Quality analysis, in-vitro antioxidant and antidiabetic effects of seaweed (*Ulva intestinalis*) supplemented bread

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Roll No: 02121/12

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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 and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

October, 2023

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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

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PLAGIARISM VERIFICATION

Title of Thesis: **Quality analysis, in-vitro antioxidant and antidiabetic effects of seaweed (*Ulva intestinalis*) supplemented bread**

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Dedicated to my beloved familyand teachers

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The Author

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Abbreviation

%: Percentage

ANOVA: Analysis of variance

AOAC: Association of Official Analytical Chemists

°C: Degree Celcius

DPPH: 2,2-diphenyl-1-picrylhydrazyl

et al: Et alii/ et aliae/ et alia

etc: Et cetera

SP: Seaweed powder

gm: Gram

GAE : Gallic acid equivalent

mg : Miligram

Ml : Milliliter

ppm: Parts per million

QE: Quercetin equivalents

SD: Standard deviation

TE: Trolox Equivalent

Abstract

Bread is a flour-based baked culinary product or a mealthat is dampened, plied, and some of the time aged. Normal wheat bread has a higher glycemic index as it contains a high amount of carbohydrates. Fortification of bread is important to increase its nutritional value and decrease its GI value. The purpose of this study is to produce a highly nutritious seaweed bread that will help to lower diabetes. Seaweeds aresupplement thick having numerous cancer prevention agents as specific nutrients (A, C, and E) and defensive shades.It has a decent amount of iodine, a trace mineral vital for the health and function of the thyroid.However, the flavor and smell of seaweed limit their utilization in foods.Therefore, In the current study, efforts have been undertaken to develop wheat-seaweed-based bread by using seaweed called *Ulva intestinalis* powder with wheat flour at 2.5%, 5%, and 7.5% levels of supplements to determine sensory acceptance and thento investigate the nutritional content, bioactive substances, antioxidant capacity, and antidiabetic activity.With the substitution of seaweed powder, the carbohydrate content will drop.The content of protein, fat, crude fiber, ash, and carbohydrate ranges was establishedas 8.43-8.86%, 1.26-2.24%, 0.17-0.67%, 0.83-1.70%, and 53.1-59.5% respectively.The total flavonoid concentration and total phenolic content of the bioactive components were higher in the seaweed-supplemented bread., ranging from 10.072-23.56 mg QE/100g and 15.5-17.47 mg GAE/100 ml respectively, in contrast with the control(2.33 mg QE/100g and 15.31 mg GAE/100ml). The antioxidant capacity was boosted thrice ranging from (13.8 to 39.27 mg TE/100g). In the sensory evaluation, bread with 5% supplementation received the best approval rate.Also,bread treated with 5% seaweed powder inhibited α-amylase activity the most.The nutritional composition obtained in this study suggests that *Ulva intestinalis*has potential food value and could be recommended as an ingredient that can be incorporated into making functional foods.

Keywords: Seaweed powder, Bread, Bioactive compounds, Antioxidant capacity,α-amylase

Chapter-1: Introduction

Due to the physical and climatic conditions of the land, Bangladesh, one of the most densely populated nations, has a large cereal-based population. Rice grain is the most well-known food ate by individuals, followed by wheat. For rural and urban residents, respectively, rice and wheat address 62% and 54% of the everyday typical admission, everything being equal,. In Bangladesh, cereal and snacks made from cereal are becoming increasingly well-liked. The processing and marketing of cereals are expanding industries. The per capita pace of individuals consuming bread goods in the nation expanded from 0.43 kg in 2008 to 2.09 kg in 2021. One of the most established and most customary things in the baking industry area is bread. It is a crucial staple food, and consumption is both steady and rising quickly.As indicated by the public authority's Public Eating routine and Nourishment Study, normal bread utilization per individual is roughly 80 grams each day, higher for men (96g) than for ladies (66g).

The expression "seaweed" alludes to various marine plants and green growth that flourish in the sea as well as streams, lakes, and different waterways.Its extracts are utilized in a variety of applications, including human meals, beauty care products, manures, and the extraction of modern gums and synthetics. According to Nayar and Bott, 2014, the majority of overall seaweed production is now for direct human consumption, mostly in Asian nations such as China, Japan, and the Republic of Korea, where seaweeds are taken as part of a regular diet, and to a considerably smaller amount in the rest of the globe.It is incredibly adaptable and may be used in a variety of recipes like sushi rolls, soups & stews, salads, supplements, and smoothies.

Seaweed has enormous potential as an elective wellspring of excellent food items that have drawn in research lately, because of their overflow and variety (Nazarudin*et al.*,2020). They contain numerous cancer prevention agents as specific nutrients such as vitaminA, vitamin C, andvitamin E and defensive pigments.Seaweedis rich in iodine, a minor element essential for the wellbeing andthyroid capability. A few seaweeds, like purple laver, contain a lot of B_{12} too.

Diabetes Mellitus is a worldwide public health issue due to the significant morbidity and death rates linked with it. Diabetes can be treated using synthetic hypoglycemic medications, albeit long-term usage has several negative effects. This has led to a shift towards the utilization of natural compounds that possess antidiabetic properties.Seaweed, a large type of benthic alga found in the marine environment, is a source of a variety of bioactive compounds and antioxidants, which can have a range of health benefits. Seaweed extract and its bioactive ingredients have the potential to help fight diabetes because they can block enzymes that break down carbs in vitro and can even lower blood sugar levels in animals when tested at random and after prandial.

Green seaweed, as the most bountiful type of large-scale seaweed, is a significant sea life natural asset. It is a rich wellspring of a few amino acids, unsaturated fats, and dietary filaments, as well as polysaccharides, polyphenols, colors, and other dynamic substances, which play essential parts in different organic cycles like cell reinforcement action, antidiabetic, immunoregulation, and calming reaction.

*Ulva intestis*is a bright green, grass-like seaweed that grows from a small, discoidal base. The fronds are irregularly inflated, irregularly narrowed, and tubular in shape. Most of the time, the fronds do not have any branches. The length of the fronds is 10-30 cm, and the diameter is 6-18 mm. The tips of the fronds are rounded.(Budd*et al*,2008).

When comparing the nutritional composition of bread and rice, bread may be regarded as the healthier alternative. This is because bread has fewer calories and carbs than rice.Bread needed to be modified to boost its nutritional value for a balanced diet.People have been using seaweed in food for thousands of years..For quite a long time, food has been utilized to advance wellbeing, however, the information on the connection between food parts and wellbeing is currently being figured out, assisting with further developing food stanmdard or finding advanced supplement origins.Seaweed can be used as a natural treatment for people and animals by adding it to their food dates back many centuries, mostlyin Asian nations.To reap the benefits of *Ulva intestinalis*, we attempted to make a seaweed bre ad with varying proportions of *Ulva intestinalis* powder and whole wheat flour both of which have high protein and fiber content. The better portion can assist with

causing us to feel fuller and lessen the sum we eat during the day. For quite a long time, food has been utilized to advance wellbeing, however, the information on the connection between food parts and wellbeing is currently being figured out, assisting with further developing food quality or finding new supplement sources. The utilization of ocean growth in human and creature feed, as normal medication, is a training that returns numerous times, chiefly in Asian nations.

Aim of the study:

The study aims to produce a healthy bread that will contain all the properties of seaweed (*Ulva intestinalis*) that will help to reduce diabetes with a great antioxidant effect. Also, itprovides a thorough investigation of the bioactive extracts derived from*Ulva intestinalis* species and how they can support the treatment as well as avoidance of difficulties related to diabetes mellitus.

Aims and objectives:

I. Using seaweed powder to make bread
II. To examine and differentiate the planned bread's healthful piece, bioactive parts, and antidiabetic action.
III. To evaluate the resulting product's palatability and general acceptance.

Chapter2: Review of literature

2.1 Overview of *Ulva intestinalis*

Ulva intestis, also referred to as Sea Lettuce, Green Bait Weed, Gutweed, and Grass Kelle, is a species of algae native to the genus*Ulvaceae*. Tubular members of the sea lettuce genus Ulva were classified as Enteromorphs until a genetic study in the early 2000s. The fronds contain branches and are completely cylindrical, reaching 15 cm or more in length and expanding to the mid-thallus in width. The cells are asymmetrically organized, and the chloroplast is hood-shaped and positioned on one side, with just one pyrenoid. The species can grow to be 10 to 30 centimeters (3.9-11.8 in) long and 6 to 18 millimeters (0.24-0.71 in) wide.

Source: https://en.wikipedia.org/wiki/ulva_intestinalis

Fig 2.1: *Ulva intestinalis*

It tends to be tracked down in the Bering Ocean close to The Frozen North, the Aleutian Islands, Puget Sound, Japan, Korea, Mexico, the Philippines, and Russia.Other than this, it very well may be tracked down in Israel, and such European nations as the Azores, Belgium, Denmark, Ireland, Norway, and Poland, and in such oceans as the Baltic, and Mediterranean Oceans. It is likewise found on the shores of the Pacific Sea remembering New Zealand.Distinct languages have different names for them, includingEntéromorphe (French), Darmtang (German), Tarmgrønske (Norwegian), Tarmalg (Swedish), and Erva-patina(Portuguese). *Ulva intestinalis*has been kept in new to saline waters from ditches, pools, rockpools, trenches, moorlands, and bedrock and its primary use is as an edible gut weed.

Scientific classification:

Kingdom: Plantae

Division: Cholophyta

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Genus: Ulva

Species: *U. intestinalis*

2.2 Nutritional properties

A new report detailed that the general synthesis of *U. intestinalis* on a dry premise was unrefined protein (12.6%) even though protein contents rely upon the species, seasons, and ecological circumstances.*U.* intestinalis is wealthy in Mg, K, Cl, Na, and Ca. *U. intestinalis* contained elevated degrees of protein ranging from 14.6-19.5% DW, and most seaweed contained lipids under 4% DW (McDermid and Stuercke, 2003). However,Certain seaweeds were found to contain elevated amounts of fatty acid. U. intestinalis contains lipid 2.1–8.7% DW.

*U. intestinalis*has an ash content of 25.9–28.6% DW.The ash contents in this ocean growth are higher than those in earthbound plants, with a typical worth of 5-10% DW. The variety in ash contents likewise relies upon ocean growth species, geological starting points, their technique for mineralization, as well as the impact of food handling by drying and canning.

Leucine, threonine, valine, and arginine are abundant in U. intestinalis. Non-Essential Amino Acids present at reasonably high levels include histidine, aspartic acid, glutamic acid, serine, proline, glycine, and alanine. Lysine is the most limited necessary amino acid. The species includes a high concentration of aspartic and glutamic acids, that contribute to the distinct aromas and tastes. Total amino acid concentration ranged from 9.5-10.6 mg/100 mg DW (613-618 mg/g protein), with *U. intestinalis* having a protein composition of 17.9% DW. The most Essential Amino Acid found in leucine. In *U. intestinalis*, the proportions of EAA to non-EAA shifted from 0.67 to 0.72, while the proportions of EAA to add up to amino acids were around 0.4.

The species' soluble, insoluble, and total dietary fiber levels varied from 25.3-39.6% DW, 21.8-33.5% DW, and 51.3-62.2% DW, respectively.Soluble dietary fiber is thought to help delay digestion and nutrient absorption, as well as decrease blood cholesterol and glucose levels. It also helps to avoid clogging, colon malignant growth, cardiovascular infection, and weight gain(Ortiz, 2006). Conversely, insoluble dietary fiber is related to waste mass increment and gastrointestinal travel time decline.

However, the nutritional makeup of seaweeds fluctuates with the seasons. *U. intestinalis* has greater Water Holding Capacity and Oil Holding Capacity. *U. intestinalis* contains a lot of B vitamins, C vitamins, iron, calcium, iodine, and potassium. According to the findings, the species could be employed as primal matter or components to elevate the nutritional content and quality of pharma foods and nourishing goods for humans.

It was viewed that *U. intestinalis* contained elevated degrees of ash, obvious protein and dietary fiber contents, and moderately elevated degrees of full-scale components, fundamental amino acids, and dissolvable and insoluble dietary filaments.

U. intestinalis separate showed impressive antimicrobial impact toward a few tried microorganisms. The methanolic concentrate of *U. intestinalis* demonstrated great movement against a Gram-positive bacterium *S. epidermidis* (Berber*et al*., 2015).

Hence, this seaweed can add to human and creature healthful necessities. Their healthful pieces along with their physicochemical properties propose that Ulva species have the expected food to be useful fixings in the food business. Besides, its utilization emphatically affects wellbeing since it can diminish blood lipid levels, corpulence, and the gamble of coronary illness (OmmeeBenjama*et al*., 2011).

2.3 Bioactive compounds

2.3.1 Flavonoids

Flavonoids are phytochemical intensifies present in many plants, organic products, vegetables, and leaves, with possible applications in restorative science.The assortment and smell of blooms as well as the limit of natural items to attract

pollinators and, appropriately, natural item dispersing to assist with seed and spore germination as well as the turn of events and improvement of seedlings are totally attributed to flavonoids in plants as per Griesbach, 2005.Flavonoids are plant compounds with an assortment of medical advantages.Flavonoids go about as exceptional UV channels (Takahashi and Ohnishi, 2004), signal iotas, allopathic substances, phytoalexins, detoxifying subject matter experts, and antimicrobial watchman fabricated materials. They moreover protect plants from a grouping of biotic and abiotic stresses (Samanta et al., 2011).

Flavonoids have a few subgroups, which incorporate chalcones, flavones, flavonols and isoflavones.Flavonoids can be artificially perceived into six essential subgroups: flavones, flavanols, flavanones, flavanonols, flavan-3-ols (catechins), and anthocyanidins. These substances (aglycones) are consistently glycosylated (somewhere around one objective with different sugars) and may in like manner be alkoxylate or esterified.This has provoked the distinctive evidence of around 5,000 undeniable flavonoids in plant materials (Harborne and Williams, 1992). The logical cycles used to conclude the flavonoid content in various plants rely upon the course of action of an aluminum chloride complex, which is used in most revealed procedures for the assessment of flavonoids (Grubesic et al., 2007).

Variance in flavonoid concentration can be attributed to differences in physicochemical characteristics such as the brininessof thebase. However, the flavonoid content of algal extracts varies from 8.048 ± 1.119 *U. intestinalis* mg RE g-1(Farasat M et al., 2014).Flavonoids are good for your body because they can help fight off bacteria, antioxidants, and free radicals. Plus, they can help with spasms. (Polterait*et al*.1997)

2.3.2 Phenolic compounds

Phenolic compounds are the most broadly dispersed assistant metabolites and are found across the plant domain, whether or not the specific sort of phenolic compound that is accessible changes relying on the phylum practical. The malonate/acetic acid derivation framework, also known as the polyketide pathway or the shikimic corrosive pathway, generates roughly 40% of the natural carbon that circulates in the biosphere (Chapman and Regan, 1980). Phenolics present in food may be classified

into threesignificant gatherings: simple phenols and phenolic acids, hydroxycinnamic corrosive subordinates, and flavonoids (Ho, 1992).

It has been demonstrated conclusively that plant phenolics safeguard many ongoing ailments related to oxidative pressure, including disease, and cardiovascular, and neurological issues (Dai and Mumper, 2010).

A literature survey reveals the phenolic compounds present in *U. intestinalis* can vary in concentration, ranging from 1.258 ± 0.126 mg GAE g^{-1}. (Farasat M *et al*., 2014).

2.3.3 Anthocyanins

Anthocyanins are a gathering of dark red, purple and blue colors tracked down in plants. They're essential for a bigger classification of plant-based synthetics called flavonoids.According to Martin et al., 2017 they are principally answerable for the engaging light yellow, orange, red, fuchsia, violet, and blue shading of an assortment of plant tissues, including blossoms, leaves, and natural products, as well as stockpiling organs, roots, tubers, stems, and grains. Notwithstanding their capability in further developing plant resistance to a few abiotic conditions like saltiness, dry season, exorbitant light, UV radiation, and cold pressure, these builds have drawn in a ton of interest as of late as food colorants that supplant counterfeit colors. Besides, earlier examination showed the significance of anthocyanins for human prosperity and their ability to defend against continuous disorders (Nassour et al., 2020).

Because of their novel compound cosmetics, anthocyanins and anthocyanidins have a more grounded cell reinforcement limit than different flavonoids. These synthetics' capacity to battle free extreme age and abatement metal-incited peroxidation is because of their capacity to tie metal particles (Dai et al., 2012; Martin et al., 2017). Anthocyanins are also especially effective patrons of hydrogen to ROS and free progressives, detoxifying them and hindering further outrageous turn of events.This is because of the positive charge, number and position of the hydroxyls and methoxys, plus the presence of electrons that give and electrons that take away. It protects important biomolecules like proteins, lipids and DNA from being damaged by oxidation, which can speed up the aging process and cause some diseases(Pojer et al., 2013; Martin et al., 2017). The most common types of anthocyanins and flavonoids

have more exploratory movement than the more prominent, potent cancer preventative agents; for example, cyanidin's cancer prevention agent limit is 4.4 times higher than ascorbic corrosion and the same as vitamin E. (Gould et al., 2002).

2.4 Antioxidant activity

Cancer prevention agents play a critical role in for killing free extremists and protecting our bodies against various afflictions connected to free revolutionaries. The inception, engendering, and end of the system are undeniably connected with the oxidative cycle that is interceded by free extremists. Cell reinforcement creation can happen both inside the body and normally in different food varieties (Alam et al., 2020).

The methanolic concentrates of U. intestinalis showed the most noteworthy DPPH rummaging action (48% hindrance) and a lower IC_{50} worth of 2.32 mg/ml.Studies have demonstrated that this consumable alga has reasonable cancer-prevention agent potential and should be taken into account for upcoming projects in medicine, dietary supplements, cosmetics,and food industries.(Srikong*et al*., 2017).

2.5 Fatty acid profile

An unsaturated fat is a carboxylic corrosive with a long side chain of hydrocarbons. Unsaturated fats are the construction blocks of the fat in our bodies and in the food we eat. During osmosis, the body isolates fats into unsaturated fats, which can then be held in the blood. Unsaturated fat particles are by and large merged in social events of three, outlining a molecule called a greasy substance. Unsaturated fats can be isolated into four general classes: soaked, monounsaturated, polyunsaturated, and trans fats. The soaked (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) segments addressed 52%, 30%, and 19% of complete unsaturated fats in *U. intestinalis*, separately. The primary SFA is introduced in *U. intestinalis* palmitic corrosive (C16:0). Concerning MUFAs, oleic corrosive (C18:1) is the prevalent unsaturated fat. Among the ω3 PUFAs, docosahexaenoic corrosive (DHA) (C22:6ω3) is the most bountiful unsaturated fat, addressing 46% of the complete PUFAs, which is trailed by linolenic corrosive (C18:3ω3) and eicosatetraenoic corrosive (EPA) (C20:5 ω3).*U. intestinalis* also contains the ω6 fattyacids (FAs) in its PUFA composition, where

linoleic acid(C18:2ω6) is detected as the predominant ω6 fatty acid. (Jannat-Alipour*et al*,2019)

2.6 Amino acid profile

Amino acids are a crucial sustenance class. They are utilized as substrates by the human body in various metabolic cycles, including protein amalgamation, cell flagging, and the making of low-sub-atomic weight nitrogenous synthetics. Since amino acids perform explicit physiological jobs, the utilization of these substances can be beneficial in the treatment of a variety of medical conditions. For instance, the use of methionine enhancement can be beneficial for individuals with multiple sclerosis (Singhal et al., 2018); Medication with arginine can help protect the brain after brain damage caused by ischemia (Chen et al., 2020); The use of histidine has the potential to further enhance insulin sensitivity and reduce hyper-insulinism; the use of glycine has the potential to reduce the effects of liver and pulmonary injury. (Lee and Kim, 2019); also, tryptophan is utilized for further develop gloom and rest problems (Wu, 2013).

Even though wheat is a huge wellspring of calories and different supplements, it is viewed as healthfully poor on the grounds that the proteins of the cereal miss the mark on amino acids includes lysine and threonine. Requests of people in immature countries for grain vegetables are a major source of protein, minerals, and essential B-complex nutrients. As well as being high in Mg, K, Cl, Na, and Ca, U. intestinalis has a high protein content (14.6-19.5% DW). The solvent, insoluble, and all-out dietary fiber levels of the species went from 25.3-39.6% DW, 21.8-33.5% DW, and 51.3-62.2% DW.

2.7 Beneficial aspects of *U. intestinalis*

The green seaweed of the genus Ulva may provide a potential platform for the development of bioactive components for the production of functional foods. In recent years, the focus has shifted to the medical applications of these bioactive components, which are derived from sulfate polysaccharides(Rahimi et al. 2016; Li et al. 2018). Historically, Ulva seaweed has been consumed in both fresh and dried form due to its high nutritional value (Yaich et al. 2011; Cofrades*et al*. 2017).

Ulva guttis is a green seaweed that's packed with protein and peptides. It's a great way to get your daily dose of nutrients. (Bodin*et al*.2020). Itcontains 263 kcal per 100 g dry weight. (Jacobsen*et al*,2023) *U. intestinalis* powder isrich in minerals (19%), protein (13.5%), and carbs (57%), but low in lipids (2.7%), however, the unsaturated fat profile contained apparent measures of unsaturated fats (49%) with docosahexaenoic to be the most elevated ω3 unsaturated fat. Amino corrosive examination showed the prevalence of fundamental amino acids, which were practically identical to FAO/WHO prerequisites. Concerning physicochemical properties, the water holding was tantamount to some rural fiber-rich items.

The number of absolute macronutrients in the concentrated seaweed was 5323.48 ± 24.53 mg (100 g DW)$^{-1}$, with K as the most elevated one followed by Ca, Mg, and Na, separately. The micronutrient commitment in U. intestinalis was significant, with the aggregate sums of 142.7 ± 4.19 mg (100 g DW)$^{-1}$ as follows: Fe > Zn > Cu (Hakimeh Jannat *et al.*,2019). A substantial admission of aluminum has been connected with medical problems like Alzheimer's infection and unfriendly consequences for the sensory system. The quantity of aluminum present in the digestive tract of Ulva intestisis somewhat low.

Many water-soluble vitamins are present in *Ulva intestinalis*. They are-

Components	Value
Ascorbic acid (Vitamin C)	<10.0 ppm *
Nicotinamide (B3)	<3.0 ppm *
Pyridoxine (B6)	<3.0 ppm *
Riboflavin (B2)	<3.0 ppm *
Thiamine hydrochloride (B1)	<3.0 ppm *

Table 2.1: water-soluble vitamins are present in Ulva intestinalis

* Below the limit of detection (LOD). (Farzanah*et al*. 2022)

Seaweeds like *Ulva intestinalis* have bioactive mixtures that show cytotoxicity in different disease cell lines. These mixtures forestall cancer development by actuating apoptotic cell demise and capture development by impeding various kinases and cell cycle pathways.Moreover, The inhibitory activity of *Ulva intestinalis* on the

carbohydrate digestive enzymes has been demonstrated to be effective in the management of diabetes. (Abo-Shady*et al*.2023)

2.8 Bread as an entire wheat bakery item

Bread is a normal food that is straightforwardly connected with individuals' day-to-day routines. It is made by blending flour, and water, and raising fixings into a mixture. Bread is perhaps one of the most seasoned foods delighted in around the world. Bread is generally viewed as a significant wellspring of starches in dietary rules. A significant part of bread is flour, and its properties are intensely affected by the characteristics of the grain from which it is ready. According toBelitz *et al*., 2004 and Hui, 2006 how much processing influences the flour's synthetic cosmetics. Processing the bark increases the amount of inorganic compounds, fiber that can't be broken down, and other nutrients, while decreasing the amount of starch. Flour is made up of protein, starch, various sugars, fats, fibers, water and other stuff.It likewise has a low degree of nutrients, minerals, and catalysts (Giannou *et al.*, 2003).The protein content decides how much gluten is produced in the flour, which influences the level of solidarity, mixture structure, and design. The protein content of hard wheat is significantly higher than that of delicate wheat, making bread is used (Andrikopoulos, 2010). In addition to the 10-12% protein content, 70-75% of the flour is composed of starch, while the remaining 14% is composed of water. The flour also contains 2-3% non-starchic polysaccharides, as well as arabinoxylan and lipids, to a similar degree.A study byBushuk, 2001 shows that these components while having insignificant amounts, are vital to the creation and nature of the bread.

The bread fills in as a phenomenal wellspring of complicated sugars, which give our body it requiredenergy and is pivotal to keeping up with glucose level(Psaltakis, 2002). Practically most bread assortments accompany plant-based proteins that are low in calories and fat. Additionally, they incorporate B nutrients, vitamin E, and minor components including iron, potassium, and calcium (Andrikopoulos, 2010).As per European regulation (Guideline (EC) 1924/2006, 2006) bread is described as an item which is characterized by its high fiber contentand thusly emphatically influences how the body works when it carries something like 6 g of fiber for each 100 g of item (Pereira and Ludwig, 2001).

2.9 Conclusion

As a result of expanded buyer wellbeing cognizance, there has been an extensive expansion in interest in adding dynamic fixings, for example, dietary fiber and phenolic cell reinforcements to normal dinners like bread. Eating this kind of food might help individuals' wellbeing execution and disease avoidance. While planning practical pastry shop things, for example, bread, it is basic to create something both physiologically helpful and buyer OK regarding appearance, flavor, and surface. Involving regular fixings in bread while holding fundamental bread quality attributes like hardness, surface, flavor and texture can hence providenumerous advantages in the advancement of human wellbeing

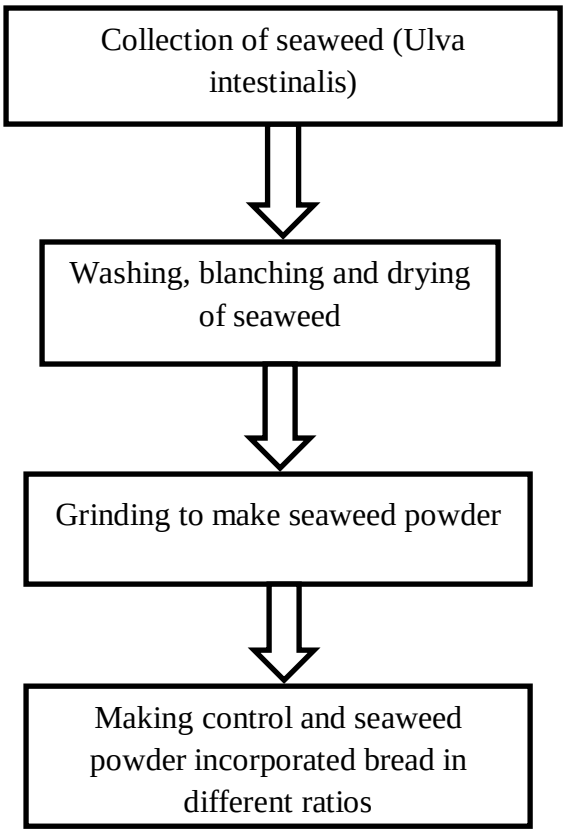
Chapter3: Materials and methods

3.1 Study area

This investigation was conducted in the labs of Chattogram Veterinary and Animal Sciences University's Departments of Applied Food Science and Nutrition, Food Processing and Engineering.The experiment was conducted for three months from 1 June 2023 to 30August 2023.

3.2 Layout of experiment

At first, the seaweed sample was collected and washed to remove sand, clay, and other particles. Then they were blanched, dried, and ground into fine powder. Then the powder was incorporated with bread ingredients to make a seaweed powder supplemented bread. The powder sample was then analyzed to determine its acceptance through sensory evaluation, proximate composition (moisture, ash, crude fat, protein, crude fiber, and carbohydrate), bioactive compounds (Total phenol, Total Flavonoids, Antioxidant), in vitro antioxidant activity and in vitro anti-diabetic activity (Figure 3.1).



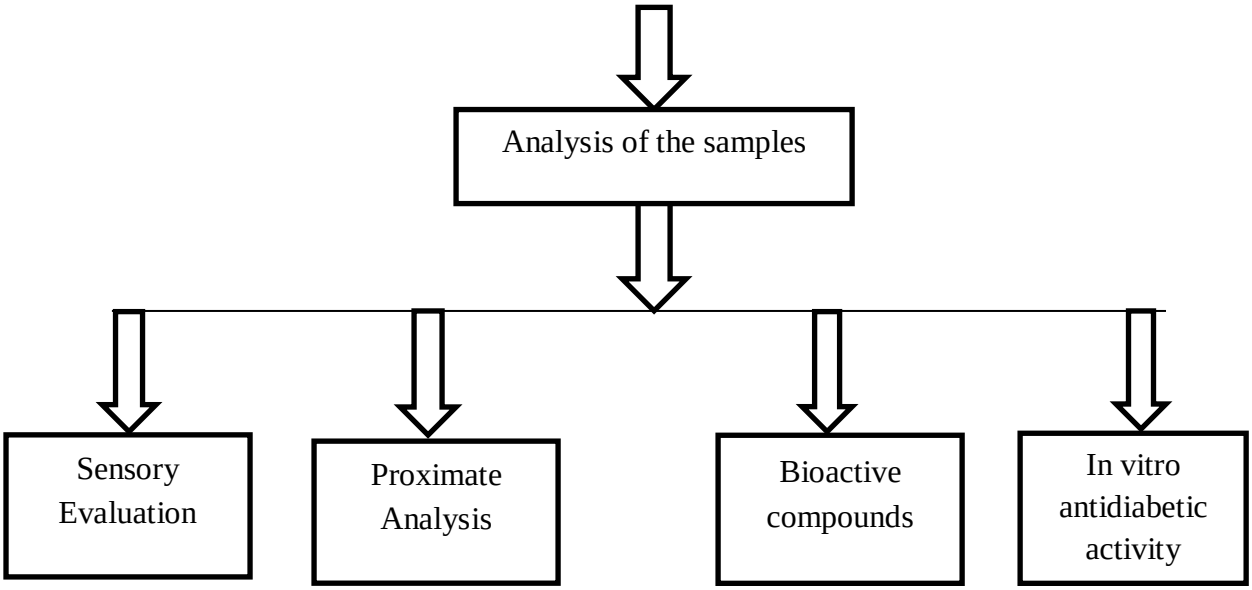


Figure 3.1: Experimental design

3.3Collection of samples

*Ulva intestinalis*was collected from the coastal side ofCoxs bazar. Other supplies for bread definition, for example, wheat flour, salt, sugar, yeast, sunflower oil, and skim milk powder were bought from the nearby local market and supermarket of Chattogram region. Other considerable materials expected for the analysis were acquired from the research center stocks.

3.4Preparation of seaweed (*Ulva intestinalis*)powder (SP)

The seaweeds(*Ulva intestinalis*) were rinsed off completelywith running tapewater in order to eliminatesalt, dust, and clay. Then they were blanched at86°C for 30 seconds. After that,they were placed on a tray and allowed to air dry in a cabinet dryer at 40°C for 12 hours. In order to produce the powder, the seaweed was ground into a fine powder in a food processor and then sieved. From that point, the powder was stuffed in a ziplock pack and put away in an impenetrable compartment for further use.

3.5Preparation of bread

Table 3.1 Formulations of bread

Ingredient	0% SP supplemented bread (Sample A)	2.5% SP supplemented bread (Sample B)	5% SPsupplemented bread (Sample C)	7.5% SPsupplemented bread (Sample D)
Wheat flour	56%	53.5%	51%	48.5%
SP	-	2.5%	5%	7.5%
Sugar	3.4%	3.4%	3.4%	3.4%
Salt	1%	1%	1%	1%
Vegetable oil	1.6%	1.6%	1.6%	1.6%
Skim milk powder	1%	1%	1%	1%
Yeast	1%	1%	1%	1%
Water	36%	36%	36%	36%

The procedure of making bread

A total of four bread samples were tested, one of which was deemed to be a control sample and the other two were deemed to be treated with seaweed powder. The proportion of whole wheat flour and seaweed powder in four samples was as follows: 100:0 (control sample), 97.5:2.5, 95:5, and 92.5:7.5 (treated samples)

The bread plans were baked utilizing the straight batter technique of Edwards, 2007 by following the recipe and baking strategy of Olaoye et al. (2006). From the beginning, the fixings were gauged by the plan given in Table 3.1. Then, at that point, weighted wheat flour, SP, salt, and skim milk powder were taken in a bowl and blended. From that point onward, a big part of the warmed water was added to the sugar and whirled to break down sugar properly. The yeast was then incorporated into the sugararrangement, blended, and left for 5 minutes. Then yeast was added to the bowl of joined fixings When it inflated to the point where it blanketed the entire surfaceof the water.The remaining water was also added to the mixture. And after that, it was thoroughly stirred for 2 to 3 minutes. Dough was made kneading the materials for around 30 minutes. Kneading the dough made the dough rise higher,

lighter, and softer. The dough was then soaked in a clean moist cloth and let aside to rise for around 1 hour at29℃.The aged batter was raised two times to the underlying volume via air catching during this proofing period. After 1 hourthe raised dough was punched to release the air pockets that had developed and scaled to 350 gm dough pieces. Then, at that point, the gauged batter was put in a bread deice for its last shape, covered with the wet towel and again positioned for 1 hour at 30℃ for second sealing to keep 85 % relative humidity. From there on, the damp cloth was taken out, and set the deice in the oven. Then, at that point, it was prepared at 220℃ for 30 minutes. Subsequent to baking, the bread was chilled, eliminated from deice, cut into cuts, and put away in a sealed shut compartment until tried.

3.6Nutritional analysis

Control endlessly bread tests comprising of SP were broken down for moisture, protein, fat, fiber, and ash content according to the techniques for AOAC 2016.

3.6.1 Moisture content

Moisturedeciding is generally significant and usually used measurement in the creation and testing of dinners. Since the amount of dry matter present in a portion of food is adversely connected with the amount of dampness it carries.The dampness content is straightforwardly significant monetarily for both the processor and the shopper. In any case, the impact of dampness on the strength and nature of food is far more noteworthy. The water level was estimated utilizing a strategy in light of the Relationship of True Scientific Physicists (AOAC, 2016) standard. A perfect cauldron was gauged and a 10 gm test was taken in the vacant pot. The heaviness of the pot with the example was noted. From that point onward, the pot was set in a hot air broiler at 105℃ and dried for 48-72 hours. The cauldron was then taken out from the broiler and set in a desiccator to cool and gauge. Along these lines, a few reiterations were finished till tracking down consistent outcomes. Moisture content was determined as follows

$$\text{Moisture\%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Sample weight}} \times 100$$

3.6.2 Crude protein

Utilizing the Kjeldahl strategy, the nitrogen centralization of tests, both natural and inorganic, is surveyed. Kjeldahl nitrogen is estimated in food sources and drinks, meat, feeds, grains, and scavenges to decide the protein level. An acknowledged strategy is depicted in numerous regularizing sources, (AOAC, 2016). This strategy's principal premise is to process the example utilizing an assimilation blend (sodium sulfate and mercuric oxide) and concentrated sulfuric acid (H_2SO_4). These outcomes in the oxidation and obliteration of protein as well as the transformation of natural nitrogen into smelling salts, which consequently stays as ammonium bisulfate in the corrosive blend. The means used to compute how much smelling salts nitrogen are to make the overview basic, distill the released alkali into a standard corrosive arrangement, and afterward measure the amount by titration.

A 0.5gm example in a debris-free channel paper piece was taken in a 100 ml perfect and dry Kjeldahl cup. After adding 10 ml of concentrated H2SO4 and a 1:1 gm processing blend (sodium sulfate and mercuric oxide), the assimilation chamber was warmed for six hours until the substance turned out to be perfect. After the absorption interaction was finished, the receptacle was permitted to cool and the fluid that had been processed was moved to a 100 ml volumetric jar and weakened sufficiently with refined water. A miniature Kjeldahl refining unit was loaded up with 10 ml of that arrangement after it had been injected with 5 ml of half NaOH and 2.5 ml of 15% Na_2S2O_3. The arrangement was steam-refined for ten minutes. The distillate was gathered in 2% boric corrosive arrangements with a pointer and afterward, it was titrated with 0.02N HCl. A similar clear processing was performed simultaneously without the substances. The evaluations for percent nitrogen or percent protein should be adjusted depending upon the sort of getting arrangement and any weakening elements utilized during the refining system. In the situation beneath, "N" represents normality. " ml clear" represents the milliliters of base expected to back titrate a reagent clear on the off chance that standard corrosive is the getting arrangement; if boric corrosive is the getting arrangement, it represents the milliliters of standard corrosive expected to titrate a reagent clear. The condition is when boric corrosive is utilized as the getting arrangement:

$$\text{Nitrogen\%} = \frac{(\text{ml standard acid} - \text{ml blank}) \times \text{N of acid} \times 1.4007}{\text{Weight of sample in gram}}$$

3.6.3 Crude fat

Fat substancesare not set in stone by putting food tests into natural solvents like chloroform or methanol and isolating the filtrate by filtration. To measure the concentrate, the filtrate is isolated into a few channels, the combination is dried, and the assessed fat percentage is then determined. Utilizing a Soxhlet device, the rough fat substance of the examples was resolved following AOAC (2016) rules. The percent of rough fat was communicated as follows

$$\text{Fat\%} = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3.6.4 Crude fiber

Around 2-5 gm of moisture and fat-free examples were weighed into a 500 ml measuring utensil and 200 ml of bubbling 0.255N (1.25% w/v) sulfuric corrosive was added to it. The blend was bubbled for 30 minutes keeping the volume steady by adding water at regular spans. A glass bar embedded in the container helps smooth bubbling toward the finish of this period. The combination was sifted through a shirting fabric and the buildup was washed with boiling water till liberated from corrosive. The buildup was then moved to a similar measuring utensil and 200 ml of bubbling 0.313N (1.25%) NaOH was added. After bubbling for 30 minutes, keeping the volume consistent as in the past, the blend was through the shirting fabric. The buildup was washed with boiling water till liberated from salt, trailed by washing with a similar measure of liquor and ether. It was then moved to a cauldron, dried for the time being at 80 to 100°C and gauged. The cauldron was warmed in a suppress heater at 650°C for 2 to 3 hours, cooled, and weighed once more. The distinction in the weight addresses the heaviness of unrefined fiber.

The crude fiber rate was determined by utilizing the accompanying recipe

$$\text{\% Crude fiber} = \frac{Weight\ of\ crude\ fiber}{\text{Weight of sample taken}} \times 100$$

3.6.5Ash content

The ash content was determined according to the AOAC(2016) procedures. Ash content is the inorganic residue remaining after the destruction of organic material. The empty crucible is taken out and left to dry for an hour in the desiccator. The crucible is dried at a temperature of 105°C. It is then weighed at a constant weight and filled with about 1 g sample. The sample is placed into the crucible. After 1 drop of nitric acid is added to the crucible, the sample is placed in the furnace. The furnace is set to a temperature of 650°C.The crucible is left in the furnace for 3 hours. After 3 hours, the crucible is removed, cooled, and held in the desiccator. The weight of the crucible with ash is measured. The ash content is calculated using the following formula:

$$\text{Ash\%} = \frac{\text{The amount of ash in the supplied sample}}{\text{Sample weight}} \times 100$$

3.6.6Carbohydrate

The total carbohydrate content was calculated using the difference method by Edeogu, 2007. The total available carbohydrate content is calculated by subtracting 100 from 100, where 100 represents the sum of 100 gm of moisture and ash, 100 gm of protein, and 100 gm of fat. The equation for carbohydrate content is as follows:

% Carbohydrate = 100 – (Moisture + Ash + Protein + Fat)

3.7Energy content

For each sample, the energy content was determined by applying the following equation: (baea et al., 1997)

Energy = (Protein × 4.1) + (Fat × 9.2) + (Carbohydrate × 4.1)

3.8 Determination of bioactive compounds

Extract preparation:

To find out what bioactive compounds were in the extracts, we followed the same approach as Zhang &Hamauzu in 2004, but with a few changes. We put the samples (10g, 0.02g) in 40 ml of 60% mixtures of methanol and CH3OH. We homogenized the mixture for 2 min, then put it in a refrigerator for 24 hours. Finally, we centrifuged it for 10 min at 4000 rpm. The mixture was then filtered through Whatman #4 filter paper. Finally, we left it to stand in a dark place at around 4°C. This served as a solution for measuring TPC and TFC levels, as well as DPPH levels. It worked great because we had the stock solution ready before we analyzed a new batch..

3.8.1 Total Flavonoid content (TFC)

To determine the TFC of the samples, we used a slightly modified aluminum chloride colorimeter method as described in Chang et al., 2002. First, we prepared a 1 mg/ml stock solution of extracts. Then, we dilute 0.5 ml of the dilute extract with 1 ml of 95% c2 H5OH. We add 0.1 ml of 10% aCl3 to the stock solution, 1 ml of potassium acetate to the 1 ml of C2 H5OH, and we add 2.8 ml of distilled water to the immixture. We then spent 30 min at room temperature with the combination. To measure the total flavonoids, present in the sample, we compared the absorbance to the standard curve for flavonoids. We measured the absorbance at UV-visible wavelengths using a UV-visit Spectro-spectrophotometer. 10% aluminium chloride replaced with d.H2 O of the same amount was used as a blank. The total flavonoids in the sample were assessed and communicated in mg QE per gram (QE/g).

3.8.2 Total Phenolic Content (TPC)

According to Da Silva et al. (2011), the Folin-Ciocalteu Phenol reagent was used to determine the total phenolic content (TPC). 8.5 ml of distilled water and 0.5 ml of the Folin-Ciocalteu Phenol reagent were combined with 0.5 ml of extract. 1 ml of a 35% sodium carbonate solution was added after the mixture had been at room temperature for 5 minutes. After being vortexed, the mixture was left at room temperature for 20

minutes. A UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan) set at 765 nm was used to measure the absorption. Deionized water was used in place of the sample as the blank. Standard Gallic acid solutions at different concentrations were read against an empty background to generate a calibration curve. Gallic acid equivalents per 100 grams (mg GAE/100) were used to express TPC.

3.8.3 Determination of Antioxidant capacity by DPPH scavenging method

The cancer prevention agent action of the concentrate was assessed utilizing the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) extremist rummaging strategy distributed by Adiletta et al. (2018). Initial, 100 l of concentrates and 1.4 ml of DPPH revolutionary methanolic arrangement (0.1 mM in methanol) were joined. The DPPH test was disintegrated in 100 ml of methanol at a centralization of 0.0039 g, and the combinations were then left in obscurity for 30 min. As the reaction continued, a drop in the complex 2,2,2-diphenyl-2-picrylhydrazyl (DPPH) concentration in the solution, as measured in a UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan) at 517 nm at 25°C (A_{sample}), resulted in a shift in color from yellow to purple.

Water ($A_{control}$) was used to prepare Blank in place of the sample, and the absorbance was measured. A sample's DPPH radical was identified. The sample DPPH radical was determined from:

% Antioxidant activity= $(Abs_{control} – Abs_{sample}) / Abs_{control} \times 100$

Where, $Abs_{control}$= the absorbance of control at the initial time

Abs_{sample}= the absorbance of the sample

3.9 Sensory analysis

3.9.1 Affective test

The outcome of this test will demonstrate the appropriateness of "SP incorporated Bread". The test was conducted by 15 panelists (all male and female) based on the CVASU. The test was administered by a panel of individuals who were not trained. Panelists were asked to classify the four bread compositions based on their level of

approval with the characteristics of the sample using a 9-point grading system. Scores ranged from -1 (extreme dislike) up to -9 (like extremely).Crust, Aroma, Shape, Internal texture, Appearance,Taste, and overall acceptance were the were the tactile properties that the panelists assessed.

Table 3.2 Grading system for sensory assessment

Ranks	Score
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

3.10In-vitro Antidiabetic activity by alpha-amylase inhibition assay

3.10.1 Preparation of extract

To prepare an aqueous extraction,in 40 ml of distilled water, 2gm of powdered sample was mixed. It was shaken for 24 hours in a rotary shaker. Following that, it was centrifuged at 8000 rpm for 10 minutes. Whatman no. 1 filter paper was used to filter the resulting supernatant. The crude extracts were kept in a freezer at -20 degrees Celsius for no longer than one week before analysis.The extract was prepared by following the method of Pinto *et al.*2010.

3.10.2 Test for α-Amylase Inhibitory Activity

Aqueous extract was tested to see if it had an effect on the activity of a substance called a "amylase". The method used was the same as the one used by Kazeem and

co. (2013), but with a few changes. For α-amylase inhibition, the seaweed extracts were diluted in buffer (PBS, 20 mM, pH 6.9) to give a final concentration of 25mg/ml, 50mg/ml, 75mg/ml. Subsequently, 1ml of the example was additionally blended in with 1mL α-amylase (0.5 mg/mL) and followed by brooding for 30 min at room temperature. Then, 1mL of 1% (w/v) dissolvable potato starch was added and followed by hatching for another 10 min. The response was ended by adding 1 mL of DNS (dinitro salicylic corrosive) reagent (12g of sodium potassium tartrate tetrahydrate in 8ml of 2M NaOH (Sodium Hydroxide) and 96mM 3, 5-dinitrosalicylic corrosive arrangement). Subsequent to warming for 5 min in a bubbling water shower, an orange-red tone was created. The absorbance was perused at 540 nm involving the spectrophotometer for estimating α-amylase exercises in a three-fold way. To take out the absorbance delivered by plant separate, fitting concentrate controls with extricate and aside from the catalyst were likewise included. Business inhibitor acarbose was utilized as a positive control. A clear cradle arrangement was utilized rather than substrate. The cylinder with compound arrangement however without plant separates/acarbose filled in as the control with absolute protein action.

Calculation: The enzyme inhibition rate expressed as a percentage of inhibition was calculated using the following formula:

$$Inhibition\ of\ \alpha - amylase\ activity\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Where the control possesses 100 % enzyme activity and the tested sample was plant extract or the standard (acarbose).

3.11 Cost analysis

The price of the bread enriched with seaweed powder was determined by taking into account the total price of the components used in the production of the bread. This price was expressed in taka and was compared to the price per packet of bread.

3.12Statistical analysis

Minitab 19.0 programming was utilized to lead measurable examinations. On the assembled information, a one-way investigation of difference was completed (ANOVA). To decide if there were any measurably tremendous contrasts between them, the Tukey test was utilized following factual programming; the degree of importance is ($p < 0.05$). Fifteen individuals from the board chose to participate in the examination, the aftereffects of which are itemized below.

Chapter-4: Results

4.1 Nutritional properties

The nutritional properties of bread supplemented with seaweed powder were evaluated by measuring the moisture content, protein content, fat content, raw fiber content, ash content, and carbohydrate content

4.1.1 Nutritional composition of bread formulations

Table 4.1 illustrates the nutritional characteristics of bread supplemented with SP; the majority of the samples differ significantly.Sample D is bread formulated with 7.5% SP contained the highest percentage of protein (8.86±.05), fat (2.24±.06), crude fiber (0.67±.04) and ash (1.70±.04). The lowest percentage of protein (8.43±.45), fat (1.26±.30), crude fiber (0.17±.02) and ash (0.83±.03) was observed in the 0% SP supplemented bread.

Table 4.1 Nutritional composition of bread formulations

Legends: Implies ± SD and values in similar sections with similar superscripts are not genuinely critical (P<0.005)

Parameter⁄ Bread sample	**A(N=3)**	**B(N=3)**	**C(N=3)**	**D(N=3)**	**P(1-ANOVA)**
Total Carbohydrate (%)	59.5±.55^{a}	56.6±.55^{ab}	54.3±2.19^{bc}	53.1±.45^{c}	0.001
Crude protein (%)	8.43±.45^{a}	8.57±.40^{a}	8.59±.05^{a}	8.86±.05^{a}	0.001
Ether extract (fat)(%)	8.86±.05a	1.50±.08^{a}	1.93±.04^{b}	2.24±.06^{b}	0.001
Ash (%)	0.83±.03^{a}	1.12±.23^{a}	1.54±.04^{b}	1.70±.04^{b}	0.001
Crude fiber (%)	0.17±.02^{a}	0.21±.03^{b}	0.38±.03^{c}	0.67±.04^{c}	0.001
Moisture (%)	28.7±.25^{a}	30.0±.08^{b}	32.5±.10^{c}	33.7±.30^{d}	0.001

A- Bread produced from 100% wheat flour

B- Bread produced from composite flour of 97.5% wheat and 2.5% seaweed powder

C- Bread produced from composite flour of 95% wheat and 5% seaweed powder

D- Bread produced from composite flour of 92.5% wheat and 7.5% seaweed powder

4.1.2 Energy content

From Figure 4.1, energy content was calculated in the highest amount (290.1 kcal/100gm) in sample A and lowest (275.61 kcal/100gm)and (276 kcal/100gm) in both samplesC and D. They have almost the same amount of energy content.

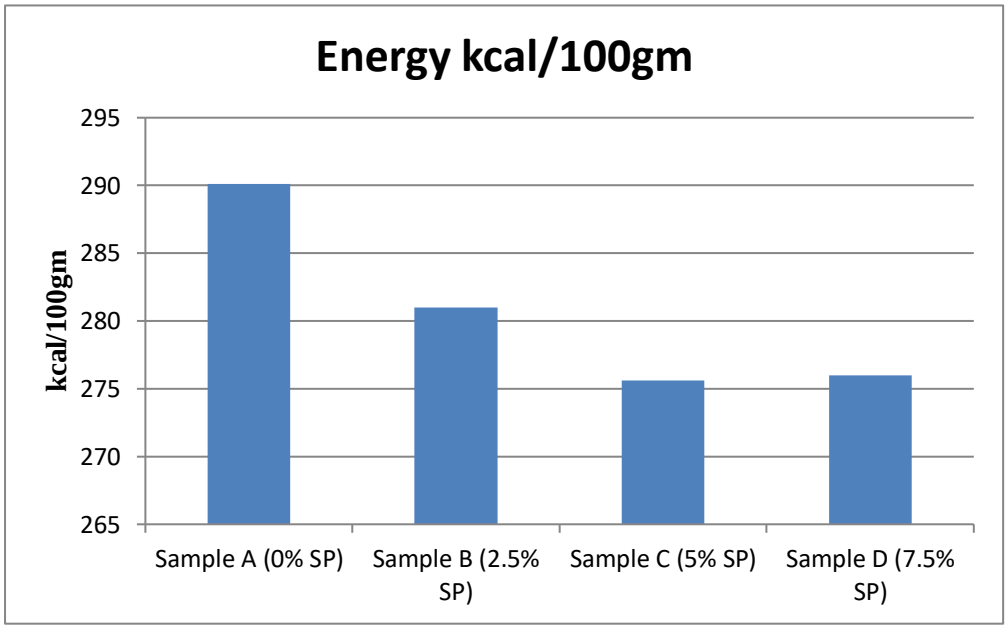


Figure 4.1: Comparison of energy content among bread formulations

4.2 Bioactive components

4.2.1 Bioactive components of bread formulations

The amount of TFC and TPC of bread are introduced in table 4.2. There are huge contrasts in the qualities. Test D conveyed the most elevated measure of all out flavonoid content (23.556±.20mg QE/100g) and total phenolic content (17.465±.20mg GAE/100 ml) and total antioxidant capacity(39.273±.02mg TA/100 ml)whereas lowest value of total flavonoid content (15.305±.20mg QE/100g) and total phenolic content (2.71±0.001 mg GAE/100 ml) and total antioxidant capacity(13.803±.10mg TE/100g) in control bread.,

Table 4.2 Bioactive components of bread formulations

Parameter⁄ Bread sample	**A(N=3)**	**B(N=3)**	**C(N=3)**	**D(N=3)**	**P(1-ANOVA)**
Total Phenolic Content (TPC) (mg GAE/100 ml)	$15.305\pm.20^{a}$	$15.459\pm.20^{b}$	$16.137\pm.12^{c}$	$17.465\pm.20^{c}$	0.001
Total Flavonoid Content (TFC) (mg QE/100g)	$2.327\pm.20^{a}$	$10.072\pm.08^{b}$	$21.869\pm.12^{c}$	$23.556\pm.20^{d}$	0.001

Legends: Implies ± SD and values in similar sections with similar superscripts are not genuinely critical (P<0.005)

4.3 Antioxidant capacity

Sample D carried the highest amount ofAntioxidant capacity(39.273±.02 mg TA/100 ml) and the lowest value oftotal antioxidant capacity is seen in sample A (13.803±.10 mg TE/100g)

Table 4.3 Antioxidant capacity of bread formulations

Variable	**Total Antioxidant (TA) (mg TE/100 ml)**	**P(1-ANOVA)**
A(N=3)	$13.803\pm.10^{a}$	0.001
B(N=3)	$16.053\pm.05^{b}$	0.001
C(N=3)	$28.547\pm.03^{c}$	0.001
D(N=3)	$39.273\pm.01^{d}$	0.001

Legends: Implies ± SD and values in similar sections with similar superscripts are not genuinely critical (P<0.005)

4.4 Sensory evaluation

Sensory characteristics of formed pieces of bread are addressed in Table 4.4, where it tends to be seen that there were no huge contrasts between tests An and B although there were tremendous contrasts for test B with tests C and D regarding every one of the qualities and generally speaking worthiness. Of all the credits, test B got the most noteworthy score. By and large example B had the most noteworthy acknowledgment rate by the specialist.

Table 4.4 Sensory score of bread formulations

Attributes/Bread Sample	A(N=3)	B(N=3)	C(N=3)	D(N=3)	P(1-ANOVA)
Crust	7.01±.50[a]	7.0±.20[a]	6.03±.15[b]	5.02±.10[c]	0.001
Aroma	8.01±.20[a]	7.06±.20[b]	6.02±.50[c]	5.03±.15[d]	0.001
Shape	8.0±.20[a]	6.70±.04[a]	6.03±.20[b]	6.02±.10[b]	0.001
Internal Texture	8.02±.10[a]	7.03±.15[b]	6.03±.25[c]	5.01±.25[d]	0.001
Appearance	8.21±.30[a]	7.01±.50[a]	6.03±.50[b]	5.06±.40[b]	0.001
Taste	7.96±.15[a]	7.03±.15[b]	6.05±.20[c]	5.01±.10[d]	0.001
General Acceptance	8.67±.40[a]	7.21±.50[b]	6.10±.10[c]	5.0±.20[d]	0.001

Legends: Implies ± SD and values in similar sections with similar superscripts are not genuinely critical (P<0.005)

4.5 Antidiabetic Activity by α-amylase inhibition assay

In this study, an anaerobic extract of four bread samples (2.5% SP, 5% SP, and 7.5% SP) was tested for inhibition of the effects of alpha-amylase on starch degradation in vitro and demonstrated strong inhibitory activity.

Table 4. 5 Antidiabetic activity by α-amylase inhibition assay

SL. No.	Conc. (µg/ml)	% inhibition of Acarbose	% inhibitionof 0% SP Bread	% inhibitionof 2.5% SP Bread	% inhibitionof 5% SP Bread	% inhibitionof 7.5% SP Bread
1	25	0.143%	0.457%	1.257%	1.718%	1.371%
2	50	0.8%	0.514%	1.342%	1.314%	0.142%
3	75	0.257%	0.942%	1.371%	0.914%	0.543%

4.6 Cost analysis

Table 4.6 Production cost of SP-supplemented bread

Heads	Tk	Quantity used	Total taka (for sample B)	Total taka (for control)
Raw materials				
Wheat flour	65tk/kg	196gm(control) 187.2gm(sample B)	12.16	12.74
Seaweed	350tk/kg	4.9gm	1.175	-
Sugar	140tk/kg	11.9gm	4.165	4.165
Skim milk powder	450tk/400gm	3.5gm	3.93	3.93
Oil	52tk/100ml	5.6ml	2.9	2.9
Salt	50tk/kg	3.5gm	0.175	0.175
Yeast	70tk/50gm	3.5gm	4.9	4.9
Sub total			29.405	28.23
Processing cost @ 15% of the expense of unrefined components			4.41	4.23
Packaging cost			3	3
Total production cost			36.8 taka	35.5 taka

Table 4.6 illustrates the total cost of production for control bread and bread products with 2.5 % SP (Sample B, highest sensory evaluation score). The raw components of both recipes are calculated for 350gm. The control bread weighed 315gm after baking, while sample B weighed 337gm.

The overall production cost for control was 35.5tk and 36.8tk for sample B.

Chapter-5: Discussions

5.1 Nutritional attributes

Seaweeds contain a critical number of solvent polysaccharides and can possibly work as a wellspring of dietary fiber. (Mamat *et al*,2013). According to Nazarudin et al *U. intestinalis*has the highest total carotenoids (162.00 μg g^{-1} DW), chlorophyll a (313.09 ± 2.53 μg g^{-1} DW), and chlorophyll b (292.52 ± 8.84 μg g^{-1} DW) concentrations.

A recent study 0f Hossain *et al*, reported that*U. intestinalis*SP has a higher quantity of ash (12.7%), crude fibers (17.1%), and proteins (12.6%).In comparison with the wheatflourit has considerably lower quantity of ash, crude fibers, and proteins, that is, 0.9%, 0.36%, and 10.55%, respectively. (Kulkarni*et al*,2012)

The data in Table 4.1 show that adding seaweed powder to wheat flour had a significant impact on the nutritional composition of wheat bread. The addition of SP to wheat flour increased the protein, fat, ash, and crude fiber content while decreasing the carbohydrate level. The larger quantity of seaweed powder in SP-integrated breads compared to wheat flour increased nutritious content.

Moisture content is an important attribute for determining the quality of bakery products.The degree of moisture in the breads progressively increased as the percentage of SP increased ranging from 28.7% in the control bread to 30% in sample B with 2.5% SP incorporated bread and 33.7% in D with 7.5% SP incorporated bread.Baking loss is the removal of moisture, which influences the texture and staling properties of baked products.(Mohibbullah*et al*, 2023).

The amount of SP supplementation somewhereelevated the protein level of the bread.The bread with 7.5%SP had a much higherquantity of protein (8.86±0.5%) than control bread (8.43±0.45%).The proportion of SP incorporated into the pieces of bread is inversely proportional to the fat, raw fiber, and ash content.Fat substance expanded from 1.26±0.30% in the control bread to 1.50±0.8% and 2.24±0.6%, crude fiber increased from 0.17±0.02% to 0.21±0.03% and 0.67±0.04%, ash content increased from 0.83±0.3% to 1.12±0.23%and 1.70±0.4% in sample B and D respectively. Similar results were found by (Mamat *et al*,2016).Because of the high

protein, fat, crude fiber, and moisture content of seaweed powder, the total carbohydrate contentof the bread decreased.

5.2 Bioactive components

Bioactive synthetic compounds are significant for supporting a safe framework and keeping away from ongoing sicknesses in people, as well as providing required nourishment. As a result, quantifying these molecules is critical, and the results of the bioactive chemicals discovered in control bread and SP-supplemented bread formulations are provided in Table 4.2.

The total flavonoid concentration differed significantly ($P<0.001$) amongst formulations (0-7.5% supplementation), ranging from 2.33-23.56 mg QE/100g. The sample with the highest flavonoid content, sample D (7.5% supplementation), assesed23.556 mg QE/100g, whereas the control bread had the lowest flavonoid level, 2.327 mg QE/100g. According to Ali *et al.*,(2020) and Farasat *et al.*,(2014)Total flavonoid content of wheat flour and seaweed powder is0.215mg QE/100gand 25.316mg QE/100grespectively.Since seaweed powder contains more flavonoid parts than wheat flour, the flavonoid content of seaweed-enhanced bread was demonstrated to be higher when contrasted with control bread.

The results showed that when wheat flour was replaced with SP in proportions ranging from 2.5 to 7.5%, the complete phenolic content of the bread improved altogether ($P<0.001$). As opposed to the control, which had the most reduced degree of all out phenolic compounds (15.305 mg GAE/100 ml), the results projected the highest concentration of total phenolics (17.465 mg GAE/100 ml) at a maximum supplementation of 7.5%. Similar outcomes were discovered byAmoriello*et al.*, (2021).

5.3 Antioxidant capacity

The DPPH test is used to determine the efficacy of natural antioxidants in scavenging free radicals. The outcomes uncovered that adding SP to bread tremendously affected their ability($P<0.001$) tremendously affected their abilityto search for free extremists. The antioxidant action (DPPH) worth of wheat flour bread was viewed as lower than

that of seaweed bread, with a direct increment.Sample D had the highest free radical scavenging activity (39.273±0.02 mg TE/100g), whereas the control had the lowest (13.803±0.10 mg TE/100g). In contrast to the control, the value of antioxidant capacity increased thrice in sample D. According to Ahmad *et al*. (2022) the expanded phenolic content of test D (supplementation at 7.5%) contrasted with the control might be credited to its higher cancer prevention agent limit.

5.4 Antidiabetic Activity by α-amylase inhibition assay

In general, human starch digestion occurs in two phases, commencing with alpha-amylase and ending with alpha-glucosidase to produce glucose before insulin penetration of the small intestine.High blood glucose levels are caused due to insulin insufficiency and malfunctioning (Boonpisuttinant*et al*., 2019).Alpha-amylase breaks down the 1, 4- and 1-oligosaccharides in starch and glycogen, making them easier to digest in the stomach. It's thought that blocking this enzyme in the digestive system can help treat diabetes by making it harder for the body to absorb glucose from starch. (Iyer, 2008).All aqueous extracts of control bread and SP-supplemented bread were investigated anti-diabetic by inhibition of α-amylase activity. Table 4.5 shows that all sample extracts could inhibit α-amylase.

Amongst the samples, 25 μg/mL concentration of 5% SP-supplemented bread had the highest amylase inhibition of 1.718% while control bread had the lowest amylase inhibition of 0.457%. Amylase inhibition is supposed to increase with increasing concentration but here we can see that it is not increasing but decreasing. Due to high concentration, UV visible cannot read it. That’s why 7.5% SP-supplemented bread has less amylase inhibition than 5% SP-supplemented bread.

Based on the outcome of the research, it can be concluded that utilizing SP-supplemented bread will help to reduce the rate of carbohydrate digestion and absorption, thereby contributing to the effective control of diabetes by lowering post-prandial hyperglycemia.

5.5 Sensory evaluation

Sensory characteristics are regarded as established quality parameters that determine the acceptability and edibility of final items.Apparance is an important qualitative aspect that attracts customers and boosts the visual appeal and acceptability of a product., was scored the highest (7.1) for sample B (2.5% supplementation), followed by sample D(5.06). The color of a baked good is determined by the physical characteristics properties of the dough (such as pH, water, amino acid, and reducing sugar content) as well as the processing factors used during the baking process (i.e., relative humidity, temperature, and air speed, and methods of heat transmission). The sensory score of aroma and internal texture indicated that sample B achieved the highest score (7.06) and sample D achieved the lowest score (5.01). The taste sensory score decreased when additional spicules were added to the bread in varying amounts, which may be attributed to the fact that seaweed can be salty and briny.Astudy by Gorman *et al.* (2023) shows a similar case where the addition of seaweed reduced the sensory quality.When wheat flour was replaced with SP up to 7.5%, the sensory qualities of the breads changed drastically. When compared to other formulations, supplementation at 7.5% exhibited an unfavorable sensory appeal.Because no improver was employed to improve the bread quality of the sample bread, aroma and flavor may improve with the addition of a commercially available bread improver compound.

Chapter-6: Conclusion

The incorporation of seaweed powder into wheat flour was found to enhance the nutritional and antioxidant properties while maintaining the quality of the bread. Additionally, the addition of the seaweed powder was found to increase the crude protein content, crude fiber content, and ash content in the bread.Similarly, when the degree of supplementation increased, so did the polyphenol, flavonoid, and antioxidant content of bread. Compounds in seaweed may also reduce diabetes risk factors, such as inflammation, high-fat levels, and insulin sensitivity. Further research in humans may help provide stronger evidence for using these compounds. According to the findings of this study, seaweed powder can be used in bread production at a level of 5% without affecting sensory quality. Also, 5% seaweed powder-supplemented bread had the highest inhibition of α-amylase activity.

Chapter7: Recommendations and future perspectives

In the present day, a significant proportion of people suffer from a variety of illnesses, including Diabetes mellitus, Lipid disorder, Obesity, Cancer, and more.At the same time, individuals have begun to rely on the benefits of natural components rather than medicines to keep them well. As a result, studies are being conducted to include these substances in food products. Existing research may be repeated to validate the experimental results.

In the current study, the quality of the seaweed bread was not up to the mark because of the hand-mixing method and it was not possible to maintain the humidity properly during the proofing time. If the dough mixing method is improved and humidity is maintained the quality of bread will increase.

The overall approval of bakery food products is usually determined by their manufacturing method, ingredients, and baking method.As a result, for consumer approval, contemporary instruments, standard procedures, and quality control assessments must be ensured.

According to the study's findings, seaweed can drastically reduce alpha-amylase activity. As a result, incorporating seaweed into bakery products improves diabetes conditions and it can reduce the occurrence of many other diseases because of its high nutritional value.

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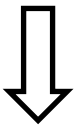
Appendices

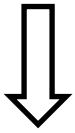
Appendix A: Flowchart for preparation of seaweed powder

Collection of seaweed (*Ulva intestinalis*)

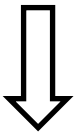
Washing to remove to remove dirt, sand and salt



Blanching of seaweed at 86^{O}C for 30 seconds



Draining of water



Drying of seaweed at 40°C for 12 hours

Grinding of dried seaweed

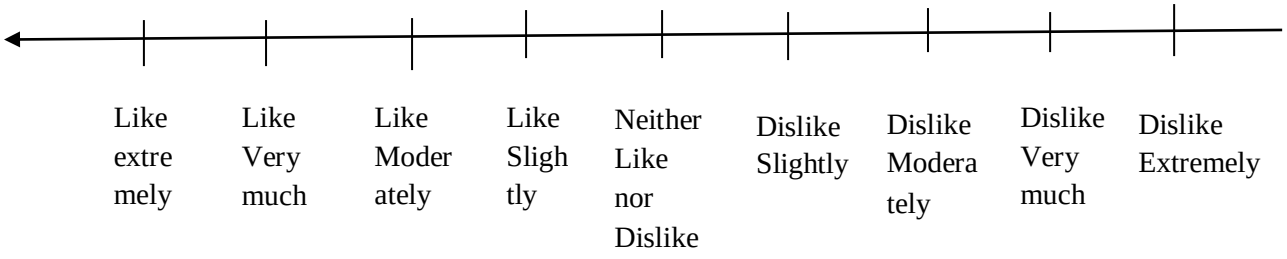
Sifting to get fine powder

Appendix B: Questionnaire for sensory test of bread

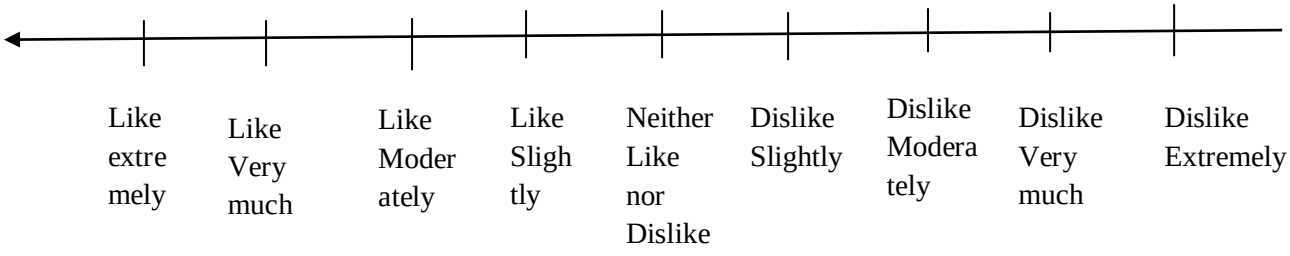
Name: **Age:**
Date:

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

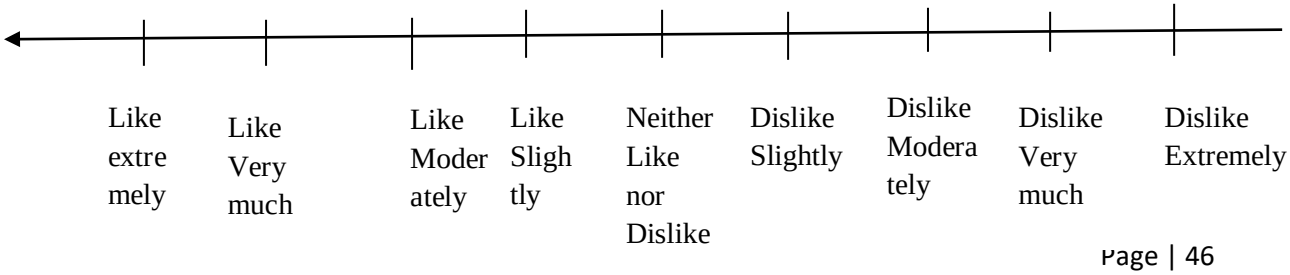
1) Crust:



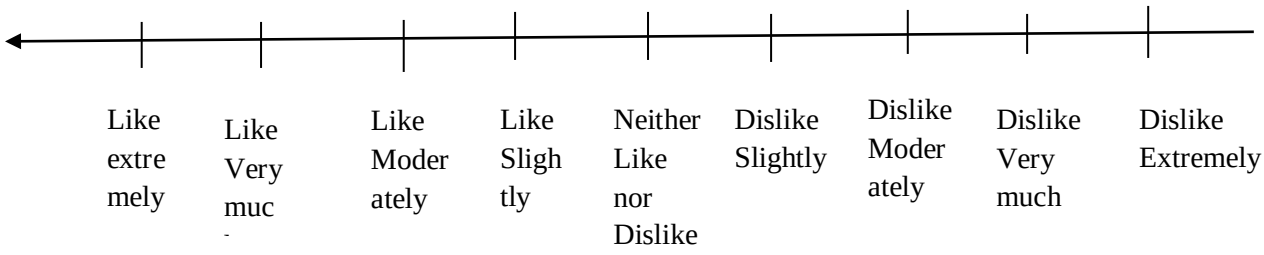
2) Aroma:



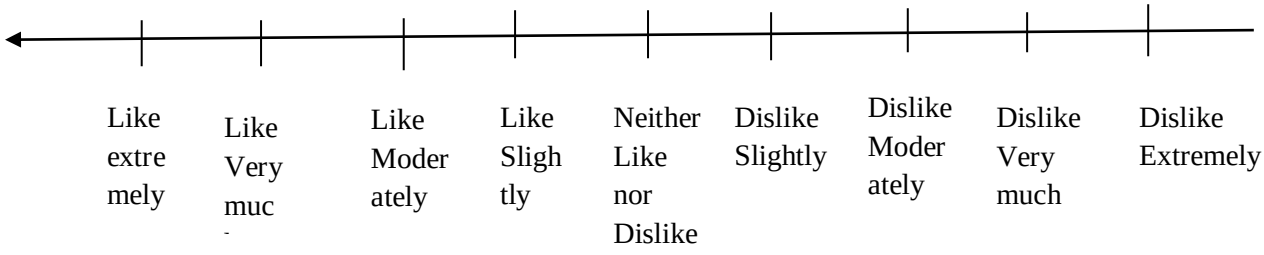
3) Shape:



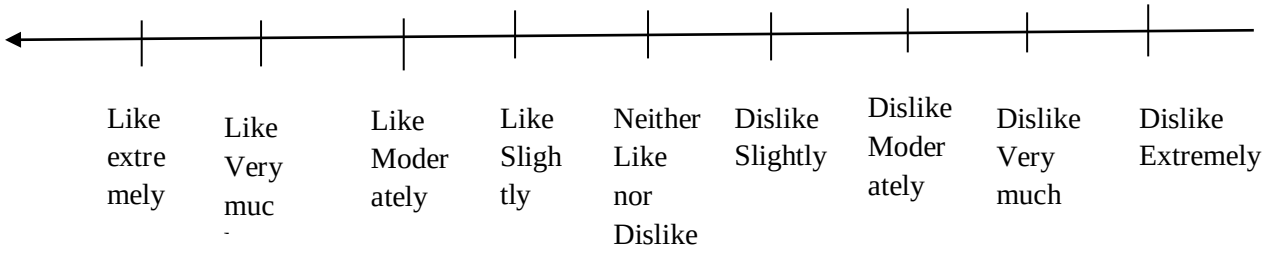
4) Internal texture:



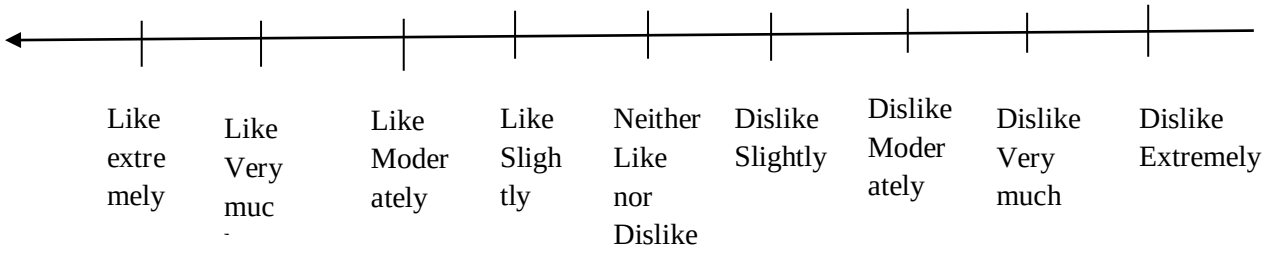
5) Appearance:



6) Taste:



7) Overall acceptance:

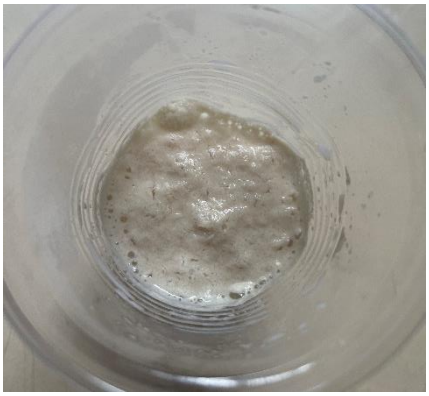


Appendix C: Procedure of making SP-supplemented bread

Drying of seaweed

Dried seaweed powder

Weighed ingredients



Activation of yeast

Kneaded dough

Proofing

Dough after first proofing

Dough after second proofing

Baked bread

Out of pan after cooling

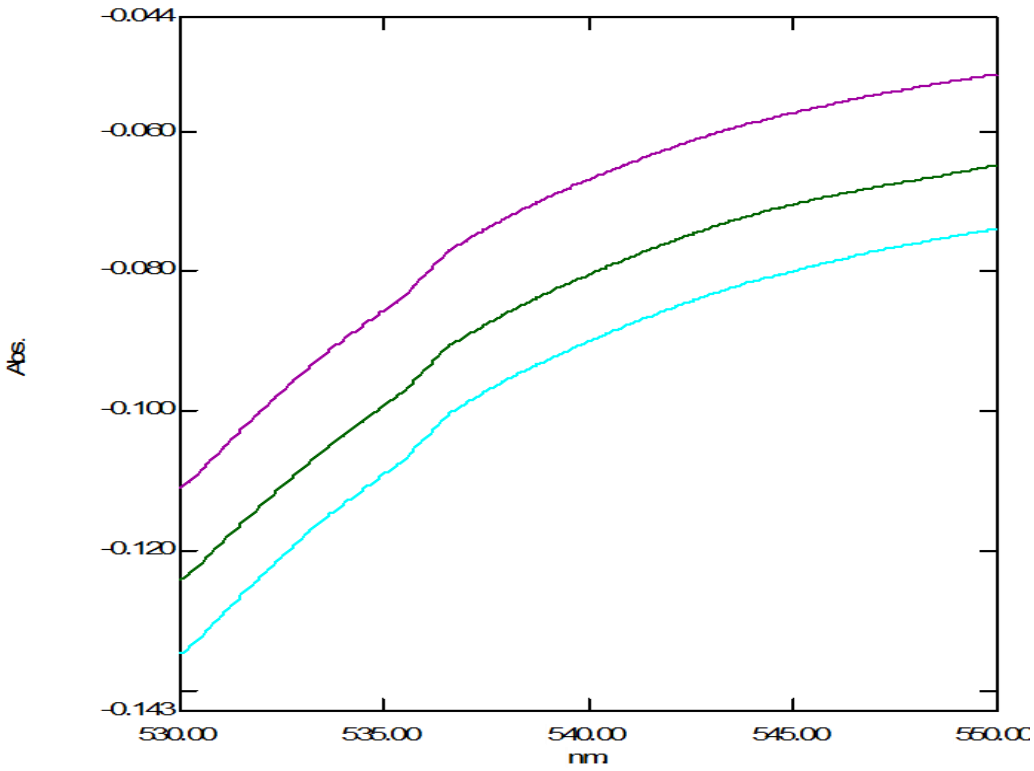
Appendix D: Pictures of analysis

Analysis of bioactive compounds

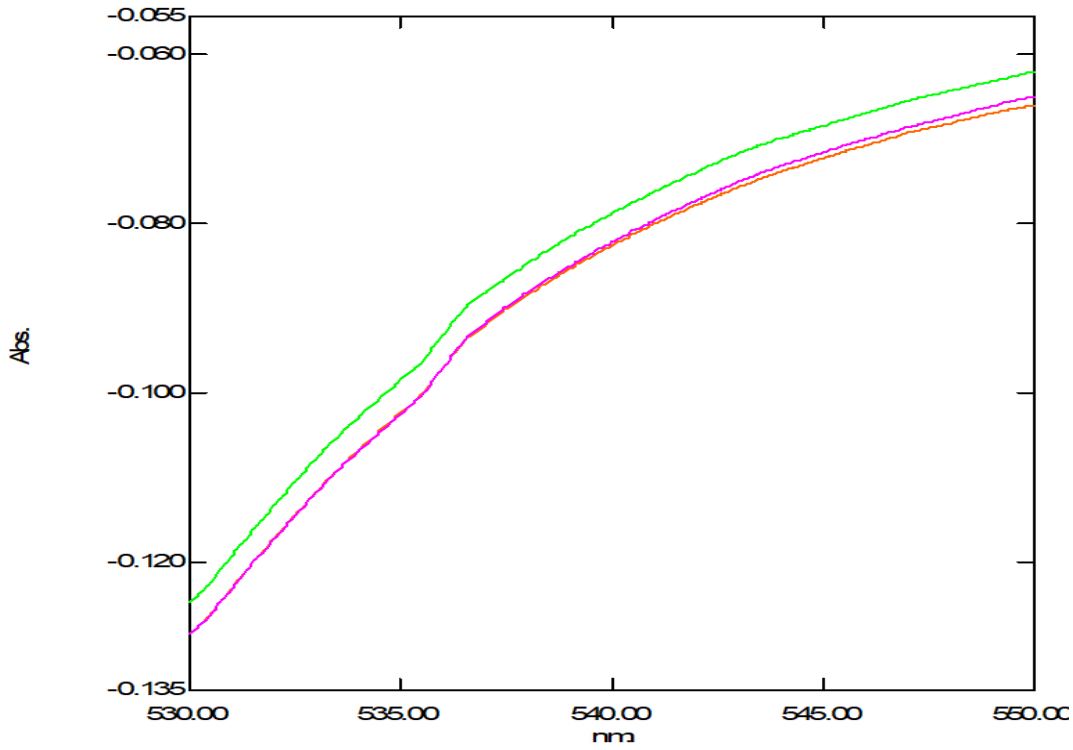
Analysis of α-amylase inhibition activity

Sensory Analysis

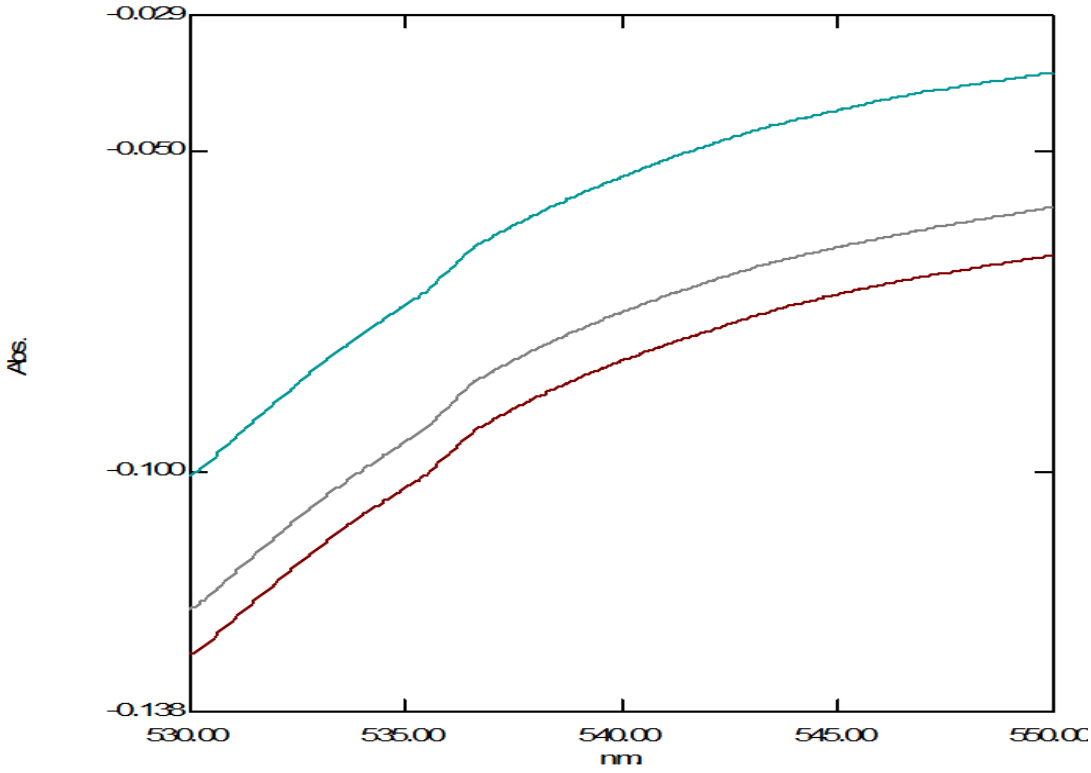
Appendix E: Inhibition of α-amylase activity graph



Graph for 2.5% SP supplemented bread



Graph for 5% SP supplemented bread



Graph for 7.5% SP supplemented bread

Brief biography

Nazifa Yeasmin passed the Secondary School Certificate Examination in 2013 from Ispahani Public School & College, and then the Higher Secondary Certificate Examination in 2015 from the same institution, Ispahani Public School & College, Chattogram. She obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest in working in the health and nutrition sector where she can utilize her knowledge and skills in imparting healthy nutritional plans for the well-being of people with various acute diseases. She wants to raise public knowledge about food safety and nutrition.