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Structural Analysis of Partial and Total Esters of Glycerol Undecenoate and Diglycerol Undecenoate

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Abstract The direct esterification reaction between glycerol and undecylenic acid or between diglycerol and undecylenic acid generates all the possible types of glycerol or diglycerol esters. Purification by silica gel chromatography resulted in the isolation of each of these types of ester in a pure form. The molecular structures of the compounds isolated were characterized and identified by mass spectrometry, ¹H NMR, ¹³C NMR and DEPT-135. We then studied the composition of esters of undecylenic acid formed with glycerol or diglycerol as a function of their reaction conditions, which constitute a highly complex system. We purified undecylenic acid esters from each polyol family to allow the structural identification of each ester of glycerol and each ester of diglycerol with undecylenic acid. We found that the polarity of these non-ionic amphiphilic esters directly affected their affinity for organic and inorganic solvents and that these esters behaved very differently from anionic amphiphilic molecules, such as undecylenic acid.

Keywords Glycerol undecenoate esters · Diglycerol undecenoate esters · Structural analysis · Direct esterification · NMR spectroscopy · Mass spectroscopy

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Introduction

Esters of glycerol and diglycerol are amphiphilic molecules widely used in industry. Glycerol monoesters (GMs) are used as emulsifiers in the food, pharmaceutical and cosmetic industries [1–3]. Glycerol diesters (GDs) play an important role in the lipid domain, because oils containing 80 % GDs are now available on the American and Japanese markets [4]. Diglycerol ester use is rapidly increasing: they are present in mixtures of polyglycerol esters, and they have been shown to reduce tension at the water–air interface [5, 6]. They are used as foaming agents [7, 8] and as coalescence agents [9].

Such esters may be produced by the esterification of fatty acids with either glycerol or diglycerol. Glycerol esters can also be produced by the glycerolysis or partial hydrolysis of plant oils. There are also other more selective pathways, such as the condensation of glycidol with an existing fatty acid [10], but they are not exploited in industry.

In this study, we used undecylenic acid as an acyl donor for the synthesis of partial esters of glycerol and diglycerol. Undecylenic acid is obtained from castor oil by pyrolysis of either the crude oil or the ricinoleic acid methyl esters [11]. This acid is potentially useful, because it has two functional groups: a carboxyl group that can be esterified, and a terminal reactive alkene group.

Partial esters of undecylenic acid, such as glycerol monoundecenoate and glycerol diundecenoate, have been studied. Pace *et al.* [12] and Cauvel *et al.* [13] studied partial esters of glycerol and described the synthesis and structural characterization of the α -monoglyceride, without taking into account the presence of the β regioisomer. Berger and Schnelder [14] proposed a method for enriching the

preparation in the α -monoglyceride; however, this study did not consider other glycerides. Chidambaram *et al.* [15] produced a stoichiometric mixture of the α and β regioisomers of glycerol monoundecenoate from acetal.

Kumar *et al.* [6], in their studies of diglycerol esters, described the synthesis of diglycerol monoundecenoate by esterification, followed by silica column chromatography purification. The structural analysis did not differentiate between the regioisomers. Brito *et al.* [16] synthesized diglycerol tetraundecenoate, with the aim of studying its lubrication properties. The structure of this molecule was confirmed by ^1H and ^{13}C NMR and by mass spectrometry.

Work characterizing and determining the properties of these glycerol and diglycerol esters should always include steps for the separation or purification of the various constituents of the reaction mixtures. Molecular distillation methods have been developed [17–19], and they yield purer ester fractions. However, they generally require the use of high temperatures (240–300 °C) for the distillation of esters with high boiling points, such as the glycerol diesters synthesized from sunflower oil, which has a high oleic acid content (87.6 % oleic acid) [19]. In such situations, there is a high risk of modifying the composition of partial esters through acyl transfer.

Temperature appears to be an important parameter and, at relatively low temperatures (23 °C), close to room temperature, acyl group migration is extremely limited [20–22]. Rapid purification methods at room temperature should make it possible to obtain samples of glycerides without the purification method causing isomerization. One possible approach involves the use of recrystallization methods for purification. However, these methods are difficult to implement when the glycerides present have similar crystallization temperatures, as is the case for partial esters of glycerol and partial esters of diglycerol [23, 24]. Separation by silica gel chromatography is widely used in organic chemistry and is the method that best preserves the structure of glycerol esters [6, 25].

Here, we report a study of the composition of esters of undecylenic acid formed with glycerol or diglycerol, based on their reaction conditions, which constitute a highly complex system. We purified undecylenic acid esters from each polyol family to allow the structural identification of each ester of glycerol and each ester of diglycerol with undecylenic acid.

Materials and Methods

Materials

Glycerol (98 %) was obtained from Sigma-Aldrich, linear diglycerol (84 % α,α' -diglycerol, 14 % α,β -diglycerol,

<1 % β,β' -diglycerol and <0.2 % cyclic isomers) was obtained from Solvay Chemicals, and undecylenic acid (99 %) was obtained from Acros Organic. Dodecylbenzene sulfonic acid (DBSA; ≥ 95 %) was obtained from Sigma-Aldrich. HPLC-quality solvents (cyclohexane, ethyl acetate, chloroform and methanol) were obtained from Aldrich.

Synthesis of Esters of Glycerol and of Diglycerol with Undecylenic Acid

The synthesis of these compounds was adapted from the procedure developed by Eychenne and Mouloungui [26]. The reactions were carried out in a 250-mL batch reactor loaded with 80 g of reaction medium and equipped with a Dean–Stark receiver and a refrigerant, with mechanical stirring at 500 rpm. The reactor was loaded with undecylenic acid and glycerol (molar ratio of 2:1) or with undecylenic acid and diglycerol (molar ratio of 3:1). DBSA was used as the catalyst (molar ratio of 0.125 with respect to undecylenic acid). The reaction was carried out for 3 h, at a temperature of 120 °C. The reaction medium was cooled and washed with saturated NaCl solution (4×100 mL), until a neutral pH was reached, to eliminate the DBSA and any residual glycerol or diglycerol. The molar composition of the glycerol esters reaction medium was 26.6 % glycerol monoundecenoate (GMU), 25.3 % glycerol diundecenoate (GDU), 4.2 % glycerol triundecenoate (GTU) and 43.9 % of residual undecylenic acid (UA). The molar composition of the diglycerol esters reaction medium was 15.2 % diglycerol monoundecenoate (DGMU), 27.6 % diglycerol diundecenoate (DGDU), 30.9 % diglycerol triundecenoate (DGTU), 5.2 % diglycerol tetraundecenoate (DGTeU) and 21.1 % of residual UA. Gas chromatography analyses are detailed in the supporting information. Esters of α,β -diglycerol, β,β' -diglycerol and cyclic glycerol were not identified by gas chromatography or by thin-layer chromatography.

Thin-layer chromatography (TLC) analyses

The analyses were carried out on plates (5×5 cm) coated with silica gel 60 F₂₅₄. The glycerol esters were separated by elution with a 60:40 (v/v) mixture of cyclohexane and ethyl acetate. The diglycerol esters were separated by elution with a 3:97 (v/v) mixture of methanol and chloroform. Esters of glycerol or diglycerol do not absorb in the visible spectrum and have very low levels of absorption for UV light (Fig. 1, supporting information). We were therefore unable to use UV lamps designed for TLC signal detection at 254 or 365 nm. GMU and DGMU absorb at wavelengths of 223 and 220 nm, respectively. We therefore used potassium permanganate detection methods on the TLC plates.

Column Gel Chromatography

We used 19.6-cm-long, 8.5-cm-wide cylindrical Pyrex glass chromatography columns, which we filled with 500 g of silica (60 μm , 60–100 mesh) in the eluent, which was cyclohexane for glycerol esters and chloroform for diglycerol esters. We placed 130 g of Fontainebleau sand on the top of each silica gel column.

Purification of Glycerol Esters

From a 30-g sample, we isolated GTU ($R_f = 0.86$) and then UA ($R_f = 0.66$), with 1.5 L of cyclohexane. GDU ($R_f = 0.52$) was purified with 3 L of a 15:85 (v/v) mixture of ethyl acetate and cyclohexane. Finally, GMU ($R_f = 0.14$) was recovered by elution in 2 L of a 40:60 (v/v) mixture of ethyl acetate and cyclohexane.

Purification of Diglycerol Esters

Thirty grams of reaction mixture was used. DGTeU ($R_f = 0.89$), DGTU ($R_f = 0.52$) and UA ($R_f = 0.47$) were purified by elution with 3 L of chloroform. DGDU ($R_f = 0.10$) and DGMU ($R_f = 0$) were recovered by elution with a 2:98 (v/v) mixture of methanol and chloroform.

Mass Spectrometry

We used a Waters GCT Premier high-resolution mass spectrometer. The samples were dissolved in chloroform. DCI CH_4 ionization techniques were used, with direct introduction and GC/MS coupling, to the determination of exact masses.

NMR

NMR spectra were recorded on a Fourier 300 (300 MHz) spectrometer (Bruker, Karlsruhe, Germany). The acquisition temperature was set to 300 K. The samples were dissolved in deuterated chloroform (CDCl_3). Chemical shifts were determined with tetramethylsilane at 0 ppm (TMS) as the reference. Standard sequences were used for the ^1H , ^{13}C and DEPT-135 NMR spectra.

Results and Discussion

All the components separated by TLC were identified. The retardation factors of glycerol esters and diglycerol esters vary linearly with $\text{Log}P$ (Table 1, and Supporting information Figs. 2 and 3). Molecules having more free OH groups are most strongly retarded by the silica stationary phase by binding to the silanols of the silica. The OH groups act as

both electron donors and hydrogen bond acceptors. Undecylenic acid has a relatively low $\text{Log}P$ value, similar to that of GMU and DGMU. It has a high retardation factor. It is retarded by the silica gel slightly less strongly than GDU or DGTU, whereas it would be expected to be retained much more strongly because it contains both an OH group capable of donating and accepting electrons for hydrogen bonding and a carbonyl group capable of acting as an electron acceptor for hydrogen bonding. Undecylenic acid would be expected to form dimers in organic solvents [27–29]. Such associations between molecules would decrease the capacity of undecylenic acid to interact with the silica stationary phase, which would therefore “see” only the lipophilic part of the undecylenic acid, accounting for the high R_f value.

Esters of Glycerol and Undecylenic Acid

The direct esterification reaction between glycerol and undecylenic acid catalyzed by DBSA can generate three different esters: glycerol mono-, di- and triundecenoate (Table 1). Glycerol has three OH groups, which accept acyl groups during esterification reactions with undecylenic acid. For each group of partial esters of glycerol, two regioisomers are possible: glycerol α -monoundecenoate (α -GMU) and glycerol β -monoundecenoate (β -GMU) for monoglycerides, glycerol α,β -diundecenoate (α,β -GDU) and glycerol α,α' -diundecenoate (α,α' -GDU) for the diglycerides. The expected overall ester was glycerol α,β,α' -triundecenoate (α,β,α' -GTU).

Determination of the Chemical Structures of Esters of Glycerol and Undecylenic Acid, in Ascending Order of Polarity

Glycerol Triundecenoate (GTU) The mass spectrum for the compound corresponding to this retardation factor is presented in Fig. 1a. The peak at m/z 590.45 corresponds to the parent ion of α,β,α' -GTU with the formula $[\text{C}_{36}\text{H}_{62}\text{O}_6]^+$. The m/z 407.32 peak corresponds to $[\text{C}_{25}\text{H}_{43}\text{O}_4]^+$, characteristic of the loss of an undecylenic acid fragment with the formula $[\text{C}_{11}\text{H}_{19}\text{O}_2]^+$. The m/z 241.18 peak $[\text{C}_{14}\text{H}_{25}\text{O}_3]^+$ corresponds to the loss of two undecylenic acid fragments with the formula $[\text{C}_{11}\text{H}_{19}\text{O}_2]^+$. The m/z 166.14 peak corresponds to a fragment with the formula $[\text{C}_{11}\text{H}_{19}\text{O}]^+$.

^1H NMR analysis of this sample (Fig. 1b, Table 2) revealed chemical shifts between 4.08 and 4.19 ppm and between 4.24 and 4.34 ppm, corresponding to the four hydrogens in the α and α' positions of glycerol. The heavily deshielded multiplet between 5.21 and 5.31 ppm corresponds to the H_β hydrogen. The chemical shifts between 0.95 and 1.46 ppm correspond to the hydrogens carried by carbons 4–8 of undecylenic acid. Between 1.53 and 1.70 ppm the chemical shifts correspond to the H_3

Table 1 Molecular structures of the esters of glycerol and diglycerol with undecylenic acid

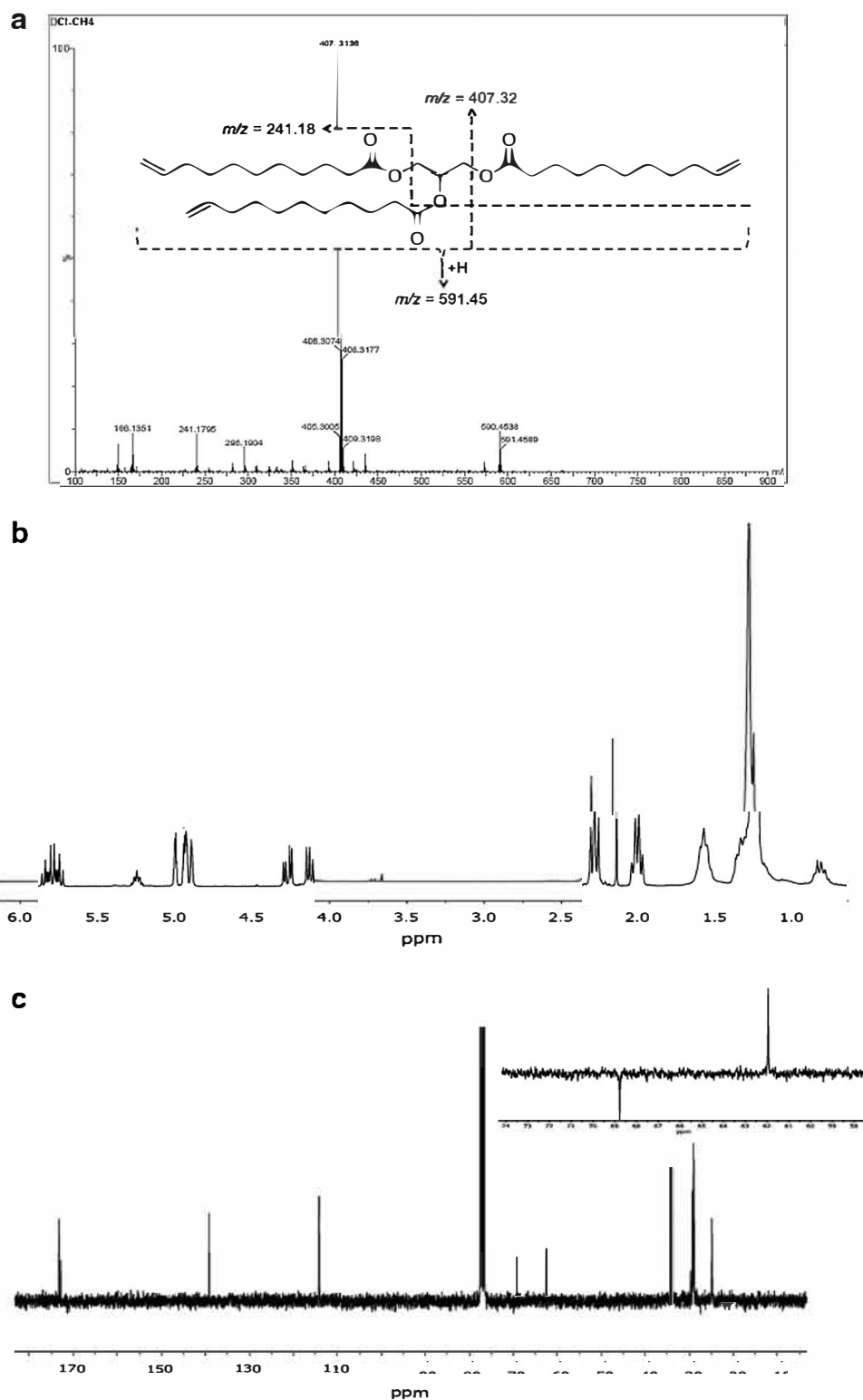
	<p>α-GMU</p> <p>β-GMU</p>	<p>GMU $R_f = 0.14$ $M_w = 258.35 \text{ g.mol}^{-1}$</p>	
<p>α, α'-GDU</p> <p>α, β-GDU</p>	<p>GDU $R_f = 0.52$ $M_w = 424.61 \text{ g.mol}^{-1}$</p>	<p>α, β, α'-GTU</p>	<p>GTU $R_f = 0.86$ $M_w = 590.87 \text{ g.mol}^{-1}$</p>
<p>α-DGMU</p> <p>β-DGMU</p>	<p>DGMU $R_f = 0$ $M_w = 332.43 \text{ g.mol}^{-1}$</p>	<p>α, α'-DGDU</p> <p>α, β-DGDU</p> <p>α, β, γ-DGDU</p>	<p>DGDU $R_f = 0.10$ $M_w = 498.69 \text{ g.mol}^{-1}$</p>
<p>α, β, α'-DGTU</p> <p>α, β, β'-DGTU</p>	<p>DGTU $R_f = 0.52$ $M_w = 664.95 \text{ g.mol}^{-1}$</p>	<p>$\alpha, \beta, \beta', \alpha'$-DGT<u>e</u>U</p>	<p>DGT<u>e</u>U $R_f = 0.89$ $M_w = 833.21 \text{ g.mol}^{-1}$</p>

GMU glycerol monoundecenoate, GDU glycerol diundecenoate, GTU glycerol triundecenoate, DGMU diglycerol monoundecenoate, DGDU diglycerol diundecenoate, DGTU diglycerol triundecenoate, DGTeU diglycerol tetraundecenoate, R_f TLC retardation factor, M_w molecular weight

hydrogen, between 1.97 and 2.09 ppm to H_9 and between 2.24 and 2.37 ppm to the H_2 hydrogen. Those between 4.88 and 5.04 ppm and between 5.72 and 5.89 ppm correspond to the hydrogens carried by the ethylenic carbon residues at the end of the undecylenic acid chain, H_{11} and

H_{10} , respectively. These characteristics of undecylenic acid were common for all glycerol esters and will not be discussed in the further sections. These findings are characteristic of triglycerides [30, 31], such as α, β, α' -GTU in particular.

Fig. 1 a Mass spectrum of GTU, b ^1H NMR spectrum of GTU, and c ^{13}C NMR and ^{13}C DEPT-135 spectra of GTU



The ^{13}C NMR spectrum of GTU (Fig. 1c, Table 3) contains the chemical shift at 62.25 ppm that is characteristic of the α and α' carbons of glycerol. The shift at 69.04 ppm is characteristic of the β carbon. This was confirmed by the DEPT-135 analysis, in which the α and α' carbons give a

positive signal, whereas the β carbon gives a negative signal. The chemical shifts characteristic of the carbons of the undecylenic acid, e.g. at 114.13 and at 139.19 ppm which are characteristic of the ethylenic carbon residues C_{11} and C_{10} , are common for all glycerol esters. They are

Table 2 ^1H NMR chemical shifts of glycerol and diglycerol esters of undecylenic acid

Molecules	Chemical shifts (ppm)	Hydrogens
GTU	4.08–4.19	α
	4.24–4.34	α'
	5.21–5.31	β
GDU	4.00–4.35	α, β, α'
	5.04–5.12	β_1
	3.69–3.75	$\alpha'1$
GMU	4.07–4.27	α
	3.54–3.75	α'
	3.87–3.98	β
DGT <u>e</u> U	3.80–3.85	α_1, α'
	3.45–3.80	γ, γ'
	4.00–4.40	α, α'
DGTU	5.10–5.25	β, β'
	3.21–4.46	$\alpha, \gamma, \gamma', \beta', \alpha'$
	5.12–5.25	β
DGDU	3.43–3.86	β, β'
	3.95–4.25	α, α'
	5.14–5.28	β_1
DGMU	5.35–5.45	β_2, β_2'
	4.12–4.24	α
	4.06–4.12	β
	3.97–4.06	β'
	3.73–3.89	α'
Common shifts of undecylenic chain	3.45–3.72	γ, γ'
	2.24–2.37	2
	1.53–1.70	3
	0.95–1.46	4–8
	1.97–2.09	9
	5.72–5.89	10
	4.88–5.04	11

GMU glycerol monoundecenoate, *GDU* glycerol diundecenoate, *GTU* glycerol triundecenoate, *DGMU* diglycerol monoundecenoate, *DGDU* diglycerol diundecenoate, *DGTU* diglycerol triundecenoate, *DGTeU* diglycerol tetratundecenoate

reported in the Table 3 and will not be discussed further. This analysis is consistent with a triglyceride [32], such as α, β, α' -GTU.

Glycerol Diundecenoates (GDU) The mass spectrum of this component (Table 4) contained a peak at m/z 424.32, with the formula $\text{C}_{25}\text{H}_{44}\text{O}_5$, corresponding to the exact molecular mass of α, α' -GDU or α, β -GDU. The fragment at m/z 407.32 resulted from the loss of a water molecule from GDU. The fragment at m/z 241.18 corresponds to the loss of an undecylenic fragment with the formula $[\text{C}_{11}\text{H}_{19}\text{O}_2]^+$ and reveals the presence of an ionized fragment of glycerol monoundecenoate with the crude formula $[\text{C}_{14}\text{H}_{25}\text{O}_3]^+$. The

Table 3 ^{13}C NMR chemical shifts of glycerol and diglycerol esters of undecylenic acid

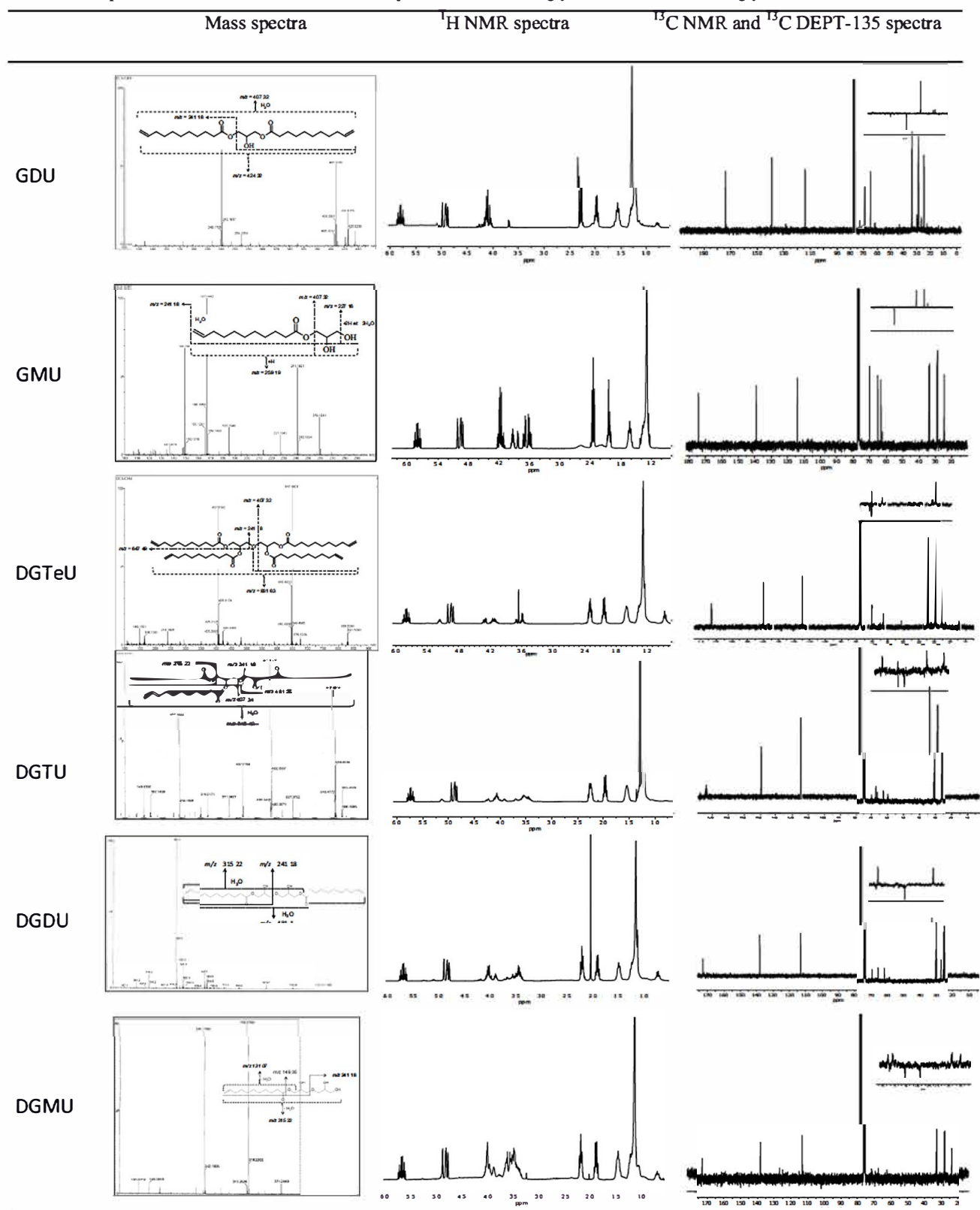
Molecules	Chemical shifts (ppm)	Carbons
GTU	62.25	α, α'
	69.04	β
GDU	65.02	α, α'
	68.37	β
	61.70	α_1
	62.16	$\alpha'1$
GMU	72.26	β_1
	70.48	α'
	65.52	β
DGT <u>e</u> U	63.70	α
	62.98	α, α'
	69.70	γ, γ'
DGTU	69.90	β, β'
	69.98	γ
	72.55	γ'
DGDU	69.93	β
	68.88	β'
	62.42	α
	65.24	α'
	68.77	α, α'
DGMU	69.06	β, β'
	72.25	γ, γ'
	72.25	α
	70.66	β
	68.77	β'
Common shifts of undecylenic chain	64.71	γ
	64.82	γ'
	63.63	α'
	72.80	β_1
	173.13–174.68	1
	24.84–34.27	2–9
	139.13–139.50	10
	114.13–114.50	11

GMU glycerol monoundecenoate, *GDU* glycerol diundecenoate, *GTU* glycerol triundecenoate, *DGMU* diglycerol monoundecenoate, *DGDU* diglycerol diundecenoate, *DGTU* diglycerol triundecenoate, *DGTeU* diglycerol tetratundecenoate

crude formula of GDU is clearly identified, but nevertheless does not differentiate between the α, α' -GDU and α, β -GDU isomers.

^1H NMR is a technique that is very suitable for the identification and quantification of regioisomers of diacylglycerol [19]. On the spectrum shown at the top of Table 4, the multiplet between 4.0 and 4.35 ppm corresponds to the five H_α , $\text{H}_{\alpha'}$ and H_β hydrogens of the glycerol of α, α' -GDU (Table 2). The chemical shifts between 5.04 and 5.12 ppm correspond to the H_{β_1} hydrogen of the α, β -GDU isomer.

Table 4 Mass spectra and ^1H , ^{13}C and DEPT-135 NMR spectra of the esters of glycerol undecenoate and diglycerol undecenoate



GMU glycerol monoundecenoate, GDU glycerol diundecenoate, DGMU diglycerol monoundecenoate, DGDU diglycerol diundecenoate, DGTU diglycerol triundecenoate, DGTeU diglycerol tetraundecenoate

The doublet between 3.69 and 3.75 ppm corresponds to the two H_{α_1} hydrogens characteristic of the presence of the α,β -GDU regioisomer. These findings are consistent with those of Hatzakis *et al.* [33]. It was thus possible to show that there was 82 % α,α' -GDU and 18 % α,β -GDU in the GDU sample of R_f 0.52.

On the ^{13}C NMR spectrum (Tables 3, 4), the chemical shifts corresponding to the undecylenic chains of GDU were found. On DEPT-135 analysis, the chemical shifts of C_α and $C_{\alpha'}$ at 65.02 ppm, C_{α_1} at 61.70 and $C_{\alpha_1'}$ at 62.16 ppm gave positive signals. This confirms the presence of the two regioisomers, α,α' -GDU and α,β -GDU, consistent with the attributions of chemical shifts published for other diacylglycerols [33].

Glycerol Monoundecenoates (GMU) The mass spectrometry analysis for this sample (Table 4) showed that the peak at m/z 259.19 corresponded to the molecular mass of the ionized molecule $[\text{C}_{14}\text{H}_{27}\text{O}_4]^+$, and the crude formula $\text{C}_{14}\text{H}_{26}\text{O}_4$ of α -GMU and β -GMU. The peak at m/z 241.18 ($[\text{C}_{14}\text{H}_{25}\text{O}_3]^+$) corresponds to the loss of a molecule of water from GMU. The peak at m/z 185.15 corresponds to a molecule with the crude formula of $[\text{C}_{11}\text{H}_{21}\text{O}_2]^+$. The loss of a molecule of water from this molecule gives rise to the peak at 167.14, with a crude formula of $[\text{C}_{11}\text{H}_{19}\text{O}]^+$. The peak at m/z 149.13 corresponds to the loss of a molecule of water from $[\text{C}_{11}\text{H}_{19}\text{O}]^+$, yielding $[\text{C}_{11}\text{H}_{17}]^+$.

^1H NMR analysis (Tables 2, 4) showed that the chemical shifts between 3.87 and 3.98 ppm corresponded to the hydrogens in positions H_α , H_{α_1} and H_1 of glycerol. H_α hydrogens display a chemical shift between 4.07 and 4.27 ppm. These hydrogens are more deshielded than the H_β hydrogen (3.87–3.98 ppm). The two $H_{\alpha'}$ hydrogens of α -GMU yield a multiplet between 3.54 and 3.75 ppm. Following esterification of the hydroxyl site in the α position of glycerol, the two H_α hydrogens become more deshielded than the $H_{\alpha'}$ hydrogens and they have a higher chemical shift (4.07 and 4.27 ppm). This difference in chemical shift between H_α and $H_{\alpha'}$ is due to the ester group, which is electron withdrawing. These results thus confirm the presence of the α -GMU regioisomer.

We can also see the doublet between 3.80 and 3.85 ppm corresponding to the four H_{α_1} and $H_{\alpha_1'}$ hydrogens of β -GMU. This finding is consistent with those of Compton *et al.* [20, 34] for other monoglycerides, making it possible to quantify the relative proportions of the two regioisomers, α -GMU and β -GMU. We found that β -GMU accounted for 7.64 %, and that α -GMU accounted for 92.34 % of the sample with R_f 0.14.

On the ^{13}C NMR spectrum (Table 4), the chemical shifts at 70.62, 65.52 and 63.70 ppm correspond to C_α , C_β and $C_{\alpha'}$, respectively (Table 3). Analysis by DEPT-135 confirms the identification of C_α at 63.48 ppm, $C_{\alpha'}$ at 65.52 and C_β

at 70.48 ppm. The chemical shifts characteristic of undecylenic chain were found. This spectrum confirms the structure of α -GMU. It was not possible to detect the shifts characteristic of β -GMU because of the limited sensitivity of the apparatus used.

Esters of Diglycerol and Undecylenic Acid The esterification reaction between undecylenic acid and linear diglycerol can generate four types of diglycerol: mono-, di-, tri- and tetraesters of diglycerol (Table 1). Several isomers of these types of linear diglycerol ester are likely to be present: (1) α -DGMU and β -DGMU for diglycerol monoesters; (2) four isomers of diglycerol diundecenoate: α,α' -DGDU, α,β -DGDU, β,β' -DGDU and α,β' -DGDU; (3) two types of diglycerol triundecenoates: α,β,α' -DGTU and α,β,β' -DGTU and (4) $\alpha,\beta,\alpha',\beta'$ -DGTeU. It is important to detect the presence of each regioisomer and to determine their relative proportions in the reaction mixture reliably, particularly as not all the molecular structures of these molecules have been published. We used silica gel chromatography for purification, with TLC as an elution control. Spectroscopic methods were used to determine the structures associated with the retardation factors and the proportions of the various regioisomers identified.

Qualitative analysis of the reaction mixture by TLC revealed the presence of various molecules with different R_f values, from the least to the most polar: $R_f = 0.89, 0.61, 0.47, 0.10, 0$. The component with an R_f of 0.47 is undecylenic acid. It was identified by comparison with a standard.

Determination of Chemical Structures on the Basis of Increasing Polarity of Esters of Diglycerol and Undecylenic Acid

Diglycerol Tetraundecenoate (DGTeU) The mass spectrum for the molecule with an R_f of 0.89 is presented in Table 4. The fragment with m/z 831.63 has a molecular mass corresponding to that of ionized diglycerol tetraundecenoate. The fragment at m/z 649.49 corresponds to the loss of an undecylenic fragment. The m/z 407.32 fragment corresponds to the loss of half the diglycerol tetraundecenoate molecule. The fragment at m/z 241.18 corresponds to an ionized fragment of glycerol monoundecenoate. The presence of these various fragments identifies the molecule obtained as diglycerol tetraundecenoate.

On the ^1H NMR spectrum (Tables 2, 4), the structure of DGTeU is identified by the presence of hydrogens H_γ and H_γ' , specific to the carbons carrying the ether group of diglycerol, for which a multiplet is observed between 3.45 and 3.80 ppm. The H_α and $H_{\alpha'}$ hydrogens are identified by the presence of a multiplet between 4.00 and 4.40 ppm. The chemical shifts between 5.10 and 5.25 ppm correspond to the H_β and $H_{\beta'}$ hydrogens.

On the ^{13}C NMR spectrum (Tables 3, 4), the DEPT-135 experiment distinguishes between CH and CH_2 . The chemical shift at 62.98 ppm is characteristic of C_α and $\text{C}_{\alpha'}$. The shift at 69.70 ppm is characteristic of C_γ and $\text{C}_{\gamma'}$, and that at 69.90 ppm is characteristic of C_β and $\text{C}_{\beta'}$. This profile corresponds exactly to the three types of carbon present in diglycerol triundecenoate. We thus find all the characteristic carbons of the undecylenic chain. No discussion of the attribution of the diglycerol carbons in diglycerol triundecenoate has ever been published. Only the carbonyl group carbons of the ester, at 173.16 ppm, and those of the terminal double bond have been identified [16].

Diglycerol Triundecenoate (DGTU) Diglycerol triundecenoate has a molecular mass of 664.952 g/mol. The loss of a water molecule thus yields a fragment at m/z 648.494 (Table 4). The fragment at m/z 481.350 results from the loss of an undecylenic chain. This fragment was also observed on the mass spectrum of DGDU. The peak at m/z 407.34 corresponds to the loss of a glycerol monoester fragment. The fragments at m/z 315.217 and 241.180 correspond to the loss of two undecylenic chains from diglycerol triundecenoate and the loss of a glycerol monoester fragment and an undecylenic chain, respectively. The presence of these fragments indicates that the molecule isolated is diglycerol triundecenoate.

On the ^1H NMR spectrum (Table 4), the multiplet between 3.21 and 4.46 ppm corresponds to the nine hydrogens of diglycerol: the H_α , H_γ , $\text{H}_{\gamma'}$, $\text{H}_{\beta'}$ and $\text{H}_{\alpha'}$ hydrogens (Table 2). The H_β hydrogen characteristic of DGTU is deshielded and visible between 5.12 and 5.25 ppm. It yields a multiplet. We observed none of the hydrogens characteristic of the α,β,β' -diglycerol triundecenoate isomer.

On the ^{13}C NMR spectrum for this sample (Table 4) the carbons carrying the ether group, C_γ and $\text{C}_{\gamma'}$, are visible at 69.98 and 72.55 ppm. The strong deshielding of the C_γ carbon results from its close proximity to the CH carbon carrying the OH group. The C_γ close to the CH of the esterified C_β is more shielded. The chemical shifts (Table 4) of the C_β and $\text{C}_{\beta'}$ carbons can be seen at 69.93 and 68.88 ppm, respectively. The esterified C_α and $\text{C}_{\alpha'}$ carbons display shifts at 62.42 and 65.24 ppm. We can also see the carbons of undecylenic chain. This spectrum is characteristic of α,β,α' -DGTU. No chemical shift indicative of the presence of the α,β,β' -DGTU was detected.

Diglycerol Diundecylenate (DGDU) The characterization of this sample by mass spectrometry (Table 4) revealed the presence of a fragment at m/z 481.1 corresponding to the loss of a molecule of water from DGDU. The fragment at m/z 315.22 corresponds to the loss of an undecylenic fragment from the symmetric diester. The fragment at m/z 241.18 corresponds to the loss of a part of DGDU consist-

ing of the glycerol and the undecylenic chain. On the basis of these fragments, this molecule was identified as DGDU.

The ^1H NMR analysis (Tables 2, 4) shows the two multiplets between 3.43 and 3.86 ppm and between 3.95 and 4.25 ppm corresponding to the two types of hydrogens on the diglycerol head. The least deshielded hydrogens, between 3.43 and 3.86 ppm, correspond to the H_β and $\text{H}_{\beta'}$ hydrogens, and the most deshielded hydrogens, between 3.95 and 4.25 ppm, correspond to the four H_α and $\text{H}_{\alpha'}$ hydrogens.

The peaks between 5.14 and 5.28 ppm and between 5.35 and 5.45 ppm are two multiplets that correspond to hydrogen $\text{H}_{\beta 1}$ of α,β -DGDU, and hydrogens $\text{H}_{\beta 2}$ and $\text{H}_{\beta' 2}$ of β,β' -DGDU, respectively.

The regioisomer composition of the isolated mixture of diglycerol diesters can be calculated on the basis of the integration of the characteristic peaks for each diglycerol ester: 73.8 % α,α' -DGDU, 22.8 % α,β -DGDU and 3.4 % β,β' -DGDU. The α,β' -DGDU form was not detected.

On the ^{13}C NMR spectrum shown in Table 4, there are six peaks corresponding to the six carbons of diglycerol (Table 3). These peaks are grouped in twos, at 68.77 ppm for the C_α and $\text{C}_{\alpha'}$ carbons, and 69.06 ppm (C_{16} and C_{13}), corresponding to the two CH carbons carrying the secondary hydroxyl groups of diglycerol. The peak at 72.25 ppm corresponds to the C_γ and $\text{C}_{\gamma'}$ carbons; this peak shows that the esterification of diglycerol with undecylenic acid occurs via the primary hydroxyl groups in positions α and α' . This analysis confirms that the molecule isolated is the symmetric molecule α,α' -DGDU. The DEPT-135 analysis confirmed the presence of the α,β -DGDU and β,β' -DGDU regioisomers, because the C_{α} , $\text{C}_{\alpha'}$, C_γ and $\text{C}_{\gamma'}$ yielded a positive signal, corresponding to CH_2 groups. The C_β and $\text{C}_{\beta'}$ carbons gave a negative signal, corresponding to CH. We did not detect the carbons corresponding to the α,β' -DGDU form.

Diglycerol Monoundecylenate (DGMU) Table 4 shows the mass spectrum for this sample. The fragment at m/z 315.21 corresponds to the loss of a water molecule from DGMU, during its ionization. The peak at m/z 241.18 corresponds to the loss of a glycerol fragment from α -DGMU and β -DGMU. The peak at m/z 149.08 results from the loss of the diglycerol fragment, and the peak at m/z 131.07 corresponds to the loss of a water molecule from this fragment, during its ionization.

On the ^1H NMR spectrum of Table 4, the multiplet between 3.45 and 4.25 ppm corresponds to the hydrogens characteristic of the linear diglycerol head of α -DGMU (Table 2). The peak between 4.12 and 4.24 ppm corresponds to the H_α hydrogens. The peaks between 4.06 and 4.12 ppm, and between 3.97 and 4.06 ppm correspond to the H_β and $\text{H}_{\beta'}$ hydrogens. The chemical shift between 3.73

and 3.89 ppm is attributed to the $H_{\alpha'}$ hydrogens, and the multiplet between 3.45 and 3.72 ppm corresponds to the four H_{γ} and $H_{\gamma'}$ hydrogens. The complexity of the multiplet between 3.4 and 4.3 ppm suggests that the β -DGMU regioisomer may be present, although its characteristic shifts are not visible. Additional analyses are required to confirm or exclude this hypothesis.

On the ^{13}C NMR spectrum (Table 4), the presence of the α -DGMU form was confirmed by the chemical shift at 72.25 ppm, corresponding to the C_{α} carbon bearing the esterified hydroxyl group of the diglycerol (Table 3). The chemical shift at 70.66 ppm corresponds to the C_{β} carbon, and that at 68.77 ppm corresponds to $C_{\beta'}$. The C_{γ} and $C_{\gamma'}$ carbons give rise to shifts at 64.71 and 64.82 ppm. Finally, the $C_{\alpha'}$ carbon bearing the primary hydroxyl group yields a shift at 63.63 ppm. The $C_{\alpha'}$, C_{γ} , $C_{\gamma'}$, $C_{\beta'}$ and C_{α} carbons gave positive signals on DEPT-135 analysis; they correspond to the CH_2 groups of α -MUG. The chemical shift at 72.82 ppm corresponds to $\text{CH}_2\text{-OH}$, confirming the presence of β -DGMU, as suggested by the ^1H NMR spectrum.

Conclusion

The direct, non-selective esterification reaction between glycerol and undecylenic acid or between diglycerol and undecylenic acid generates all the possible positional isomers of glycerol or diglycerol esters. Isolation of the isomers in high purity was achieved by silica gel chromatography. The molecular structures of the isolated compounds were characterized and identified by mass spectrometry, ^1H NMR, ^{13}C NMR and DEPT-135. The polarity of these non-ionic amphiphilic esters directly affected their affinity for organic and inorganic solvents and were found to behave very differently from carboxylic acid amphiphilic molecules, such as undecylenic acid.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

1. Van Haften JL (1976) Fat-based food emulsifiers. *J Am Oil Chem Soc* 53:831A-835A
2. Henry C (1995) Monoacylglycerides: the universal emulsifiers. *Cereal Foods World* 40:734-738
3. Young FVK, Poot C, Biernoth E, Krog N, Davidson NGJ, Gunstone FD (1994) In: Gunstone FD, Harwood JL, Padley FB (eds) *Lipid handbook*. Chapman & Hall, London
4. Wang Y, Zhao M, Song K, Wang L, Han X, Tang S, Wang Y (2010) Separation of diacylglycerols from enzymatically hydrolyzed soybean oil by molecular distillation. *Sep Purif Technol* 75:114-120
5. Holstborg J, Pedersen BV, Krog N, Olesen SK (1999) Physical properties of diglycerol esters in relation to rheology and stability of protein-stabilised emulsions. *Colloids Surf B* 12:383-390
6. Kumar TN, Sastry YSR, Lakshminarayana G (1989) Preparation and surfactant properties of diglycerol esters of fatty acids. *J Am Oil Chem Soc* 66:153-157
7. Shrestha LK, Shrestha RG, Solans C, Aramaki K (2007) Effect of water on foaming properties of diglycerol fatty acid ester-oil systems. *Langmuir* 23:6918-6926
8. Kunieda H, Shrestha LK, Acharya DP, Kato H, Takase Y, Gutiérrez JM (2007) Super-stable nonaqueous foams in diglycerol fatty acid esters-non polar oil systems. *J Disper Sci Technol* 28:133-142
9. Matsumiya K, Nakanishi K, Matsumura Y (2014) Destabilization of protein-based emulsions by diglycerol esters of fatty acids—The importance of chain length similarity between dispersed oil molecules and fatty acid residues of the emulsifier. *Food Hydrocoll* 25:773-780
10. Mouloungui Z, Rakotondrazafy V, Valentin R, Zebib B (2009) Synthesis of glycerol 1-monooleate by condensation of oleic acid with glycidol catalyzed by anion-exchange resin in aqueous organic polymorphic system. *Ind Eng Chem Res* 48:6949-6956
11. Van der Steen M, Stevens CV (2009) Undecylenic acid: a valuable and physiologically active renewable building block from castor oil. *Chem Sus Chem* 2:692-713
12. Pace S, Gonzalez P, Devoisselle J-M, Milhiet P-E, Brunel D, Cunin F (2010) Grafting of monoglyceride molecules for the design of hydrophilic and stable porous silicon surfaces. *New J Chem* 34:29-33
13. Cauvel A, Renard G, Brunel D (1997) Monoglyceride synthesis by heterogeneous catalysis using MCM-41 type silicas functionalized with amino groups. *J Org Chem* 62:749-751
14. Berger M, Schnelder M (1992) Enzymatic esterification of glycerol II. Lipase-catalyzed synthesis of regioisomerically pure 1(3)-rac-monoacylglycerols. *J Am Oil Chem Soc* 69:961-965
15. Chidambaram N, Bhat S, Chandrasekaran S (1992) A highly selective methodology for the direct conversion of acetals to esters. *J Org Chem* 57:5013-5015
16. Brito DHA, Cavalcante IM, Rocha NRC, Maier ME, Lima APD, Schanz MTGF, Ricardo NMPS (2014) Synthesis and characterization of diglycerol tetraundecylenate as potential biolubricant. In: Abstract of the IX Congresso Brasileiro de Análise Térmica E Calorimetria, Serra Negra, SP, Brasil, 2-12 November 2014
17. Fregolente LV, Fregolente PBL, Chicuta AM, Batistella CB, Maciel Filho R, Wolf-Maciel MR (2007) Effect of operating conditions on the concentration of monoglycerides using molecular distillation. *Chem Eng Res Des* 85:1524-1528
18. Szelag H, Zwierzykowski W (1983) The application of molecular distillation to obtain high concentration of monoglycerides. *Eur J Lipid Sci Technol* 85:443-446
19. Compton DL, Laszlo JA, Eller FJ, Taylor SL (2008) Purification of 1,2-diacylglycerols from vegetable oils: comparison of molecular distillation and liquid CO_2 extraction. *Ind Crop Prod* 28:113-121
20. Compton D, Vermillion K, Laszlo J (2007) Acyl migration kinetics of 2-monoacylglycerols from soybean oil via ^1H NMR. *J Am Oil Chem Soc* 84:343-348
21. Boswinkel G, Derksen J, Van't Riet K, Cuperus F (1996) Kinetics of acyl migration in monoglycerides and dependence on acyl chainlength. *J Am Oil Chem Soc* 73:707-711

22. Fureby AM, Virto C, Adlercreutz P, Mattiasson B (1996) Acyl group migrations in 2-monoolein. *Biocatal Biotransform* 14:89–111
23. Goldsmith HA (1943) Polyhydric alcohol esters of fatty acids. Their preparation, properties, and uses. *Chem Rev* 33:257–349
24. Shrestha LK, Shrestha RG, Iwanaga T, Aramaki K (2007) Aqueous phase behavior of diglycerol fatty acid esters. *J Disper Sci Technol* 28:883–891
25. Quinlin P, Weiser H (1958) Separation and determination of mono-, di-, and triglycerides in monoglyceride concentrates. *J Am Oil Chem Soc* 35:325–327
26. Eychenne V, Mouloungui Z (1999) High concentration of 1-(3-) monoglycerides by direct partial esterification of fatty acids with glycerol. *Lipid* 101:424–427
27. Bellamy LJ, Lake RF, Pace RJ (1963) Hydrogen bonding in carboxylic acids—II. Monocarboxylic acids. *Spectrochim Acta* 19:443–449
28. Excoffon P, Marechal Y (1972) Infrared spectra of H-bonded systems: saturated carboxylic acid dimers. *Spectrochim Acta Part A* 28:269–283
29. Lacaze-Dufaure C, Mouloungui Z (2000) Catalysed or uncatalysed esterification reaction of oleic acid with 2-ethyl hexanol. *Appl Catal A Gen* 204:223–227
30. Sacchi R, Addeo F, Paolillo L (1997) ¹H and ¹³C NMR of virgin olive oil. An overview. *Magn Reson Chem* 35:S133–S145
31. Knothe G, Kenar JA (2004) Determination of the fatty acid profile by ¹H-NMR spectroscopy. *Eur J Lipid Sci Technol* 106:88–96
32. Vlahov G, Angelo CS (1996) The structure of triglycerides of monovarietal olive oils: a ¹³C-NMR comparative study. *Lipid* 98:203–205
33. Hatzakis E, Agiomyrgianaki A, Kostidis S, Dais P (2011) High-resolution NMR spectroscopy: an alternative fast tool for qualitative and quantitative analysis of diacylglycerol (DAG) oil. *J Am Oil Chem Soc* 88:1695–1708
34. Compton D, Laszlo J, Appell M, Vermillion K, Evans K (2014) Synthesis, purification, and acyl migration kinetics of 2-monoricinoleoylglycerol. *J Am Oil Chem Soc* 91:271–279