Jurnal Rekayasa Kimia dan Lingkungan
(Journal of Chemical Engineering and Environment)
Volume 17, Number 2, Page 138 – 151, July - December, 2022
ISSN 1412-5064, e-ISSN 2356-1661
https://doi.org/10.23955/rkl.v17i2.27110

Microencapsulation of Fermented Red Palm Oil with *L. casei* as Nutracetical Source

Hertanti Dwi Ananda[1], Nuralang[1], Indra Lasmana Tarigan[1], Nindita Cluorisa Amaris Susanto[2], Nelson[1]*

[1]Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jl. Jambi-Ma-Bulian. Km.15, Jambi, Indonesia.
[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, East Java, Indonesia
*E-mail: nelson@unja.ac.id

Article History
Received: 24 July 2022; Received in Revision: 1 September 2022; Accepted: 12 October 2022

Abstract

Red Palm Oil (RPO) is the result of refining from Crude Palm Oil which still maintains a high level of phytonutrients that are beneficial for health. Generally, RPO can be used as a nutraceutical source due to RPO acts as a provitamin A and vitamin E. This study aims to encapsulate RPO before and after fermentation and to characterize its physical, chemical, and bioactivity properties. RPO fermentation using *L. casei* and microencapsulation using beads (emulsion) method. Characterizations were carried out to measure the physicochemical, morphology, and IR. Our finding is that fermentation of the bioactive compound ingredients in RPO using *L. casei* has a significant effect on the M1 formula. Physicochemical properties of M1 are better than RPO, moreover, RPO after fermentation using *L. casei* produces major compounds in the form of 9-Octadecenoic acid, methyl ester, (E)- Fermentation of RPO with *L. casei* increases the components of chemical compounds contained therein. Furthermore, the stability of the fermented RPO provided effective protection against oxidative damage to the oil and proved that the microencapsulation process reduced the level of damage to the oil. The finding showed that RPO fermentation broke down complex compounds and increased their bioactive compounds. In addition, microencapsulation as a nutraceutical preparation is good for increasing the stability of active compounds as delivery systems.

Keywords: Fermentation, lactic acid bacteria, *L. casei*, microencapsulation, red palm oil

1. Introduction

Red Palm Oil (RPO) is the result of refining from Crude Palm Oil (CPO) which still maintains high enough phytonutrients that are beneficial for health. The benefits of RPO can also be used as a nutraceutical source, because RPO acts as a provitamin A and also vitamin E. Nutraceuticals are food-derived commodities in the form of pills, capsules or liquids. Generally consists of dietary supplements, herbal products, probiotics and medical foods intended for the prevention and treatment of disease (Shahidi, 2012). A food product is categorized as a nutraceutical if the food has pharmaceutical activity and is useful in addition to its nutritional value to provide medical or health benefits including prevention and treatment of disease (Santini, et al, 2017).

RPO contains palmitate, α-tocopherol, oleic, α-tocotrienal, linoleate, carotene, β-tocotrienol and δ-tocotrienol compounds (Sathasivam et al., 2018). These compounds act as vitamins and antioxidants. RPO contains -carotene compounds that function as a source of antioxidants to prevent the development of atherosclerosis and other non-communicable diseases (Wallert et al., 2014). This shows that RPO has a high potential to be developed into a nutraceutical source.

Lactic Acid Bacteria (LAB) are commonly used in food and beverage fermentation which contributes as a food quality sensor and spoilage. The addition of LAB in a product functions as a probiotic with metabolite activity that can inhibit pathogenic microorganisms thereby increasing the durability of a product. The addition of LAB to Virgin Coconut Oil (VCO) shows an increase in antioxidant content which means there is an increase in the nutritional quality of VCO. The activity of VCO fermented LAB has higher antibacterial properties than without using LAB. VCO fermentation using *L. casei* significantly increased the diameter of the inhibition zone on *E. coli* and *S. aureus* more than 50% (Rahmadi et al., 2013). Several previous studies have developed essential oil fermentation using probiotic bacteria and

yeast. Research conducted by Frediansyah et al (2017), showed that fermenting black grape juice using *L. plantarum* could improve the functional properties of the juice in vitro. Fermentation using *L. casei* produces Medium Chain Fatty Acid (MCFA), a medium chain fatty acid that is potential as a candidate for drugs, antibacterial, antioxidant and other bioactivity. Therefore, the addition of LAB to RPO is expected to increase the nutraceutical value by adding probiotic properties to RPO products.

One of the active components of RPO is provitamin A (carotene) which is a compound that is easily damaged either due to oxidation or isomerization and is susceptible to oxygen, high temperature and light which causes a decrease in the nutritional quality of RPO. Microencapsulation 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., 2011). Microencapsulation aims to protect the core ingredients from loss of nutritional value, stabilize the active ingredients, facilitate the control of the release of the active ingredients and protect the active components from extreme environments (Firdaus et al., 2014).

Microencapsulation of *L. gasseri* and *B. bifidum* with alginate and chitosan coating could increase bacterial viability in simulated gastric and allow viable cells to reach favorable levels as probiotics (Chávarri et al ., 2010). Research conducted by Lee et al (2018) reported that microencapsulation of RPO as an oil-in-water emulsion (RPO, water, sodium caseinate, maltodextrin and soy lecithin) made by dispersion using supercritical carbon dioxide could increase the stability of RPO. Various methods in the microencapsulation process include spray drying and extrusion. Spray drying has a disadvantage in its operation process which requires high temperatures to produce microencapsulated powders causing the thermolabile essential oil to break down. While the extrusion method produces beads at room temperature and uses non-toxic materials so that it is one way to avoid the loss of essential substances in the oil during encapsulation (Sathasivam et al., 2018).

Utilization of fermented RPO in the form of microencapsulates contributes to the production of practical food additives, increased stability of active ingredients, increased solubility in water and efficient use of active ingredients. This research is expected to be able to provide information about the potential raw material of fermented RPO as a nutraceutical source by showing the profile of RPO before and after the fermentation process, chemical and physical properties of the microencapsulant and its bioactivity as a nutraceutical source.

2. Methodology

2.1. Materials

The main ingredient used in this research is Red Palm Oil obtained from PT. Wilmar Nabati, Padang, Indonesia. Other additives used were *Lactobacillus casei*, which is purchased from Center for Food and Nutrition Studies, Universitas Gajah Mada, Yogyakarta, Indonesia. Media deMan Rogosa Sharpe Agar (MRSA), Phosphate Saline Buffer, Phosphoric Acid, Aquadest, Media deMan Rogosa Sharpe Broth (MRSB), 96% Alcohol, PP Indicator, KOH, Chloroform, Glacial acetic acid, KI, Starch, $Na_2S_2O_3$, Tween-80, carrageenan, CMC, KCl, $CaCl_2$, Glutaraldehyde (GA), Hexane solvent, DPPH. Some of the instruments used in this study were: Glassware (Pyrex), UV-Vis (Thermo-Fischer), FT-IR, GC-MS, and SEM (Shimadzu).

2.2. Preparation of Inoculant

Inoculant preparation was initiated by culturing *Lactobacillus casei* in MRSA and incubated at 37^0C for 48 hours. The cells were harvested, pelletized, and resuspended in sterilized phosphate buffer saline at 7,02 log CFU/mL for inoculation. Calculation of the total number of colonies using the Equation 1.

$$\text{CFU/mL} = \left(\frac{\text{N}}{\text{V x F}}\right) \quad (1)$$

N: Number of colonies; V: Volume plated; F: Dilution factor

2.3. Fermentation of Red Palm Oil (RPO)

The fermentation process begins with the culture of *Lactobacillus casei* on MRSA medium and incubated at 37^0C for 24 hours. The RPO fermentation process begins with preparation using the Wet Degumming using phosphoric acid. Fermentation was carried out in a sterile 250 mL Erlenmeyer filled with RPO and MRSB media with a total reaction volume of 100 mL with three variations of fermentation concentration, M1 (1:0), M2 (1:1), and M3 (1:2) which containing RPO:MRSB (*L. casei* mediaO) with a total reaction volume of 100 mL. Three variations of concentration were produced, namely M1 (100 mL RPO: 0 ml MRSB), M2 (50 ml RPO: 50 ml MRSB), and M3 (33,3 ml: 66.7 ml MRSB) fermented RPO. The bacterial inoculum was transferred as much as

7.02 log CFU/mL into Erlenmeyer containing fermentation medium. After that the cells were cultured using a shaker for 48 hours. The results of the fermentation are separated the medium and filtered using filter paper.

2.4. Physicochemical Analysis

Physicochemical properties of Red-PalmZym were investigated in term of free fatty acid (FFA), acid, and peroxide parameter. The procedure of analysis were carried out using the titration method as previously described by research group of Suroso (Suroso & Sulistijowati, 2013).

2.5. RPO Fermented Compound

Before being fermented, RPO is prepared to remove sap or mucus using the Wet Degumming method. The degumming process is a purification process that aims to separate sap or mucus consisting of phosphatides, protein residues, carbohydrates, water, and resins in oil. In this study, degumming was carried out by using an acid method, namely adding phosphoric acid in RPO and then heating it until the phosphoric acid particles dissolved. Then the RPO was washed with water until the pH of the water became neutral. The purpose of adding phosphoric acid is to convert non-hydratable phosphatides into hydratable ones so that they are easily separated in the washing process. Figure 1 shows the degumming results of RPO with the appearance of sap or mucus.

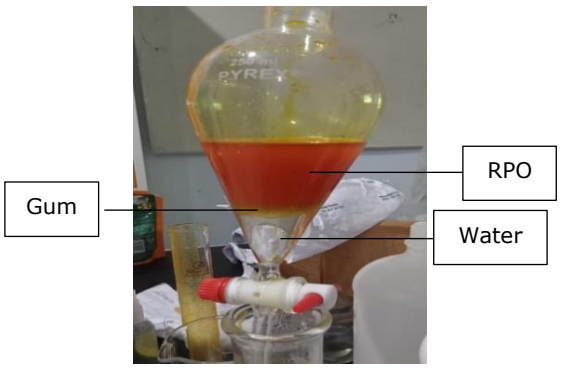

Figure 1. RPO Degumming process**.**

Fermentation of RPO with *L. casei* using three concentration variations, M1 (1:0), M2 (1:1), and M3 (1:2). The bacterial inoculum was transferred as much as 7,02 log CFU/mL into an Erlenmeyer containing a fermentation medium. After that, the cells were cultured using a 150rpm shaker for 48 hours (2 days). The results of the fermentation are separated by the medium and filtered using filter paper.

RPO components after fermentation were analyzed using GC-MS and then compared with RPO components before fermentation (Hartono et al., 2017). GC-MS was adjusted using a Phenomenex ZB-5 column with a size of 30 mm x 0.25 mm x 0.25 mm. The condition of the injector temperature was 280 ° C and the sampling time was 1 minute.

2.6. Microencapsulation

The microencapsulation was prepared by dissolving of 0.1% tween-80 emulsion in 5 mL of Fermented RPO stirred with a stirrer for 3 minutes. The microencapsulant consisting of 100 mL of distilled water mixed with 3 grams of carrageenan-CMC mixture (1:0, 1:0.5 and 1:1) (%w/w) was heated at 85^0C and stirred until the solution was homogeneous and the emulsion was added 2 ml. Beads were produced in 2M KCl-$CaCl_2$ solution, 200 mL. The microencapsulant was drained and continued with the crosslinking using 1% Glutaraldehyde (GA). The microencapsulants were drained and their physical and chemical parameters were tested (Sathasivam et al., 2018).

2.7. Percentage of Product Yield

The percentage of product yield is calculated to find out how much microencapsulation is produced from the microencapsulation process by following a previous study ((Sathasivam et al., 2018), by calculating the overall yield weight of the microencapsulated product and the total weight of the microencapsulated material (coating and emulsifying material), then calculated in percent with the Equation 2.

$$\%R = \left(\frac{W0}{WT}\right) \times 100 \qquad (2)$$

%R = Yield product; W0= Microencapsulated weight (g); WT = Total weight of microencapsulated material (g)

2.8. Microencapsulation Efficiency Test

One gram of microencapsulated added into 12 mL of n-hexane and stirred gently with a mechanical stirrer for 2 minutes. Then filtered and added 15 mL of n-hexane again through filter paper. The microencapsulated RPO was then transferred into a clean empty Erlenmeyer of known weight, then evaporated at a temperature of 69^0C to a constant weight. The surface mass of oil (SO) was determined based on the calculation of the difference between the weight of an empty Erlenmeyer and an Erlenmeyer containing microcapsules.

The total oil is 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 generated from the mass of surface oil and total oil according to the Equation 3.

$$EM=\left(\frac{Wt-\ Ws}{Wt}\right)\times 100\% \quad (3)$$

Remark:
EM : microencapsulatioan efficiency
W_t : total mass of oil (g)
W_s : surface mass of oil (g)

2.9. Solubility of Miroencapsulant

A 0.1g of microencapsulate (a) was added to 10 mL of water at 30^0C and stirred using a magnetic stirrer. Then the solution was filtered through Whatman filter paper no. 42 whose fixed weight is known. The filter paper and the unfiltered sample were oven-dried for 1 hour at 105^0C, then cooled in a desiccator for 15 min, then 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. Solubility is calculated by the Equation 4.

$$\text{Solubility in Waters (\%)} = \left(\frac{a-b}{a}\right)\times 100\% \quad (4)$$

2.10. Stability Test of Microencapsulant

Microencapsulated (1 gram), RPO oil and Red-PalmZym (as positive control) were stored in vials at 60°C for 9 days. The bottles were removed from the oven at 0, 3, 6, 9 days of storage and the peroxide value was determined by the titration method using Equation 5.

$$\text{Peroxide number} = \frac{V \times N \times 1000}{W} \quad (5)$$

V = Volume of $Na_2S_2O_3$ (ml); N= *Concentration of $Na_2S_2O_3$ (N); W = Sample weight (g)*

2.11. Fungsional Group and Morphology

The infrared spectra of the carrageenan, RPO, Red-PalmZym, Tween-80, Microencapsulant, and Glutaraldehyde were recorded in the variant 640-IR FTIR spectrophotometer at wavenumber of 500 to 4000 cm^{-1}. The shape and morphology of the microcapsules obtained from the microencapsulation process were observed by a scanning electron microscopy (SEM). Microencapsulated sample is placed in a sample holder and then coated with gold particles using a fine coater. The samples were then observed and imaged at magnifications of 500, 1000, 2000 and 5000.

3. Results and Discussion

3.1. Red-PalmZym Physicochemical

The results of the physicochemical analysis are depicted in Table 1. The FFA level is the percentage of the amount of free fatty acids contained in the oil. The FFA value is used to calculate the acid value. The acid value indicates the amount of free fatty acids contained in the oil derived from the oil hydrolysis process or due to poor processing. According to Ketaren (2005), the acid value is a measure of the amount of free fatty acids, and is calculated based on the molecular weight of the fatty acid or fatty acid mixture. A high acid value indicates a high free fatty acid content in the oil. The higher the acid value, the lower the quality of the oil. The acid value is expressed as the value of milligrams of 0.1 N KOH or 0.1 N NaOH used to neutralize free fatty acids contained in 1 gram of oil or fat.

The results of the measurement of FFA levels before fermentation obtained in the study were 4.10% while based on SNI 01-2901-1992 regarding palm oil, the maximum FFA levels were 5%. Thus, the quality of the RPO samples used still met the requirements because of low free fatty acids. Table 1 shows that fermented RPO did not significantly affect the free fatty acid content of RPO. However, the fermented RPO sample which had the lowest free fatty acid value was the M1 sample of 3.97%.

Table 1. The physicochemical properties of RPO before and after fermentation.

Test parameters	Result			
	RPO	M1	M2	M3
Free fatty acid value	4,10 **%**	3,97 %	4,22 %	4,35 %
Acid value	8, 976 mg KOH/g	8,6955 mg KOH/g	9,2565 mg KOH/g	9,537 mg KOH/g
Peroxide value	8 meq	8 meq	14 meq	20 meq

Peroxide value analysis is used to determine the degree of oxidation damage of oil or fat. Oxidation damage occurs when a certain amount of oxygen is in contact with the double bonds in the oil or fat.

The results of the analysis of the peroxide value in the RPO samples before fermentation obtained in the study were 8 meq. While the results of the analysis of the peroxide value on

the RPO sample after fermentation (Red-PalmZym) at the three concentration variations, respectively, namely 8 meq, 14 meq and 20 meq. According to the Indonesian National Standardization Agency, palm oil must have a peroxide value of less than 10 meq. This indicates that fermented RPO at a concentration of M1 (100 mL) meets the requirements because the peroxide value is less than 10 meq. Meanwhile, at concentrations of M2 and M3 the peroxide value obtained was more than 10 meq. Siddique et al (2010) stated that the peroxide value of the oil will increase after exposure to light and air at room temperature. The breakdown of fats and oils during storage can also affect the peroxide value and cause a rancid odor. Rancidity often begins to appear when the peroxide value is in the range of 20 and 40 meq. Based on the physicochemical properties testing of the fermented RPO of L. casei (Red-PalmZym) at three concentrations variation, the M1 (1:0) has good condition.

3.2. Chemical Content of Red-PalmZym

Chemical components of RPO before fermentation is tabulated in Table 2 and Table 3. There are 41 compounds founded in RPO before fermentation. The highest peak at peak number 4 with a retention time of 38,270 minutes and a percent area of 86,00% found acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a octahydronaphthal en-2-yl ester compound with molecular formula C17H26O3 (Figure 2).

Table 2. GC-MS RPO Results Before Fermentation.

Compound peak	R. Time (Min)	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	$C_{17}H_{22}F_5NO_2$	4-Methoxyphenyl)heptylamine, N-pentafluoropropionyl
9	39.375	0.15	$C_{10}H_{15}Si$	Phenyldimethylvinylsilane
10	39.601	0.92	$C_{15}H_{26}O$	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene
11	39.700	1.50	$C_{21}H_{30}O3$	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-
12	40.033	0.18	C_2F_6	Ethane, hexafluoro
13	40.151	0.21	$C_2H_3F_3O_3S$	Methanesulfonic acid, trifluoro-, methyl ester
14	40.267	0.03	$C_{15}H_{22}O_4$	Oxirane, 2-[2-(benzyloxy)-1-(1-methoxy-1-methylethoxy)ethyl]
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	$C_{32}H_{64}O_8Si_7$	1,15-Di(1-phenylpropyl)-2,2,4,4,6,6,8,8,10,10,12,12,14,14-tetradecamethyl-1,3,5,7,9,11,13,15-octaoxa-2,4,6,8,10,12,14-heptasilapentadecane
21	42.917	0.07	C_2F_6	Ethane, hexafluoro

Table 2. GC-MS RPO Results Before Fermentation (continued).

Compound peak	R. Time (Min)	Area (%)	Molecular formula	Compound name
22	43.035	0.27	$C_3H_5NS_2$	2-Thiazolidinethione
23	43.276	0.17	$C_{16}H_{16}N4_{O2}$	2,4-Diamino-8-hydroxy-5-isopropyl-5H-chromeno[2,3-b]pyridine-3-carbonitrile
24	43.483	0.10	$C_{36}H_{38}O_8$	Methyl 2,3,4-tribenzyl-6-anisoyl-.beta.-d-galactopyranoside
25	43.667	0.12	$C_{19}H_{15}NO_3S_2$	3-(4-Methylbenzoyl)-2-thioxo-4-thiazolyl 4-methylbenzoate
26	43.825	0.19	$C_{14}H_8C_{l4}O_2$	4-Methylbenzoic acid, 2,3,4,6-tetrachlorophenyl ester
27	43.908	0.28	$C_{14}H_6C_{l2}F_4O_2$	6-Fluoro-2-trifluoromethylbenzoic acid, 2,3-dichlorophenyl ester
28	44.042	0.08	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, hexamethyl
29	44.136	0.11	$C_{24}H_{20}O_2$	9-Phenanthrenemethyl 2,6-dimethylbenzoate
30	44.243	0.06	$C_{11}H_{17}F_5O_5$	2-[2-(2-Ethoxyethoxy)ethoxy]ethyl 2,2,3,3,3-pentafluoropropanoate
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	$C_{13}H_{20}O$	alpha.-Damascone
34	44.994	0.76	$C_{18}H_{14}FNO_4$	Oxazole-5-one, 2-phenyl-4-[2-fluoro-4,5-dimethoxybenzylidene]-
35	45.126	0.27	$C_{22}H_{31}OP$	Phosphine oxide, bis(pentamethylphenyl)-
36	45.250	0.24	$C_{22}H_{36}O_4Si_3$	1,7-Di(3-ethylphenyl)-2,2,4,4,6,6-hexamethyl-1,3,5,7-tetraoxa-2,4,6-trisilaheptane
37	45.378	0.08	$C_{12}H_{18}O_2Si$	Paroxypropione, TMS derivative
38	45.434	0.09	$C_{22}H_{28}O_4Si$	5-hydroxy-7-methoxyflavanone, tert.-butyldimethylsilyl ether
39	45.549	0.13	$C_{22}H_{36}O_4S_{i3}$	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	$C_{18}H_{40}O_3Si$	Silane, dodecyltriethoxy
41	45.739	0.04	$_{24}H_{52}O_3Si$	Octadecyltriethoxysilane

The second highest retention time was 42,354 and the area percentage was 4,78% contained 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one compound. The third highest peak was at retention time of 39,700 with an area percentage of 1,50% found 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)- compound.

The result of the GC-MS analysis of RPO after fermentation (Red-PalmZym) is presented in Table 3. The highest peak was detected at a retention time of 28,152 minutes and peak area of 55,03%. This indicated a 9-octadecenoic acid, methyl ester, (E)- or methyl oleate compounds available in RPO after fermentation process (Figure 3). This shows that the RPO fermented with L. casei contains a fairly high composition of methyl oleate. Additionally, from the chromatogram found also some of the most dominant peaks, namely Hexadecanoic acid, methyl ester or methyl palmitate which is one of the groups of fatty acid compounds. RPO contains palmitic acid (38-44%) and oleic acid (39-44%), this high oleic acid makes the oil more resistant to oxidation than most liquid oils.

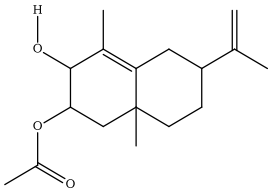

Figure 2. Compound structure of acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester.

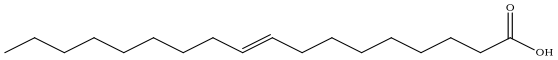

Figure 3. Compound structure of 9-Octadecenoic acid, methyl ester, (E).

Table 3. GC-MS RPO Results After Fermentation.

Compound peak	R. Time (Min)	Area (%)	Molecular formula	Compound name
1-14	12.969-15.456	0.14	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
15	15.533	0.1	$C_{20}H_{22}N_2OS$	alpha.-Piperidinomethyl-2-phenyl-6-benzothiazole methanol
16	15.567	0.06	$C_{10}H_{20}O_3$	3-Methylpentan-3-yl propyl carbonate
17-18	15.747-15.904	0.59	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
19	16.133	0.13	$C_6H_{12}O$	4-Hexen-1-ol, (Z)-
20-21	16.225-16.347	0.18	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
22	16.392	0.11	$C_{15}H_{30}O_2$	Tridecanoic acid, 12-methyl-, methyl ester
23	16.45	0.49	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
24	16.717	0.07	$C_{14}H_{28}O_2$	Tridecanoic acid, methyl ester
25-27	16.925-17.2	0.21	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
28	17.392	0.12	$C_{21}H_{15}F_{12}NO_9$	L-(-)-Fucose, tetrakis(trifluoroacetate), benzyloxime
29	17.45	0.06	$C_{13}H_{26}O_2$	Undecanoic acid, 10-methyl-, methyl ester
30-37	17.558-24.781	0.11	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
38	27.592	0.05	C_9H_{16}	Cyclooctene, 3-methyl-
39	28.152	55.03	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-
40	28.524	13.41	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-
41	28.975	0.7	$C_{17}H_{32}O_2$	7-Hexadecenoic acid, methyl ester, (Z)-
42	29.142	8.87	$C_{19}H_{38}O_2$	Methyl stearate
43	29.962	0.95	$C_{19}H_{34}O_2$	9,11-Octadecadienoic acid, methyl ester, (E,E)-
44	30.117	6.53	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-
45	30.783	1.13	$C_{19}H_{38}O_2$	Methyl stearate
46	31.406	1.15	$C_{21}H_{38}O_2$	8,11-Eicosadienoic acid, methyl ester
47	31.499	1.82	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-
48	31.961	0.18	$C_{22}H_{44}O_2$	Heneicosanoic acid, methyl ester
49	34.627	0.08	$C_{16}H_{32}O_2$	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-
50	39.191	0.36	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate

Based on the results of the GC-MS analysis in Table 2 and Table 3, there are significant differences. Where in Table 2 samples of RPO before fermentation there are 41 peaks which indicate the presence of 41 components of compounds contained in RPO before fermentation. While in Table 3 the RPO samples after fermentation there were 50 peaks indicating the presence of 50 components of the compounds contained in the RPO after Fermentation. This indicates that there is fragmentation or breakdown of fatty acid complex compounds into short fatty acid compounds in RPO samples before fermentation and RPO samples after fermentation (Red-PalmZym).

3.2. Microencapsulation Formulation of Red-PalmZym

Microencapsulation using carrageenan-CMC coating material with a variation of 1:0 (%w/w) can be seen in Figure 4. The microencapsulant produced using carrageenan-CMC coating material with a variation of 1:0 (%w/w) resulted in microencapsulated beads that had a denser texture, brighter color and perfectly round shape. According to Mawarni and Yuwono, (2018), the more concentration of carrageenan added, the harder the texture will be because the ability of carrageenan to form a gel is very strong. However, from the results obtained, there are differences in the size of the microencapsulated beads produced, this can be due to the size of the needle used (Rokka & Rantamaki, 2010) and the distance between the needle and the hardener solution (KCl-$CaCl_2$). Solanki et al (2013), resulting in different sizes of microencapsulated beads.

Carrageenan acts as a coating material for microencapsulated beads. Where the selection of the type of coating or encapsulating material (encapsulating material/wall) is one of the important factors in microencapsulation techniques. The resulting capsules can function well if using appropriate coating materials (Botrel et al., 2014). Research conducted by Distantina et al *(*2013) reported that kappa carrageenan was able to form microencapsulated beads. Carrageenan is used in microencapsulation of probiotics because of its ability to form a gel so that it can adsorb cells.

The formation of carrageenan gel is induced by temperature changes (Ggassi & Vandamme, 2012). Carrageenan to get its hydrogel structure requires modification by crosslinking method. Crosslinking process consists of 2 types, namely physical and chemical.

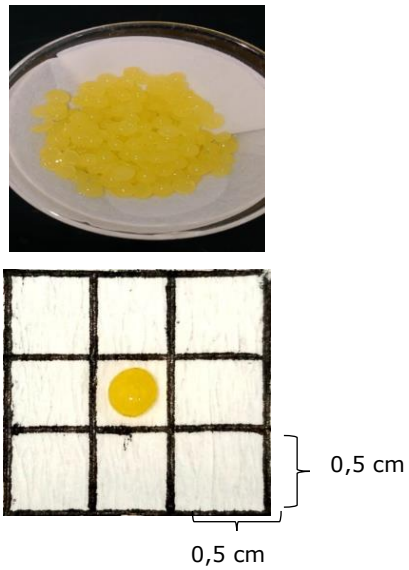

Figure 4. Microencapsulation with Carrageenan-CMC coating material (1:0).

Physically crosslinking the structure of the carrageenan hydrogel can be obtained by forming microencapsulated beads with KCl and $CaCl_2$. However, the crosslinks that occur are reversible, so chemical crosslinks are needed that are able to form irreversible bonds and strengthen the bead structure. The chemical crosslinking occurs from irreversible covalent bonds so it is necessary to strengthen the structure of the microencapsulated beads so that they are stable in aqueous media (Ebara et al 2014). Chemical crosslinking uses glutaraldehyde (GA) is widely used in research because this compound is an introductory bond between covalent molecules and polymer chains. In addition, these compounds are also easy to obtain and relatively inexpensive. The aldehyde group is very reactive and has been used as a polymer crosslink (Distantina et al., 2013). Crosslinking reaction mechanism can be seen in Figure 5.

3.3. Percent Yield of Product

Percent yield is used to determine the efficiency and effectiveness of a process. The principle of yield is to calculate the amount of product produced per material used. The yield was calculated based on the weight of the microencapsulated per weight of the total material used. RPO and Fermented RPO microencapsulated yield data can be seen in Table 4.

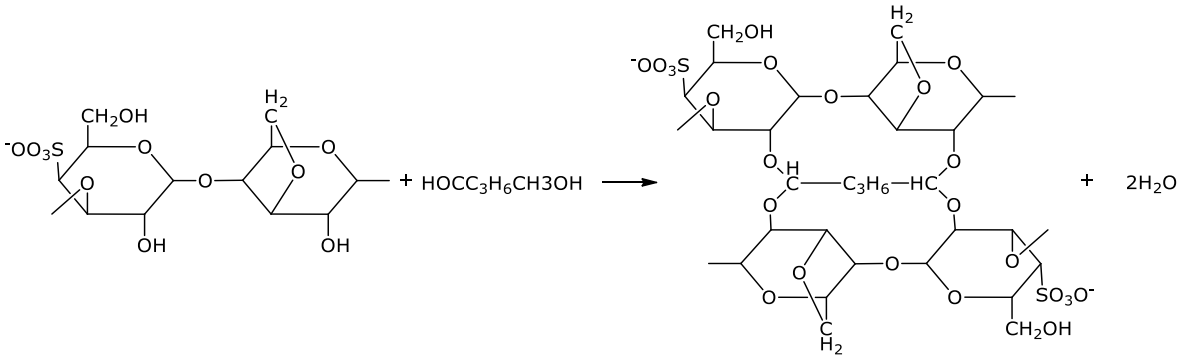

Figure 5. Mechanism of the crosslinking reaction of Carragenan and Glutaraldehyde.

Table 4. RPO and Fermented RPO micro-encapsulated yields.

Sample	Product yield (%)
RPO before fermentation	31.8
RPO afer fermentation	36.5

In Table 4 it can be seen that the RPO microencapsulated yields before and after fermentation (Red-PalmZym) were 31.80% and 36.50%, respectively. The value obtained is quite low, this is because the printing of beads into the KCl-$CaCl_2$ using a syringe takes a long time, causing the carrageenan solution to become thicker and difficult to take using a syringe.

The higher the concentration of the coating material used, the greater the yield of the microencapsulated, because the total solids will increase as the concentration of the added coating material increases.

3.4. Microencapsulation Efficiency

Efficiency test is a parameter that shows the ability of the encapsulating material to coat the core, as well as the efficiency level of the emulsification and encapsulation processes. The higher the microencapsulation efficiency value, the better the coating ability to protect the core material. The efficiency values of RPO and Fermented RPO microencapsulated can be seen in Table 5.

Table 5. Efficiency of RPO and Fermented RPO microencapsulated.

Sample	Microencapsulated efficiency (%)
RPO before fermentation	99,03
Red after fermentation	99,42

Based on the data (Table 5) shows that the microencapsulation efficiency of the RPO is lower that fermented RPO, 99.03% and 99.42% respectively. This informs that the percentage of RPO before and after fermentation is almost entirely coated in the microencapsulation. The microencapsulation efficiency produced in this study is high because the coating material used (Carrageenan) has high solubility and its ability to emulsify so that the active ingredient of red palm oil can be coated. One of the factors that can cause high efficiency of microencapsulated is the comparison between the coating material and the core material.

3.5. Solubility of Microencapsulated

The water solubility of a product is related to the release of the active ingredient upon application of the microencapsulant. If the microencapsulated solubility in water is high, it will facilitate the use of the product during application. In general, the solvent used in palm oil microencapsulated products is water. The solubility value of microencapsulated in water can be seen in Table 6.

Table 6. Solubility of Microencapsulated.

Sample	Solubility of microencapsules in water (%)
RPO before fermentation	96,11
RPO after fermentation	99,69

The solubility of the microencapsulated product in the RPO samples before and after fermentation was of high value, namely 96.11% and 99.69%, respectively (Table 6). The degree of solubility of the microencapsulated is not influenced by the type of coating material used, but is influenced by the amount of coating material used. The

more coating material used, the red palm oil as the core material will be coated perfectly, so that the microencapsulation process is effective and no oil layer is formed on the surface of the microencapsulated resulting in increased solubility (Botrel et al., 2014).

3.6. Microencapsulated Stability

The stability of RPO before and after fermentation was calculated by oxidative stability assays to obtained the oxidation rate and shelf-life of the RPO and Fermented RPO microencapsulate (Figure 6). Samples were stored in vials at 60°C for 9 days, then removed from the oven at 0, 3, 6, and 9 days of storage. Then, the peroxide value was determined by titration. Peroxide number can affect the shelf-life of a product. If the peroxide number is high, then the product cannot be stored for long and vice versa.

Peroxide value obtained based on the graph in Figure 6 shows an increasing yield from day 0 to day 9 with a range of 8-90 meq. The increase in the peroxide number of the sample at a temperature of 60°C and storage time was due to the formation of a chemical reaction between the oil and oxygen which resulted in the formation of peroxide compounds that could help the oxidation process of a small amount of saturated fatty acids in the microencapsulated sample. The peroxide formed is labile and reactive. If oxidation continues, these highly labile and reactive peroxides will rapidly degrade to form more complex second-level oxidation products such as aldehydes and ketones (Pegg, 2005). In this study, the peroxide value of the microencapsulated was lower than the peroxide value of red palm oil.

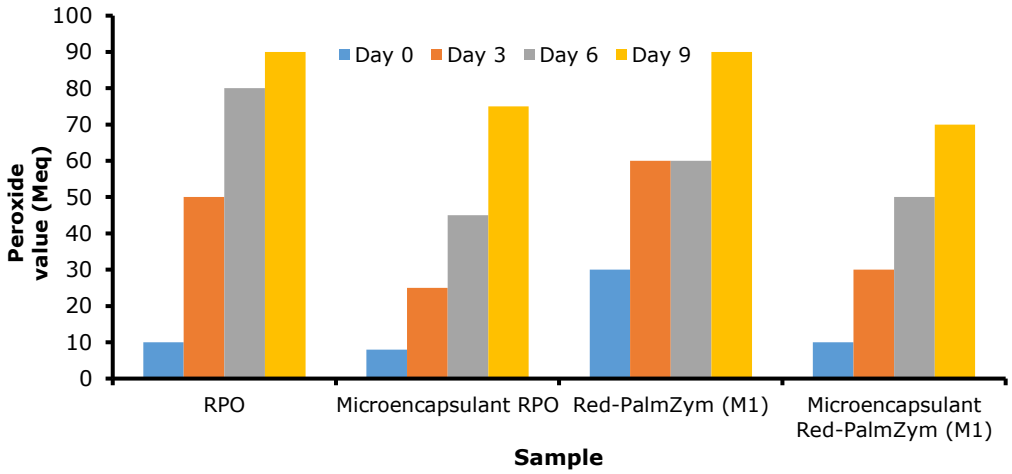

Figure 6. Stability of the sample microencapsulated.

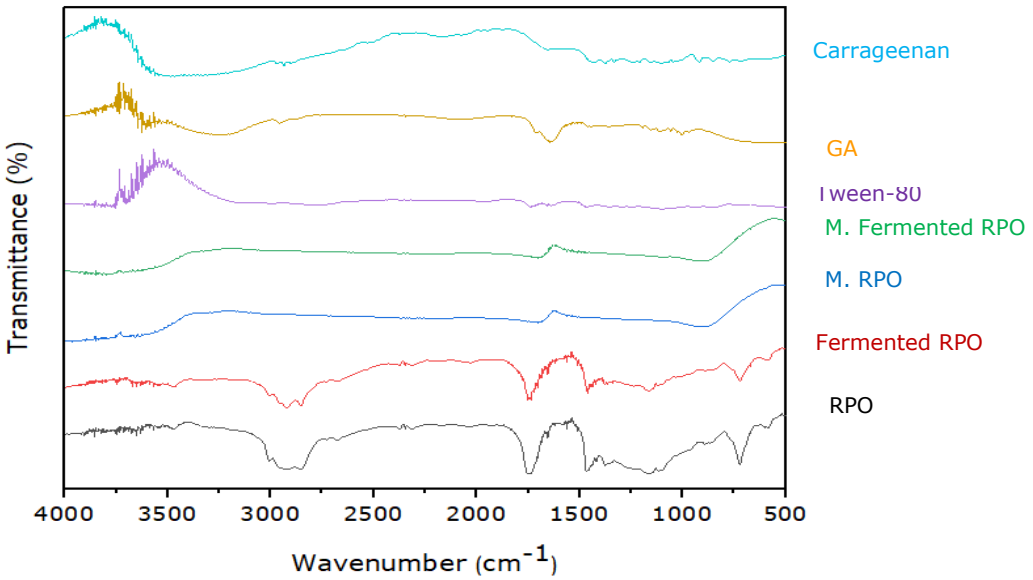

Figure 7. FTIR spectra of RPO before fermentation, RPO after fermentation, RPO microencapsulated, RPO Fermented microencapsulated, Tween-80, Glutaraldehyde (GA), and Carrageenan Spectra.

3.7. Functional Groups of RPO

A FTIR analysis was carried out to determine the functional groups contained in the sample. The results of the FTIR test for RPO before fermentation, RPO after fermentation (Red-PalmZym), RPO microencapsulants, Red-PalmZym microencapsules, Tween-80, Glutaraldehyde (GA) and Carrageenan are shown in Figure 7.

The RPO before and after fermentation has an IR spectra almost similar at wavenumbers 2850-2970 and 1340-1470 cm^{-1}, which may indicate the presence of the CH Alkane functional group which usually appears at these wavelengths. There is a wavenumber of 675-995 cm^{-1} indicating the presence of the CH Alkene functional group. Then a peak appears at a wavelength of 690-900 cm^{-1} which may indicate the presence of an aromatic ring CH functional group. Furthermore, a peak appears at a wavelength of 3200-3600 cm^{-1} indicating the presence of a hydrogen/phenol bonded -OH alcohol functional group. At a wavelength of 2500-2700 cm^{-1} possibly indicating the presence of hydrogen bonds with OH carboxylic acids. The peak appears at a wavelength of 3300-3500 cm^{-1} which may indicate the presence of an amine/amide NH functional group. At a wavelength of 1610-1680 cm^{-1}, a peak appears which may indicate the presence of a C=C alkene functional group. Furthermore, at wavelengths of 1050-1300 and 1690-1760 cm^{-1} which may indicate the presence of functional groups CO and C=O Aldehydes/ketones/carboxylic acids/esters. Then a peak appears at a wavelength of 1370 cm^{-1} which indicates the presence of nitro compounds NO_2. Based on these results it can be identified that the FTIR Spectra of RPO samples before fermentation and RPO samples after fermentation (Red-PalmZym) have CH, CO, C=O and OH, NH, C=C and NO_2.

Table 7. Results of FTIR Spectrum Analysis.

No	Functional groups	Wavelength (cm^{-1})				
		A	B	C	D	E
1	Alkane C-H	-	-	2850-2970 dan 1340-1470	2850-2970 dan 1340-1470	2850-2970 dan 1340-1470
2	Alkene C-H	675-995	675-995	675-995	675-995	675-995
3	Alkyne C-H	-	-	-	-	3307
4	Aromatic ring C-H	690-900	690-900	690-900	690-900	690-900
5	Hydrogen/phenol bonded alcohol O-H	3200-3600	3200-3600	3200-3600	3200-3600	3200-3600
6	Monomer carboxylic acids/carboxylic acids hydrogen bonds O-H	2567	2553-2607	-	-	2500-2648
7	Amine/amide N-H	3493	3496	-	3313-3388	3307-3485
8	Alkene C=C	-	-	1610-1680	1610-1680	1610-1680
9	Aromatic ring C=C	-	-	-	-	-
10	Alkyne C≡C	2106	2158	-	-	2100-2260
11	Amine/amide C-N	-	-	1180-1360	1180-1360	1180-1360
12	Nitrile C≡N	-	2268	-	-	-
13	Aldehydes/ketones/ carboxylic acids/esters C-O	1170	1170	1050-1300	1050-1300	1050-1300

A (RPO microencapsules before fermentation), B (Red-PalmZym microencapsules), C (Tween-80), D (Glutaraldehyde) and E (Carrageenan)

Furthermore, the results of the FTIR spectra on RPO (A), Red-PalmZym (B), Tween-80 (C), Glutaraldehyde (D) and Carrageenan (E) microencapsulants are shown in Figure 7. The spectrum of Tween-80 (C), Glutaraldehyde (D) and Carrageenan (E) a peak appears which indicates the possibility of the presence of the CH Alkane functional group at wavelengths 2850-2970 and 1340-1470 cm^{-1}. Then on the spectrum of RPO (A), Red-PalmZym (B), Tween-80 (C), Glutaraldehyde (D) and Carrageenan (E) microencapsulants, there are 675-995 cm-1 wavelength which indicate the presence of the CH Alkene functional group. Furthermore, a peak appears at a wavelength of 690-900 cm^{-1} which may indicate the presence of an aromatic ring CH functional group. The peak appears at a wavelength of 3200-3600 cm^{-1} which may indicate the presence of a hydrogen/phenol OH functional group. Then a peak appears at a wavelength of 1050-1300 cm^{-1} indicating the presence of a functional group CO. Alcohol/ether/carboxylic acid/ester. In the spectrum of RPO (A), Red-PalmZym (B), Tween-80 (C), and Carrageenan (E) microencapsulants, peaks appeared at a wavelength of 2100-2260 cm^{-1} indicating the presence of a C≡C alkyne functional group.

The peak appears at a wavelength of 3300-3500 cm^{-1} which may indicate the presence of an amine/amide NH functional group, except in the Tween-80 (C) spectrum. In the Tween-80 (C) and Carrageenan (E) spectra, peaks appeared at a wavelength of 1300-1370 cm which indicated the presence of nitro compounds NO_2. Fermented RPO (B), Tween-80 (C), Glutaraldehyde (D) and Carrageenan (E) have CH, OH, NH, C≡C, CN, CO, and NO_2. Fermented RPO microencapsulate (B), showed that the RPO sample and coating materials had been coated, but the % transmittance was small, so the absorption intensity was not clear enough. The results of the FTIR spectrum analysis of RPO microencapsulated before fermentation, Red-PalmZym, Tween-80, Glutaraldehyde and Carrageenan microencapsulants can be seen in Table 7.

3.8. The Morphology of RPO and Red-PalmZym microencapsulated

The shape and morphology of the RPO and Red-PalmZym microencapsulant obtained from SEM analysis are showed in Figure 8 and 9. The morphology of the RPO and Red-PalmZym microencapsulates showed the aggregate form. Aggregate is the agglomeration between the particles with one another. The addition of the carrageenan brought about an increase in the number of aggregates formed due to the double helix. The more double helixes, the more aggregates will be produced.

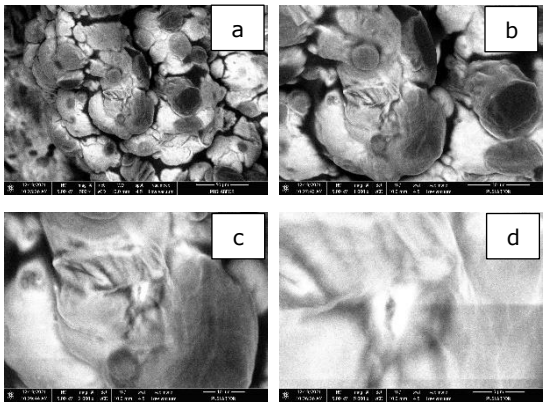

Figure 8. SEM analysis results of RPO microencapsulated samples at 500x (a), 1000x (b), 2000x (c) and 5000x (d) magnifications.

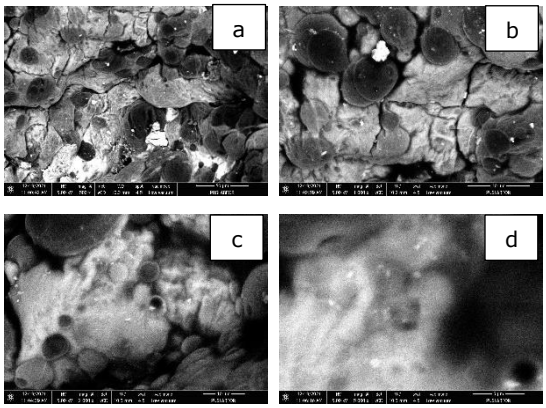

Figure 9. SEM analysis results of RPO Fermented microencapsulated samples at 500x (a) magnification 1000x (b), 2000x magnification (c) and 5000x magnification (d).

In this study, the RPO and Red-PalmZym microencapsulants were cracked and some of them were shriveled and wrinkled. This is thought to be due to the ballooning event. Jafari et al (2011) stated that ballooning can cause the loss of active ingredients from the microencapsulant due to cracks in the microencapsulated particles. When the capsule walls are not strong enough to withstand the pressure inside the microencapsulated particles, the walls will break and the microencapsulated will collapse. The shrinking and wrinkled shape of the microencapsulated is due to the fact that some of the oil and active ingredients have been

released from the microencapsulated particles.

4. Conclusion

Our finding is that fermentation of the bioactive compound ingredients in RPO using *L. casei* has a significant effect on the M1 formula. Physicochemical properties of M1 are better than RPO, moreover, RPO after fermentation using *L. casei* produces major compounds in the form of 9-Octadecenoic acid, methyl ester, (E)- Fermentation of RPO with *L. casei* increases the components of chemical compounds contained therein. Furthermore, the stability of the fermented RPO provided effective protection against oxidative damage to the oil and proved that the microencapsulation process reduced the level of damage to the oil. The finding showed that RPO fermentation broke down complex compounds and increased their bioactive compounds. In addition, microencapsulation as a nutraceutical preparation is good for increasing the stability of active compounds as delivery systems.

Acknowledgement

Thanks to the Faculty of Science and Technology, University of Jambi for research funding assistance through the PNBP of the Faculty of Applied Research Schemes in 2021. Special thanks to PT. Wilmar Nabati for the supply of CPO and RPO samples as study material in this research.

References

Botrel, D. A., Fernandes, R. V., Borges, S. V., & Yoshida, M. I. (2014). Influence of wall matrix systems on the properties of spray-dried microparticles containing fish oil. Food Res. Int. , 62, 344–352. https://doi.org/10.1016/j.foodres.2014.02.003

Chávarri, M., Marañón, I., Ares, R., Ibáñez, F. C., Marzo, F., & Villarán, M. del C. (2010). Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. Int. J. Food Microbiol, 142(1–2), 185–189. https://doi.org/10.1016/j.ijfoodmicro.2010.06.022

Distantina, S., Rochmadi, Fahrurrozi, M., & Wiratni. (2013). Preparation and characterization of glutaraldehyde-crosslinked kappa carrageenan hydrogel. Eng. J., 17(3), 57–66. https://doi.org/10.4186/ej.2013.17.3.57

Ebara, M., Kotsuchibashi, Y., Narain, R., Idota, N., Kim, Y. J., Hoffman, J., Uto, K., & Aoyagi, T. (2014). Smart Biomaterials. Spinger.

Frediansyah, A., Nurhayati, R., & Romadhoni, F. (2017). Enhancement of Antioxidant Activity , α-Glucosidase and α- Amylase Inhibitory Activities by Spontaneous and Bacterial Monoculture Fermentation of Indonesian Black Grape Juices. AIP Conf. Proc, 18(1), 1–9. https://doi.org/10.1063/1.4973149

Ggassi, G. K., & Vandamme, T. (2012). Probiotic encapsulation technology: microencapsulation to release into gut. Pharmaceutics, 4, 149–163. https://doi.org/10.3390%2Fpharmaceutics4010149

Hartono, H. S., Soetjipto, H., & Kristijanto, A. I. (2017). Extraction and Chemical Compounds Identification of Red Rice Bran Oil Using Gas Chromatography – Mass Spectrometry (Gc-Ms) Method. Eksakta, 17(2), 98–110. https://doi.org/10.20885/eksakta.vol17.iss2.art2

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2011). Encapsulation Efficiency of Food Flavours and Oils during Spray Drying. Dry. Technol., 26(1), 816–835. https://doi.org/10.1080/07373930802135972

Lee, W. J., Tan, C. P., Sulaiman, R., Smith, R. L., & Chong, G. H. (2018). Microencapsulation of red palm oil as an oil-in-water emulsion with supercritical carbon dioxide solution-enhanced dispersion. J. Food Eng., 222, 100–109. https://doi.org/10.1016/j.jfoodeng.2017.11.011

Mawarni, S. A., & Yuwono, S. S. (2018). Effect of Cooking Time and Carrageenan Concentration on Physical , Chemical , and Organoleptic Properties of Mix Fruit Sheet Jam (Starfruit and Apple). J. Food Agroind. 6(2), 33–41.

Pegg, R. B. (2005). Lipid Oxidation/Stability: Measurent of Primary Oxidation Product. Di dalam Wrolstad RE (ed.), handbook of Food Analytical Chemistry: Water, Proteins, Lipids and Carbohydrates. Wiley

Interscience.

Prakash, S., Tomaro-Duchesneau, C., Saha, S., & Cantor, A. (2011). The gut microbiota and human health with an emphasis onM the use of microencapsulated bacterial cells. Biomed Res. Int., 2011. https://doi.org/10.1155/2011/981214

Rahmadi, A., Abdiah, I., Dewi, M., & Purnaningsih, T. (2013). Physicochemical and Antibacterial Characteristics of Virgin Coconut Oil Fermented with Lactic Acid Bacteria. J. Food Technol. Industry 24(2), 178–183. https://doi.org/10.6066/jtip.2013.24.2.178

Rokka, S., & Rantamaki, P. (2010). Protecting Probiotic Bacteria by Microencapsulation: Challenges for Industrial Applications. Eur. Food Res. Technol., 231, 1-12. https://doi.org/10.1007/s00217-010-1246-2

Santini, A., Tenore, G. C., & Novellino, E. (2017). Nutraceuticals: A paradigm of proactive medicine. Eur. J. Pharm. Sci., 96, 53–61. https://doi.org/10.1016/j.ejps.2016.09.003

Sathasivam, T., Muniyandy, S., Chuah, L. H., & Janarthanan, P. (2018). Encapsulation of red palm oil in carboxymethyl sago cellulose beads by emulsification and vibration technology: Physicochemical characterization and in vitro digestion. J. Food Eng., 231, 10–21. https://doi.org/10.1016/j.jfoodeng.2018.03.008

Setijawati, D., Firdaus, M., & Kartikaningsih, K. (2014). The Effect of Lactobacillus Acidophilus Microcapsule Which Encapsulated by Kappa Caragenan Toward in Vivo Functional Test. J. Life Sci., 1(1), 27–36. https://doi.org/10.21776/ub.rjls.2014.001.01.5

Shahidi, F. (2012). Nutraceuticals, functional foods and dietary supplements in health and disease. J Food Drug Anal., 20(SUPPL.1), 226–230. https://doi.org/10.38212/2224-6614.2144

Siddique, B. M., Anes, A., Ibrahim, M. H., Hena, S., Rafatullah, M., & Omar, M. (2010). Physico-chemical properties of blends of palm olein with other vegetable oils. Grass Aceites, 61(4), 423–429. http://dx.doi.org/10.3989/gya.010710

Solanki, H. K., Pawar, D. D., Shah, D. A., Prajapati, V. D., Jani, G. K., Mulia, A. M., & Thakar, P. M. (2013). Development of Microencapsulation Delivery System for Long-Term Preservation of Probiotics as Biotherapeutics Agent. Biomed Res. Int., 1(1), 1–21. http://dx.doi.org/10.1155/2013/620719

Vrolijk, M. F., Opperhuizen, A., Jansen, E. H. J. M., Godschalk, R. W., Schooten, F. J. Van, Bast, A., & Haenen, G. R. M. M. (2015). The shifting perception on antioxidants : The case of vitamin E and β - carotene. Redox Biol., 4(1), 272–278. https://doi.org/10.1016/j.redox.2014.12.017

Wallert, M., Schmölz, L., Galli, F., Birringer, M., & Lorkowski, S. (2014). Regulatory metabolites of vitamin E and their putative relevance for atherogenesis. Redox Biol., 2(1), 495–503. https://doi.org/10.1016/j.redox.2014.02.002