NUTRITIONAL ENRICHMENT OF READY-TO-COOK FOOD- INSTANT NOODLE EXPLORING THE POTENTIAL OF MICROALGAE *ARTHROSPIRA PLATENSIS* GOMONT

Dissertation submitted in partial fulfillment of the requirements for the award of degree of

MASTER OF SCIENCE IN BOTANY

by

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DECLARATION

I, hereby declare that work which being presented in the dissertation, entitled **"Nutritional Enrichment of Ready-To-Cook Food-Instant Noodle Exploring the Potential of Microalgae *Arthrospira platensis* Gomont**" in fulfillment of requirements for the award of the degree of Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an authentic record of my own work carried out during M.Sc. period under the supervision of Dr. Aghil Soorya A.

The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this or any other University/ Institute.

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ABSTRACT

Arthrospira platensis is a microalga that can be consumed by people for its high protein content and nutraceutical compounds. The current study focuses on to identify the important benefits and protein content of *A. platensis* in order to include in the food supplements. Study was done based on three samples of noodle in which two are incorporated with the organism and the third served as control which were subjected to physical, chemical and sensory analysis. The noodle, with higher quantity of *A. platensis* percentage had high amount of phenolic and flavonoid content. This study confirms retention of nutrients post cooking revealing no significant loss in proteins and other nutrients. Proximate analysis revealed that the crude protein and ash content was high in samples with organism. In addition, *A. platensis* also affected the scores obtained from a panel for the individual parameters (texture, color, flavor, taste, and overall acceptability) in the sensory evaluation. The overall results indicated that *A. platensis* enriched noodles can be considered for the consumers.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Effective food consumption and nutrition control are essential for optimum health. Smart diet and food choices can aid in disease prevention. Eating the correct foods can help your body manage with an ongoing sickness more effectively. Understanding appropriate nutrition and paying attention to what you eat can aid in the maintenance or improvement of your health. Food and nutrition are the way that we get fuel, providing energy for our bodies. We need to fulfil nutrients to our bodies with a new supply every day. The food is one of which determines whether we have healthy body or not. It can be anything as long as it gives us with the nutrition we require, such as lipids, carbohydrate, vitamins etc. and allow us to live a healthy life and prevent disease. Without eating, our bodies lack nutrients, which can lead to many health problems. Nutrition is one of those words that can be difficult to define. It is the process by which the body digests food and utilises it to execute numerous functions. We can maintain our health, build and mend damaged tissue through diet.

Plants and animals are the two primary sources of food. We receive all of our food from one or both of these two sources. The processed foods we consume on a daily basis contain a wide range of substances sourced from either plants or animals. Plants include a wide range of nutrients that the human body requires to function properly. Humans obtain everything from fruits (apple), stem (lettuce, celery), roots (carrots and beet), seeds (wheat, rice) and so on. Animal products are used as a protein source by humans, either directly or indirectly. Animal products are high in nutrients.

Feeding a growing global population, which is expected to reach 9.8 billion by 2050, while conserving natural resources for future generations may appear contradictory at first (World population datasheet, 2020) European scientists have recently developed a test for microalgae, commonly known as phytoplankton, a type of algae composed of unicellular photosynthetic bacteria. These microscopic organisms can be utilised in animal feed, especially aquaculture, as well as a variety of foods such as pasta, vegan sausages, energy bars, bakery items etc. The majority of commercial microalgae farming focuses on the production of dried biomass, such as *Chlorella* or *A. platensis* powder, as a food with significant health advantages. Some microalgae strains not only accumulate

up to 65-70% protein but also are long-term suppliers of omega-3 fatty acids (Adarme-Vega, 2012)

Algae is available in tablet, pill, powder, and oil forms. The nutritional benefits of algal food products include being an energy source, being utilised as a food, having a high protein content (Mark *et al*. 2016). Oils from microalgae being rich in polyunsaturated fatty acids, and being a significant source of vitamins, minerals, and antioxidants. The benefits of fresh water algae include antibacterial, antiviral, and antifungal activity, a source of biologically active chemicals utilised in medications, and improved heart health. Disadvantages include hazardous metal consumption, allergies, cyanotoxins, and microbial contamination so, microalgae have so much importance in our daily life (Yoshihisa Y *et al*., 2012)

A significant group of thallophyta is called algae. These are the earliest and most basic division of the Kingdom of plants. Phycology is the systematic, orderly study of algae. The algae are primitive plants that contain chlorophyll. They may be prokaryotic or eukaryotic, and they come in a variety of cell types, from single cells to multicellular ones. Algae are ubiquitous that is, they can be found in fresh and salt water, on soil and rock, hot springs, deserts, on permanent snow-fields, etc. or as epiphytes or parasites on plants or animals. However, they are mostly found in aquatic habitats. Their cells have characteristics not found in either plants or animals and their photosynthetic pigments are more diversified and those of plants. Algal thallus exhibits a spectrum of organisation, from unicellular to highly organised multicellular habits, with the plant body lacks differentiation into root-like, stem-like, and leaf-like structure. Algae have the capacity for photosynthesis, in which they gather energy from sunlight, allows them to create organic food molecules from carbon dioxide and water. In addition to their ecological functions as oxygen providers and the primary source of nutrition for practically all aquatic life, algae play a significant economic role as a source of crude oil, food and a variety medical and industrial product for human (Emad, 2011).

Cyanobacteria is a class included in the Division chlorophytae and Kingdom Plantae which have been discovered in a variety of biological niches, including on soils, rocks, and in habitats with severe physicochemical features. Natural alkaline waters with a high mineral content and temperatures between 35 and 40°C are ideal for cyanobacteria

growth. Marine and freshwater algae and their products are in growing demand because of their nutritional and functional properties. *Chlorella, Arthrospira, Nannochloropsis* are the major microalgae suitable for human consumption. One in nine persons worldwide is malnourished thus its objective attempts to solve hunger by commercialising marine microalgae as a staple human meal. These microscopic organisms are the only source of omega-3 fatty acids and amino acids that people require in their diets (Lidiane Andrade *et.al,* 2018)

The three main subspecies of the genus *Arthrospira* are *Arthrospira platensis, Arthrospira fusiformis,* and *Arthrospira maxima* among which *A. platensis* has a wider diameter and typically longer trichomes than the other genus subspecies allows it to thrive in many climates and provide a number of health advantages (Karkos *et.al*., 2011). *Arthrospira platensis,* is a gram-negative, non-toxic species of cyanobacteria with several applications in both the natural and industrial fields. It is an oxygenic photosynthetic bacteria found worldwide in fresh and marine environments. *Arthrospira platensis* is a long, coiled cylindrical shaped algae with granulated cross walls. It develops trichomes, which are chains of vegetative cells encased in a slimy layer that allow it to reproduce through binary fission. Due to the production of the pigment phycocyanin, these microorganisms have been categorised as blue-green algae.

Systematic classification of *Arthrospira platensis* Gomont

Kingdom	:	Plantae
Division	:	Chlorophytae
Class	:	Cynophyceae
Order	:	Oscillatorales
Family	:	Oscillatorlaceae
Genus	:	*Arthrospira*
Species	:	*Platensis*
Author citation	:	Gomont

Light-harvesting chlorophyll protein complexes in *Arthrospira platensis* operate as peripheral antennae systems, allowing for more efficient light absorption. Solar energy is converted into chemical energy in the form of NADPH and ADP, which the cell can use

to carry out metabolic functions. Several producers are currently consuming and distributing *A. platensis* over the world. Many countries, including Mexico, the United States, India, Thailand, Myanmar, China, Cuba, and Japan, as well as farms in Australia, Chile, Israel, Bangladesh, the Philippines, Peru, Brazil, and elsewhere, are major producers of this cyanobacteria (Fox, 1996). *Arthrospira platensis* providing exceptional health advantages for undernourished people, various programmes to cultivate *A. platensis* in developing world villages throughout South America, Africa, and Asia have been undertaken. *A. platensis* is an ideal to balanced diet. Currently, *A. platensis* is sold primarily as a dietary supplement in the form of drinks or tablets and can be found in health food stores. *A. platensis* is largely recognized for its potential nutritional value on a global scale. Due to its low purine concentration, which negligibly increases the danger of uric acid build-up in the body, make it is as one of the common edible algae. *A. platensis* is categorised by the food industry as a single-celled protein, making it a highly valuable edible microorganism. It's abundance in vitamins, minerals, beta-carotene, fatty acids, and antioxidants has made it commercially viable to use as a food supplement for people (Wells *et al.*, 2017). As the algae are easily available, they are now widely using in ready to cook food.

Ready to cook food are the food that contain all of the ingredients for a particular dish, but some cooking or preparation is necessary according to a technique that is detailed on the packaging. Food items that are packaged as ready to eat are either fully or partially cooked. Noodle, pasta, oats are the examples of ready to cook foods. These items come with specific food handling guidelines and are typically refrigerated. Security officers, trekkers, hikers, hunters, and other people who needed nourishment quickly and they mostly consume such items due to convenience. Food preferences and cooking methods have shifted in recent years as a result of urbanisation, cultural shifts, and social development. Due to the hectic pace of our lives, we all chose quick and easy cooking methods and ready-to-cook food.

Ready to cook food can be defined as food that requires no additional preparation. It is simple to obtain and ingest. Ready-to-eat foods are easily chilled, shelf-stable, require little preparation, and are served hot. Ready to cook foods must follow strict rules to avoid contamination or the formation of bacteria after they have been cooked. They have, however, recently become more well-liked among the vast majority of busy people in

contemporary cities. Human lives are made simpler by ready-to-eat foods. People have hectic and busy life schedules so people mostly choose ready to food. Young consumers' demand for these foods is growing by the day. Which is easily targeted by manufacturing businesses. Ready to cook foods are manufactured such a way that to maintain its convenience level, texture, and pleasant taste throughout the product's whole shelf life. They are advantageous in many ways. Heat-and-eat food products are the ideal alternatives to traditional cooking. These ready-to-eat meals will only need to be cooked for a few minutes before being served. This option is ideal for working people, College students etc. so that it saves time. The availability of ready-to-eat meals has increased over the years. There are numerous options available now in these ready-to-eat categories. Ready to cook foods will be manufactured according to government protected rules and regulations so they are safe to eat. Studies and researches pointing on to *A. platensis* as most suitable algae that could be included in the diet (Wang Y, 2021)

A. platensis is a blue-green algae that can be taken as a supplement. *A. platensis* is regarded as a superfood due to its nutritional richness and potential health advantages. Its biochemical composition includes Proteins, Essential oils, fatty acids and minerals (Fetmeh, 2021). It has high amount of protein ranges between 55 to 70 percent by dry weight. Vitamins include vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), B12 (cyanocobalamin), vitamin C, vitamin D and vitamin E. *Arthrospira platensis* is rich in high quality protein, vitamins, minerals and numerous biologically active compounds. Its cell wall comprises of polysaccharide which has a digestibility of 86 percent that will be easily absorbed by the human body. There are various types of *A. platensis* meals, the most essential of which are pills and capsules prepared from dry *A. platensis* In Vietnam, *A. platensis* powder is sold as a health food tablet under the brand names "Linavina" and "Pirulamin." Another canned product called "Lactogil" is intended to increase milk secretion in moms who are experiencing a decline in lactation (Ahsan, 2008). 10 g of *A. platensis* give over 100 mg of GLA (γ-linolenic acid) which will help to heal arthritis, heart disease, obesity and zinc deficiency (Ronda, 2008).

When *A. platensis* algae cells or filaments are powdered, they can be used to make a range of culinary products such as soups, sauces, pasta, snack foods, quick drinks, and other recipes. *A. platensis* consumption of as little as 10 g per day results in rapid recovery

from malnutrition, particularly in babies (Fefe khuabi, 2016). *A. platensis* high phytonutrient content makes it a viable alternative to vitamin pills. Emphasis this fast-growing area of algae science with a specific focus on the critical studies are necessary to determine better the health benefits of an alga or algal product. In this burgeoning discipline, there are numerous prospects for phycologists, which will necessitate novel experimental and collaborative techniques.

Hence the current project work is performed with the following objectives:

- To know the culture parameters of organism.
- To identify the organism by isolating the DNA, PCR amplification of DNA, sequencing.
- To analyse the chemical composition of *A. platensis* in order to determine the chemical properties of algae.
- To identify the important benefits and protein content of *A. platensis* as food to meet the need of present and future generation
- To make instant healthy food from the algae for the convenient and easy use by the people.

CHAPTER-2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Since ancient times, people who lived in coastal region have used marine plants as food, fuel, animal feed, fertilizers etc. The most important marine plants include Phytoplanktons, red algae, kelps, seagrass and sargassum. Hence, from ancient time, algae had importance among people. It is expected that algae were used as food very early because of the identification of presence of algae traces in the ashes of prehistoric fire locations.

The first historical knowledge on use of algae is reported from Cortes who found Aztecs people isolated this substance from Lake Texcoco in the sixteenth century and named it 'tecuitlat' (Habib *et al*., 2008). The extracted algae were used as food, ritual and medical purposes. Tribes who lived in rural areas and coastal regions were used flourished algae that are easily available. Dangeard came across the Kanembu tribe, who were gathering these superb microalgae from Lake Chad in Africa (Abdulqader *et al*. 2000). Evidences were gathered by different voyages and botanists that prove people at different countries and regions were isolated and used algae for different purposes. According to archaeological findings in *Monte Verde* (Chile), people were collecting seaweeds for food and medicine 14,000 years ago (Dillehay, 2008). Seaweeds have been utilized in Asian cuisine for millennia for their nutritious benefits and distinct flavors, particularly in Korea and Japan. Seaweed usage has been documented in China since 500 BCE (Pereira, 2016). Microalgae which include cynobacteria and seaweed also known as macroalgae and sea vegetables have been consumed as food since middle Ages.

Microalgae were thought to be an important component of aquatic biodiversity; they were grown in many habitats (sea, freshwater, and desert) as various forms (Stengel *et al*., 2011). Algae have ability to grow on little fertilizer and lack in arable land, algae can contribute to the solution by serving as a viable substitute for conventional crops (Norsker *et al.* 2011). Polyphenolic chemicals were recognized to be important natural antioxidants, and microalgae included several flavonoids and other phenolic groups. Furthermore, numerous polyphenolic compounds have been linked to bio pharmacological activities such as antibacterial and antioxidant properties (Li *et al*. 2007). Algae have recently been employed as food supplements to boost nutritional value, as animal feed additives, and even for therapeutic purposes (Shahidi, 2009). Algae

can be ingested as food or as an ingredient in prepared foods, fresh, fermented, dried, or frozen, whole or milled into various sized flakes, granules, or powders (Mouristen *et al*. 2019). Problems about the environmental impact of present food production systems, as well as health and animal welfare concerns, have fueled the demand to find healthier and more sustainable food sources (caporgno *et al*. 2018). Since, there are many microalgae, the few which have high protein value and adaptability are used in food suppliments.

The common edible microalgae include, *Chlorella*, *Arthrospira*, *Dunaliella salina*, and *Aphanizomenon flos-aquae*. Among these, *A. platensis cyanobacterium* has become a trendy health food habit (Balasubramani *et al*. 2016). *A. platensis* is relatively easy to cultivate but flourishes only in alkaline lakes with an extremely high pH and in large outdoor ponds under controlled conditions. There are only a few areas worldwide that have the ideal sunny climate for production of this alga, including Greece (Nigrita, Serres), Japan, India, United States and Spain. *A. platensis* is an excellent source of proteins (60–70%), vitamins, and minerals that are added to the diets of malnourished children in impoverished nations. A kilo gramme of different veggies is equal to one gramme of *A. platensis* protein (Saranraj and Sivasakthi, 2014). While no microorganism was able to deliver on its promise of inexpensive protein, *A. platensis* continued to spark interest and production, indicating its perceived nutritional benefits (Falquet, 2000). *A. platensis* enhances IgA production and also suggesting a pivotal role of microalgae in mucosal immunity (Ishii *et al.*, 1999).

Arthrospira platensis contains diverse cAMP-dependent signal cascades which help them to sustain at several extreme environmental conditions. It have NIES-39 genome structure, believed to be a single 6.8 Mb circular chromosome with 6,630 protein-coding genes, two sets of rRNA genes, and 40 tRNA genes (Fujisawa, 2010). Whole-genome sequencing of the *A. platensis* PCC 8005 strain, which was chosen by the European Space Agency (ESA) as a nutritional product and oxygen producer of the Micro Ecological Life Support System Alternative (MELISSA) for long-term, autonomous space missions. There were 6,279,260 nucleotides with an average GC content of 44.7%, 5.856 protein-coding sequences, and 176 genes expressing RNA predicted (Janseen *et al*. 2010).

A. platensis contains a cell wall made up of easily digestible proteins, carbohydrates, and lipids, giving it more nutritional value than other vegetable food sources (Ciferri, 1983).

Unlike many other cyanobacteria that have proven toxicity, no such property has been attributed to *A. platensis*. *A. platensis* did not exhibit any potential for organ or system toxicity even though the doses given were elevated above those for expected human consumption (Chamorro *et al*., 1996). The Food and Drug Administration (FDA) and the National Sanitary Surveillance Agency (NSSA) both formally authorised *A. platensis* as a safe supplement with no toxicological effects. *A. platensis* was suggested as a fantastic future food source at the International Society for Microbiology in 1967 (Holman B and Malau Aduli, 2013). The high nutrient content of algae and its known benefits as an antiviral, antibacterial, antioxidant, anti-diabetic, anti-cancer, and anti-inflammatory material can both be used to explain their wide range of uses as a result this alga is referred to as a Super food. Baby food formulas with added *A. platensis* had an overall acceptability score in the range from 82.72 to 96.37 and the trained panellists assigned the high scores to products with *A. platensis* 5% (Sharoba, 2014). *A. platensis* is high in vitamin B groups and other possible vitamins, all of which are required by animal bodies for normal growth and health. The approach was used to determine the amounts of vitamin B complex in *A. platensis* extracts for both LCMA and Zarrouk media (Rajput G.K.,2011).

The pharmacological actions of C-phycocyanin (C-PC) from *A. platensis*, such as anti-inflammation, antioxidation, antitumor, immunological enhancement, and hepatorenal protection, have been reported, and C-PC has significant value for development and utilisation as a drug or as a functional food. C-Phycocyanin (C-PC) present in *A. platensis* can increase cell erythropoietin activity and so directly induce the creation of colony forming unit-erythroid, which stimulates bone marrow hematopoiesis (Zhang *et al*., 1996). C-PC has been shown in animal studies to increase lymphocyte activity, immunity, and the body's ability to prevent and resist disease (Peng *et al*., 1999). C-PC can promote phytohemagglutinin-stimulated lymphocyte transformation, restore T cell E-rosette formation after cyclophosphamide damage, and significantly increase the number of antibody-forming cells and their ability to produce antibodies in normal rats and immune hypofunction mouse spleen cells treated with hydrocortisone (Zhou *et al*., 1998). Research of C-PC-mediated PDT on rat tumour models, as well as the in vivo and in vitro apoptotic mechanisms of MCF-7 cells, revealed that C-PC can boost the proliferation of immune organs and immune cells (Li *et al*. 2010).

A. platensis represents a fascinating diet for numerous reasons, and it is able to be supplied to children without any risk. The World Health Organization (WHO) regards it as a very acceptable meal (Fox and Ripley, 1986). *A. platensis* is said to be "the food of the future". Its great ingredients contribute to high energy levels. Some of these elements, such as polysaccharides (Rhamnose and Glycogen) and essential fatty acids (GLA), are effectively absorbed by cells and contribute to energy production (Wuang *et al,* 2016). *A. platensis* promotes the growth of healthy lactobacillus in the intestine, allowing for the creation of Vitamin B6, which aids in energy production (Baicus and Baicus A, 2007). Eating *A. platensis* for four weeks reduces serum cholesterol levels in humans by 4.5%, according to studies (Henrikson, 1994). The extract increases tumour necrosis factor in macrophages, implying a good tumor-destroying mechanism (Shklar and Schwartz, 1988). *A. platensis* helps to improve biochemical parameters of kidney and liver (El-Sheekh et al. 2014). The effect of microalga concentration on product colour parameters was explored, as well as its stability during processing and storage duration is improved. (Ghaeni and Roomiani, 2016). Many foods marketed to children are described as containing the delectable blue-green *A. platensis*. Mix it into milkshakes, jellies, cookies, or cakes (Vedi, 2013).

A. platensis is not only helpful for human beings but also for animals and birds. Studies have revealed that the microalgae are providing many more advantages to different living organisms such as prawn, fish, hen etc. *A. platensis* is a high-quality natural feed addition that can be utilised in the nutrition of animals and poultry. Sakaida Takashi discovered that adding *A. platensis* to laying hen diets significantly improved egg yolk colour (Chirasuwan et al. 2007). *A. platensis* is less expensive than other animal meals, and China is primarily adopting it as a partial alternative for imported feed to improve prawn growth. *A. platensis* supplementation improved illness resistance in high-value fish, increasing their survival rate from 15% to 30% (Sili *et al*. 2007).

A. platensis is rich in pigments, mainly contain carotenoid and C-phycocyanin. The extraction of phycocyanin and proteins from *A. platensis* was tested using a pulsed electric field (PEF). PEF extractions were done with varied specific energies (28, 56, and 122 Jml1 of suspension) and the results were compared to bead milling extractions. The antioxidant capacity of the PEF-treated extracts was higher than that of the bead milled extracts. PEF treatment is a potential approach for obtaining blue-green antioxidant

extracts from *A. platensis* in a sustainable manner (Debora Pez Jaeschke, 2019). Green extraction technologies such as supercritical carbon dioxide fluid extraction (SFE) and microwave-assisted extraction (MAE) were employed to yield useful lipophilic chemicals from *A. platensis* biomass. For MAE, the temperature (T) factor was analysed, whereas for SFE, the pressure (P), temperature (T), and co-solvent (ethanol) (CS) factors were evaluated. For SFE and MAE, the maximum extraction yield of the recovered oleoresin was (4.07% 0.14%) and (4.27% 0.10%), respectively (Diego, 2019). A variety of extraction procedures have been investigated in order to isolate this blue pigment from *A. platensis.* Phycocyanin is extracted from either wet or dry material. Intensification procedures may be employed as a pretreatment or concurrently with the extraction to boost the extraction yield (Vernes *et al.*, 2015). Ammonium sulphate fractionation was used to prepare the crude phycobiliprotein extract. Then, hydroxylapatite was selectively adsorbed by crude phycobiliprotein extract dissolved in 20 mM phosphate buffer (pH 7.0), while C-phycocyanin (C-PC) was not. APC was recovered from the crude phycobiliprotein extract after collecting the hydroxylapatite. The enhanced APC was then washed away from the hydroxylapatite with a 100 mM phosphate buffer (pH 7.0). It was simple to remove C-PC and isolate Allo Phycocyanin in significant quantities using this straightforward extraction procedure. (Hai Nan Su, 2009) Ammonium sulphate fractionation was used to extract, purify and concentrate crude C- Phycocyanin and Allophycocyanin present in *A. platensis* at saturations of 25% and 60%, respectively, before purification on a DEAE-Sepharose Fast Flow chromatography column with continuous pH gradient elution from pH 5.0 to 3.6. C-PC and APC with high purity and recovery were obtained simultaneously as a result of this single-step chromatography. Electrophoresis and fluorescence emission spectroscopy were used to confirm their purity (Shi Van Yan *et al.*, 2010). The cell lysis of cyanobacterium combined with repeated freezing and thawing cycles is a straightforward process for the extraction and purification of food-grade C-phycocyanin from *Arthrospira platensis*. The crude extract of C-phycocyanin was purified using ammonium sulphate precipitation followed by ion exchange chromatography, yielding a purity of 2.5. UV-visible spectrophotometry was used to test the purity of the phycocyanobilin chromophore by monitoring absorption after each stage of purification (Maria Kissoudi, 2017). Extraction with a phosphate buffer yielded higher Phycocyanin (65.5 mg g1) with a purity of 0.53. Purity was

increased to 0.76 when the UF was followed by one step of infiltration (Vandre Barbosa Briao, 2020)

A. platensis have high role in regulating immune system of human beings. It has ability to suppress certain viruses, anti-cancerous etc. The active ingredient in *A. platensis* water extract is a sulfated polysaccharide called calcium spirulan (Ca-Sp). Ca-Sp suppresses the in vitro replication of various enveloped viruses including Herpes simplex type I, human cytomegalovirus, measles and mumps virus, influenza A virus and human immunodeficiency virus-1 virus (HIV-1) (Hayashi et al., 1996). In a recent study, an aqueous extract of *A. platensis* suppressed HIV-1 replication in human T-cells, peripheral blood mononuclear cells, and Langerhans cells *in vitro* (Ayehunie *et al.*, 1998). The benefit of employing herbs and algae products with proven antiviral capabilities in battling specific viruses is that they can be utilised even after the illness has been established (through immunomodulation). *A. platensis* may have a tumor-destroying mechanism and hence play a role in cancer prevention. While there have been several animal and in vitro investigations, there has only been one trial with human volunteers. This study focused on the effects of *A. platensis* on oral carcinogenesis, specifically leukoplakia (Mathew *et al.*, 1995). They reported that 45% of their research population demonstrated complete remission of leukoplakia after consuming *A. platensis* supplements for 1 year. A more recent study found that *A. platensis* supplementation reduced blood cholesterol, triglycerides, and LDL cholesterol while increasing HDL cholesterol in patients with ischemic heart disease. More research is required before *A. platensis* may be recommended for cholesterol reduction (Ramamoorthi and Premakumari, 1996). *A. platensis* delivered at a dose of 3 g day−1 did not relieve tiredness more than the placebo in any of the four patients and maybe it has no effect on chronic fatigue (Baicus and Baicus, 2007). Japanese research uncovered the molecular mechanism of the human immunological capacity of *A. platensis* by studying blood cells of volunteers with pre- and post-oral administration of hot water extract of *A. platensis*. After administering microalga extracts to male volunteers, IFN-γ production and Natural Killer (NK) cell damage increased (Hirahasi *et al*., 2002). In a recent double-blind, placebo-controlled study from Turkey evaluating the effectiveness and tolerability of *A. platensis* for treating patients with allergic rhinitis, *A. platensis* consumption significantly

improved the symptoms and physical findings compared with placebo ($P < 0.001$), including nasal discharge, sneezing, nasal congestion and itching (Cingi *et al.*, 2008).

Nanofiber scaffolds can mimic the structure and function of the native extracellular matrix, they have the potential to be used in tissue engineering. The synthetic polymers normally used to produce nanofiber scaffolds can be replaced by *A. platensis* biopolymers, which are biodegradable and biocompatible with cells and tissues (*De* Morais *et al*., 2010). *A. platensis* biomass can be added to the polymer solutions used in nanofiber production to produce scaffolds that incorporate *A. platensis* properties; this is possible because electrospinning does not involve extreme temperatures or pH that would reduce the biological activity of the biomass or its nutrients (Ramier *et al*., 2014). Depending on the solvent used to manufacture the polymer, internal biomass components (proteins, fatty acids, and biopolymers) become available within the scaffold and can stimulate cells or tissues (Khan *et al*., 2005)

A. platensis is an underutilised protein source suitable for human nutrition, and little is known about the usage of *A. platensis* as a food and the associated consumer opinion. For the success of new products, early and active consumer participation is required as a part of this, a mixed method approach was used to conceptualise (sensory profiling of *A. platensis* extrudates and expert interviews) and then evaluate consumer willingness to try (consumer survey) three innovative products: pasta filled with *A. platensis,* maki-sushi filled with *A. platensis* jerky. Influenced by this, alternative protein-based convenient food products such as *A. platensis* burger and meatballs are now commercially accessible in the Netherlands (J. House, 2016) It is also considered as a biological technique for reducing unpleasant odour by using algal biomass in complicated foods. Two alternative treatments were used individually to minimize the microbial contamination on the biomass. The first was a 15-minute UV light irradiation to reduce the microbial total charge using a Faster BH-EN 2004 Class II Microbiological Safety Cabinet (S/N 1113) (Richmond Scientific, Chorley, UK) with a lamp emitting light at an intensity of 253.7 nm in the UV-C (UV) spectrum, while the second was a 20-minute thermal treatment in an autoclave (3870MLV, Tuttnauer, NY) these methods will help to sterilize contamination.

The digestibility is considered to determine Net Protein Utilisation (NPU) by the proportion of protein nitrogen absorbed, and the amino acid composition (along with other factors such as age, sex, and physiological status), determine protein utilisation. The NPU value is experimentally determined by calculating the percentage of nitrogen retained when the only limiting nutritional factor is the protein source under consideration. This value is generally studied in various situations, such as: active development, adulthood, and convalescence (WHO, 1973). Unlike other microorganisms proposed as protein sources, such as yeast *A. platensis* cells lack cellulose walls and instead have a relatively fragile murein envelope. This explains why its proteins are so easily digestible (Dillon & Phan, 1993). Beta-carotene accounts for 80% of the carotenoids found in *A. platensis*, with the remainder primarily consisting of Fucoxanthin and cryptoxanthin (Palla and Busson, 1969). A study on 5000 Indian pre-school children revealed that a single daily dose of one gramme of *A. platensis* was surprisingly effective in treating chronic Vitamin A deficiency.

Experimenting the enhanced protein quantity of food by incorporating *A. platensis S*had been already done in other food items like cookies, Pasta etc. When compared to the control sample, the integration of microalgae leads in an increase in quality parameters. After cooking, the colour of microalgae pastas remained relatively constant. When compared to the control sample, the addition of microalgae resulted in an increase in uncooked pasta stiffness. An increase in biomass concentration (0.5-2.0%) resulted in a general tendency for an increase in pasta stiffness across all microalgae tested. Sensory investigation found that microalgae pastas are delicious (Monica Fradique *et al*., 2010). SP with 2.6% *A. platensis*. Nutritional content (protein, lipids, ash, carbohydrates, carotenoids, and in vitro protein digestibility), physical properties (expansion index, bulk density, hardness, water absorption index, water solubility index, microstructure and colour parameters), sensorial characteristics (flavour, colour, taste, texture, overall acceptance, and purchase intention), and microbiological analyses were performed on these snacks. This formulation received an 82% sensory acceptability index. It was concluded that *A. platensis* can be employed at the concentration of 2.6% resulting in snacks with high nutritional value and sensory appeal (Barbara Franco Lucas *et al*., 2018). A study was carried out to evaluate the technological and nutritional features of dry pasta made with oatmeal and *A. platensis* and assessed using the response surface

methodology (RSM). The chemical and physical properties of the mixes were investigated. In general, *A. platensis* influenced the soluble solids content and colour of the pasta, whereas oats primarily affected the acid content. The oatmeal raised the levels of crude protein and total dietary fibre (13.06%) when compared with the commercial pasta (2.40%) and may be considered as a source of fibre (Fernanda Arnhold *et al*., 2014). Therefore, various studies reveal that *A. platensis* is most suitable algae to be included in the diet and it have so much advantages to both animal and human. They will play an important role is maintaining health and adaptability.

CHAPTER 3
MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. MICROALGAL CULTURE

Microalgal species selected for the current study was a strain of *Arthrospira platensis* obtained from Scire Science R & D laboratory, KINFRA Kalamassery, Kerala, India.

3.1.1. Culture media and composition and culture parameter

The algae culture broth was prepared and the culture tubes were incubated under optimum conditions as given below:

Parameter	Value
Working volume	250 ml
Temperature	24±1°C
Light intensity	700-800 lux
Photo period	16/18h (light/dark)
Time	20 days
Salinity	20
pH	8

3.1.2 Microscopic observation

After 5 days of incubation, the cultures were observed microscopically under 40X magnification using a light microscope (ZEISS primo star) on a daily basis in order to examine growth and multiplication of microalgal cells.

3.1.3 Maximum absorbance determination

The efficiency of biomass growth was controlled by measuring the optimal density (OD), which is defined as the absorption of visible radiation. The optical absorbance was measured at various wavelengths such as 620 nm, 700 nm in order to determine maximum absorbance using a spectrophotometer (LAB India).

3.1.4 Cell counting using Neubauer Haemocytometer

The microalgae concentration in the mixed culture was counted using the improved Neubauer haemocytometer counting chamber. A clean Neubauer cover glass was

attached to the counting chamber by pressing it carefully in space. Then, the sample was collected using a pipette and gently allowed to run quickly through the leading edge of the coverslip into the chamber, exactly filling it. After that, the counting chamber was allowed to stand on the bench for two minutes before counting using the light microscope (ZEISS primo star). The grid under the microscope was examined using 10X objective for distribution of the cells and refocused at 40X objective before counting cells in the four corner squares (Absher, 1973).

$$\text{Cell number of Cell density} \quad x \quad \frac{\text{Counted cells}}{\text{Volume of square}} \quad x \text{ Dilution factor}$$

3.1.5 Determination of chlorophyll content

The chlorophyll content of the microalgal cells was determined by using spectrophotometric technique. Sample of microalgal suspension was centrifuged for 10 minutes at 13000rpm (Centrifuge HERMLE-Z 3242). The supernatant was decanted and the pellet resuspended in 90% methanol. Chlorophyll was then extracted from the sample during one hour of incubation in a water broth (Rotek) at 50°C. The sample was again centrifuged for 10 minutes at the same speed. For the spectrophotometric determination of chlorophyll. The absorbance of light green supernatant was measured at two wavelengths 405 nm and 450 nm, using the UV spectrophotometer blanked with methanol (Dere *et al.,* 1998).

3.2 IDENTIFICATION OF MICROALGAE USING MOLECULAR SEQUENCING

At the molecular level, the rRNA genes are the most widely used markers for the identification of algae due to their conserved function and universal presence. Several researchers have exploited the conserved regions of the 16s rRNA gene for phylogenetic analysis. Here, we explored the possibility of 16s forward and reverse primer for amplification (Grada *et al*., 2013)

3.2.1 DNA isolation

DNA isolation method by Doyle and Doyle (1987), using CTAB yielded good quality DNA for PCR.

DNA isolation using CTAB (Doyle and Doyle,1987)

Freshly prepared CTAB (Cetyl Trimethyl Ammonium Bromide) buffer was preheated at 65°C. 1g of the microalgae sample was ground in 16 mL of CTAB buffer and homogenized. The ground tissue incubated at 65°C in a water bath for 30 minutes followed by incubation at room temperature. Equal volume of Phenol: Chloroform: Isoamylalcohol (25:24:1) was added and mixed thoroughly to form an emulsion. The aqueous layer was collected after centrifugation at 10000 rpm for 10 minutes and transferred to a new 50 mL centrifuge tube using a cut tip. Phenol: Chloroform: Isoamylalcohol extraction was repeated and the aqueous layer was collected in Eppendorf tubes (1 mL in each tube). 3M ammonium acetate (pH 5.2) was added to the aqueous phase and DNA was precipitated by the addition of the two by third volume of ice-cold isopropanol and thoroughly mixed by inverting. The samples were kept for overnight incubation at -20C. The supernatant was decanted off and the pellet was washed with cold 70% ethanol. The DNA was further pelleted by centrifugation at 12000 rpm for 10 minutes. The ethanol was decanted off and the pellet was air dried to remove all traces of ethanol and resuspended in 100µl TE buffer.

3.2.2 Amount and purity of DNA

The yield of DNA extracted was measured using a UV spectrophotometer (Lab India) at 260 nm. The purity of DNA was also determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA concentration and purity was also determined by running the sample in 1.0% Agarose gel. The intensities of the band obtained by staining with (0.5μg/mL). Ethidium bromide was compared with a 250bp DNA marker from Chromous Biotech. The gel documentation system (BIORAD- Molecular imager) was used for DNA visualization on the gel (Boesenberg *et al*., 2012)

3.2.3 PCR amplification

The 16s region was amplified by polymerase chain reaction (PCR) with specific primers (forward and reverse primers). Amplification of the conserved regions of the 16s rRNA gene was conducted in a reaction mixture with a final volume of 20μl that contained about 20mg of template DNA and primers using the PCR Master Mix (Fermentas, USA) and a thermal cycler. The reaction consisted of initial denaturation at 94°C for 3 minutes

followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes.

Amplicons were checked for appropriate size by 2% agarose gel visualization. Amplicons were gel purified using commercial column-based purification kits (Invitrogen, USA) and sequencing was performed with forward and reverse primers in ABI 3730 XL cycle sequencer. Forward and reverse sequences were assembled and contig was generated after trimming the low-quality bases. Sequence analysis was performed by using online tool BLAST of NCBI database and based on maximum identity score E value top most sequences was utilized for multiple sequence alignment (Van *et al*., 2008)

3.3 PREPARATION OF NOODLES

Procurement of raw materials

Maida or Atta, quinoa, beetroot powder, guar gum, sodium bicarbonate, calcium bicarbonate is procured from supermarket, Kalamassery.

3.3.1 Noodle preparation

To prepare noodle sample at laboratory conditions formulations (250g). For noodle preparation quinoa flour were used to replace wheat flour in the formulation 5%, 9%, 13.6% respectively. Water levels of the noodle formulations where 75, 82.5 & 180mL/kg respectively.

Ingredient	F1 gram	F2 gram	F3 gram
Atta	225g	212.5g	200g
Quinoa	12.5g	22.5g	34g
Organism	3g	6g	-
Salt	7.5g	7.5g	3.75g
Guar gum	0.75g	1.25g	1.75g
Baking powder	0.25g	0.25g	0.25g
Sodium bicarbonate	0.125g	0.125g	0.125g
Water	75ml	82.5ml	180ml

The ingredients were mixed manually for 10 to 15 minutes. The dough was rounded and allowed to rest at ambient temperature for one hour. The dough was passed through the

reduction rolls of a noodle extruder machine. The thickness of the dough sheet was reduced and dough sheets were rested at ambient temperature for 5 minutes. After that dough sheets were cut into noodle strips and the strips were dried for 15 to 20 hours. The dried noodle samples (MBQ1, MBQ2, MBQ3) were packed in polyethylene bags and stored at ambient temperature prior to analysis. Cooking properties, sensory characteristics, functional properties of the flour and the proximate analysis and the chemical analysis of the noodle samples (MBQ1, MBQ2, MBQ3) were determined. (Rathod, 2017)

3.3.2 Functional properties of flour

3.3.2.1 Bulk density

50g of flour was taken into a 100mL volumetric cylinder. The cylinder was tapped several times on a laboratory bench to attain a constant volume. The bulk density (g/cm^3) was then calculated as weight of wheat flour (g) divided by flour volume (cm^3) (Berton *et al*., 2002).

3.3.3 Cooking properties of noodle

3.3.3.1 Water absorption

It was calculated as the percentage difference in weight of noodle before and after cooking divided by the weight of the noodle before cooking. Sosulski *et al*. method is used as reference (Oladunmoye *et al*., 2010)

3.3.3.2 Swelling volume

It was expressed as the percentage difference in volumes of the cooked and uncooked noodle samples divided by the volume of uncooked noodle (Berton *et al*., 2002)

3.3.4 Sensory analysis

Noodle samples (100g) were cooked for 18 minutes in 1L unsalted water and drained. Sensory tests were applied after 10 minutes of draining. The sensory test of noodles was performed with an evaluation panel of 20 members, (10 female and 10 male age ranges 17 – 50 years) familiar with noodle. Information on the nature of this evaluation was given to panellist. Panellist received one sample at a time with a 2-minute break with a sample. Each panelist individually evaluated the noodles in the laboratory.

- Surface properties (wetness, slipperiness and roughness)

- Chewing properties (hardness, cohesiveness and sensation of starch between teeth after each chew)
- Mouth feels after chewing chalkiness and stickiness)
- Taste and overall acceptability

Panellists were asked to score the cooked noodle in terms of these above-mentioned properties. Using a 9 – point hedonic scale with 9 – like extremely, 5 – neither like nor dislike, 4 – dislike slightly and 1 – dislike extremely (Mason *et al*.,2002).

3.3.5 Chemical analysis

3.3.5.1 Determination of total phenolic compounds

The total phenolic compounds were determined using the folin-ciocalteu (Folin-phenol reagent) procedure with slight modifications. 0.2 ml of sample (25mg/ml) was mixed with 0.5ml of folin-ciocalteu (diluted 1:10with deionized water) and 25 ml of distilled water and it was allowed to stand for 3 to 8 minutes. 0.8ml of Na_2CO_3 solution (7.5%) was added and the resultant solution was allowed to stand at room temperature for 30 minutes in a dark place. The absorbance was measured at 765nm using spectrophotometer. The total phenolic content was expressed as Gallic acid equivalence (GAE) in mg/100g of the extract. (Lamuela-Raventos, 2018)

3.3.5.2 Determination of flavonoid content

0.1mL of sample (25mg/ml) was added to 0.1ml of 2% $AlCl_3$ solution in methanol. The mixture was allowed to stand at room temperature for 10 minutes in a dark place. The absorbance of the mixture was measured at 415nm against a blank sample without $AlCl_3$ using spectrophotometer. The flavonoid-aluminum complex has an absorptivity maximum at 415 nm. The total flavonoid content was expressed as Quercetin equivalence (QE) in mg/100g of the extract (Pekal, 2014)

3.3.6 Proximate analysis

3.3.6.1 Determination of moisture content (AOAC, 2000)

1. Dry the empty dish and lid in the oven at 105°C for 3 hours and transfer to desiccator to cool. Weigh the empty dish and lid.
2. Weigh about 3g of sample to the dish. Spread the sample to the uniformity.
3. Place the dish with sample in the oven. Dry for 3 hours at 105°C.

4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample

Calculation

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \text{ X } 100$$

W1 = Weight (g) of sample before drying

W2 = Weight (g) of sample after drying

3.3.6.2 Determination of protein content (AOAC, 2000)

Reagents

- Kjeldahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) with 1 part of coppersulphate ($CuSO_4$)
- Sulphuric acid (H_2SO_4)
- 40% NaOH solution
- 0.2 N HCl solution
- 4% H_3BO_3
- Indicator solution: Mix 100 ml of 0.1%methyl red (in 95% ethanol) with 200ml of the 0.2% bromocresol green (in 95% ethanol)

Methods

1. Place sample (0.5 -1.0g) in digestion flask.
2. Add 5g Kjeldahl catalyst and 200ml of conc. H_2SO_4.
3. Prepare a tube containing the above chemical except sample as blank. Place flask in inclined position and heat gently unit frothing ceases. Boil briskly until solution clears.
4. Cool and add 60ml of distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser and titrate excess standard acid distilled with standard NaOH solution.

$$\text{Protein (\%)} = \frac{(A-B)\times N\times 1.4007\times 6.25}{W}$$

where, A = volume (ml) of 0.2 N HCl used sample titration

B = volume (ml) of 0.2N HCl used in blank titration

N = Normality of HCl

W = weight (g) of sample

14.007 = atomic weight of nitrogen

6.25 = the protein-nitrogen conversation factor for fish and its by-products

3.3.6.3 Determination of ash content (AOAC, 2000)

Method

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible and burned off.
2. Cool the crucible in the desiccator (30 min).
3. Weigh the crucible and lid to3 decimal places.
4. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.
5. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
6. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for the further ashing.

Calculation

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

CHAPTER 4
RESULTS

4. RESULT

4.1 MICROALGAL CULTURE

Inoculation of organism is done on the first day of project. The organism *Arthrospira platensis* is inoculated in to a suitable medium, F/2. Sample is incubated in sterile condition (Plate Ia.)

4.1.1 Maximum absorbance determination

The inoculated culture in the conical flask showed gradual colour changes from first day of inoculation to 15 days of inoculation. The colour of culture is changed from pale green to dark green which indicates the increased cell count and chlorophyll content. (Plate Ib.)

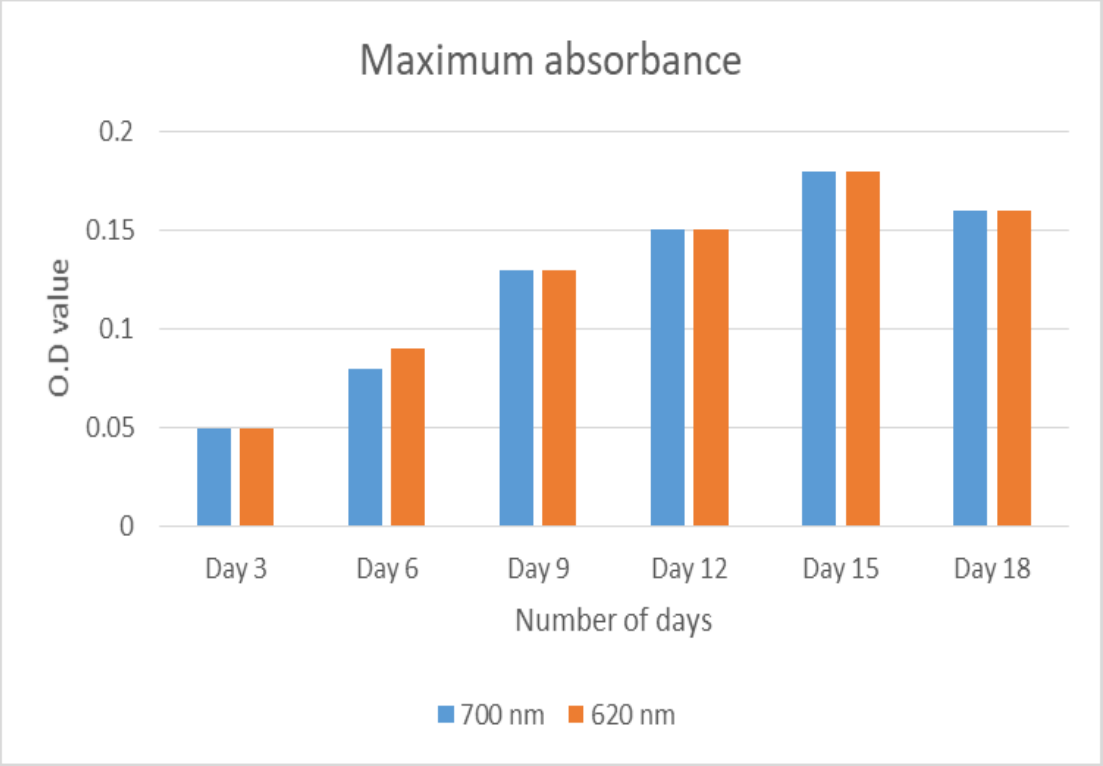

Figure 1: Absorbance at 700nm and 620nm

The optical absorbance was measured at two wavelengths 620nm and 720nm using spectrophotometer. O.D value is checked at an interval of 3 days. Gradual increase in O.D value represents the increase in number of cells. Maximum absorbance was observed on 15th day of inoculation and 18th day shown decline in O.D value. Culture at 15th day is taken for further experiments (Figure 1).

4.1.2 Cell counting using Neubauer Haemocytometer

Under sterile condition and suitable growth medium the algal number will be increased. Using the instrument haemocytometer the increased cell count can be observed and calculated. (Plate Ic.)

Neubauer Haemocytometer determine the cell count by microscopic observation of *Arthrospira platensis* under light microscope. Light green coloured dots could be seen, which are the cells present in the sample. Gradual increase in the number of cells is shown at each counting intervals.

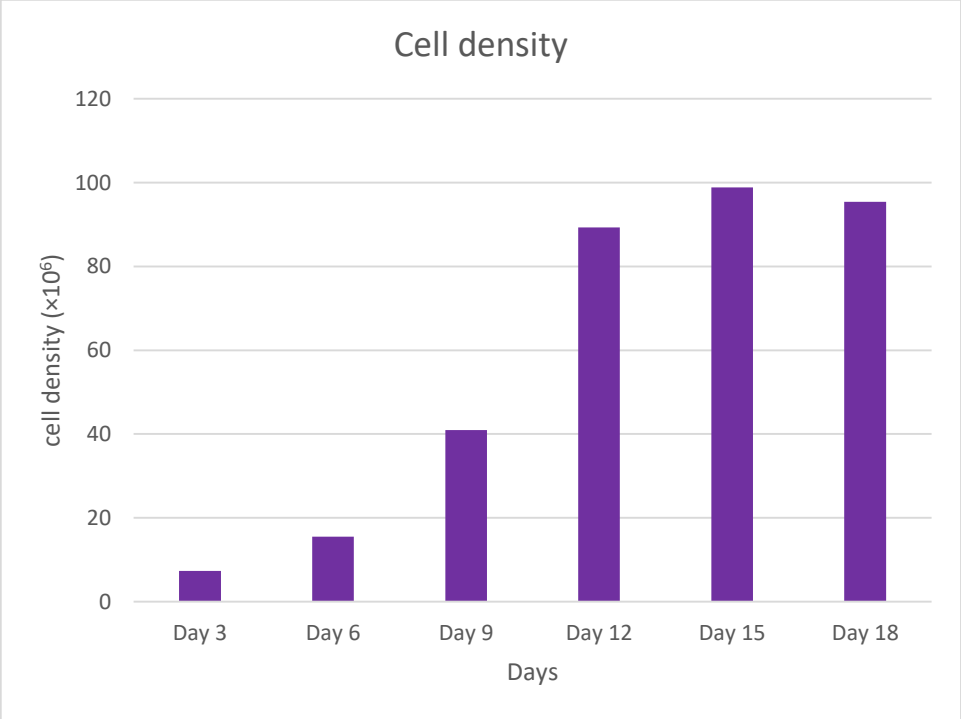

Figure 2: Cell density of organism at regular intervals

The cell density has been checked at regular intervals of 3 days. It is identified that the cell density is increased at each interval. Maximum cell density is shown at 15th day of inoculation and on 18th day of examination the cell density has been declined. So, the maximum cell division occurred at a range of 15 days (Figure 2)

4.1.3 Determination of Chlorophyll content

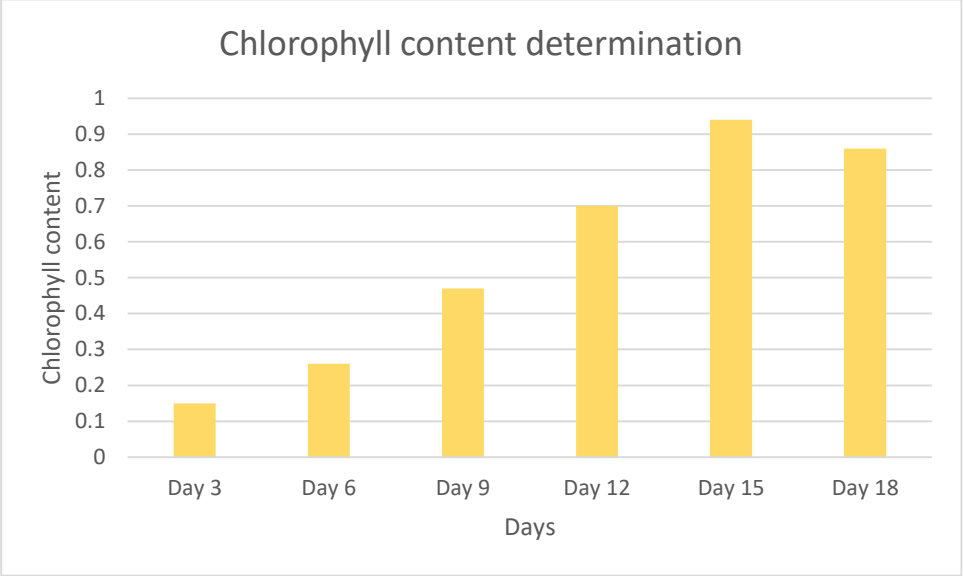

Figure 3: Chlorophyll content determination

Chlorophyll content was measured at two wavelengths 405nm and 450nm using spectrophotometer. O.D value is taken at an interval of 3 days. Enhanced chlorophyll content indicated the growth of organism. Maximum chlorophyll content is obtained on 15^{th} day and after that slight decline is identified (Figure 3).

4.2 IDENTIFICATION OF MICROALGAE USING MOLECULAR

4.2.1 DNA isolation using CTAB

Organisms can be differentiated and identified based on conserved regions on gene. The first step done for this is DNA isolation using CTAB which yield good quality DNA for PCR.

In gel electrophoresis the DNA fragments are got separated based on their molecular size. Smaller fragments with low molecular size will easily be migrated towards positively charged anode. The band pattern has specificity. By comparing the specificity of banding pattern, the organism could be identified. (Plate IIa.)

4.2.2 BLAST Search

The obtained sequence is compared with formerly sequenced database to determine the similarity between them. BLAST is the tool used to identify the similar sequence from

the database. BLAST helps to identify the phylogenetic relationship between organisms and to determine the matching sequences (Plate IIb). The sequence show similarity with *Arthrospira platensis* 16s ribosomal RNA gene, partial sequence. Many sequences that are similar to query sequence was identified using blast search (Plate III).

4.2.3 Graphic summary of alignment

The graphic summary of alignment represents sequence similarity of query sequence with that of sequence in the database. Each of the horizontal lines are representation of sequence that is in the database matches. The query length is greater than 500 nucleotides. Alignment scores greater than 200 indicates these is high similarity between the sequences (Plate IV).

Phylogenetic tree

The phylogenetic tree shows that the *A. platensis* 16S ribosomal RNA gene partial sequence shows close similarity and relationship with *Arthrospira platensis* PV14 16S ribosomal RNA gene partial sequence. Other phylogenetically related organisms were identified by the analysis of phylogenetic tree (Plate V).

4.3 NOODLE PREPARATION

The ingredients for three noodle samples (F1, F2 and F3) are arranged. F1, F2 contains the organism and F3 is taken as control. (Plate VI, VII and VIII).

4.3.1 Functional properties of flour

Bulk density is found to be 0.57gcm^3. Particle size and the initial moisture content affect bulk density. Flour's high bulk density indicates that it is suitable for use in food preparations.

4.3.2 Cooking properties of noodle

4.3.2.1 Water absorption capacity

By analysing the water absorption capacity of F1, F2 and F3 it is found that the water absorption capacity is higher for the control. Compared to F1, F2 sample have less water absorption capacity. Results were such that F1 sample have 80%, F2 sample have 74% and F3 have 85% water absorption capacity. Among these the sample with less water absorption capacity will have high shelf life (Figure 4)

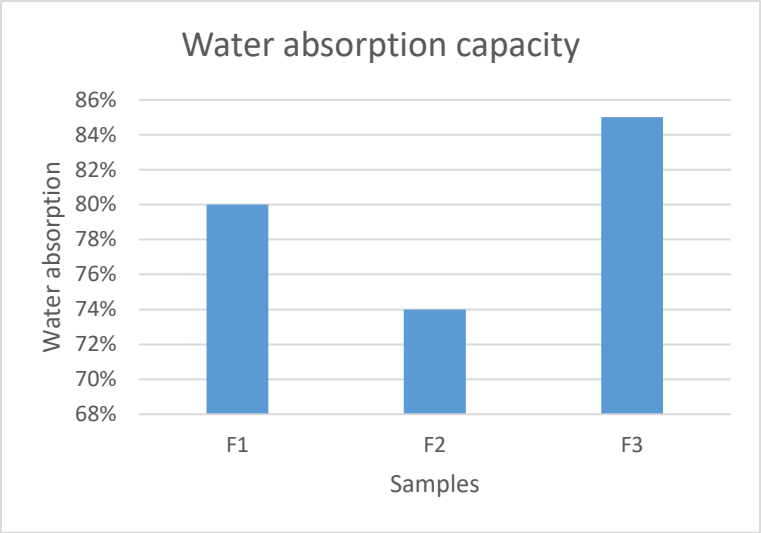

Figure 4: Water absorption capacity

4.3.2.2 Swelling capacity

Swelling capacity of three samples are calculated. The F1 and F2 samples shown high swelling capacity compared to F3 sample. As the swelling volume increases the quantity of food increases after cooking so sample with high swelling capacity is more convenient to use (Figure 5)

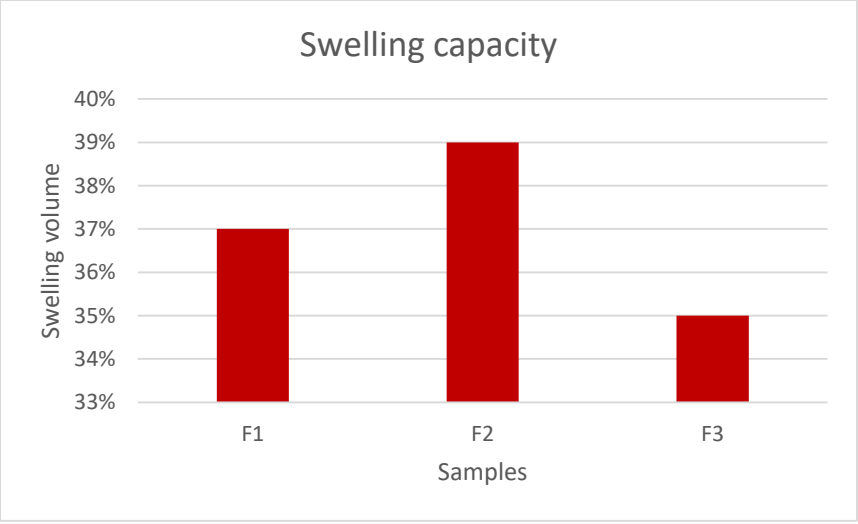

Figure 5: Swelling capacity

4.3.3 Sensory analysis

Sensory evaluation is done by panellist of 10 members. Panellists evaluated the sample based on four parameters and given an overall acceptance score. Each colour in the graph

represents a specific parameter of evaluation. For the F1 sample most of them given 7 marks for the colour of noodle. Five of them given 8 score for the texture. Most of them given score six for the taste. The overall acceptance was 7 on 9- point scale (Plate IXa).

On sensory evaluation of sample F2, most panellists were given 6 or 7 for the taste. Many given 6 scores for the appearance. For the colour of noodle panellists were given scores above 6. Six of the panellists were given 7 marks for the texture. Most of them given scores above 5 for the overall acceptance in a 9-point scale (Plate IXb).

The F3 sample is the control. Most panellists scored 7 for the texture and taste. Scores from panel for the colour was above 5 and below 8. The average of overall acceptance by the panel was 7 in a 9-point scale (Plate IXc).

From the sensory evaluation of three samples, done by 10 panellists reveals the wide acceptance of microalgal noodles as that of normal noodles. The overall acceptance given by the panel for the three samples are nearly same so, microalgae incorporated noodle can be replaced as a nutritional food for a healthy individual and it also provides high nutritional value as compared to the normal noodle

4.3.4 Chemical analysis

4.3.4.1 Determination of phenol and flavonoid content

Phenol and Flavonoid content determination tests reveals that the samples having *A. platensis* have more phenol and flavonoid content than the control. The phenol content in F3 was only 3.7 while the F1 and F2 have 6.2 and 7.5 respectively. While considering the flavonoids contents of the samples the control had less content value than the samples containing microalgae. Therefore, as the content of *A. platensis* is increased, the total phenolic and flavonoid value get increased. (Table 1)

Table 1: Results of Chemical analysis

Sample code	Total Phenol content (mg/ml)	Total Flavonoid content(mg/ml)
F1	6.2	10
F2	7.5	12
F3	3.7	8

4.3.5 Proximate chemical composition

Proximate analysis is done on the dried samples of the prepared noodle (Plate Xa, Xb,Xc) They were arranged on cleaned plastic sheet and allowed to dry so that to remove the water content in them. Proximate chemical analysis (g/100g) of Noodles with 90%, 85%, 80% and 75% composition (W/W). Proximate chemical analysis determines the difference of samples in moisture content, Total ash content, crude protein content and crude fat content. This moisture content analysis aims to quantify how much water *A. platensis* adds to dry noodles. Here, the control had more samples containing micro algae. Noodles with low moisture content will have high shelf life. Ash content is higher in the F2 sample as compared to other two samples due to the increased amount of *A. platensis*. There is a great variation in the crude protein contents of F1 and F2 with that of F3. (Table 2)

Table 2: Results of Proximate chemical analysis

Sample code	**Moisture content (g/100g)**	**Total ash content (g/100g)**	**Crude protein content (g/100g)**
F1	3.3	1.4	8.2
F2	3.1	1.6	9.5
F3	3.4	1.2	6.6

CHAPTER 5
DISSCUSSION

5. DISCUSSION

The present study aims to identify the benefits of *A. platensis* in food supplements. The microalgal species was chosen due to their faster cell divisions, higher biomass index and the commercial importance of strains produced by species.

A. platensis has been marketed as "food for the future" because it has "exceptional components" that boost energy. Several of these components, such the polysaccharides rhamnose and glycogen and the important fatty acid GLA, are readily absorbed by human cells and aid in the release of energy. These algae include a significantly lower percentage of total fat and cholesterol as compared to other food sources, as well as essential fatty acids like omega-3 and omega-6 (AlFadhly *et al*., 2022). Vitamins are essential micronutrients, but since humans cannot create them in sufficient amounts, they must be obtained from the food that they eat and their lack is associated with a wide range of illnesses. Food made from algae are very vitamin-rich.

Inoculation of organism is done F/2 media. The maximum absorbance determination is done in order to evaluate the growth of organism. The optical absorbance was measured at two wavelengths 620nm and 720nm using spectrophotometer. Neubauer Haemocytometer determine the cell count by microscopic observation of *Arthrospira platensis* under light microscope. The cell density has been checked at regular intervals of 3 days. It is identified that the cell density is increased at each interval. Chlorophyll content was measured at two wavelengths 405nm and 450nm using spectrophotometer. Enhanced chlorophyll content indicated the growth of organism. Maximum cell density, chlorophyll content was shown at 15^{th} day of inoculation and on 18^{th} day of examination these have been declined.

DNA isolation using CTAB method was conducted and DNA fragments are separated using gel electrophoresis. Then the fragments are sequenced using BLAST tool. The query sequence is compared with databases and evaluated the similarity and dissimilarity between organisms. Other phylogenetically related organisms were identified by the analysis of phylogenetic tree. The study species was identified and confirmed as *A. platensis.*

The ingredients for three noodle samples (F1, F2 and F3) are arranged. F1, F2 contains the organism and F3 is taken as control. Corresponding ingredients are mixed and noodle is made using noodle extruder machine.

The bulk density of the sample is evaluated by tap density. The bulk density is observed as $0.57 gcm^3$. The bulk density depends on particle size and initial moisture content. The high bulk density of flour suggests their suitability for use in food preparations (Akapata and Akubor, 1999). It aids in reducing paste thickness, which is a crucial element in convalescent and paediatric feeding.

On experimental evaluation it is found that the cooking properties of samples i.e., the water absorption capacity and swelling volume. Water absorption capacity is lower for the F1 and F2 samples compared to F3. The increased water absorption capacity will lead to high moisture content which decrease the shelf life as they provide satisfiable cooking and physical features. These outcomes could be explained by the algae's capacity to take in water and hold it in the protein-starch net (*Prabhasankar et al*., 2009). The Swelling volume is shown similarity in values. As the swelling volume increases the quantity of food increases after cooking. The swelling capacity (index) of flours are influenced by the particle size, species variety and method of processing or unit operations (Suresh and Samsher, 2013). When compared to noodles made with wheat flour, the other two varieties of microalgae enriched noodles were found to have higher *in vitro* availability of calcium, iron, and zinc. It might be because *A. platensis* powder lacks phytic acid, which is known to bind to divalent cations and limit their bioavailability (Chaudhary 2011)

On sensory evaluation it is found that the noodle containing micro algae have as much as sensory acceptance with that of normal noodle. Noodle developed a vibrant green colour as a result of the inclusion of *A. platensis* which enhanced its look. The surface is smooth, and the shape of the cooked dry noodles is symmetrical. In case taste most of them chosen the F3 sample may be because of the slight bitter taste due to the addition of *A. platensis* it can be improved by additing additives. The texture acceptance of dry noodles fortified with *A. platensis* ranged in value from 2.65 to 3.28, with dry noodles fortified with 6.5% *A. platensis* receiving the highest grade (Dinda *et al*., 2022) Samples strengthened by *A.*

platensis were found to be substantial differences in the look, taste, colour, odour, hardness, and overall acceptability (Youssef *et al*., 2020)

Chemical analysis done by the determination of phenol and flavonoid contents in the sample. Samples having *A. platensis* have more phenol and flavonoid content than the control. Phenol and flavonoid content add its bioactivity to the consumers through the food.

The high protein content in both samples containing *A. platensis*. Due to the inclusion of *A. platensis* biomass, the protein and fibre values for both types of flour demonstrated higher nutritional enrichment. Given the parameters examined, the presence of 10% biomass will significantly alter the situation, conferring functional features (Ailton *et al*. 2012). The proximate analysis shows that the moisture content is higher in the control compared with samples containing organism. It will provide the F1 and F2 sample high shelf life and less vulnerable to fungal infections. On basis of total ash concentration tested on three samples it is found that the samples containing *A. platensis* have more ash content concentration compared to the control. There is an increase in ash content by the addition of *A. platensis* because the ash content of dried noodles without the addition of *A. platensis* is 1.37% and dried noodles with the addition of 9% *A. platensis* is 2.46%. Based on this finding, adding paste made from *A. platensis* caused a noticeable change in the dried noodle's ash level. An essential aspect of dietary nutrition is ash content. Ash content indicates an inorganic substance that is produced when an organic material burns. The high mineral content of *A. platensis* contributed to the high result of ash content. Protein has a crucial role for the production enzyme, antibody and hormones it also contains essential amino acids for the growth. The dried noodle protein content significantly changed with the addition of *A.platensis* paste. 55-75% percent protein is present in dried *A. platensis* (Agustini *et al*. 2015).

To assess the fermentative and aromatic capabilities, LAB (L. Casei 2240 and L. Rhamnosus GG) was used to ferment *A. platensis* biomass that had undergone UV treatment or sterilisation at 121 °C. The applied treatment had no effect on the LAB growth, indicating that *A. platensis* is a fermentable substrate that can be exploited to create new fermented foods and supplements with significant functional benefits (Martelli *et al*., 2020). Also, fermented *A. platensi*s shown no harmful bacterial presence

and had a lower pH, indicating an extension of its shelf life. As a result, fermented *A. platensis* shown the potential to be employed as a food additive or as a nutritional supplement.

CHAPTER 6

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

The goal of this study was to determine how different *A. platensis* at different concentrations affected the physical, chemical, and sensory qualities of dried noodles. The microalgae *A. platensis* is inoculated and cultured in F/2 medium. The culture is allowed to grow in the medium for the further experiments. Chlorophyll content, salinity, pH and cell count is checked at intervals of 3 days. pH value was 8 using pH indicator paper and the salinity was measured as 20 using refractometer. On 15^{th} day maximum growth is shown and extracted for further experiments. The culture is centrifuged and made in to pellets. Pellets are then dried to get powdered form. Three samples are taken, of which two are samples with microalgae and the third served as control (F1, F2 and F3 respectively). F1 had 3g and F2 ad 6g of alga. Ingredients of each sample are mixed well, made into dough and then to sheets after which they are passed through noodle extruder to get noodles. Cooking properties of three noodle samples are analysed by measuring the initial and final weight of noodles before cooking and after cooking. The results shown that both water absorption and swelling volume is higher in samples containing microalgae. Sensory evaluation of the noodle is done by providing a 9-point scale with different parameters to a panel. They were asked to evaluate the three samples based on their colour, texture, appearance and taste. The panellists were given satisfied result that the algae containing samples have as much as sensory quality with that of the control. The presence of microalgae made the F1 and F2 samples attractive than the control. As the *A. platensis* improves the water absorption capacity the F1 and F2 samples were soft compared to the F3. Appearance scored more for the noodle containing microalgae. Comparing three samples the samples containing microalgae got overall acceptance to the control.

Various chemical analysis is conducted to check whether the chemical properties of *A. platensis* is stable or not. Phenolic and flavonoid contents are determined using spectrophotometer by taking the O.D value. Phenolic and flavonoid content is determined by considering the O.D. value, mass and volume taken. The final values reveals that the F1 and F2 samples contain phenolic content and flavonoid content in adequate amount.

Proximate analysis includes the detection of moisture content, protein content, crude fat content and total ash content in three samples (F1, F2 and F3). Results shown that the moisture content, crude fat content and total ash is somewhat similar in three. The crude protein content is higher in both samples containing *A. platensis* as compared with that of control. In dry noodles, the higher the *A. platensis* fortification content, the higher will be the elongation factor and tensile power. The test conducted proves that the normal noodle commonly used can be improved in protein content by adding the *A. platensis* powder as an ingredient.

Algae are easily available and with less side effects thus they are more convenient to include in the diet. Proper understanding about the growth requirements of *A. platensis* will lead to mass cultivation. People prefer green- bio product than the chemical products because they can be recycled and improved. As *A. platensis* is rich in protein, they can be incorporated with so that to promote a healthy diet. *A. platensis* content can be added in different food forms such as energy bars smoothies etc. Studies reveal that having *A. platensis* will prevent the metabolic problems. It reduces high blood pressure, cholesterol and are source of antioxidant, anti- inflammatory, anti-cancerous etc. So, the microalgae can be used to meet the food necessity of present and future generations. *A. platensis* have many more applications other than the food supplement. *A. platensis* has the potential to be a bioactive component in the creation of safe and effective cosmetics. It has peptides, polyunsaturated fatty acids, vitamins, minerals, and antioxidant phytonutrients are just a few of the components that work in concert to give appropriate healthy formulation. *A. platensis* is also being used as a biodegradable packing material. It is also used for medicinal purposes. Hence, the study species *A. platensis* can be incorporated in noodles and can serve as a good source of nutrition to the consumers.

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ABBREVIATIONS

APC	:	Allophycocyanin
BLAST	:	Basic local alignment search tool
CTAB	:	Cetyltrimethylammonium bromide
DMSO	:	Dimethyl sulfoxide
EDTA	:	Ethylenediamine tetraacetic acid
GLA	:	Gamma linolenic acid
MAE	:	Microwave- assisted extraction
O.D.	:	Optical density
PDT	:	Photodynamic therapy
PEF	:	Pulsed electric field
QE	:	Quercetin equivalence
rRNA	:	ribosomal ribonucleic acid

APPENDIX

APPENDIX 1: F/2 MEDIA

$NaNO_3$	:	1 ml
Na_2HOP_4	:	1 ml
Vitamin solution	:	0.5 ml
Trace metal solution	:	1 ml

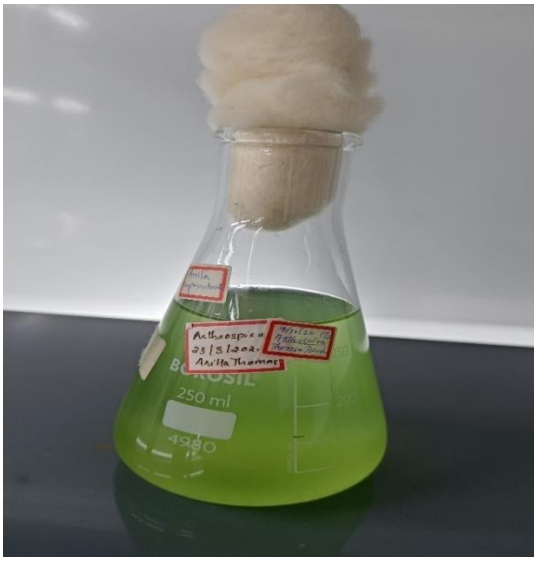
APPENDIX 2: CTAB BUFFER

1M Tris HCl	:	1.576 g
4M NaCl	:	2.3376 g
05 M EDTA	:	1.8612 g
2% CTAB	:	0.2 g

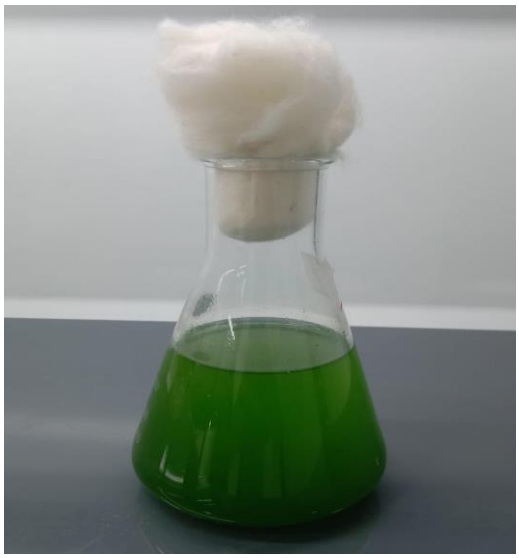
APPENDIX 3: PREPARATION OF MASTER MIX

Molecular biology grade water	:	15µl
10X assay buffer	:	2.5µl
Template DNA	:	1µl
Forward primer	:	0.5µl
Reverse primer	:	0.5µl
$MgCl_2$	:	2.5µl
DNTPS	:	2.5µl
Taq DNA polymerase	:	0.5µl

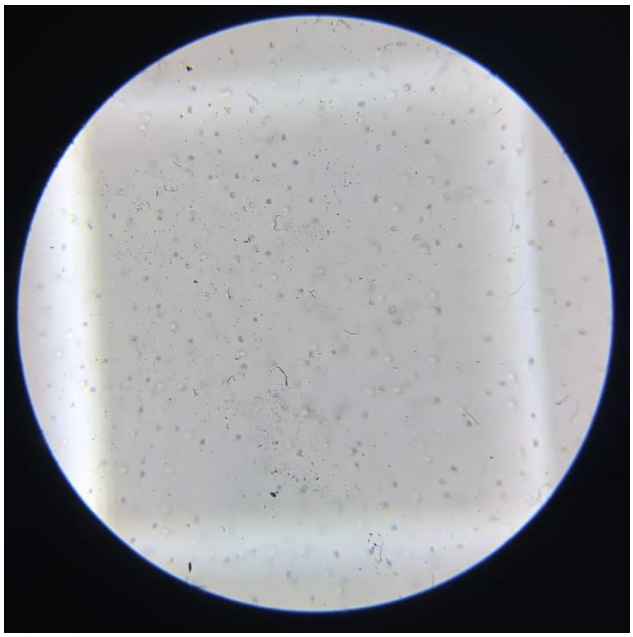
PLATE – I



a) Microalgal culture- first day of inoculation

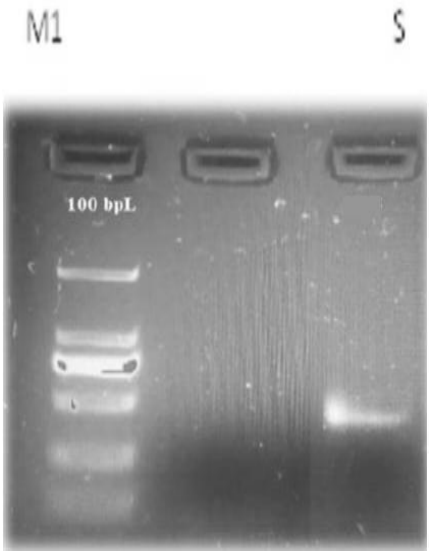


b) Microalgal culture- 15th day of inoculation



c) Microscopic observation of *Arthrospira platensis* using haemocytometer

PLATE – II



Lane (M1): 100 bp marker.

Lane (S1): 16s rRNA PCR amplicon of DNA

a) An Ethidium bromide stained 2% agarose gel showing 16s rRNA PCR amplificationof DNA.

>AF527460.1 Spirulina platensis 16S ribosomal RNA gene, partial sequence
GGAAACTTCTGCTAATCCCGGATGAGCCGAAAGGAAAAGATTTATCGCCGGGAGATGAGCTCGCGTCTGA
TTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGCAA
GCCTGACGGAGCAAGACCGCGTGGGGGAGGAAGGCTCTTGGGTTGTAAACCTCTTTTCTCAAGGAAGAAC
ACAATGACGGTACTTGAGGAATAAGCCTCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGAGGC
AAGCGTTATCCGGAATGATTGGGCGTAAAGCGTCCGTAGGTGGCTGTTCAAGTCTGCTGTCAAAGACAGT
GGCTTAACTACTGAAAGGCAGTGGAAACTGAACAGCTAGAGTACGGTAGGGGCAGAGGGAATTCCCGGTG
TAGCGGTGAAATGCGTAGATATCGGGAAGAACACCGGTGGCGAAAGCGCTCTGCTGGGCCGTAACTGACA
CTGAGGGACAAAGCTA

b) *Arthrospira platensis* 16S ribosomal RNA gene partial sequence

PLATE - III

BLAST search

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Arthrospira platensis spk 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1064	1064	100%	0.0	100.00%	576	MK839189.1
Arthrospira platensis pk 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1064	1064	100%	0.0	100.00%	576	MZ215785.1
Arthrospira platensis PV14 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1064	1064	100%	0.0	100.00%	576	MW042889.1
Spirulina platensis 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1064	1064	100%	0.0	100.00%	576	AF527460.1
Arthrospira platensis BEA1257B 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1050	1050	100%	0.0	99.48%	1476	MT426015.1
Limnospira fusiformis SAG 85.79 chromosome, complete genome	Limnospira fusif...	1050	2100	100%	0.0	99.48%	6423694	CP051185.1
Limnospira fusiformis PMC 917.15 16S ribosomal RNA gene, partial sequence	Limnospira fusif...	1050	1050	100%	0.0	99.48%	1321	MF579877.1
Limnospira fusiformis PMC 894.15 16S ribosomal RNA gene, partial sequence	Limnospira fusif...	1050	1050	100%	0.0	99.48%	1321	MF579875.1
Limnospira fusiformis PMC 851.14 16S ribosomal RNA gene, partial sequence	Limnospira fusif...	1050	1050	100%	0.0	99.48%	1321	MF579872.1
Arthrospira sp. TJSD092 chromosome, complete genome	Arthrospira sp. T...	1050	2100	100%	0.0	99.48%	6434389	CP028914.1
Arthrospira sp. PMC738.11 16S ribosomal RNA gene, partial sequence	Arthrospira sp. ...	1050	1050	100%	0.0	99.48%	1306	KX840361.1
Arthrospira sp. PMC737.11 16S ribosomal RNA gene, partial sequence	Arthrospira sp. ...	1050	1050	100%	0.0	99.48%	1306	KX840360.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT4B_B10	uncultured bacte...	1050	1050	100%	0.0	99.48%	805	LT800456.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT4S_B2	uncultured bacte...	1050	1050	100%	0.0	99.48%	998	LT800335.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT2W_H9	uncultured bacte...	1050	1050	100%	0.0	99.48%	1027	LT800231.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT2W_C12	uncultured bacte...	1050	1050	100%	0.0	99.48%	1064	LT800197.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT1W_H2	uncultured bacte...	1050	1050	100%	0.0	99.48%	682	LT800183.1
Arthrospira platensis IPPAS B-256 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1050	1050	100%	0.0	99.48%	1486	KX262886.1
Arthrospira sp. 'Nigrita M1' 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic s...	Arthrospira sp. '...	1050	1050	100%	0.0	99.48%	1844	KU605609.1
Uncultured bacterium clone HF33 16S ribosomal RNA gene, partial sequence	uncultured bacte...	1050	1050	100%	0.0	99.48%	1453	KR188909.1
Arthrospira platensis RRGK-AP 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1050	1050	100%	0.0	99.48%	1340	KT250929.1
Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1050	1050	100%	0.0	99.48%	1481	KJ463625.1
Uncultured bacterium partial 16S rRNA gene, clone TOWC-20	uncultured bacte...	1050	1050	100%	0.0	99.48%	1010	LN650529.1
Arthrospira platensis DM06 16S ribosomal RNA gene, partial sequence	Arthrospira plate...	1050	1050	100%	0.0	99.48%	640	ON856535.1

PLATE – IV

Graphic summary of alignment score

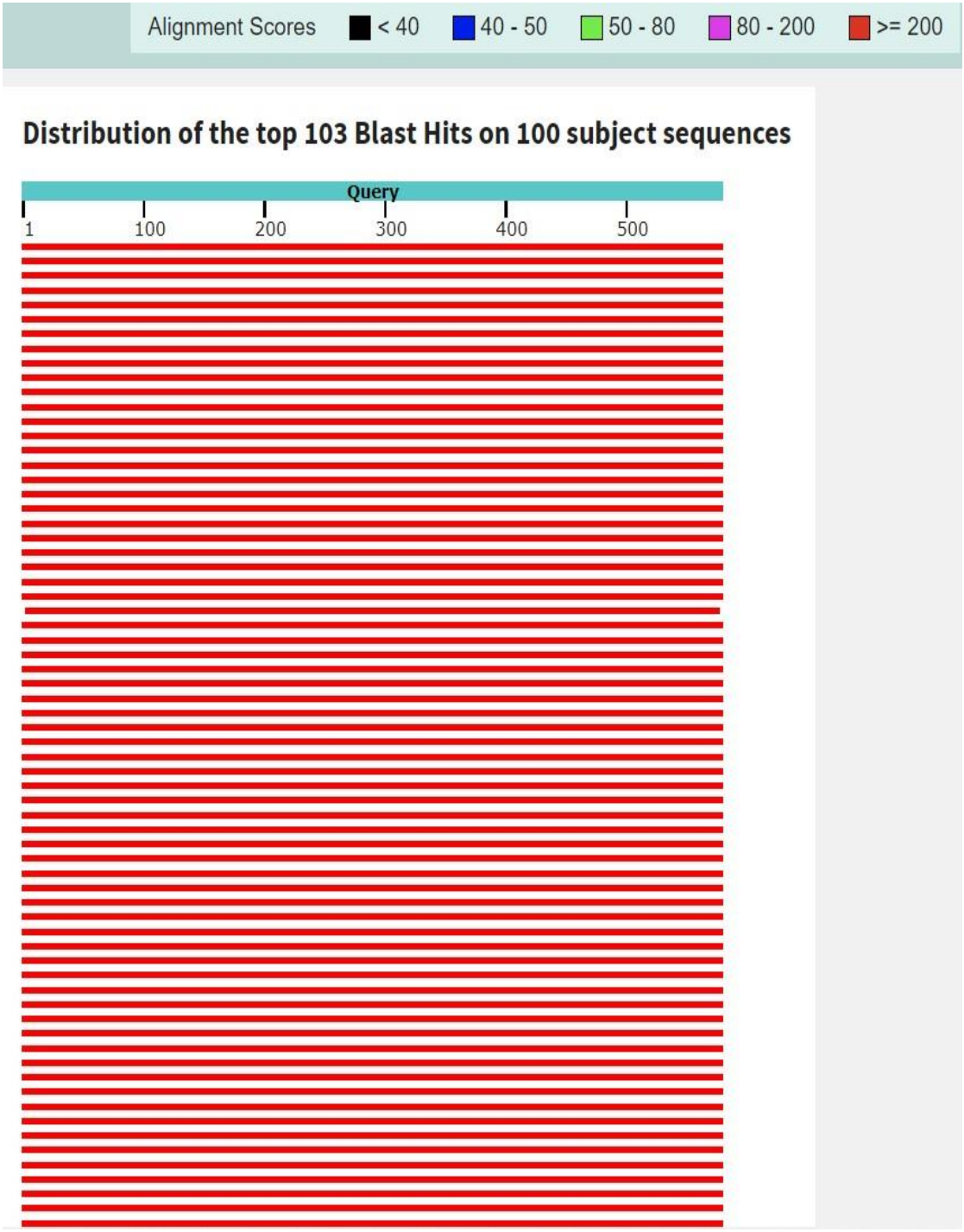


PLATE – V

Phylogenetic tree

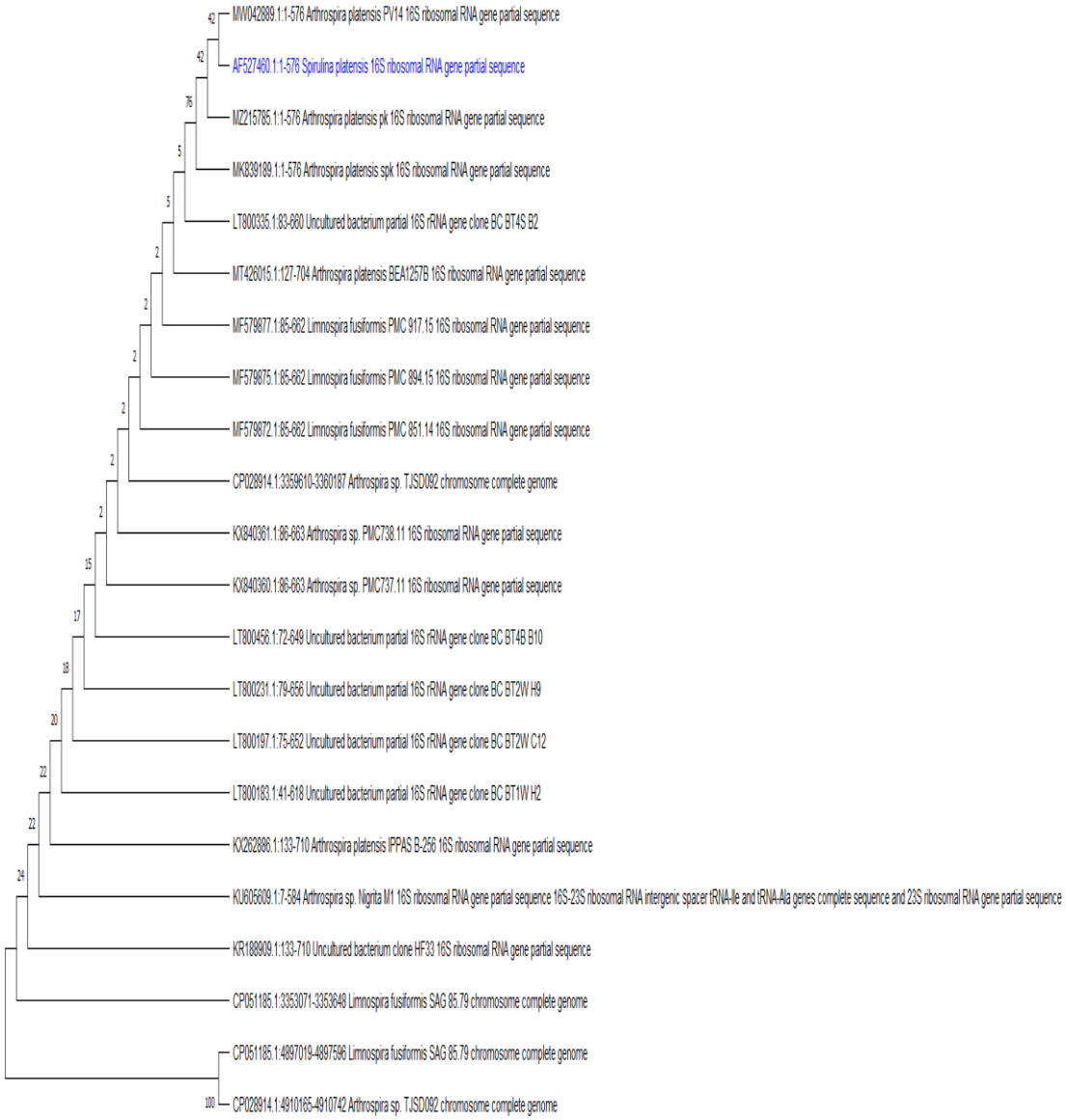


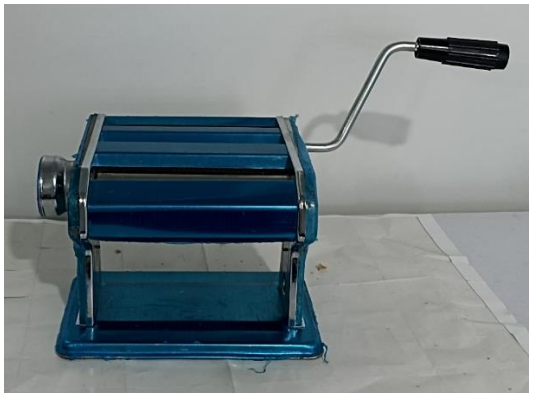
PLATE – VI

F1 Sample

Ingredients

Rounded dough

Dough sheet



Noodle extruder machine

Sample noodle

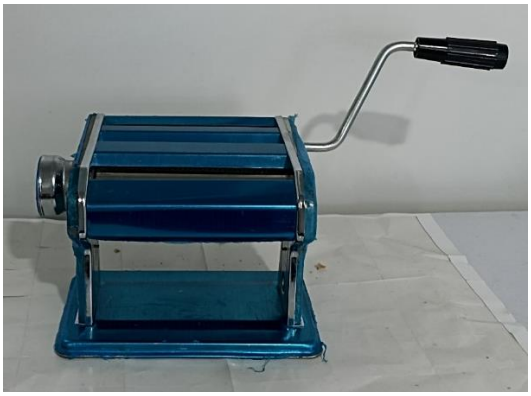
PLATE – VII

F2 Sample

Ingredients

Rounded dough

Dough sheet



Noodle extruder machine

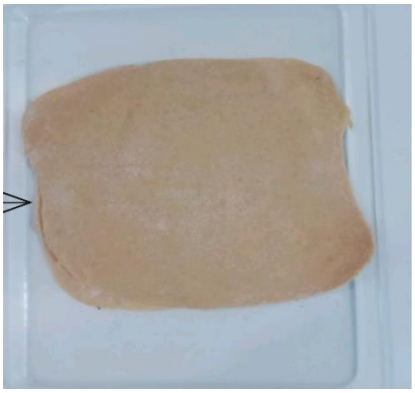
Sample noodle

PLATE – VIII

F3 Sample

Ingredients

Rounded dough



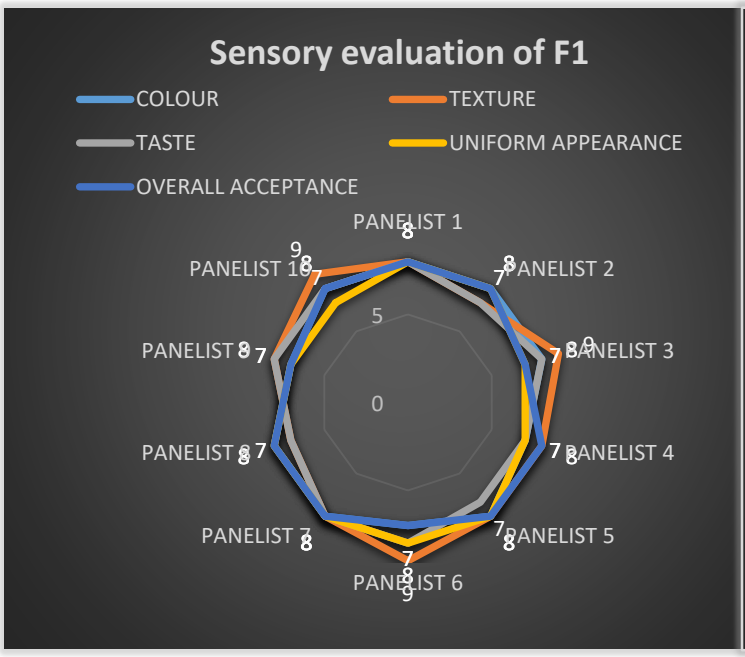
Dough sheet

Noodle extruder machine

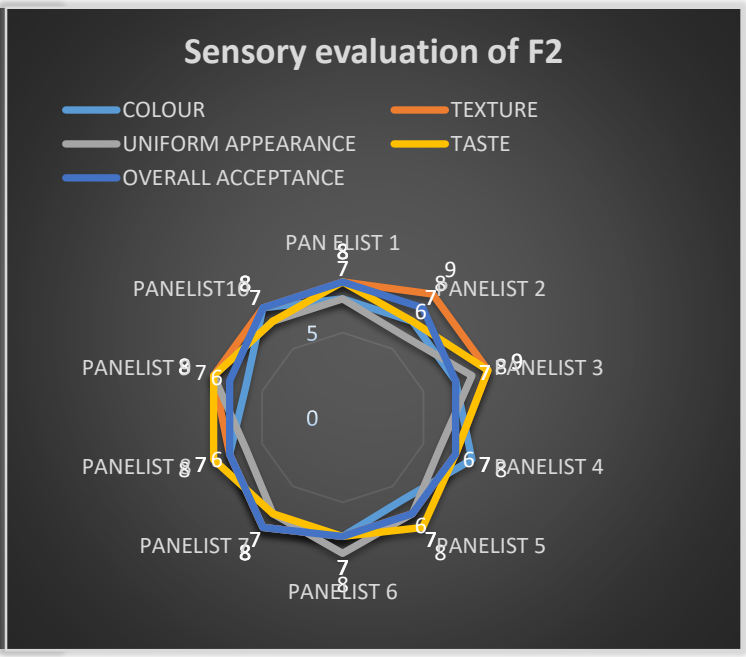
Sample noodle

PLATE – IX

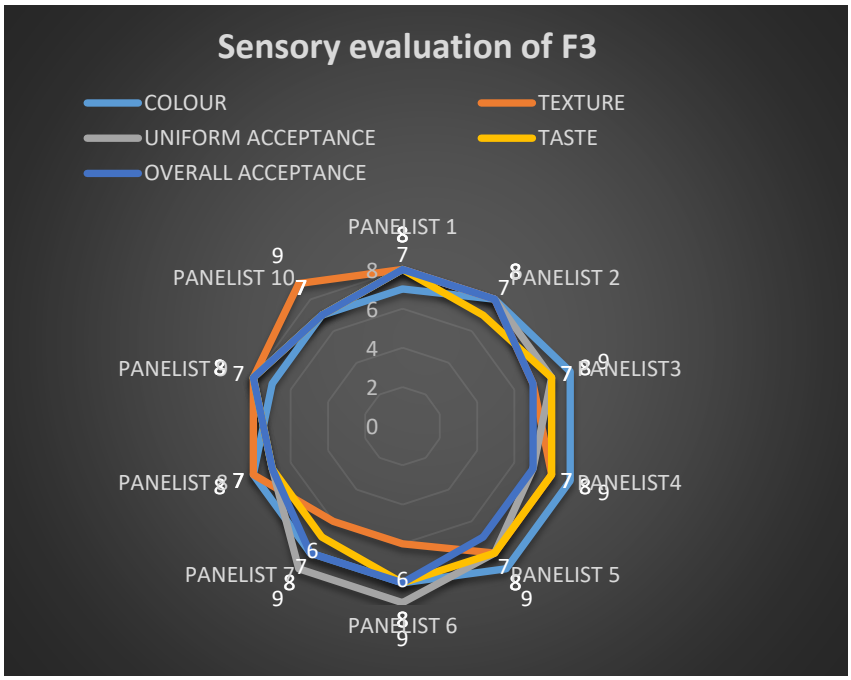
Sensory evaluation



a) Sensory evaluation of F1



b) sensory evaluation of F2



c) Sensory evaluation of F3

PLATE – X

Dried noodles

a) F1 Noodles

b) F2 Noodles

c) F3 Noodles