Bioactive Glyceroglycolipids from Marine Macroalgae: Isolation, Purification, and Potential Applications

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Abstract

Macroalgae are valuable marine resources, rich in bioactive glyceroglycolipids with antioxidant and anti-inflammatory properties. This study focused on optimizing extraction processes for glyceroglycolipids from *Palmaria palmata*, *Chorda filum* and *Enteromorpha clathrate*. Using single-factor and response surface experiments, optimal extraction conditions were established, yielding 69.96 mg g^{-1}, 65.42 mg g^{-1}, and 86.81 mg g^{-1} glyceroglycolipids extracts, respectively. Then the extracts were analyzed for antioxidant and hygroscopic-moisturizing activities. The glyceroglycolipid extracts demonstrated significant effects in scavenging DPPH, hydroxyl, and ABTS radicals with rising concentrations, though their efficacy was lower than that of Vitamin C. The extracts showed hygroscopic and moisturizing properties superior to hyaluronic acid but inferior to glycerol under different humidity conditions. Further purification through liquid-liquid extraction, thin-layer chromatography, and silica gel column chromatography led to the isolation of specific glyceroglycolipids, including sulfoquinovosyl diacylglycerol, digalactosyl diacylglycerol, and monogalactosyl diacylglycerol. Additionally, *Channa argus* fillets treated with glyceroglycolipids exhibited improved physicochemical indices compared to the control, effectively delaying spoilage by inhibiting growth of *S. marcescens.* These findings highlight the potential applications of marine macroalgae derived glyceroglycolipids in various fields, especially in meat preservation.

1 Introduction

Macroalgae are an important group of multicellular algae in marine ecosystems. They typically grow attached to rocks or the seabed, providing shelter and food sources for marine organisms (Alstyne et al. 2007). Macroalgae exhibit diverse morphologies and structures and are primarily classified into red algae (Rhodophyta), green algae (Chlorophyta), and brown algae (Phaeophyta) (Wan et al. 2018). Due to their rich variety and diverse structures, macroalgae contain different nutritional elements and bioactive substances (Verland et al. 2019). In recent years, many scholars have noted that some macroalgae possess medicinal value in addition to their nutritional value (Leandro et al. 2019; Vicente et al. 2021; Guo et al. 2022a). The diverse macroalgae in marine ecosystems are undoubtedly a reservoir of various natural compounds, among which glyceroglycolipids are included. Some of the bioactivity of macroalgae may be attributed to the presence of glyceroglycolipids. Various macroalgae have been detected to contain glyceroglycolipids, which is closely related to their biodiversity (Sun et al. 2021).

Glyceroglycolipids are natural products composed of lipids and sugars, exhibiting various bioactivities and typically found in cell membranes and different organisms (Iván et al. 2018). Glyceroglycolipids can be divided into three major categories: glyceroglycolipids, sphingolipids, and glycolipids with atypical lipid components. The distinction between glyceroglycolipids and other glycolipids lies in their glycerol-based backbone, consisting of glycerol, sugar molecules, and fatty acids. SQDG (sulfoquinovosyl diacylglycerol), DGDG (digalactosyl diacylglycerol), and MGDG (monogalactosyl diacylglycerol) (Al-Fadhli et al. 2006; Xu et al. 2020) are common glyceroglycolipids in macroalgae, differing mainly in the fatty acid chains and the sugars attached at the sn-3 position (Hoyo et al. 2016). Macroalgae are important

sources of various glyceroglycolipids. In recent years, numerous researchers have successfully isolated glyceroglycolipids from different macroalgae, such as *Ahnfeltia tobuchiensis* (Sanina et al. 2012), *Chondria armata* (Al-Fadhl et al. 2006), *Gracilaria verrucosa* (Khotimchenko 2005), and other rhodophytes species (Song et al. 2015; Guo et al. 2017); *Fucus vesiculosus* (Deal et al. 2003), *Laminaria gurjanovae* (Shevchenko et al. 2007), *Sargassum wightii* (Arunkumar et al. 2005), and other phaeophytes species (Sanina et al. 2000; Kim et al. 2007; Nunes et al. 2020); *Codium tomentosum* (Rey et al. 2020), *Ulva rigida* (Lopes et al. 2019), *Ulva pertusa* (Fang et al. 2017), *Tydemania expeditionis* (Jiang et al. 2008), and other chlorophytes species (Williams et al. 2007; Kostetsky et al. 2018; Sun et al. 2019). Mancini (1998) analyzed the polar metabolites of *Caulerpa taxifolia* and found glyceroglycolipids among them. Thanh (2021) used thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to identify complex lipids in green algae, revealing the presence of multiple glyceroglycolipids. It is reported that the saturation and chain length of fatty acids influenced the bioactivity of glyceroglycolipids (Hoyo et al. 2016). In recent years, various bioactivities of glyceroglycolipids in macroalgae, such as anti-algal (Slattery et al. 2014), anti-oxidant (Nova et al. 2023), antiviral (Plouguerné et al. 2013), anti-inflammatory (Guo et al. 2022b) and anti-bacterial (Treyvaud et al. 2011) properties, have attracted significant interest from researchers. The rich bioactivity of glyceroglycolipids endows them with great potential for applications in the medical, food, and cosmetic industries.

P. palmata is a well-known economic macroalgae primarily distributed along the eastern coasts of the Atlantic and Pacific Oceans (Le et al. 2023). Its thick, rectangular fronds radiate from a central point, resembling a hand or palm, and it is deep red in color. *P. palmata* is rich in nutritional value and contains active substances such as polysaccharides, phytosterols, and fatty acids (Pierrick et al. 2023). Banskota (2014) discovered that the methanol and chloroform extracts from *P. palmata*, when dissolved in ethyl acetate, significantly inhibited NO production in mouse cells. These polar lipids, identified as including SQDG and several galactosyl glyceroglycolipids, suggested that *P. palmata* contained complex glycolipids with anti-inflammatory activity, potentially serving as natural drugs for inflammation treatment in the future. Lopes (2019) found that *P. palmata* contained many polar lipids, including DGDG and MGDG, and these extracts exhibited certain antioxidant activities. *C. filum* is one of brown algae species in the family Laminariaceae, resembling long ropes and mainly distributed in temperate and cold-temperate regions of the Northern Hemisphere (Chizhov et al. 1999). Gerasimenko (2016) discovered that extracts from *C. filum* collected off the coast of Japan also contained glyceroglycolipids. Studies have shown that SQDG and other glyceroglycolipids in these extracts possessed antioxidant properties, protecting organisms from oxidative damage. *E. clathrata* is a common aquatic green alga along the eastern coastal regions of China. Yu (2022) found that polysaccharides from *E. clathrata* could reduce the weight and incidence of colonic bleeding in diseased mice, showing anti-inflammatory and gut-protective effects. These three types of macroalgae are all edible economic algae, abundant in temperate and cold-temperate marine areas, making them easily accessible raw materials. Additionally, as renewable resources, macroalgae are environmentally friendly and ideal for experimental materials. Preliminary investigations by our team have identified glyceroglycolipids in *P. palmata* and *C. filum*, making them ideal materials for glyceroglycolipid extraction and preparation. Unfortunately, due to the

unique and similar structures of glyceroglycolipids, they are difficult to be isolated and purified, and there is limited research on the extraction and purification processes for glyceroglycolipids from macroalgae. This has resulted in high prices and somewhat compromised quality of glyceroglycolipids in the international market. Therefore, it is urgent to develop efficient methods for isolating and purifying glyceroglycolipids from macroalgae. In recent years, glyceroglycolipids have been recognized as important natural active substances in macroalgae with significant application potential. Studies focused on glyceroglycolipids and their bioactivities can not only enhance the value of macroalgae but also provide technological support for future developments in the medical, food, and cosmetic industries.

Although researchers began exploring glyceroglycolipids in marine macroalgae as early as the last century (Pohl et al. 1979), the study of these compounds remains insufficient due to the vast variety and abundance of macroalgae. Most glyceroglycolipids in marine macroalgae have not yet been discovered (Guo et al. 2022b). Structural differences, such as variations in fatty acid chain lengths and diverse glycosyl structures, may alter the biosynthesis and metabolic pathways of glyceroglycolipids, ultimately affecting their bioactivity. Glyceroglycolipids can also be obtained through artificial synthesis (Pagano et al. 2016). Despite the controllable purity and stability advantages of artificial synthesis, it faces challenges such as high costs, environmental pollution, high technical requirements, and low bioactivity. Additionally, the similarity in the structures of glyceroglycolipids makes their separation and purification difficult. Therefore, from the perspectives of environmental friendliness, better bioactivity, and the research value of glyceroglycolipids, naturally extracted glyceroglycolipids are more advantageous. It is necessary to extract, separate, and conduct related research on glyceroglycolipids from more types of macroalgae. *P. palmata*, *C. filum*, and *E. clathrata* are edible economic macroalgae, but research on the extraction and activity of glyceroglycolipids from these three macroalgae is lacking. Therefore, this study aims to establish extraction processes for glyceroglycolipids and evaluate the antioxidant, hygroscopic, moisturizing, and preservative activities of the extracts. This will help explore the potential for developing natural preservatives from macroalgae glyceroglycolipids and expand the application range of macroalgae.

2 Materials and methods

2.1 Macroalgae and reagents

The dried samples of *P. palmata*, *C. filum*, and *E. clathrata* were purchased from Jiangsu Blue Ocean Marine Biotechnology Co., Ltd. After being cleaned and oven-dried, the dried seaweed samples were pulverized using a grinder and sieved through a 40-mesh screen for later use. The glycolipid standards, SQDG, DGDG, and MGDG, were obtained from Avant Polar Lipids (USA). *S. marcescens* was preserved in our laboratory and cultured in LB medium at 37°C. The snakehead fish (*Channa argus*) were purchased from the seafood market in Lianyungang, Jiangsu Province.

2.2 Extraction, iaolation and purification

5 g of dried macroalgae powder was placed in an Erlenmeyer flask and extracted under ultrasonic assistance according to the factors and levels set in Table S1. After the extraction, the extract was poured out and the same amount of methanol solution was added to the residue for further extraction. The combined extracts were then centrifuged, and the supernatant was collected. The precipitate was washed with pure water and combined with the previously collected supernatant. Methanol was removed by filtration and rotary evaporation under reduced pressure, resulting in glyceroglycolipids extracts. The yield of the extracts was calculated using the following formula, where m_1 was the mass of the extract (g), and m_2 was the mass of the macroalgae powder (g).

$$\text{Yield}\ (\%) = 100\% \times m_1/m_2 \qquad (1)$$

The liquid-solid ratio (A), extraction temperature (B), methanol volume fraction (C) and extraction time (D) were set as response factors, with glyceroglycolipid content as the response value. A response surface with a 4-factor, 3-level design in Table S2 was used to optimize the extraction conditions of glyceroglycolipids from three types of macroalgae. The glyceroglycolipid content was determined using a colorimetric method. A glucose standard solution with a concentration gradient of 0.1-1.0 mg mL^{-1} was prepared, using pure water as a blank control. In a colorimetric tube, 1 mL of 5% phenol solution and 2 mL of concentrated sulfuric acid were added, mixed, and then incubated in a water bath at 80°C for 20 min. After cooling to room temperature, the absorbance at 490 nm was measured, and a standard curve was plotted. The absorbance at 490 nm of the prepared glyceroglycolipid extract solution (1 mg mL^{-1}) was measured using the same method, and the glyceroglycolipid content was calculated.

The liquid-liquid extraction and separation of glyceroglycolipid extracts from macroalgae were carried out as follows. Five grams of the extract was dissolved in 250 mL of deionized water and thoroughly shaken before being poured into a separatory funnel. The solution was extracted three times with dichloromethane at twice the volume of the aqueous phase. After evaporation under reduced pressure, the dichloromethane fraction was obtained. The remaining aqueous phase was then extracted three times with ethyl acetate to obtain the ethyl acetate fraction after removing dichloromethane. Finally, n-butanol was added, and the extraction was performed as described above to obtain the n-butanol fraction. Each extraction fraction was spotted on a silica gel G plate, with the developing solvent being chloroform: methanol: distilled water (75:25:3, v/v/v). After development, the plate was removed, dried, sprayed with a 50% ethanol-sulfuric acid solution, and heated at 110°C for 10 min for color development, followed by natural cooling. Glyceroglycolipid standards (SQDG, DGDG, MGDG) were used as controls. For NMR analysis, the dried glyceroglycolipid fractions were mixed with an appropriate amount of potassium bromide, ground finely, and pressed into tablets for infrared spectroscopy measurement, with a wavelength range of 400–4000 cm^{-1}. The extracts obtained from the liquid-liquid extraction were dissolved in a small amount of dichloromethane and subjected to silica gel column chromatography (100–200 mesh, 4.0 × 40 cm) for separation. The flow rate was 1.0 mL min^{-1}, and the elution solvents used sequentially were petroleum ether, ethyl acetate, ethyl acetate-methanol solution (1:1, v/v), and methanol. Elution was performed in order of increasing solvent polarity, changing to the next eluent after

twice the column volume. The fractions obtained from the silica gel column chromatography and the glyceroglycolipid standards were spotted on a silica gel G plate using a streak method. The developing solvent was chloroform: methanol: water (75:25:3, v/v/v). The developed plate was visualized using iodine vapor, and the spots corresponding to the target glyceroglycolipids were scraped off to obtain the desired glyceroglycolipids. DPPH (1,1-Diphenyl-2-picrylhydrazyl radical), hydroxyl free radical and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) free radical scavenging tests were performed according to the kit instructions.

2.3 Measurement of hygroscopic and moisturizing activity

0.1 g of glyceroglycolipid extracts, glycerol, and hyaluronic acid was accurately weighed and placed into weighing bottles, immediately covering each bottle with a lid. Then, each weighing bottle was placed into two pre-prepared desiccators (with controlled RH (relative humidity) of 43% and 81%). Open the lids of the weighing bottles and promptly seal the desiccators. The samples were weighed at 4 h, 12 h, 24 h, 36 h, 48 h, and 60 h. The experiments were conducted at room temperature, with three replicates per test group. The hygroscopicity of the samples was calculated using the following formula, where M_1 was the mass of the sample at different times (g) and M_0 was the mass of the sample before being placed in the desiccator (g).

$$\text{Moisture absorption rate } (\%) = 100\% \times (M_1 - M_0)/M_0 \qquad (2)$$

The glyceroglycolipid extracts, glycerol, and hyaluronic acid were prepared into 10% solutions, respectively. The mass of each weighing bottle before and after adding the samples was then recorded. Place the bottles into desiccators, open the lids of the weighing bottles, and seal the desiccators. The samples were weighed at 4 h, 12 h, 24 h, 36 h, 48 h, and 60 h. The experiments were conducted at room temperature, with three replicates per test group. The moisture retention rate of the samples was calculated using the following formula, where M_2 was the initial mass of the sample (g), and M_3 was the mass of the sample at different times (g).

$$\text{Moisture retention rate } (\%) = 1 - (M_2 - M_3)/M_2 \times 100\% \qquad (3)$$

2.4 Antibacterial activity and preservative property of glyceroglycolipids extracts

Glyceroglycolipid extracts was prepared into solutions of different concentrations (4, 6, 8 mg mL^{-1}) and their inhibitory activity against *S. marcescens* was determined. Fresh *C. argus* was processed by removing the head, tail, scales, and viscera, and then rinsed with distilled water to remove residual blood. The fish were then drained and sliced (each slice approximately 35 g). The *C. argus* slices were soaked for 20 minutes in glyceroglycolipid extracts solutions from the three types of macroalgae and their extraction fractions (concentration of 1%). The control group was soaked in distilled water. After treatment, the *C. argus* slices were drained, and excess water was removed with filter paper. The samples were then packaged in PE ziplock bags and stored at 4°C. Physicochemical parameters, including conductivity, were measured every 48 h until the end of the 10-day storage period. To measure conductivity, 2 g of minced fish was placed in a centrifuge tube, and 20 mL of deionized water was

added. After standing for 20 min, the supernatant was filtered, and conductivity was measured and recorded using a conductivity meter. The TBA (thiobarbituric acid) value was determined as follows: 2.5 g of the sample was placed in 10 mL of pure water, and 12.5 mL of 20% trichloroacetic acid solution was added. The mixture was allowed to stand for 40 min, then centrifuged at 8000 rpm for 10 min. The supernatant was filtered and diluted to 25 mL. 5 mL of the supernatant was mixed with 5 mL of TBA solution (0.02 mol L^{-1}) and heated in a boiling water bath for 20 min. After cooling the samples in cold water, the absorbance of the supernatant was measured immediately at 532 nm. The TVB-N (Total Volatile Basic Nitrogen) value and total bacterial count were determined according to Chinese National Standards GB 5009.228–2016 and GB 47892 – 2010, respectively.

2.5 Statistical analysis

All experiments were performed in triplicate. The results were presented as means with standard deviations (mean ± SD, n = 3). Data processing and graphical illustrations were carried out using Origin 2021 and SPSS 14.0. Response surface methodology was designed using Design-Expert version 13.1, which was also employed for statistical analysis and experimental design. To explore the relationships between independent factors and the response variable, the Box–Behnken experimental data were subjected to multiple regression analysis in Design-Expert. A second-order polynomial equation was constructed and assessed based on fit quality indicators, including R^2, adjusted R^2 (R_{adj}^2), and predicted R^2 (R_{pred}^2), ensuring the reliability of the model. Model validity and the significance of regression terms were evaluated through analysis of variance (ANOVA) at a 95% confidence level, followed by Tukey's post hoc tests when appropriate. Statistical significance was established at a P-value threshold of < 0.05. The assumptions of normality and homoscedasticity were verified through residual diagnostics conducted in the Design-Expert software.

3 Results

3.1 Optimization of the extraction process of glyceroglycolipids from macroalgae

P. palmata, *C. filum*, and *E. clathrata* were used as raw materials to explore the optimal extraction process for glyceroglycolipids. The optimization results of glyceroglycolipid extraction from *P. palmata* have already been published. Therefore, this discussion focused primarily on the optimization of extraction processes for glyceroglycolipids from *C. filum* and *E. clathrata*. As shown in Fig. 1a, with the increase of the liquid-solid ratio, the yield and content of glyceroglycolipid extracts initially increased and then gradually decreased. As for *C. filum*, the maximum yield and content of glyceroglycolipid extracts were achieved at a liquid-solid ratio of 25 mL g^{-1}, with yield and content of 13.53% and 55.14 mg g^{-1}, respectively. For *E. clathrata*, the maximum yield and content of glyceroglycolipids extracts were obtained at a liquid-solid ratio of 20 mL g^{-1}, with values of 8.47% and 92.34 mg g^{-1}, respectively. An appropriate liquid-solid ratio facilitated the improvement of glyceroglycolipid yield. When the amount of

extraction solvent reached a certain level, the glyceroglycolipids in macroalgae were almost dissolved, and further increase of the solvent would not improve the dissolution of glyceroglycolipids.

The impact of extraction temperature on the yield and content of glyceroglycolipids from *C. filum* and *E. clathrata* was shown in Fig. 1b. Within a certain range, the extraction yield and content of glyceroglycolipids increased with the rise in extraction temperature. For *C. filum*, the maximum yield and content of glyceroglycolipids were achieved at 55°C, with values of 6.01% and 56.13 mg g^{-1}, respectively. For *E. clathrata*, the maximum yield and content of glyceroglycolipids were obtained at 65°C, with values of 8.34% and 78.43 mg g^{-1}, respectively. Beyond these optimal temperatures, both the yield and content of glyceroglycolipids exhibited a decreasing trend with further increases in extraction temperature. These results indicated that the extraction temperature significantly affected the yield and content of glyceroglycolipids from macroalgae.

Then, we investigated the effect of various methanol volume ratios on the extraction yield and content of glyceroglycolipids from *C. filum* and *E. clathrata*, as shown in Fig. 1c. With the increase in methanol volume ratio, both the yield and content of glyceroglycolipid extracts increased. At 80% methanol volume ratio, the glyceroglycolipid extraction yield and content for *C. filum* and *E. clathrata* reached their maximum values, with the former achieving 8.2% and 56.96 mg g^{-1}, and the latter achieving 9.01% and 88 mg g^{-1}, significantly higher than those obtained at other methanol concentrations.

Additionally, the effects of extraction time on the yield and content of glyceroglycolipids from *C. filum* and *E. clathrate* were explored. As shown in Fig. 1d, the yield and content of glyceroglycolipids generally increased with extraction time, followed by a gradual decline. For *C. filum*, the optimal extraction time was 2.5 h, achieving maximum yields and contents of 5.6% and 52.13 mg g^{-1}, respectively. For *E. clathrata*, the maximum yield and content of glyceroglycolipids were obtained at 2 h, with values of 5.77% and 82.51 mg g^{-1}, respectively. The increase in extraction time facilitated the release of glyceroglycolipids from the macroalgae cells. However, the extraction efficiency of glyceroglycolipids showed a decline beyond the optimal extraction time.

Response surface method is often used to optimize the extraction process of various active substances from marine macroalgae. With liquid-solid ratio (A), extraction temperature (B), methanol volume ratio (C), and extraction time (D) as variables, and glyceroglycolipids content as the response value, a Box-Behnken experiment was designed using Design-Expert 13 software. The specific factors and results were shown in Table S3. A multiple regression fit was performed on the response results, yielding the following quadratic regression equation:

$$Y = 63.46 + 1.19\mathrm{A} + 4.02\mathrm{B} + 1.06\mathrm{C} + 5.49\mathrm{D} - 2.78\mathrm{AB} - 0.078\mathrm{AC} +$$

$$0.88\mathrm{AD} - 1.75\mathrm{BC} + 1.89\mathrm{BD} - 1.20\mathrm{CD} - 6.40A^2 - 6.50B^2 - 7.84C^2 - 9.61D^2 \quad (4)$$

The variance and significance of the regression model were analyzed, and the results were shown in Table S4. The model's P-value was less than 0.0001, indicating high significance. The lack-of-fit term had

a P-value greater than 0.05, suggesting a good model fit. The model's R^2 was 0.9769, R_{adj}^2 was 0.9538, and R_{pred}^2 was 0.8806, indicating a strong correlation between the actual experimental values and the model's predicted values. Response surfaces and contours of the effects of different factors on the glyceroglycolipids content from *C. filum* was shown in Fig. 2. The maximum predicted value of 64.9363 mg g^{-1} could be achieved under the conditions of a liquid-solid ratio of 25.74 mL g^{-1}, extraction temperature of 57.37°C, methanol volume ratio of 80.21%, and extraction time of 2.64 h. Based on practical considerations, these conditions were adjusted to a liquid-solid ratio of 26 mL g^{-1}, extraction temperature of 57°C, methanol volume ratio of 80%, and extraction time of 2.6 h. Under these optimized conditions, the glyceroglycolipids extraction from *C. filum* resulted in a yield of 65.42 mg g^{-1}, which was close to the predicted value, demonstrating the accuracy and reliability of the model.

A response surface experiment with four factors and three levels was designed using Design-Expert 13 software to investigate the effects of liquid-solid ratio (A), extraction temperature (B), methanol volume ratio (C), and extraction time (D) on glyceroglycolipids content. The experimental design and results were shown in Table S5. A multiple regression fit was performed using the glyceroglycolipids content as the response value, resulting in the following quadratic regression equation:

$$Y = 85.88 + 1.35\mathrm{A} + 0.88\mathrm{B} + 1.00\mathrm{C} + 1.51\mathrm{D} + 3.24\mathrm{AB} - 0.20\mathrm{AC} -$$

$$3.44\mathrm{AD} - 0.63\mathrm{BC} - 1.68\mathrm{BD} - 1.71\mathrm{CD} - 4.59A^2 - 2.69B^2 - 3.13C^2 - 3.09D^2 \quad (5)$$

The variance and significance of the regression model were analyzed, with the results presented in Table S6. The model's P-value was less than 0.0001, indicating high significance. The lack-of-fit term had a P-value greater than 0.05, suggesting a good model fit. The model's R^2 was 0.9535, R_{adj}^2 was 0.9071, and R_{pred}^2 was 0.7506, indicating a strong correlation between the actual experimental values and the model's predicted values. As shown in Table S4 and Fig. 3, there were highly significant (P-value < 0.0001) interactions between the liquid-solid ratio and extraction temperature, and between the liquid-solid ratio and extraction time. According to the response surface design and various parameters, the maximum predicted glyceroglycolipids content in *E. clathrata* was 86.2465 mg g^{-1}, obtained under the conditions of a liquid-solid ratio of 21.32 mL g^{-1}, extraction temperature of 68.16°C, methanol volume ratio of 81.26%, and extraction time of 1.99 h. Based on practical considerations, these conditions were adjusted to a liquid-solid ratio of 21 mL g^{-1}, extraction temperature of 68°C, methanol volume ratio of 81%, and extraction time of 2 h. Under these optimized conditions, the glyceroglycolipids extraction from *E. clathrata* resulted in a yield of 86.81 mg g^{-1}, which was close to the predicted value, demonstrating the accuracy and reliability of the experimental parameters.

3.2 Antioxidant activity of glyceroglycolipids extracts from macroalgae

The antioxidant activity of glyceroglycolipids extracts from *P. palmata*, *C. filum*, and *E. clathrata* was assessed in this study to explore the potential of macroalgae glyceroglycolipids as natural preservatives.

The results in Fig. 4a demonstrated that the glyceroglycolipids extracts from the three macroalgae exhibited a certain capacity to scavenge DPPH radicals, with a general trend of increasing activity as the concentration increases. Among them, the positive control, Vitamin C (Vc), showed the best effect. The glyceroglycolipids extract from *E. clathrata* followed, achieving a DPPH radical scavenging rate of 58.12% at 8 mg mL^{-1}. The extracts from *P. palmata* and *C. filum* showed slightly lower antioxidant activity, with scavenging rates of 54.26% and 40.16% at 8 mg mL^{-1}, respectively. However, when the concentration of glyceroglycolipids extracts continued to increase, the antioxidant activity did not improve significantly. These results indicated that the glyceroglycolipids extracts from the three macroalgae have a certain scavenging activity against DPPH radicals. According to reports, the DPPH radical scavenging activities of extracts from different brown algae were all lower than 30% at 50 mg mL^{-1} (Zubia et al. 2009). In comparison, the antioxidant effect of the glyceroglycolipids extracts in this study was superior.

Hydroxyl radicals are a type of reactive oxygen species generated within cells, which readily react with biological macromolecules such as amino acids and proteins. We evaluated the hydroxyl radical scavenging activity of the glyceroglycolipids extracts from the three macroalgae at different concentrations. As shown in Fig. 4b, similar to the DPPH radical scavenging rates, the hydroxyl radical scavenging activity of the extracts increased with higher concentrations. The extract from *E. clathrata* reached a hydroxyl radical scavenging rate of 52.11% at 4 mg mL^{-1}, while the extracts from *P. palmata* and *C. filum* achieved scavenging rates of 53.12% and 56.2% at 16 mg mL^{-1}, respectively. Overall, the order of hydroxyl radical scavenging effectiveness was: Vc > extracts from *E. clathrata* > extracts from *C. filum* > extracts from *P. palmata*.

Additionally, as shown in Fig. 4c, the ABTS radical scavenging activity of the glyceroglycolipids extracts from the three macroalgae and Vc increased with higher concentrations. Among them, Vc showed the best effect. The glyceroglycolipids extracts from *C. filum* had the second highest ABTS radical scavenging activity, reaching a scavenging rate of 58.7% at a concentration of 4 mg mL^{-1}. The extracts from *P. palmata* and *E. clathrata* had scavenging rates of 56.2% and 55.48% at 8 mg mL^{-1}, respectively. Compared to the ABTS radical scavenging rate of about 50% for *Sargassum binderi* extracts at a concentration of 5.29 mg mL^{-1} (Balboa et al. 2013), the glyceroglycolipids extracts from the three macroalgae in this study exhibited superior effects. In summary, the glyceroglycolipids extracts from the three macroalgae exhibited good antioxidant activities. The order of DPPH and hydroxyl radical scavenging rates was Vc > extracts from *E clathrata* > extracts from *P. palmata* > extracts from *C. filum*. For ABTS radical scavenging rates, the order was Vc > extracts from *C. filum* > extracts from *P. palmata* > extracts from *E. clathrata*.

3.3 Hygroscopic and moisturizing activity of glyceroglycolipids extracts from seaweed macroalgae

Our previous study demonstrated that extracts from four types of red macroalgae exhibited significant moisturizing abilities, often outperforming hyaluronic acid. In this study, the moisture absorption and retention activity of glyceroglycolipid extracts was evaluated to explore their potential as natural preservatives and natural cosmetic ingredients. As shown in Fig. 5a, the glyceroglycolipid extracts from the three types of macroalgae exhibited moisture absorption at RH = 43%. The moisture absorption rates increased over the first 48 h and then plateaued between 48 to 60 h. The moisture absorption efficacy ranked as follows: glycerol > extracts from *P. palmata* > extracts from *C. filum* > extracts from *E. clathrate* > hyaluronic acid. The maximum moisture absorption rates for the glyceroglycolipid extracts were 26.14%, 22.75%, and 18.96%, respectively. These rates exceeded the moisture absorption rates of crude fucoidin extracts from *Sargassum fusiforme* at 60 h.

When the RH was 81%, the glyceroglycolipids extracts from the three macroalgae showed a noticeable increase in moisture absorption within 48 h (Fig. 5b). The moisture absorption rates of glyceroglycolipids extracts from *C. filum* and *E. clathrate* tended to stabilize between 48 and 60 h, reaching maximum values of 25.07% and 27.19%, respectively. The glyceroglycolipids extracts from *P. palmata* continued to show a slight increase between 48 and 60 h, with a final moisture absorption rate of 37.07%. The order of moisture absorption effectiveness for the tested substances was as follows: glycerol, extracts from *P. palmata*, extracts from *E. clathrate*, extracts from *C. filum*, and hyaluronic acid. The moisture absorption rates of these glyceroglycolipids extracts were all superior to that of different sulfated polysaccharides from from five algae (Wang et al. 2013). These results indicated that the glyceroglycolipids extracts from the three macroalgae exhibited significant moisture absorption under both RH = 43% and RH = 81%. Although the effectiveness was lower than that of glycerol, the natural products derived from macroalgae offered the advantages of being non-toxic and environmentally friendly. Additionally, the moisture absorption performance of the glyceroglycolipids extracts from macroalgae surpassed that of hyaluronic acid, another natural product. Compared to other natural macroalgae extracts, such as the polysaccharides from *Saccharina japonica* and *Codium fragile* (Wang et al. 2013), the glyceroglycolipids extracts from these macroalgae in this study demonstrated superior moisture absorption activity.

According to Fig. 5c, all three glyceroglycolipids extracts from macroalgae exhibited moisturizing effects. Within 48 h, all five tested substances showed a rapid decline in moisturizing effect. At 60 h, the moisturizing rates were as follows: glyceroglycolipids extract from *P. palmata* was 24.41%, from *C. filum* was 19.54%, from *E. clathrate* was 18.45%, glycerol was 26.39%, and hyaluronic acid was 16.13%. Overall, the moisturizing effectiveness ranked as follows: glycerol > extracts from *P. palmata* > extracts from *C. filum* > extracts from *E. clathrate* > hyaluronic acid.

3.4 Isolation and purification of glyceroglycolipids from crude extracts

The glyceroglycolipids extracts from three macroalgae were subjected to liquid-liquid extraction using dichloromethane, ethyl acetate, and n-butanol as solvents, with the results shown in Table 1. It was observed that the yields of separated components from the glyceroglycolipids extracts were highest for

the n-butanol extracts, followed by the dichloromethane extracts, and then the ethyl acetate extracts. As shown in Fig. 6, the dichloromethane extracts (A1), ethyl acetate extracts (A2), and n-butanol extracts (A3) from the *P. palmata* extract exhibited similar chromatographic behaviors, containing the target compounds DGDG and MGDG, with SQDG not being prominent. The dichloromethane extracts (B1), ethyl acetate extracts (B2), and n-butanol extracts (B3) from the *C. filum* extract contained the target compound DGDG. The dichloromethane extracts (C1), ethyl acetate extracts (C2), and n-butanol extracts (C3) from the *E. clathrate* extract contained the target compound MGDG.

Table 1
The yield and extraction rate of fractions from crude extracts

Macroalgae	Yield (%)	Extraction rate (%)		
		Dichloromethane extracts	Ethyl acetate extracts	N-butanol extracts
P. palmata	16.97	5.12 (A1)	4.31 (A2)	7.54 (A3)
C. filum	21.76	5.86 (B1)	5.64 (B2)	9.26 (B3)
E. clathrate	19.34	5.79 (C1)	4.42 (C2)	9.13 (C3)

HPLC was employed to analyze the separated fractions of glyceroglycolipid extracts from three macroalgae species and three standard compounds (SQDG, DGDG, and MGDG). The retention times were determined to be 8.0 min for MGDG, 10.6 min for SQDG, and 14.3 min for DGDG. Comparison with the standards revealed that the liquid-liquid extracted fractions from the macroalgal samples exhibited retention times closely matching those of the standards, indicating the presence of glyceroglycolipids in the analyzed components (Table 2). These results further demonstrated that each of the nine liquid-liquid extracted fractions contained one or two types of glyceroglycolipids.

Table 2
HPLC analysis of the separated fractions of the liquid-liquid extraction from glyceroglycolipids extracts

Macroalgae	Fractions	MGDG	SQDG	DGDG
P. palmata	Dichloromethane extracts (A1)		√	
	Ethyl acetate extracts (A2)	√	√	
	N-butanol extracts (A3)			√
C. filum	Dichloromethane extracts (B1)	√		
	Ethyl acetate extracts (B2)	√		√
	N-butanol extracts (B3)		√	√
E. clathrate	Dichloromethane extracts (C1)	√		
	Ethyl acetate extracts (C2)		√	
	N-butanol extracts (C3)		√	

The absorption characteristics of infrared light can be analyzed by infrared spectroscopy based on different chemical bonds or functional groups within a molecule. Glyceroglycolipids are complex lipids containing sugar moieties and fatty acid chains, which include specific functional groups. The presence of hydroxyl groups (-OH), ester groups (-COOR), and carbon-carbon double bonds (C = C) can be determined by characteristic peaks in the spectrum. Based on the TLC analysis, the infrared spectroscopy analysis was performed on the ethyl acetate and n-butanol fractions from *P. palmata*, the dichloromethane and ethyl acetate fractions from *C. filum*, and the dichloromethane fraction from *E. clathrate*.

From Fig. 7, it could be observed that the five tested samples exhibited multiple absorption peaks at 1000–1200 cm^{-1}, likely corresponding to the C-O stretching vibrations of the sugar rings in glyceroglycolipids. Notably, the region from 1000–1100 cm^{-1} was also a potential peak area for ether bonds (C-O-C). If the sugar moieties in the glyceroglycolipids were connected to the glycerol backbone via ether bonds, absorption peaks would appear in this region. Based on this feature, it could be confirmed that all five tested samples contained a glycerol backbone. Additionally, smaller absorption peaks at 1741.97 cm^{-1}, 1701.54 cm^{-1}, and 1713.70 cm^{-1} were detected in the ethyl acetate fraction of *P. palmata*, the ethyl acetate fraction of *C. filum*, and the dichloromethane fraction of *E. clathrate*, respectively, suggesting the presence of ester bonds in these samples. Absorption was observed in the 3200–3600 cm^{-1} range for all five tested samples, which corresponded to hydroxyl (O-H) absorption peaks, an important feature for identifying glyceroglycolipids. Moreover, weak absorptions at 2923.50 cm^{-1}, 2925.27 cm^{-1}, 2920.00 cm^{-1}, and 2916.77 cm^{-1} were found in the ethyl acetate fraction of *P. palmata*, the dichloromethane fraction of *C. filum*, the ethyl acetate fraction of *C. filum*, and the dichloromethane fraction of *E. clathrate*, respectively. These absorptions were attributed to C-H stretching vibrations. Glyceroglycolipids, connected via ester bonds to multiple sugar moieties and fatty acid chains of varying lengths and saturation levels, commonly include methyl and methylene groups in the fatty acid chains. Attention should also be paid to absorptions around 1400 cm^{-1}, which may be due to the asymmetric bending vibrations of methyl groups or symmetric bending vibrations of methylene groups, or potentially due to the presence of free carboxyl groups or their symmetry in the glyceroglycolipids. Interference from aromatic compounds or stretching vibrations of C-C single bonds in the glyceroglycolipids might also be considered. Based on the infrared spectroscopy results indicating the presence of glycerol backbones, ether bonds, and hydroxyl groups, it could be determined that the ethyl acetate extracts of *P. palmata*, the ethyl acetate extracts of *C. filum*, and the dichloromethane extracts of *E. clathrate* exhibited the structural characteristics of glyceroglycolipids, providing preliminary evidence of their glyceroglycolipid content. Furthermore, the n-butanol extracts of *P. palmata* and the dichloromethane extracts of *C. filum* may also contain glyceroglycolipids, which will require further identification.

Through liquid-liquid extraction, TLC, HPLC, and infrared spectroscopy, the ethyl acetate extracts from *P. palmata* and *C. filum* were selected for glyceroglycolipid separation and purification using silica gel column chromatography (100–200 mesh, 4.0×40 cm). The eluted fractions were analyzed by TLC (Fig. 8). The ethyl acetate extract from *P. palmata* was further separated into four sub-fractions: HP1 (0.431

g), HP2 (0.528 g), HP3 (0.537 g), and HP4 (0.477 g). The ethyl acetate extract from *C. filum* was separated into four sub-fractions: SZ1 (0.422 g), SZ2 (0.498 g), SZ3 (0.505 g), and SZ4 (0.394 g). Comparison with glyceroglycolipid standards revealed that HP3 contained MGDG, SQDG, and MGDG3; HP2 contained SQDG and MGDG; SZ3 contained DGDG and MGDG; and SZ4 contained only MGDG (Fig. 8a). Subsequently, sub-fraction HP3 was selected for preparative TLC, resulting in the isolation of three samples: SQDG (11.9 mg), DGDG (9.3 mg), and MGDG (7.1 mg) (Fig. 8b). Katsuoka (1990) used column separation to prepare 30 mg and 33 mg of MGDG and other glycreoglycolipids. Thus, this study successfully isolated and prepared three types of glyceroglycolipids from *P. palmata*. Their specific structures will be further determined by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).

Based on the preparation process of glyceroglycolipids from macroalgae described above, a preliminary cost estimation was performed for the preparation of glyceroglycolipids using *P. palmata* as the raw material. In this study, 250 g of *P. palmata* was used to prepare SQDG, DGDG, and MGDG through extraction, liquid-liquid extraction separation, silica gel column chromatography, and thin-layer chromatography purification. The yields were 11.9 mg of SQDG, 9.3 mg of DGDG, and 7.1 mg of MGDG. The costs of raw materials, solvents, and separation media were calculated, resulting in the following unit costs for the three glyceroglycolipids: SQDG was 704 RMB mg^{-1}, DGDG was 450 RMB mg^{-1}, and MGDG was 613 RMB mg^{-1}. In comparison, the market prices for purchased standard glyceroglycolipids are approximately 788 RMB mg^{-1} for SQDG, 500 RMB mg^{-1} for DGDG, and 680 RMB mg^{-1} for MGDG, all of which are higher than the costs of the glyceroglycolipids prepared in this study.

3.5 Antibacterial activity and preservative property of glyceroglycolipids extracts from macroalgae in *Channa argus* fillet

During the storage and spoilage processes of meat products, various microorganisms are involved, which can cause spoilage under different conditions. Common spoilage microorganisms in meat products include *Lactobacillus* spp., *Pseudomonas* spp., *Enterobacteriaceae*, and *Serratia marcescens* among others. To investigate whether the glyceroglycolipids extracts prepared above have food preservation properties, their inhibitory activity against *S. marcescens* was tested. Glyceroglycolipids extracts from *P. palmata* and *C. filum* exhibited certain inhibitory activity against *S. marcescens* at a concentration of 8 mg mL^{-1}. In contrast, the glyceroglycolipids extract from *E. clathrate* did not show significant antibacterial activity at 8 mg mL^{-1} or lower concentrations (data not shown). Subsequently, *C. argus* fillets were used as samples to assess the preservation performance of the glyceroglycolipids extracts during storage. The impact of *C. filum* and *E. clathrate* glyceroglycolipids extracts on various indicators was shown in Fig. 9, while the relevant data for glyceroglycolipids extracts from *P. palmata* have been published.

During refrigeration, microbial growth degrades meat fats and proteins, causing spoilage (Zhu et al., 2022). Total bacterial count, a key hygiene indicator, should remain below 6 lg CFU g^{-1} for aquatic products. As shown in Fig. 9a, bacterial counts in C. argus fillets increased over time, exceeding the safety limit by day 6 in the control group. In contrast, fillets treated with macroalgae glyceroglycolipid extracts delayed microbial growth. On day 10, four groups remained below the limit: ethyl acetate extracts from *C. filum* (5.94), *P. palmata* (5.96), butanol extracts from *P. palmata* (5.98), and dichloromethane extracts from E. clathrate (5.99 lg CFU g^{-1}), effectively extending shelf life by 4 days. Previous studies have shown the antimicrobial potential of glyceroglycolipids, such as MGDG and SQDG, against pathogens including *E. coli* and *H. influenzae* (Erwan et al., 2014; Ahamed et al., 2017; Furukawa et al., 2007).

During refrigeration, microbial decomposition of proteins and fats in *C. argus* produces ionic small molecules, increasing the fillets' electrical conductivity—a marker of spoilage. Figure 9b illustrates that conductivity rose daily, with lower values indicating better freshness. In the *C. filum* group, ethyl acetate extracts showed the lowest conductivity on day 10, while crude extracts had the highest. Similarly, in the *E. clathrate* group, dichloromethane extracts resulted in the lowest conductivity. Overall, glyceroglycolipid extracts from *C. filum* and *E. clathrate* reduced conductivity compared to the control, though their crude form may limit activity.

Fish freshness is closely linked to pH, which initially drops postmortem due to lactic acid buildup, then rises as microbial degradation produces alkaline compounds (Abbas et al., 2008). Figure 9c shows that *C. argus* fillet pH values first declined slightly, then increased during refrigeration. By day 10, the control group's pH reached 7.43, while treated groups generally had lower pH values. Dichloromethane, ethyl acetate, and butanol extract treatments from the three macroalgae all showed pH values below the control, suggesting that glyceroglycolipid extracts helped slow spoilage and have potential as natural preservatives.

TVB-N reflects protein degradation into ammonia and amines during spoilage, with higher values indicating reduced fish freshness. Figure 9d shows that TVB-N levels remained stable early on, then rose significantly from day 2. By day 6, the control group exceeded the 20 mg 100 g^{-1} limit for level II freshness, marking spoilage. In contrast, some treated groups remained below this threshold until days 8 or 10. On day 10, TVB-N values for selected extracts (e.g., ethyl acetate from *P. palmata* and *C. filum*, dichloromethane from *E. clathrate*) hovered just above 20 mg 100 g^{-1}. These results suggest that macroalgae glyceroglycolipid extracts can delay protein breakdown and preserve freshness.

Lipid oxidation, driven by unsaturated fatty acid degradation, produces malondialdehyde (MDA), with TBA values indicating oxidation levels. A TBA value above 1.0 mg kg^{-1} signals spoilage. As shown in Fig. 9e, TBA values increased during refrigeration, with the control group exceeding the safety limit by day 10. Several treatment groups remained below this threshold, notably extracts from *P. palmata*, *C. filum*, and *E. clathrate*. The lowest values were observed in ethyl acetate and butanol extracts from *P. palmata*.

These results, combined with antioxidant activity assessments, confirm that glyceroglycolipid extracts from macroalgae effectively inhibit lipid oxidation and hold strong potential as natural preservatives.

Discussion

This study investigated the key factors influencing the yield of glyceroglycolipids extracted from *C. filum* and *E. clathrata*. The results demonstrated that extraction variables, including the liquid-to-solid ratio, temperature, methanol volume ratio, and extraction time, significantly impact glyceroglycolipid yield. These findings were consistent with previous research aimed at optimizing the extraction of bioactive compounds from marine macroalgae (Sun et al. 2024; Wei et al. 2025). An optimal liquid-to-solid ratio was identified, beyond which increased solvent volume did not enhance efficiency, due to solubility saturation, as noted by Sun et al. (2021). Extraction temperature exhibited a parabolic effect, where moderate heat improved solubility and penetration, whereas higher temperatures led to compound degradation (Zwerger et al., 2022). The ideal methanol ratio was found to be 80%, in agreement with studies that highlight the importance of solvent polarity in matching target compounds (Sun et al., 2021). Longer extraction times initially improved diffusion but ultimately resulted in compound degradation and excessive energy consumption. These results underscore the importance of optimizing extraction conditions to balance efficiency, stability, and cost-effectiveness. Using Box–Behnken design and response surface methodology, predictive models for glyceroglycolipid extraction were developed, with the optimized conditions closely aligning with theoretical predictions, thereby confirming the model's practical applicability for industrial-scale processes. Overall, this study provides optimized protocols for glyceroglycolipid extraction that are relevant for future industrial applications.

Recently, macroalgae have attracted extensive attention due to their natural, non-toxic properties and unique bioactivities (Ercan et al. 2013; Catarino et al. 2023). Several studies have reported that active substances in macroalgae possessed significant antioxidant potential, making macroalgae an ideal source of natural antioxidants (Jacobsen et al. 2019; Tziveleka et al. 2021). In this study, the antioxidant activities of glyceroglycolipids extracted from *P. palmata*, *C. filum*, and *E. clathrate* were measured. The antioxidant activities of the glyceroglycolipids extracts from the three macroalgae increased with higher concentrations. Compared to other macroalgae extracts (Balboa et al. 2013), the glyceroglycolipids extracts in this study exhibited superior antioxidant activities. Although the antioxidant activities of the extracts in this study were lower than those of Vc, this could be attributed to the extracts being crude fractions. If the extracts were further purified, their antioxidant activities might improve. Moreover, while the antioxidant effect of the macroalgae glyceroglycolipids crude extracts was not as high as Vc, they possess unique advantages over Vc. Vc is unstable under natural conditions, leading to a decrease in its antioxidant performance over time (Giannakourou et al. 2021). In contrast, due to the unique hydrophilic and lipophilic structure of glyceroglycolipids (Yuan et al. 2024), they may exhibit better oil solubility and stability. This means that compared to Vc, glyceroglycolipids extracts can penetrate cell membranes or lipid environments more easily, providing longer-lasting antioxidant protection. Term (2018) first reported the free radical scavenging activity of lipid substances extracted from algae, which aids in the development of natural antioxidants from macroalgae glyceroglycolipid extracts for use in cosmetics

and food industries. Researchers analyzed the composition of polar lipids in the Atlantic red algae Grateloupia turuturu and assessed their antioxidant and anti-inflammatory activities (Costa et al. 2021). Additionally, MGDG, DGDG, and SQDG have showed significant anti-inflammatory activity in mouse inflammation models (Bruno et al. 2005). Graciliana (2014) also found that glyceroglycolipids, including MGDG extracted from edible brown algae, possessed anti-inflammatory activities. Moreover, marine algal compounds have long been utilized in antiviral drugs. These results indicated that glyceroglycolipids from macroalgae have a broader application range and are potential sources of natural antioxidants.

In addition, the glyceroglycolipid extracts from *P. palmata*, *C. filum*, and *E. clathrata* exhibited significant moisture absorption and retention properties, positioning them as potential natural humectants for use in cosmetics and preservatives. At relative humidities (RH) of 43% and 81%, extract from *P. palmata* demonstrated the highest moisture absorption, outperforming crude fucoidan from *Sargassum fusiforme* (Wang et al., 2013). These extracts also surpassed hyaluronic acid, a commonly used moisturizing agent, and were comparable to glycerol in performance. Although all extracts experienced a rapid decline in moisture retention after 48 h, extract from *P. palmata* exhibited the highest retention rate, followed by those of *C. filum* and *E. clathrata*. Glyceroglycolipids, being non-toxic, environmentally friendly, and derived from renewable sources, meet the growing demand for natural ingredients in personal care products. Their hydrophilic and long-chain structures contribute to moisture retention, positioning them as effective natural humectants for use in cosmetics and pharmaceuticals. Therefore, glyceroglycolipids from these species show considerable potential as natural alternatives to synthetic humectants.

Glyceroglycolipids were successfully isolated and purified from crude extracts through liquid-liquid extraction and silica gel column chromatography, with the highest yield obtained from the n-butanol fraction, in accordance with their polarity and solubility characteristics. Key glyceroglycolipids, such as DGDG and MGDG, were identified via HPLC analysis, and infrared spectroscopy confirmed the presence of characteristic hydroxyl and ester groups. Ether bonds observed in some extracts suggest a complex structure, warranting further analysis using NMR and MS. Silica gel chromatography effectively separated the glyceroglycolipids into pure components, including MGDG, SQDG, and DGDG, with promising yields and low extraction costs, making the production of glyceroglycolipids from *P. palmata* economically viable. This study demonstrated the feasibility of extracting and purifying glyceroglycolipids from macroalgae for potential applications in food and pharmaceuticals, although further structural analysis is needed to optimize extraction and purification.

Glyceroglycolipid extracts from *P. palmata*, *C. filum*, and *E. clathrata* exhibited promising preservative properties for seafood, demonstrating antibacterial activity, particularly against *S. marcescens*, which helped inhibit microbial spoilage. Treated fillets showed reduced total bacterial counts, lower TVB-N values, decreased electrical conductivity, and stabilized pH levels, all indicative of reduced spoilage. Moreover, the extracts effectively inhibited lipid oxidation, as evidenced by lower TBA values. These findings suggested that glyceroglycolipids could extend the shelf life of seafood by slowing microbial

growth, protein degradation, and lipid oxidation, offering a natural and eco-friendly alternative to synthetic preservatives. Further optimization and testing on additional food products could expand their potential applications in food preservation.

Conclusions

This study highlighted the extraction, characterization, and application of glyceroglycolipids from marine macroalgae, emphasizing their potential in various fields. The extraction conditions were optimized for maximum yield, and the antioxidant, humectant, and preservative properties of the glyceroglycolipids were thoroughly evaluated. The results indicated these glyceroglycolipids possess considerable potential as natural alternatives to synthetic antioxidants, humectants, and preservatives in food, cosmetic, and pharmaceutical industries. Further refinement and purification of the extracts could enhance their properties and broaden their application potential.

Declarations

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Conceptualization: Yingying Sun; Methodology: Xin Wei, Yang Mu, Tianle Li, Xiujing Jiang, You Yu; Formal analysis and investigation: Haonan Wang, Mengjiao Yang, Chenwei Mao; Writing - original draft preparation: Xin Wei; Writing - review and editing: Xin Wei and Yingying Sun; Funding acquisition: Yingying Sun; Resources: Mingxuan Pan and Yadong Hu; Supervision: Yingying Sun.

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Conflicts of Interest

The authors declare no conflict of interest.

Availability of data and material

The original contributions presented in this study are included in the article and supplementary material.

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Figures

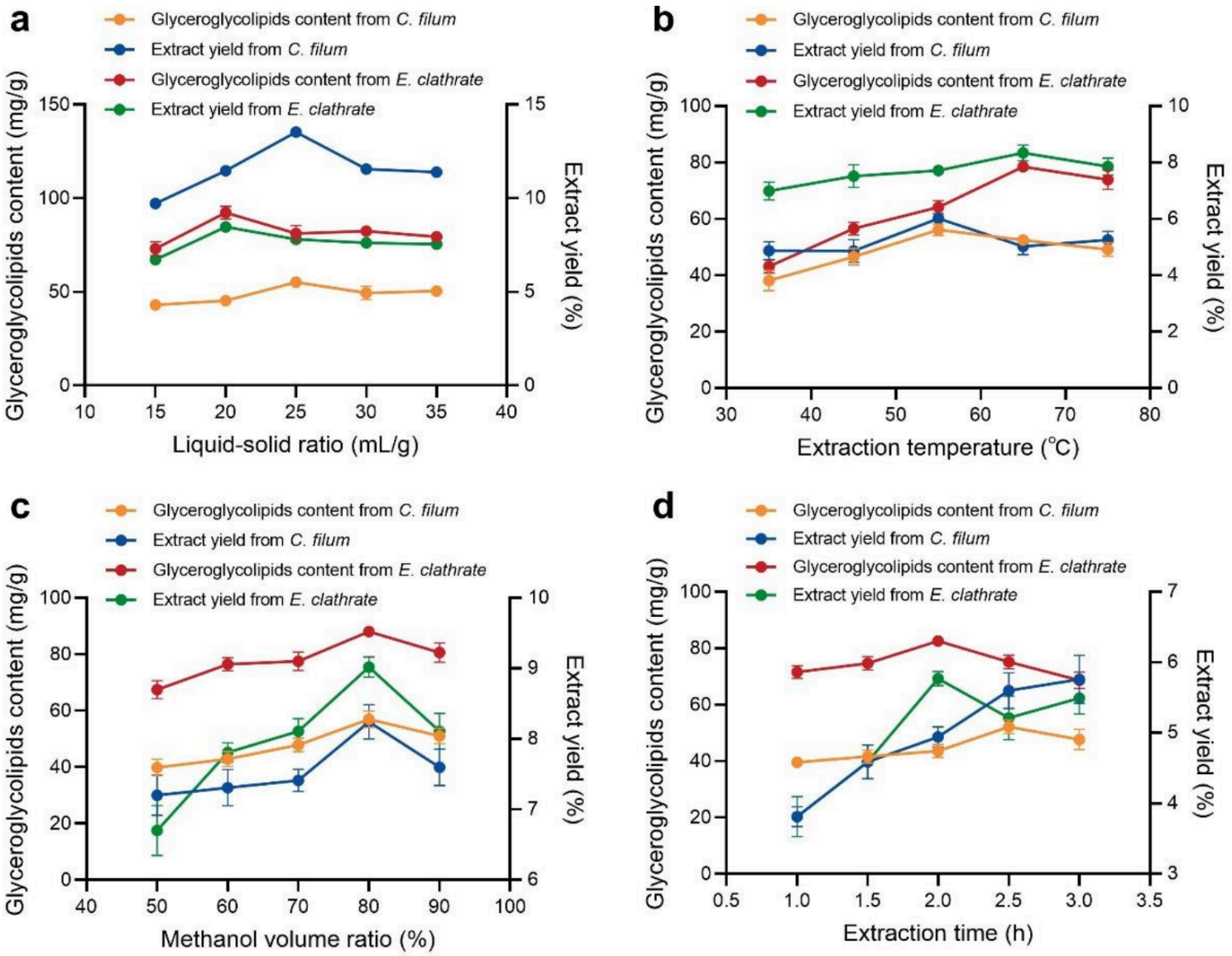

Figure 1

Effects of liquid-solid ratio (a), extraction temperature (b), methanol volume ratio (c) and extraction time (d) on the extraction efficiency of glyceroglycolipids from *C. filum and E. clathrate* (Data were given as mean ± SD, n = 3)

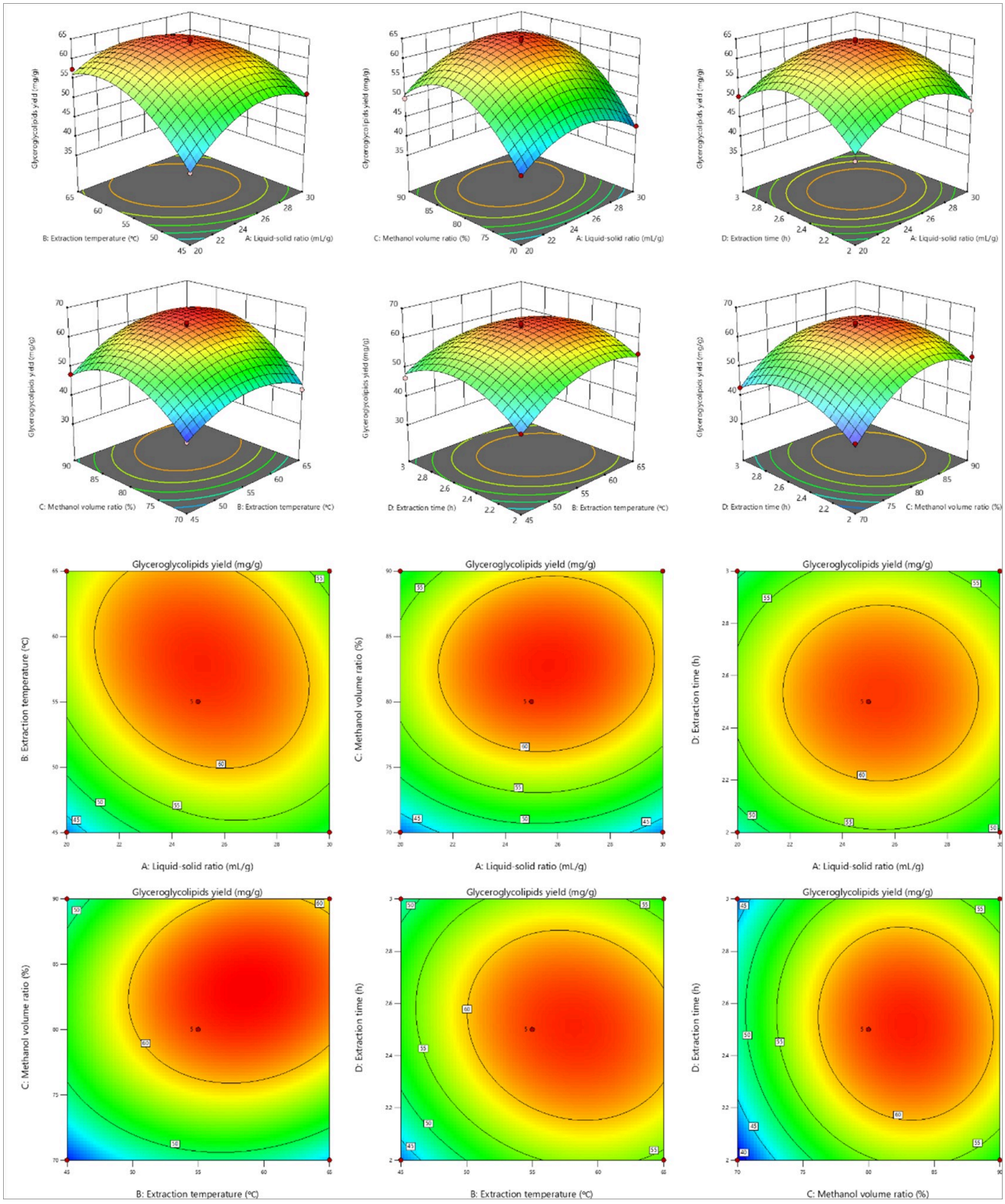

Figure 2

Response surfaces and contours representing the effects of different factors on the glyceroglycolipids content from *C. filum*

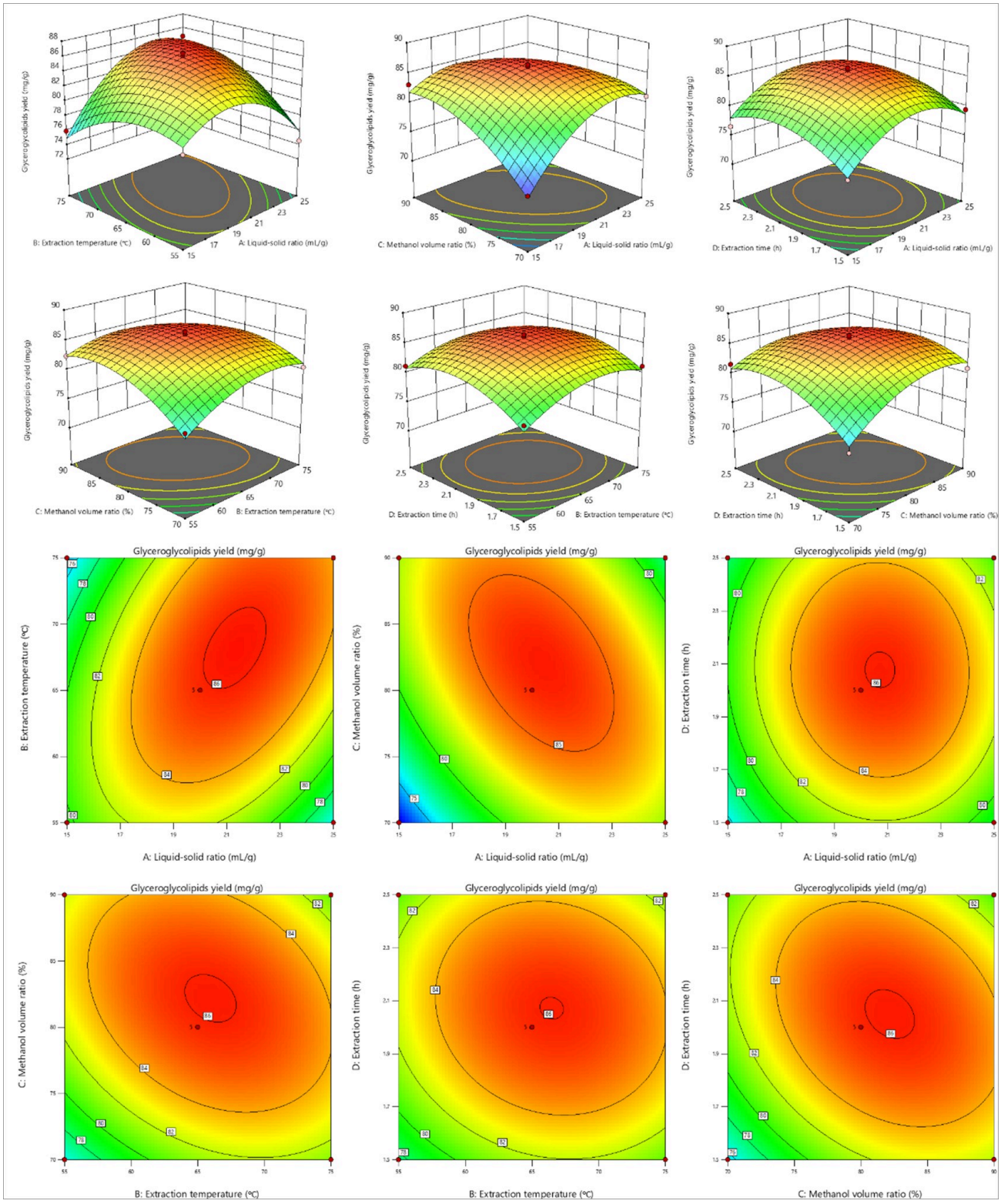

Figure 3

Response surfaces and contours representing the effects of different factors on the glyceroglycolipids content from *E. clathrate*

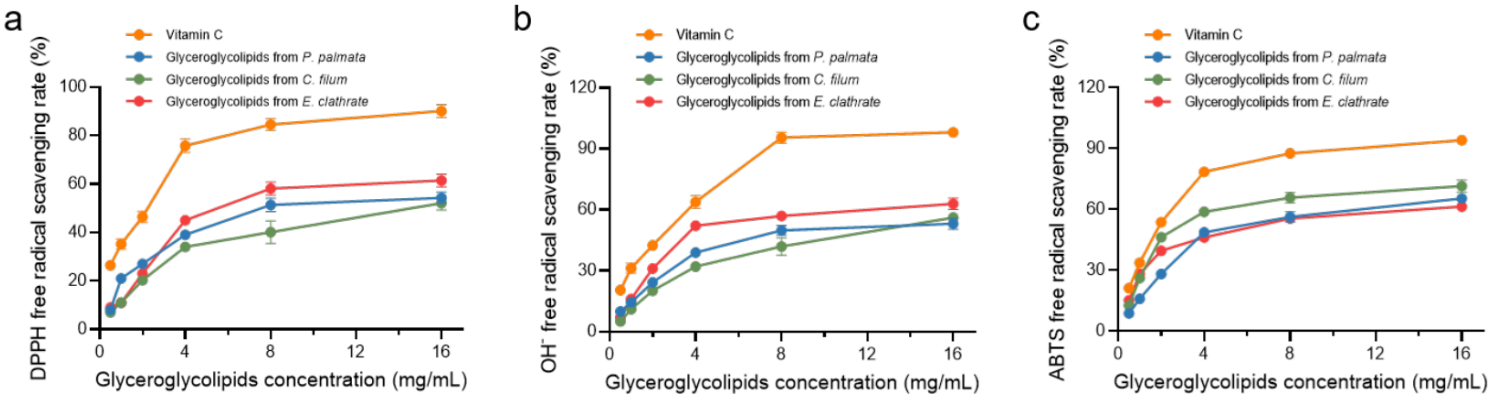

Figure 4

Scavenging activities of glyceroglycolipids extracts from three marine macroalgae on DPPH free radicals (a), hydroxyl radicals (b) and ABTS free radicals (c) (Data were given as mean ± SD, n = 3)

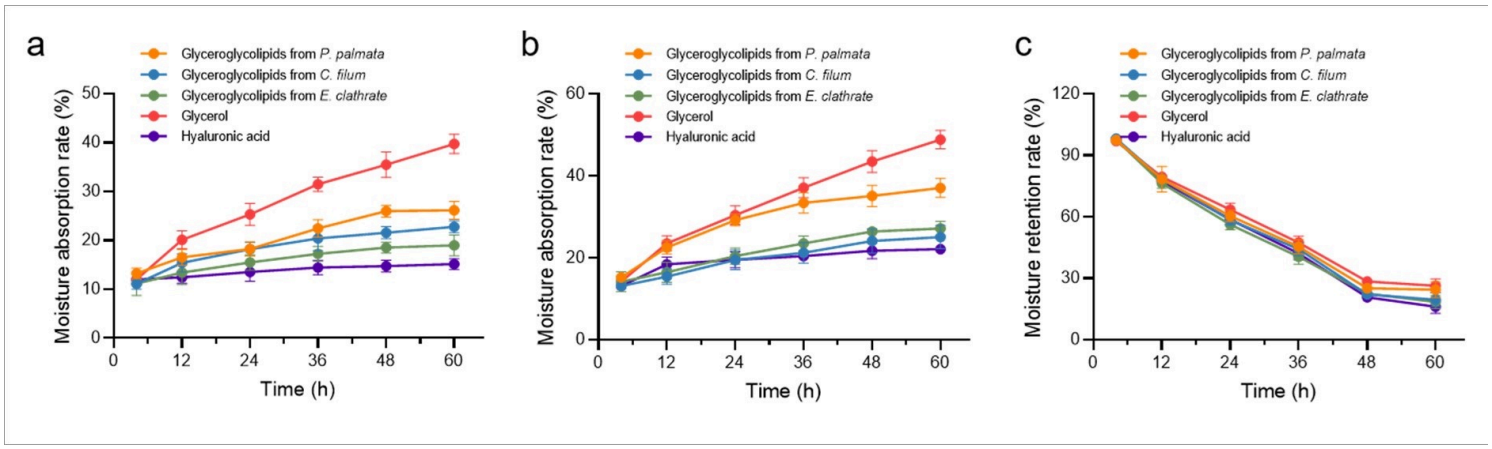

Figure 5

Moisture absorbing rate (a: RH=43%; b: RH=81%) and retention rate (c) of glyceroglycolipids extracts from three macroalgae (Data were given as mean ± SD, n = 3)

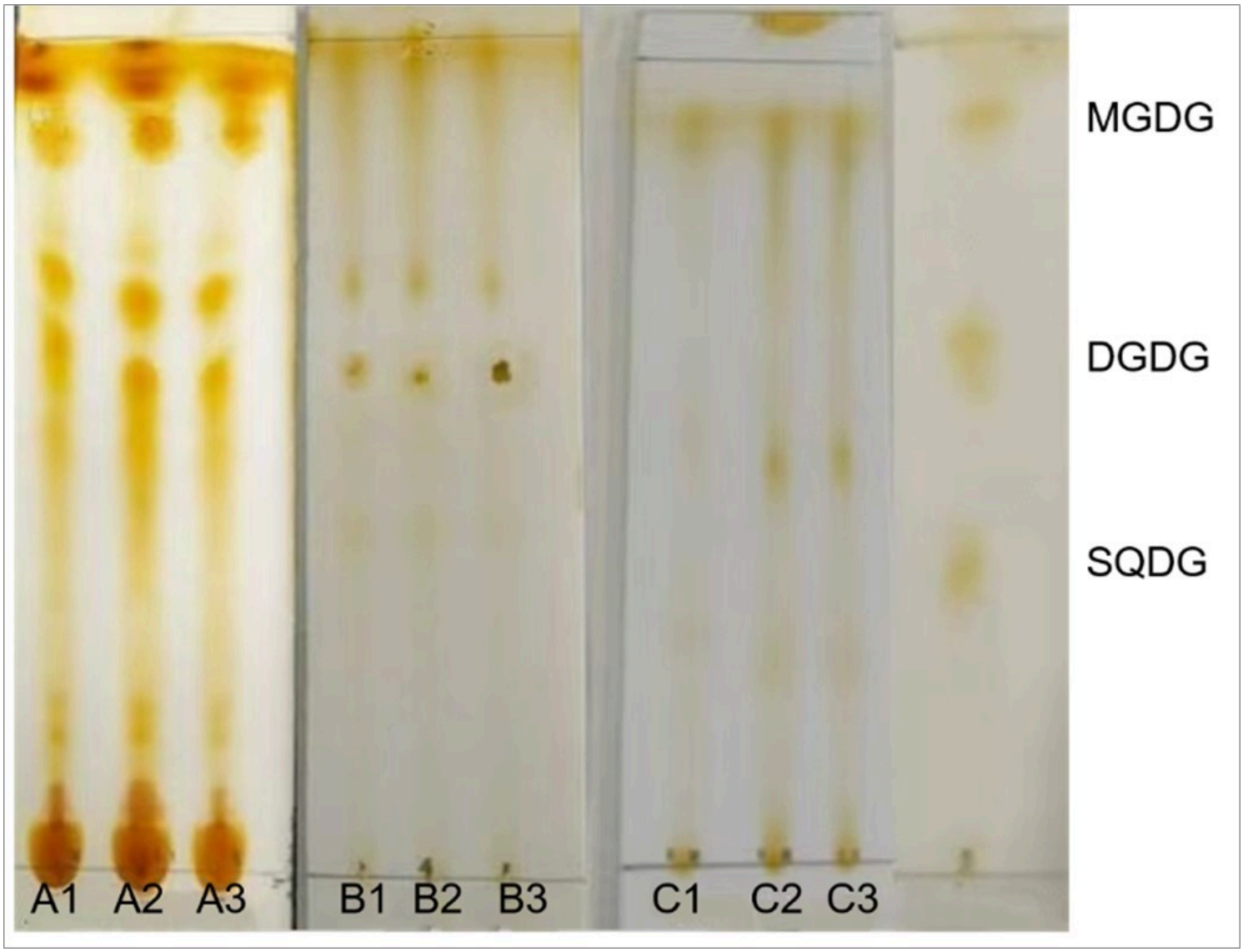

Figure 6

TLC analysis of different fractions from liquid-liquid extraction

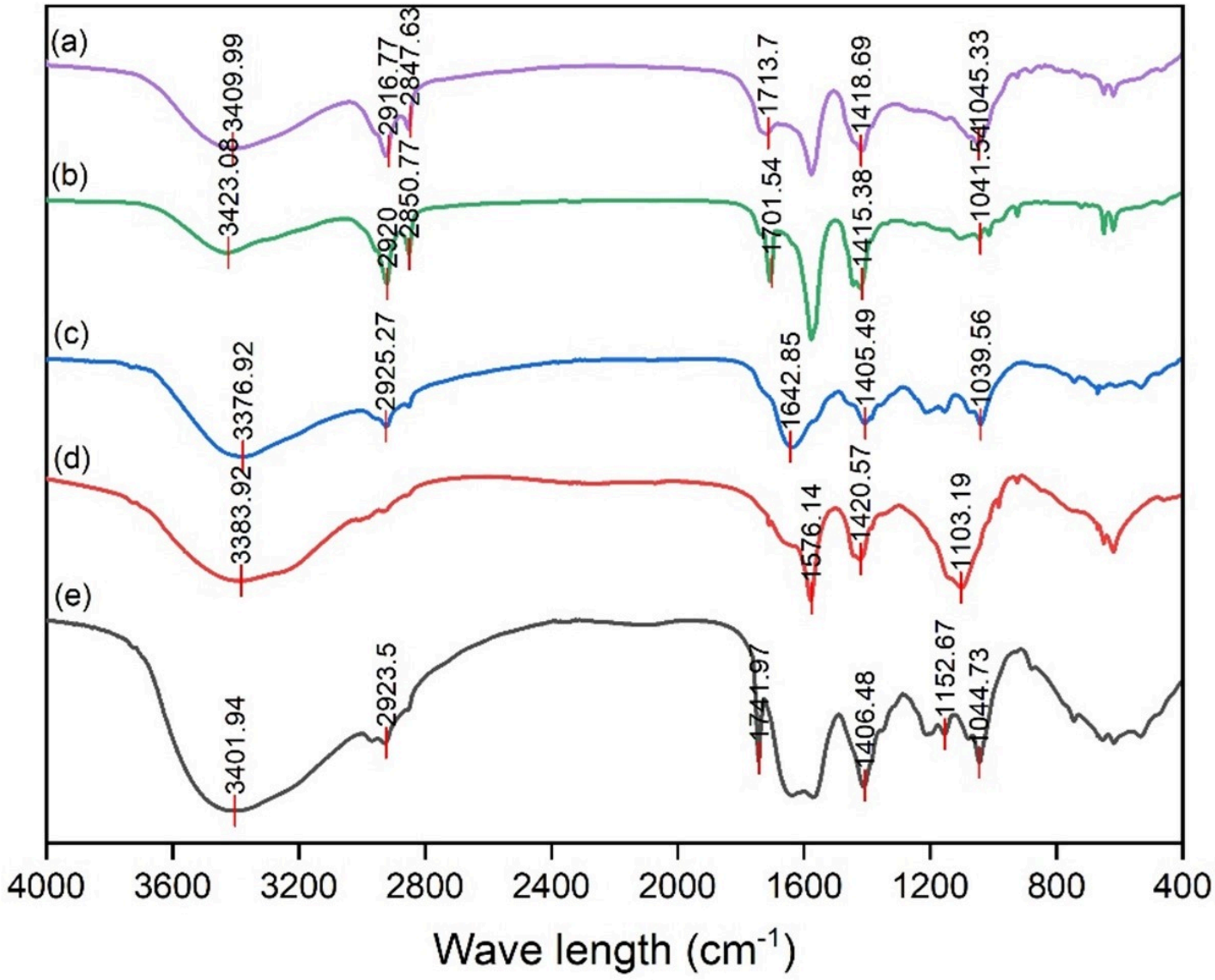

Figure 7

Infrared spectroscopy analysis of different extracts (a: dichloromethane extracts from *E. clathrate*; b: ethyl acetate extracts from *C. filum*; c: dichloromethane extracts from *C. filum*; d: n-butanol extracts from *P. palmata*; e: ethyl acetate extracts from *P. palmata*)

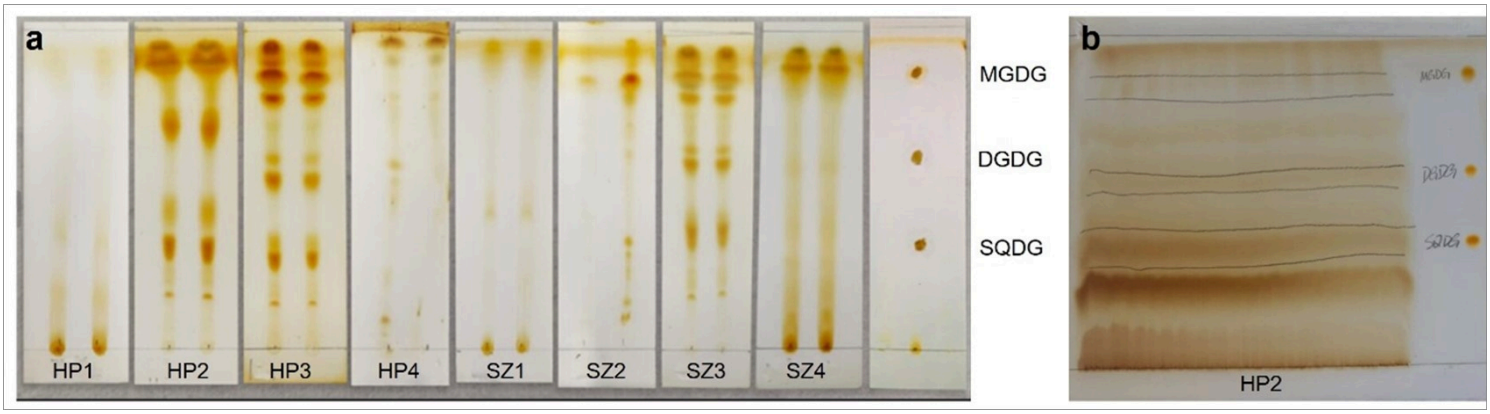

Figure 8

TLC analysis (a) and preparation of glyceroglycolipids (b)

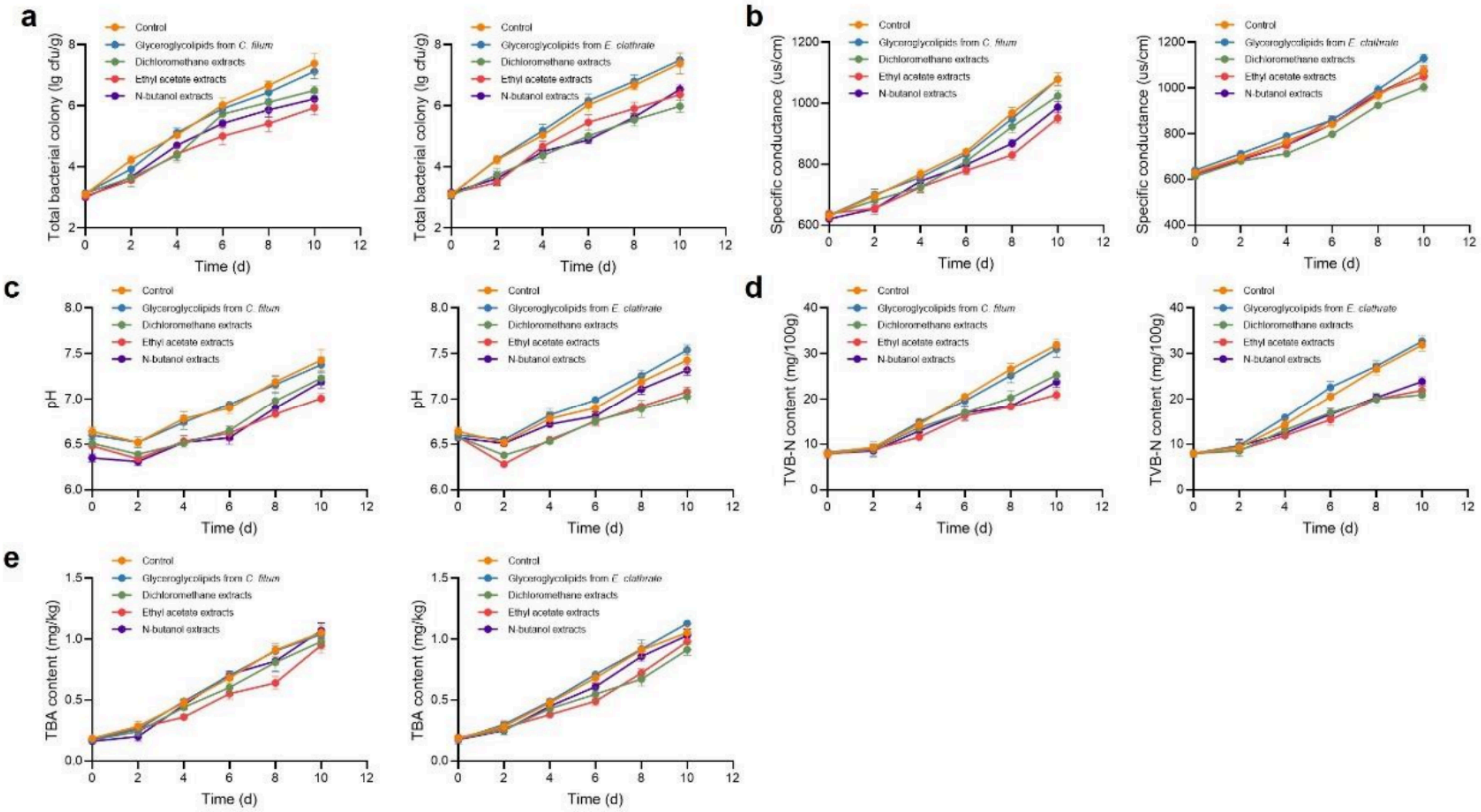

Figure 9

Effects of glyceroglycolipids extracts on total bacterial count (a), electrical specific conductance (b), pH (c), TVB-N content (d) and TBA content (e) of *C. argus*fillets during storage (Data were given as mean ± SD, n = 3)

Supplementary Files

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