

The Effect of the Mole Ratio of Ethoxy Solution and Palm Kernel Oils (PKO) on the Pattern of Monoglycerides (MG), Diglycerides (DG), and Triglycerides (TG) Content of the Level I, II and III PKO Ethanolysis Products

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Abstract

Ethanolysis reactions of palm kernel oil (PKO) had been carried out in stages, as follows: Level I ethanolysis was carried out at 60°C, at a stirring speed of 1000 rpm for 3 minutes, in the treatment of the mole ratios of ethoxy solution (ethanol 90%-NaOH 1%) to PKO of the 10, 12, 14, 16, 18 and 20, resulting in ethanolysis product I and the residual reaction I. Level II ethanolysis was ethanolysis of the residual reaction I, resulting in ethanolysis product II and residual reaction II. Level III ethanolysis was ethanolysis of the residual reaction II, resulting in ethanolysis product III. The mean yield of all treatments for PKO Ethanolysis Levels I, II, and III were 12.26±0.46%, 11.20±2.61%, and 11.78±2.99%, respectively. The highest total yield of PKO ethanolysis level I, II, and III was 41.04±9.74% (w/w) from the mole ratio treatment of 20, while the lowest yield was 27.54±3.39% (w/w) from the mole ratio treatment of 10. The average amount of monoglycerides (MG), diglycerides (DG), and triglycerides (TG) from all treatments were 9.740%, 12.801%, and 2.675%, respectively. The highest MG content was obtained from the mole ratio treatment of 20 at Ethanolysis Level I (11.83%).

Keywords: ethoxy, PKO, ethanolysis, monoglycerides, diglycerides.

1. Introduction

Oil palm fruit can produce two types of oil, namely crude palm oil (CPO) and palm kernel oil (PKO). CPO is reddish orange in color, obtained from the pulp (mesocarp) of oil palm fruit, while PKO is yellow, obtained from the kernel (endosperm) of oil palm fruit. Palm kernel contains about 50 percent oil (PKO) whose fatty acids are dominated by lauric acid (12:0) and myristic acid (14:0) (Gurr, 1992). The total amount of fatty acids in PKO extracted using hexane was 99.42g per 100g PKO (99.42%). The types of fatty acids contained in PKO consists of 9 types of fatty acids, which are divided into 7 types of saturated fatty acids, which are dominated by lauric acid (49.39%) and myristic acid (15.35%), and 2 unsaturated fatty acids. dominated by oleic acid (15.35%) and linoleic acid (3.10%), and the rest (0.07%) in the form of unidentified minor fatty acids (Murhadi, 2010). Research resulted by Lestari and Murhadi (2008); Murhadi (2009a); Murhadi (2009b); Murhadi (2010); Murhadi *et al.* (2012); Nendela *et al.* (2012); Khasbullah *et al.* (2013); Murhadi *et al.* (2017) proved that PKO and/or a mixture of PKO and CPO had the potential to be a derivative product that has a function as an antimicrobial compound and also an emulsifier that can be used as a preservative for emulsion food products.

The potential content of lauric fatty acids and myristic acid in palm kernel oil (PKO), totaling around 65% of the total fatty acids (Murhadi, 2010), have not been optimally utilized as antimicrobial agents. On the other hand, PKO can be reacted with ethanol through ethanolysis process to produce new products as food additives that have a dual functional role as a preservative and as a food emulsifier. Yunggo *et al.* (2016) reported that the difference in ethanolysis reaction time did not affect product yield, emulsion stability, and coconut milk durability carried out at room temperature (28-30°C).

The ethanolysis process of PKO had been carried out by mixing ethoxy ion solution with 100 g of PKO at a ratio of 1.0, 1.2, and 1.4 (w/w), added with 10% glycerol (w/w of PKO weight), heated at 55-60°C and stirred at 1000 rpm for 3, 6, 9, or 12 minutes. Such ethanolysis process produced ethanolysis products with an average content of glycerides, esters, monoglycerides, internal standards, diglycerides and triglycerides of 6.09%, 55.02%, 7.07%, 2.55%, 23.54%, and 5.73%, respectively. The treatment that produced the highest monoglycerides (MG) and diglycerides (DG) content was the ethanolysis process which was carried out for 3 minutes with a ratio of ethoxy solution to PKO of 1.0 (w/w), in which the percentages of MG and DG resulted were 7.74% and 30.60% (w/w), respectively (Murhadi *et al.*, 2019).

2. Materials and Methods

2.1. Materials and Equipment

Crude palm kernel oil (PKO) was obtained directly from the palm oil mill of the PTPN VII Bekri Central Lampung, Chemicals consist of: absolute ethanol, technical ethanol, NaOH, HCl 35%, aquades, n-hexane, indicator pp, N- Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), THF, and heptane. The equipments used: a set of 3-necked glass reflux flask for PKO ethanolysis process, hot plate-magnetic stirrer, separating funnel, Gas Chromatography (GC-2010 Shimadzu; Column DB 5 HT serial US Number 1491822).

2.2. PKO Preparation

Crude PKO was filtered and then stored in a cool, dark, dry place. Some of the PKO were analyzed for water content (gravimetry method), acid number (method of SNI 01-3555-1998), iodine number (method SNI 01-3555-1998), free fatty acid content, and fatty acid profile (C8 to C22).

2.3. Preparation of 90% Ethanol Solution Containing 1% NaOH

Preparation of 90% ethanol solution containing 1% NaOH followed the method of Murhadi *et al.* (2019) with modifications. The calculation to produce an ethoxy solution in the treatment of the mole ratio of 90% ethanol solution-1% NaOH (w/w PKO) to PKO equal to 10, as follows: It is known that MW of ethanol is 46 and MW of PKO (as trilaurine) is 638. For the experimental unit of PKO 120 g or 0.1881 mol and a mole ratio equal to 10, it required ethanol solution as much as 1.881 mol or 86.5 g of ethanol 100%. Furthermore, to make an ethoxy solution containing 90% ethanol-NaOH 1% (w/w PKO) which would be mixed with 120 g PKO (mol ratio of 10) was 1.2 g NaOH pellets dissolved in 9.6 g of distilled water until completely dissolved (heat will arise), then add 86.5 g of absolute ethanol (100%), resulting in 97.3 g ethanol 90% containing 1% NaOH (w/w PKO). With the same calculation (proportional), the above calculation was used for experimental units with different mole ratios (12, 14, 16, 18 and 20).

2.4. Ethanolysis of PKO

PKO ethanolysis was followed the method of Murhadi *et al.* (2019) with modifications, which was carried out in a 3-necked boiling flask (1,000 mL capacity) equipped with a condenser. The boiling flask was placed on a hot plate with a magnetic stirrer. Level I ethanolysis was carried out at 60°C at a stirring speed of 1000 rpm for 3 minutes, resulting in ethanolysis product I (top layer) and the residual reaction I (bottom layer). Level II Ethanolysis was ethanolysis of the residual reaction I with the same procedure and treatment as Level I Ethanolysis, resulting in ethanolysis product II and residual reaction II. Ethanolysis Level III was ethanolysis of the residual reaction II, with the same procedure and treatment as Level II ethanolysis, resulting in ethanolysis product III. PKO ethanolysis level I was carried out for all mole ratios of ethoxy solution to PKO (10, 12, 14, 16, 18, and 20), with the basic calculation being 120 g of PKO weight. With the same procedure, it was carried out for the PKO Ethanolysis Level II (PKO residual reaction from level I ethanolysis which was free of water and solvent, an amount of 70 g) and Level III (PKO residual reaction from level II ethanolysis an amount of 40 g). PKO ethanolysis level II and level III are carried out at the same mole ratio as PKO ethanolosis level I (10, 12, 14, 16, 18, and 20).

2.5. Analysis of Ethanolysis Products

PKO ethanolysis products at each ethanolysis reaction level were analyzed for the yield and composition of MG, DG and TG compounds, as well as fatty acid profiles using Gas Chromatography carried out at the Laboratory of Food Chemistry at the Indonesian Palm Oil Research Institute (IOPRI) Medan, North Sumatra. The yield of PKO ethanolysis products was calculated based on the weight of the PKO ethanolysis product to the initial weight of PKO.

3. Results and Discussion

3.1. Chemical Analysis in Crude PKO

The results of the analysis of the iodine number, acid number, and free fatty acid content in crude PKO were 19.2 mg/g; 4.15 mg/g; and 1.39 percent, respectively. While the analysis of the fatty acid profile in crude PKO was: C6:0 (0.17%), C8:0 (2.90%), C10:0 (2.91%), C12:0 (48.99%), C14:0 (15.77%), C16:0 (8.68%), C18:0 (1.98%), C18:1 (16.13%), C18:2 (2.28%), C20:0 (0.10%), and C20:1 (0.10%). There were two dominant fatty acids, namely lauric acid (C12:0) and myristic acid (C14:0), with a total of 64.76% which contribute to the properties of antimicrobial activity and are very potential as agents for food preservatives. Murhadi (2010) showed that there were at least 4 types of acids that dominated PKO fatty acid profile, which consisted of 2 types of saturated fatty acids, lauric acid (49.39%) and myristic acid (15.35%) and 2 unsaturated fatty acids, oleic acid (15.35%) and linoleic acid (3.10%). The levels of lauric and myristic fatty acids in crude PKO from this study were relatively the same as levels of lauric and myristic fatty acids in PKO obtained from palm kernel extraction with hexane (Murhadi. 2010).

3.2. Yield of PKO Ethanolysis Products

Yields of PKO Ethanolysis Levels I, II, and III for the mole ratios of 10, 12, 14, 16, 18, and 20, respectively, are presented in Figure 1. Average yields for PKO Ethanolysis Levels I, II, and III were $12.26 \pm 0.46\%$, $11.20 \pm 2.61\%$, and $11.78 \pm 2.99\%$, respectively.

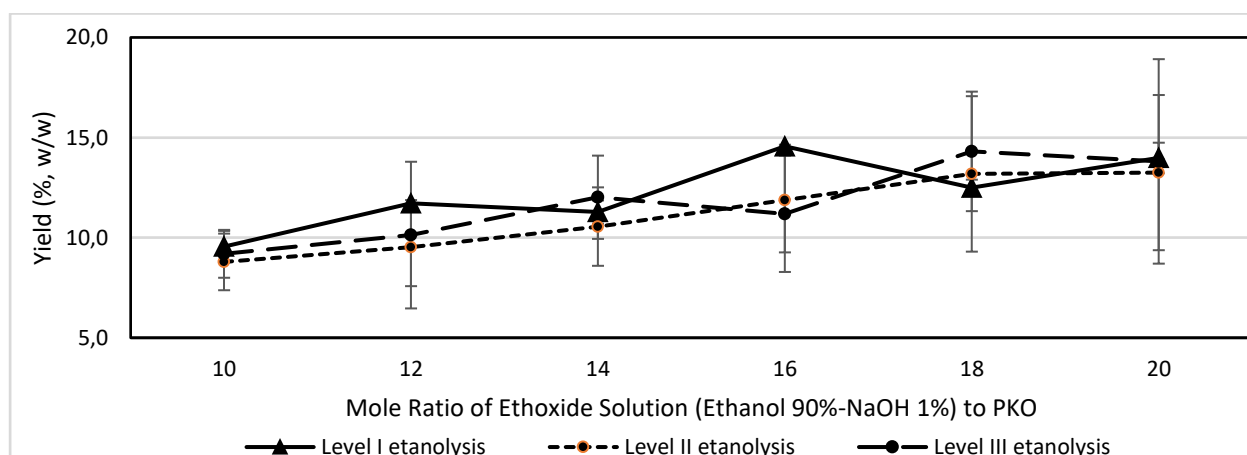


Figure 1. Yield of level I, II, and III PKO Ethanolysis products for the mole ratios of 10, 12, 14, 16, 18, and 20, respectively.

There was a relative increase in the yield of PKO ethanolysis products at ethanolysis level I, II or III with the increasing mole ratio of ethoxy solution to PKO from a ratio of 10 to 20. Treatment with weight ratio of 90% ethanol solution-1% NaOH (w/w PKO) to PKO equal to 1.4 (w/w) was better than treatment with the ratio of 1.0 (w/w) and 1.2 (w/w) (Murhadi *et al.*, 2019). Need to know that a weight ratio of 90% ethanol solution-1% NaOH (w/w PKO) to PKO equal to 1.0; 1.2; and 1.4 (w/w) is equivalent to a mole ratio of 13.87; 16.64; and 19.42, respectively. The increases the yield of PKO ethanolysis products, presumably because the increase presence of ethoxide ion solution which increases the ethanolysis reaction to

produce PKO ethanolysis products (Murhadi et al., 2019). The highest yield of PKO ethanolysis levels I, II, and III was $41.04 \pm 9.74\%$ (w/w) for treatment of the mole ratio of 20, while the lowest yield was $27.54 \pm 3.39\%$ (w/w) for treatment of mole ratio of 10. The results of this study were in line with previous study (Murhadi et al., 2019).

3.3. Pattern of MG, DG and TG Compounds in PKO Ethanolysis Products

The chromatogram showing the identification results of MG, DG, TG and other components in the level I PKO ethanolysis product for the mol ratio of 10 is presented in Figure 2.

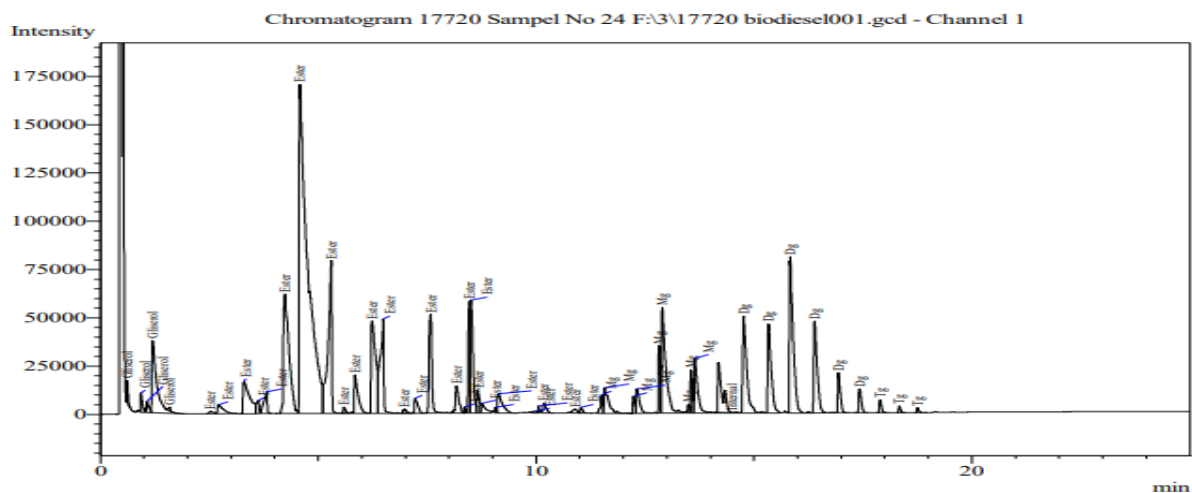


Figure 2. GC chromatogram as a result of identification of MG, DG, TG and other components in level I PKO Ethanolysis products for a mole ratio of 10.

A recapitulation of all data on MG, DG, TG, and other components contained in PKO ethanolysis products levels I, II, and III for each treatment ratio (10 up to 20) is presented in Table 1.

Table 1. Data recapitulation of MG, DG and TG compounds, and other components in PKO ethanolysis products for all treatments

No	Code	Compounds (%)						
		MG	DG	TG	Glycerol	Ester	IS	TOTAL
1	N10T1U1	11,3	20,67	0,65	3,37	61,81	2,21	100
2	N12T1U1	6,2	22,28	1,75	4,71	62,93	2,13	100
3	N14T1U1	10,55	21,55	0,58	3,87	60,93	2,52	100
4	N16T1U1	11,3	24,01	0,72	3,31	58,12	2,54	100
5	N18T1U1	10,54	20,76	0,49	4,56	61,18	2,47	100
6	N20T1U1	4,53	14,77	0,37	4,86	72,54	2,92	100
7	N10T2U1	10,13	13,41	0,58	4,36	69,57	1,95	100
8	N12T2U1	5,13	7,74	0,61	4,21	80,67	1,65	100
9	N14T2U1	6,72	12,58	0,89	3,6	74,51	1,71	100
10	N16T2U1	7,87	11,01	0,62	3,34	75,37	1,78	100
11	N18T2U1	7,82	14,02	0,74	3,52	71,99	1,9	100
12	N20T2U1	5,69	10,51	0,8	3,69	78,24	1,07	100
13	N10T3U1	7,24	11,15	0,57	4,07	74,96	2,01	100
14	N12T3U1	4,52	8,82	0,75	1,67	83,05	1,19	100
15	N14T3U1	6,54	12,52	0,97	3,39	74,92	1,65	100
16	N16T3U1	10,22	12,04	0,56	0,76	74,24	2,19	100
17	N18T3U1	5,15	10,49	1,02	3,08	79	1,26	100

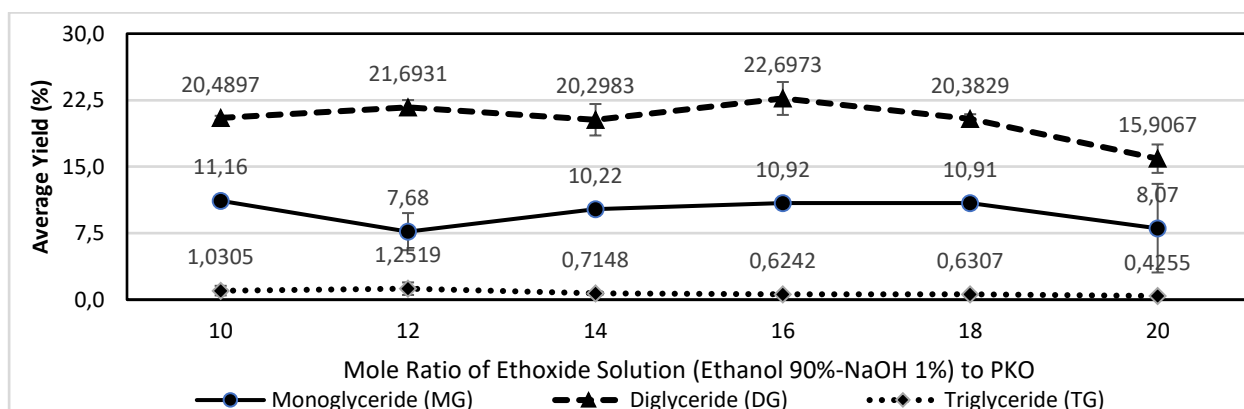
18	N20T3U1	7,57	12,78	0,73	0,83	76,71	1,38	100
19	N10T1U2	11,03	20,31	1,41	6,37	58,51	2,37	100
20	N12T1U2	9,16	21,11	0,75	4,89	61,94	2,14	100
21	N14T1U2	9,89	19,04	0,85	4,96	62,99	2,27	100
22	N16T1U2	10,54	21,38	0,53	4,57	60,74	2,24	100
23	N18T1U2	11,29	20	0,77	4,66	60,76	2,52	100
24	N20T1U2	11,6	17,04	0,48	4,29	64,48	2,11	100
25	N10T2U2	11,32	14,88	1,23	5,24	65,37	1,97	100
26	N12T2U2	7,02	10,85	0,97	4,61	74,97	1,59	100
27	N14T2U2	6,81	12,06	0,76	4,59	74,66	1,12	100
28	N16T2U2	5,3	9,29	0,89	5,55	76,95	2,02	100
29	N18T2U2	8,9	15,57	0,92	3,46	68,95	2,19	100
30	N20T2U2	7,31	9,38	0,62	5,77	75,29	1,64	100
31	N10T3U2	9,04	10,92	0,71	3,39	74,16	1,78	100
32	N12T3U2	8,53	10,35	0,61	1,98	76,56	1,96	100
33	N14T3U2	8,25	12,28	0,77	2,1	74,76	1,84	100
34	N16T3U2	6,75	9,74	0,52	1,52	79,67	1,8	100
35	N18T3U2	8,2	11,69	0,58	2,8	74,65	2,08	100
36	N20T3U2	7,24	11,53	0,88	3,63	74,46	2,26	100
Average		8,26	14,4	0,77	3,77	70,85	1,96	100

Note: N10 s.d. N20 = Mole ratio of ethoxy solution to moles of PKO (10, 12, 14, 16, 18 or 20); T1, T2, or T3 = Level I, Level II, or Level III Ethanolysis.; U1 and U2 = Repetition 1 or Repetition 2; IS = Internal standard.

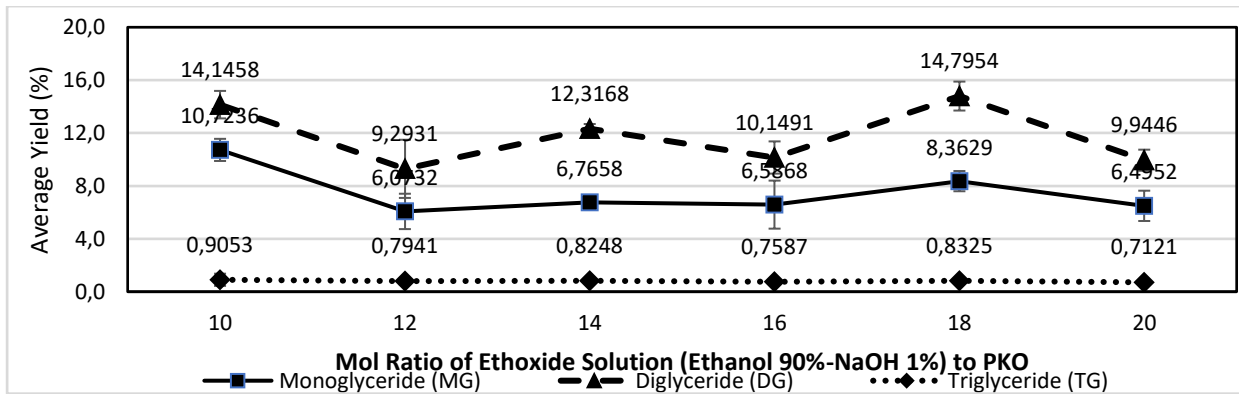
Based on the data in Table 1, the average content of monoglycerides (MG), diglycerides (DG), and triglycerides (TG) for all treatments were $8.26 \pm 2.17\%$, $14.40 \pm 4.66\%$, and $0.77 \pm 0.27\%$, respectively. Murhadi et al. (2019), found that ethanolysis carried out at a temperature of 55–60°C with a stirring speed of 1000 rpm for 3, 6, 9, and 12 minutes with the weight ratio of ethoxy ion solution to crude PKO of 1.0, 1.2, and 1.4 (w/w) produced an average content of glycerides, esters, monoglycerides, internal standards, diglycerides, and triglycerides in PKO ethanolysis products, each of 6.09; 55.02; 7.07; 2.55; 23.54; and 5.73 percent, respectively. The types of monoglycerides that dominated ethanolysis products were monolaurin and monomyristin, which amounted to 7.07 percent of the total glycerides in PKO ethanolysis products (Oh and Marshall, 1994; Cotton and Marshall, 1997).

3.4. Composition Patterns of MG, DG, and TG in Ethanolysis Products

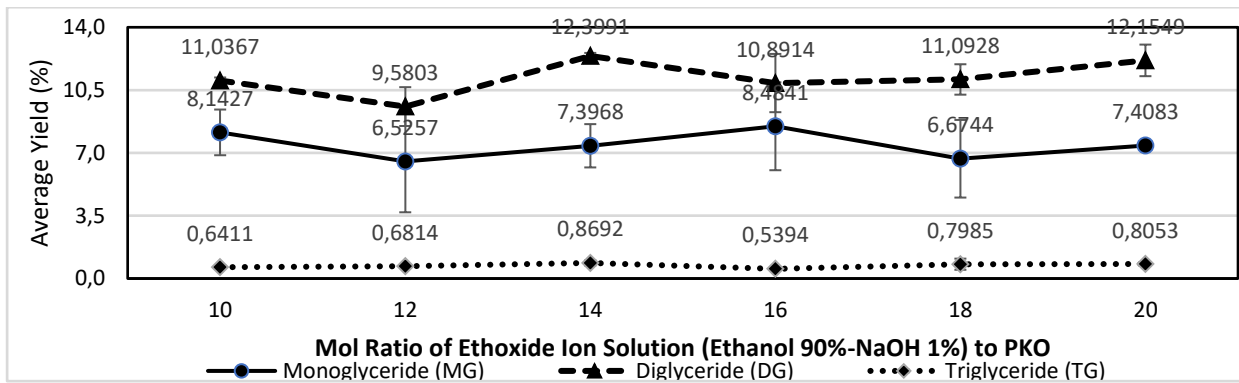
The Graphs of the composition patterns of MG, DG and TG of the PKO ethanolysis products for each mole ratio (10, 12, 14, 16, 18, and 20) are presented in Figure 3 (A, B, and C).



(A)



(B)



(C)

Figure 3. Pattern composition of MG, DG and TG in PKO ethanolsis level I (A), level II (B) and level III (C) at mol ratios of 10, 12, 14, 16, 18, and 20.

The average MG, DG, and TG content of the level I PKO ethanolsis were $9.83\pm 1.55\%$, $20.24\pm 2.32\%$, and 0.78 ± 0.30 , respectively. Furthermore, the average content of MG, DG, and TG of the level II PKO ethanolsis were $7.50\pm 1.76\%$, $11.77\pm 2.33\%$, and $0.80\pm 0.07\%$, respectively. And the average MG, DG, and TG content for the level III PKO Ethanolsis were $7.44\pm 0.78\%$, $11.19\pm 1.01\%$, and $0.72\pm 0.12\%$, respectively.

At the ethanolsis level I, the highest average percentage of MG content was obtained by the treatment of mole ratio of 10 (11.16 ± 0.19 percent), while the highest percentage of DG was produced the treatment of the mole ratio of 16 (22.70 ± 1.86 percent). At the ethanolsis level II, the highest percentage of MG was obtained by the mole ratio treatment of 10 (10.72 ± 0.84 percent) and the highest percentage of DG was obtained by the mole ratio treatment of 18 and 10 (14.80 ± 1.09 percent and 14.15 ± 1.04 , respectively). In terms of efficiency of using ethoxy solvent, it is recommended to use treatment with a mole ratio of 10 only. The percentage of MG obtained resulted in all ethanolsis level III products were all relatively the same, based on the standard deviation value, so it is more efficient to use the smallest mole ratio treatment, such as mole ratio of 10.

4. Conclusion

The fatty acids that make up crude PKO were dominated by 11 types of fatty acids, namely: C6:0 (0.17%), C8:0 (2.90%), C10:0 (2.91%), C12:0 (48.99%), C14:0 (15.77%), C16:0 (8.68%), C18:0 (1.98%), C18:1 (16.13%), C18:2 (2.28%), C20:0 (0.10%), and C20:1 (0.10%). Of the 11 types of fatty acids contained in PKO, there were two dominant fatty acids that contribute to the properties of antimicrobial activity, that were lauric acid (C12:0) and myristic acid (C14:0) which are 48.99 percent and 15.77 percent, respectively, so that the total of the two types of acids reached 64.76 percent.

The average amounts of monoglycerides (MG), diglycerides (DG), and triglycerides (TG) for all treatments were $8.26 \pm 2.17\%$, $14.40 \pm 4.66\%$, and $0.77 \pm 0.27\%$, respectively. The average MG, DG, and TG content of PKO ethanolysis level I were $9.83 \pm 1.55\%$, $20.24 \pm 2.32\%$, and $0.78 \pm 0.30\%$, respectively. Furthermore, the average MG, DG, and TG content for ethanolysis level II were $7.50 \pm 1.76\%$, $11.77 \pm 2.33\%$, and $0.80 \pm 0.07\%$, respectively. The average MG, DG, and TG content for ethanolysis stage II were $7.44 \pm 0.78\%$, $11.19 \pm 1.01\%$, and $0.72 \pm 0.12\%$, respectively.

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References

- Cotton, L.N. and Marshall, D.L. (1997). Monolaurin preparation methods effects activity against vegetative cell of *Bacillus cereus*. *Journal of Food Science and Technology*, 30(8), 830-832.
- Gurr, M.I. (1992). *Role of Fats in Food and Nutrition*. New York: Elsevier Applied Science.
- Lestari, M. and Murhadi. (2008). Effect of ethanol - PKO ratio and reaction time on the yield and antibacterial activity of palm kernel oil (PKO) ethanolysis products. *Jurnal Teknologi & Industri Hasil Pertanian*, 13(2), 95-107.
- Murhadi. (2009a). The emulsifying power of ethanolysis products from a mixture of palm kernel oil (*Elaeis guineensis* Jacq) and noni seed oil (*Morinda citrifolia* L.) in fresh coconut milk, presented at one-day seminar of research results and community service, Bandar Lampung, October 05, 2009. Lampung University Research Institute. Proceeding: B-66 - B-71.
- Murhadi. (2009b). Anti-yeast activity and preservative power of ethanolysis products from a mixture of palm kernel oil (*Elaeis guineensis* Jacq) and noni seed oil (*Morinda citrifolia* L.). *TEGI Magazine (Agroindustry Technology Scientific Magazine, ISSN 2085 – 6067)*, 1(2), 17-25.
- Murhadi. 2010. The Emulsion Stability of Coconut (*Cocos nucifera* L) Milk Added with Ethanolysis Product from Palm Kernel Oil (*Elaeis guineensis* Jack). Presented at International Seminar on Horticulture to Support Food Security, Bandar Lampung, June 22-23, 2010. Preceeding: B-223 – B-229.
- Murhadi, Zuidar, A.S., and Fanny, R.L. (2012). Yield and functional characteristics of crude ethanolysis products from PKO and CPO mixtures. Presented at The SMAIP III National Seminar at The Faculty of Mathematics and Natural Sciences University of Lampung, Bandar Lampung, June 28-29, 2012. Proceeding ISBN 978-602-98559-1-3: 546-552.
- Murhadi, M., Hidayati, S. and Kurniawan, R. (2017). Effect of acid types and heating reaction times on the characteristics of PKO (palm kernel oil) ethanolysis products *Journal Agritech*, 37(1), 69-76.
- Murhadi, Hidayati, S. and Sugiharto, R. (2019). Profile of monoglyceride and diglyceride compounds of the ethanolysis products from palm kernel oil (PKO). Presented at International Conference on Food Science & Technology, Semarang, Central Java, 2018. IOP Conference Series: Earth and Environmental Science 292 (2019) 012002; IOP Publishing (Scopus); doi:10.1088/1755-1315/292/1/012002.
- Nendela, C.S., Murhadi, and Hidayati, S. (2012). Study of yield value, antibacterial activity and emulsion stability of ethanolysis products from PKO and CPO mixtures through multistage reactions. *TEGI Magazine (Agroindustry Technology Scientific Magazine, ISSN 2085 – 6067)*, 4(1), 28-38.
- Oh, D.H. and Marshall, D.L. (1994). Enhanced inhibition of *Listeria monocytogenes* by glycerol monolaurate with organic acids. *Journal of Food Science*, 59(6), 1258-1261.
- Khasbullah, F., Murhadi and Suharyono, As. (2013). Study of functional characteristics of ethanolysis products of mixture of CPO (crude palm oil) and PKO (palm kernel oil) in level two ethanolysis reactions. *Jurnal Teknologi & Industri Hasil Pertanian*, 18(1), 13-27.
- Yunggo, J., Murhadi, and Hidayati, S. (2016). Effect of ethanolysis time at room temperature on yield and emulsion stability of palm kernel oil (PKO) ethanolysis products. *Jurnal Teknologi Industri & Hasil Pertanian*, 21(2), 97-106.