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Protein Extraction from Spirulina Platensis

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Abstract*: The extraction of protein from green spirulina microalgae platensis was carried out by centrifugation, ultra sound assisted extraction and combination of centrifugation and ultrasound assisted extraction spirulina platensis was carried out using ultrasound-assisted extraction and statistical optimization method is used to obtain the optimum conditions. A Box–Behnken design method was used to optimize the process conditions affecting protein extraction. This conditions investigated on microalgae cell disruption method by the combination of both ultrasonication and centrifuge. Different buffer solutions were used and the time of the ultrasonication was also varied. The protein extraction quantity was evaluated. The obtained results confirm that mixed buffer III showed the high concentration of protein but low quality. The study on the effect of ultrasonication period, the concentration of protein increased and remains constant also when duration of sonication is elongated and this result was observed during continuous ultrasonication in similar manner. The protein concentration is one of the important aspect, the protein quality must be satisfied. The results confirms the optimum condition of protein extraction from green microalgae requires the combination of the use of buffer solution and a proper duration of ultrasonication to maximize the protein quantity.

Index Terms: Protein, Extraction, Ultrasonication, Centrifuge, Spirulina.

I. INTRODUCTION

A. *Spirulina:*

Spirulina microalgae is one of the microalgae which produce more amount of oxygen in the planet's atmosphere. Spirulina is a human and animal food or nutritional supplement made primarily from two species of cyanobacteria: Arthrospira platensis and Arthrospira maxima. The shape of Spirulina is of spiral shaped algae which is one of the most nutrient-rich foods on Earth. Spirulina contain " among 50 and 75% protein, eight essential and more than ten non-essential amino acids, also greater levels of gamma-linolenic acid (GLA), beta-carotene, linoleic acid, arachidonic acid, vitamin B12, iron, calcium, phosphorus , nucleic acids RNA & DNA, chlorophyll, and phycocyanin, in blue green algae a pigment-protein complex is found." (Morton, M.2016)

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B. *Benefits of Spirulina*

Spirulina has many of health benefits when almost immediately upon ingestion. It provides good power for the Person, and while helping to enhance continuance and lessen weakness. It have many od advantage to improve the immune system, and provides support for the heart and kidneys. Spirulina is also a characteristic detoxifier, oxygenating the blood, and purging the group of poisons and different polluting influences that might cause diseases or other wellbeing complexities. (Morton,M.2016)

Recently, there has been a revival of interest in Solid state fermentation (SSF) techniques due to the fact that SSF has many advantages over submerged fermentation (SmF), such as less energy requirements, small reactors volumes, and the product is obtained in concentrated form. The greatest advantages of SSF is about requirement of less quantity of water, which reduces the energy consumption in the recovery of products, and production of low volumes of effluents (Parimi et al., 2015).

Health Benefits of Spirulina Protein (Leech, 2017):

a) Spirulina Is Extremely High in Many Nutrients: A single tablespoon (7 grams) of dried spirulina powder contains:

- Protein: 4 grams.
- Vitamin B1 (Thiamin): 11% of the RDA.
- Vitamin B2 (Riboflavin): 15% of the RDA.
- Vitamin B3 (Niacin): 4% of the RDA.
- Copper: 21% of the RDA.
- Iron: 11% of the RDA.
- It also contains decent amounts of magnesium, potassium and manganese, and small amounts of almost every other nutrient.

b) Spirulina Has Powerful Antioxidant and Anti-inflammatory Properties

c) Spirulina Protects LDL Cholesterol from Becoming Oxidized

d) Spirulina Appears to Have Anti-Cancer Properties, Especially Against Oral Cancer

e) Studies Show That It May Reduce Blood Pressure

f) Spirulina Improves Symptoms of Allergic Rhinitis

g) Spirulina May Be Effective Against Anemia

h) Muscle Strength and Endurance May Improve

i) Spirulina May Help With Blood Sugar Control

C. Protein

Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. Proteins perform a vast array of functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another. Proteins are the basic foundation of the human body (Becker, 2007).

Proteins are complex molecules that are made up of smaller units called amino acids and help build muscle, blood, and internal organs. The enzymes are made up of proteins and the latter are also the protein units involved in the synthesis of enzymes (Salma Said, 2018). Protein has structural or mechanical roles. It forms the stents and joints of the cellular structure. It also carries out other vital functions of being an important member of the immune response and the transport and storage of biological molecules (Stephen Bleakley, 2017). A source of amino acids for organisms that cannot produce these amino acids themselves (Sarode, Pawar and Walke, 2013)..

C. *Manufacture of Proteins:*

The Spirulina (Arthrospira) comprises a group of filamentous multicellular cyanobacteria (blue-green microalgae). Spirulina almost contains average 60% (51–71%) protein and about 15-25% carbohydrates of its dry weight. Spirulina is a human and animal food or nutritional supplement made primarily from two species of cyanobacteria: Arthrospira platensis and Arthrospira maxima. Spirulina – cyanobacteria also used as a food from decades by different species and only rediscovered in recent years. Apart from nutritional values, Spirulina has a balanced protein composition, presence of rare essential lipids, vitamin, B1, B2, B3, B6, B9, vitamin C, vitamin D, vitamin A and vitamin E B12 and even numerous minerals. Through the project, protein tablets were produced from algae found in water (LUBITZ, 1963). In this process the proteins of the algae have been separated with the help of some chemicals and Ultrasonic device.

D. *Uses and Benefits of Proteins:*

Proteins are found in many of the foods we eat on a daily basis, where they are distributed in plant sources and animal sources. The benefits of proteins protect the body from diseases, builds cells and muscles of the body properly and quickly, gives the body energy, vitality and activity, strengthens and nourishes hair, and protects it from falling, damage and dehydration, burns a lot of fat and calories in the body, It builds the teeth, protects them from decay, and finally contributes to building body hormones.

II. PROCESS DESCRIPTION

The main aim of this project is to produce a protein by solid state cultivation of Spirulina using date seed powder as a support. Sultanate of Oman have variety of algae's cultivated in different wadis. As Oman has a great value for production of Spirulina algae in most of the areas of Wadi's.

I. Selection of Spirulina Seeds for cultivation.
II. Preparation of microalgae suspension,
III. Agitation,
IV. Chemical Extraction of Protein by optimum operating parameter selection.
V. Washing with clean water for removing impurities.
VI. De watering.
VII. Drying.
VIII. Grinding/Crushing the dried spirulina.
IX. Tablet making

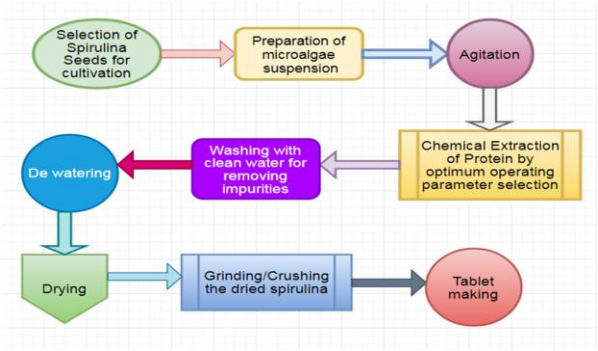


Fig.1. Spirulina Production Flow Chart

I. Selection of Spirulina Seeds for cultivation

A suitable seeds of spirulina is selected for cultivation, Microalgae Coelastrum sp. microalgae was used in this study. It was cultured in the lab and was harvested by gravity sedimentation method.

(a) (b) (c)

Fig. 2. (a): suspension spirulina; (b) slurry spirulina algae (c) Concentrated spirulina.

II. Preparation of microalgae suspension

The freeze microalgae suspensions was prepared at the concentrate (slurry) microalgae (a) and water (ml) ratio of 1:4 and kept at 6°C. (Figure1) Similarly, the fresh microalgae suspensions (Figure1a) was prepared at the concentrate (slurry) microalgae, after that water is removed by using vacuum pump, and finally the microalgae is concentrated (Figure1b). the microalgae and other solvents were mixed in different proportions (ml) ratio of 1:4 and kept at 6 °C. Different solvents are used in this research such as: Water; Mixed buffer I (pH 7.5, 50 mM Tris, 10 mM EDTA (Ethylenediaminetetraacetic Acid) and 50 mM NaCl); Mixed buffer II (50 mM NaCl, 1% Triton X (Triton TM X-100) and 20mM Tris pH 7), Mixed buffer III (50 mM NaCl, 1% Triton X, 20 mM Tris pH 7.5 and 0.1% SDS (Sodium Dodecyl Sulfate)), SDS lysis buffer (pH7, 50 mM Tris, 5 mM EDTA and 50 mM NaCl added SDS buffer. In this experiment, an evaluation on the effect of the concentration of SDS and Triton x lysis buffers and duration of cell disruption by ultrasonication and centrifugation was performed. Comparison of the concentration of protein and the quality of protein after extracted from the different solvents during ultrasonication and centrifugation was also studied.

III. AGITATION

The microalgae sample is agitated for a period of 30 minutes for maintaining uniformity of the solution.

IV. CHEMICAL EXTRACTION OF PROTEIN BY OPTIMUM PARAMETER SELECTION.

i. Centrifugation

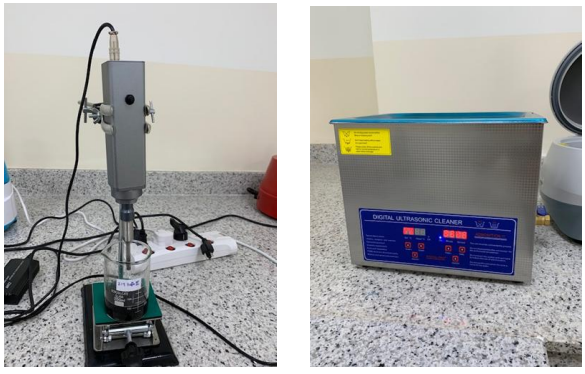
Microyn Digital Bench-top Centrifuge, (100-5000rpm (Max. 3074xg), 6x15ml) is used for centrifugation of the microalgae. The samples are tested for different speed such as 1000 rpm, 2000 rpm, 3000 rpm, 4000 rpm, 5000 rpm the time of centrifuge is also varied for different samples. The time selected is in the interval of 1 minutes starting from 1 minute to maximum 5 minutes.

(a) (b)

Fig. 3. (a) Centrifuge (b) Sample testing in centrifuge

ii. Ultrasonication

Cells disruption method was done by ultrasonication device (MXBAOHENG Lab Equipment Handheld Portable Ultrasonic Homogenizer mixer processor Sonicator 80W 150uL-80mL 220V-240V). The ultrasonic processors was operated at 80 Watt 30 kHz.



(a) (b)

Fig. 4. (a) Ultrasonic Probe (b) Ultrasonic bath

The ultrasonic horn is made of titanium alloy material, which has the characteristics of high strength, high sound Speed, good corrosion resistance and high heat resistance, thus prolonging the service life of the instrument. The product circuit is made by patch technology, featuring automatic frequency tracking, automatic amplitude control, stable load, high electro-acoustic conversion efficiency and over-temperature protection. The sonicator was operated for 20 s and stop for 5 s and worked at 60 % amplitude. In the experiments, the generated ultrasonic heat was absorbed by ice.

iii. Ultrasonication and centrifugation

The samples are tested for combination of ultrasonication for different period of interval ranging from 1 minute to 5 minutes with an interval of 1 minute, followed by centrifugation for different rpm ranging from 1000 rpm to 5000 rpm at an interval of 1000.

iv. Quality and quantity of the product

A liquid phase was measured concentration of protein by Bradford assay and quality of protein was determined by Sodium Dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In this work, the test was triplicated and reported in mean value ± SD.

(a) (b) (c)

Fig. 5. (a), (b), (c). Solvents at different conditions.

V. RESULTS AND DISCUSSION

The main target of this study was to find out the amount of protein presence in the spirulina micro algae. According to this it can be easily said that our results is positive .And this research is successful. Although our main objective was to set up a definite process for ethanol production from micro algae spirulina by using its carbohydrate content. The Spirulina (Arthrospira) comprises a group of filamentous multicellular cyanobacteria (blue-green microalgae). Spirulina almost contains average 60% (51–71%) protein and about 15-25% carbohydrates of its dry weight. Spirulina is a human and animal food or nutritional supplement made primarily from two species of cyanobacteria: Arthrospira platensis and Arthrospira maxima. Spirulina – cyanobacteria also used as a food from decades by different species and only rediscovered in recent years. Apart from nutritional values, Spirulina has a balanced protein composition, presence of rare essential lipids, vitamin, B1, B2, B3, B6, B9, vitamin C, vitamin D, vitamin A and vitamin E B12 and even numerous minerals.

i. Protein Content Analysis

The concentration of protein is shown in figure 4.1 and figure 4.2. The experiments are carried out keeping constant rpm for 5 samples and varying the time from 1 minute to 5 minutes with an interval of 1 minutes. The results shows that the protein concentration is high at 5000 rpm as compared to 1000 rpm at time 3 minutes.

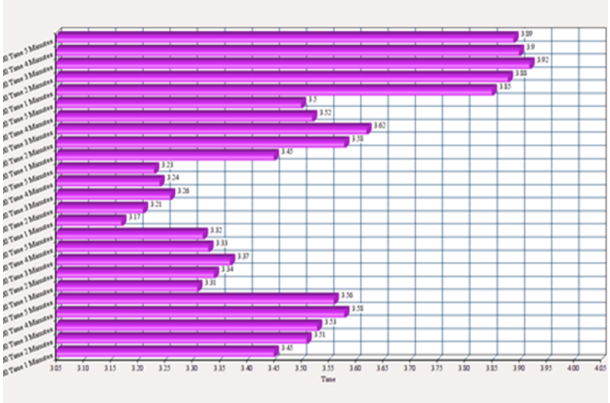
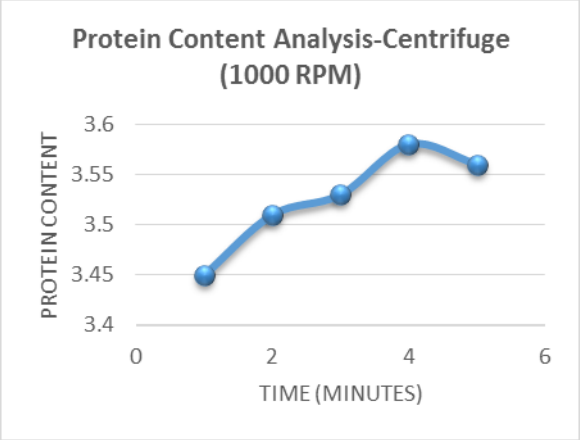
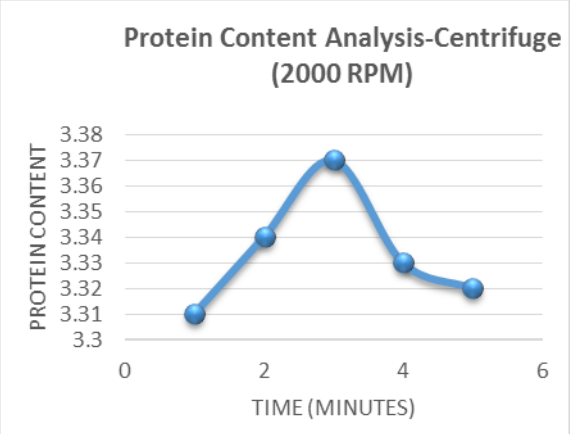


Fig. 6. Protein Content Analysis using Centrifuge



(a)



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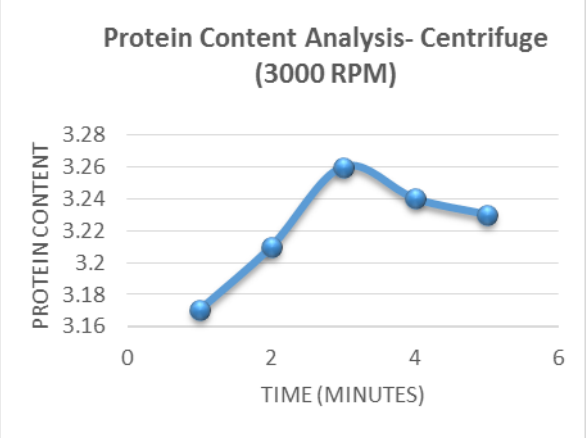
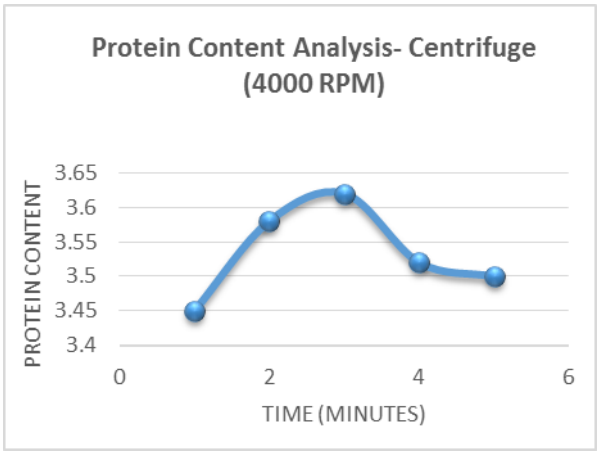
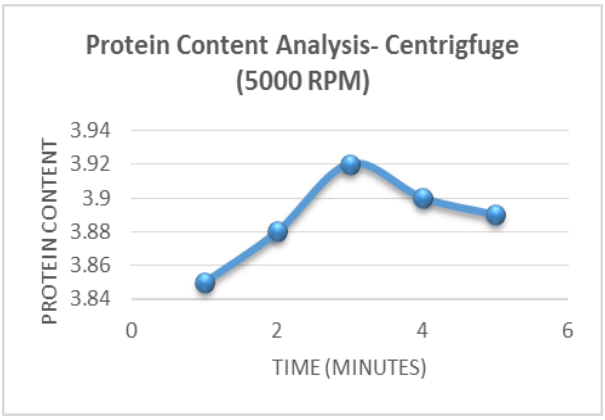


(c)



(d)



(e)

Fig. 7: Protein Content Analysis using Centrifuge (a) Centrifuge Speed 1000 RPM (b) Centrifuge Speed 2000 RPM (c) Centrifuge Speed 3000 RPM (d) Centrifuge Speed 4000 RPM (d) Centrifuge Speed 5000 RPM

The protein content analysis is shown for samples of spirulina microalgae at different range of time starting from 1 minute to 5 minutes of ultrasonication. The result shows that at 4 minutes of ultrasonication the protein extraction was maximum followed by 3 minutes.

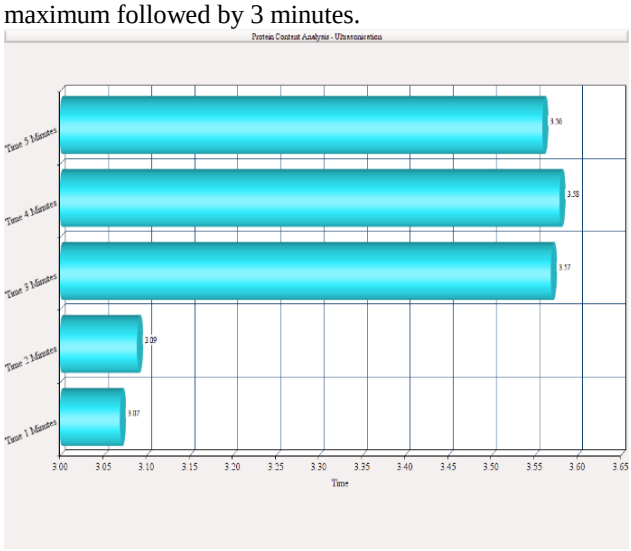


Fig. 8: Protein Content Analysis using Ultrasonication

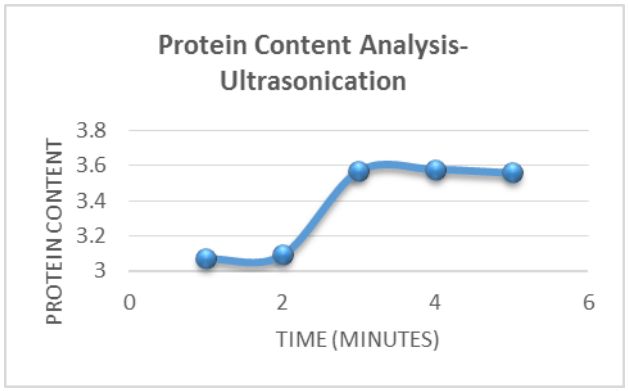


Fig. 9: Protein Content Analysis using Ultrasonication at different time interval.

The protein content analysis is shown for samples of spirulina microalgae at different range of time starting from 1 minute to 5 minutes of ultrasonication followed by centrifuge for different speed ranging from 1000 RPM to 5000 RPM. The result shows that at 4 minutes of ultrasonication the protein extraction was maximum followed by 3 minutes.

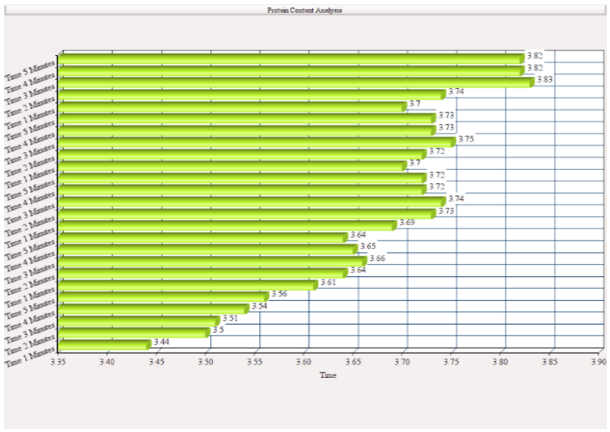
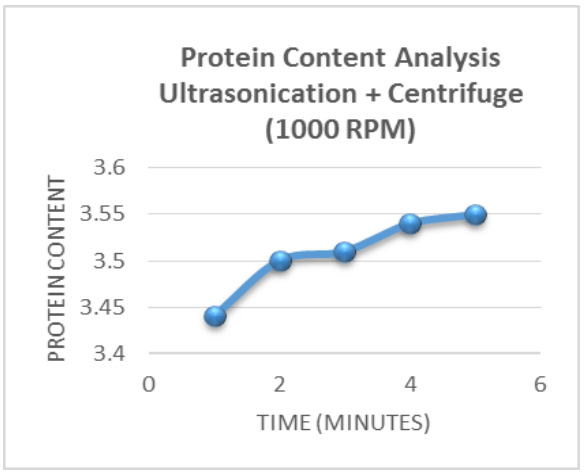
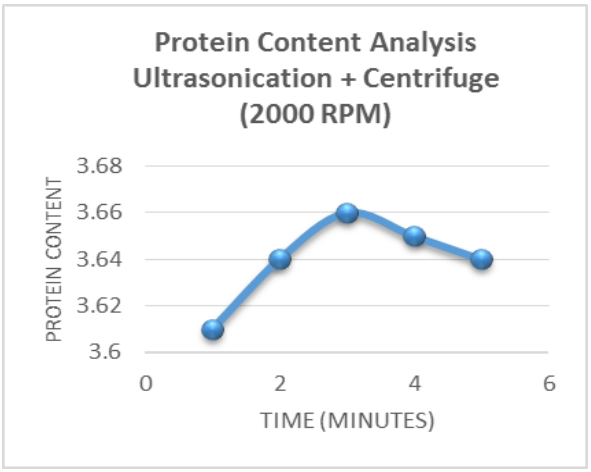


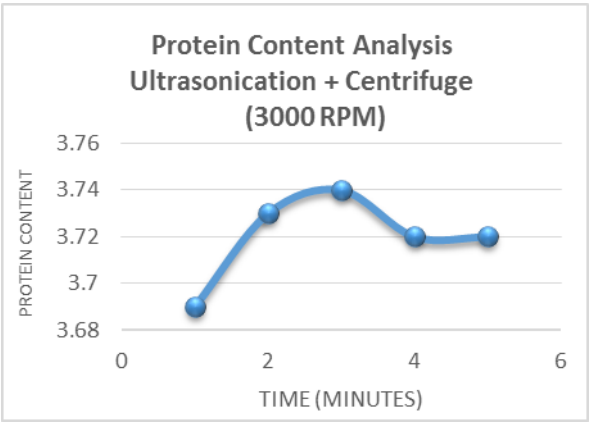
Fig. 10: Protein Content Analysis using Ultrasonication followed by centrifugation



(a)



(b)



(c)

Fig. 11: Protein Content Analysis using Centrifuge (a) Centrifuge Speed 1000 RPM (b) Centrifuge Speed 2000 RPM (c) Centrifuge Speed 3000 RPM

ii. Optimization Studies

A statistical analysis tool used for obtaining optimization parameters. RSM is used for study of extraction parameters such as Centrifugation time, ultrasonication time and pH. In this work, independent
variables (temperature 25–35°C, pH 7–9, and time 1–5 min) were used in the experimental design. Extraction parameters were normalized as coded variables. Variables were coded according to the Equation (1):

$$A = (a_i - a_0)/\Delta a \quad (1)$$

where: ai – corresponding actual value; a0 – actual value in the center of the domain;
Δa increment of ai corresponding to a variation of one unit in A
The response functions (Z) were extraction yield (%). The response variables were fitted to a second-order polynomial model to obtain the regression coefficients (β). The generalized second-order polynomial model used in the response surface analysis is as follows

$$Z = \beta 0 + \beta 1\ X1 + \beta 2\ X2 + \beta 3\ X3 + \beta 11X12 + \beta 22X22 + \beta 33X32 + \beta 12X1\ X2 + \beta 13X1\ X3 + \beta 23X2\ X3 + \phi \quad (2)$$

where: β0 – constant term; βi – linear effects; βii – quadratic effects; βij – interaction effects;
ϕ – random error term.

A mathematical model was established to describe the influence of a single process parameter and/or the interaction of multiple parameters on each investigated response (Walke and Sathe, 2012). To visualize the relationships between the responses and the independent variables, surface response and contour plots of the fitted polynomial regression equations, optimal conditions for the targeted responses were generated using the trial version of Design software.

VI. CONCLUSION

The major objectives of this research work is to cultivate Spirulina in high alkaline water liquid mineral medium by using microorganism Platensis and covert it in to dried spirulina powder, to extract the protein from dried spirulina powder using chemical extraction process, to study the optimum operating parameter for maximization of protein extraction and to reduce the environmental impact. In this study we have cultivated Spirulina in the laboratory condition and then it is converted to dry powder. The extraction of protein was carried out by using ultrasonication, centrifugation and combination of both ultrasonication and centrifugation. Different samples were tested using buffer-I, buffer-II and buffer-III solution. The time for ultrasonication, centrifugation and RPM are varied and good number of data is collected after completion of experiments. The samples are tested in a reputed certified laboratory for estimation of protein content in the samples. The test method used AOAC 920.87 for finding out the amount of protein content. The statistical analysis is carried out after results obtained the three parameters were studied for estimation of optimization parameters. From the results it is observed that Spirulina platensis protein concentrate is a cheap and novel source of protein with high protein digestibility, could be used as an additive to improve the antioxidant property and increase the protein content of food products.

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