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Research Article

Fermentation and Microencapsulation of Red Palm Oil as a Nutraceutical Source

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ABSTRACT

Red palm oil (RPO) has various bioactive and nutritional components with high potential to be developed as a source of nutraceuticals in addition to its potential as a functional food. The fermentation technology is a processing process that affects metabolite activity, increasing the durability of a product. *Staphylococcus epidermidis* is one of the bacteria that is generally used in the sugar and oil fermentation process. RPO fermentation by *Staphylococcus epidermidis* is supposed to increase nutraceutical value by adding probiotic properties to RPO products. Moreover, the instability of bioactive compounds in RPO needs to be protected with coating technology microencapsulation. So, this study aims to formulate a microencapsulation procedure for fermented RPO using *Staphylococcus epidermidis* ATCC 12228 as a potential nutraceutical. RPO was fermented using *S. epidermidis* with three variations of MRS media and RPO comparison, then microencapsulated using emulsification and extrusion methods. The physicochemical properties and bioactivity of the product, microcapsule, were then analyzed. Our data shows that RPO fermentation was able to increase the chemical components. RPO fermentation produced more 1,2-Benzenedicarboxylic acid (2-Ethylhexyl) ester. Furthermore, microencapsulation of fermented RPO has better yield, efficiency, and solubility in water than non-fermented RPO microencapsulation. Moreover, our work also shows that the microencapsulation process increased RPO stability.

1. Introduction

Palm Oil (*Elaeis guineensis*. Jacq) is a monocot plant belonging to the Palmae family. Oil harvested from palm oil has two fractions: crude palm oil (CPO) and crude palm kernel oil (CPKO). The olein fraction from the refining of CPO produces red palm oil (RPO), which has nutritional components such as carotenoids, vitamin E, tocopherols, sterols, squalene, tocotrienols, and polyunsaturated fatty acids (PUFAs) (Cazzonelli 2011; Sathasivam *et al.* 2018; Goon *et al.* 2019). However, this compound has not been utilized optimally because it is red and has a less familiar 'palmy' smell; people are more accustomed to consuming cooking oil with a golden yellow color (Purnama *et al.* 2020). In addition, in the cooking oil production process from CPO, the oil color compounds are degraded to increase stability against oxidation (Wu *et al.* 2019). Some countries use RPO as food products such as fortified biscuits (Van Stuijvenberg *et al.* 2001), local snacks (spring rolls, pastels, and donuts), curries and cakes (Ng *et al.* 2012), instant noodles, brown sugar (Dwiyanti *et al.* 2014), dry bread, and alternative additive for snack food products (Harianti *et al.* 2018).

Red palm oil, RPO, contains phytonutrient compounds, including carotene (as provitamin A), tocopherol, tocotrienols (as vitamin E), phytosterols, phospholipids, squalene, ubiquinone, aliphatic alcohol,

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triterpene alcohol, methyl sterols, and aliphatic hydrocarbons (Mba *et al.* 2015). Other research study reported that RPO contains palmitate, α-tocopherol, oleic, α-tocotrienol, linoleate, carotene, β-tocotrienol and δ-tocotrienol compounds (Sathasivam *et al.* 2018). These compounds act as vitamins and antioxidants. The carotenoid group is used as vitamin A, and the tocopherol and tocotrienol are used as vitamin E (Ayeleso *et al.* 2012). Phytonutrient compounds in RPO are beneficial for health as antioxidants, anti-cancer, anti-hypertensive, anti-diabetic, and lower cholesterol (Monjotin *et al.* 2022). RPO compositions such as polyunsaturated fatty acids (PUFA), carotenoids, tocopherols, tocotrienols, sterols, phospholipids, squalene, and tripterpenic/aliphatic hydrocarbons are reported to improve human health (Cazzonelli 2011). RPO has a high potential to be developed as a nutraceutical source in addition to its potential as a source of functional food. Nutraceuticals generally consist of dietary supplements, herbal products, prebiotics, probiotics, and medical foods intended to prevent and treat disease (Khorasani *et al.* 2018). To improve the human body's absorption of the compound of RPO, the initial breakdown of complex fatty acid compounds through fermentation can be done (Aprea *et al.* 2023).

Fermentation can break down large organic molecules via the action of microorganisms and contribute to increase food's accessibility and sensory quality (Ananda *et al.* 2022). The activity of microorganisms plays a vital role in food fermentation by exhibiting changes in food's chemical and physical properties. The fermenting microorganisms mainly involved like *Leuconostoc mesenteroide, Lactococcus lactis, Streptococcus thermophilus, Lactobacillus helveticus, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus kefiranofaciens, Lactobacillus plantarum, Acetobacter lovaniensis, Acetobacter orientalis, Saccharomyces cerevisiae, Saccharomyces unisporus, Candida kefyr*, and *Kluyveromyces marxianus* (Soemarie *et al.* 2021). One of the bacteria as a fermentation agent is *Staphylococcus epidermidis* (*S. epidermidis*), a skin microorganism in the human fingerprint microbiome that can ferment glycerol and create a zone of inhibition to expel overgrown Propionibacterium acnes colonies (Wang *et al.* 2014). Fermentation generally uses microorganisms by breaking down complex compounds into simple compounds easily digested by the human body as nutrients, such as probiotic bacteria (Nuraida 2015). Adding probiotics can inhibit pathogenic microorganisms, increasing a product's durability (Silva *et al.* 2020). A previous study showed that *S. epidermidis* ATCC 12228 could ferment glycerol, sucrose or polyethylene Glycol (PEG) to produce Short-chain fatty acids (SCFAs), such as acetic, butyric, lactic, and succinic acids, and successfully inhibited the growth of opportunistic *Cutibacterium acnes* (Shu *et al.* 2013; Yang *et al.* 2019; Marito *et al.* 2020). Using *S. epidermidis* as a fermentation agent in RPO is intriguing to study because it can break down complex lipid compounds into SCFA and increase their bioavailability in the body.

Additionally, the bioactive components have low stability and are sensitive to oxidation, which causes a decrease in the nutritional quality of RPO (Ezhilarasi *et al.* 2013) and perishable (Lee *et al.* 2018). Coating technology via encapsulation technology can protect the bioactive content of essential oils against unwanted chemical interactions with other components and provide increased stability during processing (Prakash *et al.* 2018). Encapsulation of bioactive compounds through the chemical process will have a higher nutraceutical value because encapsulation increases their stability and bioactivity as well as the delivery process of active compounds as functional and nutraceutical foods (Ezhilarasi *et al.* 2013; Hasrini *et al.* 2017). In this study, we microencapsulated the fermented RPO that is targeted to be easily absorbed by the body as a source of nutraceuticals and pharmaceuticals. Microencapsulation technology was developed about 70 years ago for the pharmaceutical sector, then extended to the agri-food, biotechnological, textiles, and cosmetic fields (Tolve *et al.* 2023). Microencapsulation with alginate and chitosan as coating could increase bacterial viable cells to reach favorable levels as probiotics (Chávarri *et al.* 2010). Moreover, microencapsulation as a nutraceutical preparation is suitable for increasing the stability of bioactive compounds as a delivery system (Ananda *et al.* 2022).

2. Materials and Methods

2.1. Chemical and Reagents

Red palm oil was obtained from PT. Wilmar Nabati Indonesia. The fermenter used is Staphylococcus epidermidis [ATCC 12228], which was purchased from the Microbiology and Molecular Biology Laboratory,

Food and Drug Supervisory Agency, Indonesia (Certificate Analysis: PP.05.02.10.104.06.21.616). The bacterial media was Nutrient Agar (NA) (Merck, Darmstadt, Germany), Nutrient Broth (NB) (Merck, Darmstadt, Germany), De Man, Rogosa and Sharpe Broth (MRS Broth) (Merck, Darmstadt, Germany), phosphate saline buffer, phosphoric acid (Merck), Carrageenan, Carboxymethyl cellulose (CMC), KCl, $CaCl_2$, Tween-80, and Glutaraldehyde (GA) (Sigma-Aldrich St. Louis, MO, USA). Other reagents were analytical grade or better.

2.2. Methodst

2.2.1. *Staphylococcus epidermidis* Starter Preparation

Stock culture of *S. epidermidis* was prepared by scratching several needle loops in a zig-zag scratching on a sterile petri dish containing NA media, then incubated at 37°C for 24 hr. One colony of *S. epidermidis* was taken from the stock culture, then inoculated in 10 ml of NB media (incubated at 37°C for 48 hr in the shaker). The cell population in the suspension of S. epidermidis was calculated by dilution of 1 ml of bacterial suspension into 9 ml of Phosphate Saline Buffer. The diluted bacterial suspension was then taken about 100 ml using a micropipette, put into a sterile petri dish containing NB media, and scraped. The Petri-dishes incubated for 48 hours at 37°C. Growing colonies were counted using the colony counter. The total number of colonies was calculated using the following equation (Frediansyah *et al.* 2021):

$$N = \sum C / [(n1 + - 0.1.n2)d$$

N : number of colonies in plate
∑C : the sum of plates containing 15 to 300 colonies
n1 : the number of plates retained in the first dilution
n2 : the number of plates retained in the second dilution
d : the first dilution factor. The viable colonies were then converted into log CFU/ml

2.2.2. Fermented Red Palm Oil (FRPO)

Non-fermented RPO (NFRPO) and Fermented RPO (FRPO) were prepared using the Wet-Degumming method with phosphoric acid (Nidzam *et al.* 2022). After the degumming process, it was washed with hot water at 60°C using a separator flask to remove the gum until the pH was around 6-7. RPO was fermented using *S. epidermidis* with three variations of MRS Broth media and RPO comparison, i.e., M1 (1:0), M2 (1:1), and M3 (1:2). The bacterial (Figure 1) inoculum was transferred with an initial starter of 7.02 log CFU/ml into Erlenmeyer containing fermentation medium. After that, the cells were cultured using a 100 rpm shaker and incubated aerobically for 48 hours at 37°C before being harvested for subsequent analysis. The fermentation results are separated by the medium and filtered using filter paper.

2.2.3. Physicochemical Properties of Fermented Red Palm Oil

2.2.3.1. Organoleptic Properties

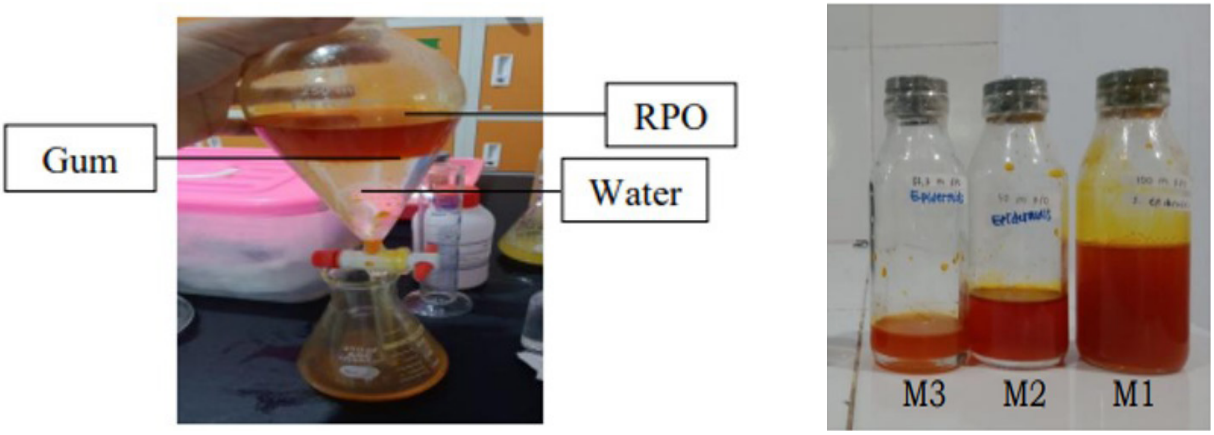
Organoleptic testing was carried out using 15 panels. Panelists were asked to respond to NFRPO and FRPO regarding color and odor. The test uses the senses of sight and smell, and the assessment of this hedonic test is (5) Very much like, (4) Like, (3) Slightly like, (2) Dislike, and (1) Very dislike. Then, the acid number and free fatty acid number analysis were carried out following previous studies, and peroxide number analysis was performed using the titration method (Tarigan *et al.* 2020).

2.2.3.2. GC-MS Analysis

GC/MS has long been used for the selective analysis of non-polar compounds. The carboxylic fatty acids require prior hydrolysis from their glycerolipid sources and derivatization to a respective ester form for separation on capillary chromatographic columns. The bioactive components of RPO before and after fermentation were analyzed using GC-MS. GC-MS was adjusted using a Phenomenex ZB-5 column of 30 mm × 0.25 mm × 0.25 mm. The condition of the injector temperature was 250°C, and the sampling time was 1 minute. Column Oven Temperature 80°C, with Pressure 100.0 kPa, total Flow: 588.8 ml/min, Column Flow:1.46 ml/min, Linear Velocity:44.5 cm/sec, Purge Flow:3.0 ml/min dan Split Ratio:400.0 (Riski *et al.* 2020).

2.2.4. Microencapsulation

The formulation method of bead microencapsulation was modified from Distantina *et al.* (2018). The 0.1% tween-80 emulsion was prepared in 5 ml of Fermented RPO (FRPO) and stirred with a stirrer for 3 minutes. Then, 100 ml of distilled water mixed with 3 g of carrageenan-CMC mixture (1:0, 1:0.5, and 1:1) (%w/w) was heated at 85°C and stirred until the solution was homogeneous. An emulsion was added with variations in volume: 1 ml, 2 ml, and 3 ml. Beads were printed into



A B

Figure 1. Preparation of red palm oil, RPO. (A) Degumming of RPO, (B) FRPO: media fermentation ratio = M1 (1:0), M2 (1:1), and M3 (1:2)

200 ml KCl-$CaCl_2$ 2 M solution and allowed to stand for 15 min. The microcapsules were drained and continued with the crosslinking step using Glutaraldehyde (GA) with a concentration of 1% w/w. The microcapsules were drained, and their physical and chemical parameters were tested (Sathasivam *et al.* 2018).

2.2.5. Physicochemical Properties and Characterization of Microcapsule

2.2.5.1. Percent Yield of Microcapsule

The percentage yield of the microcapsule was calculated to determine how large the microencapsulation products were following Frediansyah *et al.* (2021). The calculation was conducted by calculating the overall yield weight of the product and the total weight of the material (coating and emulsifying material).

$$\% R = \frac{MW\ (g)}{TW\ (g)} \times 100\%$$

MW : weight of microcapsule (gr)
TM : the total weight of the microencapsulated material

2.2.5.2. Microencapsulation Efficiency

One g of microencapsulant was added to 12 ml of *n*-hexane and stirred for 2 minutes. Then, 15 ml of *n*-hexane was added through filter paper. The microencapsulated portion was transferred to a clean, empty Erlenmeyer of known weight, then evaporated at a temperature of 69°C to a constant weight. Surface oil (SO) was determined based on the calculation of the difference between the weight of the empty Erlenmeyer and the Erlenmeyer containing the microencapsules. Total oil was determined based on the mass of the microencapsulated product from the ratio of the coating material (1:0). Thus, the microencapsulation efficiency (EM) of RPO is derived from the mass of surface oil and total oil according to the equation below (Ananda *et al.* 2022):

$$EM = \frac{Wt - Ws}{Wt} \times 100\%$$

EM : microencapsulation efficiency
Wt : total mass of oil (mg)
Ws : mass of Surface oil (mg)

2.2.5.3. Solubility of Microcapsule

The solubility was determined by following the previous studies (De Marco *et al.* 2013) with minor modifications. 0.1 g of microcapsules (a) was put into 10 ml of water at 300C and stirred using a magnetic stirrer. Then, the solution was filtered through filter paper, the constant weight of which was known. The filter paper and the unfiltered sample were oven-dried for 1 hour at 105°C, cooled for 15 minutes, and weighed. The weight of the unfiltered sample (b) was obtained from the difference between the weight of the final filter paper and the weight of the initial filter paper. The equation calculates solubility:

$$\text{Solubility } (\%) = \left(\frac{a - b}{a}\right) \times 100\%$$

2.2.5.4. Microcapsule Stability

One g of microencapsulated NFRPO oil and FRPO (as positive control) were stored in vials at 60°C for 9 days (1 day equivalent to 1 month at room temperature).

The vials were removed from the oven at 0, 3, 6, and 9 days of storage, and the peroxide value was determined. The solubility test was carried out to determine the solubility value of microcapsules in the test solution related to the release of the active ingredient

2.2.5.5. IR Spectrum and Morphology of Microcapsule

The infrared spectrum of NFRPO, FRPO, and microcapsules was recorded in the Varian 640-IR, FTIR spectrophotometer at wave numbers 500 and 4,000 cm^{-1}. The morphology of the microcapsule was observed using a Scanning Electron Microscopy (SEM). In the SEM analysis, the microencapsulated sample is placed in a sample holder and then coated with gold particles using a fine coater. The samples were then observed, and their morphology was seen at magnifications of 500x, 1,000x, 2,000x, and 5,000x (Yulaev *et al.* 2017).

2.2.6. Statistical Analysis

The mean and standard error (mean ± SE) was determined for each data. The analysis of variance (ANOVA) and student t-test were employed using SPSS 16 to determine the level of statistical differences between control and FRPO with *S. epidermidis*. Differences at $p<0.05$ were considered statistically significant.

3. Results

3.1. Physicochemical Properties of FRPO

The results of the hedonic sensory test on NFRPO showed that the average value of the panelists' preference for color ranged from 2-3 (dislike-little likes), and the color of NFRO was yellow-orange. As for the color in FRPO for the three concentration variations, it shows that the M1 (100 ml) and M2 (50 ml) concentrations tend to be favored by panelists who have a value of 4 (likes) and have the same level of preference for panelists, which is indicated by reddish-orange color. From variations in MI to M3 concentrations, the panelists' preference level tends to be lower due to the decrease in the brightness level of the microencapsule is caused by the oxidation of carotene due to the increase in the peroxide value. The results of the hedonic test on RPO before fermentation showed that the average value of the panelists' preference for aroma was around 3 (slightly like), indicated by a robust, distinctive aroma of the palm. As for the aroma in FRPO for three variations of concentration, the highest preference value is at the concentration of M1 (100 ml), with a distinctive palm aroma that is not too strong and a brighter color (Figure 2).

The acid number of the NFRPO and the M1 FRPO samples was still lower than the quality requirements of SNI 01-2901-1992 regarding palm oil, with a maximum level of 28.42 meq/kg. The results of measuring FFA levels on NFRPO obtained in the study were 4.10%. The measurement of FFA levels on the M1RPO sample was 4.86%. The FFA levels of the two degumming samples were still low compared to the quality requirements of SNI 01-2901-1992 regarding palm oil, with a maximum ALB content of 5%. The comparison in Table 1 shows that FRPO has no significant effect on the free fatty acid content of NFRPO. The refined RPO peroxide used for the microencapsulation process still meets the requirements of International Food Standards.

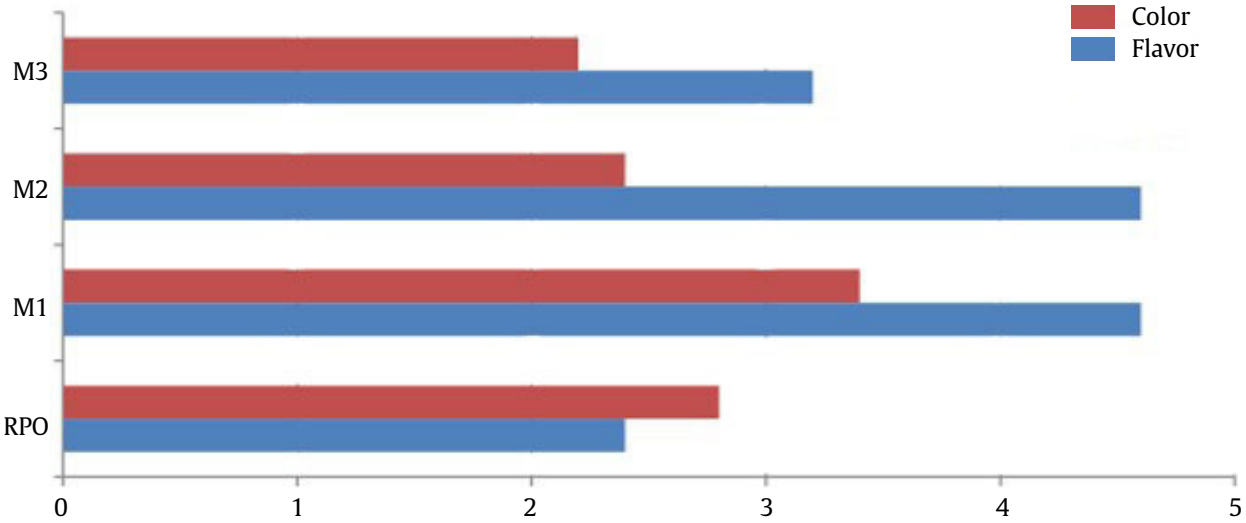


Figure 2. Hedonic score of non-fermented red palm oil (RPO) and fermented RPO (M1, M2, M3); 5:Very much like; 4: Like; 3:Slightly like; 2: Dislike, and 1:Very dislike

3.2. GC-MS Analysis of Chemical Compounds

GS-MS was used to identify chemical compounds of both NFRPO and FRPO. Based on the results of the GC-MS analysis (Table 2 and 3), the NFRPO chromatogram shows that 41 detected peaks were produced in varying percentage areas (Figure 3). The highest peak was seen by the compound Acetic acid,3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a octahydronaphthalen-2-yl ester ($C_{17}H_{26}O_3$) with time retention of 38.270 minutes and the percentage of the area of 86.00%: acetic acid compound, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester. On the other hand, the results of the GC-MS analysis of FRPO show there are 50 peaks, indicating the presence of more than 50

Table 1. Free Fatty Acid (FFA) test, acid number, and peroxide value of red palm oil (RPO)

Test parameters	Test results			
	RPO	M1	M2	M3
FFA number	4.10%	4.86%	5.25%	6.14%
Acid number				
Peroxide number	8.976 mg KOH/kg 8 mEq/kg	10,659 mg KOH/kg 9 mEq/kg	11.5005 mg KOH/kg 13 mEq/kg	13,464 mg KOH/kg 18 mEq/kg

Table 2. Compound profile of non-fermented red palm oil (NFRPO) by GC-MS analysis

Compound peak	R. Time (minutes)	Area (%)	Molecular formula	Compound name
1	36,775	0.39	$C_{17}H_{24}O_2$	Hydratropic acid, oct-3-en-2-yl ester
2	36,833	0.04	$C_4H_5N_3$	1H-1,2,4-Triazole, 1-vinyl
3	36,892	0.07	$C_{10}H_9NO_4$	Dinocap
4	38.270	86.00	$C_{17}H_{26}O_3$	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester
5	38.958	0.01	$C_{25}H_{36}O_2$	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)
6	39.058	0.17	$C_{14}H_{22}O_2$	4,6-di-tert-Butylresorcinol
7	39.211	0.16	$C_{19}H_{19}NO_4$	p-Cyanophenyl p-(2-propoxyethoxy)benzoate
8	39.291	0.19	$C_{18}H_{16}ClFO_5$	Succinic acid, 2-chloro-6-fluorophenyl 4-methoxybenzyl ester
9	39.375	0.15	$C_{14}H_{21}ClO_2$	Chloroacetic acid, 2-(1-adamantyl)ethyl ester
10	39.601	0.92	$C_{14}H_{22}O_2$	2-(2,6,6-Trimethylcyclohex-1-enyl)cyclopropanecarboxylic acid, methyl ester
11	39.700	1.50	$C_{21}H_{30}O_3$	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-
12	40.033	0.18	$C_{22}H_{24}O_4$	1,3-Benzenediol, o-(2-methylbenzoyl)-o'-(3-cyclopentylpropionyl)-
13	40.151	0.21	$C_2H_3F_3O_3S$	Methanesulfonic acid, trifluoro-, methyl ester
14	40.267	0.03	$C_2H_5NO_3$	Acetic acid, (aminooxy)-
15	40.654	0.23	$C_{21}H_{30}O_2$	p-Toluic acid, tridec-2-ynyl ester
16	41.634	0.15	$C_5H_9NaO_2$	Propanoic acid, 2,2-dimethyl-, sodium salt
17	42.122	0.06	C_9H_9NO	Phenethyl isocyanate
18	42.354	4.78	$C_{18}H_{26}O$	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one
19	42.675	0.32	$C_{18}H_{26}O$	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one
20	42.842	0.20	$C_{14}H_8Cl_4O_2$	4-Methylbenzoic acid, 2,3,4,6-tetrachlorophenyl ester
21	42.917	0.07	$C_{14}H_{10}Cl_2O_2$	4-Methylbenzoic acid, 2,3-dichlorophenyl ester
22	43.035	0.27	$C_{24}H_{22}O_4$	Phthalic acid, di(3-ethylphenyl) ester
23	43.276	0.17	$C_{24}H_{22}O_4$	Isophthalic acid, di(2-ethylphenyl) ester
24	43.483	0.10	$C_{17}H_{15}NO_3$	2-Formylamino-3-phenylacrylic acid, benzyl ester
25	43.667	0.12	$C_{14}H_8Cl_4O_2$	Benzoic acid, 3-methyl-, 2,3,4,6-tetrachlorophenyl este
26	43.825	0.19	$C_{14}H_8C_{14}O_2$	4-Methylbenzoic acid, 2,3,4,6-tetrachlorophenyl ester

Table 2. Continued

Compound peak	R. Time (minutes)	Area (%)	Molecular formula	Compound name
27	43.908	0.28	$C_{14}H_6Cl_2F_4O_2$	6-Fluoro-2-trifluoromethylbenzoic acid, 2,3-dichloro-phenyl ester
28	44.042	0.08	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, hexamethyl
29	44.136	0.11	$C_{24}H_{34}O_4$	Terephthalic acid, propyl tridec-2-yn-1-yl ester
30	44.243	0.06	$C_{11}H_{17}F_5O_5$	2-[2-(2-Ethoxyethoxy)ethoxy]ethyl 2,2,3,3,3-pentafluo-ropropanoate
31	44.308	0.05	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, hexamethyl
32	44.500	0.26	$C_7H_{22}O_2Si_3$	1,1,1,3,5,5,5-Heptamethyltrisiloxane
33	44.756	0.82	$C_{10}H_{11}NO_3$	2-propenamide, N-(2,6-dihydroxyphenyl)-2-methyl
34	44.994	0.76	$C_{17}H_{14}FN_3O_3$	Benzoic acid N'-[1-(2-fluorophenyl)-2,5-dioxopyrro-lidin-3-yl]hydrazide
35	45.126	0.27	$C_{22}H_{31}OP$	Phosphine oxide, bis(pentamethylphenyl)-
36	45.250	0.24	$C_{22}H_{28}O_4Si$	5-hydroxy-7-methoxyflavanone, tert.-butyldimethylsilyl ethe
37	45.378	0.08	$C_{20}H_{26}O_5Si_2$	4-Trimethylsilyloxybenzoic anhydride
38	45.434	0.09	$C_{22}H_{28}O_4Si$	5-hydroxy-7-methoxyflavanone, tert.-butyldimethylsilyl ether
39	45.549	0.13	$C_{22}H_{36}O_4Si_3$	1,7-Di(3-ethylphenyl)-2,2,4,4,6,6-hexamethyl-1,3,5,7-tetraoxa-2,4,6-trisilaheptane
40	45.663	0.08	$C_{24}H_{22}O_4$	Phthalic acid, methyl 4-(2-phenylprop-2-yl)phenyl ester
41	45.739	0.04	$C_{24}H_{52}O_3Si$	Octadecyltriethoxysilane

Table 3. Compound profile of fermented red palm oil (FRPO) by GC-MS analysis results

Compound peak	R. Time (minutes)	Area (%)	Molecular formula	Compound name
1	24,886	2.18	$C_7H_{14}O_2$	Hexanoic acid, 2-methyl-
2	26.175	0.34	$C_8H_{16}O_2$	2-Methylheptanoic acid
3	28.000	1.22	$C_9H_{11}NO$	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl
				2-Ethylformanilide
4	30.143	0.67	$C_6H_{12}O_2$	Pentanoic acid, 2-methyl-
			$C_6H_{15}B$	Borane, ethylisopropylmethyl
			$C_9H_{16}O$	2-Heptanone, 6-methyl-5 methylene
			C_6H_{12}	1-Pentene, 3-methyl
5	36.131	3.21	$C_{22}H_{42}O_4$	Hexanedioic acid, dioctyl ester
6	38.977	5.27	$C_{22}H_{34}O_4$	Phthalic acid, di(hept-2-yl) ester
7	39.186	36.31	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
8	39.367	4.71	$C_{24}H_{38}O_4$	Bis (2-ethylhexyl) phthalate
9	39.550	0.59	$C_{16}H_{24}O_2$	Propanoic acid;
			$C_{12}H_{20}O_6$	1,2,3-Propanetriol
10	39.843	0.49	$C_8H_{16}OS$	Tert-Butylcycloprophylmethil sulfoxide,
			C_4H_9ClO	1-Propanol, 2-chloro-2-methyl
11	41.073	3.14	$C_{21}H_{36}O$	Phenol, 3-pentadecyl
			$C_{19}H_{32}O$	3-Tridecylphenol
12	41.340	0.59	$C_{13}H_{17}ClO_2$	6-Chlorohexanoic acid,
			$C_{12}H_{15}ClO_2$	5-Chloropentanoic acid
13	41.437	0.92	$C_9H_{10}O_2$	Acetic acid, 4-methylphenyl ester
			$C_{13}H_{17}ClO_2$	6-Chlorohexanoic acid,
14	41.538	2.38	$C_9H_{11}NO_2$	Carbamic acid, 3-methylphenyl ester
15	41.956	0.38	$C_{17}H_{30}Osi$	4-tert-Octylphenol,
			$C_{15}H_{24}O_2Si$	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone
16	42.508	0.43	$C_8H_{24}O_4Si_4$	Cyclotetrasiloxane, octamethyl
17	42.617	0.35	$C_{24}H_{36}O_2Si_2$	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene,
			$C_6H_{18}O_3Si_3$	Cyclotrisiloxane
18	42.860	0.55	$C_5H_9NaO_2$	Propanoic acid, 2,2-dimethyl- sodium salt

Table 3. Continued

Compound peak	R. Time (minutes)	Area (%)	Molecular formula	Compound name
19	42.958	0.81	$C_6H_8N_2O$	2,6-Dimethylpyridazin-3-one
			$C_6H_8N_2O$	N-Propionylimidazole
20	43.043	3.14	$C_{12}H_{15}BrO_3$	5-Bromovaleric acid , 4-methoxyphenyl ester
			$C_{14}H_{20}O_3$	Heptanoic acid, 4-methoxyphenyl ester
21	43.160	0.67	$C_8H_{13}Cl_3O_3$	Carbonic acid, 2,2,2-trichloroethyl neopentyl ester
22	43.200	0.30	$C_{24}H_{22}O_4$	Isophthalic acid, di(2-ethylphenyl) ester
23	43.333	1.29	$C_{13}H_{17}N$	Deprenyl
24	43.432	1.52	$C_{32}H_{54}O_4$	Phthalic acid, nonyl pentadecyl ester
25	43.508	0.29	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, hexamethyl
26	43.542	0.26	$C_{14}H_{24}O_3Si_2$	2-Hydroxybenzeneacetic acid, 2TMS derivative
27	43.586	0.51	$C_{16}H_{26}O_5Si$	1-(4-Hydroxy-3-methoxyphenyl)-1-ethoxyacetic acid ethyl ester
28	43.692	0.54	$C_{24}H_{22}O_4$	Phthalic acid, di(3-ethylphenyl) ester
			$C_{24}H_{22}O_4$	Isophthalic acid, di(2,5-dimethylphenyl) ester
29	43.748	0.35	$C_{13}H_{22}OSi_2$	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl
30	43.933	0.90	$C_{14}H_6Cl_2F_4O_2$	4-Fluoro-2-trifluoromethylbenzoic acid, 2,3-dichloro-phenyl ester
31	43.983	0.51	$C_{14}H_6Cl_2F_4O_2$	6-Fluoro-2-trifluoromethylbenzoic acid, 2,3-dichloro-phenyl ester
32	44.092	0.31	$C_{21}H_{14}F_4O_3$	2-Fluoro-3-trifluoromethylbenzoic acid
33	44.168	0.64	$C_{14}H_{22}O_2Si$	m-Toluic acid
			$C_{11}H_{16}O_2Si$	o-Toluic acid
			$C_{14}H_{22}O_2Si$	p-Toluic acid
34	44.309	0.77	$C_{21}H_{24}O_7$	d-arabinoitol 1,5-o-di-p-toluate
35	44.419	1.97	$C_{11}H_{15}NO_2$	Carbamic acid, 3-methylphenyl-, propyl ester
36	44.458	0.34	$C_{10}H_9NO_6$	1,4-Benzenedicarboxylic acid, 2-nitro-, dimethyl ester
37	44.493	0.51	$C_{12}H_{16}O_2$	Valeric acid
38	44.577	1.10	$C_{32}H_{28}O_4$	2,2'-Dimethyl-alpha,alpha'-bis(O-toluoyloxy)-trans-stilbene
39	44.611	0.79	$C_{17}H_{12}BrNO_2$	4-(2-Bromobenzylidene)-2-(o-tolyl)-5(4H)-oxazolone
40	44.684	10.58	$C_{21}H_{34}O$	(Z)-3-(pentadec-8-en-1-yl)phenol
41	44.900	1.24	$C_{11}H_{12}O_4$	3,4-Dimethoxycinnamic acid
				3,5-Dimethoxycinnamic acid
42	44.933	0.25	$C_{11}H_{16}N_2O_2$	Neostigmine
43	45.017	0.99	$C_{35}H_{68}O_4$	Hexadecanoic acid, (2-pentadecyl-1,3-dioxolan-4-yl) methyl ester
44	45.067	0.78	$C_{23}H_{50}Osi$	1-Triethylsilyloxyheptadecane
45	45.175	0.65	$C_{12}H_{36}O_4Si_5$	Pentasiloxane, dodecamethyl
46	45.242	0.92	$C_9H_{27}AsO_3Si_3$	Arsenous acid, tris(trimethylsilyl) ester
47	45.351	0.52	$C_{22}H_{28}O_4Si$	5-hydroxy-7-methoxyflavanone, tert.-butyldimethylsilyl ether
48	45.476	1.50	$C_6H_{18}SSi_2$	Disilathiane, hexamethyl
49	45.611	0.92	$C_{17}H_{16}O_4$	Phthalic acid, 3,5-dimethylphenyl methyl ester
			$C_{11}H_{12}O_3$	3-Methyl-2-propionyl-benzoic acid
50	45,683	0.41	$C_4H_{10}O_3$	1,2,4-Butanetriol

chemical components contained in the FRPO (Figure 4). Five main compounds have the highest peaks, at peaks 7, 40, 6, 8, and 5. The highest peak was at a retention time of 39,186 minutes and a percentage area of 36.31%. 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester found, a group of phthalic compounds (phthalic acid) (Table 3).

3.3. Microencapsulation

The results of the three variations of the emulsion on the microencapsulation of NFRPO and FRPO are presented in Figures 5 and 6. The texture appears denser, and the color brighter. In addition, the microencapsulated carboxymethyl cellulose mixed coating material has an irregular capsule shape (flat and elongated). So, in the following procedure, only 3 grams of carrageenan coating material will be used. The results obtained from emulsions with volume variations of 1 ml, 2 ml, and 3 ml show that those used with 3 ml emulsion variations have a rounder shape, denser texture, and brighter color brightness compared to 1 ml and 2 ml volume variations (Figure 6).

3.4. Characterization of Microcapsules

3.4.1. Percent Yield of Products

The percentage yield of microencapsulated products is presented in Table 4. Based on the results data obtained, the percentage value of the product yield to the NFRPO and FRPO is similar 31.80% and 32.30%, respectively. The data indicates that the microencapsulated NFRPO and FRPO characteristics did not significantly affect the yield produced. The yield of the FRPO product obtained was higher than that of NFRPO.

3.4.2. Microencapsulation Efficiency

The results of the microencapsulation efficiency (Table 4) show that the NFRPO and FRPO had percentage values of 99.03% and 99.13%, respectively. The data indicates that the characteristics of the

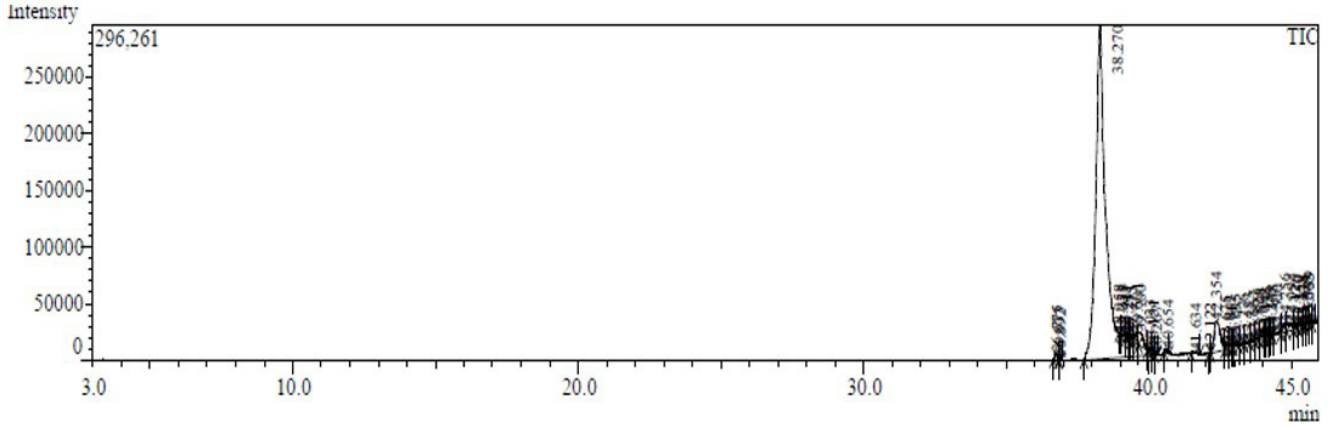


Figure 3. Chromatogram of non-fermented red palm oil (NFRPO) from GC-MS analysis

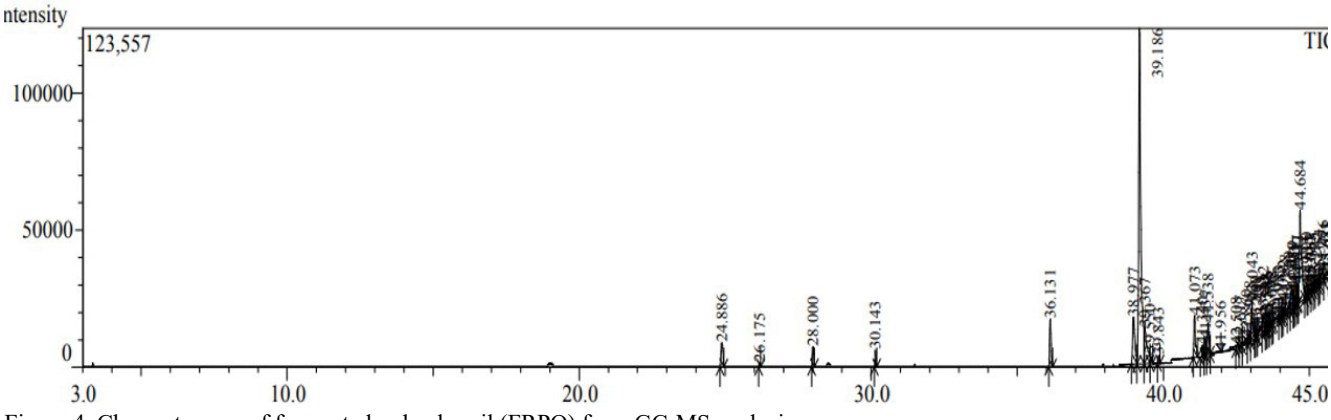


Figure 4. Chromatogram of fermented red palm oil (FRPO) from GC-MS analysis

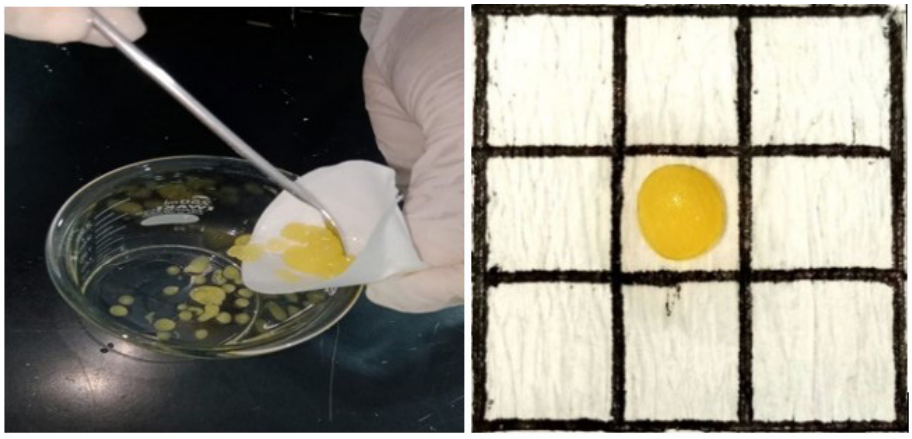


Figure 5. Microcapsule of non-fermented red palm oil (NFRPO). (A) mixture of carrageenan-CMC (1:0.5 and 1:1), (B) carrageenan-CMC (1:0)

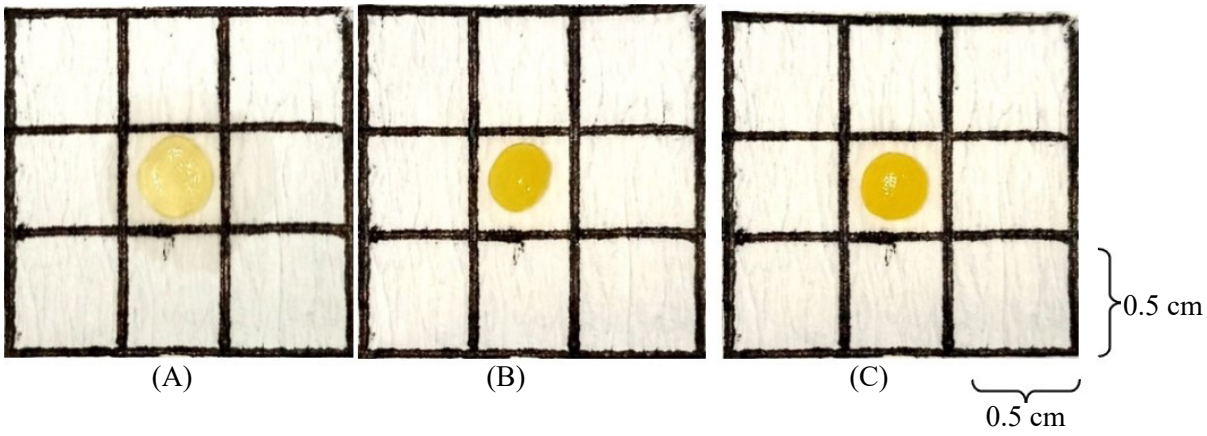


Figure 6. Microcapsule of fermented red palm oil (FRPO) with carrageenan coating and various emulsions (A) 1 ml, (B) 2 ml, and (C) 3 ml

Table 4. The Characterization of microcapsules

Sample	%Yield	Efficiency (%)	Solubility (%)
Non-fermented RPO (NFRPO)	31.80±0.201[a]	99.03±0.189[b]	96.1±0.192[c]
Fermented RPO (FRPO)	32.30±0.221[a]	99.13±0.231[b]	99.1±0.197[d]

[a,b]Superscripts with different capital letters in the same column indicate very significant differences (P<0,05).

microencapsulated before and after fermentation also do not significantly affect the efficiency of the resulting microencapsulation. It can be seen in Table 4 that the results of NFRPO and FRPO were not very different. The microencapsulation efficiency of the FRPO product obtained is not significantly different from NFRPO.

3.4.3. Solubility of Microcapsules

In addition, the solubility of microcapsules was tested between fermented and unfermented water (Table 4). The solubility values obtained in microencapsulated NFRPO and FRPO in water were 96.1% and 99.1%, respectively. The solubility data indicated that the microencapsulate NFRPO and FRPO characteristics had a slight but insignificant effect on the solubility of the microencapsulated water, and the data indicated that NFRPO and FRPO showed similar results (Table 4).

3.4.4 Microcapsule Stability

The stability test of the microcapsule is presented in Table 5. Based on the data obtained, during the storage process, there was an increase in the value of the peroxide number in each sample in the range of days: 0.3, 6, and 9. The sample encapsulated with carrageenan coating had a lower peroxide value than the unencapsulated sample (Figure 7). The microencapsulation process can provide adequate protection against oxidative damage to oil or fat and proves that the microencapsulation process can reduce the level of damage to the oil by suppressing the oxidation rate so that the microencapsulation remains stable.

Table 5. Stability test of microencapsulated red palm oil (RPO) before fermentation and Red-PalmZym

Sample	Peroxide number analysis (mEq/kg)			
	0 day	3rd day	6th day	9th day
NFRPO	10±0.322[a]	50±0.665[a]	80±0.542[a]	90±0.672[a]
FRPO	25±0.452[b]	50±0.627[a]	70±0.422[a]	100±0.732[a]
NFRPO Microcapsule	8.0±0.550[c]	25±0.572[b]	45±0.592[b]	75±0.372[c]
FRPO Microcapsule	10±0.672[a]	35±0.572[b]	45±0.632[b]	75±0.352[c]

[a,b]Superscripts with different capital letters in the same column indicate very significant differences (P<0,05). Non-fermented RPO (NFRPO), Fermented RPO (FRPO)

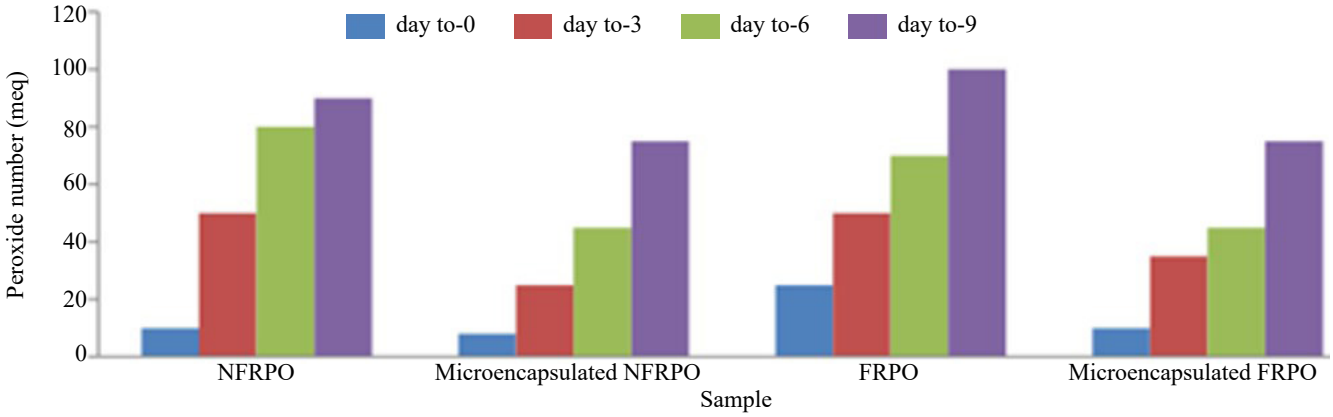


Figure 7. Stability test of microencapsulated NFRPO and FRPO by peroxide number analysis. Non-fermented red palm oil (NFRPO), fermented red palm oil (FRPO)

3.4.5. IR-Spectrophotometer Analysis

Characterization using FTIR spectrophotometer in this study was carried out to determine the functional groups contained in NFRPO and FRPO, to determine the presence of functional groups in microencapsulated coating materials (Tween-80, Carrageenan, Glutaraldehyde, and RPO microencapsulated samples encapsulated with coating materials) microencapsulation (Figure 8). From the results obtained, the overall visible spectrum between NFRPO and RPO has functional groups that are not very different, and there are only differences in wave numbers (Table 6). The functional groups of RPO encapsulated with coating materials were also tested using an FTIR instrument. The tests carried out on RPO microencapsulated were compared with the FTIR spectra of tween-80, carrageenan, and pure glutaraldehyde (Table 7).

3.4.6. Microencapsules Morphological Analysis

The microstructure of microencapsulated NFRPO is presented in Figure 9. The microstructure of the NFRPO microcapsule is slightly irregular, primarily spherical. It may be affected by the stirring speed in the emulsion homogenization process, which could be more optimal. In addition, high viscosity can also cause the particle size to become more prominent if the stirring is not optimal, as the homogenization is crucial for a uniform particle shape. The distribution of microencapsulated RPO particles is quite even, and there is no accumulation of particles in one part.

The microstructure of microencapsulated FRPO is presented in Figure 10, shows a slightly wrinkled and dented surface, thus forming small pores that affect the microstructure. The core material is entirely well-encapsulated and round, with few pores and a small portion having dents on the surface. The microencapsulated FRPO structure was partially spherical and wrinkled, so some carrageenan used as a coating material has coated well (Figure 5). In this study, the shape of the microcapsules was slightly dents or indentations formed.

4. Discussion

S. epidermidis ATCC 12228 is one of the fermenting agent bacteria belonging to human skin commensal bacteria, the human skin microbiome in an AD lesion that is able to produce antibiotics (Kong *et al.* 2012; Wang *et al.* 2014). Previous studies used S. epidermidis ATCC 12228 to ferment glycerol to butyric acid and

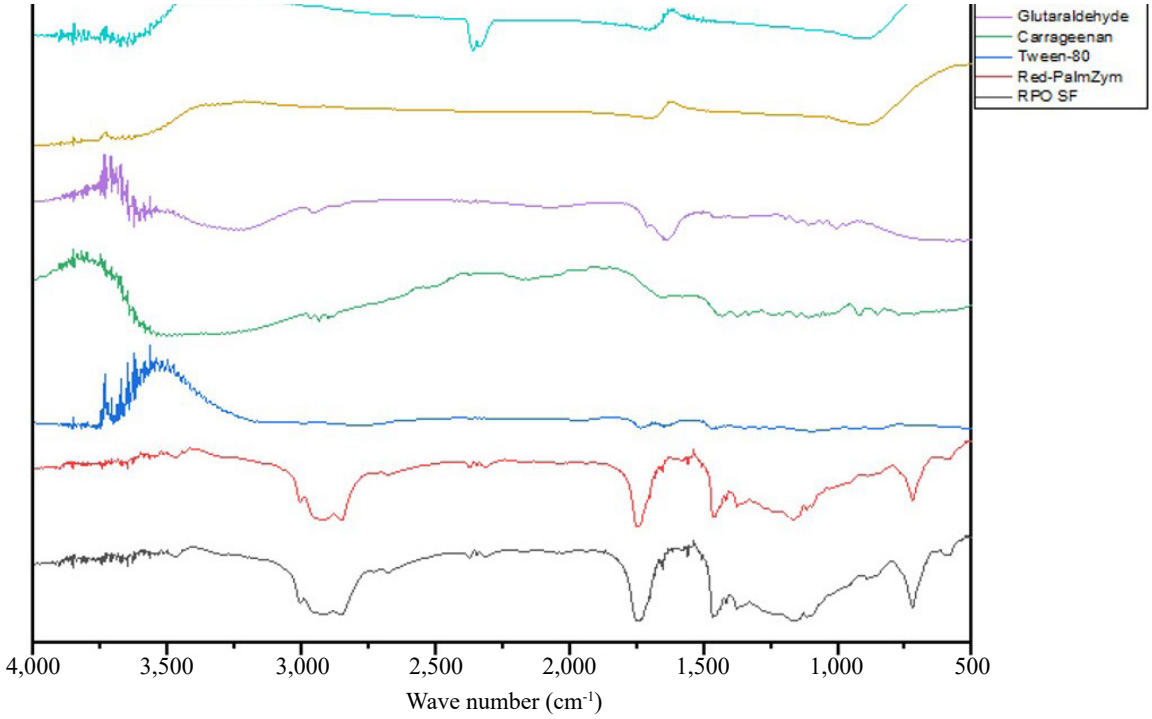


Figure 8. IR-Spectrum of microencapsulated NFRPO, FRPO, NFRPO and FRPO coating material. Non-fermented red palm oil (NFRPO), fermented red palm oil (FRPO)

Table 6. FTIR analysis

Functional groups	Wave number (cm^{-1})	
	NFRPO	FRPO
Alkanes CH	2850-2970 and 1340-1470 cm^{-1} 675-995 cm^{-1}	2850-2970 and 1340-1470 cm^{-1} 675-995 cm^{-1}
Alkene CH		
Alkyne CH	-	-
Aromatic Ring CH	690-900cm^{-1}	690-900cm^{-1}
Hydrogen bonding alcohol/phenol OH	3200-3600cm^{-1}	3200-3600cm^{-1}
Carboxylic acid monomer/carboxylic acid hydrogen bond OH	2500-2700cm^{-1}	2500-2700cm^{-1}
Amine/amide NH	3300-3500cm^{-1}	3300-3500cm^{-1}
Alkene C=C	1610-1680cm^{-1}	1610-1680cm^{-1}
Aromatic Ring C=C	-	-
Alkyne C≡C	-	-
Amine/amide CN	1180-1360cm^{-1}	1180-1360cm^{-1}
Nitrile C≡N	-	-
Alcohol/ether/carboxylic acid/ester CO	1050-1300cm^{-1}	1050-1300cm^{-1}
Aldehydes/ketones/carboxylic acid/ester C=O	1690-1760cm^{-1}	1690-1760cm^{-1}
Nitro Compounds NO_2	1370cm^{-1}	1370cm^{-1}

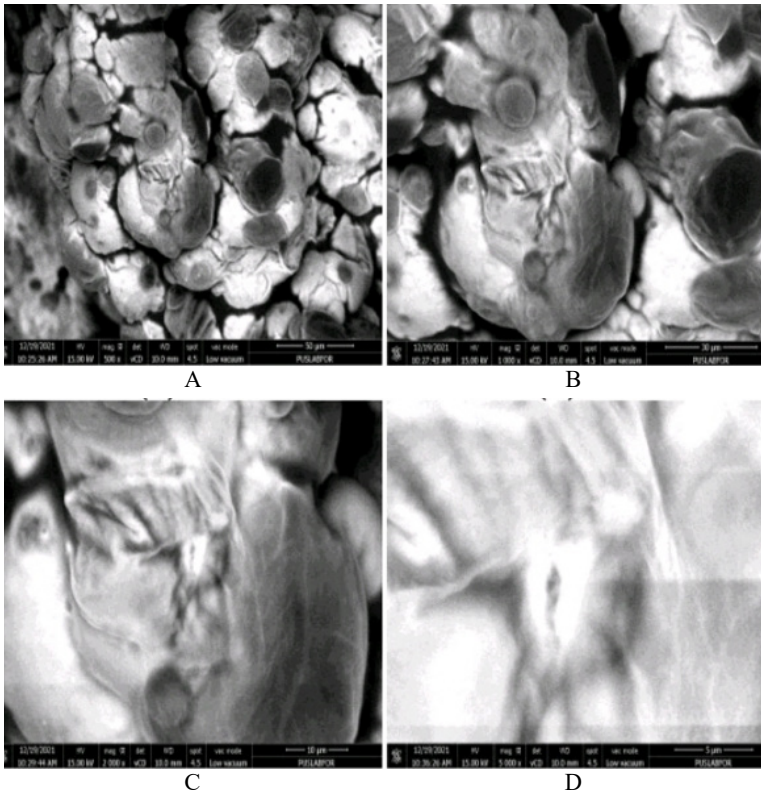
Table 7. FTIR analysis

Functional groups	Wave number (cm^{-1})				
	I	II	III	IV	V
Alkanes CH	-	-	2850-2970 and 1340-1470	2850-2970 and 1340-1470	2850-2970 and 1340-1470
Alkene CH	675-995	675-995	675-995	675-995	675-995
Alkyne CH	-	-	-	3307	-
Aromatic ring CH	690-900	690-900	690-900	690-900	690-900
Hydrogen bonding alcohol/Phenol OH	3200-3600	3200-3600	3200-3600	3200-3600	3200-3600
Carboxylic acid monomer/carboxylic acid hydrogen bond OH	2567	2553-2607	-	2500-2648	-
Amine/amide NH	3493	3496	-	3307-3485	3313-3388
Alkene C=C	-	-	1610-1680	1610-1680	1610-1680
Aromatic ring C=C	-	-	-	-	-
Alkyne C≡C	2106	2158	2100-2260	2100-2260	-
Amine/amide CN	-	-	1180-1360	1180-1360	1180-1360
Nitrile C≡N	-	2268	-	-	-
Alcohol/ether/carboxylic acid/ester CO	1170	1170	1050-1300	1050-1300	1050-1300
Aldehydes/ketones/carboxylic acid/ester C=O	1751	1718	1690-1760	-	1690-1760
Nitro Compounds NO_2	-	-	1300-1370	1130-1370	-

Wave number: I (microencapsulated NFRPO), II (microencapsulated FRPO), III (tween-80), IV (carrageenan), V (glutaraldehyde); non-fermented red palm oil (NFRPO), Fermented RPO (FRPO)

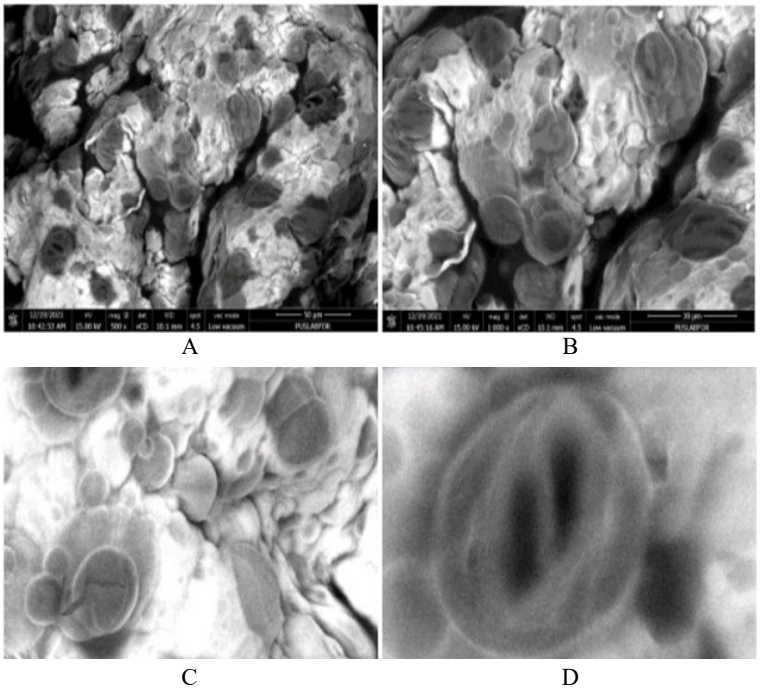
acetic acid, which exerts a growth-suppressing effect on USA300, community-associated methicillin-resistant S. aureus (MRSA) (Kao *et al.* 2017). In another study reported by previous research, *S. aureus* ATCC 122288 fermented with PEG-8 Laurate-induced pro-inflammatory macrophage-inflammatory protein 2 (MIP-2) (Marito *et al.* 2020). Fermentation by *S. epidermidis* produces Short Chain Fatty Acids (SCFAs) that have the potential to be candidates for drugs, antibacterial, antioxidant, and other bioactivity (Keshari *et al.* 2019; Traisaeng *et al.* 2019). Previous studies have developed essential oil fermentation using probiotic bacteria and yeast. The FRPO contains a high level of β-carotene contained in it, which functions as an antioxidant (Rao & Rao 2007; Cazzonelli 2011). The RPO contains a high carotenoid pigment, which is up to 500-700 ppm. Fat or oil in tissues generally binds with color pigments such as carotenoids, making them easily damaged (Mezzomo & Ferreira 2016).

The acid number test measures the amount of free fatty acids and is calculated based on the molecular weight of the fatty acid or fatty acid mixture (Tarigan *et al.* 2020). A high acid number indicates a high free fatty acid content in the oil, which means the quality of the oil is reduced. The number of fatty acids in RPO was calculated using palmitic acid because it was the most dominant fatty acid in RPO. The quality of the oil or fat is affected by the free fatty acids (FFA) levels. The amount of FFA is estimated by determining the amount of alkali that must be added to the fat to make it sufficiently neutral. High FFA content in M2 and M3 samples indicates poor-quality oil or fat. High FFA content can increase the risk of further oil damage due to oxidation. The hydrolysis reaction influences the oil's high and low content of FFA. This reaction is accelerated in acid, heat, water, and enzymes (triacylglycerol acyl hydrolase) (Emebu *et al.* 2022). Moreover, the level of peroxide in fat or oil begins to increase, and after reaching the maximum value, the percentage of oxygen in the oil will also increase gradually (Gotoh & Wada 2006).



A B C D

Figure 9. Microstructure of microencapsulated non-fermented red palm oil (NFRPO), with various magnifications: (A) 500x; (B) 1,000x; (C) 2,000x; and (D) 5,000x



A B C D

Figure 10. Microstructure of microencapsulated fermented red palm oil (FRPO), with various magnifications: (A) 500x; (B) 1,000x; (C) 2,000x; and (D) 5,000x

From the results of the GC-MS analysis, the chromatograms of NFRPO and FRPO contained 41 and 50 peaks, respectively, indicating the presence of more than 50 chemical components in RPO after fermentation. There is an addition of nine peaks, which indicates the increase in the chemical components of FRPO. The major compounds in FRPOin, the form of a 1,2-benzene dicarboxylic acid (2-ethylhexyl) ester identified through GS-MS, belong to the antioxidant group. Similar results are reported by previous studies that found the chemical fermentation product 1,2-benzene dicarboxylic (40-50%) (Romeh & A 2013; El-Beltagi *et al.* 2019). Those compounds have biological activity as antifungal, antitumor, anti-diabetic, anticancer, antioxidant, anti-inflammatory, and antimicrobial (Balachandran *et al.* 2012; Ezhilan & Neelamegam 2012). It seems that the nine compounds are SCFA compounds that were successfully broken down by *S. epidermis* from the RPO complex. Furthermore, the compound 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (CAS) bis(2-ethylhexyl) phthalate is efficacious as an antimicrobial, antioxidant and anti-inflammatory with a large percentage of the area (Zhao *et al.* 2018). The compound found in FRPO in the form of a 1,2-benzene dicarboxylic acid, bis(2-ethylhexyl), also has antifungal properties against *A. rolfsii*. Similar result results of the research by (Akpuaka *et al.* 2013) obtained a compound 1,2-benzene dicarboxylic acid bis (2-ethylhexyl), which belongs to the phthalate group. The compound 1,2-benzene dicarboxylic acid, bis(2-ethylhexyl) isolated from the leaves of Thevia peruviana was an anticancer activity on PC3, MCF, HCT-116, A549, and MIAPACA cell lines (Save *et al.* 2015). A previous study found a compound of 1,2-benzene dicarboxylic acid, 1,2-bis(2-ethylhexyl) ester of 5.05% isolated from the *Leonotis nepetifolia* plant (Oliveira *et al.* 2015). The extract's ability is also an antifungal on *Aspergillus* spp., *Candida albicans*, and *Trichophyton* spp. The difference in the chromatogram of the NFRPO indicated that the fermentation succeeded in producing new compounds from the RPO. In addition, the breakdown of complex compounds into simple compounds through fermentation will increase the body's ability to absorb (Nkhata *et al.* 2018).

Gel strength is the main physical property of carrageenan, which shows the ability of carrageenan in gel formation (Fateha *et al.* 2021). The higher the concentration of carrageenan added, the stronger the gel formed (Gustaw & Mleko 2003). Microcapsules show a promising future in increasingly complicated applicated applications such as drug delivery, supported by previous research that the higher the concentration of carrageenan added, the harder the solution will be due to carrageenan binding water in large quantities, which causes the space between particles to become narrower so that more water is bound and trapped into a hard solution (Loret *et al.* 2009). In addition, carrageenan is a type of hydrocolloid that has gel-forming properties, is stable and elastic, and can be consumed (Abdou & Sorour 2014). Hydrocolloids are polymer components derived from vegetables, animals, microbes, or synthetic components that generally contain hydroxyl groups. This polymer component is soluble in water, able to form colloids, and can thicken or form a gel from a solution (Jayakody *et al.* 2023). The chemical reactivity of carrageenan is shown by the presence of a highly anionic sulfate ester group in each carrageenan disaccharide unit, which contributes to its hydrophilic. The sulfate ester anionic group on a different chain from carrageenan can induce electrostatic repulsion and increase the distance between carrageenan chains so that it causes swelling (Distantina *et al.* 2018). Microencapsulation without the crosslinking agent is easier to leak than that added with a crosslinking agent. This phenomenon causes low yield and efficiency in the microencapsulated obtained. Physical crosslinking needs to be done to make bead gel with KCl-CaCl2 (Distantina *et al.* 2018). Chemical crosslinking uses glutaraldehyde (GA) as a crosslinker. Glutaraldehyde crosslinker compounds are widely used in research because they introduce bonds between covalent molecules and polymer chains so that the polymer becomes more rigid (Migneault *et al.* 2004).

The yield of microcapsules was calculated by comparing the initial weight of the material used to manufacture microcapsules with the weight of the microcapsules obtained. The low yield obtained in the NFRPO and FRPO could be due to the coating material used in the microencapsulation process. The coating material in the form of carrageenan has hygroscopic properties (Abdou & Sorour 2014); it might cause the resulting microcapsules to lose a lot of water content, resulting in small microcapsule yields. Furthermore, the difference in yield is influenced by the water content of a food ingredient. The lower the water content, the smaller the weight of the water contained in the material. Removing water molecules will make the material denser and lighter, affecting the final product's yield. The added carrageenan can bind water in the material

and maintain the water content so that the large amount of carrageenan added will affect the yield. The increase in yield and the proportion of carrageenan indicate that carrageenan has a better ability to coat oil droplets, in this case, the ability to form emulsions, film formation, and flexibility in coating oil droplets. The higher the total solids in the dried material, the higher the yield. The higher the number of proportions added, the greater the total solids. In addition, the greater the ratio of the RPO core material, the greater the yield produced. The larger the core material coated, the greater the total solids produced (Nwaogu & Tiedje 2011). One factor that might affect the % yield of microencapsulation is the suitable method chosen for the degumming process. The wet degumming method is simpler and easier to do than the dry degumming method but produces lower yields. In the wet degumming method, oil components such as dyes, a small amount of metal, free fatty acids, and hydratable phosphatides are also wasted along with the washing water, reducing oil yield (Tarigan *et al.* 2022).

The results show that carrageenan has a good coating ability (Table 4). Somehow, the inlet temperature did not affect the microencapsulation efficiency because the extrusion method did not use high temperature in the microencapsulation process. The value of the encapsulation efficiency of the results is higher than before microencapsulation (Li *et al.* 2019). Microencapsulation efficiency will decrease with the increase in the encapsulated filling material when microencapsulated β-carotene concentrate using different drying treatments. The amount of unencapsulated oil strongly influences the encapsulation efficiency (Jyothi *et al.* 2010). Encapsulation efficiency is strongly influenced by the amount of unencapsulated oil used. The presence of non-encapsulated oil is related to the stability of the encapsulation during storage. The high amount of non-encapsulated oil tends to correlate with the formation of off-flavor in the encapsulated and low stability when exposed to environmental conditions (Dubey *et al.* 2009), following the theory, where one of the advantages of the microencapsulation technique is to increase the solubility of the resulting product (Choudhury *et al.* 2021).

Solubility is one of the important characteristics that determine the quality of a product because RPO microcapsules are expected to be applied in food fortification of nutraceutical sources (Choudhury *et al.* 2021). The solubility value obtained is higher than the solubility value reported by a previous study; the solubility of the microencapsulated red fruit oil produced is 50-80%, which is influenced by the type and concentration of the coating material used (Sarungallo *et al.* 2019). The solubility of several microcapsules from other studies that were processed by spray drying were, among them, crude palm oil microcapsules with maltodextrin and sodium caseinate coating materials having a solubility of 70% (Saputri & Ngatirah 2019), then Tectona grandis leaves natural dye microcapsules with maltodextrin, kappa-carrageenan, and maltodextrin coatings. The high and low percentage of solubility of the resulting microencapsulated products is thought to be due to the influence of the coating material given in the form of polysaccharide compounds with different water solubility properties (Choudhury *et al.* 2021). The nature of the coating affects the microencapsulant's ability to deliver the active substances contained therein. In this study, the same coating material was used for NFRPO and FRPO in the form of carrageenan. The mixture of polysaccharides used as a coating causes the product to be very soluble in water (Ezhilarasi *et al.* 2013; Yi *et al.* 2014). Carrageenan is a type of hydrocolloid that can be dissolved in water. The microencapsulated has the appropriate water absorption and solubility if this product is to be used as a premix. The solubility of a material in water is influenced by the water content of the material in question (Yi *et al.* 2014). The high water content in the material causes the material to be difficult to spread in water because the material tends to stick so that no pores are formed. As a result, the material is unable to absorb water. The higher the water content contained in the microencapsulation, the more open the coating structure will be. This causes an increase in the contact between oxygen and oil contained in the microencapsulated, and an auto-oxidation reaction occurs, affecting the increase of peroxide (Perez-Palacios *et al.* 2022).

The stability of the microencapsulated NFRPO and FRPO through the analysis of the peroxide number shows that the length of storage of the sample in the oven has a significant effect on the peroxide value (Table 4). This is because the higher the temperature used, the faster the oxidation process occurs. Oil contains a number of unsaturated fatty acids in the triglyceride molecule. The reactions during th0e heating process are based on the decomposition reaction of fatty acids so that they will form decomposition products that can evaporate and are released with steam when heated) (Li *et al.* 2019). The oxidation process can take place when there is contact between a certain amount of oxygen

and oil or fat. The occurrence of this oxidation reaction will result in a rancid odor in oils and fats. Oxidation usually begins with the formation of peroxides and hydroperoxides (Ezhilarasi *et al.* 2013). The next stage is the breakdown of fatty acids, accompanied by the conversion of hydroperoxides into aldehydes, ketones, and free fatty acids. Rancidity is formed by aldehydes, not by peroxides. Increasing the peroxide value is only an indicator and a warning that the oil will soon smell rancid. Heating up to 60°C does not cause carotene decomposition, but stereoisomer changes can occur. Heat will decompose carotene and cause a change in stereoisomers (D'evoli *et al.* 2013).

The results of the analysis of the IR-spectrum of NFRPO and FRPO (Table 6) show that peaks appear at wavelengths of 2850-2970 and 1340-1470 cm^{-1}, which indicate the presence of the C-H alkane, which usually appears at these wavelengths. At a wavelength of 675-995 cm^{-1}, a peak appears, which may indicate the presence of a C-H Alkene functional group, which usually appears at wavelengths of 3010-3095 and 675-995 cm^{-1}. Then, a peak appears at a wavelength of 690-900 cm^{-1} for the aromatic ring C-H functional group, 3,200-3,600 cm^{-1} indicates the presence of the O-H, and at 2,500-2,700 cm^{-1} for carboxylic acid monomer O-H functional group/carboxylic acid hydrogen bonds. Several other peaks at wavelengths 3,300-3,500 cm^{-1} (N-H amine/amide), 1,610-1,680 cm^{-1} (C=C alkene), 1,180-1,360 cm^{-1} (C-N amine/amide), 1,050-1,300 cm^{-1} (C-O of alcohol/ether/carboxylic acid/ester), 1,690-1,760 cm^{-1} (C=O of aldehydes/ketones/carboxylic acids/esters), and 1,370 cm^{-1} (NO_2).

The IR spectrum of carrageenan shows that a cross-linking reaction and the appearance of the carrageenan functional group are formed. The absorption peak at a wavelength 3,200-3,600 cm^{-1} (O-H bond/hydroxyl stretching), 2,850-2,970 and 1,340-14,700 cm^{-1} indicates stretching of the C-H bond (Table 7). The IR spectrum of carrageenan shows a broad absorption at a wave number of 3,423.4 cm^{-1}. The wave numbers at 3,500-3,200 cm^{-1} and 2,950-2,870 cm^{-1} are considered stretches of the hydroxyl and C-H groups, respectively (Sathasivam *et al.* 2018). The presence of functional groups in the IR spectrum is associated with RPO, coating components, and encapsulation (Nandiyanto *et al.* 2019). The microcapsules of both NFRPO and FRPO have the same IR spectrum pattern; NFRPO and FRPO showed absorption in the form of wide peaks with varying intensity in the area of 2,106 and 2,158 cm^{-1}, which indicated the absorption area of the C≡C Alkyne functional group derived from tween-80, carrageenan and glutaraldehyde. Based on the results of the IR-spectrum interpretation (Table 7) indicate that the NFRPO and FRPO have been encapsulated or physically trapped in the tween-80-carrageenan-glutaraldehyde complex completely (Sathasivam *et al.* 2018). The characteristics of crosslinked using glutaraldehyde carrageenan membrane indicated absorption peaks at wave numbers 2,500-2,700 cm^{-1} and 1,610-1,680 cm^{-1}, indicating the formation of interactions between glutaraldehyde-carrageenan crosslinking (Silitonga *et al.* 2022). However, the absorption intensity shown is low and has a weak vibration signal (Figure 8).

The morphological structure of the RPO microencapsulated products NFRPO and FRPO was observed. Cracks and shrinkage in the RPO microencapsulation might be caused by the weak physical strength of the capsule walls (Figure 9). The surface morphology of the FRPO microencapsule affects the microencapsulated characteristics, such as the active ingredient's release rate and non-encapsulated oil (Figure 10). The shape of the microcapsules is slightly dented, or indentations are formed. The indentation on the microencapsulated surface is probably due to the uneven shrinkage during storage and the high surface protein content (Lee *et al.* 2018; Li *et al.* 2019). The dents in the microcapsule product could be caused by the rapid evaporation of water during the microencapsulation process. Some of the microencapsulants also form aggregates with each other, but some are also evenly distributed (Dubey *et al.* 2009). This aggregate state indicates that the microencapsulated has a relatively high viscosity (Gharsallaoui *et al.* 2012). Microencapsulated NFRPO have a smooth surface and do not have cracks on the surface, so they have low permeability to gases. They can protect the core material from oxidative processes and unwanted leakage (Purnomo *et al.* 2014). A good result of microencapsulation is a round shape without wrinkles, which means the active ingredients are well encapsulated (He *et al.* 2020). In the FPRO microencapsulated beads, the surface shape is flat and round, although several shapes shrink, do not accumulate, and are spread less evenly. Meanwhile, the RPO microencapsulated granules before fermentation had an unfavorable shape because the surface particles were not scattered or piled up (agglomerated) (Calvo *et al.* 2011).

In conclusion, the results showed that FRPO with *S. epidermidis* was able to increase the chemical components. RPO fermentation produces major compounds in the form of 1,2-Benzenedicarboxylic acid (2-Ethylhexyl) ester. In addition, there were differences in the physicochemical characteristics of the microencapsulated NFRPO and FRPO. Microencapsulated FRPO has a higher percent product yield, microencapsulation efficiency, and microencapsulation solubility in water, which is better than that of microencapsulated NFRPO. Moreover, encapsulation can increase RPO stability.

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