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# Effects of thermal treatments on the characterisation of microencapsulated chlorophyll extract of *Caulerpa racemosa*

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**Abstract**

*Caulerpa racemosa* is a macroalga that has a green pigment, that is, chlorophyll. Chlorophyll is highly sensitive to damage during heat processing. In the present work, *C. racemosa* chlorophyll extract was microencapsulated with fish gelatine and Arabic gum coatings, using a freeze-drying technique, to protect against heat damage. The microcapsules were subjected to high temperatures (120, 140, and 160°C) for 5 h. The protective effect of microcapsules on chlorophyll stability was assessed by measuring chlorophyll a and b degradation, total phenolic content, antioxidant activity, functional group analysis, colour, particle size, and morphology *via* scanning electron microscopy. Chlorophyll b significantly decreased by 87.78% in comparison with chlorophyll a (61.49%) during heating; the characteristic green colour of chlorophyll changed to brownish-green following heat exposure. However, chlorophyll was still present in the microcapsules as detected by the presence of the functional group C=O bond at 1600 nm wavelength. The heat treatment did not affect microcapsule particle size and morphology. Particle size distribution ranged from 91.58 to 112.51 µm, and the microcapsule was flake-shaped. The activation energy of chlorophyll a was 19336.96 kJ/mol·K; this was higher than that of chlorophyll b, which was 1780.53 kJ/mol·K. Based on the results, microcapsules produced using fish gelatine and Arabic gum as coating materials were able to protect chlorophyll in *C. racemosa* extract from heat damage.

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## Introduction

The natural green pigment chlorophyll has been proposed as a functional food health supplement in recent years. Chlorophyll is the main green pigment component in the macroalga *Caulerpa racemose* (Sihono *et al.*, 2018). Chlorophylls and flavonoids are the main components in *C. racemosa* that are responsible for antioxidant activity. Chlorophyll pigments from seaweed are used in the food and beverage, cosmetics, and pharmaceutical industries (Aryee *et al.*, 2018; Jiao *et al.*, 2020). Food and beverage companies are becoming more aware of the nutritional and health values of chlorophylls. Industrial production of chlorophyll is mainly extracted from plants and microalgae such as *Chlorella*, and is rarely extracted from macroalgae. *C. racemosa* is easily found in tropical zones along the coasts of Indonesia, and commonly consumed fresh by the locals. Natural pigment extraction generally uses solvents. The solvent used depends on the polarity of the material to be extracted. Ethanol is a safe solvent for extracting natural components, and is applied to foods and medicines (Hikmawanti *et al*., 2021). Ethanol has also been used to extract the natural dye curcumin, which produces the weight, total phenolic content, and highest antioxidant activity when compared with other solvents such as methanol, acetone, and water (Popuri and Pagala, 2013; Array *et al*., 2019). The same results were also shown in anthocyanins (Thao, 2015) and quercetin (Pertiwi *et al*., 2020).

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Chlorophyll pigments are easily damaged during processing, such as heating and the use of vacuum pressures (Armesto *et al.*, 2017). The stability of chlorophyll are influenced by several factors including temperature, heating duration, pH, oxygen, and light exposure (Zheng *et al.*, 2014; Hsiao *et al.*, 2020). Stability or chlorophyll retention has been used as a measure of quality in green vegetables. Therefore, microencapsulation is an attempt to preserve chlorophylls during storage and processing.

Microencapsulation is the process of packaging by coating an active component in a tiny capsule (2 – 5,000 μm). The standard methods used in microencapsulation are spray- and freeze-drying (da Silva *et al*., 2014; Jouki *et al*., 2021a). Spray-drying converts emulsion samples in liquid form into powder, with specific operating standards, using a spray-dryer. This method requires high temperature to evaporate the water; hence, it obtains results in the form of powder. Microencapsulation with spray-drying is a flexible method, and provides a good quality particle. Spray-drying is also known to be a flexible and more cost-efficient method. As compared to spray-drying, freeze-drying operates without oxygen, and uses a lower temperature (Guo *et al*., 2020). Freeze-drying is a drying method utilising evaporation without the use of heat. Microencapsulation by freeze-drying alters the liquid emulsion substance or suspended component in the water that has been frozen, and then sublimated to the gas phase. This method produces low depreciation rates, and maintains a coated bioactive substance (da Veiga *et al*., 2019; Jouki *et al*., 2021a). This method is suitable for bioactive components that are sensitive to heat, especially natural dyes. Freeze-drying methods have been used for microencapsulation processes in natural dyes such as phycocyanin (Dewi *et al*., 2017), anthocyanins (Yamashita *et al*., 2017), and curcumin (Guo *et al*., 2020). Therefore, in the present work, the microencapsulation process of chlorophylls was conducted using the freeze-drying method.

Several studies have used microencapsulation methods to protect plant chlorophylls against damage. Coatings that have been used in microencapsulation include Arabic gum-maltodextrin (Kang *et al.*, 2019), whey protein isolate (Zhang *et al.*, 2019), and polycaprolactone (Hsiao *et al.*, 2020). Microcapsules produced using a mixture of hydrophobic and hydrophilic coatings provide stability to sensitive compounds during processing, especially during exposure to high temperatures. The present work is the first study reporting the stability of *C. racemosa* chlorophyll microcapsules upon heat treatment.

The stability of chlorophylls during the microencapsulation process is affected by the coating material applied. Dang *et al.* (2017) used corn starch and gelatine as coating materials to produce vitamin C microcapsules; vitamin C was protected against degradation during the microencapsulation process at 180°C inlet temperature. Muhoza *et al.* (2019) used a combination of pectin-alginate coating materials to produce cinnamaldehyde microcapsules that were stable after 80°C hot water dilution for 120 min. Arepally and Goswami (2019) used an Arabic maltodextrin-gum coating to produce probiotic microcapsules that were stable at 150°C inlet temperature. However, no explanation of the factors affecting the stability of the microcapsule was offered. The coating materials used in the microencapsulation process are suggested to affect the stability of chlorophyll microcapsules upon heat treatment. Therefore, the present work aimed to determine the stability of chlorophyll microcapsules from *C. racemosa* using gelatine-Arabic gum as coating material.

## Materials and methods

### *Materials*

The raw material *C. racemosa* was obtained from a local market (Karimun Java Island, Indonesia). Chlorophyll extraction was performed using ethanol 96%, Tween 80, and Arabic gum from Merck (Darmstadt, Germany). Chlorophyll a and b standards were purchased from Sigma-Aldrich (Steinheim, Germany). Tilapia scales were supplied by PT. Aquafarm (Semarang, Indonesia).

### *Gelatine extraction from tilapia scales*

The gelatine extraction from tilapia scales was performed following Irwandi *et al*. (2009) with slight modifications. Tilapia scale was soaked in 2% sodium hydroxide (Merck Darmstadt, Germany) for 12 h at room temperature (28°C), and washed with distilled water. The scales were then extracted with distilled water by heating at 60°C in a water bath (Memmert, Schwabach, Germany) for 2 h at a ratio of 1:3 (w/v). The filtrate obtained was dried at 40°C for 48 h, and ground into 80 mesh size. The gelatine was analysed for gel strength (128.20 bloom), viscosity (2.6 cps), pH (5.3), and water content (7.7%). The

gelatine was of Type B with gel strength of 110 - 130 bloom. These characteristics signified the safety of the gelatine to be added to foodstuffs (GMIA, 2019).

*Chlorophyll extraction*

The chlorophyll extraction was performed following Derrien *et al.* (2017) with slight modifications. Fresh *C. racemosa* was cut with a size of 1 cm and then soaked in ethanol (Merck, Darmstadt, Germany) at a ratio of 1:4 (w/v) for 48 h at room temperature. The filtrate was then evaporated with a rotary vacuum evaporator (Tmax Battery Equipments Ltd., China) at 40°C to remove the solvent. The chlorophyll extract was stored at 5°C until further processing.

*Chlorophyll microencapsulation*

The microencapsulation was conducted using a freeze-dryer (Ningbo Yinzhou Sjia Lab Equipment Co., Ltd.) following Lee *et al.* (2020) and Yamashita *et al.* (2017). Approximately, 10% chlorophyll extract, 1% Tween 80 (Merck, Darmstadt, Germany), 2% fish gelatine, and 8% Arabic gum (Merck, Darmstadt, Germany) were dissolved in distilled water until a volume of 100 mL was reached, after which it was homogenised using an Ultra-Turrax homogeniser (IKA Werke, Labortechnik, Staufen, Germany) at 10,000 rpm for 3 min. The homogenised solution was placed into a baking dish, and frozen at -35°C for 24 h. Then the sample was dried for 48 h using freeze-drying at a condenser temperature of -50°C, and at a chamber pressure of < 0.07 mbar. The final sample temperature was -24°C. The chlorophyll powder was directly stored at -20°C. Samples of chlorophyll microcapsules that had been packaged in aluminium foil were then heated using an oven (Memmert, Germany). The obtained chlorophyll microcapsules were then heated at 120, 140, and 160°C for 5 h in air-free conditions, and analysis was conducted hourly. The encapsulation efficiency of chlorophyll a and b was calculated on the basis of the percentage of chlorophyll trapped against the initial chlorophyll in the extract (Jouki *et al*., 2021b).

*Chlorophyll analysis*

The chlorophyll was analysed following Scheepers *et al*. (2011). Standard curves for chlorophyll a and b were prepared by dissolving chlorophyll in acetone and double distilled $H_2O$ ($ddH_2O$) at a ratio of 1:1. Next, 100 µL solution was added to 900 µL $ddH_2O$, and 5 µL microcapsule sample was eluted on ethyl acetate and methanol (32:68). The sample was then injected into HPLC (Agilent 1100, Germany) at a flow rate of 1 mL/min (Millipore Co., Milford, USA). A detector was set to 254 and 665 nm for the detection of chlorophyll pigments.

*Total phenolic content*

The total phenolic content was measured following Milani *et al*. (2020). A total of 1 g microcapsules was dissolved in 10 mL $dH_2O$, and homogenised using a vortex. Next, 1 mL sample solution was mixed with 1 mL Folin-Ciocalteu reagent (Merck, Germany), and homogenised. Then, 3 mL 3% sodium carbonate was added to the solution. The mixture was then set aside for 30 min with stirring. Absorbance was read using a UV-Vis spectrophotometer (Shimadzu, Japan) at 760 nm, and gallic acid was used as a standard. The total phenolic content was expressed as microgram gallic acid per milligram of the dry sample against a gallic acid standard curve.

*Antioxidant activity*

The antioxidant activity was measured following a modified method by Milani *et al.* (2020). The test was performed using 2,2-diphenyl-1-picrylhydrazil (DPPH) (Merck, Germany). Approximately, 1 g microcapsules was dissolved in 10 mL $dH_2O$, and homogenised using a vortex. Next, 1 mL chlorophyll microcapsule solution in methanol was mixed with 2 mL methanolic DPPH solution (0.1 mM); the mixture was then homogenised and stored in a dark room at room temperature for 30 min. Then, absorbance was read using a UV-Vis spectrophotometer (Shimadzu, Japan) at 517 nm. The percent DPPH free radical was calculated by dividing the difference between absorbance of the sample and the control over absorbance of the control, and then multiplied by 100%. DPPH solution is used as a control.

*Colour*

The chlorophyll colour intensity was assessed using a Minolta Chromameter (Model CR-400 Osaka Japan). Results were expressed as L*, a*, and b* values (Anthonissen *et al.*, 2018). To evaluate the colour change during heating, the parameters L*, a*, and b* were calculated on the basis of formulation.

L* indicated brightness, a* (+) indicated red pigment, a* (-) indicated green pigment, b* (+) indicated yellow pigment, and b* (−) indicated blue pigment.

*Fourier transform infrared spectroscopy analysis*

The functional group analysis was carried out using Fourier transform infrared spectroscopy (Shimadzu FTIR 8400, Japan) at a wavelength range of 400 - 4000 $cm^{-1}$.

*Particle size distribution*

The chlorophyll microcapsules were analysed for particle size in the form of dry material powder. The particle distribution was analysed using a particle size analyser (Laser Particle Sizer Testing LLPA C10, England) (Du *et al.*, 2019). The span was measured by dividing the difference between D90 and D10 with D50 (Hamishehkar *et al.*, 2010).

*Scanning electron microscopy*

The particle microstructure was measured following Du *et al.* (2019) using scanning electron microscopy (Jeol JSM-6510LA, Japan) at 3 kV. The samples were coated with platinum before analysis.

*Degradation kinetics*

The thermal degradation kinetics were assessed following Kim *et al.* (2018) by calculating first-order reaction (Eq. 1):

$$ln\ C = ln\ C_0 - kt \quad \text{(Eq. 1)}$$

The activation energy was calculated on the basis of the Arrhenius equation (Eq. 2):

$$k = k_0.e^{-Ea/RT} \quad \text{(Eq. 2)}$$

The kinetic parameters are essential to predict quality loss during thermal processing. The microcapsules were heat-treated at different temperatures: 120, 140, and 160°C for 5 h. Chlorophyll degradation analysis was performed every 5 h.

*Statistical analysis*

Experiments were performed in triplicates, and the data were analysed using analysis of variance followed by Tukey's tests if there was a significant difference ($p < 0.05$). Data analysis was performed using SPSS 23 software (Chicago, USA).

## Results and discussion

*Chlorophyll stabilisation*

Chlorophyll is a green pigment formed from carbon and nitrogen atoms with a magnesium ion in the centre. Chlorophyll a and b are found in algae and other marine species (Pareek *et al.*, 2018). In the present work, chlorophyll a and b extracted from *C. racemosa* were microencapsulated, with an encapsulation efficiency of chlorophyll a of 91.11% and chlorophyll b of 95.67%. Chlorophyll microcapsules were then subjected to heating. Chlorophyll a and b contents after microencapsulation were 13.01 and 55.40 mg/L, with a total chlorophyll of 68.41 mg/L. This was higher as compared to a study by Kang *et al.* (2019) which reported the chlorophyll content of spinach extract microcapsules as 46.78 mg/L. Upon heat treatment, chlorophyll a and b contents decreased to 5.01 and 6.77 mg/L, respectively. Chlorophyll b content decreased significantly in the first hour of heating across all temperatures. Chlorophyll a decreased by 61.49%, while chlorophyll b decreased by 87.78%. The large decrease in chlorophyll a and b after heating indicated their susceptible nature towards heat.

Chlorophyll b is more elastic than chlorophyll a (Indrasti *et al*. 2018); however, both types of chlorophyll are susceptible to heat treatments. During processing, heat damages the isocyclic pheophytin ring, thus resulting in the formation of pheoforbides. Hence, the green colour of chlorophyll is lost, and instead, a bright or olive-brown colour resulting from pheoforbides, is produced. The decrease in chlorophyll a and b contents in the microcapsules was expected to be accompanied by a decrease in the antioxidant activity of microcapsules given that chlorophyll is an antioxidant.

*Total phenolic content*

The phenolic component in algae can either be found as phenolic acids or phlorotannin complexes. Phlorotannins are products of phloroglucinol polymerisation that are formed through the acetate-malonate pathway (Mekinić *et al.*, 2019). Heat treatment of the microcapsules increased the total phenolic content, which could have been due to the alteration of polyphenol complexes into simpler phenolic components. A previous study by Gunathilake *et al.* (2018) stated that heating degrades complex polyphenol components such as tannins into

simple polyphenols, by changing the structure and matrix of the compounds, and inactivating the enzyme polyphenol oxidase. Heating also frees the polyphenols from complex intracellular proteins to be converted into simple polyphenols. Previous studies by Shaimaa *et al.* (2016) have also reported an increase in total phenolic content in Sina green chili following the application of heat, especially via boiling. During boiling, heat dehydrates the food matrix, thus degrading polyphenols in the process. The total phenolic content in microcapsules following heating treatment increased three to four times as compared to that without heating. These results concur with those reported by Leng *et al.* (2017), whereby total phenolic content in tamarind leaves increased by four times when the heat was applied by frying, as compared to tamarind leaves without heat treatment.

*Antioxidant activity*

The antioxidant activity of microcapsules was analysed using the DPPH method. This method is simple, inexpensive, can be performed at room temperature, reproducible, and accurate. DPPH radicals do not efficiently react with oxidisers and cation radicals from the environment, thus affecting the analysis results. This method is advantageous as compared to other methods such as ABTS (Munteanu and Apetrei, 2021). The DPPH method is suitable for measuring antioxidant activity on hydrophobic media, such as foodstuffs containing pigments. While ABTS is more suitable for use in hydrophilic media (Floegel *et al*., 2011; Jing *et al.*, 2012). Chlorophyll is a green pigment which, based on its structure, has a hydrophobic phytol group, so that it can only be dissolved in organic solvents (Indrasti *et al*., 2018). Therefore, in the present work, the DPPH method was used to measure the antioxidant activity. It was found that, the higher the temperature and the longer the heating time, the lower the antioxidant activity. This could have been due to the decrease in the contents of chlorophyll a and b during heat treatment. Chlorophyll is a green pigment that undergoes discoloration from green to slightly yellowish when degraded (Christ and Hörtensteiner, 2014). Heating oxidises chlorophyll, and reduces its antioxidant activity. The results observed in the present work concur with Moser *et al*. (2017) who stated that the decrease in antioxidant activity in microcapsule Violeta grape juice was positively correlated with a decrease in anthocyanin content. Antioxidant activity is also influenced by the formation of Maillard reaction product on microcapsules, which affects the colour change of microcapsules. Maillard reaction products are known to have the potential as antioxidants. Antioxidant activity is usually positively correlated to the total phenolic content. However, the present work showed different results. The decrease in antioxidant activity observed was suspected to be related to the Maillard reaction product that provides antagonism effects that decrease antioxidant activity. Maillard reaction products sometimes act as prooxidant that increases free radicals (Zhao, 2014).

*Colour*

The chlorophyll microcapsule colour was indicated by the values L*, a*, and b*. Positive L* value (+) indicates brightness, negative a* value (-) indicates green colour, and positive b* value (+) indicates yellow colour. Chlorophyll microcapsule, with and without heat treatment, had a bright green colour with the value of 39.67, -18.00, and 35.33 for L*, a*, and b*, respectively (Table 1). Chlorophyll a gives a blue-green colour, whereas chlorophyll b appears as a yellow-green colour (Pareek *et al.*, 2018). During heating at 160°C, the colour of total chlorophyll in the microcapsules changed from green to an olive-brown colour (Figure 1); the discoloration was indicative of heat damage and the formation of pheophytins (Aamir *et al.*, 2014). During heating, $Mg^{2+}$ ion in the central porphyrin ring is replaced by $2H^{+}$ ions to produce pheophytins. Continuous heating converts the pheophytins into pyro-pheophytins. Erge *et al.* (2008) reported similar results, where green peas underwent discoloration from green to greenish-yellow during heating, thus indicating a loss of green chlorophyll in the process.

The higher the temperature and the more prolonged heat treatment indicated a decrease in L* value, and change from a* (-) to a* (+); a* and b* also decreased. This indicated an increase in dark red colour. This change occurred since during warming, Maillard reaction would form Maillard products. This agrees with Lee *et al*. (2020) who stated that a high temperature and storage duration caused a change in the colour of the red palm oil microcapsule to dark red, which indicated the Maillard reaction. The same results were also shown by Purnama *et al*. (2020) in *Spirulina* microcapsule that underwent discoloration

**Table 1.** Discoloration of microcapsules during heating.

| Microcapsule | L | a* | b* | ΔE |
|---|---|---|---|---|
| Without heating | 39.67 ± 1.15$^{ef}$ | -18.00 ± 0.00$^{a}$ | 35.33 ± 0.58$^{fg}$ | – |
| 120°C, 1 h | 41.67 ± 2.08$^{fg}$ | -15.00 ± 0.00$^{b}$ | 38.67 ± 0.58$^{h}$ | 5.50 ± 1.67$^{a}$ |
| 120°C, 2 h | 42.00 ± 1.00$^{fg}$ | -14.00 ± 0.00$^{b}$ | 39.33 ± 1.15$^{h}$ | 6.36 ± 1.68$^{ab}$ |
| 120°C, 3 h | 41.00 ± 1.00$^{fg}$ | -12.33 ± 0.58$^{c}$ | 39.33 ± 0.58$^{h}$ | 7.16 ± 0.40$^{abc}$ |
| 120°C, 4 h | 41.33 ± 0.58$^{fg}$ | -12.00 ± 0.00$^{c}$ | 38.33 ± 0.58$^{h}$ | 7.04 ± 0.75$^{abc}$ |
| 120°C, 5 h | 42.33 ± 0.58$^{g}$ | -11.67 ± 0.58$^{c}$ | 39.33 ± 0.58$^{h}$ | 8.09 ± 0.59$^{abc}$ |
| 140°C, 1 h | 38.67 ± 0.58$^{ef}$ | -8.67 ± 0.58$^{d}$ | 37.33 ± 0.58$^{gh}$ | 9.73 ± 0.63$^{cde}$ |
| 140°C, 2 h | 37.33 ± 0.58$^{e}$ | -7.33 ± 0.58$^{d}$ | 36.00 ± 0.00$^{fg}$ | 10.96 ± 0.45$^{def}$ |
| 140°C, 3 h | 35.00 ± 1.00$^{d}$ | -6.00 ± 0.00$^{e}$ | 35.00 ± 1.00$^{f}$ | 13.03 ± 0.88$^{ef}$ |
| 140°C, 4 h | 34.00 ± 1.73$^{d}$ | -6.00 ± 1.00$^{e}$ | 34.33 ± 1.15$^{f}$ | 13.48 ± 2.31$^{ef}$ |
| 140°C, 5 h | 34.00 ± 1.00$^{d}$ | -4.33 ± 0.58$^{f}$ | 34.33 ± 0.58$^{f}$ | 14.95 ± 0.42$^{f}$ |
| 160°C, 1 h | 25.67 ± 0.58$^{c}$ | 1.67 ± 0.58$^{g}$ | 27.33 ± 0.58$^{e}$ | 25.45 ± 0.27$^{g}$ |
| 160°C, 2 h | 23.33 ± 1.15$^{c}$ | 3.67 ± 0.58$^{gh}$ | 24.67 ± 0.58$^{d}$ | 29.19 ± 1.99$^{g}$ |
| 160°C, 3 h | 15.33 ± 0.58$^{b}$ | 6.00 ± 0.00$^{j}$ | 16.33 ± 0.58$^{c}$ | 39.11 ± 1.43$^{h}$ |
| 160°C, 4 h | 10.00 ± 1.00$^{a}$ | 5.00 ± 0.00$^{i}$ | 9.67 ± 0.58$^{b}$ | 45.48 ± 0.70$^{i}$ |
| 160°C, 5 h | 9.33 ± 2.08$^{a}$ | 2.33 ± 0.58g$^{h}$ | 5.67 ± 0.58$^{a}$ | 47.07 ± 3.06$^{i}$ |

Values are mean ± standard deviation. Means followed by different lowercase superscripts in the same column indicate significant differences (α 0.05).

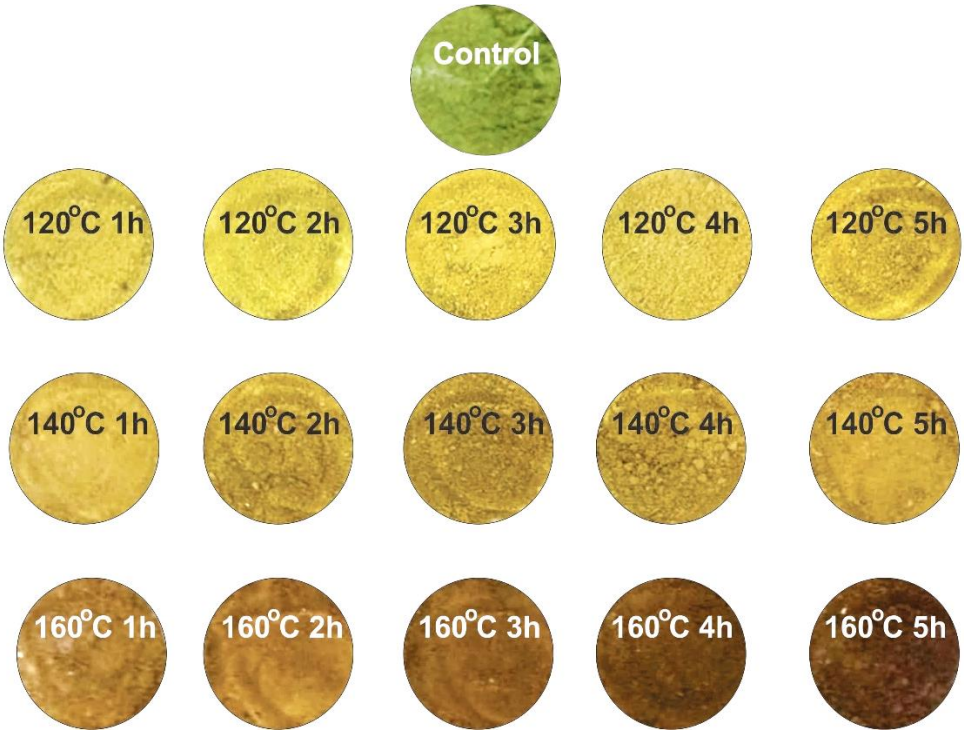


**Figure 1.** Colour of microcapsules at different temperatures and heating times.

from green to brown due to Maillard reaction between D-glucose in maltodextrin and amino acids in the ingredients included. In the present work, the Maillard reaction occurred between the reduced sugar in Arabic gum and the amino acid in gelatine.

*Degradation kinetics*

Thermal degradation of chlorophyll a and b follow a first-order Arrhenius reaction kinetic model (Indrasti *et al*., 2018). Activation energy is the minimum kinetic energy required by a molecule to react. Therefore, at lower activation energy, the presence of slight temperature changes can cause damage (Indrasti *et al*., 2018; Oancea, 2021). In the present work, the stability of the chlorophyll extract from *C. racemosa* was assessed via the activation energies of chlorophyll a and b derived from the Arrhenius equation: 19,336.96 and 1,780.53 kJ/mol·K. From the kinetic activation value, it was deduced that chlorophyll b was more sensitive to heat damage when compared with chlorophyll a.

The kinetic parameters for thermal degradation of total phenolics in chlorophyll followed the zero Arrhenius order. The activation energy of total phenolics was 8,004.75 kJ/mol, and increased with temperature. Kim *et al*. (2018) reported the effects of kinetic parameters on kiwi purée: the increased temperature was followed by decreased total phenolics following the first order with an activation energy of 28.15 kJ/mol. Nevertheless, in the present work, the opposite was observed because of the protective effects of microencapsulation on the polymerisation of phenolic compounds (Moser *et al*., 2017). In a study by Yu and Lv (2019), microencapsulated particles with a phenolic core had higher retention in comparison with non-coated particles due to the protective effects of encapsulation.

Heat treatment resulted in a degradation of chlorophyll a and b, thus causing a decrease in antioxidant activity. The decrease in antioxidant activity followed a first-order Arrhenius kinetic model. This agrees with reports by Kim *et al*. (2018), where kiwi fruit subjected to high temperature resulted in a decrease in antioxidant activity following the Arrhenius first-order kinetics. In a study by Wu *et al.* (2018), heat treatment was applied to rosella flowers. Rosella contains anthocyanin as the major pigment. Increased temperature decreased antioxidant activity, with an activation energy of 74.9 kJ/mol. The antioxidant reaction activation energy of chlorophyll in the present work (47,673.37 kJ/mol) (Table 2) was higher than anthocyanin in rosella.

Thermal degradation is also indicated in the colour parameters L*, a*, and b*. The a* value had the lowest activation energy of 27,484.8 kJ/mol·K based on its activation energy. This inidcated that heat treatment could give a change in the value of a*. There was a change in value a* from negative (green) to positive (red) during heating. This could have been due to the Maillard reaction during heating as earlier mentioned (Lee *et al*., 2020).

*Description of functional groups with FTIR*

According to Yalcin *et al.* (2012), chlorophyll has the following functional groups: C–O aldehydes at 1583 - 1709 $cm^{-1}$, C–H at 2809 - 3012 $cm^{-1}$, and O–H at 3029 - 3639 $cm^{-1}$. Arabic gum has O–H at 3413 $cm^{-1}$, C–H at 2930 $cm^{-1}$, C=O and N–H at 1613 $cm^{-1}$, and C–O at 1141 $cm^{-1}$ (Figure 2). Meanwhile, fish gelatine has C=O at 1633 $cm^{-1}$, N–H at 1547 $cm^{-1}$, and C–N at 1239 $cm^{-1}$ (Kang *et al.*, 2019; Stevenson *et al.*, 2020). Based on FTIR spectral data, the C=O bond representing chlorophyll, and the coating materials were found at a wavelength of approximately 1600 $cm^{-1}$ following heat treatment.

*Particle size and morphology of microcapsules*

The average particle size following heating treatment ranged from 91.58 to 112.51 µm (Figure 3). The size of microcapsules was determined using the microencapsulation method. In the freeze-drying method, the sample droplets were frozen to maintain their size and shape; this influenced particle aggregation. Particle size is closely related to the morphology of microcapsules because it affects the appearance, solubility, and flow properties of the microcapsules (Parthasarathi and Anandharamakrishnan, 2016).

The span value indicates particle size distribution; low span values indicate uniformity of particle size distribution (Parthasarathi and Anandharamakrishnan, 2016). In the present work, microcapsules that underwent heating treatment maintained the flaky and porous morphology before heating (Figure 4). This agrees with Parthasarathi and Anandharamakrishnan (2016), whereby microcapsules produced via freeze-drying had flaky and porous morphology. Jouki *et al*. (2021b) stated that the microcapsule structure is affected by the

**Table 2.** Stability of microcapsules based on Arrhenius equation.

| Parameter | Storage temperature (T, °C) | Storage temperature (T, K) | 1/T | Selected order reaction | k | ln k | Slope Arrhenius | $E_a$ (kJ/mol·K) |
|---|---|---|---|---|---|---|---|---|
| Chlorophyll a | 120 | 393 | 0.0025 | y = -0.1021x + 2.5911 | 0.1021 | -2.28 | 2377.3 | 19,336.96 |
| | 140 | 413 | 0.0024 | y = -0.0996x + 2.5202 | 0.0996 | -2.31 | | |
| | 160 | 433 | 0.0023 | y = -0.1804x + 2.4338 | 0.1804 | -1.71 | | |
| Chlorophyll b | 120 | 393 | 0.0025 | y = -0.3341x + 3.6235 | 0.3341 | -1.10 | 218.90 | 1780.53 |
| | 140 | 413 | 0.0024 | y = -0.2898x + 3.3726 | 0.2898 | -1.24 | | |
| | 160 | 433 | 0.0023 | y = -0.3537x + 3.4701 | 0.3537 | -1.04 | | |
| Total phenolic content | 120 | 393 | 0.0025 | y = 176.63x + 771.430 | 176.63 | 5.17 | 984.11 | 8004.75 |
| | 140 | 413 | 0.0024 | y = 216.54x + 758.810 | 216.54 | 5.38 | | |
| | 160 | 433 | 0.0023 | y = 222.00x + 993.000 | 222.00 | 5.40 | | |
| Antioxidant activity | 120 | 393 | 0.0025 | y = -0.1738x + 3.8784 | 0.1738 | -1.75 | 5861.00 | 47,673.37 |
| | 140 | 413 | 0.0024 | y = -0.5401x + 4.1741 | 0.5401 | -0.62 | | |
| | 160 | 433 | 0.0023 | y = -0.6800x + 3.9729 | 0.6800 | -0.39 | | |
| L | 120 | 393 | 0.0025 | y = 0.0079x + 3.7017 | 0.0079 | -4.84 | 15408 | 125329 |
| | 140 | 413 | 0.0024 | y = -0.0349x + 3.6811 | 0.0349 | -3.36 | | |
| | 160 | 433 | 0.0023 | y = -0.2995x + 3.639 | 0.2995 | -1.21 | | |
| a* | 120 | 393 | 0.0025 | y = -0.0847x + 2.8269 | 0.0847 | -2.47 | 3379 | 27484.4 |
| | 140 | 413 | 0.0024 | y = -0.2408x + 2.6173 | 0.2408 | -1.42 | | |
| | 160 | 433 | 0.0023 | y = -0.1836x + 1.9506 | 0.1836 | -1.70 | | |
| b* | 120 | 393 | 0.0025 | y = 0.0146x + 3.6106 | 0.0146 | -4.23 | 13405 | 109036 |
| | 140 | 413 | 0.0024 | y = -0.0121x + 3.5962 | 0.0121 | -4.41 | | |
| | 160 | 433 | 0.0023 | y = -0.3623x + 3.7183 | 0.3623 | -1.02 | | |

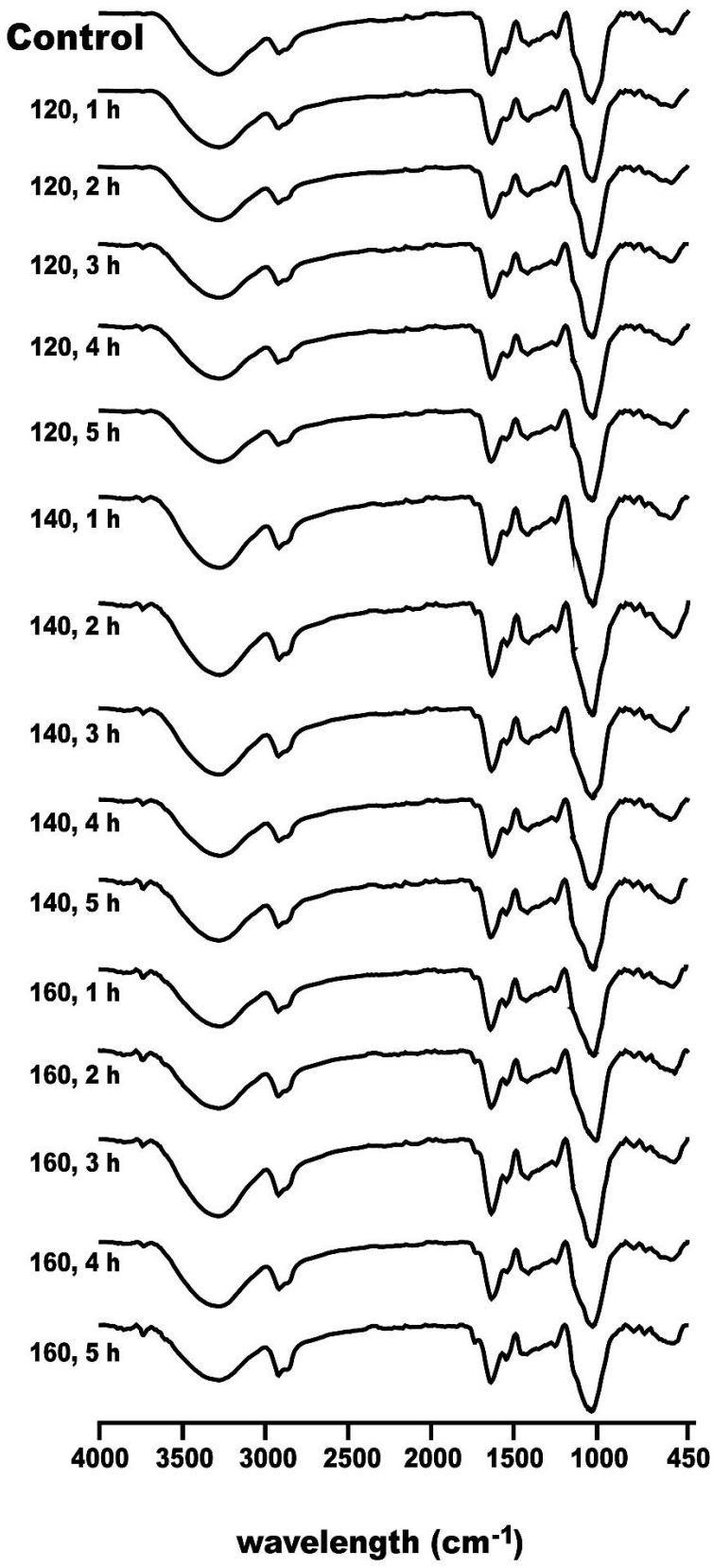


**Figure 2.** FTIR analysis of microcapsules' functional groups at different temperatures and heating times.

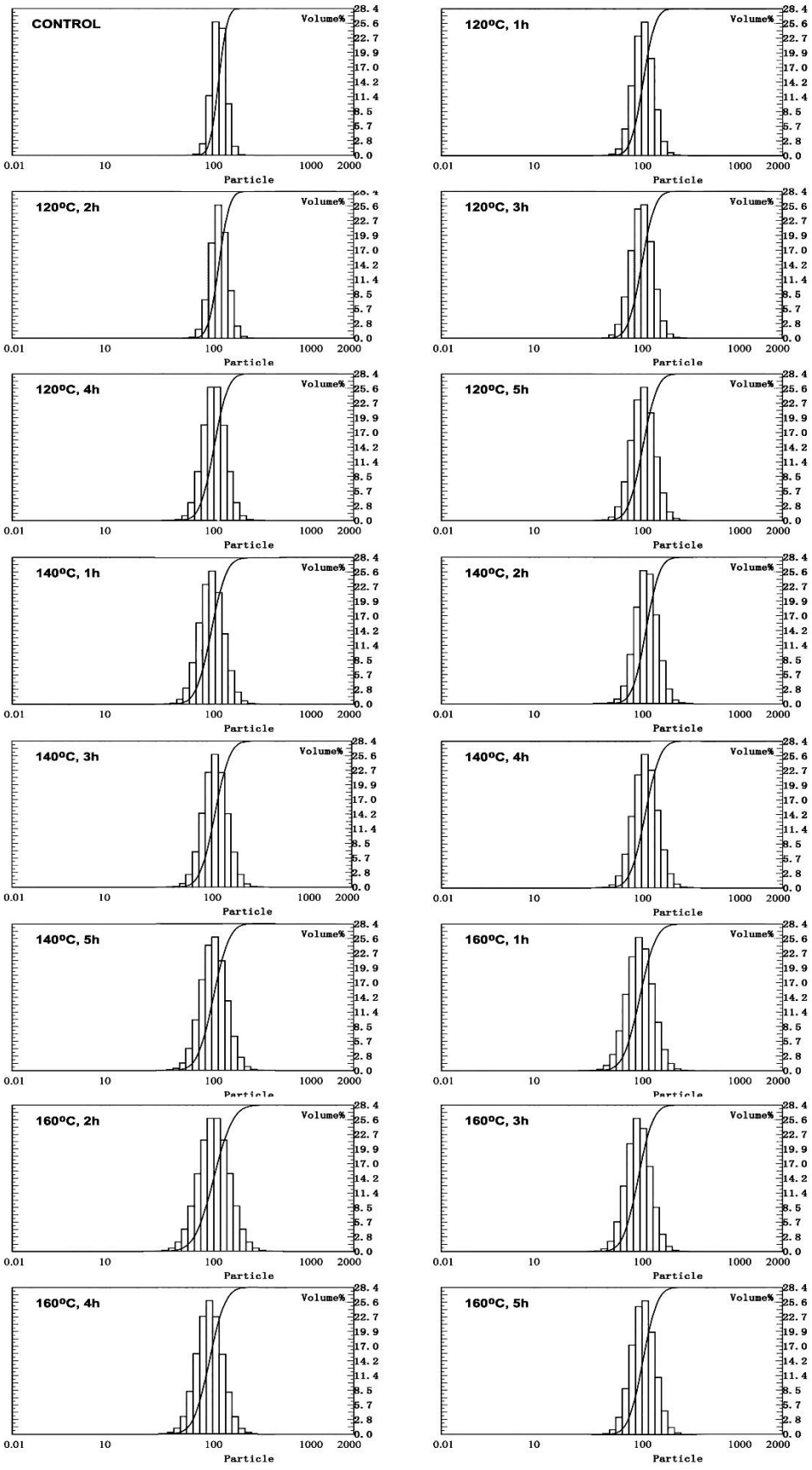


**Figure 3.** Particle size of microcapsules at different temperatures and heating times.

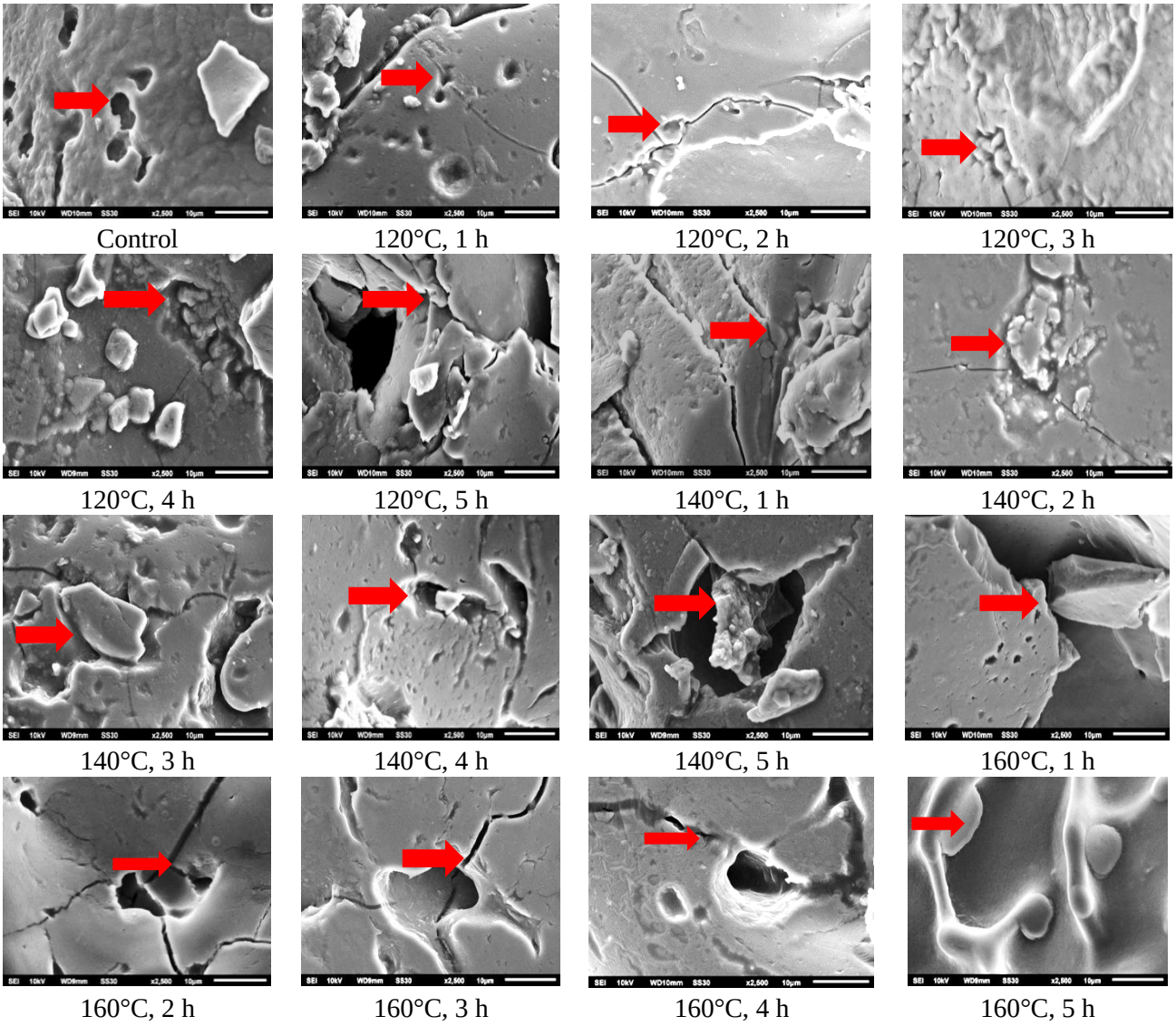


**Figure 4.** SEM morphology analysis of microcapsules at different temperatures and heating times.

drying method used. Microcapsule drying using the freeze-drying method usually produces microcapsules with large and elliptical structures.

## Conclusion

The application of heat treatment between 120 and 160°C caused more damage to chlorophyll b when compared with chlorophyll a, observed as a colour change from green to olive-brown. Heat treatment also lowered the total phenolic content and antioxidant activity of chlorophylls. The microencapsulation of chlorophylls provided protective effects towards them. However, the interaction between chlorophyll extract and coating materials was observed. O–H groups were present at a wavelength of 3400 $cm^{-1}$. The chlorophyll microcapsules had uniform particle size with flaky and porous morphology. Based on the results obtained, microcapsules protected chlorophylls from heat damage between temperatures of 120 and 160°C.

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