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The effect of the mole ratio of ethanol solution and palm kernel oil on the pattern of glycerides content of the level I, II and III PKO ethanolysis products

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Abstract

The specific purpose of this scientific article is to obtain scientific information about the pattern composition of monoglycerides (MG), diglycerides (DG) and triglycerides (TG) in palm kernel oil (PKO) ethanolysis products at three stages and of the mole ratios of ethanol in ethoxy solution (ethanol 90% - NaOH 1%) to PKO (assumed as trilaurin). Ethanolysis reactions of PKO had been carried out in three stages, as follows: Level I ethanolysis was carried out at 60°C, at a stirring speed of 1000 rpm for 3 mins, in the treatment of the mole ratios of ethanol in 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% (w/w), 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 MG, DG, and 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%).

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 the oil palm fruit. Palm kernel contains about 50% 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 99.42 g per 100 g PKO (99.42%). The types of fatty acids contained in PKO consists of nine types of fatty acids, which are divided into seven types of saturated fatty acids, which are dominated by lauric acid (49.39%) and myristic acid (15.35%), and two 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). The research 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

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(w/w of PKO weight), heated at 55-60°C and stirred at 1000 rpm for 3, 6, 9, or 12 mins. 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 three mins 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).

The specific purpose of this scientific article is to obtain scientific information about the pattern composition of MG, DG and TG in palm kernel oil (PKO) ethanolysis products at three stages and of the mole ratios of ethanol in ethoxy solution (ethanol 90% - NaOH 1%) to PKO (assumed as trilaurin).

2. Materials and methods

2.1 Materials and equipment

Fresh 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%, aquadest, n-hexane, indicator pp, N- Methyl-N -trimethylsilyl-trifluoroacetamide (MSTFA), THF, and heptane. The equipment 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

The peparation 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 the 90% ethanol-1% NaOH solution to PKO equal to 10 (w/w), as follows: It is known that MW of ethanol is 46 and MW of PKO (as trilaurine) is 638. For the experimental unit of 120 g or 0.1881 mol of PKO and a mole ratio of the ethanol solution to PKO equal to 10, it required the ethanol solution of 1.881 mol or 86.5 g of ethanol 100% and 1.2 g of NaOH pellets. Furthermore, 1.2 g of NaOH was dissolved in 9.6 g of distilled water until the NaOH completely dissolved, then added 86.5 g of ethanolto obtain an ethoxy solution of 90% ethanol containing 1% of NaOH. The solution was then mixed with 120 g PKO with mol ratio of 10. 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

Ethanolysis of PKO has 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 mins, 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 have 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.

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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%, 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±0.46%, 11.20±2.61%, and 11.78±2.99%, respectively.

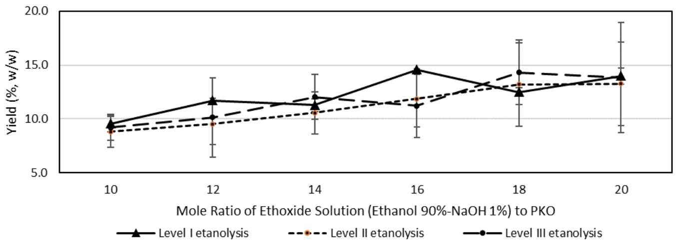

Figure 1. Yield of level I, II, and III PKO Ethanolysis products for the mole ratios of 10, 12, 14, 16, 18, and 20, respectively.

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. The treatment with the weight ratio of 90% ethanol solution-1% NaOH (w/w PKO) to PKO equal to 1.4 (w/w) was better than the treatment with the ratio of 1.0 (w/w) and 1.2 (w/ w) (Murhadi *et al*., 2019). It is noted 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 in the yield of PKO ethanolysis products presumably was because of the increased presence of ethoxide ion solution which increased 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± 9.74% (w/w) for the treatment of the mole ratio of 20, while the lowest yield was 27.54±3.39% (w/w) for the treatment of mole ratio of 10. The results of this study were in line with a 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.

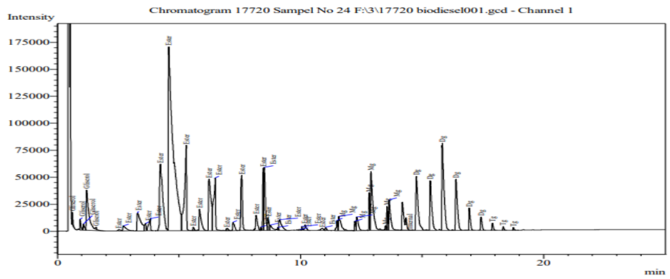

Figure 2. GC chromatogram as a result of identification of MG, DG, TG and other components in level I PKO Ethanolysis products for a mole ratio of 10.

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.

Based on the data in Table 1, the average content of monoglycerides (MG), diglycerides (DG), and triglycerides (TG) for all treatments were 8.26±2.17%, 14.40±4.66%, and 0.77±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 mins 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%, respectively. The types of monoglycerides that dominated ethanolysis products were monolaurin and monomyristin, which amounted to 7.07% 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

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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	11.30	20.67	0.65	3.37	61.81	2.21	100
2	N12T1U1	6.20	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	11.30	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	3.60	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	1.90	100
12	N20T2U1	5.69	10.51	0.80	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	79.00	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	20.00	0.77	4.66	60.76	2.52	100
24	N20T1U2	11.60	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	5.30	9.29	0.89	5.55	76.95	2.02	100
29	N18T2U2	8.90	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	2.10	74.76	1.84	100
34	N16T3U2	6.75	9.74	0.52	1.52	79.67	1.80	100
35	N18T3U2	8.20	11.69	0.58	2.80	74.65	2.08	100
36	N20T3U2	7.24	11.53	0.88	3.63	74.46	2.26	100
Average		8.26	14.40	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.

ratio (10, 12, 14, 16, 18, and 20) are presented in Figure 3 (A, B, and C).

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

At the ethanolysis level I, the highest average percentage of MG content was obtained by the treatment of mole ratio of 10 (11.16±0.19%), while the highest percentage of DG was produced the treatment of the mole ratio of 16 (22.70±1.86%). At the ethanolysis level II, the highest percentage of MG was obtained by the mole ratio treatment of 10 (10.72±0.84%) and the

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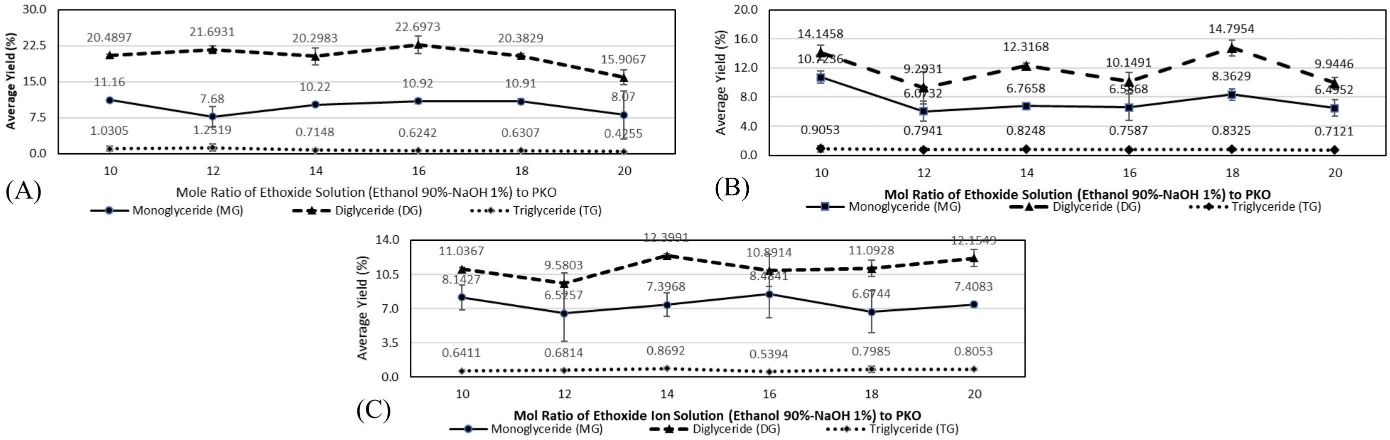

Figure 3. Pattern composition of MG, DG and TG in PKO ethanolysis level I (A), level II (B) and level III (C) at mol ratios of 10, 12, 14, 16, 18, and 20.

highest percentage of DG was obtained by the mole ratio treatment of 18 and 10 (14.80±1.09% and 14.15±1.04, respectively). In terms of the 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 ethanolysis level III products were relatively the same, based on the standard deviation value, so it is more efficient to use the smallest mole ratio treatment, such as a mole ratio of 10.

4. Conclusion

The fatty acids that make up crude PKO were dominated by eleven 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 eleven types of fatty acids contained in PKO, two dominant fatty acids contribute to the properties of antimicrobial activity, that were lauric acid (C12:0) and myristic acid (C14:0) which are 48.99% and 15.77%, respectively, so that the total of the two types of acids reached 64.76%.

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

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