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Development and Evolution of Spirulina Enriched Biscuits as an Immunomodulator

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Abstract: *The goal of the current study was to evaluate the nutritional makeup of spirulina powder, the creation of value-added goods based on spirulina, and the nutritional makeup and shelf life of these products. Spirulina, flour combination, Amul butter, sugar, baking powder, and five, ten, and fifteen percent levels of Spirulina powder were used to make value-added biscuits. On a nine-point hedonic ranking scale, the mean score for the overall acceptability of value of cookies with 10% spirulina added was 6.2 compared to the control group. On a dry weight basis, the value-added cookies had 4.29 percent moisture, 12.43 gram of protein, 27.26 gram of fat, and 1.99 gram of ash. F-carotene, vitamin C, iron, and potassium are all present in biscuits that have been fortified with spirulina. After three months of storage, fat acidity showed that the value-added biscuit was of satisfactory grade. Because of its high nutritional value, value-added products based on spirulina may be advantageous for groups that are more susceptible. Due to their therapeutic properties, these might also be beneficial for people with degenerative disorders.*

Keywords: Spirulina, Biscuits, Immunomodulator, Refined wheat flour, Organoleptic property

I. INTRODUCTION

Spirulina:

The Dutch terms "Koekje" or "koekie," from which the word "cookie" is derived, mean "small cakes." With the exception of a higher ratio of sugar and fat to flour and a lower ratio of liquid, cookies share many ingredients with cakes. Cookies are a common baked food around the world, with a high sugar and fat content and a low water content. Cookies with less moisture have a longer shelf life and are more resilient to microbial deterioration. In contrast to bread, biscuits and pastries only need a little amount of gluten development. The resultant dough is easily rolled out and cut, and it is cohesive without being unduly elastic. Consumption of spirulina, a healthy and non-toxic meal made from minute filamentous cyanobacteria now known as Arthrospira, has a long history. Interest in the possible health effects and nutritional advantages of spirulina has recently increased due to its chemical makeup, which includes proteins (which make up 55-70% of any natural food), carbohydrates, essential amino acids, minerals (particularly iron), essential fatty acids, vitamins, and pigments. During their early years, children that experienced chronic malnutrition would have grown up shorter. One of the healthiest nutrients is spirulina. In its normal state, it comprises 65–71 percent complete protein. Compared to almost all other unprocessed foods, this is higher.

Fig No-1 Spirulina Powder

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What is spirulina?

The blue-green algae known as spirulina grows in fresh, alkaline water and has a high nutritional content. A filamentous cyanobacterium multicellular algae, spirulina is referred regarded as "super food." Because these algae contain nutrients and bioactive chemicals, NASA uses them as a food supplement for astronauts on space missions. These active substances include protein, vitamins, pigments, and long-chain polysaturated fatty acids. Consuming adequate amounts of complete proteins is crucial. A nutrient-dense superfood that supports extraordinary health is spirulina. Super-foods are characterised by their enhanced health benefits and disease-preventing qualities in addition to their usual nutritional content. All together, it is the most nutrient-dense natural food source. One kind of blue-green algae that can be taken as a dietary supplement is spirulina. Spirulina is regarded as a superfood because of its high nutritional content and many health advantages. Because of its high protein and vitamin content, spirulina is a great dietary supplement for vegans and vegetarians. Eleven possible health advantages of spirulina consumption are covered in this article

Because of its distinct biochemical makeup and high levels of proteins, essential amino acids, vitamins (particularly B12), mineral salts, and colours (carotenoids, phycocyanins, and xanthophylls), spirulina is used in food technology procedures. Chlorophylls and polyunsaturated fatty acids, particularly Omega-3 fatty acids, are additional physiologically active compounds. The micronutrients that are most noticeable include copper, zinc, manganese, and iron. Beta-carotene is 10 times more abundant in carrots than in any other nutrient. The world's food scarcity has led to a notable surge in interest in algal biomass production over the last 60 years. The rate at which microorganisms reproduce and the degree to which their growth circumstances may be controlled are two elements to take into account when assessing the role of spirulina biomass in the human diet.

Products that support excellent health are in high demand because health is a major priority in our everyday lives. Cookies have a longer shelf life than other food items, are portable, and are always ready to consume. Sugar, butter, and refined wheat flour are typical cookie ingredients. Due of its rheological qualities, wheat is typically used. An edible algae called Spirulina platensis is used as a raw material to make wholesome goods that are beneficial to health. It has adequate levels of single-cell proteins (60–70%) as well as all other necessary elements, including pro-vitamin A, magnesium, zinc, ions, and the important fatty acid gamma-linolenic acid. The plant is characterized by the presence of several secondary metabolites, such as flavonoids, terpenoids, and chemical groups called polyphenols. Among its many therapeutic benefits include the inhibition of digestive enzymes such as lipases, glucosidase, and amylase, as well as anti-inflammatory and anti-arthritic properties. The prevention and treatment of cardiovascular diseases, type 2 diabetes, and their associated co-morbidities, such the metabolic syndrome, may benefit from such advantages.

Since therapeutic diets can be used to prevent and control many diseases, there is a great demand for nutritional and health-promoting goods in our daily lives. Cookies are among the foods that people of all ages can easily carry, keep, and eat. These are also thought to be a better way to enrich macro and micronutrients. The cookies made for this study are high in carbohydrates and proteins, which are both nutritious.

Because of their wide range of flavours, textures, digestibility, affordability, and long shelf life, cookies are among the most widely consumed bakery goods by practically every segment of society. The three primary ingredients used to make cookies are sugar, shortening oil, and wheat flour. However, additional possible ingredients include flavoring, aeration, milk, salt, and other culinary additives. Cookies are a wonderful source of nutrients and give energy because they are high in fat, protein, and carbohydrates. The most common cereal and one of the most significant staple food crops in the world is wheat (Triticumaestivum L). The carbohydrate content of wheat is high. Along with vitamins and minerals including salt, potassium, calcium, magnesium, iron, phosphorus, copper, zinc, and manganese, it also contains protein, fat, ash, and fiber. Wheat flour is typically made by grinding whole wheat kernels and stands apart from other cereals because of its special ability to form dough. The primary component used to make cookies is wheat flour. Cakes, biscuits, puffins, pie crusts, and other baked goods are typically made with low-protein flour.

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What makes it special?

- Rich in Anti-oxidant
- High in Fiber
- 0% Maida
- No refined Sugar
- No added preservatives, Flavours and Colours

Advantages of Spirulina-

1. Rich inAntioxidants: Spirulina contains antioxidants like phycocyanin, which help protect cells from damage and reduce inflammation.
2. Boosts Immune System: Spirulina stimulates the production of antibodies and activates immune cells, such as macrophages and natural killer cells.
3. Antiviral and Antibacterial Properties: Spirulina has been shown to exhibit antiviral and antibacterial properties, helping to prevent infections.
4. Anti-Inflammatory Effects: Spirulina's anti-inflammatory properties can help reduce inflammation and alleviate symptoms associated with various diseases.

DRUG PROFILE

Parameter	Information
Plant Name	Spirulina powder
Synonyms	Blue green algae
Image	
Origin	For generations, people have utilised cyanobacteria, or blue-green algae, as sustenance. It is thought to have started in lakes with high alkalinity levels in Africa and the Americas.
Biological Source	It consists of dried biomass of *Cyanobacterium Arthrospira*. Arthrospira are filamentous microscopic blue green algae or algal form in high alkaline lakes with high ph.The most use are *ArthrospiraMaxima* and *ArthrospiraPlaetensis.*
Family	Phormidiaceae or Oscillatoriaceae
Chemical Constituents	Phycocyanin, chlorophyll, carotenoids, polysaccharides, vitamins, proteins, omega-6 fatty acids, anti-oxidant
Uses	Anti-inflamatory,Anti-oxidant, Anti-diabetic, Neuroprotection, Anti-viral property, Immunomodulator,Detoxification, Anti-agening.
Kingdom	Monera
Phylum	Cyanobacteria
Genus	Spirulina (Arthrospira)
Class	Cyanophyceae

Table no-1 Drug Profile

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II. MATERIAL AND METHODS

Selection of Plant Material-

In the present study, I have selected the spirulina.

Collection of plant Material-

Amazon Shopping provided the spirulina powder utilised in this study, whereas the local market in Manchar, Pune, provided the wheat flour, sugar, baking soda, butter, and milk powder.

Formulation of Spirulina Biscuits-

Ingredients	Quantity	Role
Wheat flour	250gm	Base
Spirulina	According to ratio(90/10)	Immunomodulator
Baking Soda	1tsp	Leavening agent
Butter	30gm	Binding agent
Sugar	100gm	Sweetning agent
Cardamom	15gm	Flavouring agent

Table no 2: Formulation table

Fig No-2 Formulation

Preparation of samples

Sample preparation

Four samples are taken for experimental in this study, for fortification of cookies.

Sr.No	Sample Ingredients	Ratio Ingredients
1	Flour without spirulina	100%
2	Flour with spirulina powder	95%+5%
3	Flour with spirulina powder	90%+10%
4	Flour with spirulina powder	85%+15%

Table no- 4 Sample preparation

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Preparation of control sample-

Take out 250 gram of wheat flour

Add 25g of butter and well mixed

100 gram of powdered sugar.

Add one teaspoon of baking powder to the soft dough after it is ready.

Knead it once more after adding 10g of cardamom powder and pineapple essence.

Shape the dough into a round. Bake the cookies till golden brown, 180 degrees Fahrenheit

Fig no-3 Preparation of Control sample

Preparation of Test Sample-

A mixture of flour, sagar, shortening oil, and baking powder was used to make cookies (as a control). The cookies were baked for 15 minutes at 180 °C. When making cookies, the following ingredients were used:

- Flour mixture
- Amul Butter
- Sugar
- Baking Powder
- Spirulina according to ratio

The ratio of sugar, baking powder, and spirulina Four treatments were used to produce the cookies: T-0%, T-5%, T-10%, and T-15%. Spirulina was added to the total weight of the dough in place of flour. Spirulina powder, which is high in bioactive chemicals, should not be consumed in excess of 2.5 grammes daily. After adding the sugar, the dough was agitated for five more minutes. After combining the remaining dry ingredients, the dough was kneaded until it had a consistent consistency.

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Fig no-4 Prepared cookies samples

Parameter	Batch 1	Batch 2 Batch 2	Batch 3
Ratio	95%- Wheat flour 5%- spirulina	90%- Wheat flour 10%- spirulina	85%- Wheat flour 15%-Spirulina
Colour	Dark Brown	Greenish brown	Muted green
Odour	Sweet yeast	Musty sea water	Fishy odour
Hardness	14 kg	12 kg	11.0 kg
Texture	Flaky	Flaky	Flaky

Table no- 5 Batch Preparation

Procedure-

Take 250gm wheat flour add spirulina powder according to ratio

↓

Add 100gm grinded sugar +25gm butter into

↓

Prepare the soft dough and add 1 tsp of baking powder

↓

Add 10gm elachi and pineapple essence into it and kneaded again Cut the dough in round shape

↓

Bake the cookies at 180 F until turns golden brown

Fig No-5 Final Product

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Evaluation Test for Biscuits:

Determination of ash value- Add around 3 g of the precisely weighed material or the amount recommended in the monograph. in an appropriate coated dish that has been previously fired, cooled, and weighed (for instance, silica or platinum). Heat the material progressively up to 450 °C to incinerate it until it is carbon-free, then let it cool and weigh it. If this method is unable to produce carbon-free ash, use hot water to expel the charred material, gather the residue on ash-free filter paper, burn the residue and filter paper together, add the filtrate, evaporate until dry, and ignite at a temperature no higher than 450 °C. Determine how many milligrammes of ash there are in one gram of air-dried material. It is discovered that the biscuit's overall ash value is 1.99%.

Sr.No	Physical Constant	Results
1	Total ash	1.99%
2	Acid insoluble ash	0.3%
3	Water soluble ash	1.2%

Table no-6 Ash value

%Ash Content= Weight of Ash / Weight of Sample

Moisture Content- Five gram of crushed biscuits were dried in a lab convection dryer set to 130 °C for one hour. The materials were weighed both before and after drying. Based on the difference, the moisture content of the cookies was calculated and shown as a percentage. Three separate runs of the test were conducted. The control cookie's moisture level was 4.30 percent, and it gradually dropped once spirulina powder was added. 4.27 is the final biscuit's total moisture content.

$$\%LOD = \frac{\text{Mass of water in sample}}{\text{Total mass of wet sample}} 100$$

3. Fat Content- The control biscuits had a fat level of 27.23 percent. It was discovered that the spirulina-containing biscuits' fat content was similar to that of the control sample. T_{2} had the least amount of fat, while T_{3} had the most. The fat contents of T_{1}, T_{2}, and T_{3} are 28.39, 27.26, and 29.51 percent, respectively.

Fig no-6 Fat content

4. Protein Analysis- (a) Biuret test: 1 mL of extract was mixed with two drops of 3% copper sulphate and a few drops of 10% sodium hydroxide; the production of a violet or red colour indicates the presence of proteins.

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Fig No-7 Protein Analysis

5. Microbial Testing-

In order to guarantee food safety and quality, microbial testing of biscuits entails determining the quantity and kinds of microorganisms present in the product. Determining shelf life and avoiding spoiling depend on this. High mineral concentration can occasionally be used to stop some microorganisms from growing.

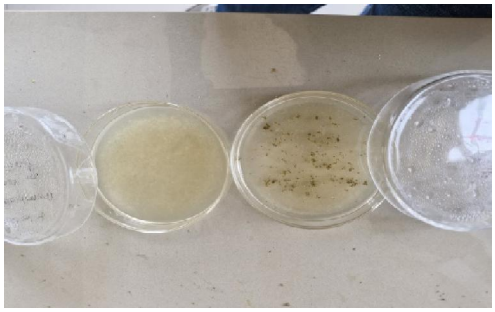

Fig no-8 Microbial Testing

Phytoconstituents Analysis-

Sr.No	Phytochemical Test	Procedure	Observation	Inference
1	Molischs Test-	Filtrate +2drop of alcoholic sol of alpha napthol +shake +1ml H2SO4+H2O	Violet Ring at the junction	Carbohydrates is present
2	Fehlings Test-	1ml filtrate+boil+1ml fehlings reagent nd B	Red precipitate	Carbohydrates is present
3	Barfoeds Test-	1ml filtrate+1ml barfoedsreagent+heat for 2 min	Red precipitate	Carbohydrates is present
4	Mayers Test	Filtrate+2 drop of Mayers reagent along with test tube	White or Creamy precipitate	Alkaloids is present
5	Hagers Test	Filtrate+2 drop of Hagers reagent along with test tube	Prominent Yellow colour	Alkaloids is present
6	Wagners Test	Filtrate+2 drop of Wagners reagent along with test tube	Reddish brown ppt	Alkaloids is present
7	Dragendroffs Test	Filtrate +2 drop of dragendroffs reagent is added	Reddish brown precipitate	Alkaloids is present

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8	Borntragers Test	2ml filtrate+3ml ethylacetate added+ shaken then add 10% ammonia solution	Pink colour	Glycosides is present
9	Legals test	20mg extract dissolve in pyridine+sodiumnitroprusside solution added and make 10% sodium hydroxide	Pink colour	Glycosides is present
10	Foam Test	Small quantity of extract diluted with water and shake graduated for 15 min and then measure the foam	Foam is present	Saponin is present
11	Ferric Chloride test	50mg extract dissolved in water+few drop of 5%ferric chloride solution added	Blue, Green, Violet colour form	Tannin is present
12	Lead Acetate Test	Extract dissolve in water+3ml of 10%lead acetate added	Bulky white Precipitate	Tannin is present
13	NH4OH Test	3ml extract + NH4OH solution	Yellow fluorescence	Flavonoids is present
14	Ninhydrin Test	1ml sample+5 drop reagent heatfor 2 min	Purple colour form	Amino acid is present
15	Millions Test	1ml Sample add 1 ml reagent heated for 3 min then add 1%sodium nitrate is added	Red colur form	Amino acid is present

Table no-7 Phytochemical Test

Sensory Evalution of Biscuits by untrained panel-

Fig No-9 Sensory evalution by untrained panel

Sensory evaluation was done by untrained Panel. There was six untrained panel. Sensory evaluation of composite flour biscuits was carried out on the basis of Parameters consider for sensory evaluation was Appearance, Color, Flavor, Taste and Texture.

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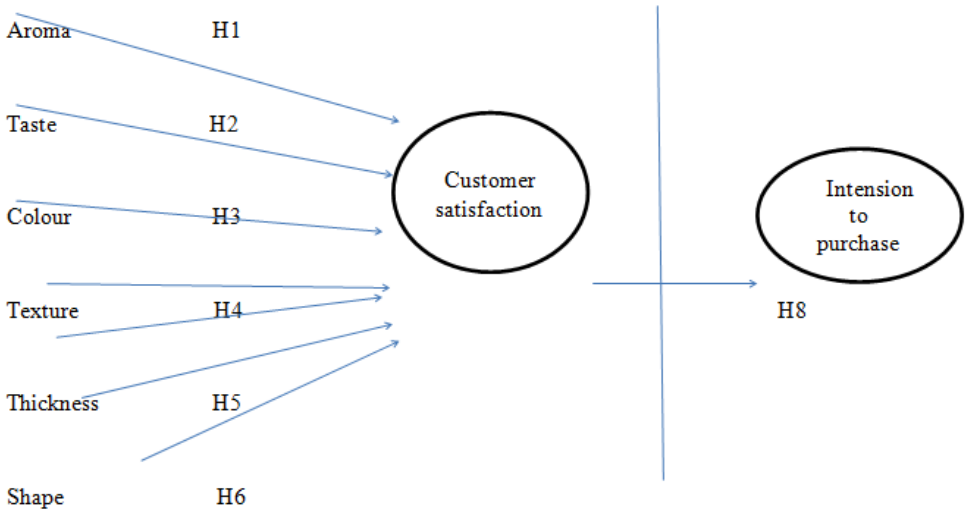
The Research Model-



Stimulus **Organism** **Response**

Expectation Confirmatory Theory- Therefore, consumers are more receptive to food products that promote healthiness. The new cookies enriched with spirulina contain ingredients that boost human health. This will satisfy consumer needs.

H7: The perceived healthiness of the cookies has a positive effect on customer satisfaction. Previous studies also found the effect of customer satisfaction on the intention to buy in various contexts such as a hotel shopping apps and juice among others. Therefore, the last hypothesis proposed in this study is as follows.

H8: Customer satisfaction has a positive effect on purchase intention. "Based on the hypotheses above, the research model is presented in Figure.

Research method-

This study employed a survey method, which is used to collect data by asking the respondents structured questions related to behaviour, intention, attitudes, knowledge, motivation, and other characterist. The survey was carried out by sending questionnaires directly to the respondents after they were given samples of the cookies. Considering that the product in this study, which is cookies enriched with spirulina, was not yet available on the market, samples of the cookies was produced. Then, the respondents were given the prototypical product to taste before they answered the questionnaires. This type of survey was considered the most appropriate for this study. Respondents had opportunities to touch and taste the new cookies. Then, they answered the questionnaire. The measured items or questionnaires in this study for the attributes of aroma, taste, colour, texture, thickness, and shape

III. RESULT

Preliminary Phytochemical Test-:

Sr.No	Test Name	Observation
1	Molisch Test	+
2	Fehling test	+
3	Barfoed Test	-
4	Mayer test	+
5	Hager test	+

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6	Wagner test	+
7	Dragendroffs test	-
8	Borntrager test	+
9	Legal test	+
10	Foam test	+
11	Ferric chloride test	+
12	Lead acetate test	-
13	NH4OH Test	+
14	Ninhydrin Test	+
15	Millions Test	+

Preliminary phytochemical test
(+):Presence of test
(-):Absence of test

Physical Evalution

Sr.No	Parameter	Observation
1	Colour	Greenish Brown
2	Odour	Musty sea water
3	Texture	Flaky
4	State	Solid

Chemical Evalution-
Ash value(%w/w)

Sr.No	Physical Constant	Results
1	Total ash	1.99%
2	Acid insoluble ash	0.3%
3	Water soluble ash	1.2%

Moisture Content-The total moisture content of biscuits found tobe 4.27%

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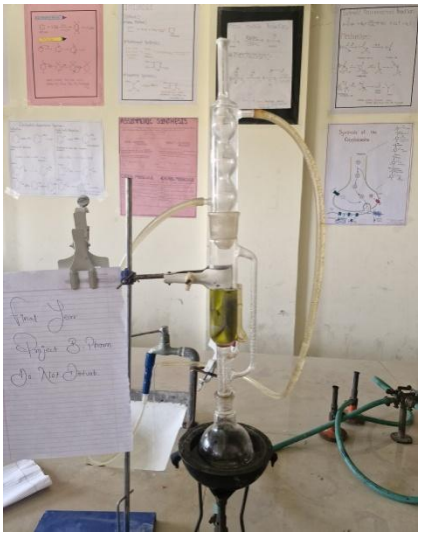
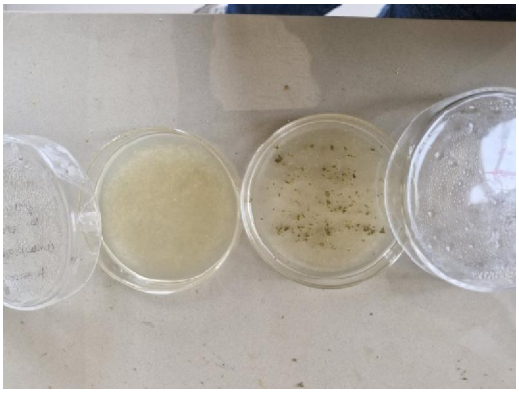
Fat Content-Fat content found to be 27.26



Microbial Testing



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Protein test-

IV. CONCLUSION

In this study, spirulina-enriched biscuits with 10% spirulina were judged satisfactory based on proximate analysis and organoleptic characteristics. According to the findings, the created cookies were noticeably healthier than the control samples. The community benefits greatly from the higher quality of spirulina-enhanced cookies because this useful product has a high extrusion potential and increased acceptability on organoleptic metrics. The goal of the study, which was to create nutritious cookies with therapeutic bio-ingredients, was accomplished. In addition to increases in minerals, the proportion of protein in the cookies increased when the percentage of S. platensis increased compared to cookies made solely with refined wheat flour. In terms of protein and mineral percentages, cookies containing 10% S. platensis stood out, and they were comparable to the entire cookies in terms of the minerals, lipids, and highest energetic value

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all those who supported me throughout the journey of developing and evaluating spirulina-enriched biscuits as an immunomodulator.

First and foremost, I would like to thank my guide, Prof.Shelkemaam, for their continuous support, valuable guidance, and encouragement throughout the research project. Their expertise and constructive feedback were instrumental in shaping the direction and outcome of this study.

I extend my heartfelt thanks to the Samarth Institute of pharmacy, belhe, junnar for providing the necessary infrastructure and resources to carry out this work.

My appreciation also goes to the laboratory staff and technical assistants for their help with sample analysis, experimental procedures, and maintaining quality standards throughout the development process.

I am deeply grateful to my family and friends for their unwavering support, motivation, and understanding during this project.

Lastly, I acknowledge the contribution of Spirulina suppliers and sensory evaluation panel members whose inputs were vital to the completion of this study.

This project has been a valuable learning experience, and I am truly thankful to everyone.

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