



Characterization of Hydrogel Beads Based on Sodium Alginate and Bacterial Cellulose as Encapsulants for Red Palm Oil Emulsion (*Elaeis guineensis* Jacq)

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Abstract. Red palm oil is a carotenoid-rich lipid source with functional potential, but its bioactive compounds are susceptible to oxidative degradation during processing and storage. This study aimed to develop red palm oil emulsion hydrogel beads using sodium alginate and bacterial cellulose as encapsulating materials and to evaluate the effect of their ratios on physicochemical and encapsulation properties. Beads were prepared by emulsion ionotropic gelation using CaCl_2 as a crosslinking agent. A completely randomized design with five treatments and three replications was used, and data were analyzed by ANOVA followed by Duncan's New Multiple Range Test at a 5% significance level. The sodium alginate-to-bacterial cellulose ratio significantly affected yield, bead size, sphericity factor, swelling capacity, total carotenoid content, and encapsulation efficiency. Increasing bacterial cellulose proportion improved bead yield, diameter, carotenoid retention, and encapsulation efficiency. Treatment E, containing 50 mL sodium alginate, 25 mL bacterial cellulose, and 25 mL red palm oil emulsion, showed the best characteristics, with 63.17% yield, 3.55 mm bead size, 0.004 sphericity factor, 44.47 $\mu\text{g/g}$ total carotenoid content, and 83.21% encapsulation efficiency. These findings indicate that sodium alginate–bacterial cellulose hydrogel beads are promising encapsulants for carotenoid-rich red palm oil emulsion.

Keywords: bacterial cellulose; encapsulation; hydrogel beads; red palm oil emulsion; sodium alginate.

Type of the Paper: Regular Article.

1. Introduction

Palm oil is an important plantation commodity in Indonesia and has long supported export-oriented downstream development. However, palm oil-derived products are still largely dominated by conventional crude palm oil (CPO) and its refined fractions, whereas several value-added fractions remain less optimally utilized [1]. One promising fraction is red palm oil (RPO), which retains important phytonutrients, particularly carotenoids and vitamin E compounds, including tocopherols and tocotrienols. These bioactive compounds contribute to the functional potential of RPO and support its possible application in food-based delivery systems [2].

Despite its nutritional and functional value, RPO is susceptible to quality deterioration during processing and storage. Carotenoids are highly sensitive to oxidation due to their conjugated double-bond structure, and their degradation may reduce nutritional quality as well as

affect product stability [3–5]. Therefore, protecting carotenoid-rich lipid systems from oxidative degradation is an important strategy to broaden the utilization of RPO as a functional ingredient

Encapsulation is widely applied to improve the stability of oxidation-sensitive oils and lipophilic bioactive compounds. This approach can reduce the exposure of active compounds to environmental stressors and improve their handling in food matrices [5–7]. In hydrogel-based encapsulation systems, sodium alginate is frequently used because of its biocompatibility and ability to form gel networks through ionotropic gelation with divalent cations such as Ca^{2+} [8]. Alginate beads have been reported as suitable matrices for entrapping lipid-based emulsions, including palm oil-related systems, through ionic gelation [9].

The incorporation of hydrophobic oils such as RPO into a hydrophilic alginate matrix generally requires the formation of an oil-in-water (O/W) emulsion prior to gelation. Emulsion characteristics, including droplet dispersion, viscosity, and pH, may influence the stability of the emulsion and the physical properties of the resulting beads. Nonionic emulsifiers such as Tween-type surfactants are commonly used to reduce interfacial tension, suppress droplet coalescence, and support the formation of more uniform oil droplets in aqueous polymer systems [7,10,11].

Although alginate can form hydrogel beads effectively, its structural performance may be improved through composite formulation [12,13,18,19]. Bacterial cellulose was selected as a reinforcing component because its nanofibrillar three-dimensional network can strengthen the alginate matrix, improve matrix compactness, and support bead integrity during ionotropic gelation [13,18,19]. In addition, its high hydrophilicity and fibrous structure may increase suspension viscosity and facilitate the retention of RPO emulsion droplets and carotenoids within the hydrogel network [16–19]. Therefore, variation in the sodium alginate-to-bacterial cellulose ratio is an important formulation factor that may affect the physicochemical and encapsulation properties of RPO-loaded hydrogel beads [16–19].

Previous studies have reported the encapsulation of RPO or carotenoid-rich oils using polysaccharide-based systems and alginate-based matrices [9,14,15]. However, studies focusing specifically on sodium alginate–bacterial cellulose composite hydrogel beads for RPO emulsion encapsulation remain limited. In particular, systematic evaluation of how different sodium alginate and bacterial cellulose proportions affect bead yield, bead size, sphericity, swelling behavior, carotenoid retention, and encapsulation efficiency is still needed.

Therefore, this study aimed to develop red palm oil emulsion-loaded hydrogel beads using sodium alginate and bacterial cellulose through an emulsion ionotropic gelation method. The study further evaluated the effect of different sodium alginate-to-bacterial cellulose ratios on the physicochemical characteristics and encapsulation performance of the resulting hydrogel beads.

This work is expected to provide a formulation basis for developing hydrogel bead systems as encapsulants for carotenoid-rich red palm oil emulsion.

2. Materials and Methods

2.1. Materials

The materials used in this study were Harvist red palm oil, Tween 80 (pure analytical grade), sodium alginate, calcium chloride (CaCl_2) p.a., sodium chloride (NaCl) p.a., 2% sodium hydroxide (NaOH), 3% hydrogen peroxide (H_2O_2), 5 mM sodium phosphate buffer solution (pH 7), n-hexane, distilled water, *Acetobacter xylinum* bacteria, mature coconut water, 0.5% acetic acid, zwavelzure ammoniac (ZA) or ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), 10% granulated sugar, tissue paper, aluminum foil, and labeling paper.

2.2. Research Design

This study employed a Completely Randomized Design (CRD) with five treatments and three independent replicates per treatment. The treatments consisted of different proportions of sodium alginate and bacterial cellulose, as summarized in Table 1. The experimental data were analyzed using one-way analysis of variance (ANOVA) to evaluate the effects of the treatments on the measured response variables. Statistical significance was established at $p < 0.05$. When significant differences were detected, Duncan's New Multiple Range Test (DNMRT) was performed for multiple comparisons among treatment means

Table 1. Treatment matrix

Material	Composition (ml)				
	A	B	C	D	E
Emulsion (ml)	25	25	25	25	25
Sodium alginate 3% (ml)	70	65	60	55	50
Bacterial cellulose (ml)	5	10	15	20	25
CaCl_2 100 mM (ml)	100	100	100	100	100

2.3. Preparation of Red Palm Oil Emulsion

The red palm oil emulsion was prepared by dispersing Tween 80 as an emulsifier at a concentration of 10%, using an oil-to-water ratio of 7:3. First, Tween 80 at 10% concentration was added to 27 mL of water, then stirred and heated using a magnetic stirrer. Next, 63 mL of red palm oil was slowly added dropwise into the Tween–water mixture. After all components were well mixed, the emulsion was homogenized for 7 minutes using a homogenizer at 10,000 rpm. The formation of the emulsion could be observed from the appearance of the palm oil and water mixture turning turbid. The resulting emulsion was then stored at 25°C for ten days. If no separation or other signs of degradation occurred during this period, the emulsion was considered stable.

2.4. *Synthesis of Bacterial Cellulose from Nata de Coco*

One liter of mature coconut water was filtered, then mixed with 10% sugar and 0.5% ammonium sulfate and heated to boiling. After heating, 0.5% acetic acid was added, and the solution was aseptically cooled before 10% *Acetobacter xylinum* starter was introduced. The mixture was poured into sterilized trays and incubated for about 21 days to form bacterial cellulose. The formed cellulose was soaked for 24 hours, washed, cut, and purified by boiling 250 g of cellulose in 250 mL of 2% NaOH at 85°C, followed by rinsing and soaking in 3% H₂O₂. It was then blended with water (1:4), allowed to settle, filtered, and pressed [20].

2.5. *Preparation of Sodium Alginate–Bacterial Cellulose Hydrogel Beads as Encapsulants for Red Palm Oil Emulsion*

A 3% sodium alginate solution and a 1 % bacterial cellulose suspension were placed into a beaker until the total volume reached 75 mL. Then, 25 mL of red palm oil emulsion was added into the sodium alginate–bacterial cellulose mixture and stirred until homogeneous. The resulting encapsulant solution was dripped using a 60 mL syringe pump into a calcium chloride (CaCl₂) solution while being stirred with a magnetic stirrer at 30 rpm. The beads formed in the CaCl₂ solution were allowed to stand for 30 minutes [21]. Next, the beads were filtered and rinsed with distilled water. The resulting beads were stored in a refrigerator. Additional variations of formulations were also tested.

2.6. *Droplet Size*

Droplet size was determined using a particle size analyzer (Horiba SZ-100). Approximately 0.1 mL of emulsion was diluted with distilled water in a centrifuge tube at a 1:500 dilution ratio. The sample was placed into a cuvette suitable for the PSA, and the instrument analyzed particle size distribution through the Horiba SZ-100 software [22].

2.7. *Specific gravity*

A cleaned and dried empty pycnometer was weighed (A g). It was then filled with distilled water, weighed again (A1 g), cleaned, and refilled with emulsion before re-weighing (A2 g). Specific gravity was calculated using Eq. (1).

$$\text{Specific gravity (g/ml)} = \frac{A2-A}{A1-A} \quad (1)$$

2.8. *Viscosity*

Viscosity was measured using a Brookfield viscometer. The emulsion was poured into a beaker up to 100 mL, then spindle No. 3 was immersed completely and the viscosity reading in cPs was recorded [23].

2.9. pH measurement

Ten grams of sample was placed in a beaker. The pH meter electrode was calibrated using pH 4 and 7 buffer solutions until stable readings were obtained. The electrode was then immersed into the emulsion and the pH was recorded.

2.10. Yield

The yield calculation is performed to determine the percentage of the material after processing compared to the material before processing [24]. The work steps involved weighing the input material and the resulting sodium alginate hydrogel beads. The yield was calculate using Eq. (2).

$$\text{Yield} = \frac{\text{weight of hydrogel beads(g)}}{\text{weight of material for making beads(g)}} \times 100\% \quad (2)$$

2.11. Bead size

Bead size was determined using a digital caliper. Twenty sodium alginate hydrogel beads were randomly sampled from each treatment. The beads were placed in the digital caliper. The diameter of the beads was then displayed on the digital caliper screen. The results were then recorded [24].

2.12. Sphericity factor

Sphericity factor analysis was conducted to quantitatively evaluate the roundness of the hydrogel beads. The analysis was performed by measuring the maximum diameter passing through the bead center (D_{max}) and the perpendicular diameter passing through the bead center (D_{per}). A total of 20 beads were randomly selected from each treatment for measurement [25]. The sphericity factor was calculated using Eq. (3).

$$SF = \frac{D_{\text{max}} - D_{\text{per}}}{D_{\text{max}} + D_{\text{per}}} \quad (3)$$

2.13. Swelling power

The capacity of beads to swell and absorb water was measured through a swelling test. The swelling test was observed by soaking beads in three different media, namely distilled water, phosphate buffer (5 mM; pH 7), and NaCl solution (30 mM). A total of 50 beads were weighed first, then soaked in 25 ml of each medium at 25°C for 24 hours. The soaked beads were then weighed to obtain their weight [26]. The swelling percentage was calculate using Eq. (4).

$$\text{Swelling power} = \frac{W_1 - W_0}{W_0} \times 100\% \quad (4)$$

Description:

W1: weight of hydrated beads (g)

W0: weight of dry beads (g)

2.14. Total carotenoid test

Total carotenoid content was determined using the UV-Vis spectrophotometry method with n-hexane as the solvent at a maximum absorption wavelength of 446 nm. A 1 g sample was

weighed and placed into a test tube, followed by the addition of 10 mL of n-hexane. The mixture was homogenized using an Ultrasonic Cleaner BK-1200 at 60°C for 10 minutes under light-protected conditions. The extract was then transferred into a 25 mL volumetric flask and diluted with n-hexane up to the calibration mark, followed by homogenization. The absorbance of the solution was measured using a UV-Vis spectrophotometer at 446 nm, with n-hexane used as the blank [27]. The total carotenoids was calculate using Eq. (5).

$$\text{Total carotenoid } (\mu\text{g/g}) = \frac{25 \times \text{absorbance} \times 383}{100 \times \text{weight sample (g)}} \times 100 \quad (5)$$

2.15. Encapsulation efficiency

The presence of oil on the outer surface (SO) of the beads was identified after hexane extraction by dispersing 0.5 g of beads in 10 mL of hexane and homogenizing the suspension for 1 min. The resulting mixture was then filtered, diluted, and the palm oil concentration was quantified using a spectrophotometer at 228 nm. For total oil (TO) determination, 0.5 g of beads was first treated with 5 mL of hot water (60 °C) and stirred for 3 h to disintegrate the bead matrix. Subsequently, 20 mL of hexane was added and the mixture was agitated for 60 min, followed by 5 min of sonication (Ultrasonic cleaner BK-1200) to enhance oil recovery. The sample was centrifuged at 8,000 rpm for 15 min, and 5 mL of the supernatant was collected, diluted with 100 mL of distilled water, and analyzed again at 228 nm [14]. Encapsulation efficiency (EE%) was then calculated using Eq. (6).

$$\text{EE (\%)} = 1 - \frac{\text{SO}}{\text{TO}} \times 100\% \quad (6)$$

3. Results and Discussion

3.1 Red palm oil analysis

The analysis conducted on the red palm oil emulsion included droplet size, specific gravity, viscosity, and pH. The results of the red palm oil emulsion analysis are presented in Table 2.

Tabel 2. Red Palm Oil Analysis Result

Analysis Parameters	Analysis Results (Average)
Droplet Size (μm)	0.8421 ± 0.0227
Specific Gravity (g/ml)	0.9566 ± 0.0013
Viscosity (cPs)	1364 ± 0.58
pH	5.27 ± 0.05

The droplet size of the red palm oil emulsion obtained in this study was 0.8421 ± 0.0227 μm, indicating that the homogenization process at 10,000 rpm for 7 min using 10% Tween 80 was effective in producing a relatively fine dispersion. Smaller droplet sizes increase the interfacial surface area between oil and water phases, thereby reducing the probability of coalescence and improving emulsion stability. Droplet size in emulsions is strongly influenced by homogenization intensity, emulsifier concentration, and the physicochemical properties of the oil and aqueous

phases. Previous studies have reported that high shear homogenization combined with non ionic surfactants such as Tween 80 can significantly reduce droplet size and improve the stability of oil-in-water emulsions [28]. Complete data regarding the results of the emulsion particle size using a particle size analyzer can be seen in Fig. 1.

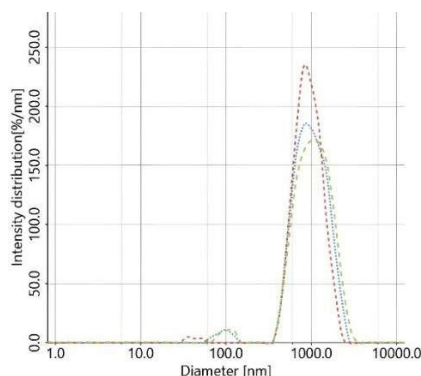


Fig. 1. Results of Red Palm Oil Emulsion Particle Size

The specific gravity (0.9566 ± 0.0013 g/mL), viscosity (1364 ± 0.58 cPs), and pH (5.27 ± 0.05) obtained in this study indicate that the formulated emulsion possesses suitable physicochemical characteristics for a stable oil-in-water system. The specific gravity value close to unity reflects the combined contribution of the oil and aqueous phases, as palm oil typically has a density of approximately 0.91–0.93 g/mL, while water has a density of 1 g/mL. Meanwhile, the relatively high viscosity suggests stronger droplet–droplet interactions and a greater resistance to flow, which may contribute to improved physical stability by slowing droplet movement and reducing phase separation. Previous studies have also reported that increasing lipid concentration and droplet surface area can lead to higher viscosity in emulsified systems [29]. Furthermore, the pH value of 5.27 falls within the typical range of food emulsions and contributes to maintaining chemical stability while minimizing microbial growth and potential degradation reactions. Therefore, the combination of small droplet size, moderate viscosity, appropriate density, and suitable pH indicates that the red palm oil emulsion system produced in this study exhibits favorable physicochemical properties and potential stability during storage. The red palm oil emulsion is shown in Fig. 2.



Fig. 2. Red Palm Oil Emulsion

3.2 Analysis Results of Sodium Alginate and Bacterial Cellulose Hydrogel Beads Containing Red Palm Oil Emulsion

3.2.1 Yield

The yield was determined by comparing the total mass of hydrogel beads obtained with the total mass of sodium alginate and bacterial cellulose used in the preparation of hydrogel beads for the encapsulation of red palm oil emulsion. The yield of sodium alginate–bacterial cellulose hydrogel beads obtained in this study is presented in [Table 3](#).

Table 3. Yield of Hydrogel Beads

Treatment	Yield (%)	
A	46.93 ± 0.112	a
B	49.85 ± 0.188	b
C	53.66 ± 0.318	c
D	55.22 ± 0.379	d
E	63.17 ± 0.208	e

Analysis of variance (ANOVA) at a significance level of $\alpha = 5\%$ indicated that the sodium alginate to bacterial cellulose ratio had a significant effect on the yield of hydrogel beads. Duncan's multiple range test further showed that the mean yield differed significantly among all treatment groups. The yield ranged from 46.93% to 63.17%.

The results indicate that the yield of hydrogel beads was influenced by the sodium alginate-to-bacterial cellulose ratio used in the formulation. Increasing the proportion of bacterial cellulose, accompanied by a reduction in sodium alginate content, resulted in a higher hydrogel bead yield. This increase can be attributed to the higher amount of total solids incorporated into the bead formulation. Bacterial cellulose acts as a structural component of the resulting gel matrix [30]. As the proportion of bacterial cellulose increased, more solid material was entrapped within the alginate–CaCl₂ gel network, thereby increasing the mass of the harvested final product. The sodium alginate–bacterial cellulose hydrogel beads are shown in [Fig. 3](#).

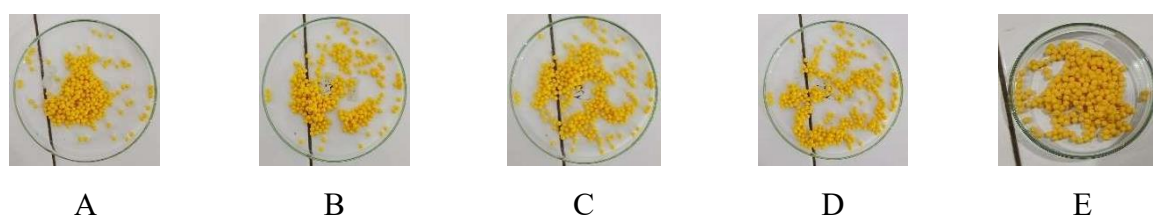


Fig. 3. Sodium Alginate–Bacterial Cellulose Hydrogel Beads Containing Red Palm Oil Emulsion

3.2.2 Bead Size

Bead size is a physical evaluation parameter used to determine the average diameter of hydrogel beads, usually expressed in millimeters or micrometers. This parameter also reflects bead uniformity, which is assessed by measuring the diameter of several beads and calculating the mean value. The bead size results are presented in [Table 4](#).

Table 4. Bead Size of Hydrogel Beads

Treatment	Beads Diameter (mm) $\pm$ SD		
A	2.84 $\pm$ 0.067	a	
B	2.92 $\pm$ 0.005	b	
C	3.22 $\pm$ 0.035	c	
D	3.39 $\pm$ 0.030	d	
E	3.55 $\pm$ 0.030	e	

Analysis of variance at a significance level of $\alpha = 5\%$ showed that different sodium alginate-to-bacterial cellulose ratios had a significant effect on the diameter of the hydrogel beads. Duncan's multiple range test further indicated that the mean diameter of sodium alginate–bacterial cellulose hydrogel beads differed significantly among all treatments.

The bead diameter ranged from 2.84 to 3.55 mm, which is consistent with the diameter range previously reported for alginate hydrogel beads, namely 2–5 mm [24,31]. The bead size tended to increase as the volume of bacterial cellulose suspension added to the sodium alginate and red palm oil emulsion mixture increased. A higher proportion of bacterial cellulose in the formulation increased the viscosity of the suspension. This higher viscosity delayed droplet detachment from the nozzle tip, resulting in the formation of larger droplets during the bead production process. Consequently, the resulting hydrogel beads had larger diameters [31].

3.2.3 Sphericity Factor

The sphericity factor test was used to quantitatively evaluate bead roundness. A value of 0 indicates a perfectly spherical bead, whereas higher values indicate greater deviation from sphericity. Sphericity was determined by measuring $D_{\max}$, the maximum diameter passing through the bead center, and D_{per} , the perpendicular diameter passing through the bead center, using 20 randomly selected beads from each treatment. The results of the sphericity factor test are presented in Table 5.

Table 5. Sphericity Factor of Hydrogel Beads

Treatment	Sphericity Factor $\pm$ SD		
E	0.004 $\pm$ 0.002	a	
D	0.016 $\pm$ 0.008	a	
C	0.036 $\pm$ 0.011	b	
B	0.053 $\pm$ 0.010	c	
A	0.084 $\pm$ 0.006	d	

Based on the analysis of variance, the bacterial cellulose-to-sodium alginate ratio had a significant effect on the sphericity factor of the hydrogel beads at $\alpha = 5\%$. Duncan's multiple range test showed that treatments D and E were not significantly different from each other, whereas treatments A, B, and C differed significantly. The mean sphericity factor ranged from 0.004 to 0.084. These values are comparable to those reported by Chan et al. [25], who found sphericity factor values of 0.02–0.06. According to Chan et al. [25], lower values indicate beads that are

closer to a perfect spherical shape, while values below 0.05 indicate spherical beads. The results indicate that increasing the proportion of bacterial cellulose decreased the sphericity factor, suggesting improved bead roundness. This improvement may be attributed to the nanofibrillar structure of bacterial cellulose, which forms a stable three-dimensional network and increases suspension viscosity. Higher viscosity enhances droplet stability during extrusion into CaCl_2 solution, reducing deformation caused by gravitational and collision forces. Therefore, a higher bacterial cellulose ratio contributed to the formation of more spherical and structurally stable hydrogel beads [32].

3.2.4 Swelling Capacity

Swelling capacity analysis was conducted to evaluate the ability of hydrogel beads to absorb water under different environmental conditions, including immersion in distilled water, 30 mM NaCl solution, and phosphate buffer. A total of 50 beads were immersed in 25 mL of each medium at 25°C for 24 h. The results of the swelling capacity analysis are presented in Table 6.

Table 6. Swelling Capacity of Hydrogel Beads

Treatment	Swelling Capacity (%) $\pm$ SD		
	Distilled Water	NaCl 30 mM	Buffer phospat 5 mM
A	64.49 $\pm$ 0.265 e	90.50 $\pm$ 0.653 a	170.12 $\pm$ 0.681 e
B	55.99 $\pm$ 0.465 d	108.16 $\pm$ 0.525 b	162.49 $\pm$ 0.757 d
C	45.00 $\pm$ 0.813 c	110.92 $\pm$ 0.472 c	152.06 $\pm$ 0.484 c
D	36.64 $\pm$ 0.434 b	119.13 $\pm$ 0.200 d	145.39 $\pm$ 0.826 b
E	25.84 $\pm$ 0.349 a	124.58 $\pm$ 0.407 e	140.96 $\pm$ 0.825 a

Analysis of variance at a significance level of $\alpha = 5\%$ showed that the sodium alginate-to-bacterial cellulose ratio had a significant effect on the swelling capacity of hydrogel beads in all immersion media. Duncan's multiple range test further indicated that the mean swelling capacity differed significantly among all treatments after immersion in distilled water, 30 mM NaCl solution, and phosphate buffer.

The swelling capacity of the hydrogel beads varied depending on both the formulation and the immersion medium. In distilled water and phosphate buffer, beads with a lower bacterial cellulose ratio, particularly Treatment A, exhibited higher swelling capacity than those with a higher bacterial cellulose ratio, such as Treatment E. In contrast, the opposite trend was observed in 30 mM NaCl solution, where swelling capacity increased with increasing bacterial cellulose content.

In distilled water, the swelling capacity decreased from 64.49% in Treatment A to 25.84% in Treatment E. A similar trend was observed in phosphate buffer, where Treatment A showed the highest swelling capacity of 170.12%, whereas Treatment E showed the lowest value of 140.96%. Conversely, in 30 mM NaCl solution, Treatment A had the lowest swelling capacity of 90.50%, while Treatment E exhibited the highest swelling capacity of 124.58%.

Swelling occurs when water penetrates the hydrogel matrix, causing relaxation and loosening of the polymer network [26,33]. The lower swelling capacity observed in distilled water may be attributed to the absence of ions that can interact with the functional groups of sodium alginate. In contrast, when sodium alginate–bacterial cellulose hydrogel beads were immersed in NaCl solution or phosphate buffer, ions present in the medium interacted with alginate functional groups and promoted water uptake into the hydrogel matrix [34]. This water absorption increased bead swelling and may have enlarged the bead diameter. Furthermore, the loosening of the hydrogel network due to water penetration can reduce stiffness and affect the mechanical properties of the beads [26,33].

3.2.5 Total Carotenoid Content

Total carotenoid content analysis was conducted to determine the amount of carotenoids retained in sodium alginate–bacterial cellulose hydrogel beads. This analysis reflects the ability of the encapsulating matrix to retain carotenoids after the encapsulation process. The total carotenoid content values are presented in Table 7.

Table 7. Total Carotenoids in Hydrogel Beads

Treatment	Total Carotenoid Content ($\mu\text{g/g}$) $\pm$ SD		
A	8.53 $\pm$ 0.1101	a	
B	13.66 $\pm$ 0.3343	b	
C	14.1632 $\pm$ 0.053	c	
D	14.57 $\pm$ 0.0640	d	
E	44.47 $\pm$ 0.0787	e	

Based on the analysis of variance presented in Table 7, the sodium alginate-to-bacterial cellulose ratio had a significant effect on the total carotenoid content of the hydrogel beads at $\alpha = 5\%$. Duncan's multiple range test further showed that the mean total carotenoid content differed significantly among all treatments. The total carotenoid content ranged from 8.53 to 44.47 $\mu\text{g/g}$.

These findings indicate that total carotenoid retention in hydrogel beads was influenced by the bacterial cellulose-to-sodium alginate ratio. As the proportion of bacterial cellulose increased, the total carotenoid content of the hydrogel beads also increased. This increase may be attributed to the role of bacterial cellulose in forming a denser and more stable hydrogel matrix, thereby improving the ability of the system to retain and protect carotenoids within the emulsion [35].

A compact hydrogel matrix can reduce oxygen diffusion and limit excessive carotenoid release. Consequently, encapsulated carotenoids are better protected from degradation caused by environmental factors, such as heat, light, and oxygen [16]. Carotenoids are highly susceptible to oxidation because of the presence of conjugated double bonds in their molecular structure, and their degradation can be accelerated by metals, peroxides, and high temperatures [36].

In addition, total carotenoid content may be related to the size of the hydrogel beads. A greater amount of encapsulated carotenoids is generally associated with larger bead diameters, as

a larger bead volume allows more material to be retained within the hydrogel network formed by sodium alginate and bacterial cellulose. Therefore, increasing bacterial cellulose content strengthened the hydrogel matrix and enhanced carotenoid retention by protecting carotenoids from oxidation and degradation [16]. This finding is also supported by previous studies reporting that hydrophilic polymers with dense network structures can retain lipophilic compounds, including carotenoids [14].

3.2.6 Encapsulation Efficiency

Encapsulation efficiency was determined to evaluate the success of red palm oil emulsion encapsulation in sodium alginate–bacterial cellulose hydrogel beads. In this study, encapsulation efficiency refers to the ratio of total carotenoid content entrapped within the hydrogel beads to the total carotenoid content initially present in red palm oil. The encapsulation efficiency results are presented in Table 8.

Table 8. Encapsulation Efficiency of Hydrogel Beads

Treatment	Encapsulation Efficiency (%) $\pm$ SD	
A	37.00 $\pm$ 0.980	a
B	47.97 $\pm$ 0.539	b
C	52.70 $\pm$ 0.634	c
D	65.49 $\pm$ 0.883	d
E	83.21 $\pm$ 0.225	e

Analysis of variance at a significance level of $\alpha = 5\%$ showed that the sodium alginate-to-bacterial cellulose ratio had a significant effect on encapsulation efficiency. Duncan's multiple range test further indicated that the mean encapsulation efficiency differed significantly among all treatments.

The encapsulation efficiency ranged from 37.00% to 83.21%, with the highest value observed in Treatment E and the lowest in Treatment A. The increase in encapsulation efficiency indicates that the sodium alginate-to-bacterial cellulose ratio plays an important role in determining the ability of the encapsulating matrix to retain active compounds within the hydrogel bead structure [14].

Increasing the proportion of bacterial cellulose may enhance system viscosity and promote the formation of a denser and more stable hydrogel matrix. This matrix can improve the retention of red palm oil emulsion, allowing more active compounds, particularly carotenoids, to be entrapped within the beads. Thus, a higher bacterial cellulose content strengthened the hydrogel network and increased encapsulation efficiency [37].

Encapsulation efficiency is also closely related to the total carotenoid content retained in the hydrogel beads. A greater amount of encapsulated carotenoids indicates a higher ability of the system to protect active compounds from degradation or loss during the encapsulation process

[16]. Therefore, optimizing the sodium alginate-to-bacterial cellulose ratio is essential for improving the efficiency and effectiveness of carotenoid encapsulation in hydrogel beads.

4. Conclusions

This study demonstrated that sodium alginate–bacterial cellulose hydrogel beads can be used as encapsulating matrices for red palm oil emulsion through an emulsion ionotropic gelation method. The variation in sodium alginate-to-bacterial cellulose ratio significantly affected the physicochemical and encapsulation properties of the hydrogel beads, including yield, bead size, sphericity factor, swelling capacity, total carotenoid content, and encapsulation efficiency. Increasing the proportion of bacterial cellulose generally improved bead formation, carotenoid retention, and encapsulation efficiency, indicating its role in strengthening the hydrogel matrix and enhancing the ability of the beads to retain red palm oil emulsion. Treatment E, with a formulation of 50 mL sodium alginate, 25 mL bacterial cellulose, and 25 mL red palm oil emulsion, produced the most favorable characteristics, including a yield of 63.17%, bead size of 3.55 mm, swelling capacity of 25.84% in distilled water, 124.58% in NaCl solution, and 140.96% in phosphate buffer, total carotenoid content of 44.47 µg/g, sphericity factor of 0.004, and encapsulation efficiency of 83.21%. Therefore, this formulation can be considered the best treatment and may serve as a potential basis for developing hydrogel bead systems as encapsulants for carotenoid-rich red palm oil emulsion.

Data availability statement

The data sets generated and/or described during the research are included in this paper

CRedit authorship contribution statement

Fitri Zuzilla Maha Rilmi: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Visualization, Writing –original draft, and Writing –review and editing. **Ira Desri Rahmi:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Validation, Writing –original draft, and Writing –review and editing. **Deivy Andhika Permata:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, and Writing –original draft.

Declaration of Competing Interest

The authors declare no conflict of interest in preparing this paper.

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