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FULL PAPER

The analysis of the glyceride components on the treatment variation of refined bleached deodorized palm oil by gas chromatography method

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Abstract

The glyceride components such as glycerol, ester, mono and diglyceride are useful components for food ingredients like food emulsifiers. One of the natural resources of the glyceride component is refined bleached deodorized palm oil (RBDPO). This research was aimed to analyze the glyceride component of the treatment variation of RBDPO. The design of the research was completely randomized design (CRD) non-factorial with three variables of treatment specifically the 9 g of RBDPO with 5 g glycerol (A), RBDPO (B) and RBDPO with 0.7 g lipase enzyme *Thermomyces lanuginosus* immobilized (TLIM). The concentration of glycerol, ester, mono- and diglyceride was tested by the gas chromatography method. The data will be analyzed by using a descriptive method with a boxplot and histogram. The results showed that the highest concentration of glycerol, ester, mono- and diglyceride, respectively were shown in treatment B (1.5922%), C (9.5699%), C (0.1783%), C (3.3329%). The boxplot graphic described that there was statistically significant difference among the treatments.

1. Introduction

The Refined Bleached Deodorized Palm Oil (RBDPO) is a derivative product of crude palm oil after having undergone a process of removing non-oil components such as free fatty acids, colloids, metals, pigments and aromas. The RBDPO is a triglyceride which at room temperature has two fractions, namely the liquid fraction (olein) and the solid fraction (stearin) (Subroto *et al*., 2018). The RBDPO was hydrolyzed naturally by heating treatment or adding other components like glycerol and lipase. Every one mole of the triglyceride that reacts with glycerol or lipase enzyme can produce monoglyceride (MG), diglyceride (DG) and the presence of excess glycerol. The processing of triglyceride at a high temperature leads to an equilibrium mixture of glycerol and esters (Affandi *et al*., 2011). The important use of the glyceride component such as glycerol, ester, mono and diglyceride are good emulsifiers for various food emulsions, such as margarine, shortening, mayonnaise and chocolate products. The alteration of glyceride components in RBDPO is obtained through hydrolysis, glycerolysis, esterification reactions using triglycerides, water, glycerol or alcohol as reactants with TLIM lipase as catalyst.

So, it is important to know the variation of the glyceride alteration in RBDPO both naturally, the addition of glycerol or lipase components. The variations in the treatment of the RBDPO need to be done to determine the various derivative products that can be obtained from the RBDPO especially in glyceride components (Santoso, 2016). Therefore, this research will study the analysis of the glyceride components on the treatment variation of refined bleached deodorized palm oil by gas chromatography method.

2. Materials and methods

2.1 Research design

The research was carried out from June - October

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2020. The place where the research was conducted was the Plantation Agribusiness College (STIPAP) and Universitas Sumatera Utara (USU).

The equipment used was a water bath shaker (Memmert), analytical scales (Sartorious), Erlenmeyer 100 mL (Pyrex), test tube, centrifuge (Centurion), centrifuge tube, refrigerator (Samsung), mesh filtration, Gas Chromatography (GC Series 2010 Plus, Shimadzu). Meanwhile, the materials used were Refined Bleached Deodorized Palm Oil (RBDPO), TL IM lipase enzyme (Novozyme), glycerin (Merck), alcohol (Technical), silica gel.

This study used a completely randomized design (CRD) Non-Factorial, with three variable treatments namely the 9 g of RBDPO with 5 g glycerol (A), RBDPO (B) and RBDPO with 0.7 g lipase enzyme TLIM. The products were tested by using gas chromatography apparatus and the results were analyzed.

2.2 Analysis of data

The results were analyzed descriptively using a chromatogram of GC, boxplot graph, then the differences were described by histograms.

2.3 Research procedure

The raw material was 9 g of RBDPO with a mixture of 5 g of glycerol in treatment A, without mixture in treatment B and a mixture of 0.7 g of TL IM lipase enzyme in treatment C. Then, 5 mL of alcohol (85%, technical) was added to each treatment. Then, the mixture was agitated at 60^{o}C using a water bath shaker at 170 rpm (Kaewthong *et al*., 2005) for 24 hrs (Zakwan *et al*., 2017). The suspension formed was diluted with 10 mL of technical alcohol and separated using a centrifuge at 1000 rpm (Zakwan *et al*., 2017) for 5 mins (Palacios *et al*., 2019).

2.4 Gas chromatography

The glyceride component was tested using the AOCS Cd 11b-91 method. Approximately 10 mg of homogenized test sample pipetted into a 2.5 mL screw-cap vial with Teflon^{TM}-face septa. At about 0.2 mL BSTFA (N, N-bis-trimethylsilyl-trifluoroacetamide) and 0.1 mL TMCS (Trimethylchlorsilane) and 0.1 mL of internal standard solution (tricaprin) was added. The moisture had to be excluded strictly. The vial was closed and shook vigorously. The reaction mixture was heated at 70^{o}C for approximately 20 mins. At about 1-5 µL of the reaction mixture was injected into the gas chromatography and analyzed (avoid the delay). The reaction was carried out two times and two injections are made. The reference solution: approximately 0.10 mL of the reference solution and the silylating agent, 0.2 mL BSTFA and 0.1 mL TMCS (no internal standard solution) was added. The reaction mixture was heated and injected into gas chromatography. The concentration range of reference standards similar to the range of the components was used to be quantified in the test solution. A plot of response vs. concentration of reference substances was useful to check linearity. The response factors had to be checked periodically (AOCS, 2020).

3. Results and discussion

3.1 Determination of glyceride components using GC analysis

Figure 1 was three chromatograms that verified the presence of the internal standard namely tricaprin used in this study at the same concentration, identified by its retention time. The result showed that the glycerol, esters and diglyceride were present at all chromatogram types, while the monoglyceride was only at chromatogram type C. According to Hobuss *et al.* (2020) that esters eluted between 10 and 16 mins retention time, monoglyceride appear in the range 17 mins, diglyceride appears after 27 mins and triglyceride was detected in chromatogram after 35 mins.

3.2 The result of glyceride component tested

The treatment had different levels of glycerol, esters, mono- and diglyceride that could be seen in Table 1.

Table 1. The result of glyceride components tested by the Gas chromatography method

Treatment	Glyceride components (%)			
	Glycerol	Ester	MG	DG
A	1.5129	5.4753	Undetected	0.4763
B	1.5922	3.1583	Undetected	1.3491
C	0.6981	9.5699	0.1783	3.3329

Treatment A: RBDPO with glycerol, Treatment B: RBDPO, Treatment C: RBDPO with lipase enzyme, MG: Monoglyceride, DG: Diglyceride.

Table 1 shows that the monoglyceride component was detected in treatment C at about 0.1783% but undetected in treatments A and B. The glycerol, ester, and diglyceride components could be detected in all treatments. The presence of esters was due to a heating treatment at 60^{o}C. Even without a catalyst, esters could still appear in heated carboxylic acids. Meanwhile, the use of a catalyst such as a lipase enzyme caused the formation of esters more consistently and higher. Mono and diglycerides were the products obtained before the reaction of ester formation ran completely. The glycerol was formed with esters formation (Otera and Nishikido, 2010).

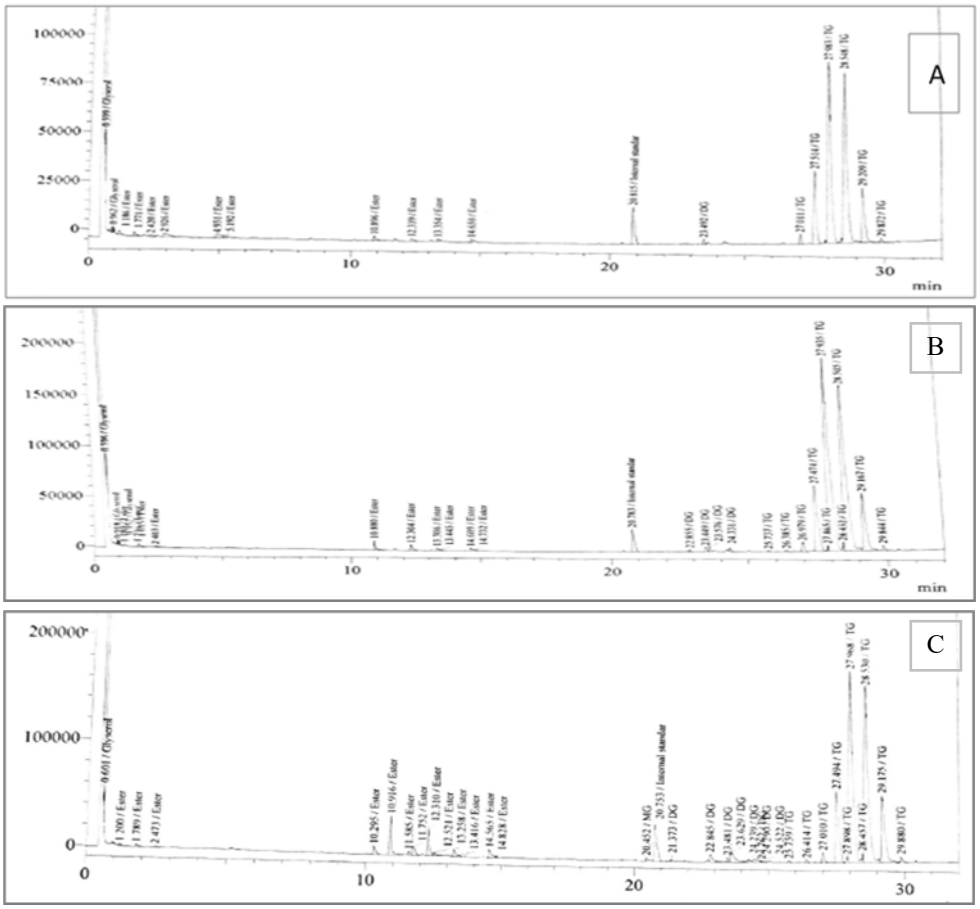

Figure 1. Chromatograms of (A) the RBDPO with glycerol, (B) the RBDPO, (C) the RBDPO with lipase enzyme.

In Figure 2, the boxplot in treatment C had a wide distribution (the highest interquartile) followed by treatment A and B, respectively. The upper position of the boxplots was in treatment C, which means that they generally had larger glyceride components in comparison to treatments A and B. Such evidence was also supported by the fact that the minimum value in treatment C was above the maximum value of treatment A and B.

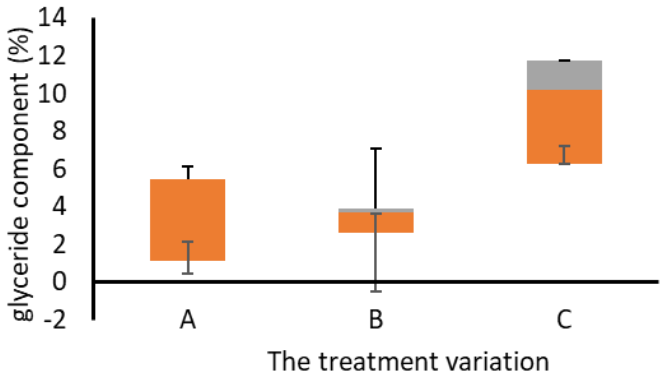

Figure 2. Boxplots for the variation of treatments of A: RBDPO with glycerol, B: RBDPO and C: RBDPO with lipase enzyme.

In the median of each treatment variation, there were differences in the levels of glycerol, ester, monoglyceride, and diglyceride. Furthermore, from Figure 2 could be seen that there were no outliers. The boxplot of the 'C' treatment showed that the vertical box was the highest one. It means that The 'C' treatment had the more widespread data in the glyceride components (Ferreira *et al*., 2016). Figure 1 describes that the amount of the glyceride picks in the 'C' chromatogram was the most one at about 17 peaks.

3.3 The glyceride components and the treatment variation

Figure 3 shows the differences of glycerol, ester, monoglyceride, and diglyceride in the variation of RBDPO treatment.

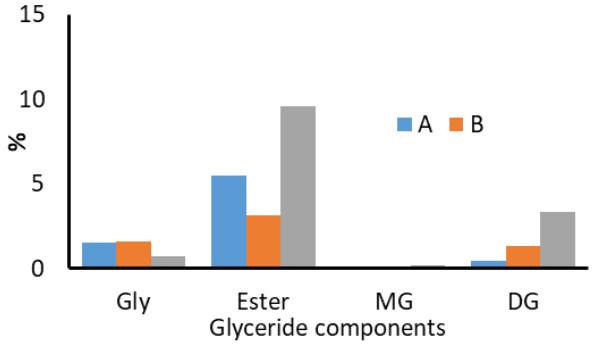

Figure 3. The concentration of glycerol (Gly), esters, monoglycerides (MG) and diglycerides (DG) in the variation of treatments of A: RBDPO with glycerol, B: RBDPO and C: RBDPO with lipase enzyme.

The glyceride components were glycerol (0.7%), ester (9.6%), monoglyceride (0.2%) and diglyceride (3.3%) in the 'C' treatment (the RBDPO with lipase

enzyme). Meanwhile, in the other treatments, monoglyceride components were not detected. It meant that the presence of the lipase enzyme was able to catalyze the RBDPO to produce monoglycerides. According to Wei *et al.* (2020), lipase could catalyze the formation of monoglycerides and diglycerides in triglyceride hydrolysis reactions.

The esters concentration in treatments C, A and B were 9.57%; 5.48% and 3.16%, respectively. The highest ester component was found in treatment C (the RBDPO with lipase enzyme). This was according to the opinion of Otera and Nishikido (2010) who reported the use of the lipase enzyme caused the formation of esters to be faster in the equilibrium reaction.

4. Conclusion

The adding of the lipase enzyme to the refined bleached deodorized palm oil could catalyze the emergence of the glyceride components such as glycerol, ester, monoglyceride and diglyceride components. The ester level in the reaction using the lipase enzyme was higher than the reaction using the glycerol.

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