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Production mono-diglyceride (MDG) from refined deodorized palm oil (RBDPO) by enzymatic process

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Abstract

The objective of this research was to know the best reaction time of glycerolysis processing. Esterification of glycerol and RBDPO using lipase as catalyst was used to synthesize MDG. Eight batch reactions consisting of refined bleached deodorized palm oil (RBDPO), glycerol, and commercially lipase were carried out. Production yield, glyceride (MG, DG and TG) composition, Free Fatty Acid (FFA), peroxide value, iodine value, melting point, and HLB value were used as parameters. Monoglyceride (MG), diglyceride (DG) and triglyceride (TG) composition in the product were analyzed using thin layer chromatography (TLC) and TLC Scanner. The best reaction time identified at about 24 hours. Under this condition production yield was 78,70% with MG, DG, TG was 40,16%; 49,92%; 10% respectively. The Analysis of physicochemical properties showed that the average FFA, peroxide value, iodine value, melting point, and HLB value were 0.16%, 5.33 meq/kg, 46.33, 59.83oC, and 8.97, respectively. The product melting point was higher and the iodine value was lower than RBDPO i.e. 53.5˚C and 53.99, respectively with FFA content was 0.14%.

Introduction

The total production of vegetable oil/fat in the world on 2013 is about 189.5 million tons which expectedly, it will reach 236 million tons on 2020 (Direktorat Jenderal Perkebunan, 2012). The palm tree has the main product that called Crude Palm Oil (CPO). Indonesia is one of the CPO production country in the world that produces CPO up to 26 million tons on 2012 (Direktorat Jenderal Perkebunan, 2012).

According to Hasan (2013), the production of CPO in Indonesia reach 28 million tons on 2013. In Indonesia, at about 75% CPO is exported and only 25% for domestic requirement. It means that the prospect of palm oil industry would be still potential by which processing CPO to become the high value products is required. Indonesian government responded this condition by increasing the export tax to limit the exportation of CPO, in order to develop the downstream industries of CPO (An, 2008).

Palm oil is resulted by extracting the mesocarp of palm fruit to become CPO. The cooking oil is obtained by next processing to produce refined deodorized palm oil (RBDPO). Many derivative products of palm oil, such as emulsifier are used as food stabilizers. Emulsifier is a product with special characteristic which able to mix water and oil.

Mostly, the emulsion products use emulsifier, for example margarine, mayonnaise, drugs, and cosmetics. Because of that, the emulsifier has the high economic value. The import dependence of emulsifier can be reduced by domestic production. Approximately, 70% of food emulsifier is the mixture of mono- and diglycerides. MDG is produced by glycerolysis process of both oil and glycerol or fatty acid esterification and glycerol (O'Brien, 1998).

The MDG needs in food industry is high, but it is still imported. So, there is a good chance to develop and produce the MDG product in Indonesia. According to Anggirasti (2008), MDG is able to be obtained by glycerolysis process and using lipase catalyst with hexane as the solvent. This research investigates the production of MDG with different solvent and concentration of catalyst. Therefore, the aim of this research is to determine the best time to produce MDG from RBDPO by glycerolysis process.

Saberi *et al.* (2011) has conducted research about glycerolysis enzymatic reaction on a mixture of palm oil and diglycerides. From this research known that the glycerolysis enzymatic process of palm oil did not change fatty acid composition significantly. It's proved by the analysis results of fatty acid composition palmitic;Oleic (as the main fatty acid) of oil before and after the process gliserolisis respectively 44.7;38.6% and 45.6;38.4%.

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According to Kaewthong (2005), the glycerolysis process of using enzyme lipase TLIM mole ratio of 1: 3 between palm olein oil with glycerol will produce MDG with MG composition 24%. According to Watanabe (2003), before starting the reaction, the glycerol to be adsorbed by silica gel to obtain high yields and optimum reaction rates. The reaction time effect on the levels and types of MG formed (Myrnes, 1995).

Material and Method

The raw materials of this research are RBDPO and MDG standard (dimodan, MG=96%) from PT. Pacific Medan Industry (Medan, Indonesia), Lipozyme TLIM from Novozyme A/S (Bagsvaerd, Denmark), Glycerol (Bratachem, Indonesia), Silica Gel 60 H (Merck, Germany), n-alcohol (Bratachem, Indonesia) and chemicals for analysis.

Gas Chromatography (GC) system (Varian CP 3800), Thin Layer Chromatography (TLC) (Merck, TLC SG F254), chamber glass, desikator, waterbath shaker (Stuart,SBS 40), analitical balance (Sartorious, TE 2145), sentrifuse (Hitachi), refrigerator, oven (Memmert, 854 Schwabach), TLC-Scanner (Camag), TLC-photoviewer (Camag), filtration, hotplate (Maspion) and glass equipment (pyrex) are using in this research. This research was done to determine the optimal time of glycerolysis process in producing MDG. The temperature used was 60^{o}C (Anggirasti, 2008) and time (6, 12, 18 and 24 hour).

Chemical properties of raw material

The analysis of RBDPO quality was moisture content (AOAC, 1995), free fatty acid content (AOAC, 1995), peroxide value (AOAC, 1995) and iodine value (SNI, 01 3555 1998).

The synthesis of MDG

The substrate (14 g) was RBDPO and glycerol with a ratio of 1:5 (mol/mol) (Damstrup *et al*., 2005). The glycerol mixed with silica gel (ratio 1:1, w/w) before reacting with RBDPO. The lipase enzyme (7%, w/w oil) and 5 ml alcohol (85%, technical) was added to the reaction (Torres *et al*., 2002; Yang *et al*., 2005). Then, the mixture was agitated at 60^{o}C by using waterbath shaker at 200 rpm (Kaewthong *et al.*, 2005) until the reaction time over. The suspension was diluted with alcohol 10 ml and separated by using sentrifuse at 1.000 rpm (Anggirasti, 2008). The product was fractionated through winterization at 7^{o}C for 16 h (Zaelani, 2007). The solid fraction was MDG and was sparated from liquid by filtration.

Table 1. The composition of raw material (RBDPO)

Parameter	Reference	Result
Moisture content (%)	Max. 0.10 (SNI, 1987)	0.13
FFA content (%)	Max. 0.15 (SNI, 1987)	0.17
Peroxide value (meq/kg)	Max. 5.00 (Willis *et al.*, 2002)	4.63
Iodine value	50-55 (SNI, 1987)	53.99

The parameter analysis of MDG

The MDG product was analyzed using parameters : yield, gliceride composition (TLC Method) (Anggirasti 2008), moisture content (AOAC 1995), free fatty acid content (AOAC 1995), peroxide value (AOAC 1995), iodine value (SNI, 01 3555 1998), melting point (AOAC 1995), hydro-lipophilic balance value (HLB) (modified from Barus, 2014).

Results and Discussion

Chemical properties analyzed of raw material

The chemical properties of RBDPO, like moisture and FFA content, peroxide and iodine value are showed in Table 1. Moisture content was a quality parameter of palm oil that could affect FFA content in product. The higher moisture content caused the hydrolysis reaction that effected breaking and increasing FFA in oil. So, it took effect in the gliserolysis processing. Moisture content in RBDPO in the present research was about 0.13%. According to SNI 1987, the moisture content standard of RBDPO maximum 0.1%. The moisture content of RBDPO is higher than standard because the processing in laboratorium is not exactly same with SNI Standard. The FFA of RBDPO in this research was about 0.14%. According to SNI (1987), FFA in oil maximum 0.15%, this mean that FFA in RBDPO was still in the standard range. FFA was an important factor in transesterification effectivity. The higher FFA caused the reduction of pH value. The lower pH value could decrease the lipase activity (Willis and Marangoni, 2002).

The PV in RBDPO used was approximately 4.49 meq/kg. According to Willis and Marangoni (2002), peroxides (PV>5) could influence interesterification reaction and increase the oxidation activity. The higher oxidation caused the RBDPO quality become lower. The IV indicate the saturation level of fatty acids in oil. The more iod adsorbed by oil, the more unsaturated it is. The IV of RBDPO was about 53.99, it was still in the range of SNI (1987) that IV standard of RBDPO within 50-55.

Determination of the optimal reaction time

The result of parameters analysis of MDG

Table 2. The result analysis of MDG products

Treatment / Parameter	RBDPO	Temperature 60°C			
		6 h	12 h	18 h	24 h
Yield (%)	-	65.98	73.48	77.48	78.70
MG (%)	0	23.98	36.48	38.35	40.16
DG (%)	0	29.88	31.53	39.89	49.92
TG (%)	100	46.14	31.99	21.76	10
FFA (%)	0.14	0.15	0.16	0.16	0.16
PV (meq/kg)	4.63	4.67	4.66	5.32	5.33
IV (mg iod/g)	53.99	47.77	47.50	47.41	46.33
MP (°C)	53.5	54.75	57.42	58.25	59.83
HLB value	-	9.92	9.50	9.32	8.97

*) MG:Monoglyceride, DG:Diglyceride, TG: Trigliceride, FFA:Free Fatty Acid, PV:Peroxide Value, IV:Iodine Value, MP:Melting Point, HLB:Hydro-Lipofilik Balance.

product such as MG, DG and TG composition, FFA content, PV, IV, melting point and HLB value are shown in Table 2. The reaction time is an important factor in producing MDG because it directly relates to reversible reaction of glycerolysis. This research evaluated the optimal reaction time of glycerolysis to produce high MDG. The result of MDG product analysis is shown in Table 2 and Figure 1, the highest yield (78.70%), MG (40.16%) and DG (49.42%) was reached at 24 hours. This condition was expected nearly equilibrium, because all TG almost reacted with glycerol in MDG product. It was proven that the least content of TG (10%) was reached at 24 hours (Table 2).The best MDG product showed by high yield and low TG value. The solvent ratio used to elute the MDG's band at TLC plate was respectively petroleum ether : diethyl ether : acetic acid glacial (70 : 30 : 0.2 ; v/v/v) (Gunstone *et al.*, 1994). The TG is more nonpolar fraction compared to DG and MG. The elution time of MDG's band on TLC plate was at about 10 minute.

Commercially, MDG product had various content (MG : 40, 50, and 90%) that depended on the product processing (O'Brein 1998). The emulsifier with high MG was more compatible for oil/water emulsion because it could be dissolved perfectly in oil/fat and dispered in water with certain condition (Gunstone *et al.,* 1994). Table 2 shows that MDG product had the content of MG 40% while MDG standard had MG 90% (dimodan, from PT. Pacific Medan Industry).

The FFA of MDG product was about 0.16% (Table 2). It means that the MDG product had fulfilled the food additive standard (FFA max. 0.2%). The high FFA content would increase the forming of peroxide compound, aldehide, ketone, and polymer. The oxidation could change the flavour composition of oil/fat. The peroxide, aldehide, and ketone cause rancidity and browning (Ketaren, 2005).

Table 2 shows IV of MDG product was about 47.06. According to O'Brein (1998) this MDG was clasified in hard emulsifier form, that suitable for various product like bakery, candy, paste, peanut

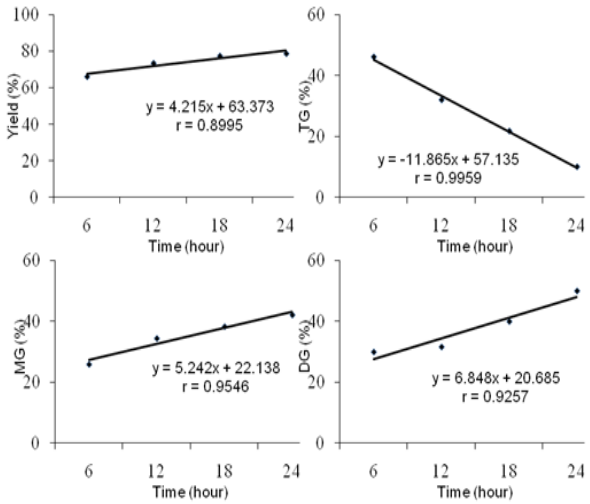

Figure 1. The relationsip between the best reaction time and parameters of MDG product (MG, DG, and TG).

butter, margarine and frozen foods. The MP is the temperature at which it changes state from solid to liquid at atmospheric pressure (O'Brien, 1998).

The MP is the important parameter in MDG application, and customer acceptance. Table 2 shows the MP of MDG at about 59.83°C and MP of RBDPO at about 53.5oC. It means that the higher MG, the higher MP of MDG product would be. According to Stauffer (1996), MG is classified in lipophilic emulsifier (HLB 4-9), suitable at water in oil (w/o) emulsion (required HLB at 2-6.5) and oil in water (o/w) emulsion (required HLB at 8.5-16.5). The HLB value of MDG product was about 8.97 (Table 2). It means that the MDG product is classified in o/w emulsion (used to produce mayonnaise).

Conclusion

The RBDPO which is used in this research has moisture content (0.13%), FFA (0.17%), PV (4.63 meq/kg), and IV (53.99). The best reaction time to produce MDG was about 24 hours with yield, MG, DG and TG content 78.70, 40.16, 49.92 and 10%, respectively. The best MDG product has FFA, PV, IV, MP and HLB, respectively at about 0.16%, 5.33 meq/

kg, 46.33, 59.83^{o}C, and 8.97.

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