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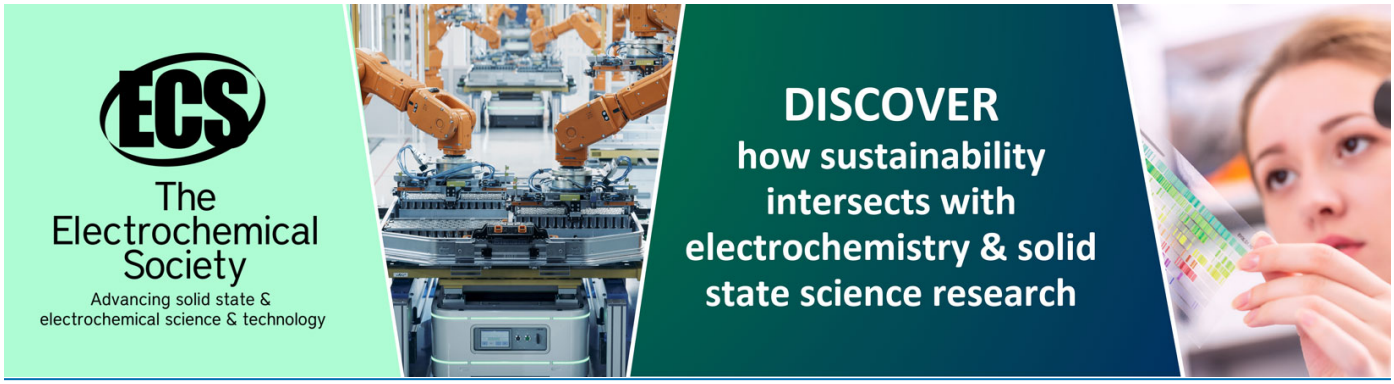

Synthesis of glycerol mono-laurate from lauric acid and glycerol for food antibacterial additive

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Abstract. Synthesis of glycerol mono-laurate (GML) has been performed using esterification reaction of glycerol and lauric acid. The reaction was performed at the condition of temperature of 120-140 ^{o}C within 7 hour, variation of molar ratio of glycerol - lauric acid, and was using heterogeneous catalyst of zeolist Y. Without catalyst dealumination the maximum acid conversion was 78%, with GML contained in the sample was 38.6%, and it was obtained at the reaction condition of 140 ^{o}C, 15wt% catalyst, and 8:1 molar ratio of glycerol - lauric acid. At the same condition, using dealuminated catalyst, the maximum acid conversion was increased up to 98%, with GML contained in the sample was 50.4%. The GML antibacterial activity was examined. It was observed that the GML has antibacterial activity against gram positive bacterial such as *B. cereus* and *S.aureus*.

1. Introduction

Glycerol Mono-laurate (GML) (2,3-dihydroxypropyl dodecanoic) that also called monolaurin is lauryl esters of glycerol. It is important to use in the food and cosmetic industries due to its properties such as emulsifying properties, complexing, and polyfunctional state[1]. GML is considered as a natural surfactant. It has antimicrobial activity against *enveloped-virus* and bacteria including gram negative bacteria and gram positive bacteria (*Streptococcus* spp., *Staphylococcus aureus* dan *Bacillus* spp)[2]. Unlike most of antibiotic that have antibacterial activity against a certain bacteria. GML has some target antibacterial activity through interaction with plasma membrane.

Synthesis of GML can be performed trhough esterification of lauric acid and glycerol using acid catalyst [3], transesterification of palm kernel oil or coconut oil or glycerolysis of methyl laurate using base catalyst[4]. At industrial scale generally the esterification and transesterification process utilize homogeneous catalyst, such as KOH, $NaHCO_3$ or Ca $(OH)_2$. It is reported that the process which used the homogeneous catalyst resulted unpurified product contain 40-60% monoglyceride, 30-40% diglyceride , 5-15% triglyceride, 1-5% free fatty acid and 2-10% glycerol[5]. Further purification usually using molecular distillation process which need high cost production[6].

The greater public interest in natural food products has encouraged manufacturers to develop natural preservative. Fatty acid ester compound is not only act as flavoring and emulsifying agents but also has potential of antimicrobials. Various experiments on GML as an antimicrobial effectiveness test has been conducted by several researchers[7,8,2,9]. Moreover, the study of antibacterial activity of GML have been done for *S. aureus, S. hominis, B. cereus, Streptococcus pyogenes, Haemophillus influenzae, P. aeruginosa, Escherichia coli.* GML more effective against gram positive bacteria than gram negative bacteria[10,11,12,13,14]. GML can be more effective when combined with antibiotics such as gentamycin and streptomycin or natural antimicrobial such as subtilosin, lauric acid and poly L-lysine[15,16,11]. It has been reported that a common mechanism of action for fatty acids that involves disruption of the cell membrane, leading to interference with energy production within the bacterial cell[17]. GML might facilitate to disrupt the matrix-embedded bacteria, resulting the release of cell compounds that affecting the cell metabolism[11].

In this work, the synthesis of GML using esterification of lauric acid and glycerol has been performed. More over the antibacterial activity of the GML has been examined.

2. Materials and methods

2.1. Materials

The materials for glycerol mono-laurates synthesis were lauric acid (94% purity) and Glycerol (98% purity) which obtained from Sinar Mas and Bratachem respectively were used as recieved. The catalyst was Zeolite Y CBV 712 (supplied by Zeolyst International in NH_4 form; mole ratio SiO_2 / Al_2O_3 = 12; surface area = 730 m^2 / g.). Microbe test which is used in the examination were *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aerginosa.* Antibacterial examination materials were Nutrient *agar* (NA), *Nutrient broth* (NB), absolute alcohol and distillate water. The standard microbiological apparatus was used for the examination.

2.2. Experimental procedures

The glycerol mono-laurate was synthesized from lauric acid and glycerol. The lauric acid and glycerol was reacted in a batch reactor using zeolist Y as a catalyst. The reaction was performed with mole ratio of glycerol to lauric acid of 2:1, 5:1 and 8:1 at the temperature of 110-140 C for 7 hour. The catalyst was varied from 10-20wt% of the lauric acid. The reaction conversion was observed with acid number analysis. The reaction conversion percentage was calculated using equation 1. Sample of GML was analyzed using GC-MS analysis. The GC-MS analysis of the GML sample showed that the reaction not only resulted GML but also glycerol di-laurate (GDL) and glycerol tri-laurate (GTL). The synthesis was also performed using modified zeolist Y. The catalyst was modified using dealumination method with difference condition of time, temperature and acid normality.

$$Reaction\ conversion = \frac{acid\ number\ of\ lauric\ acid\ (N_i) - acid\ number\ of\ reaction\ product\ (N_t)}{acid\ number\ of\ lauric\ acid\ (N_i)} \times 100\% \quad (1)$$

Preliminary examination of antibacterial activity of the GML was observed. The purpose of the examination was to identify the potential of the GML antimicrobial activity. The microbe of 10^6 CFU/ml was inoculated at the surface of NA solid media for about 0.1 mL. Then, a paper disc that had been immersed in GML solution (in absolute alcohol) was laid on the NA media. The GML solution concentration were 6,25, 12.5 mg/mL, 25 mg/mL, 50 mg/mL, 100 mg/mL.

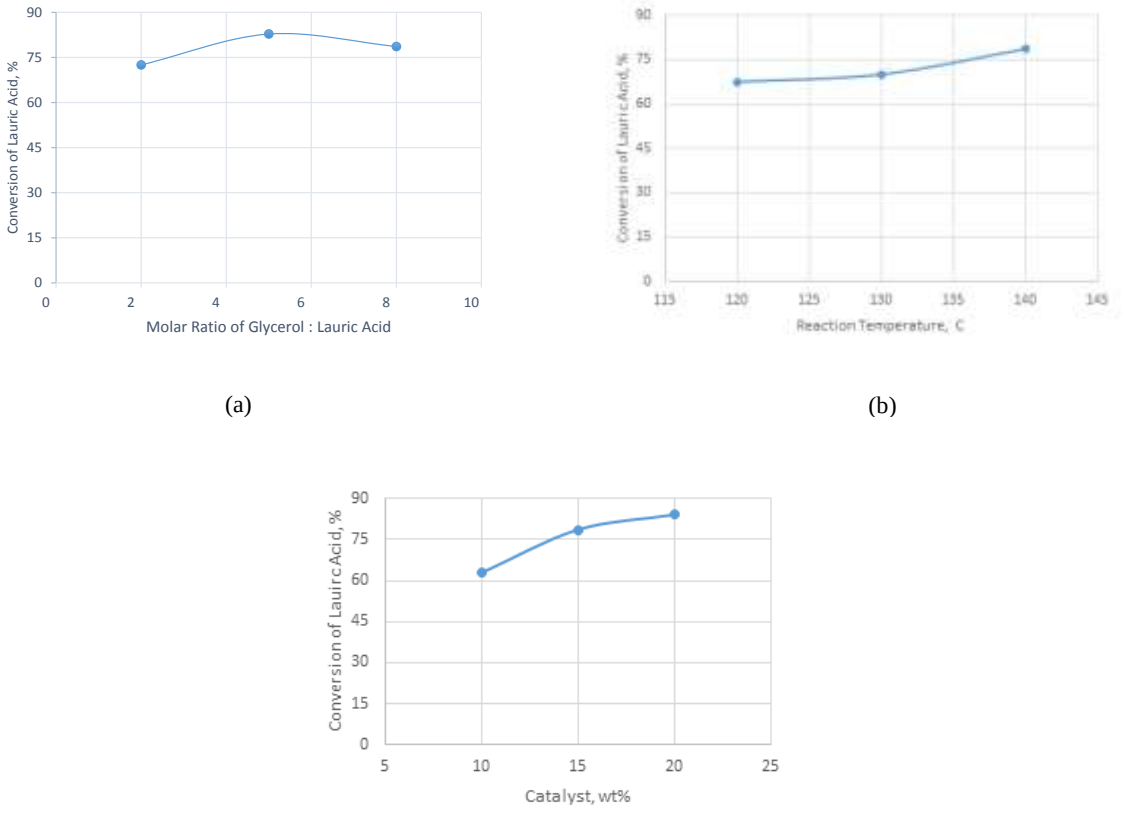
Minimum Inhibitory Concentration (MIC) determination was performed using nutrient broth (NB) dilution media method. Various concentration of GML in NB was inoculated with 0.1 mL microbe suspension. The tubes were incubated for 24 hours at 36 ^oC. Then, the incubated tubes were streaked on nutrient agar plate. MIC was determined by observation of the lowest concentration of the GML that did not show the colony formation.

3. Results and discussion

3.1. Glycerol Mono-laurate synthesis

In order to examine the effect of glycerol/lauric acid (2:1, 5:1, 8:1) molar ratio on the synthesis of GML, the reactions were performed at the constant temperature of 140 ^{o}C and at the same catalyst loading of 15wt%. It can be seen in the figure 1a that the effect of glycerol/lauric molar ratio on the conversion reaction from 5:1 to 8:1 was insignificant. It has been reported their work on synthesis of GMO using SBA-15 mesoporous catalyst. It was reported that the effect of glycerol/lauric acid molar ratio on the lauric acid conversion was insignificant when the glycerol/lauric acid molar ratio was increased from 2:1 to 4:1, especially after 3–7 h of reaction. It is also reported that the reaction product, monoglyceride yield significantly increased from 58% to 68%, while diglyceride yield experienced a decrease from 35% to 23% while triglyceride yield remained stable at very low level (below 2%)[18].

The synthesis GML was carried out at various temperature of 120, 130 and 140 ^{o}C using a constant glycerol/lauric acid molar ratio (8:1) and at a constant of catalyst loading of 15wt%. In the figure 1b, it was observed that the increasing temperature condition from 110 to 140 ^{o}C enhanced reaction conversion from 62.9 to 84.1%.



(a) (b)

(c)

Figure 1. The effect of reaction conditions on reaction conversion

The effect of temperature to the reaction conversion can be observed at the figure 1b. The lauric acid conversion increased with increasing temperature. This could be due to the increase of kinetic energy

of glycerol and lauric acid and it make the number chemisorption on the catalyst active site was increased and enhance the interaction of the molecules of reactant[19].

The effects of loading of catalyst were examined with a constant temperature (140 ^{o}C) and glycerol/lauric acid mole ratio (8:1). This observation suggested that the increase in catalysts loading from 10 to 20 wt% increased the number of active site that available for the reaction.

It can be observed from the figure 1c that increasing the amount of catalyst from 10 to 20 wt% increased the reaction conversion from 62.9 to 84.1. However the increasing of the reaction conversion due to the additional catalyst did not give significant improvement of reaction conversion. Moreover, the greater amount of catalyst will make difficulties in the separation process of the GML product and the catalyst.

Due to the low reaction conversion, other experiment of the GML synthesis was conducted using dealuminated catalyst. The catalyst was dealuminated using the method as presented in the International Conference on Chemical Process and Product Engineering 2016 that was organized by Department of Chemical Engineering, Diponegoro University at 14-15th September 2016[21]. In the comparison of GML synthesis using non dealuminated and dealuminated catalyst (table 1), it was observed that conversion of lauric acid that using non dealuminated catalyst was 78%, and according to GCMS resulted GML, GDL and GTL content were 38.6, 19,0 and 9.1% respectively. Meanwhile, the experiment using dealuminated catalyst resulted GML, GDL and GTL content were 59.5, 31.9 and GTL 0.2% respectively. The selectivity of GML on the process using non dealuminated and dealuminated catalysts were 57.9 and 65.0% respectively. The results showed that the dealuminated catalysts could enhance reaction rate. However, reaction time for 7 hour lead the increasing of formation of GDL and GTL.

Table 1. Comparison of the GML synthesis with different treatment of catalyst zeolist Y T = 140 ^{o}C, Molar ratio 8:1 (glycerol : lauric Acid), 15 wt% catalysts

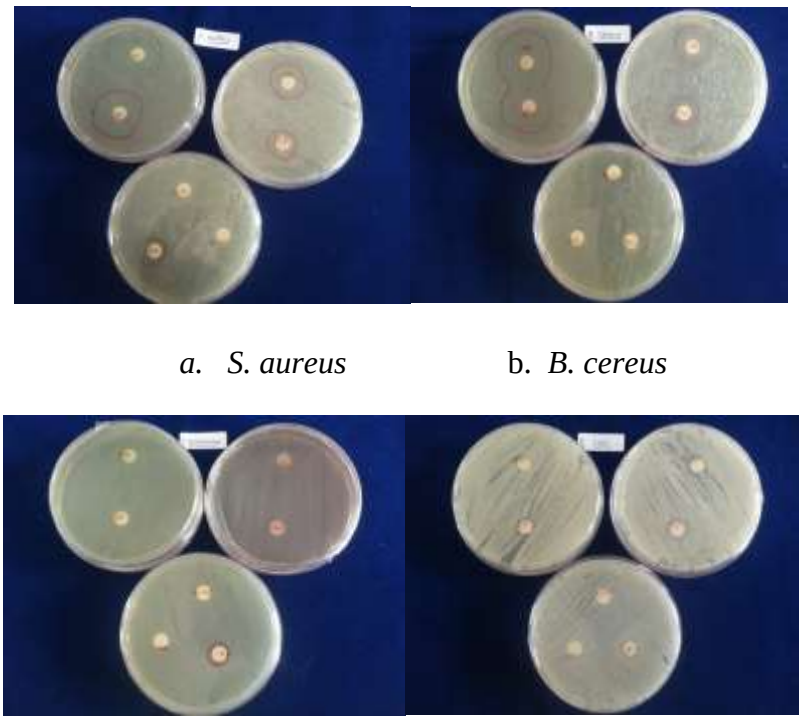
Catalyst treatment	Reaction conversion (%)	Selectivity (%)	Glyceride content (%)		
			Glycerol Mono-laurate (GML)	Glycerol Di-laurate (GDL)	Glycerol Tri-laurate (GTL)
Without dealumination	78.0	57.9	38.6	19.0	9.1
With dealumination	97.8	65.0	59.5	31.9	0.2

3.2. Antibacterial activity examination

The antibacterial Activity examination showed that GML has a potential effect to inhibit pathogen bacterial. The biggest inhibition was obtained at *S. aureus* plate with clear zone of 33.44 mm. followed by *B. cereus* plate with clear zone of 32.75 mm. that was found at the concentration of GML was 100 mg/ml (figure 2). The results indicate that GML has more effective on the inhibition of gram positive bacteria. (*S. aureus* and *B. cereus*) than gran negative bacteria (*E.coli* and *P. aeruginosa*). However, the result also showed that E. coli was also inhibited by GML antibacterial activity. Potential antibacterial activity of GML is greater than that of diacyl glycerol[10]. It was also reported that the GML has no antibacterial activity against *S. typhymurium* and *E. coli* which are gram positive bacteria[10].

GML or monolaurin was categorized as indirect antimicrobial or multifunctional food additives, because of this product are added to foods used primarily as emulsifier other than antimicrobial. Monolaurin, the medium-chain fatty acid, is effective at high pH (5.0 – 8.0) different with the sort-chain fatty acids. The application of GML as preservative in food industry will be more effective when

combined with another "indirect antimicrobials" because of the fatty acid and esters have a narrow range of effectiveness and generally recognized as safe (GRAS) substances such as *EDTA (*Ethylene diamine tetra acetic acid), citrate, and phenolic antioxidants also have limitations as antimicrobial agents when used alone[22]. GML was more effective inhibit *S. aureus* growth when its combined with pH lowering and *EDTA* addition [14,2]. GML acted synergistically with gentamicin and streptomycin to eliminate detectable viable biofilm bacteria (*S. aureus*)[11].



a. S. aureus b. *B. cereus*

c. P. aeruginosa d. *E. coli*

Figure 2. Clear zone of GML antibacterial examination

The antibacterial activity examination of GML showed that the minimum inhibitory concentration of GML for *S. aureus* and *B. cereus* growth was 25 mg/ml. *Minimum Inhibitory Concentration* (MIC) value from MDAG-CNO from coconut oil for *S. aureus* and *B. cereus* growth was 17,5 mg/ml[10]. The MIC value for *S. aureus* increase when GML was combined with 1 µg/ml Gentamycin. In the future GML is very applicable for medicinal industry to inhibit biofilm microbial growth[11].

4. Conclusions

Synthesis of glycerol mono-laurate from lauric acid and glycerol could be performed by esterification reaction using zeolist Y catalysts. The catalyst can be used either without dealumination or with dealumination. The conversion reaction of the process using dealuminated zeolist Y catalyst resulted higher than that of the process using non dealuminated one, which were 97.8 and 78.0% respectively. The process using dealuminated zeolist Y at 140 ^{o}C, Molar ratio of 8:1 glycerol:lauric acid and catalysts loading of 15wt% for 7 hours reaction time also gave GML selectivity i.e. 59.5%. The GML antibacterial activity was pre-examined. It was observed that the GML has antibacterial activity against gram positive bacterial such as *B. cereus* and *S.aureus*.

Acknowledgements
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