MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Detection of active ingredients in brown algae probiotic fermentation broth**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
Xinyue CHEN

Scientific supervisor
Tetiana SHCHERBATIUK, Dr. Sc., Prof.

Reviewer
Liubov ZELENA, Ph.D., Assoc. Prof.

Kyiv 2024

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Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA
_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Xinyue Chen

1. Thesis topic **Detection of active ingredients in fermentation broth of brown algae probiotics**
scientific supervisor Tetiana Shcherbatiuk, Dr. Sc., Prof.

approved by the order of KNUTD "__"_____2024, №____

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student _____ Xinyue CHEN

Scientific supervisor _____Tetiana SHCHERBATIUK

SUMMARY

Detection of active ingredients in brown algae probiotic fermentation broth – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2024.

As a rich marine resource, the bioactive components in brown algae, such as polysaccharides, polyphenols, proteins, cellulose and minerals, etc., have very positive effects on human health, and therefore have broad scientific prospects and practical applications in the pharmaceutical industry. Adding probiotics to brown algae can enhance the release and efficacy of the active ingredients in brown algae through the fermentation of probiotics. The efficacy of the content of functional components in the fermentation broth of brown algae varies with different forms of fermentation. In this thesis, based on the optimization of the fermentation process in the laboratory, the content and efficacy of the active ingredients in the brown algae fermentation were examined.

This experiment was divided into two parallel control groups with four samples in each group. Samples 1 and 2 were cultured with lactobacilli added to the culture; samples 3 and 4 contained only *Bacillus sp*. Samples 1 and 3 constituted one set of controls and samples 2 and 4 constituted the other set of controls. The polysaccharide and alginate contents, as well as the antioxidant activity (via DPPH content) of the brown algae and their fermentation broth were examined. The results showed that the polysaccharide content of fucoidan increased with the addition of lactic acid bacteria in control groups 2 and 4, but no enhancement of antioxidant activity was observed, whereas in control groups 1 and 3, the antioxidant activity was enhanced despite the decrease in the polysaccharide content of fucoidan.

Key words：*Brown algae; Fermentation broth; Active components; Efficacy*

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INTRODUCTION

Brown algae are a group of macroalgae belonging to the phylum Brown Algae that grow primarily in marine environments, usually thriving in cold, shallow waters. Brown algae are usually brown or greenish-brown in colour and can range in size from tiny millimetres to huge tens of metres. They are important biological components of marine ecosystems and provide many ecological and economic values. Brown algae contain a variety of bioactive components (Liu, 2016), such as polysaccharides, polyphenols, proteins, vitamins and minerals, which have a positive impact on human health (Li, 2024). In particular, the active substances in brown algae, such as fucoidan polyphenols and fucoidan polysaccharides (Xiao, 2016), are attracting extensive attention from researchers and industry for their remarkable physiological activities such as antioxidant, blood pressure-lowering, lipid-lowering and anti-inflammatory activities (Kusaykin, 2008). In particular, brown algal polyphenols, which are compounds with unique chemical structures and strong antioxidant activities, are able to effectively scavenge free radicals and reduce oxidative stress, thus demonstrating potential health benefits in terms of anti-aging, anti-inflammation, and anti-tumour effects (Xu, 2014).

The focus on the active ingredients in the fermentation broth of brown algae probiotics addresses a critical area of research with substantial scientific and practical implications. By exploring the interactions between probiotics and brown algae, this work aims to contribute valuable knowledge to the fields of biotechnology, nutrition, and pharmaceuticals.

CHAPTER 1 LITERATURE REVIEW

Brown algae are a group of macroalgae belonging to the phylum Brown Algae that grow primarily in marine environments, usually thriving in cold, shallow waters. Brown algae are usually brown or greenish-brown in colour and can range in size from tiny millimetres to huge tens of metres. They are important biological components of marine ecosystems and provide many ecological and economic values. Brown algae contain a variety of bioactive components (Liu, 2016), such as polysaccharides, polyphenols, proteins, vitamins and minerals, which have a positive impact on human health (Li, 2024). In particular, the active substances in brown algae, such as fucoidan polyphenols and fucoidan polysaccharides (Xiao, 2016), are attracting extensive attention from researchers and industry for their remarkable physiological activities such as antioxidant, blood pressure-lowering, lipid-lowering and anti-inflammatory activities (Kusaykin, 2008). In particular, brown algal polyphenols, which are compounds with unique chemical structures and strong antioxidant activities, are able to effectively scavenge free radicals and reduce oxidative stress, thus demonstrating potential health benefits in terms of anti-aging, anti-inflammation, and anti-tumour effects (Xu, 2014).

In order to better study the active substances in fermentation broth, we can learn some detection methods from some past studies. Hu Shunqiang6 (Hu, 2013)mentioned that the research on the active components of brown algae has been more intensive, and active components such as polysaccharides, polyphenols, fucoxanthin, sterols and terpenoids with various physiological activities such as antioxidant, anti-tumour and hypoglycemic have been successfully prepared by aqueous extraction, extraction with organic solvents, ultrasound-assisted, microwave-assisted and enzymatic hydrolysis methods.

Although the assay methods for each active substance were not described in detail, the methods for evaluating the biological activity of some active substances were mentioned. For example, for the antioxidant activity of polysaccharides, it can be evaluated by in vitro models such as the ability to scavenge diphenylpicrylhydrazyl radicals (DPPH) and hydroxyl radicals. The antioxidant capacity of polyphenols can also be determined by similar free radical scavenging assays and also evaluated by assessing their inhibitory effect on metal ion-induced lipid peroxidation.Chakraborty (Chakraborty, 2017) 7studied the antioxidant activity of three species of brown algae collected from the Gulf of Mannar region of India and found that they were rich in polyphenols, which had significant free radical scavenging capacity and antioxidant potential. The antioxidant properties of the brown algae were evaluated by using various in vitro test systems such as DPPH, free radical scavenging capacity assay, hydroxyl radical scavenging, hydrogen peroxide scavenging, inhibition of TBARS formation and Fe2+ ion chelation. In particular, Anthophycus longifolius showed strong antioxidant activity, suggesting that brown algae can be used as a natural source of antioxidants for the development of food supplements and functional foods (Guo, 2018). The study also analysed the phenolic constituents of brown algae using RP-HPLC technique, which further confirmed the contribution of these constituents to the antioxidant activity(Chkhikvishvili,2000). Overall, brown algae are a multifunctional bio resource whose research covers a wide range of disciplines and has a wide range of applications in pharmaceutical, industrial and ecological fields. With the continuous progress of science and technology, brown algae research will continue to contribute to the development of human society.

In this experiment, antioxidant activities, also known as free radical oxidants, are extensively discussed. Free radical oxidants include reactive oxygen, nitrogen, and sulfur substances, which can be either beneficial or harmful. When antioxidant levels in the body are lower than free radical

oxidants, free radical stress occurs, leading to potential harm. Notably, free radical oxidants can damage DNA by causing cross-linking and cleavage, disrupting DNA synthesis, transcription, and translation. This disruption can trigger immune responses, activate inflammatory pathways, and lead to cell damage and apoptosis, increasing the risk of cancer (Dizdaroglu, 2012).

Alginic acid, a naturally occurring polysaccharide found in brown algae, consists of glucuronic and mannuronic acid units linked by glycosidic bonds (Sreekumar, 2020). It is used as a thickener, gelling agent, and stabilizer in the food industry, enhancing taste, texture, stability, and shelf life. In pharmaceuticals, alginate's biocompatibility and biodegradability make it suitable for drug delivery systems, biomedical materials, wound dressings, and oral care products (Li,2011). Despite showing some antioxidant activity, alginic acid's DPPH radical scavenging activity is not very significant. However, it exhibits a high intensity of antioxidant activity in other mechanisms (Zhou, 2002).

In recent years, the attention to the activity of brown algal polysaccharides has mainly focused on antitumour and antioxidant activities, while relatively few studies have been carried out on antibacterial, anti-inflammatory and humectant activities (Wijesinghe, 2011). Although many novel active substances have been successfully extracted from brown algae, and during the extraction process, it has been found that the addition of appropriate amounts of fungi for multiple fermentations can increase the active substances in the extract(Fang, 2024), and at the same time, the addition of some of the fungi can reduce the fermentation time and make the efficacy of the products more significant(Wang, 2024), these substances have been proved to have a high utilisation value and play an important role in human health and nutrition, but we still know little about the active mechanism of action of most fucoidan. Therefore, more in-depth studies and research are necessary to better understand and utilise these valuable resources.

Despite the well-documented benefits of brown algae, the mechanisms through which fermentation enhances the activity of their bioactive components remain underexplored. Given the increasing interest in natural and effective health-promoting substances, understanding these mechanisms is essential for developing new functional products (Shahidi, 2018). This research aims to fill this gap by examining the effects of probiotic fermentation on the active ingredients in brown algae. The primary purpose of this research is to investigate the active components in the fermentation broth of brown algae probiotics and assess their potential as novel health products. The specific objectives include analyzing the polysaccharide content in the fermentation broth, evaluating the antioxidant activity through DPPH radical scavenging assays, and determining the efficacy of other bioactive components present in the broth (Qu,2014).

The novelty of this research lies in its comprehensive approach to understanding the synergistic effects of probiotics and brown algae during fermentation. By optimizing the fermentation process and analyzing the resulting bioactive components, this study provides new insights into enhancing the efficacy of brown algae through biotechnological methods.The practical implications of this research are significant. The findings can inform the development of new health products and functional foods that leverage the enhanced bioactive components of fermented brown algae. Additionally, this research can contribute to the sustainable utilization of marine resources, promoting the broader application of brown algae in various industries.

In conclusion, this study's focus on the active ingredients in the fermentation broth of brown algae probiotics addresses a critical area of research with substantial scientific and practical implications. By exploring the interactions between probiotics and brown algae, this work aims to contribute valuable knowledge to the fields of biotechnology, nutrition, and pharmaceuticals.

CHAPTER 2. RESEARCH MATERIALS AND METHODS

2.1 Seaweed treatment:

For the first step in the preparation of the experimental materials was seaweed treatment, which was done by soaking dried kelp in purified water until it was fully soaked. After soaking, the kelp was rinsed repeatedly with purified water to remove sediment and salt. The kelp is dried at low temperature. After drying, the kelp was ultramicrocrushed and the particle size was controlled at 1-100 μm. the ultramicrocrushed seaweed powder was irradiated and sterilized(Xu, 2024).

2.1.1.Strain activation and culture with fermentation process:

Because the experiment needs to be studied for the brown algae probiotic fermentation broth, the activation and cultivation of the strain is also very important. Strain activation and cultivation was performed by aspirating 100 μL of glycerol-preserved Lactobacillus and Bacillus into 5 mL of MRS liquid medium at -80°C, and incubating them in a shaker at 37°C for 16-20 h to activate them for two generations. The seaweed powder was mixed with the Bacillus fermentation solution for the first fermentation. After removing the bacillus organisms, lactobacilli were inoculated for the second fermentation. At the end, solid-liquid separation was carried out by centrifugation and membrane filtration, and the liquid phase components were collected to obtain the seaweed fermentation broth.

2.2. Detection of polysaccharides:

For the detection of polysaccharides using the phenol-sulfuric acid method, 1 mL of each sample was accurately pipetted into a 10 mL centrifuge

tube after dilution (up to 200-fold to ensure that the measured absorbance values were between 0.2 and 0.8). Next, 1.0 mL of phenol solution 5% (v/v) was added to the cuvette, and 5 mL of concentrated sulfuric acid was added quickly. Then the reaction was allowed to stand for 10 min, and the colorimetric tube was placed in a water bath at 3 0 ℃ for 20 min. finally, the absorbance value of the mixture was measured as OD490. the absorbance value was determined by reference to the method of standard curve plotting, and a pure water blank was made as a control, and the polysaccharide content of the fermentation broths of each group was calculated by the standard curve with the zero adjustment of a spectrophotometer (Dong, 1996).

2.3. Detection of alginate:

Alginic acid was detected by mesotriphenol spectrophotometry, which required 1g/100mL of mesotriphenol, 0.2g/L sodium alginate standard reserve solution, hydrochloric acid and sodium carbonate. The experimental method for plotting the standard curve was as follows: take ten 25 mL colorimetric tubes, add 0.00 mL,0.50 mL,0.75 mL,1.00 mL,1.25 mL,1.5 mL,1.75 mL,2.00 mL,2.25 mL,2.50 mL of standard use solution of sodium alginate in order, and then add 10.00 mL of hydrochloric acid, respectively, and then add each of them after mixing well. 0.70 mL of resorcinol solution, mix well, placed in a boiling water bath color reaction for 65 min, remove and use running water to reduce to room temperature, fixed to the scale, with a blank tube as a reference, at 440 nm to determine its absorbance. For the determination of the sample, 2.00 mL of bird's nest sample was pipetted into a 100 mL volumetric flask, and 1.000 g of solid sodium carbonate was added and heated at 85 ℃ for 3 h. The sample was removed and cooled, and then filtered. Pipette 1.00 mL of filtrate into a 25 mL colorimeter. Make it follow the previous steps and determine the absorbance. The content of sodium alginate can be obtained by substituting the absorbance into the standard curve.

2.4. DPPH free radical scavenging:

Prepare 0.1 mM DPPH solution first. The specific operation is to take 0.002g of DPPH dissolved in 50mL of ethanol and keep it away from light. Next, a 0.5 mg/mL solution of vitamin C (Vc) was prepared, at least 2 mL. vitamin C is usually used as a positive control to verify the validity of the experiment. According to the experimental requirements, a certain concentration of sample solution was prepared, which included the solution during the first fermentation and the solution obtained after the second fermentation, at least 2 mL was used as the mother solution. The plate-up operation was carried out under light-avoiding conditions. After mounting the plate, the plate was placed at room temperature and protected from light for 30 minutes. This step was to avoid the effect of light on DPPH radicals and to ensure the accuracy of the experimental results. The absorbance was measured at 517 nm using a spectrophotometer and the average value was taken. This wavelength is the wavelength at which the absorbance of the DPPH radical changes most significantly in the free radical state and in the steady state after being scavenged.

2.5. Determination of scavenging capacity of superoxide anion radicals

In this experiment, a total of five reagents were used, namely Tris-HCL (Tris solution conditioned with HCL) 1.817g/100mL, PH=8.2 (around 80 water + weighing Tris; diethylenetriaminepentaacetic acid solution 0.118g/100mL; o-benzyltriol solution 0.0151g/100mL; bacterial fluid samples (diluted 2 times; VC Ascorbic acid positive control 0.1 g/mL. 1 mL of 150 mM Tris-HCL solution 1 mL of 3 mM diethylenetriaminepentaacetic acid solution, 1 mL of 1.2 mM o-triphenyltriol solution and 0.5 ml of Streptococcus gelatinosus bacterial solution were mixed well and put into a 25 electrically heated thermostatic sink to react for 10 min, and then centrifuged for 10 min at 8,000 r/min and measured

at 325 nm. The absorbance was measured at 325 nm. Ascorbic acid (Vc) was used as a positive control.

A0 was 1mL Tris-HCL solution and 1mL diethylenetriamine pentaacetic acid solution.

A1 is 1 mL Tris-HCL, 1 mL diethylenetriamine pentaacetic acid solution and 1 mL o-triacontanol solution

A2 is 1 mL Tris-HCL solution, 1 mL diethylenetriaminepentaacetic acid solution and 0.5 mL Bacillus coagulans bacterial solution (VC for positive control).

A3 is 1 mL Tris-HCL solution, 1 mL diethylenetriaminepentaacetic acid solution, 1 mL o-benzenetriol solution, and 0.5 ml Bacillus coagulans bacteriophage solution (positive control with VC.)

Clearance = [1-(A3-A2)/(A1-A0)

Positive control = [1-(A3 positive - A2 positive)/(A1-A0)]

2.6. Hydroxyl radical scavenging capacity assay

In this experiment, a total of five reagents were used, which were ①: 1mL 0.02M PBS (PH7.4); 0.5mL 2.5mM o-diphenyl solution 0.0451g/100mL; 0.5mL 2.5mM ferrous sulphate solution 0.0379 g/100mL; 20mM hydrogen peroxide (100μL+50mL H2O); ddH2O; VC Ascorbic acid positive control 0.1 g/mL.

After mixing 1 mL of 0.02 M PBS buffer (pH=7.4), 0.5 mL of 2.5 mM o-diazophene, 0.5 mL of 2.5 mM ferrous sulfate, 0.5 mL of 20 mM hydrogen peroxide, and 0.5 mL of Streptococcus gelatinosus, the solution was placed into an electrically heated water bath at 37℃ for 1 h, and then centrifuged at 8000 r/min for 10 min, and measured at 536 nm. 536 nm wavelength to determine the absorbance control group with an equal volume of ddHzO instead of Streptococcus pyogenes bacterial fluid, and blank group with an equal volume of ddH20 instead of hydrogen oxide and Streptococcus pyogenes bacterial fluid. Ascorbic acid (Vc) was used as positive control.

Clearance = (sample-control)/(positive-control)

2.7. Determination of total antioxidant capacity:

In the determination of total antioxidant capacity, 28 mM of sodium phosphate, 4 mM of ammonium molybdate tetrahydrate and 0.6 M of concentrated sulfuric acid were used. 3 mL of ammonium molybdate system (28 mM sodium phosphate, 4 mM ammonium molybdate tetrahydrate, and 0.6 M concentrated sulfuric acid were individually formulated, and then 1 mL of each was taken and mixed well for use) and 0.3 mL of Streptococcus pyogenes bacterial fluid were mixed, and the reaction was carried out in a 95 thermostatic water bath for 90 min, and then, after it was cooled down to room temperature, centrifugation was carried out at 8,000 r/min for 10 min, and then absorbance was measured at 695 wavelength, with ascorbic acid (Vc) as a positive control, and compare the size of the total finding ability according to its absorbance value.

2.8. Mannitol determination

For mannitol determination, acetylacetone reagent and potassium permanganate solution were used. The preparation method of acetylacetone reagent is to weigh 150 g of ammonium acetate, add appropriate amount of distilled water to dissolve, and then add 3 mL of glacial acetic acid and 2 mL of acetylacetone, transfer to 1,000 mL measuring flask, add distilled water for volume, and shake well. Store the prepared reagent in a brown reagent bottle and keep it in the refrigerator after 12h at room temperature (the color of the reagent will be darkened in the first 12h, so it should be placed in a stable condition before use), and the prepared reagent can be used in 6~8 weeks. Potassium permanganate solution was prepared by weighing 3.2 g of potassium permanganate, dissolving it with 0.12 mol-L1 HCl and condensing it to 1,000 mL to obtain potassium permanganate solution with a concentration of 0.015

mol-L1, mixing it well, and then setting it aside. Then, the standard solution was prepared by weighing 10 mg of dried mannitol standard to constant weight, placing it in a 10 mL measuring flask, adding water and shaking it well to obtain the standard stock solution with a concentration of 1 mg-mL1. Precisely absorb 4 mL of Mannitol Standard Stock Solution into a 100 mL measuring flask, add water and shake well, the concentration of 40 μg-mL1 standard working solution was obtained.

Take mannitol standard solution, test solution in 25 mL flask, add 1 mL of 0.015 mol-L1 potassium periodate solution, room temperature reaction for 10 min, add 4 mL of newly prepared acetylacetone reagent, 50 ℃ water bath heating for 15 min, cooling, and distilled water to 25 mL, shaking well. The spectra were scanned by UV-visible spectrophotometer, and the results showed that both the standard solution and the test solution had the maximum absorption at 412 nm.

2.9. Sulfate of fucoidan

Take 0.25g of gelatin and dissolve it in 50ml of water at 60-70℃ to make gelatin solution, and leave it at 4℃ overnight. Then take 0.25g BaCl2 (try to saturate the excess) dissolved in 50ml gelatin solution to make BaCl2-gelatin solution, stored at 4℃. Sulfate standard is 400μg/ml K2SO4 aqueous solution, then make the standard curve, respectively, 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 ml of K2SO4 standard solution, add water to 2.5 ml, and then add 2.5 ml of BaCl2 gelatin solution, shaking well, colorimetric at 360nm.

CHAPTER 3 RESULTS

3.1.Determination of polysaccharide content

In the determination of polysaccharide content, it was taken that all the samples had maximum absorption at 490 nm using UV-Vis spectrophotometer, and the maximum absorption of sample 1 was 0.263, and the polysaccharide content was 2.218 mg/mL; the maximum absorption of sample 2 was 0.244, and the polysaccharide content was 2.315 mg/mL; the maximum absorption of sample 3 was 0.557, and the polysaccharide content was 6.040 mg/mL; Sample 4 had a maximum absorbance of 0.395 and a polysaccharide content of 3.799 mg/mL. (See Figures 2-1 and 2-2 for details.)

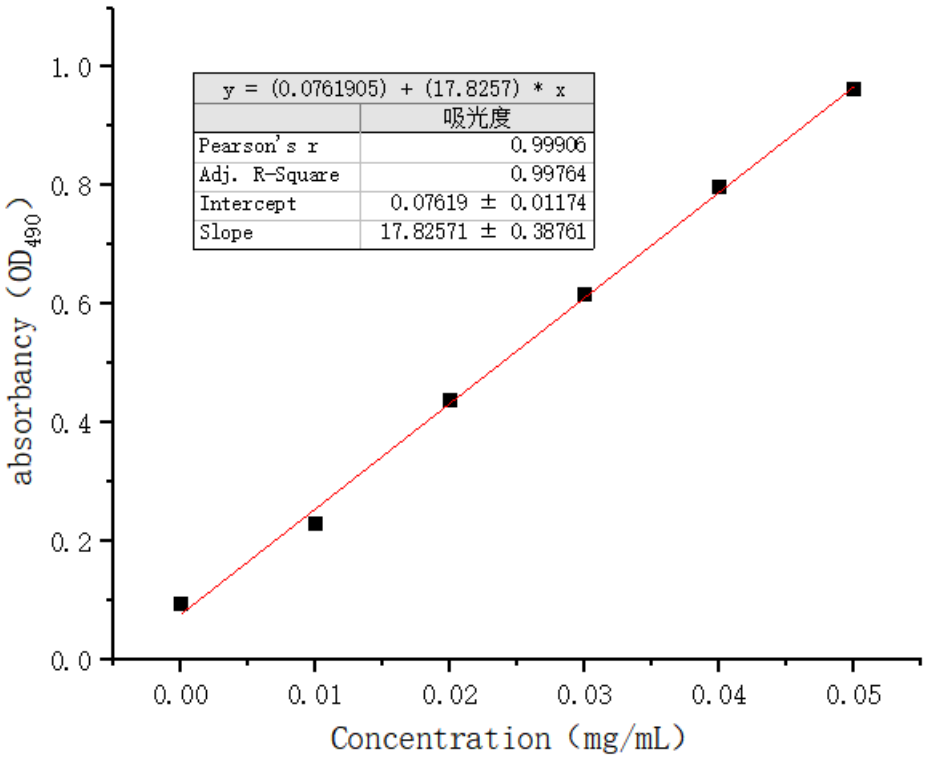

Figure 2-1 Glucose Standard Curve

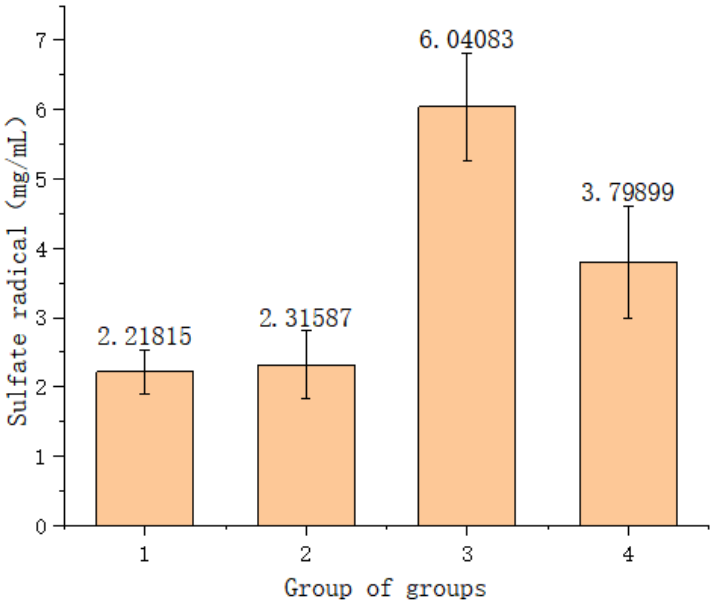

Figure 2-2 Polysaccharide content in seaweed fermentation broth

3.2. Determination of DPPH

In the determination of free radical scavenging capacity of DPPH, in samples 1 and 2 with the addition of lactobacilli, the free radical scavenging rate of DPPH in both of them was 83.19% and 82.30%, respectively. In samples 3 and 4 without the addition of lactobacilli, the free radical scavenging rate of DPPH was higher compared to samples 1 and 2, which were 86.28% and 85.84%, respectively. (Details can be seen in Table 2-1)

Groups	DPPH free radical scavenging
Experiment （1）	83.19%
Experiment （2）	82.30%
Blank （3）	86.28%
Blank （4）	85.84%
VC	97.79%

Table 2-1 DPPH free radical scavenging rate

3.3. Determination of superoxide anion radical scavenging ability

In the experiment of superoxide anion radical scavenging capacity, in the samples 1 and 2 with the addition of lactic acid bacteria, the superoxide anion radical scavenging rate was 37.82% and 37.54% for samples 1 and 2, respectively. In contrast, in Sample 3 and Sample 4, where no lactic acid bacteria were added, the superoxide anion radical scavenging rates of Sample 3 and Sample 4 were 27.48% and 28.73%, respectively, which were lower than the superoxide anion radical scavenging rates of Sample 1 and Sample 2, respectively, where lactic acid bacteria were added. (Details can be found in Table 2-2)

Groups	Superoxide anion radical scavenging
Experiment（1）	37.82%
Experiment（2）	37.54%
Blank（3）	27.48%
Blank（4）	28.73%
VC	95.23%

Table 2-2 Superoxide anion radical scavenging rate

3.4. Determination of hydroxyl radical scavenging ability

In the determination of hydroxyl radical scavenging capacity, in samples 1 and 3, the scavenging rate of hydroxyl radicals was 75.68% in sample 1 with the addition of lactobacilli and 54.90% in sample 3 without the addition of lactobacilli. In samples 2 and 4, the scavenging rate of hydroxyl radicals in sample 2 with the addition of lactobacilli was 83.33%, and the scavenging rate of hydroxyl radicals in sample 4 without the addition of lactobacilli was 66.37%.

By comparing the data of control and blank groups, the scavenging rate of hydroxyl radicals in the samples without the addition of lactobacilli was lower than the scavenging rate of free radicals in the samples with the addition of lactobacilli. (Details can be seen in Table 2-3)

Groups	Hydroxyl radical scavenging rate
Experiment（1）	75.68%
Experiment（2）	83.33%
Blank（3）	54.90%
Blank（4）	66.37%
VC	95.23%

Table 2-3 Hydroxyl radical scavenging rate by group

3.5. Determination of total antioxidant capacity

In the experiment to determine the total antioxidant capacity of each group, the experiment was mainly used to determine the strength of antioxidant capacity by absorbance. The absorbance of Sample 1 and Sample 2 were 0.478 and 0.488, respectively; the absorbance of Sample 3 and Sample 4 were 0.556 and 0.517, respectively. (Details can be found in Tables 2-4.)

Groups	Absorbancy（DO695nm）
Experiment（1）	0.478
Experiment（2）	0.488

Blank（3）	0.556
Blank（4）	0.517

Table 2-4 Total antioxidant capacity by group

3.6. Determination of sodium fumarate

The concentration of sodium fucoidan and the proportion of sodium fucoidan to the raw material in each sample were also determined in this experiment, and the concentration of sodium fucoidan and the proportion of sodium fucoidan to the raw material did not differ much among the four samples. The maximum absorbance of sample 1 was 2.017, the concentration was 0.433 mg/mL, and the ratio of sodium alginate to raw material in sample 1 was 1.41%; the maximum absorbance of sample 2 was 1.922, the concentration was 0.419 mg/mL, and the ratio of sodium fucoidan to raw material in sample 2 was 1.32%; the maximum absorbance of sample 3 was 1.967, the concentration was 0.421 mg/mL, and the ratio of sodium fucoidan accounted for 1.36% of the raw material ratio; the maximum absorbance of Sample 4 was 1.938, the concentration in Sample 4 was 0.414 mg/m, and the sodium fucoidan in Sample 4 accounted for 1.33% of the raw material ratio. (See Table 2-5 and Figure 2-3.)

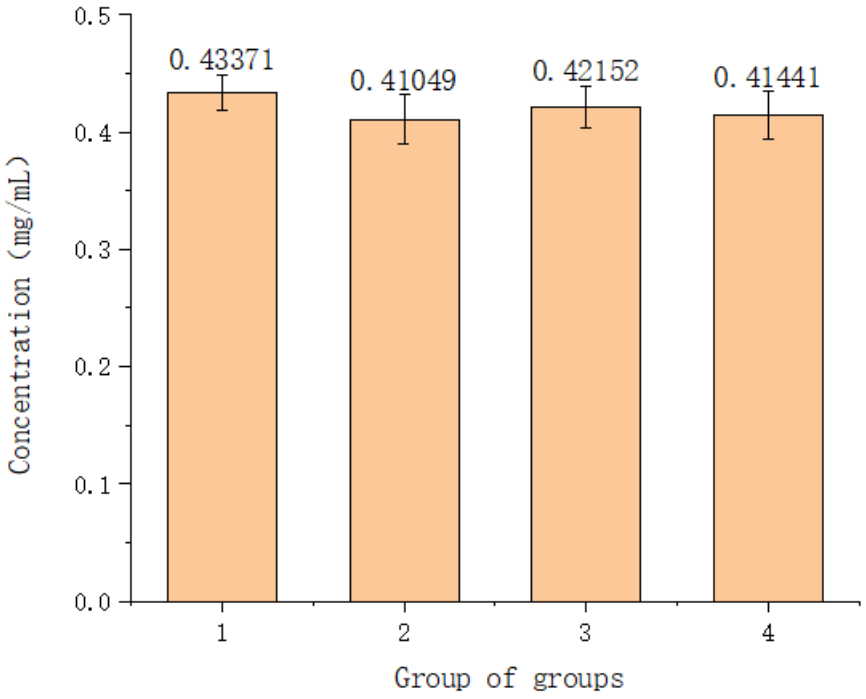

Figure 2-3 Alginic acid as a proportion of raw materials

Groups	Concentration (mg/mL)
Experiment (1)	0.4233
Experiment (2)	0.3957
Blank (3)	0.4088
Blank (4)	0.4003

Table 2-5 Alginate Concentration by Groups

3.7. Determination of mannitol

In this experiment for the determination of mannitol, a UV-Vis spectrophotometer was used for the determination of mannitol concentration, and the ratio of mannitol content in the fermentation broth to the dry weight of the seaweed raw material was also determined. The ratio of mannitol content in the fermentation broth to the dry weight of the raw seaweed in samples 1-4 were

0.49%, 0.54%, 0.43% and 0.45%, respectively. (Details can be seen in Table 2-4.) In the determination of mannitol concentration by UV-Vis spectrophotometer, all the samples had the maximum absorption at 413 nm, the maximum absorption of sample 1 was 0.257, and the concentration of mannitol was 0.146 mg/ml; the maximum absorption of sample 2 was 0.296, and the concentration of mannitol was 0.162 mg/ml; the maximum absorption of sample 3 was 0.219, and the concentration of mannitol was 0.130 mg/ml; the maximum absorption of sample 3 was 0.219, and the concentration of mannitol was 0.130 mg/ml. mannitol concentration of 0.130mg/ml; Sample 4 had a maximum absorbance of 0.229 and a mannitol concentration of 0.135mg/ml. (For more details, see Table 2-7, Figure 2-4, and Figure 2-5.)

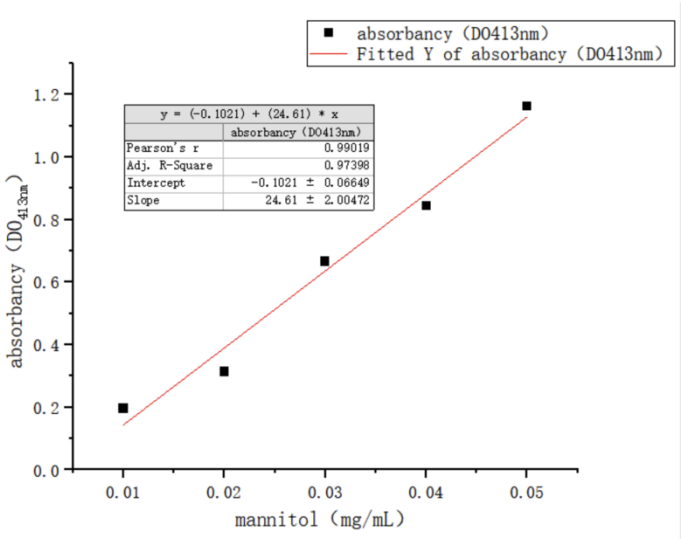

Figure 2-5 Mannitol Standard Solution Curve

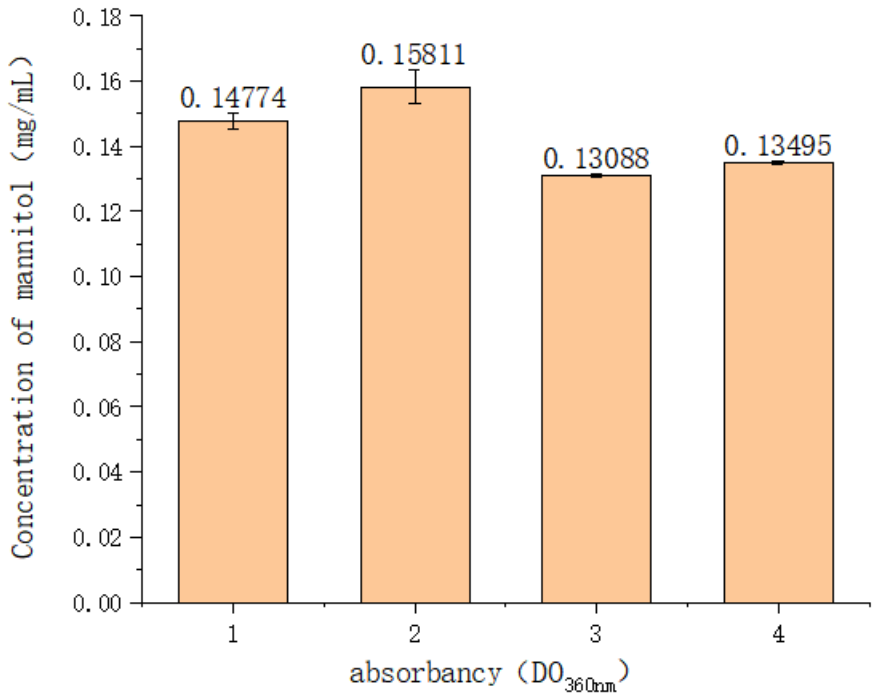

Figure 2-4 Mannitol Concentration by Groups

Groups	Ratio of mannitol content of fermentation broth to seaweed raw material
Experiment（1）	0.49%
Experiment（2）	0.54%
Blank（3）	0.43%
Blank（4）	0.45%

Table 2-6 Mannitol content of each group as a ratio of seaweed raw materia

3.8. Determination of Fucoidan Sulfates

In the determination of fucoidan sulfate, similar to the determination of mannitol and polysaccharides, the same not only used UV-visible spectrophotometer for the determination of the concentration of fucoidan sulfate,

but also determined the ratio of fucoidan sulfate content in the fermentation broth to the dry weight of the raw material of the seaweeds in the also early. The ratio of fucoidan sulphate content in fermentation broth to dry weight of seaweed raw material in samples 1-4 were 0.86%, 0.81%, 0.48% and 0.54% respectively. Sample 2 had a maximum absorbance of 0.561 and a fucoidan sulfate concentration of 0.243 mg/mL; Sample 3 had a maximum absorbance of 0.321 and a fucoidan sulfate concentration of 0.155 mg/mL; and Sample 4 had a maximum absorbance of 0.363 and a fucoidan sulfate concentration of 0.159 mg/mL. (For more details, see Table 2-7, Figure 2-6, and Figure 2-7.)

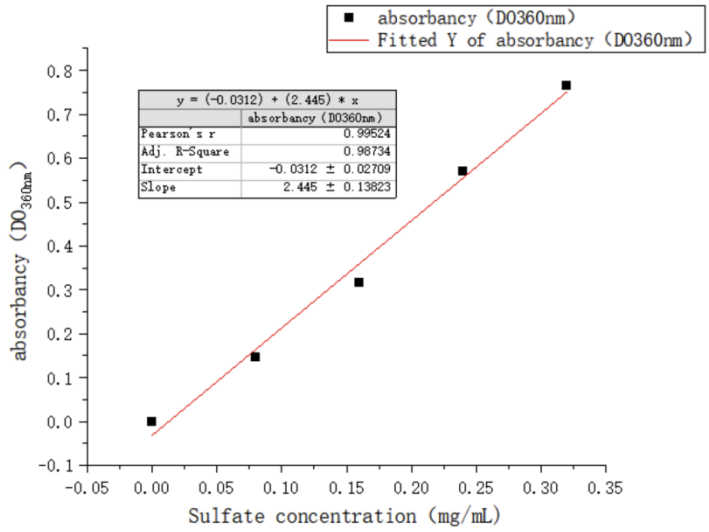

Figure 2-7 Curve of standard solution of fucoidan sulfate

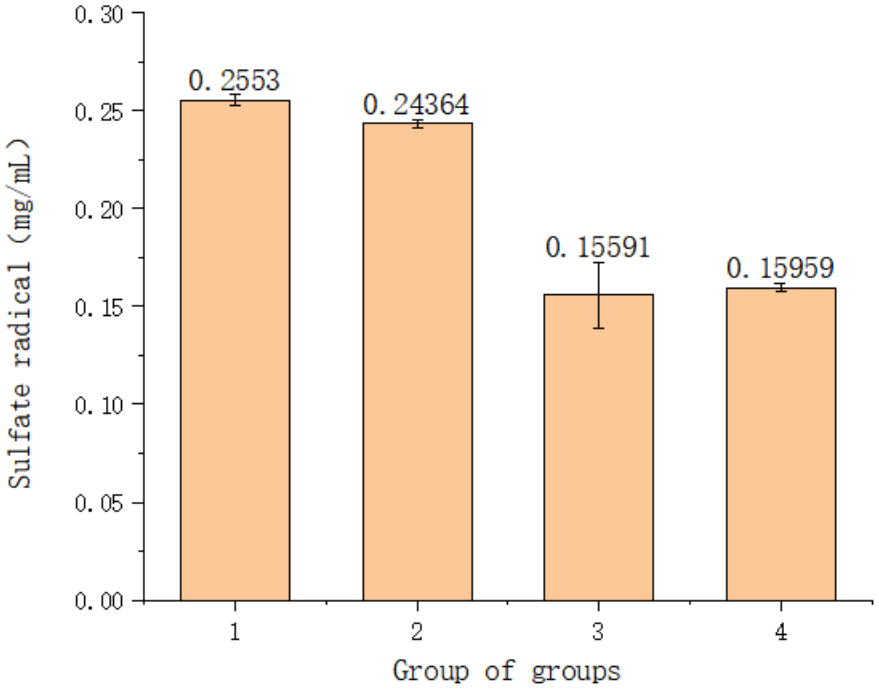

Figure 2-6 Fucoidan Sulfate Concentrations by Groups

Groups	Fucoidan sulfate content of fermentation broth as a ratio of seaweed raw material
Experiment（1）	0.86%
Experiment（2）	0.81%
Blank（3）	0.48%
Blank（4）	0.54%

Table 2-7 Fucoidan sulfate content of each group as a ratio of seaweed raw material

CONCLUSION

The DPPH content mainly determined the antioxidant activity of the active components in brown algae and its probiotic fermentation broth, while the determination of polysaccharide content proved that polysaccharides are one of the active components in brown algae and brown algae probiotic fermentation broth. According to the literature, polysaccharides in brown algae and their probiotic fermentation broth exhibit the antioxidant activity function of polysaccharides by neutralizing DPPH free radicals. From the content of DPPH in samples 1 and 3, it is known that the content of DPPH increased in both samples 1 and 3 at 24-48 hours. Whereas, when lactic acid bacteria were added to Sample 1 at the 48th hour, at the 72nd hour, which was 24 hours after inoculation with lactic acid bacteria, the content of DPPH decreased by about 8% compared to the 48th hour. When Sample 1 was compared to Sample 3 without the addition of lactobacilli, the content of DPPH in Sample 3 only decreased by only 1% between hours 48 and 72. This is because the antioxidant capacity can be measured by the amount of DPPH scavenged, i.e., if the DPPH decreases more, it means that the antioxidant capacity is increased and vice versa. In this experiment, the amount of DPPH radicals in Sample 1 decreased more after the addition of Lactobacillus than in Sample 3, which suggests that the addition of Lactobacillus enhanced the scavenging antioxidant activity. The antioxidant capacity can be judged by the scavenging rate of DPPH radicals, but also by the scavenging rate of superoxide anion radicals, hydroxyl radical scavenging rate, and total antioxidant capacity measurement.

The scavenging rate of superoxide anion radical and hydroxyl radical in fermentation broth can also be used to determine the antioxidant capacity of superoxide anion radical and hydroxyl radical, which are highly oxidizing and active free radicals. We can find that the superoxide anion radical scavenging

rate of both samples in samples 1 and 2 with the addition of lactobacilli increased significantly after the addition of lactobacilli at the 48th hour, so the trend of superoxide anion radical scavenging rate of samples 1 and 2 was with the addition of lactobacilli. The superoxide anion radical scavenging was the first to increase and then decrease, which also indicates that the addition of lactobacilli enhanced the antioxidant activity and capacity of the fermentation broth. Then for the samples 3 and 4 in which no lactic acid bacteria were added, the superoxide anion radical scavenging continued to decrease for both samples. In the experiment for determination of hydroxyl radicals, sample 1 with the addition of lactobacilli had a higher hydroxyl radical scavenging rate compared to 3 without the addition of lactobacilli. Similarly in samples 2 and 4, sample 4 without the addition of lactobacilli had a lower hydroxyl radical scavenging rate than sample 2. This suggests that the addition of lactobacilli enhances the scavenging of hydroxyl radicals and thus exhibits enhanced antioxidant activity. The total antioxidant capacity was determined mainly by the absorbance of the sample fermentation broth at 695 nm. The smaller the absorbance, the higher the antioxidant capacity. In Sample 1 and Sample 3, the absorbance of Sample 1 was smaller than the absorbance of Sample 3 and lactic acid bacteria were added to Sample 1 while no lactic acid bacteria were added to Sample 3. In Sample 2 and Sample 4, the absorbance of Sample 2 was less than the absorbance of Sample 4 and Lactobacillus was added to Sample 2 but not to Sample 4. This result proved that the addition of lactic acid bacteria increased the total antioxidant capacity of the fermentation broth. These several antioxidant capacity experiments lead to the conclusion that the addition of lactobacilli increases the antioxidant activity of the fermentation broth.

Theoretically, the concentration of polysaccharides should have a negative correlation with DPPH content but a positive correlation with DPPH radical scavenging rate, i.e., if the concentration of polysaccharides is higher, more polysaccharides will be able to neutralize the DPPH radicals, so the lower the

DPPH radical content should be but the higher the DPPH radical scavenging rate should be. However, in the control group of samples 1 and 3, although the addition of lactobacilli proved to enhance the antioxidant activity, the polysaccharide content was significantly reduced by the addition of lactobacilli. The reasons for this result are complex and still unknown, but one of the conjectured mechanisms that could lead to this result is that pH affects the polysaccharide activity. At hour 72, the pH of Sample 1 with lactic acid bacteria was greater than the pH of Sample 2 and both were acidic. It is possible that the decrease in polysaccharide in Sample 1 was due to the fact that the acid concentration in Sample 1 was not strong enough, resulting in a decrease in polysaccharide concentration. Even though the concentration of polysaccharides was reduced, there were enough polysaccharides to neutralize the DPPH radicals. In addition, because there are many active components other than polysaccharides in brown algae and their fermentation broth that can have antioxidant activity capacity, such as polyphenols. It is also due to the fact that the acid concentration is not strong, which results in no inactivation of the other active components, thus allowing the polysaccharides to synergize with other actives, such as polyphenols, to exhibit antioxidant activity capacity, which results in a decrease in the concentration of DPPH radicals.

In the determination of the polysaccharide content for samples 2 and 4, the polysaccharide content of sample 2 with the addition of lactobacilli was greater than the polysaccharide content in sample 4 without the addition of lactobacilli. From the results of DPPH content in samples 2 and 4, it can be seen that the DPPH content of both samples 2 and 4 was decreasing at 24-48 hours, while when lactobacilli were added, at the 72nd hour, the DPPH content of both samples 2 and 4 increased, but the increase in DPPH content in sample 2 was more than that in sample 4. This suggests that although the polysaccharides rose after the addition of lactic acid bacteria, the rising polysaccharides did not exhibit antioxidant activity. This is because if the polysaccharides were

exhibiting antioxidant activity, the DPPH content in Sample 2 should have decreased or risen less than Sample 4, not risen more than Sample 4. In this control group, pH decreased significantly after the access of lactobacilli, but it was also because of the increased acidity that the polysaccharide concentration in Sample 2 was higher than that in Sample 4. However, despite this, at hour 72, the DPPH content of sample 2 was greater than the DPPH content of sample 4. This is most likely due to the fact that the acid concentration was too high, which led to the inactivation of other active ingredients, and thus the polysaccharides could not have the antioxidant synergistic effect with the other active ingredients, which led to the antioxidant activity of sample 2 was not as good as that of sample 4. From the above results, we can learn that the proper concentration of acidity and alkalinity plays a very important role for the activity of the active ingredients of the brown alga probiotic fermentation broth. In the future research and analysis, it should be devoted to find the optimal point of pH, at which the polysaccharide and other active substances can have the strongest activity, so that the polysaccharide can have synergistic effect with other active substances to produce the maximum antioxidant activity. Through the determination of sodium alginate, it was found that the sodium alginate content of the four samples was nearly the same, which can be speculated that it may be because Bacillus consumed sodium alginate, while lactobacillus did not directly decompose sodium alginate, so the results of the four samples were about the same.

Through the determination of polysaccharides, sodium alginate and the antioxidant activity of polysaccharides, we can detect the active ingredients in the fermentation broth of brown seaweed and have a good characterization of the content of active ingredients in different fermentation methods.

LIST OF REFERENCES

1. Liu Ning, Pan Ren, Yang Tingting, et al. Research progress on the application of bioactive substances in brown algae in functional foods [J]. Chinese Seasoning Journal, 2021, 46 (8): 5. DOI: 10.3969/j.issn.1000-9973.2021-08.041
2. Li Zhijun, Jia Xiaowei, Lv Zhaolong. Research status of health active ingredients in brown algae [J]. Food and Drug (8): 5-8 [2024-03-23] DOI: 10.3969/j.issn.1672-979X.2003.08.02
3. Xiao Lei, CHEN Gang, et al. Research progress on the application of brown algae active ingredients in cosmetics [C]//The First International Symposium on seaweed active substance science and technology; Qingdao Mingyue Seaweed Biotechnology Center, China, 2016
4. Kusaykin M, Bakunina I, Sova V, et al. Structure, biological activity, and enzymatic transformation of fucoidians from the brown saweeds [J] Biotechnology Journal: Healthcare Nutrition Technology, 2008, 3 (7): 904-915
5. Xu Yaru. Study on antioxidant activity of brown algae polyphenols [D]. Ningbo University, 2014. DOI: 10.76666/d. D604448
6. Hu Shunqiang, Zheng Enping, Zhao Tingxue, et al. Progress in the preparation and stability of active ingredients from brown algae [J]. Zhejiang Agricultural Science, 2023, 64 (8): 2018-2027
7. Chakraborty K, Maneesh A, Makkar F. Antioxidant activity of brown saweeds [J] Journal of Aquatic Food Product Technology, 2017, 26 (4): 406-419
8. Guo Jingjing, Song Yuefan, He Yunhai, et al. Research progress on antihypertensive active substances in brown algae and their extraction processes [J]. Food Industry Technology, 2018, 39 (19): 8. DOI: 10.13386/j.issn1002-0306.2018.19.056

9. Chkhikvishvili, I. D., & Ramazanov, Z. M. (2000). Phenolic substances of brown algae and their antioxidant activity. Applied Biochemistry and Microbiology, 36, 289-291.
10. Dizdaroglu M, Jaruga P. Mechanisms of free radial induced damage to DNA [J] Free radial research, 2012, 46 (4): 382-419
11. Li, Y. X., Wijesekara, I., Li, Y., & Kim, S. K. (2011). Phlorotannins as bioactive agents from brown algae. Process biochemistry, 46(12), 2219-2224.
12. Sreekumar, K., & Bindhu, B. (2020). Alginic acid: A potential biopolymer from brown algae. Mater. Int, 2, 433-438.
13. Zhou Linzhu, Yang Xiangliang, Zhou Jingyan, et al. Research progress on antioxidant effects of polysaccharides [J]. Chinese Journal of Biochemical Medicine, 2002, 23 (4): 3. DOI: 10.3969/j.issn.1005-1678.2002.04.028
14. Wijesinghe W, Jeon Y J. Biological activities and potential Cosmetic applications of bioactive components from brown seeds: a review [J] Phytochemistry Reviews, 2011, 10:431-443
15. Fang Guozhong, Wu Keqiang, Wang Yuke, et al. A brown algae fermentation composition and its preparation method and application: CN202110238469.9 [P] CN202110238469.9 [2024-05-06]
16. Wang Mingpeng, Wang Xuejiang, Liu Zhengyi, et al. A sugar loving Bacillus subtilis for degrading alginic acid and its application method: 201610382846 [P] [2024-05-06]
17. Shahidi, F., & Rahman, M. J. (2018). Bioactives in seaweeds, algae, and fungi and their role in health promotion. Journal of Food Bioactives, 2, 58-81.
18. Qu, G., Liu, X., Wang, D., Yuan, Y. I., & Han, L. (2014). Isolation and characterization of fucoidans from five brown algae and evaluation of their antioxidant activity. Journal of Ocean University of China, 13, 851-856.

19. Xu Chao, Wang Ting, Zhao Lin, et al. A method for preparing seaweed fermentation broth through probiotic fermentation and its application in cosmetics: 201810638230 [P] [2024-04-11]
20. Dong Qun, Zheng Liyi. Study on the improved phenol sulfuric acid method for determining the content of polysaccharides and oligosaccharides [J]. Chinese Journal of Pharmacy, 1996, 031 (009): 550-553. DOI: CNKI: SUN: ZGYX. 0.1996-09-013