

**APPLICATION OF GREEN ADDITIVE FROM SELECTED SEaweEDS TO
INCREASE THE SHELF LIFE OF THE SELECTED FOOD PRODUCT**

**NITYASHREE N
(21PFD018)**

A thesis submitted to



**Avinashilingam Institute for Home science and Higher Education for Women,
Coimbatore - 641043**

**In partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE IN FOOD SERVICE MANAGEMENT AND
DIETETICS**

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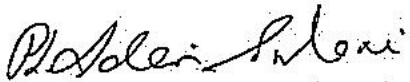
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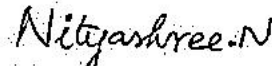

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DECLARATION

This is to certify that the thesis entitled "Application of Green Additive from Selected Seaweeds to Increase the Shelf Life of the Selected Food Product" submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for the award of degree of Master of Science in Food Service Management and Dietetics is a record of original work done by Ms. Nityashree. N with Register Number 21PFD018 during the period of this study under the supervision and guidance of Dr. PL. Sridevi Sivakami, Associate Professor, Department of Food Service Management and Dietetics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore - 641 043, and it has not been submitted for the award of any Degree/ Diploma/Associateship/Fellowship etc., of any other University or Institute.



SIGNATURE OF THE SUPERVISOR



SIGNATURE OF THE CANDIDATE

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Introduction

I. INTRODUCTION

Food additives are compounds that are purposefully added to food to maintain the nutritional worth of food and to improve the product's quality, that includes shelf-life, appearance, flavor and composition. While preparing the food, substances of natural or synthetic origin that are often of minimal nutritional value are added in modest doses (Silva and Lidon, 2016)

The food sector is extremely concerned about microbial food deterioration. According to estimates, microbial activity causes up to 25 percent of food wastage after harvest. Although the development of spoilage microbiota in food poses no threat to human health, it has a detrimental effect on the finished goods overall quality, shelf life and textural attributes. It also influences customer preferences and causes considerable economic losses. As a result, for the contemporary worldwide food production, prevention or suppression of microbial development in foods is of paramount importance (Bondi *et al.*, 2017)

Natural food preservatives can be used in foods for great safety. They are often derived from sources that include plants, animals and microorganisms. Preventing the growth of unwanted bacteria is the primary use of natural preservatives in the food business. It is feasible to include plant antimicrobials into packaging, add them to food surfaces or incorporate them into product composition (Teshome *et al.*, 2022)

Additives are the most effective for a longer shelf life and prevent the loss of some essential amino acids and some vitamins, as well as delaying or stopping bacterial growth, also suppressing reactions when food is exposed to oxygen or heat. Additives enhance the flavors and colors of food. There are some negative consequences of utilizing chemicals for preservation, such as the possibility of adverse effects. Sulfites, a typical preservative found in many fruits and related products causes headaches, palpitations, allergies and even cancer as side effects. Nitrates and Nitrites are used to cure meat products. It is believed to cause stomach cancer since it converts to nitrous acid when ingested. Foods are preserved using benzoates, an antibacterial preservative that has been linked to allergies, asthma and skin rashes. As an antibacterial preservative, sorbates and sorbic acid are added to foods. While sorbate reactions are uncommon, urticaria and contact dermatitis have been reported. Moreover, nuclear radiation used to preserve food does not render food radioactive but may modify the color or texture of food (Sharma, 2015)

Artificial preservatives address some of these issues by extending the freshness of foods, but they may also have unfavourable side effects. To stop botulism, sodium nitrite is a preservative added to lunch meats, hams, sausages, hot dogs and bacon. Sodium nitrite performs the crucial task of preventing the growth of the bacteria that cause botulism, but it can also interact with proteins or create carcinogenic N-nitrosamines when food is cooked at high temperatures. \ Sodium benzoate use may also result in hyperactivity. The possibility that preservatives and other food additives might result in hyperactivity (Mirza *et al.*, 2017)

Preservatives, nutritional additives, flavoring agents, coloring agents, texturizing agents and miscellaneous additives are the six main categories into which additives can be divided. Several additives typically fulfill more than one role in meals. Depending on their frequency, origin and role in food, additives are categorized in a variety of ways. Some of these ingredients, such as the sweetener and the colors annatto and curcumin are naturally occurring. Aluminum silicate, amino acid compounds, ammonium carbonates, sodium nitrate, propyl gallate, butylated hydrozyl toluene (BHT), butylated hydroxyanisole (BHA), monosodium glutamate, white sugar, salt, potassium bromate, potassium sorbate and sodium benzoate are a few of the frequently used food additives, also recognized preservatives. Erythrosine (red), cantaxanthin (orange), amaranth (Azoic red), tartrazine (Azoic yellow) and annatto bixine (yellow orange) are a few of the coloring agents which are artificially produced and causes allergy for some consumers (Alemu, 2022)

The use of food additives is only acceptable when it does not deceive customers, there is a technological necessity for it and provides well defined technological function, such as to promote the stability of the food or protect the nutritional integrity of the meal (Awuchi *et al.*, 2020)

Seaweeds are a fantastic source of chemicals that are ideal for use as functional additives. Algae offer the potential to be incorporated into foods like meat products and cereal based products to improve its functional and nutritional properties. The incorporation of seaweeds into food products improve the sensory and nutritional quality by enhancing functional proteins and polyunsaturated fatty acids. It has been included into a variety of foods including pasta, meat, fish, dairy and bread. Given the plethora of knowledge about the useful characteristics of seaweed and seaweed extracts, it will be fascinating to examine how successful these substances have been when they have been added to a variety of food products (Ainsa *et al.*, 2022)

More than 386 species of marine seaweeds have been identified in Malaysia and several Malaysian seaweeds have been discovered to exhibit potential antibacterial, antioxidant and antiviral activities (Yap *et al.*, 2019). Particularly in Asian nations, where 90 percent of global output comes from, several algae species are utilized as food for both people and animals. Due to their abundance in vitamins, minerals, proteins, fibers and functional substances, they stand out as a distinct diet (Ferrara, 2020)

A possible source of the wholesome meals with enhanced nutrition needed to feed the world's expanding population can be achieved through seaweed. They can be added to diets as salads, wholesome snacks, sushi wraps, gelatin and additives to enhance the nutritional profiles, functions and tastes of common meals (Nor *et al.*, 2020)

It is well known that seaweeds are abundant in both primary and secondary metabolites. The primary metabolites can be divided into several classes, including carbohydrates like fucoidan, alginate, laminarin in brown seaweeds, agar, carrageenan in red seaweeds, ulvan in green seaweeds and lipids. Seaweeds contain minerals like microelements, macro elements and trace elements, in addition to the main metabolites, which are necessary for their survival in seawater (Alipour *et al.*, 2019)

Seaweeds naturally contain more of B complex vitamin like vitamin B1, B2, B3, B6, B9 and B12. Vitamin C and fat-soluble vitamin like A, D, E and K are present in seaweeds (Vinothkumar *et al.*, 2015). It is well known that seaweeds are a magnificent source of natural and bioactive substances that include polyphenols, vitamins, polysaccharides, peptides and fatty acids with a variety of functional characteristics and structural variations that provide living things with a wide range of health benefits (Shafay *et al.*, 2021)

The dry bulk might include up to 36 percent of mineral content. Hence, eating seaweed can aid in getting the required daily intake of minerals, such as iodine. Because of its high mineral concentration, seaweed has a salty flavor (Slegers *et al.*, 2021). Seaweeds are used for their functional advantages beyond their nutritional value and are extensively advertised as "functional foods" or "nutraceuticals" (Thiviya *et al.*, 2022)

Agar, agroses, alginate and carrageenan are the four mainly used seaweed polysaccharide isolates. In the food sector, these polysaccharide isolates are frequently employed for a variety of functions, including stabilizing and gelling agents to maintain the structure of food. The

usage of agar depends on its purity; low-quality agar is used in the industrial manufacturing of many culinary items, including ice cream, fruit juice, sweets and baked goods. Three major kinds of carrageenan utilized in commercial applications are kappa, iota and lambda. Due to its thickening and suspending capabilities, carrageenan is primarily utilized in milk-based goods such as chocolate, ice cream, dessert gels and evaporated milk. Carrageenan can also behave as aqueous gels, which are crucial for the creation of juices, jelly candies, fruit gels and marmalades (Kumarasinghe *et al.*, 2022)

The biological activities of seaweeds have been the subject of several investigations in recent years and they have been suggested as possible sources of natural antioxidants. At various locations around our nation, several academics looked into the antibacterial properties of marine algae. Recent years have seen a huge increase in the usage of seaweeds for a variety of purposes, including antimicrobial, antiviral, antifungal, anti-allergic, anti-coagulant, anti-cancer, antifouling and antioxidant effects (Kausalya and Rao, 2015).

The bioactive substances extracted from algae have increased interest in seaweeds as a food supplement. Phycobiliproteins are a kind of protein. Beta-carotene and fucoxanthin are examples of carotenoids. Important minerals such as calcium, iron, iodine, magnesium and potassium and polyunsaturated fatty acids (primarily ω -3 fatty acids) compose the intriguing group of chemicals. These chemicals, which were extracted from seaweeds, have been investigated for their potential to treat a variety of pathologies and illnesses that directly impact people, including cancer, diabetes, metabolic disorders, osteoporosis, neurodegenerative diseases and cardiovascular diseases (Cotas *et al.*, 2020).

Instead of using complete sources of macronutrients, there has been a significant increase in interest recently in using seaweed as a source of functional components. The phenolics found in seaweed serve as complementing substitutes for aquaculture uses synthetic additives with a wide range of bioactive qualities, including antibacterial, antiviral, antifungal, anti-stress, antioxidant, anti-inflammatory and hunger stimulants (Gunathilake *et al.*, 2022).

Seaweeds have long been used as a food source in a variety of ways including raw ingestion, incorporation into meals that include meat, dairy, baked goods, confections, space foods and low-calorie dietetic foods. In the dairy sector, seaweed derived hydrocolloids are widely used and the inclusion of carrageenan gives a quality improvement that is noticeable even in relatively tiny quantities. Alginate, carrageenan and agar have also long been used as gelling,

thickening, emulsifying, stabilizing and restructuring agents because of their unique qualities. The commercial worth of seaweed derives from uses in the nutraceuticals and pharmaceutical sectors in addition to its widespread usage in cuisine (Pandey *et al.*, 2023).

Seaweeds has been suggested as a food source that would secure future food security since it provides a diversified supply of raw materials for the production of foods, drinks, cosmetics, fertilizers and chemicals (Ristivojević *et al.*, 2021).

The production of the crucial agar, which is extensively utilized as a growth medium for microorganisms and other biotechnological processes use red algae *Gelidium*, *Gracilaria* and *Pterocladis*. It is generally regarded as safe (GRAS), which authorizes their use in food items. Even at low concentrations, this component, which is used to make jellies and fruit sweets, form a hard gel in water at ambient temperature without the addition of potassium and calcium salts (Penalver *et al.*, 2020).

With 287 species, the genus *Gracilaria* is both the most numerous and the biggest in the Rhodophyta category of macroalga. Given its richness, this genus serves as a valuable source of agar to the food industry, selling for 246 million USD which requires generation of 14,500 tons of agar per year (Deepika *et al.*, 2022).

Red algae (*Rhodophyta*) belonging to the genus *Gracilaria* are renowned for their commercial value as agarophyte and for being used as food by humans and numerous shellfish species. *Gracilaria* is frequently consumed as a salad or uncooked for dietary and therapeutic reasons, creating demand for manufacturing of agar (Andriani *et al.*, 2016). Moreover, *Gracilaria* is a rich source of soluble and insoluble dietary fibre, making it a possible substitute for cereal based fibre in Western nations. *Gracilaria* have a mineral concentration that is 10 to 20 times higher than that of terrestrial plants because they can concentrate minerals from saltwater. Hence, they serve crucial nutritional roles in human nutrition and are a great source of minerals (Rosemary *et al.*, 2019).

Together with improving the nutritional benefits, adding seaweed to food also transmits its technical capabilities. The behaviour of food components during food processing is described by their techno-functional qualities, such as viscosity, foaming, gel-forming ability, emulsion-forming ability, plasticity, dough-forming ability and gelatinization. The compositional, conformational and physicochemical characteristics of the three main food components such as protein, fat and fibre as well as the manufacturing technique used to prepare the meal,

determine the functional aspects of a food. These characteristics influence how food components behave during preparation and cooking, which in turn impacts the final product's quality (Kumar *et al.*, 2023).

Due to consumer's awareness on the link between nutrition, health and illness, functional component and nutraceutical research, development and commercialization have increased on a global scale. Consumers from many nations are drawn to items that include seaweed as they are healthier. The general acceptance of seaweeds as a possible source of proteins is extremely high as a result of the rapidly growing public understanding of their excellent nutritional benefits. Making functional meals with seaweed as a foundation or concentrating protein isolates from extracted seaweeds are two ways to encourage the intake of seaweeds (Raja *et al.*, 2022).

In recent years, extracts from plant and marine sources have replaced synthetic preservatives such as benzoic acid, nitrite and sulphur dioxide in food products. Natural marine algae called seaweeds have numerous qualities that can be used as a natural preservative to increase the shelf life of perishable goods without having any negative side effects (Ramani and Murugan, 2020).

The use of natural antimicrobials as food preservatives can aid in preventing the certification of microbiological safety by excessive physical processing of food, which commonly modifies the organoleptic qualities of food. Consequently, discovering natural antimicrobials, employing them, designing them for safety and efficacy are significant objectives in the development of food and medicine (Karmakar *et al.*, 2022).

Seaweeds have the potential to be renewable sources of bioactive substances with a range of beneficial benefits. Bioactive secondary metabolites that were extracted from different seaweeds have been shown in several studies to have the potential to be employed as antibacterial agents (Hafez *et al.*, 2022)

Recent studies on seaweeds polysaccharides-based packaging revealed that alginate and carrageenan-based film or coating for meat and its products can prevent shrinkage, microbial contamination, oxidative changes, surface discoloration by delaying the transport of moisture and oxygen. These studies also revealed that these materials are effective for maintaining postharvest quality of fruits like tomatoes, cherries, berries, etc. by delaying the ripening and storage life. Alginate or essential oil preservation coatings with water repellent, gas barrier properties might stop the quality deterioration in fresh cut fruits and processed meat kinds.

Combinations of carrageenan or fruit seed extract, carrageenan or organically modified nano clay were created to extend shelf life and retain quality attributes (Perera *et al.*, 2021).

Algae polysaccharide films are frequently fragile, lack flexibility and are not robust enough to be used as edible films for commercial purposes. The concentrations of the polymer, additives, the spreading of the film solution, its density, viscosity and surface tension all have an impact on the thickness of the film, which in turn affects the biological characteristics and shelf life of food goods (Trindade *et al.*, 2022).

The possible bio preservative qualities of algae on seafood have been noted by several researchers, with the main goal of increasing the shelf-life of fisheries products while simultaneously preventing the growth and presence of hazardous compounds such biogenic amines. Consuming seafood maintained under circumstances that encourage the growth of biogenic amines is linked to negative consequences, including death and poses a major health risk to people (Arulkumar *et al.*, 2020).

Seaweeds have a number of qualities that allow them to preserve foods naturally without degrading their quality or having negative consequences (Umashree *et al.*, 2023) Concerns have been raised about the spread of antibiotic resistance genes (ARG) and the development of antimicrobial resistance (AMR) in bacteria as a result of the increased use of antimicrobials. These issues may make it more difficult to successfully treat bacterial infections (Blikra *et al.*, 2021).

Interest in brown macroalgae has been extensively discussed due to their unique composition in polyphenols like phlorotannins, polysaccharides like fucoidans, pigments like fucoxanthin, which have been widely documented to be active against pathogenic and spoilage bacteria, albeit the antibacterial action from pure extracts is not often observed (Rubino *et al.*, 2022)

Seaweeds have acquired a few antibacterial qualities. It is believed that acceptable methods exist to stop the spread of disease resistance to traditional antibiotics. In several publications that have been published, seaweeds have been suggested as potential sources of antimicrobial chemicals that fight illnesses. The discovery of bioactive natural compounds and the creation of cutting-edge antibiotics have both been the subject of numerous studies to date (Gomes *et al.*, 2022).

The production of nanoparticles utilizing green chemistry technologies makes considerable use of seaweeds. Many macroalgae are capable of the effective bioproduction of nanoparticles in a variety of sizes, forms and morphologies that are employed in a variety of industries, notably for antibacterial activity, as well as the removal of contaminants. Comprehensive research has been done on seaweeds to find novel treatment options that have antimicrobial, antiviral and antibacterial properties (Mofeed *et al.*, 2022).

Food firms are seeking for novel compounds to utilize as an alternative option to existing preservatives in order to preserve microbiological food safety and enhance quality in accordance with customer demands for "clean label" goods. According to several investigations, extracts from various edible algae species have antimicrobial efficacy against the primary foodborne pathogens, including *Salmonella spp.*, *L. monocytogenes*, *E. coli*, *S. aureus* and *B. cereus*. Also, a remarkable antibacterial activity against *L. monocytogenes* was demonstrated by an *A. platensis* extract when it was used *in situ*. The observed antibacterial action was not necessarily correlated with the tested seaweed's high variety in phenolic content, highlighting the possibility of a synergistic impact involving other chemicals (Martelli *et al.*, 2020).

Marine algae *U. intestinalis* and *G. fisheri* have been shown to be possible sources of bioactive chemicals that are effective against a variety of human illnesses. These compounds can be employed as natural, non-toxic preservatives and may be more appealing to consumers (Srikong *et al.*, 2015).

The antibacterial activities of macroalgae defined macroalgal extracts made up of a variety of substances with different chemical properties have been extensively studied in the scientific literature. To expand the market and to boost the potential of natural antimicrobials in food, further research is required on the extraction, chemical characterization and inclusion of these promising compounds against foodborne pathogens into actual food matrices. The effectiveness of these substances against various food pathogens must also take into account an assessment of the realistic doses that can be used of these natural products in food without negatively impacting their sensory qualities, as this may have an adverse effect on consumer preference for food containing natural products from macroalgae. In order to comprehend their impact on the storage conditions, shelf-life, quality and health characteristics of novel, fortified food products containing them, it is also necessary to further explore the chemical structure of

seaweed antimicrobials and the structure-function relationship of these molecules (Cabral *et al.*, 2021).

The many geographical regions where fermented foods are produced have a diversity of customs and cultural preferences. Since then, they have been devoured due to their longer shelf life, smaller volume, quicker cooking times and higher nutritional content as compared to non-fermented products. In order to preserve food, fermentation techniques are regarded. In essence, fermentation is the gradual breakdown of organic compounds, which is sped up by bacteria or enzymes. Carbohydrates are basically broken down into alcohols or organic acids or other simpler compounds (Sathe and Mandal, 2016)

Sugars, starches and fibre are the components of carbohydrates. Similar to digestible fibres, gut bacteria in the colon ferment them. The composition of the human microbiome has been examined for a variety of functional potentials, such as an increase in Firmicutes to Bacteroidetes leading to an increase in enzymatic pathways involved in phospholipid metabolism in the gut (Chelliah *et al.*, 2019).

Fermented cereal-based products could be investigated as promising sources of prebiotic compounds because they may contain soluble fibres (glucans and arabinoxylans and their oligomeric products), galacto-oligosaccharides (GOSs), fructo-oligosaccharides (FOSs), resistant starch, phenolics, peptides, depending on the cereal used as a substrate and the extent of fermentation. Fermented cereal products have a significant impact on the microbiota in the human gut. In addition to the prebiotic fibres found in cereals, bacteria utilized in fermentation may also excrete chemical compounds (Tsafrakidou *et al.*, 2022).

Idly is a well known culinary product made from an autofermented batter made of rice and black gram dhal, generally in a 3:1 ratio. Idly originally came from South India, but because of its distinctively soft, spongy texture, sour flavor and alluring flavor, it has rapidly acquired popularity throughout India. It is now a staple of international cuisine. In addition to its organoleptic qualities, it has substantial number of calories, proteins and minerals. The batter is fermented, which results in a higher protein efficiency ratio, more important amino acids, vitamins like niacin, riboflavin and thiamine, as well as less anti-nutrient content than the unfermented (Shrivastava and Ananthanarayan, 2015).

Furthermore, natural fermentation in idly batter increases Vitamin B, amino acids and reduces anti-nutrients like phytates. Idly is traditionally made using a natural (spontaneous) fermentation technique. Micronutrients such as B-vitamins like riboflavin, folate are important

for human growth and metabolism. Fermentation-based biofortification is a useful method for reducing B-vitamin deficiency (Rajendran *et al.*, 2017).

Leuconostoc mesenteroides, *Enterococcus faecalis* and *Pediococcus cerevisiae* are the only heterofermentative bacteria that produce the acid and gas needed for batter leavening, based on the basic study defining the role of microorganisms in the fermentation of idly. Early in the fermentation process, low-acid producers *L. mesenteroides* and *E. faecalis* coexisted with high-acid producers *P. cerevisiae*. Similar to sour dough, the fermentation of idly uses bacteria rather than yeast for the leavening process (Sarma and Gupta., 2022).

When Xanthan is added to the idly batter at a concentration of 0.1%, the texture is enhanced, resulting in higher sensory analysis ratings than when idly are made without additives. Prolonged fermentation and storage lead to the flaw where idly batters begin to collapse. Whey gets separated, giving idly an unfavorable hard feel. The viscosity of the batter can be increased by adding various hydrocolloids, which will enhance the end product's texture (Singh and Saini, 2017).

The aim of this research is to analyse the shelf life of perishable food product with the application of seaweed as a green additive. This study contributes to the evidence that seaweeds have antimicrobial activity and can inhibit the growth of microorganisms, in order to prevent spoilage in food products. This study sought to determine if the formulated additive improved the food product or not by assessing the effect of the additive in the included food product. Hence, the present study was undertaken emphasising on the following objectives

- To formulate a green additive from *Gracilaria edulis*, a red algal species
- To incorporate the formulated green additive into Idly batter
- To analyse the microbial growth in the incorporated product to analyse the shelf life

Review of Literature

II. REVIEW OF LITERATURE

The review of Literature concerning to the thesis topic “**Application of Green Additive from Selected Seaweeds to Increase the Shelf Life of the Selected Food Product**” is reviewed under the following

A. GREEN ADDITIVES IN FOOD INDUSTRY

B. CONSUMPTION PATTERN OF SEAWEEDS AS FOOD PRODUCTS

C. MICROBIAL GROWTH CONTROL IN FOOD WITH SEAWEEDS

D. ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF VARIOUS SEAWEEDS AGAINST FOOD PATHOGENS

A. GREEN ADDITIVES IN FOOD INDUSTRY

For the bio-preservation of meat and animal products, Demir (2021) employed pomegranate and green tea extracts. Three distinct food pathogens were used to gauge antimicrobial activity. The research discovered that meatballs stored at +4 °C retained their chemical and microbiological qualities thanks to the bioactive ingredients (14 days). Before usage, 3 of the 14 samples that were stolen from Sigma-Aldrich in St. Louis, Missouri, USA, were cultured in Tyriptic soy broth and Mueller Hinton Broth. Using a Soxhlet extractor, green tea powder (GTP) and pomegranate peel powder (PPP) were extracted for 24 hours. For the extraction powder sample extraction time and solvent amount were subjected to six distinct circumstances. The utilization of HPLC grade EP and PN in comparison to natural extracts. Salt and ground beef minced meat were used to make the samples. The quality deterioration during storage was postponed for at least 7 days.

Gassara *et al* (2016) explained the use of nitrates and nitrites in the curing of meat products is crucial. They serve as food preservatives as well as fertilizers and explosives. Nitrate's Acceptable Daily Intake (ADI) of nitrate ion is 0–3.7 mg/kg bw/day. Risks to the environment and to human health can result from it. The most common ingredients in curing combinations are salt (sodium, potassium), salts of nitrite and nitrate and spices. In addition to serving as a colour fixative, sodium nitrite also prevents the growth of microorganisms like *Clostridium botulinum*, the causative agent of botulism toxin. The high concentration of salts (including nitrate) used during the curing process is responsible for food preservation. Consumer health may suffer as a result of the usage of nitrites and nitrates as preservatives. There are substances

that prevent nitrite and/or nitrate from producing nitrosamines and nitroamides. In order to prevent the transformation of meat products containing nitrites, these items might be utilized. Ascorbate is used to lower nitrosamine production in meat products. Additionally, ascorbate interacts with "nitrite" and binds the produced NO. Due to their well-known antibacterial and antifungal characteristics, spices are regarded as suitable nitrite substitutes. Since the dawn of time, people have been curing meat in an effort to extend its shelf life. Due of the component's easily fluctuating levels of oxidation, the curing agent's nitrite and nitrate react. Nitrites and nitrates have antibacterial, antioxidant and preservation effects.

Edla *et al* (2022) used holy basil oil and clove oil as bio-additives in formulation of rice bran oil-based cutting fluid using food-grade emulsifiers like Tween 80 and Span 80. Addition of herbal oils is found to improve the oxidative and corrosive stability of the base oil. Performance test on the lathe during the turning operation revealed that the formulated green cutting fluid had shown lower cutting forces when compared with that of the commercial cutting fluid. According to ASTM D 4627, the oil's resistance to corrosion is assessed. Pouring the oil-in-water emulsion over filter paper and 4 g of cast iron chips in a petri dish. It shows the oil has strong corrosion resistance if both the weight loss of the chip and the stain marks on the filter paper are minimal. Consider MRBO as a prospective contender for use in industry.

Leal *et al* (2022) conducted research with *Annona muricata* leaves as antioxidants source to extract bioactive chemicals from soursop leaves, deep eutectic solvents (DES) containing alcohols and sugars as HBD and cholinium chloride as HBA were coupled with ultrasound-assisted solid-liquid extraction (UA-SLE). DES showed a protective effect against the bioactive compound's heat degradation. They were used as an addition in soybean crude oil, which produced an acceptable level of oil preservation. The *Annona muricata L.* (soursop) leaves were procured and vacuum-packed in the dark. The leaves were ground, vacuum-packed with crude soy oil, then oven-dried at 45 °C for 24 hours. The total phenolic content (TPC), antioxidant activity of the extracts and process energy cost were all taken into account while determining the optimal process parameters, which were 45 °C and 2 hours. Finally, taking into account the extracts' significant antioxidant activity and the DES's protective impact, they were used as a natural addition in soybean crude oil, which resulted in a successful preservation of the oil. Natural extracts with antioxidant qualities can be obtained using the Deep eutectic solvents (DES) in conjunction with the ultrasonic technology and used as a natural supplement in the food sector.

According to Pyne *et al* (2022), spirulina nanodots can be utilized as a natural preservative. Food preservatives are crucial to reducing food deterioration and preserving its nutritional content. Traditional chemical preservatives seldom operate as food chemical contaminants while having negative health effects on people. The enormous, singular nano size, the surface area/volume ratio and the anti-microbial characteristics of nanoparticles are only a few of its amazing qualities. Food and beverage shelf life can be extended by spirulina nanoparticles during putative antibacterial and antifungal activity. For the aforementioned and other reasons, further research is needed to determine the ideal strategy for food preservation. To provide the groundwork for next research projects, it is necessary to conduct more in-depth investigations with accurate chemical analysis. *Saccharomyces cerevisiae* and *Lactobacillus plantarum*, two spoilage bacteria, can be prevented from growing in drinks by adding sodium benzoate (SB). According to reports, SB is most effective against yeasts on the first day of storage (reduction of 2–2.5 log CFU) and the rates of microbial growth decrease peaked on the fifteenth day. Organic nanodots generated from spirulina may also be suggested as the optimal food and beverage preservative.

Natural preservatives for prolonging the shelf life of fish were evaluated by Mei *et al* (2019). Due to contamination or the quick microbial growth that occurs naturally in fish, seafood is very perishable. Bacteriocins and bacterial organic acids have demonstrated potent antibacterial properties against spoilage microorganisms. Antimicrobials produced from plants could reduce lipid oxidation and increase fish shelf life. Fresh fish is extremely perishable and although while refrigeration or freezing might lengthen its shelf life, these measures might not be enough to stop bacterial development, rancidity or lipid oxidation. Numerous bioactive properties, such as antibacterial, antioxidant, antiviral, anti-inflammatory and other health advantages, are present in both algae and mushrooms. Algae and mushrooms have typically been evaluated for their antibacterial properties *in vitro*, yielding accurate estimates of the minimum inhibitory concentrations (MIC) for a variety of samples. Pediocins, lacticin, nisin and reuterin are a few of the natural preservatives used to preserve fish. Wood smoke, plant extracts and essential oils are examples of plant-based preservatives. Potential sources of novel natural antibacterial agents can be found in the wide variety of algae and mushroom species

Meena *et al* (2021) investigated on natamycin, also known as pimaricin, an antimycotic polyene macrolide produced by submerged fermentation of actinomycetes strains such *Streptomyces chattanogenesis* and *S. natalensis*. Natamycin is a commonly acknowledged as a

safe component for usage in a variety of food applications and has been utilized as a bio preservative. By exposing natamycin to acid, alkali and hydrogen peroxide for 4 hours, all of its active properties were destroyed. In a fermenting medium with a pure inoculum of *Streptomyces* species, natamycin is made *S. chattanoogensis*, *S. gilvosporeus* and *S. lydicus* are some of the most popular strains. The ideal medium conditions and process control are the main bottleneck. The quality and shelf life of dairy products are greatly improved and maintained by preservatives and food additives. Due to its poor solubility, natamycin has been widely utilized in dairy products all over the world. It is often sprayed to food goods' surfaces to lengthen their shelf lives.

Amiri *et al* (2021) conducted research to improve food security using antimicrobial peptides or in conjunction with other substances. *A. hydrophila*, *B. cereus*, *Enterobacter aerogenes*, *E. coli*, *K. pneumonia*, *Proteus vulgaris*, *S. typhimurium* and *Yersinia enterocolitica* were all suppressed by cranberry extract at a concentration of 15%. Carvacrol and thymol exposure impairs the function of aromatic and phenolic substances. Significant sources of EOs include ginger, oregano, rosemary, sage, thyme and peppermint. EOs contain antioxidants such alcohols, aldehydes, phenylpropanoids, terpenes and ketones. Low oxygen levels, low pH and low temperature can all harm EOs. Flavonoids and other phenolic components are found in herbs and spices, which are bioactive substances. cationic antimicrobial peptides, which guard against bacterial infection, are abundant in mammalian leukocytes. Several of them are capable of quickly destroying the lipid layer that protects microorganism cell membranes. Fish derived pleurocidin and protamine exert antibacterial effects against *G* bacteria. Both kinds of bacteria have been effectively combated by amphibian macrogainins. Antimicrobials are cysteine-rich, cystoietin-rich peptides (molecular weight 2–9 kDa) They have antimicrobial effects against *L. monocytogens*, *S. Typhimurium* and *E. coli* O157:H7. Lactoferrin is an iron-binding glycoprotein with antimicrobial effect on both types of bacteria, such as *Salmonella* and *e. coli* and fungi.

Ritota and Manzi (2020) investigated a study in cheese making with the help of natural preservatives from plant. Natural additives contain antioxidant capabilities that raise the amounts of bioactive molecules and improve the food products' microbiological stability. Because natural preservatives may produce sensory features that consumers may not find appealing, the impact of plant-based preservatives on the sensorial qualities of new goods must be taken into account. Natural compounds with antibacterial properties appear to work by

permeabilizing or rupturing the cytoplasmic membrane. *Penicillium* and *Aspergillus* are typically found in cheese contamination. Spices and herbs can be used as carriers for nutraceuticals in food items. The majority of herbs' and spices' beneficial benefits on a number of ailments may be mediated by their potent antioxidant properties. Some spices, such as black pepper, black cumin and clove, have more antioxidant activity *in vitro* than spicy cheeses do. Due to exposure to heat, enzymes, transition metal ions, oxygen and light during storage, food quality is often sensitive to alterations. Dairy products and other water-oil emulsions are susceptible to oxidative and hydrolytic rancidity. The majority of these substances are what give cheese its characteristic smell and flavour. By delaying changes brought on by microbial growth or maintaining the physical characteristics, chemical makeup and initial nutritional value throughout storage, preservatives can extend the shelf life of cheese.

Putra *et al* (2022) investigated a brown seaweed species as a carrier in natural food preservative. Allyl isothiocyanate (AITC) is a chemical compound in vegetables from Cruciferae with strong antimicrobial activity. It is currently used as an additive to preserve food, but its safety to human health is under discussion. Brown seaweed *Saccharina japonica* was proposed to be a natural delivery matrix for AITC. The porous structure of *S. japonica* changed significantly after SC-CO₂ extraction. A simple green process has shown that high-shaped mesoporous materials can be formed from fresh seaweed. This kind of work has also been demonstrated by Dodson *et al.* to produce shaped materials from a variety of fresh seaweed species. *S. japonica* could be used as a natural and safe alternative carrier for food preservatives in order to avoid chemical contaminant and increase food safety. Processed seaweed adsorbed more AITC compared to the basic seaweed powders. The removal of lipid by SC-CO₂ could enhance the porosity of the processed seaweed powder.

Matsuo *et al* (2022) evaluated citrus peels as natural food additive. Without the use of any pesticides, three distinct *C. natsudaidai* fruits were raised in Japan and gathered every year from May to July. Citrus peels (20 g) that had been ground and frozen were extracted in 200 ml of hexane for 24 hours at room temperature using a magnetic stirrer. The main flavonoids in CN peels were naringin and neohesperidin, both of which had positive effects on health and industry. Peel waste from CN plants may be used to make components and additives for food, medicine and cosmetics. The CN peel extract was identified as a 379 natural food odorant with consumer approval based on a sensory assessment and technical testing.

Novais *et al* (2022) reviewed on natural food colorants and preservatives. The colours used in food up to the middle of the 20th century were all natural, like saffron (obtained from the stigma and styles of the flower) or orcein (extracted from certain lichens). There are now techniques for extracting, purifying, stabilizing and standardizing a variety of natural food colors. The yellow to orange carotenoids bixin and norbixin, which give annatto (E160b) its somewhat redder hue, are derived from the seeds of the *Bixa Orellana* L. tree. Jams, jellies, bread goods, dairy goods and drinks all utilize it. To create colours that botanical sources cannot, phycocyanin (synthetic pigments) can be produced from bacteria, fungus and other organisms like *E. coli*. Food items might deteriorate due to microbiological, enzymatic, physical or chemical processes. Reducing the amount of chemicals in food or substituting new, natural additives for them are two trends that are on the rise. Natural substances are often less dangerous than those made synthetically, although research on toxicity, carcinogenicity and other factors is necessary. The future of food preservation, which allows for shelf-life extension and prevents food loss, is thought to lay with additives of natural origin. Some substances may not mix well with other substances natural or synthetic or with food-related ingredients, which might make them difficult to utilize. Better, safer and more effective natural additives will be made possible by overcoming the restrictions.

Preetha and Iyer (2021) developed a packaging film using carrageenan from red and green seaweed. Sulphated polysaccharide was used to extract carrageenan from red and green *Kappaphycus alvarezii* seaweed, then gelatin, lemon oil and glycerol were added to create films. The film's thickness, solubility, transparency, antioxidant, anticoagulant, antibacterial, cytotoxic and shelf-life characteristics were examined. The film comprises functional groups of sulphate and other added components, according to the FTIR data and other analyses showed that it was totally safe to use as a food packaging material. Fruits and vegetables may be efficiently packaged with it as food, but organic, nontoxic, biodegradable and environmentally friendly film was also created. The films are a good material for food packaging, as shown by the characteristics analysis research of SEM, FTIR, tensile strength and elongation at break, films transparency, antioxidant, antibacterial, anticoagulant and cytotoxicity.

Chaudhari *et al* (2019) discussed the use of nanoencapsulation technology to increase the stability and efficacy of essential oils (EOs) and their bioactive constituents against mycotoxigenics, this paper describes the antifungal and antimycotoxigenic mechanism of action of EOs. It also proposes using them widely to safely preserve food and lengthen its shelf

life while being stored. *Anethum graveolens*, *Mentha Spicata* L. EO, *Thymus vulgaris*, *Syzygium aromaticum* and other mycotoxigenic fungal infections are affected by certain compounds. Different factors, such as decreased ergosterol production and increased cellular ion leakage, have been measured to support the plasma membrane's role as an EO target site. Strong antifungal and antimycotoxigenic potential for EOs and their bioactive components against fungus and Nanoencapsulation technology can assist in overcoming obstacles in large-scale.

Multiwalled carbon nanotubes (MWCNTs) were used to modify a biosensor that Arvand *et al* (2020) presented for the detection of *S. aureus* PTCC 1112, which was inoculated in PBS. Three typical chemical cosmetic preservatives, euxyl (phenoxyethanol:ethylhexylglycerin), benzyl alcohol and benzalib (alkyl benzyl dimethyl ammonium chloride), were compared against three herbal essential oils, tea, bergamot and lemon, for their antibacterial effects. According to the findings, the essential oils reduced the corresponding currents by 93.30% (lemon), 80.49% (bergamot) and 65.83% (green tea) respectively, which is consistent with the inhibitory zones of the microbiological test. This study compares the antibacterial effects of certain herbal essential oils to chemical cosmetic preservatives in order to identify *S. aureus* PTCC1112 using an easy, quick and sensitive electrochemical approach. The findings might lead to a return to conventional herbal medicine practices as a natural alternative for clinical care issues with bacterial infection.

Citrus (*Citrus aurantium*) peel and mint (*Mentha arvensis*) leaf extract's ability to prevent Indian mackerel quality alterations during refrigerated storage was examined by Viji *et al* (2015). LDPE pouches were used to package and store the gutted mackerel, which was then dipped in mint extract (0.5%, w/v) and citrus extract (1%, w/v) before being kept at 0–2 °C. Citrus peel extract was outperformed by mint leaf extract in terms of in-vitro antioxidant activity and phenolic content. Mint leaf and citrus peel were used as the raw materials, which were cleaned in tap water and dried over night at 50 ° in an oven. A shaking water bath at 50 °C was used to extract the powder for 5 hours using 60% ethanol. After cooling, the solution was filtered using Whatman No. 1 filter paper and the extracted again using new solvent. The filtrate was collected and a rotary evaporator was used to evaporate it. 10 cc of water were added to the finished thick solution and evaporated. The mint leaf extract and citrus peel extract were vacuum-dried at 45 °C and kept in the refrigerator for usage.

Rai *et al.* (2016) conducted research on antimicrobial peptides. Animals, plants, insects and microbes all contain a variety of natural proteins known as antimicrobial peptides (AMPs). They may be used alone or in conjunction with other antibacterial, essential oils and polymeric nanoparticles to extend food's shelf life. They have been demonstrated to be an effective substitute for chemical preservatives. Antimicrobial peptides like hiolbiotics, colicin, antibiotic and microcin came from bacteria and are known as peptides from prokaryotes. The majority of antimicrobial peptides are produced by insects and these peptides have been shown to have antimicrobial action against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Aero coccus viridians*, *Micrococcus luteus* and *Bacillus megaterium*. Plants produce cysteine-rich peptides with molecular weight in the range of 2-9 kDa. Food preservation with antimicrobial peptides is promising, but new ones still need to be developed. The only way to prevent the formation of food-spoilage organisms is through bio-preservation, thus it's important to both improve the current antimicrobial peptides and look for new ones. The ability to synthesize new peptides and preserve food using computational understanding of peptide structure may prove to be a useful tool. When considering the investigation of new peptides, the chemical approach of peptide synthesis is advantageous.

B. CONSUMPTION PATTERN OF SEAWEEDS AS FOOD PRODUCTS

Pandey *et al* (2020) researched on seaweeds for its protein source. Seaweeds include bioactive substances with known health advantages, including peptides, minerals, phlorotannins, carotenoids and sulfoxides. Seaweeds have long been used as traditional medicine to treat conditions like goiter, cuts, rashes, inflammation and diabetes. They have also caught the interest of the pharmaceutical industry. A typical seaweed's lipid content falls between 4.6percent in *E. clathrata* and 1.33 percent in *Enteromorpha intestinalis*. According to certain research, *Utricularia rigida* and *Kappaphycus alvarezii* had lipid contents of 12 percent and 1.09 percent, respectively. When compared to the fiber content of higher plants, edible seaweeds have a substantially dry mass content, up to 62 percent. To enhance the quality and lengthen the shelf life of food goods, seaweed isolates are utilized. Seaweeds isolates of the polysaccharide's agar, agroses, alginate and carrageenan are the most popular. Agar is a stabilizing and gelling ingredient that helps preserve food's structural integrity. Because it can chelate metal ions, alginate finds extensive usage in the food and pharmaceutical industries for creating very viscous solutions.

Young *et al* (2022) took research on seaweeds influencing health among young adults. Asian and Pacific Islander tribes have long eaten seaweed. Seaweeds that are edible provide unique nutritional advantages to crops grown on land, especially in terms of mineral and fibre content. For the creation of food products and consumer marketing techniques, it is essential to comprehend the reasons why young Australians consume seaweed. More than half of the young responders involved in the research get their micronutrients from seaweed. Flavour, vitamin content and health benefits were the benefits of seaweed eating that were most frequently mentioned. Iodine is important for many aspects of healthy growth and metabolism, especially during the development of the foetus. In order to allay worries about the under- and overconsumption of micronutrients as a barrier to intake, the nutritional makeup of seaweed products should be listed on the container. Young Australians are requesting more seaweed product marketing and promotion, as well as a replacement for the excessive single-use plastic packaging. Young consumers are encouraged by flavour, nutrient content and health advantages, but they are discouraged by accessibility issues, high prices and a lack of variety in the available alternatives.

Cherry *et al* (2019) had discussed about the risks and benefits of consuming seaweeds. The growing interest in seaweeds has drawn attention to potential uses in the functional food and nutraceutical sectors, with a focus on reducing the risk factors for noncommunicable illnesses including obesity, type 2 diabetes and cardiovascular diseases. Polyphenols are extremely intricate, structural elements of the cell wall that serve as antioxidants and may have anticancer properties. In diabetic mice models, the seaweed extracts *Sargassum polycystum*, *Ecklonia stolonifera* and *Fucus vesiculosus* have been demonstrated to improve glucose and insulin metabolism. Despite the lack of a clear mechanism of action, recent research points to a function in the inhibition of digestive enzymes such as α -amylase, α -glucosidase and lipase. In premenopausal and postmenopausal Japanese women (n=14 1002), consumption of *Porphyra* spp., *Undaria pinnatifida* and *Sargassum fusiforme* was negatively related to breast cancer risk, as well as the incidence of allergic rhinitis in pregnant women. As evidenced by reports of norovirus contamination and the presence of polycavernoside, a toxin in *Gracilaria edulis*, worries over the likelihood that seaweed may contribute to foodborne diseases. A plentiful and sustainable supply of macronutrients, including dietary fiber and other nutrients are present in edible seaweeds.

Bouga and Combet (2015) evaluated on seaweed and seaweed-containing foods in the UK focusing on labelling, iodine content, toxicity and nutrition. Using seaweed to combat dietary iodine deficiency may be beneficial. A risk of toxicity from excessive iodine levels has been linked to the consumption of particular seaweed species and sources. There is room to make product labeling better (species, source, processing, content). Seaweed goods or seaweeds isolated components fit within the European Union's definition of new foods. The European Register on Nutrition and Health Claims presently lists five illegal claims. It is challenging to draw reliable findings due to the lack of information on the type of seaweed utilized, its source and its iodine level.

Nova *et al* (2020) conducted a review on how microalgae and seaweeds foster the health of the consumers. Polyunsaturated fatty acid (PUFA), polysaccharides and phlorotannin are only a few of the unique bioactive substances and metabolites that microalgae create. Some of these creatures can survive in unfavorable environments. Through their adaptability to age-related nutritional and sensory changes, functional foods may play a significant role in enhancing nutrient intake as well as lowering the prevalence of chronic debilitating illnesses in the aged. A popular food item like bread becomes a suitable carrier for beneficial substances derived from micro- and macroalgae. Recently, the microalgae *C. vulgaris* and *A. platensis* were effectively used to create functional breadsticks that contain significant levels of iron and selenium. Using seaweeds and microalgae as components in the creation of novel food items that prioritize health, nutrition and environmental sustainability is a great way to use sustainable marine resources. Future research should concentrate on determining the bioaccessibility and bioavailability of marine bioactive compounds, particularly when these compounds are included in various dietary matrices.

Koh *et al* (2022) formulated seaweed-infused noodles. The sensory, physicochemical and cooking qualities of the noodles were identified. The cooking yield, loss and time were all greatly improved with the addition of 7.5–15 percent seaweed powder, while the pH and water activity were also dramatically lowered. Compared to control noodles, seaweed noodles were denser and greener. The three seaweed noodles (F2, F5 and F12) chosen through a ranking test were liked by panelists F2 and F5 more than F12. To get rid of the salt, epiphytes and other undesirable particles adhered to the surface, the seaweed (*E. denticulatum*) was washed under running tap water. To get seaweed powder, it was then processed using a grinder and sieved using a sieve shaker. By improving the cooking yield and lowering the cooking loss, the

addition of red seaweed powder (SP) improved the cooking characteristics of noodles. Noodle's pH value and water activity were decreased and their colour was darkened by the addition of SP. However, the textural characteristics of noodles were adversely impacted by the addition of SP. The best noodles sample in terms of cooking quality was F5, which had a Wheat flour (WF): Potato starch (PS) ratio of 9: 1 and 15 percent Seaweed powder (SP).

C. MICROBIAL GROWTH CONTROL IN FOOD WITH SEAWEEDS

Fresh chicken meat was utilized by Nayana and Stanley (2021) in their investigation. In order to reduce the impacts of dangerous chemicals, preservatives must be made from natural components. The extract of *Gracilaria sp.* and *Ulva sp.* is one of the natural marine preservatives. Along with other settings, the texture was displayed but did not change. Some of the natural nutrients found in seaweeds are not found in terrestrial plants. Bioactive substances with demonstrated health advantages against a number of disorders include sulfated polysaccharides, peptides, minerals, phlorotannins, carotenoids and sulfolipids. A meat sample and *Gracilaria corticata* antibacterial investigation showed that *S. cerevisiae*-inhibiting bacteria were present. 5.69 log colony forming unit (CFU) g⁻¹ is the upper limit for the total bacterial count for beef products. Meat from chicken that was stored for a longer period of time had a higher *total viable bacteria* (TVB) value. In low-temperature storage, *Gracilaria sp* two percent (2%) extracts were able to prolong the shelf life of poultry meat by four days. The usage of extracts was discovered to have antibacterial potential based on the information in *total viable bacteria* (TVB) and total plate count (TPC).

According to Sipahutar *et al* (2019), *Sargassum polycystum* ethanol extract is recommended as an option in the study of using seaweed extracts as a preservative. The study exhibited durability for up to a year, no unpleasant smell, antioxidant activity (IC₅₀) of 105.42, Sun Protection Factor (SPF) of 2.00 and moderate to acceptable consumer approval. Seaweeds *S. polycystum* and *E. cottonii* were collected for study from the waters of Banten Bay in Indonesia's Serang Regency. Plate Count Agar (PCA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder, aquades, NaCl, ethanol 96%, CaO and alcohol 70% made up the raw material. Referring to earlier study of *cottonii* seaweed porridge, washing, bleaching, soaking and draining were the four steps in the process of preparing *S. polycystum* porridge. *Staphylococcus aureus* and *Escherichia coli* microorganisms were used in the processing. With the inclusion of seaweed porridge, seaweed extract from *S. polycystum* has the ability to take the place of methylparaben as a sufficient natural preservative in sunscreen cream.

Lekshmi *et al* (2021) described that thousands of species of photosynthetic, mostly watery, living things referred to as algae, are capable of being a preservative. Seaweeds are consumed in many cultures, particularly in Asia and have long been used in traditional medicine to treat a variety of illnesses (tuberculosis, colds and influenza). Numerous algae have been used to extract phytosterols, which have proven to have antibacterial, analgesic and antioxidant properties. A diverse set of chemicals known as phenolic compounds can be categorised in several ways. Flavonoids, phenolic acids, lignans and tannins and out among them. Humanity depended on plants and plant extracts to heal illnesses before the invention of modern antibiotics. Because certain bacteria have become resistant to modern antibiotics, society is facing a serious danger to the health of the populace. The industry now shows a strong trend toward consumers choosing natural and ecofriendly items. Algal extracts appear to work well as antibacterial agents depending on the circumstances of the extraction and the choice of solvent. Considering that algal extracts have been shown to be effective against foodborne infections, they may potentially contribute to food safety. A global trend is the need for natural foods and because of their nutritional value, algae are a valuable resource.

According to Asmad *et al* (2021), the edible coating from red seaweeds to fruits improved the postharvest quality. Fruit preservation techniques that are edible covering are successful. A fruit covering made of red macroalgae (*Kappaphycus alvarezii*) could be edible. Seaweed extracts also play a significant role in the post-harvest care of Fuji apples that have had little processing. Mature macroalgae might be utilised as a delectable covering to extend the shelf life of produce. Additionally, the use of this extract can delay ripening, preserve nutritional value and provide effective barrier characteristics against bacterial infection. It can replace the synthetic fruit covering and is a cost-effective source of natural antioxidants.

Lin *et al* (2023) produced a sodium alginate-carnauba wax food packaging film. Alginic acid and gum are primarily taken from the cell walls of brown algae to create sodium alginate, which has chelating action. By combining the antioxidant and nutritional enhancer sodium ascorbate with the water-blocking agent carnauba wax and the plasticizer glycerin, a novel edible composite film was created. Fresh-cut apples covered with this composite film retained their colour and firmness better than apples coated with other films. Through tests and response surface design research, the ideal sodium alginate edible composite film formula was discovered to be: 1% sodium alginate, 0.3% carnauba wax, glycerol and 0.4% calcium ascorbate. In order to create the film, the film liquid was poured into a plastic plate, dried in

oven at 50 °C. Due to its high level of food safety and environmental friendliness, edible film may eventually replace conventional plastic packaging. High solubility and low mechanical strength are the two main drawbacks of edible films. In order to create an edible film with improved performance, sodium alginate, carnauba wax, glycerine and calcium ascorbate were mixed in this study.

The antibacterial activity of seaweeds, according to Galan *et al* (2022) was much greater than that of the other seven antibiotics examined in this study and equivalent to that of cefixime, trimethoprim and novobiocin. Promisingly, the mortality of *C. auratus* infected with *S. arizonae* was dramatically reduced after in vitro therapy with *K. striatus*. After 10 days post-infection, the treated group had a 40% mortality rate compared to 100% for the positive control group. *Salmonella spp.* and *S. arizonae* were highly inhibited by *K. striatus*, *H. edulis* and *E. denticulatum* extract. However, this extract has a greater zone of inhibition and is similar with that of commercial antibiotics when compared to the antibacterial activity of *K. striatus* in *S. typhi*. Compared to methanol extracts, *K. striatus* extract had a greater zone of inhibition. The best solvent for preserving the active chemicals in virtually all seaweed species was ethanol extraction.

Algal extracts were utilised in antibacterial testing against food spoilage bacteria such *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Enterobacter cloacae* and *Pantoea agglomerans*, by Manguntungi *et al* (2022). Morphological characteristics were used to identify the algae on the Luk Coast and then samples were prepared and secondary metabolites were extracted (bioactive compounds). Pathogenic bacteria such *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae* and *Salmonella typhi* were able to be inhibited by the *Galaxaura rugosealgae*, *Padina sp.*, *Halimeda opuntia*, *Sargassum crassifolium* and *Halimeda opuntia*. At 100% extract concentration, the inhibition zones for the five species of algae range from 19 to 27 mm, has the strong ability to suppress. They might therefore be utilised as organic food preservatives.

According to Lomartire *et al* (2022) explanation, bioactive chemicals made from macroalgae are very important because of their qualities, which make them the best candidates for the creation of environmentally friendly packaging. Alginates and carrageenan's, two seaweed polysaccharides, contribute to biopolymeric film and biodegradable packaging in addition to offering health advantages. Plastic and microplastic pollution in the waters causes significant environmental problems that have an impact on aquatic life and ecosystems. It is essential to

replace plasticizers with biodegradable materials and so safeguard the environment through the sustainable usage of biopolymers obtained from seaweed. A natural, edible plastic-like polymer that may biodegrade in four to six weeks was developed by the French firm Notpla. The seaweed used to make the membrane is grown in northern France, where it is dried, processed into a powder and then turned into a viscous fluid. The global pollution from plastics could decline as a result of this natural plastic.

Balti *et al* (2020) made edible coatings from microalgal exopolysaccharides (EPS) that were enhanced with varying amounts of red seaweed extract (*Gracilaria gracilis*) (RSE) (0.5 and 1.5% (w/v)) total volatile basic nitrogen (TVB-N), trimethylamine (TMA) and *Thiobarbituric acid reactive substances* (TBARS) levels were considerably ($p < 0.05$) decreased by the coating of an *Extracellular polymeric substances* (EPS) and Reference Standard Endotoxin (RSE) combination. Overall, the findings demonstrated that the use of EPS-based coating treatments improved the quality and lengthened the shelf life of chilled shrimp. With a shrimp/ice ratio of 1:2 (w/w), whole shrimp were maintained in ice and brought to the lab in iceboxes within 30 minutes. An active coating made of EPS and RSE might result as a safe preservative for frozen shrimp.

Seaweeds were employed as natural preservatives by Banu *et al* (2020). Perishable foods can have their shelf life increased with no negative consequences on the food's quality. A fantastic option, inexpensive natural preservative that is widely accessible on the Indian coastlines is seaweed gel coating. The maximum antibacterial activity was demonstrated by *K. alvarezarezzarezzii* and *S. tenerrimum* extracts against *Salmonella typhi*, *Bacillus spp.*, *S. aureus*, *E. coli*, *Proteus*, *Pseudomonas*, *Streptococcus pyogenes* and *Candida albicans*. Pathogens such *S. anginosus* and *M. racemosus* were responsive to extracts of chloroform, ethanol, methanol and water

Lovdal and Skipnes (2022) performed a thorough analysis of food safety with an emphasis on microbiological concerns. Red seaweeds contain halogenated chemicals like bromoform that minimize greenhouse gas (i.e., methane) production from ruminants, making them a potential alternative to feed for cattle. When bromo form is excreted into milk, it can be poisonous and possibly hazardous to both cattle and people who consume cow milk. The long-term effects of red seaweed on animal health, ruminal alterations, feed intake, milk production and milk composition require more study. Vitamin C is a powerful antioxidant and seaweed is a great

source of vitamin C. Considering the iodine and heavy metal concentration of seaweed as diet, it has been studied previously.

Perez *et al* (2016) have proposed that substances derived from seaweed such as alginates, agar and carrageenan are employed in the food industry. Seaweeds also offer bioactive compounds used in the treatment of human diseases and also act as antimicrobial agents, replacing synthetic antibacterial agents utilized in the food industry. The application of seaweed antimicrobials has increased the quality, safety and stability of food products. Also, the inclusion of seaweed extracts containing nutraceuticals is recommended for utilization in place of synthetic additives. Bioactive compounds such as polysaccharides, derived oligosaccharides, lipids, fatty acids, sterols, phenolic compounds, pigments, terpenes, alkaloids, lectins, halogenated compounds are naturally found in seaweed, which are deemed nutraceuticals and offer additional health advantages to the human body.

Brown seaweeds were employed by Etemadian *et al* (2018) as a flavouring for maize snacks. Brown seaweeds, such as *Sirophysalis trinodis* and *Polycladia myrica* were used. The study assessed the sensory qualities of fortified corn snacks seasoned with four percent (4%) *P. myrica* extracts, as well as the antioxidant, microbiological properties, total phenol content, fatty acid composition, chemical analyses such as thiobarbituric acid, peroxide value, colour, water activity and microbial count. The acceptable snacks tasted comparable to the control snack and had a little seaweed flavor. They also contained a 2.66:1 ratio of n-3 to n-6 fatty acids. The study has shown that making maize snacks with seaweed extract and other additions might be a healthy substitute for the traditional snacks is available. The author discovered that, in comparison to the antibiotic amoxicillin, *S. aureus* shown modest sensitivity to *S. trinodis* algal extract (5 mg/ml) with a diameter of the non-growth zone (9.68 ± 0.16 mm). The effects of *S. enteritidis* and *B. subtilis* on *S. myrica* differed noticeably from one another.

The shelf life of minced tilapia was tested in the study by Ribeiro *et al* (2014) to see whether Hijiki and Nori seaweed extracts may substitute synthetic preservatives. Prior to the creation of ethanol extracts, the dehydrated forms of the brown alga Hijiki (*Hijikia fusiformis*) and the red seaweed Nori (*Porphyra tenera*) were purchased from vendors of oriental food goods. The fish met the microbiological requirements established by Brazilian legislation and only little variations in the items' colors could be seen. The extracts were placed in amber vials and kept at 8°C after being filtered via quality filter paper. The Mineral Solubilizing Microorganisms (MSM) was manually homogenized after being rinsed in water with a minimal free chlorine

residual of 0.5 mg/L at 10°C. Using the Folin Ciocalteu technique, the extracts' gallic acid equivalents (GAE) were calculated. Seaweed extracts were not added to the control sample and BHT at a concentration of 100 g Butylated hydroxytoluene (BHT)/g minced tilapia was added to the positive control sample to make it positive. The addition of seaweed extracts was discovered to be microbiologically safe and had no impact on the moisture, protein, fat or ash content of minced fish.

D. ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF VARIOUS SEAWEEDS AGAINST FOOD PATHOGENS

Lovdal *et al* (2021) has proposed the microbiological food safety of seaweeds through his work. Edible seaweed is a heterogenous product including species within the green, red and brown macroalgae. The use of seaweed in the human diet has a long history in Asia and now also in the western world. There are emerging trends to improve on the safety, utilization, quality and storability of seaweeds. The global seaweed market is valued more than USD 6 billion annually or over 12 million tons/year in volume and about 85% of this market is made up of food products intended for human consumption. Protein-rich dietary sources will be more and more necessary as the population of the globe is expected to increase. Since seaweed is a non-toxic and non-microbial source of protein, it is a sustainable food source. *Bacillus* spp., *Vibrio* and *Aeromonas* spp. are the main inherent bacteria that are of special concern for the food safety of seaweeds. *E. coli*, *Salmonella*, Norovirus and Hepatitis A virus are considered as potential food safety concerns, predominantly by virtue of recontamination during processing. Traditional technologies like drying, freezing and heat treatments, like blanching and pasteurization are still the most obvious ways to achieve food safety.

Kilinc *et al* (2013) had discussed about the application of seaweeds in food and industries. Their photosynthetic mechanism is comparable to that of terrestrial plants. They generally convert solar energy into biomass more effectively than other methods. In the biochemical polysaccharide structure, which is a naturally non-toxic colloidal fluid that has been utilised as a slimy substance called a gel, all seaweed contains significant levels of carbohydrates (sugars and starches). This sea vegetable has an intriguing nutritional profile because to its low-calorie count and high vitamin, mineral and fibre content. Additionally, it serves as a fertiliser and an animal feed additive in addition to being a component of the worldwide food and cosmetics industries. Algae can be obtained natural but is also increasingly being grown. The use of algae in various industrial applications, including food, cosmetics, paints, medicine, etc., is now being explored. The biologically active substances found in algae, such as phlorotannin's,

carotenoids, alginic acid, fucoidan and peptides, have been demonstrated to be crucial in the prevention of some degenerative disorders. Seaweed's polysaccharides serve as the foundation for a significant and rapidly growing global industry.

The antioxidant and anti-inflammatory activity of seaweed extracts as functional components has been studied by Amaro *et al* (2022). More focus has been placed on using seaweed as a source of bioactive extracts. The author assessed the use of biomass and extracts from three Portuguese seaweeds as nutraceuticals. All things considered, *L. ochroleuca* ethanol water (EW) extract showed the most promise as an antioxidant and anti-inflammatory. Seaweed extracts can be utilized as bioactive or functional substances. In this investigation, the author has looked at the ability of seaweed extracts to fight inflammation and free radicals. In methanol, acetone and ethyl acetate extracts, *Desmarestia viridis* (Ochrophyta) was recognized as having a strong antioxidant potential. The ethanol water (EW) extract from *L. ochroleuca* has a lot of promise to replace artificial food preservatives. Seaweed polysaccharides have been linked to potential for antithrombotic, antibacterial, anti-inflammatory and antioxidant effects. It was determined that *L. ochroleuca ethanol water* (EW) was the best extract for inhibiting the COX-2 enzyme. In a cell line investigation, it was revealed that Laminaria species have the ability to reduce inflammation. The extract worked by preserving the nc 886 gene expression levels, which thus prevented the inflammatory response from being produced. The antioxidant capability of several substances, including proteins, peptides and low-molecular-weight polysaccharides, may be influenced.

Surendhiran *et al* (2021) discussed the present and future uses of antibacterial chemicals generated from marine algae. Historically, algae have been utilised as food or to make hydrocolloids like alginate and carrageenan. The secondary metabolites of many algal groups have been found to include antibacterial chemicals using modern screening techniques. These substances contain phlorotannins, fatty acids, polysaccharides, peptides, polyacetylenes, sterols and aromatic organic acids as functional groups having antibacterial action. Phlorotannins have been found to have antibacterial activity because they impede oxidative phosphorylation and can attach to bacterial proteins including enzymes and cell membranes to lyse cells.

Almeida *et al* (2011) have investigated that the hydrocolloids agar, carrageenan and alginate are produced industrially from macroalgae in western nations including Venezuela, the United States and Canada. The *Gracilaria* species are crucial for biotechnological and industrial

applications since they represent the primary agar source. Oceans are full with algae, which are a major source of unidentified secondary metabolites. Mice were given oral and intraperitoneal administration of 4.0 mg/animal of *G. verrucosa* polysaccharide fractions, which demonstrated immunopotential activity and stimulated phagocytosis. There comes the conclusion that the genus *Gracilaria* contains several properties that might be used to create novel natural therapies.

El-manawy *et al* (2019) examined the antioxidant, total phenolic content and nutritional composition of six seaweed species which were chosen from the Red sea shore near Hurghada, Egypt and the activities in their crude extracts were evaluated. While all of the algal extracts were effective against *Candida albicans* and *Pseudomonas aeruginosa*, only the extract of *H. cuneiformis* was effective against *Enterococcus faecalis*. The bacterial strains that were utilised were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. *Aspergillus niger* and *Candida albicans* were two of the fungi strains. By assessing the α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging activity at various doses of the crude extract (0.5, 1, 1, 2 and 2.5 mg/ml), the antioxidant activities of the seaweed extracts were assessed. The EC50 value of each seaweed extract was also determined. *Hormophysa cuneiformis* has been demonstrated to have a particular phenolic content, antioxidant and antibacterial capabilities; as a result, it may be exploited as a source for substances that are biologically active.

Sobuj *et al* (2020) have explained that seaweed has garnered global attention as a source of functionally bioactive components due to its benefits on human health. The methanol extracts prepared from *Sargassum coriifolium* and *H. pannosa* were the most effective, followed by those made from ethanol and water. These discoveries will be useful for functional food and pharmaceutical applications. The seaweed can be used as a useful feed or as a significant supply of antioxidants from natural sources. Extract composition and antioxidant activity have been found to substantially correlate with one another. The researcher also stated that more study has to be done on the active principles that cause bioactivity, including their discovery, isolation and characterisation.

The pigments phycoerythrin, phycocyanin and allo- phycocyanin are found in the crude extract, according to Sudhakar *et al* (2023). It came from 100 g of freshly harvested thallus of *Gracilaria corticata* from Gulf of Mannar coastal region. After being divided into small pieces, Thallus was centrifuged for 15 minutes at 10,000 rpm. The pathogens utilized were *Candida albicans*, a fungus, *Shigella sonnei*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella*

typhi and *Staphylococcus aureus*. Less antibacterial activity against pathogens has been reported due to purified phycoerythrin (PE) protein composition and probable ineffectiveness against infections. The phycoerythrin isolated from the *Gracilaria corticata* plant has remarkable antioxidant activity and cytotoxicity against colon cancer cell lines. The protein structure of PE may make it less effective against infections, as seen by the observed reduction in phycoerythrin's antibacterial action.

According to Cmikova *et al* (2022), the antioxidant activity of numerous seaweeds, including *wakame*, *arame*, *dulse*, *laminaria*, *kombu* and *hijiki*, was assessed using the DPPH technique. The activity varied from 0.00 to 2641.34 *trolox equivalent antioxidant capacity* (TEAC). By using the Minimal inhibitory concentration (MIC) technique and the disc diffusion method, antimicrobial activity was examined. As model organisms, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*, three gram positive bacteria, were chosen. Aluminum, cadmium and arsenic were identified. Arsenic levels in all seaweed samples ranged from 6.6546 to 76.48 mg/kg. Finding the active components in algae that are responsible for their antioxidant and antibacterial action requires more research. The kombu seaweed extract was the most effective seaweed extract for inhibiting gram positive *E. faecalis*. The extract against *C.albicans* was from *wakame* seaweed, whereas *S. aureus* was from *laminaria* seaweed. Seaweeds can be used as an indicator of environmental pollution since they absorb heavy metals from the environment. It is expected that developments in algal biotechnology will significantly affect our knowledge of human health.

Begum *et al* (2016) developed a *T. conoides* seaweed extract from seaweed harvested in the month of December from Rameswaram coastal region of Tamil Nadu. The antifungal activity of the extract at various doses, including 5, 10, 15 and 20 percent, against *Fusarium oxysporum* was assessed. Using the approach of poisoned food, the impact of fungicides and plant extracts on the pathogen's development was investigated. In the control and increasing concentrations of 15 and 20% treated Petri plates, fungal growth of *F. noxysporum* was suppressed. Plates treated with carbendazim and 5 percent Swimming motility (SWE) did not exhibit mycelial development until the fourth day.

A Research was done by Rizwan *et al* (2020) to determine the *Ulva*'s antibacterial efficacy in the coastal region of Karachi. Seaweed extracts were made using ethanol, methanol, n-hexane and distilled water as the first four solvents in a series of preparations. A limited zone of inhibition was found to exist between seaweeds and both gram-positive and gram-negative

bacterial strains. *Ulva intestinalis*, *Ulva fasciata* and *Ulva lactuca* have antibacterial properties. *Shigella sp* was highly adversely affected by *lactuca's* comparable biofilm-associated bacterium species. *U. intestinalis* extracts in 70% ethanol, however, shown efficacy against *S. typhimurium*, *S. aureus* and *B.subtilis*. N-hexane and chloroform were unsuccessful against all types of bacteria.

Freitas *et al* (2022) carried out research with twelve red seaweed species gathered in central Portugal for the study that aims to reveal their antibacterial and antioxidant properties. Results from the ABTS and TPC experiments demonstrated the coralline algae's great ability for scavenging and *Porphyra umbilicalis'* high phenol concentration. This study made people aware of the untapped bioactive potential of lesser-known *Rhodophyta* species as *Amphiroa rigida* and *Mesophyllum lichenoides*. The only algal species that did not prevent the development of *B. subtilis* or *S. cerevisiae* was *E. elongata*. Every positive control showed a microbiological growth rate of more than 100 percent. Full transparency for *M. lichenoides* was reported at a concentration of 18.02 ± 2.97 percent of *B. subtilis* and 11.91 ± 2.9 percent of *S. cerevisiae*, respectively. Twelve aqueous seaweed extracts were examined for their antioxidant activity and the results revealed diverse and intriguing patterns among species. Only a few of the seaweed species have been evaluated.

Rhodophyta, the biggest class of seaweeds, were the subject of a study by Carpena *et al* (2022). The most important and widely used bioactive substances are sulfated polysaccharides, primarily agar and carrageenan. Fatty acids, phycobiliproteins, vitamins, minerals and other secondary metabolites are other possible compounds. Carrageenan and phenolic compounds have been proposed as the catalysts for red algae's growth of bioactivity. Different red seaweed-derived chemicals have been linked to antimicrobial action. Red algae species have demonstrated appropriate efficacy against Gram negative and non-Gram negative bacteria. Sulpholipid-enriched extracts from *Galaxoura cylindrica* and *Laurencia papillosa* were efficient against *Bacillus subtilis* and *E. coli*. As shown by the efficacy of *Delisea pulchra* extracts against *Escherichia coli* and *Campylobacter jejuni*, furanones are another important class of chemicals with related antibacterial action from red algal sources. It has been demonstrated that *Porphyra yezoensis* prevents *Staphylococcus aureus* from growing. The biggest class of seaweeds, red algae, may contain bioactive substances. Lectins have been suggested as antibacterial substances and mediators of cell signalling. Red algae's lipid profile reveals a low metabolic rate, which suggests that it is helpful.

A pathogen was tested in a study by Bhuyar *et al* (2020) using red seaweed. The biggest species of red algae, *Kappaphycus*, has a rapid rate of growth. According to disc diffusion experiments, *K. alvarezii* red alga extract was more effective than control against *B. cereus*. Future studies on red seaweed will be crucial for the pharmaceutical and medical fields as well as industrial uses as a homogenizer in milk products, toothpaste and other items. With certain changes, ethanol and hot water were used to extract the powdered algal sample. The red alga *Kappaphycus alvarezii* was chosen because it showed promise for producing bioactive chemicals that may function as harmful bacterial inhibitors.

Methodology

III. METHODOLOGY

The methodology of the study entitled as “**Application of Green Additive from Selected Seaweeds to Increase the Shelf Life of the Selected Food Product**” constitutes of the following headings

A. SELECTION OF SEaweEDS

B. FORMULATION OF THE GREEN ADDITIVE FROM THE SEaweED

C. SELECTED FOOD PRODUCT TO INCORPORATE THE GREEN ADDITIVE

D. TREATMENT OF PREPARED ADDITIVE TO THE FOOD PRODUCT

E. ORGANOLEPTIC EVALUATION OF THE TREATED FOOD PRODUCT

F. MICROBIAL ANALYSIS OF THE TREATED FOOD PRODUCTS

A. SELECTION OF SEaweEDS

Seaweeds are main plants that lack flowers, roots, stalks and leaves. They are mostly found on solid substrates at depths of 30 to 40 meters and can be found at the bottom of the ocean up to 180 meters. They grow on estuaries and cling to stones, pebbles, shells and other plant matter (Kumar *et al.*, 2021). In order to commercialize algal extracts, it is crucial research on the potential of algae to serve as antioxidants and antimicrobials. Natural antioxidants and antibacterial agents may be found in abundance in seaweeds, macroalgae and microalgae. All the essential nutrients for a balanced diet can also be found in algae extracts. The addition of algae to food items enhances both the nutritional value and quality of the food products. Seaweeds have a number of qualities that make them an excellent natural preservative for extending the shelf life of perishable goods without having any negative side effects (Banu *et al.*, 2020)

The seaweeds used for study is *Gracilaria edulis* (*Rhodophyta*), a red algal species shown in figure - 1



Figure 1

Gracilaria edulis (Lende and Tendal, 2017)

TAXONOMIC CLASSIFICATION OF *GRACILARIA EDULIS*

Table I

Taxonomic Classification of *Gracilaria edulis*

(Taxonomic reference Guiry, 2023)

KINGDOM	<u>Plantae</u>
SUBKINGDOM	<u>Biliphyta</u>
PHYLUM	<u>Rhodophyta</u>
SUBPHYLUM	<u>Eurhodophytina</u>
CLASS	<u>Florideophyceae</u>
SUBCLASS	<u>Rhodymeniophycidae</u>
ORDER	Gracilariales
FAMILY	Gracilariaceae
SUBFAMILY	Gracilarioideae
TRIBE	Gracilarieae
GENUS	<i>Gracilaria</i>
SPECIES	<i>Gracilaria edulis</i>

Gracilaria edulis is a group of warm water seaweeds which has a very important economic value in the preparation of food products. *Gracilaria edulis* is known for its high antioxidant and antimicrobial property which authorizes it in utilizing it as a preservative in foods. *Gracilaria* and other red algae have a number of health advantages. It has been demonstrated

to improve blood flow, control blood glucose and reduce low density lipoproteins also known as bad cholesterol. Additionally, it has been shown to strengthen the entire immune system.

High levels of secondary metabolites are produced by marine red algae, including sesquiterpenes, diterpenes, triterpenes and many more kinds that have biological effects such as antifungal, antibacterial and anticancer properties (Lomartire et al., 2021). *Gracilaria edulis* displayed antibacterial efficacy against *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis* when extracted with ethanol (Dayuti, 2018)

Gracilaria edulis is chosen for the development of green additive to extend the shelf-life of food items in light of the findings of earlier research on the species and its antibacterial properties.

The approval for the proposed study was cleared by the Institutional Human Ethics Committee (IHEC) of Avinashilingam Institute for Home science and Higher Education for Women. (Annexure – I)

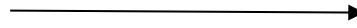
B. FORMULATION OF THE GREEN ADDITIVE FROM THE SEAWEED

Food additives are compounds with either a natural or synthetic origin that are added to food to fulfill a specific technical or sensory need. The concept of food additive has been evolved through time and is now defined as any material that is not typically employed as an ingredient in food or ingested as a food on its own, regardless of whether it is nutritive. The use of chemical food additives is becoming less and less popular with consumers, despite the fact that they are permitted in many nations. As a result, it is necessary to look into alternative sources of natural additives that are safe, efficient and acceptable (Abdelghany et al, 2015).

Seaweeds were gathered from the coastal region of Mandapam located in Ramanathapuram District, Tamil Nadu. To get rid of the dirt and salts that were stuck to the outer surface of the seaweeds, it was rinsed with tap water many times before cleaned with bi-distilled water. The washed seaweeds were dried in sun until the moisture was removed and the seaweeds had become totally dry. In a mixer grinder, the cleaned and dried seaweeds were ground into powder. A planetary ball mill was used to ball-mill the seaweeds that had been crushed (Mohamed et al., 2022). Without wasting any seaweeds, this precise grinding produced green seaweed additive into fine powder.



Live *Gracilaria edulis*



(Washed and sundried)



Dried *Gracilaria edulis*



Ball milled seaweed powder



(Ballmilling)



Grinded dried Seaweed powder



(Sieved with cloth)



Seaweed powder

Plate 1

Formulation of green additive from *Gracilaria edulis*

Characterization of Seaweed powder

To analyse the characteristics of the resulting seaweed powder, the following tests were carried out.

- Particle size analysed by Particle Size Analyser (PSA)
- Zeta potential
- UV spectroscopy
- Fourier Transform Infrared spectroscopy (FTIR)

Particle size analyser and zeta potential

Particle size analyser was used to measure the powder's average diameter, size distribution and zeta potential. At 25 °C, a sample of powder was dispersed in bi-distilled water (used as the solvent). Photons were used to expose the sample, which prompted the particle to react. A sensitive detector picked up on changes in distribution intensity that were clumped in the targeted corner as a result of the particle ray's dispersion. Just before to evaluation, the sample suspension was subjected to sonication for around 60 minutes (Mohamed *et al.*, 2022)

UV- visible spectroscopy

An examination of the methanolic extracts of the seaweed powder UV-visible spectrum was performed. For UV spectrophotometer analysis, the samples were centrifuged at 3000 rpm for 10 minutes and then filtered through Whatmann No. 1 filter paper using a high-pressure vacuum pump. Using the same solvent, the sample was diluted to a ratio of 110. Using a Perkin Elmer Spectrophotometer, the samples were scanned in the wavelength range of 200-800 nm and the distinctive peaks were found (Rajeshkumar , 2016)

Fourier Transform Infrared spectroscopy (FT-IR)

With just minor modifications, past techniques were used to produce the spectra using an OMNI sampler attenuated total reflectance (ATR) accessory on an FT-IR spectrophotometer. With constant pressure applied, a small amount of *Gracilaria edulis* liquid was placed directly on the sample container of the infrared spectrometer. Data of infrared absorbance were collected over the wave number ranged from 4000 cm⁻¹ to 400 cm⁻¹ and computerized for evaluations using the 21 CFR part 11 software. Before each sample duplicate was run, the reference spectra were obtained from the cleaned blank crystal. The FT-IR peak values were

recorded. The results of each and every analysis were verified twice (Rajeswari and Jeyaprakash, 2019)

C. SELECTED FOOD PRODUCT TO INCORPORATE THE GREEN ADDITIVE

Fresh fruits and vegetables are considered perishable, as very few can be stored for long periods of time at room temperature. Fresh foods like meat, fruits, vegetables and airy products can get contaminated when they include pathogenic microbes, undesirable metabolites, organic acids and CO₂. Sulphur derivatives or volatile components are created quickly. Foods' colour, taste or pH are altered later in an irreversible manner. These microbiological and chemical events have a direct impact on the quality of these foods (Luo *et al.*, 2023). Foods may develop pathogenic germs without any discernible odour, look or flavour alteration. Certain types of bacteria, yeasts and moulds that cause spoilage can thrive in temperatures as low as 40°F. So, it has to be refrigerated for extended shelf life.

Five variations were formulated with one variation as standard idly batter to evaluate the effect of seaweed powder in idly batter. Variation I, II, III and IV were treated with 0.25 g, 0.50 g, 0.75 g and 1 g respectively. Standard idly batter remained untreated for the comparison of changes in batter of untreated and treated.

The selected product for the application of the additive is Idly batter as it is a perishable food product. Idly is a popular and extensively consumed culinary item in India that is a long-standing, traditional fermented product made from steamed grains or legumes. Because to the fermentation process, idly serves as a significant source of vitamins, particularly B-complex vitamins, protein and calories for individuals. Idly is another name for "rice cake." Due to its appealing look, flavour, enticing taste and spongy texture, idly has become one of the most popular morning foods both inside and outside of India. Comparing the fermented batter of idly to the unfermented batter, there is an improvement in the nutritional values with a decrease in the antinutritional component and an increase in the vitamin content (Sonawane *et al.*, 2019)

According to Times of India (February Month, 2023), Idly is referenced in several ancient Indian books, notably the Kannada classic "Vaddaradhane," which was written in the seventh century and it has discussed about the preparation of "Iddalige." This meal is also described in the Tamil book "Periya Puranam," written in the tenth century, describing the lives of 63 Shaivite saints.

Idly's were traditionally prepared with rice and black gram. Rice and black gram were first soaked separately, followed by grinding steps with water added intermittently. The rice and black gram batters were then combined with a small amount of salt and the mixture was allowed to ferment overnight at room temperature. Finally, the fermented batter was poured into idly pans and allowed to steamed for 5-8 minutes.

D. TREATMENT OF PREPARED ADDITIVE TO THE FOOD PRODUCT

Food additives may be classified as antimicrobial agents, antioxidants, artificial colours, flavour enhancers, bleaching agents, chelating agents, nutrient additives which includes vitamins, minerals, thickening and stabilizing agents.

The developed green additive fall under the category of antimicrobial agents, which stop food from becoming spoiled by mould or other microorganisms. Here, the additive was immediately mixed into the chosen food product which is idly batter in various proportions.

Five variations were chosen. One was left untreated as standard to be compared with the treated green additive. The green additive was weighed in varied amounts and added to the idly batter. 0.25g, 0.50g, 0.75g, 1g of seaweed were weighed and added to 100 ml batter for each variation.

i. PHYSICOCHEMICAL ANALYSIS OF IDLY BATTER

Physicochemical analysis is described as the branch of chemistry concerned with the interaction of matter's composition and characteristics. The properties of physicochemical analysis change with regard to the nature of the product. The selected food product idly batter includes physicochemical characteristics like pH, acidity, increase in batter volume.

a. pH

pH meter is a scientific tool that monitors the hydrogen-ion activity in water-based solutions, revealing its acidity or alkalinity which is represented as pH. The pH meter is frequently referred to as a "potentiometric pH meter" because it detects the difference in electrical potential between a pH electrode and a reference electrode. The pH or acidity of the solution has an impact on the difference in electrical potential.

A digital pH meter with a microprocessor was used to determine the idly batter's pH for every four hours till the 12th hour of fermentation to check the pH scale of the batter (Bernard *et al*, 2021

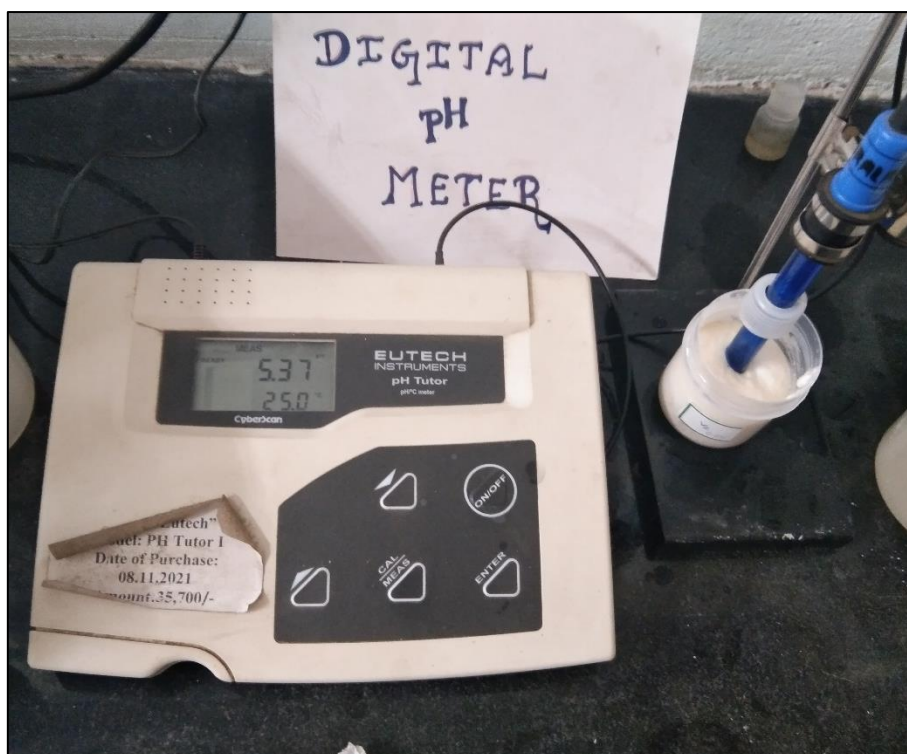


Plate 2

Analysis for pH of the Formulated Idly Batter

b. TITRATABLE ACIDITY

The titrimetric approach was used to measure the amount of acid generated during fermentation. Five grams of substance or batter was dissolved in ten millilitres of distilled water and titrated using phenolphthalein as an indicator against 0.1N NaOH (Sharma and Chand, 2015) The following formula was used to determine titratable acidity

$$\text{Percentage of lactic acid / gram} = \frac{\text{ml of 0.01N NaOH} * 0.009}{\text{Weight of the batter(g)}} * 100$$



Plate 3

Determination of Total Acidity for the Formulated Idly Batter

c. BATTER VOLUME

The freshly prepared batter of thirty millimeter was poured into a hundred milliliter measuring cylinder, left unattended at room temperature, and then measured out. In order to analyze the increasing of batter and identify the size of the pores, the growth in batter volume was measured every four hours in milliliters (ml) until the 12th hour.



Plate 4

Measuring the Volume of Idly Batter

b. QUALITY ANALYSIS OF IDLY

With the treated idly batter, idlys were prepared to check for sensory analysis and to analyse the porosity of the prepared idly.

The five variation including standard, variation I, II, III and IV with no treatment, 0.25 g, 0.50 g, 0.75 g, 1 g respectively, were added to 100ml of idly batter. With each variation idlys were made and evaluated for its quality with porosity assessment and organoleptic evaluation.

i. POROSITY ASSESSMENT

The ink print of the cross-section was used to estimate the pores in the interior structure of the idly. Ink print test was used to capture the internal structure and porosity of the idly. Idly was cut into half in the middle and stamped on an ink pad. The stamped idly with ink was pressed on a graph sheet to test to length, width, porosity and its inner structure.

E. ORGANOLEPTIC EVALUATION OF THE TREATED FOOD PRODUCT

The evaluation of a food product's flavor, aroma, appearance and mouthfeel is known as organoleptic testing. Food items must undergo organoleptic testing to ensure they meet organizational and customer criteria. (Kumar, 2021)

Research and development as well as quality control both benefit greatly from sensory assessment. Organoleptic evaluations are a crucial part of any Quality Control assessments since customers judge a product's quality using their senses.

The purpose of the sensory evaluation is to characterize the item. Finding disparities between two or more items qualities, their magnitude and their directions. Therefore, pleasure is the culmination of organoleptic attributes.

With the help of 10 semi-trained panel members, the organoleptic features of the food items containing the additive are examined to see if the additive modifies the nature of food product. Ten female students from Avinashilingam University, Coimbatore were chosen as panel members between the age 18 to 25 years. The panel members assessed the combined culinary products and provided grades for each feature.

The scoring was done with composite score card. In this analysis, panellists were given the samples to evaluate for each sensory attribute with ten point hedonic scale. (Annexure – II)



Plate 5

Panellists evaluating the formulated idlys for sensory attributes

F. MICROBIAL ANALYSIS OF THE TREATED FOOD PRODUCT

For the treated food products, a microbiological assay was carried out to determine the level of microbial activity. The measurement of the levels of microbe contamination in finished consumer items and throughout the production process is known as microbial testing of food. Utilizing biochemical and molecular techniques, microbial testing entails finding, identifying or counting microorganisms in the product. (Matthews *et al.*, 2020)

The treated food product was kept for a stipulated time for the observation of the microbial activity in the treated food product. Later, they were analysed for the microbial action using following tests,

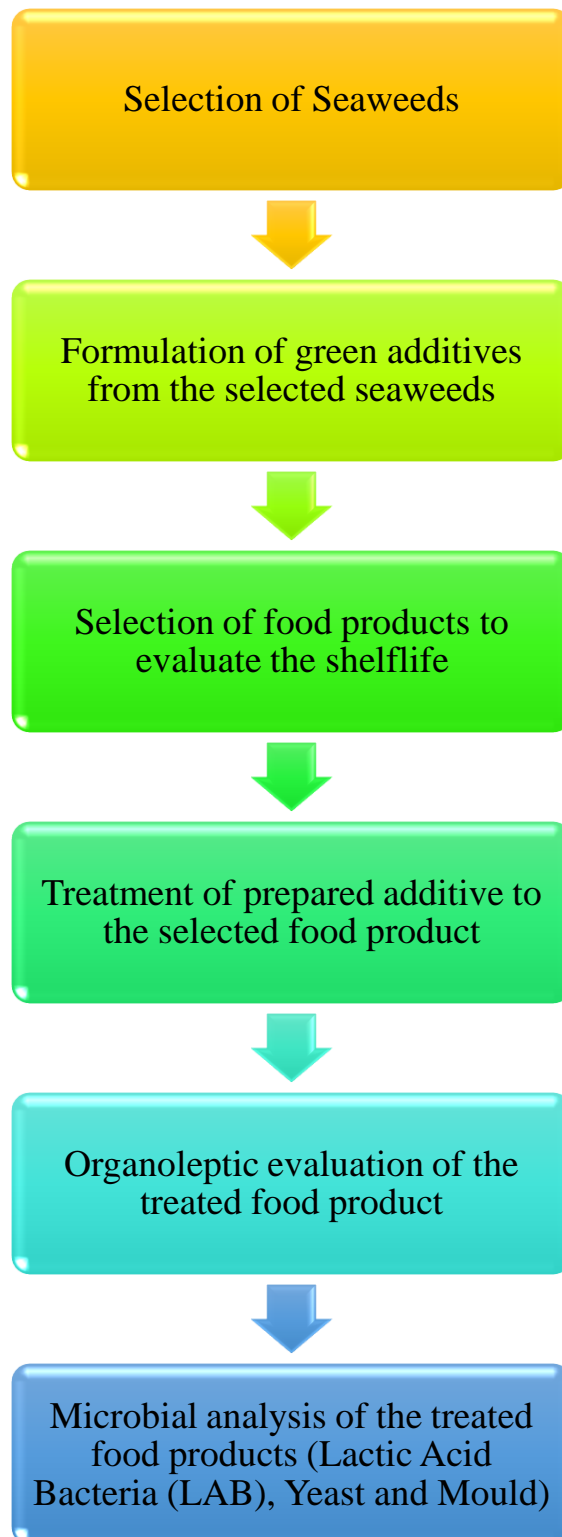
- Lactic acid bacteria (LAB) count
- Yeast and mould

i. Microbial analysis to determine LAB, Yeast and Mould

The batter samples were homogenized in an aseptic environment for 60 seconds using a high-speed blender (REMI), 225 ml of the diluent (physiological saline) were used to dissolve 25 g of the material, which was then combined and serially diluted in the ratio of 19. The pre-treatment of the batter is necessary before performing a microbiological study since the batter is a pseudoplastic fluid in nature. For the Lactic acid bacteria (LAB) count, samples were spread plated on De Man, Rogosa and Sharpe agar (MRS) and incubated at 37°C for 24 to 48 hours. Spread plating on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (1%), incubation at room temperature of 25 °C for 3 to 5 days and representation of counts as colony forming units per gram (cfu/g) of batter were used to determine the yeast and mould count (Kannan *et al.*, 2015)

The shelf-life quality of the food is determined by analysing the LAB count, yeast and mould development in the idly batter using the aforementioned food analysis.

FLOW CHART DEPICTING THE METHODOLOGY OF THE STUDY



Results and Discussion

IV. RESULTS AND DISCUSSION

The results obtained from the present study entitled “**Application of Green Additive from Selected Seaweeds to Increase the Shelf Life of the Selected Food Product**” is discussed under the following headings

A. FORMULATION OF SEAWEED POWDER

B. CHARACTERIZATION OF SEAWEED POWDER

C. PHYSICOCHEMICAL ANALYSIS OF TREATED IDLY BATTER

D. QUALITY ANALYSIS OF IDLY

E. MICROBIAL ANALYSIS OF THE IDLY BATTER

A. FORMULATION OF SEAWEED POWDER

The study aimed at formulating green additive from seaweed to increase the shelf life of a food product. *Gracilaria edulis* was chosen for the formulation of additive due to its high composition of nutrients and its high efficiency of antimicrobial property. Mahendran *et al* (2021) has stated that *Gracilaria edulis* polyphenol compound showed maximum inhibition zone against pathogens like *Bacillus subtilis* and *H. valentiae*. Also, *Gracilaria edulis* had the high antioxidant capacity.

Piras *et al* (2019) has described that ball milling is a mechanical process that is frequently used to combine materials and grind powders into very small particles. It has found extensive use in industry around the globe as a cost and environmentally friendly method. Cost-effectiveness, dependability, simplicity of use, consistent results owing to energy and speed control and suitability for use in both wet and dry circumstances on a variety of materials are all advantages of this technology. Contrarily, potential drawbacks include the potential for contamination, the development of nanomaterials with irregular shapes, noise, lengthy milling and cleaning time.

To formulate the green additive, it was provided as powder for easy incorporation and to reduce the wastage of the seaweed. No chemicals were involved in the production process which made the resulting additive natural and green. Ball milling was used for the precise grinding of seaweed.

As the texture of dried seaweed powder of *Gracilaria edulis* was fibrous, it was difficult to grind it further small with the help of a mixer. With the help of ball milling the fibrous form of

powder was ground and sieved with a cloth to obtain the fine powder. Thus, the green additive powder was formed.

B. CHARACTERIZATION OF SEAWEED POWDER

a. NUTRIENT PROFILE OF THE SEAWEED *GRACILARIA EDULIS*

Ganesan *et al* (2020) claimed that underutilized seaweeds such *Gracilaria edulis*, *Acanthophora spicifera*, *Padina gymnospora*, *Enteromorpha flexuosa* and *Ulva fasciata* might be a substitute source of micronutrients such as iron, iodine and zinc that may guard against micronutrient deficiencies that is regarded as a safe meal for all age groups and pregnant women during pregnancy and illnesses. Furthermore, *Ulva fasciata* and *Gracilariaria edulis* include all of the Essential Amino Acids (EAA) and Essential Fatty Acids (EFA), which are ideal sources for pregnant women to consume to support their fetus's cognitive development. Therefore, the findings from the underutilized seaweeds would be a viable diet for people to increase therapeutic qualities and decreases deficiencies, which may be thought of as a futuristic functional meal.

Table II
Nutrient Composition of *Gracilaria edulis*

NUTRITENTS (% DRY WEIGHT BASIS)	ANALYSED VALUES
Moisture	87.14
Protein	14.26
Fat	0.93
Ash	7.63
Carbohydrate	32.39
Total dietary fibre (TDF)	63.175

Table I shows that the major nutrient composition of *Gracilaria edulis* from the study conducted by Debbarma *et al* (2016). The evaluated *Gracilaria edulis* yielded the following values: 87.14 for moisture, 14.26 for protein, 0.93 for fat, 7.63 for ash, 32.39 for carbohydrate and 63.175 for total dietary fiber. Highest Total Dietary Fibre (TDF) was found in *G. edulis* with the value of 63.175 ± 0.46%. The lipid content varied from 0.73 ± 0.001 to 0.93 ± 0.00%, with *Gracilaria edulis* having the greatest amount which was 0.93 ± 0.00% and *Sargassum sp.*

having the lowest value of $0.73 \pm 0.001\%$. From the findings of the nutritional values, it was stated that *Gracilaria edulis* had the highest nutritional value than that of *Sargassum wightii* and *Ulva lactuca* from the above mentioned study.

Table III
Mineral Composition of *Gracilaria edulis*

NUTRIENTS	ANALYSED VALUES (mg/ 100g)
Sodium	423
Potassium	283
Zinc	1.7
Manganese	3.96
Copper	1.78
Calcium	223
Iron	65.28
Selenium	3.70

Table II shows the mineral composition of *Gracilaria edulis* from the study conducted by Gamero-vega *et al* (2020). Minerals such as sodium, potassium, zinc, manganese, copper, calcium, iron and selenium were found in *Gracilaria edulis* with the proportion of 283 mg, 1.7 mg, 3.96 mg, 1.78 mg, 223 mg, 65.28 mg and 3.70 mg respectively. Due to the interaction with seawater, red algae have an extremely high sodium content higher than the terrestrial plants. The highest value was discovered with the average of 882 mg/100 g in *Gracilaria changii*. On average, 3.0 mg/100 g of zinc was present in red algae, which is a significant quantity. It was stated that red seaweeds are abundant in nutrients compared to other kind of seaweeds and can be used as food for better health. It was found that *Gracilaria sp* had very high concentrations of manganese which was 15.7, when compared to other species of red algae. Manganese is essential for the immune system, influences bone formation and reproduction and has a protective impact against reactive oxygen species.

Table IV**Fatty Acid Composition of *Gracilaria edulis***

FATTY ACIDS	ANALYSED VALUES (% PER GRAM)
Total fatty acids	11.41
Palmitic acid	2.06
Margaric acid	0.11
Stearic acid	2.33
Oleic acid	1.98
Linolenic acid	2.56
Alpha linolenic acid	18.3

Table V**Amino Acids Composition of *Gracilaria edulis***

AMINOACIDS	ANALYSED VALUES (mg/g)
Glutamic acid	13.1 ± 0.65
Serine	0.84 ± 0.04
Histidine	3.3 ± 0.16
Glycine	0.73 ± 0.03
Threonine	2.98 ± 0.14
Alanine	2.64 ± 0.13
Tyrosine	0.56 ± 0.02
Valine	1.71 ± 0.08
Methionine	1.69 ± 0.08
Phenyl alanine	1.31 ± 0.06
Isoleucine	1.10 ± 0.05
Aspartic acid	4.64 ± 0.23
Leucine	0.37 ± 0.01
Lysine	1.91 ± 0.09
Arginine	3.27 ± 0.16

Table IV and V shows the fatty acids and amino acids content of *Gracilaria edulis* from the study of Sakthivel and Devi (2015) respectively. The researchers have revealed that *Gracilaria*

edulis have an optimal quantity of sulphate content and polysaccharides. It has been discovered that *G. edulis* contains significant amounts of both saturated and unsaturated fatty acids with total fatty acids of 11.41%, palmitic acid of 2.06%, margaric acid of 0.11%, stearic acid of 2.33%, oleic acid of 1.98%, linolenic acid of 2.56% and alpha linolenic acid of 18.3%. Fatty acids help in the prevention of heart diseases, stroke, help control lupus, eczema and rheumatoid arthritis. Fatty acids play protective roles in preventing cancer.

From the findings, *Gracilaria edulis* has all the essential amino acids in it. The amino acids such as glutamic acid of 13.1 mg, serine of 0.84 mg, histidine of 3.3 mg, glycine of 0.73 mg, threonine of 2.98 mg, alanine of 2.64 mg, tyrosine of 0.56 mg, valine of 1.71 mg, methionine of 1.69 mg, phenyl alanine of 1.31 mg, isoleucine of 1.10 mg, aspartic acid of 4.64 mg, leucine of 0.37 mg, lysine of 1.91 mg and arginine of 3.27 mg were estimated in per gram of the *Gracilaria edulis*. Amino acids plays an important role in protein synthesis, tissue repair and nutrient absorption.

b. PARTICLE SIZE ANALYSER AND ZETA POTENTIAL OF SEAWEED POWDER

Particle size analysis is used to determine the size of the particle which is usually very small. Dynamic light scattering (DLS) is currently a widely accepted technique for particles in the lower nanometres to lower micrometre range. The zeta potential's magnitude provides an indication as to the colloidal system's possible stability. The tendency for the particles to attract each other and come together will be absent if all the particles in suspension have a strong negative or positive zeta potential.

Table VI

Analysis of Particle Size of *Gracilaria edulis*

MEASUREMENTS	ANALYSED VALUES
Z-Average (r.nm)	1637
Pdl (Polydispersity index)	0.462
Size(r.nm)	1038
Width (r.nm)	181.3

Table VII
Analysis of Zeta Potential of *Gracilaria edulis*

MEASUREMENTS	ANALYSED VALUES
Zeta potential(mV)	-2.34
Zeta deviation (mV)	3.81
Conductivity (mS/cm)	0.149
Width (mV)	3.81

Using the dynamic light scattering (DLS) method, which is most common approach for determining the size distribution profile of nano powders, particle size or size distribution were observed. The *Gracilaria edulis* powder has physiochemical characteristics with a particle size of 1038 ± 180 nm which will be helpful during the addition of the additive into food products. With the particle size of 1038 nm, small amount of powder would provide a good result in the experiments. As the particle size was size, it would be easy to dissolve and the addition of powder would not be noticeable.

An important indicator of the stability of the micellar system is the zeta potential. When the surface charge is quite large, the particles can resist one another strongly enough to increase the stability of the system. Zeta potential is an indicator of the strength of charges on powders; values higher than +30 mV and lower than 30 mV show a significant resistance to coalescence. The *Gracilaria edulis* powder particle had a zeta potential of around -2.34 ± 3.8 mV in this test, indicating that it has a high rate of coagulation or flocculation. This rapid coagulation or flocculation would help the formulated green additive to disperse in the food medium rapidly. Zeta potential varies on the method and material parameters, which may explain why different studies on the link between zeta potential and particle size have produced inconsistent results.

The qualities of the material, such as particle size, concentration, surface characteristics and ionic concentration, as well as ambient factors like pH and temperature, have an impact on the zeta potential's absolute value and positive/negative charge. Superfine grinding technique has evolved in order to enhance the attributes that the raw material lacks, such as solubility, dispersion, surface effects and chemical reactivity. High energy ball milling has several benefits, including adaptability to a variety of materials, ease of handling, simplicity, capability for large production runs and cheap cost (*Pohshna et al., 2020*)

c. UV SCAN OF *GRACILARIA EDULIS* SEAWEED POWDER

Picollo *et al* (2018) described that the study of interactions between matter and electromagnetic radiation in the ultraviolet-visible range is known as ultraviolet-visible spectroscopy. The phrase "electronic spectroscopy" is frequently used to describe UV-Visible spectroscopy because it is connected to the excitation of the atom's outermost electrons, which play a role in the synthesis of molecules. Transmittance, reflectance and photoluminescence modes are frequently used for UV-Visible measurements. While photoluminescence measurements can be viewed as absolute measurements, transmittance and reflectance measurements must be made in comparison to a reference material.

Table VIII

Intensity of *Gracilaria edulis* Seaweed Powder

Wavelength (nm)	Intensity (OD)
800	0.303
700	0.372
600	0.434
500	0.545
400	0.712
300	1.11
250	1.538
220	2.335

The spectrum shows the band edge absorption peak which was found to be at 220 nm. In UV–Visible, high energy electromagnetic radiation in the wavelength range of 200 to 800 nm was utilized to promote electrons to higher energy orbitals. From the UV spectra, it was clear that the absorbance decreases with increase in wavelength. Sharma *et al* (2019) has stated that the decrease in absorption indicates the presence of optical band gap in the material. Similar to the above mentioned statement this study provided the information that the absorption decreased with the increase in intensity which proved that the seaweed powder has optical band gap. With this optical band gap, it was concluded that the green additive's optical properties such as absorption, complex refractive index and transission were strongly depended on

materials band gap. The UV visible spectroscopy showed the absorbance rate of the seaweed powder

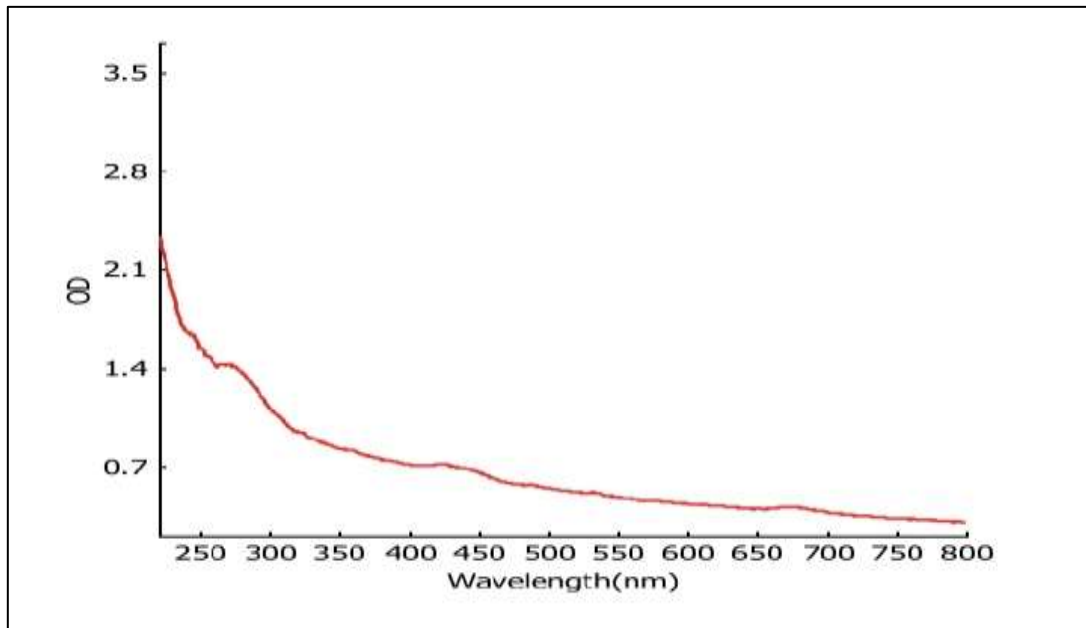


Figure 2

Water-diluted Suspension of *Gracilaria edulis* Seaweed Powder by UV Scan

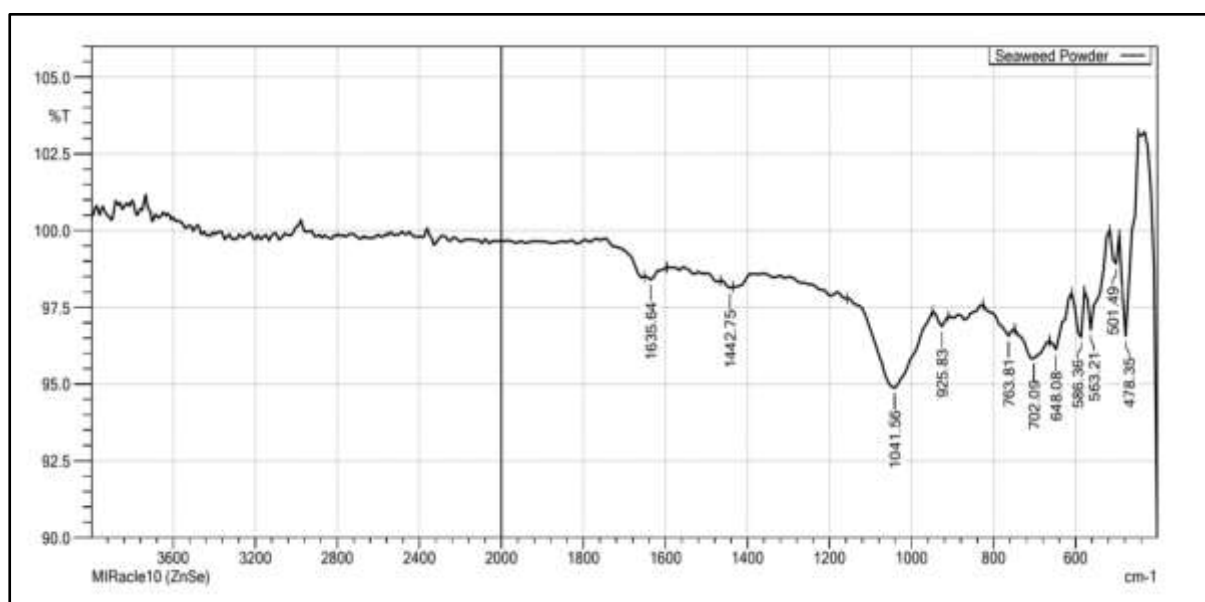
Rajeswari and Jeyaprakash (2019) assessed the bioactive compounds found in the brown seaweed *Sargassum wightii* using UV-VIS and FT-IR. The findings demonstrated that the flavonoids' characteristic spectral bands consisted of two absorption spectra with peak values in the ranges of 230 to 290 nm and 300 to 360 nm. These maxima's actual position and perceived intensities provide extremely useful information about the nature of flavonoids. The existence of phenolic and alkaloids chemicals in *Sargassum wightii* was revealed by the peak that occurred at 234–676 nm.

d. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR) OF SEAWEED POWDER *GRACILARIA EDULIS*

According to Dutta (2017), an effective method for identifying organic materials has been carried out by infrared spectroscopy. The introduction of Fourier transform infrared (FTIR) spectroscopy has brought about a well-liked technique for the quantitative examination of complex mixtures as well as for the evaluation of surface and interfacial phenomena.

Table IX**FT-IR Fingerprint (Wavenumber, cm⁻¹) of *Gracilaria edulis* Seaweed Powder**

PEAK POSITION ON FTIR SPECTRA (cm ⁻¹)	ASSIGNMENT OF BONDS	MODE OF VIBRATION
478	C-I	Bend
501	C-I	Bend
563	C-I	Bend
586	C-I	Bend
648	=C-H	Bend
702	=C-H	Bend
764	=C-H	Bend
926	=C-H	Stretch
1041	C-F	Bend
1443	C=C	Stretch
1636	C=C	Stretch

**Figure 3****FT-IR Spectra of the Transmittance of the *Gracilaria edulis* Seaweed Powder**

The figure 3 shows the typical FTIR spectra of the seaweed powder used in this study. Al- Arikki *et al* (2021) study was taken as the standard value for the present study to plot the typical FT-

IR spectra of the seaweed powder. The findings revealed the characteristic major peaks appearing at 478.35, 501.35, 563.21, 586.36, 648.08, 702.09, 763.81, 925.83, 1041.56, 1442.75, 1635.64 cm^{-1} . The vibrations along with their assigned bonds and the mode of vibration were mentioned in the table VIII. Most of the vibrations were seen between 600-1500 cm^{-1} , which was considered as fingerprint region. With the assigned bonds it is known that carbon, iodine, fluorine and hydrogen are present in the seaweed powder as functional groups. The C=C represents the presence of alkenes, aromatic compounds, anhydride and carboxylic acid. The =C-H indicates the presence of alkane and aldehyde. The bend in the vibration 478 was the strongest bend of all vibrations. The band wavelength was weak at its increased wavelength which is 1636.

With the help of FT-IR spectroscopy, the presence of organic materials such as carbon, iodine, fluorine, hydrogen, alkenes, aromatic compounds, anhydride, carboxylic acid, alkane and aldehyde were found in the seaweed powder.

C. PHYSICOCHEMICAL ANALYSIS OF IDLY BATTER

a. PREPARATION OF IDLY BATTER

Parboiled rice (*Oryza sativa*) and dehulled black gram (*Phaseolus mungo*) were purchased from a local market to conduct the study. Rice and dehulled black gram were cleaned in a 3:1 ratio with potable water before being soaked in sterile water for 8 hours. The soaked items were finely and coarsely ground individually, with water added on a regular basis. The batters were combined together and later mixed the seaweed powder and salt in varied proportions. The batter was allowed to ferment for 12 hours and idly was cooked using the wet steaming method in an idly cooker for 10 to 15 minutes.

Table X

Variations of Idly Batter Prepared with Seaweed Powder

FORMULATED VARIATIONS	PROPORTION OF SEAWEED POWDER (g)
Standard	-
Variation I	0.25
Variation II	0.50
Variation III	0.75
Variation IV	1.0

With the above mentioned procedure for the preparation of idly batter, the product was prepared. For each variation, 100 ml of batter was taken in separate containers. Standard idly batter remained untreated as for to compare this standard with the treated idly batter. As the table X depicts, Variation I, II, III, IV were treated with the varying amount of seaweed powder of 0.25g, 0.50g, 0.75g and 1g respectively. The variation of 0.25 g to 1g were added in varying proportions as 1g was set as limitation as seaweeds are rich source of iodine. The proposed recommended daily amount for persons 19 years of age and older without a thyroid issue is 150 micrograms, with a maximum of 1,100 micrograms. This was taken into consideration while determining the ratio of seaweed powder, which ranged up to 1g.

b. pH

pH often lowers during fermentation but improves after an extended period. This is because the pH decreased as a result of microbes consuming the nutrients and releasing organic acids into the medium.

Table XI

Measurement of pH for the Formulated Idly Batter

FORMULATED VARIATIONS	0th HOUR	4th HOUR	8th HOUR	12th HOUR
Standard	5.79	5.34	4.60	4.19
Variation I	5.76	5.37	4.64	4.25
Variation II	5.79	5.36	4.71	4.34
Variation III	5.77	5.41	4.80	4.46
Variation IV	5.88	5.65	4.93	4.54

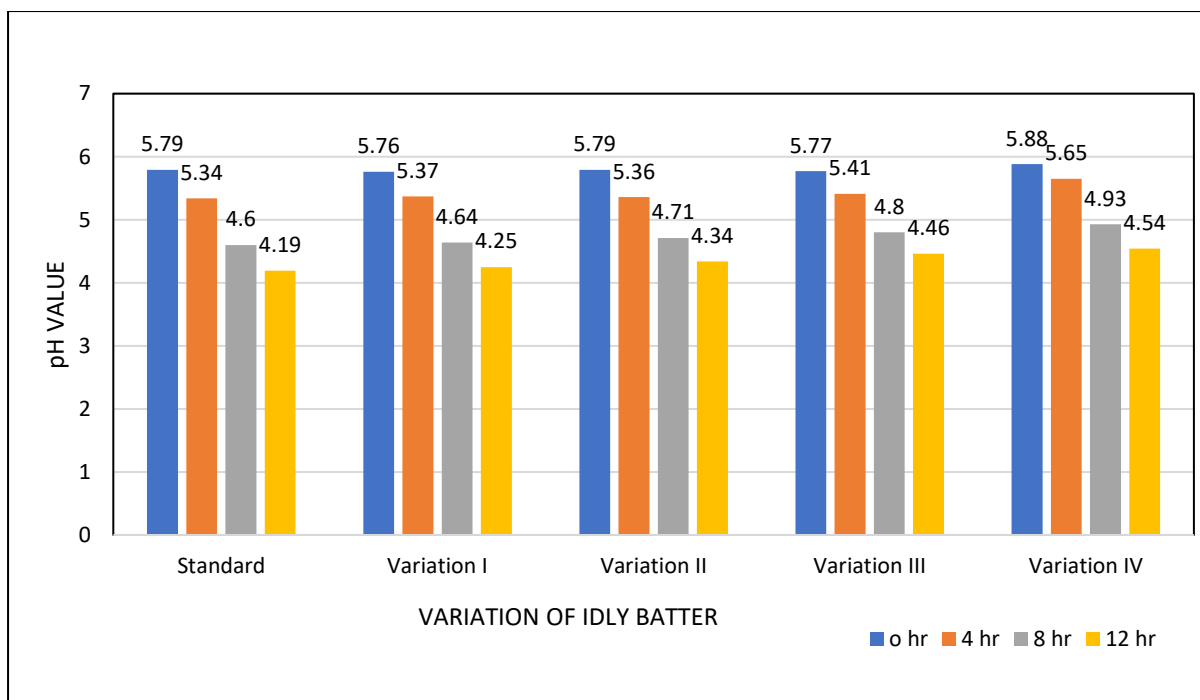


Figure 4

Change in pH of Formulated Idly Batter

The results on the pH change of idly batter throughout fermentation are reported in table XI and figure 3 displays the diagrammatic representation of pH change. The batter became increasingly acidic as a result of growth of lactic acid bacteria.

Addition of seaweed powder to the freshly prepared batter reduced the rate of fermentation as there was decrease in the pH values as the proportion of powder increases in the batter. The addition of seaweed powder at the beginning of fermentation which was at 0th hour was another factor that inhibited fermentation and caused a gradual decrease in pH. The decreased fermentation rate caused by the inhibition of bacterial growth may be related to anti-microbial action of *Gracilaria edulis*. When seaweed powder was added to the batter, a pH range of 5.76 to 4.54 was recorded. Variation IV, which contained of 1g of seaweed powder, showed a higher pH at the end of fermentation which was 4.54. Standard, which was untreated had the normal range of fermentation and also this had the lowest pH value of 4.19 at the end of fermentation.

Shaikh *et al* (2021) has reported that the pH of the freshly prepared batter was 6 and the fermented batter was 4.5. Chelliah *et al* (2016) has declared that addition of curry leaves powder in idly batter has decreased the pH value which has resulted in delayed rate of fermentation. Gaikwad *et al* (2022) has stated that the pH of the stored ready to cook (RTC) idly batter will be decreased by yeast activity as a result of growth of heterofermentative Lactic

acid bacteria (LAB) in order to spoil the batter. Bhaskar and Ananthanarayan (2019) has found that the pH of freshly prepared batter exhibited 6.15 ± 0.07 and at the end of fermentation all sets of batter showed 4.6 to 4.9. With using different starter cultures for fermentation of idly batter, Momin and Prasad (2019) has found that the pH ranged from 4.68 to 4.83.

c. TITRATABLE ACIDITY

Tyl and Sadler (2017) has stated when determining the organic acids in food affecting the flavour, titratable acidity was a better predictor. Food acids are only partially ionised, as opposed to strong acids, which are entirely dissociated. This ionised percentage of the acid molecules affects some food attributes exclusively, whilst the overall acid content affects other food properties.

Table XII

Total Acidity in Standard and Seaweed Powder treated Idly Batter

FORMULATED VARIATIONS	PERCENTAGE OF LACTIC ACID (%)	
	0 th HOUR	12 th HOUR
Standard	0.541	1.062
Variation I	0.378	1.044
Variation II	0.313	0.954
Variation III	0.275	0.828
Variation IV	0.253	0.432

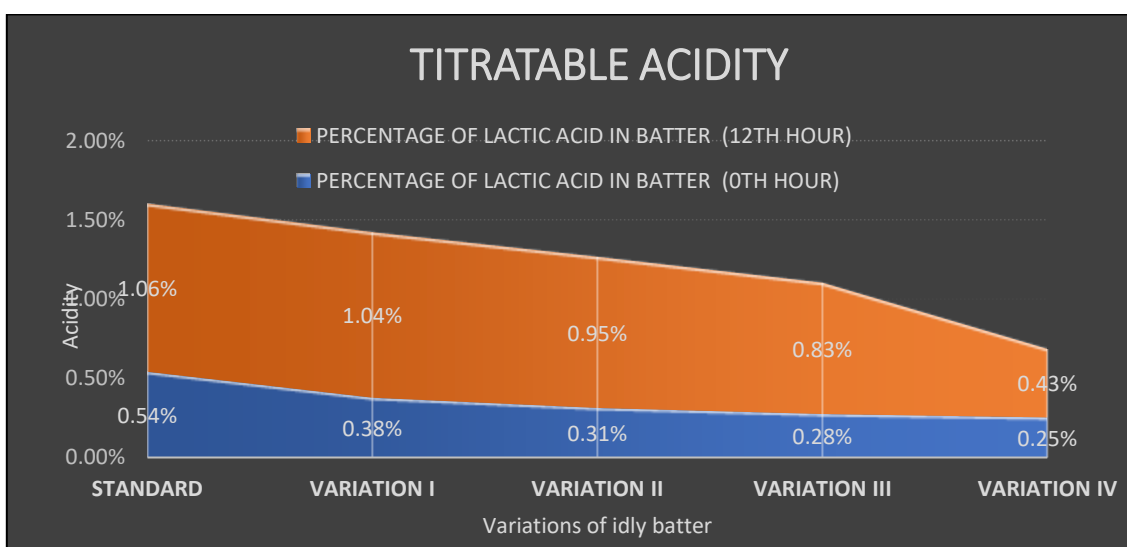


Figure 5
Change in Total Acidity for the Formulated Idly Batter

Increase in acidity indicates the growth of LAB and shows that fermentation has started in the batter. Acidity can be seen through pH in quantitative attribute but titratable acidity gives the qualitative attribute. At the start of fermentation, the acidity was relatively very low compared to the acidity rate at the end of fermentation. Acidity increases with increase in LAB activity. The highest range of acidity was seen in Standard which is untreated. The one without any treatment had the normal rate of fermentation and high acidity of 1.062% compared to the treated variations. The variation with the highest proportion of the seaweed powder showed the less amount of acidity which was 0.432% overall. Standard, which was untreated had the highest level of acidity among all the variations which is 1.062% at the end of the fermentation. The acidity in percentage shows the percentage of lactic acid in the batter.

d. BATTER VOLUME

Vanniarajan *et al* (2022) has reported that the increase in idly batter volume will increase in the number of idly and has provided the information that with limited amount of black gram it is possible to produce more batter volume by using black gram mutants. The idly batter volume was measured with the help of measuring cylinders.

Table XIII
Batter Volume for the Formulated Idly Batter

FORMULATED VARIATIONS	0th HOUR (ml)	4th HOUR (ml)	8th HOUR (ml)	12th HOUR (ml)
Standard	0	4.3	8.5	12.3
Variation I	0	1.3	2.3	3.4
Variation II	0	0.9	1.6	2.4
Variation III	0	0.5	0.9	1.5
Variation IV	0	0.2	0.9	1.3

In 100ml measuring cylinder, 30 ml of idly batter was filled for the five variations in separate measuring cylinders. To evaluate the rise of volume of idly batter during the fermentation process, the volume of each variation in measuring cylinder was noted. Initially all the variations were filled with the idly batter of 30ml to analyse a noticeable change in the volume.

The texture of an idly greatly depends on the batter volume. The hardness of idly rises as batter volume decreases. The variants using seaweed powder demonstrated a considerably lower volume increase during fermentation as compared to Standard. This was due to the addition of seaweed powder which had antimicrobial activity against Lactic acid bacteria. The volume of the standard idly batter increased by 2.4 ml at the end of fermentation, which was considerably less than that of the other variations. It was because of no treatment in the standard idly batter. The rise in volume of idly batter hour by hour was due to the process of fermentation. In this study, the addition of seaweed powder to the batter for idly prevented the batter from collapsing during fermentation, leading to a reduced percentage drop in batter volume compared to the batter that wasn't treated.

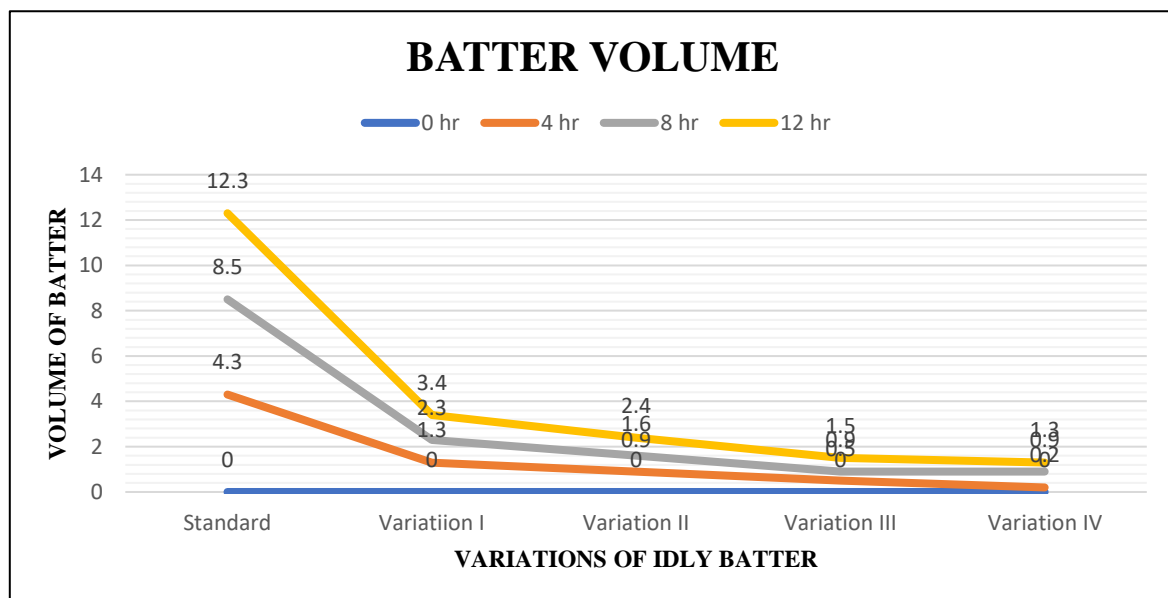


Figure 6

Change in the Formulated Idly Batter Volume

The addition of seaweed powder decreased the pH and acidity of batter according to the proportion of batter. The amount of the batter increased by 12.3 ml in Standard, which was left untreated. The idly batter that had high amount of seaweed powder added to it which was variation IV with 1g, showed a lower percentage increase in volume which was 0.6%. The standard with no treatment had the highest volume increase of batter which was 5.3% and the variation IV with highest proportion of seaweed powder had the lowest rise of batter of 0.6%.

According to Momin and Singh (2020), the batter volume treated with various starter cultures showed from 11.31 to 103.99. The highest recorded percentage was 103.99% and the lowest was 96.87.

To conclude the best variation with regard to the lowest rise in batter volume, variation IV with 1g of seaweed powder was suitable as it had the lowest rise in volume which was 0.6% which was notably very less compared to standard with 5.3% which was untreated.

C. QUALITY ANALYSIS OF IDLY

a. POROSITY ASSESSMENT

To record the internal structure, height, length and the porosity of the prepared idlys, the idlys were cut into half, stamped on an inkpad and were imprinted on graph sheet.

Porosity increases as the fermentation rate of the idly batter increases. The height that is rise of idly was measured with the imprinted structure of idly in graph. The length was also measured with the help of imprinted idly structure in graph. Both the length and height were measured in the unit of centimetres (cm). Porosity was determined in terms of the ink structure imprinted in the graph.

Table XIV

Imprint Characteristics of Formulated Idlys

FORMULATED VARIATIONS	HEIGHT (cm)	LENGTH (cm)	POROSITY
Standard	3.8	7	<ul style="list-style-type: none"> • Porous
Variation I	3.6	6.8	<ul style="list-style-type: none"> • Porous • Thick at the edges
Variation II	3.4	6.5	<ul style="list-style-type: none"> • Less porous • Thick internal structure
Variation III	3.4	6.3	<ul style="list-style-type: none"> • Very less porous • Thick internal structure all over
Variation IV	3.3	6.3	<ul style="list-style-type: none"> • Porous at the bottom • Thick internal structure at the top

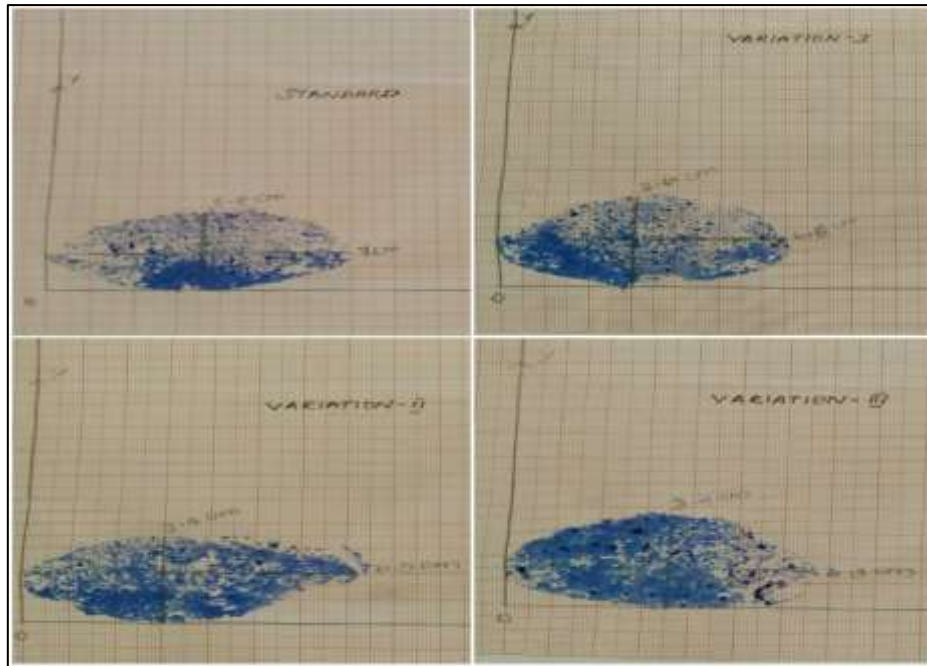


Plate 6

Graph Imprint of Formulated Idlys

The pores were determined by the dots seen on the ink print of idlys. The idly made from standard idly batter had more pores compared with the idlys treated with seaweed powder. Also, the height and length of standard idly batter were high which was 3.8 and 7 respectively, rather than other variations. This is due to the proper fermentation of the idly batter. Variation I treated with 0.25g of seaweed powder was porous when compared to other seaweed powder treated variations. The pores were less at the edges of the idly. Variation II treated with 0.50g of seaweed powder was thick and the pores were very less. Variation III treated with 0.75g of seaweed powder was thicker than variation II and the pores were less. The internal structure had less pores resulting in thick texture of the idly. The final variation treated with 1 g of seaweed powder which had the highest proportion, was thick at the bottom of the idly and was slightly porous at the top.

The porosity changes were due to the fermentation rate of the idly batter. The highest proportion of seaweed powder which was 1g, delayed the fermentation rate resulting in hard texture, less porosity and low rise of idly while steaming of idly.

b. ORGANOLEPTIC EVALUATION OF IDLI

Organoleptic evaluation was carried out to assess the acceptability of the incorporation of green additive into idly batter. Totally five variations were analysed in this study. Standard remained

untreated and acted as standard idly batter. Variation I, II, III and IV had 0.25g, 0.50g, 0.75g, 1 g of the seaweed powder respectively. Appearance, colour, texture, flavour, taste and overall acceptability were the attributes that were evaluated for the idlys at different varied proportions.

The sensory evaluation was carried out with the help of composite score card. Composite score card helps in the evaluation of food products with minor to major variations. Each and every attribute could be evaluated with multiple variations and each and every detail could be analysed with the help of composite score card.

Table XV
Sensory Attributes of Formulated Idlys

FORMULATED VARIATIONS	SENSORY ATTRIBUTES (Mean \pm S.D)					
	APPERANCE	COLOUR	TEXTURE	FLAVOUR	TASTE	OVERALL ACCEPTABILITY
Standard	8.7 \pm 0.48	8.5 \pm 0.52	8.2 \pm 0.63	7.6 \pm 0.96	7.7 \pm 0.82	8.1 \pm 0.56
Variation I	8.5 \pm 0.52	8.3 \pm 0.67	8 \pm 0.66	7.8 \pm 0.63	8 \pm 0.47	8.2 \pm 0.42
Variation II	8.5 \pm 0.70	7.9 \pm 0.70	7.5 \pm 0.70	7.7 \pm 0.48	8.1 \pm 0.56	7.8 \pm 0.63
Variation III	8.5 \pm 0.70	8.1 \pm 0.56	7.5 \pm 0.70	7.7 \pm 0.94	8.1 \pm 0.87	7.9 \pm 0.73
Variation IV	8.5 \pm 0.52	7.9 \pm 0.56	8.1 \pm 0.73	7.9 \pm 1.10	8.1 \pm 0.99	8.1 \pm 0.73

The results of the sensory analysis indicated the overall acceptability of *idly prepared with the standard and seaweed powder treated idly batter*. As standard was untreated and acted as control idly batter, the fermentation process was not affected and it was carried out without any delay. Due to this, the appearance, colour and the texture was very good. The taste and flavour were sour when compared to other variations. Considering the appearance, colour and flavour of standard, the overall acceptability of the variation was very good with the mean score 8.1, although the idly had sour flavour and taste.



Plate 7

Formulated Idlys for Sensory Evaluation

Similar to standard, the appearance and colour of variation I was good. Unlike standard, the flavour and taste were better. The sourness was less, compared to the standard idly batter. This variation had the highest score for the overall acceptability which was 8.2 ± 0.42 . As this variation had the less proportion of seaweed powder which was 0.25 g, it doesn't affect the taste and flavour of the idly. The texture was quite similar to standard. The mean score of 8.2 shows that the addition of lowest proportion of seaweed powder is acceptable considering all the attributes.

The variation II scored a good appearance and colour which showed the mean score of 8.5 and 7.9 respectively, but the texture was hard with a score of 7.5. This variation had 0.50 g of seaweed powder which made the fermentation process delay than the natural fermentation. Due to this, delay in fermentation affected the texture of the idly resulting in the hardness. The taste and flavour were better than the Variation II. The addition of seaweed powder doesn't express the sour flavour and taste in the idly because of the delayed fermentation. The overall acceptability was fair which scored 7.8 as the texture was hard.

In variation III, the proportion of seaweed powder was 0.75g which was quite high compared to Variation I and II. Variation II and III almost had the same scores in all the attributes as there was only 0.25 g difference between them. As mentioned before, the appearance and colour were same as other variations. Similar to Variation II, the texture was quite hard and there was complete absence of sour flavour and taste which was due to the composition of seaweed powder. Due to this absence of sourness, the idly was bland. The overall acceptability of the

variation was affected due to its hardness which was declared by the mean score 7.5. Bland taste was observed which was not acceptable as the flavour of idly.

Variation IV, which had the highest amount of seaweed powder did not show any flavour of the seaweed powder and the texture was hard and not fluffy like standard. The more amount of seaweed powder had affected the texture of the idly but had improved its taste and flavour. More the amount of seaweed powder, more the change in texture was observed. Although the texture was hard, the overall acceptability was good and fair as it had improved the taste and flavour.

With the results of the organoleptic evaluation, the overall acceptability was high in variation I which had 0.25g of the seaweed powder where the proportion was low when compared to other variations. In standard, which was untreated had a sour taste due to its normal fermentation process resulting in good appearance, colour and texture compared with other variations. Variation I had the highest overall acceptability compared with other variations. Variation II and Variation III had almost the same level of scores in all attributes. Although the taste does not affect much, the hardness in texture was observed. The appearance and colour were normal and similar to the other variations.

The difference in texture of the idly in various variations was due to the addition of seaweed powder. The untreated variation had a good texture of idly. The treated variations exhibited slow fermentation process and as a result, the idly's texture was altered. Referring to the taste and flavour of the idlys, the untreated variation was sour. In this case, the addition of seaweed powder which slowed the fermentation process decreased the sour taste and flavour. But with the mean scores variation I treated with 0.25g of seaweed powder was accepted by the panellists for the consumption.

D. MICROBIAL ANALYSIS OF THE IDLY BATTER

Fermented food products develop lactic acid bacteria as it plays an important role in preservation process. Also, fermentation acts as an efficient process in the production of wholesome foods. In the process of fermentation, the mould secretes liquefying enzymes for the process of saccharification and liquefaction when the yeast undergoes fermentation. According to Saravanan *et al* (2015), The idly batter's microbial load increased from 3 to 9 hours, ranging between 8.6 to 8.8 and 8.3 to 9.2 Log CFU/g for lactic acid and aerobic bacteria, respectively. Due to the formation of acid because of lactic acid bacteria, the pH of the idly batter decreased from 5.64 to 4.53.

Table XVI**Microbial Counts in Idly Batter of Standard and Added Seaweed Powder**

FORMULATED VARIATIONS	REFERENCE VALUE*	LACTIC ACID BACTERIA (LAB) (CFU/g)	YEAST & MOULD (CFU/g)
Standard	89,40,000	24,54,000	2400
Variation I	-	20,00,000	1900
Variation II	-	12,90,000	1700
Variation III	-	10,00,000	400
Variation IV	-	9,18,000	340

*Saravanan *et al.*, 2015

Microbial analysis was carried out to check whether the seaweed powder have the potential in controlling fermentation process. Though Lactic Acid Bacteria (LAB) are naturally produced in the process of fermentation, they are industrially used in fermentation to improve the taste and texture of food. Lactic Acid Bacteria (LAB), Yeast and mould were examined for the variations of idly batter to analyse the effectiveness of the seaweed powder in controlling the growth of microbes.

From the table XVI, the results of the enumeration of Lactic Acid Bacteria, yeast and mould in the variations of idly batter were revealed. Standard, which was untreated idly batter had the highest colonies of Lactic acid bacteria, yeast and mould. Variation IV, which had the highest proportion of seaweed powder exhibited less colonies of LAB, yeast and mould which was 918000 colony forming units/gram (CFU/g) and 340 CFU/g respectively. The Variation II, III and IV had the intermediate levels of colonies decreasing from the standard. The colonies of Lactic acid bacteria were high in standard and the lowest colonies were observed in the variation IV. Seaweed has helped in the microbial growth control in the fermentation process of idly batter by reducing the formation of LAB, Yeast and mould to extend its shelf life by extending the fermentation process.

To conclude the best variation for enhances the shelf life of the idly batter, the microbial analysis clearly depicted that variation IV, treated with 1g of seaweed powder was effective in controlling the Lactic Acid Bacteria (LAB), Yeast and mould. Thus, variation IV was regarded as the proper proportion of seaweed powder to improve the shelf life of idly batter thereby extending the rate of fermentation process.

Summary and Conclusion

V. SUMMARY AND CONCLUSION

Food additives are compounds that are added to food in order to preserve or improve its safety, freshness, flavor, texture or appearance. Before food additives are utilized, they are tested for any adverse effects on human health. Some food additives, such as salt in meats such as bacon or dried salmon, sugar in marmalade and sulfur dioxide in wine, have been used for ages to preserve food. These synthetic additives are the outcome of a chemical or enzymatic process. These synthetic additives have ill effects on health of the consumers like allergy, cancer, asthma, heart diseases and the effects are exposed to the next generation.

Natural food additives are gaining popularity among consumers and food businesses. Organic compounds taken from plants, animals or minerals are examples of natural additives. They are either absolutely similar to natural equivalents or pure inventions that do not exist in nature. Rosemary extract, neem oil, citrus oils, citric acid, grapeseed extract and buffered vinegar are some of the most popular and efficient natural preservatives available. Plant extracts act as an excellent source of natural antimicrobials and helps in enhancing the shelf life of a food product. Thus, by natural incidence they provide organic additives without any harmful effects.

Fermentation techniques are considered for food preservation. In essence, fermentation is the progressive decomposition of organic substances, which is sped up by bacteria or enzymes. Natural fermentation increases nutrient profile of a food in idly batter increases Vitamin B, amino acids and reduces anti-nutrients like phytates. Idly is a well-known culinary product made from a fermented batter made of rice and black gram dhal, generally in a 3:1 ratio. Idly originally came from South India, but because of its distinctively soft, spongy texture, sour flavor and alluring flavor, it has rapidly acquired popularity throughout India.

Seaweeds, being an underutilized source acts an incredible source of phenolic compounds with potent antimicrobial and antioxidant properties. Seaweeds have a multitude of properties that enable them to naturally preserve meals without compromising their quality or having detrimental repercussions. Agar, Alginate and carrageenan are some of the additives made from seaweeds. Algae are classified into brown, red and green based on the pigment present in the seaweed.

Gracilaria edulis, a red algal species is an agarophyte that produces an algal galactan that can be extracted in hot water and gels at room temperature. *Gracilaria* is widely ingested as a salad or raw for nutritional and medicinal purposes, creating a need for agar manufacture. They have

mineral concentrations that are 10 to 20 times greater than terrestrial plants due to their ability to concentrate minerals from seawater. As a result, they play critical nutritional roles in human nutrition and are an excellent source of minerals.

This study “**Application of green additive from selected seaweeds to increase the shelf life of the selected food product**” involved in the formulation of green additive to incorporate in the selected food product which is idly batter to analyze the shelf life and to study the effect of the green additive on idly batter. The main objectives of the study involve

- To formulate a green additive from *Gracilaria edulis*, a red algal species
- To incorporate the formulated green additive into idly batter
- To analyse the microbial growth in the incorporated product to analyse the shelf life

For this study, fresh and live seaweed *Gracilaria edulis*, which has high antimicrobial efficiency against bacteria was selected for the formulation of the green additive. The live algal species *Gracilaria edulis* was collected from Mandapam coastal region. The collected seaweeds were washed with water to get rid of the dirt and salts that were stuck to the outer surface.

The washed seaweeds were sun dried and ground into powder with the help of mixer grinder. The powdered seaweed was again ground into fine powder with the help of Ultra fine grinding technique in the name of ball milling. Thus, the green additive was obtained as a fine powder. The resulting seaweed powder underwent several characterisation analysis like particle size analysis, Zeta potential, UV visible spectroscopy and Fourier transform infrared spectroscopy.

The food product that was chosen for the treatment of the formulated additive was Idly batter. The shelf life of the fermented idly batter is very short and it can be stored under refrigerated conditions for about 5 days. So, to extend the life of idly batter, this formulated green additive was incorporated to check the effect of the additive in the fermentation rate of the idly batter. Variations were prepared with varying proportions of seaweed powder. Standard idly batter was made to compare the effect of normal rate of fermentation with the treated seaweed powder idly batter. Variation I, II, III and IV had 0.25g, 0.50g, 0.75g and 1g in per 100 ml idly batter respectively.

The idly batter was tested for its physicochemical properties like pH, total acidity and rise in batter volume. To determine the fermentation rate, the physicochemical analysis was not sufficient. To strongly determine the rate of fermentation, the idly batter was checked for Lactic

Acid Bacteria (LAB), Yeast and Mould. Thus, microbial analysis was carried out on idly batter to determine the increasing shelf life.

Idlys were prepared with the standard and seaweed powder treated idly batter. With 30 ml of batter each idly was prepared. To test the acceptability, the prepared idlys were analysed with organoleptic evaluation with the help of ten semi-trained panellists. The mean scores were calculated for the acceptability of the proportion of seaweed powder in idly batter. The quality of the idly was tested with the help of graph imprint of each idly with inkpad. With the imprint, height, length and the porosity of the idlys were evaluated. Thus, with the help of sensory evaluation and graph imprint, the quality and characteristics of the idly were analysed.

The major findings of the study are summarised as follows,

- *Gracilaria edulis*, the red algae have antimicrobial properties and has enough nutritional profile that could be included in the diet to meet the nutritional needs of human body
- The formulated green additive from the process of ball milling was effective in utilising the whole seaweed without wastage
- Green additive formulated as powder was analysed for particle size analyser, zeta potential, UV visible spectroscopy and Fourier transform infrared spectroscopy where the particle size which was 1038 r.nm with the width of 181 r.nm was seen. The zeta potential of the green additive revealed that the additive will exhibit a rapid coagulation or flocculation. The UV visible spectroscopy showed the band absorption spectra of the *Gracilaria edulis* seaweed powder. Finally, the FT-IR spectroscopy showed the presence of alkenes, aromatic compounds, anhydride and carboxylic acid compounds in the additive.
- Idly batter with varied proportions of seaweed powder were done and analysed for physicochemical properties which included pH, titratable acidity and batter volume. The physicochemical properties showed the quality and characteristics of the idly batter.
- The formulated five variations of idly batter including standard which was untreated, variation I, II, III, IV with seaweed powder weighing 0.25g, 0.50g, 0.75g and 1 g respectively were tested for the pH every four hours till the end of fermentation which was 12 hours. The pH of idly batter which was standard had the high pH at the end of fermentation which was 4.19. The variation IV which had the highest proportion of

seaweed powder exhibited low pH of 4.54 compared to other variations. This showed that the green additive lengthened the process of fermentation thereby reducing the acidity of the idly batter.

- Titratable acidity of standard was the highest which was 1.062% and the lowest 0.432% was seen in variation IV. With this it is evident that the green additive added with highest proportion had the lowest acidity level compared with standard and other variations.
- Batter volume of standard was seen the highest at the end of fermentation. The variation IV had the less rise in volume of the idly batter which shows that the fermentation rate was slow in the variation with high proportion of green additive.
- To assess the quality of idly, sensory evaluation and porosity assessment was done. The highest overall acceptability with the mean score of 8.2 ± 0.42 was exhibited by variation I which had the less amount of seaweed powder in it. The appearance, colour were excellent with the texture which was very good. The taste and flavour were fair which was due to the addition of seaweed powder. The seaweed powder reduced the sourness in the idly as the rate of fermentation was slow than in standard.
- With the mean scores of sensory evaluation, the highest mean score of 8.2 as overall acceptability was scored by variation I with 0.25g of seaweed powder and was accepted by the semi-trained panellists.
- Porosity assessment was done with the help of graph imprint of the idly. The idly made with standard idly batter has the spongy texture with more pores. The variation IV was very less porous and had a hard texture due to the effect of fermentation in the idly batter due to the addition of seaweed powder. The idly didn't rise well as much as standard. Due to this effect, it was confirmed that the green additive increased the time of fermentation which increased proportions.
- With porosity assessment, the standard idly batter with no treatment had the best texture and more pores.
- Microbial analysis was carried out to analyse the microbial growth. Lactic Acid Bacteria (LAB), Yeast and Mould were analysed. LAB plays an important role in the process of fermentation. The LAB count was high in standard which was 24,54,000 CFU/g and the lowest in variation IV which was 9,18,000 CFU/g. This shows that the higher the addition of seaweed powder lesser the LAB count in the idly batter.

- With the results of microbial analysis, the effective variation to extend shelf life of idly batter was variation IV with the proportion of 1g of seaweed powder
- Similarly, Yeast and mould were also less in variation IV which exhibited a count of 340 CFU/g. Higher count of 2400 CFU/g in standard as it was untreated.
- The addition of seaweed powder had gradually decreased the microbial count as the proportion increased. Similarly, the physicochemical properties of the idly batter also showed the positive results like less acidity, lesser pH and less rise in batter volume in the variation IV which had had the highest proportion of the green additive. This showed that the green additive has the potential in improving the shelf life of idly batter by lengthening the fermentation process and by controlling the LAB, Yeast and mould count.

CONCLUSION

Seaweeds being an excellent source of nutrients and antimicrobials, is remaining as an underexploited resource of marine wealth. They can be utilised in many ways for the betterment of human health such as nutraceuticals, pharmaceuticals, natural antimicrobials and production of natural food additives as they exhibit enormous antimicrobial and antioxidant activity. They can also be included in the diet of human in various forms to obtain nutritional needs. These seaweeds could also be incorporated in various food products as flavour enhancers, natural additives and to make it nutritionally rich. From the findings of this study, the formulated green additive from *Gracilaria edulis* was helpful and extended the shelf life of the idly batter by slow fermentation process and with the control of microbial counts especially LAB, Yeast and mould. Hence, the following study entitled “**Application of green additive from selected seaweeds to increase the shelf life of the selected food product**” concluded that *Gracilaria edulis* seaweed powder can be used as a natural additive to increase the shelf life of idly batter by decreasing the rate of fermentation.

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Appendices

VII. APPENDICES

APPENDIX – I

ESTIMATION OF pH

SCOPE

To determine the pH of the sample using digital pH meter.

PROCEDURE:

- Ensure that the instrument is on a clean and dry platform.
- Ensure that the instrument is clean.
- Switch on the main power supply to the instrument.
- Press 'ON / OFF' switch, it will display "MEAS" pH and temp." mode on the screen.
- Ensure that the instrument is calibrated with the buffer solution.
- Remove the pH electrode from the storage solution and rinse it with purified water 2-3 times. Soak the excess water with tissue paper and insert the pH electrode.
- Stir the electrode sample solution keeping the pH
- Allow stabilizing the pH and then record the pH. The display shows the pH of your sample, the temperature in °C.
- Rinse the electrode with purified water and soak the excess water with tissue paper between measurements.
- After completion of pH measurement rinse the electrode and temperature sensor with the purified water, soak the excess water with tissue paper and insert the pH with temp. sensor electrode in the storage solution (saturated potassium chloride solution).

APPENDIX – II

TITRATABLE ACIDITY

SCOPE

To determine the total acidity of the sample

PROCEDURE

- Five grams of sample dissolved in 10 ml of distilled water in a 100ml conical flask
- Add few drops of phenolphthalein
- Fill the burette with 50ml of 0.1 N NaOH solution
- Titrate the sample dissolved in 10 ml of distilled water against the 0.1 N NaOH solution until permanent pale pink colour appears.

CALCULATION

Percentage of lactic acid/gram = ml of 0.1 N NaOH * 0.009/ weight of the sample (g) * 100

APPENDIX – III

METHOD FOR YEAST AND MOULD COUNT OF FOODSTUFFS

(IS 5403:1999)

SCOPE

This standard specifies the method for viable yeast and mould count in products intended for human consumption for foodstuffs by means of the colony count technique at 25°C.

PROCEDURE

- Take two sterile petri dishes. Transfer to each dish, by means of a sterile pipette, 1 ml of the test sample if liquid, or 1 ml of the initial suspension in the case of other products.
- Take two further sterile petri dishes. Transfer, by means of another sterile pipette, 1 ml of the 10^{-1} dilution to each dish (liquid product), or 1 ml of the 10^{-2} dilution (other product).
 - Repeat the procedure described above using further dilutions, if necessary.
 - Pour about 15 ml of the yeast extract dextrose-chloramphenicol-agar medium, previously melted and maintained at $45 \pm 1^\circ\text{C}$ in a water-bath, from a culture bottle into each petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min.
 - Carefully mix the inoculum with the medium and allow the mixture to solidify, by leaving the petri dishes to stand on a cool horizontal surface.
 - Prepare a control plate, with 15 ml of the medium, to check its sterility.
 - Invert the plates and place them in the incubator at $25 \pm 1^\circ\text{C}$.
 - Count the colonies on each plate after 3, 4 and 5 days of incubation. After 5 days, retain those plates containing fewer than 150 colonies. If parts of the plates are overgrown with moulds, or if it is difficult to count well-isolated colonies, retain the counts obtained after 4 or even 3 days of incubation. In this event, record the incubation period of 3 or 4 days in the test report.

APPENDIX – 1V

MICROBIOLOGY OF FOOD: METHOD FOR THE ENUMERATION OF MESOPHILIC LACTIC ACID BACTERIA - COLONY-COUNT TECHNIQUE (IS 16068:2013)

SCOPE

This International Standard specifies a horizontal method for the enumeration of viable mesophilic lactic acid bacteria by counting the colonies growing in a solid medium after incubation at 30 °C for 3 days.

PROCEDURE

- Prepare the initial suspension and dilutions
- Take two sterile Petri dishes. Using a sterile pipette, transfer to each dish 1 ml of the test sample if the product is liquid, or 1 ml of the initial suspension in the case of other products.
- Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution of the test sample if the product is liquid, or 1 ml of the first decimal dilution of the initial suspension in the case of other products.
- Repeat the procedure described with the further dilutions, using a fresh sterile pipette for each decimal dilution.
- Pour into each Petri dish approximately 15 ml of the MRS medium which has been prepared then cooled to approximately 47 °C in the water bath.
- Carefully mix the inoculum with the medium and allow the mixture to solidify.
- Invert the prepared dishes and incubate them in the incubator set at 30 °C for 72 h ± 3 hour. Avoid desiccation of the agar during incubation so that the medium does not become too inhibitory
- After the specified period of time, count the colonies in each dish.
- Retain dishes containing fewer than 300 colonies at two successive dilutions, and more than 15 colonies on at least one dish.

CALCULATION

Calculate the number N of mesophilic lactic acid bacteria present in the test sample, as the weighted mean from two successive dilutions, using the equation:

$$N = \frac{\sum C}{V (n_1 + 0.1 n_2) d}$$

Annexures

VIII. ANNEXURES

ANNEXURE – I

INSTITUTIONAL ETHICAL APPROVAL

INSTITUTIONAL HUMAN ETHICS COMMITTEE



Avinashilingam

Institute for Home Science and Higher Education for Women
(Deemed to be university under Category 'A' by MHRD, Estd. u/s 3
of UGC Act 1956) Re-accredited with 'A++' Grade by NAAC.
Recognised by UGC Under Section 12 B
Coimbatore- 641043, Tamil Nadu, India

Chairman

Dr. Sudha Ramalingam
Director – Research and Innovation
Professor- Community Medicine,
PSG Institute of Medical Sciences
& Research, Coimbatore.

Member Secretary

Dr A Thirumani Devi
Professor
Department of Food Science
and Nutrition

Members

Mr. K Arulmoli (Legal Expert)
Dr. Subashini K. Sripathi
Dr. A Saraswathy (Medical Officer)
Ms. D. Kavitha
Dr. A R Sudamani Ramasamy
Dr. G. Victoria Naomi
Dr. Judith Justin
Dr. Anitha Subash
Dr. K Sampath Rani

06.01.2023

To
Ms. Nityashree, N.
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore- 641043

Dear Nityashree,

Ref: Your proposal No. IHEC/22-23/FSMD-14 entitled
"Application of Green Additive from Selected Seaweeds to Increase
the Shelf Life of the Selected Food Products" submitted for approval
of IHEC 21.11.2022

The Institutional Human ethics Committee of our University
hereby grants approval to your research proposal No. IHEC/22-23/
FSMD-14 entitled "Application of Green Additive from Selected
Seaweeds to Increase the Shelf Life of the Selected Food Products"
submitted by you. The Approval number for the same is
AUW/IHEC/FSMD- 22-23/XPD-14.

We wish you all the best in your research endeavours.

Regards


Dr. A. Thirumani Devi
Member Secretary
6.1.23


ANNEXURE – II

SENSORY EVALUATION SCORE CARD

NAME:		DATE:				
CLASS:						
PRODUCT:						
QUALITY	POSSIBLE SCORE	SAMPLE SCORES				
		Standard	Variation 1	Variation 2	Variation 3	Variation 4
Appearance	10					
Color	10					
Texture	10					
Flavor	10					
Taste	10					
Overall acceptability	50					

Signature:

Date:

ANNEXURE – III

PARTICLE SIZE ANALYSING OF *Gracilaria edulis* SEAWEED POWDER

Size Distribution Report by Intensity

v2.1



Sample Details

Sample Name: seaweed powder size 1

SOP Name: mansettings.nano

General Notes:

File Name: EXP 2 PHASE 2.dts	Dispersant Name: Water
Record Number: 472	Dispersant RI: 1.330
Material RI: 1.59	Viscosity (cP): 0.8872
Material Absorbtion: 0.010	Measurement Date and Time: Wednesday, April 05, 2023 8:...

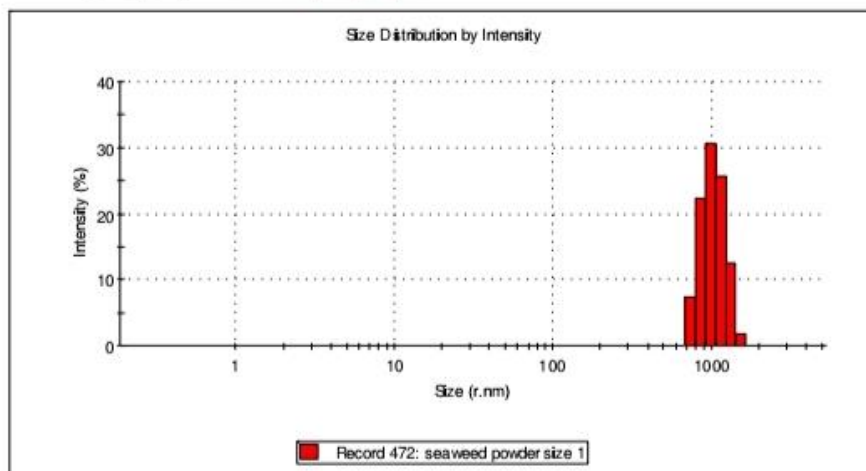
System

Temperature (°C): 25.1	Duration Used (s): 80
Count Rate (kcps): 188.0	Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette	Attenuator: 11

Results

	Size (r.nm):	% Intensity	Width (r.nm):
Z-Average (r.nm): 1637	Peak 1: 1038	100.0	181.3
Pdl: 0.462	Peak 2: 0.000	0.0	0.000
Intercept: 0.643	Peak 3: 0.000	0.0	0.000

Result quality : **Refer to quality report**



ANNEXURE – IV

ZETA POTENTIAL OF *Gracilaria edulis* SEAWEED POWDER

Zeta Potential Report

v2.2



Malvern Instruments Ltd. © Copyright 2008

Sample Details

Sample Name: SEAWEEDV POWDER ZETA 1

SOP Name: mansettings.nano

General Notes:

File Name: EXP 2 PHASE 2.dts Dispersant Name: Water
Record Number: 477 Dispersant RI: 1.330
Date and Time: Wednesday, April 05, 2023 9:30:... Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

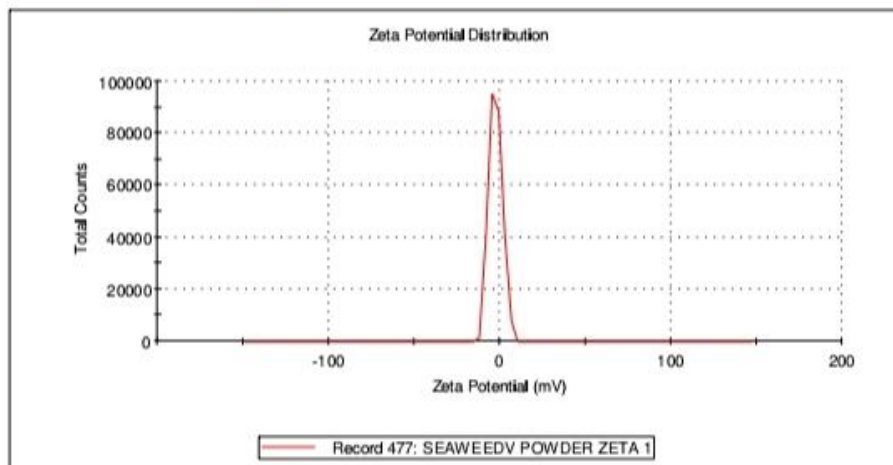
System

Temperature (°C): 25.0 Zeta Runs: 12
Count Rate (kcps): 75.3 Measurement Position (mm): 2.00
Cell Description: Clear disposable zeta cell Attenuator: 7

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -2.34	Peak 1: -2.34	100.0	3.81
Zeta Deviation (mV): 3.81	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.149	Peak 3: 0.00	0.0	0.00

Result quality : **Good**



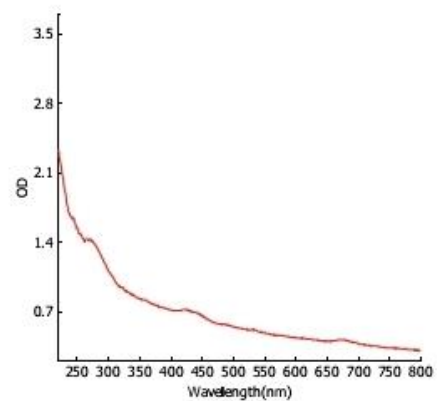
ANNEXURE – V

UV VISIBLE SPECTROSCOPY OF *Gracilaria edulis* SEAWEED POWDER

No. : 29
Sample Name : SEA WEED POWDER
Measurement Mode : Photometric
Wavelength(nm) : 800 700 600 500 400 300 250 220
DateTime : 23-04-05 14:54:52

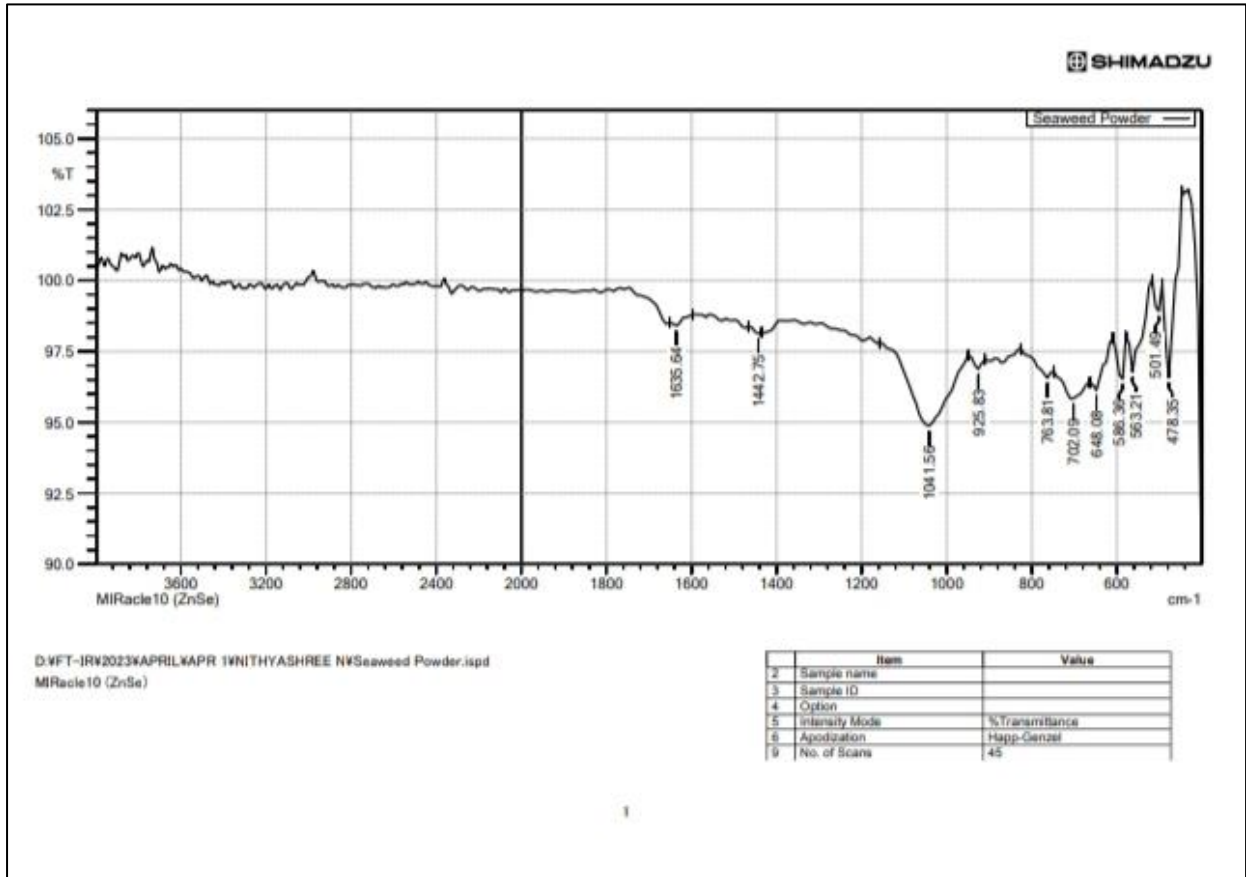
OD800 : 0.303
OD700 : 0.372
OD600 : 0.434
OD500 : 0.545
OD400 : 0.712
OD300 : 1.110
OD250 : 1.538
OD220 : 2.335

Item	Result
Pathlength (mm)	0.651
Dilution	1.000




ANNEXURE – VI

FOURIER TRANSFORM INFRARED SPECTROSCOPY OF *Gracilaria edulis* SEAWEED POWDER



ANNEXURE – VII

MICROBIAL ANALYSIS OF FORMULATED IDLY BATTERS



41 B, Muthiyalayam Road, Villakona Pithu, Thudikkai, Coimbatore - 641 034, Tamil Nadu, INDIA
 Ph: +91 22720 9666, 91 20539 26999, Email: info@srihakthi.com

Accredited by NABL Through Certificate Number TC-10001 dated 18.10.2021 as per ISO / IEC 17025 : 2017
 Sponsored by Ministry of Food Processing Industries, Government of India.

TEST REPORT

Test Report No: 2023/04/21/SSFTL/23-24/NN-029-001	Issue Date: 02.05.2023	Page 1 of 1
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CUSTOMER DETAILS	
Customer Name & Address	Ms. Nithyashree.N No.2, Raja Annamalai Road, Subbavacolony, Coimbatore.
Customer Reference	Test Request dt: 21.04.2023


SAMPLE DETAILS			
Product Category	Food and Agricultural Products	Sample Code	SSFTL/23-24/NN-029-001
Sample Name	Idly Batter - V1	Sample Conditions at Receipt	Good
Sample Description	Received in a Sterile Container	Sample Received on	21.04.2023
Sample Quantity	75ml	Test Commenced on	21.04.2023
Sampled by	Not Drawn by Lab	Test Completed on	26.04.2023
Sampling Procedure	-----	Testing performed at	Sri Shakthi Food Testing Laboratory, Coimbatore

TEST RESULTS – BIOLOGICAL PARAMETERS				
Sl. No.	PARAMETERS	TEST METHOD	UNIT	RESULT
1	Yeast and Mould	IS 5403 : 1999	CFU/g	2400
2	Mesophilic Lactic Acid Bacteria	IS 16068 : 2013	CFU/g	24,54,000


Remarks:
Result Related Only to the Sample Tested.

End of Report

Checked By: 
 Name : Nithiya.S
 Designation : Junior Microbiologist



Authorized Signatory
 Name : M.Karthikeyan
 Designation : Technical Manager – Microbiology



41 B, Muthiyalayam Road, Villakona Pithu, Thudikkai, Coimbatore - 641 034, Tamil Nadu, INDIA
 Ph: +91 22720 9666, 91 20539 26999, Email: info@srihakthi.com

Accredited by NABL Through Certificate Number TC-10001 dated 18.10.2021 as per ISO / IEC 17025 : 2017
 Sponsored by Ministry of Food Processing Industries, Government of India.

TEST REPORT

Test Report No: 2023/04/21/SSFTL/23-24/NN-029-002	Issue Date: 02.05.2023	Page 1 of 1
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CUSTOMER DETAILS	
Customer Name & Address	Ms. Nithyashree.N No.2, Raja Annamalai Road, Subbavacolony, Coimbatore.
Customer Reference	Test Request dt: 21.04.2023


SAMPLE DETAILS			
Product Category	Food and Agricultural Products	Sample Code	SSFTL/23-24/NN-029-002
Sample Name	Idly Batter - V2	Sample Conditions at Receipt	Good
Sample Description	Received in a Sterile Container	Sample Received on	21.04.2023
Sample Quantity	75ml	Test Commenced on	21.04.2023
Sampled by	Not Drawn by Lab	Test Completed on	26.04.2023
Sampling Procedure	-----	Testing performed at	Sri Shakthi Food Testing Laboratory, Coimbatore

TEST RESULTS – BIOLOGICAL PARAMETERS				
Sl. No.	PARAMETERS	TEST METHOD	UNIT	RESULT
1	Yeast and Mould	IS 5403 : 1999	CFU/g	1900
2	Mesophilic Lactic Acid Bacteria	IS 16068 : 2013	CFU/g	20,00,000


Remarks:
Result Related Only to the Sample Tested.

End of Report

Checked By: 
 Name : Nithiya.S
 Designation : Junior Microbiologist



Authorized Signatory
 Name : M.Karthikeyan
 Designation : Technical Manager – Microbiology



41 B, Muthiyalayam Road, Villakona Pithu, Thudikkai, Coimbatore - 641 034, Tamil Nadu, INDIA
 Ph: +91 22720 9666, 91 20539 26999, Email: info@srihakthi.com

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TEST REPORT

Test Report No: 2023/04/21/SSFTL/23-24/NN-029-003	Issue Date: 02.05.2023	Page 1 of 1
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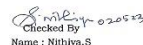
CUSTOMER DETAILS	
Customer Name & Address	Ms. Nithyashree.N No.2, Raja Annamalai Road, Subbavacolony, Coimbatore.
Customer Reference	Test Request dt: 21.04.2023


SAMPLE DETAILS			
Product Category	Food and Agricultural Products	Sample Code	SSFTL/23-24/NN-029-003
Sample Name	Idly Batter - V3	Sample Conditions at Receipt	Good
Sample Description	Received in a Sterile Container	Sample Received on	21.04.2023
Sample Quantity	75ml	Test Commenced on	21.04.2023
Sampled by	Not Drawn by Lab	Test Completed on	26.04.2023
Sampling Procedure	-----	Testing performed at	Sri Shakthi Food Testing Laboratory, Coimbatore

TEST RESULTS – BIOLOGICAL PARAMETERS				
Sl. No.	PARAMETERS	TEST METHOD	UNIT	RESULT
1	Yeast and Mould	IS 5403 : 1999	CFU/g	1700
2	Mesophilic Lactic Acid Bacteria	IS 16068 : 2013	CFU/g	12,90,900


Remarks:
Result Related Only to the Sample Tested.

End of Report

Checked By: 
 Name : Nithiya.S
 Designation : Junior Microbiologist



Authorized Signatory
 Name : M.Karthikeyan
 Designation : Technical Manager – Microbiology



41 B, Muthiyalayam Road, Villakona Pithu, Thudikkai, Coimbatore - 641 034, Tamil Nadu, INDIA
 Ph: +91 22720 9666, 91 20539 26999, Email: info@srihakthi.com

Accredited by NABL Through Certificate Number TC-10001 dated 18.10.2021 as per ISO / IEC 17025 : 2017
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TEST REPORT

Test Report No: 2023/04/21/SSFTL/23-24/NN-029-004	Issue Date: 02.05.2023	Page 1 of 1
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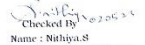
CUSTOMER DETAILS	
Customer Name & Address	Ms. Nithyashree.N No.2, Raja Annamalai Road, Subbavacolony, Coimbatore.
Customer Reference	Test Request dt: 21.04.2023


SAMPLE DETAILS			
Product Category	Food and Agricultural Products	Sample Code	SSFTL/23-24/NN-029-004
Sample Name	Idly Batter - V4	Sample Conditions at Receipt	Good
Sample Description	Received in a Sterile Container	Sample Received on	21.04.2023
Sample Quantity	75ml	Test Commenced on	21.04.2023
Sampled by	Not Drawn by Lab	Test Completed on	26.04.2023
Sampling Procedure	-----	Testing performed at	Sri Shakthi Food Testing Laboratory, Coimbatore

TEST RESULTS – BIOLOGICAL PARAMETERS				
Sl. No.	PARAMETERS	TEST METHOD	UNIT	RESULT
1	Yeast and Mould	IS 5403 : 1999	CFU/g	400
2	Mesophilic Lactic Acid Bacteria	IS 16068 : 2013	CFU/g	10,00,000

Remarks:
Result Related Only to the Sample Tested.

End of Report

Checked By: 
 Name : Nithiya.S
 Designation : Junior Microbiologist



Authorized Signatory
 Name : M.Karthikeyan
 Designation : Technical Manager – Microbiology



SRI SHAKTHI FOOD TESTING LABORATORY

43-B, Mettupalayam Road, Velakinar Pirkku, Thudiyalur, Coimbatore - 641 034, Tamil Nadu, INDIA.
Mob : +91 72220 96666, +91 89030 26999, E-mail : srishakthi@gmail.com

Accredited by NABL Through Certificate Number TC-10001 dated 18.10.2021 as per ISO / IEC 17025 : 2017
Sponsored by Ministry of Food Processing Industries, Government of India.

TEST REPORT

Test Report No:2023/0421/SSFTL/23-24/NN-029-005	Issue Date: 02.05.2023	Page 1 of 1
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CUSTOMER DETAILS

Customer Name & Address	Ms.Nithyashree.N No.2, Raja Annamalai Road, Saibabacolony, Coimbatore.
Customer Reference	Test Request dt 21.04.2023

SAMPLE DETAILS

Product Category	Food and Agricultural Products	Sample Code	SSFTL/23-24/NN-029-005
Sample Name	Idly Batter - VS	Sample Conditions at Receipt	Good
Sample Description	Received in a Sterile Container	Sample Received on	21.04.2023
Sample Quantity	75ml	Test Commenced on	21.04.2023
Sampled by	Not Drawn by Lab	Test Completed on	26.04.2023
Sampling Procedure	-----	Testing performed at	Sri Shakthi Food Testing Laboratory, Coimbatore

TEST RESULTS - BIOLOGICAL PARAMETERS

Sl. No.	PARAMETERS	TEST METHOD	UNIT	RESULT
1	Yeast and Mould	IS 5403 : 1999	CFU/g	340
2	Mesophilic Lactic Acid Bacteria	IS 16068 : 2013	CFU/g	9,18,200

Remarks:
Result Related Only to the Sample Tested.

End of Report

Checked By *Nithiya* 020523
Name : Nithiya.S

Designation : Junior Microbiologist



M. Karthikeyan 02.05.23
Authorized Signatory
Name : M.Karthikeyan

Designation : Technical Manager - Microbiology