



Research article

Alginate modification using n-butanol for red fruit (*Pandanus conoideus*) oil encapsulation

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Abstract: Red fruit oil, rich in fatty acids, is prone to oxidation, reducing its shelf life. Encapsulation using modified alginate can enhance its stability. Alginate, a natural emulsifier, requires hydrophobic modification due to its hydrophilic nature for oil encapsulation. This research aims to modify alginate through esterification with n-butanol to enhance its hydrophobicity, thereby improving the binding of red fruit oil. The study examines the effects of the mole ratio of n-butanol to alginate, esterification time, and pH of alginate on the modification of alginate, emulsification, and the beads produced. Results showed that the highest degree of substitution (DS), that is 0.283, was obtained by 15 h esterification of 40:1 mole ratio of n-butanol:alginate in pH = 4 conditions. The attachment of butyl chain to alginate backbone was identified from their IR spectra. Increasing DS led to the molecular weight reduction ($M_v = 22\text{--}27$ kDa). The thermal stability of alginate is maintained after modification with n-butanol. Ability of modified alginate by attaching the C4 chain increased stability of red fruit oil emulsion up to 40 min without any separation. The emulsion was stabilized using modified alginate produced red fruit oil beads with $\pm 30\%$ encapsulation efficiency. The formed beads prevented the oxidation of red oil (oxidation degree $< 3\%$) after 5 days of storage. This research emphasizes better handling and storage of the red fruit oil.

Keywords: gelation; beads; esterification; amphiphilic; emulsion; oxidation; degree of substitution

1. Introduction

Red fruit (*Pandanus conoideus*), commonly found in Papua (spread in Indonesia and Papua New Guinea), has been widely used as food coloring and as a source of traditional medicinal compounds with various health benefits [1]. Red fruit comprises active components, including carotenoids like alpha-carotene, beta-carotene, and beta-cryptoxanthin, along with the antioxidant alpha-tocopherol. It is also rich in unsaturated fatty acids, such as oleic acid, linoleic acid, linolenic acid, and palmitoleic acid [2]. The high fatty acid content in red fruit oil, particularly oleic and linoleic acids, makes it susceptible to oxidation, which can reduce the quality of the oil. Encapsulation had been conducted to protect the beneficial content of red fruit oil, as well as ease the handling and storage of red fruit oil handling and storage [3].

Volić et al. [4] encapsulated thyme oil in alginate-whey protein matrix to prevent oxidative reaction. The encapsulated thyme oil was added to polymer film by Marcovich et al. [5] to enhance its strength and antioxidant properties. Apart from protecting and facilitating storage, oil encapsulation also supports drug delivery system [6]. Salvia-Trujillo et al. [7] formed β -carotene nano-emulsion which increased its bioavailability. The nano-emulsion system converts the oil compound into nano-sized droplets which improves bioavailability and water solubility of oil [8].

Encapsulation of oil compounds requires an oil stabilizer/emulsifier so that it can be spread uniformly in the encapsulant. There are diverse food emulsifiers that are widely used, from natural ingredients like proteins, polysaccharides, and phospholipids to synthetic compounds like small molecule surfactants and solid particles [9]. Alginate, as a natural polysaccharide, has the ability to act as an emulsifier and stabilizer in the food and pharmaceutical industries. Alginate's reactive hydroxyl and carboxyl groups make it ideal for chemical modification, enabling the creation of derivatives with altered properties like solubility, hydrophobicity, and biological behavior [10]. The use of alginate also supports the bead formation in encapsulation as it easily solidifies upon exposure to divalent cations, especially Ca^{2+} ions [11]. Besides, alginate is widely available worldwide polysaccharide, cheap, and non-toxic, which may attract industrial interest [12]. However, pure sodium alginate has hydrophilic properties that limit its use in applications requiring hydrophobic stability [13].

Preparation of amphiphilic alginate had been studied by several researchers. Wardhani et al. [14] modified the alginate using stearic acid which used as o/w emulsifier to stabilize fish oil emulsion, but did not produce solid particle. Yang et al. [9] attached alkyl chain from dodecanol to alginate with the help of coupling agent EDC-HCl. However, this coupling agent needed other chemicals and processes which made the process more complicated. Broderick et al. [15] increased hydrophobic properties of alginate by attaching butyl groups using esterification reaction with sulfuric acid catalyst. This esterification process is simple and more economical.

This study aims to modify alginate by adding n-butanol groups through an esterification method, using concentrated hydrochloric acid as a catalyst. It will also study the effects of n-butanol and alginate mole ratio and reaction time on the degree of substitution (DS), emulsion stability, and other physical characteristics of the modified alginate. The results of this study are expected to contribute to the development of encapsulation technology to improve the stability of red fruit oil and expand its applications in the industry.

2. Materials and methods

2.1. Materials

Sodium alginate (HiMedia, Mumbai, India) was used as primary material to be modified. N-butanol as alginate modifier was in analytical grade (Merck KGaA, Darmstadt, Germany). Red fruit oil extracted from *Pandanus conoideus* was purchased from local seller in Sorong, Indonesia. Other chemicals used were in analytical grade.

2.2. Alginate modification

Alginate was modified through direct esterification with n-butanol in the presence of hydrochloric acid as a catalyst under continuous stirring. Sodium alginate was dissolved in distilled water to form homogeneous solution (1% w/v; 100 mL) before n-butanol and concentrated hydrochloric acid was added (3% v/v). N-butanol was added in excess with various mole ratios of n-butanol to alginate (10:1, 20:1, and 40:1, v/w). The reaction was conducted for 5, 10, and 15 hours at room temperature ($\pm 25^\circ\text{C}$). The resulting Alg-C4 was filtered, washed with ethanol, dried, and stored at room temperature.

2.3. Determination of DS

The DS of the modified alginate was determined using a titration method [16]. A sample of Alg-C4 (0.1 g) was dissolved in 10 mL of distilled water and 10 mL of NaOH solution. The solution was added with phenolphthalein indicator then titrated with 0.1 M HCl until the color changed from deep purple to light pink, nearly disappearing. The DS was calculated using Equation (1).

$$\text{DS} = \frac{194 \text{ M} \times (\text{B} - \text{S})}{1000 \text{ W}} \quad (1)$$

where M is the molarity of HCl, B and S are the titration volumes of NaOH and sample solution, respectively, and W is the sample weight.

2.4. Molecular weight (MW) determination

MW was determined by dissolving 0.01, 0.02, 0.03, 0.04, and 0.05 g of Alg-C4 in 10 mL of distilled water and measuring the flow time using a viscometer for viscosity (η) determination, following the method of Wardhani et al. [17]. The MW was calculated using the Mark-Houwink equations where $K = 7.3 \times 10^{-5}$ and $\alpha = 0.92$ (Equation [2]) [18].

$$\eta = K \times \text{BM}^\alpha \quad (2)$$

2.5. Functional group

Fourier transform infrared spectroscopy (FTIR) analysis was performed to identify functional group changes in alginate after modification with n-butanol. The analysis was conducted using FTIR spectrometer (Perkin-Elmer Spectrum Two UATR FT-IR spectrometer, Perkin-Elmer, Waltham, USA) in the range of 4000 to 450 cm^{-1} .

2.6. Thermal properties

Differential scanning calorimetry (DSC) was performed to evaluate the thermal properties of the modified alginate. The analysis used a DSC-60 Plus Shimadzu apparatus (Shimadzu Co., Tokyo, Japan). The samples were heated from 30 °C to 600 °C at a rate of 10 °C per minute. The DSC data provided insight into the phase transitions, such as thermal stability of the alginate after modification with n-butanol.

2.7. Surface tension

Surface tension was determined using the drop method [19]. The sample density was measured using distilled water as a comparison fluid. The drop apparatus was filled with 10 mL of sample, and the number of drops until empty was counted. Surface tension of the sample (γ_x) was calculated using Equation (3):

$$\gamma_x = \frac{\rho_x \times n_w}{\rho_w \times n_x} \times \gamma_w \quad (3)$$

where ρ_x and ρ_w are the density of the sample and distilled water, n_x and n_w are the number of drops of sample solution and distilled water, respectively, and γ_w is the surface tension of distilled water obtained from reference.

2.8. Preparation of red fruit oil emulsion and beads

The red fruit oil-alginate emulsion was prepared by dissolving Alg-C4 in 10 mL of distilled water at 60 °C while stirring continuously for 30 minutes. Red fruit oil (0.1 mL) was added and the mixture was homogenized using IKA RW homogenizer (IKA, Staufenim Breisgau, Germany) at 25,600 rpm for 2 min at room temperature. Red fruit oil beads were produced by dropping the oil-alginate emulsion into calcium chloride solution (1% w/v). The beads were left in the immersion solution for 30 min before being filtered.

2.9. Emulsion stability and optical microscope

Emulsion stability of the red fruit oil was assessed by placing the emulsion in a closed test tube. The emulsion stability was determined from the value of creaming index as the phase separation occurred during storage as shown in Equation (4). The emulsion stability was observed for 48 h (2880 min) at room temperature (± 25 °C). The creaming index was calculated using Equation (4), with H_{serum} is the height of serum produced during storage and H_{total} is the total height of the emulsion.

$$\text{Creaming index (\%)} = \frac{H_{\text{serum}}}{H_{\text{total}}} \times 100\% \quad (4)$$

Meanwhile, the microscopic image of emulsion was captured using trinocular microscope (Boeco, Hamburg, Germany) with a digital camera (MDCE-5a) at 400x magnification.

2.10. Red fruit oil beads analyses

2.10.1. Syneresis [20]

Syneresis, defined as the expulsion of liquid from the gel network, was assessed by weighing the alginate beads after certain times from their preparation (0, 12, 24, 36, and 48 h). The excess water was wiped out using dry and clean cloth before sample weighing. The initial weight of the sample (W_{initial}) and the sample weight after storage (W_{stored}) were used in syneresis calculation using Equation (5).

$$\text{Syneresis} = \frac{W_{\text{initial}} - W_{\text{stored}}}{W_{\text{initial}}} \quad (5)$$

2.10.2. Carbonyl content

The carbonyl content was determined by dissolving Alg-C4 in distilled water, adjusting the pH to 3.2 with HCl. Hydroxylamine reagent (15 mL), prepared by dissolving 25 g of hydroxylamine hydrochloride in 100 mL of 0.5 M NaOH solution and then diluted to 500 mL, was added to the Alg-C4 solution. After stirring for 4 h at 40 °C, the sample solution was titrated using HCl [21]. The carbonyl content was calculated using Equation (6).

$$\text{Carbonyl content (\%)} = \frac{(V_{\text{blank}} - V_{\text{sample}}) \times C_{\text{HCl}} \times 0.028}{m} \times 100\% \quad (6)$$

where V_{blank} and V_{sample} are the HCl volumes used for hydroxylamine and sample solution titration, respectively, C_{HCl} is the concentration of HCl, and m is the sample mass.

2.10.3. Encapsulation efficiency (EE)

The EE of the oil was determined by measuring the surface oil content and total oil. To determine the amount of surface oil, beads sample (1 g) was stirred in 20 mL n-hexane for 1 min. The sample was filtered and moved to a closed bottle containing 20 mL of n-hexane. The sample was shaken vigorously for 1 min and separated by filtration. The filtrates were combined and heated at 75 °C for solvent evaporation. The unevaporated oil was weighed as surface oil.

Meanwhile, total oil was extracted from the beads by dispersing the beads in 20 mL of n-hexane and performing sonication at 20 kHz frequency for 10 min. Subsequently, Soxhlet extraction was performed on the same sample. The obtained filtrate was evaporated at 75 °C. The unevaporated oil was weighed as total oil. The amounts of surface oil (O_{surface}) and total oil (O_{total}) were used to calculate the EE using Equation (7).

$$\text{EE\%} = \frac{(O_{\text{total}} - O_{\text{surface}})}{O_{\text{total}}} \times 100\% \quad (7)$$

2.11. Statistical analysis

The laboratory analyses, except instrument tests, were conducted in triplicate and were expressed as mean \pm standard deviation. Statistical analysis was tested using Microsoft Office Excel 2019

(Microsoft Corp., Washington, US) performing a one-way analysis of variance (ANOVA) to compare the groups. Differences in the data were considered to be significant at a level of $p < 0.05$.

3. Results and discussions

3.1. DS

The DS describes the number of hydroxyl groups of alginate that are replaced with the C4 chain of n-butanol. Figure 1a shows that an increase in the n-butanol concentration and esterification time increased the DS. According to Broderick et al. [15], excessive use of alcohol in alginate modification promoted the formation of ester bonds between n-butanol and the carboxyl groups in alginate molecules. This is supported by chemical reaction theory, where a higher concentration of n-butanol increases the likelihood of substitution reactions. HCl as a catalyst reduced activation energy and enhanced the efficiency of the reaction, especially at high n-butanol concentrations. Additionally, longer esterification duration allowed for better diffusion of n-butanol into the alginate matrix and significantly increased the DS [22,23].

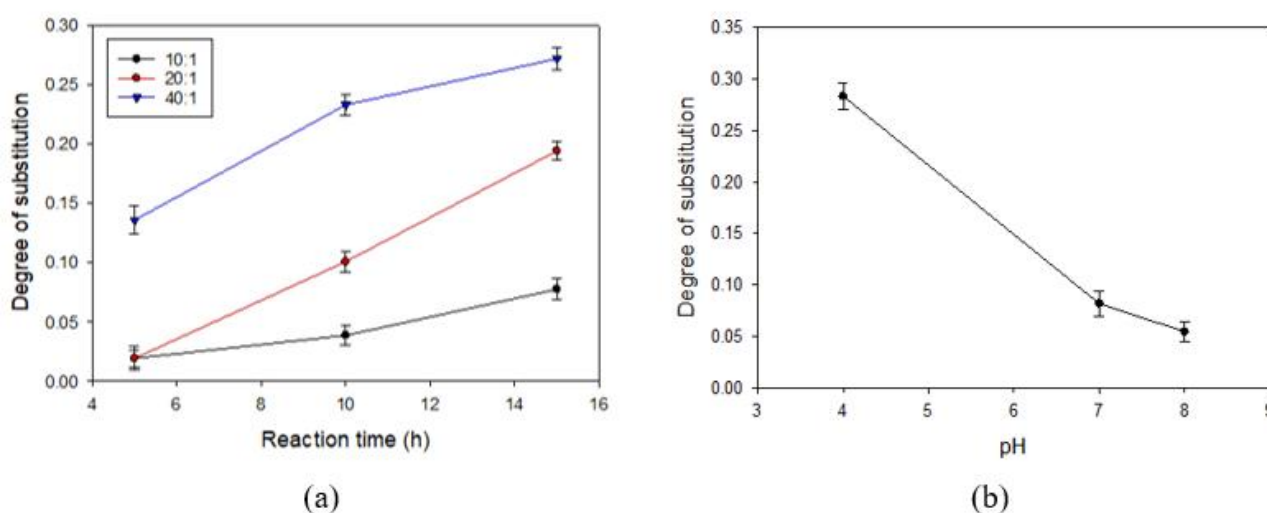


Figure 1. DS of Alg-C4 with the variation of (a) n-butanol:alginate ratio, reaction time and (b) reaction pH.

The effect of reaction pH was shown in Figure 1b. The data indicate that alginate conditions with a more acidic pH (pH = 4) are more effective in increasing the DS during alginate modification. Acid-base chemistry explains that pH plays a key role in alginate reactivity, particularly by influencing the ionic form of functional groups like carboxyl ($-\text{COOH}$). At lower pH (acidic conditions), carboxyl groups are protonated, making them more reactive for substitution due to better interaction with H^+ ions from the HCl catalyst, resulting in higher DS, as observed at pH 4. In contrast, at higher pH, carboxyl groups deprotonate ($-\text{COO}^-$), reducing reactivity and lowering DS [23]. Thus, more acidic conditions (pH 4) are more effective in increasing DS during alginate modification.

3.2. Functional groups

The functional groups of n-butanol and alginate samples were shown in Figure 2. The IR spectra of n-butanol revealed peaks corresponding to specific functional groups, particularly the C-O group at $\sim 1030\text{ cm}^{-1}$ and C-H group at $\sim 3000\text{ cm}^{-1}$, which are the characteristic of alcohol and indicative of n-butanol's chemical structure. Sodium alginate shows specific functional groups, such as -OH stretching bonds at $\sim 3400\text{ cm}^{-1}$ and -COO- groups at $\sim 1600\text{ cm}^{-1}$ [24]. In general, Alg-C4 samples with low DS exhibit similar group intensities to those of native alginate. However, increasing the DS to 0.28 gave significant increase in the peak intensities of Alg-C4 with high DS. The high DS value shifted and strengthened the peak of C-O at $\sim 1125\text{ cm}^{-1}$. This result supports the esterification reaction which breaks the -OH bond of carbonyl groups of alginates with C-O bonds of ester products. Increasing DS also increased the -OH ($\sim 3400\text{ cm}^{-1}$) and -CH groups ($\sim 3000\text{ cm}^{-1}$) of Alg-C4 as more butanol chain attached to alginate ester [25]. Meanwhile, the C=O group of alginates, observed at $\sim 1600\text{ cm}^{-1}$, should be present in the same amount, as esterification cannot break the double bond of carbonyl groups. The stronger peaks of IR spectra of Alg-C4 with high DS might be affected by more water produced during esterification. Drying process dehydrated the water content which left the ester product and intensified its functional groups' spectra.

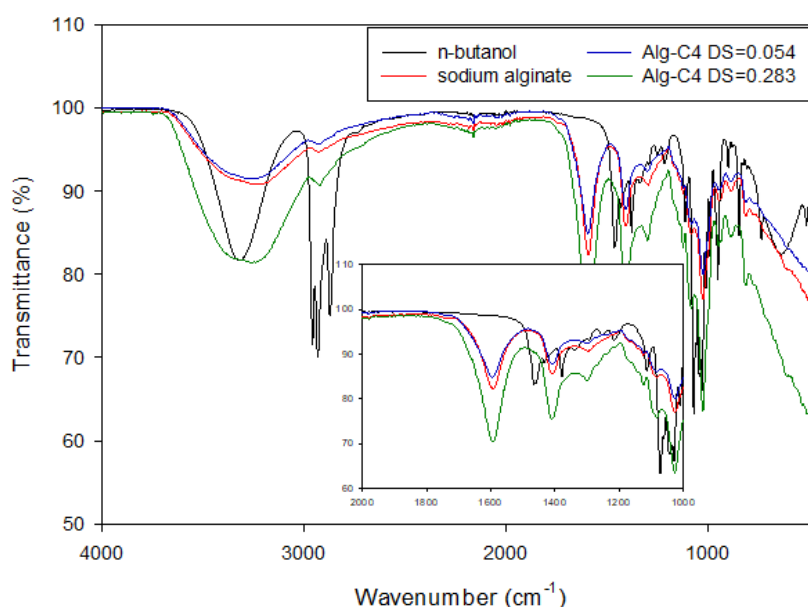


Figure 2. IR spectra of alginate samples and n-butanol, with magnified view of the region between $2000\text{--}1000\text{ cm}^{-1}$.

3.3. Viscosity average MW (Mv)

Figure 3 illustrates the effect of substitution reaction on MW of alginate (Mv). A higher DS tended to decrease the Mv. At higher concentrations, n-butanol acts as a solvent and an active agent, modifying polymer side chains and enhancing hydroxyl group reactivity, accelerating substitution. Besides substituting hydroxyl groups and forming ester bonds, the introduction of n-butanol also caused larger chain segment breaks and significantly reduced alginate MW [26]. The use of HCl as

catalyst also promoted the breakdown of glycosidic bonds in alginate, which can lead to the formation of smaller chain fragments and a subsequent decrease in MW [27].

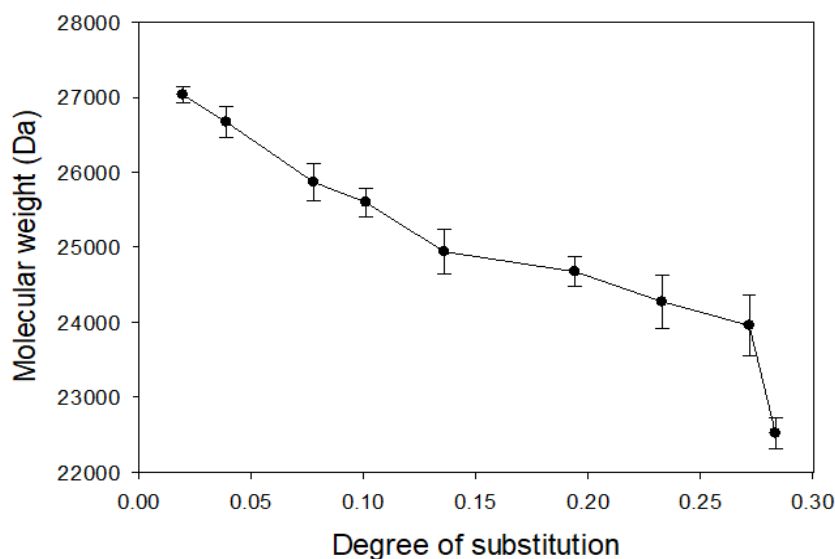


Figure 3. MW of Alg-C4 of different DS.

3.4. Thermal properties

Figure 4 shows the DSC results for native alginate, revealing endothermic peaks at 117.82 °C and exothermic peaks at 238.93 °C. Meanwhile, the thermogram also presents the DSC analysis for Alg-C4 with low DS, with an endothermic peak at 116.77 °C and exothermic peak at 230.72 °C. Similarly, Alg-C4 with high DS shows endothermic peaks at 86.79 °C and exothermic peaks at 239.62 °C. Endothermic peaks between 63 and 134 °C are associated with water loss due to alginate's hydrophilic functional groups, while the reduced endothermic temperatures in Alg-C4 (low and high DS) indicated a decrease in hydrophilicity, likely due to n-butanol modification [15]. The prominent exothermic peaks result from the thermal decomposition of carboxylate groups, causing CO₂ evolution and promoting oxidation. The temperature of exothermic peaks between sodium alginate and alginate esters is not significantly different. The exothermic peaks of sodium alginate and Alg-C4 with high DS are sharp, while Alg-C4 shows a broader peak with low DS. The broad peak is influenced by the functional group heterogeneity, which indicates the presence of more types of functional groups attached to polymer chain [28,29]. The heterogeneity of alginate structure is possible to be occur due to the acid/alkaline addition during esterification reaction [23]. Thus, the lower endothermic peaks in Alg-C4 suggest reduced hydrophilicity, while higher DS in Alg-C4 does not exhibit significant difference in thermal degradation.

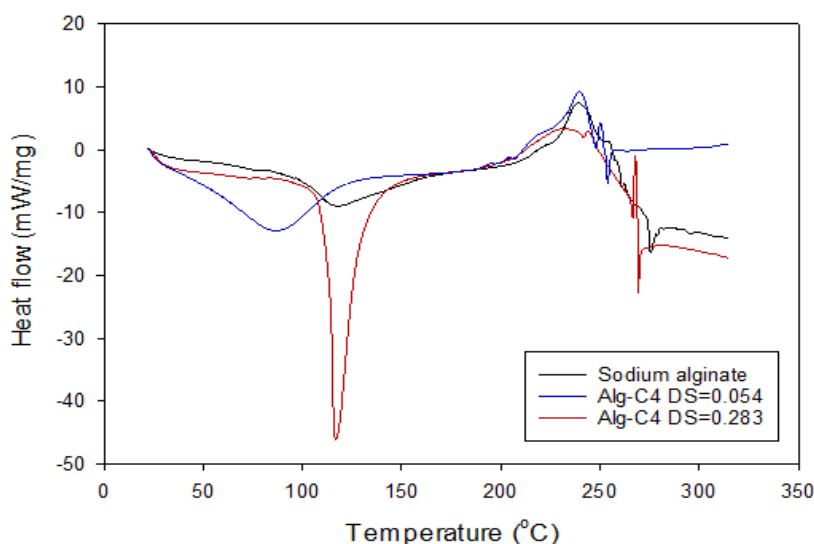


Figure 4. Thermogram of native alginate and Alg-C4.

3.5. Emulsion stability

The stability of red fruit oil emulsion was determined using creaming index analysis, which observed the separated serum or cream during the emulsion's storage. Figure 5 illustrates the effect of n-butanol:alginate ratio, reaction time, and reaction pH on the creaming index. A higher ratio of n-butanol:alginate, longer reaction duration, and pH reaction increased the DS value, which increased the o/w emulsion stability. This phenomenon can be attributed to the esterification reaction, which enhances the hydrophobic characteristics of alginate by incorporating butyl groups into the polymer chain [15]. A higher DS indicates that a greater number of carbonyl groups have been replaced by butyl groups, thereby increasing the hydrophobic properties and contributing to the stability of the emulsion [14]. Although the highest DS value was obtained by Alg-C4 which was produced by combination of the highest ratio of n-butanol:alginate (40:1), 15 h esterification time, and pH reaction of 4, the most stable emulsion was obtained by Alg-C4 with similar reaction conditions without pH adjustment. To adjust the reaction pH to 4, more HCl solution was added. However, more acid added to the reaction could accelerate the cleavage of alginate chain, which degraded the viscosity of alginate [30]. The viscosity of continuous phase is important to maintain the oil droplet in its state and prevent droplet coalescence [31]. Hence, the Alg-C4 with uncontrolled pH had better emulsion stability as it has higher viscosity.

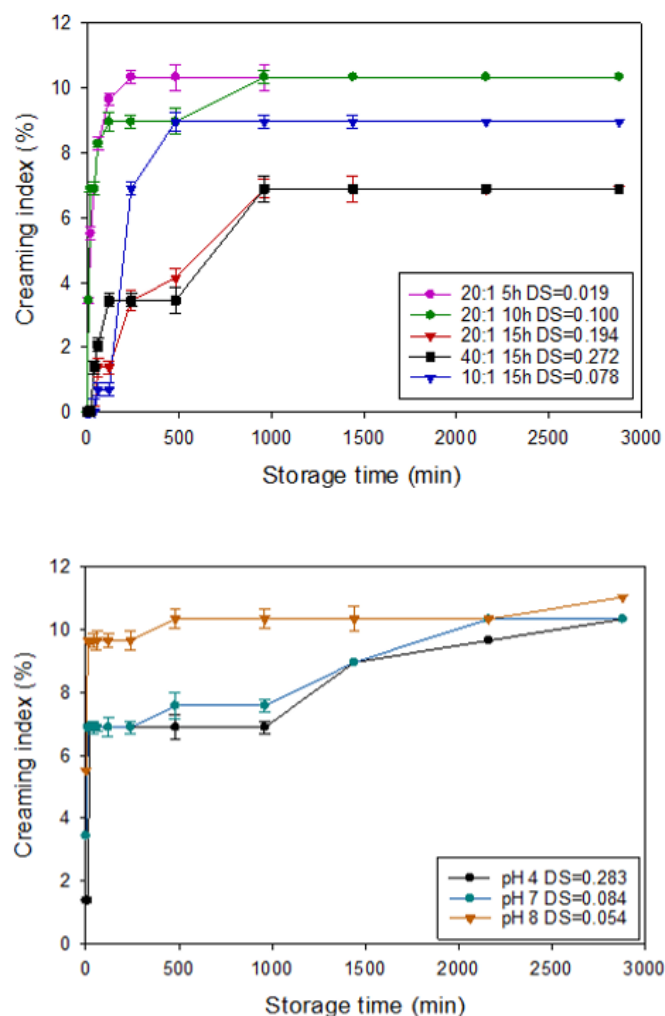


Figure 5. Stability of red fruit oil emulsion using Alg-C4 with different esterification conditions of n-butanol:alginate ratio and reaction time (top) and reaction pH (bottom).

3.6. Microscopic image of emulsion

Figure 6 shows optical microscope images at 400x magnification of red fruit oil emulsions formed using native sodium alginate, Alg-C4 with DS = 0.019, and Alg-C4 with DS = 0.272. Initial emulsion stabilized using native alginate and Alg-C4s showed oil droplets of nonuniform size. After 48 h of storage, fewer oil droplets of smaller size were observed. In native alginate emulsion, the oil is separated faster than in the Alg-C4 emulsion. Red fruit oil is gathered on the top layer of the emulsion, which cannot be observed in the remaining emulsion. In Alg-C4 emulsions, the coalescence process began the separation, which formed bigger oil droplets. However, the higher DS of Alg-C4 slowed down the coalescence process, hence the combined oil droplets are smaller than those with the lower DS. Increased droplet size over time indicates emulsion instability, but a higher DS in modified alginate enhances hydrophobicity, reducing interfacial tension and improving stability [32].

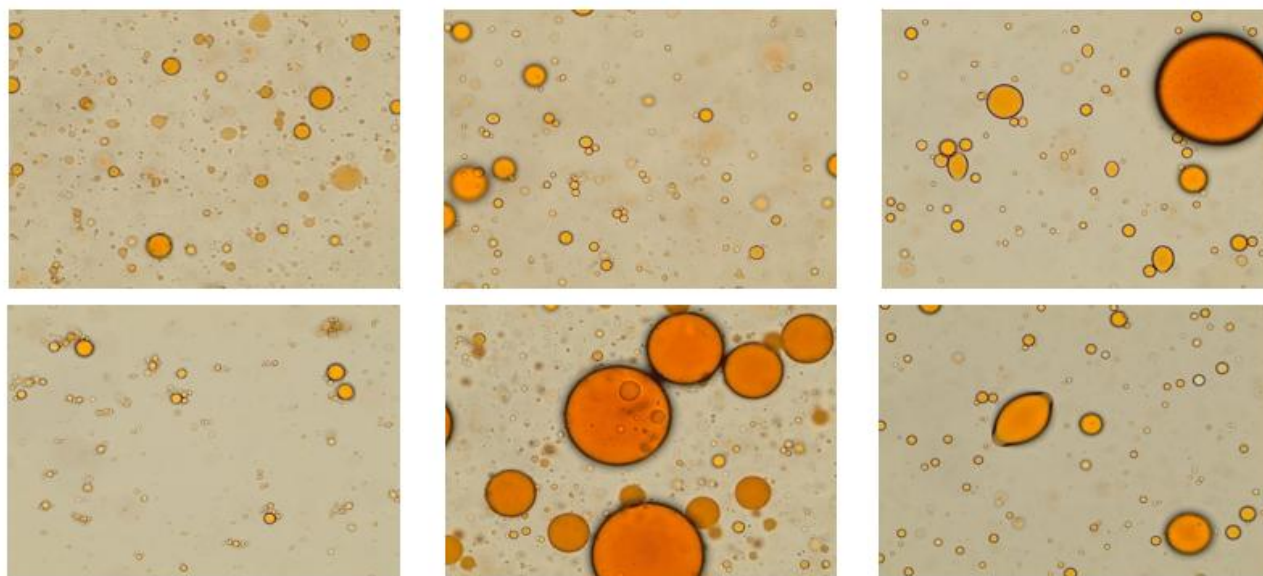


Figure 6. Microscopic images at 400x magnification of red fruit oil emulsion stabilized using (from left to right) native alginate, Alg-C4 DS = 0.0194, and Alg-C4 DS = 0.272 at initial storage time (top) and after 48 h of storage (bottom) in ambient conditions.

3.7. Bead syneresis

Syneresis is identified as the ability of the beads to hold water. Water holding ability of alginate beads was strongly influenced by -COOH group, which can be ionized and swelled at $\text{pH} > 4.4$ [33]. This property of alginate supported the beads formation in neutral pH of CaCl_2 solution ($\text{pH} = \pm 7$), which begins with the binding of water by alginate, followed by the crosslinking process between Ca^{2+} ions and alginate [34].

High DS increased the syneresis of alginate-red fruit oil beads (Figure 7). In general, esterification replaces the hydroxyl groups of alginates, including those of the -COOH group. Higher DS value indicates that more hydroxyl group replacements occurred. Hydroxyl groups were essential to form hydrogen bonds to attract water to alginate. Therefore, a lower content of hydroxyl groups led to a lowering of the water holding ability of alginate beads. This, in turn, results in greater syneresis due to faster water loss [35].

Besides the DS value, the pH adjustment of esterification process also affected the syneresis process of alginate beads. Acidic and alkaline solutions may promote the acetylation/deacetylation of alginate, which usually occurs frequently in the mannuronic unit [23]. However, the egg-box structure that holds water during gelation process is strongly influenced by the ratio of mannuronic and guluronic units of alginate [36]. Therefore, further study is needed to explain how esterification pH affects the syneresis phenomenon of alginate beads.

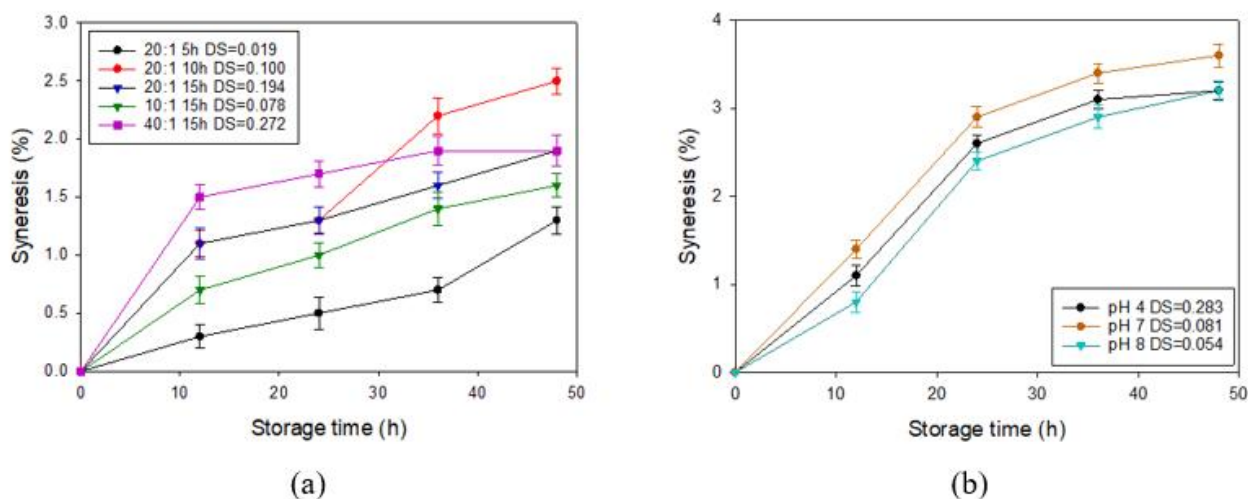


Figure 7. Syneresis of red fruit oil beads encapsulated using Alg-C4 with different preparation conditions: (a) n-butanol:alginate ratio, reaction time, and (b) reaction pH.

3.8. EE and storage stability

The esterification reaction influenced the EE of red fruit oil beads (Table 1). As the ratio of n-butanol to alginate increases, the EE decreases. Similar trends were also observed by prolonging the reaction time. A longer esterification time could result in greater degradation, which in turn reduced viscosity. This, in turn, gives rise to the formation of iron beads with a lower EE [37]. The EE value increases with increasing pH since acidic conditions cause degradation, which in turn results in a decrease in MW and solution viscosity [37].

Oxidation is defined as the process of an organic substrate gaining or losing oxygen [38]. During the oxidation process, the hydroxyl group in the molecule is oxidized to a carbonyl group [39]. The observed increase in the carbonyl group in alginate is indicative of an oxidation reaction having occurred. The rise of carbonyl content after 5 days of storage of red fruit oil beads is shown in Table 1. Increasing ratio of n-butanol:alginate slowed down the formation of carbonyl content. A higher content of n-butanol promotes the hydroxyl group replacements, consequently limiting the number of hydroxyl groups available for the oxidation reaction [40]. However, prolonging reaction time accelerated the formation of carbonyl content. A more broken chain of alginate with a longer acidic introduction formed more hydroxyl groups, hence increasing the formation rate of carbonyl content. The alkaline pH reaction also provided more hydroxyl groups through the direct addition of OH^- ions. Therefore, carbonyl groups formed more rapidly.

Table 1. EE and the increase of carbonyl content (ICC) after 5-days storage of red fruit oil beads encapsulated with Alg-C4s.

Parameters	20:1 5 h	20:1 10 h	20:1 15 h	10:1 15 h	40:1 15 h	pH 4	pH 7	pH 8
EE (%)	33.33	32.97	30.00	30.11	28.21	30.11	30.53	31.58
ICC (%)	1.4	1.4	1.68	1.68	1.4	2.24	2.52	2.8

4. Conclusions

The Alg-C4 was successfully prepared from alginate through esterification with n-butanol using HCl as a catalyst. The DS was increased by increasing mole ratio of n-butanol:alginate and prolonging the reaction time but decreased by increasing reaction pH. Higher DS decreased the viscosity average MW but did not significantly change the thermal stability of Alg-C4. Overall, the performance of Alg-C4 in stabilizing red fruit oil emulsion was in line with its value of DS. Increasing the mole ratio of n-butanol:alginate, reaction duration, and reaction pH increased the syneresis of the beads but decreased their EE. The ability of the bead to prevent oxidation was high and had different performances as various esterification conditions were applied. The esterification of alginate broadens the use of modified alginate, especially for plant-based food emulsifiers. The encapsulation of red fruit oil in bead form holds promise for future applications in food preservation, nutraceuticals, and pharmaceuticals. By minimizing storage and shipping risks, this method enhances the stability and shelf life of red fruit oil, making it more viable for large-scale distribution

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

DH Wardhani: Conceptualization, Funding acquisition. Ratnawati: Supervision, Writing—Review & Editing. HN Ulya: Writing—Original Draft, Project administration. D Savitri: Investigation, Resources. OT Sihombing: Investigation, Resources. N Aryanti: Validation. K Anam: Writing—Review & Editing. NA Handayani: Formal analysis. JA Vázquez: Methodology, Validation.

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