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Inhibitory effects of capsulated Sargassum ilicifolium extract on lipid oxidation in fish oil and pathogenic microorganisms

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ABSTRACT

This research investigates the optimal formulation of microencapsulated Sargassum algae extract to improve the oxidative stability of fish oil and its potential use as an antimicrobial agent. Given the oxidative sensitivity of fish oil due to its specific fatty acid composition, the use of antioxidants is essential to increase its shelf life. Microencapsulated extract, pure extract, and a synthetic antioxidant (TBHQ) were added to fish oil at three concentrations: 0, 1.25, and 2.5 percent, and examined over a 15-day period. Response surface methodology was used to evaluate the effect of independent variables on peroxide and anisidine values as oxidation indices and to optimize the process. Oxidation increased with increasing storage time, while the values of the indices decreased with increasing concentration. For selecting the optimal treatment, microencapsulated algae extract and synthetic antioxidant at a concentration of 2.5% and a time of 15 days were the best suggested conditions. Furthermore, investigation of the antimicrobial effects of the microencapsulated treatments revealed that the treatments prepared with a chitosan wall composition, followed by the treatment with a wall composition of whey protein and maltodextrin at a 50:50 ratio, exhibited the highest antimicrobial activity against the pathogenic bacteria tested, suggesting that these treatments could be used to prepare alternative to standard antibiotics.

1- Introduction

In recent years, food quality and safety have become major concerns for consumers, manufacturers, and regulatory agencies. Changes in dietary habits and consumer behavior, such as increased demand for fresh, minimally processed, natural, and convenient foods, can be attributed to this trend. Therefore, the production of safe and high-quality foods, particularly in spoilage-prone products such as fish oil, has received considerable attention. Given its high nutritional value, fish oil consumption has significantly increased in recent decades, making the development of natural and acceptable methods for its safe delivery to the market essential. Simultaneously, ensuring both chemical and microbial stability of food products can create optimal conditions and minimize the need for synthetic preservatives [1].

One of the most important and widely used preservative compounds in the food industry is antioxidants. Due to the health and environmental concerns associated with synthetic antioxidants over recent decades, there has been a growing demand for the development of safe and natural alternatives [2, 3]. This issue is particularly evident in foods such as fish oil, which is highly susceptible to oxidative deterioration due to its fatty acid composition, making the use of antioxidants indispensable [4]. Fish oil is a rich source of unsaturated omega-3 fatty acids, whose beneficial effects on human health have been well established. These fatty acids are highly unstable and easily oxidize during storage, leading to a reduction in nutritional value, the development of off-flavors, and the formation of toxic compounds [5]. The use of antioxidants is essential to extend the shelf life of fish oil; however, synthetic antioxidants may compromise the beneficial properties associated with omega-3, whereas the application of natural antioxidants does not have this drawback [6, 7].

Algae are rich sources of various antioxidant pigments, including fucoxanthin, chlorophyll a and b, beta-carotene, xanthophylls, flavonoids, and carotenoids. Among these, phenolic compounds exhibit remarkable antioxidant activity, and a group of polyphenols, such as catechins, flavonols, and flavonol glycosides, has been observed in methanolic extracts of brown and red algae [8, 9]. Therefore, brown algal pigments, such as those from *Sargassum*, containing phenolic compounds and flavonoid pigments, have high potential as natural antioxidants capable of reducing oxidative processes in food products [10].

Moreover, microencapsulation, as a proven protective technique, can effectively enhance the stability and safety of these compounds. Microencapsulation can significantly improve the efficacy of plant and algal extracts against oxidation by controlling the release of active compounds and increasing their stability in food matrices [11, 12].

Modeling of chemical and biological processes is highly valuable for process system engineering due to its critical role in process design, optimization, and control operations [13]. Mechanistic models, which are based on the physics of the reactions, generally provide

reasonable accuracy but often face limitations due to high complexity and convergence issues. Computational or software-based "black-box" models, such as artificial neural networks (ANN) and adaptive neuro-fuzzy inference systems (ANFIS), have been widely applied to overcome these challenges [14, 15]. Response surface methodology (RSM) is an effective statistical technique and a key tool for modeling and analyzing the effects of multiple process parameters. It allows estimation of complex interactions among process variables and is also employed for process optimization [16].

In this study, *Sargassum* algae extract, after extraction and microencapsulation, was used as a natural antioxidant in fish oil. Its anti-oxidative effects were compared with unencapsulated samples and synthetic antioxidants. To investigate trends and determine the influence of independent variables, as well as to optimize the anti-oxidation process, response surface methodology was applied. Additionally, the antimicrobial activity of the microencapsulated extract was evaluated and compared with standard antimicrobial agents for controlling the growth of nine Gram-positive and Gram-negative pathogenic microorganisms.

2-Materials and Methods

2.1 Collection, Extraction, and Microencapsulation of *Sargassum* Algae

Details regarding the collection, extraction, and microencapsulation of *Sargassum* algae have been fully described in a previous study [17]. Among various microencapsulation treatments, an optimized formulation (microencapsulated *Sargassum* extract with a 50:50 ratio of whey protein to maltodextrin as the wall material and an extract-to-wall ratio of 1:12) was selected for evaluating antioxidant activity. Two treatments—the optimized antioxidant treatment and a formulation using 100% chitosan as the wall material with an extract-to-wall ratio of 1:4—were used for antibacterial assessment.

2.2 Antioxidant Activity

The antioxidant potential of the microencapsulated *Sargassum* extract treatments in protecting fish oil against oxidation was evaluated. Briefly, three types of preservatives—microencapsulated *Sargassum* extract, crude (unencapsulated) extract, and a synthetic antioxidant (TBHQ; Sigma-Aldrich, USA)—were added to fish oil at concentrations of 0, 1.25, and 2.5%. The samples were stored at room temperature (25°C) for 15 days. The main parameters assessed during the oxidation process were peroxide and anisidine values at 0 and 15 days.

2.2.1 Peroxide Value Determination

Peroxide values (PV) were measured using 100 mg of the sample, to which 8.9 mL of a chloroform:methanol mixture (7:3, v/v) was added. Subsequently, 0.1 mL of 0.02 M ferrous chloride in 10% hydrochloric acid was added. After mixing, the samples were left undisturbed for three minutes, followed by the addition of 0.1 mL of 30% ammonium thiocyanate. Finally, the absorbance was recorded at

500 nm, and the peroxide value was calculated according to Equation 1 [18, 19].

$$PV = \frac{C \times (V - V_0) + 12.69 \times 78.8}{m} \quad (1)$$

where PV is the peroxide value, C is the concentration of sodium thiosulfate (mol/L), V and V_0 represent the volumes of thiosulfate consumed by the sample and the blank (mL), respectively, and m is the mass of the oil (g).

2.2.2 Anisidine Value Determination

To determine the para-anisidine value (AV), 0.25 g of para-anisidine was dissolved in 100 mL of acetic acid. Between 0.5 and 0.7 g of the oil sample was accurately weighed in a 25 mL flask (m), and the para-anisidine–acetic acid solution was added. The resulting solution was then diluted with iso-octane to the desired volume and thoroughly mixed. The absorbance of the resulting solution (iso-octane + oil) was recorded at 350 nm against iso-octane as the blank (Ab). Subsequently, 5 mL of the oil solution in iso-octane was transferred to one test tube, and 5 mL of iso-octane to another. One milliliter of the para-anisidine solution was added to each tube and mixed thoroughly. After 10 minutes in the dark, the absorbance of the sample solution (iso-octane + oil + para-anisidine) was measured against the blank (iso-octane + para-anisidine) (As). Finally, the anisidine value was calculated according to Equation 2 [20].

$$AV = \frac{(25 \times (1.2\,As - Ab))}{m} \quad (2)$$

where AV represents the anisidine value.

2.3 Response Surface Modeling

In this study, response surface methodology (RSM) was applied to analyze and optimize the antioxidant activity of microencapsulated Sargassum extract in fish oil. This method allows evaluation of linear, interaction, and quadratic effects of the variables on the responses [16]. The experimental design was conducted as a full factorial design using historical data capabilities in Design-Expert software, version 13. A qualitative variable, namely the type of preservative, was considered at three levels (microencapsulated Sargassum extract, crude unencapsulated extract, and a synthetic antioxidant), while two quantitative variables included preservative concentration at three levels (0, 1.25, and 2.5%) and storage time at two levels (0 and 15 days). The response variables for the experimental design were peroxide and anisidine values. Process optimization aimed to identify the best combination of process variables to achieve the highest antioxidant activity (i.e., minimum values of the evaluated indices). The relationship between input and output variables was modeled using a second-order polynomial response surface model, described by Equation 3.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n-1} \sum_{j=2}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_{ii} X_i^2 \quad (3)$$

In this equation, Y represents the predicted response for each variable, β_0 is the constant coefficient, β_i, β_{ii}, and β_{ij} are the coefficients of the linear, quadratic, and interaction effects, respectively, and X_i and X_j represent the independent process variables.

2.4 Antimicrobial Activity

The antimicrobial effects of the optimized microencapsulated treatments were evaluated using the well diffusion method. In this approach, Mueller-Hinton agar plates inoculated with microorganisms were used. Sterile Pasteur pipettes, specifically designed to create wells, were employed to form a cavity in the agar medium, and 0.5 mg of each treatment was added separately into each well. The plates were then incubated at 37°C for 24 hours.

The antimicrobial activity was assessed against both Gram-positive and Gram-negative microorganisms, using appropriate standard synthetic preservatives for comparison, as well as treatments containing maltodextrin–whey protein and chitosan as wall materials with the encapsulated extract. Figure 4 shows the preservatives used for each microorganism. Each test was performed in triplicate, and the diameter of the inhibition zone was measured in millimeters and reported [21].

3-Results and Discussion

The linear and interaction effects of each independent variable (preservative concentration, storage time, and preservative type) on the response variables (peroxide and anisidine values) were analyzed using response surface methodology. As shown in Table 1, in the evaluation of linear effects, both preservative concentration and storage time had significant effects on both response variables (p-value < 0.05), whereas the effect of preservative type was not significant at the 5% level. Among the interaction effects, only the interaction between concentration and storage time was significant. Furthermore, based on the F-values, storage time had a stronger impact on peroxide and anisidine values compared to the other two variables.

Accordingly, the terms that should be included in the mathematical models for each output parameter were determined (Table 2). The greater the effect of an independent variable on the process, the higher its coefficient in the corresponding model (in the models presented in Table 2, the coefficients for storage time are considerably higher than those for the other two factors).

The significance of the models for both response indices was confirmed not only by p-values, which were both below 0.05, but also by correlation coefficients greater than 0.9 and standard deviations of 4.26 and 3.20, indicating acceptable accuracy. If the difference between the adjusted and predicted correlation coefficients is less than 0.2, the model demonstrates acceptable precision and reproducibility; in this study, these values were 0.1 and 0.05, respectively. To adequately cover the design space, the Adeq Precision should be greater than 4 [22], which, according to Table 1, was achieved in this study.

Table 1: Analysis of variance for the model for Peroxide value and Anisidine value

Source	peroxide value			Anisidine value		
	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value
Model	1438.69	8.81	0.0027	1682.12	18.24	0.0002
A-Concentration	243.99	13.45	0.0063	134.00	13.08	0.0068
B-Time	900.44	49.62	0.0001	1381.80	134.84	< 0.0001
C-Treatment	18.60	0.5126	0.6173	32.14	1.57	0.2664
AB	255.12	14.06	0.0056	105.14	10.26	0.0126
AC	4.21	0.1160	0.8920	3.91	0.1908	0.8299
BC	16.34	0.4502	0.6527	25.13	1.23	0.3432
Residual	145.16			81.98		
Cor Total	1583.85			1764.10		
R^2	0.9083			0.9535		
Adjusted R^2	0.8052			0.9012		
Adeq Precision	8.2084			11.5868		
Std. Dev.	4.26			3.20		
C.V. %	32.12			20.49		

Table 2: Final Equation in Terms of Actual Factors

indexes	treatments	Equations
peroxide value	Encapsulated sargassum extract	6.19+0.02 Concentration+1.46 Time-0.49 Concentration * Time
	TBHQ	6.65-0.46 Concentration+1.46 Time-0.49 Concentration * Time
	UnEncapsulated sargassum extract	5.41+0.68 Concentration+1.73 Time-0.49 Concentration * Time
Anisidine value	Encapsulated sargassum extract	7.04-0.65 Concentration+1.58 Time-0.31 Concentration * Time
	TBHQ	7.78-0.60 Concentration+1.36 Time-0.31 Concentration * Time
	UnEncapsulated sargassum extract	6.89+0.34 Concentration+1.74 Time-0.31 Concentration * Time

3.1 Evaluation of Linear and Interaction Effects of Parameters

Figures 1 and 2 illustrate the linear and interaction effects of the independent variables on the peroxide and anisidine values of fish oil during storage. At first glance, the combined plots indicate that increasing the concentration of different preservatives led to a reduction in both peroxide and anisidine values, whereas increasing the storage time resulted in an increase in these indices.

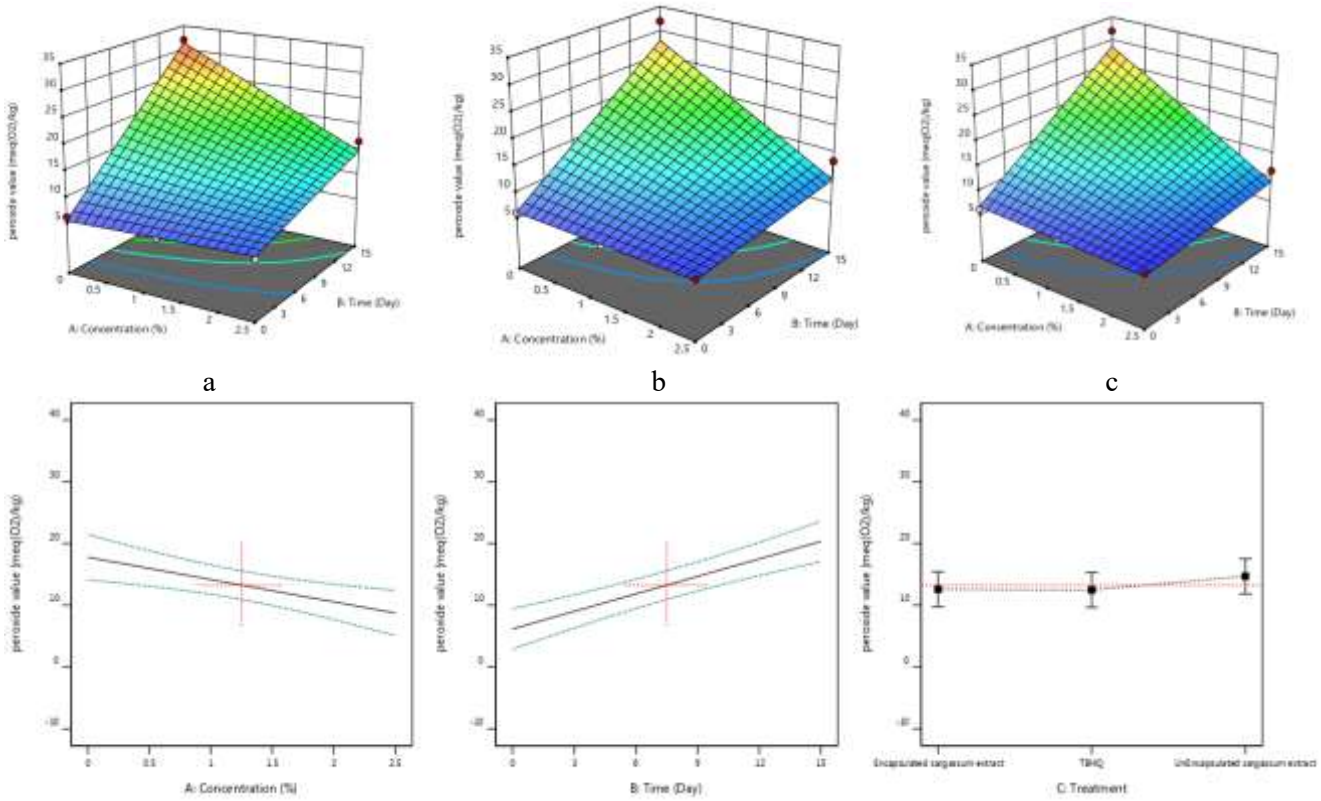
Comparing the preservatives, it can be observed that the synthetic antioxidant and the microencapsulated extracts exhibited similar effects, whereas the unencapsulated extract provided lower preservation, resulting in higher peroxide and anisidine values. In the evaluation of the combined effects of storage time and preservative concentration, the slope of

the 3D plots along the time axis was consistently steeper than along the concentration axis, indicating that storage time had a greater impact than concentration on these oxidation indices; this was also reflected in the F-values of the ANOVA table. The 3D surface plots clearly show that the highest peroxide and anisidine values occurred at the longest storage time and the lowest preservative concentrations. In these plots, the unencapsulated extracts consistently performed worse than the other two preservatives, with higher values for both indices.

Overall, given the increased oxidation indices in fish oil compared to oil enriched with microencapsulated particles, it can be concluded that the encapsulation matrix was effective in controlling oxidative processes. Peroxides in oxidizing oils are unstable and gradually convert into other oxidized compounds [23]. During the early stages of oxidation, peroxide levels increase and are subsequently converted into aldehydes and ketones [24]. Therefore, it is possible for the initially increasing trend of peroxide formation to transition into a declining trend with prolonged storage, as observed in some cases up to day 15.

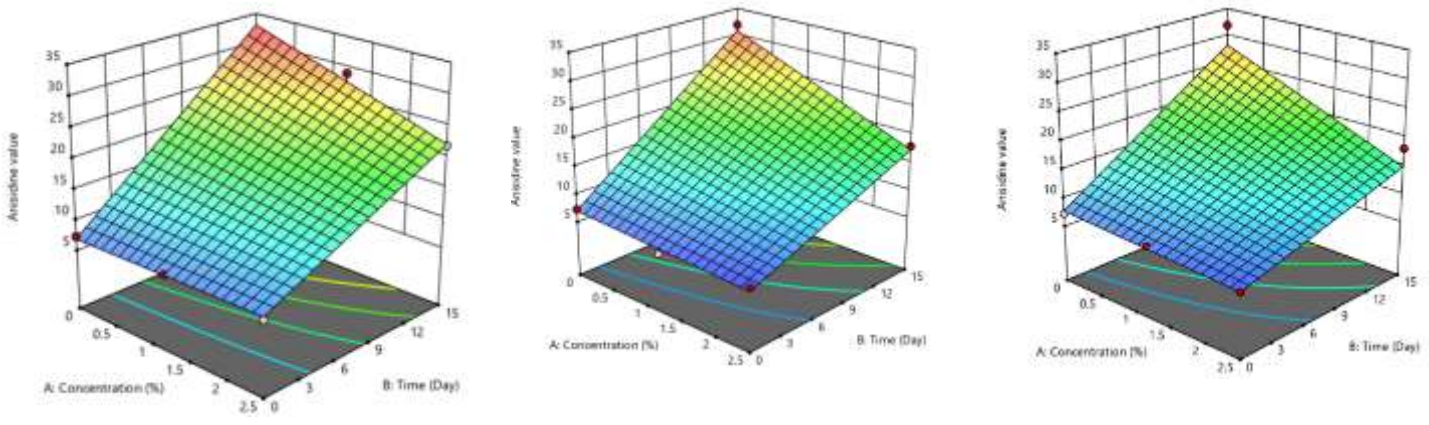
The type of wall material used for microencapsulation can play a crucial role in the oxidative protection conferred by the final preservative [25]. Considering the limited emulsifying capacity of maltodextrin and the importance of emulsion properties on the characteristics of the resulting powders, it can be concluded that the addition of whey protein significantly reduced powder oxidation. It is expected that increasing the proportion of whey protein in the wall material would further enhance the oxidative protection of the microcapsules. Examination of the results indicates that fish oil containing microencapsulated algal extracts with a whey protein–maltodextrin wall combination, especially when fortified with the synthetic antioxidant TBHQ, exhibited superior oxidative stability compared to other treatments. Therefore, it can be stated that microcapsules containing *Sargassum* extract demonstrated acceptable performance and have potential as natural antioxidants for protecting fish oil. The high antioxidant activity of *Sargassum* extracts has been confirmed in several studies [10, 26, 27].

Furthermore, the anisidine value is used to quantify secondary oxidation products, including aldehydes, ketones, and other oxidized compounds [28]. Lipid oxidation is influenced by multiple factors such as water activity, oxygen availability, and the presence or absence of antioxidants [29]. In this study, similar trends were observed for anisidine values as for peroxide values. The lowest anisidine values were recorded for the TBHQ treatment and the microencapsulated extract with a whey protein–maltodextrin wall, consistent with findings reported by Dalal et al. (2021) on the antioxidant properties of seaweed extracts. These results are unsurprising, as the simultaneous presence of carbohydrate and protein components in the wall structure enhances emulsifying properties and oxidative stability of food compounds [30]. Therefore, it can be concluded that microcapsules of *Sargassum* extract exhibit significant antioxidant activity, comparable to that of synthetic antioxidants [10].



a b c

Fig. 1. Linear and simultaneous effect of process variables on the peroxide changes in fish oil; a) UnEncapsulated sargassum extract, b) Encapsulated sargassum extract and c) TBHQ



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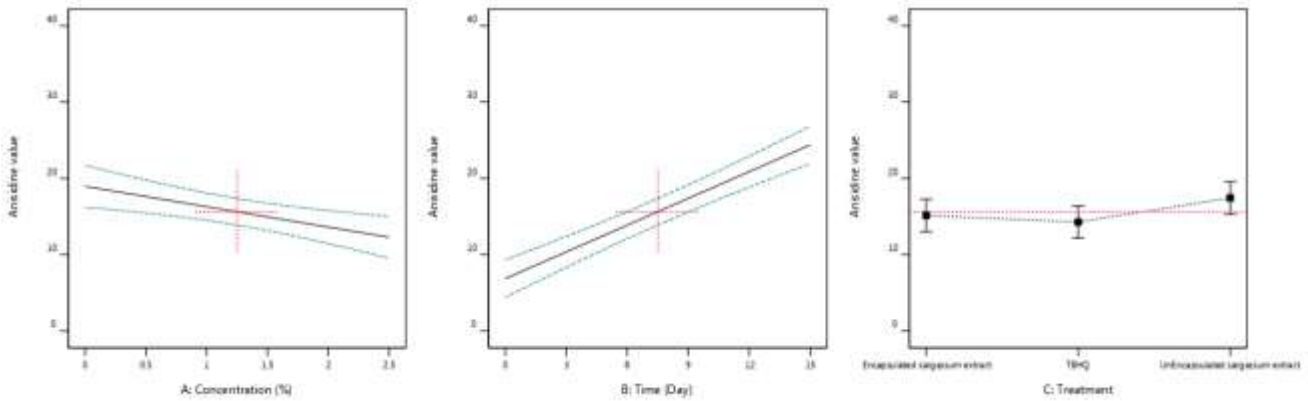


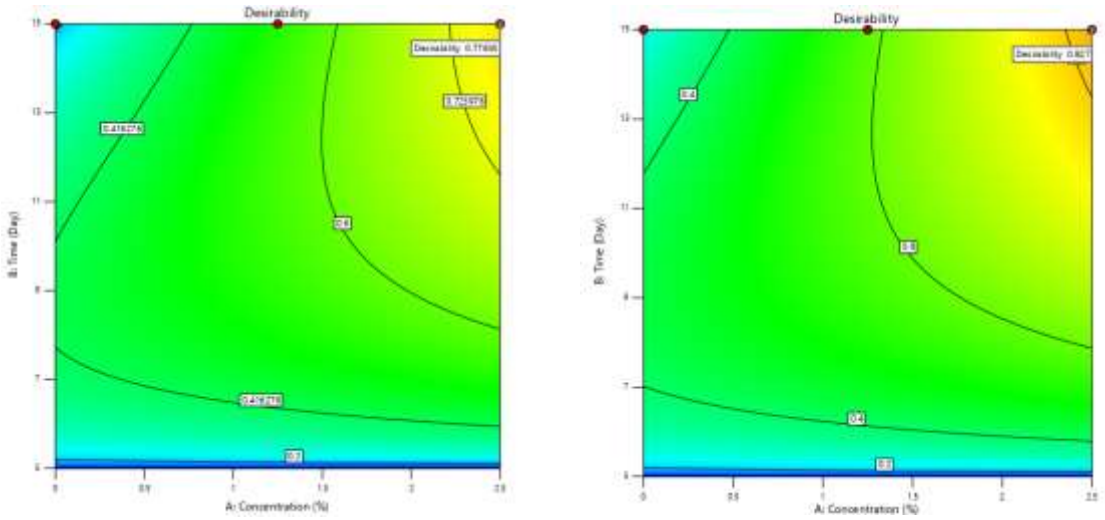
Fig. 2. Linear and simultaneous effect of process variables on the Anisidine value changes in fish oil; a) UnEncapsulated sargassum extract, b) Encapsulated sargassum extract and c) TBHQ

To determine the optimal process conditions for achieving the lowest level of oxidation in fish oil during the storage period, the optimization module of Design-Expert software was used, and the conditions presented in Table 3 were identified. Accordingly, to minimize the peroxide and anisidine values, the following criteria were set: preservative concentration within the tested range, maximum possible storage time, and the type of treatment selected from the evaluated options.

Ultimately, the suggested solutions indicated a slightly lower preference for TBHQ compared to the microencapsulated *Sargassum* extract. However, the desirability of the selected solutions (0.77–0.82) and the minimal values of peroxide and anisidine indices (particularly peroxide) showed only minor differences (Figure 3). Therefore, it can be concluded that using either of these two treatments at a concentration of 2.5% over approximately 15 days of storage can achieve relatively optimal results.

Table 3: Numerical optimization of Peroxide and Anisidine Value

Name	Constraints	Solution 1	Solution 2	Solution 3	Solution 4
A:Concentration	Is In Range	2.5	2.500	2.5	2.5
B:Time	Maximize	15	14.725	15	14.91
C:Treatment	Is In Range	TBHQ	TBHQ	Encapsulated Sargassum Extract	Encapsulated Sargassum Extract
Peroxide Value	Minimize	9.094	9.028	9.797	9.776
Anisidine Value	Minimize	14.845	14.688	17.332	17.262
Desirability	-	0.827	0.822	0.776	0.776



a b

Fig. 3. Numerical optimization of Peroxide and Anisidine Value for a) Solution 3 and b) Solution 1

2.3. Evaluation of Antimicrobial Properties

To assess antimicrobial activity, two wall formulations—one containing a 50:50 ratio of whey protein and maltodextrin, and the other pure chitosan—were evaluated against six Gram-negative and three Gram-positive bacterial strains using the well diffusion assay (24 h incubation at 37°C) with standard antibiotics as controls (Figure 4). Brown seaweeds possess secondary bioactive metabolites and other natural compounds responsible for their antibacterial activity [31]. Previous studies have shown that methanolic extracts of *Sargassum dentifolium* exhibit moderate effects against Gram-negative bacteria such as *Escherichia coli* [32]. Several researchers have also reported the antimicrobial activity of *Sargassum* extracts against Gram-positive bacteria [33]. However, limited information is available regarding the antibacterial effects of their microencapsulated forms.

In the present study, two optimized *Sargassum* extract formulations with different wall materials (chitosan at a 4:1 ratio with the extract, and whey protein–maltodextrin at a 50:50 ratio with a 12:1 extract-to-wall ratio) were evaluated for antimicrobial activity against nine pathogenic Gram-negative and Gram-positive bacterial strains. The results, expressed as the inhibition zone diameter (mm) using the well diffusion method, indicated that the treatments at a concentration of 0.5 mg/mL exhibited varying antibacterial effects against the tested Gram-positive and Gram-negative bacteria. In some cases, no inhibition zones were observed on the plates.

Comparison with the inhibition zones of standard antibiotics revealed that the Gram-positive bacterium *Bacillus subtilis* was highly sensitive to the chitosan wall treatment, showing a larger inhibition zone (19 mm) than the corresponding standard antibiotic (17 mm), which was statistically significant ($P < 0.05$). Conversely, Gram-positive bacteria such

as *Streptococcus* and *Bacillus cereus* showed lower sensitivity to this treatment, with inhibition zones smaller than those of their respective standard antibiotics. Among Gram-negative bacteria, *Proteus*, *Citrobacter*, and *Klebsiella* exhibited the highest sensitivity to the chitosan wall treatment. Notably, *Klebsiella* also showed higher sensitivity to the whey protein–maltodextrin treatment compared to its standard antibiotic, indicating effective growth inhibition in the culture medium. Moreover, *Pseudomonas* (Gram-negative) demonstrated better inhibition with this treatment compared to its standard antibiotic ($P < 0.05$).

These findings indicate that the wall composition significantly influences the antimicrobial efficacy of the microcapsules. The chitosan wall treatment showed superior antimicrobial activity compared to the whey protein–maltodextrin formulation. Chitosan and its derivatives are known for their biodegradability, biocompatibility, antimicrobial activity, non-toxicity, and favorable chemical and physical properties, making them valuable in food applications [34]. Several studies have reported that chitosan-based edible films play a crucial role in preserving food from contamination by pathogenic Gram-positive and Gram-negative bacteria, aligning with the present results [34–37]. Similarly, Goharkhani et al. (2020) demonstrated that microcapsules prepared with a higher proportion of chitosan (70%) showed stronger antimicrobial effects than those with whey protein, confirming chitosan's dominant role in antimicrobial activity [38].

It is noteworthy that among all Gram-negative and Gram-positive strains tested in this study, *Escherichia coli* and *Salmonella* were completely resistant to all treatments. This can be attributed to the higher structural resistance of Gram-negative bacterial cell walls against the penetration of external compounds, resulting in reduced efficacy of the extracts [39].

In conclusion, *Sargassum* brown seaweeds from the southern coasts of Iran are potential sources of bioactive compounds and could be utilized for the production of natural antibiotics. Nevertheless, further studies are required to identify the specific bioactive constituents in these marine algae.

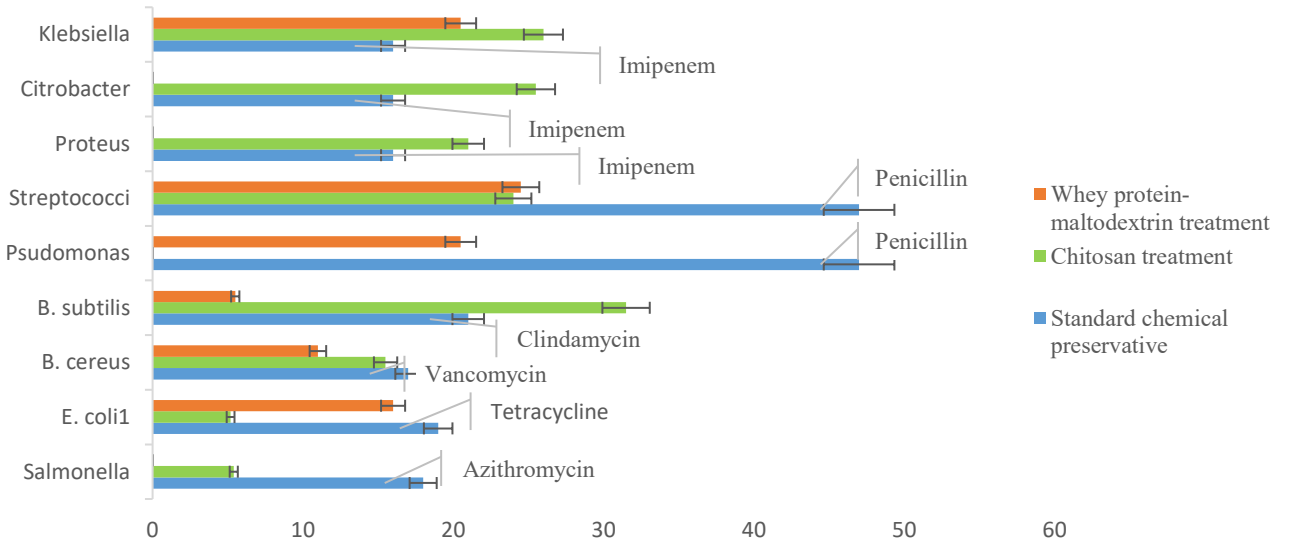


Fig. 4. Diameter of bacterial growth inhibition halo in culture medium in millimeters by well method

3.3. Conclusion

In this study, *Sargassum* seaweed extract was microencapsulated to evaluate its antioxidant protection in fish oil as well as its antimicrobial properties. The extract was converted into microcapsules using a freeze-drying system with different wall materials, including maltodextrin and whey protein, and tested for its ability to protect fish oil against oxidation (assessed by peroxide and anisidine indices). The results demonstrated that the resulting microcapsules, particularly those with a 50:50 whey protein–maltodextrin wall composition, exhibited significant antioxidant activity comparable to the commercial antioxidant TBHQ. Furthermore, microcapsules at a concentration of 2.5% were more effective than those at 1.25%, indicating that increasing the concentration enhances antioxidant efficacy. Beyond antioxidant properties, the study revealed that the microencapsulated extract, especially with a chitosan wall, was capable of inhibiting the growth of multiple Gram-positive and Gram-negative pathogenic bacteria, and in some cases, performed better than standard antibiotics. Overall, the findings indicate that microencapsulated *Sargassum* extract possesses remarkable antioxidant and antimicrobial activities, making it an effective approach for preserving the quality of fish oil and controlling microbial growth.

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مقاله علمی_پژوهشی

ارزیابی قدرت مهارکنندگی عصاره ریزپوشانی شده جلبک سارگاسوم بر اکسیداسیون چربی‌ها در روغن ماهی و میکروارگانیسم‌های پاتوژن

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چکیده

در این پژوهش ترکیب بهینه عصاره ریزپوشانی شده جلبک سارگاسوم جهت بهبود پایداری اکسیداتیو روغن ماهی و استفاده به عنوان ترکیب ضد میکروبی مورد بررسی قرار گرفت. با توجه به حساسیت اکسیداتیو روغن ماهی به دلیل ترکیب اسیدچرب خاصی که دارد، استفاده از آنتی‌اکسیدان جهت افزایش مدت ماندگاری آن اجتناب‌ناپذیر است. عصاره ریزپوشانی شده، عصاره خالص و آنتی‌اکسیدان سنتزی (TBHQ) در سه غلظت ۰، ۱/۲۵ و ۲/۵ درصد به روغن ماهی افزوده شد و در بازه زمانی ۱۵ روزه مورد بررسی قرار گرفت. از روش سطح پاسخ جهت ارزیابی تاثیر متغیرهای مستقل بر میزان پراکسید و آنیزیدین به عنوان شاخص‌های اکسیداسیون و بهینه‌سازی فرایند استفاده شد. با افزایش زمان ماندگاری میزان اکسیداسیون افزایش و با افزایش غلظت میزان شاخص‌های مورد بررسی کاهش یافت. در انتخاب تیمار بهینه نیز عصاره ریزپوشانی شده جلبک و آنتی‌اکسیدان سنتزی در غلظت ۲/۵ درصد و زمان ۱۵ روز بهترین شرایط پیشنهادی بودند. همچنین با بررسی اثرات ضدمیکروبی تیمارهای ریزپوشانی شده مشخص شد تیمارهای تهیه شده با ترکیب دیواره کیتوزان و پس از آن تیمار با ترکیب دیواره پروتئین آب پنیر و مالتودکسترین با نسبت ۵۰:۵۰ بیشترین قابلیت ضدمیکروبی را بر علیه باکتری‌های بیماری‌زای مورد بررسی از خود بروز دادند به گونه‌ای که می‌توان از این تیمارها به منظور تهیه آنتی بیوتیک‌های استاندارد جایگزین بهره جست.