DEVELOPMENT AND QUALITY EVALUATION OF VALUE ADDED PRODUCT: MANDARIN JELLY INCORPORATED WITH SEAWEED *(Gracilaria tenuistipitata)*.

Rajia Sultana Eshita

Roll No: 01-20/02

Registration No: 831

Session: 2020-2021

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics.

Department of Applied Food Science and Nutrition

Faculty of Food Science & Technology

Chattogram Veterinary and Animal Sciences University,

Chattogram-4225, Bangladesh

August 2022

Authorization

I hereby declare that I am the sole author of the thesis. I authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of Scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, and author of this work, declare that the electronic copy of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

Rajia Sultana Eshita

August 2022

DEVELOPMENT AND QUALITY EVALUATION OF VALUE ADDED PRODUCT: MANDARIN JELLY INCORPORATED WITH SEAWEED *(Gracilaria tenuistipitata)*.

Rajia Sultana Eshita

Roll No: 01-20/02

Registration No: 831

Session: 2020-2021

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made.

.....	.....
(Taslima Ahmed)	**(Dr. Md Asaduzzaman)**
Supervisor	Co-supervisor
Assistant Professor	Associate professor
Department of Applied Food Science and Nutrition, CVASU	Department of Marine Bioresource Science CVASU

(Kazi Nazira sharmin)

Chairman of the Examination Committee

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

August, 2022

PLAGIARISM VERIFICATION

Title of Thesis: Development and quality evaluation of value added product: Mandarin jelly incorporated with seaweed (*Gracilaria tenuistipitata*)

Name of the Student: Rajia Sultana Eshita

Roll number: 01-20/02

Reg. no: 831

Department: Applied Food Science and Nutrition

Faculty: Food Science and Technology

Supervisor: Taslima Ahmed, Assistant Professor, Department of Applied Food Science and Nutrition

This is to report that as per the check……% of the content of the above thesis is stated to be plagiarized and is covered/not covered as per plagiarism policy and institutions which has been issued from CASR, Chattogram Veterinary and Animal Sciences University. The report has been sent to the coordinator, CASR via email

The thesis may/may not be considered for the evaluation.

Taslima Ahmed

Assistant Professor

Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University

DEDICATED

To

MY

BELOVED

PARENTS

And Teachers

Acknowledgements

First and foremost, I would like to express my gratitude to the **"Almighty Allah"** from my deepest sense of gratitude, whose blessing has enabled me to complete the thesis for the degree of Masters of Science (MS) in Applied Human Nutrition and Dietetics.

I express my sincere and deepest gratitude to supervisor **Taslima Ahmed**, Assistant professor, Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University, for her effective steerage during my whole study period who ploughed through several preliminary versions of my text, making critical suggestions and posing challenging questions. Her expertise, invaluable guidance, constant encouragement, affectionate attitude, understanding patience, and healthy criticism added considerably to my experience.

I also offer my special gratitude and heartfelt thanks to my Co-supervisor Dr. Md Asaduzzaman, Associate professor, Department of Marin Bioresource Science, CVASU, for his guiding support and wholehearted cooperation during my research work.

I owe my special thanks to the director and the scientists associated with this research work of Poultry Research and Training Center (PRTC), Department of Applied Food Science and Nutrition, Department of Applied Chemistry and Chemical Technology, Department of Animal Science and Nutrition, Department of Food Processing and Engineering, CVASU for their Constant inspiration and kind cooperation in performing the research activities precisely in those laboratory.

Finally, I must express my very profound gratitude and cordial thanks to my loving family, friends, and well-wishers for their cooperation, cheerfulness and inspiration during the study. I gratefully acknowledge thanks to my beloved parents for their Understanding, inspirations, moral support, kindness and blessings, forbearance and endless love to complete my study.

The Author

August, 2022

Table of Contents

List of Tables

List of figures

List of Abbreviations

g/L	gram per liter
mg/dl.	Mili gram per deciliter
mmol/L	Mili mol per liter
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
TSS	Total Soluble Solids
$^{\circ}$B	Degree Brix
$^{\circ}$C	Degree Celsius
et al	Et alii/ et aliae/ et alia
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalent
TE	Trolox equivalent
QE	Quercetin equivalents
PPM	Parts per Million
Ros	Reactive oxygen species
Cfu	Colony forming unit
SDA	Sabouraud Dextrose Agar
spp.	Species
Eos	Essential oils
COX-2	Cyclooxygenase 2
PGE2	Prostaglandin E2
TNF-α	Tumour Necrosis Factor alpha
IL-1β	Interleukin-1β
MCF-7	Michigan Cancer Foundation-7

Abstract

Mandarin fruit and Seaweed (*Gracilaria tenuistipitata*) are rich in nutrients. This study was aimed to create value added mandarin jelly with seaweed extract and to evaluate the physicochemical properties, microbiological analysis, and sensory attributes. Four formulations of jelly such as sample A, sample B, sample C, sample D were prepared varying the amount of mandarin and seaweed extract. Proximate composition, mineral content (Na, K, Ca, Mg, and Fe), bioactive profile, antioxidant capacity, microbiological analysis, and sensory evaluation were performed on the formulated jellies. One-way analysis of variance (ANOVA) was used to compare the results in order to determine their significance level at $P< 0.05$. The findings showed that the prepared jellies differed significantly ($P< 0.05$) in terms of moisture, protein, fat, fiber, ash, carbohydrate, net amount of energy, Na, K, Ca, Mg, and Fe, total phenolic content, total flavonoid content, antioxidant capacity, and sensory characteristics. The analysis showed the range from (26.34% -31.74%) moisture content, (64.11% -66.38%) carbohydrate content, (0.96% -2.73%) protein content, (1.06% -2.70%) fat content, (0.99% -1.65%) fiber content, (0.38% -1.11%) ash content for four formulation of mandarin-seaweed jelly where the highest value was obtained in containing maximum seaweed extract jelly. Titratable acidity was found 0.59%, 0.55%, 0.54%, 0.64% and Total soluble solid was ranged from (65ºBrix - 68ºBrix), pH ranged from (2.91-3.1) in the variations of these jelly sample. In jelly containing maximum seaweed extract, Potassium (193.83mg/100g) and Calcium (68.27mg/100g) were found higher than other minerals. Up to 15 days, the total viable count was within acceptable bounds, and after three months of storage at a cold temperature, no signs of fungal activity were seen.

Keywords: Seaweed, Mandarin, Gracilaria, Bioactive compound, Antioxidant

Chapter 1: Introduction

In contrast to actual plants with true roots, stems, and leaves, seaweeds and other marine algae are ancient non-flowering organisms. They are a valuable renewable resource that can be found in the ocean. Vitamins, minerals, trace elements, protein, iodine, bromine, and several bioactive compounds are found in these foods. Several types of seaweed can be used for both nutrition and health benefits. All phytochemicals, including agar, agarose, carrageenan, and algin, must be extracted from seaweed, and there is no other source. Many different industries rely on phycocolloids for various purposes, including those dealing with food, medicines, confectionery, dairy, textiles, paper, paint, varnish, and more. Many additional compounds, including mannitol, iodine, bromine, laminarin, and fucoidin, are made from seaweeds or marine algae. Several different types of consumable foods can be made from seaweed, including jelly, payasam, jam, chocolate, salad, soup, curry, etc. Additionally, they can be used in the production of seaweed meal and seaweed liquid fertilizer. Seaweeds have a wide variety of therapeutic uses. In a number of studies, researchers found that seaweeds contained bioactive chemicals (Chennubhotla et al., 1987).

There are three major categories of seaweed, based on the color of their photosynthetic pigments such as green (Chlorophyta), brown (Phaeophyta) and red (Rhodophyta). In accordance with this, they contain pigments such as chlorophyll a and b in Chlorophyta, chlorophyll a and c, carotene, xanthophylls, and fucoxanthin in phaeophyta, and phycoerythrin in Rhodophyta. The best dietary supplies of vital minerals can be found in seaweeds. Seaweeds provide a complete mineral balance in the diet. Potassium, sodium, calcium, magnesium, zinc, copper, chlorine, sulfur, phosphorus, cobalt, manganese, selenium, bromine, iodine, arsenic, iron, and fluoride are some of the elements found in abundance in seaweeds. It's important for the human body to have a wide variety of minerals because they're used in so many different ways. For example, lack of iodine in the diet causes thyroid dysfunction, inadequate calcium absorption in the body causes osteoporosis (Maehre et al., 2014).

Gracilaria as a genus is significant as a marine bio-resource because it provides over 80% of the world's agar supply from only a few species. Many *Gracilaria* species are also consumed or used in traditional medicine by people all over the world. The

Gracilaria genus as a whole shows promise as a rich resource for several high-value chemicals and extracts. The most common application of seaweeds is the isolation of phycocolloids. The polysaccharides called phycocolloids are a feature of the cell walls of several types of seaweed (Yang et al., 2015). Major seaweed phycocolloids include agar, carrageenan, and alginate (Ma et al., 2019). Carrageenan is derived from Kappaphycus and Hypnea, while agar comes from *Gracilaria*, and alginate comes from Sargassum. The genus *Gracilaria* is the most prolific source of agar among farmed seaweeds. *Gracilaria* is the best agar source because it grows quickly and contains a high concentration of agar. Production of agar around the world increased to 14,500 tons in 2016, bringing about $246 million at retail (Porse and Rudolph, 2017).

Citrus is the genus that includes both sweet and sour oranges, including mandarins, tangerines, tangors, and tangelos (Moulehi et al., 2012; Cassano et al., 2009). Organic acids, phenols, and sugars, among others, are abundant in citrus fruits and contribute to the fruit juice's palatable flavor and aroma (Legua et al., 2014).Citrus juice is one of the most consumed beverages worldwide because of its high nutritional value. Flavonoids, phenolic acids, limonoids, and adrenergic amines are all examples of bioactive chemicals that contribute to the potent antioxidant effects (Ye et al., 2011).

Jelly is a fruit-flavored condiment that is clear, translucent, sparkling, quivers, tasty, shelf-stable, and prepared from fruit or fruit juice, sugar, and pectin. When fruit is crushed and the solid, lumpy remnants are taken out, jelly is created, which has the bald texture. The only thing left is the crushed fruit, which is mixed with pectin and boiled to create the gelatinous spread with 65% sugar. It must contain at least 62% water-soluble solids in order to be termed jelly if it is made from fruit, fruit juice, or a fruit juice concentrate. The main components required to manufacture jellies are pectin, sugar, and acid, and the proper proportions of each must be used to ensure the best gel formation (Al-Noori et al., 1984).

Mandarin jelly is a very popular and well-known food. In this study the manufacturing of mandarin jelly has been employed with sea weed (*Gracilaria tenuistipitata)*. This study is conducted to examine the value addition of different formulation of mandarin and seaweed extract. The nature of value added seaweed-mandarin jelly was determined on premise on physico-chemical characteristics

namely: moisture content, fat content, protein content, crude fiber content, mineral content, bioactive profile and, antioxidant capacity, microbial analysis and organoleptic test to oversee the sensory attributes as well as consumer acceptance.

1.1 Justification and importance of the study:

Seaweeds are popular as dietary item in Japan, Korea, China, Far East, and the Hawaiian Islands. Although there are many edible kinds of seaweed present in the coastal zones in Bangladesh, seaweed has not yet made its place on the Bangladeshi peoples' dining table. *Gracilaria tenuistipitata* is a nutrient-dense and healthful sea vegetable due to its high protein, carotene, mineral, and moderate amounts of crude fiber, ash, moisture, and total calorie contents. It has also low levels of lipids and heavy metals and a balanced amino acid profile (Aziz et al., 2021). Therefore, particular consideration is needed to conduct scientific research on such macroalgae for its efficient and environmentally sustainable exploitation given the economic significance and health-benefitting potentials.

With this goal, the present study has been conducted. Mandarin jelly is a very popular food among people of all age groups. So the present study has combined this known food with seaweed and reviewed its nutritional value, phytochemical profile, antioxidant property, microbial activity and consumer acceptability of value added mandarin-seaweed jelly.

1.2 Aims and Objectives:

- To develop a value added mandarin jelly with seaweed
- To evaluate proximate composition of mandarin-seaweed jelly
- To determine bioactive profile and antioxidant capacity of mandarin-seaweed jelly
- To determine mineral content of mandarin-seaweed jelly
- To assess the sensory evaluation

Chapter 2: Review of Literature

2.1 Overview of Seaweeds:

Seaweeds also known as marine macro algae which include Chlorophyta, Rhodophyta, and Ochrophyta or Phaeophyceae, are thought to be one of the plant-based foods of the future. This is due to the fact that seaweeds can grow without making use of cultivable land or fresh water resources, meaning that they do not compete with conventional agriculture. Consuming them regularly has been linked to lowering one's risk of developing a number of ailments and they provide a wealth of beneficial nutrients and bioactive phytochemicals (Brown et al., 2014). Although direct use of macroalgae as food is still in its infancy in Europe compared to Asiatic countries, there is a tendency towards growing intake of these "sea vegetables. In addition, the food and nutraceutical sectors are becoming increasingly interested in including macroalgae as ingredients in functional foods, further contributing to its incorporation into the diets of Europeans. The stated health benefits of macroalgae are in large part owing to its high content of beneficial nutrients like dietary fiber, minerals, proteins, steroids, and phenolic compounds (Lordan et al., 2011).

Seaweed's mineral content can make up as much as 40% of its dry weight. Furthermore, many types of seaweed have a high potassium-sodium ratio, which can assist to counteract the harmful intake of sodium that is common in the modern diet (Hanjabam et al., 2017). Furthermore, red and green macroalgae are portrayed as an alternative to or supplement to conventional protein sources, since their protein content can reach as high as 47% and 26% of the fresh and dry weight (dw) respectively, in some seaweed species (Wells et al., 2017).

There are only about 221 types of seaweed utilized around the world. These include 125 different types of Rhodophyta (red algae), 64 different types of Phaeophyceae (brown algae), and 32 different types of Chlorophyta (green algae). About 145 of these species (66% of the total) are consumed directly as food. There are 79 Rhodophyta, 38 Phaeophyceae, and 28 Chlorophyta. There are 101 species utilized in the phycocolloid business, including 41 alginophytes (algae that make alginic acid), 33 agarophytes (algae that produce agar), and 27 carrageenophytes (algae producing carrageenan). About 12 species are farmed in marine agronomy; 24 species are used

in traditional medicine; 25 species are used in agriculture, animal feed, and fertilizers; and so on (Pereira et al., 2009).

From the months of October to April, the Bangladeshi coast of the Bay of Bengal is home to at least 150 different types of seaweed, 19 of which are of commercial importance. St. Martin's Island harvesters collect and smuggle 250–500 metric tons of dried seaweed into Myanmar each year, out of a total of 5,000 metric tons available. The Mog and Rakhyine people, who inhabit the region, use the surviving seaweeds for subsistence purposes (Sarkar et al., 2016a).

Benthic varieties of seaweed grow naturally in intertidal areas on pneumatophores of mangrove tree, other wooden logs and barks of trees thanks to the favorable climatic, environmental, and waterway conditions found throughout the entire Sundarbans mangrove forest. Approximately 60 different types of seaweed, including *Boodliopsis sundarbanensis*, *Ulvalactuca*, *Catenellarepen*, *Gelidium*, *Polysiphonia* etc can be found in the Sundarbans. Cox's Bazar is home to around 155 different types of seaweed. The Shilkhali-Shaplapur coast, Jaillapara, the Shahparirdip area of Teknaf, Nuniarchara, Nazirartek of the Bakkhali-Moheshkhali river estuary, Moheshkhali Island, and the planted mangrove forest or Parabon section of Cox's Bazar all have an abundance of seaweeds. Natural seaweed beds can be found in the Moheshkhali Channel estuary, on Moheshkhali Island, and along the Bakkhali River in Cox's Bazar, from its mouth to the village of Nuniarchara. The two most common types of seaweed found in seaweed beds are *Hypnea musciformis* and *Enteromorpha intestinalis*. St. Martin's Island is home to over 140 types of seaweed. St. Martin is an island with four different coastlines: west, east, south, and north. Northern coasts don't have access to seaweeds. Southern coastlines are home to a wide variety of seaweeds, including *Sargassum coriifolium, Chaetomorpha moniligera, Gracilaria verrucosa,* and many more. The seaweeds found on the western coast include *Gracilaria textorii, Hypnea musciformis, H.pannosa, Gracilaria verrucosa, Padina arborescens, Chaetomorpha moniligera* and others. When comparing the western and eastern coasts of St. Martin Island, seaweeds are more prevalent on the western coast (Sarkar et al., 2016).

2.1.1 Potential health benefits from marine algae supplements and their

Biological activities:

Changing one's diet and lifestyle can prevent 33 percent of diseases including cancer, diabetes, and chronic disorders related to inflammation. Natural dietary supplements have potential as disease preventatives as well. As will be seen below, marine algae phytochemicals like peptides, amino acids, lipids, fatty acids, sterols, polysaccharides, carbohydrates, polyphenols, photosynthetic pigments, vitamins, and minerals can serve as powerful antioxidants and have positive effects as anti-diabetic and chemotherapeutic drugs (Conlon and Bird, 2015).

- **Peptides and Amino Acids**

Bioactive peptides, which are produced when proteins are hydrolyzed, have the potential to improve health and alter disease progression. Phyto-peptides with biological activity have 3–20 amino acid residues and exhibit anti-oxidant, anti-cancer, anti-inflammatory, and immune modulatory effects. Proteins in both large and small algae have all nine essential amino acids, which work together to protect cells from harm.

- **Lipids and Fatty Acids**

Medicinal efficacy of lipids and fatty acids' can be attributed, in part, to the great structural diversity among these substances. Some research suggests that consuming a moderate amount of saturated fatty acids may reduce the risk of cardiovascular disease. The polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) found in marine algae are thought to be good for human health and may even play a role in lowering the risk of cardiovascular disease (Ruxton et al., 2004).

- **Polysaccharides and Carbohydrates**

According to Misurcova et al., (2012), red algae are a common source of the polysaccharides. Numerous algal polysaccharides exhibit bioactivity and have the potential to be used as therapeutic options for the treatment of a variety of human health problems. Some polysaccharides, such as soluble and insoluble fibers can be included in a healthy diet and should be considered dietary fibers. To a greater extent than in other produce categories, seaweeds (25–75%) contain dietary fibers relative to

their dry weight. The antitumor, anticancer, anticoagulant, and antiviral properties of algal dietary fibers are only a few of the ways they improve human health. Glucose and starch are two examples of carbohydrates that can be found in great quantities in microalgae. Microalgal species' carbohydrate content is ascribed for a broad range of biological functions, including immune-modulatory effects and lowering of blood lipids and the ability to halt cell proliferation (Bin et al., 2013).

- **Polyphenolic Compounds**

Bioactive chemicals derived from marine algae are powerful antioxidants that protect cells from the harm caused by oxidation. The antioxidant activity of bioactive substances derived from marine algae has been linked to protection against cancer, inflammatory conditions, diabetes, and a number of disorders connected to ROS. Both microalgae and macroalgae are the most common sources of polyphenolic chemicals. Hydroxycinnamic acids, phenolic acids, simple phenols, xanthones, coumarins, naphthoquinones, stilbenes, flavonoids, anthraquinones, and lignins are all examples of phenolic components (Fernando et al., 2016).

- **Vitamins and Minerals**

Human growth and development rely on vitamins, which are a type of micronutrient. Vitamin B_1, B_2, and B_{12} are plentiful in seaweeds and microalgae. In contrast to brown algae, green and red algae have significantly more vitamin B_{12} or cobalamin per cell. Megaloblastic anemia and neuropsychiatric problems are just two of the illnesses that a lack of cobalamin can bring on. All types of seaweed-brown, red, and green; contain vitamin C (ascorbic acid)Vitamin C's antioxidant, anti-aging, and immune-stimulating properties are just a few of the ways it helps human health. It is the tocopherols that make up vitamin E. Green, red, and brown seaweeds all contain tocopherol (Kim and Taylor, 2011)

Minerals, trace elements, and inorganic atoms are all preserved in seawater by seaweeds and macroalgae. Trace minerals and elements are essential for human nutrition. Seaweeds as dietary supplements provide the body with some of the major minerals like Na, K, Ca, and Mg, and some of the trace minerals like Fe, Zn, Mn, and Cu. As a result of their ability to lessen the likelihood of Ca insufficiency in pregnant

women and adolescents and to slow the onset of premature aging in young people, seaweeds are also significant Ca sources (Lordan et al., 2011).

2.1.2 Seaweed based products and their uses in daily life:

From ancient times, people in places as diverse as Costa Rica, Japan, China, and Egypt have been eating specific types of seaweed (Dillehay et al., 2008). Traditional commercial products recovered from seaweed biomass focus on the phycocolloids (Carbohydrates) such agar, carrageenan, and alginate. Baghel et al., (2021) reviewed that the food, beverage, dairy, animal feed, cosmetics, personal care, pharmaceutical, healthcare, textile, printing, and paper coating sectors are just some of the many that rely on hydrocolloids derived from seaweed as an essential element. Ulvan and porphyran, two types of storage sulfated polysaccharides, are abundant in Ulva and Porphyraspecies. As a result of their wide range of biological activities, such polysaccharides hold great promise as new drug and nutraceutical ingredients. The minerals found in seaweed are yet another ingredient in commercial fertilizers.

Seaweed as a human food:

Seaweeds, botanically referred to as "algae,"are used as a staple food source in both Eastern and Western diet. Sea-vegetable is the Chinese name for marine algae, and it is typical to see locals in Japan, Malaya, China, and the Philippines. Algae farms are located primarily in South Korea and Japan. Japan is the only country where seaweed is cultivated on a large scale. To a lesser extent, Indians eat seaweeds; one species in particular (*Gracilaria edulis*) has been used for decades to make gruel in the coastal parts of Tamil Nadu. (Kaladharan and Kaliaperumal, 2000 a)

It is possible to make and eat a wide variety of seaweed food products and recipes like salad, seaweed masala, seaweed pickle, seaweed wafer, seaweed porridge, seaweed jam, jelly, soup, ice-cream, yogurt, seaweed singara, seaweed samosa etc. Nori or parple laver (*Porphyra spp.*), Aonori or green laver (*Monostroma spp*. and *Enteromorpha spp*.), kombu or haidai (*Laminaria japonica*), wakame (*Undaria pinnatifida*), Hiziki (*Hizikia fusiforme*), dulse (*Palmaria palmate*), ogo or ogonori or sea moss (*Gracilaria spp.*) etc are the mostly used seaweed for human consumption (Chennubhotla et al., 1987a).

According to Sarkar et al., (2019) powdered *Hypnea sp*. was boiled in water to extract phycocolloids, which were then utilized to make seaweed jelly, soup, ice cream, and yogurt due to their gelation property. Traditional methods were used to make seaweed singara and samosa, however *Hypnea sp*. powder was mixed into the filling. In present study, seaweed (*Gracilaria tenuistipitata*) has been incorporated with mandarin jelly to evaluate the quality of jelly product whether it has been value added or not as well as consumer acceptance.

Seaweed as medicine:

From marine algae, more than 600 secondary metabolites have been discovered, including terpenes, alkaloids, fatty acids, and nitrogenous substances. Many of these substances have undergone significant research utilizing pharmacological and laboratory testing and are therapeutically effective. *Sargassum* species were employed to cool and purify the blood. *Sargalin*, a substance that lowers blood sugar, is present in them. As a vermifuge, *Hypnea musciformis* is used, and as a cathartic, *Centroceras clauulatum* is used. Goitre disease can be controlled by eating iodine-rich seaweeds like *Sarconema furcellatum* and *Asparagopsis taxiformis*. Different stomach and intestinal problems have been treated using a variety of red algae, including *Gracilaria*, *Gelidium*, and *Pterocladia*. By stretching the uterus during labor, *Laminaria cloustonistipes* have been utilized to assist in child birth. Alginates have been discovered to extend the rate of activity of some medications, and carrageenan is helpful in the treatment of ulcers. Numerous marine algae species have been discovered to possess antimicrobial and anticoagulant activities. Although the use of many seaweed products in pharmacology is well established, research and development on the production of antibacterial, antifungal, and antiviral compounds from seaweeds are still in their early stages (Chennubhotla et al., 1987a).

2.2 Overview of *Gracilaria tenuistipitata*:

2.2.1 General biology of *Gracilaria tenuistipitata*:

Gracilaria, with 190 species, is the most populous genus in the order Gracilariales; it comprises the major genera of Rhodophyta (Guiry et al., 2018).The taxonomy of *Gracilariat enuistipitata*is given below:

Kingdom: Plantae

Phylum:Rhodophyta

Class: Florideophyceae

Order: Gracilariales

Family:Gracilariaceae

Genus: *Glacilaria*

Species*: G. tenuistipitata*

According to Guiry et al., (2018) Gracilaria species can be found all over the world, from the tropics to the subarctic, with the greatest diversity in the Indo-Pacific and Western Atlantic Still, much remains unknown about these species, and their global range is extremely restricted. Although most species of Gracilaria can be found in the subtidal and intertidal zones between 0.5 and 10 m deep, there are a few exceptions that live exclusively in deeper water. The species of this genus are typically found growing on solid substrata (such as rocks) or anchored in sandy or sandy-muddy substrata, with sand covering at least some of the thallus. According to (Lyra et al., 2021) Some species may live in the wild, where they can grow to enormous, dense mats even in relatively calm waters. Although Gracilaria species are found in many different maritime environments, they are most commonly discovered in protected places such as estuaries, bays, mangroves, reefs, and mudflats.

G. tenuistipitata plants are 10-20 cm in length, slender, and 2-3 orders of branched, and they are easily separated from the sub-stratum due to their short holdfast and delicate stipe. The main axis are densely and irregularly branched, with shorter, more delicate branchlets (ranging in diameter from 1.0 to 1.5 mm) near their tips. Each

medulla cell is about 10-25 µm in diameter, but grows to be 130-200 µm in size as it moves toward the center. The cortex has two layers of circular cells ranging in size from 14 to 22µm in diameter (Yang et al., 2012)

2.2.2 Traditional applications and economic significance:

The economic value of Gracilaria species has been extensively exploited by humans. People living in the area around Chile's Monte Verde started using it as a food and medicine source as early as prehistory (about 14,000 years ago) (Dillehay et al.,2008). Using Gracilaria plants for their benefits is an ancient practice in China. These algae were used for both nourishment and as a binder to enhance the fixation of lime in wall paintings. The popularity of these algae appears to have spread from China to other eastern nations. Consumption of Gracilaria species which are classified as macroalgae or sea vegetables is concentrated in Japan, Southeast Asia, Hawaii, and the Caribbean. These algae, for instance, are a popular topping for the Hawaiian fish salad-Poke recognized as ogonori (or ogo) in Japan, they are commonly used as a salad topping or as an additional decorative element when serving sashimi globally (Gordon, 2017).

The Gracilaria species are often used as a source of the phycocolloid agar which is typically found in certain types of red algae. The Food and Agriculture Organization of the United Nations reports that gracilaria species are responsible for > 80% of the world's agar production. Agar has become popular in Western diets as a replacement for gelatin which comes from animals. Agar is a common ingredient in soups, snacks, and regional specialties like "Tokoroten" (Japanese) and "Anmitsu" (Chinese) and "Halo-halo" (Philippine) (Japan). Several remedies based on Gracilaria species are utilized in traditional medicine. Constipation, enteritis, dysentery, urinary abnormalities, thyroid ailments, and respiratory diseases are only some of the conditions that various species of Gracilaria are used to treat in China (Santelices, 2014).

The baking and confectionery industries use Gracilaria agar, a food-grade agar, as a thickening, stabilizing, or gelling agent when making sweets like pies, icings, and jelly candies. One of the most widely grown macro algae in southern China's marine aquaculture is *Gracilaria tenuistipitata* Chang & Xia, is widely cultivated because of its high agar content, fast growth rate, and broad resistance to environmental variables (Zhongneng et al., 2002). Although Gracilaria agar is abundant, it is of lower grade

than Gelidium agar. To this end, producing Gracilaria based products with high value added and increasing agar quality are the two most pressing concerns. Since enhancing the quality of Gracilaria agar is unlikely, researchers are instead concentrating on creating new value-added products from *Gracilaria tenuistipitata* to maximize the output's potential (Armisen,1995).

According to Glazer et al., (1983) both agar and phycobiliproteins (PBPs) have been found in *Gracilaria tenuistipitata*, however PBPs are far less common.The primary PBPs are phycoerythrin(PE,565), allophycocyanin (APC,651), and phycocyanin (PC,621). Phycoerythrin is one such protein that has been widely employed as a fluorescent tag in a variety of biochemical procedures including immunology, cell biology, and flow cytometry due to its exceptionally bright fluorescence. In addition, it finds applications in the cosmetics, food, and dyestuff sectors (Raja et al.,2008). Cyanobacteria (blue-green algae) and various red algae are known to produce R-phycoerythrin (R-PE), according to numerous studies published over the past decade. However, there is a dearth of research describing the isolation of R-PE from *G. tenuistipitata*, a species commonly used in commercial marine aquaculture (Sonani et al., 2018).

2.2.3 Potential health effect of *Gracilaria tenuistipitata*:

Anticancer activities:

WHO (2018) reports that cancer is responsible for the deaths of over 8 million people annually around the world. Chemotherapy is widely used to treat cancer; nevertheless, the treatment is not without its drawbacks, and drug resistance is becoming common. Therefore, the vigorous and warranted hunt for new anticancer medications should come as no surprise. Extractions using ethanol or methanol have been categorized as the most common method for obtaining the bioactive chemicals found in the genus Gracilaria (Armisen, 1995). For example, methanolic extracts of *G. edulis* were reported to inhibit the proliferation of MDA-MB231 cells, whereas aqueous extracts of *G. tenuistipitata* inhibited the growth of oral squamous cell cancer (Yang et al., 2012).Anticancer activity may be due to the medicinal value and rich phytoconstituent content of the ethyl acetate extract of *G. tenuistipitata* seaweed. In an in vitro study of cytotoxic reactivity against MCF-7 breast cancer cells, it was found to play a critical role in lowering cancer activity after 24 hours of interaction (Vivek et al., 2022).

Effects on inflammation and pain perception:

Compounds with anti-inflammatory properties work to alleviate some of the symptoms associated with inflammation including pain, fever, and edema. Half of the research on Gracilaria species' anti-inflammatory properties has been done with aqueous extracts or sulfated agarans (Chaves et al., 2013;Chen at al.,2013). These tests generally demonstrated promising anti-inflammatory effects. The expression of key inflammatory chemicals was decreased and edema and leukocyte migration were reduced by the aqueous extracts and sulfated agarans (Sugiura et al., 2006).

Chen et al., (2013) reported that anti-inflammatory chemicals and alkaloids with established biological activity are abundant in red algae of the genus Gracilaria. In an in vitro model of Hepatitis C Virus (HCV)-induced inflammation, the aqueous extract of *Gracilaria tenuistipitata* demonstrated anti-inflammatory action. This extract was able to decrease COX-2 activity and PGE2 generation in HCV-infected cells, as well as NF-B p65 translocation to the cell nucleus and TNF-α, IL-1β, and iNOS gene expression after treatment with the extract.

Antimicrobial activities:

Extracts from seaweed have been utilized extensively in recent years for the treatment and prevention of bacterial and viral infection in fish and other aquatic animals. According to Thanigaivel et al., (2014) in few studies, seaweed extracts were found to have significant antibacterial action against waterborne pathogens. Large numbers of shrimp, crabs, and other aquatic species have died since the white spot syndrome virus (WSSV) was originally discovered in 1992 in Taiwan. Sirirustananun et al., (2011) found that several kinds of seaweed have been shown to exhibit anti-WSSV viral activity in recent research. They also found that Extracts of the plant *Gracilaria tenuistipitata* dramatically raised Litopenaeusvannamei's immunological activity against WSSV, resulting in lower mortality rates for the shrimp.

Gastrointestinal effect:

According to Charoensiddhi et al., (2020) the majority of seaweed polysaccharides, including alginates, fucoidans, laminarins, ulvans, agars, and carrageenans, may be considered dietary fiber. They are resistant to the digestive enzymes found in the human digestive system, promote the growth of good bacteria in the gut, prevent

pathogens from adhering and evading the immune system, and alter metabolic processes in the intestines, such as fermentation.

Several studies have shown that prebiotics produced from seaweed cause positive changes in gut microorganism, adding to the body of evidence supporting the use of prebiotics. Many types of seaweed, particularly those are red or green in color, have polysaccharides that could be used as prebiotics. Total dietary fibre (TDF) was measured in red seaweeds *Gracilaria fisheri* and *G. tenuistipitata*from, Pattani Bay in southern Thailand by Benjama and Masniyom (2011, 2012), and in green seaweeds *Ulva pertusa* and *U. intestinalis* by same authors (51.3-62.2% DW). The potential gut health benefits of polysaccharides from green and red seaweeds have been reported on in only a small number of published research, despite the recent growth in commercial goods and patent actions on seaweed-derived bioactive chemicals (Hu et al., 2006; Zhang et al., 2020).

2.2.4 Nutritional composition of *Gracilaria tenuistipitata:*

Gracilariaceae (Rhodophytceae) seaweeds are economically significant due to their high concentrations of protein, fiber, fatty acids, vitamins, macro- and trace-elements, and key bioactive substances, such as agar-agar (Sousa et al., 2008). The health benefits of eating edible seaweeds include lower blood lipid levels, decreased body fat percentage, and decreased chance of developing cardiovascular disease (Benjama and Masniyom, 2011). However, Seaweeds differ in their nutrient makeup depending on their species, level of maturity, the location in which they grow, and the season. Nutrient synthesis is impacted by changes in ecological circumstances (Lobban et al., 1985).

Gracilaria tenuistipitata, commonly known as"sea lettuce,"contains significant amounts of protein, ash, and dietary fiber, as well as reasonably high quantities of minerals and important amino acids. This Gracilaria species therefore seems to be a possible source for or ingredient in functional food products and animal feed. The nutrient composition of *Gracilaria tenuistipitata* are listed below:

Table 2.1: Proximate composition (%) of *Gracilaria tenuistipitata:*

Carbohydrate	45.93±1.53
Moisture	12.10±0.25
Crude protein	21.6±2.6
Crude lipid	2.8±0.90
Crude fiber	5.65±0.13
Ash	17.±0.10

Table 2.2: Mineral (mg% DM) content of *Gracilaria tenuistipitata*

Na	291.8
K	5791.8
Mg	580.8
Fe	12.75
Cu	0.60
Zn	4.5
Ca	218.5
P	311.5

Source: (Benjama and Masniyom, 2012).

2.2.5 Phytochemical component:

During normal cellular physiological or biochemical processes, reactive oxygen species (ROS) are produced. These ROS include free radicals such as superoxide anion radical, hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, and lipid peroxides. They have a crucial role in the pathophysiology of many diseases such as aging, inflammation, carcinogenesis, atherosclerosis, cardiovascular diseases, rheumatoid arthritis, and neurodegenerative disorders, by inducing oxidative stress that damages cells (Sasikumar and Kalaisezhiyan, 2014). The course of diseases caused by oxidative stress can be halted by supplementing with natural antioxidants, which protect cells from damage by either inhibiting the creation of free radicals or by scavenging free radicals. Microflora, macroalgae, and vascular plants are only a few

examples of marine flora that they are being used from ancient times for medical motives. (Boopathi and Kathiresan, 2010). Due to its powerful antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, and anticancer capabilities, marine alagae, also known as seaweeds, have received interest in recent years, and research is focused on discovering phytochemicals from marine algae. Marine macroalgae have been found to have phytochemicals with potent antioxidant activity making them an important source of natural antioxidants. Padmanabhan, (2022) reviewed on phytochemical of *Gracilaria tenuistipitata* and they found the presence of tannins,phenols, flavonoids, Saponins, glycosides, steroids, carbohydrates and reducing sugars, proteins and amino acids in the extract of *G. tenuistipitata.*

Table 2.3: Quantitative analysis of profound phytochemical and antioxidant capacity of *Gracilaria tenuistipitata*:

Parameter	**Value**
Total phenolic content (mg GAE/g extract	57.20±0.59
Total Flavonoids content (mg Catechin Equivalent/100 g extract)	9.28±0.27
Antioxidant capacity; DPPH (IC50, mg/ml)	0.510 ± 0.019

Source: Padmanabhan, (2022).

2.3 Overview of Mandarin orange:

Mandarin oranges (Citrus reticulate) have the shortest storage life of all citrus fruits but are rich in healthy nutrients such organic acids and phenols (Betoret et al., 2017). They can be distinguished from oranges by their form, size, and wide open cores .Late fall and midwinter are prime times for harvesting mandarin oranges. As a result of its high moisture content (80-85%), it rots in the face of unfavorable environmental conditions while being stored (Hong et al., 2007). Sugars, organic acids, amino acids, pectin, minerals, and volatile organic compounds are all abundant in mandarins, in addition to the antioxidants (such as ascorbic acid, carotenoids, and phenolic compounds) (Legua et al., 2014). Additionally, the distinct scent of mandarin is developed through the interaction of multiple volatile components; in fact, isolated 32

volatile compounds from the juice of newly picked mandarins. The concentration of volatile chemicals responsible for mandarin's distinctive flavor and aroma varies across stages of ripeness, and it has been demonstrated that juice made from unprocessed, unpeeled fruit has a higher concentration of these components. Juice is the most researched type of Mandarin product, suggesting that it is the most popular value-added product made from mandarins (Barboni et al., 2010).

Nam et al., (2019) reported that total soluble solids (TSS), titratable acidity (TA), ripening index, titratable acidity to total soluble solids ratio and vitamin C concentration are the most important quality control metrics used in the juice industry to create a high-quality product. Ye et al., (2011) reported that mandarin oranges have a high concentration of bioactive compounds, and even the immature fruits that are normally thrown away as a result of physiological dropping have been recognized as a potential source of nutraceutical compounds that contribute to the improvement of human health, with the free and ester form of phenolic acids predominating over the bound phenols.

2.3.1. Nutritional fact of Mandarin:

Minerals including calcium, potassium, phosphorus, and magnesium, as well as vitamins C and A, proteins, and dietary fiber, can all be found in a good amount in mandarin. Furthermore, they contain little amounts of vitamins B_1, B_2, B_3, B_5, B_6, B_9. Sugars, acids, carotenoids, polyphenols, limonoids, and vitamin C all play a role in determining the flavor of the fruit. Vitamins, fiber, and other substances found in plants that are beneficial to human health, such as flavonoids, will be of great use to people who consume the fruits and the byproducts of the fruits.

The vitamin B complex, for instance, aids in maintaining a healthy heart, providing energy, ensuring proper nerve function, producing hormones and cholesterol, maintaining healthy cells, and preventing off infections. Cryptoxanthine is a xanthophyll with pro-vitamin A activity, and mandarin fruits have very large concentrations of it. Consumers enjoy mandarins for their delicious taste and high phytochemical content.Mandarins and other citrus fruits and their derivatives are in high demand as more people become health-conscious (Putnik et al., 2017).

Mandarins have a high concentration of beneficial nutrients. The following nutrients can be found in one medium mandarin (88 grams):

- **Calories:** 47
- **Carbs:** 13.3 grams
- **Protein:** 0.81 grams
- **Fat:** 0.38 grams
- **Fiber:** 0.31 grams
- **Vitamin C:** 26% of the Daily Value (DV)
- **Magnesium:** 2.5% of the DV
- **Potassium:** 3% of the DV
- **Copper:** 4% of the DV
- **Iron:** nearly 1% of the DV

2.4 Therapeutic Uses of mandarin:

In addition to vitamin C, carotenoids, and phenolic compounds, the edible part of the mandarin fruit is rich in antioxidants. The fruit is also rich in a variety of other nutrients, including minerals, sugars, organic acids, amino acids, pectins, and volatile chemical compounds. These parts protect the body from long-term diseases and provide essential nutrients for it to function normally. The dietary fiber and phenolic compounds found in mandarins are useful for making nutrient dense dishes. Moreover the coumarinsin of mandarins, are known to increase the skin's photosensitivity (Al-Snafi, 2016).

Yeung, (1995) reviewed that the peel of this fruit is reported to have laxative, aphrodisiac, antiemetic, astringent, and tonic effects while also regulating moisture levels, softening hard, rough skin, and cleansing oily skin. Dyspepsia, bloating of the stomach or intestines, a persistent cough with mucus production, hiccups, and vomiting are all conditions that can be treated and managed with their help. The unripe green exocarp is used to treat hepatic cirrhosis, as well as enlarged organs like the liver and spleen, as well as bloated organs like the digestive tract, the hypo-chondrium and the chest. Because of its analgesic and carminative characteristics, the seed is used to treat a variety of ailments, including but not limited to: hernia, lumbago, mastitis, and testicular pain and swelling.

2.5 Pharmacological effects of mandarin:

Antimicrobial characteristics of mandarin:

Mathur et al., (2011) found that the antibacterial properties of citrus fruit products are effective against bacteria and fungi. Several different foods containing citrus extracts have demonstrated antibacterial efficacy. Many polymethoxylated flavones, which are extremely uncommon in plants, are found in citrus fruit peel. Ortuno et al., (2006) reported that, phytochemicals and plant extracts, both of which have antibacterial properties, can be quite useful in medical practice. They appear to have a protective function in plants against microbial, fungal, and viral invaders. Several studies have shown that Eos from citrus fruits, as well as the components of Eos are effective against molds and yeasts. Citric acid, coumarins, flavonoids, and flavonones, all with antibacterial and anti-inflammatory properties, can be found in the seed, peels, and pulp of mandarin fruits (Habib et.al., 1986). Both the leaves and peel of mandarin fruit were found to have potent antibacterial activity, inhibiting the growth of a wide variety of microorganisms, including common Gram-positive and Gram-negative bacteria as well as some fungi (Hamdan et al., 2013).

Cardiovascular effect of mandarin:

Cardiovascular disease (CVD) is a prominent source of morbidity and mortality in both developed and developing countries. Lin et al., (2018) reported that, due to inactivity, smoking, and bad eating habits, the global prevalence of CVD has risen considerably). Fruits and other plant-based foods have been shown to help lower cholesterol and lower the risk of cardiovascular disease (Hemler, 2019). Mandarin orange juice is a complex food matrix full of beneficial components including vitamins, minerals, and phyto-chemicals that neutralize the effects of simple carbohydrates which triggers CVD (Sakaki et al. 2019).

Effect on blood pressure:

Maintaining a healthy blood pressure is crucial for a steady flow of blood. Mandarin oranges are good for managing high blood pressure because of the fiber, antioxidants, and potassium they contain. This mandarin orange fruit is a good source of potassium, which regulates the body's fluid levels. It helps the kidneys to excrete excess sodium

(salt) which in turn relaxes the smooth muscle lining the blood vessels. When the kidneys regulate the quantity of fluid the body stores, they regulate blood pressure. (Wartenberg and Richter, 2020).

Anticancer and antioxidant effect:

When compared to other CF juices, mandarin orange juice has been investigated the most due to the widespread interest in its purported health advantages and widespread consumption around the world. As a matter of fact, it has a lot of vitamin c and flavonoids, which gives it antioxidant properties. Recently, it was examined that the antioxidant activity of an mandarn juice (MJe) extract high in flavonoids in both cell-free and cell-based experimental paradigms. (Ferlazzo et al., 2015) claimed that it can protect human lung epithelial A549 cells from the oxidative stress caused by hydrogen peroxide H_2O_2 by reducing ROS formation and membrane lipid peroxidation, boosting mitochondrial performance, and protecting against DNA-oxidative damage.

Effect of mandarin on skin:

Wartenberg and Richter, (2020) said that Mandarin oranges are a great source of vitamin A, Vitamin C, which help to protect the skin from conditions like acne and pimples. Rebuilding damaged skin tissues is one of vitamin A's primary actions, making it crucial for healing wounds and other skin damage. Vitamin A is an excellent nutrient for keeping wrinkles, fine lines, and dull skin at bay, thanks to its anti-aging qualities. They also said that vitamin C, which helps boost collagen formation and keep skin looking firm and young. Strengthening the skin is collagen's job, and this protein is essential.

2.6 Characterization of mandarin jelly:

Since fruit juice or a fruit water extract is used to create jellies, the final product is transparent. On the other hand, jams are made using whole, crushed, macerated, or pureed fruit, thus they contain all or most of the insoluble solids of the fruit. The only difference between jams and preserves is that the latter typically includes entire fruit while the former does not. When sugar is added to pectin in the presence of acid, the result is a gel. At least in part, the sturdiness of fruit jellies can be imposed to the

hydrogen bonding that occurs between hydroxyl groups and between hydroxyl and carboxyl groups Relationships between pectin, sugar, and acid are crucial to the final product's success. As an example, if there isn't enough pectin or acid, the jelly won't gel, and if there isn't enough sugar, the jelly will be rough.

Not overall acidity but active acidity or pH determines how much acid is needed. Pectin's carboxyl groups are negatively charged, thus the acid must be neutralizing those charges in order to have an effect on the molecular level and increase the pectin's propensity to associate with itself and create a gel. Gels only develop at pH levels lower than 3.5.Below 3.5, the jelly becomes more solid. To preserve the fruit's fresh flavor, acidic ingredients such vinegar, lemon juice, lime juice, citric acid, lactic acid, malic acid, and tartaric acid are frequently used (Woodroof, 1986).

2.6.1 Value addition by Seaweed:

Seaweed is a comprehensive source of nourishment. Seaweed can contain as much as 440,000 times its weight in marine nutrients. Seaweed is an excellent source of macrominerals like nitrogen, oxygen, calcium, and selenium and microminerals like iron, magnesium, and sodium, as well as essential nutrients like carbs, proteins, lipids, and fiber. Seaweed is 10-20 times more concentrated in amino acids, vitamins, and minerals than terrestrial plants. Due to its unique nutritional profile, seaweed has a colossal range of potential applications in areas such as food and beverage production, pharmaceuticals, agriculture, and industry, where it is expected to play a significant role in the generation of value (Wijesinghe, 2012). Although there is a value-added study for some seaweed derivatives, such as pharmaceutical grade, industrial grade, and food grade, value-added study is not available for all. According to the previous review of literature Mandarin jelly, also known as orange jelly has been developed with the incorporation of seaweed known as *Gracilaria tenuistipitata* to add value to the culinary product.

Chapter 3: Materials and Methods

3.1 Study area:

The whole experiment was carried out in the laboratory of Applied Food Science and Nutrition, Applied Chemistry and Chemical Technology, Food Processing and Engineering, Animal Science and Nutrition, and Poultry Research and Training Centre at Chattogram Veterinary and Animal Sciences University (CVASU).

3.2 Duration of study:

The experiment was conducted for a period of six months from June-November 2022.

3.3 Sample Collection:

Fresh mandarin was collected from the local market in Chattogram and the seaweed sample named *Gracilaria tenuistipitata,* was collected from the ECOFISH (World Fish, USAID funded seaweed project) which is located at Nuniachara (21º28´26.1´´ N and 91º57´51.5´´ E), Cox's Bazar Sadar, Cox's Bazar.

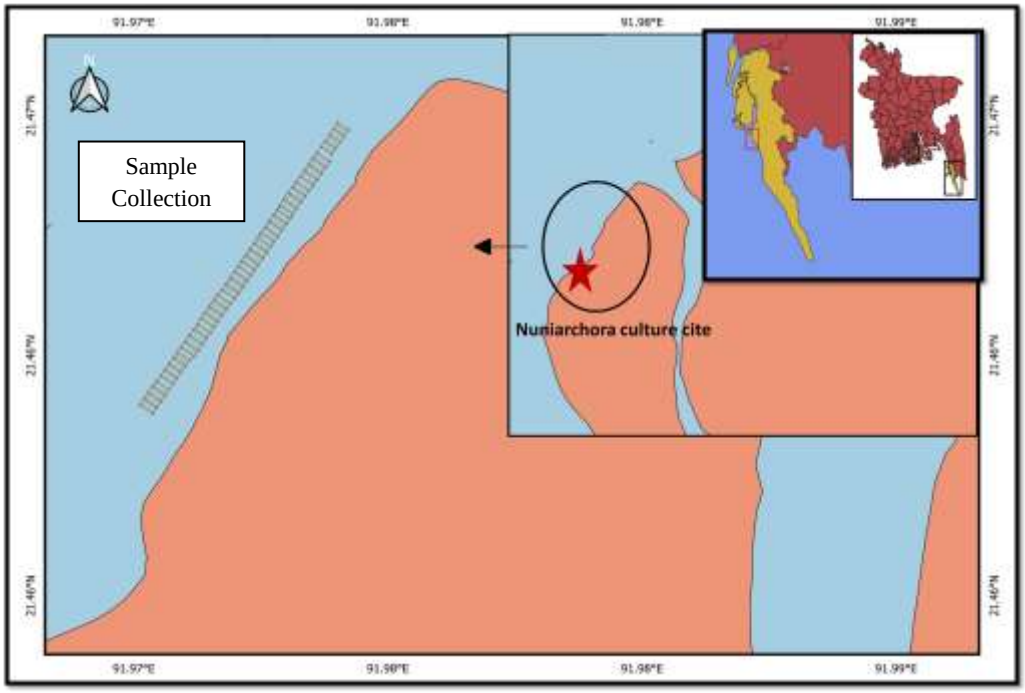


Figure 3.1 Seaweed sampling from Nuniachara, Cox's Bazar.

3.4 Methodological framework of study:

The four different type of formulation of fresh mandarin and dry seaweed was developed to create a value added mandarin-seaweed jelly product, slightly modified from Jayasinghe et al., (2019). Extract of dry seaweed and juice from fresh mandarin was used with other relevant materials such as sugar, pectin and citric acid. After making jelly, panel were asked to rate their satisfaction with four different examples of a given product (A, B, C and D). Physichochemical test (pH, Acidity, TSS) Proximate analysis, mineral content, bioactive component, microbiological analysis, and organoleptic test were conducted for each product category. One of the controls (sample D) was subjected to an analogous analysis

3.4.1 Preparation of jelly sample:

In each sample of 500 grams, the quantity of seaweed extract and mandarin had been changed and the other materials had been used in equal quantity.

3.1 Formulation of mandarin-seaweed jelly:

Ingredients	**Sample (500 gram in each)**			
	Sample A	**Sample B**	**Sample C**	**Sample D**
Mandarin juice	37.5%=187.5g	25%=125g	12.5%= 62.5g	50%=250g
Seaweed extract	12.5%= 62.5g	25%=125g	37.5%=187.5g	0%=0g
Sugar	48.5%=242.5g	48.5%=242.5g	48.5%=242.5g	48.5%=242.5g
Pectin	1%=5g	1%=5g	1%=5g	1%=5g
Citric acid	0.5%=2.5g	0.5%=2.5g	0.5%=2.5g	0.5%=2.5g
In Total	100%=500g	100%=500g	100%=500g	100%=500g

Legends:

Sample-A: 75% mandarin juice with 25% seaweed extract

Sample-B: 50% mandarin juice with 50% seaweed extract

Sample-C: 25% mandarin juice with 75% seaweed extract

Sample-D: 100% mandarin juice with 0% seaweed extract

3.4.2 Manufacturing process of mandarin-seaweed jelly:

Mandarin juice extract was incorporated with seaweed extract in three types of different formulation where the amount of mandarin juice extract and seaweed extract had been used in vice versa in two samples and kept equal in another sample. The last formulation was made from pure mandarin extract because consumer are familiar with mandarin jelly, so it will be helpful for overseeing the consumer acceptance comparing with the incorporation of seaweed extract in other mandarin jelly.

The manufacturing process of jelly is given below:

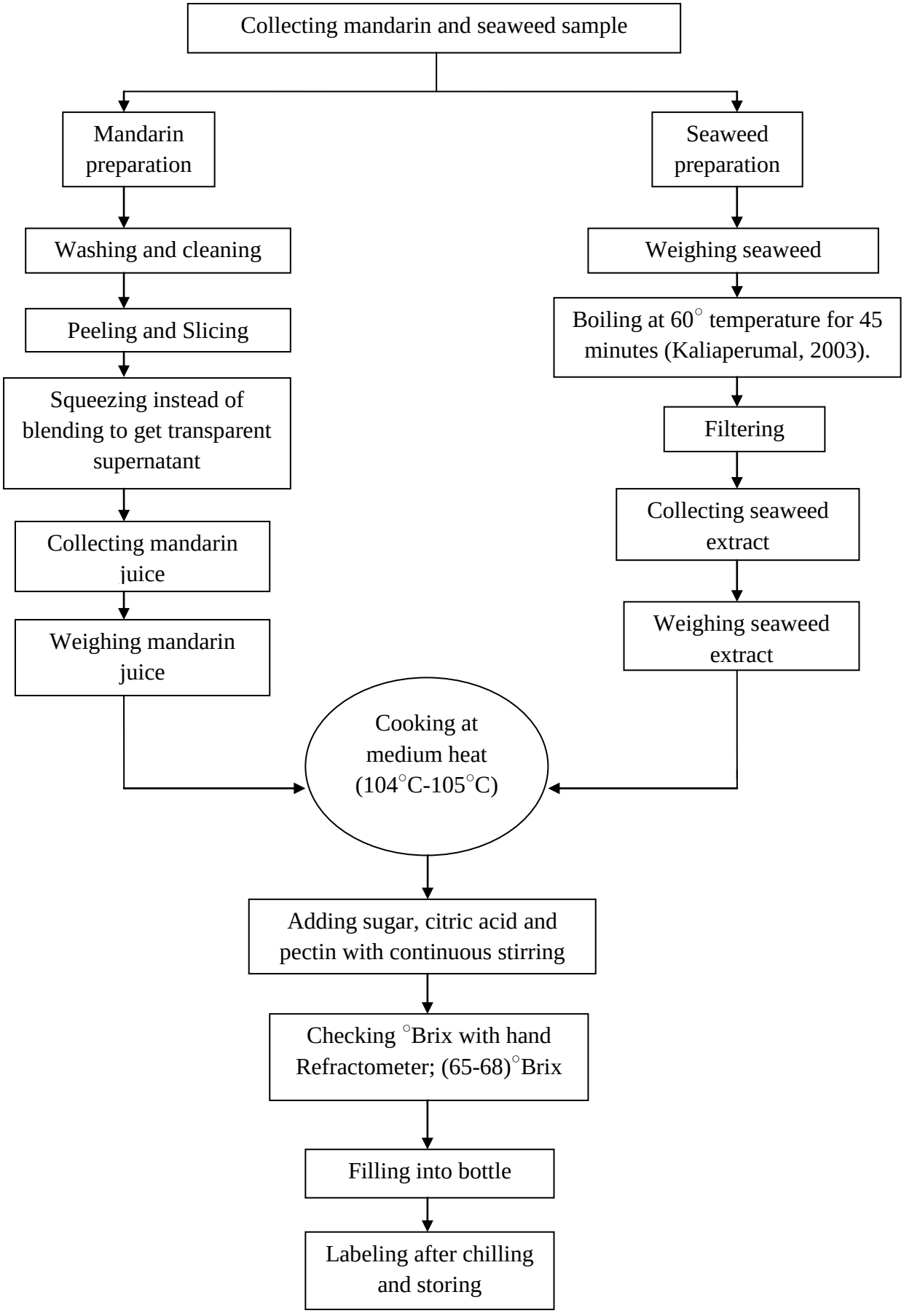


Figure 3.2: Flowchart of manufacturing process of mandarin-seaweed jelly

3.5. Physicochemical analysis of jelly:

3.5.1 Measurement of pH:

The pH scale is used to determine how acidic or basic a given aqueous solution is in the field of chemistry. pH is defined as the negative logarithm of the activity of the (solvated) hydronium ion or, more commonly, the concentration of hydronium ions. International agreements have been reached to develop a set of standard solutions whose pH can be used as a reference for the pH scale. Using a concentration cell with transference, primary pH standards are calculated by comparing the potent distinction between a hydrogen electrode and a standard electrode, like a silver chloride electrode. When determining the acidity or basicity of aqueous solutions, a glass electrode and a pH meter, or indicators, can be employed. The reciprocal of the hydrogen ion activity in a solution, expressed as a decimal logarithm, is known as pH (McClements and Decker, 2010).

3.5.2 Total Soluble Solids:

In order to determine the total soluble solids of the jelly sample, a hand refractometer was used. The (AOAC, 2016) recommended method was followed for measuring total soluble solids (TSS), called for a digital refractometer (Atago RX 1000) and the findings were reported as a percentage of soluble solids (Brix).

3.5.3 Titratable acidity:

The percentage of acidity level was calculated in terms of anhydrous citric acid via titration with regard to N/10 NaOH with phenolphthalein indicator. When titrating against N/10 NaOH using phenolphthalein as an indicator, 10 ml of diluted juice was added to a 100 ml volumetric flask containing 100 ml of juice. When a pink color develops, it indicates the end point of titration. On three separate occasions, the average titration value was recorded (AOAC, 2016).

Titratable acidity can be determined as below:

$$(\%) \text{ of TA} = (\text{T.V} \times \text{Factor})/\text{W}$$

Where,

TV = Titer value of the sample in ml

W = Quantity of the sample taken for the test in ml

Factor - Citric acid: 0.0064 (Citrus Fruit); Malic Acid: 0.0067

3.6 Nutritional composition of jelly:

3.6.1 Moisture content:

Principle:

The determination of moisture is one of the most fundamental and commonplace tests in the food industry. The moisture content of food has direct economic significance for both the processor and the consumer because dry matter is inversely proportional to moisture. However, the impact of moisture on the durability and quality of meals is of even greater importance. Association of Official Analytical Chemists Method was used to calculate moisture content (AOAC, 2016).

Calculation: The moisture percentage was calculated as follow:-

Percentage of Moisture= [(Initial weight-Final weight) ÷Sample weight] ×100

3.6.2 Estimation of Ash content:

The ash content was determined using AOAC protocols (2016).Ash is an inorganic by product of decomposing organic substances. Pre-weighed crucible was used to measure out 10 grams of dried jelly. Next, it was turned to charcoal by burning intofire. After this, muffle furnace was used to heat the charcoal for 4 hours at a temperature of about 600ºC, at which point the charcoal was totally removed. After then, the crucible was removed from the kiln. It was kept in a desiccator and let it cool for a while before weighing it.

Calculation:

The following expression was used to determine the percentage of ash present:

Percentage of Ash = $\frac{W_2 - W_1}{W} \times 100$

Where

W_1 = weight of the empty crucible

W_2 = weight of the crucible with ash

W= weight of the sample

3.6.3. Estimation of Crude fiber content:

Principle:

The water-insoluble portion of carbohydrates found in crude fiber includes the macromolecules cellulose, hemicellulose, and lignin. For this purpose, the known amount of fat-free food is boiled in a dilute acid solution (1.25% H_2SO_4) for 30 minutes, then boiled in a dilute alkali solution (1.25% NaOH) for 30 minutes, a constant volume needs to maintain and the ash is then subtracted from the resulting residue to obtain an estimation. In this study the AOAC (2016) technique was used to calculate the crude fiber content. The ash was created by burning the waste in a muffle furnace for 4-6 hours at 550-600 degrees Celsius.

Calculation: The following expression was used to estimation of the percentage of Crude fiber content:

Percentage of Crude fiber $=\frac{w1-w2}{w} \times 100$

Where,

W_1= Weight of crucible, crude fiber and ash

W_2=Weight of crucible and ash

W= Weight of sample

3.6.4. Estimation of fat content:

Principle:

Estimating fat content requires such food samples which is dissolved in organic solvents (chloroform: methanol) and then filtering out the filtrate. The filtrate is then separated using the separating funnels, after that the mixture is dried for the extract

measurement, and the fat percentage is determined. The crude fat content of the samples was calculated using the AOAC (2016) procedures was described for the soxhlet apparatus.

A Soxhlet apparatus was used to calculate the total amount of unrefined fat. The dried, weighted sample was stored in a thimble that had been sealed with fat-free cotton. Thimble was used to place fat extraction tube into Soxhlet flask. The flask was filled with 75 ml of anhydrous ether and the fat extraction tube's top was secured to the condenser. Within a time frame of no more than 16 hours, the required content was retrieved. When the separating process was done, the thimble was removed and the Soxhlet tube was used to distill and collect the ether. When all of the ether was poured out, the tube would be nearly full. After the volume of ether holding the fat granules of the sample was reduced to an extremely low value, the ether was transferred through a funnel into a beaker. Ether was used to wash and filter the flask. The ether was then evaporated in a steam bath maintained at a low temperature.

Calculation: The following expression was used to estimation of the percentage of fat content:

Percentage of fat = $\frac{w1}{W} \times 100$

Where,

W_1= weight of extracted portion

W= Weight of the sample

3.6.5 Estimation of Crude protein content:

Principle:

The Kjeldahl method is applied in order to calculate the amount of nitrogen present in both organic and inorganic samples. For the purpose of calculating the amount of protein present in foods and drinks, as well as in meat, feeds, cereals, and forages, the Kjeldahl nitrogen content is measured. The Kjeldahl method is also utilized for the purpose of determining the amount of nitrogen present in wastewaters, soils, and several other materials. It is an approved method, and its description may be found in a variety of normative sources, including (AOAC, 2016).

It is important to consider the kind of receiving solution and any dilution factors used in the distillation process when calculating the nitrogen or protein content. According to following equations Normality is denoted by "N". If standard acid is the receiving solution, "ml blank" is the amount of base needed to back titrate the reagent blank; if boric acid is the receiving solution, "ml blank" is the amount of standard acid needed to titrate the reagent blank. Boric acid known as a popular choice for the receiving solution, and the corresponding equation is

Nitrogen % = {(ml standard acid-ml blank) × N of acid × 1.4007} ÷ Weight of sample in gram

Protein % = Nitrogen % × 5.85 (plant origin)

3.6.6 Determination of Carbohydrate:

Carbohydrate content was calculated by subtracting the Nitrogen Free Extractive (NFE) Value from the Total Protein Value. It was expressed as a percentage that was subtracted from 100 to account for all other nearby factors.

Calculation: Therefore, it was determined using the following formula.

% CHO = 100% - % (Protein + Fat + Fiber + Ash + Moisture content).

3.7 Determination of mineral content:

According to AOAC (2010), this method uses digestion to extract the minerals from the food source. Jelly was dissolved in a 2:1 HNO_3/$HCIO_4$ acid solution and then digested. One gram of sample was weighed and placed in a conical flask. After adding 7 ml of HNO3 and 3 ml of HCIO4, the conical flask was set on a hot plate set to 200W for 3 minutes to ensure thorough digestion. After being cooled, the prepared solution was filtered into a 100 ml standard flask, via filter paper, and then it was diluted to volume with distilled water. The AAS method of mineral content analysis was applied to this solution. (Humalyzer-3000, Origin Germany)

3.7.1 Determination of Sodium (Na):

The sodium is cast down as a triple salt with the help of magnesium and uranylacetate. Ferrocyanide reacts with excess uranyl ions in an acidic medium to produce a brownish color. The color development is inversely proportional to the

amount of sodium present in the sample. Pipettes were utilized to add 0.02 ml of sodium standard and 1 ml of the precipitating reagent to the cuvette during the precipitation process. Just 0.02 ml of sample and 1 ml of precipitating reagent were placed in the cuvette. After letting the ingredients sit for 5 minutes, they were mixed completely and shaken vigorously. After that, centrifugation at 2500 to 3000 RPM was used to separate the clear supernatant. In the color development phase, 1 ml of acid reagent for the blank was used. The cuvette was then injected with 0.02 ml of the precipitating reagent and 0.1 ml of the coloring reagent using a pipette. To make standards and samples, 1 ml of acid reagent, 0.02 ml of supernatant, and 0.1 ml of color reagent into a cuvette were stuffedinto into a cuvette. The ingredients were mixed and then incubated them at room temperature for 5 minutes. The absorbance of the blank, standard, and sample were all tested against distilled water within 15 minutes. Multiplying the sample absorbance by the standard absorbance at a given concentration (mmol/L) yielded the sodium concentration in mmol/L.

3.7.2 Determination of potassium (K):

Mixing potassium and sodium tetraphenylboron produces a fine turbidity of potassium tetra-phenyl-boron. The turbidity level is inversely proportional to the potassium concentration in the sample. The blank solution was made by pipetting 1 ml of potassium reagent and 0.02 ml of deionized water into a cuvette. The sample solution consisted of 1 ml of potassium reagent, 0.02 ml of potassium standard, and 1 ml of sample extract placed to a cuvette. These were mixed and then incubated at retention time for 5 minutes. Absorbance was measured against a blank and the Standard within 15 minutes. Potassium concentration was determined in millimoles per liter (mmol/L) by multiplying the ratio of sample absorbance to standard absorbance by the concentration standard (mmol/L).

3.7.3 Determination of Calcium (Ca):

O-Cresolphthalein forms a violet compound with calcium ions in an alkaline environment.A reagent blank solution was made by adding 1 mL of working reagent to 25 µL of distilled water in a cuvette. The standard was adjusted by adding 25 µL of (Ca++) standard and 1 ml of the working reagent. The sample solution was made by combining 25 µL of sample extract with 1 ml of the working reagent. Absorbance measurements were taken of both the sample and the standard. The concentration of

calcium was calculated in milligrams per deciliter by multiplying the standard concentration (mg/dL) by the ratio of sample absorbance to standard absorbance.

3.7.4 Determination of magnesium (Mg):

An alkaline pH is employed because it causes a specific binding of the metallochromic indicator calmagite to magnesium. This shift in absorption wavelength is the basis of the method. It has been found that the strength of the resulting chromophores is proportional to the magnesium concentration in the sample. To create the reagent blank solution, 1 ml of the reagent was placed in the cuvette. The sample solution, which took 10 L to make, was placed in a cuvette with 1 ml of reagent. The standard solution was prepared by adding 1 ml of reagent to 10 ml of magnesium standard in the cuvette. After mixing the cuvettes were allowed to keep at room temperature for two minutes. Each sample and standard's absorbance at 520 nm was compared to that of the reagent blank. Magnesium concentration was expressed as milligrams per deciliter (mg/dl) by multiplying the absorbance value of the sample by the standard concentration.

3.7.5 Determination of iron (Fe):

The iron is released from the transferring complex when it is dissolved in a mildly acidic solution. The free iron is converted back to the bivalent form with the help of ascorbic acid. When combined with iron ions, ferrozine creates a bright molecule. The amount of iron in the sample determines the vibrancy of the resulting hue. By using a pipette, 1 ml of reagent was deposited into a cuvette to make a blank solution. 200 µL of standard and 1 ml of reagent were combined to prepare the standard. In order to make the sample solution, 200 µL of the sample extract and 1 mL of the reagent were combined. A ten-minute incubation period at room temperature followed the mixing. Absorbance was determined by comparing a standard and a sample to a blank. The iron concentration was calculated in µg/dL.

3.8 The DPPH radical scavenging assay for measuring antioxidant activity: Preparation of extract:

A Felcon tube containing 1 gram of sample was used. Next, 10 ml of absolute methanol was added and the mixture was allowed to sit for 72 hours. The straining

process was repeated every 4 hours. After 72 hours, methanoic extract was discovered in the collected filtrate.

Procedure:

The DPPH assay was used to measure the extracts' antioxidant mobility in a manner similar to that published by Azlim et al. (2010). Methanoic DPPH solution was made by dissolving around 6 mg of DPPH into 100 ml of pure methanol.

Then, 2 ml of DPPH solution were added to 1 ml of methanoic acid extract. After 30 minutes of resting in the dark at room temperature, the mixture was given a light shake before being used. Using a UV-VIS spectrophotometer (model UV-2600, Shimadzu Corporation, USA), the absorbance was measured at 517 nm. To make the control, combined 1 ml of methanol with 2 ml of DPPH solution; this served as blank. By comparing the absorbance of the samples to that of a DPPH standard solution, it was able to calculate the scavenging mobility of the samples. Antioxidant capability was determined based on the DPPH free radical scavenging mobility of extracts and estimated using the following equation:

% of inhibition = [(Blank absorbance - Sample absorbance) ÷ Blank absorbance] × 100

As the standard, trolox was utilized, and the TEAC composite (Trolox equivalent antioxidant mobility) served as the basis for the calibration standard curve. On a dry weight (DW) basis, the results were presented as milligrams per one hundred grams of trolox equivalents (TE) for each gram of powder.

3.9 Determination of Bioactive component:

Preparation of extract:

A Felcon tube containing 1 gram of sample was used for TPC and TFC. Next, 10 ml of absolute ethanol was added and the mixture was allowed to sit for 72 hours. The straining process was repeated every 4 hours. After 72 hours, ethanoic extract was discovered in the collected filtrate.

3.9.1 Total Phenolic content (TPC):

The total phenolic content of the extracts was calculated using a modified version of the Folin-Ciocalteu reagent technique (Al-Owaisi et al., 2014). The total polyphenol content (TPC) of the jelly was calculated using a modified version of the Folin-Ciocalteu method. A falcon tube containing 1 ml of ethanoic extract was mixed with 1.5 ml of FC reagent and kept for 3 minutes at room temperature. After waiting an additional 60 minutes, 1.5 ml Na_2CO_3 (7.5%) was added to the mixture. Using a UV-VIS Spectrophotometer (UV2600, Shimadzu Corporation, USA), the absorbance was measured at 760 nm with C_2H_5OH serving as the blank. The total phenolic content (TPC) was determined and shown in milligrams of gallic acid equivalents (mg GAE/g) per gram of extracts.

3.9.2 Total flavonoid content (TFC):

The total flavonoid content (TFC) of the samples was calculated by using the aluminum chloride colorimetric method, described by Chang et al. (2002). Aliquots of 0.5 mL of diluted extract were diluted with 1.5 ml of 95% C2H5OH in a cuvette from a stock solution of extracts (1 mg/mL). A total of 2.8 ml of distilled water, 0.1 ml of 10% $AlCl_3$, and 0.1 ml of 1 mol/L potassium acetate were added to the cuvette's immixture. After 30 minutes of sitting at room temperature, the immixture was ready to be used. With a UV-visible spectrophotometer (UV2600, Shimadzu Corporation, USA), the absorbance was measured at 415 nm, using a blank solution comprising of the same volume of distilled water and 10% aluminum chloride. Total flavonoid content was calculated and shown as milligrams of quercetin equivalents per milligram of extract (mg QE/g).

3.10 Microbiological analysis:

3.10.1 Aerobic plate count (Bacterial plate count):

A sample's bacterial population can be estimated with the help of the Aerobic Plate Count. Aerobic Plate Count is also known as the Aerobic Colony Count (ACC), Standard Plate Count (SPC), Mesophilic Count (MC), and Total Plate Count (TPC) (APC). The Total Viable Bacterial Count (TVC) was calculated using the Standard Plate Count (SPC) method.

When the cells are combined with agar that contains the required nutrients, the test is predicated on the idea that they will each eventually form a visible colony. It is not a measurement of all of the bacteria in the population but rather a general test for organisms that grow aerobically at mesophilic temperatures (25 to 40 degrees Celsius). APC is unable to differentiate between different types of bacteria, which makes it impossible for it to be used as a measure of organoleptic acceptability, sanitary quality, adherence to good manufacturing procedures, or as an indicator of safety. APC is able to provide information regarding a food's shelf life as well as an approaching change in its organoleptic properties (Banwart, 2012).

Requirement:

1.Plate count Agar

2.PBS (Phosphate buffer saline)

3.Test tube

4.Glass bid

5.Colony count machine.

Preparation of sample:

The accuracy of the analysis and interpretation of the data relies largely on the precision with which the sample was taken. The selection of data should accurately reflect the entire population. This was done by giving the entire batch of product a good stir so that the sample would accurately reflect the total amount. In a 250 ml flask, 25 g of jelly sample were weighed out. The sample was diluted with phosphate buffer saline that had a pH of 7.2 and a concentration of 0.6 M KH_2PO_4. After adding approximately 100 ml of the buffer saline to the beaker, it was thoroughly combined using a to-and-fro motion. The volume was brought up to its original state using the same buffer water. It is imperative that every piece of equipment, solution, and other instrument must be sterilized by being heated to 121º Celsius for fifteen minutes. After the sample had been prepared, it was diluted 10 times, which is equal to a 1×10-1 time's dilution, and utilized as stock solution (Andrews, 1992).

Dilution:

A series of dilution were done as follows using 9 ml blanks. The initial 1/10 dilution (1 ml in 9 ml) was performed which was labeled as ‘a’. This was mixed in a vortex mixer, labeled for ‘b’.1 ml from (a) was taken, then added to the next tube and mixed well. It was become 10-2 time’s dilution. The final dilution factor was thus increased by a factor of 10-6.

Standard plate counts

The number of microorganisms present in the preserved samples was estimated using an SPC. One possible application of these measurements is as markers of food quality or forecasters of product freshness. Finally, at 45º Celsius, 1 ml of the diluted sample was pipetted into each of the empty, sterile petri dishes containing nutrient agar medium (Plate count agar).On a level surface, plates were stirred to combine the contents. Once the media had set, the plates were inverted and left in an incubator at 37 degrees Celsius for 24 hours (AOAC, 1990; Sharf, 1966).

Counting and recording:

Depending on the amount of colonies and the easy counting the incubated plates were assigned for colony counting. After that depending on the amount of colonies and the easy with which they could be counted. It was decided to not go with the plate of colonies that were separated, overlapping, and confusing. Plates with 30–300 bright, visible, and countable colonies met the criteria.

The number of Colony-forming units was calculated by the following formula;

Colony-forming units (cfu) per g or ml = Average cfu per plate × dilution factor

Total viable bacterial count (TVC) was determined after the procedures of sample preparation, sample dilution, standard plate counts, and counting and recording. The incubation period lasted 24 hours at 37º Celsius (AOAC, 1990; Sharf, 1966).

3.10.2 Fungal analysis.

Media preparation:

65 g of the SDA medium were initially dissolved in 1 liter of clean water. It was then heated while being stirred frequently and cooked for one minute to completely dissolve the medium. It was kept for 15 minutes at 121 °C in an autoclave. The mixture was then placed into petri dishes after cooling to 45° to 50°C. To process the sample, isolated colonies were obtained by streaking the sample onto the medium using a sterile inoculating loop. The plates were then incubated at 25–30°C with elevated humidity while they were upside down (agar side up). Weekly fungal growth checks were performed on the cultures, which were kept for 4-6 weeks before being declared negative (Aryal, 2015).

Interpretation

There should be solitary colonies in streaked areas of the plate after enough incubation and confluent development in areas of strong injection. Look for fungus colonies on plates that have the expected color and form. Yeast colonies will develop in shades of cream to white. Molds will develop into filamentous colonies of different hues (Aryal, 2015).

3.11 Cost analysis:

The price of the jelly made from the mandarin and seaweed (*Gracilaria tenuistipitata*) was derived from the total price of the items used to make the jelly. The jelly price per kilogram was calculated and reported in taka.

3.12 Sensory evaluation:

Sensory evaluation was conducted for the adduction of overall acceptance of the final product by the consumers. A taste-testing panel evaluated the consumer's acceptability of developed product. The panel test was done in the CVASU premises and there were not only teachers but also students as panelists. Panelists of 15 persons were given the product that has been developed from the mandarin and seaweed. There were four formulations which were encoded with sample A, sample B, sample C, sample D. All the panelists tasted four samples without knowing their formulation. Panelists allotted eligible score for manifold sensory attributes of appearance, color,

smell, taste, sweetness, thickness and overall acceptance of jelly, as requested. The panelist marked four samples based on their opinion after tasting. Sensory evaluation of qualitative parameters (taste, appearance, smell, thickness, sweetness and overall acceptance) of the four samples was carried out using nine point Hedonic scales (Larmond, 1977).

The scale was organized in such a way that:

Table 3.2: Rating Scale for sensory evaluation

Rank	Scores
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

3.13 Statistical analysis

Collecting and storing data for statistical analysis was done in a Microsoft Excel 2019 spreadsheet. Descriptive statistics (mean and standard deviation) were calculated for jelly samples. The information was organized, coded, and recorded using MINITAB 19.The results of these experiments were then analyzed statistically. Data on proximate and physiochemical composition, mineral content, phytochemical content, and sensory evaluation were analyzed using one-way ANOVA to estimate the amount of significant variance at a 95% confidence interval. The statistical analysis was done with a level of 5% significance ($p < 0.05$).

Chapter 4: Result

4.1 Physicochemical properties of mandarin-seaweed jelly:

Traditional jams and jellies rely on the sugar content and final product's pH to inhibit the growth of dangerous microorganisms. The ideal gelling conditions also depends on jellies pH. According to table 4.1 the highest pH was found in sample C (3.1±0.057) and the lowest pH was found in sample D (2.91±0.005). Samples B and C had the highest total soluble solids (TSS; 68 degree brix), whereas sample D had the lowest total soluble solids (65 degree brix). Sample D had the highest acidity (0.64±0.010%) and Sample C had the lowest (0.54±0.015%).

Table 4.1 Result of Physicochemical parameter analysis of jelly

Formulation	**pH**	**TSS(ºB)**	**Acidity (%)**
Sample A	2.97 ± 0.005^{c}	67 ± 0.00^{c}	0.59 ± 0.005^{b}
Sample B	3.00 ± 0.005^{b}	68 ± 0.00^{ab}	0.55 ± 0.010^{c}
Sample C	3.1 ± 0.057^{a}	68 ± 0.00^{ab}	0.54 ± 0.015^{c}
Sample D	2.91 ± 0.005^{c}	65 ± 0.00^{d}	0.64 ± 0.010^{a}

Legends: Means ± SD and values in the same column with the same superscripts are not significantly different (P>0.05).

4.2 Nutritional Composition of mandarin-seaweed jelly:

The nutritive value of mandarin-seaweed jellies are displayed in Table 4.2. Practically all of the samples were significantly different from one another. The highest percentage of crude protein, crude fiber, crude fat and ash was found in sample C, respectively 2.73±0.005%, 1.65±0.011%, 2.70±0.005% and 1.11±0.010% while the lowest percentage of crude protein, crude fat and ash was found in sample D respectively 0.96±0.005%, 1.06±0.010% and 0.38±0.010%.

Table 4.2: Nutritional composition of jelly:

Parameter	Formulation of jelly sample			
	Sample A	**Sample B**	**Sample C**	**Sample D**
Moisture(%)	30.87 ± 0.062^{b}	28.60 ± 0.020^{c}	26.34 ± 0.045^{d}	31.74 ± 0.025^{a}
CHO(%)	64.11 ± 0.005^{d}	66.38 ± 0.010^{a}	65.47 ± 0.010^{b}	64.31 ± 0.010^{c}
Crude protein(%)	1.65 ± 0.010^{c}	2.01 ± 0.005^{b}	2.73 ± 0.005^{a}	0.96 ± 0.005^{d}
Crude fat(%)	1.12 ± 0.026^{c}	1.18 ± 0.020^{b}	2.70 ± 0.005^{a}	1.06 ± 0.010^{d}
Crude fiber(%)	1.24 ± 0.025^{c}	0.99 ± 0.037^{d}	1.65 ± 0.011^{a}	1.55 ± 0.015^{b}
Ash (%)	0.56 ± 0.010^{c}	0.83 ± 0.015^{b}	1.11 ± 0.010^{a}	0.38 ± 0.010^{d}

Legends: Means ± SD that do not share a letter (superscripts; a, b, c, d) are significantly different ($P<0.05$).

4.3 Mineral Content of mandarin-seaweed jelly:

The mineral content of mandarin-seaweed jellies is shown in Table 4.3. It is observed that the amount of all minerals in sample C was significantly higher than other samples while the least amount was found in sample D.

Table 4.3 Result of mineral content analysis of jelly

Formulation	Mineral content (mg/dl)				
	Na	**K**	**Ca**	**Mg**	**Fe**
Sample A	1.49 ± 0.002^{c}	65.77 ± 0.010^{c}	9.53 ± 0.057^{c}	8.09 ± 0.010^{d}	0.0052 ± 0.001^{d}
Sample B	1.72 ± 0.001^{b}	79.97 ± 0.021^{b}	27.10 ± 0.100^{b}	14.17 ± 0.068^{c}	0.0078 ± 0.058^{a}
Sample C	5.68 ± 0.010^{a}	193.83 ± 0.015^{a}	68.27 ± 0.020^{a}	22.46 ± 0.010^{a}	0.018 ± 0.010^{b}
Sample D	0.98 ± 0.010^{d}	57.90 ± 0.005^{d}	8.90 ± 0.005^{d}	6.32 ± 0.010^{b}	0.0039 ± 0.058^{c}

Legends: Means ± SD that do not share a letter (superscripts; a,b, c, d) are significantly different ($P<0.05$).

4.4 Phytochemical composition of mandarin-seaweed jelly:

Bioactive compound data (TFC and TPC) is shown in table 4.4. It was found that all the samples varied significantly from one another except sample A and sample D in case of TPC. The highest levels of total Phenolic content (53.06±0.149 mg GAE/100 mL) was found in sample D, whereas the highest level of total flavonoid (3.25±0.013mg QE/100 g) was found in sample C.

Table 4.4: Analysis of Phytochemical component of jelly

Formulation Of sample	Total Phenolic Content (TPC) (mg GAE/100g)	Total Flavonoid Content (TFC) (mg QE/100 g)
Sample A	52.89 ± 0.025^{a}	3.02 ± 0.007^{c}
Sample B	51.43 ± 0.144^{b}	2.48 ± 0.007^{d}
Sample C	50.31 ± 0.010^{c}	3.25 ± 0.013^{a}
Sample D	53.06 ± 0.149^{a}	3.16 ± 0.007^{b}

Legends: Means ± SD and values in the same column with the same superscripts are not significantly different (P>0.05).

4.5 Antioxidant capacity of mandarin-seaweed jelly:

According to the data in table 4.4, the antioxidant capacity of sample D was the highest (2.97±0.001 mg TE/100 g), whereas the antioxidant capacity of sample C was the lowest (1.45±0.014 mg TE/100 g).

Table 4.5: Analysis of Antioxidant capacity of jelly

Formulation Of sample	Anti-oxidant Capacity (mg TE/100 g)
Sample A	2.46 ± 0.001^{b}
Sample B	2.32 ± 0.005^{c}
Sample C	1.45 ± 0.014^{d}
Sample D	2.97 ± 0.001^{a}

Legends: Means ± SD that do not share a letter (superscripts; a, b, c, d) are significantly different (P<0.05).

4.6 Microbial analysis:

Total viable count and fungal count are shown in Table 4.6.1 and 4.6.2, and they were evaluated from 15 days to 3 months following the manufacture of the jelly. For the evaluation, samples were kept at 4° temperature for a three-month period. When the products were made, yeast and mold did not exist, and three months later, their presence had not been detected.

Table 4.6.1 Microbial analysis (TVC):

Formulation Of sample	**Evaluation of TVC(cfu/ml)**	
	15 days	**After 3 months**
Sample A	7.7×10^{1}	9.5×10^{5}
Sample B	9.4×10^{1}	7.6×10^{6}
Sample C	9.1×10^{1}	8.2×10^{4}
Sample D	7.3×10^{1}	9.1×10^{5}

Table 4.6.2 Microbial analysis (Mold and Yeast):

Formulation Of sample	**Evaluation of mold and yeast**			
	15 days	After 1^{st} month	After 2^{nd} month	After 3^{rd} month
Sample A	No growth	No growth	No growth	No growth
Sample B	No growth	No growth	No growth	No growth
Sample C	No growth	No growth	No growth	No growth
Sample D	No growth	No growth	No growth	No growth

4.7: Energy content of mandarin-seaweed jelly:

According to figure 4.1, sample C had the highest energy content (304.46 kcal/100g) and sample D had the lowest energy content (277.35 kcal/100g).

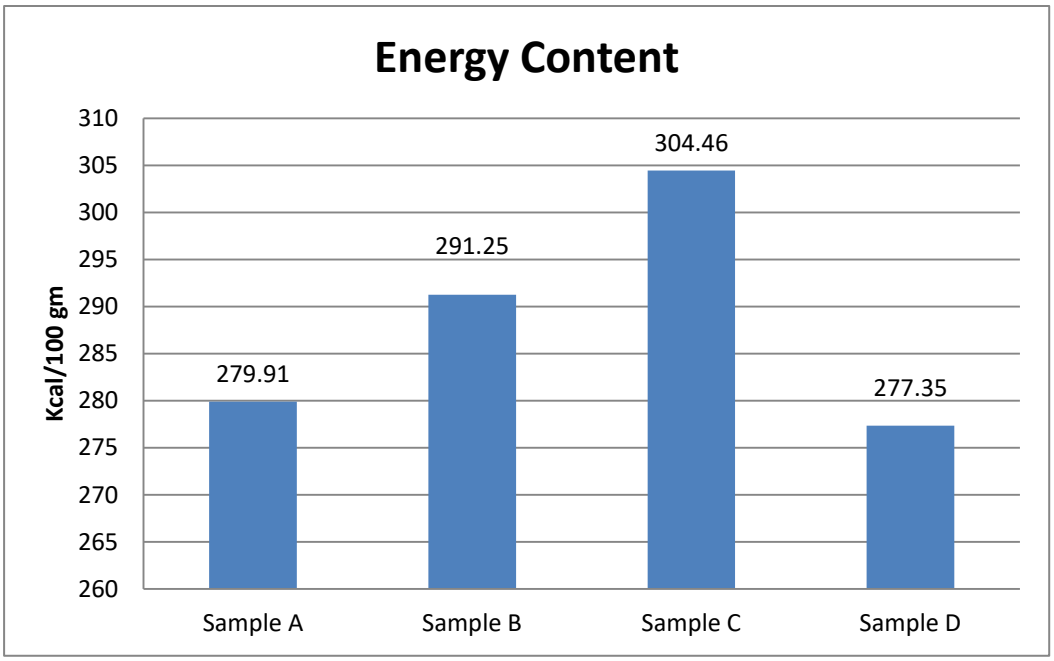


Figure 4.1: Energy content of the four formulation of mandarin-seaweed jelly

Legends:

Sample-A: 75% mandarin juice with 25% seaweed extract

Sample-B: 50% mandarin juice with 50% seaweed extract

Sample-C: 25% mandarin juice with 75% seaweed extract

Sample-D: 100% mandarin juice with 0% seaweed extract

4.8 Cost analysis:

Table 4.8: Production cost of mandarin-seaweed jelly

Head	Tk./Kg	Quantity Used kg/2 kg product	Total Cost	Total tk for Sample A	Total tk for Sample B	Total tk for Sample C	Total tk for Sample D
1)Expendi-ture of Raw materials							
Mandarin	280	4	1120	336	224	112	448
Seaweed	600	0.02	12	2	4	6	0
Sugar	90	0.97	87.3	21.825	21.825	21.825	21.825
Pectin	25000	0.02	500	125	125	125	125
Citric acid	180	0.01	1.8	0.45	0.45	0.45	0.45
Sub total				485.275	375.275	265.275	595.275
2)Processing cost @15% of raw				25	25	25	25
3)Bottling costing	40 tk/ piece	4 piece	160	40	40	40	40
Total production cost of 2 kg jelly				550.275	440.275	330.275	660.275

Table 4.6 shows that the price per half kilogram of jelly for Sample A (containing 75% mandarin juice and 25% seaweed extract) was 550.275 tk, whereas the price for Sample B (containing 50% seaweed extract and 50% mandarin juice) was 440.275 tk.

Sample C containing 75% seaweed extract and 25% mandarin juice, was significantly cheaper than pure mandarin jelly at a price of 330.275 tk for 1/2 kg. Sample D, containing 100% mandarin juice, costs 660.275 tk. According to the results of this research, jelly containing a higher concentration of seaweed extract had greater positive health effects than processed mandarin jelly at a lower price.

4.9 Sensory evaluation:

Sample C differed significantly from the others in terms of taste, sweetness, and thickness, as shown in Table 4.7. All of the samples differed significantly in color, and samples A and B also seemed different in appearance. Sample B also differed significantly in smell from the other samples. Sample C had the highest overall acceptance, whereas sample B scored lowest.

Table 4.9: Hedonic rating test for sensory evaluation

Parameter for sensory evaluation	**Formulation Of sample**			
	Sample A	**Sample B**	**Sample C**	**Sample D**
Appearance	6.70 ± 0.675^{b}	5.80 ± 0.422^{c}	7.40 ± 0.516^{a}	7.50 ± 0.527^{a}
Color	6.74 ± 0.483^{c}	6.10 ± 0.568^{d}	7.30 ± 0.483^{b}	7.80 ± 0.422^{a}
Smell	7.60 ± 0.483^{a}	7.10 ± 0.316^{b}	7.90 ± 0.316^{a}	7.70 ± 0.516^{a}
Taste	7.20 ± 0.422^{bc}	7.50 ± 0.527^{b}	8.20 ± 0.422^{a}	7.11 ± 0.316^{c}
Sweetness	6.70 ± 0.483^{bc}	7.00 ± 0.471^{b}	7.90 ± 0.361^{a}	6.30 ± 0.483^{c}
Thickness	6.70 ± 0.483^{b}	6.80 ± 0.422^{b}	7.60 ± 0.516^{a}	7.00 ± 0.471^{b}
Overall acceptance	6.60 ± 516^{b}	6.55 ± 0.422^{c}	8.00 ± 0.568^{a}	7.10 ± 0.516^{b}

Legends: Means ± SD that do not share a letter (superscripts; a, b, c, d) are significantly different ($P<0.05$).

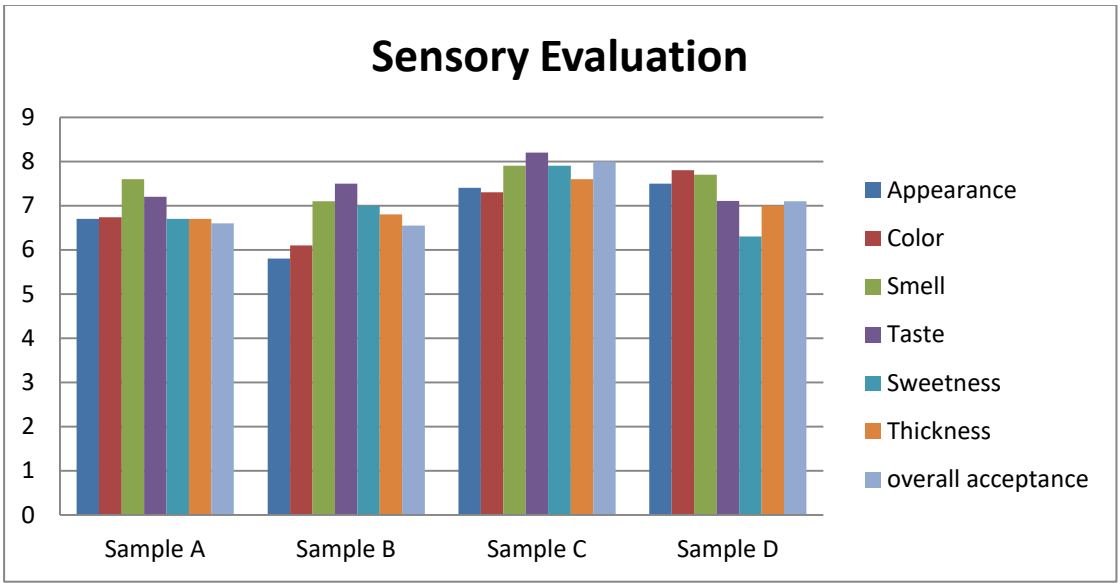


Figure 4.2: Sensory Quality Evaluation of Mandarin-seaweed Jelly

Legends:

Sample-A: 75% mandarin juice with 25% seaweed extract

Sample-B: 50% mandarin juice with 50% seaweed extract

Sample-C: 25% mandarin juice with 75% seaweed extract

Sample-D: 100% mandarin juice with 0% seaweed extract

4.10: Food labeling: Based on overall acceptance and nutrition value, food labeling of sample C (Containing 75% seaweed extract) has been prepared.

4.10: Food labeling

Nutrition Facts		
Serving Size		**100 g**
Amount per serving energy	**304.46 kcal**	
	DV	**%DV**
Total fat-2.70g	65g	4.15%
Total protein-2.73g	50g	5.46%
Total carbs-65.47g	300g	21.82%
Fiber-1.65g	25g	6.6%
Total sugar-242.5g	-	-
Sodium (Na)-5.68 mg	2400 mg	0.23%
Potassium(K)-193.83 mg	3500 mg	5.53%
Calcium (Ca)-68.27 mg	1000 mg	6.82%
Magnesium(Mg)- 22.46 mg	400 mg	5.61%
Iron (Fe)- 0.018 mg	18 mg	0.1%
The % of daily value tells you how much a nutrient in a serving of food contributes to a daily diet.2000 calories a day is used for general nutrition advice(based on FDA)		

Chapter 05: Discussion

5.1 physicochemical characteristics of mandarin-seaweed jelly:

pH: For the best gel condition, jellies pH is a crucial consideration. Besides formation of gel, low pH in food also prevents microbial growth. According to Anuar and Salleh (2019), the pH value plays a significant role in determining the gel consistency, and a pH range of 2.8 to 3.3 is necessary to achieve the best jelly-like consistency and spreadability. In present study pH value of mandarin-seaweed jellies were recorded 2.97, 3.0, 3.1, 2.91 with respectively sample A, sample B, sample C and sample D which are slightly less than the pH of commercial pine apple jelly (3.22) (Sikder and Ahmed, 2019). According to Ismawati et al., (2021) the seaweed jelly candy had pH of 4.88, and according to that, pH 4.6-7.0 is the optimum condition for bacterial growth. So this jelly candy was in an optimum condition for bacterial growth. In present study the low pH was recorded due to use of acidic fruit and citric acid. Desrosier, (1977) stated that the low pH value of commercial jelly is due to the high amount of citric acid used during preparation. He also said that near about 3.2 is an optimum pH for gel formation. According to Ahmmed et al., (2017) the overall range of pH was 2.0 to 5.0 for common fruits whereas pH for mandarin-seaweed jellies and pure mandarin jelly was ranged from 2.91-3.1.

Total soluble solid (TSS):

The TSS values for mandarin-seaweed jellies were 67, 68, 68 and 65º Brix for pure mandarin jelly. TSS levels rise, most likely as a result of hydrolysis of polysaccharide. According to Muresan et al., (2014), fruit jelly made from banana and ginger had a total soluble solid content of 66-69 ºBrix which is in line with present findings. Jayasinghe et al., (2019) found 65º Brix for seaweed jam made from *Gracilaria edulis* whereas in present study, TSS for the jelly incorporated with *Gracilaria tenuistipitata* are slightly higher than that of. According to Aksay et al., (2018) TSS in fresh mandarin fruit is 11.5 ºBrix while they found 70 ºBrix for mandarin jam which is also close to the present findings. They reported that high ºBx values decrease food's water activity, making it less susceptible to microbial and certain biochemical deteriorations. They also suggested that, the addition of sugar and water loss during the boiling procedure may have caused the rise in ºBx for their jam sample. This information may account for the differences in the results of the present study.

Titratable acidity:

The titratable acidity of the produced mandarin-seaweed jellies and pure mandarin jelly were ranged between 0.54 ± 0.015% to 0.64 ± 0.01%. These values are comparable to the value of pineapple jelly made with china lemon pectin (0.91±0.03%) (Sikder and Ahmed, 2019). In present study the maximum value (0.64±0.010%) was obtained in sample D which is pure mandarin jelly and the least value (0.54 ±0.015%) was found in sample C which has the highest seaweed concentrations and lowest mandarin concentrations. Acidity may be primarily caused by the addition of citric acid during the jelly-making process. The causes of getting higher value of acidity in sample D may be due to uses of acidic fruit, mandarin. Timilsina and Tripathi, (2019) reported that pH for mandarin juice is 3.23-3.24 and TA is (0.74-0.1.06%), though these chemical properties of mandarin are affected by the altitude and fruit bearing position. Jayasinghe et al., (2019) found 1.045% acidity for seaweed jam and the results of the present study found less than their results. According to Agarwal and Mangaraj (2005), apple and olive jam had an acidity of about 0.6 and increased in acidity with time. They stated that the breakdown of pectic materials, the oxidation of reducing sugar, and the breakdown of polysaccharides-all of which create acids were to blame for the increase in acidity. It's probable that the decrease in acidity is only partially due to the co-polymerization of natural acids.

5.2 Nutritional composition of mandarin-seaweed jelly:

5.2.1 Proximate analysis

Moisture:

In order to regulate the food's quality and shelf life, moisture content analysis is crucial. The physical characteristics of a food product, such as its shape, color, texture, and taste, as well as factors affecting the product's shelf life, freshness, quality, and resistance to bacterial contamination, all are greatly influenced by its moisture content. Weight (which can have an impact on price) is another characteristic that is strongly influenced by moisture content. The moisture content for mandarin-seaweed jelly along with three different formulations like Sample A, Sample B, sample C and the pure mandarin jelly, sample D was found 30.87%, 28.60%, 26.34%, 31.74%, respectively. The highest value found in sample D and the

lowest value found in sample C. All of these value are comparable to bocaiuva orange pulpe jelly (27.19%) and bocaiuva yellowish pulpe jelly (28.84%), reported by Silva et al., (2018).

Sarkar et al., (2019) found 42.75% moisture in seaweed jelly made from *Hypnea sp.* which is significantly higher than the three mandarin-seaweed formulation of jelly sample in present study. The moisture content obtained in this study was extremely low when compared to the percentages published by Umeh and Nwadialu, (2010) for orange and Cola pachycarpa jams, respectively (90.8% and 96.3%). They claimed that a food's moisture content may be utilized to predict how long it would persist. Moisture is a crucial factor that affects the freshness and shelf life of products. Foods containing a high level of moisture content have a limited shelf life. So it can be anticipated that sample C which has the highest seaweed concentrations and lowest mandarin concentrations would have a longer shelf life comparing to rest of formulations because of the low moisture content of this present research.

Carbohydrate (CHO):

The obtained value of CHO for sample A, sample B, sample C and Sample D are 64.11%, 66.38%, 65.47%,64.31% , respectively, which are comparable to orange jelly (60.71%), pineapple jelly (61.60%), commercial pineapple jelly (63.59%), reported by Ahmmed et al., (2017) and these are less than the present findings. Silva et al., (2018) found 70.20% for bocaiuva orange pulpe jelly and 68.12% for bocaiuva yellowish pulpe jelly which are significantly higher than present findings. This variation may be due to addition of sugar and pectin substances. Brejnholt, (2009) stated that Pectin and gelatin are two ingredients that enhance a product's CHO profile.

Crude protein:

The percentage of crude protein were recorded 1.65, 2.01, 2.73, 0.96 for sample A, sample B, sample C and sample D respectively where the maximum value was found in sample C which had the highest seaweed extract and the lowest mandarin juice extract and the least value was found in sample D which was made with mandarin juice extract without incorporating seaweed extract. Jayasinghe et al., (2019) reported 0.59% protein for jam, made from *Gracilaria edulis* which is significantly lower than

the present findings. Silva et al., (2018) found 0.58% protein for bocaiuva orange pulpe jelly and 0.62 % protein for bocaiuva yellowish pulpe jelly and the obtained value of present findings are higher than those of.

Ash:

Ash content is a measure of the total amount of minerals in food. Ash content can be used to determine a nutritional value of food. The ash content was recorded 0.56%, 0.83%, 1.11%, 0.38% for sample A, sample B, sample C, sample D, respectively. It has been mentioned before that sample A, B, C are three formulation, developed with mandarin-seaweed extract while sample C had the highest concentration of seaweed extract and the lowest concentration of mandarin juice extract and sample A is vice versa of sample C and sample B contents with equal amount of mandarin juice and seaweed extract whereas sample D had been formulated with only mandarin juice extract. It has been noticed that the amount total ash content in sample C was higher than other sample especially sample A and sample D. The ash content of orange, pineapple and commercial pineapple jellies were 0.43%, 0.53% and 0.32%, respectively, reported by Ahmmed et al., (2017) which are significantly lower than the present findings though commercial pineapple jelly (0.32%) can be compared mildly to mandarin jelly (0.38%) in the present study. Jayasinghe et al., (2019) found 0.42% total ash for seaweed jam and they did not get much ash even though they used pulpe instead of extract in case of jam. The seaweed used in present study (*Gracilaria tenuistipitata*) is rich in mineral content having (14.6-28.47%) ash content. Compared to this, the present study found much less amount of ash. This variation in mineral concentration was considered due to usage of extract instead of pulpe. Ahmmed et al., (2017) stated that the amount ash depends on the puple and they found higher ash content where higher pulpe was used in case of jelly.

Crude fiber:

Crude fiber is a measurement of the quantity of indigestible food ingredients such as lignin cellulose. After solvent extraction and digestion with diluted acids and alkalis, there are still plant materials left over. While having minimal nutritional value, these substances still contribute the bulk required for the intestinal tract to function properly during peristaltic motion. Because of its capacity to bind water and thereby soften the stool, fiber is believed to aid in the elimination of waste from the gastrointestinal tract.

Fiber has several health advantages and is helpful in lowering the risk of chronic diseases like diabetes, obesity, and cardiovascular disease. In the present study the obtained value for crude fiber was 1.24%, 0.99%, 1.65%, 1.55% for sample A, sample B, sample C, sample D respectively. Here it is also seen that the crude fiber content in sample C is higher than other sample. The findings in present study are higher than bocaiuva orange pulpe jelly (0.66%) and bocaiuva yellowish pulpe jelly (0.46%), reported by Silva et al., (2018). Jayasinghe et al., (2019) reported 4.89% for seaweed jam though it was total dietary fiber (TDF) instead of crude fiber.

Crude fat:

Crude fat was recorded 1.12%, 1.18%, 2.70%, 1.06% for sample A, sample B, sample C, sample D, respectively. The maximum value was found in sample C which had the highest seaweed extract and the lowest mandarin juice extract and the least value was found in sample D which was made with pure mandarin juice extract. Sarkar et al., (2019) found 6.76% fat in seaweed jelly made from *Hypnea sp*. According to Jayasinghe et al., (2019) 5.23% fat was obtained from *Gracilaria edulis* jam. The values obtained in these mentioned reference are relatively higher than the values found in the present study. So it can be claimed that jellies made from *Gracilaria tenuistipitata* are lower in fat while comparing to other seaweed products. However these findings are higher than the bocaiuva orange pulpe jelly (0.73%) and bocaiuva yellowish pulpe jelly (0.63%), reported by Silva et al., (2018).

5.2.2 Mineral Content of mandarin-seaweed jelly:

The elements in food known as minerals are those that our body needs in order to grow and function normally. A variety of minerals are needed by the human body on a daily basis to maintain healthy bones and muscles. Additionally, it supports a number of biological processes. As a result, we get these nutrients through eating meals high in minerals. Certain nutritional deficiency illnesses may develop when the body does not acquire adequate minerals. Sodium, potassium, calcium, phosphorus, magnesium, and iron were the most prevalent minerals. The body's fluid balance, blood volume, blood pressure, and cellular osmotic pressure are all maintained by sodium. Potassium supports the body's fluid equilibrium and ensures that the muscles and neurological system continue to function properly. Calcium is necessary for the development of robust and healthy bones. Magnesium gives strong bones structure.

In the present study, the presence of some minerals including Na, K, Ca, Mg, Fe which are essential for human body, has been reviewed in the prepared jelly products. In this research the main raw materials for the prepared jelly were seaweed and mandarin where seaweed is a very good source of minerals. *Gracilaria tenuistipitata* had been used here in a form of extract. *Gracilaria tenuistipitata* are good source for macro minerals, reported by Benjama,O. and Masniyom,P,(2012). Table 4.3 shows that sodium (Na) content was found 1.49, 1.72, 5.68, 0.98 in mg/dl, potassium (K) content was found 65.77, 79.97, 193.83, 57.90 in mg/dl, Calcium (Ca) content was found 9.53, 27.10, 68.27, 8.90 in mg/dl, Magnesium (Mg) content was found 8.09, 14.17, 22.46, 6.32 in mg/dl, and Iron (Fe) content was found 0.0052, 0.0078, 0.018, 0.0039 in mg/dl for sample A, sample B, sample C, sample D respectively. It appears that there are significant variations among the samples and the highest values of all minerals were found in sample C, where the concentration of seaweed extract was the highest. The minimal value was found was found in sample A where the concentration of seaweed extract was the lowest. While comparing with sample A, B, C sample D had the lowest value of all minerals which was made from only mandarin juice extract.

According to Czech et al., (2020) mandarin contains 1.19 mg sodium, 133 mg potassium, 30 mg calcium, 11.1 mg magnesium, 0.29 mg iron. These values are higher than sample D which was formulated with only mandarin juice extract in present study. This variation in mineral content was initially attributed to mineral losses that occurred during fruit washing and cooking. Dandago (2009) claims that certain minerals found in raw materials are absent from processed meals because they leak into the water during processing, thus reducing their availability. On the other hand, if the present study is compared with the natural colours incorporated agar jelly, significant variation can be observed where obtained value for Na, K, Ca, Mg was 340, 445.03, 510.13, 77.76 in mg/dl, respectively, reviewed by Jayasinghe et al., (2016). According to Benjama and Masniyom, (2011), several environmental parameters, including water temperature, salinity, light, and nutrients, are linked to variations in the nutritional content of seaweeds. They reported that chemical composition of *Gracilaria tenuistipitata* varies seasonally.

5.3. Bioactive component:

Bioactive substances have physiological effects that could advance health. They are being researched for the purpose of preventing diseases including heart disease, cancer, and others. Due to their significance in preventing a number of chronic diseases, bioactive chemicals such polyphenols, carotenoids, vitamins, omega-3 fatty acids, organic acids, nucleosides and nucleotides, and phytosterols have received a great deal of attention. Examples of phenolic substances that may be present in plants and have the capacity to scavenge free radicals include simple phenolic compounds, phenolic acids, anthocyanins, cinnamic acid derivatives, flavonoids, and tannins.

Table 4.4 shows total phenol content for sample A, sample B, sample C, sample D as 52.89 mg GAE/100g, 51.43 mg GAE/100g, 50.31 mg GAE/100g and 53.06 mg GAE/100g, respectively. This is considerably less than seaweed-jelly candies (360 mg GAE/100g), reported by Faridah, (2019). According to Aksay et al., (2018) mandarin jam had 201.60 mg GAE/100g of total phenol content which is significantly higher than present findings. Flavonoids were found in the highest concentration in sample C which had a value of 3.25 mg QE/100g and 3.02 mg QE/100g, 2.48 mg QE/100g, 3.16 mg QE/100g for sample A, sample B, sample D, respectively, which are higher than grape fruit jellies (1.54 mg QE/100g) and lower than blood orange jellies (9.06 mg QE/100g), reported by (Kopjaret al., 2016).

The kind of processing and the conditions during processing have an impact on the bioactive chemicals of seaweed in a manner similar to how they do on vegetables. The drop in total phenolics during jelly production, as observed by Patras et al. (2009), may be due to the disruption of cell structure during fruit processing and the increased risk of non-enzymatic oxidation. Rajauria et al., (2010) reported that the degradation of bioactive content is related with temperature and time effect.

5.4 Antioxidant capacity:

According to table 4.5, the highest antioxidant capacity was found in sample D (mandarin jelly). It was observed that along with reducing the amount of mandarin, the values for antioxidant capacity in the rest of the samples also decreased gradually**.** 2.46 mg TE/100g in sample A, 2.32 mg TE/100g in sample B, 1.45 mg TE/100g in sample C, 2.97 mg TE/100g in sample D were obtained. while comparing in sample

sample A and sample C the maximum value was found in sample A, which content highest amount of mandarin and lowest amount of seaweed extract whereas sample C was vice versa in formulation of sample A. According to Aksay et al., (2018), mandarin is rich in antioxidant, having 164.87 mg/100g in fruit sample, seaweed specifically *Gracilaria tenuistipitata* on the other hand is much less than mandarin reviewed by Padmanabhan, (2022).

5.5 Microbial analysis:

Microbiological evaluation (Total viable count, yeast and mold count) were performed for each of four samples of mandarin-seaweed jelly. Table 4.6.1 shows that, between 15 days and 3 months TVC was found to be 7.7×10^{1}-9.5×10^{5} cfu/ml in sample A, 9.4×10^{1}-7.6×10^{6} cfu/ml in sample B,9.1×10^{1}-8.2×10^{4} cfu/ml in sample C, 7.3×10^{1}- 9.1×10^{5} cfu/ml in sample D. The total bacterial counts till 15 days period were in range between 7.3×10^{1} - 9.4×10^{1} and it was within the range of consumption while comparing with seaweed agar jelly reviewed by Jayasinghe et al., (2016). The total bacterial count fluctuation during storage was noticeably larger. This may be due to cross contamination when jelly samples were stored in the refrigerator.

Table 4.6.2 shows that Yeast and mold were not detected in the four formulations of jelly samples. According to Muck (2010), mold is an aerobic microorganism and cannot grow in environments with insufficient oxygen. On the other hand, yeast can grow in both aerobic and anaerobic environments. Jellies were preserved in airtight bottles, which prevented the formation of yeast and mold.

In the present study each jelly contains mandarin extract, equal amount of sugar and citric acid. Because of the strong acidity of the fruit and the preservation effect of the sugar, jelly and jam typically don't grow mold on their own. However, a contaminated utensil that was previously used on another food source could occasionally introduce mold spores into a jelly jar. On the other hand water activity can be reduced by freezing, dehydrating, or mixing with dissolved materials like sugar, salt, or citric acid. Because the sugar in jams and jellies dissolves in the water and therefore make it unavailable to microorganisms and the high sugar level prevents pathogenic microorganism from forming. As a result each jelly samples in the present study can be stored in the refrigerator for 3 months without adding additional preservatives.

5.6 Sensory evaluation:

Sensory analysis of mandarin-seaweed jelly was performed to attain the most organoleptically admissible rate among all jelly sample. Table 4.8 shows that jelly made from highest seaweed extract and lowest mandarin juice extract (sample C) scored highest (8.00±0.568) in overall acceptability. It may be due to taste, sweetness and thickness. In some parameters jelly made from only mandarin juice extract (sample D) scored slightly close to sample C and in appearance and color, it scored higher than all other samples. The overall acceptability level of sample A (containing highest amount of mandarin concentration and lowest seaweed extract concentration), sample B (containing equal amount of mandarin concentration and seaweed extract concentration) was nearest with the value of each other. In the present study the thickness of all samples was may be affected by the composition of pectin and citric acid in jelly. Basu et al., (2010) also reviewed the results of using pectin and citric acid in jam and jelly. Highest mean score of acceptability 8.00 in sample C in hedonic rating scale denoted “Like Very Much”, Whereas mean score of acceptability 7.10 in sample D denoted “Like moderately” and sample A and B scored 6.60 and 6.55 respectively, denoted “Like slightly”. According to the results of the investigation, jelly with the highest seaweed extract was determined to have greater organoleptic properties than other samples.

Chapter 06: Conclusion

Considering nutritional value, mandarin jelly is a well-known item in ready-to-eat dishes. This study shows that the popular mandarin jelly when mixed with seaweed, it has gained the highest consumer acceptance in terms of sensory perception as well as its value addition. The physicochemical analysis was carried out for mixed mandarin-seaweed jelly with its rest of two formulations and also for mandarin jelly as control which showed significant variations. Products made from seaweed have a promising approximate composition. According to proximate analysis, the jelly with the highest seaweed extract concentration was high in carbohydrates as well as a good source of protein, fat, fiber, and minerals. It qualifies as a functional food because it contains bioactive elements like flavonoids, which are regarded to be among the substances that could prevent cancer. Commercial seaweed jelly was not sampled for the current investigation since it was not available in the local markets. The consumer can use this technique because manufacturing jelly is cheap and simple.

As seaweed is still not a common food in Bangladesh, this study highlights the potential for success in processing mandarin jelly with seaweed extract. The discovery of seaweeds' nutritional benefits has made them appealing to health-conscious customers all over the world. Therefore, this item may become a new food and export for Bangladesh. To market these goods, widespread education on their nutritional benefits is necessary, therefore this study will be useful for Bangladeshi growers, processors, and consumer alike. Additionally, it can be shown that exporting international standard, best-quality jelly can bring in foreign currency that can help Bangladesh's economy. There is a need for more research in order to prepare seaweed jelly with various fruits and other necessary materials.

Chapter 07: Recommendations and Future Perspectives

The saying "Good food, good health" has a lot of significance. People today experience a lot of stress in their daily lives as a result of their hectic schedules. They experience a variety of bodily problems as a result of such high stress. Alleviating this stress requires a ready-to-eat meal that will simultaneously re-energize the body and meet nutritional needs. In this situation mandarin-seaweed jelly can be a better option for people. Regarding the mandarin-seaweed jelly a successful conclusion can be drawn through present study. The manufacturing process from medium to large scale production can be adopted by modern food enterprises. For future research recommendations and prospects are depicted in this study given based on the current investigation.

.

- For confirmation of the experimental results, the present research can be performed repetitively.
- The formulation can further be renovated and anyone can try making mixed seaweed jelly using multiple recipes with varying fruit ratios.
- Other available fruits like banana, apple, pineapple olive, guava, and those, particularly during the off-season, should be the subject of this kind of research.
- The findings will be helpful to develop different types of seaweed based products such as bread, biscuit, curd, ice-cream, soup etc.
- It is also recommended for off-season use and may be maintained for a long time. Conversely, as its preparation is simple and cost effective it will benefit individuals who fall into the economically weaker sector from an economic standpoint.
- Modern packaging and storing techniques would be developed to enhance jelly.
- To provide additional nutritional value to mandarin jelly sold commercially, sufficient actions should be done.

Reference

Agarwal, G.S., Mangaraj. (2005). Studies on physicochemical changes in selected fruits characteristics of jams made from fresh and frozen strawberries. Pakistan Journal of Agricultural Research. 2(1), 51-60.

Ahmmed, R., Inam, A.K.M.S., Alim, M.A., Sobhan, M.M., and Haque, M.A. (2017). Extraction, Characterization and Utilization of Pectin from Jackfruit (Artocarpus heterophyllus L.) Waste. Journal of Environmental Science and Natural Resources. 32, 57–66.

Aksay, S., Tokbaş, H., Arslan, R., and Çınar, F. (2018). Some physicochemical properties of the whole fruit mandarin jam. Turkish Journal of Agriculture-Food Science and Technology. 6(5), 632-635.

Al-Owaisi, M., Al-Hadiwi, N., and Khan, S.A. (2014). GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves. Asian Pacific Journal of Tropical Biomedicine. 4 (12), 964–970.

Al-Noori, F., Yousif, A.K., and Khalil, E.M. (1984). Use of dates in the formulations of some bakery products. Arab Gulf Journal of Scientific research. 3, 45.

Al-Snafi, A.E. (2016). Nutritional value and pharmacological importance of citrus species grown in Iraq. IOSR Journal of Pharmacy. 6(8), 76-108.

Anuar, N.A., Salleh, R.M. (2019). Development of fruit jam from Averrhoa bilimbi L. Journal of Food Processing and Preservation. 43(4), e13904.

AOAC. (1990). Official methods of analysis of AOAC. In International. Washington, DC: Association of Official Analytical Chemist International.

AOAC. (2010). Official Methods of Analysis, Association of Official Analytical Chemists. Washington DC, USA.

AOAC. (2016). Official Methods of Analysis, Association of official Analytical Chemists.

Andrews, W. (1992). Manual of food quality control 4. Microbiological Analysis (rev. 1).Food and Drug Administration, FAO Consultant, Washington. DC: 1-12.

Armisen, R. (1995). World-wide use and importance of Gracilaria. Journal of applie Phycology. 7(3), 231-243.

Aryal, S. (2015). Sabouraud Dextrose Agar (SDA)–Composition, Principle, Uses, Preparation and Colony Morphology.

Aziz, A., Hassan, A., Roy, S.K., Haque, M.Z., Saha, B.K., Ahmed, S., and Mashuk, O.F. (2021). Potential of Gracilaria tenuistipitata var. liui grown in Nuniachara, Cox's Bazar, Bangladesh. Bangladesh Journal of Scientific and Industrial Research. 56(1), 39-46.

Azlim, A.A., Khan, A.J., Zahir, S.J.I., Suleiman, K.M., Aisyah, M.R., and Rahim, K.R. (2010). Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. International Food Research Journal. 17(4), 1077-1084.

Baghel, R.S., Reddy, C.R.K., and Singh, R.P. (2021). Seaweed-based cellulose: Applications, and future perspectives. Carbohydrate Polymers. 267, p.118241.

Banwart, G. (2012). Basic food microbiology. Springer Science and Business Media.

Barboni, T., Muselli, A., Luro, F., Desjobert, J.M., and Costa, J. (2010). Influence of processing steps and fruit maturity on volatile concentrations in juices from clementine, mandarin, and their hybrids. European Food Research and Technology. 231(3), 379–386.

Basu, S., Shivhare, U.S. (2010). Rheological, textural, micro-structural and sensory properties of mango jam. Journal of Food Engineering. 100(2), 357-365.

Benjama, O., Masniyom, P. (2011). Nutritional composition and physicochemical properties of two green seaweeds (*Ulva pertusa* and *U. intestinalis*) from the Pattani Bay in Southern Thailand. Sonklanakarin Journal of Science and Technology. 33, 575–583.

Benjama, O., Masniyom, P. (2012). Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand. Sonklanakarin Journal of Science and Technology. 34, 223–230.

Betoret, E., Mannozzi, C., Dellarosa, N., Laghi, L., Rocculi, P., and Dalla Rosa, M. (2017). Metabolomic studies after high pressure homogenization processed low pulp mandarin juice with trehalose addition. Functional and technological properties. Journal of Food Engineering. 200, 22–28.

Bin, S.H.A.O., Zhi-ping, W.A.N.G., Xin-ying, L.I.U., Jin-xin, Y.U., Jin-jin, L.A.N., Jing-mei, W.A.N.G., and Zi-yuan, C.H.E.N. (2013). Breeding of a Chlorella strain with high yield of polysaccharide and its effect on growth and immunoregulation of Litopenaeus vannamei. Journal of Nuclear Agricultural Sciences. 27(2), 168.

Boopathy, N.S., Kathiresan, K. (2010). Anticancer Drugs from Marine Flora: An Overview. Journal of oncology.

Brejnholt, S.M. (2009). Pectin. Food stabilisers, thickeners and gelling agents. 237-265.

Brown, E.M., Allsopp, P.J., Magee, P.J., Gill, C.I., Nitecki, S., Strain, C.R., and Mcsorley, E.M. (2014). Seaweed and human health. Nutrition reviews. 72, 205–216.

Cassano, A., Tasselli, F., Conidi, C., and Drioli, E. (2009). Ultrafiltration of Clementine mandarin juice by hollow fibre membranes. Desalination. 241(1–3), 302–308.

Chang, C.C., Yang, M.H., and Wen, H.M. (2002). Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of Food and Drug Analysis. 10, 178-182.

Charoensiddhi, S., Abraham, R.E., Su, P., and Zhang, W. (2020). Seaweed and seaweed-derived metabolites as prebiotics. Advances in Food and Nutrition Research. 91, 97–156.

Chen, K.J., Tseng, C.K., Chang, F.R., Yang, J.I., Yeh, C.C., Chen, W.C., and Lee, J.C. (2013). Aqueous extract of the edible *Gracilaria tenuistipitata* inhibits hepatitis C viral replication via cyclooxygenase-2 suppression and reduces virus-induced inflammation. PloS one. 8(2), e57704.

Chennubhotla, V.S., Kaliaperumal, N., and Kalimuthu, S. (1987). Common seaweed products. CMFRJ Bulletin. 41: 26-30.

Chennubhoda, V.S.K., Kaliaperumal, N. (1999). Seaweeds and their importance. Proc. Workshop Natl. Aquaculture Week, Aquaculture Foundation of India, Chennai. pp. 54-57.

Chaves, L.D.S., Nicolau, L.A.D., Silva, R.O., Barros, F.C.N., Freitas, A.L.P., Aragão, K.S., and Medeiros, J.V.R. (2013). Antiinflammatory and antinociceptive effects in mice of a sulfated polysaccharide fraction extracted from the marine red algae *Gracilaria caudata*. Immunopharmacology and Immunotoxicology. 35(1), 93-100.

Conlon, M.A., Bird, A.R. (2015). The impact of diet and lifestyle on gut microbiota and human health. Nutrients.7, 17–44.

Czech, A., Zarycka, E., Yanovych, D., Zasadna, Z., Grzegorczyk, I., and Kłys, S. (2020). Mineral content of the pulp and peel of various citrus fruit cultivars. Biological Trace Element Research.193(2), 555-563.

Dandago, M.A. (2009). Changes in nutrients during storage and processing of foods. Techno Science Africana Journal. 3(1), 24–27.

Desrosier, N.W. (1977). Elements of Food Technology. Westport, Connecticut.: AVI Publishing Co. Inc.

Dillehay, T.D., Ramírez, C., Pino, M., Collins, M.B., Rossen, J., and Pino-Navarro, J.D. (2008). Monte Verde: seaweed, food, medicine, and the peopling of South America. Science. 320(5877), pp.784-786.

FAO, Fishery and Aquaculture Statistics - 2016, FAO Fisheries and Aquaculture Departament, 2018

Faridah, A. (2019). The effect of Water extract of Brown Seaweed on the Characteristic of Jelly Candy as a Functional Food. International Journal of Research and Review. 6(11), 148-152.

Ferlazzo, N., Visalli, G., Cirmi, S., Lombardo, G.E., Lagana, P., and Di Pietro, A. (2016c). Natural iron chelators: protective role in A549 cells of flavonoids rich extracts of Citrus juices in Fe (3+)-induced oxidative stress. Environmental toxicology and pharmacology. 43, 248–256.

Fernando, I.S., Kim, M., Son, K.T., Jeong, Y., and Jeon, Y.J. (2016). Antioxidant activity of marine algal polyphenolic compounds: a mechanistic approach. Journal of medicinal food. 19(7), 615-628.

Glazer, A.N., Lundell, D.J., Yamanaka, G., and Williams, R.C. (1983). The structure of a simple phycobilisome. In Annales de l'Institut Pasteur/Microbiologie. (Vol. 134, No. 1, pp. 159-180). Elsevier Masson.

Gordon, A. (2017). Case study: Improving the quality and viability of a traditional beverage—Irish Moss. In Food Safety and Quality Systems in Developing Countries (pp. 47-79).

Guiry, M.D., Guiry, G.M. (2018). World-wide electronic publication, National University of Ireland, Galway. Algae Base.

Habib, M.A., Hammam, M.A., Sakr, A.A., and Ashoush, Y.A. (1986). Chemical evaluation of Egyptian citrus seeds as potential sources of vegetable oils. Journal of the American Oil Chemists' Society. 63(9), 1192-1196.

Hamdan, D.I., Abdulla, R.H., Mohamed, M.E., and El-Shazly, A.M. (2013). Chemical composition and biological activity of essential oils of Cleopatra mandarin (*Citrus reshni*) cultivated in Egypt Journal of Pharmacognosy and Phytotherapy .5(5), 83-90.

Hanjabam, M.D., Zynudheen, A.A., Ninan, G., Panda, S. (2017). Seaweed as an ingredient for nutritional improvement of fish jerky. Journal of food processing and preservation. 41(2), e12845.

Hemler, E.C, Hu, F.B (2019). Plant-based diets for cardiovascular disease prevention: All plant foods are not created equal. Current Atherosclerosis Reports 21 (5), 18.

Hong, S.I., Lee, H.H., Kim, D. (2007). Effects of hot water treatment on the storage stability of satsuma mandarin as a postharvest decay control. Postharvest Biology and Technology 43(2), 271–279.

Hu, B., Gong, Q., Wang, Y., Ma, Y., Li, J., and Yu, W. (2006). Prebiotic effects of neoagaro-oligosaccharides prepared by enzymatic hydrolysis of agarose. Anaerobe.12, 260–266.

Ismawati, R., Romadhoni, I.F., Nurul, Q.T., and Ratna, S.T. (2019). Storability of seaweed jelly candy based on chemical, physical and microbiology characteristics. In IOP Conference Series: Earth and Environmental Science (Vol. 347, No. 1, p. 012021). IOP Publishing.

Jayasinghe, P.S., Ganegama, Arachchi, G.J., and Perera, R. (2019). Development of seaweed based palmyra (Borassus flabellifer) and determination of its quality parameters. NARA.

Kaladharan, P., Kaliaperumal, N. (2000a). Seaweed recipes. In : Training Manual on Seaweed culture, Processing and Utilisation. CMFRI, Cochin. pp. 32-35.

Kaliaperumal, N. (2003). Products from seaweeds. SDMRI Research Publication. 3, 33-42.

Kim, S.K., Taylor, S. (2011). Marine medicinal foods: implications and applications, macro and microalgae (Vol. 64). Academic Press.

Kopjar, M., Pichler, A., Turi, J., Piližota, V. (2016). Influence of trehalose addition on antioxidant activity, colour and texture of orange jelly during storage. International Journal of Food Science & Technology. 51(12), 2640–2646.

Larmond, E. (1977). Laboratory methods for sensory evaluation of food. Research Branch, Canada Dept. of Agriculture.

Legua, P., Forner, J.B., Hernandez, F., Forner-Giner, M.A. (2014). Total phenolics, organic acids, sugars and antioxidant activity of mandarin (Citrus clementina Hort. ex Tan.): Variation from rootstock. Scientia Horticulturae.174(1), 60–64.

Lin, J.S., Evans, C.V., Johnson, E., Redmond, N., Coppola, E.L., and Smith, N. (2018). Nontraditional risk factors in cardiovascular disease risk assessment. JAMA 320 (3), 281–97.

Lobban, C.S, Wynne, M.J. (1981). The biology of seaweeds (Vol. 17). Univ of California Press.

Lobban, C.S., Harrison, P.J., and Duncan, M.J. (1985). Physiological ecology of seaweeds. Cambridge University Press.

Lordan, S., Ross, R.P., Stanton, C. (2011). Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Marine drugs. 9(6), 1056-1100.

Lyra, G.D.M., Nunes, J.M.D.C., Pestana, E.M.D.S., DE MATOS, J.C.G., Caires, T.A., DE JESUS, P.B., and Oliveira, M.C. (2021). Diversity of Gracilariaceae (Rhodophyta) in Brazil: integrating morphological and molecular data. Phytotaxa, 496(1), 1-53.

Ma, Z., Khalid, N., Shu, G., Zhao, Y., Kobayashi, I., Neves, M.A., Tuwo, A., Nakajima, M., (2019). Fucoxanthin-Loaded Oil-in-Water Emulsion-Based Delivery Systems: Effects of Natural Emulsifiers on the Formulation, Stability, and Bioaccessibilit. ACS Omega 4, 10502–10509

Maehre, H.K., Malde, M.K., Eilertsen, K.E., and Elvevoll, E.O. (2014). Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. Journal of the Science of Food and Agriculture. 94(15), 3281-3290.

Mathur, A., Purohit, S., Gupta R., Prasad V, Mathur D, Singh S. (2011). Evaluation of in vitro antimicrobial & antioxidant activity of peels & pulp of some citrus species. International journal of biotechnology & biotherapeutics. (1)2 :2229-2278.

McClements, D.J., Decker, E.A. (2010). Lipídeos. In: Química de Alimentos de Fennema. Porto Alegre. 7, 131-178.

Mišurcová, L., Škrovánková, S., Samek, D., Ambrožová, J., and Machů, L. (2012). Health benefits of algal polysaccharides in human nutrition. Advances in food and nutrition research. 66, 75-145.

Moulehi, I., Bourgou, S., Ourghemmi, I., Tounsi, M.S. (2012). Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (Citrus reticulate Blanco) and bitter orange (Citrus aurantium L.) seeds extracts. Industrial Crops and Products. 39(1), 74–80.

Muck, R.E. (2010). Silage microbiology and its control through additives. Revista Brasileira de Zootecnia. 39, 183-191.

Muresan, C., Pop, A., Muste, S., Scrob, S., and Rat, A. (2014). Study concerning the quality ofJelly products based on banana and ginger. Journal of Agroalimentary Processes and Technologies. 20(4), 408–411.

Nam, H.A., Ramakrishnan, S.R., Kwon, J.H. (2019). Effects of electron-beam irradiation on the quality characteristics of mandarin oranges (Citrus unshiu (Swingle) Marcov) during storage. Food Chemistry. 286(February), 338–345.

Ortuno, A.A., Baidez, P., Gomez, M.C., Arcas, I., Porras, A.G., and Del Rio, J.A. (2006). Citrus paradise and Citrus sinensis flavonoids: Their influence in the defence mechanism against Penicillium digitatum.Food Chem.98(2), 351-358.

Patras, A., Brunton, N.P., Tiwari, B.K., Butler, F. (2009). Stability and degradation kinetics of bioactive compounds and color in strawberry jam during storage. Food Bioprocess Technology. 5, 1308-1455.

Padmanabhan, K.L.S. (2022). Phytochemical analysis and antioxidant activity of seaweed extracts.

Pereira, L., Critchley, A.T., Amado, A.M., and Ribeiro-Claro, P.J. (2009). A comparative analysis of phycocolloids produced by underutilized versus industrially utilized carrageenophytes (Gigartinales, Rhodophyta). Journal of Applied Phycology. 21(5), 599-605.

Porse, H., Rudolph, B. (2017). The seaweed hydrocolloid industry: 2016 updates, requirements, and outlook J. Appl. Phycol. 29, 2187–200.

Putnik, P., Barba, F.J., Lorenzo, J.M., Gabrić, D., Shpigelman, A., Cravotto, G., and Bursać Kovačević, D. (2017). An integrated approach to mandarin processing: Food safety and nutritional quality, consumer preference, and nutrient bioaccessibility. Comprehensive Reviews in Food Science and Food Safety, 16(6), 1345-1358.

Raja, R., Hemaiswarya, S., Kumar, N.A., Sridhar, S., and Rengasamy, R. (2008). A perspective on the biotechnological potential of microalgae. Critical reviews in microbiology. 34(2), 77-88.

Rajauria, G., Jaiswal, A.K., Abu-Ghannam, N., and Gupta, S. (2010). Effect of hydrothermal processing on colour, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds. International journal of food science and technology. 45(12), 2485-2493.

Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A., and Millington, K.J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. Journal of human nutrition and dietetics. 17(5), 449-459.

Sakaki, J.R., Melough, M.M., Li, J., Tamimi, R.M., Chavarro, J.E., Chen, M.H., and Chun, O.K. (2019). Associations between 100% orange juice consumption and dietary, lifestyle and anthropometric characteristics in a cross-sectional study of US Children and adolescents. Nutrients 11 (11), 2687. 2687.

Santelices, B. (2014). Cultured aquatic species information programme. *Gracilaria spp.* Cultured Aquatic Species Information Programme, FAO Fisheries and Aquaculture Department, Rome.

Sarkar, M., Kamal, M., Hasan, M., and Hossain, M. (2016a). Present status of naturally occurring seaweed flora and their utilization in Bangladesh. Research in Agriculture Livestock and Fisheries 3, 203–216.

Sarkar, M.S.I., Kamal, M., Hasan, M.M., Hossain, M.I.(2019). Quality aspects of some value added seaweed food and functional food products. Fundamental and Applied Agriculture. 4(2), pp.798-805.

Sasikumar, V., Kalaisezhiyen, P. (2014). Evaluation of Free Radical Scavenging Activity of Various Leaf Extracts from Kedrostis Foetidissima (Jacq.) Cogn. Biochemistry and Analytical Biochemistry. 3(2), 1.

Sharf, J.M. (1966). Recommended methods for the microbiological examination of foods (Vol. 147). Washington, DC: American Public Health Association.

Sikder, M.B.H., Ahmed, S. (2019). Extraction, characterization and application of three varieties of Citrus limon L. pectin in jelly product. Food and Applied Bioscience Journal. 7(1), 31-50.

Silva, V.M.D., Campos, R.P., Borsato, A.V., Candido, C.J., and Donadon, J.R. (2018). Bocaiuva jelly: preparation, physicochemical and sensory evaluation. Revista Brasileira de Fruticultura. 40.

Sirirustananun, N., Chen, J.C., Lin, Y.C., Yeh, S.T., Liou, C.H., Chen, L.L., and Chiew, S.L. (2011). Dietary administration of a *Gracilaria tenuistipitata* extract enhances the immune response and resistance against Vibrio alginolyticus and white spot syndrome virus in the white shrimp Litopenaeus vannamei. Fish & shellfish immunology. 31(6), 848-855.

Sonani, R.R., Roszak, A.W., Ortmann de Percin Northumberland, C., Madamwar, D., Cogdell, R.J. (2018). An improved crystal structure of C-phycoerythrin from the marine cyanobacterium *Phormidium sp*. A09DM. Photosynthesis research. 135(1), 65-78.

Sousa, M.B., Maria-dos, S., Pires, K., Barroso de Alencar, D., Holanda-Sampaio, A.H., and Saker-Sampaio, S. (2008). α,β- carotene and α tocopherol in fresh seaweeds, Food Science and Technology. 28(4), 3-4

Thanigaivel, S., Vijayakumar, S., Mukherjee, A., Chandrasekaran, N., and Thomas, J. (2014). Antioxidant and antibacterial activity of Chaetomorpha antennina against shrimp pathogen Vibrio parahaemolyticus. Aquaculture. 433, 467-475.

Timilsina, K., Tripathi, K.M. (2019). Chemical quality attributes of mandarin (Citrus reticulata Blanco) as affected by altitude and fruit bearing position in Kavre, Nepal. Archives of Agriculture and Environmental Science. 4(3), 319-325.

Umeh, A.S., Nwadialu, M.A. (2010). Production and Proximate Analysis of Jam (food spread) prepared from Cola Pachycarpa Fruit. Journal of Home Economics Research.13, 152-158.

Vivek, S., Kumar, Y.A.S., Palanisamy, M. (2022). Phytochemical Analysis and Anticancer Activity of *Gracilaria tenuistipitata* CF Chang & B.-M. xia against Breast Cancer MCF-7 Cells. Nelumbo. 64(1), pp.363-371.

Wartenberg, L., Richter, A. (April 2020). Mandarin orange: Nutrition facts, benefits, and types. Health line.

Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S., Helliwell, K.E., and Brawley, S.H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. Journal of applied phycology. 29(2), 949-982.

Wijesinghe, W.A.J.P., Jeon, Y. (2012). Int. J. of Food Sciences and Nutrition. 63, 225–235

Woodroof, J.G. (1986). History and growth of fruit processing. In Commercial fruit processing. (pp. 1-24).

Yang, M.Y., Dong, J.D., Kim, M.S. (2012). Taxonomic notes on five species of Gracilariaceae from Hainan, China. Algal research. 27(3), pp.175-187.

Yang, Y., Chai, Z., Wang, Q., Chen, W., He, Z., and Jiang, S. (2015). Cultivation of seaweed Gracilaria in Chinese coastal waters and its contribution to environmental improvements. Algal research. 9, 236–44

YANG, J.I., YEH, C.C., LEE, J.C., YI, S.C., HUANG, H.W., TSENG, C.N., and CHANG, H.W. (2012). Aqueous Extracts of the Edible *Gracilaria*

tenuistipitata are Protective Against H2O2-Induced DNA Damage, Growth Inhibition, and Cell Cycle Arrest, Molecules. 17, 7241-7254.

Ye, X.Q., Chen, J.C., Liu, D.H., Jiang, P., Shi, J., Xue, S., and Kakuda, Y. (2011). Identification of bioactive composition and antioxidant activity in young mandarin fruits. Food Chemistry. 124(4): 1561-1566.

Yeung, H.C. (1995). Handbook of Chinese herbs and formulas. Institute of Chinese Medicine.

Zhang, Y.J., Li, S., Gan, R.Y., Zhou, T., Xu, D.P, and Li, H.B. (2015). Impacts of gut bacteria on human health and diseases. International Journal of Molecular Sciences.16, 7493–7519.

Zhongneng, X., Xiaotao, L., Jihui, L., Longchu, X., and Changjiang, H. (2002). The effects of nutrient availability on the uptake of nitrogen and phosphorus by *Gracilaria tenuistipitata* var. liui Zhang et Xia. Acta Ecologica Sinica. 22(3), 366-374.

Appendices

Appendix-A: Preparation of mandarin-seaweed jelly:

Mandarin Sample

Peeling Mandarin

Seaweed sample

Weighing seaweed

Boiling seaweed

Weighing seaweed extract

Cooking jelly with all ingredients

Checking °Brix by using refractometer

Final products

Appendix-B: Laboratory works:

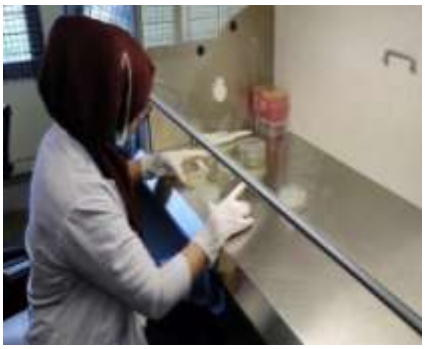
pH determination

Acidity determination

Sample preparation for proximate analysis

Minerals determination

Working in UV Visible Spectrophotometer



Microbial Analysis

Appendix-C: Sensory evaluation of mandarin-seaweed jelly






Appendix-D: Questionnaire for Hedonic test of mandarin-seaweed jelly:

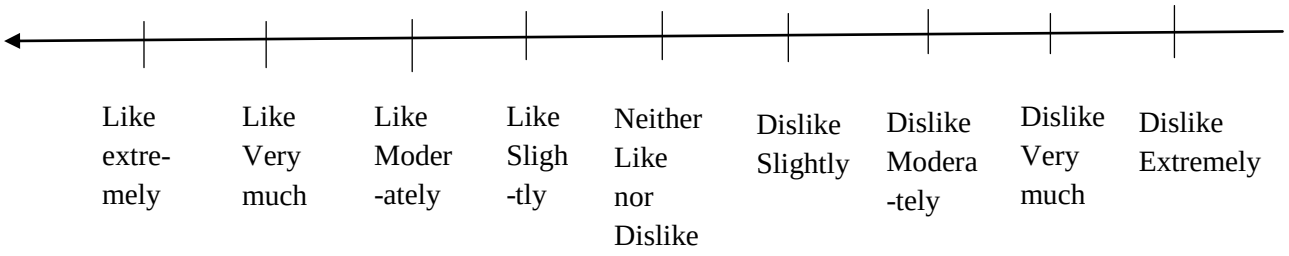
I am Rajia Sultana Eshita, Student of Applied Food Science and Nutrition, CVASU. This designed questionnaire is required for the organoleptic test of my developed product for my thesis work.

I request to the panel judges to developed product, your cooperation will be highly appreciated

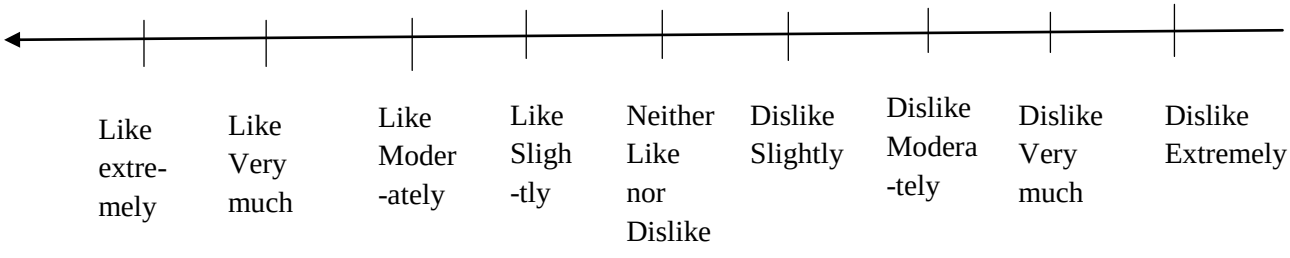
Name: **Age** **Date:**

You are receiving the coded samples. Please taste them from left to right and circle them on the scale which best represents how much you liked or disliked each sample with respect to overall acceptance.

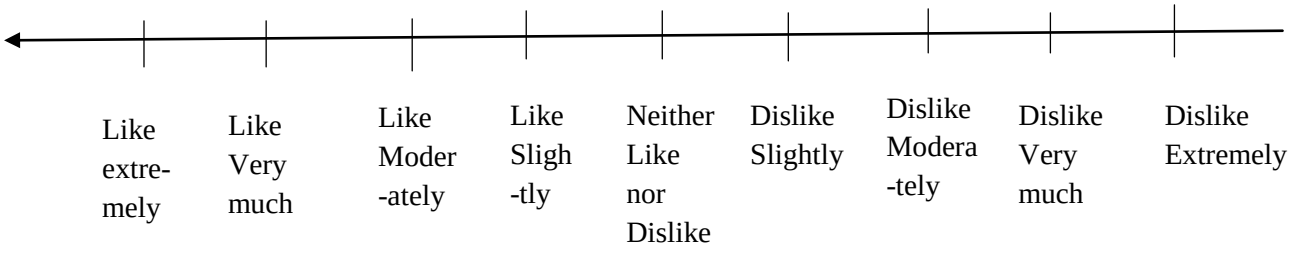
1) **Appearance:**



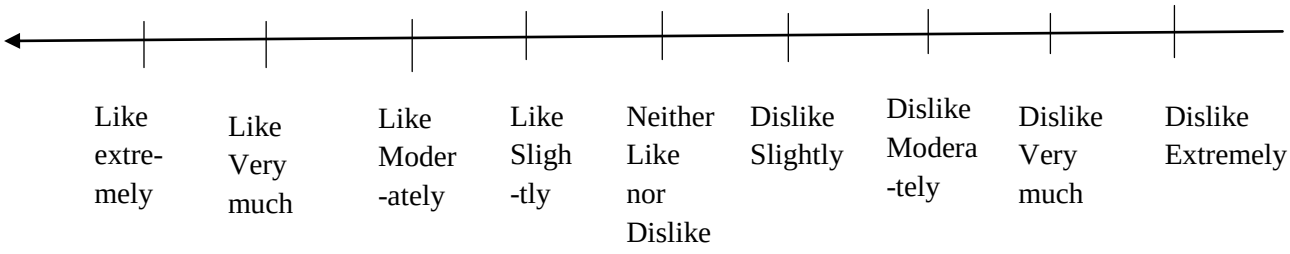
2) **Color:**



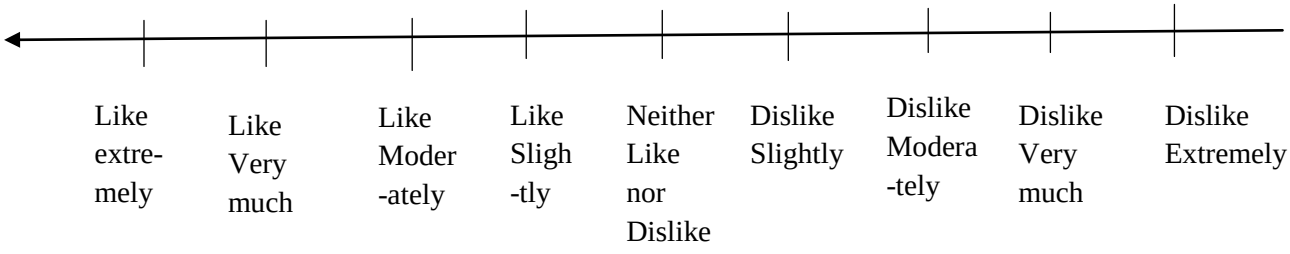
3) **Smell:**



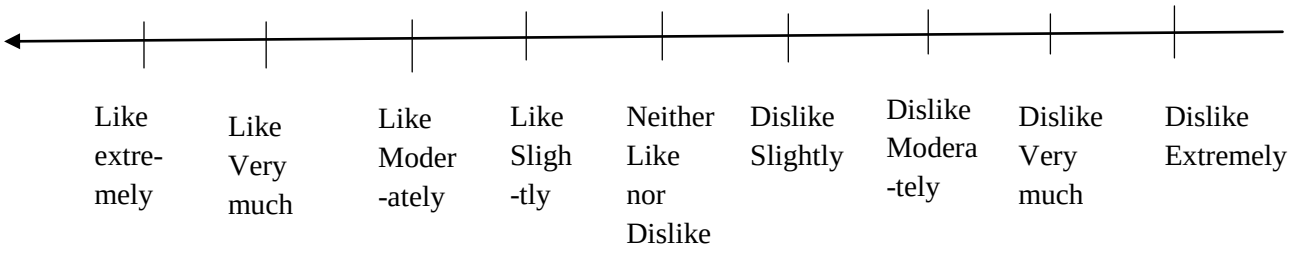
4) Taste:



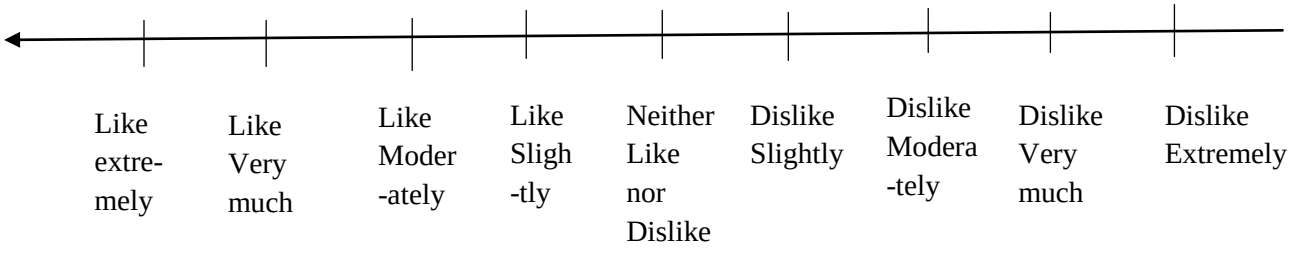
5) Sweetness:



6) Thickness:



7) Overall acceptance:



Brief biography

Rajia Sultana Eshita graduated with a B.Sc. (Hons.) in Food Science and Technology from the Chattogram Veterinary and Animal Sciences University (CVASU), in Chattogram, Bangladesh, with a 3.54 cumulative grade point average on a 4.00 scale. She previously completed the Higher Secondary Certificate (HSC) Examination in 2013 with a GPA of 5.00/5.00 after passing the Secondary School Certificate (SSC) Examinations in 2011 with a Grade Point Average (GPA) of 5.00/5.00. She briefly declared herself a candidate for the MS in Applied Human Nutrition and Dietetics degree under Department of Applied Food Science and Nutrition, the Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. She has a strong passion for sharing information and academic research, and she would appreciate any further opportunity to do so.