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Effect of chlorophyll in alginate-based edible film in inhibiting spoilage of fish snacks

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Abstract

Edible films are environmentally biodegradable materials used for food packaging. The edible green alga *Caulerpa racemosa* has antimicrobial properties; however, its chlorophyll-based bioactive compounds can be damaged when heated so it is prepared in microcapsules. Our research evaluated the effect of *Caulerpa* microcapsules on an alginate-based edible film on film properties and food spoilage. The microcapsules were used at concentrations of 0%, 0.5%, 1%, and 1.5%. The edible film was measured for film properties, total phenolic content (TPC), antioxidant activity (DPPH), functional group (FTIR) and microstructure (SEM). Food spoilage was evaluated on a popular fish-based product (fish bubble snacks). Bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were used to evaluate antimicrobial effectiveness of the edible films. The addition of *Caulerpa* microcapsules had no significant negative effect on physical properties of the alginate-based film, while the smoother and more homogenous surface should enhance the barrier properties of the film. The slow and evenly distributed release of active compounds from the microcapsules increased resistance to *Rhizopus* sp. and significantly reduced the proliferation of *E. coli* but not *S. aureus* on coated fish snacks. *Caulerpa racemosa* can be used to enhance the effectiveness of alginate-based films in delaying spoilage and could extend product shelf-life.

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1. Introduction

An edible film is a thin edible layer that is used to coat food and can be consumed together with this food. The role of the edible film is to prevent external contamination and retard the deterioration of food so that food quality and safety can be maintained (1). Alginates are complex polysaccharides containing mannuronic and guluronic acids (2). They can form stable gels at high and low temperatures and in low pH environments and can be used for numerous applications in food processing (3). Glycerol is one of the plasticizers that can be added to alginate-based edible films to improve the strength, solubility and elasticity (4).

Ideally, a high-quality edible film will also contain substances that can retard food spoilage, for example through enrichment with natural antimicrobial agents (5,6). Chlorophyll

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extracts from green algae have shown antibacterial activity (7). The bioactive compounds in crude phenolic extract of the green alga *Caulerpa racemosa* have antibacterial properties (8–11). Furthermore, the bioactive compounds from the methanolic fraction have bactericidal and larvicidal properties (9,12). However, bioactive chlorophyll compounds are easily damaged when heated, for example during food processing or cooking (13–15).

Microencapsulation is a nanotechnology method which can be used to prevent damage to bioactive compounds by protecting the encapsulated compounds during edible film processing (16). This technology has applied in several industries, including the pharmaceutical industry, and in medical applications (17,18). Microencapsulation methods used to increase the stability of bioactive compounds include spray drying and freeze drying (16,19). While spray drying is arguably the most popular method (16), and has shown advantages for some compounds, in particular greater regularity in the size and shape of the nanoparticles produced (19,20), this method can damage compounds sensitive to elevated temperatures (21). Freeze drying, also known as lyophilisation, is relatively simple, carried out at low temperatures, and also avoids oxidation or chemical modification (21). Freeze dried microencapsulated bioactive compounds can also be more stable and less prone to damage in cold or hot temperatures (22,23).

The presence and growth of fungi is related to the moisture content of food and that many fresh food products are readily susceptible to microbial spoilage (24), while the range of water activity (aw) of foods with intermediate moisture content is conducive for fungal growth (25). According to Nagaraj et al., *Caulerpa racemosa* could be an antifungal and/or antibacterial agent, as it contains phenolic compounds (9). Freeze dried microencapsulated chlorophyll extracts from *C. racemosa* are stable and can release potentially antimicrobial compounds in a slow and controlled manner (26).

Based on these considerations, encapsulated chlorophyll from *C. racemosa* could be applied as a potential solution to prevent some types of microbial food spoilage by enhancing the antimicrobial activities of protective edible film. This study evaluated the physical properties of an alginate-based edible film enriched with microcapsules containing chlorophyll from *C. racemosa* and its effectiveness as a barrier for preventing microbial growth. The rationale was that the enriched edible film would contain antimicrobial agents that could be released gradually over time and thus extend the shelf-life of the product.

2. Materials and Methods

2.1. Collection of Materials and Preparation

Fresh *Caulerpa racemosa* was harvested from Karimunjawa Island, Central Java, Indonesia. The macroalgal sample was dried in a shady place for 4-5 days to reduce its water content to 80-20%. Alginate (Merck, Germany) was obtained from CV. Total Equipment Pharmacy in Semarang, Indonesia. Glycerol (Merck, Germany) and distilled water were purchased from Indrasari Chemical Agencies, Semarang, Indonesia. The ingredients for fish bubble snacks were purchased from the local market in Semarang, Indonesia.

The extraction of active compounds from fresh *Caulerpa racemosa* was based on (27) with modification. The *C. racemosa* was first dried and then a weighing 100 grams was chopped and macerated at room temperature through total immersion in 400 ml of industrial ethanol (Merck, Germany) as the solvent. Ethanol is safe for consumption so it is used as a solvent in this study. Ethanol is a solvent known to be able to extract bioactive components such as curcumin and anthocyanins with the highest total phenol content and antioxidant

activity compared to methanol, water, and acetone (28–30). The sample was macerated for ± 48 h then filtered. Residual ethanol was removed from the *C. racemosa* extract through rotary evaporation (RE-2000E, China). The extract was then freeze-dried (Ningbo Yinzhou Sjia Lab Equipment Co, Ltd).

The microencapsulation process was performed using a solution of 10% Caulerpa extract, 1% Tween 80 (polyoxyethylene sorbitan monooleate, a non-ionic and non-toxic surfactant) (Merck, Germany), 10% coating materials, and distilled water to reach 100%. The coating materials used were fish gelatine and Arabic gum in the ratio 0.5:9.5 (0.5% and 9.5% of the mixture). The mixture was homogenized (Ultra Turrax Homogenizer WiseTis HG-15D, Germany) at 10,000 rpm for 3 minutes. The homogenized solution was then frozen at -35°C for 24 h (23). The frozen samples were then freeze dried at -100°C vacuum pressure for ± 48 h.

The edible film was prepared by adding 2% alginate to distilled water. The alginate was dissolved on a hot plate with a magnetic stirrer to prevent coagulation. Glycerol (2.5 %) was added when the temperature reached 50°C, and the mixture was then stirred at a moderate speed for 30 min. *Caulerpa* microcapsules were added in three different concentrations: 0.5%, 1%, and 1.5%, with 0% as a control. Edible film composition was prepared based on Table 1. The mixture was homogenized and then poured onto a glass plate and oven dried at 60°C for 4 h.

Table 1. Edible film composition for each of the (100 ml) solutions prepared.

	Control	A	B	C
Alginate (%)	2	2	2	2
Glycerol (%)	1.5	1.5	1.5	1.5
Caulerpa mirocapsules (%)	0	0.5	1	1.5
Distilled water	97.5	97	96.5	96

A dough was made by combining the dough ingredients (mashed potatoes, wheat flour, eggs, garlic, pepper, and sugar) in a mixer. Small balls of dough (called bubbles) with a diameter of approximately 2 cm were formed and stuffed with raw barracuda fish filleted. These bubbles were then fried, coated in beaten egg and covered with breadcrumbs and kept at -18°C for 24 hours.

2.2. Edible Film Analysis

The analysis for edible film including several tests. The thickness of the edible film was measured at five points using a screw micrometre (Mitutoyo, Japan). Tensile strength and percent elongation were measured using a Universal Testing Machine (Texture Analyser CT3 Brookfield, USA). Small rectangular strips (5 mm wide x 50 mm long) were cut from the edible film. Tensile strength and the Young Modulus (MPa) were determined based on maximum force per unit area (Newton/m^2) exerted on the film before it failed (ripped). Percent elongation (in %) was obtained by dividing the difference between the maximum (just before failure) and initial length (50 mm) by the initial film length then multiplying the result by 10 following (31).

The gravimetric method was applied to measure Water Vapour Transmission Rate (WVTR). The edible film samples, together with 10 grams of silica gel (RH=0%), were placed in a jar containing NaCl 40% (w/v) (RH=75%) at 25°C. The silica gel absorbed the water vapour transmission escaping through the film (32).

Total phenolic content was measured based on (33). Folin-Ciocalteu reagent was applied to the sample and the results expressed in Gallic acid equivalent per gram of extract. Antioxidant activity (DPPH) was measured by adding 1 mL of 2.2-diphenyl-1-picrylhydrazil (DPPH) to a sample which was placed in methanol, then homogenized and stored in a dark room at room temperature for 25 minutes. The absorbance was then read using a UV-Vis spectrophotometer (Shimadzu, Japan) at 760 nm. The percentage of DPPH free radicals was calculated following (11). The functional group was analysed through Fourier Transform Infrared Spectroscopy (FTIR) at wavelengths of 400-4000 cm^{-1} following (11).

The edible film microstructure was subjected to scanning electron microscopy (SEM) according to (11). The samples were attached to a SEM unit (Jeol JSM I 00 651 OLA, Japan) using a two-sided adhesive tape and then coated. The SEM was operated at 20 kV with a magnification of 1000X and the surface morphology was recorded as a scaled digital image.

Bacterial strains of *Escherichia coli* and *Staphylococcus aureus* were used to evaluate the antimicrobial effectiveness of edible film in this study. Bacterial samples were prepared from beef (10 g) and placed in Stomacher bags with 90 ml of Mueller Hinton solution in a Colworth Stomacher 400 (Seward Ltd., England). These were then incubated for 18 h at 37°C before the test, following (34). Circles of edible film with a diameter of 5 mm were laid on top of agar medium (set in a petri dish) which had been spread with 0.1 ml of fungal culture. The petri dish then was incubated at a temperature of 25°C for 72 h. After the incubation period, the diameter of the inhibition zone and the diameter of the clear zone formed were measured following (35).

2.3. Statistical Analysis

All procedures were performed in triplicate (3 replicates) and results presented (tabulated) as mean ± standard deviation (SD). Data were analysed through analysis of variance (ANOVA). If there were any significant differences between treatments, a post-hoc Tukey test was conducted. Significance was evaluated at the 95% confidence level ($\alpha = 0.05$). Statistical analyses were performed in SPSS Software version 21.

3. Results and Discussion

3.1. Edible Film Physical Characteristics

The addition of *C. racemosa* microcapsules increased the thickness of the edible film and affected several other properties (Table 2). The microcapsules were in powder form and increased the total mass.

Table 2. Thickness, tensile strength, elongation, young modulus, and WVTR (mean ± standard deviation) of the edible film enriched with chlorophyll microcapsules and the control.

Sample type (treatment)	Thickness (mm)	Tensile strength (Mpa)	Elongation (%)	Young modulus (Mpa)	WVTR (g/mm^2s)
Control	0.02 ± 0.01^{a}	4.70 ± 0.07^{d}	13.57 ± 0.03^{c}	0.32 ± 0.01^{b}	253.93 ± 0.01^{b}
Microcapsules 0.5% (A)	0.03 ± 0.01^{b}	2.96 ± 0.02^{c}	10.51 ± 0.44^{b}	0.27 ± 0.01^{a}	280.13 ± 0.01^{c}
Microcapsules 1% (B)	0.04 ± 0.02^{c}	2.85 ± 0.01^{b}	7.27 ± 0.00^{a}	0.39 ± 0.01^{d}	253.92 ± 0.56^{b}
Microcapsules 1.5% (C)	0.05 ± 0.03^{d}	2.46 ± 0.08^{a}	7.17 ± 0.02^{a}	0.34 ± 0.01^{c}	228.53 ± 0.67^{a}

The mean value followed by different letters showed a significant difference at the 5% level (p-value < 0.05).

The thickness of the edible film is influenced by the raw materials and the addition of active ingredients, as stated in (31). The addition of *C. racemosa* increased the soluble solids,

and therefore thickness was also increased. A similar effect was described from the addition of oregano essential oil (36). It is likely that the increased thickness affected the tensile strength, elongation, young modulus, and WVTR.

Tensile strength, elongation, and WVTR decreased with increased microcapsule content. Furthermore, the glycerol used as a plasticizer also increased the film matrix in the edible film, as found by (37). The alginate in an edible film influence both the elongation and tensile strength (38). Savoury Essential Oil (SEO) added to nanocomposites can decrease tensile strength and Young modulus and increase percent elongation (17). The WVTR is influenced by the ratio between hydrophobic and hydrophilic materials, as well as the thickness: the thicker the edible film, the lower the WVTR, thought to be due to the increased time needed for the diffusion of water vapour (39).

The total phenolic content in edible film enriched with *C. racemosa* microcapsules (Table 3) showed that the TPC and antioxidant activity also increased at higher enrichment rates. The control (unenriched) edible film had a TPC of 0.89±0.01 mg GAE/g.

Table 3. Total Phenolic Content (TPC) and antioxidant activity (DPPH) of the edible film enriched with chlorophyll microcapsules and the control.

Samples	Total Phenolic Content (TPC)(mg GAE/g)	DPPH Imbibition (%)
Control	0.89±0.01[a]	9.34±0.50[a]
Microcapsules 0.5% (A)	4.29±0.01[b]	21.74±0.02[b]
Microcapsules 1% (B)	6.67±0.20[c]	56.57±0.10[c]
Microcapsules 1.5% (C)	8.17±0.01[d]	61.72±0.01[d]

The mean value followed by different letters showed a significant difference at the 5% level (p-value < 0.05).

The TPC level increased in line with addition of chlorophyll microcapsules. This is similar to the results of (40) who found that both the total phenol content and the antioxidant content of edible film increased with the addition of oregano oil or black cumin oil. TPC likely affected the antioxidant activity, which also increased with higher microcapsule enrichment. Measuring the DPPH is a widely accepted quantitative test for antioxidant activity (41). The antioxidant activity reached an optimum DPPH scavenging activity level of around 62% with the highest *C. racemosa* microcapsule enrichment level (1.5%).

3.2. Antimicrobial Activity

An inhibition zone size of 1.30–2.95 mm was observed for the bacterium *E. coli*. However, no inhibition zone was observed for *S. aureus* (Table 4).

Table 4. Inhibitation Zone of *S. aureus* and *E. Coli* on the edible film enriched with chlorophyll microcapsules and the control.

Samples	Inhibitation Zone of *S. aureus* (mm)	Inhibitation Zone of *E. coli* (mm)
Control	Resistant	0.75±0.01[a]
Microcapsules 0.5% (A)	Resistant	1.30±0.02[b]
Microcapsules 1% (B)	Resistant	1.85±0.01[c]
Microcapsules 1.5% (C)	Resistant	2.95±0.01[d]

The mean value followed by different letters showed a significant difference at the 5% level (p-value < 0.05).

The phenolic extract of *Caulerpa* showed some activity against *E. coli*. Gram-positive microorganisms (*S. aureus* and *B. cereus*) have been found more susceptible to the Articoat DLP 02 (AR) than the Gram-negative group (34). High antibacterial activity against *Erwinia amylovora* and *Pseudomonas* sp. is reported from films enriched with Gallic phenolic acids (42). The addition of savoury essential oil (SEO) to agar-cellulose nanocomposite films was less effective against Gram-negative bacteria (E. coli) than Gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*) (17). Alginate film enriched with essential oil of oregano has also been reported as significantly more effective against Gram-positive than against Gram-negative bacteria (36). Alginate-based edible film combined with cinnamon oil obtained an inhibition zone of 36.00 mm against *S. aureus* (43), while edible alginate can prolong shelf-life of fresh fruit for up to 28 days (44).

3.3. Antifungal Activity

With respect to antifungal activity, *Rhizopus* type fungi were observed on the surface of fish bubble snacks after storage at room temperature (Table 5). Fungal growth appeared much more rapidly on the control (no microcapsule enrichment) than on the product treated with chlorophyll microcapsule enriched film, indicating an antifungal activity of the chlorophyll microcapsules at all levels (0.5%, 1%, 1.5%) tested in this study.

Table 5. The growth of fungi on fish bubble snacks coated with edible film with and without chlorophyll microcapsule enrichment.

Type of Product	Day 3	Day 5
Control (no chlorophyll microcapsule enrichment)	Dark coloured fungi began to grow 	The fungi spread over the entire surface of the product, with a fibrous appearance. Positive: *Rhizopus* sp. 
Fish potato bubble snack with chlorophyll microcapsule enriched edible film coating	Negative (no fungal growth) 	Fungi began to grow and spread, fibrous appearance 

3.4. Edible Film Physical Characteristics

Fourier Transform Infrared Spectroscopy (FTIR) detected the presence of the C-H functional group in the three edible film formulations with chlorophyll microcapsules on the edible film (Figure 1 a,b,c), but not in the control (Figure 1d).

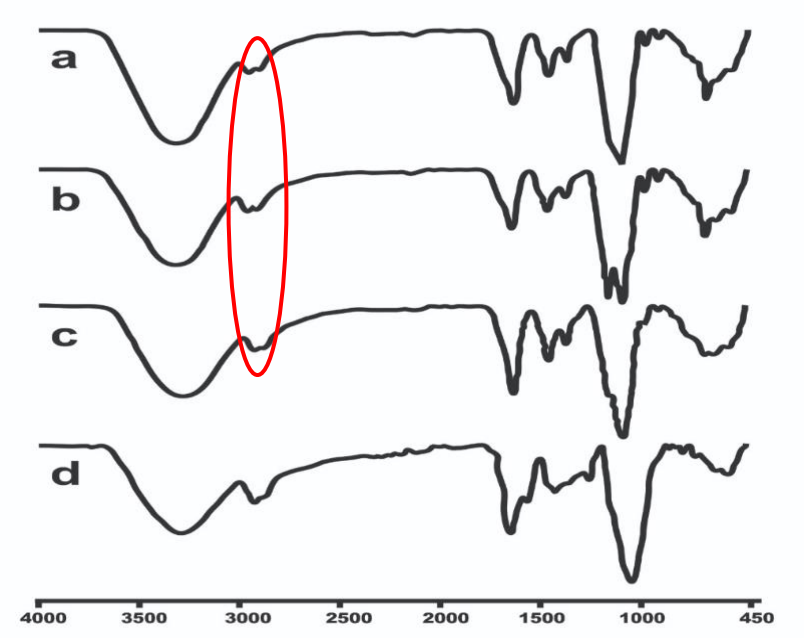
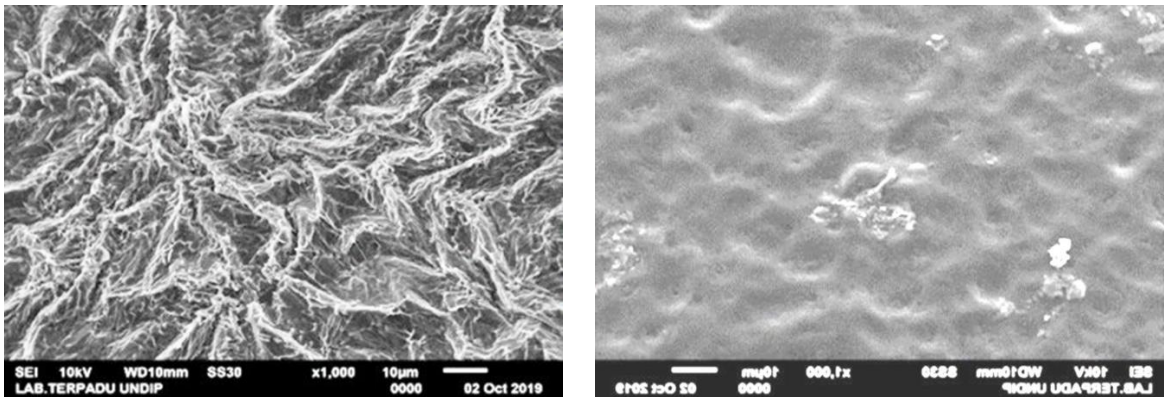


Figure 1. FTIR Spectra of Edible Films. Chlorophyll microcapsule treatments a = 0.5%, b = 1%, and c = 1.5%.

Chlorophyll has a C-O aldehyde functional group at a wavelength of 1583-1709 cm^{-1}, C-H groups at 2809-3012 cm^{-1}, and O-H groups at 3029-3639 cm^{-1} (45). The C-H group is a determining group for chlorophyll compounds because it is located close to the tetrapyrrole ring bonded to metal ions (46). Based on the FTIR spectra, the edible film contained chlorophyll microcapsules, which were detected by the presence of a C-H group expressing chlorophyll and appeared at a wavelength around 2900 cm^{-1}. This group was not detected in control (d). The interaction of chlorophyll microcapsules with edible films is indicated by a shift in wavelength, especially for the C-O and O-H groups (47). The O-H group shifted from 1413 to 1414 cm^{-1} while the C-O shifted from 1590 to 1594 cm^{-1}. This indicates that there was an interaction between the chlorophyll microcapsules and the edible film.

3.5. Film Microstructure - Scanning Electron Microscopy (SEM)

The addition of microencapsulated chlorophyll affected the microscopic morphology of the edible film. The SEM images (Figure 2) show a clearly visible difference between the surface texture of edible film with and without chlorophyll microcapsule enrichment. The control film (no enrichment) had a rough surface (Figure 2a), while the addition of microcapsules produced a much flatter, smoother and more homogenous surface (Figure 2b). This characteristic should enhance the barrier properties of the edible film (48). The surface of the film was homogeneous and smooth, indicating that there was no agglomeration and the microcapsules were evenly distributed on the surface of the film. The results of this study are in accordance with that shown by (49) who found that microcapsules with gelatine and gum Arabic coating materials can form cross-links with the film matrix so that they can be dispersed into the film. This causes the mechanical properties of the film to be better than the control.



(a) (b)

Figure 2. Scanning electron microscopy of edible films: (a) without chlorophyll microcapsules; (b) with the addition of microcapsules.

4. Conclusions

The enrichment of alginate-based edible film with chlorophyll microcapsules enabled the chlorophyll microcapsules to blend into the alginate-based edible film where they migrated and slowly released compounds which were able to prevent or retard some microbial growth on the fish bubble snack product tested. The enrichment increased the film thickness, improved the surface texture of the film, increased the resistance of the coated food (fish bubble snacks) to the growth of the mould *Rhizopus* sp., and increased resistance to the proliferation of *E. coli* but not of *S. aureus*. At room temperature the antifungal effect was twice as strong as for the same product without enrichment of the edible film coating. The antifungal properties of the enriched edible film extended the shelf-life of the product tested.

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Author Contributions

E.N.D. and A.C.M.A.R.T. conceived and designed the experiments; L.P. performed the experiments; M.Y. and E.A.S. analyzed the data; E.N.D. and L.P. contributed reagents/materials/analysis tools; E.N.D. and J.F.A. wrote the paper.

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Institutional Review Board Statement

Available data are presented in the manuscript.

Data Availability Statement

Not applicable.

Conflicts of Interest

Authors may declare no conflict of interest.

References

1. Ramos M, Valdés A, Beltran A, Garrigós MC. Gelatin-based films and coatings for food packaging applications. Coatings [Internet]. 2016;6(4):41. Available from: https://doi.org/10.3390/coatings6040041
2. Ertesvåg H. Alginate-modifying enzymes: biological roles and biotechnological uses. Front Microbiol [Internet]. 2015;6:523. Available from: https://doi.org/10.3389/fmicb.2015.00523
3. Senturk Parreidt T, Müller K, Schmid M. Alginate-based edible films and coatings for food packaging applications. Foods [Internet]. 2018;7(10):170. Available from: https://doi.org/10.3390/foods7100170
4. Togas C, Berhimpon S, Montolalu RI, Dien HA, Mentang F. Characteristic of edible film from composite carrageenan and bee wax by nanoemulsion. J Pengolah Has Perikan Indones. 2017;20:468–477.
5. Tavassoli-Kafrani E, Shekarchizadeh H, Masoudpour-Behabadi M. Development of edible films and coatings from alginates and carrageenans. Carbohydr Polym [Internet]. 2016;137:360–74. Available from: https://doi.org/10.1016/j.carbpol.2015.10.074
6. Bustos C RO, Alberti R F V, Matiacevich SB. Edible antimicrobial films based on microencapsulated lemongrass oil. J Food Sci Technol [Internet]. 2016;53(1):832–9. Available from: https://doi.org/10.1007/s13197-015-2027-5
7. Kustov A V, Belykh D V, Smirnova NL, Venediktov EA, Kudayarova T V, Kruchin SO, et al. Synthesis and investigation of water-soluble chlorophyll pigments for antimicrobial photodynamic therapy. Dye Pigment [Internet]. 2018;149:553–9. Available from: https://doi.org/10.1016/j.dyepig.2017.09.073
8. Chan YS, Ong CW, Chuah BL, Khoo KS, Chye FY, Sit NW. Antimicrobial, antiviral and cytotoxic activities of selected marine organisms collected from the coastal areas of Malaysia. J Mar Sci Technol [Internet]. 2018;26(1):13. Available from: https://jmstt.ntou.edu.tw/journal/vol26/iss1/13
9. Nagaraj SR, Osborne JW. Bioactive compounds from Caulerpa racemosa as a potent larvicidal and antibacterial agent. Front Biol (Beijing) [Internet]. 2014;9(4):300–5. Available from: https://doi.org/10.1007/s11515-014-1312-4
10. Rusli A, Metusalach M, Tahir MM, Salengke S, Syamsuar S. Analysis of bioactive compounds of Caulerpa recemosa, Sargassum sp. and Gracillaria verrucosa using different solvents. J Teknol [Internet]. 2016;78(4–2). Available from: https://doi.org/10.11113/jt.v78.8146
11. Yap W-F, Tay V, Tan S-H, Yow Y-Y, Chew J. Decoding antioxidant and antibacterial potentials of Malaysian green seaweeds: Caulerpa racemosa and Caulerpa lentillifera. Antibiotics [Internet]. 2019;8(3):152. Available from: https://doi.org/10.3390/antibiotics8030152
12. Alshalmani SK, Zobi NH, Bozakouk IH. Antibacterial activity of Libyan seaweed extracts.

Int J Pharm Sci Res [Internet]. 2014;5(12):5425. Available from: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(12).5425-29
13. Cervantes-Paz B, Yahia EM, de Jesús Ornelas-Paz J, Victoria-Campos CI, Ibarra-Junquera V, Pérez-Martínez JD, et al. Antioxidant activity and content of chlorophylls and carotenoids in raw and heat-processed Jalapeño peppers at intermediate stages of ripening. Food Chem [Internet]. 2014;146:188–96. Available from: https://doi.org/10.1016/j.foodchem.2013.09.060
14. Benlloch-Tinoco M, Kaulmann A, Corte-Real J, Rodrigo D, Martínez-Navarrete N, Bohn T. Chlorophylls and carotenoids of kiwifruit puree are affected similarly or less by microwave than by conventional heat processing and storage. Food Chem [Internet]. 2015;187:254–62. Available from: https://doi.org/10.1016/j.foodchem.2015.04.052
15. Aparicio-Ruiz R, Mínguez-Mosquera MI, Gandul-Rojas B. Thermal degradation kinetics of chlorophyll pigments in virgin olive oils. 1. Compounds of series a. J Agric Food Chem [Internet]. 2010;58(10):6200–8. Available from: https://doi.org/10.1021/jf9043937
16. Umaraw P, Verma AK. Comprehensive review on application of edible film on meat and meat products: An eco-friendly approach. Crit Rev Food Sci Nutr [Internet]. 2017;57(6):1270–9. Available from: https://doi.org/10.1080/10408398.2014.986563
17. Atef M, Rezaei M, Behrooz R. Characterization of physical, mechanical, and antibacterial properties of agar-cellulose bionanocomposite films incorporated with savory essential oil. Food Hydrocoll [Internet]. 2015;45:150–7. Available from: https://doi.org/10.1016/j.foodhyd.2014.09.037
18. da Silva BV, Barreira JCM, Oliveira MBPP. Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies. Trends Food Sci Technol [Internet]. 2016;50:144–58. Available from: https://doi.org/10.1016/j.tifs.2015.12.007
19. Rezende YRRS, Nogueira JP, Narain N. Microencapsulation of extracts of bioactive compounds obtained from acerola (Malpighia emarginata DC) pulp and residue by spray and freeze drying: Chemical, morphological and chemometric characterization. Food Chem [Internet]. 2018;254:281–91. Available from: https://doi.org/10.1016/j.foodchem.2018.02.026
20. Dias MI, Ferreira ICFR, Barreiro MF. Microencapsulation of bioactives for food applications. Food Funct [Internet]. 2015;6(4):1035–52. Available from: https://doi.org/10.1039/C4FO01175A
21. Ozkan G, Franco P, De Marco I, Xiao J, Capanoglu E. A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. Food Chem [Internet]. 2019;272:494–506. Available from: https://doi.org/10.1016/j.foodchem.2018.07.205
22. Cortés-Rojas DF, Souza CRF, Oliveira WP. Encapsulation of eugenol rich clove extract in solid lipid carriers. J Food Eng [Internet]. 2014;127:34–42. Available from: https://doi.org/10.1016/j.jfoodeng.2013.11.027
23. Wilkowska A, Ambroziak W, Czyzowska A, Adamiec J. Effect of microencapsulation by spray-drying and freeze-drying technique on the antioxidant properties of blueberry (Vaccinium myrtillus) juice polyphenolic compounds. Polish J Food Nutr Sci [Internet]. 2016;66(1):11. Available from: https://doi.org/10.1515/pjfns-2015-0015
24. Garnier L, Valence F, Mounier J. Diversity and control of spoilage fungi in dairy products: An update. Microorganisms [Internet]. 2017;5(3):42. Available from:

https://doi.org/10.3390/microorganisms5030042
25. Leyva Salas M, Mounier J, Valence F, Coton M, Thierry A, Coton E. Antifungal microbial agents for food biopreservation—A review. Microorganisms [Internet]. 2017;5(3):37. Available from: https://doi.org/10.3390/microorganisms5030037
26. Kurniasih RA, Dewi EN, Purnamayati L. Effect of different coating materials on the characteristics of chlorophyll microcapsules from Caulerpa racemosa. In: IOP Conference Series: Earth and Environmental Science [Internet]. IOP Publishing; 2018. p. 12030. Available from: https://10.0.4.64/1755-1315/116/1/012030
27. Tarman K, Madduppa H, Januar HI. Metabolite profiles and antioxidant activity of Caulerpa racemosa with different handlings. Squalen Bull Mar Fish Postharvest Biotechnol [Internet]. 2018;13(3):93–100. Available from: https://doi.org/10.15578/squalen.v13i3.355
28. Array EJ, Tonfack Djikeng F, Kingne Kingne F, Kinge EE, Womeni HM. Effect of different extraction solvents on the phenolic content and antioxidant activity of turmeric (Curcuma longa) from South-West Region, Cameroon. Food Res. 2018;3:86–90.
29. Hikmawanti NPE, Fatmawati S, Asri AW. The effect of ethanol concentrations as the extraction solvent on antioxidant activity of Katuk (Sauropus androgynus (L.) Merr.) leaves extracts. In: IOP Conference Series: Earth and Environmental Science [Internet]. IOP Publishing; 2021. p. 12060. Available from: doi:10.1088/1755-1315/755/1/012060
30. Thao NL, Dao TKT, Le PT, Than TUX, DSM NTNT. Effect of ethanol on the anthocyanin extraction from the purple rice of Vietnam. J Food Nutr Sci [Internet]. 2015;3(1–2):45–8. Available from: htpps://doi.org/10.11648/j.jfns.s.2015030102.18
31. Arham R, Mulyati MT, Metusalach M, Salengke S. Physical and mechanical properties of agar based edible film with glycerol plasticizer. Int Food Res J. 2016;23(4):1669.
32. Adjouman YD, Nindjin C, Tetchi FA, Dalcq A-C, Amani NG, Sindic M. Water vapor permeability of edible films based on improved cassava (Manihot esculenta Crantz) native starches. J Food Process Technol [Internet]. 2017;8:665. Available from: htpps://doi.org/10.4172/2157-7110.1000665
33. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants [Internet]. 2019;8(4):96. Available from: https://doi.org/10.3390/plants8040096
34. Clarke D, Molinaro S, Tyuftin A, Bolton D, Fanning S, Kerry JP. Incorporation of commercially-derived antimicrobials into gelatin-based films and assessment of their antimicrobial activity and impact on physical film properties. Food Control [Internet]. 2016;64:202–11. Available from: https://doi.org/10.1016/j.foodcont.2015.12.037
35. Utami R, Nurhartadi E, Putra AYT. Pengaruh penambahan minyak atsiri kunyit putih (Kaempferia rotunda) pada edible film pati tapioka terhadap aktivitas antimikroba dan sensoris. J Teknosains Pangan. 2013;2(2).
36. Benavides S, Villalobos-Carvajal R, Reyes JE. Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. J Food Eng [Internet]. 2012;110(2):232–9. Available from: https://doi.org/10.1016/j.jfoodeng.2011.05.023
37. Sinaga LL, Sinaga MS. Karakteristik edible film dari ekstrak kacang kedelai dengan penambahan tepung tapioka dan gliserol sebagai bahan pengemas makanan. J Tek Kim USU. 2013;2(4):12–6.
38. Galus S, Lenart A, Voilley A, Debeaufort F. Effect of oxidized potato starch on the

physicochemical properties of soy protein isolate-based edible films. Food Technol Biotechnol. 2013;51(3):403–9.
39. Wulandari Y, Warkoyo NH. Characterization of edible film from starch of taro (Colocasia esculenta (L.) Schott) with addition of chitosan on dodol substituted seaweed (Eucheuma cottonii L.). Food Technol Halal Sci J [Internet]. 2018;1(1):22–32. Available from: https://doi.org/10.22219/fths.v1i1.7544
40. Suput D, Lazic V, Pezo L, Markov S, Vastag Z, Popovic L, et al. Characterization of starch edible films with different essential oils addition. Polish J Food Nutr Sci [Internet]. 2016;66(4). Available from: https://doi.org/10.1515/pjfns-2016-0008
41. Nguyen VT, Ueng J, Tsai G. Proximate composition, total phenolic content, and antioxidant activity of seagrape (Caulerpa lentillifera). J Food Sci [Internet]. 2011;76(7):C950–8. Available from: https://doi.org/10.1111/j.1750-3841.2011.02289.x
42. Alkan D, Yemenicioğlu A. Potential application of natural phenolic antimicrobials and edible film technology against bacterial plant pathogens. Food Hydrocoll [Internet]. 2016;55:1–10. Available from: https://doi.org/10.1016/j.foodhyd.2015.10.025
43. Frank K, Garcia C V, Shin GH, Kim JT. Alginate biocomposite films incorporated with cinnamon essential oil nanoemulsions: Physical, mechanical, and antibacterial properties. Int J Polym Sci [Internet]. 2018;2018. Available from: https://doi.org/10.1155/2018/1519407
44. Guerreiro AC, Gago CML, Faleiro ML, Miguel MGC, Antunes MDC. The effect of alginate-based edible coatings enriched with essential oils constituents on Arbutus unedo L. fresh fruit storage. Postharvest Biol Technol [Internet]. 2015;100:226–33. Available from: https://doi.org/10.1016/j.postharvbio.2014.09.002
45. Duygu DY, Udoh AU, Ozer TB, Akbulut A, Erkaya IA, Yildiz K, et al. Fourier transform infrared (FTIR) spectroscopy for identification of Chlorella vulgaris Beijerinck 1890 and Scenedesmus obliquus (Turpin) Kützing 1833. African J Biotechnol [Internet]. 2012;11(16):3817–24. Available from: https:doi.org/ 10.5897/AJB11.1863
46. Younis U, Rahi AA, Danish S, Ali MA, Ahmed N, Datta R, et al. Fourier Transform Infrared Spectroscopy vibrational bands study of Spinacia oleracea and Trigonella corniculata under biochar amendment in naturally contaminated soil. PLoS One [Internet]. 2021;16(6):e0253390. Available from: https://doi.org/10.1371/journal.pone.0253390
47. Chavoshizadeh S, Pirsa S, Mohtarami F. Conducting/smart color film based on wheat gluten/chlorophyll/polypyrrole nanocomposite. Food Packag Shelf Life [Internet]. 2020;24:100501. Available from: https://doi.org/10.1016/j.fpsl.2020.100501
48. Zhang R, Wang X, Cheng M. Preparation and characterization of potato starch film with various size of nano-SiO2. Polymers (Basel) [Internet]. 2018;10(10):1172. Available from: https://doi.org/10.3390/polym10101172
49. Wang H, Li M, Dong Z, Zhang T, Yu Q. Preparation and characterization of ginger essential oil microcapsule composite films. Foods [Internet]. 2021;10(10):2268. Available from: https://doi.org/10.3390/foods10102268