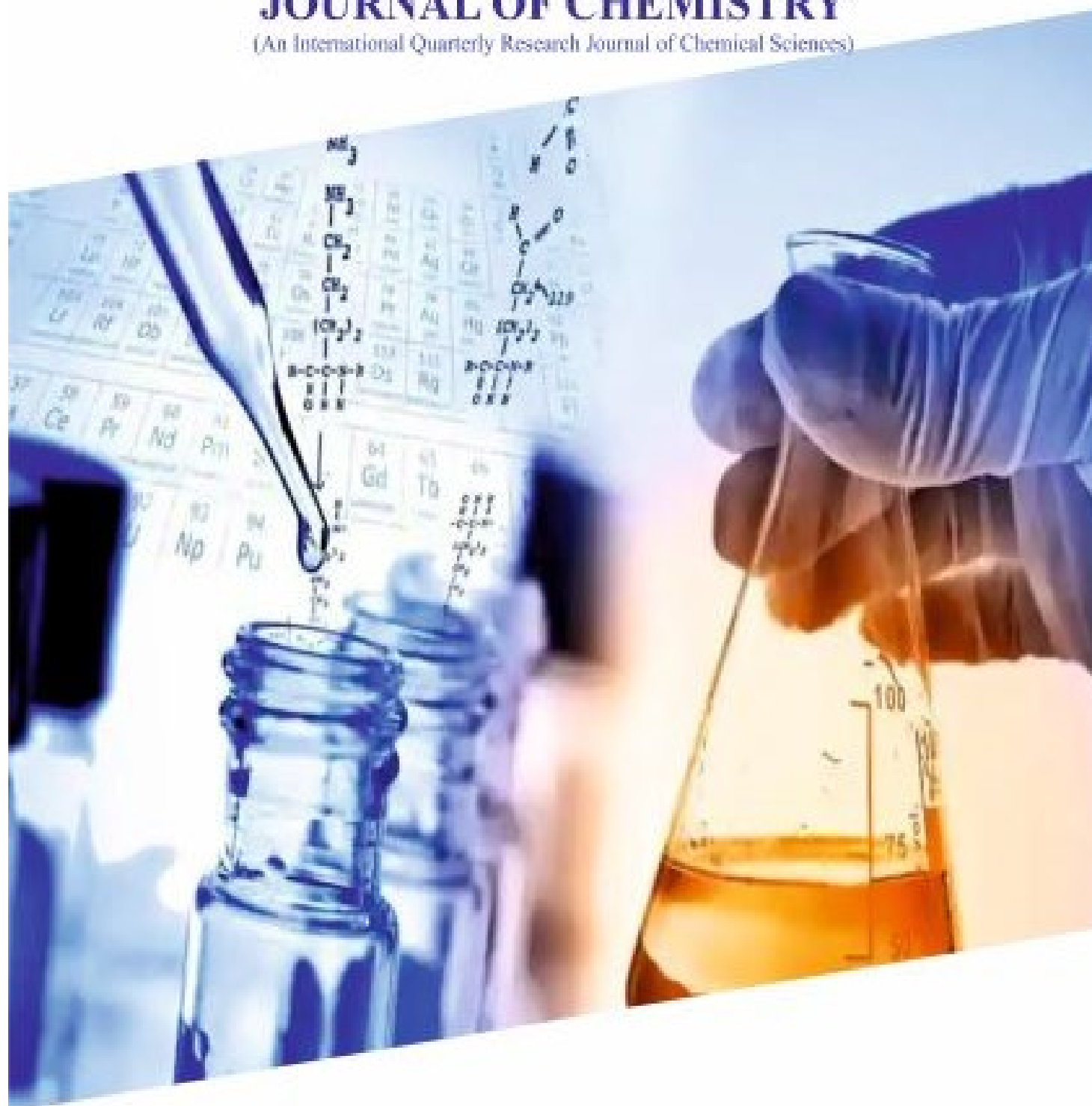


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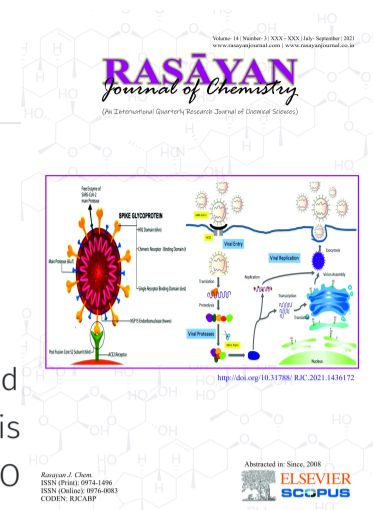


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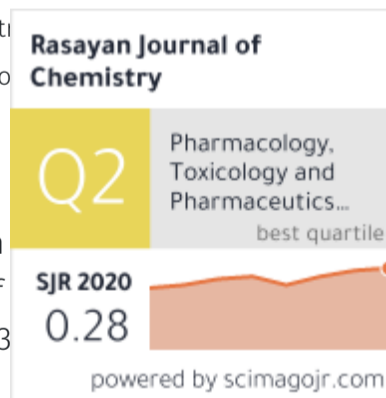
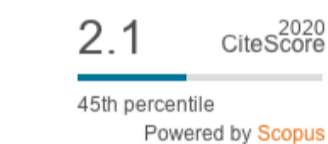
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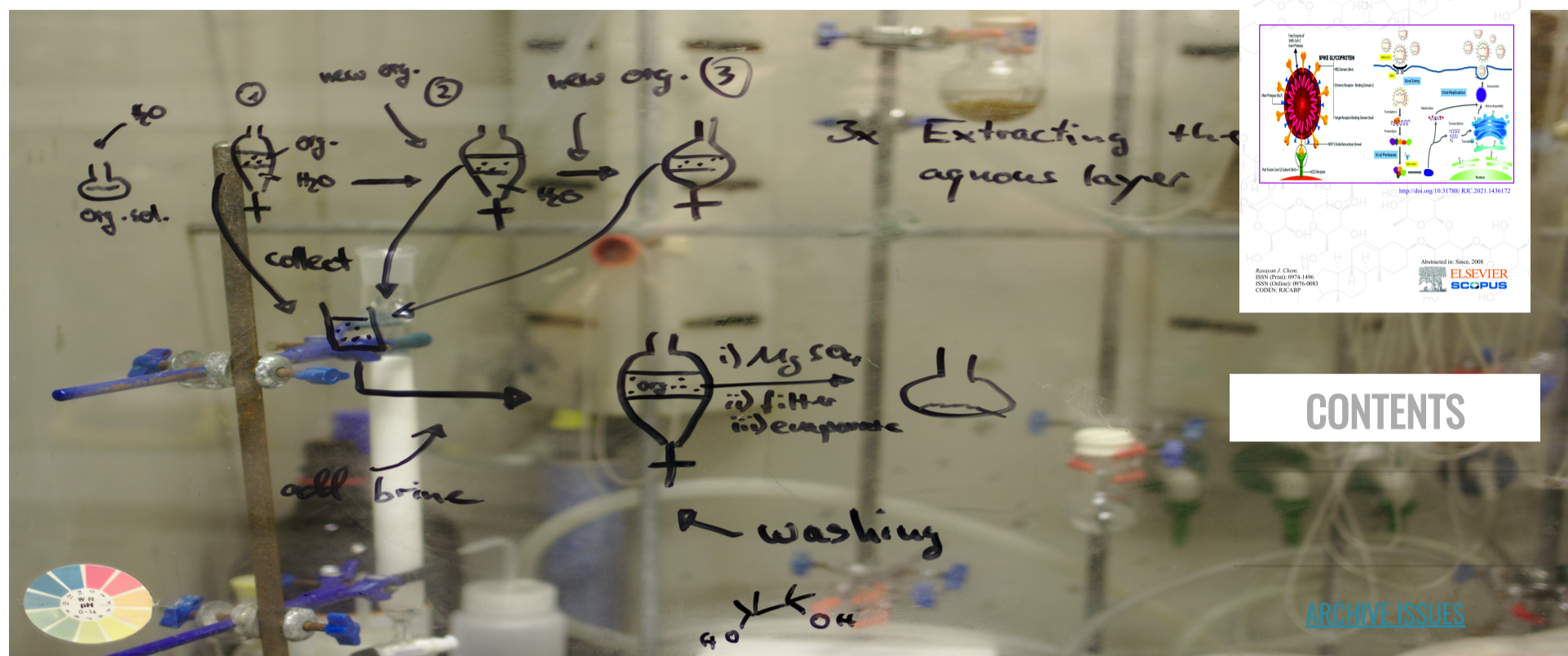
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OILS AND FATTY ACIDS ENCAPSULATED IN MICROPARTICLES AS ANTIBACTERIALS: A REVIEW

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ABSTRACT

Essential oil is a component that can be obtained from plants and animals that have various biological activities such as antibacterial activity for various diseases. Some antibacterial activities come from the unsaturated fatty acid content of essential oils. However, due to the various limitations possessed by essential oils such as volatile, unstable to heat and light, and quickly evaporated, therefore encapsulation of essential oil using a polymer as the wall material can maintain the stability of oils. This review focuses on the encapsulation of oils, lipids, and fatty acids.

Keywords: Essential Oils, Encapsulation, Antibacterial, Fatty Acids, Diseases

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INTRODUCTION

Essential oil from the medicinal plant is a rich source of bioactive components, which has various biological activities for various diseases, including anti-tumor, antioxidant, anti-diabetic, insecticide, and antimicrobial properties. Since a long time ago, essential oil and its bioactive components have been used in the food industry as flavorings and preservatives to enhance taste and stability, prevent food spoilage, and extend the shelf life.¹

Antibacterial activity has attracted much attention, but due to some of the limitations of this essential oil, such as quickly evaporated, a strong odor, and physically and chemically unstable, an encapsulation technique is developed to solve the problem with this essential oil.² Microencapsulation is a delivery system consisting of two parts (core and shell) that functions as a protective layer consisting of polymers, while the core is an active material such as *essential oil*.^{3,4}

Microencapsulation

Microencapsulation has been used as a technique that can increase the biological activity and characteristics of essential oil⁵. Microencapsulation is a process in which small and sensitive particles such as fatty acids, also known as the core, are packed into an encapsulation matrix (Fig.-1).⁵ Several encapsulation methods include lyophilization, solvent evaporation, gelation ionic, spray dry, and coacervation.

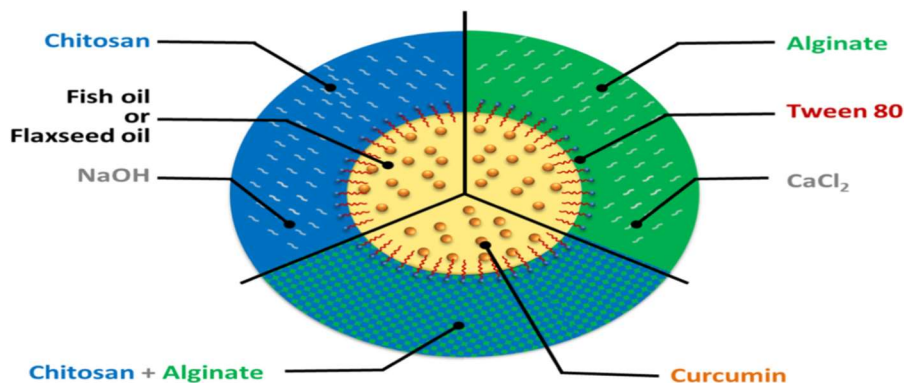


Fig.-1: Illustration of Encapsulation of Omega-3 rich Oil and Curcumin incorporated into a Hydrophilic Polymer: a Combination of Chitosan and Alginate using a NaOH or CaCl₂ Cross-linker⁵.

Lyophilization

Lyophilization method is the most widely used method for microencapsulation of essential oil ingredients because it can prevent or prolong the occurrence of evaporation, such as protecting sweet orange essential oil (SOEO) using maltodextrin polymer.³

Lyophilization or freeze-drying is a process that removes the water from the product after being frozen and placed in a vacuum which causes ice to change from the solid phase to the vapor phase⁶.

Based on the studies, it is known that particle morphology using the lyophilization method forms irregular size and non-spherical microparticles. Contrarily, with microparticles made using the atomization technique, the particles formed are more spherical and have an irregular size.⁷ This happened because the fluid is frozen in lyophilization process, then water in the form of ice crystals is removed by sublimation, resulting in a porous and brittle structure.⁸

However, this process has several disadvantages, such as it takes a long time, high operational costs and the dry microparticle structure tends to be porous due to the sublimation process.⁹ Therefore, the porous microparticle structure is the most critical factor that must be considered for applying this technique and the high operational costs.¹⁰

Solvent Evaporation Technique

Solvent evaporation technique is encapsulation method to efficient most compounds, especially those that are insoluble or have slight solubility in water.⁴

Ionic Gelation

The ionic gelation method is a simple encapsulation method and does not require high temperature in the process therefore it is suitable for encapsulating volatile materials.⁷ Ionic gelation is a chemical method based on the interaction between polymers with different charges, or between polymers and polycation or polyanion.¹¹

The ionic gelation method is based on the ability of polyelectrolytes to form cross-linked bonds in the presence of cross-linker ions forming a hydrogel. This method is prepared by dropping a drug-polymer solution into a solution of a cross linker polyvalent cation. The cation will diffuse into the drug-polymer solution droplets to form a three-dimensional cross-link formation.¹²

The natural or semisynthetic polymers used are alginates, gellan gum, chitosan, and carboxymethyl cellulose. Natural polyelectrolyte contains anion/cation in its chemical structure. Anion/cation will form woven structures with ion counter and induce gelation through cross linking.¹²

Spray Drying

Spray drying is the oldest encapsulation method and is an economical and flexible process that results in high encapsulation efficiency.¹³ Microparticles with the atomization process show a spherical shape, one of the characteristics of the microparticles produced by this process. However, this process has a drawback, such as microparticles tend to be damaged in the atomizer, which may be caused by air pressure and high temperatures.¹⁴

The microencapsulation study of echium oil using gelatin and cashew gum / gum arabic as the wall material and synapic acid as a crosslinker dried by spray drying method showed spherical microparticles successfully produced by this process. However, after rehydration showed agglomeration and lost its original form (Fig.-2). This can be explained by the conditions in the drying process that uses high temperature and pressure.¹⁴

Coacervation Complex

Microencapsulation through the coacervation complex method is used in the separation of two phases of the biopolymer, which has an opposite charge to the formation of the conservative as a layer and the oil-in-water emulsion droplet as the core. The action of the cross linker agent will make the coacervate layer membrane stiffer.¹⁵

This method is made by dispersing the drug into a polymer solution, then reducing the polymer's solubility by adding a third component to the system. The coacervation process consists of three stages,

first, separation of the coating polymer solution, second, the adsorption of the coacervate around the drug particles, and third, the compaction of the microspheres. Coacervation is widely used to manufacture water-soluble drug microspheres such as proteins, peptides, and vaccines.¹⁶ The weakness of this method is that the resulting microspheres tend to aggregate and the scale-up process for production becomes more difficult. These problems can be solved by adjusting the stirring rate, temperature, or adding additives. This method does not use toxic organic solvents and cross-linking as advantages of the retention of the active agent.¹⁷

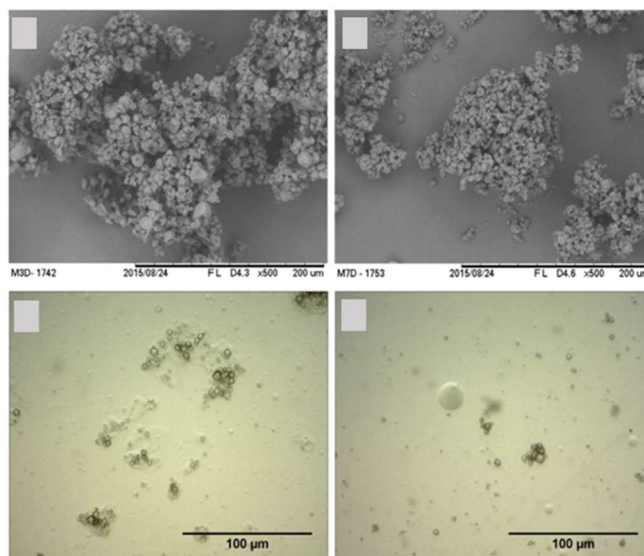


Fig.-2. Optical Microscopy of Echium Oil after atomized and Rehydration¹⁴

Types of Oils as Antibacterial Agents

Essential oils as antibacterial agents and characterization of microparticles are shown in Table-1 and Table-2.

Table-1: Essential Oils and Antibacterial Activities

Oils	Compositions	Functions	Reasons of Encapsulation	Bacteria	Activities	Ref.
Essential oil	Cuminaldehyde Isoeugenol	Antibacterial Antioxidant	Poor bioavailability Difficult to dissolve in water Evaporates easily	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	Each bacteria decreases after given essential oil by $100 \pm 0.6\%$ (<i>S.aureus</i>) and $100 \pm 0.2\%$ (<i>E.coli</i>)	1
Star anise essential oil (<i>Illicium verum</i>)	<i>Trans-anethole</i> After micro-encapsulated, the content increases from 91.38% to 95.36%	Antioxidant Antibacterial liver cancer prevention	Unstable Evaporates quickly	<i>Rhizopus stolonifer</i> <i>Saccharomyces cerevisiae</i> <i>E. coli</i>	Inhibits <i>Rhizopus stolonifer</i> > <i>Saccharomyces cerevisiae</i> > <i>E. coli</i>	2
Sweet orange essential oil	D-limonene (96.02%) Hydrocarbons	Antibacterial Antioxidant	Easily oxidized Evaporates easily	<i>S. aureus</i> <i>E. coli</i>	Effectively inhibits bacteria with an inhibition zone between 13.0 – 18.5 mm	3
Essential oil and	Linalool Anethole	Antibacterial	Unstable Evaporates easily	<i>Staphylococcus aureus</i>	Inhibits the growth of	4

other component (EOCs)	4-allylanisole			<i>Escherichia coli</i>	<i>S.aureus</i> and <i>E. coli</i>	
Omega-3 rich oil	Omega-3 rich oil	Antibacterial Antioxidant	Easily oxidized	<i>Escherichia coli</i> <i>Salmonella typhimurium</i> <i>Yersinia enterocolitica</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Bacillus cereus</i> <i>Listeria monocytogenes</i>	Inhibits bacterial growth	5
Thyme Essential Oil	Thymol carvacrol	Antibacterial	Unstable to high temperatures, light, and oxygen	Gram positive bacteria (<i>S.aureus</i> & <i>L. monocytogenes</i>) Gram negative bacteria (<i>E.coli</i> & <i>S.enteritidis</i>)	Gram-positive bacteria show a larger inhibition zone than gram-negative bacteria	11-13
Mustard seed essential oil	Allyl isothiocyanate (AITC) Isothiocyanate Penetil (PEITC)	Antibacterial	Unstable Evaporates easily	Gram positive and negative bacteria	The inhibition zone of gram-positive bacteria is greater than that of gram-negative bacteria	15
Cinnamon oil	Limonen Cinnamaldehyde	Flavor Antibacterial	Evaporates quickly Sensitive to light, heat, and pressure Difficult to dissolve Unstable	<i>E. coli</i> <i>Y.enterocolitica</i> <i>B.thermospacta</i> <i>L.monocytogenes</i>	Effectively inhibits at a concentration of 500 ppm except for thermospacta which is at the concentration of 125 ppm	22
Babchi essential oil	Bakuchiol (65.37%) 2-phenyl-4-anilino-6 (1H) – pyrimidinone (1.47%), Octadecanoic acid (1.29%), 2-5- (2-methyl benzoxazole-7-yl) –	Anti-tumor anti-inflammatory Immunomodulator Antioxidant Antifungal Antibacterial	Very soluble in water Sensitive to light Very thick Easily degraded	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i>	Inhibition zone of <i>Staphylococcus aureus</i> (12.67 ± 1.49) <i>Escherichia coli</i> (11.33 ± 0.98) <i>Pseudomonas aeruginosa</i>	23

	(1H-pyrazol-3-yl) - phenol (1.15%), sigmast-5-en-3-ol (1.04%).				(11.67 ± 0.66)	
Clove essential oil	Eugenol β-caryophyllene α-humulene	Antioxidant Antimicrobial	Unstable Evaporates easily Difficult to dissolve in water	<i>S.aureus</i> <i>E. coli</i>	Inhibits the growth of <i>S.aureus</i> and <i>E. coli</i>	25
Garlic oil	Garlic oil	Antibacterial	Evaporates easily Strong scent Insoluble in water	<i>S. aureus</i> <i>E. coli</i>	Not show activity against <i>E. coli</i> , at a concentration of 150-200 µg/ml it has an inhibition zone towards <i>S.aureus</i> bacteria	29
Satureja Hartensis essential oil (SEO)	Carvacrol (48.51%) γ-terpinene (36.63%)	Antibacterial Antioxidant	Evaporates easily	<i>E.coli</i> <i>S.</i> <i>Typhimurium</i> <i>S. aureus</i> <i>L. monocytogenes</i>	Inhibits bacterial growth	32, 55
Perilla essential oil	2-Hexanoylfuran (17%) thymoquinone (32%)	Antibacterial Antioxidant Anti-inflammation Anti-cancer	Sensitive to light Evaporates easily	Gram positive and negative bacteria	Inhibits bacterial growth	35
Pomegranate oil	Punicic acid (ClnA, omega 5) 81.29% Linoleic acid (omega 6) Linolenic acid (omega 3) Eicosapentanoate	Antimicrobial	Unstable Evaporates easily	<i>Escherichia coli</i> <i>Salmonella typhi</i> <i>Shigella sonni</i> <i>Staphylococcus aureus</i> <i>Listeria monocytogenes</i> <i>Bacillus cereus</i>	Gram negative component is less sensitive than gram positive	56
Pumpkin seed oil	Unsaturated fatty acids	Antibacterial	Evaporates easily Unstable to heating	<i>Staphylococcus aureus</i>	Inhibits bacterial growth	57
Sour cherry oil	Mono and polyunsaturated fatty acids (oleic acid)	Antibacterial	Easily oxidized	<i>E. coli</i> <i>S.aureus</i> <i>P.</i> <i>Aeruginosa</i> <i>E.faecalis</i> <i>A.brasiliensis</i>	Has antibacterial activity against pathogenic bacteria except <i>E. coli</i> which is evaluated using well-	58

					diffusion assay method Gram negative bacteria are more resistant to antibiotics because they have a distinctive lipopolysaccharide membrane layer	
Pepper seed oil (PSO)	Tocopherol Carotenoids Kapsaicin Unsaturated fatty acids	Antibacterial	Easily oxidized	<i>E. coli</i> <i>P. aeruginosa</i> <i>S. aureus</i> <i>E. faecalis</i>	no antibacterial activity except <i>S. aureus</i> bacteria = 13 mm Encapsulation increases the antibacterial activity of PSO against <i>P. aeruginosa</i> and <i>E. faecalis</i>	59

Table-2: Characterization of Microparticles of Essential Oils

Oils	Polymers	Methods	Characteristics of Microparticles	Ref.
Star anise (<i>Illicium verum</i>)	HPCD (Hidroxypropyl- β -cyclodextrin) HPCD can increase the solubility of star anise in water for the antibacterial components easily penetrate the microbial cell membrane	Freeze dry	The sulfur in the encapsulation is significantly lower which results in the bad smell of star anise essential oil and can be overcome by the encapsulation technique. Encapsulation with HPCD increases the solubility of star anise essential oil which results in antibacterial components that can easily penetrate the cell membranes of microorganisms so as to provide an inhibitory power towards the growth of these microorganisms.	2
Sweet orange essential oil	Maltodextrin and Gel	Lyophilization	Encapsulation Efficiency; 74.75 \pm 1.06% Thermogravimetry: SOEO Maltodextrin: 30 and 265 $^{\circ}$ C SOEO: 30 and 198 $^{\circ}$ C The thermal degradation of maltodextrin SOEO is slower than SOEO	3
Essential oil	Poly(lactic acid (PLA) Poly (methyl methacrylate) (PMMA)	Solvent evaporation	Encapsulation efficiency; 44.5 – 81.7%	4

Omega-3 rich oil	Chitosan Alginate	Nanoemulsion -in- microbeads Oil is made of nano emulsion and then inserted into the micro-sized wall material	- Encapsulation efficiency; 83-99% - Oil derived from plant seeds: The first peak appears at temperature approaching 200 °C (weight loss of 8.09 – 15.89%) The second peak appears at temperatures of 200-390 °C (weight loss of 12.62-39.14%) The third peak is at 390-590 °C (14.99-22.34%)	5
Thyme essential oil	Casein maltodextrin	Spray dry	- Encapsulation Efficiency; 88.9% - Thermogravimetric: TEO = close to 200 °C TEO encapsulation > 300 °C	13
Mustard seed essential oil	Gelatin Arabic gum	Coacervation complex	Decomposition begins at 250 °C	15
Satureja Hartensis essential oil (SEO)	Alginate	Ionic gelation	- Loading capacity; 20.27±0.94% - 26.13±0.71% - Encapsulation Efficiency: 52.40±0.65% - 66.37±1.00 - Thermogravimetric: SEO without encapsulation: 128-178 °C SEO with encapsulation: 178-189 °C	32
Perilla essential oil	Sodium alginate	Ionic gelation	- Encapsulation efficiency: 33-57%, where the highest EE is obtained with a PEO concentration of 2% - Loading capacity: 25-36% with a maximum loading capacity that is also obtained with a PEO concentration of 2% - The increase in PEO concentration does not make EE and LC increase, but it is probably due to the saturation of EO that enters the sodium alginate particles.	35
Pepper seed oil (PSO)	Arabic gum Maltodextrin	Spray dry	- Yield is 34.99-57.77% The increase of maltodextrin concentration in the emulsion increases the yield of microparticles The drying temperature has a good effect on the microparticle yield, but has a bad effect on the oil content of the emulsion. - Encapsulation efficiency: 67.45-87.14% A higher inlet temperature decreases the presence of surface oil, thereby increasing the high encapsulation	59

			efficiency - Morphology The particle size distribution varies from 0.5-80 μm	
Fish oil	casein-pectin and gum 1687teara, maltodextrin	Coacervation complex, spray drying (casein-pectin-maltodextrin microparticles) Suspension, spray drying (gum 1687teara-maltodextrin)	- EE Arabic gum microparticles: 51.2-56.79%; Casein pectin microparticles: 64.74-67.89%; 10% higher than Arabic gum microparticles - Microencapsulation with the coacervation complex method is an efficient method The percentage of solid used is 30% because the high concentration of solid can reduce oil migration to the capsule surface and at this concentration the polymer is not too viscous so that it does not inhibit the atomization process (not clogged at the nozzle)	60
	Hydroxypropyl cell ulose	conventional spray drying the ratio of FO and HPC used varies up to a ratio of 1: 4.3 the solvents used to disperse the HPC are also different (methanol, ethanol, and acetone)	- Encapsulation using FO/acetone has the lowest percentage of oil on the surface, followed by ethanol, methanol, and water - The use of solvents does not affect the release of FO	61
Patchouli oil	maltodextrin	Spray dry Flow rate 300 mL/min Inlet temperature 110 °C Outlet temperature 68 °C	The smaller the maltodextrin used, the microparticles are formed, the more spherical it is and there is no agglomeration	62

Star Anise Essential Oil (SAEO)

Star anise (*Illicium verum*) is a plant in the Magnoliaceae family. This fruit is a spice found in Southeast China. Their usages include as an antioxidant, antibacterial, prevention of liver cancer, and insect repellent. This oil is obtained from star anise fruit ranging from 3-3.5% in fresh fruit and > 8% in dry fruit obtained by steam distillation. Due to its biological activity, star anise essential oil is used in food and medicine. This star anise essential oil is also used in baked food, candy, alcoholic beverages, and soda. It can also be used to relieve the inflammatory response, as medicinal tea flavor, and as cough medicine mixture.²

Gas Chromatography-Mass Spectrometry (GC-MS) analysis is method to identify the essential oil components. A study on star anise essential oil has been conducted by comparing star anise essential oil

composition before being encapsulated with after being encapsulated. GC-MS identifies 13 main components. The main components of SAEO before it is encapsulated are *trans-anethole* (91%), then followed by estragole (2.5%), and *trans-foeniculin* (2%). In contrast, after encapsulation, *trans-anethole* (95%), followed by estragole (2.23%), and *trans-foeniculin* (0.91%) are obtained. Based on the data above, it is known that the contents contain no changes in the essential oil before and after encapsulation.² The contents obtained after the encapsulated star anise essential oil decrease, except for *trans/cis-anethole*, indicate that these two components are suitable when encapsulated in hydroxypropyl- β -cyclodextrin (HPCD). This decrease/change is due to differences in structure and polarity in the molecules that make up star anise essential oil and different HPCD encapsulation. The effect of HPCD encapsulation on essential oil may be closely related to the molecular structure of the essential oil.² Star anise essential oil has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. However, the inhibition of oil only was lower than encapsulated star anise essential oil.¹⁸

Sweet Orange Essential Oil (SOEO)

SOEO is obtained from orange fruits. Orange fruits have good economic value with their production mainly used to manufacture juices that contain ingredients that have biological activity. Orange fruit oil can be used in agriculture, pharmaceuticals, cosmetics, and the food industry. Orange oil contains D-limonene, a large amount of monoterpene hydrocarbons, as well as small amounts of oxygenated monoterpenoids and sesquiterpenes.³

In a study conducted by JSF de Araújo et al, the polymers used as wall material for the microspheres are SO1 (Maltodextrin only); SO2 (Maltodextrin : gel 2:1), and SO3 (Maltodextrin : gel 1:1). Those three formulas have an inhibition zone against the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria of 13.0-18.5 mm. Based on the antibacterial activity test against the sweet orange essential oil microsphere, it can be said that the microencapsulated SOEO is able to diffuse through the culture medium.³

Omega-3 Rich Oil

Fish oil is the most important source of omega-3 unsaturated fatty acids such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). These two components can reduce the risk of certain chronic diseases such as inflammation, cardiovascular, immune response disorders and mental disorders. Some of the shortcomings of these unsaturated fatty acids are the high lipophilic molecules that will cause difficulty in absorption and poor bioavailability. In addition, unsaturated fatty acids are also easily oxidized, which will cause reduced nutritional value and the formation of toxic products.¹⁹ Microencapsulation can prevent omega-3 fatty acids from oxidizing and extend the shelf life (expired date); therefore, it can be a solution to maintain the stability of the oil.⁵

The antibacterial activity of omega-3 rich oil (which comes from plants and fish) is carried out by the agar diffusion method (well). The result shows that omega-3 rich oil derived from fish has more significant antibacterial activity than that from plants. This study also found that omega-3 rich oil (derived from fish), which is included in the alginate polymer and added with curcumin as an antioxidant, showed gram-positive and gram-negative antibacterial activities. Fish oil incorporated into alginate particles showed better antibacterial activity than fish oil loaded in chitosan particles and chitosan-alginate combination.⁵

Thyme Essential Oil (TEO)

Thyme essential oil (*Thymus vulgaris*) contains thymol and carvacrol, phenols which have antibacterial activity.²⁰ However, specific problems such as volatility, difficulty dissolving in water can prevent this oil from being dispersed in the food matrix. To solve this problem, thyme essential oil can be encapsulated to increase bioactivity.²¹ Research conducted by Radunz *et al* states that there are differences in the chemical compound components found in thyme essential oil without encapsulation with encapsulated TEO with the main components of thymol and carvacrol, which also shows that the contents of these two components increase after the encapsulation process. This can be due to the formation of a hydrogen bond between the hydroxyl groups and the active site of the protein molecule, thereby increasing volatile retention during encapsulation.¹³ The yield obtained from TEO microencapsulation process is relatively low, which is between 60.2 - 62.3%. This is due to the emulsion loss during the removal of the emulsion

from the container and syringe. Besides that, it is also during the drop dripping process through the syringe.¹¹

Several oils encapsulated in polymer show inhibitory and bactericidal effects, for examples Thyme essential oil with encapsulation has an inhibitory and bactericidal effect against *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* bacteria with a minimum inhibition zone of 0.1 mg/ml, which can be said that TEO is a potential antimicrobial agent.¹³

Cinnamon Essential Oil (CEO)

In this study, the kinetics of CEO encapsulation in cyclodextrin nanosponge (CD-NS) in a polar solvent such as ethanol incubated for 96 hours is performed. The equilibrium point is reached at an incubation time of 24 hours and after that time, it does not show significant cinnamaldehyde entrapped in CD-NS.²²

In antibacterial testing against CEO, minimum inhibitory concentration (MIC) is obtained for gram-positive bacteria of *B. thermospacta* and *L.monocytogenes* and gram-negative bacteria of *E.coli* and *Y. enterolitica*. Both CEO without encapsulation and CEO with encapsulation can inhibit bacterial growth. For its working action, CEO with encapsulation does not affect the mechanism of action against bacterial cells, which means that the bactericidal and bacteriostatic effects of the CEO are still maintained.²²

Babchi Essential Oil

Babchi essential oil is obtained from the extraction of *Psoralea caryfolia* L. Dried fruit of *P.caryfolia* is known as traditional Chinese medicine registered in the Chinese Famakopea. This herb is also reported to be used to treat skin damage such as leukoderma, leprosy, and psoriasis.²³ Encapsulation is a method to overcome this problem because it is easy to be photodegradable at a constant rate. After the Babchi essential oil was entrapped in the nanoparticles, a photodegradation test was carried out and a reduction in the rate of degradation was obtained.²⁴

Clove Essential Oil

Clove oil comes from *Eugenia caryophyllata* plant from the Myrtaceae family that is used as a herbal plant. Clove oil contains eugenol and its derivatives.²⁵ The phenol content in this oil has antibacterial activity that can inhibit bacteria growth, which works by poisoning the cytoplasm, damaging and penetrating bacterial cell walls²⁶. The clove essential oil microparticles were successfully obtained by the spray dry method, which increased their solubility and stability.²⁷ Clove essential oil microparticles using maltodextrin with a ratio of 1: 8 showed the microparticles were round but slightly wrinkled. This causes the oil not wholly to fill the wall material.²⁸

Garlic Essential Oil

Garlic is a natural ingredient used throughout the world for cooking, so it has attracted researchers to conduct further research. The main components of this garlic oil are diallyl sulfide (DAS) 5.69%, diallyl disulfide (DADS) 60.12%, and diallyl trisulfide (DATS) 14.18% which have many pharmacological activities.^{29,30} The method used affects the content of garlic essential oil. The Clevenger laboratory hydrodistillation method obtained DADS and DATS of 20.8% and 33.4% where this content was not significantly different from the results obtained from industrial steam distillation, which gave an increase in the content of DAS and DADS.³¹

Satureja hortensis Essential Oil (SEO)

Saturejo hortensis is a medicinal plant belonging to the Lamiaceae family, widely used in the Mediterranean area as a spice and traditional medicinal ingredients. Besides that, it is also known for its biological activity as antibacterial and antioxidant due to its high phenolic content in this plant.³²

This essential oil also has activity against gram-positive and gram-negative bacteria in the order of *P. aeruginosa* > *E. coli* = *S. typhimurium* > *S.aureus* > *L. monocytogenes*, which is strongly suspected to come from the content of carvacrol which is the main ingredient in SEO with 48.51% content. In addition, SEO also contains γ -terpinene, p -cymene, terpinolene, α -terpinene, α -pinene, and α -thujene compounds. The place to grow affects the compound content contained in SEO. SEO originating from Egypt does not contain thymol which is inversely proportional to SEO from French.³³

Storage conditions such as temperature, storage time and interaction of content also play an essential role in maintaining the quality and quantity of active compounds. The carvacrol content stored in the freezer was more stable than SEO stored in the refrigerator and at room temperature, respectively.³⁴

Perilla Essential Oil (PEO)

Perilla frutescens (L.) Britt is a seasonal aromatic plant widely used in Asian countries such as China, Japan, North Korea, and India for cooking and medicine. PEO consists of a mixture of volatile compounds with antioxidant, antimicrobial, anti-inflammatory, insecticidal, anticancer, and antidepressant activities.³⁵

Encapsulation of Oils in Polymer

A schematic overview of the encapsulation of the oils in the polymer is shown in Fig.-1.

Evaluation of Essential Oil Encapsulation

Morphology

The difference in morphology and capsule diameter is influenced by the characteristics of encapsulation and wall material used¹³. Encapsulation of *Pimenta dioica* essential oil in chitosan microparticles protected with κ -carrageenan showed irregular microparticles (Fig.-3a). At higher κ -carrageenan concentrations, the consistency of the gel formed was higher and would form irregular aggregates of microparticles (Fig.-3b). Whereas microparticles using glutaraldehyde as a crosslinker showed irregular microparticles with a hard and rigid surface full of holes and cracks (Fig.-3c, d)³⁶.

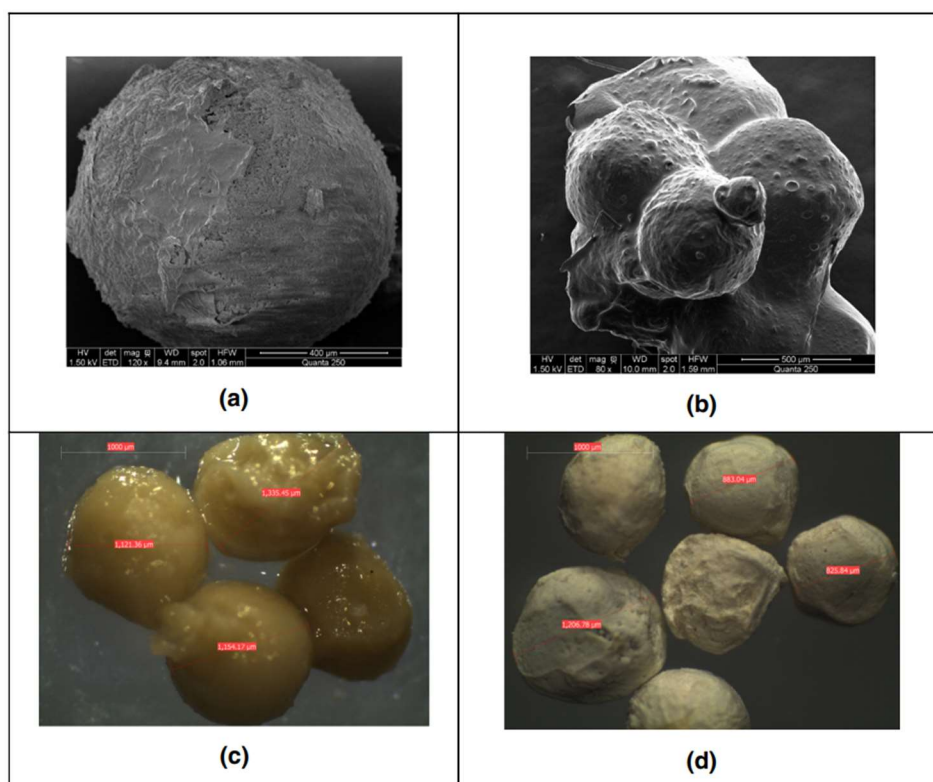


Fig.-3: Morphology of *Pimenta dioica* Essential Oil-Chitosan Microparticle covered with κ -Carrageenan³⁶

Microparticle Size

Microencapsulation of essential oil with polylactic acid (PLA) and Polimethyl methacrylate (PMMA) with particle sizes using PLA polymers ranging from 1.5-9.5 μm has a polydispersity index (PDI) of 1.6-2.7 which indicates that these microparticles have a broad particle size distribution due to PDI in this study, that is >0.7 ⁴. The ratio of the mixture used has little effect on microspheres size, although it does not have a significantly different dispersity.⁴ Microparticles of *Pimenta dioica* essential oil using chitosan,

which κ -carrageenan protects, have an average diameter of $1224 \pm 6.56 \mu\text{m}$. Meanwhile, microparticles using a crosslinker obtained a lower average diameter. The size of these oil microparticles decreases along with the increase in chitosan, which is used as a result of tensioactive chitosan reducing the interfacial tension to support the formation of microparticles.³⁶

Encapsulation Efficiency

According to Benavides et al, the increase of TEO amount decreases encapsulation efficiency in the microsphere with alginate as the wall material. This can be explained due to the limitation of the microsphere in accommodating oil. When the oil concentration increases, the oil will stick a lot or be close to the surface of the microsphere; therefore, when drying, this oil will be lost due to its volatile nature, which will reduce the encapsulation efficiency. 2% TEO, which is distributed at a speed of 18000 rpm has an encapsulation efficiency of 85%.¹¹ In addition, another factor that can affect the encapsulation efficiency is the polymers used, such as the study using casein and maltodextrin, which results in an encapsulation efficiency of 88.9%. The amount of encapsulation efficiency obtained is probably because the spray dry method can minimize mechanical damage compared to other methods and the oil component's affinity with a higher wall material.¹³

High encapsulation efficiency is also obtained in the microencapsulation of linalool using polylactic acid (PLA) polymer, which is 81.7% because linalool has a larger surface area polar topology¹⁰. The polarity of the essential oil and polymers is an important factor in the absorption process because those absorbed easily are those with the same polarity (polar PLA, polar linalool).⁴

Thermogravimetric Analysis (TG)

Thermogravimetric analysis (TG) is used to evaluate the thermal properties of the microsphere about mass loss compared to its single component (without microencapsulation). SOEO without microencapsulation shows thermal degradation at temperatures between 30°C and 198°C, which means that thermal degradation occurs faster, where the most significant degradation occurs at a temperature of 98.61°C with almost 90% of mass loss. In contrast, SOEO microspheres using maltodextrin show slower thermal degradation. The degradation of SOEO without microencapsulation starts at 60.44°C, while in SOEO microsphere the temperature is $>250^\circ\text{C}$.²³ This is also the case with the encapsulation of thyme essential oil (TEO) without encapsulation, showing mass loss at temperatures approaching 200°C while TEO is encapsulated at a temperature of $>300^\circ\text{C}$ which states that the encapsulation process increases the thermal stability of the essential oil.¹² In *Satureja hortensis* essential oil (SEO), an increase in temperature of decomposition occurs after SEO is encapsulated with alginate polymer. From the explanation above, it can be concluded that encapsulation of essential oils can increase the thermal stability of essential oils.³²

Mustard seed essential oil encapsulated with gelatin and genipin as a crosslinker agent decomposes at a temperature of 250°C. This is because, under neutral and weak alkaline environmental conditions, genipin as a crosslinker and amino group polymers such as gelatin will form a cross-linked network.²² On the other hand, in strongly alkaline conditions, the microparticle stability of mustard seed essential oil is reduced. This may be due to a strong alkaline condition that makes the microparticles prone to swelling.¹⁵

Differential Scanning Calorimetry (DSC)

Based on the analysis using DSC, it is known that SOEO without microencapsulation undergoes an endothermic process at temperatures between 30 °C and 87.8 °C, in which it is characterized at this temperature that SOEO experiences evaporation and at a temperature of 126.4 °C oxidative degradations begins to occur. In wall material using maltodextrin polymer, the endothermic process occurs at a temperature of 92.5 °C which means that the system will lose moisture at that temperature, which will also be followed by the loss of SOEO from the system. The use of the microencapsulation technique effectively prolongs the thermal degradation time and the evaporation time of SOEO.³

Fatty acid

Oils contain fatty acids and their antibacterial activities are demonstrated in Table-3. Fatty acid is a molecule that is usually found attached to other compounds such as sugar, glycerol, or phosphate, which

is the main group forming lipid. Lipid is an important component of cell structure, for example, for cell membrane, which is the main component of phospholipid.³⁷

Table-3: Oils containing Active Fatty Acid as Antibacterial

Oils	Compositions	Antibacterial Activities	References
<i>Crotalaria juncea</i> seed oil (CJSPE)	Linoleic acid 62% Palmitic acid 18% Stearic acid 10%	Inhibition zone against bacteria: <i>S. aureus</i> (18 mm) <i>E. coli</i> (17 mm) <i>K. pneumonia</i> (16 mm) <i>P. aeruginosa</i> (8 mm) <i>S. flexneri</i> (16 mm) <i>V. cholera</i> (14 mm)	39
<i>Tamarindus indica</i> seed oil	Asam lemak jenuh : Asam kaprilat 12.66% Asam laurat 25.18% Asam lemak tak jenuh : Asam linoleat (21.91-38.68%) Asam oleat (17.76%)	Inhibition zone with acetone extract : <i>Escherichia coli</i> 7.9 mm <i>Staphylococcus epidermidis</i> 7.1 mm No antibacterial activity at n-hexane and DMSO extract	40
<i>Crotalaria pallida</i> leaf oil	Linolenic acid (34.06%) Palmitic acid (24.47%)	Minimum inhibitory and inhibition zone diameter: <i>B. subtilis</i> (80 µg/ml and 15 mm) <i>E. coli</i> (10 µg/ml and 18 mm) <i>Acinetobacter junii</i> (10 µg/ml and 18 mm)	43
Mastic tree oil (<i>Pistacia lentiscus</i> L.)	Oleic acid > 56% Palmitic acid 27% Linoleic acid 16%	The pressing methode and growing sites in Byzerte has abtibacterial activity against <i>Clostridium perfringens</i> with inhibition zone 13 mm There is no antibacterial activity against <i>E.coli</i> and <i>S.typhimurium</i>	44
<i>Swietenia macrophylla</i> seed oil	Linoleic acid 37.5-39.21% Oleic acid 18.82-22.03% Stearic acid 16.57-17.65% Palmitic acid 14.62 – 15.47 %	Inhibition zone of seed oil at concentration 10-1000 µg/mL : <i>S. aureus</i> 5-11 mm <i>S. typhimurium</i> 4 – 20 mm <i>P.aeruginosa</i> 5-11 mm No activity against <i>E.coli</i>	45
Virgin Coconut Oil	Lauric acid Caprylic acid Capric acid	1.2% VCO has been diluted to inhibit <i>Clostridium difficile</i> bacterial growth by 99.9% 0.15% VCO lipolysis inhibits 50% growth of <i>C. difficile</i> bacteria	47
Red palm oil and Palm kernel oil	Fatty acid salt	Inhibitor zone diameter : Mixture red palm oil : palm kernel oil 20% pada konsentrasi 10-50% <i>Propionibacterium acne</i> 8.83-11.73 mm <i>Staphylococcus epidermidis</i> 8.93-10.23 mm Red palm oil fatty acid salt showed no inhibition zone diameter	52
Algerian seed oil (<i>Pistacia lentiscus</i>)	41.22% Oleic acid 19.94% Linoleic acid	Only provides an inhibition zone of 1 mm towards MRSA bacteria (Methicillin resistant <i>Staphylococcus aureus</i>) and has no antibacterial activity towards <i>E. coli</i> and	55

		<i>P.aeruginosa</i> bacteria	
Algerian seed oil (<i>Opuntia ficus indica</i>)	12.92% oleic acid Linoleic acid 55.81%	Has no antibacterial activity towards MRSA, <i>E. coli</i> , and <i>P.aeruginosa</i> bacteria	55
Algerian seed oil (<i>Argania spinosa</i>)	28.53% Oleic acid 39.10% Linoleic acid	Has no antibacterial activity against MRSA, <i>E. coli</i> , and <i>P.aeruginosa</i> bacteria	55
Virgin coconut oil is hydrolyzed by enzymes	Lauric acid Caprylic acid Capric acid	Formulas inhibit <i>P. aeruginosa</i> , <i>S.aureus</i> , <i>S.epidermidis</i> , <i>P.acnes</i> after incubated for 12 hours. The growth of bacteria with VCO hydrolysis is more active against gram-negative bacteria (<i>P.aeruginosa</i>) than gram-positive bacteria (<i>S.aureus</i>).	63
Fixed oil (<i>Orbignia speciosa</i>)	Monounsaturated fatty acids Lauric acid (54.15%) Myristic acid (10.62%) Caprylic acid (9.13%)	MIC of formulas: <i>E. coli</i> (MIC 32 µg/mL) <i>S.aureus</i> (MIC 256 µg/mL) <i>E. coli</i> ATCC 25922 (MIC 512 µg/mL)	64
Fixed oil (<i>Mauritia flexuosa</i>)	Oleic acid (72.14%) Palmitic acid (19.3%)	MIC of formula against <i>S.aureus</i> (MIC 256 µg/mL) The other strains have MICs of 512 µg/mL	64
<i>Schinus terebinthifolius</i> essential oil	The composition of fatty acid (leaves and twigs) Linolenic acid 43.14% and 24.39% Palmitic acid 20.27% dan 21.27% Other composition : terpenoid dan phenylpropanoids group	Inhibition zone (leaves and twigs) : <i>E. coli</i> 10.5 and 7.83 mm <i>S. typhimurium</i> 10 mm and 8.33 mm <i>E. faecium</i> 31.83 mm and 25.5 <i>S. agalactiae</i> 27.5 and 7.67 mm Antibacterial activity is derived from terpenoid and phenylpropanoids	65
<i>Kleinhovia hospital</i> leaf oil	Asam palmitat 17.97% Asam linoleat 8.05% Asam oleat 7.87% Asam stearat 7.79%	Inhibition zone : <i>B. subtilis</i> 19.5 mm <i>E. coli</i> 18.5 mm Minimum inhibitory concentration : <i>B. subtilis</i> 61.75 µg/ml <i>B. licheniformis</i> 60.02 µg/ml <i>E. coli</i> 35.75 µg/ml <i>A. junii</i> 38.04 µg/ml	66

Fatty acids are classified according to chain length and degree of unsaturation. In biological systems, fatty acids have a number of carbon atoms between 4-28. Fatty acids that have <8 carbon chains are referred to as short chain fatty acids, 8-12 carbon chains are called medium chain fatty acids, while > 12 are long chain fatty acids.³⁸ The fatty acid content is very important in oil; it is usually used to determine the identity and purity of the oil.³⁹

In unsaturated fatty acids, the increase in double bonds will increase the solubility in water, increasing antibacterial activity.⁴⁰ In antibacterial tests against various bacteria, it is known that unsaturated fatty acids show more significant inhibition than saturated fatty acids by inhibiting biosynthesis. bacterial protein (enoyl-acyl carrier protein reductase/FabI) which in turn will affect the composition of the bacterial cell membrane.⁴¹

Cacay oil shows antibacterial activity (in vitro) against *B. cereus*, *E. faecalis* and *S. aureus*. Based on the evaluation results, this antibacterial activity is due to the high content of polyunsaturated fatty acids (58.3%).⁴² Similarly, *Crotalaria pallida* leaf oil which contains fatty acids such as linolenic acid (34.06%), palmitic acid (24.47%), linoleic acid (13.50%) and oleic acid (4.6%) are known to have

antibacterial activity against *B. subtilis* (MIC = 80 µg / ml), *E. coli* (MIC = 10 µg / ml) and *Acinetobacter junii* (MIC = 10 µg / ml).⁴³

Mastic tree oil contained in *Pistacia lentiscus* L. contains 5 types of fatty acids, namely: oleic acid, palmitic acid, linoleic acid, palmitoleic acid and stearic acid. The extraction method used to obtain mastic tree oil does not significantly affect the fatty acid content contained therein. From the antibacterial test (in vitro), the inhibition zone diameter obtained varies according to the growing site, extraction method, and bacterial strain used.⁴⁴

Crotalaria juncea seed oil (CJSPE) contains 3 main fatty acids components, namely linoleic acid, palmitic acid and stearic acid. Based on the antibacterial test against gram-positive and gram-negative bacteria, it is known that CJSPE has good antibacterial activity against *S. aureus*, *E. coli*, *K. pneumonia* and *S. flexneri* bacteria. However, the antibacterial activity of CJSPE was lower than that of ciprofloxacin (5 µg / disc) which was used as a positive control.³⁹

Crotalaria juncea seed oil (CJSPE) contains 3 main fatty acids components, namely linoleic acid, palmitic acid and stearic acid. Based on the antibacterial test against gram-positive and gram-negative bacteria, it is known that CJSPE has good antibacterial activity against *S. aureus*, *E. coli*, *K. pneumonia* and *S. flexneri* bacteria. However, the antibacterial activity of CJSPE was lower than that of ciprofloxacin (5 µg / disc) which was used as a positive control.⁴⁵

The hydrophobic group influences the antibacterial activity of saturated fatty acids, the longer the saturated fatty acid chain, the hydrophobicity will increase so that it can reduce the solubility in water. Thus, the hydrophobic group can not interact with hydrophobic protein or lipids on the bacterial cell surface.^{40,46}

Saturated fatty acids are a significant component of coconut oil (78.4%), particularly lauric acid (38.4%).⁴² Lauric acid is most effective at inhibiting *Clostridium difficile* bacterial growth. Lauric acid inhibits almost 100% of *Clostridium difficile* bacterial growth at a concentration of 1000 µM. Lauric acid also produces strong inhibition even at a small concentration of 250 µM, which can reduce bacterial growth by almost 90% compared to controls.⁴⁷ Lauric acid with a concentration of 15% and 20% has a larger zone of inhibition than 0.5% ciprofloxacin which is tested towards *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, and *Escherichia coli* bacteria.⁴⁸

Antibacterial activity of lauric acid depends on the presence of food particles in bacteria culture because gives a site for adsorption of lauric acid into bacteria and inhibits bacterial growth⁴⁹. When lauric acid is digested, then release from triglyceride form and can enter the liver via a portal vein or can be changed into new triglyceride and go to the lymphatic system.³⁸ Lauric acid is also a component of fatty acid found in samples of murumuru, palm kernel, babassu, and coconut oil.⁵⁰

Virgin Coconut Oil (VCO) contains 99% triglycerides with less than 0.2% free fatty acid. VCO triglycerides can be lipolyzed with lipase and water to form monoglycerides, diglycerides, glycerol, and free fatty acid. Monoglycerides and free fatty acid are reported to have antimicrobial activity while triglycerides, diglycerides, and glycerides have lower antimicrobial activity.⁴⁷

Virgin coconut oil (VCO) does not have antibacterial activity against *Clostridium difficile* bacteria when added to bacterial culture after grown overnight. However, after VCO is hydrolyzed with lipase, it shows inhibition of bacterial growth by 99.9% at a concentration of 1.2%, while at a concentration of 0.15% VCO lipolysis inhibits 50% of bacterial growth.⁴⁷ VCO must be metabolized first; therefore, it can release medium chain fatty acid components such as caprylic acid, capric acid, and lauric acid for its antimicrobial activity. From the metabolites mentioned earlier, lauric acid has the greatest antibacterial activity.⁴⁷ Capric acid in VCO can inhibit 90% of *Clostridium difficile* bacterial growth at twice a concentration than lauric acid.⁴⁷ Capric acid can inhibit *Propionibacterium acnes* bacterial growth at a concentration of 1 mM, whereas using lauric acid with a concentration of 0.25 mM alone can inhibit *Propionibacterium acnes* bacterial growth. Caprylic acid in VCO requires five-time concentration to achieve the same bacterial growth inhibition as lauric acid.⁴⁷ Based on these data, it can be said that lauric acid is more potential as an antibacterial than capric acid.⁵¹

In an antibacterial study of palm kernel oil and red palm oil, which are rich in fatty acid content, fatty acids are extracted first using potassium hydroxide, which will form fatty acid salts. After the antibacterial test against *Propionibacterium acnes* and *Staphylococcus epidermidis*, it was found that a

mixture of red palm oil: palm kernel oil 20% at a concentration of 10% had a good antibacterial activity with an inhibition zone diameter of 18.03 mm.⁵² The antibacterial activity of the mixture comes from the fatty acid salt content of palm kernel oil because after the activity test against red palm oil with a concentration of 10-50%, it does not show any diameter of the inhibition zone.⁵²

Chaidir *et al* stated that the fatty acids and fatty acids methyl esters contained in the microalgae *Nannochloropsis oculata* had lower antibacterial activity than using positive controls (streptomycin sulfate) against *E. coli* and *S. aureus* bacteria. This is due to the mixing of fatty acids and fatty acids methyl esters with other ingredients. In this study it was also known that the content contained in *N. oculata* microalgae was more sensitive to gram-positive bacteria.⁵³

The acid number of oil is an important factor that determines the nutrition of the oil. CJSPE has a high acid value of around 9.66, making this oil unsuitable for use as a nutrient.³⁹ It is as same as *crotalaria pallida* leaf oil which has an acid number of 19.63.⁴³

The iodine number is closely related to the concentration of saturated and unsaturated fatty acids of an oil. Coconut oil has a low iodine number due to the high concentration of saturated fatty acids (69.1%). This contrasts with cacay oil with a high iodine number, indicating that the saturated fatty acid concentration in cacay oil is low (23.6%).⁴² The ratio of saturated / unsaturated fatty acids contained in mastic tree oil is 0.4, indicating that this oil has a high content of unsaturated fatty acids, which makes this oil more attractive to consume.⁴⁴ *Crotalaria pallida* leaf oil also contains unsaturated fatty acids which is higher than saturated fatty acids with a ratio of 1.22: 1.⁴³ This is different from the fixed oil from *Orbignia speciosa* which contains a high content of saturated fatty acids (91.38%).⁵⁴ Therefore, the high percentage of unsaturated fatty acids in *cacay oil* indicates that this oil can be used for food, pharmaceutical and cosmetic products because unsaturated fatty acids have bioactive content for industrial purposes.⁴²

CONCLUSION

The essential oil encapsulation technique can overcome some limitations, including a high percentage of the compound in the encapsulated essential oils compared to the unencapsulated essential oils. In addition, encapsulation also can extend the shelf life of essential oils, as proven by thermogravimetric analysis where the encapsulated essential oils experience longer degradation than unencapsulated essential oils. One of the antibacterial activities of essential oils is due to their content of essential fatty acids. This demonstrates the potential of encapsulation of oils, fatty acids and lipids for antibacterial drugs.

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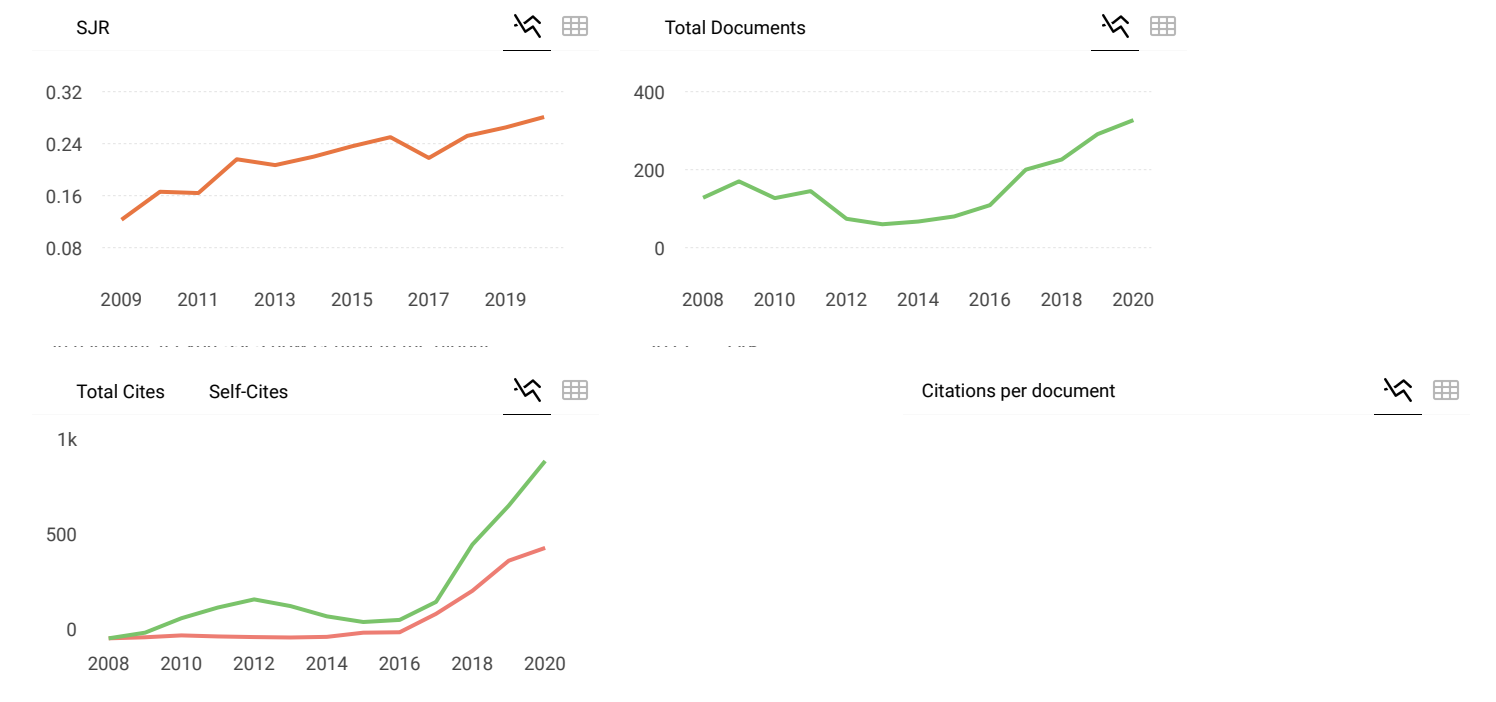
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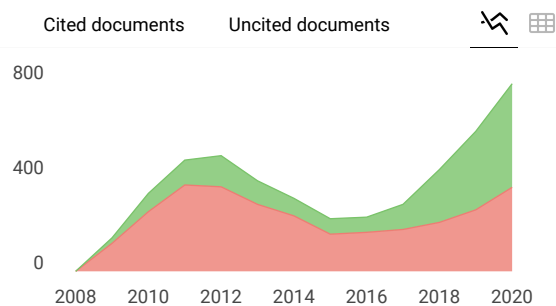
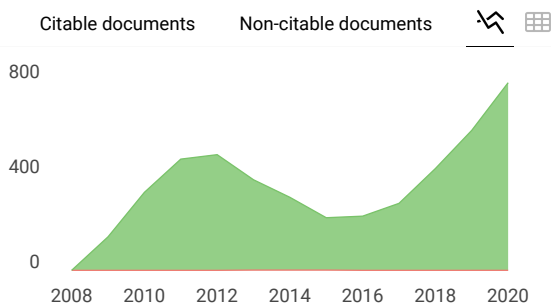
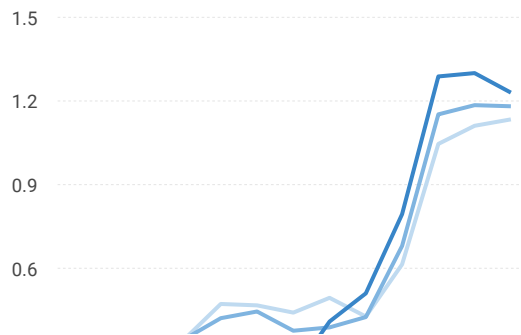
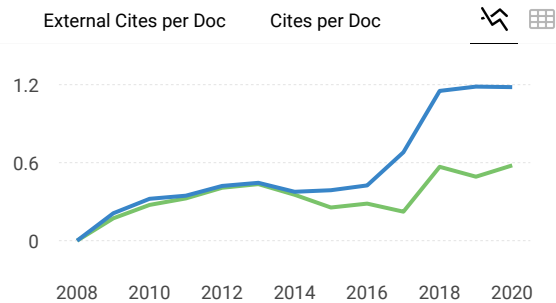
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