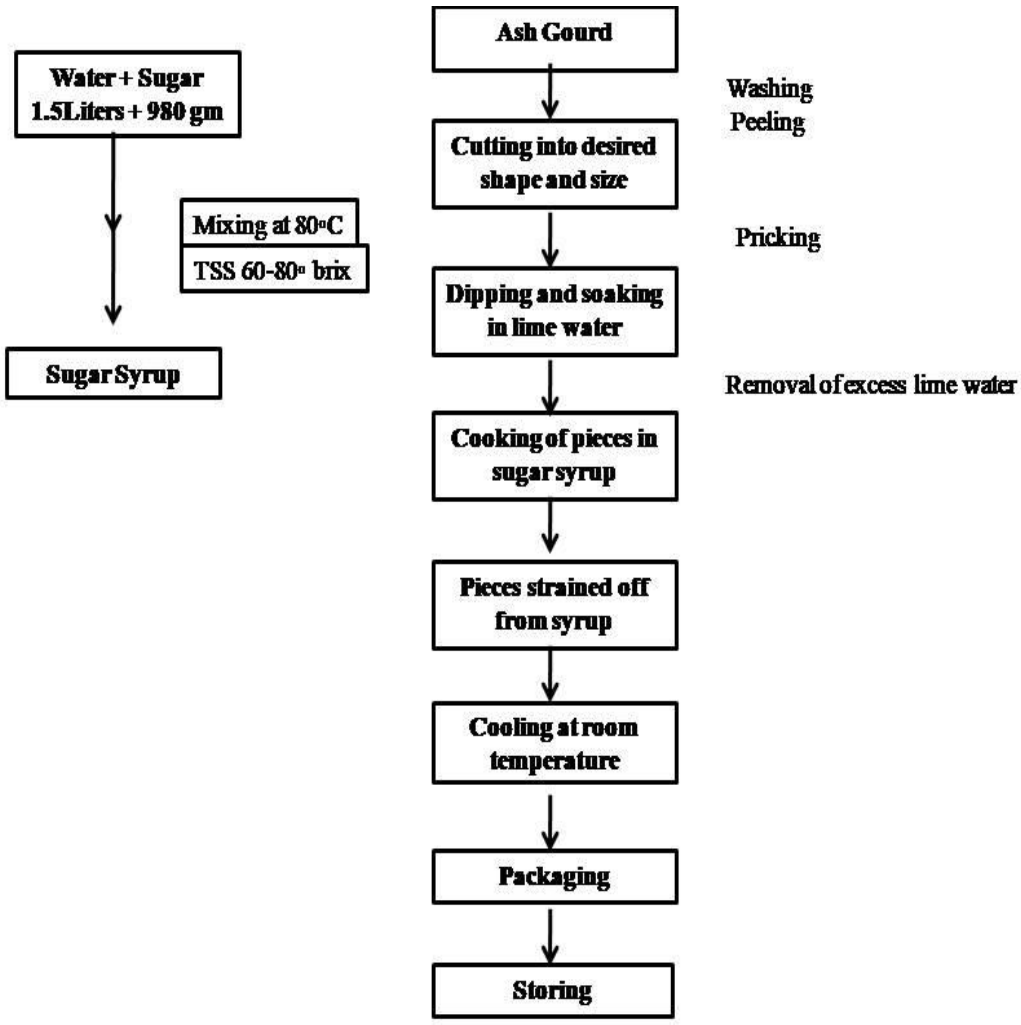

Figure 3.1:- Standard preparation of petha

3.8. Spirulina powder preparation

3.8.1. *Arthrospira platensis* (*Spirulina*) Preparation.

Zarrouk's Media was used for the growth of *Spirulina*. The optimal growth yield of the Spirulina were obtained at pH level of 9.5, while maintaining temperature of 25±5°C and light intensities of 2000 lux with time interval of 10 to 14 hour of lightness and darkness respectively.

3.8.2. Growth Study

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

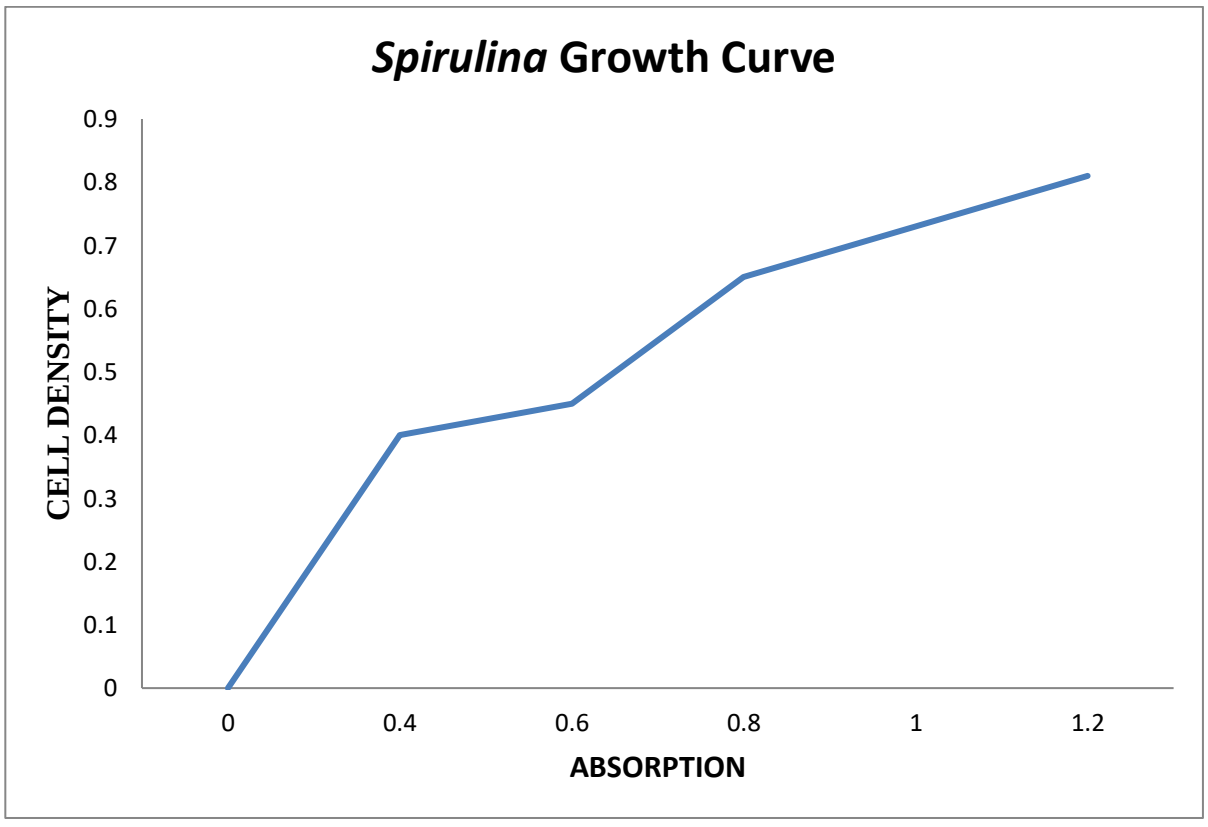

Figure 3.2:- *Spirulina* growth curve

3.9. Dry *Spirulina* biomass preparation

The dry algal biomass was prepared by using the steps shown in figure:

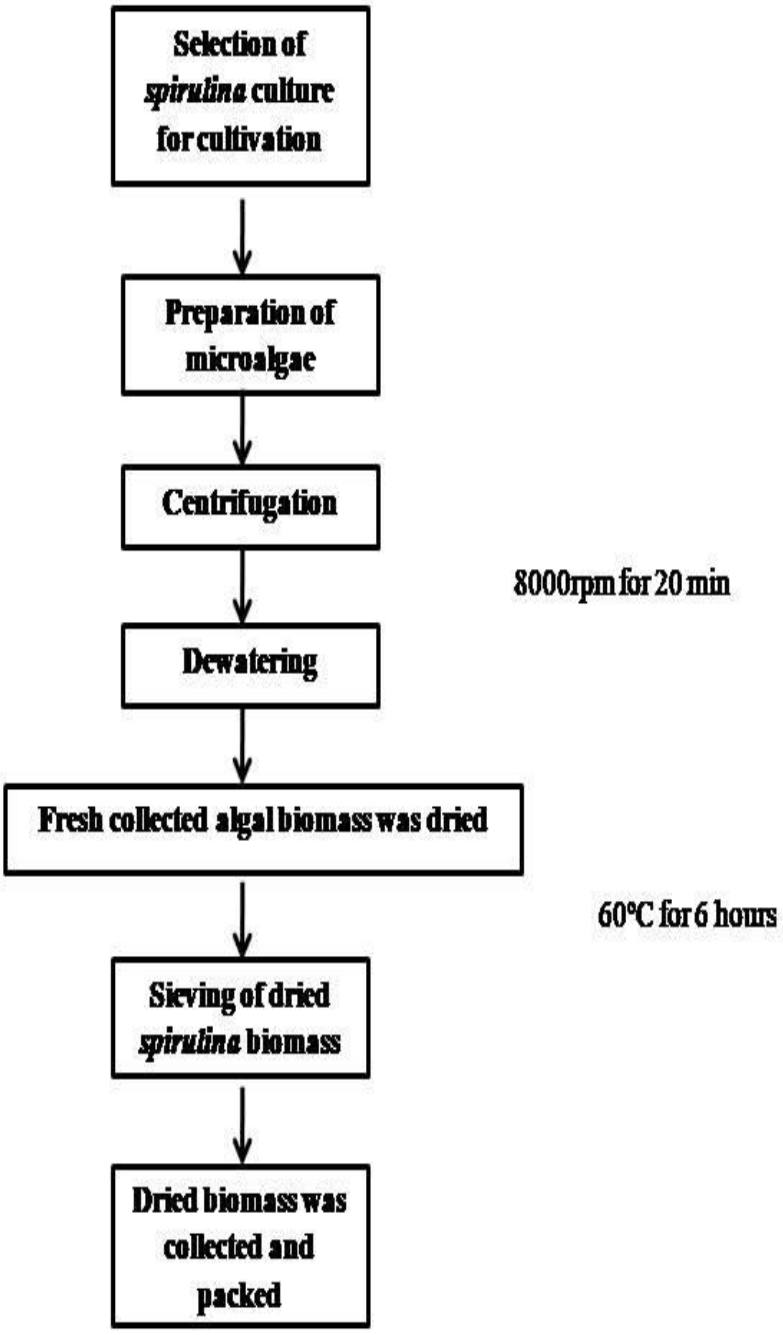

Figure 3.3:- *Spirulina* powder preparation

3.10. Incorporation of *Spirulina* powder in petha – Powdered *Spiruilna* was added during the sugar syrup preparation. Once the powdered is completely homogenized with the sugar syrup the chopped pieces of ash gourd were added into it for further cooking.

3.11. Optimization of process parameters -

3.11.1. Sugar content - Different concentration (98, 88, 78, 68, 58, 48gms) of sugar were taken for the optimization of sweetness in petha. The selected amounts were mixed into 150ml of water.

3.11.2. Substitution of lime water with Alum –To optimize the toughness and texture of the cooked petha lime water was replaced with alum. Different concentration (20, 40, 60, 80, 100, 120gms) of alum was dissolved in 150ml of water

3.11.3 Effect of skimmed milk -Skimmed milk is added to the sugar syrup to give it a clear solution .The protein in the skimmed milk binds with impurities in the boiling syrup and rise on top.

3.11.4 Different dosage of *Spirulina* - Different dosages (0.1, 0.2, 0.4, 0.8, 1.0gms) of *Spirulina* powder were mixed with 15ml distilled water and 7.8gm sugars and homogenized using a magnetic stirrer for 15–20 minutes.

3.11.5. Size- Prepared petha was reduced to different sizes of 5x5, 3x3, 2.5x2.5, 2x2 and 1x1 in order see the better absorption of *Spirulina*, when it is dipped in the solution

Table: - 3.7 Optimized parameters

Raw Material	**Different concentration / dosage (150ml)**					
1. Sugar	98gm	88gm	78gm	68gm	58gm	48gm
2.Lime water replacement with alum	20gm	40gm	60gm	80gm	100gm	120gm

3. Spirulina Dosage	0.1gm	0.2gm	0.4gm	0.6gm	0.8gm	1.0gm
4. Size Reduction of petha	1x1	2x2	2.5x2.5	3x3	4x4	5x5

3.12. Cooking of petha at optimized condition

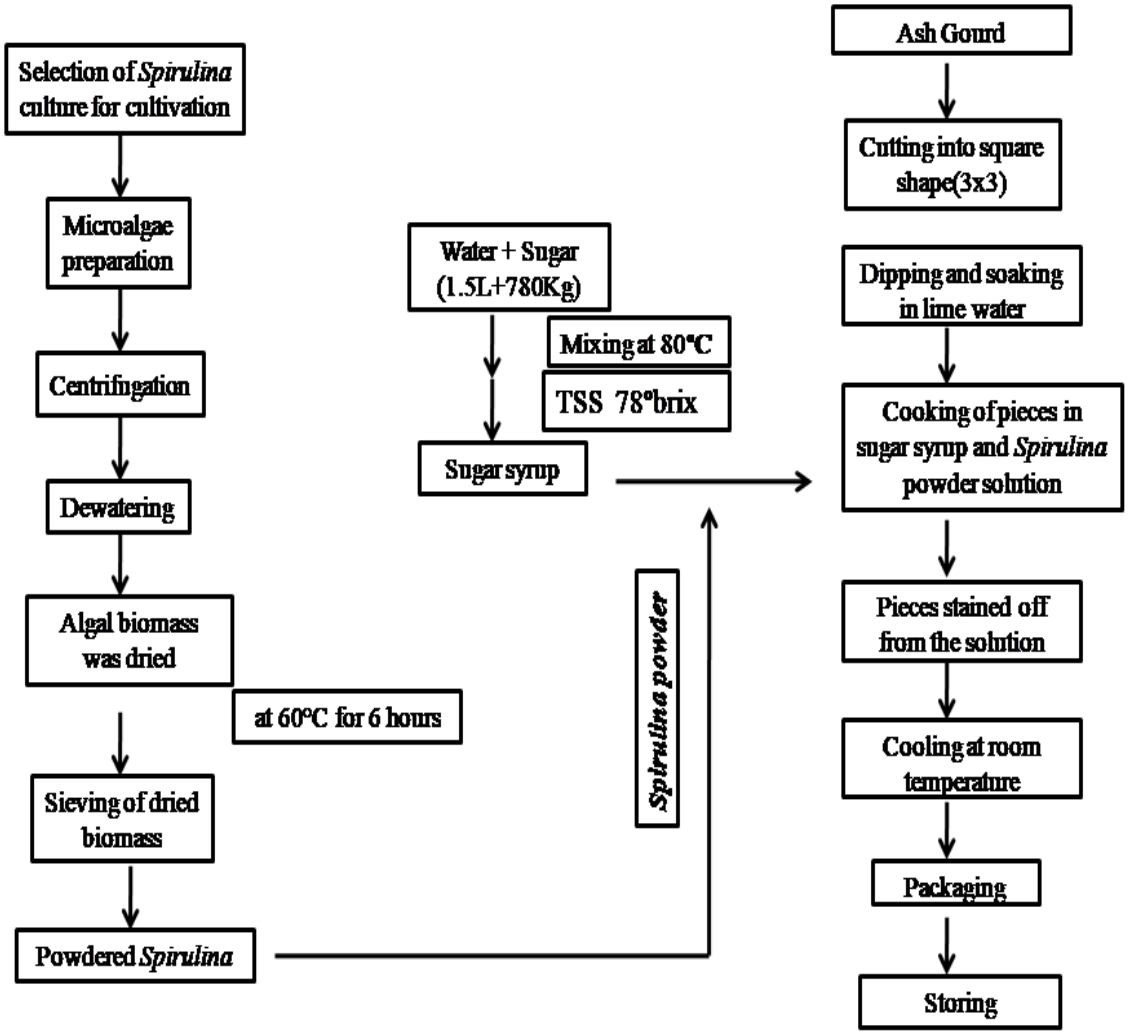

Figure: - 3.4 Optimized petha preparation

3.13. Quality Analysis - The product was prepared in the month of March 2022. The quality analysis of Petha was started in April – May 2022 and continued for 90 days at 30days interval.

3.13.1. Physiochemical Analysis

3.13.1.1. Moisture Content - Initially, the weight of empty Petri plates was determined using an electronic balance. In flat bottom Petri plates, 5g of each sample were weighed. The dish and its contents were placed in a hot air oven that was thermo statistically controlled at 105ºC and heated for 1 hour and then the readings were calculated. Further samples were again kept at 30 minutes intervals until no further weight loss was observed. Finally, the dish was removed from the oven and placed in desiccators to cool before being weighed again. The following formula was used to calculate the moisture content of petha sample.

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

(Lomauro *et al.*,2004)

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

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

Marshall, M. R. (2010)

3.13.1.3. Fat Content- The soxhlet method was used for extraction of fat of petha sample which is described below: 5g of sample was taken and a thimble was made with the help of Whatman paper number one. The thimble was placed into the extracting tube and this tube was connected with the weighted flask and also the condenser. The heat vaporized the volatile solvent, which passed up the side arm and was condensed in the condenser. The condensed

solvent fell drop by drop onto the thimble. When a sufficient amount of solvent had been transferred to the extracting tube to fill the siphon arm, it was siphoned back into the weighed flask. This process was continued for a few hours until the extraction was completed. Then the bottom flask was removed, the volatile solvent was evaporated and the fat extracted was obtained as crude fat. The following formula was used to express the fat content of the sample. Apparatus used in this method are Mortar and pestle, Chemical

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

Dobush, G. R.,et.,(2015)

3.13.1.4. Protein Content- For protein estimation in the Petha sample, the Kjeldahl method was used, as described below:

This method was being performed in 3 major steps:

(a) Digestion

The food sample to be analyzed was weighed into a *digestion flask* and then digested by heating it in the presence of sulfuric acid (an oxidizing agent which digests the food), anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, titanium, or mercury (to speed up the reaction). Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia and other organic matter to C02 and H20. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH4+) which binds to the sulfate ion (SO42-) and thus remains in the solution:

$$\text{N (food sample)} \longrightarrow (NH_4)_2SO_4$$

(b) Neutralization

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

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

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

$$NH_3 + H_3BO_3 \text{ (boric acid)} \longrightarrow NH4^+ + H_2BO_3^- \text{(borate ion)}$$

(c) Titration

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

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

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

$$\mathbf{N\ (\%)} = \frac{\text{(Sample titration – blank titration)x (N of HCl)x (14)}}{\text{Weight of sample (g) x 1000}} \text{ X 100}$$

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

$$\boldsymbol{Proteincontent}(\%) = (\%\boldsymbol{N}\mathbf{X6.5})$$

Gornall, A. G(2010**)**

3.13.2. Anti- microbial effect

3.13.2.1 Nutrient Agar media- It is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients

needed for the bacterial growth.

3.13.2.2. Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 liter of distilled water. Media was prepared for 250 ml water.

2. Heat this mixture while stirring to fully dissolve all components.

3. Autoclave the dissolved mixture at 121ºC for 15 minutes.

4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.

5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.

6. Replace the lid of each Petri dish and store the plates in air laminar flow.

3.13.2.3.Preparation of Potato dextrose agar media

1. Dissolve 24g of the potato dextrose powder in 1liter of distilled water. Media was prepared for 250ml water.

2. Heat this mixture while stirring to fully dissolve all components.

3. Autoclave the dissolved mixture at 121ºC for 15 minutes.

4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.

5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.

6. Replace the lid of each Petri dish and store the plates in air laminar flow.

3.13.2.4. Preparation of Petri plates

Sterilized Petri-plates were taken to the laminar air flow cabinet. Warm media was poured into each plate. This was done near flame to prevent contamination of the plates by microbes. Plates were kept for cooling and solidification.

3.13.2.5. Procedure

After the plates were solidified, sample was placed on the petri-plate. Then the plates were placed into the incubator for 48 hours. The colonies were observed and counted.

3.13.2.5. Preparation of Zarrouk's media

In a plastic bottle, all the chemicals were dissolved in 2 liters of distilled water and were autoclaved at 121◦C and 15psi for 30 minutes and then allowed to cool at room temperature. After attaining a normal temperature media was poured into large plastic tub followed by culture and left for a few days for growth.

3.13.2.6. Sensory Analysis -The sensory attributes were observed for 90 days at 30 day intervals. Hedonic Ratting Test was used to evaluate Petha sensory attributes such as colour, flavour, texture, taste, size etc… The Hedonic Ratting test was used to evaluate sensory characteristics. This test is used to assess consumer acceptance of a product. The methodology is presented in detail below.

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

Table 3.8:- Hedonic Scale table

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

CHAPTER 4

RESULTS AND DISCUSSION

This chapter present the experimental results conducted to enhance the protein content of petha with the incorporation of *Spirulina* powdered under ambient condition.

During the analysis 3 different samples were taken and their comparative study is done in order to get the best results.

Sample 1(S_1) – Market Petha

Sample 2 (S_2) – Standard Petha prepared in lab

Sample 3 (S_3)- Optimized Petha

The experiments were carried out to determine various analysis of the petha. The result of this investigation was discussed under the following heads:-

4.1 Physical characteristics of Ash gourd

The physical characteristics like shape, color, average diameter and the average length of the fully matured Ash gourd obtained from the local vegetable market of Tedhi puliya, Lucknow, Uttar Pradesh are presented in the table 4.1:-

Table 4.1: Physical characteristics of Ash Gourd

S .No	Morphological characters	Parameters of Ash Gourd
1.	Shape	Round and Oblong
2.	Color	Green in color
3.	Diameter	15 cm
4.	Length	23 cm
5.	Weight	2.60 kg
6.	Shelf life	4-6 months

Effect on Physico-chemical characteristics of Petha

4.2. Moisture Content

The storage period and *Spirulina* powder effected moisture content of packed petha is presented in Fig4.2.The moisture content decreased considerably due to *Spirulina* powder used. (4.2).

Table 4.2 Moisture Content (%) of Petha

Samples	0days	30days	60days
S_1	3.4	2.6	1.3
S_2	2.9	2.1	1.1
S_3	2.1	1.4	0.7

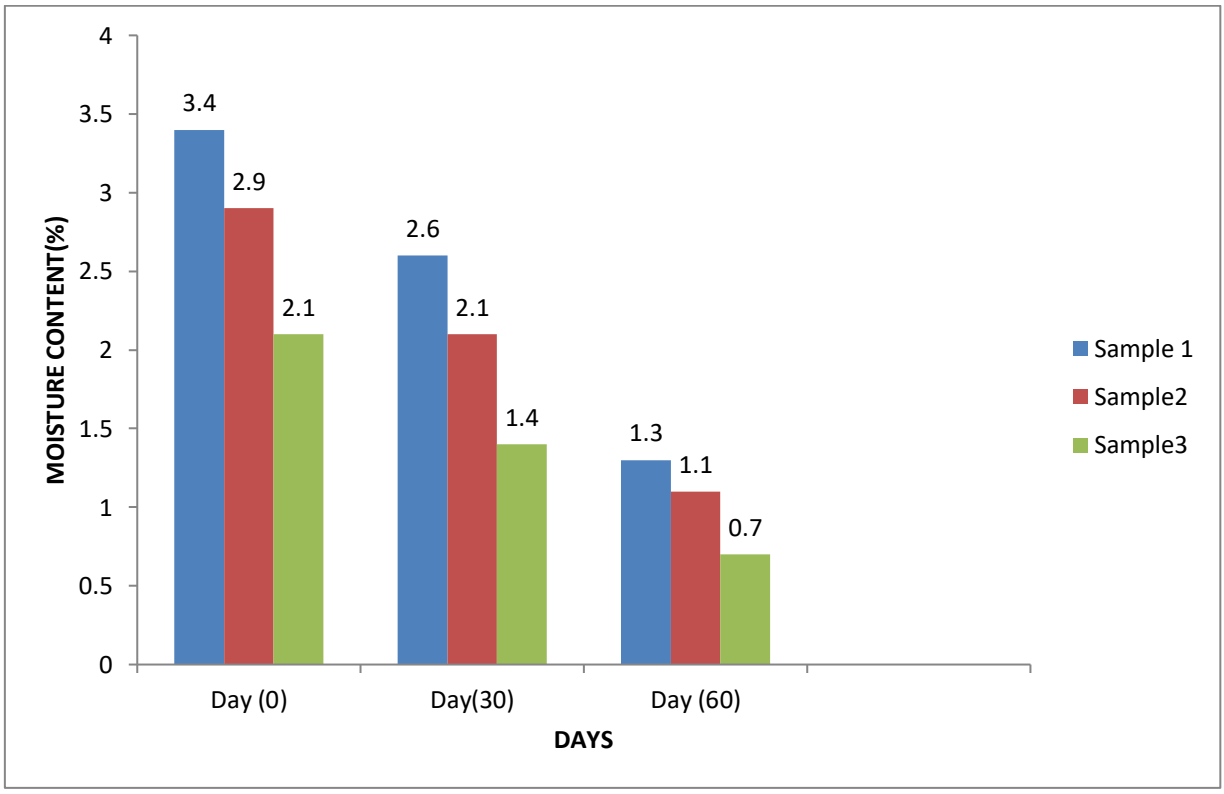

Figure- 4.2 Moisture Content (%) of Petha

4.3. Ash Content

The effect of storage period and *Spirulina* powder on ash content of packed petha is presented in table 4.3

On critical evaluation of results, it was found that the ash content of petha was considerably increased. The storage period considerably reduced the ash content of petha, probably due to increase in moisture content with increase in storage period. The packaging material had no significant effect on ash content.

Table4.3 Ash Content (%)of Petha

Samples	0days	30days	60days
S_1	2.3	2.1	2.06
S_2	2.1	1.97	1.84
S_3	1.84	1.86	1.56

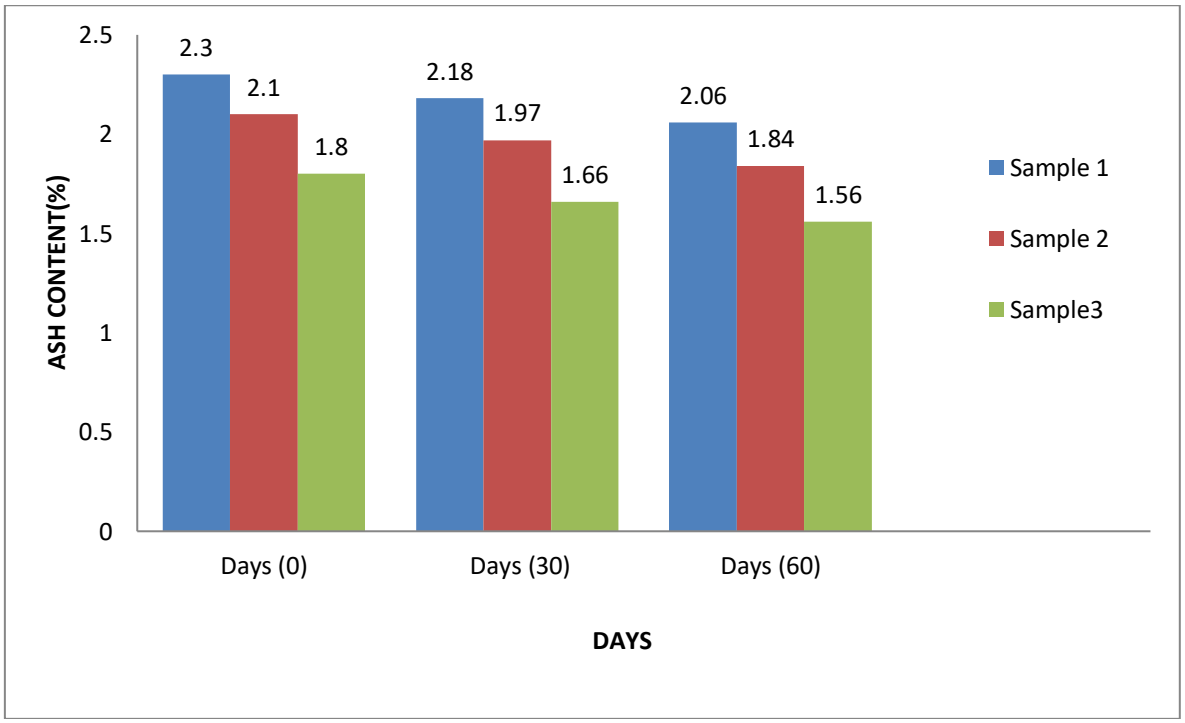

Figure – 4.3 Ash content (%) of petha

4.4 Fat Content

The effect of storage period and *Spirulina* powder on fat content of packed petha is presented in table 4.4

Fat content is very less in *Spirulina* incorporated petha. The overall results clearly revealed that fat content of packed petha decreased considerably with the increase in storage period..

Table4.4 Fat content (%)of Petha

Samples	0days	30days	60days
S_1	5.3	4.4	3.5
S_2	4.5	3.1	1.9
S_3	3.9	2.5	1.6

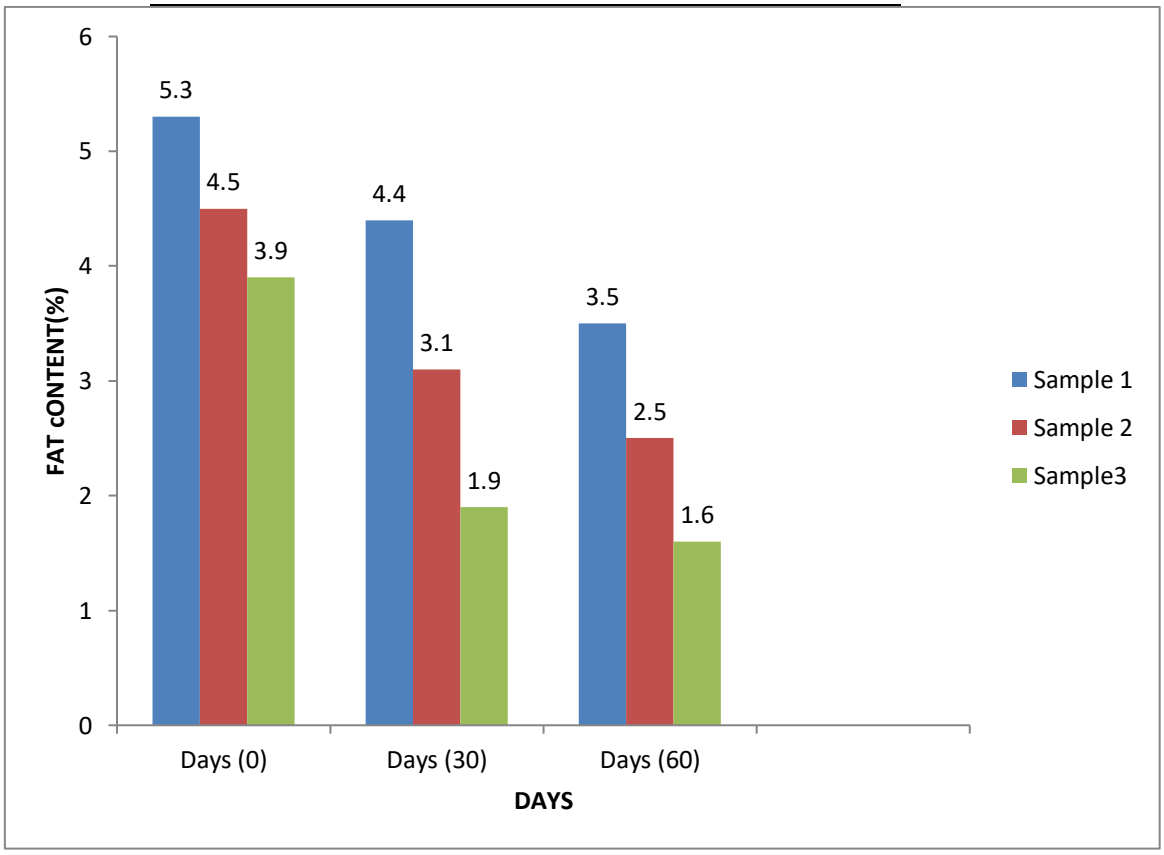

Figure- 4.4 Fat content (%) of Petha

4.5. Protein content

The effect of storage period and *Spirulina* powder incorporated in packed petha on protein content is presented in Fig 4.5. Protein content is very high in *Spirulina* incorporated petha. The overall results revealed that the protein content increased considerably with the increase in *Spirulina* powder in Petha.

Table4.5 Protein content (%) of petha

Samples	0 Days	30Days	60 Days
S_1	3.4	3.4	3.4
S_2	8.6	8.5	8.5
S_3	22.41	22.86	22.88

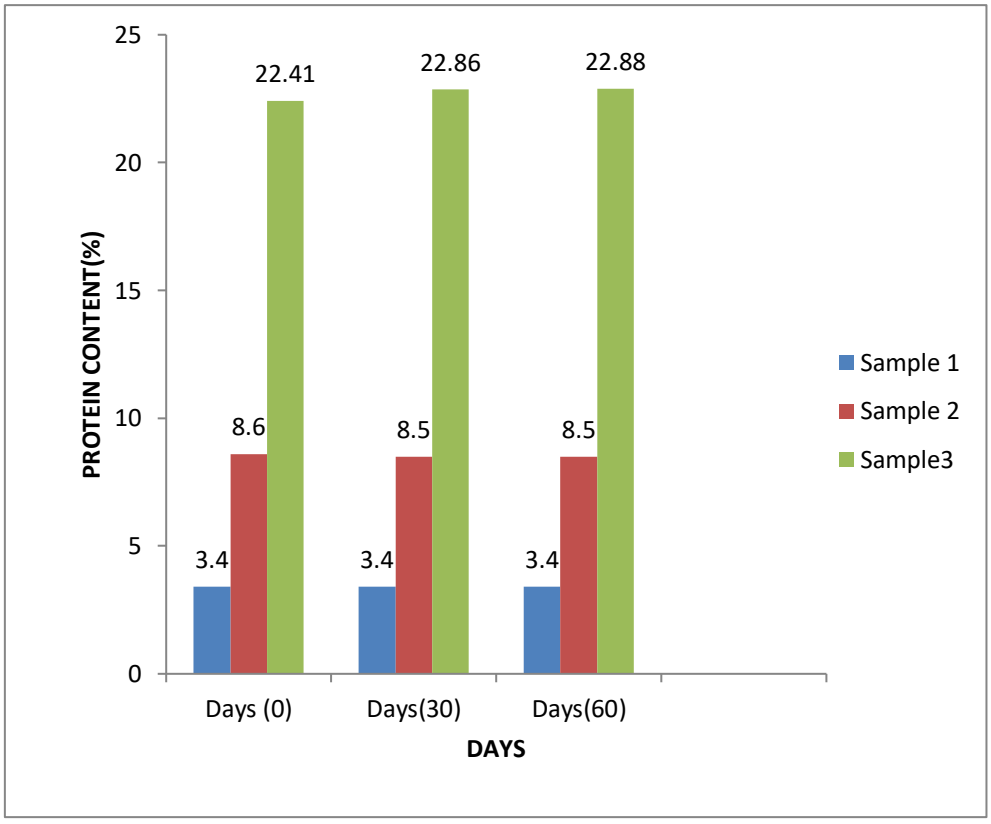

Figure - 4.5 Protein content (%) of Petha

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

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

4.6 Colour

The packaging materials, storage period and *Spirulina* showed an effect on *Spirulina* incorporated petha are presented in Fig 4.6.Thecolor is an important sensory attribute for any new developed product. The color of petha varied due to addition of *Spirulina* powder. The color of petha decreased Slightly with storage period. Green colour was observed due to *Spirulina* powder, which was actually a new development as people were attracted towards it.

Table 4.6 Color of petha

Samples	0days	30days	60days
S_1	9	8.5	8
S_2	8	7.5	7
S_3	9	8.5	8

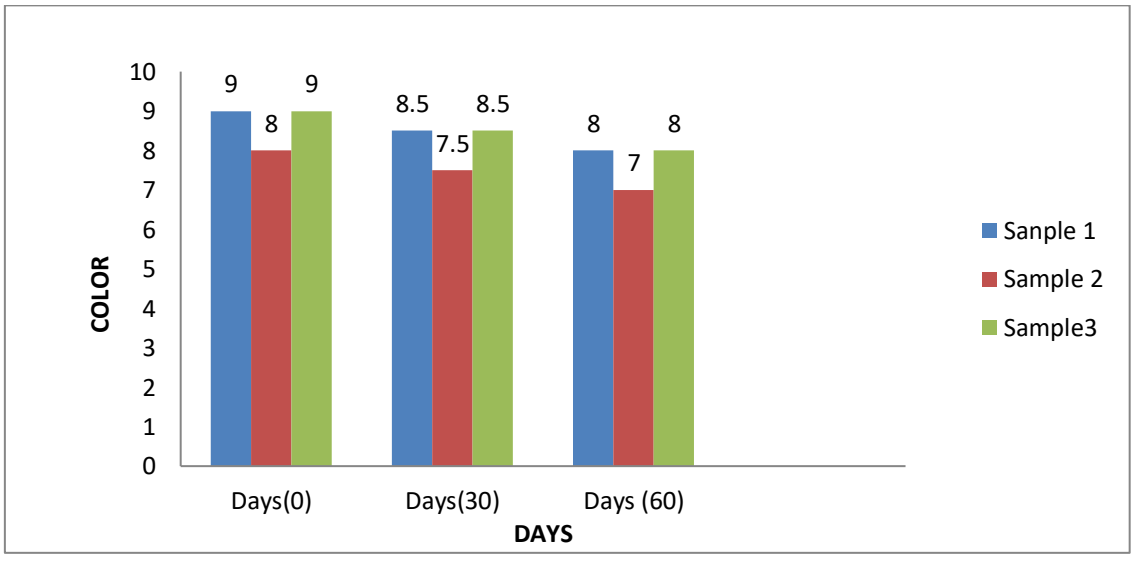

Figure-4.6 Color of petha

4.7 Taste

The packaging material, storage period and *Spirulina* powder affected on taste of *Spirulina* incorporated petha are presented in Fig 4.7. The taste of petha varied due to incorporation of *Spirulina* powder. After some time tastes change and it minor affected the sample, incorporated with *Spirulina* powder. The taste of the petha was slightly reduced with increase in storage period.

Table 4.7 Taste of petha

Sample	0days	30days	60days
S_1	8.5	7.5	7
S_2	8	7	6.5
S_3	9	8	8

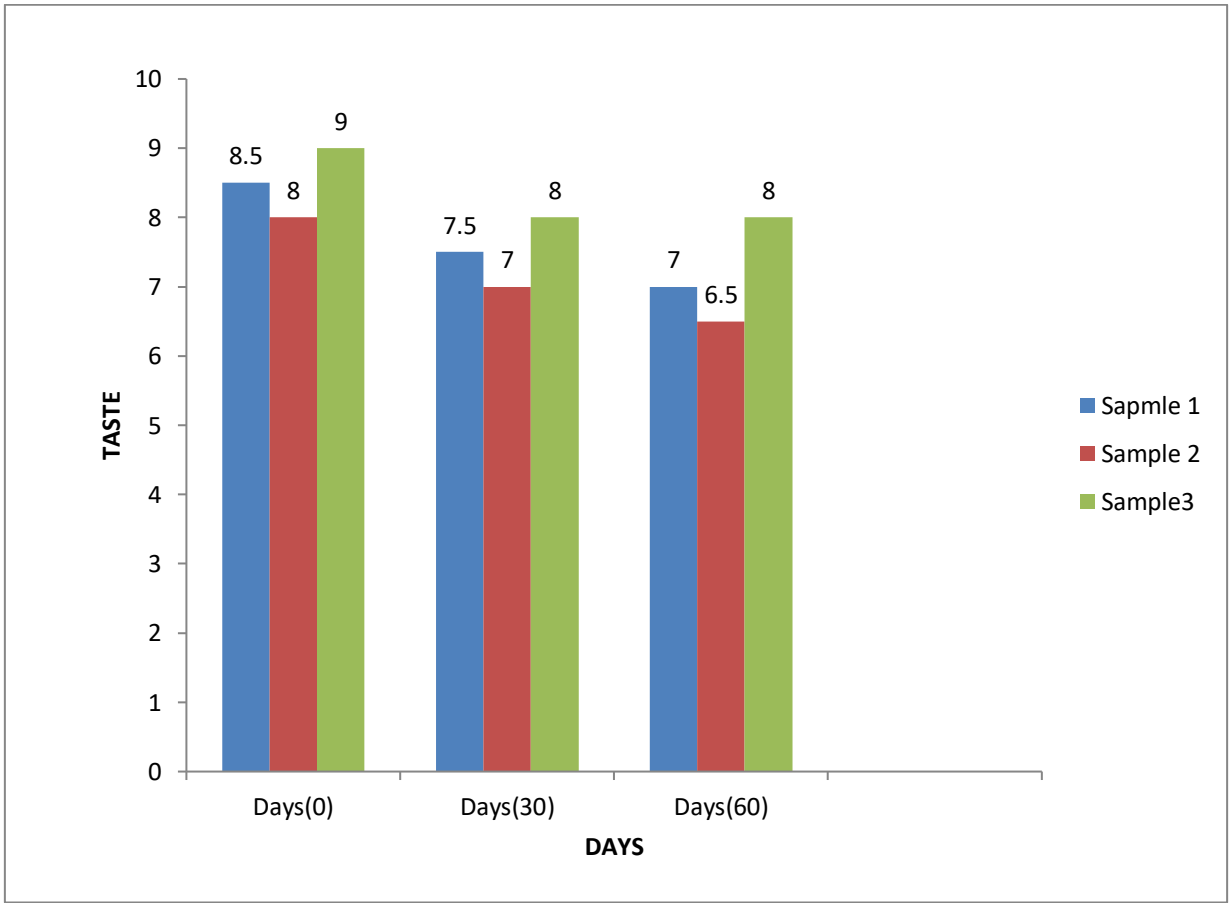

Figure- 4.7 Taste of Petha

4.8. Aroma

The effect of packaging material, storage period and *Spirulina* powder on aroma of Spirulina incorporated petha is presented in Fig 4.8. The aroma of Petha reduced with increase in storage period.

Table 4.8 Aroma of petha

Sample	0days	30days	60days
S_1	7	7	7.5
S_2	8	7.5	6.5
S_3	9	8.5	7.5

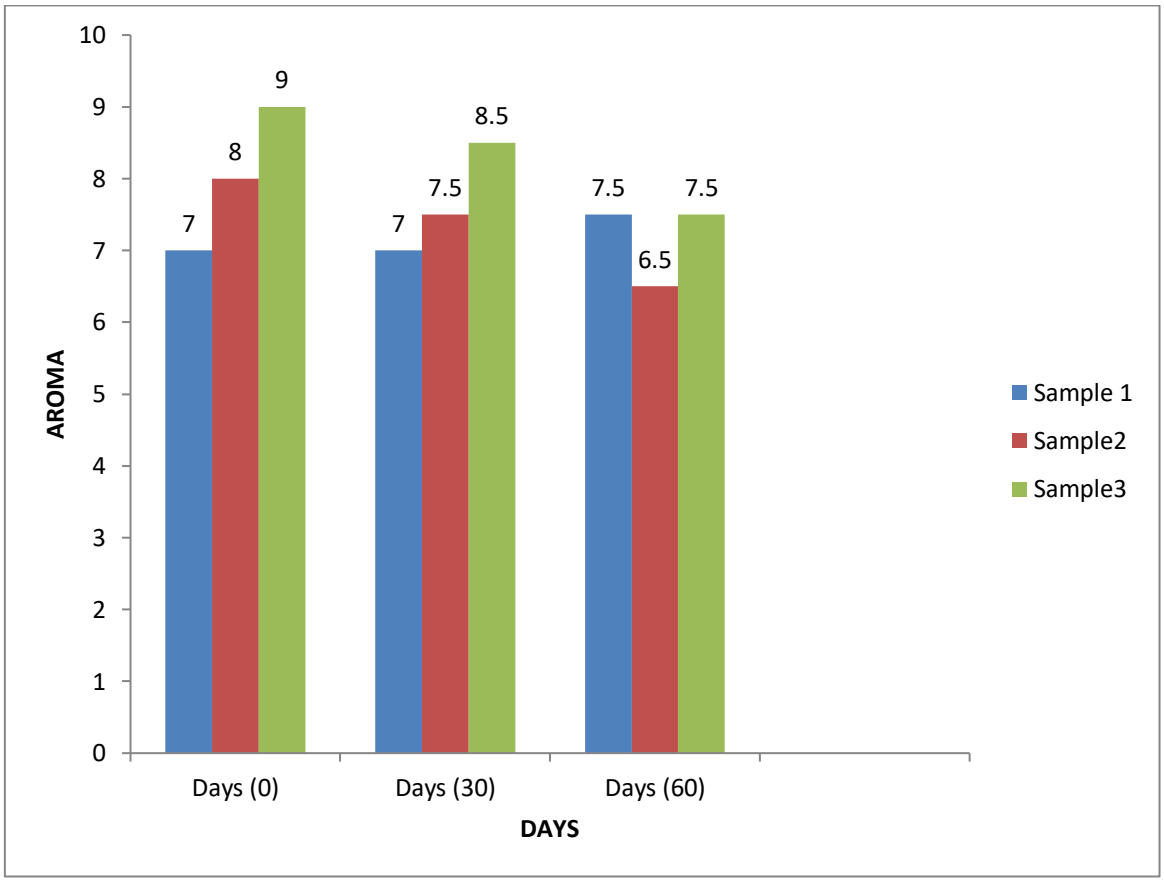

Figure -4.8 Aroma of petha

4.9 Flavor

The effect of packaging material, storage time and *Spirulina* powder on flavor is presented in figure 4.9.The flavor of petha decreased slightly with increases in storage period.

Table 4.9 Flavor of petha

Sample	0days	30days	60days
S_1	8.5	8	8
S_2	8.5	8	7.5
S_3	9	8.5	8.5

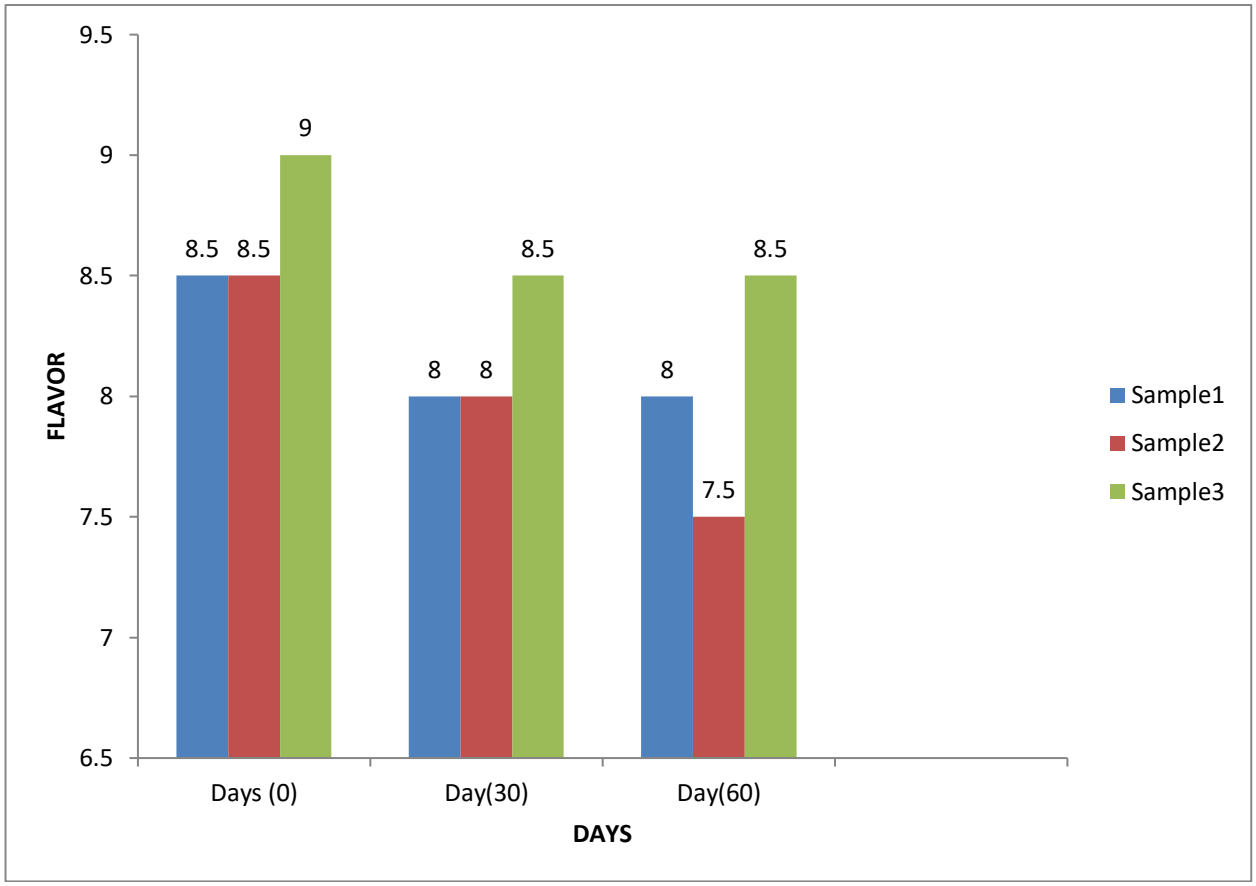

Figure - 4.9 Flavor of petha

4.10 Texture

The effect of packaging materials, storage period and *Spirulina* powder on texture of petha is presented in Fig 4.10.There was a slight decrease in texture due to less moisture present packed petha. After 2 months of storage the score for texture of different samples are given below.

Table 4.10 Texture of petha

Sample	0days	30days	60days
S_1	9	7	5
S_2	8	7.5	5.5
S_3	9	8.5	6.5

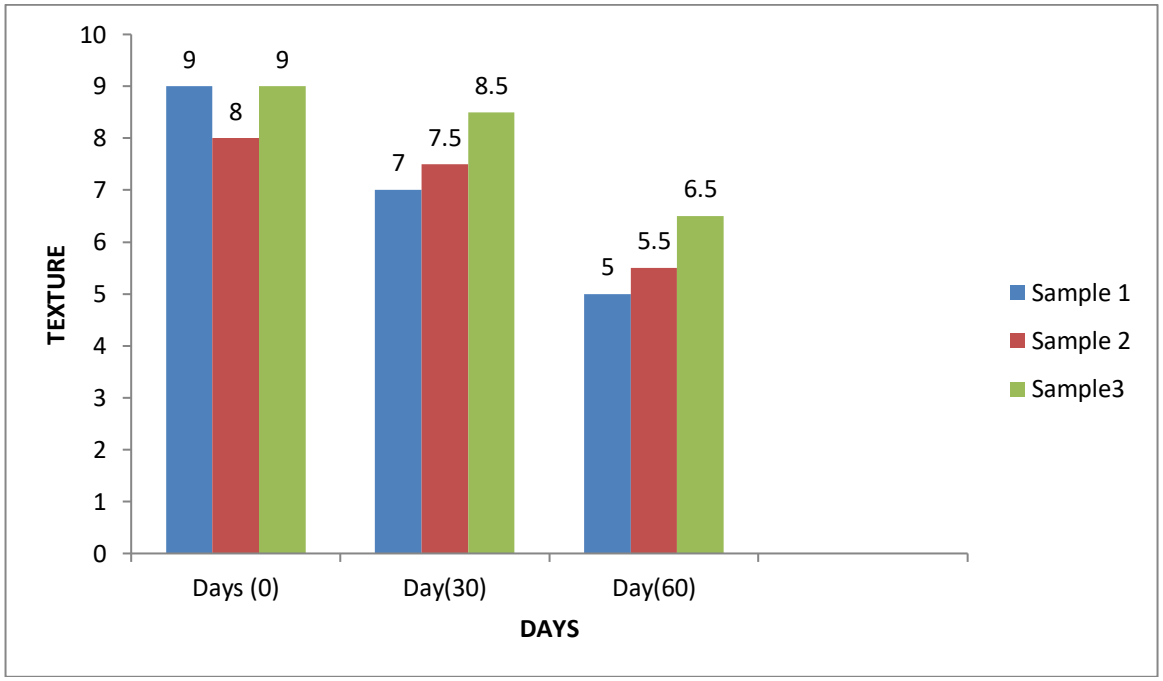

Figure - 4.10 Texture of petha

4.11 Appearance

The effect packaging materials, storage period and *Spirulina* powder on appearance of petha are presented in Fig 4.11. The highest score for appearance was obtained in Sample S3. There was a slight decrease inappearance score for storage of petha

Table4.11 Appearance of petha

Sample	0days	30days	60days
S_1	8.5	8	8
S_2	8.5	8	7.5
S_3	9	8.5	8

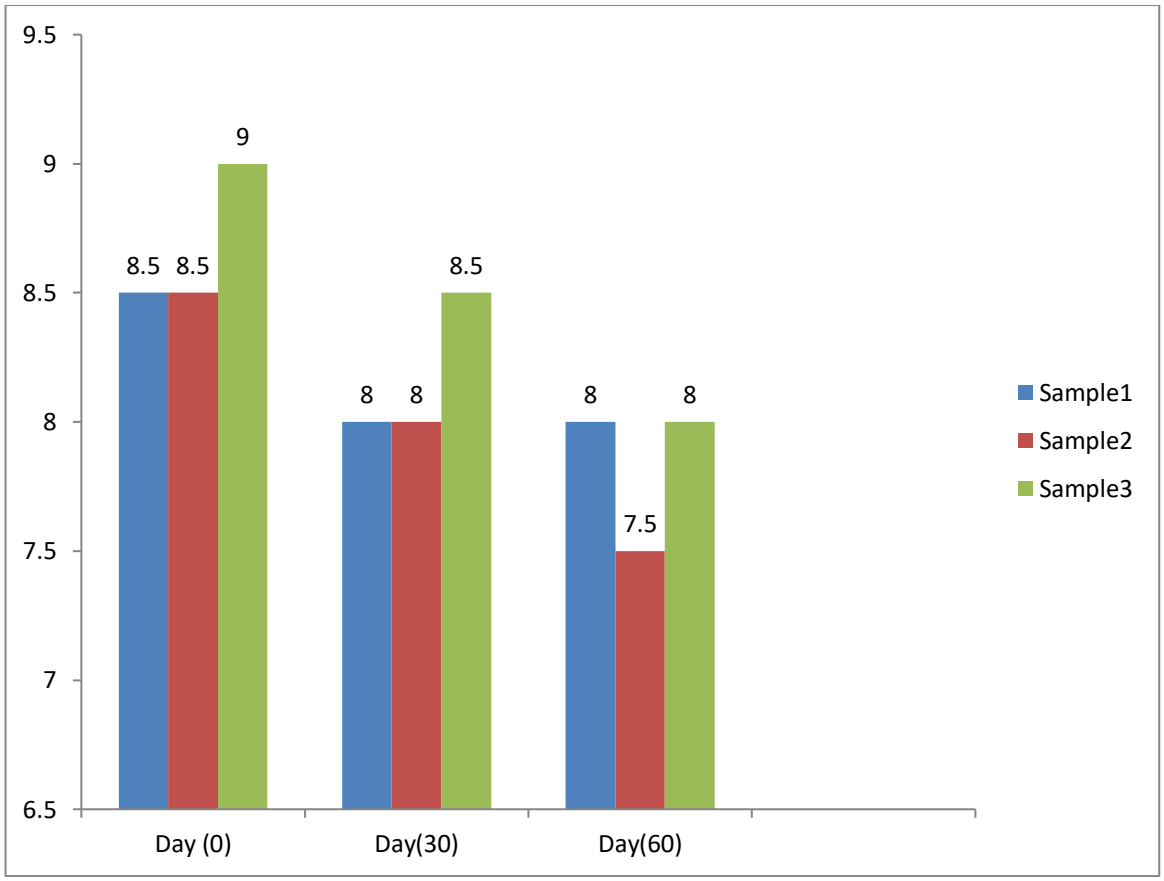

Figure:-4.11 Appearance of petha

4.12 Overall Acceptability

The effect of packaging materials, storage period *Spirulina* powder on appearance of petha is presented in Fig 4.12. The overall acceptability of petha decreased slightly with increase in storage period.

Table4.12 Overall acceptability of petha

Sample	0days	30days	60days
S_1	8.5	7.5	7
S_2	8.5	8	7.5
S_3	9	8	8

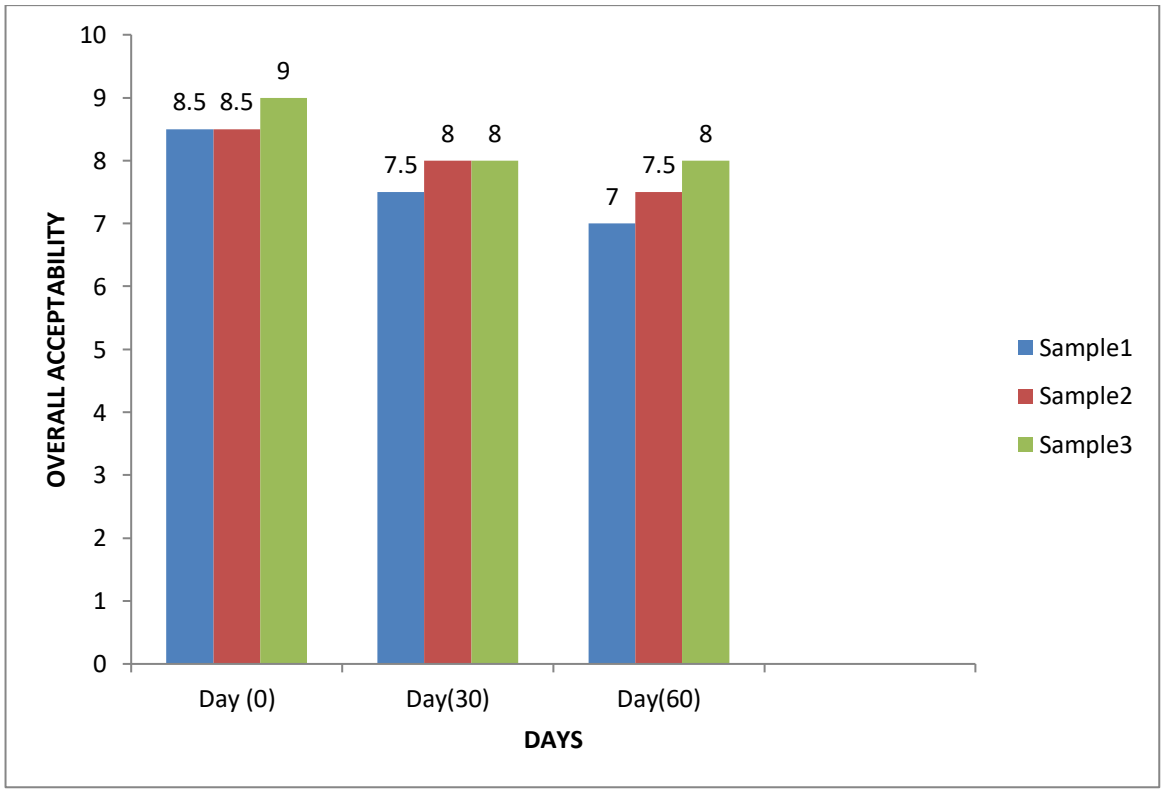

Figure - 4.12 Overall acceptability of petha

4.13. Antimicrobial Activity

The antimicrobial activity of petha was examined based on the diameters of the clear zone of inhibition surrounding the Petri plates. 12 samples were taken out of which 6 were treated with alum and the rest 6 were treated with lime water. Nutrient agar and Potato dextrose agar media were used. In 3 Petri plate nutrient agar media was poured and alum treated samples were kept, again 3 Petri plates were taken and potato dextrose media was poured and alum treated samples were kept. A similar procedure was done for lime water-treated samples. After all the samples were kept in the media the lid of the Petri plate was closed and the plate was kept for a few days to see the zone inhibition.

After few days it was observed that inhibition zone for nutrient agar media in which alum treated petha was kept showed the maximum zone of 1.145cm where as the minimum zone 0.75 cm was observed in potato dextrose agar media in which lime water treated petha was kept as shown in Fig:- 4.13(a) and (b)

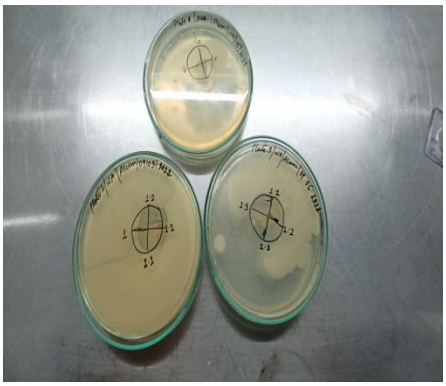

Figure:- 4.13(a) anti microbial activity of alum treated petha

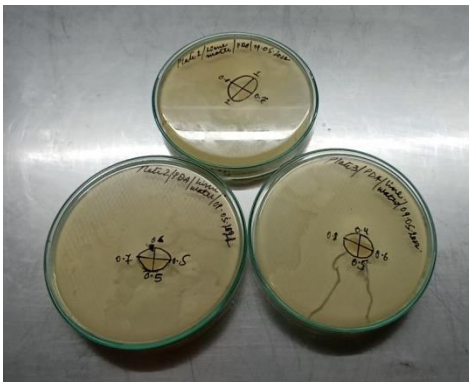

Figure:- 4.13(b) Anti-microbial activity of lime water treated petha

CHAPTER 5

SUMMARYANDCONCLUSION

During this project it was observed that when petha was incorporated with *Spirulina* powder there was no increase in protein content and fat content after 30days. Fat content was decreased with storage. The following conclusions were obtained.

- It was obtained that as we increased the content of *Spirulina* powder there was a huge increase in protein content and decrease in fat content and the developed product was ready to be served as proteinatious food.
- The observation also concluded that the product can be stored till 90 days for better sensory characteristics.
- The developed product is a proteinatious food which can be consumed on daily basis.
- The sensory analysis showed that due to addition of *Spirulina* the color and the taste ratings were minimum. So by adding natural flavors and colors the product can be modified in a certain manner.

Future aspects of the research-

The value added petha can be further modified and tastier by adding natural flavors and colors in a certain proportion. It could serve as the healthiest food for which can be consumed on daily basis and can also be stored for long term. *Spirulina* if grown in hygienic conditions can be used as space food. For the betterment of food, it can be mixed with other ingredients and after further testing it can be used as **Space Food.**

CHAPTER 6

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