

Characterization of Epoxidized and Non-Epoxidized Fatty Diethanolamides by High Performance Liquid Chromatography and Gas Chromatography

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Abstract: Problem statement: This study was carried out for the characterization of epoxidized and non-epoxidized fatty diethanolamides by High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). **Approach:** The fatty diethanolamides were synthesized by reacting 40% (w/w) of Epoxidized Palm Olein (EPO_o) with 60% (w/w) Refined Bleached Deodorized Palm Kernel Olein (RBDPKO_o) with Diethanolamine (DEA) at 110°C for 5 h. **Results:** The identities of the peaks were established by secondary standards that were derived from pure methyl esters. HPLC separated major non-epoxidized diethanolamide compounds, namely C_{10:0}, C_{12:0}, C_{14:0}, C_{16:0}, C_{18:1} and C_{18:0} and the molecular weights of the compounds were identified by Liquid Chromatography-Mass Spectrometry (LC-MS). **Conclusion:** GC provided a more complete separation for the non-epoxidized diethanolamides, epoxidized diethanolamides, glycerol, Diethanolamine (DEA) and some minor compounds from the self-condensation of DEA and diethanolamides. Gas Chromatography-Mass Spectrometry (GC-MS) elucidated the chemical structure of the epoxidized diethanolamides.

Key words: Diethanolamides, gas chromatography, gas chromatography-mass spectrometry, high performance liquid chromatography, liquid chromatography-mass spectrometry

INTRODUCTION

Reaction of fatty acids or fatty acid methyl esters with alkanolamine produces fatty alkanolamides, such as monoalkanolamide and dialkanolamide (Shapiro, 1968). Fatty alkanolamides were prepared by reacting animal or vegetable-based triacylglycerols with different classes of alkanolamines, such as ethanolamine and diethanolamine (Feairheller *et al.*, 1994; Kolancilar, 2004). The fatty alkanolamides are versatile oleochemicals that usually used as non-ionic surfactant in cosmetic, personal care and house-hold products. Besides, they can also be used as polyols for the production of rigid polyurethane foams. The fatty alkanolamides for polyurethane were prepared by reacting natural oils and fats, i.e., tallow, soybean, linseed, peanut, safflower oil and palm oil with alkanolamines at elevated temperature (Khoe *et al.*, 1973; Khoe and Frankel, 1976; Badri *et al.*, 2004; Rahman *et al.*, 2003; Lee *et al.*, 2007; Bononi and Tateo, 2007). As the world progress, the industries are paying more attention to produce polyols from

vegetable oil considering that petroleum is diminishing rapidly. Epoxidized diethanolamides as starting material of rigid polyurethane foam were prepared from epoxidized palm olein (Lee *et al.*, 2009)

In this study, fatty diethanolamides were synthesized from the mixture of Refined Bleached Deodorized Palm Kernel Olein (RBDPKO_o) and Epoxidized Palm Olein (EPO_o) with Diethanolamine (DEA) reagent. The synthesized diethanolamides were mixture of epoxidized and non-epoxidized fatty acid diethanolamides ranging from C₈-C₂₀ (Fig. 1). The epoxides in fatty diethanolamides provide additional cross-linking sites by forming oxazolidone linkages in the polyurethane network and improved the properties of the rigid polyurethane foam, in terms of thermal and chemical properties (Lee *et al.*, 2007).

The potential of these fatty diethanolamides are no doubt noticeable and to prepare the production of these fatty diethanolamides in plant scale, a reliable method for the analysis and characterization of the fatty diethanolamides is needed, for the purpose of process development and quality control.

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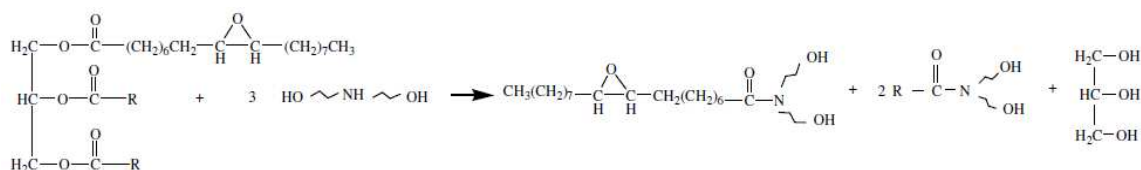


Fig. 1: Synthesis of epoxidized and non-epoxidized fatty diethanolamides

Common analyses are hydroxyl value determination, Fourier Transform Infrared spectroscopy (FTIR) and Thin Layer Chromatography (TLC) to determine the completion of the reaction (Badri *et al.*, 2004; Rahman *et al.*, 2003). Gel Permeation Chromatography (GPC) had been used to analyze the average molecular weight of the vegetable oil-based polyols (Badri *et al.*, 2001). The chemical identification of the fatty diethanolamides by spectroscopy methods are not widely used. In this study, we used HPLC and GC chromatographic method followed by LC-MS and GC-MS spectrometric method for the simultaneous analysis of fatty diethanolamides polyols.

MATERIALS AND METHODS

Materials: RBDPKOo (specifications: iodine value = 26 mg I₂/100 g sample, free fatty acids = 0.10 mg KOH/g sample, saponification value = 250 mg KOH g⁻¹ sample) was obtained from Kempas Oil Sdn. Bhd. and EPOo (specifications: oxirane oxygen contents = 3.0%, acidity = 1.1 mg KOH/g sample, iodine value = 0.65 mg I₂/100 g sample) was obtained from the pilot plant of Advanced Oleochemicals Technology Division (AOTD) of Malaysian Palm Oil Board (MPOB). Reagents: Diethanolamine (DEA) (BASF), dimethylformamide (J.T. Baker), N, O-bis(trimethylsilyl)-trifluoroacetamide, 98+% (BSTFA) (ACROS ORGANICS), were used as purchased.

Synthesis of fatty diethanolamides: The reaction was carried out using a 1 litre Pyrex glass reaction flask submerged in a silicone bath. The reaction flask was equipped with a condenser, thermometer and a magnetic stirrer. 40 % EPO_o (w/w) was blended with 60% (w/w) RBDPKOo to react with DEA at molar ratio of 1:3 [palm oil Triacylglyceride (TAG): DEA]. The reaction was carried out at 110°C for 5 h. The blend of starting materials and reaction condition were the optimal percentage and reaction condition based on previous study on the synthesis of fatty diethanolamides.

HPLC analysis:

Test samples: The samples were taken from the synthesis of fatty diethanolamides from 40% (w/w) EPOo and 60% (w/w) RBDPKOo.

Validation: There were no standard for fatty diethanolamides, therefore secondary standards were synthesized from pure methyl esters, such as methyl laurate (C_{12:0}), methyl myristate (C_{14:0}), methyl palmitate (C_{16:0}), methyl stearate (C_{18:0}) and methyl oleic (C_{18:1}). These esters were reacted with DEA at 110°C for 5 h.

Instrument and condition: The HPLC system was equipped with a binary gradient pump, an automatic, three-line degasser, a column oven and an ELSD. Data were recorded and analysed using Windows-based Intuitive Software for Chromatography (Borwin, Version 1.2; JBMS Developments, Le Fontanil, France). A packed Inertsil ODS 3 column (4.6×250 mm; GL science Inc, Rockford, IL) was used for separation. The column was maintained at 30°C and the mobile phase 87 used was a mixture of 95:5 Acetonitrile (ACN) and water at a flow rate of 0.5 mL min⁻¹. The ELSD detector was set at 40°C and run under 2.3 bar air pressure. The sample solution (approximately 0.02 g in 10 mL CAN) was passed through a 0.45 μm disc filter to an auto sampler vial. A aliquot of about 20 μL was injected into the HPLC system.

LC-MS analysis: The analyses were performed on a Perkin Elmer LC-200 pump fitted with a Rheodyne 8125 20 μL loop injector. The LC column was connected to a single quadrupole Perkin Elmer Sciex API 100 LC-MS system equipped with a heated nebuliser interface. The separations were performed on a packed Inertsil ODS 3 column (4.6×250 mm; GL Science Inc., Rockford, IL). The separation was carried out with acetonitrile-water at 95:5. The flow rate was set at 0.08 mL min⁻¹ and 10 μL of the sample was injected into the LC-MS system.

GC analysis:

Standard solutions: The samples were prepared by adding in 1 mL of DMF into 0.03 g samples to dissolve the samples, 0.1 mL Bis(Trimethyl Silyl)tri Fluoro Asetanida (BSTFA) was then added. The mixture was shake vigorously and warmed at 60°C for 15 min before analyzing by gas chromatography. This procedure allowed the silylation of the samples to take place. The sample was then transferred to a 2 mL auto-sampler vial for injection into the GC system.

Instrument and condition: The SGE HT-5, Polyimide clad, 12 m×0.53 mm×0.15 µm column was used in the gas chromatograph study. The injection was made at 80°C, hold for 8 min and the temperature of the injector and column oven were raised by 5°C min⁻¹ to 220°C. The inlet temperature and the detector temperature were 250°C. Helium gas was used as the carrier gas and the flow rate for the column was 27.0 mL min⁻¹. Nitrogen gas was used as the makeup gas.

GC-MS analysis: Mass spectrometric data was carried out on an Agilent 5973 Network Mass Selective detector interfaced to an Agilent 6890 Series gas chromatograph with a septum-equipped programmable Agilent 783 Series injector. The HP-5 ms column, 30 m×0.25 mm id, 0.25 micron m film was used in the gas chromatographic study and was connected directly to the Electron Impact (EI) source (70 eV). The injection was made at 80°C, hold for 50 min and the temperature of the injector and column oven was raised by 5°C min⁻¹ to 270°C. The inlet pressure of the helium carrier gas was 8.67 psi and the flow rate was 1 mL min⁻¹ with the MSD detector was used. The inlet temperature was 280°C whereas the auxiliary set point was 290°C. The

samples were dissolved in THF and derivatized by BSTFA before analyzing by mass spectrometry.

RESULTS

Table 1 shows the retention time of the fatty diethanolamides by HPLC analysis. The analysis was continued by using LC-MS and the molecular weights of the fatty diethanolamides are shown in Table 2.

Table 1: Components of the diethanolamides and retention time by HPLC analysis

Component	Retention time (min)
DEA	5.6
Decanoic acid bis-(2-hydroxy-ethyl)-amide / C _{10:0} diethanolamide	6.5
Dodecanoic acid bis-(2-hydroxy-ethyl)-amide/ C _{12:0} diethanolamide	8.9
Tetradecanoic acid bis-(2-hydroxy-ethyl)-amide/ C _{14:0} diethanolamide	11.8
Hexadecanoic acid bis-(2-hydroxy-ethyl)-amide/ C _{16:0} diethanolamide	16.3
Octadec-9-enoic acid-bis-(2-hydroxy-ethyl)-amide/ C _{18:1} diethanolamide	17.2
Octadecanoic acid bis-(2-hydroxy-ethyl)-amide/ C _{18:0} diethanolamide	23.6

Table 2: Selected LC-MS fragment ions of the fatty 321 diethanolamides

Diethanolamides	Molecular weight of diethanolamides	Fragment ions (m/z)
Decanoic acid bis-(2-hydroxy-ethyl)-amide	259	488.5, 323.0, 282.5, 260.0 [(M-H) ⁺ , 100%], 142.0
Dodecanoic acid bis-(2-hydroxy-ethyl)-amide	287	488.5, 392.0, 350.5, 288.0 [(M-H) ⁺ , 100%], 146.0
Tetradecanoic acid bis-(2-hydroxy-ethyl)-amide	315	419.5, 378.0, 316.0 [(M-H) ⁺ , 100%], 232.5, 147.0
Hexadecanoic acid bis-(2-hydroxy-ethyl)-amide	343	447.0, 365.5, 344.0 [(M-H) ⁺ , 100%], 288.5, 147.0
Octadec-9-enoic acid-bis-(2-hydroxy-ethyl)-amide	369	472.5, 391.0, 370.0 [(M-H) ⁺ , 100%], 217.5, 147.0
Octadecanoic acid bis-(2-hydroxy-ethyl)-amide	371	472.5, 393.5, 372.0 [(M-H) ⁺ , 100%], 288.5, 146.0

Table 3: Composition of trimethylsilyl derivatives of fatty diethanolamides and 351 retention time

Component	Retention time (min)	w/w (%)
Glycerol	0.846	21.76
DEA	1.554	0.79
Unknown	2.383	0.28
C ₁₂ fatty acid	2.984	1.26
Unknown	4.065	0.48
C ₁₄ fatty acid	4.729	0.21
C ₆ diethanolamide	5.155	0.20
C ₁₆ fatty acid	6.356	0.76
C ₈ diethanolamide	6.744	2.34
Monolauroyl glycerol	7.996	0.85
C ₁₀ diethanolamide	8.262	1.68
Unknown	9.200	0.52
Unknown	9.375	0.45
C ₁₂ diethanolamide	9.847	19.46
Unknown	10.736	0.27
C ₁₄ diethanolamide	11.109	6.22
Unknown	11.809	0.26
C ₁₆ diethanolamide	12.447	13.53
C _{18:2} + C _{18:1} diethanolamide	13.395	9.28
C _{18:0} diethanolamide	13.564	2.10
8-(3-octyl-oxiranyl)-octanoic acid bis-(2-hydroxy-ethyl)-amide	14.513	8.38
Unknown	14.670	0.23
Dilauroyl glycerol	15.166	1.03
8-[3-(3-Pentyl-oxiranylmethyl)-oxiranyl]-octanoic acid bis-(2-hydroxy-ethyl)-amide	15.454	1.11
Unknown	16.628	0.50
Dimyristoyl glycerol	16.738	0.44
Dipalmitoyl glycerol	17.833	4.38
Unknown	18.198	0.89
Unknown	18.397	0.34

Table 4: Selected GC-MS fragment ions of trimethylsilyl derivatives of 362 diethanolamides

Trimethylsilyl derivatives of diethanolamide polyols	Retention time (min)	Molecular weight (g mol ⁻¹)	Fragment ions, m/z
N,N-bis(2-(trimethylsilyloxy)ethyl)hexanamide	24.51	347	347 (M+TMS) ⁺ , 332, 214, 146, 73
N,N-bis(2-(trimethylsilyloxy)ethyl)octanamide	27.99	375	375 (M+TMS) ⁺ , 360, 242, 146, 73
N,N-bis(2-(trimethylsilyloxy)ethyl)decanamide	31.30	403	403 (M+TMS) ⁺ , 388, 270, 146, 73
N,N-bis(2-(trimethylsilyloxy)ethyl)dodecanamide	34.43	431	431 (M+TMS) ⁺ , 416, 298, 146, 73
N,N-bis(2-(trimethylsilyloxy)ethyl)Tetradecanamide	37.33	459	459 (M+TMS) ⁺ , 444, 326, 146, 73
N,N-bis(2-(trimethylsilyloxy)ethyl)palmitamide	40.08	487	487 (M+TMS) ⁺ , 472, 354, 146, 73
N,N-bis(2-(trimethylsilyloxy)ethyl)octadec-9-enamide	42.31	513	513 (M+TMS) ⁺ , 498, 380, 146, 73
N,N-bis(2-(trimethylsilyloxy)ethyl)stearamide	42.64	515	515 (M+TMS) ⁺ , 500, 382, 146, 73
8-(3-octyl-oxiran-2-yl)-N,N-bis(2-(trimethylsilyloxy)ethyl)octanamide	44.99	529	529(M+TMS) ⁺ , 514, 396, 146, 73
8-(3-(3-pentylloxiran-2-yl)methyl)oxiran-2-yl)-N,N-bis(2-(trimethylsilyloxy)ethyl)octanamide	47.55	543	543 (M+TMS) ⁺ , 528, 410, 146, 73

Table 3 summarizes the composition and retention time of trimethylsilyl derivatives of fatty diethanolamides, glycerol, DEA, monoglycerides, diglycerides and some minor unknown compounds. Table 4 shows the results of GC-MS analysis by showing the selected GC-MS fragment ions of trimethylsilyl derivatives of diethanolamides.

DISCUSSION

HPLC and LC-MS analyses: To date, the characterization of diethanolamides using HPLC and LC-MS analyses have not been reported yet. Therefore, this study attempted to use a compatible solvent, Acetonitrile (ACN), to dissolve the sample for HPLC analysis. As the diethanolamides consisted of hydroxyl and amide groups that are rather polar, the solvent system of 90:10 (ACN: water) was the most suitable for use in the separation. When the fatty diethanolamide mixtures are analyzed, the retention time between the products and the prepared secondary standards was comparable and most of the major diethanolamide compounds, namely C_{10:0}, C_{12:0}, C_{14:0}, C_{16:0}, C_{18:1} and C_{18:0} were successfully identified as in Table 1. Subsequent characterization of the diethanolamides was continued using LC-MS analysis and the molecular weight of the products were identified (Table 2). HPLC is a convenient method in separation of fatty diethanolamides by only dissolving the samples with mobile phase without any derivatization. However, only non-epoxidized fatty diethanolamides were identified by HPLC analysis, the separation was still not

complete. Different solvent systems, condition of the system had been modified; unfortunately the epoxidized fatty diethanolamides were still failed to separate. Studies may be continued in future by using other type of columns.

GC and GC-MS analyses: GC method has been reported not only in separating diethanolamides products (O'Connell, 1977) but also in obtaining better results when compared with the HPLC method in analyzing palm oil-based polyol esters (Yunus *et al.*, 2002). Thus, GC method was attempted in order to obtain a better and comprehensive separation of the diethanolamide mixtures. A number of organic solvents were used to dissolve the synthesized diethanolamides. Results showed that DMF and THF were the most suitable solvents. The fatty diethanolamides with hydroxyl and amide functionalities are rather polar with low volatility and hence were converted to the less polar trimethylsilyl derivatives prior to injection. Piekos and co-workers reported the conversion of ethanolamines to trimethylsilyl derivatives before gas chromatographic determination by reacting the monoethanolamines, diethanolamines and triethanolamines with N,O-bis(trimethylsilyl)acetamide (BSA) (Piekos *et al.*, 1975). In this study, N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was used to convert diethanolamides to the trimethylsilyl diethanolamide products. The retention time between the products and the prepared secondary standards was comparable and most of the trimethylsilyl-diethanolamides, namely C_{6:0}, C_{8:0}, C_{10:0}, C_{12:0}, C_{14:0},

C_{16:0}, C_{18:2}, C_{18:1}, C_{18:0}, epoxidized C_{18:1} and epoxidized C_{18:2} diethanolamides, were successfully identified. Besides, Glycerol, DEA, fatty acids, monoglycerides and diglycerides were also identified (Table 3). The percentage of the fatty diethanolamide products and glycerol by-products contained in the sample were 64.30 and 21.76%; respectively. Some unknown compounds (3.22%) were detected in the product mixtures. These unknown compounds were most likely diesteramine, N,N-bis(2-hydroxyethyl) piperazine(I), monoesteramine, monoesteramide or diesteramide produced from the self-condensation of DEA and diethanolamide products at elevated temperature (Trowbridge *et al.*, 1955). This observation was consistent with the report on condensation of coconut oil with DEA that yielded 2.8% (w/w) of diesteramine by-products through gas chromatographic analysis (O'Connell, 1977). The molecular weight and structure of the trimethylsilyl-173 derivatized diethanolamides were further characterized using GC-MS analysis (Table 4) The molecular ion (M+TMS)⁺. For the epoxidized diethanolamides, namely 8-(3-octyl-oxiranyl)-octanoic acid bis-(2-trimethylsilyloxyethyl)-amide and 8-[3-(3-Pentyl-oxiranylmethyl)-oxiranyl]-octanoic acid bis-(2-trimethylsilyloxyethyl)-amide, were recorded at 529 and 543 m/z respectively. GC and GC-MS analyses gave complete separation to the reaction mixture containing epoxidized and non-epoxidized fatty diethanolamides. These chromatographic methods are more suitable to be used in monitoring the products of the reaction. The only disadvantage for this method is the conversion of fatty diethanolamides to trimethylsilyl derivatives prior to injection.

CONCLUSION

HPLC and LCMS analyses can be used to analyze fatty diethanolamides from the synthesis of natural triacylglycerols with diethanolamine. GC and GC-MS analyses provide a more complete separation to fatty diethanolamide mixture with epoxidized and non-epoxidized fatty diethanolamides. These chromatographic and spectrometric methods are suitable for reaction monitoring, particularly reactions involving epoxidized oil.

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